CHAPTER - III

MATERIALS AND METHODS
The present investigation was planned with the following objectives:

1) To assess the nutritional status of a sample of Institutionalised elderly drawn from Daycare centres, Non-paying and Paying homes for the aged in Chittoor district of Andhra Pradesh and in a sample of Non-Institutionalised (home-bound) elderly drawn from the same localities.

   The assessment of the nutritional status had the four components of dietary survey, biochemical analysis, anthropometric measurements and clinical examination for nutrient deficiency symptoms.

2) To assess the nutritional status of the elderly in the sample as influenced by living style, economic status and gender.

3) To assess the taste acuity in a sub-sample of the elderly and compare it with that of younger subjects and

4) To study the inter-relationships and associations among dietary constituents; biochemical parameters like haemoglobin, serum total protein, serum albumin and zinc levels; anthropometric data; clinical examination data and taste perception data in the sample of the study.

A purposive sample of 340 elderly subjects was drawn from the Institutionalised and the non-institutionalised (Home bound). The subjects were men and women, above 60 years of age.
In the institutionalised category, the subjects were selected from a) day care centres at Gudimallam, Gundlakandriga and Marrimanda villages, all run by a voluntary organisation, known as Rayalaseema Seva Samithi (RASS), b) Non-paying old age homes at Krishi Vignana Kendra at Karakambadi and Chittoor run by RASS. Another home exclusively for women run by the Ministry of Social Welfare Govt. of A.P., Chittoor was also included to select the sample and c) Paying old age homes known as 'Vridhashramam' run by another voluntary organization, Kanchi Peetham at Srikalahasti of Chittoor district. Photographs of old people from institutions are presented in Plates 1 and 2.

In the non-institutionalised (Home bound) category, the subjects were drawn on the basis of their income and categorised into two groups i.e. Lower Income Group (LIG) and Upper Middle Income Group (UMIG) Low Income Groups consisted of those families drawing less than Rs.1000 a month and those from Upper Middle Income Groups drawing above Rs.5000 a month.

Among these subjects the required number was selected. The details of the stratification of sample chosen and the schematic plan of the study is presented in Figure 4a. The design of the experiment to assess the taste acuity is given in Figure 4b.

A suitable questionnaire was formulated with short, specific and direct questions to elicit information on

1) General information consisting of name, address and age.
PLATE - 1: The Aged Subjects from Day Care Centres
PLATE - 2: The Aged Subjects from Non-paying Homes
Fig. 4a: Schematic plan of the study
Fig. 4b: Schematic Plan for the Assessment of the Taste Acuity
2) Information on economic status, educational level, occupation of the subjects and their family members.

3) Specific dietary habits.

4) Dietary intake for three consecutive days.

5) Anthropometric data and

6) Clinical signs and symptoms and blood pressure of the subjects.

All the subjects were requested to give true facts of information in the questionnaire and were assured that the information given would be kept strictly confidential. The subjects were explained about the objectives of the study and requested to extend their full cooperation, without which, studies of this nature lose their practical significance. In the case of illiterates and very old people who could not write the responses were recorded by the investigator herself, by conducting the interview directly. The formulated questionnaire is given in Appendix (i).

Diet Survey

A diet survey was carried out for three consecutive days. Each subject was provided with a standard cup and spoon and were requested to measure and record the quantity of each and every food item they had consumed, in the questionnaire, in terms of the cup and spoon. For such breakfast recipes like idli, poori, dosai, vada etc, the information with regard to the number and thickness of recipe was requested for. The kitchens of the institutions were visited to note down the cooking practices adopted, the quality of oil used in curries and seasonings and the other relevant information. With regard to home bound subjects, this information was elicited in the interview and recorded in the questionnaire. The raw weight equivalents for the
The collected blood samples were allowed to clot. After 2 to 3 hours, when serum had separated, it was transferred to centrifuge tubes and centrifuged for 20 minutes at 3000 rpm. The supernatant layer of serum was taken into clean, dry, labelled tubes. The serum was stored under refrigeration for the subsequent estimation of proteins, albumin and zinc.

1) Total proteins and albumins were estimated by the Reinhold Method (1966)\(^1\)

2) Zinc was estimated by Atomic Absorption Spectrophotometer as per the method of Butrimovitz and Purdy (1977)\(^2\)

3) Haemoglobin was estimated by the modified method of cyanmethaemoglobin to suit the needs of field investigators. Twenty µl of blood were measured accurately from a Hb Pipette and delivered on to a Whatman No.1 filter paper disc. The filter paper is air dried, labelled and brought to the laboratory for analysis (Annual Report, NIN, 1974).

The preparation of reagents for the estimation of serum total proteins, albumins, zinc and haemoglobin are given in Appendix iii.

**Estimation of Total Proteins and Albumins (Method of Reinhold, 1953)**

**Principle:** The diluted plasma or serum is treated with a special biuret reagent. Light absorption of the coloured solution at 545-560 nm was compared with that of a standard albumin solution treated similarly. By suitable fractionation of serum before


treatment with the biuret reagent, albumin may be determined separately. The method described permits the estimation of total protein and albumin in a single serum sample.

**Procedure:** 7.5 ml of sulphate sulphite reagent was transferred into a standard test tube. To this, 0.5 ml serum was added slowly, with continuous stirring. It was stoppered and mixed by, inversion. Two ml of the mixture was removed and added to 5 ml of biuret reagent in a cuvet. This was set aside until completion of the albumin/globulin separation. Three ml anhydrous, reagent grade, ether was added to the tube containing the remainder of the sulphate - sulphite mixture. It was stoppered, shaken exactly 40 times in 20 seconds, and the mixture was centrifuged at 2000 rpm for five to ten minutes. Two millilitres of the bottom layer was removed from the separated, centrifuged mixture and placed in a cuvet. This is best accomplished by tilting the tube and inserting the pippete down the lower side holding one finger over the pippete until into the lower layer. This will prevent the ethereal solution from entering into the pipette. Five ml of biuret reagent was added. All tubes containing biuret reagent were placed into a waterbath at 30º to 37ºC for ten minutes. They were cooled at room temperature for five minutes and read in a spectrophotometer, at wave lengths from 540 nm to 565 nm, zeroing the instrument with the biuret blank.

**Plotting of Standard Curve**

A series of standards were prepared by measuring 0.1, 0.2, 0.3, 0.4 and 0.5 ml standard protein solution into separate test tubes and above procedure was followed for colour development. A standard curve was plotted using five different dilutions of the stock solution. The corresponding concentrations were 1.5, 3.0, 4.5, 6.0 and 7.5 g/dl
of the working standard. The data was plotted as optical density versus concentration of the standard. The plotted curve for protein is presented in Figure 5.

Calculations:

\[
\text{Total protein} = \frac{\text{Optical Density of test sample}}{\text{Optical Density of standard}} \times \text{conc. of standard proteins} = \text{g of total proteins/100 ml serum}
\]

\[
\text{Density of test sample (after ether)}
\]

\[
\text{Albumins} = \frac{\text{Density of standard}}{\text{Density of standard}} \times \text{conc. of standard} = \text{g of albumins/100 ml serum}
\]

The standard protein was obtained from Armour and Co. Ltd., Madras.

The cyanmethaemoglobin method has been modified to suit the needs of field investigators. 20 \(\mu\)l of blood are measured accurately from a Hb pippette and delivered on to a whatman No. 1 filter paper disc. The filter paper is air dried, labelled and brought to the laboratory.

In the laboratory, the portion of filter paper containing the blood is cut out and dipped in five ml of drabkins solution taken in a test tube, waited for 30 minutes before taking the reading. The filter paper containing blood stain can be stored upto one week.
Fig. 5: Standard curve for total proteins
Estimation of haemoglobin (Cyanmethaemoglobin method)

Haemoglobin is converted into cyanmethaemoglobin by the addition of KCN and Ferricyanide. The colour of cyanmethaemoglobin is read in a photoelectric colorimeter at 540 nm against a standard solution. Since cyanide has the maximum affinity for Hb, this method estimates the Total Hb.

Procedure: Blood 20 µl of was transferred with the help of haemoglobin pipette into a test tube containing five ml of Drabkins solution. The tubes were shaken thoroughly and readings were taken in a spectronic-20. The reagent blank was (Drabkins diluent) adjusted to zero. Percentage of haemoglobin in the standard is 60.

Plotting of Standard Curve for the Estimation of Haemoglobin

A standard curve was drawn using five different concentrations of reagent solution into separate test tubes with five ml of standard haemoglobin in each tube. The corresponding percent concentration of standard haemoglobin were 100, 80, 60, 40 and 20. The data was plotted as percent optical density versus concentration of the standard. The plotted standard curve for haemoglobin is shown in Figure 6.

Calculations

\[
\text{Blood haemoglobin} = \frac{\text{OD of test sample}}{\text{OD of standard}} \times \text{conc. of std. (60)} \times 0.250
\]

The cyanmethemoglobin standard was obtained from Span Diagnostics Private Limited, Madras.
Fig. 6: Standard curve for haemoglobin
Estimation of Zinc by Atomic Absorption Spectrophotometer

Principle: Atomic Absorption is the absorption of light by activated atoms. Atomic absorption takes place on extremely narrow spectral lines, the so-called absorption of resonance lines, which have a spectral width of the order of 0.001 Å. These lines are characteristic for each element, and no two elements possess identical resonance lines. Monochromatic light of the wavelength of such a resonance line will be absorbed only by atoms of that element, whose resonance line is identical with the wavelength of the light source. The amount of absorption is proportional to the total number of absorbing atoms.

In the Atomic Absorption Spectrophotometer (AAS), the light from special sources emitting the resonance line of the element to be determined is passed through a flame which contains a vapour of neutral atoms. The amount of absorption is measured by a light sensitive detector and recorded by a read out system.

Procedure: For the determination of serum Zinc, dilute the sample with 1:5 deionized water.

Zinc standards were prepared by diluting the stock standard solution, described in the "Standard Conditions" for zinc, with 5% (V/V) glycerol. A 5% (V/V), glycerol solution was used as a blank in determining zinc.

Zinc standards: Deliver 10 ml of 10 μg/ml zinc standard into a 100 ml volumetric flask and made to 100 ml with 5% glycerol. This contains 1 μg zinc/ml. Deliver 2 ml, 4 ml, 6 ml, 8 ml and 10 ml of this solution into 100 ml volumetric flasks and dilute to 100 ml with 5% glycerol to produce 0.02, 0.04, 0.06, 0.08 and 0.1 μg/ml of zinc working standards. Plot day to day standard working graph by taking absorbance on 'Y' axis and standard concentration on 'X' axis. Read concentrations of test samples from Figure 7.

The estimations of serum zinc were estimated at "Micronutrients Laboratory", Agricultural Research Institute, Andhra Pradesh Agricultural University, Rajendra nagar, Hyderabad. The model of Atomic Absorption Spectrophotometer used for this study is Spectra AA-20-Varian.
Fig. 7: Standard curve for zinc drawn on the basis of AAOS value.
Assessment of Taste Acuity

In the second phase of the study, taste acuity to the four basic tastes namely sweet, salt, sour and bitter was assessed both in the young and old subjects. A total number of 120 subjects, comprising of 60 young and 60 old, were selected. In each category, there were men and women of two age groups. In the young category, the age groups were 25-30 and 35-40, where as in the old group they were 60-70 and 70-80 years. The details of the stratification of sample is presented in Figure 4b. The subjects in the young group were mostly teaching staff and research scholars of Sri Padmavathi Women’s College and S.V.University. The old subjects belonged to the upper middle income group and were well educated.

The educated or the literate subjects only were selected as they can communicate and express the different scores of detection and recognition thresholds more accurately than the less educated. Apart from this, the personal cleanliness, especially the oral hygiene, is most important aspect in finding out taste acuity. Chewing tobacco and betelnuts is a habit of most of the illiterates which may be a hindrance in finding out the taste acuity. Even among the educated subjects, those with the habit of smoking cigarettes are excluded. The participants had not consumed any food or beverage within one hour of taste assessment.

Taste acuity was determined by assessment of the detection and recognition thresholds. The detection threshold was defined as the concentration at which the participant could detect accurately that the solution was different from water. The concentration at which the specific taste could first be identified or recognised accurately was defined as the recognition threshold. At the point of accurate
recognition, no samples of greater concentration were presented to the participant. Assessments for each participant were repeated until a consistent concentration was detected or recognised.

**Preparation of the solutions**

Taste solutions were prepared from double distilled, deionized water and reagent grade Sucrose (Su), Sodium Chloride (NaCl), Citric Acid (CA) and Quinine Sulphate (QS). For the detection thresholds, the solutions were prepared at the following concentrations.

<table>
<thead>
<tr>
<th>Type</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>For Sweet</td>
<td>2% Sucrose</td>
</tr>
<tr>
<td>For Salt</td>
<td>0.2% Sodium chloride</td>
</tr>
<tr>
<td>For Sour</td>
<td>0.07% Citric Acid</td>
</tr>
<tr>
<td>For bitter</td>
<td>0.04% Quinine Sulphate</td>
</tr>
</tbody>
</table>

The score sheet used for this purpose is given in Appendix - iv.

**Recognition thresholds**

The subjects were given four series (A,B,C,D) of four basic tastant solutions. Each series contained seven different concentrations of the tastest. For each series the tastant quality was identified and the intensity scores were given as in the score sheet given in Appendix - iv.

The seven concentrations of each tastant were used in the study were as presented in Table 21 (Gellinik, 1964). The number of subjects who recognised the tastant at a particular concentration was tabulated for both young and elderly.
Table-21: The Concentrations (µm) of Different Tastants used in the Recognition Threshold Tests

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sweet Mol. Wt. 342.30</th>
<th>Salt Sodium Chloride Mol.Wt. 58.45</th>
<th>Sour Citric Acid Mol. Wt. 210.15</th>
<th>Bitter Quinine Sulphate Mol. Wt. 442.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>1.6 x 10^{-3}</td>
<td>1.6 x 10^{-3}</td>
<td>0.6 x 10^{-4}</td>
<td>0.8 x 10^{-4}</td>
</tr>
<tr>
<td>2.</td>
<td>3.2 x 10^{-3}</td>
<td>3.2 x 10^{-3}</td>
<td>0.8 x 10^{-4}</td>
<td>1.0 x 10^{-3}</td>
</tr>
<tr>
<td>3.</td>
<td>6.4 x 10^{-3}</td>
<td>6.4 x 10^{-3}</td>
<td>1.0 x 10^{-3}</td>
<td>1.2 x 10^{-3}</td>
</tr>
<tr>
<td>4.</td>
<td>1.28 x 10^{-2}</td>
<td>1.28 x 10^{-2}</td>
<td>1.2 x 10^{-3}</td>
<td>1.4 x 10^{-3}</td>
</tr>
<tr>
<td>5.</td>
<td>2.56 x 10^{-2}</td>
<td>2.56 x 10^{-2}</td>
<td>1.4 x 10^{-3}</td>
<td>1.6 x 10^{-3}</td>
</tr>
<tr>
<td>6.</td>
<td>5.12 x 10^{-2}</td>
<td>5.12 x 10^{-2}</td>
<td>1.6 x 10^{-3}</td>
<td>1.8 x 10^{-3}</td>
</tr>
<tr>
<td>7.</td>
<td>1.024 x 10^{-1}</td>
<td>1.024 x 10^{-1}</td>
<td>1.8 x 10^{-3}</td>
<td>2.0 x 10^{-3}</td>
</tr>
</tbody>
</table>

Source: Jellinik (1964)

Statistical Methods

The analysis on quantitative variables was carried on by calculating all descriptive statistics namely mean, median and standard deviation etc. to understand the nature of distribution. To test various hypothesis formulated, 't' test and 'F' test were employed appropriately. Finally, correlational techniques were employed to study the relationships among different variables of the assessment of nutritional status. Multiple Regression Analysis was also done to predict variance in the dependent variables by the independent variables. The details of analysis of data and the interpretations of the results are presented in the next chapter.