Subjects and Methods

CHAPTER 3

SUBJECTS AND METHODS

The details concerning selection of subjects, study design and methods used in the study are presented in this chapter under the following heads:

* Area Selected for the Study
* Sample Selection
* Experimental Design
* Dose Levels of Iron and Folic Acid
* Form of Iron Used and the Source of Supplements
* Distribution and Administration of Supplements
* Parameters and Techniques Used for Data Collection
* Statistical Analysis.

Area Selected for the Study

The study was conducted in the city of Baroda (Gujarat State, India) from July 1991 to May 1993. The estimated population of Baroda city as per 1981 Census was 1.32 million, of which approximately 1/3rd were expected to be residing in slums (UNICEF/WHO 1975). As per the information provided by the Municipal Corporation of Baroda, there were a total of 118 slums in the city of Baroda in 1991. The underprivileged pregnant women of these slums received basic

nutrition and health services from the State financed Family Welfare Bureau which included immunization against tetanus, iron supplements, and antenatal care. Besides, most of these slums were also covered by the Integrated Child Development Services scheme (ICDS) which in addition to the above mentioned services provided supplementary food to the pregnant women and nutrition health education to all women of reproductive age. The subjects for the present study were drawn from the slums that were catered to by the ICDS scheme.

Sample Selection

Of the 160 ICDS Centers (Anganwadis) covering the slum population of Baroda city, 15 were selected purposively using the following criteria:

1) There should be an anganwadi center with an anganwadi worker and a helper
2) The ICDS authorities in Baroda should be willing to co-operate and permit the anganwadi worker to assist in identifying and enrolling pregnant women for the study.

Each selected ICDS Center catered to a population of approximately 1000.

A census survey was carried out in all the 15 centres at the commencement of the study and all available pregnant women between 20-24 weeks of gestation were enlisted. Women who consented to participate in the supplementation trial and who were not likely to move out during the study period were included in the study. The enrollment of subjects was carried out over a period of one and half years. In all 171 pregnant women, 20-24 weeks of gestation formed the sample for the study. The gestational age of 20-24 weeks at entry was dictated by the field situation where identifying the pregnant women prior to 20 weeks was very difficult.

Experimental Design

The pregnant women as they were enrolled were sequentially and randomly alloted to three treatment groups as shown below:

Gp I - 60 mg elemental iron + 1.5 mg folic acid daily

Gp II - 120 mg elemental iron and 1.5 mg folic acid daily

Gp III- 180 mg elemental iron and 1.5 mg folic acid daily

The subjects received the supplements for a period of 12-16 weeks, i.e. from 20 to 24 weeks of gestation till delivery. Group II and Group III were double blind as the investigator received coded tablets identical in shape, size and color that were distributed to the women once a month.

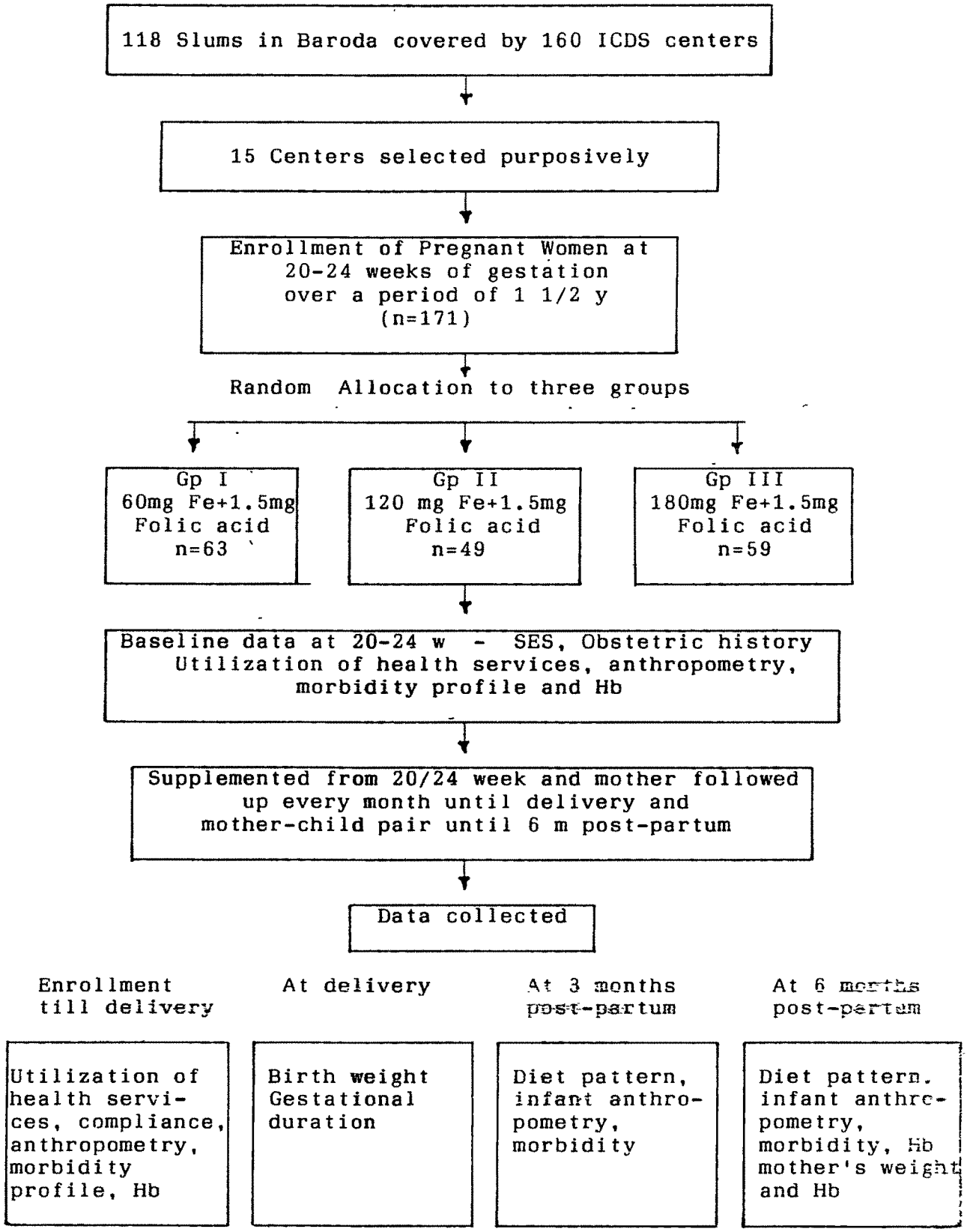
Neither the investigator nor the subject knew the dose level of iron they were receiving. Decoding was done at the end of supplementation. This was however not possible with the 60 mg group, the reason for which is elaborated in the next section.

At baseline i.e. 20 or 24 weeks of gestation data were collected from all the subjects on socio-economic status, obstetric history, health services utilization, anthropometry and hemoglobin. Morbidity profile prior to 20-24 weeks of gestation was also collected at enrollment. The women were followed up every month and data on health services utilization, compliance with supplement consumption, anthropometry, morbidity profile and hemoglobin were collected until delivery. The birthweight of the infant was recorded within 48 h of delivery. Following delivery the mother and infant pairs were followed up for 6 months and data were collected at 3 months and/or 6 months post-partum on maternal weight and Hb, and the infant height, weight, Hb, diet pattern and morbidity profile. The experimental protocol is depicted in Fig 3.01.

Dose Levels of Iron and Folic Acid

The iron cost of a pregnancy is estimated to be about 1000-1200 mg (DeMaeyer 1989, INACG 1981). As increased

Fig 3.01 Experimental Protocol



maternal erythropoiesis and transfer of iron to the fetus occur chiefly in the last two trimesters, the demand for iron is relatively high in the 2nd and 3rd trimester (6.3 mg/day) compared to the first trimester (0.8 mg/day) (DeMaeyer 1989).

In non-anemic pregnant women with adequate iron stores (500 mg) about half of the requirements will be met from the iron stores. Providing 60 mg elemental iron per day for a period of 100 days to these women would be expected to contribute 480 mg absorbable iron as 8% absorption has been reported in non-anemic women (Narasinga Rao 1991). Thus 60 mg elemental iron daily for 100 days will meet the other half of the requirements for non-anemic pregnant women.

However for women who begin their pregnancy with inadequate or no iron stores but with normal Hb levels, the total iron requirement will have to be met chiefly by supplemental iron. These women will require approximately 120 mg iron per day for 100 days which at 8% absorption would provide about 960 mg of iron which is close to the estimated requirements for pregnancy.

Anemic pregnant women with no iron stores will have to be provided additional iron to correct anemia and if possible build some iron stores to prevent them from lapsing into anemia at a later stage. Computations for absorbable

iron would be dependant on the anemic status of the individual. In the present study, it was found that the initial mean Hb of the pregnant women was approximately 9 g/dl. To raise this level to 11 g/dl, an additional 340 mg absorbable iron will required taking the blood volume as approximately 5 liters at term (Rajalakshmi and Raman 1985). Therefore the total requirement for absorbable iron would be about 1500 mg iron (1000 mg for pregnancy need, 340 mg for correcting anemia and a small store of iron of about 160 mg). In order to provide 1500 mg of absorbable iron, the dose level of iron daily will have to be > 120 mg/day, about 180 mg if an absorption of 8% is considered. Although iron absorption is reported to be about 10% in anemic pregnant women, as the Hb levels rise, percent absorption decreases reaching about 8% (Narasinga Rao 1991).

The level of folic acid used in pregnancy supplementation trials has ranged from 0.5 mg to 5 mg daily, with the effect on Hb being not dose dependent (Sood et al 1975, Aung Than Batu et al 1976).

Based on the above considerations three dose levels of iron 60, 120 and 180 mg daily were selected with 1.5 mg of folic acid. It was not ethically feasible to include a placebo control group due to the high prevalence of anemia in these pregnant women initially at 20 weeks of gestation.

Form of Iron Used and the Source of Supplements

Oral iron was provided in the form of ferrous sulphate tablets. Tablets of 60 mg elemental iron containing iron as ferrous sulphate and 0.5 mg of folic acid were provided by the Family Welfare Bureau Medical Officer. These tablets were manufactured by Eupharma Laboratories, Bombay. Subjects in Group I received one tablet of 60 mg iron and 0.5 mg folic acid daily, while subjects in Group III received three tablets of 60 mg iron and 0.5 mg folic acid daily. For Group II tablets of 40 mg elemental iron as ferrous sulphate, containing 0.5 mg of folic acid were specially manufactured for the study by Alembic Chemicals, Baroda, according to the specifications identical to the Eupharma tablets (colour, weight and disintegration characteristics). The subjects in Group II received three tablets of 40 mg iron and 0.5 mg folic acid daily. Thus

Group I received 60 mg, Group II 120 mg and Group III 180 mg iron.

Tablets containing 0.5 mg of folic acid, identical in appearance and other characteristics to the Eupharma tablets of iron folic acid were specially manufactured by Alembic Chemicals. Group I received two of these tablets daily so that all groups received 1.5 mg folic acid daily.

Distribution and Administration of the Supplements

Supplements were distributed every month by the investigator at the homes of the mothers. Tablets were packed in autoseal polythene covers. A supply of 90 tablets was given every month to women in Groups II and III. They were instructed to consume three tablets a day : one in the morning, one in the noon and one in the evening, preferably after meals.

Supplements for the women in Group I were provided in two separate polythene covers: one cover contained 30 tablets each containing 60 mg elemental iron and 0.5 mg folic acid; the other contained 60 tablets of folic acid only, each containing 0.5 mg folic acid. Women in Group I were instructed to consume one tablet a day from the packet

containing the iron tablets and two tablets a day from the packet containing folic acid tablets. This was necessary in order to keep the folic acid level the same for all three groups i.e. 1.5 mg/day. Although this appeared to be a little cumbersome, in practice few problems were encountered, the chief one being the mother's questions concerning why two separate packets were provided. When the purpose of this was explained, adherence to the instruction was generally good. The reason for providing the 60 mg iron as a single dose rather than three divided doses was to make it consistent with the programme situation where 60 mg iron is usually delivered as a single dose.

Initially a visit was made 10 days after the distribution of the first supply of tablets to confirm whether the supplements were being consumed in the required dose levels.

Women who reported serious side effects were counselled and encouraged to continue with the supplementation at a lower dose level for one or two days and then to increase the dose level gradually (after 2-3 days). Subsequently the next visit was made at the end of one month of starting the supplements and thereafter follow up visits were made only once a month. At these monthly follow up visits, mothers

were provided simple counselling on the importance of regular consumption of the tablets and how to overcome the problems related to poor compliance.

At each monthly visit, the autoseal covers in which the supplements were provided were collected back and a fresh supply of 90 tablets for the next month was given. The number of tablets consumed during the past one month was determined by counting the remaining tablets in the polythene covers at the end of the month.

In addition to the measure of compliance obtained by counting left-over tablets, a calender type of device was also used to record compliance. This is described under the section on parameters.

In order to ensure that the subjects did not receive or consume iron from other sourses, a letter of request (Appendix I) was provided to the subjects, which was taken by them during their visits to the ante-natal clinic to ensure that the physician did not prescribe or recommend other iron supplements. This was complied well and in the event of the physician prescribing some supplements and drugs, the subjects usually made it a point to show the investigator the prescriptions given by the doctor or other

supplements provided free of cost. This facilitated the investigator to ensure that iron supplements from any other source was not consumed by the subjects by explaining to them that the iron supplement prescribed by the doctor was the same as that provided to them in the study and they needed to consume only one or the other. The few subjects who insisted on following only the doctor's prescription were dropped from analysis.

Parameters and Techniques Used for Data Collection

Parameters which were measured at different points during the course of the study are shown in Table 3.01 and 3.02.

Details of Parameters

Maternal parameters

Socioeconomic and demographic information

This was collected using a pretested interview schedule and included information on family characteristics, family type, educational level of the subject and the spouse, occupation of the subject and the spouse, total income from all sources, type of construction of the house, source of

Table 3.01 Parameters Measured Prior to Delivery

PARAMETER	20 w	24 w	28 w	32 w	36 w
SES	_/				
Obstetric history	_/				
Utilisation of services	_/		_/		_/
Compliance with iron supplements	_/	_/	_/	_/	_/
Anthropometry -					
Height	_/				
Weight	_/	_/	_/	_/	_/
MUAC	_/		_/		_/
Hb	_/	_/	_/	_/	_/
Morbidity profile	_/	_/	_/	_/	_/

Table 3.02 Parameters Measured Postnatally
(Delivery to 6 m post-partum)

PARAMETER	AT DELIVERY	3 m	6 m
Infant anthropometry			
Height		_/	_/
Weight	_/	_/	_/
Hb			_/
Feeding practices		_/	_/
Morbidity profile		_/	_/
Maternal Data			
Weight			_/
Hb			_/

drinking water, toilet facility and environmental sanitation (Appendix II). A scoring system was developed for categorizing environmental sanitation into four classes (Appendix II), excellent, good, fair and poor.

Obstetric history

Information on age of the subject, age at menarche, age at marriage and age at first conception were collected by subject's recall (Appendix III).

During the time of interview it was observed that some women could not recall their age at marriage or age at menarche. This was traced by determining the age of the first born child, and then asking the woman the period between her marriage and the birth of the first child, and the period between her menarche and marriage. The women were able to estimate the period between attaining menarche and marriage, and the period between the marriage and birth of the first child. The subjects were able to recall their current age easily.

Data on parity, previous history of miscarriages, incidence of premature deliveries, still-births and low birth weight babies were obtained by personally interviewing the mothers. Parity was defined as the number of full term births the mother had gone through, live or dead. A

miscarriage was defined as the termination of pregnancy prior to 28 weeks of gestation, counting from the first day of the last normal menstrual period (Royston and Armstrong 1989). Both natural and induced miscarriages were probed for. Premature delivery was defined as delivery occurring between 28-36 completed weeks of gestation, still-birth as a full term infant who showed no evidence of life after birth. Lowbirth weight babies were defined as infants with birthweight less than 2.5 kg.

In the absence of any records concerning these past events, the investigator had to rely on mothers reports and therefore a certain amount of overlap between these events, could not be ruled out.

Utilization of maternal health services

A personal interview schedule was used to ellicit information on available health facilities and their utilization which included gestational age at first antenatal visit, total number of ante-natal visits, whether or not the subject received supplementary food, gestational age at which 1st, 2nd and 3rd injections of tetanus toxoid were received and any illnesses for which medical aid was sought. Since these data were collected every two months

during the present pregnancy, obtaining reliable information was not a problem (Appendix IV).

Compliance with iron supplements

Information regarding the receipt and consumption of iron supplements from all sources, dosage and type of supplement received prior to 20 weeks of gestation (i.e. prior to enrollment) was recorded using a compliance schedule (Appendix V). This also included information on whether the women felt any beneficial effect after consumption of the iron supplements, incidence and nature of side effects, remedial action taken for side effects, and receipt and consumption of other supplements such as B-Complex, multivitamins, calcium and others.

Data on compliance with consumption of iron folic acid supplements during the study period were collected using two methods. One was to count the number of the leftover tablets in the autoseal polythene covers. The investigator carefully questioned the mothers concerning the tablet consumption. In no instance did a mother report of sharing the tablets or giving it to others in the family.

As an additional measure for recording compliance and as a memory aid a calendar type of device was also evolved which was a simple strip of chart paper with 30 circles in

it. It was explained to the women that each circle represented one day. The women were requested to hang it in a place where they could see it easily and make a mark or punch holes inside a circle each time they took a tablet in a day. The calendar was distributed along with the supplements every month and the marked ones collected at the end of the month.

Anthropometry

Height : Height is a linear measurement made up of the sum of four components: legs, pelvis, spine and skull (Jelliffe, 1966). A non-flexible fiber glass measuring tape was fixed vertically on a smooth wall, perpendicular to the ground taking care to see that the floor area was even. After removing the footwear the subject was asked to stand straight with her feet parallel and with heels, buttocks, shoulders and back of head touching the wall. The subject was asked to look in front with her arms hanging loosely by the side. A smooth thin ruler was held on top of the head in the center, crushing the hair at right angle to the scalp and the height read off from the lower edge of the ruler to the nearest 0.1 cm (Jelliffe 1966). Measurements were recorded in duplicate.

Weight : The most common assessment of nutritional status of women during pregnancy is by monitoring weight gain. Maternal weight gain is considered a sensitive indicator to acute nutritional stresses during pregnancy and also provides a general indicator of fetal growth, and has been shown to be a critical determinant to the pregnancy outcome of the mother and the infant (Krasovec and Anderson 1991).

For weighing the mothers, an adult bathroom type weighing scale was used, since it is portable and convenient to use in the field. The weighing scale was periodically checked for accuracy with known weights. The scale was adjusted to zero before each measurement and the subjects were weighed with minimum clothing. Weights were measured to the nearest 0.5 kg and the measurements were made in duplicate.

Mid-upper-arm circumference : Muscle and fat constitute the soft tissues that vary with a deficiency of protein and calories. Measurement of the mid-upper-arm circumference is the most useful, practical method for assessing muscle mass, as this region is easily accessible and measurement requires only a flexible fiber glass tape (Jelliffe 1966).

The subject was asked to flex her left arm at the elbow such that the lower arm was at a right angles to the upper

arm. The length between the acromion process of the scapula and olecranon process of the ulna was measured with a flexible fiber glass tape and the site of measurement, exactly mid way down the upper arm, was marked with a pen. The subject was then asked to hang her arm relaxed by the side and the tape was then passed gently, but firmly around the arm at the selected mid-point taking care to avoid compression of the soft tissues of the arm. The arm circumference was measured to the nearest 0.1 cm. Each measurement was made in duplicate (Jelliffe 1966).

Morbidity profile

Types of morbidities : A proforma for recording morbidities was developed in consultation with the Professor of Obstetrics and Gynaecology at the Sri Sayajirao Gaekwad Medical College, Baroda. The morbidity proforma consisted of three categories of morbidities, which were pregnancy related morbidity, anemia and work related morbidity and infectious episodic morbidities (Appendix VI).

For infectious episodic morbidities the following definitions were used:

Diarrhea	Defined as passing of $\geq$ 3 loose stools in a day
Cold	Characterized by running or blocked nose
Cough	Based on subject history
Fever	Based on subject history
Malaria	Characterised by high fever, shivering and pain in abdomen and as diagnosed by physician
Typhoid	As diagnosed by physician
Throat pain, ear pain	Based on subject history
Conjunctivitis	By observation and subject history

This proforma was pretested first on a sample of pregnant women who were not subjects for the present study. After ensuring that the women comprehended the items in the proforma and were able to respond, the proforma was utilized for collection of morbidity data for the present study.

The morbidity data were collected by the investigator through personal interviews. The subjects were visited in their homes once every month and were requested to report the morbidities they had experienced specifically for the one month period between two visits of the investigator. This procedure helped in avoiding overlap between successive reference periods. After the free listing of the morbidities by the subjects, the women were asked to describe in detail the nature of morbidities experienced to determine whether they were consistent with the above definitions. Other infectious morbidities that were not listed above but reported by the subjects were also recorded.

Episodes of morbidity : An episode of morbidity was defined as one or more days of a particular morbidity preceded by at least one symptom-free day in the last one month. This was explained to the subjects and they were asked to recall carefully the number of episodes of each morbidity that they had experienced in the past one month. When two or more types of morbidities occurred in a subject

at the same time, each type of morbidity was recorded as one episode.

Severity of morbidity : The severity of the morbidity experienced was categorized as mild, moderate and severe, based on the criteria listed below:

Mild	..	If the mother complained of a morbidity, but was able to carry out normal work activity.
Moderate	..	Normal work activity was reduced.
Severe	..	Subject was bed-ridden or resting without being occupied in any work activity.

Duration of morbidity : Days of morbidity were defined as the number of days the mother suffered from one or more illnesses.

Validation of the reference period : Records of morbidity have been shown to be taken at various periods in studies, which range from daily, once a week, once in 15 days, once a month, once in three months or even once in twelve months duration (Andelman and Sered 1966, Reeves et al 1984, Hussain et al 1988, Gopaldas et al 1991). Long periods of morbidity recall, however are considered to be less valid as individuals tend to forget the number of morbidities or episodes due to the lapse of time.

In the present study a reference recall period of one month was selected as it was not feasible to make weekly visits to all the women. This was validated against a weekly recall of morbidity data on a subsample of ten mothers. Morbidity data using the pretested proforma were collected for the same group of women once a week and at the end of 4 weeks they were requested to recall the morbidity for the whole month. Mean episodes of morbidity and mean number of morbidities were similar for the recall over a reference period of one month and one week as shown in Table 3.03. Thus the one month reference period appeared reasonably valid in the present study.

Table 3.03 Validation of morbidity recall

Time period	Mean number of episodes	Mean number of days
Weekly recall (4 weeks)	0.5	2.1
1 month recall	0.5	1.9

Blood hemoglobin

The preferred method is that in which hemoglobin is measured photometrically after conversion to

cyanmethemoglobin or hemiglobincyanide (H_1CN). The advantages of the cyanmethemoglobin technique are that it entails dilution with a single reagent, measures all forms of circulating hemoglobin (with the exception of sulphemoglobin), produces a relatively broad absorption band at 540 nm that can be measured in both filter and narrow band spectrophotometers and employs standards with exceptionally long stability (INACG, 1985).

In solution the ferrous ions (Fe-II) of the hemoglobins are oxidized to the ferric state (Fe-III) by potassium ferric cyanide to form hemiglobin, methemoglobin or ferrihemoglobin. In turn, hemiglobin reacts with the cyanide ions (CN) provided by potassium cyanide to form HiCN. The time necessary for full color development is shortened to 3 minutes if dihydrogen potassium phosphate is substituted for sodium bicarbonate in the classic Drabkin's reagent. The addition of a non ionic detergent enhances erythrocytic lysis and minimizes turbidity resulting from lipoprotein precipitation.

The initial standardization of the method was done by collecting blood samples in duplicate in the households of the pregnant women. A finger other than the thumb or the little finger was first wiped with alcohol and then dried with a clean filter paper. A bold prick was made using a

sterile disposable blood lancet, so as to obtain free flowing blood. The first drop of blood was wiped off using filter paper. Then, 0.02 ml of blood was pipetted into a graduated hemoglobin pipette taking care to avoid the formation of air bubbles. The tip of the pipette was wiped free of blood with a filter paper and the blood was transferred into a test tube containing 5 ml of Drabkin's reagent. The contents were mixed by whirling the tube and allowed to stand for a minimum period of 30 minutes. The reading was taken in a Spectrophotometer (Spectronic 21) at a wave-length of 540 nanometers. The instrument was set at zero using 5 ml Drabkin's solution as the reagent blank.

A series of standards were run using the Cyanmethemoglobin (H_1CN) reference solution obtained from Span Diagnostics. A caliberation curve was prepared using the standard. A series of 5 tubes were labelled 1 through 5 respectively. 10 ml of the standard was pipetted into tube 1 and 5 ml of the reagent was pipetted into tubes 2, 3, 4 and 5. Tubes 2, 3, and 4 were serially diluted as follows :

Tube No.	Dilution
1	10 ml standard
2	5 ml reagent + 5 ml of standard
3	5 ml reagent + 5 ml solution from Tube 2
4	5 ml reagent + 5 ml solution from Tube 3
5	5 ml reagent (Blank)

The readings were recorded at a wavelength of 540 nm using the Spectrophotometer (Spectronic 21) after setting the instrument to zero with the reagent blank. A factor of 36.82 was established from the standardization and each Spectronic reading was multiplied by this value to obtain Hb concentration in g/dl (see Appendix VII for standard curve). After this procedure, the same was modified so that the samples from the field could be more easily transported to the lab. All Hb estimations for the present study were done using the filter paper technique.

Modified filter paper technique : As mentioned earlier 0.02 ml of blood in duplicate was pipetted and was blown out, in concentric circles onto a Whatman Grade 1 filter paper (1.5 x 1.5 cm). This was allowed to dry. Each piece of filter paper was labelled and stored between butter paper, in a box. In the laboratory, the blood stained portion of the filter paper was cut and immersed into a test tube containing 5 ml of Drabkin's reagent for elution. To enable complete elution it was allowed to stand for 30-45 minutes. The reading was taken in the Spectrophotometer at 540 nm after shaking the tubes well. The spectrophotometer readings were multiplied by the factor 36.82 to obtain Hb (g/dl) values. To the Hb values, a correction factor of 4.4% was applied as suggested by NIN (1974) where it was observed that the filter paper method underestimated the actual Hb

content (as measured by direct method by 4.4%), which was also the finding in this laboratory.

Infant parameters

Anthropometry

Height : Recumbent length (crown - heel length) of the infants was measured in duplicate using an infantometer. The infant was laid on the flat surface of the board with minimal clothing. The head was positioned firmly against the fixed head board, with the eyes looking vertically. The knees were extended using firm pressure and the feet were flexed at right angles to the lower legs. The upright sliding footpiece was moved to obtain firm contact with the heels and the length was measured to the nearest 0.1 cm (Jeliffe 1966).

Weight : Weight is a measurement of the body mass, and its deficiency appears to be the best indicator for assessing the prevalence of protein-energy malnutrition in the population. Weight measurements when repeated at intervals gives a better index of actual growth or growth failure. Comparison of weight for age values with regional standards at corresponding ages will help determine the degree of underweight in a community (Jelliffe 1966). Weight

for age indicates the current nutritional status of the subject.

For obtaining accurate body weight of young children the Salter scale was used, calibrated to the nearest 100 g. It was hung freely from a hook along with trousers. The pointer was brought to zero by adjusting the screw. The child was then placed in the trousers with minimum clothing and weight measurements in duplicate were recorded to the nearest 0.1 kg. The Salter scale was checked for its reliability against standard 10 kg weights every week.

Feeding pattern

This was recorded using a semi structured interview schedule (Appendix VIII). Duration of breast-feeding, age at introduction of top feed and semi-solids, type of weaning foods, frequency of feeding, quantity and duration were recorded by interviewing the mothers.

Morbidity profile

Morbidity of the infants in the preceding 3 months was recorded by mother's recall using a pretested proforma. This included the presence/absence of infectious morbidity (cold, cough, fever, diarrhoea, malaria, measles, skin infections, eye/ear infections, jaundice and pneumonia),

number of episodes, severity as perceived by the mother, duration and treatment received. Definitions were the same as indicated earlier.

Blood hemoglobin

The methodology used for estimating the Hb of the infant was identical to the one used for the mothers.

Statistical Analysis

Data were entered into a PC, cleaned and verified. The statistical package for the social sciences (SPSS/PC+) was utilized for various analyses as indicated below:

* Percentages and frequency distributions were computed for parameters that were expressed in a dichotomous or rank order fashion.
* Mean and standard errors (SE) were calculated for all the quantitative parameters that were expressed numerically.
* Chi-square tests were carried out to test the percentages and frequency distributions of various parameters between the three groups at baseline and follow ups.

* One way ANOVA followed by t' test was applied to compare differences between the means of different parameters in the three experimental groups at baseline and at various points of the study.

* Paired t' test was used for parameters that were measured on the same subjects initially and finally.

* Correlation coefficients were computed between the level of iron intake on the one hand and the rise in haemoglobin, side effects and beneficial effects on the other.

* Analysis of covarience was done to determine the effect of iron supplementation on rise in hemoglobin after controlling for initial hemoglobin. Mathematical model for ANCOVA is given by

$Yij = \mu + \tau_1 + B\,Xij + \varepsilon ij$ $\quad i = 1,2,t$ (treatment)

t = levels of iron $\quad$ j = 1,2,n (number of subjects)

ni = number of subjects

Yij = Hb increment of j^{th} subject from i^{th} group

μ = Overall mean effect

τ_i = True effect of i^{th} class

B = regression coefficient

Xij = Initial Hb of j^{th} subject from i^{th} class

εij = Error term $E - N(0, 6^2)$

$Yi_{adi} = Yi - b\,(Xi - X)$

Yi = adjusted average Hb for i^{th} group

$\overline{Xi}$ = observed average initial Hb for i^{th} group

$\overline{X}$ = observed average initial Hb for all groups

i = 1,2,---

* Multiple Regression analysis was carried out to determine the various factors significantly affecting birthweight and gestational duration. The model for multiple regression was:

$$y = \alpha + B_1 X_1 + B_2 X_2 + B_3 X_3 \text{ --- } E$$

B_1, B_2, B_3 are partial regression coefficients

E = Error term

α = Regression constant

y = Birthweight/gestational duration dependent variable

X = Independent variables