III EXPERIMENTAL DESIGN

The experimental design is presented under the following heads:

A. Site And Target Selection
B. Benchmark Survey
C. Study Of Food Beliefs
D. Assessing The Nutritional Status Of The Expectant Mothers
   1. Recording the food and nutrient intake
   2. Clinical examination
   3. Biochemical analysis of selected nutrients and body metabolites
   4. Microbiological assay of folic acid
   5. Recording the weight gains and height
E. Recording Details Related To Past And Present Pregnancy
   1. Assessment of gestation age
   2. Obstetric performance
   3. History of previous pregnancies
   4. Estimating the cost of pregnancy wastage
   5. Health status of the elder children in the family
   6. Recording the amenorrheal period
F. Recording The Anthropometric Measurements Of The Newborn
G. Assessing The Nutritional Status Of The Nursing Mothers
H. Lactation Performance Of Nursing Mothers

I. Growth Performance Of The Extramemostate Fetus

1. Anthropometric measurements
2. Physical and motor development
3. Health status of the infants
4. Weaning foods given

J. Impact Of Selected Nutritional Intervention Programmes

1. Studying the impact of food supplements distributed in the Applied Nutrition Programme, to the expectant mothers
2. Impact of iron and folic acid therapy
3. Impact of vitamin A and iron therapy

A. Site And Target Selections

The following hospitals in the Coimbatore urban and villages in the rural area were selected as sites for carrying out the work.

1. Urban:

The following five municipal Maternity Centres in Coimbatore city namely:

a. Jail Road Maternity Centre
b. Seethalakshmi Maternity Centre
c. C.T.M.O. Maternity Centre
d. Vaisyal Street Maternity Centre and
e. P.L.M. Home
and four hospitals, namely:

a. Government Medical College Hospital

b. Kuppuswamy Naidu Memorial Hospital for Women and Children

c. Isaiah Nursing Home and

d. Balaji Nursing Home

were chosen to draw out the sample of expectant mothers

2. Rural:

Pannirvelai village was chosen to select the sample of expectant mothers for studying the impact of the food supplements provided in the Applied Nutrition Programme and poonchisur village served as a control village.

The selection of maternity centres in the Municipal area, the hospitals in the Coimbatore urban area and the two villages in the rural area were based on the following criteria.

a. The municipal maternity centres were under the jurisdiction of the Coimbatore Municipality and hence had easy approach

b. The maternity centres had an antenatal clinic and expectant mothers came regularly for health check up.

c. Expectant mothers of different income levels and dietary intake were available and the authorities and doctors were cooperative. The low income group was mainly from the Government Hospital and maternity Centres, while the high income group was from the private hospitals
The selection of the expectant mothers was based on the following criteria.

1. Age of the expectant mothers
2. Stage of pregnancy
3. Parity of pregnancy
4. Socio economic status

1. Age of expectant mothers:

Expectant mothers between the ages of 21-30 years were included in the study. On the whole 2063 mothers were studied. Because of the problems of anxiety and lack of understanding on the part of the mothers, it took a long time to develop rapport and establish good relationship with these mothers, before actually recording the required data and collection of food and biological samples.

2. Stage of Pregnancy:

Significant changes in weight gain and in the growth of the foetus takes place only in the second and third trimester of pregnancy, according to Iyengar (1969), Ramalingaswamy (1975) and Singh and Borkotoky (1975). Because of time limitations and reluctance on the part of the expectant mothers to disclose the fact that they were expecting, the study could be started only when the mother was in the 5th or 6th month of pregnancy.
3. **Parity of pregnancy**

In the selection of the expectant mothers care was taken to see that all the mothers were in the first, second or third para of pregnancy. There are evidences in the studies carried out by Maitra *et al.* (1970), Mukherjee and Sethna (1970) and Beal (1971) to prove that if there is a vast difference in the para of pregnancy it may affect the nutritional status of the newborn.

All the mothers were residents of Coimbatore city, cooperative and accessible to the investigator.

4. **Socio-economic status**

Mothers in the low income group (per capita income less than Rs. 100/month) and high income group (per capita income above Rs. 200/month) were studied. (Government of India, 1977/75). In other words, the target expectant mothers were grouped into two categories, namely, low income and high income groups.

5. **Rashmi Mark Survey**

Information pertaining to the total income of the family, size and type of family, educational qualifications of the parents and their occupation was collected through an interview schedule. The proforma used for the interview schedule is presented in Appendix I.
C. Study Of Food Beliefs

It has been observed time and again that expectant mothers have strong food habits, beliefs and attitudes about certain foods. It may be worth finding out these food beliefs to help in the long run to find out as to whether they really have any beneficial or untoward effects on the health of the mother. This information would also help to understand the cultural and socio-economic dimensions to be ensured in future nutrition educational efforts. Hence, the interview schedule contained questions regarding the food beliefs of the expectant mothers.

D. Assessing The Nutritional Status Of The Expectant Mothers

1. Recording the food and nutrient intake

One of the best ways of finding out the nutritional status of the expectant mothers is to find out quantitatively the various kinds of foods consumed by the mothers. For this purpose, a three-day food weighing survey was carried out for a sub-sample of 66 mothers in the low income and 34 mothers in the high income group. The mean that the investigator had to be with the target families throughout the period of weighing. Four families could be covered at a stretch. All the raw foods used for cooking were weighed in
the raw form and the total cooked food weight was recorded. Feed was served to the expectant mothers and the remaining items of the food were weighed again to find out the exact amount of food consumed by the expectant mothers. Raw equivalents for these food items were calculated next. The nutrients available from this food intake was computed using the 'Nutritive value of Indian Foods by Gopalan et al., (1978). The proforma used for the food weighing survey is presented in Appendix II.

2. Clinical examination:

As most of the expectant mothers in India are reported to suffer from a variety of deficiency diseases, a clinical examination was conducted in the third trimester of pregnancy on all the expectant mothers with the help of a trained gynecologist using the proforma prepared by the investigator in accordance with the guidelines given by Jelliffe (1966).

3. Biochemical analysis of selected nutrients and body metabolites:

Selected nutrients and metabolites present in the serum, urine and amniotic fluid were analysed for a sub-sample of cooperative expectant mothers. The details of the biochemical analysis of the nutrients and metabolites and the sample size are presented in Table I.
TABLE I

NUTRIENTS AND METABOLITES ANALYSED AND SAMPLE SIZE

<table>
<thead>
<tr>
<th>Nutrients and metabolites</th>
<th>Number of mothers on whom analysis was done</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low Income mothers</td>
</tr>
<tr>
<td>1. Total protein, albumin and globulin</td>
<td>86</td>
</tr>
<tr>
<td>2. Haemoglobin</td>
<td>499</td>
</tr>
<tr>
<td>3. Serum iron</td>
<td>63</td>
</tr>
<tr>
<td>4. Total iron binding capacity</td>
<td>63</td>
</tr>
<tr>
<td>5. Percentage saturation</td>
<td>63</td>
</tr>
<tr>
<td>6. Vitamin A</td>
<td>39</td>
</tr>
<tr>
<td>7. Urinary Oestrogen</td>
<td>15</td>
</tr>
<tr>
<td>8. Creatinine in amniotic fluid</td>
<td>15</td>
</tr>
</tbody>
</table>

a. Selection, handling and storage of blood samples:

Blood samples were taken preferably before breakfast to avoid the influence of food digestion on blood composition. Twenty mg. of potassium oxalate were used to prevent coagulation. Samples were transported in an iced flask to the laboratory and kept in deep freeze. Analysis were made within a week after the blood was drawn.
In the collection of the blood for serum, care was taken to avoid hemolysis. The syringe and needle were kept clean and dry. Minimum amount of constriction was applied to the arm and the blood drawn from the cubital vein. After removing the needle, the freshly drawn blood was placed directly into a test tube without any anticoagulant. The mechanical breakdown of red cells was thus avoided to the extent possible. Blood was allowed to clot at room temperature, for three hours. The clot was separated from the walls of the tube by carefully running a clean applicator, centrifuged immediately and the supernatent serum removed with a rubber bulb pipette. During the interval between drawing and analysis, the serum was kept in well stoppered test tubes in a freezer.

Laboratory techniques were selected for their accuracy, sensitivity, reproducibility and practicability and having in mind the technical resources available. Methods commonly used and of proved value were chosen.

b. Estimation of total serum protein, albumin and globulin.

The serum proteins of the expectant mothers, especially albumin, is much reduced in dietary protein depletion according to Jelliffe (1964). Protein nutrition
can be assessed by estimating the total protein, serum albumin and globulin values. In the last trimester of pregnancy three ml. of blood were drawn from 72 expectant mothers in the high income group and 86 mothers in the low income group and analysed for total proteins, albumins and globulins by the Biuret method suggested by Varley (1975). Help was taken from a technician for drawing the blood and the estimations were carried out in the college laboratory. Details of the procedure are presented in appendix III.

C. Estimation of haemoglobin

Anaemia occurs due to series of nutrient deficiencies. In field situations, this can be best assessed by haemoglobin estimations associated with the examination of the blood films and haematocrit estimations. Haemoglobin values are of great practical value in the assessment of the severity of anaemia in the expectant mothers (Jelliffe, 1966). Haemoglobin values were estimated for 274 expectant mothers in the high income group and 499 expectant mothers in the low income group. Cyanmethaemoglobin method was followed (Varley, 1975). A field modification of this was done as suggested by National Institute of Nutrition (1971)
in which 0.02ml of fresh blood obtained by finger prick, 
without squeezing, was run on to a strip of Whatman filter 
paper (2cm x 4cm) and allowed to dry. Such specimens were 
transported in small individual envelopes and ultimately 
dissolved for 10 minutes in the Drabkins solution and 
haemoglobin estimated.

4. Estimation of serum iron, total iron-binding capacity 
and percentage saturation.

There is a small amount of ionic iron in the plasma 
in addition to that bound as haemoglobin. This ranges from 
1-3 per cent of the total blood iron. Quantification of 
this serum iron levels is of value in differentiating the 
anemias and indicating their etiology and possible therapy. 
The concomitant determination of serum iron binding capacity 
and percentage saturation facilitates such diagnostic 
procedures.

Two ml. of blood from each of the 49 expectant 
mothers in the high income group and 63 mothers in the low 
income group were collected and used for estimating the serum 
iron. The serum samples were analysed for their ionic iron 
and iron binding capacity using the dipyridyl method of 
Ramsay (1958). From these values, it was possible to 
calculate the percentage saturation of transferrin.
e. Estimation of serum vitamin A

Blood samples of 2 to 2 ml were drawn and collected in centrifuge tubes and kept for 3 hours, centrifuged, labelled and stored in a freezer. Hemolysed samples were discarded. The procedure of Roels and Mahadevan (1963), using trifluoroacetic acid was adopted, as this method has the advantages of simplicity, widespread use and single direct reading and analysis.

f. Collection and preservation of the urine samples

For dependable data regarding the quantitative composition of the urine, the examination of the mixed excretion collected for 24 hours is absolutely necessary. In the collection of the urine the mothers were requested to empty the bladder at a given hour, the urine discarded and all the urine from that hour upto and including that passed till the next day at that time, collected thoroughly mixed and a sample was taken for analysis and labelled. Toluene, a very safe preservative was used to preserve urine for 24 hours. A thin layer of toluene was spread on top of the urine surface and kept in a refrigerator.
3. **Estimation of urinary oestrogens**

Urinary oestrogens are highest in women during the active ovulatory phase of life. The expectant mother's urine contains a number of oestrogens, the three most important being oestrone, oestrodial and oestriol. Oestrogen excretion in the urine is influenced by the nutritional status of the mother (Banerjee, 1962). Hence urinary oestrogen was estimated by the quinol sulphuric acid method of Brown (1960).

Fifteen expectant mothers from each group contributed 24 hours urine samples for this purpose. The total volume of urine was measured and a known amount taken for analysis.

4. **Analysis of amniotic fluid for creatinine contents**

Prasad *et al.* (1974) observe that the creatinine levels of amniotic fluid indicate the foetal viability, nutritional status of the newborn and correlate well with the infants birth weight. Hence 2.0ml of amniotic fluid were taken by a syringe with the help of a physician for estimation of creatinine from 13 mothers in the high income group and 15 from low income group according to the procedure of Folie (1914).
4. **Microbiological assay of folic acids**

From 20 mothers belonging to high income group and 40 mothers from low income group 3.0ml. of blood (each) were collected and utilized for the estimation of serum folate and RBC folate levels. L. Casei was used for the microbiological assay using the procedure of Clegg et al (1952).

5. **Recording the weight gains and heights**

Body weight of the expectant mothers was recorded with the help of a spring balance with an accuracy of ± 250g. Since transport of heavy lever type balance to different hospitals was difficult and lever balances which are portable were not available, Gurney (1969) points out that a spring balance is adequate to measure the weight provided the balance is checked constantly against standard weights. Before weighing, the balance was checked for its accuracy with the help of standard weights. The weights of the expectant mothers were recorded at an interval of 30 days from the beginning of the study, until delivery. The weights were taken preferably in the mornings after emptying the bowel and bladder and before meals.
One week after the delivery, the nonpregnant weight of the mother was recorded so that this weight deducted from the full term weight would give the total increase in body weight in pregnancy.

Standing height was measured using a fibre glass tape fixed to a wall. The mothers were made to stand erect on the flat floor barefooted against the scale with the arms hanging at the sides in a natural manner. A wooden scale was placed gently on the head, perpendicular to the wall and the height was measured from the scale correct to 0.1 cm. This measurement was taken to enable the investigator to correlate the height of the mother with that of the offspring.

E. Recording Details Related To Past And Present Pregnancy

The following details related to past and present pregnancy were collected.

1. Assessment of gestation age
2. Obstetric performance
3. History of Previous pregnancies
4. Estimating the cost of pregnancy wastage
5. Health status of elder children in the family
6. Recording the amenorrheal period
1. Assessment of gestation age

Accurate assessment of gestational period is of practical importance for therapeutic purposes and in research work. Though many biochemical parameters based on amniotic fluid analysis have been suggested as indicators of foetal maturity, their application is not possible in routine practice. The most frequently used method of assessment of foetal age, based on last menstrual date has been the subject of considerable debate, since it is influenced by a variety of factors like time of ovulation, irregularity of cycles, and the mothers' ability to recall accurately the date. Because of ease, it however continues to be one of the important methods of calculating the foetal maturity.

Also it is generally thought that the educational level of the subject influences her ability to judge the time of conceiving by recalling the last menstruation accurately. In this investigation, data were collected on the gestation weeks to determine the extent to which this was true in the population sample. The mothers were requested to recall the last menstrual period and one week was omitted as non ovulation period and gestation weeks calculated at
the time of delivery. Advice was taken from the physician regarding the foetal maturity and she based her conclusions on the birth weight, nail growth, hair growth and the size of fontanelle and firmness of the skull bone.

2. Obstetric performance:

The obstetric performance of the expectant mothers was studied with reference to the duration of labour, type of delivery and complications if any, at the time of delivery and maternal and perinatal deaths if any. These data were collected from all the expectant mothers with a view to find out any correlation between them and the nutritional status of the expectant mothers.

3. History of previous pregnancies:

The performance of the mother in her previous pregnancies, with reference to total number of pregnancies, miscarriages, still births neonatal and infant mortality cases and details regarding breast feeding were collected with a view to assess the performance of the mothers in the earlier pregnancies.
4. **Estimating the cost of pregnancy wastage.**

An attempt was made in the present study to calculate the amount of energy, protein and calcium that are lost when there is a miscarriage or pregnancy gets aborted intentionally at different stages of pregnancy based on the data of Widdowson (1979). Table II presents the amount of energy, protein and calcium that is being utilized for maintenance and growth at different stages of foetal life.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Foetal age in months</th>
<th>Amount required/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy in K.cal</td>
<td>3-6</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>6-7</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>7-9</td>
<td>178</td>
</tr>
<tr>
<td>Protein in g.</td>
<td>3-5</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>5-7</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>7-9</td>
<td>3.7</td>
</tr>
<tr>
<td>Calcium in mg.</td>
<td>3-5</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>5-7</td>
<td>139.0</td>
</tr>
<tr>
<td></td>
<td>7.9</td>
<td>317.0</td>
</tr>
</tbody>
</table>
Pregnancy wastage could be calculated only from 3rd month onward as the data was available from that month only. The total amount of energy, protein and calcium utilised for one day as estimated by Widdowson (1979) was multiplied by 30 (days in a month) to obtain the amount utilised for each month and the values added to arrive at a cumulative figure for a particular month when pregnancy might have resulted in a failure or terminated, or miscarried. The cumulative figures thus obtained represent the amount of energy, protein and calcium that is wasted when a pregnancy is wasted, and the amount of money required to purchase food commodities to provide these amounts of energy, protein calcium would represent the cost of pregnancy wastage.

5. Health status of the elder children in the family

The health status of the elder children in the family may throw light on the nutritional status of the family in general and that of the expectant mothers in particular. Hence information regarding, number of children dead and alive, sickness of the children, reasons for death and details of vaccinations done on the children were collected on the elder children in the family.
6. Recording the amenorrheal periods

Length of the breast feeding affects the anovulatory lactation amenorrheal period according to Scrimshaw et al., (1975) and Jelliffe (1976). According to them the longer the breast feeding the longer is the amenorrheal period. Hence an attempt was made to record the amenorrheal period and correlate the same with the duration of lactation.

7. Recording The Anthropometric Measurements Of The Newborn

The birth weight of the infant was recorded after delivery and the other anthropometric measurements of the newborn infants were recorded within 10 to 24 hours after delivery. The following measurements were recorded:

1. Birth weight
2. Crown heel length
3. Head circumference
4. Chest circumference and
5. Mid arm circumference

1. Birth weight

Birth weight was recorded with the help of a UNICEF Detecto beam balance nearest to 0.01 kg. The balance was checked with standard weights everytime before use, for accuracy as suggested by Pachuria and Manwah (1970) and Gupta (1971).
2. **Crown heel length**

Crown heel length was recorded with the help of an infantometer which is a specially prepared measuring board provided with two wooden cross-pieces, one fixed and the other capable of sliding. The infant was placed on the table with legs completely stretched and head positioned in such a way that a line drawn from the infra-orbital margin to the auditory meatus was perpendicular to the horizontal surface. The fixed cross-piece of measuring board just touched the most protruding part of the head and the sliding cross-piece just touched the heels to give the crown-heel length (Venkatachalam and Singh, 1962). Length was taken to the nearest 0.1 cm.

3. **Head circumference**

Head circumference was measured using a soft fibre glass tape passing round the supraorbital ridges in front and occipital protrusion behind (Desilva and Baptist, 1969), and Mayer and Thomas (1972) and American Academy of Pediatrics (1973).

4. **Chest circumference**

Chest circumference was measured with the same fibre glass tape. A helper held the baby lightly so that the thorax did not present any skin folds. The measurements
of the girth of the thorax were done at the level of the "
"xiphisternum and in a place at right angles to the vertebral "
"column below the inferior angle of the scapula. Sufficient "
"tension was applied to enable the tape to rest against the "
"perimeter of the thorax without slipping (Venkatachalam, "
"1962, Jelliffe (1966) and Pachuria and Marwah, 1970). "

3. **Mid arm circumference**

   The arm girth was measured at the level midway "
"between the acromial and olecranon process with the arm "
"hanging freely relaxed with the tape applied at right angles "
"to the long axis of the humerus (Mayer and Thomson, 1972). "

6. **Assessing The Nutritional Status Of The Nursing Mothers**

   For assessing the nutritional status of the nursing "
"mothers a record of food and nutrient intake of selected "
"nursing mothers in the low income and high income groups was "
"collected according to the procedures already described. "
"Clinical examination and biochemical profile of selected "
"nutrients were carried out utilizing the procedures already "
"included.

   It has been stressed time and again that the nursing "
"mother loses her weight due to the demand of lactation. "
"This weight reduction may or may not be related to the
Quantitative evaluation of the mother's diet during pregnancy and lactation. Hence the investigator undertook to record the weight pattern of a subsample of mothers after delivery and followed them for 12 months after delivery. Weight was recorded at 3-month intervals with the help of a spring balance.

2. Lactation Performance of Nursing Mothers

i. Quantification of breast milk

Studies on the volume of breast milk are exceedingly difficult to undertake, because of interference with the emotionally labile let down reflex and problems of measuring throughout the 24 hours. However, quantification of breast milk was undertaken in this study, on nursing mothers who were cooperative in both the income groups. The infant was weighed before and after all the feedings in one day and the total volume computed, wherever possible. In some cases the infant was weighed before and after feeding three times on a given day and the milk intake computed by finding out the total number of feeds.

ii. Analysis of breast milk

Breast milk of randomly selected 10 mothers from each income group was analysed for total solids, energy, protein, fat, calcium, and iron. Estimation of protein was done by the
mikrokjeldahl method (Hawk et al., 1966). Estimation of energy was done with the help of a bomb calorimeter according to the method suggested by Swaminathan (1974). The lipids present in the breast milk were analysed according to the method suggested by Davies (1939). The calcium and iron contents of breast milk were found out by adopting the procedure published by Hart and Fisher (1971).

I. Growth Performance Of The Intercaste Patters:

1. Anthropometric measurements:

   Infants who could be reached from both the income groups were followed for a period of 12 months and their weight, height, head, chest and mid arm circumferences were recorded every three months in accordance with the procedures already described. Table II presents the number of infants thus measured at different stages of life in the first year in the two income groups. They were also further classified into breast fed and non breast fed infants.
2. Physical and motor development

The various milestones in the development of the child, namely, fixing of the eyes, following objects, smiling, recognising the mother, oral exploration, sitting with and without support, transport of the object, tonic neck reflex, head and plane with symmetric posture and complete head control were observed and carefully recorded by the investigator herself and the exact period of acquiring that skill was checked with the mother.
3. Health status of the infants

The health status of all the infants included in the study was recorded with respect to the signs of malnutrition exhibited, presence of worms and reported sickness in the last two months. The clinical signs of malnutrition were recorded with the help of a trained physician. For finding out the presence of worms, the faeces of the infants were collected and analysed for the presence of cysts or worms, in the Municipal Health Laboratory situated near the college. Any history of repeated sickness since birth were enquired and recorded. The common ailments among the infants were diarrhoea, fever, measles and common cold. Morbidity scores were calculated following the method adopted by Arroyave and Pineda (1974).

<table>
<thead>
<tr>
<th>Degree of morbidity</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0-15</td>
</tr>
<tr>
<td>II</td>
<td>16-50</td>
</tr>
<tr>
<td>III</td>
<td>50 and above</td>
</tr>
</tbody>
</table>

Morbidity scoring system was as follows:

1. Diarrhoea for 15 days 30
2. Diarrhoea for 7-15 days 20
3. Diarrhoea for 4-7 days 10
4. Diarrhoea for 4 days 5
II. 1. Fever for 15 days and above 20  
   2. Fever for 5-10 days 10  
   3. Fever below 5 days 5  

III. Bronchitis 5  
IV. Cold 5  

V. Other ailments for 10 days or more 20  
VI. Other ailments for less than 10 days 10  

The pattern of immunisation such as vaccination against smallpox, polio myelitis, diphtheria, pertussis and tetanus and the age at which it was given and the details about the booster dose were elicited from the parents through informal discussion.

4. Weaning foods given:

The weaning foods used by the mothers to wean the child from the breast were recorded for the infants belonging to both the income groups.
3. Impact of Selected Nutritional Intervention Programmes

1. Studying the impact of the supplements distributed in the Applied Nutrition Programme to the expectant mothers:

   a. Introduction of supplements in the Applied Nutrition Programme

   After obtaining the necessary permission from the Panchayat Union Commissioner the feeding programme was observed for 25 available expectant mothers all of whom belonged to the low income group in the Applied Nutrition Programme Village (Pannimadai). The feeding programme consisted of distributing about 44.2 g of skim milk daily and eggs three times a week (30g/day) for each expectant mother.

   From the date of starting the feeding, care was taken to see that the milk was reconstituted properly, eggs were boiled to the right extent and distributed to the expectant mothers whose attendance was recorded. At times when the mothers were too busy, supplements were covered and kept for them to come and collect. In the beginning the women felt bad to eat the supplements without giving their children or husband, but got used to it later on.
The feeding started at the beginning of the third trimester and was continued for a period of 100 days. Twenty five expectant mothers from Poochiyur village, who did not receive any supplements served as control.

b. Assessing the impact of the supplements on the expectant mothers:

The impact of the supplements was assessed adopting the following procedures:

i. Food and nutrient intake before and after supplementation

ii. Weight gain during pregnancy

iii. Haemoglobin estimation

iv. Estimation of urinary creatinine level

v. Clinical examination at term of pregnancy and

vi. Anthropometric measurements of the newborn

1. Food and nutrient intake before and after supplementation

The food and nutrient intake of ten of the twenty five mothers who were willing to let to investigator weigh the food stuffs was determined by the precise food weighing method for seven days using a portable food weighing balance, according to procedures, already detailed. The nutrient intake was calculated from the raw food equivalents using,
'The Nutritive value of Indian Foods' by Gopalan et al.,
(1976) Food and nutrient intake of 10 mothers of the control
group were also recorded and nutritive value calculated.

ii. Weight gain during pregnancy

The total weight gain during pregnancy was obtained
as per procedures already outlined for both the control
and supplemented mothers.

iii. Estimation of haemoglobin

0.02ml of blood was obtained by finger prick and
the haemoglobin level estimated by cyanmethaemoglobin method
for mothers of both the groups, a t the beginning and end of
the study.

iv. Estimation of urinary creatinine level

Urine samples were collected from all the mothers
and analysed for creatinine level by the method of Hawk, et al.,
Sumamaran (1966) at the beginning and at the end of the study,
for the mothers belonging to both the groups.

v. Clinical examinations

All the expectant mothers in both the villages
were examined clinically by a physician and the clinical symptoms
assessed.
vi. Assessing the impact of the supplements on the newborn by anthropometric measurements.

Anthropometric measurements, namely, birth weight, crown heel length, head, chest and arm circumference were measured according to the procedures already described, for the newborn babies of both the groups of mothers.

2. Impact of Iron and folic acid therapy
   a. Introduction of the supplements:

   The State Family Welfare Bureau is carrying out the prophylaxis against anaemia programme since 1970. Mothers are provided with medical care through the maternity centres. One hundred Ferrous Sulphate tablets each containing 200mg Ferrous sulphate of iron (360mg of elemental iron) and 500 mg of folic acid are supplied to the mothers in three instalments (30, 30, 40) in the third trimester of pregnancy.

   The mothers are also given antenatal and postnatal care and treatment for minor ailments. These facilities were availed to study the impact of iron and folic acid supplementation through the prophylaxis against anaemia programme.
The mothers who were attending the antenatal clinics were divided into two groups. While both the groups received the same dose of iron and folic acid supplied by the programme, the consumption of the tablets was supervised for one group by the investigator and the same not supervised for the second group.

Since the dose given in the prophylactic programme was one and the same for all irrespective of their haemoglobin levels or body weights, the dose of iron and folic acid to be supplemented to the expectant mothers based on their haemoglobin levels and body weight were computed and the same were fed to two more groups of expectant mothers, consumption in one group being supervised and in the other not being supervised. The dose calculation was done as follows for these two groups.

b. Iron calculation

The women were first seen at 24 weeks of pregnancy. Supplementation was planned for 100 days and the mean body weight was found to be 45 kg. The total amount of iron required was computed as follows, according to the guidelines given in the report of the International Nutritional Anemia Consultative group (INACU, 1977).
Basal daily losses 1mg x 100 (days) ... 100 mg (excluding menstrual loss)

Iron requirements for foetus and placenta ... 250 mg

Iron for expansion of haemoglobin mass during pregnancy ... 450 mg

Iron to increase Hb concentration from 6.5g - 10.5g ... 200 mg

Total iron requirements ... 1000 mg

Amount of iron which must be absorbed daily \[
\frac{1000}{100} \]

... 10.0 mg

The absorption of iron from the standard supplement namely ferrous sulphate is as much as 40 per cent for expectant mothers according to Sushalatha and Reddy (1980). However leaving a margin for differences in the absorption, and assuming the absorption level to be 25 per cent, it was decided to supplement the mothers with 120mg of ferrous sulphate (containing 43.26 mg of elemental iron) and 200 mg of folic acid so that about 10.0 mg of iron would be available to each of the expectant mothers every day. A group of mothers who did not get any of these supplements served as control.

Thus out of the 110 expectant mothers available for participation in the present study, 30 mothers served as control. The rest 89 mothers formed the following therapeutic groups.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of mothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Control</td>
<td>30</td>
</tr>
<tr>
<td>B 120mg of ferrous sulphate + 200 µg of folic acid (unsupervised)</td>
<td>15</td>
</tr>
<tr>
<td>C 120mg of ferrous sulphate + 200 µg folic acid (supervised)</td>
<td>25</td>
</tr>
<tr>
<td>D 200mg of ferrous sulphate + 500 µg of folic acid (unsupervised)</td>
<td>23</td>
</tr>
<tr>
<td>E 200mg of ferrous sulphate + 500 µg of folic acid (supervised)</td>
<td>26</td>
</tr>
<tr>
<td><strong>Total number of mothers</strong></td>
<td><strong>119</strong></td>
</tr>
</tbody>
</table>

*Represents mothers participating in the prophylaxis programme*

As the expectant mothers participating in this study had different haemoglobin levels ranging from 8-10.4g/100ml, they were distributed between all the groups so that no group had mothers with only the lower or the higher levels in the given range.

The expectant mothers were requested to visit the antenatal clinics in the maternity centres every month and the tablets were given to them. The supplementation continued for a period of 100 days and regular visits to the home and
Maternal care was made to ensure regular consumption of the supplements, in the supervised groups.

6. **Recording the impact of the supplementation on the expectant mothers**

The impact of the supplements on the expectant mothers was recorded with reference to their haemoglobin levels, packed cell volume, serum iron, total iron binding capacity and serum and red cell folate levels according to the procedures already mentioned in the experimental design for estimating packed cell volume, blood was collected in micro haemocrit tubes, the capillary tube of blood was centrifuged at 3000 rpm on an electrical microhaematocrit centrifuge and the packed cell volume readings were compared with normal values.

4. **Assessing the impact of the mothers' nutritional status on the off-springs**

The nutritional status of the off-spring born to the mothers who consumed the supplements were assessed through anthropometric measurements as per procedures already described in the experimental design.
8. Impact of vitamin A and iron therapy

a. Introduction of vitamin A and iron therapy:

Serum vitamin A concentrations of 20 μg/100ml or less, are closely correlated with inadequate body stores of vitamin A and with clinical manifestations of vitamin A deficiency. National Institute of Nutrition (1977) reports that vitamin A may play an important role in haemopoiesis. Vitamin A deficiency induced in human volunteers has been shown to result in anaemia and hypoferrernaia (Hodges et al 1975) Hypovitaminosis A and anaemia coexist in many poor communities. Hence an effort was made in the present study to assess the role of vitamin A in the haemopoietic activity of the expectant mothers. The serum vitamin A levels of randomly selected expectant mothers were analysed and those whose serum levels exhibited a low concentration of vitamin A in addition to that of haemoglobin were chosen for the vitamin A supplementation studies.

Twenty two mothers were administered 2 ml of vitamin A solution orally (2 lakhs international units) in accordance with the guidelines given by WHO (1976). In addition to this one tablet containing 120mg of ferrous
sulphate (=to 43.26mg of elemental iron)/day was supplemented for 100 days to a second group of 30 expectant mothers. A third group of 30 expectant mothers were given iron tablets alone for hundred days. Thirty mothers who did not receive any of the aforesaid supplements served as control.

2. Assessing the impact of supplements on the mothers:

The impact of supplementation of vitamin A and iron together, and iron and vitamin A separately were estimated by carrying out a biochemical analysis of blood for serum vitamin A, iron, haemoglobin, total iron binding capacity and percentage saturation. The procedures adopted in the analysis of these nutrients have already been discussed earlier in the chapter.