METHODS AND MATERIALS
CHAPTER III

METHODS AND MATERIALS

The general and specific objectives of the study were:

1. To screen pearl millet for amylase activity:
   i To screen several varieties of locally available pearl millet for their relative amylase content.
   ii To identify the variety under study yielding the highest amylase activity.
   iii To study the effect of processing conditions on amylase activity.

2. To develop wheat ARP:
   i To estimate the amylase activity of wheat purchased from the local market.

3. To study the catalytic action of ARP:
   i To study the reduction in dietary bulk of cereal gruels, namely, those of rice and wheat.
   (a) Reduction in the dietary bulk of rice gruels at 10, 15, 20 and 25 per cent cooked slurries.
iii To study the reduction in dietary bulk of donated weaning food namely Soya Fortified Bulgar Wheat (SFBW).

(a) To obtain a SFBW slurry of highest possible solid concentration and to study the reduction in dietary bulk by wheat ARF added in catalytic amounts (1-7 g% of total solids).

(b) Effect of various methods used for milling SFBW on viscosity and viscosity reduction.

(c) Reduction in dietary bulk and increase in energy density on addition of fat and jaggery.

(d) Effect of time of addition of ARF viz. prior to or after cooking on viscosity reduction.

iv To study the reduction in dietary bulk of commercially and traditionally processed foods.

Commercial Foods

(a) Reduction in viscosity of 20% cooked slurries of biscuits (low, medium and high fat) and bread by the addition of wheat ARF.

Traditionally Processed Foods

(b) Reduction in viscosity of 20% cooked slurries
of "Chapati" and "Khichdi" by the addition of wheat ARF.

4. To evaluate the acceptability and intake of gruels or porridges with and without ARF on infants and toddlers:
   i Development of the gruels or porridges (wheat, sago and SFBW) for feeding trials.
   ii Acceptability of the gruels among mothers and their children.
   iii Intake of Control and Experimental gruels by infants and toddlers.
   iv Mean calorie intake of Control and Experimental gruels by infants and toddlers.

5. To monitor the intake of gruels prepared with and without ARF by infants and toddlers (6-24 months of age) over a period of 6 months. And also to document their effect on the morbidity profile of these children:
   i To monitor the daily gruel intake of subjects for a period of six months
   ii To study the type of morbidity and its duration among the child subjects during the feeding trials of six months.
6. To evaluate the calorie and protein intake of infants and toddlers subjected to the above feeding trial:
   i Calorie and protein intake from home diets
   ii Additional calorie and protein intake from the gruels.

7. To investigate the impact of gruels with ARF and those without ARF on the growth and nutritional status of the child subjects (6-24 months) in the six months growth trial.

The study was completed in three phases:

Phase I - Objectives 1 to 3
Phase II - Objective 4
Phase III - Objectives 5 to 7

**Phase I**

**Parameters of Objectives 1, 2 and 3**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearl millet ARF</td>
<td>Method as standardized by Nayak (1983) and modified</td>
</tr>
<tr>
<td>Wheat ARF</td>
<td>Method as standardized by Deshpande (1987)</td>
</tr>
<tr>
<td>Amylase activity</td>
<td>Bernfield (1955)</td>
</tr>
</tbody>
</table>

Note: Please see later for details.
Experimental Plan

- ARF preparation from pearl millet & wheat
  - Amylase activity of pearl millet & wheat ARF
    - Viscosity measurements of gruels with and without ARF/takadiastase
      - Action of pearl millet ARF on rice gruels
        - Cereal gruels
          - Rice
        - Non-cereal gruels
          - Wheat
          - Sago
      - Action of wheat ARF on donated food
        - SPBW
          - Biscuit and bread
          - Chapati and Khichdi
        - Commercially and traditionally processed foods
ARF PREPARATION FROM PEARL MILLET

A simple method for malting which can be implemented at household or village level was used. The method initially standardized by Nayak (1983) for the preparation of pearl millet malt was followed.

**Steps involved in the preparation of pearl millet ARF**

**Selection of grain:**

Samples of different varieties of pearl millet were procured from the local market. The grains were picked, cleaned and stored in clean dry aluminium cans.

**Steeping of grains:**

The clean grains were soaked in equal volume of water (V/V) at room temperature for 2 hours.

**Germination:**

The steeped grains were drained to remove excess of water spread on filter paper and wiped lightly with muslin cloth to remove excess surface moisture. These grains were then wrapped in a moist muslin cloth and were placed in open petridishes for germination. The samples were germinated for 72 hours.
Drying and roasting of grains:

Germination was terminated by drying. The germinated grains were spread on filter paper and divided into three batches and were dried in an oven at 50°C or under bright sunlight 40°C±2°C or were roasted in an iron pan at 70-80°C.

Milling:

Sprouts were removed from the grains by hand abrasion and the grains were ground to a fine powder (about 80 mesh) in a hand operated grinder.

ARF PREPARATION FROM WHEAT

The method initially standardized by Deshpande (1987) for wheat ARF preparation was followed:

Selection of grain

Commonly available variety of wheat was purchased from the local market. The grains were picked, cleaned and stored at room temperature in clean, dry, aluminium cans.

Steeping:

The clean grains were soaked in triple the volume of water for 12 hours at room temperature.
**Germination:**

The steeped grains were drained to remove excess water, spread on filter paper and wiped lightly with muslin cloth to remove excess of surface moisture. These grains were then wrapped in moist muslin cloth and were placed in open petri-dishes for germination. The samples were germinated for 48 hours.

**Drying:**

The germinated grains were spread on filter paper and dried in an oven at 50°C for 5 hours.

**Milling:**

Sprouts were removed from the grains by hand abrasion and the grains were ground in the electrically operated 'Baby Prince mini grinder' to a fine powder (about 80 mesh).

The following table (7) shows the steps involved in pearl millet and wheat ARF preparation:
### Table 7 Preparation of ARF

<table>
<thead>
<tr>
<th>Steps involved</th>
<th>Time required (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearl Millet</td>
</tr>
<tr>
<td>Steeping</td>
<td>2</td>
</tr>
<tr>
<td>Germination</td>
<td>72</td>
</tr>
<tr>
<td>Oven drying</td>
<td>5</td>
</tr>
<tr>
<td>Sun drying</td>
<td>5</td>
</tr>
<tr>
<td>Roasting</td>
<td>10 minutes</td>
</tr>
</tbody>
</table>

**Amylase Activity of ARF**

Amylase activity of ARF was estimated using the method of Bernfield (1955).

This method is based on the phenomenon of enzyme substrate action resulting in a product. Amylase catalyses the hydrolysis of starch into low molecular weight dextrins and maltose. This maltose liberated reacts with DNSA reagent to give a coloured compound. The intensity of colour is measured in a colorimeter at 540nm and enzyme activity expressed as milligrams of maltose liberated in 30 minutes at 37°C by 1 g of ARF acting on 1 ml of 1% starch solution.
Reagents:

(i) Dinitrosalicylic Acid Reagent (DNSA): 30 g of sodium potassium tartrate (Rochelle's salt) was dissolved in 50 ml of distilled water.

To this was added 1 g of 3,5-dinitro salicylic acid and 20 ml of 2 N NaOH. The solution was heated slightly, cooled and the volume made up to 100 ml filtered and stored in a well stoppered bottle.

(ii) 0.2 M Acetate Buffer: 4.1 g of anhydrous or 6.8 g of hydrous sodium acetate was dissolved in distilled water and 3.1 ml of glacial acetic acid was added to it. Volume was made up to 1 litre with distilled water. The pH was adjusted to 4.8 using a very dilute solution of sodium acetate or acetic acid.

(iii) 1% starch solution: 1 g of soluble potato starch was stirred with 20 ml of distilled water. It was added to 80 ml of boiling distilled water and boiled for 2 minutes. The volume was made up to 100 ml after cooling. This solution was prepared freshly.
(iv) Standard maltose solution: 100 mg maltose was dissolved in distilled water and then volume was made up to 100 ml with water.

Preparation of Enzyme Extract

0.1 g of ARF was suspended in 100 ml of 0.2 M acetate buffer (pH 4.8) for 30 minutes at room temperature with occasional stirring. It was then filtered through Whatman filter paper No.1 and the clean filtrate was used for enzyme assay.

Method of Enzyme Assay

Following tubes were set up and treated as described:

Reagent blank: 1 ml buffer + 2 ml DNSA + 1 ml starch
heated in boiling water bath for 5 minutes
and cooled under running water.

Sample blank: 1 ml enzyme extract and 2 ml DNSA + 1 ml starch and treated as above.

Sample: 1 ml enzyme extract and 1 ml starch
solution incubated at 37°C for 30 minutes
followed by addition of 2 ml DNSA. The contents were heated in a boiling water bath for 5 minutes and cooled under running water. The volume was made up to 20 ml and the colour was measured at 540 nm in a Spectronic 20. Samples and
sample blanks were read against a reagent blank. The difference in the absorbance of the experimental sample and the sample blank was taken for calculating the reducing sugars released. A calibration curve established with maltose was used to convert the colorimeter readings to milligram of maltose.

**Standard**: 0.5, 1.0, 1.5 and 2 ml of standard maltose solution was taken and the volume was made up to 2 ml using distilled water. To it 2 ml of DNSA was added and it was heated in boiling water bath for 5 minutes and cooled.

**For reagent blank**: 2 ml water + 2 ml DNSA was taken and treated as above. Standards were read against this reagent blank.

**Calculation:**

Amylase activity was expressed in terms of milligrams of maltose released in 30 minutes at 37°C by 1 g of ARF acting on 1 ml of 1% starch solution.

**VISCOSITY MEASUREMENTS**

**Measurement of cooked paste viscosity of gruels**

(i) **Standardization of viscometer:**

Brookfield Viscometer (RVT model) was standardized using glycerol. Starting with Spindle No. 1, viscosity was measured
using different spindles and speeds. A time interval of one minute and speed of 50 rpm was standardized and used for all viscosity measurements. The viscosity was calculated in centipoise units using the scale given on the factor finder supplied with the viscometer (Appendix I).

(ii) **Preparation of cooked slurries** (common for all flours)

Slurries of 10, 15, 20, 25 and 30 per cent solid concentrations were prepared. Composition of 400 ml slurries was as follows:

Table 8 Composition of 400 ml slurries

<table>
<thead>
<tr>
<th>Per cent slurry concentration (W/V)</th>
<th>Amount of flour (g)</th>
<th>Amount of water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>40</td>
<td>360</td>
</tr>
<tr>
<td>15</td>
<td>60</td>
<td>340</td>
</tr>
<tr>
<td>20</td>
<td>80</td>
<td>320</td>
</tr>
<tr>
<td>25</td>
<td>100</td>
<td>300</td>
</tr>
<tr>
<td>30</td>
<td>120</td>
<td>280</td>
</tr>
</tbody>
</table>

Control gruels:

Accurately weighed flour was mixed with measured amounts of cold water in a 500 ml beaker and stirred well.
**Experimental gruels:**

To the weighed flour and water mixture ARF/takadiastase were added at different levels at the cost of flour. The mixture (Control and Experimental) was then incubated at 37°C for 10 minutes for the enzyme action to occur with stirring after every 2 minutes. The slurry was then cooked in a boiling water bath for 5 minutes. The temperature of slurry was maintained between 72°C-82°C and was stirred continuously to prevent lump formation. The slurries were brought to a temperature of 45-46°C before measuring the viscosity. At this temperature, viscosity was measured using appropriate spindles at a speed of 50 rpm. The slurry was stirred well before taking the reading and an average of 3 readings was taken.

To study the effect of jaggery and oil in lowering paste viscosity, both these ingredients were added at levels that were suitable for child feeding gruels. These levels were added by the weight of flour. They were:

- **Sago** – 70% level jaggery and 10% level fat.
- **Wheat** – 100% level jaggery and 10% level fat.
- **SBFW** – 100% level jaggery and 10% level fat.

Sago required only 70% of jaggery as this level was enough to establish a suitable proportion of sweetening for the sago gruel.
(iii) **Special treatment with reference to the following:**

(a) **Sago**

Sago bought in the market was cleaned and hand picked. It was weighed and then spread on a clean filter paper and oven dried for 2 hours at 70°C till completely dry. It was cooled to room temperature and again weighed. Then it was ground in an electrical grinder to a fine powder.

Cooked slurries of sago flour and of whole sago of 5, 10, 15 and 20 per cent solid concentrations were prepared and ARF/takadiastase was added at 1-7% level at the cost of the flour either prior to cooking or to the cooked slurry (70°C). Jaggery and fat were incorporated at 70% and 10% levels respectively. Cooked paste viscosity of all the slurries cooled to 45-46°C was measured using a Brookfield Viscometer RVT model.

(b) **Soya Fortified Bulgar Wheat (SFBW)**

Ten kg of SFBW was supplied by the World Food programme, New Delhi Office for the study.

**Milling of SFBW**

The SFBW was milled by the following methods:

1. By hand operated stone mill which is available in every tribal and rural home and some urban slums.
(2) Commercial plate mill which is available in every village, town and city.

(3) 'Baby Prince' electric grinder which is a smaller version of commercial plate mill for laboratory use.

Preparation of slurries

Slurries of 10, 15, 20 and 25 per cent solid concentrations were prepared and ARF at 1-7% of total solids was added at the cost of SFBW flour either prior to cooking or to the cooked slurry (70°C). Jaggery and fat were incorporated at 100% and 10% levels respectively. Cooked paste viscosity of all the slurries cooled to 45-46°C was measured using a Brookfield Viscometer RVT model.

(c) Biscuits

Three varieties of biscuits manufactured by Windsor Foods Limited were purchased from the local market. These varieties were:

Marie biscuits .. .. low fat
Glucose biscuits.. .. medium fat
Avanti salty biscuits.. high fat

All the three types of biscuits were powdered individually in the electrical grinder and used for viscosity measurements.
A slurry of 20 per cent solid concentration was prepared from each biscuit powder. ARF at 4% level of total solids was added to the slurry at the cost of the biscuit powder. The slurry was cooked at 70°C. Cooked paste viscosity of all the slurries was measured at 45-46°C using a Brookfield Viscometer RVT model.

(d) Bread

Locally made sandwich bread was purchased from the market, dried in the oven at 70°C for 3 hours and powdered in the electrical grinder. A slurry of 20 per cent solid concentration was prepared from the bread powder. ARF at 4% level of total solids was added to the slurry and cooked. Cooked paste viscosity was measured at 45-46°C using a Brookfield Viscometer RVT model.

(e) Khichdi

'Khichdi' from rice and dal in the ratio of 3:1 (w/w) was prepared in the laboratory. It was then dried in the oven and powdered and used for viscosity measurements.

Slurries of 10, 15 and 20 per cent solid concentrations were prepared from the Khichdi flour. ARF at 4% level was incorporated into the slurry and cooked upto 70°C. Cooked paste viscosity of all slurries cooled to 45-46°C was measured using the Viscometer.
(f) Chapati

Wheat flour 'Chapati' was prepared in the laboratory. It was then dried in the oven as was the bread, powdered and used for viscosity measurements.

Slurries of 10, 15 and 20 per cent solid concentrations were prepared from the powdered Chapati flour. ARF at 4% level of total solids was incorporated into the slurry at the cost of the flour and cooked up to 70°C. Cooked paste viscosity of all the slurries cooled to 45-46°C was measured in the Viscometer.

(g) Whole Chapati and Khichdi

For visual observation, whole Chapati was broken down into smaller pieces. A 20% slurry was prepared and ARF was added at 4% level. ARF at 4% level was also added to 20% whole Khichdi. Both the slurries were heated to 70°C cooled and observed for viscosity reduction visually.
Experimental Plan

PREPARATION OF PORRIDGE

ACCEPTABILITY TRIALS

Mothers
n=60

Infants & Toddlers
n=60

INTAKE TRIALS
Infants and Toddlers

Wheat Porridge
C
n=30
E
n=30

Sago Porridge
C
n=30
E
n=30

SFBW Porridge
C
n=30
E
n=30

C = Control porridge (Gruel without ARF)
E = Experimental porridge (gruel with ARF)
DEVELOPMENT OF PORRIDGE

The recipe for the preparation of snack porridge was standardized in the laboratory. The feeding trials were initially carried out among mothers and their children to judge the acceptability of the porridge. The subjects were from the low socio-economic groups of 2 Anganwadis* namely Kamatipura of Fatehgunj and Govindnagar of Karelibaug.

(1) **Preparation of porridge:**

Table 9 Composition of 100 ml porridge

a. Control porridge

<table>
<thead>
<tr>
<th>Staple</th>
<th>Flour (g)</th>
<th>Jaggery (g)</th>
<th>Fat (g)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (20%)</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>Sago (10%)</td>
<td>10</td>
<td>7</td>
<td>1</td>
<td>90</td>
</tr>
<tr>
<td>SFBW (20%)</td>
<td>20</td>
<td>20</td>
<td>2</td>
<td>80</td>
</tr>
</tbody>
</table>

b. Experimental porridge (ARF at 4% level of total solids)

<table>
<thead>
<tr>
<th>Staple</th>
<th>Flour (g)</th>
<th>Jaggery (g)</th>
<th>Fat (g)</th>
<th>ARF (g)</th>
<th>Water (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat (20%)</td>
<td>19.2</td>
<td>20</td>
<td>2</td>
<td>0.8</td>
<td>80</td>
</tr>
<tr>
<td>Sago (10%)</td>
<td>9.6</td>
<td>7</td>
<td>1</td>
<td>0.4</td>
<td>90</td>
</tr>
<tr>
<td>SFBW (20%)</td>
<td>19.2</td>
<td>20</td>
<td>2</td>
<td>0.8</td>
<td>80</td>
</tr>
</tbody>
</table>

*Note: An Anganwadi is the smallest operational unit of the Integrated Child Development Services program. It serves approximately 1000 total population in a slum, rural of tribal setting and approximately 200 mother-child units.
A porridge with the above mentioned solid concentration was prepared and ARF was added to the experimental porridge at 4% levels of total solids in the porridges at the cost of the flour.

Method:

Jaggery was grated and dissolved in half the amount of water. Flour was roasted in fat till a roasted aroma developed. The rest of the water was made lukewarm and added to the roasted flour along with ARF and jaggery syrup and the mixture stirred well to prevent lump formation. The mixture was stirred occasionally for 10 minutes to allow the enzyme action to take place and then it was cooked on the fire thoroughly with continuous stirring. The same procedure was followed for the control porridge where only the ARF was excluded.

(ii) ACCEPTABILITY TRIALS

About 60 infants and toddlers and their mothers were selected on the basis of their willingness to serve as subjects. The following criteria were used to judge the acceptability of the porridge:

(1) The porridge should not cause any ill effects like diarrhoea and vomiting.

(2) The amount served to them should be consumed in addition to their home diet.
By the end of the study, only 55 subjects formed the total study population out of which 21 pairs were complete. This was due to intermittent absence of the subjects and consequent inability to feed them continuously for 180 days.

EXPERIMENTAL DESIGN

Anthropometric measurements were carried out on all the subjects that were enrolled. The same children were then pair matched for age and nutritional status. One subject from each pair was then randomly assigned to either of the groups namely the Control group or the Experimental group. Each group consisted of 34 subjects.

The experimental plan is summarized in Figure 4.

The study was conducted over 6 months period. The subjects were fed for 180 days continuously. The Control group received wheat porridge (gruel) of 20% solid concentration without ARF while the Experimental group received wheat porridge (gruel) of 20% solid concentration with 4% ARF (of total solids). Wheat porridge was selected because it is widely consumed in this region and 'Sheera', a similar preparation from wheat flour but with a much higher consistency, is a common delicacy among this population.

Porridge was prepared in the laboratory and taken to the slum. A 3-day pilot study helped in determining the total
Fig 4  EXPERIMENTAL PLAN FOR PHASE III

Experimental Plan

URBAN SLUM OF A LOW INCOME GROUP

INFANTS AND TODDLERS 6-24 months of age (n=82)

BASELINE DATA (ANTHROPOMETRY) (Weight, Height and Arm Circumference)

PAIR MATCHED FOR AGE AND NUTRITIONAL STATUS

CONTROL GROUP (n=34) EXPERIMENTAL GROUP (n=34)

6 months feeding and growth trials

Gruel intake Morbidity profile Dietary survey Anthropometric measurements
amount of porridge that was needed to be prepared. Five kg of control gruel and 10 kg of experimental gruel was prepared daily. The composition of gruel was as under:

**Wheat gruel of 20% solid concentration**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (5 litres)</th>
<th>Experimental (10 litres)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>1000 g</td>
<td>1920 g</td>
</tr>
<tr>
<td>Jaggery</td>
<td>1000 g</td>
<td>2000 g</td>
</tr>
<tr>
<td>Fat</td>
<td>100 g</td>
<td>200 g</td>
</tr>
<tr>
<td>Water</td>
<td>4000 ml</td>
<td>8000 ml</td>
</tr>
<tr>
<td>ARF</td>
<td>-</td>
<td>80 g</td>
</tr>
</tbody>
</table>

Gruel was served to each subject in their home by the investigator between 10 AM and 11.30 AM. A subject was served 100 ml of the gruel initially and fed by the mother or the grandparents and in some cases by older siblings or even neighbours fed the subjects. If the child wanted more, a second 100 ml was served. Daily records were maintained of the amount served and the amount wasted per child.
Other parameters studied were:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>Jelliffe (1966)</td>
<td>Weekly</td>
</tr>
<tr>
<td>Height</td>
<td>Jelliffe (1966)</td>
<td>Monthly</td>
</tr>
<tr>
<td>Arm circumference</td>
<td>Jelliffe (1966)</td>
<td>Monthly</td>
</tr>
<tr>
<td>Dietary data</td>
<td>24 hour recall</td>
<td>Mid-term</td>
</tr>
<tr>
<td></td>
<td>(Pasricha 1959)</td>
<td></td>
</tr>
<tr>
<td>Morbidity profile</td>
<td>Observation and questionnaire</td>
<td>Daily</td>
</tr>
</tbody>
</table>

**Description of the Methods**

**Weight for age:**

Weight is a measurement of body mass. Weight deficiency appears to be the best indicator of the prevalence of protein-energy malnutrition in children of all age groups. Comparison of weight for age values with regional standards at corresponding ages will help determine degrees of underweight in a community (Jelliffe 1966). It is important that the age of the subject is correctly known and presence of pathological weight (e.g.) due to oedema is ruled out.
Methodology:

For obtaining body weights of infants and toddlers, a Salter scale was used as it is portable and convenient to use in the field. The scale was hung freely from a hook along with the trousers. The pointer was brought to zero by adjusting the screw. A standard weight was then used to check its accuracy.

The scale was adjusted to zero before each measurement. The child was placed in the trousers with minimum clothing on and the weight was recorded to the nearest 0.1 kg. The subjects were weighed every week.

Height for age

Height is a linear measurement made up of the sum of four components: legs, pelvis, spine and skull (Jelliffe 1966). The extent of height deficit in relation to age may be regarded as a measure of the duration of malnutrition. Height for age gives a picture of the past nutritional status. A given deficit in height may represent a short period of growth failure at an early stage or a longer period of growth failure at a later stage (Waterlow 1976). Waterlow defined the deficit in height for age as stunting.
Methodology:

Infantometers were used for children below 18 months of age as the measurement of standing height was impossible to take and inaccurate among this age group. The infant was laid on the board which is itself on a flat surface. The head was positioned against the fixed headboard with the eyes looking vertically. The knees were extended by firm pressure and the feet were flexed at right angles to the lower legs. The upright sliding foot-piece was moved to obtain firm contact with the heels and the length was read to the nearest 0.1 cm.

For children above 18 months of age, a measuring tape was fixed vertically on a smooth wall perpendicular to the ground, taking care to see that the floor area was even and not rough. After removing their sandals (if any) each child was measured as he stood with head erect and heels, buttocks, upper part of the back and occiput against the tape, the heels were close together with the arms hanging loosely at the sides. Firm pressure was exerted on the knees to obtain an accurate erect height. A ruler was placed firmly on the head of the child, crushing the hair at right angle to the scale and the height read off from the lower edge of the ruler to the nearest 0.1 cm. Each reading was taken twice to ensure correctness of the measurement.
Weight for height:

While an assessment of the age must be attempted at, an accurate estimate is not always possible. In such cases weight may be expressed in relation to height.

Weight/height relationship is age dependent because the value of the ratio of median weight/median height increases with age. However, this does not invalidate the proposal that weight for a given height is age independent. An age effect on the relationship between height and weight becomes apparent only at the extremes of the range in children who are very tall or very short for their age. Thus standards of expected weight at a given height from a well nourished population are essentially age independent. Further, weight for height is an index of current nutritional status and a deficit in it is termed as wasting (Waterlow 1976).

Methodology:

The weight of each subject for his height was compared with the standard weight at the same height and percentages of subjects falling in 4 categories of nutritional grading as given below was calculated (WHO 1976):
### Mid-Upper Arm Circumference for Age (MUAC)

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 the measurement requires only a flexible fibre glass tape (Jelliffe 1966).

#### Methodology:

The subject's left arm was flexed at the elbow such that the lower arm was at right angle to the upper arm. The length between the acromion process of the scapula and the olecranon process of the ulna was measured with a flexible fibre glass tape and the site of measurement, exactly midway down the upper arm was marked with a pen. The subject was
then made to hang his/her arm relaxed by the side and the tape was 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 then measured to the nearest 0.1 cm (Jelliffe 1966).

Each measurement was made twice to ensure accuracy.

**Standard Employed**

Mean weight, height and weight for height were compared with the National Centre for Health Statistics (NCHS) standard for children 6-24 months of age (Gopaldas and Seshadri 1987).

Mean arm circumference was compared with standards from Health and Nutrition Examination Survey (HANES) (Gopaldas and Seshadri 1987).

**Diet Survey by the Interview Method**

**Questionnaire Method:**

The nutritional status of an individual is dependent on the dietary intake and therefore, nutrient intake of various foods and nutrients with inadequate consumption leading to undernutrition. Diet surveys provide relevant information
on dietary adequacy/inadequacy and the causes of inadequacies in the population group under study.

Several methods of diet surveys exist but the most widely used one is the 7-day-weighment method in which actual quantities of foodstuffs consumed by a family are weighed and recorded daily for a period of 7 days. This method though fairly accurate is tedious. Its significance decreases in a community of the poor socio-economic status where the day-to-day variation in intake is minimal (Madhavan and Swaminathan 1966). An alternative method is, therefore, necessary. Pasricha (1959) did a comparative study of the oral questionnaire method and 7-day weighment method of diet surveys. The reliability of the oral questionnaire method in assessing the dietary intake of individuals was demonstrated. It was reported that the oral questionnaire technique was as good as the weighment method when applied to individuals in the clinic or the field.

Therefore, it was decided to use the 24-hour dietary recall method in the present study to obtain information regarding the calorie and protein intake of the subjects in both Control and Experimental group.

**Methodology:**

All the foods generally consumed by the low socio-economic group were standardized in the laboratory using
standard cups, glasses and spoons (Appendix II). The same utensils were used for the survey. Each child's mother was explained the purpose of the survey and she was requested to carefully observe what her child, who was a subject of the study, ate for the subsequent 24 hours. She was requested to quantify her observation with her own domestic plates and utensils. On the following day the mother was asked to show the investigator the amount of food consumed by her child by using her own domestic utensils. Standard cup, glass and spoon helped the investigator to translate the indicated food quantities into grams or millilitres. All responses of the mother were recorded on a structured and pretested questionnaire (refer Appendix III).

Thereafter, the calorie and protein intake of each item was calculated using values reported by Gopalan et al (1978). Breast milk output and calorie + protein intake from breast milk was calculated using the data of Belavady and Gopalan (1959) (Appendix IV). Qualitative information about the feeding pattern was obtained using the same questionnaire.

MORBIDITY PROFILE

Synergistic role of infection in childhood and malnutrition is well recognised. Reports from different parts of the world have shown that infants and young children have high risk of
illness and death during the weaning period (Morley 1973).

In the present study, the type, frequency and duration of illness of Control and Experimental gruel fed children was documented.

The mother was asked about the illness of her child daily and a record on each subject was maintained. The investigator also observed the child daily for any visible symptoms of disease. The common illness for which observation was made were as follows:

1) Diarrhoea
2) Fever
3) Cough and cold
4) Skin infection
5) Eye and ear infection
6) Mouth infection
7) Vomitting
8) Measles
9) Other illness.

The prevalence and the nature of illness were recorded daily for both the groups.

Statistical Analysis

(1) Mean, standard deviation (SD) and standard error (SE) were calculated for all quantitative parameters.
(2) Per cent prevalence was calculated for qualitative parameters like morbidity profile.

(3) Student's 't' test was applied while making comparisons of quantitative parameters.

Levels of significance selected were $P \leq 0.05$ (significant) and $P \leq 0.01$ (highly significant) and $P \leq 0.005$ (very highly significant).

Formulae used were from Snedecor and Cochran (1967).