Biochemical Profile of Selected Nutrients Among Normal Healthy Adult Women

BY

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A DISSERTATION SUBMITTED TO THE BHARATHIAR UNIVERSITY COIMBATORE, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

MAY - 1986

Acknowledgement

A C K N O W L E D G E M E N T

The author records her deep felt thanks to Dr.(Tmt.) VIJAYALAKSHMI PURUSHOTHAMAN, M.Sc., Ph.D. (Madras), Professor of Department of Food Science, Sri Avinashilingam Home Science College for Women, Coimbatore, for her valuable and inspiring guidance and timely encouragement at every stage of this study.

She wishes to record her most reverential and sincere gratitude to Tmt. M. AMIRTHAVENI, M.Sc., Dip.Ed., Assistant Professor of Foods and Nutrition, Sri Avinashilingam Home Science College for Women for her dynamic guidance rendered throughout the study.

The author wishes to express her thanks to Dr. (Tmt.) USHA CHANDRASEKHAR, M.Sc. (Madras), Ph.D. (Purdue), Professor of Department of Foods and Nutrition for the deep interest shown by her in the course of the investigation.

Grateful acknowledgement are due to the Director and Dean of Post Graduate Studies Dr.(Tmt.) RAJAMMAL P. DEVADAS, M.A., M.Sc., Ph.D. (Ohio State), D.Sc. (Madras), and Dr.(Tmt.) LAKSHMI SANTA RAJAGOPAL, M.S. (Tennessee), Ph.D.(Madras), Principal, Sri Avinashilingam Home Science College for Women, for the facilities provided for the investigation.

The investigator wishes to express her sincere and heartfelt gratitude to the Tamil Nadu Agricultural University for the help rendered during the course of the study.

With deep sense of gratitude the investigator expresses her heartfelt thanks to the students of Sri Avinashilingam Home Science College for their kind co-operation as volunteers for the study.

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Introduction

I INTRODUCTION

"He who has health has hope, and he who has hope has everything"

- Arab Proverb

Nations talk about what they lack. America talks about peace, Germany talks about unity, France about glory, Russia about Freedom and India about Food. Our country has been plagued with chronic food scarcity and periodic famines which have aggrevated the situation for atleast two centuries. For generations our people have been getting inadequate food which was ill-balanced as well.

Thimmayamma (1983) points out that the importance of a nutritionally balanced diet has been well recognised long ago and is referred to even in ancient Hindu scriptures. In Mahabharatha, it has been mentioned that 'He who takes food in proper measures, lives a long life and lives without any disease, gets strength and has alertness of mind. Food nourishes our bodies and people always have known that they must eat to live, to grow normally and to keep strong. But food can do more than satisfying hunger. Parimala (1984) opines that food contributes to physical mental and emotional health.

According to Anand (1981) improvement in health has to be considered in its totality as a part of the overall strategy of human development. A greater degree of confluence is needed between the social welfare and economic development objectives. The Health plan seeks to establish linkages with integrated rural development, education, social welfare, agriculture, water, sanitation and drug development. The chief focus is an achievement of the goals of Health for all by 2000 A.D. through the universal provision of primary health care.

The Central Government guides, sponsors and supports major schemes, for improving the health of the people. Gopalan (1979) exhorts, 'If we are to successfully attack the problems of poverty and social injustice, the concept that human resources represent a most valuable national asset, becomes the central theme of our national development strategy. He drew attention to the fact that most observers over rate the economic importance of land and greatly under-rate the importance of the quality of human agents.

According to the 1981 census, the total population of India is 68.3 crores. The Nation's development depends to a great extent on the way this population acts. If this huge human resource can be developed properly, the nation can look up with hope for further progress.

There are various factors which influence human resources in great many ways, some of them being knowledge, skill, attitudes, aptitudes and nutrition. The ability of an individual to contribute the maximum possible to a nation is dependent on his nutritional and health status. Gopalan (1979) and other nutrition scientists in our country frequently stress the urgency of improving the nutritional status and health of both children and adults, if we are to derive benefit from our vast human endowment.

Nutrition cuts across the whole fabric of society in that it affects all individuals at all stages of their lives. Improvements and developments in the field would only be meaningful in so far as the people may apply scientific knowledge in everyday living situations in the home and community. Understanding the community and its people is basic to any planned change. Gopalan et al (1982) points out that dietary habits in different regions of the world have been determined mainly by the local availability of foods and dietary patterns necessary to sustain reasonably good health have perhaps been evolved after a good deal of trial and error.

According to Devadas (1983) food practices are deeply rooted in their culture, which controls the choice and use of food in the context of their life-styles,

while income, food availability, home food production and marketing facilities influence the food preferences of the individual, customs and traditions dictate the manner in which food should be procured, stored, cooked served and eaten. Eventually distinctive food preferences and prejudices are developed.

The nutritional well being is a prerequisite for health, full functional capacity and physical fitness of individuals and populations. Optimal nutritional status is the foundation for socio-economic development. Hence development programmes place emphasis on the improvement of nutritional status of the people as the principle goal. Assessment of nutritional status lies as the basis of all efforts to control nutritionally determined factors and assessment of human needs for various nutrients is done based on the amount of food actually eaten and the resulting nutritional status.

Hence nation wide efforts have been made to assess the nutritional status of population groups through the various means available like anthropometry, food and nutrient intake, clinical examinations and biochemical picture. The Indian Council of Medical Research has developed the Recommended Allowances for Foods and Nutrients which are widely used to assess the nutritional status of the population. The National Institutes of Nutrition has produced data on anthropometric

measurements which is used for purposes of comparison. Of equal importance is the need to develop local norms for biochemical profile of our population. Gauberlich (1974) points out that biochemical measurements represent the most objective assessment of the nutritional status of an individual frequently providing pre or subclinical information. Nevertheless we have not yet developed biochemical norms representing the levels of various nutrients in blood for our population groups.

Hence a humble effort has been made in the present study, to determine the biochemical profile of selected nutrients among young healthy adult women, which it is hoped will be a step towards having our own data for the biochemical profile of our population groups.

Review of Literature

II REVIEW OF LITERATURE

The Review of Literature pertaining to this study, "Bio-chemical Profile of Normal Healthy Adult Women", is discussed under the following heads.

1. Need for assessing the nutritional status
2. Methods of assessing the nutritional status
3. Need for developing norms
4. Studies related to biochemical profile of nutrients
5. Need to have local data on biochemical profile of nutrients.

1. Need For Assessing The Nutritional Status

Obert (1911) defines nutritional status as the physical health of a person as it results from consumption and utilisation of food in the body. It is determined by the kind and amount of nutrients supplied to the body and how completely they are used to meet body needs. Maintenance of the health is dependent on good nutrition (Food and Nutrition Board 1979). According to Panerjee (1962) and Gopalan (1966) food production and demographic data indicates low availability of many food groups including the major source of energy. This low availability of most foods is reflected through different degrees of malnutrition prevalent in the large sector of Indian population.

Srikantia (1973) opines that the health and longevity of the mothers are far from satisfactory. The mortality among child bearing women is eight to ten times higher in India than in developed countries.

Jelliffe (1978) points out that the principle aim of the nutritional assessment of a community is to map out the magnitude and geographical distribution of malnutrition as a public health problem, to discover and analyse the ecological factors that are directly or indirectly responsible and where possible suggest appropriate corrective measures preferably capable of being applied with continuing community participation.

According to Shankaranand, (1981) improvement in the health status of the people has to be considered in its totality as a part of the overall strategy of human resource development. The nutritional well being of a community or nation is an important determinant of its health status. Protein Advisory Group (1976) opines that in short the nutritional assessment of a community should aim at discovering facts and guiding action intended to improve nutrition and health.

2. Methods of Assessing the Nutritional Status

Jelliffe, (1966) Davidson et al (1974), Swaminathan, (1974) Blackburn, (1977) have listed dietary survey, physical

anthropometry, biophysical and biochemical tests and vital statistics as yardsticks in assessing the nutritional status.

Meisell and Francois, (1931) opine that anthropometric data have been used primarily as a measure of nutritional status and health. Bowler et al, (1983) state that anthropometry is used as a proxy for nutritional status and are shortened measurements of body nutrient stores and previous growth patterns.

Bugine, (1972) reports that there is a general agreement that anthropometric measurements properly taken and properly evaluated represent a useful basis for the assessment of nutritional status. According to Devadas (1982) measurements of heights and weights are important tools in assessing the nutritional status of a population. If properly obtained and interpreted they would serve as useful indicators in the evaluation of nutritional status. Emmerseifrit (1984) points out that height once gained cannot be lost and it is believed that it is relatively less affected by acute and short episodes of malnutrition but is affected by chronic malnutrition of long duration.

According to Pechives, (1974) weight measurement is by far the most common and best known anthropometric

method. It is also most valuable for basic health services for it provides the greatest amount of information.

Combination of anthropometric measurements and indices like height, weight and weight/height have been suggested for assessment of nutritional status by NIN (1979). Laboratory tests provide a precise objective method of determining levels of nutrients in blood or urine that indicates the status of the body with respect to specific nutrients, Obert (1911).

According to Rao et al, (1969) and Whitehead, (1969) the most objective means for assessing the nutritional status and to evolve some normal pattern of deficiency will be based on biochemical analysis of materials such as blood and urine. Bauberlich & Dowdy, (1974) suggest that biochemical measurements represent the most objective assessment of the nutritional status of an individual frequently providing pre or subclinical information.

Blood samples do permit the investigation a slightly greater latitude than urine since the former can be partitioned into whole blood, serum, plasma and/or red blood cells.

Biochemical techniques as with clinical examinations, anthropometric measurements and dietary surveys can be of considerable value in assessing nutritional status of a person.

According to Swaminathan (1974) dietary surveys constitute an essential part of any complete study of nutritional status of individuals or groups, providing essential information on nutrient intake levels, sources of nutrients, food habits and attitudes. Berg (1981) states that malnutrition in large populations is measured primarily by comparing people's actual diets with what nutritionists regard as adequate diet.

3. Need for Developing Norms

Varley et al (1980) suggest that it has long been customary to quote "normal ranges" for the concentration of constituents in body fluids. These are intended to indicate the values found in persons who are in good health and by inference to define results which indicate that some abnormality is present.

Bauberlich (1974) opines that depending on the measurement employed information may be obtained as to an individual's present or recent and sometimes long-range nutritional status.

Jelliffe (1966) opines that the standards for a community are usually obtained by measuring a statistically adequate sample of a healthy, well-fed segment of the

population whose ages are known with certainity. Local anthropometric standards should be prepared and used wherever possible because they may often be considered a more realistic goal.

4. Studies related to Biochemical Profile of Nutrients

a. Iron and Haemoglobin

According to Finch, (1977) iron deficiency is considered to be a state in which the iron supply is inadequate to permit normal synthesis of essential iron compounds. Cullimbine, (1969) found a correlation between the haemoglobin level and muscular function as shown by a seep of movement and ability to sustain effort.

Sandra and Bradfield, (1971) showed that children with relatively low concentration of haemoglobin made less efficient use of oxygen than did children with higher concentrations. Iron deficiency anaemia affects the physical capacity by reducing the availability of oxygen to the tissues which in turn affects cardiac output and the heart eventually leading to death in severe cases Vijayalakshmi et al, (1983)

Surveys conducted by WHO (1972) throughout the world have shown a prevalence of iron deficiency in menstruating women varying from 10-50 percent. These data are sufficient

to establish the prevalence of iron deficiency in hundreds of millions of people in the world today.

In developing countries where the amount of available dietary iron is extremely low and particularly when there is also an increase in iron losses from hookworm infestations, anaemia of moderate to severe degree is frequent. This condition reduces the work production and ability to resist infection and increases mortality risk during pregnancy.

According to Cohen, (1969) WHO, (1972) although the measurement of haemoglobin levels in man is not difficult, the accurate diagnosis of anaemia is far from easy, especially when a particular level of haemoglobin is the only criterion of normality.

Rebecca, (1992) opines that serum iron and transferrin are needed to diagnose iron status of an individual along with iron and haemoglobin estimations. Swaminathan, (1974) has suggested a value ranging from 300-480 mcg/100 ml. for TIBC. Letsky, (1982) suggests that in health the serum iron of adult non pregnant women lies between the range of 13-27 p mol/lt. It shows immense individual diurnal variation and fluctuates even from hour to hour.

The total iron binding capacity (TIBC) in the non-pregnant state lies in the range of 45-72 µgm./lt. In the non-anaemic individual the TIBC is approximately 1/3 saturated with iron.

According to NIN report, (1978) haemoglobin and PCV either singly or together have been used to determine iron nutritional status of population groups. It is generally assumed that haemoglobin and PCV have a high degree or correlation and a simple ratio in fact has been used for converting haemoglobin into corresponding PCV values.

b. Protein

Maclean et al, (1977) reported that protein deficiency might interfere with the optimum utilisation of energy by interfering with fat absorption. According to Stein et al (1993) for subjects in nitrogen balance the protein synthesis rate, the protein turnover rates are equal. Gopalan et al, (1984) reports that the daily protein needs of an adult are adequately met if the diet provides about one gram of protein per kg. of body weight. Protein deficiency is difficult to assess but serum albumin concentrations are recognised as the index of protein status (Fluet et al,(1979).

c. Vitamin A

According to Srikantia, (1978) Vitamin A deficiency, another type of malnutrition poses a serious threat as it causes blindness. Scrimshaw et al, (1981) reports that the interaction between hypovitaminosis A and infection is well recognised. Vitamin A deficiency diminishes the resistance to infection and infection also can impair Vitamin A nutriture.

Peterson et al, (1973) indicates that a population is considered to have a Vitamin A nutritional problem when 15% or more of the persons surveyed have serum values less than 10 mcg/100 ml. Persons with less than 10 mcg/100 ml. of Vitamin A are likely to have significantly developed low liver stores and can be considered as potential cases. According to Sauberlich, levels of plasma Vitamin A correlate closely with plasma retinol binding protein levels. Levels of 10-20 mcg/100 ml. are considred "low" while levels below 10 mcg/100 ml. reflect a defecient state.

Agnikar and Flores, (1979) opine that the setting of an "adequate" or "acceptable" range for serum Vitamin A which is universally applicable poses many problems since few manifestations of Vitamin A deficiency are evident

in individuals with serum values of 20 mcg/100 ml. or more, this level has been proposed as a cut off for the adequate category however and when applied to young children may over estimate the magnitude of the problem.

According to [illegible], (1973) the realisation has grown that Vitamin A defeciency and the blindness that results from its acute form in young children remains a major public health problem in many parts of the world.

Calcium

The small part of the body calcium present in plasma and other extra-cellular fluid is vitally important in maintaining the correct conditions for normal neuromuscular transmission, glandular secretion and for activity of enzyme system particularly those involved in blood coagulation (Linkswiller et al (1974).

Tanaka and Deluca (1973) opine that the level of calcium in serum is maintained remarkably constant at a concentration of about 10 mg. per 100 ml. since it is regulated by parathyroid hormone, calcitonin and metabiologically active Vitamin D.

According to Varley et al (1963) although the total plasma calcium level varies little in health, the homeostatic mechanisms of the body are primarily directed towards

maintaining a constant concentration of the ionised calcium fraction which is the biologically active component. For many years the normal range for the serum calcium was accepted as being 2.25 to 2.75 m.mol./l (9.0 to 11.0 mg./100 ml.) with some workers allowing an upper limit of 2.87 m.mol./l. (11.5 mg./100 ml.)

Zinc and Copper

According to Iyengar (1986), take 15 mg. of zinc daily and try to maintain a trace-element balanced diet. This advise is being increasingly given in the west by physicians and health workers to the people, especially to pregnent women and to the very young people. Fell and Burns (1978) report that zinc is a component of numerous enzyme systems which help to regulate most stages of nucleic acid and protein synthesis. Defeciency therefore has effect upon cell regeneration and overall growth of animals and man. Acute signs of zinc defeciency are seen only rarely, in complete parenteral nutrition is one instance. Prasad (1979), reports that according to their techniques plasma zinc concentration (mean ± SD) in normal subjects is 112 ± 12 mg.

Fisher et al (1984) opine that the ingestion of moderately high amounts of zinc for a 6 week period by human results in a decreased copper status. Dietary zn/cu ratios of greater than 10:1 can produce an impact on copper nutriture. According to Lefeure et al (1985) HDL from

zinc deficient rats contained more free cholesterol and less triglyceride than HDL from rats fed higher amount of zinc. In zinc defeciency lipolysis of chylomicrons would be defective, preventing the transfer of surface lipids and apoproteins to the HDL fraction.

6. Need to have local Data on Biochemical Profile of Nutrients

Jelliffe (1966) points out that it should be the ultimate aim of nutritionists to prepare and use local standards for different patterns of growth. Body proportions appear to vary in different groups of peoples. This is partly genetic, possibly being related in some instances to climatic adaptation.

Francois & Weisell (1982) warn that it may be misleading to use reference data from developed countries, particularly as the population approaches adulthood. In many developing countries the mean adult height and weight is the only 3rd centile of the developed country reference population. The weight comparisons are made with international standards which is the 50th percentile data.

Though we have developed local standards for certain criteria such as anthropometry, height, weight daily food and nutrient intake, we lack norms regarding the biochemical picture of various nutrients. This forms the basis for this study.

Experimental Procedure

III EXPERIMENTAL PROCEDURE

The experimental procedure pertaining to this study on the "Biochemical Profile of Selected Nutrients among Normal Healthy Adult Women" included the following steps.

A. Selection of the venue

B. Selection of volunteers

C. Recording anthropometric measurements

D. Determination of the food and nutrient intake of the volunteers

E. Determination of blood pressure and clinical examination

F. Determination of the bio-chemical profile of selected nutrients

1. Collection of blood and separation of serum

2. a) Determination of haemoglobin level and packed cell volume

   b) Estimation of serum iron and total iron binding capacity

3. Estimation of serum copper and zinc

4. Estimation of serum calcium

5. Estimation of serum Vitamin A levels

and 6. Estimation of total proteins and its fractions

A. Selection of the venue

Sri Avinashilingam Home Science College premises was selected as the venue for the study. It proved to be convenient since ample number of students belonging to the age group of 21-23 years on whom the study ought to be conducted were available. The laboratory in which the experiments should be conducted was also situated in the college premises. Hence the college was selected as the venue of the study.

B. Selection of volunteers

The volunteers for the study were so chosen that they were in the age group of 21-23 years. The number of volunteers were one hundred. All the volunteers were doing moderate work and as defined by ICMR (1981) for reference woman they were free from disease and physically fit, as revealed by the clinical examination. The fact that the volunteers were all literate, proved to be useful since they were themselves interested in the study and co-operated with the investigator. There was no difficulty in getting the correct information regarding their past and present health status.

Regular alcohol consumption affects nutrient intake and modifies the nutrient level in the blood according to Carol (1978). According to Joseph et al (1978) smoking

affects the availability of the nutrient to the body thus affecting the biochemical profile. Hence precaution was taken to see that the volunteers were non-smokers and non-alcoholics.

Out of the hundred volunteers 35 belonged to the middle income group (Rs.1000-1500) and 65 belonged to the high income group (Above Rs.1500/-). The volunteers were selected after ensuring that they were free from diseases and enjoying good health. This was made sure with the help of anthropometric measurements and clinical examination.

C. Recording anthropometric measurements

Weight is the anthropometric measurement most in use and is the key anthropometric measurement. The weights were taken in the morning for all the volunteers with ordinary light clothes on, and after removing their foot wear. The weights were measured in a level operated beam balance capable of reading upto 0.1 kg.

Jelliffe (1966) opines the height of an individual is made up of the sum of four components-legs, pelvis, spine and skull. The height was recorded with the help of a stadiometer which reads nearest to 0.1 cm. The volunteers were made to stand on a flat floor by the scale after removing the foot wear, feet parallel with heads, buttocks,

shoulders and back of head touching the upright. The head should be held comfortably erect, with the lower border of the orbit in the same horizontal plane as the external auditory meatus. The arms should be hanging at the sides in a natural manner. The head piece is gently lowered crushing the hair and making contact with the top of the head. The height and weight were measured for all the volunteers.

D. Determination of food and nutrient intake of the volunteers

Berg (1981) states that malnutrition in large populations is measured primarily by comparing people's actual diets with what nutritionists regard as adequate diet.

To determine the food and nutrient intake of the volunteers, twenty four hour recall was conducted by gathering details on the foods consumed as recalled by the individuals and from the data, raw weights of various food stuffs were computed. The nutrients available to the volunteers were calculated with the help of food consumption tables of ICMR (1994). Persons consuming adequate food as compared with the Recommended Dietary Intake of ICMR (1981) were chosen for the study.

E. Determination of Blood Pressure and Clinical Examination

Blood pressure is the lateral pressure exerted by blood on the vessel walls. The pressure of the brachial artery was measured with the help of a sphigmomanometer. The Auscultatory method of measurement was made. Keeping at the level of the heart the cuff is tied round the upper arm, pressure raised to 200 mm Hg. and then gradually released. Variations of sound are heard with the stethescope placing the chest piece on the brachial artery, a little below the cuff. As the pressure is released, variations of sounds are heard, a sudden appearance of a clear tapping sound indicated the systolic pressure the tap sound is replaced by the murmer which was replaced by a clear loud sound which suddenly becomes muffled and rapidly fades. This indicates the diastolic pressure.

Clinical assessment of hair, face, eyes, lips and mouth, tongue, teeth, gums, skin, nails and general appearances were taken into account. The above took care to indicate any defeciency symptoms on tongue and lips like cheilosis and angular stomatitis, majenta tongue or any changes on the skin like dermatosis and poor or good musculature. Volunteers who did not have any deficiency symptoms were considered healthy.

Details of the proforma used for clinical examination is given in Appendix I.

F. Determination Of The Bio-Chemical Profile of Selected Nutrients

1. Collection Of Blood And Separation Of Serum

The only type of blood sample which can ordinarily be considered for clinical and biochemical tests are venous and capillary blood (Rieterich 1969). For the study 10 ml. of the blood was drawn from the vein, taking care that no air bubbles were present and using a 10 ml. syringe. Two ml. of the whole blood was stored at 4°C using ammonium oxalate as anticoagulant.

Eight ml. of the whole blood was centrifuged serum separated and stored at 4°C. The estimations were carried out within a day or two.

2. a. Determination Of Haemoglobin And Packed Cell Volume (PCV)

Haemoglobin level and heamtocrit values are useful indices in finding the state of nutrition. Hence haemoglobin status of all the selected volunteers was estimated using the wong's method (NIN 1971) and packed cell volume by the usual centrifugation method as per the procedure of National Institute of Nutrition (NIN 1971).

Details of the procedure followed are given in Appendix II.

b. Estimation Of Serum Iron And Total Iron Binding Capacity (TIBC)

Serum iron and TIBC are dependable indicators of iron nutritional status. Since there is only a very small amount of iron in the serum, great care has to be taken to avoid any form of contamination. Letsky (1982) points out that serum iron shows immense individual diurnal variation and fluctuates even from hour to hour.

The whole blood was centrifuged, serum seperated out and immediately stored in the refrigerator, estimations carried out within a few hours. Many colorimetric methods have been advised of which Dipyridyl method is reliably used. For all the volunteers serum iron and TIBC were estimated by Dipyrdyl method of Ramsay (1964 and 1958).

Details of the procedure are given in Appendix III.

For the estimation of serum Vitamin A level, serum was seperated out and stored in the refrigerator. The analysis was done within 24 hours.

3. Estimation of serum copper and zinc

Many investigators have utilised plasma zinc and copper for biochemical assessment of certain diseases like

pulmonary tuberculosis. It is an indication of health status. So the serum zinc and copper levels were estimated for all the volunteers by the Atomic absorption spectrophotometer method of Parkeh*at al (1967) details of which are given in Appendix IV.

4. Estimation of serum calcium

The small part of the body calcium present in plasma and other extra cellular fluids is vitally important in maintaining the correct conditions for normal neuromuscular transmission, glandular secretion and for activity of enzyme systems particularly those involved in blood coagulation (Linkswiler at al 1974). The serum was seperated out and was used for the estimation of calcium by the modified method of Kramer tisdall as given in NIN Manual (1971).

The details of the procedure are given in Appendix V.

5. Estimation of Serum Vitamin A Levels

The serum retinol was estimated by the trifloureacetic acid method by Neeld and Pearson as modified and suggested by Reel quoted by Gyorgy and Pearson (1967). The details of the procedure are given in Appendix VI.

For the estimation of serum Vitamin A level, serum was seperated out and stored in the refrigerator. The analysis was done within 24 hours.

6. Determination of total protein and its fractions

Protein nutritional status is difficult to assess but serum proteins, albumin and globulin concentrations are recognised as the index of protein status according to Dehra Mfluit et al (1979). Hence along with total protein estimation, albumin and globulin estimations were carried out on all the volunteers by the Biurette method of Hawk and Oser as given in NIN manual (1971).

Details of the procedure are given in Appendix VII.

Results and Discussions

IV RESULTS AND DISCUSSION

The results and discussion pertaining to the study on "Bio-chemical Profile of Selected nutrients Among Normal Healthy Adult Women" is presented under the following headings:

1. Background details of volunteers
2. Clinical examination, pulse rate and blood pressure of volunteers
3. Anthropometric measurements of volunteers
4. Mean food and nutrient intake of the volunteers
5. Mean and range of haemoglobin and packed cell volume
6. Mean and range of serum iron and total iron binding capacity
7. Mean and range of serum copper and zinc levels
8. Mean and range of serum calcium levels
9. Mean and range of serum retinol levels
10. Mean and range of serum proteins - Albumin and globulin levels

and 11. Suggested reference values for different nutrients

1. Background details of volunteers

All the 100 volunteers selected for the present study came from well-to-do families with an income of Rs.1200 and above. Their past healthy history was good, they enjoyed good health and were doing moderate physical activity.

They did not suffer from any genetic or metabolic disorders and were free from serious illness for the past 3 years. All the volunteers belonged to the age group of 21-23 years. The volunteers were non-smokers and non alcoholics.

2. Clinical examination, pulse rate and blood pressure of volunteers

Table I highlights the health status of the volunteers as revealed by the Clinical Assessment.

TABLE I

CLINICAL ASSESSMENT OF ALL THE SELECTED VOLUNTEERS

S.No.	Details	Symptoms	Deficiency symptoms	Healthy and normal in percentage
1.	Hair	Lack, lusture, thin and sparse	Nil	100
2.	Face	Moon face Pale look	Nil	100
3.	Eyes	Night blindness Bitot's spot Pallor of eye other symptoms of deficiency	Nil	100
4.	Lips and mouth	Angular stomatitis cheilosis	Nil	100
5.	Tongue	Red, Swollen, Majenta Tongue	Nil	100
6.	Teeth	Mottled enamel caries		
7.	Gums	Spongy bleeding gums	Nil	100
8.	Skin	Dry skin hyperkeratosis dermatitis	Nil	100
9.	Nails	Koilonychia	Nil	100
10.	General Apperance	Oedema Thin and emaciated	Nil	100

From Table I it is evident that none of the volunteers selected for the present study suffered from any deficiency symptoms as shown through the clinical examination of their hair, face, eyes, lips, tongue, teeth, gums, skin, nails and general appearance. Thus all the volunteers were considered healthy as revealed by the clinical examination. The clinical assessment was carried out by the investigator herself after taking necessary guidance from a medical doctor. Further details recorded in the proforma indicated that the volunteers had normal bowel movement and also did not suffer from any allergic conditions or any chronic illness like ulcer, diabetes, liver disease and other such problems.

Clinical examination also revealed that none of the volunteers were anemic.

The pulse rate and blood pressure were normal for all the volunteers, and the mean and range values of pulse rate and blood pressure are presented in Table II.

TABLE II

MEAN AND RANGE VALUES OF PULSE AND BLOOD PRESSURE

S.No.	Details	Pulse rate per minute	Blood pressure mm Hg
1.	Mean	71.9	120/80
2.	Range	69 - 72	-

3. Anthropometric measurements of the volunteers

Table III presents the mean and range values of height and weight of the volunteers.

TABLE III

ANTHROPOMETRIC MEASUREMENTS OF THE VOLUNTEERS

S.No.	Details	Height in Cms.	Weight in Kg.
	Present study		
1.	Mean	155.47	49.22
	Range		
2.	ICMR (1965-66)	147.30	47.30

From Table III it is noticed that the volunteers recorded a mean height of 155.47 Cm. and weight as 49.22 kg., as against.

The ICMR standard values of 147.3 cm. for height and 47.8 kg. for weight.

Thomson and Billewicz (1963) opine that in general there is no reason to suppose that adults of either short or tall stature have a health risk attributable to their stature, except perhaps in relation to pregnancy and child birth.

The height of the volunteers in the present study was higher by 8.2 cms. and heavier by 1.4 kg. when compared with the ICMR value.

The individual values are given in Appendix VIII.

4. Mean food and nutrient intake of the volunteers

Table IV represents the mean food intake of volunteers as compared to the recommended dietary allowances for Indians by the ICMR (1981).

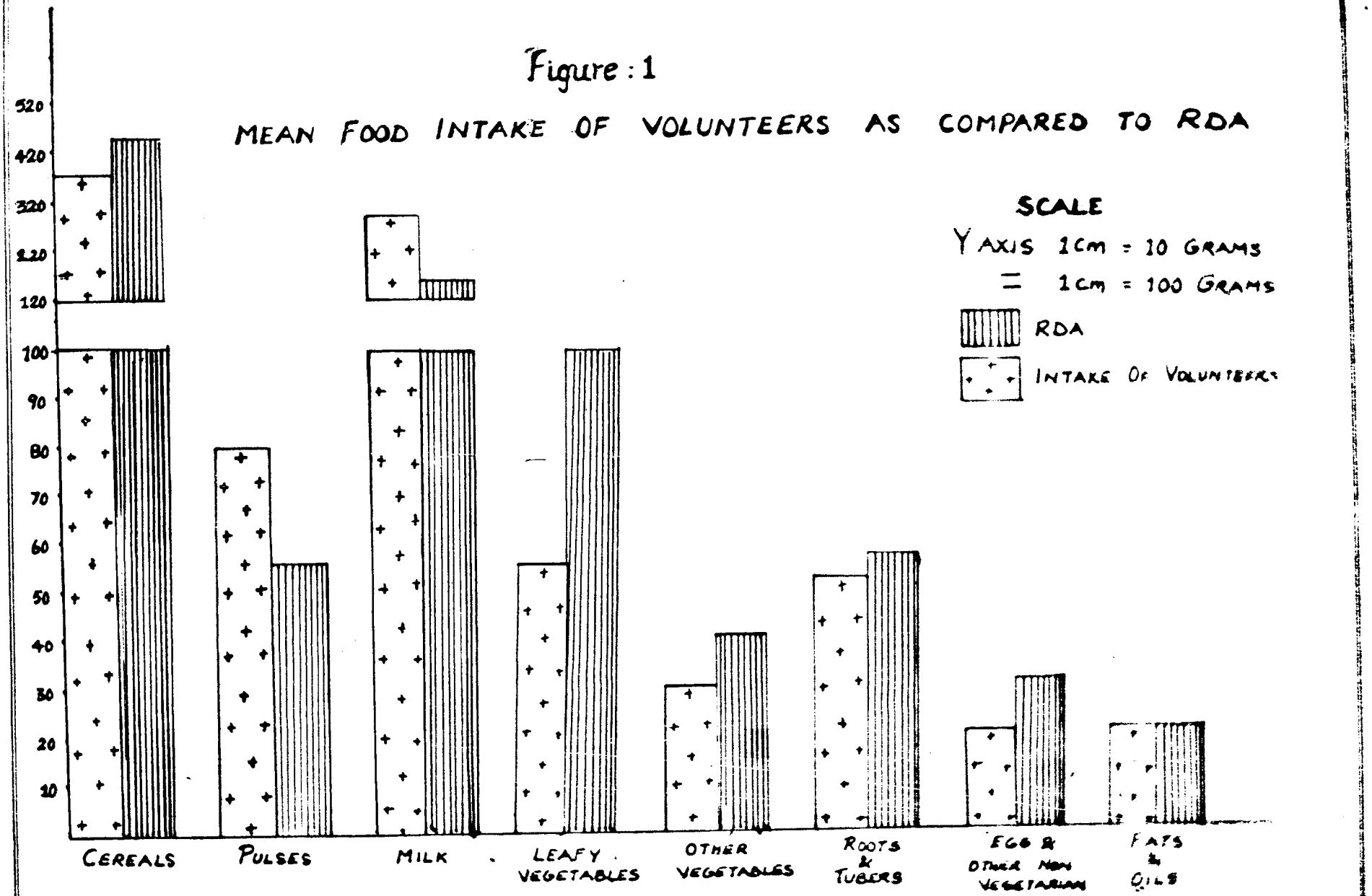
TABLE IV

MEAN FOOD INTAKE OF THE VOLUNTEERS

S.No.	Food items	Mean intake of volunteers in Grams	Recommended allowance for moderate work ICMR (1981)
1.	Cereals	380	440
2.	Pulses	30	45
3.	Green leafy vegetables	65	100
4.	Other vegetables	30	40
5.	Roots and Tubers	55	50
6.	Milk and Milk Products	306	150
7.	Fats and Oils	20	20
8.	Sugar and Jaggery	13	20
9.	Egg and other Non Vegetarian items	207	30
10.	Fruits	90	30

Figure : 1

MEAN FOOD INTAKE OF VOLUNTEERS AS COMPARED TO RDA



From Table IV it is evident that the mean food intake of the volunteers was adequate with reference to all the foods except cereals, green leafy and other vegetables. However the inadequacy with reference to these foods was only marginal. The mean nutrient availability was calculated for these foods in an effort to find out if these intake were adequate nutritionally.

Fig. 1 presents the mean food intake of volunteers as compared with RDA (1981).

Table V presents the details regarding the mean nutrient intake in comparison with RDA (1981).

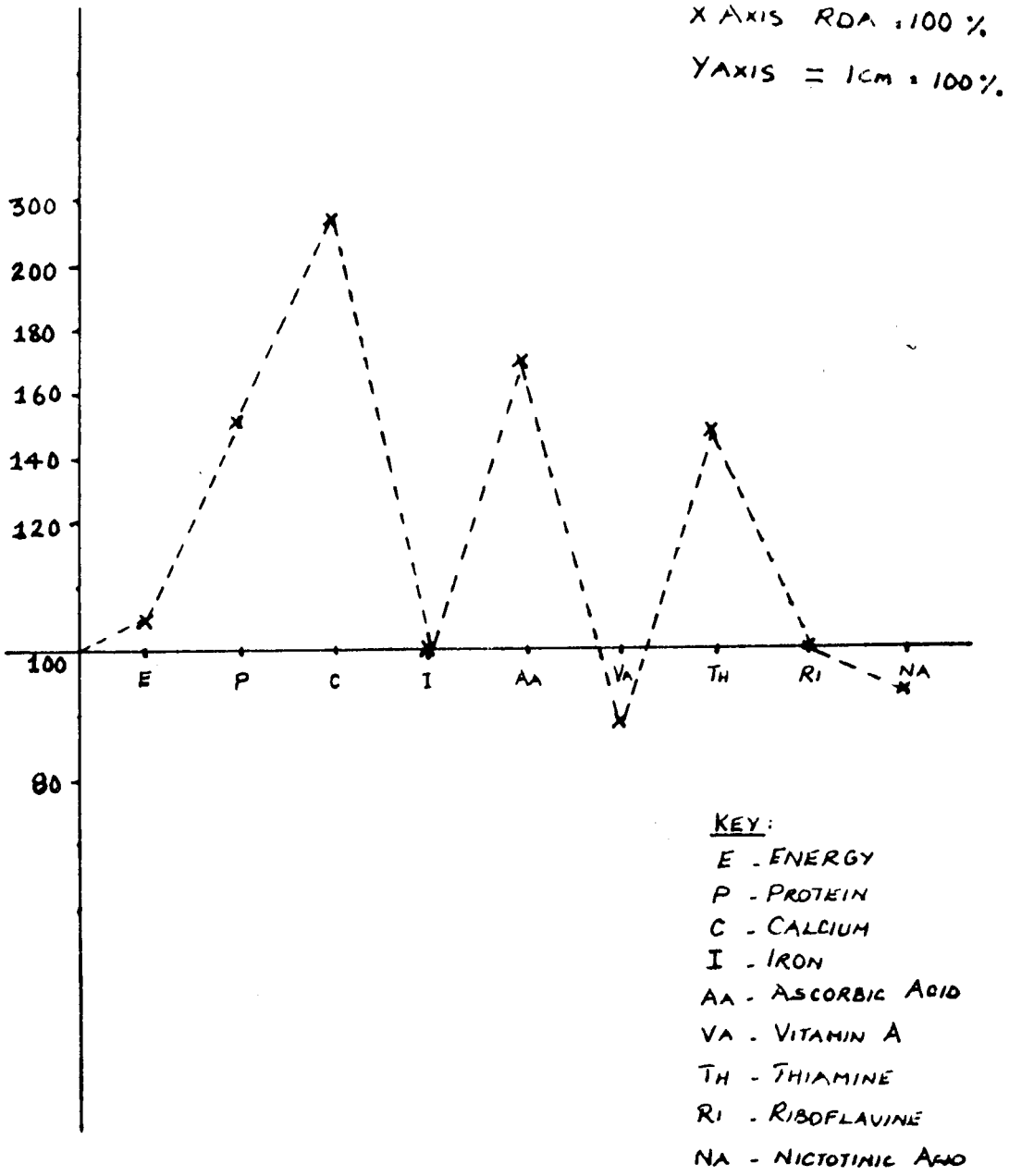
TABLE V

MEAN NUTRIENT INTAKE OF THE VOLUNTEERS

S.No.	Nutrients	Mean intake by volunteers	Recommended Allowances of ICMR (1981)
1.	Energy (Kcal)	2301	2200
2.	Protein (g)	69.89	45
3.	Calcium (g)	1039	0.4 - 0.5
4.	Iron (mg)	32	32
5.	Ascorbic Acid (mg)	68	40
6.	Vitamin A (μ g) (retinol)	662	750
7.	Thiamine (mg)	1.64	1.1
8.	Riboflavin (mg)	1.3	1.3
9.	Nicotinic acid (mg)	15	16

Figure 2

MEAN NUTRIENT INTAKE OF VOLUNTEERS AS COMPARED TO RDA IN PERCENTAGE



Though the mean food intake was slightly inadequate with reference to cereals and leafy and other vegetables. The calculation of nutrient intake revealed that the intake was on par with the recommended nutrient allowances and this justified the inclusion of these volunteers in this study.

Fig.2 presents the mean nutrient intake of volunteers as compared with the RDA.

The individual values of food and nutrient intake of volunteers are presented in Appendix IX.

5. Mean and range of haemoglobin and packed cell volume levels

Table VI shows the levels of Haemoglobin and PCV of the volunteers.

TABLE VI

MEAN AND RANGE OF HAEMOGLOBIN AND PCV LEVELS

S.No.	Details	Present study	
		Haemoglobin g/100 ml.	Packed cell volume %
1.	Mean	12.9	38.8
2.	Range	11.6 - 14.8	37-47

The mean haemoglobin level recorded was 12.9 g/100 ml. The range varied from 11.6 - 14.6 g./100 ml.

According to Cook et al (1971) haemoglobin determination is useful in indicating the severity of the deficiency state, but is too insensitive and non specific to serve as an initial screen for iron deficiency.

According to WHO (1975) the haemoglobin values lower than 12 g./100 ml. were considered anemic.

The mean value for PCV levels obtained in the present was 38.5 percent as against the standard range of value of 37-47 percent.

According to O'Neal et al (1976) 38 percent PCV is an acceptable value.

Best and Taylor (1967) suggest that the volume of cells (packed cell volume) is 46 percent of the total volume of the specimen of blood.

The CDC Nutrition surveillance (1976) regards 12 g/100 ml. for haemoglobin and 37 percent for PCV level as acceptable.

The individual values of haemoglobin and PCV are presented in Appendix X.

6. Mean and range of serum iron and total iron binding capacity of volunteers

Table VII depicts the mean and range of serum iron and total iron binding capacity (TIBC) of volunteers as compared with the values given by Varley (1980).

TABLE VII

MEAN AND RANGE OF SERUM IRON AND TIBC OF VOLUNTEERS

S.No.	Details	Present study mcg/100 ml.	Varley(1980) mcg/100 ml.
1.	Serum Iron		
	Mean	94.26	-
	Range	60-145	80-175
2.	TIBC		
	Mean	320.98	
	Range	260-378	249-387

The mean serum iron and TIBC levels were 94.26 mcg/100 ml. and 320.98 mcg/100 ml. respectively. The ranges were 60-145 mcg./100 ml. for serum iron and 260-378 mcg/100 ml. for TIBC respectively.

The ranges suggested by Cornn and Cornn (1980) is 75-175 mcg/100 ml. for serum iron and 240-410 mcg/100 ml. for TIBC which are slightly higher than the values noted in the present study. Varley (1980) gives a range of 30-175 mcg/100 ml. for serum iron and 240-397 mcg/100 ml. for TIBC. Serum iron values of the present study were lower when compared to other studies.

However Letsky (1982) opines that, in adult non-pregnant women the serum iron shows immense individual diurnal variationsand fluctates even from hour to hour. According to O'Neal et al (1976) serum iron of 40 mcg/100 ml. is considered acceptable.

The individual values of serum iron and TIBC are presented in Appendix XI.

7. Mean and range of serum copper and zinc levels

Table VIII shows the mean and range values for serum copper and zinc levels of volunteers.

TABLE VIII

MEAN AND RANGE OF SERUM COPPER AND ZINC LEVELS

S.No.	Details	Zinc mcg/100 ml.	Copper mcg/100 ml.
1.	Mean	124.3	110.6
2.	Range	102-178	82-163

Table VIII highlights the mean and range for serum copper values as 110.6 mcg/100 ml. and 92-163 mcg/100 ml. respectively.

The normal range for copper according to Hambridge, et al (1970) is 73-150 mcg/100 ml. Varley (1980) indicates a range of 75-160 mcg/100 ml. for copper which justifies the values of the present study.

According to the present study the mean level of serum zinc was 124.3 mcg/100 ml. According to Saubürlich (1976) the normal range for serum zinc was 100-150 mcg/100 ml. This value is comparable with the values of the present study.

Swaminathan (1974) has reported a range of 110-130 mcg/100 ml. for zinc levels. The values of the present study were comparable with those of the above.

The individual values of serum zinc and copper are given in the Appendix XII.

8. Mean and range of serum calcium levels

Table IX shows the mean and range for serum calcium levels of the volunteers as compared with that of Varley and Gauberlich standards.

TABLE IX

MEAN AND RANGE OF SERUM CALCIUM LEVELS

S.No.	Details	Present study mg/100 ml.	Varley (1983) mg/100 ml.	Gauberlich (1976) mg/100 ml.
1.	Mean serum calcium levels	10.15	-	10.0
2.	Range for serum calcium levels	9.1-11.3	9.0-11.0	9.0-11.0

From Table IX it is seen that the mean serum calcium levels were 10.15 mg/100 ml. and the range was 9.1-11.3 mg/100 ml. for the volunteers. The findings of the present study are identical with the range of 9-11 mg/100 ml. suggested by Varley (1983) Cornn and Cornn (1980) and Gauberlich (1976).

The ranges arrived at in the present study is comparable with studies conducted by Hawk and Oser (1967) who have suggested a range of 9-11.5 mg/100 ml.

The individual values of serum calcium of volunteers are presented in Appendix XIII.

9. Mean and range of serum retinol levels of the volunteers

Table X shows the mean and range for serum retinol levels of the volunteers, as compared with the standard given by Varley (1983).

TABLE X

MEAN AND RANGE FOR SERUM RETINOL

S.No.	Details	Present study mcg/100 ml.	Varley (1983) mcg/100 ml.
1.	Mean serum retinol levels	30.5	-
2.	Range for serum retinol levels	21-42	20-60

Table X shows the mean and range values for serum retinol for the volunteers. The mean value was 30.5 mcg/100 ml. for the volunteers and the range was 21-42 mcg/100 ml.

Cornn and Cornn (1980) has suggested a range of 20-30 mcg/100 ml. for Americans whereas the ranges suggested by Varley (1980) was 20-60 mcg/100 ml. The acceptable range obtained from the present study lies within the ranges suggested by the above authors.

Based on the Sheffield Experiment in England the following guidelines for serum retinol were set up by the ICNND in (1963).

Deficient 10 mcg/100 ml.
Low 10-19 mcg/100 ml.
Acceptable 20-49 mcg/100 ml.
High 50 mcg/100 ml.

As suggested by Agnikar and Flores (1979) serum values of 20 mcg/dl can be proposed as a cut off for the adequate category, below which manifestations of Vitamin A deficiency are evident. Correspondingly in the present study none of the sample showed a retinol value of 20 mcg/dl and this reflected in the absence of clinical manifestations of Vitamin A deficiency.

The individual values of serum retinol for volunteers are presented in Appendix XIV.

10. Mean and range for serum protein, albumin and globulin levels of the volunteers

Table XI depicts the total serum protein, albumin and globulin levels of the volunteers as compared with that of Varley and Sauberlich standards.

TABLE XI

MEAN AND RANGE OF TOTAL PROTEINS, ALBUMIN AND GLOBULIN LEVELS

S.No.	Nutrients in g/100 ml.	Present study g/100 ml.	Varley (1983) g/100 ml.	Sauberlich (1976) g/100 ml.
	Total Proteins			
1.	Mean	7.02	-	7.5
2.	Range	6.38 - 7.74	5.7-7.3	6.5 - 8.0
	Albumin			
3.	Mean	4.51	-	4.4
4.	Range	3.66 - 5.30	3.5 - 5.6	4.0 - 5.5
	Globulin			
5.	Mean	2.51	-	2.1
6.	Range	2.05 - 3.15	2.6 - 3.1	-

The mean total serum protein was 7.02 g/100 ml. for the volunteers who participated in this study.

Mean serum albumin and globulin levels were 4.61 g/100 ml. and 2.51 g/100 ml. respectively.

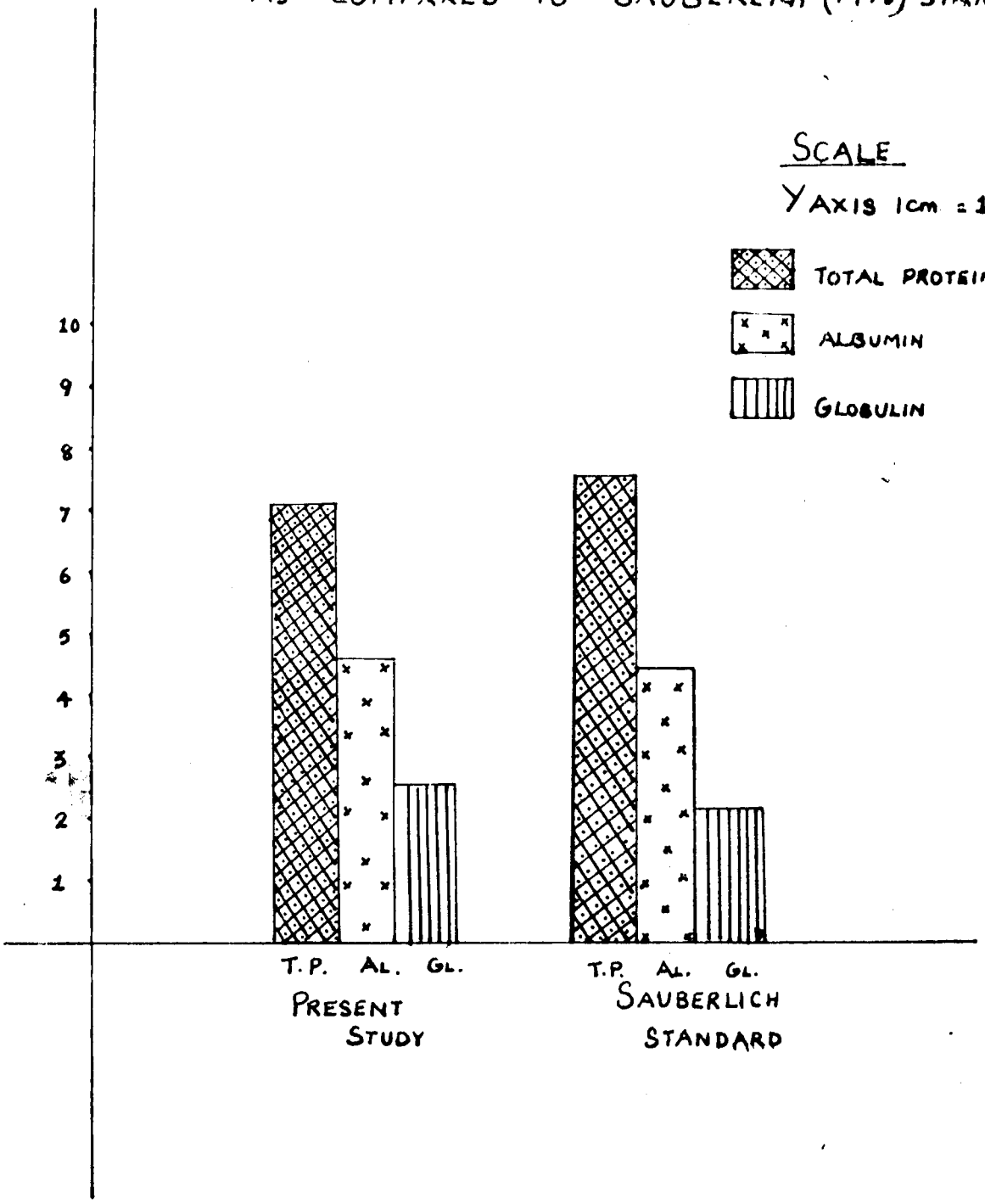
The range of levels with reference to total serum proteins was 6.33 - 7.74 g/100 ml. for aluminium albumin 3.65 - 5.30 g/100 ml. and for globulin 2.05 - 3.15 g/100 ml.

The ranges assigned at in the present study with reference to total protein, albumin and globulin were similar to the values suggested by Cornn and Cornn (1930) for the people of the United States. The range suggested by this another was 6-8 g/100 ml., 3.5-5.5 g/100 ml. and 2.5-3.5 g/100 ml. for protein, albumin, globulin respectively. Varley (1980) indicates a range of 5-8 - 7-8 g/100 ml. for protein, 3.5 - 5.6 g/100 ml. for albumin and 2-6 - 3-1 g/100 ml. for globulin. The range and mean values obtained in the present study appear to be similar to these values though the lower limit for aluminium was slightly higher than what is reported by Varley.

Sauberlich (1976) indicates acceptable value for serum protein as 7.5 g/100 ml. for aluminium as 4.4 g/100 ml. globulin 3.1/100 ml. respectively. The values obtained in the present study are slightly lower for total protein and albumin and slightly higher with reference to globulin.

Figure : 3

SERUM PROTEIN ALBUMIN GLOBULIN VALUES AS COMPARED TO SAUBERLICH (1976) STANDARD



Individual values are represented in Appendix XV.

Fig. 3 represents the serum protein in Albumin globulin ratio in comparison with Sauberlich (1976) standard.

11. Suggested reference values for different nutrients

Table XII reveals the range values obtained from the present study and suggested as possible reference values.

TABLE XII

SUGGESTED REFERENCE VALUES FOR DIFFERENT NUTRIENTS

Nutrients	Range suggested	Mean suggested
Haemoglobin g/100 ml.	11.6 - 14.6	12.9
Packed cell volume per cent	37 - 47	38.5
Serum iron mcg/100 ml.	60 - 145	94.26
Total iron binding capacity mcg/100 ml.	260 - 378	320.98
Serum copper mcg/100 ml.	82 - 163	110.6
Serum zinc mcg/100 ml.	102 - 178	124.3
Serum calcium mg/100 ml.	9.1 - 11.3	10.15
Serum retinol mcg/100 ml.	21 - 42	30.5
Total serum proteins g/100 ml.	6.38 - 7.74	7.02
Serum albumin g/100 ml.	3.65 - 5.30	4.51
Serum globulin g/100 ml.	2.03 - 3.15	2.51

Many more studies of this kind on larger samples need to be undertaken to arrive at norms or guidelines for reference values which will be of immense use as guidelines.

Summary and Conclusion

V SUMMARY AND CONCLUSION

The study was undertaken in view of the need to have local biochemical norms with reference to selected nutrients among adult women. The study included 100 healthy young adult women in the age group of 21-23 years belonging to middle and high income groups.

Clinical examination was carried out to ensure that the volunteers were not suffering from any deficiency diseases. The adequate intake of food and nutrients by the volunteers was ensured by conducting a 24 hour recall survey and by comparing their food and nutrient intake with the Recommended Food and nutrient allowances suggested by the Indian Council of Medical Research (1994).

Ten millilitres of venous blood was drawn from the volunteers which was used for the estimation of different nutrients. The results of the estimated levels of various nutrients were compared with those of the United States and British guidelines suggested by Corrn and Corrn (1980), Bauberlich (1976) and Varley (1980). The ranges of values obtained in the present study are as follows.

Haemoglobin levels ranged from 11.6-14.6 g/100 ml. The mean level was 12.9 g/100 ml. This was little low when compared to the standard of WHO (1975) which

considers haemoglobin values lower than 12 g/100 ml. as anemic. However clinical examinations revealed that these volunteers were not anemic.

The packed cell volume as observed in the present study ranged from 37-47 percent and ONeal et al (1976) considers a PCV level of 38 percent as acceptable.

The ranges of serum iron and TIBC as observed in the present study were 60-145 mcg/100 ml. and 360-378 mcg/100 ml. and when compared to the acceptable standards of Varley (1980) the value of serum iron was slightly low and the value of TIBC was slightly higher.

Serum copper and zinc ranged from 88-163 mcg/100 ml. and 108-178 mcg/100 ml. respectively. Serum copper levels recorded in the present study was comparable with the suggested ranges of Varley (1980) and serum zinc levels were compared with the suggested range of Sauberlich (1976).

Serum calcium levels in the present study ranged between 9.1-11.3 mg/100 ml. which is similar to the values suggested by Varley (1963) and Sauberlich (1976) who suggest a range of 9-11 mg/100 ml. The values of the present study are comparable to the values suggested by Hawk and Oser (1967) (9-11.6 mg/100 ml.) Serum retinol as observed in the present study was found to be 21-42 mcg/100 ml.

This could be compared with the studies of Agnikar and Flores (1979) who suggest serum values of 20 mcg/100 ml. as a cut off point for below which manifestations of Vitamin A deficiency are evident.

Total serum proteins recorded in the present study showed a range of 6.38 - 7.74 g/100 ml. which could be compared with the range suggested by Varley (1963) as 6.7 - 7.8 g/100 ml. But when compared to that of the standard suggested by Sauberlich (1970) as 6.5 - 8.0 g/100 ml., the values of the present study was slightly low.

Studies similar to this needs to be conducted on larger population groups and different regions thus correlating their food intake with the nutrient levels in blood. The results of which could be a reliable suggesting guideline for our population groups of India.

Bibliography

B I B L I O G R A P H Y

1. ACHILAR and FLORES (1979) .. Evaluation of sugar fortification with Vitamin A at the National Level PAHO Scientific Publication. No.384, Washington D.C.

2. BANERJEE (1936) .. Food Intake and Nutrient Adequacy of Rural Population of Andhra Pradesh 1982. Human Nutrition, Applied Nutrition 36-A, 293-301.

3. BERG, A. (1981) .. Malnourished people on Policy View World Bank. p.1.

4. BERGREN, G.G. (1982) .. Questions and Answers about meaning Food & Nutrn. Bulletin

5. BEST, C.H. and TAYLOR, N.B. (1967) .. The Physiological basis of medical practice Eighth Edition, Second Indian Reprint Calcutta (1967) Scientific Book Agency, p.435.

6. BLACK BURN, G.L. (1974) .. Nutritional assessment and support during infection American Journal of Clinical Nutrition. Vol.30, p.1483-1500.

7. BUGINE (1972) .. Assessment of nutritional status and food composition surveys Anthropometry measurements and indices. Assessment of nutritional status and food composition surveys Kongyi I.C. Reschil Konzurich Szezygieth p.62.

8. CARL L. KEECH et al (1985) .. Different effects of zinc and copper defeciency on composition of plasma high density lipeproteins in rats. The journal of Nutrition March 1985 Vol.115, No.3, p.359-366.

9. COHEN (1969) .. The Concentration of Haemoglobin in Circulating Blood. Ecology of Food & Nutrition Vol.2, No.4 (1973)

10. COOK, J.D., ALVARADO, J., GUTNISKY, A., JAMSA, M., LABARDINI, J., LAYRISSE, M., VELEZ, J. and VITERI, F. (1971) .. Nutritional defeciency and Anemia in Latin America. A collaborative study. Blood 38: 591-603 (1971).

11. CORN, H.F. and CORNN, R.S. (1990) .. Adapted by American Dietetic Association. Handbook of Clinical Dietetics. pp.119, 121, 122.

12. CENSUS (1981) .. India Year Book 1984 A Reference Annual compiled and edited by Research and Reference Dimension Ministry of Information And Broadcasting Govt.of India, Publications Division.

13. DAVIDSON, S., PASSMORE, P., BROCK, J.F. and TRUSWELL, A.S. (1974) .. Human Nutrition and Dietetics 5th Edition. The English Language Book Society Churchill Living Stone Ltd. Edinburgh, p.366.

14. DEVADAS (1983) .. Food comes first Home Science Vol.21, No.9

15. DOLLER, H. LIZABETH, A and THOMSON, A. (1983) .. Nutritional status indicators. Interpretation and Policy making role. Nutrition Planning Vol.6, No.1 pp.17-18

16. [illegible] (1984) .. Average, Ideal, Desirable A brief overview of height weight tables in United States. Journal of American Dietetic Association. Vol.84, No.4, p.417.

17. FELL, G.S. and BURNS, R.R. (1978) .. Zinc and other trace elements. In advances in parenteral nutrition ed. IDA Johnston. pp.241-250. Lancaster: MTP Press.

18. FINCH, C.A. .. IRON Nutrition
(1977) Food & Nutrition
Vol.3, No.4, p.13
FAO of the United Nations.

19. GOPALAN, C. .. Green Power and Freedom from Hunger - M. Swaminathan
(1979) Proceedings of Nutrition Society of India. No.26 - 1980 p.1.

20. GOPALAN, C., .. Nutritive value of Indian Foods
SASTRI, B.B.V. National Institute of Nutrition
and ICMR Hyderabad, India.
BALASUBRAMANIAM, S.C.
(1984)

21. IYENGAR, G.V. .. Trace elements - For Monitoring
(1986) Health, Science Today. p.32.

22. JELLIFFE, D.B. .. The Assessment of Nutritional
(1966) status of the community - Geneva (1966)

23. JOSEPH et al .. Alcohol and nutrient intakes of
(1978) elderly men.
Journal of the American Dietetic Association.
Vol.72, No.5, p.493.

24. LATHAM, M.A. .. Human Nutrition: Applied Nutrition
(1982) Vol.36-A, No.4, August 1982
pp.241-320. ISSN 0303-4399.

25. LINKSWILLER, H.M. .. Calcium retention of young adult
(1974) males and calcium intake.
Trans N.Y. Academic Science
No.36, p.333.

26. MACLEAN, W.C., DE ROMANA, G.L. and GRAHAM, G.G. (1977) .. Effect of the level of dietary protein intake on fat absorption in children. Effect of the level of dietary Paediatric Res.11: 774-778 (1977).

27. M FLUIT et al (1979) .. Foods and Nutrition Notes and Reviews - 36 p.558.

28. FOOD AND NUTRITION BOARD (1979) .. Food and Nutrition situation in the country. Manual of Nutrition for mobile Food & Nutrition Extension Science, Ministry of Agriculture & Irrigation. Govt. of India, pp.118-123.

29. FRANCOIS, P. and WEISELL (1982) .. Need for health review in developing countries Yojana, Vol.25, No.11, p.6.

30. NEELD and PEARSON (1967) .. Macro and micro methods for estimation of serum Vitamin A levels. Journal of Nutrition. 73, pp.454-462.

31. NIN REPORT (1979) .. NIN Annual Report 1979 - ICMR pp.146-147.

32. NIN MANUAL (1982) .. A Manual of Leboratory Techniques. National Institute of Nutrition Indian Council of Medical Research. p.113, 116, 125, 141, 172.

33. ODERT, J.C. (1911) .. Community Nutrition Consulting Nutritionist. John Wiley and Sons. p.15.

34 P.A.O. BULLETIN (1976) .. Anthropometry in nutritional surveillance. An over view PAO Bulletin Vol.VI, No.2, p.43.

35 PARIMALA, A.N. (1984) .. Problem eaters Home Science Vol.22, No.3

PARKKA et al

36. PRASAD, A.S. (1979) .. Zinc in human nutrition CRC Press Inc. Boca Raton Florida 33431

37. PETER W.F. FISHER ALEXANDRE GRIOUX MARY R.L. ABBS (1984) .. Effect of zinc supplementation on copper status in adult man. The American Journal of Clinical Nutrition, Vol.40, No.4. Oct.1984. p.No.743-746.

38. STEIN, T.P., SCHLUTER, M.D., and DIAMOND, C.E. (1983) .. Nutrition, Protein turn over and physical activity in young women. The American Journal of Clinical Nutrition. Vol.38, No.2, August, 1983, p.223.

39. SWAMINATHAN, M.S. (1974) .. Essentials of Food and Nutrition Ganesh and Co., Madras p.217-233 and 301-311.

40. RAO, N.P., SINGH, O. and SWAMINATHAN, M.S. (1969) .. "Nutritional status of pre school children of rural communities near Hyderabad City" Indian Journal of Medical Research. Vol.57, pp.2132-2146.

41. REBECCA (1982) .. Discussions of Techniques in Anthropometry, Part II Nutritional support services Vol.2, No.1, pp.11-14.

42. SAUBERLICH, H.E. SKALA, R.P.D. (1974) .. Laboratory Tests for the Assessment of Nutritional Status. Arnold E. Schaefer Pan American Health Organisation, WHO Washington D.C. p.1.

43. SCRIMSHAW, N., TAYLOR and GORDON (1963) .. Interactions of Nutrition and Infection WHO Geneva, 1963.

44. SENGAL and MEHTA (1984) .. Fight against hunger Home Science Vol.22, No.3

45. SHANKARANAND, B. (1981) .. Improving Health Status in totality Swasthhind. p.229,230.

46. SHEFFIELD EXPERIMENT ICNND (1963) .. United Kingdon Research Council Vitamin A Requirements of Human Adults Special Report No.264.

47. STEIN, T.P., SCHLUTER, M.D. and DIAMOND, C.E. (1983) .. Nutrition, Protein turn over and physical activity in young women. The American Journal of Clinical Nutrition. Vol.38, No.2, August, 1983, p.223.

48. SWAMINATHAN, M.S. (1974) .. Essentials of Food and Nutrition Ganesh and Co., Madras p.217-223 and 301-311.

49. SWASTHMANTIA (1973) .. Swasthhind (1973) October Three dimensional Approach to Combat Malnutrition. p.249.

50. TANAKA, Y. HF FRANK and F. DELUCA (1973) .. Role of 1, 25 - Dihydroxy - Cholecalciferol in Calcification of Bone and Maintenance of Serum Calcium concentration in the Rat

51. THIMMAYAMMA, B.V.S. (1983) .. Nutrition and Welfare of the family Nutrition, July 1983. NIN, ICMR.

52. THOMSON, A.M. and BILLEWICZ, W.Z. (1963) .. Proceedings of the Nutrition Society, 22: pp.55-60. (1963)

53. WALSELL, R.C. and FRANCOIS, P.J. (1981) .. Reference Weight for height standards. An earlier approach of computerisation Food and Nutrition Bulletin Vol.17, No.1, p.13.

54. WHITEHEAD, R.G. (1969) .. The assessment of nutritional status of protein and nourished children. The Proceedings of the Nutrition Society of India. Vol.28, p.1-5.

55. WHO (1972) .. Nutritional Anaemias Report of a WHO Group of experts, Geneva WHO Technical Report. Series No.503.

56. VARLEY, H. (1983) .. Practical Biochemistry IV Edition. pp.23-259.

Appendices

APPENDIX I

CLINICAL ASSESSMENT OF THE VOLUNTEERS

NAME :

AGE :

SEX :

1. HAIR

   a) Pluckability - Easy pluckability/ slightly brittle/normal

   b) Nature - Thin and sparse/Moderately dense/Dense and glistening

   c) Colour - Dispigmented/Partial dispigmentation/Natural colour

2. FACE

   a) Moon face

   b) Pale look

3. EYES

   a) Vision - Night blindness/Diminished vision/clear vision

   b) Conjunctiva - Wrinkled thickened and pigmented/ slightly dry on exposure to sunlight/glistening moist.

4. LIPS AND MOUTH

   a) Cheilosis/Angular stomatitis/Normal

5. TONGUE

   a) Red raw ulcerated/Pale and fissured/normal

6. TEETH

   a) Mottled enamel

   b) Caries

7. GUMS

a) Spongy bleeding gums/retracted and loose/firm non bleeding.

8. SKIN

Pellagrous flaky paint dermatosis/Dry and desquamated/ Natural, smooth, soft, elastic skin.

9. NAILS

a) Koilonychia

10. GENERAL APPEARANCE

a) Musculature - Poor/Moderate/Good

b) Subcutaneous - Severely emaciated/moderate/normal

c) Oedema - Severe/on dependent parts/absent

11. WOUNDS - Delayed healing of wounds/ Moderately rapid healing/rapid healing.

12. CONSTIPATION - Problem of constipation often/ Sometimes/No problem of constipation.

13. ACTUAL INFECTION - Very frequent/once in a while/Rarely

14. HISTORY OF ILLNESS - 1-6 months back/6-2 months back/ Before one year.

15. ALLERGY - Long term allergy/short term/absent

16. ANEMIA - Severe/Mild or moderate/Normal

17. ADEQUACY OF DIET - Inadequate/Moderate/Adequate

- -

NAME OF INVESTIGATOR

APPENDIX II

DETERMINATION OF HAEMOGLOBIN AND PACKED CELL VOLUME

Haemoglobin Estimation By Cyanmeth Hb - Method

Estimation of Hb. by this method was recommended by Xth International Hematology congress and WHO Expert Committee on Nutritional Anaemias. This method measures not only oxy haemoglobin but also carbon-monoxide haemoglobin and methaemoglobin except sulpha - haemoglobin with filter type photoelectric colorimeters. The single relatively broad band of Cyanmethaemoglobin in the green spectral region, has a distinct advantage. This method can be modified to determine haemoglobin in dry blood or filter paper also.

Reagents:

Drabkin's Diluent Solution (Carteright 1963)

Sodium Bicarbonate - 1 gm.

Potassium Cyanide - 0.05 gm.

Potassium Ferricyanice - 0.20 gm.

Distilled water to make one litre.

This solution should not be used after it forms a precipitate on the bottom of the storage bottle. The solution is preserved in a dark brown bottle and preferably under cold storage. Its preparation and handling should be done with great care.

Procedure

Exactly 5 ml. of Drabkins solution is measured into a dry test tube from a burette or a pipette with suction bulb.

2. Exactly 0.02 ml. of blood is transferred from a standardised Hb pipette into the diluent solution. Usual care in filling and cleaning of loaded haemoglobin pipette must be observed.

3. The pipette is rinsed three times with the diluent solution, without allowing the formation of air bubbles in the solution.

4. The blood and the diluent are thoroughly mixed by rotating the tube.

5. 10 minutes time is allowed for the formation of cyanmethaemoglobin.

6. 5 ml. of diluent solution is used as blank.

7. With green filter No.540 the readings are taken in a photoelectric colorimeter.

Calibration Procedure

1. Total blood iron is determined by Wong's method. This determination would give absolute amount of Hb.

2. Exactly 0.02 ml. of this known blood sample is measured as above into 5.0, 7.5, 10.0, 12.5 and 15 ml. respectively of diluent solution and mixed by rotating the tubes. These solutions are now equivalent to blood samples containing respectively 100, 67, 50, 40, and 30 percent that of the original solution.

3. The intensity of the colorimeter is read using green filter 540 against diluent as blank set at zero.

4. On a graph paper standard graph is drawn using these Rb concentration and corresponding density values are determined.

[redacted]

A sample of blood is digested with conc. H_2SO_4 to get iron in the free form in the presence of potassium per sulphate. The digest is then [redacted] with sodium tungstate solution, [redacted] and a known amount of [redacted] is treated with potassium [redacted]. The colour developed is then [redacted] using a filter of 540 mμ.

[redacted]

1. 10% Sodium tungstate
2. [redacted] potassium [redacted] solution
3. Potassium [redacted] 3 ml. solution
   Dissolved 146 grams of potassium [redacted]
4. Standard Stock solution

Prepared standard iron solution 100 mg. of iron present in 70.2 mg. of ferrous ammonium sulphate in 100 ml. of distilled water.

[redacted]

Diluted 10 ml. of the sock standard to 100 ml. with distilled water so that 1 ml. of the solution contains

Procedure

0.3 ml. of the blood is whirled with 2 ml. of [redacted] H_2SO_4 in a 50 ml. standard flask for 2 minutes

2 ml. of saturated potassium per phosphate solution was added and shaken well. To this 2 ml. of 10% sodium tungstate was added, cooled and the volume was made up to the mark filtered. This is the experimental solution I.

To another 50 ml. standard flask 2 ml. of standard potassium persulphate solution, 2 ml. conc - H_2SO_4, 2 ml. of 10% sodium tungstate was added, volume was made upto the mark with distilled flask. This is experimental II solution.

1 ml. to 5 ml. of the standard was taken in different test tubes added 0.3 ml. of conc. H_2SO_4 and 0.4 ml. of saturated potassium persulphate to each of the test tube. Finally added 1.6 ml. of potassium thiocyanide and made up the volume to 10 ml. in each test tube with distilled water. 5 ml. of the filterate of each solution was taken. To each of the tubes was added 0.3 ml. of conc. H_2SO_4, 0.4 ml. of saturated potassium persulphate and 1.6 ml. of 5 N potassium thiocyanate and the volume made upto 100 ml. with distilled water. The intensity is measured in a colorimeter, using a green filter of wavelength 540 mg. within 10 minutes. Then iron content of blood can be calculated from these values.

Iron content of the blood - Iron content of the experimental solution I - Iron content of the Experimental Solution II.

From the amount of iron present, the haemoglobin can be calculated by multiplying the value of iron by $\frac{100}{0.34}$.

HAEMOTOCRIT DETERMINATION

(Micro Method)

The haematocrit or packed cell volume of blood is determined using heparinized capillary tubes (75 x 1 mm) and microhaematocrit centrifuge.

Procedure

Blood from the finger tip is allowed to run about $\frac{1}{4}$ to $\frac{3}{4}$ length of the tube. The tube is sealed on the opposite end using sealing wax or plasticine. The tubes are then transferred to the high speed microhaematocrit centrifuge, and placed in grooves of the centrifuge head. They are centrifuged for five minutes at 11000 r.p.m. and read on the reader which gives the direct haematocrit value.

APPENDIX III

DETERMINATION OF SERUM IRON

1. Dipirydyl Method: (Ramsay 1954, 1963)

Ferous iron gives a pink colour with 2-2' dipyridyl. A solution of dipyridyl in acetic acid is added to serum followed by a reducing agent. Proteins are removed by heating in boiling water and then centrifuging or filtering.

Reagents:

1. 2-2' dipyridyl, 0.1% in acetic acid 3 percent V/V

2. Sodium sulphite, 0.1m. Dissolve 1.26 gms. of anhydrous sulphite or 2.52 gms. of $Na_2 SO_3 7H_2O$ in water and make up to 100 ml. Prepare freshly every few days.

3. Chloroform

4. Standard solution containing 100 micro grams iron per ml. Dissolve 0.493 grams of ferrous sulphate in water add 1 ml. concentrated $H_2 SO_4$ and make to a litre.

Alternatively use a solution of ferrous ammonium sulphate $(NH_4)_2 SO_4$, $Fe SO_4$, $6H_2O$, containing 0.702 from per litre.

Technique

Mix equal volumes of serum 0.1 m. sodium sulphate and dipyridyl reagent in a glass stoppered tube which can be centrifuged. Heat in boiling water for five minutes.

Cool, add 1 ml. of chloroform stopper and shake vigorously for thirty seconds. Remove the stopper and centrifuge for five minutes at 300 r.p.m. If the supernatant fluid is not completely clear repeat the shaking and centrifuging. Read at 520 milli microns or using a green filter. As blank use water instead of serum. For the standard put thro' the working standard in the same way.

Clean tubes used by placing them boiling 5 N HCL. The wash with glass distilled water and keep for this determination duly.

Calculation

$$\text{Micrograms iron per 100 ml. of serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 300$$

The readings are linear with concentration to atleast 500 mg per 100 ml. To obtain a calibration curve dilute 5 ml. of the stock standard to 100 ml. with water and set up tubes containing 0.4, 0.8, 1.2, 1.6 and 2.0 ml. of this, make each to 2 ml. with water and develop the colour as described above and read against the blank. These correspond to 100, 200, 300, 400 and 500 mg./100 ml.

Note: If the iron binding capacity is being done by Ramsay's method at the same time as sirum iron it may be more convenient to use the double strength sulphite & dipipyridyl

reagent for both. Then for serum iron use 1 volume of serum, 1 volume of water and 0.5 volume of each of 0.2 M sulphite and 0.2 percent, dipyridyl in 3% acetic acid.

Ramsay's Dipyridyl Method

Reagents:

1. Ferric Chloride solution 5 mg. iron per ml. in 0.005 N HCl. Prepare a stock solution containing 145 mg. of $FeCl_3$ per 100 ml. of 0.5 N acid and dilute 1 to 100 with water.
2. Magnesium Carberate "light" for absorption
3. Sodium sulphite (0.2 M) 2.52 grams of the anhydrous salt per 100 ml.
4. 2.2' dipyridyl, (0.2%) in acetic acid 3 per cent V/V
5. Chloroform and stand solutions as for the method (serum iron)

Technique

Add 4 ml. of the ferric chloride solution to 2 ml. of serum. After standing for five minutes add 400 mg. of magnesium carborate (100 mg. for each ml. of ferric chloride). Shake frequently and vigorously for thirty to sixty minutes. Centrifuge and pipette of 4 ml. of the supernatant fluid for iron determination. If the dipyridyl method is used add 1 ml. each of the 0.2 M sulphite and 0.2 per cent dipyridyl and proceed as described previously for determing serum

iron. The result gives the total iron binding capacity. If the serum iron is determined at the same time the percentage saturations is easily calculated.

Calculation

Since in this case the volume of serum in the 4 ml. of supernatant fluid is 1.33 ml. if the same proportions are used for the standard as in Note (i.e. 2 ml. of standard containing 3 mg. iron per ml., 2 ml. water and 1 ml. each of dipyridyl and sulphite).

TIBC in micrograms/100 ml. solution

$$= \frac{\text{Reading of unknown}}{\text{reading of standard}} \times 450$$

APPENDIX IV

ESTIMATION OF ZINC AND COPPER

One ml. of the sample to the analysed was digested with 10 ml. of triple acid (nitric, sulphuric, perchloric acids in the ratio 9:2:1) and made upto 50 ml. The make up sample was then fed into the Atomic absorption spectro photometer for the analysis of Cu & Zn.

APPENDIX V

DETERMINATION OF SERUM CALCIUM

Principle

Calcium is precipitated from serum as oxalte. The magnesium is not precipitated as the conditions are selected to increase the solubility of magnesium oxalate. The precipitate dissolved in acid and the oxalate is determined litrametrically by litrating against $KMnO_4$.

Reagents

1. Ammonium Oxalate - 4% solution
2. Ammonia 2% solution
3. $KMnO_4$
4. $2NH_2SO_4$

Technique

Take 2 ml. of serum, 2 ml. of distilled water 1 ml. of 4% ammonium oxalate in centrifuge tubes. Then centrifuged, tested with calcium chloride solution, the supernatant liquid in which ppt should not be obtained. Add 2 ml. of 2% ammonia to the ppt. and centrifuged. Discard the supernatant finally and add 2 ml. of 2 NH_2SO_4 kept in the boiling water bath. In the hot condition titrated against N/100 $KMnO_4$ to pale pink colour. As a blank, titrated 2 ml. of $2NH_2SO_4$ against N/100 $KMnO_4$.

APPENDIX VI

DETERMINATION OF SERUM VITAMIN A LEVELS

The most commonly used biochemical test to assess Vnit A nutritional status has been the determination of serum Vnit A levels (Srikantia 1975). Hence, the Vnit A levels of the target.

Estimation of Serum Vnit A (Retinol)

The serum Vnit A was estimated by the trifluoro acetic acid method of Neels and Pearson as modified and suggested by Neels et al quoted by Gyorgy and Pearson (1967).

Procedure

Reagents

1. Absolute ethanol: Purified for spectrophotometry
2. Hexane : Fisher Certified reagent special for spectrophotometry
3. Chloroform: Merck reagent special for spectrophotometry
4. Trifluoro acetic acid: Reagent grade (Sigma)
5. IN alcholic KOH
6. Stock Unit A Solution: 344 mg. of Vnit A acetate (300 mg. of Vnit A) was dissolved in chloroform and made upto 100 ml. 1 ml. of Stock contains 3000 mcg. of retinol.

b. Intermediate Standard

1. 0.1 ml. of stock diluted to 100 ml. with chloroform (3 mlg. 1 ml.)

2. 0.1 ml. of stock diluted to 50 ml. with chloroform (6 mlg. 1 ml.)

3. 0.15 ml. of stock diluted to 60 ml. with chloroform (9 mlg. 1 ml.)

4. 0.1 ml. of stock diluted to 25 ml. with chloroform (12 mlg. 1 ml.)

c. Working standard

Each intermediate standard was again diluted in the ratio 1:10 and from each standard finally 1.0 ml. was taken.

Method

The serum (0.5 ml. or less) is saponified with an equal volume of 1 N ethanolic KOH in a water bath at 60°C for 20 minutes. The mixture is cooled and vigorously shaken in a glass stoppered tube with an equal volume (1 ml.) of n - hexane for 10 minutes. The tube is centrifuged for 1 minute at 100 RPM to seperate the layers. An aliquots (0.8 ml.) of the n - hexane layer is pipetted off for of the determination of retinol. The n - hexane is evaporated from this aliquot in a water bath at 60°C in a stream of oxygen free nitrogen. The last traces of n - hexane are removed by nitrogen blowing at room temperature. The residue is taken up in (0.5 ml.) chloroform, 1 drop of acetic anhydride is added followed by (0.1 ml.) trifluoroacetic

acid. The mixture is shaken vigorously and the optical density at 620 mp is determined exactly 30 seconds after addition of the trifluoro acetic acid.

Correction of Unit A. Transmission reading at 620 mp for Carotene interference at that wavelength

Tribuoro acetic acid in chloroform solution reacts with carotene and the absorption of the reaction product at 620 mp interference with the Unit A determination done at the same wavelength.

A series of the solutions of the B-carotene standard in petroleum ether are made and aliquot portions of it are evaporated and the residue is taken up in chloroform and optical density is read at 452 mp. A second series of solutions of the B-carotene standard in petroleum ether is made, aliquot portions of it are evaporated from centrifuge tubes and exactly the same procedure is applied to the residues remaining in the centrifuge tubes as was described from serum. The optical density at 620 mp. contributed.

APPENDIX VII

DETERMINATION OF TOTAL SERUM PROTEIN AND ITS FRACTIONS

Proteins form a purple coloured sample with cupric ions in alkaline solution. The reaction has taken its name from the simple compound Biuret which reacts in the same way.

Reagents

1. Sodium chloride dilutent: Dissolved 9 gms. of sodium chloride in water and made upto one litre.

2. Stock Biuret Reagent: Dissolved 45 gms. potassium sodium tastarate (Rochelle salt) in 400 ml. of 0.2 N NaoH Added 15 g. cupric sulphate and stirred until all was dissolved to one litre with 0.2 N NaoH.

3. Dilute Biuret Reagent: Diluted 200 ml. stock biuret Nagent to ore litre with 0.2 N NaoH containing 5 g. potassium inside per litre.

4. Standard Protein Solution: Weighed 400 ml. alumin and dissolved in 0.9% salire solution (Sodium Chloride) Then made upto the volume (100 ml.) so that 1 ml. of the solution contains 4.0 mg. of protein.

5. 09% sodium chloride

6. 22.6% sodium sulphite

Procedure

Into a series of test tubes 0.5 ml., 1.0 ml., 1.5 ml. 2.0 ml. and 2.5 ml. of standard protein solution were pipetted out. With water into another test tube pipetted out 0.2 ml. serum and diluted with 0.9% saline into 5 ml. From this solution 2 ml. was taken, made upto 3 ml. and treated as unknown. Now added 3.0 ml. of dilute Biruet reagent to all the test tubes. Along with this a blank was prepared. The colour developed into it was read colorimetrically at 500 mp. after 30 minutes. The amount of protein present in the serum was calculated.

PRECIPITATION OF GLOBULIN

Globulin is precipitated by mixing 0.2 ml. of serum with 4.8 ml. of 22.8% sodium sulphate solution. Stoppered the tubes and inverted it several times and left in the incubator at 40°C, over night. Filtered the solution next day using what was (many) No.42 filter paper.

Took 2 ml. of the filterate and carried out the experiment as for Total Proteins. The concentration in g/albumin present in the globulin free filtrate is determined from the standard graph.

Globulin value can be obtained by deducting albumin value from the total proteins.

APPENDIX VIII

MEASUREMENT OF HEIGHT AND WEIGHT OF THE VOLUNTEERS

No.	Height (Cms)	Weight (Kg.)
1.	153	47.750
2.	159	40.5
3.	160	50.5
4.	160	53.25
5.	159	45.5
6.	160.3	58.25
7.	157.0	49.75
8.	157.0	52
9.	164	50.45
10.	168	53.5
11.	159	42
12.	149	50
13.	155	42.5
14.	167	44.25
15.	155	41.75
16.	155	59.25
17.	160	53
18.	156.2	46
19.	153	57
20.	156.2	41.0
21.	157	43.25
22.	152	62.5
23.	151	46.5
24.	159.2	42.5
25.	159.6	52.5

Sno.	Height (Cms.)	Weight (Kg.)
26.	170.5	55
27.	155	47
28.	159	54.5
29.	153.3	44.75
30.	153	44.25
31.	144	32
32.	157	46.5
33.	151.5	43.5
34.	162	53.75
35.	153.2	46.5
36.	149	50
37.	148	52
38.	153	43.5
39.	154	44.75
40.	160.3	52.05
41.	153.5	47
42.	160.2	53.75
43.	150.9	45
44.	152.2	47.25
45.	153.4	46.5
46.	151.7	42.3
47.	152.3	43.9
48.	15461	55
49.	149	47
50.	150	46.25
51.	155.2	49
52.	153	47.25

S.No.	Height (Cms.)	Weight (Kg.)
53.	150	46.2
54.	162	43.2
55.	160.3	50.3
56.	153.3	49.5
57.	159	54.5
58.	157.2	47
59.	149	44.5
60.	151.5	49
61.	160	53.5
62.	161	58.25
63.	152.5	43.6
64.	149	47
65.	153.4	56.25
66.	155.2	52
67.	159	46
68.	152	43.5
69.	156	52
70.	154.2	55
71.	148	50.25
72.	149	50.5
73.	154.3	48
74.	159.4	52.5
75.	156	55.75
76.	153.2	57
77.	153.5	45
78.	149	42.5

S.No.	Height (Cms.)	Weight (Kg.)
79.	162	53
80.	160.2	50.8
81.	161	54.5
82.	160.5	53
83.	163.5	44.5
84.	162.7	48
85.	159	55
86.	154	45
87.	153.3	46.25
88.	156.2	46
89.	151.3	47
90.	160.2	53
91.		
92.	150.1	45
93.	153.5	49
94.	162	54.5
95.	161.7	48.75
96.	160.3	50.25
97.	154	41.75
98.	159.2	53.25
99.	151.7	46.75
100.	153.9	48.25

APPENDIX IX

INDIVIDUAL FOOD INTAKE IN GRAMS

S.No.	Cereals (a)	Pulses (b)	Green leafy vegetables (c)	Other vegetables (d)	Roots and Tubers (e)	Milk and Milk Products (f)	Fats and Oils (g)	Sugar and Jaggery (h)	Egg and other Non Veg. items (i)	Fruits (j)
1.	360	80	40	30	30	320	20	20	30	90
2.	375	100	20	40	60	300	20	20	-	100
3.	325	90	75	35	20	290	20	10	30	30
4.	400	30	10	30	50	360	20	20	30	90
5.	325	75	100	25	-	300	20	30	-	90
6.	350	90	100	25	40	350	22	20	30	110
7.	375	90	50	70	15	375	20	25	-	90
8.	455	90	50	60	-	300	20	10	30	-
9.	325	75	50	40	60	320	25	15	30	150
10.	400	35	75	40	-	290	40	10	-	100
11.	405	100	75	60	10	295	10	10	-	-
12.	400	60	10	40	50	250	30	20	40	-
13.	400	85	75	35	40	310	20	10	-	90
14.	345	65	100	20	55	160	20	10	30	100

No.	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
15.	410	60	10	10	35	300	25	20	30	90
16.	365	90	45	30	35	275	15	25	40	80
17.	330	100	20	35	105	310	30	20	30	-
18.	340	85	65	30	45	290	20	10	30	90
19.	420	45	75	45	60	320	10	15	30	-
20.	390	70	35	25	40	275	15	20	30	80
21.	395	65	55	30	40	290	20	10	30	90
22.	410	30	50	10	300	80	10	15	-	90
23.	355	95	40	35	20	310	20	20	30	90
24.	375	90	55	70	30	250	25	20	30	100
25.	380	75	40	20	90	340	25	25	-	90
26.	360	95	60	40	10	320	30	15	30	80
27.	390	70	40	25	35	315	-	10	-	-
28.	375	85	20	45	55	270	20	-	30	70
29.	350	80	30	35	10	325	30	15	40	-
30.	295	105	75	10	50	315	35	10	30	90
31.	405	85	20	15	55	305	25	20	-	90
32.	325	75	10	30	15	300	10	10	45	90
33.	370	65	110	15	90	295	30	20	-	100
34.	385	80	100	20	35	260	10	40	30	90
35.	365	65	30	45	40	300	15	15	35	90
36.	390	80	50	30	45	310	20	15	-	90
37.	345	85	70	35	25	290	20	35	30	90
38.	355	100	25	30	50	270	25	20	30	90
39.	310	90	40	50	50	305	30	10	-	90
40.	280	125	60	25	20	295	30	15	15	80
41.	340	95	35	20	35	240	10	10	25	100
42.	400	55	20	10	25	250	10	20	40	90

S.No.	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
43.	415	85	45	40	45	110	15	25	-	100
44.	355	85	65	35	45	315	20	20	30	90
45.	360	65	45	40	25	325	15	20	-	90
46.	325	95	80	45	55	340	25	15	35	90
47.	380	75	55	40	30	280	20	10	-	100
48.	390	65	60	30	50	275	15	20	-	80
49.	330	90	60	10	65	290	20	10	25	-
50.	320	35	40	25	60	300	25	20	30	90
51.	295	100	55	40	55	340	35	15	20	80
52.	405	60	40	25	35	220	10	20	-	100
53.	325	75	60	45	60	300	30	20	30	90
54.	370	65	40	35	10	295	10	25	20	80
55.	385	80	20	10	15	290	15	15	30	50
56.	365	85	30	15	10	260	20	10	-	90
57.	390	100	75	30	20	300	25	15	-	90
58.	345	90	20	15	55	275	20	10	25	80
59.	355	125	10	20	50	315	30	20	-	125
60.	310	55	110	45	65	325	10	10	10	90
61.	290	85	100	20	60	320	30	20	30	80
62.	390	65	30	35	50	300	15	40	35	90
63.	415	55	50	30	20	260	10	15	-	90
64.	400	60	20	50	30	175	10	20	20	90
65.	295	35	40	40	55	305	15	15	35	90
66.	405	70	50	10	35	210	20	20	-	100
67.	390	65	45	25	50	260	25	25	30	50
68.	325	90	75	35	20	290	20	10	30	90
69.	400	80	10	30	50	350	20	20	30	90

S.No.	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)
70.	325	75	100	25	-	300	20	30	-	90
71.	405	100	75	60	10	295	10	10	-	50
72.	400	60	10	40	50	250	30	20	40	-
73.	346	65	100	20	55	160	20	10	30	100
74.	405	95	75	40	10	290	40	10	-	90
75.	375	90	50	70	15	340	20	10	30	-
76.	405	75	70	40	-	290	40	10	30	95
77.	390	65	60	30	50	275	15	20	-	85
78.	330	90	60	10	65	280	20	10	25	-
79.	325	95	50	45	55	340	25	15	35	90
80.	355	85	65	35	45	315	20	20	30	90
81.	370	90	40	50	305	50	30	10	-	90
82.	340	95	35	20	240	35	10	10	25	100
83.	415	85	45	40	140	45	15	25	-	20
84.	385	90	100	20	260	35	10	40	-	80
85.	410	80	50	10	200	60	10	20	-	70
86.	380	75	40	20	340	90	25	25	30	50
87.	360	90	40	50	305	50	30	10	-	90
88.	390	95	65	35	45	315	20	20	30	90
89.	296	95	40	40	55	305	15	15	35	90
90.	370	90	40	50	220	40	10	20	20	60
91.	375	90	55	70	250	30	25	20	30	100
92.	410	80	50	10	230	60	10	15	-	90
93.	360	75	40	20	340	90	25	25	-	90
94.	355	95	40	35	310	20	20	20	30	90
95.	350	80	30	35	325	10	20	15	30	90
96.	405	65	20	15	325	55	25	20	-	80
97.	345	85	70	35	280	25	20	35	30	90
98.	360	95	35	20	340	35	10	10	20	100
99.	370	65	110	15	295	90	30	20	-	80
100.	365	65	30	45	300	40	15	15	35	90

INDIVIDUAL NUTRIENT INTAKE OF VOLUNTEERS

S.No.	Protein gm.	Fat gm.	Fibre gm.	Carbo-hydrates gm.	Energy Kcal.	Calcium (mg.)	Phosphorus (mg)	Iron (mg)	Carotene mg	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin C (mg)
	1	2	3	4	5	6	7	8	9	10	11	12	13
1.	66.3	55.4	6.06	402.3	2145	360.3	1390	40.2	630.4	2.12	1.3	16.8	34
2.	70.2	52.3	4.35	436.6	2559	768.7	1083	27	772	1.6	1.05	17.2	59
3.	68.9	71	3.3	381	2414	2252	1483	45.3	3215	1.4	1.2	17	99
4.	72.2	57.4	8	455.7	2341.8	836	1534	43	637	2.3	1.7	18.6	34
5.	64.3	68.7	3.06	390	2368	2100	1403	48	3726	1.3	1.1	17.2	39
6.	62.7	52.3	5.31	327	2248	1227	1429	46	4096	1.26	1.27	19.5	120
7.	73.6	58.3	5.4	110	2148	1799	1390	22	1095	1.7	1.3	31.6	88
8.	63.4	53.1	5.7	682	2644	1003	1401	36.7	1068	1.6	1.1	19.6	57
9.	63.9	71	3.7	381.9	2416	2051	1469	39	3213	1.0	1.1	17.013	99
10.	75.9	72.6	10.2	496	2719	1046	2384	47	3210	1.2	1.2	31.35	90
11.	72.4	49.4	6.015	437.3	2255	993	1340	36.4	1127	1.9	1.16	17.4	59
12.	72.39	70.03	3.6	455.73	2592	986	1433	45	3215	1.4	1.2	17	90
13.	63.4	63.73	5.7	682.4	2644	1008	1401	36.7	1063	1.62	1.0	19.6	57
14.	73.57	53.3	5.42	110.14	2148	1099	1390	22.6	1095	1.265	1.2	19.7	72
15.	64.27	76.11	1.9	380.01	2523	1291	1297	37.43	5362	1.4	1.0	19.8	58

	1	2	3	4	5	6	7	8	9	10	11	12	13
16.	74.05	87.6	6.34	423.0	2045.32	500.32	1352	238.8	710.99	8.46	1.313	17.72	160.
17.	72.31	87.4	8.02	455.7	2341.8	886.25	1534.7	43.91	637.74	8.78	1.72	13.64	74
18.	66.31	35	10.22	492.7	2719.4	1046.9	2384	493	3 10.1	1.42	1.249	31.3	89
19.	75.85	83.03	8.32	331	2439	1123.1	974.6	22.3	1385	1.45	1.37	22.4	78
20.	66.57	62.2	4.35	272.66	2296	1112	1309	23.9	2086	1.34	1.18	21.27	85
21.	60.99	76.11	1.8	330.01	2439	1011	1106	18.6	1077	1.06	0.94	31.6	110
22.	70.2	82.3	4.83	426.6	255.8	768.6	1083	26.39	772	1.8	1.04	17.2	59
23.	69.7	81.4	7.06	402.13	2203.2	1304.3	1080	36.61	1578	2.0	1.26	17.6	94.6
24.	63.43	83.13	5.68	692.4	2644	1003.9	1401	36.77	1068	1.6	1.102	10	57.9
25.	64.27	68.73	3.05	390	2368	2000	1403	49	3726	1.2	1.091	22.3	110.514
26.	70.2	82.3	4.93	4266	2558	763.67	1083	26.39	772.62	1.549	1.04	17.2	58
27.	65.93	83.05	6.19	4996	2523	1291.12	1206.2	37.43	5052.0	1.491	1.079	19.7	3 .58
28.	60.99	76.11	5.42	110.14	2147.3	1099.6	1390	22.02	1095	1.69	1.33	31.6	88.1
29.	70.4	70.77	5.024	396.6	2075.5	617.15	1300	22.03	766.14	1.412	1.511	16.13	89.3
30.	69.77	81.436	7.06	402.8	2203.2	1304.35	1080	36.61	1513.79	2.013	1.26	17.166	94.62
31.	61.75	70.03	5.38	358	2214	860	1387	45	2630	2.12	1.3	16.3	34
32.	45.99	78.92	8.20	272	2300	901	1077	43.95	7979.4	4.2	1.23	13.6	180
33.	41.3	22.7	7.315	230.6	1624	602.2	874.15	35.62	5633.6	1.06	0.9	13.66	40
34.	36.78	22.13	2.61	230.13	1840.5	430.3	838	16.75	5839.9	0.9265	0.259	11.2	72
35.	41.34	20.73	3.4	276.34	1432.7	357.6	861.5	8.395	4015	0.4475	0.543	12.67	104
36.	36.93	15.165	4.9	233.6	1234.15	219.1	1156	26.21	1179.3	1.515	0.401	4.26	88
37.	55.15	22.64	3.8	304.7	1600.45	301.94	945.5	34.2	247.71	1.71	1.53	19.3	73
38.	43.77	21.47	5.7	309.2	1742	523	932.2	39.2	392	1.82	1.35	17.2	88

	1	2	3	4	5	6	7	8	9	10	11	12	13
38.	43.77	21.47	3.7	300.2	1742	823	932.2	30.2	392	1.82	1.35	17.2	85
39.	39.7	40.9	7.12	130.9	2464	1131	1569	42.3	7052.1	1.69	0.96	11.2	154
40.	42.4	23.9	3.15	264.7	2044	667	1782	43.2	5386.4	1.04	0.79	11.1	110
41.	45.3	25.4	2.17	431.7	2617	1743	136.0	31.62	1163.0	1.7	1.2	17.6	67
42.	67.15	73.4	8.67	464.15	2581	1634	1478	34.67	1763	1.7	1.0	19.1	78
43.	59.2	23.64	8.02	234.32	2552	1583	999.6	21.97	3452	1.15	0.174	13.06	107
44.	29.2	40.03	6.09	323.7	2236	1092	1225.5	42.35	7236	1.11	1.257	34.57	30
45.	33.8	43.55	7.43	423.2	2240	1432	1915	44.00	5324	1.09	0.97	19.7	54
46.	52.2	38.05	6.64	304.5	2556.	1674	124.5	43.40	4394	1.25	1.25	12.3	120
47.	47.3	32.32	5.54	264.7	2443	1562	145.30	39.82	3732	1.92	1.34	14.3	110
48.	41.7	41.8	4.52	130.9	2484	1444	121.50	45.32	5836	1.45	1.39	11.75	98
49.	43.8	42.32	3.92	431.3	2300	1132	1300	47.00	7904	1.72	1.11	11.23	97
50.	52.8	44.4	2.96	353.14	2382	1240	143.00	24.09	3002	1.32	1.07	13.3	79
51.	66.9	53.05	6.19	410.2	2523	1291	1296	37.48	5062	1.52	1.079	19.7	85.33
52.	41.3	22.7	7.315	230.6	1624	2143	1099	45.7	3726	2.2	1.21	13.6	67
53.	36.93	52.1	7.20	304.7	304.7	410.5	1398	36.8	772.5	1.32	1.02	12.2	58
54.	55.16	51.4	6.27	339.2	937	1255	2003	32.9	1077	1.06	1.19	13.7	49
55.	43.77	53.05	8.67	400.9	210	1272	2597	41.2	3210	1.72	1.09	19.1	64
56.	65.93	53.13	6.015	330.01	330	1561	2227	52.3	2086	1.51	1.07	10.7	50
57.	45.3	25.4	2.17	431.7	2260	1131	1569	42.8	7052	1.69	0.93	11.2	65
58.	59.8	43.5	7.03	423.3	2279	1003	1429	46	4090	1.26	1.27	19.5	62
59.	43.2	42.3	3.92	431.3	2300	1132	1303	46.7	1127	1.9	1.16	17.4	59
60.	63.9	71	8.1	380	2416	2051	1469	39	3213	1.62	1.07	13.1	34

No.	1	2	3	4	5	6	7	8	9	10	11	12	13
61.	75.9	56.1	7.1	360.4	2363	336	1662	22.6	2143	1.21	1.01	17.2	88
62.	63.4	52.3	6.9	338.9	2414	728	1428	37.49	3169	0.96	0.912	14.6	57
63.	61.73	45.1	6.36	401	2145	1206	2018	34.67	3826	1.05	0.916	16.12	42
64.	75.6	37.9	5.32	292.1	2859	943	1928	22.9	2215	6.97	1.02	15.9	80
65.	67.15	29.9	8.02	376.4	2719	842	1621	36.7	1063	0.912	0.927	14.9	57
66.	62.9	41.6	7.09	342.9	2252	629	1262	46.2	1763	1.001	0.913	15.2	72
67.	63.4	43.9	6.82	327	2562	521	1043	43.1	3021	0.975	1.12	16.7	34
68.	68.9	44.5	4.9	298.2	2149	1082	2011.9	28.7	2691	0.961	0.916	13.1	61
69.	64.3	52	5.2	390.2	2523	927	1337	31.62	2172	0.893	0.675	13.9	34
70.	72.2	61.8	6.34	450.7	2617	1004	2014	34.6	3031	0.912	0.921	17.2	69
71.	61.7	53.1	5.8	292	2561	896	1621	43.2	3009	1.05	0.712	23.1	39
72.	66.2	57	6.07	331	2343	932	1831	29.7	3215	1.009	0.766	22.3	36
73.	72	40.9	7.26	420	2410	669	1296	31.3	1269	0.976	0.810	19.9	39
74.	63.3	35.4	6.06	402.3	2145.4	860.3	1309	40.2	630.4	2.12	1.3	16.3	34
75.	70.2	52.3	4.85	456.6	2559	768.7	1093	27	772	1.6	1.05	17.2	59
76.	68.9	71	8.3	391	2414	2252	1493	45.3	3215	1.4	1.2	17	89
77.	72.2	57.4	8	455.7	2341.8	896	1534	43	637	2.8	1.7	13.6	34
78.	64.3	63.7	8.05	390	2303	100	1403	43	3726	1.3	1.1	17.2	39
79.	62.7	53.3	6.31	347	2242	1007	1429	46	4036	1.26	1.27	19.5	72
80.	73.5	52.3	5.4	310	2143	597	1300	22	1095	1.7	1.3	31.6	88
81.	63.4	53.1	5.7	632	2644	903	1401	36.7	1063	1.6	1.1	19.6	57
82.	63.9	71	8.7	331.9	2413	2051	1429	39	3213	1.0	1.15	17.013	89
83.	75.9	72.6	10.2	495	2719	1046	2304	47	3210	1.21	1.20	31.25	90
84.	72.4	49.4	6.015	4373	2255	393	1340	36.4	1127	1.90	1.16	17.4	59

S.No.	1	2	3	4	5	6	7	8	9	10	11	12	13
85.	72.39	70.03	8.6	455.73	2252	886	1488	45	3215	1.45	1.21	17	90
86.	63.4	63.73	5.7	382.4	2644	1008	1401	36.7	1063	1.62	1.0	19.6	57
87.	73.57	53.3	7.42	110.14	2143	1099	1390	22.6	1095	1.265	1.0	19.7	73
88.	64.27	76.11	7.8	330.01	2523	1291	1297	37.48	5052	1.4	1.2	19.8	58
89.	61.73	71.17	6.72	43170	2617	1743	1369	31.62	1163	1.7	1.20	17.6	67
90.	67.15	75.40	3.67	464.15	2561	1694	1473	34.67	1768	1.73	1.01	19.1	78
91.	63.9	52.3	5.1	392	2143	629	1230	27	1095	1.41	1.21	31.6	34
92.	64.3	55	5.7	450	2290	928	1369	40.2	3210	1.02	1.15	17.4	39
93.	62.7	62.1	5.41	215	2315	1100	1970	43	2251	0.96	1.29	19.5	42
94.	63.4	71.2	6.09	682	2308	946	1812	45.8	3076	0.821	1.23	31.3	92
95.	75.9	52.4	8.05	455	2401	663	1249	46	3516	1.624	1.16	19.8	61
96.	77.2	49.7	6.15	277	2296	1105	1916	48	1063	1.072	1.07	14.2	41
97.	63.4	51.06	6.50	396	2644	961	1551	22	1126	1.059	1.09	12.1	57
98.	64.27	63.7	5.42	271	2561	842	1657	36.7	1100	1.082	1.22	13.9	77
99.	67.15	71.3	6.98	348	2253	649	1292	45.1	5052	0.732	0.941	14.6	66
100.	61.73	70.03	7.21	367	2363	721	1567	36.5	3210	1.061	1.76	17.4	39

APPENDIX X

S.No.	Haemoglobin g/100 ml.	PCV Percent	S.No.	Haemoglobin g/100 ml.	PCV Percent
1.	11.61	37	26.	12.555	40
2.	14.53	47	27.	12.15	39
3.	13.5	43	28.	11.61	37
4.	13.095	42	29.	13.905	44
5.	12.56	40	30.	14.31	46
6.	13.095	42	31.	13.23	42
7.	11.6	37	32.	14.175	45
8.	11.6	37	33.	11.61	37
9.	12.015	38	34.	13.905	42
10.	12.56	40	35.	12.285	39
11.	12.28	39	36.	13.095	42
12.	12.06	41.1	37.	12.15	39
13.	11.61	37	38.	12.555	40
14.	12.285	39	39.	13.095	42
15.	12.285	39	40.	12.42	39
16.	12.015	38	41.	13.095	42
17.	12.28	39	42.	11.61	37
18.	14.53	47	43.	12.15	39
19.	13.905	44	44.	13.635	44
20.	13.5	43	45.	12.825	41
21.	13.745	37.5	46.	12.15	39
22.	13.905	42	47.	11.61	37
23.	12.555	40	48.	14.38	47
24.	12.15	39	49.	14.175	45
25.	11.83	38	50.	13.5	43

S.No.	Haemoglobin g/100 ml.	PCV Percent
51.	13.905	42
52.	13.77	44
53.	13.23	42
54.	12.015	38
55.	12.69	41
56.	12.285	39
57.	14.31	46
58.	14.985	47
59.	11.6	37
60.	12.825	41
61.	13.905	42
62.	12.42	39
63.	12.555	40
64.	12.285	39
65.	14.04	45
66.	14.04	45
67.	12.555	40
68.	14.31	46
69.	13.095	42
70.	13.095	42
71.	12.42	39
72.	14.31	46
73.	12.285	39
74.	14.175	45
75.	12.285	39
76.	12.825	41
77.	13.905	42
78.	11.61	37
79.	12.285	39
80.	11.61	37
81.	12.69	31
82.	12.285	39
83.	12.285	39
84.	11.88	33
85.	12.42	39
86.	11.99	38
87.	13.23	42
88.	13.77	44
89.	13.905	42
90.	13.5	43
91.	13.905	42
92.	12.015	42
93.	13.365	42
94.	12.285	39
95.	13.77	44
96.	13.23	42
97.	12.825	41
98.	14.175	45
99.	12.015	38
100.	12.69	41

APPENDIX XI

SERUM IRON AND TIBC mcg/dl.

No.	Serum Iron	TIBC	No.	Serum Iron	TIBC
1.	60	362	26.	77	335
2.	140	289	27.	65	359
3.	90	329	28.	131	291
4.	80	334	29.	129	279
5.	90	312	30.	94	301
6.	90	307	31.	140	272
7.	60	339	32.	72	335
8.	60	346	33.	64	340
9.	90	326	34.	92	321
10.	90	316	35.	85	314
11.	98	331	36.	72	305
12.	94	313	37.	79	320
13.	77	347	38.	74	319
14.	88	332	39.	81	261
15.	88	336	40.	76	340
16.	73	342	41.	89	306
17.	94	309	42.	69	328
18.	145	268	43.	77	317
19.	140	260	44.	104	301
20.	120	238	45.	87	321
21.	68	380	46.	76	336
22.	130	280	47.	68	340
23.	77	348	48.	120	289
24.	68	378	49.	68	320
25.	68	361	50.	130	291

Cells.	Grain Area	Yield	Cells.	Grain Area	Yield
51.	104	302	76.	90	307
52.	94	310	77.	93	302
53.	65	316	78.	69	349
54.	78	316	79.	94	333
55.	90	309	80.	69	315
56.	82	314	81.	90	300
57.	120	296	82.	84	342
58.	124	281	83.	94	331
59.	78	320	84.	74	326
60.	81	307	85.	86	320
61.	112	280	86.	68	349
62.	80	325	87.	98	313
63.	85	315	88.	106	290
64.	73	343	89.	110	278
65.	116	292	90.	94	313
66.	120	281	91.	100	306
67.	85	320	92.	74	312
68.	120	273	93	94	321
69.	108	299	94.	82	317
70.	80	271	95.	110	320
71.	94	263	96.	90	319
72.	120	269	97.	84	307
73.	88	328	98.	126	293
74.	103	291	99.	74	334
75.	80	320	100.	90	310

APPENDIX XII

SERUM ZINC µg/dl

S.No.	Serum Zinc µg/dl	S.No.	Serum Zinc µg/dl
1.	102	26.	110
2.	121	27.	124
3.	104	28.	118
4.	126	29.	126
5.	107	30.	122
6.	126	31.	92
7.	131	32.	127
8.	117	33.	122
9.	119	34.	121
10.	120	35.	126
11.	94	36.	120
12.	118	37.	121
13.	122	38.	121
14.	127	39.	123
15.	120	40.	109
16.	126	41.	178
17.	105	42.	122
18.	123	43.	136
19.	121	44.	122
20.	122	45.	120
21.	123	46.	105
22.	115	47.	122
23.	122	48.	131
24.	129	49.	111
25.	106	50.	115

S.No.	Serum Zinc µg/dl	S.No.	Serum Zinc µg/dl
51.	121	76.	120
52.	120	77.	102
53.	116	78.	104
54.	120	79.	101
55.	122	80.	97
56.	141	81.	107
57.	122	82.	174
58.	116	83.	119
59.	124	84.	120
60.	131	85.	121
61.	118	86.	116
62.	128	87.	108
63.	124	88.	120
64.	128	89.	136
65.	125	90.	143
66.	132	91.	130
67.	143	92.	122
68.	120	93.	136
69.	113	94.	107
70.	115	95.	102
71.	106	96.	129
72.	121	97.	106
73.	133	98.	123
74.	120	99.	134
75.	119	100	140

APPENDIX XII

SERUM COPPER mg/dl

S.No.	Serum Copper mg/dl	S.No.	Serum Copper mg/dl
1.	109	26.	92
2.	114	27.	109
3.	107	28.	103
4.	97	29.	117
5.	106	30.	130
6.	85	31.	86
7.	119	32.	124
8.	114	33.	124
9.	117	34.	114
10.	116	35.	122
11.	90	36.	113
12.	116	37.	115
13.	119	38.	112
14.	117	39.	117
15.	115	40.	100
16.	101	41.	102
17.	82	42.	118
18.	104	43.	127
19.	101	44.	118
20.	99	45.	140
21.	116	46.	121
22.	107	47.	117
23.	114	48.	104
24.	130	49.	101
25.	94	50.	82

S.No.	Serum Copper mg/dl	S.No.	Serum Copper mg/dl
51.	111	76.	116
52.	103	77.	101
53.	97	78.	98
54.	102	79.	163
55.	118	80.	85
56.	132	81.	101
57.	115	82.	109
58.	106	83.	110
59.	113	84.	117
60.	125	85.	114
61.	104	86.	99
62.	116	87.	83
63.	114	88.	112
64.	121	89.	129
65.	119	90.	126
66.	122	91.	120
67.	139	92.	113
68.	107	93.	122
69.	102	94.	96
70.	107	95.	91
71.	89	96.	112
72.	101	97.	100
73.	118	98.	116
74.	112	99.	123
75.	109	100.	141

APPENDIX XIII

SERUM CALCIUM VALUES.

S.No.	Serum Calcium (mg/100 ml.)	S.No.	Serum Calcium (mg/100 ml.)
1.	9.25	26.	10.73
2.	11.1	27.	9.505
3.	9.71	28.	11.285
4.	9.25	29.	9.8375
5.	10.175	30.	10.175
6.	11.56	31.	9.62
7.	9.25	32.	10.73
8.	10.64	33.	9.9075
9.	10.175	34.	9.3425
10.	10.64	35.	10.915
11.	9.25	36.	11.1
12.	9.71	37.	9.99
13.	9.1	38.	10.36
14.	9.435	39.	10.0825
15.	10.545	40.	10.64
16.	9.16	41.	9.25
17.	10.36	42.	9.1
18.	10.545	43.	9.435
19.	9.6275	44.	9.1573
20.	9.7125	45.	9.62
21.	9.8275	46.	10.915
22.	10.73	47.	9.305
23.	10.78	48.	11.0075
24.	10.36	49.	10.0825
25	9.99	50.	10.8255

S.No.	Serum Calcium (mg/100 ml.)	S.No.	Serum Calcium (mg/100 ml.)
51.	9.18	74.	10.914
52.	9.09	75.	9.792
53.	10.2	76.	11.016
54.	9.588	77.	10.404
55.	9.18	78.	9.99
56.	10.404	79.	10.302
57.	10.608	80.	9.384
58.	9.09	81.	10.098
59.	9.894	82.	10.608
60.	11.118	83.	9.384
61.	10.914	84.	10.818
62.	10.098	85.	9.486
63.	10.506	86.	10.71
64.	10.098	87.	9.486
65.	10.2	88.	11.32
66.	9.792	89.	10.914
67.	10.098	90.	9.588
68.	10.098	91.	10.302
69.	9.996	92.	9.996
70.	10.914	93.	10.20
71.	10.608	94.	10.404
72.	10.506	95.	10.914
73.	9.996	96.	9.486
74.	10.914	97.	9.894
75.	9.792	98.	10.098
		99.	10.71
		100.	11.016

APPENDIX XIV

SERUM VITAMIN A mcg/100ml.

S.No.	Serum Vitamin A	S.No.	Serum Vitamin A
1.	33	26.	32
2.	29	27.	27
3.	31	28.	27
4.	27	29.	21
5.	24	30.	23
6.	28	31.	34
7.	37	32.	32
8.	36	33.	28
9.	26	34.	37
10.	31	35.	21
11.	24	36.	42
12.	33	37.	29
13.	24	38.	32
14.	37	39.	28
15.	39	40.	27
16.	24	41.	31
17.	35	42.	39
18.	26	43.	26
19.	31	44.	21
20.	27	45.	37
21.	25	46.	30
22.	36	47.	38
23.	29	48.	39
24.	38	49.	32
25.	37	50.	22

S.No.	Serum Vitamin A	S.No.	Serum Vitamin A
51.	43	76.	25
52.	26	77.	29
53.	28	78.	29
54.	29	79.	32
55.	23	80.	38
56.	33	81.	41
57.	36	82.	33
58.	34	83.	36
59.	37	84.	30
60.	37	85.	29
61.	22	86.	39
62.	23	87.	27
63.	32	88.	28
64.	30	89.	31
65.	24	90.	37
66.	37	91.	24
67.	30	92.	29
68.	26	93.	23
69.	40	94.	32
70.	32	95.	29
71.	28	96.	23
72.	32	97.	24
73.	25	98.	34
74.	27	99.	25
75.	22	100.	34

APPENDIX XV

SERUM PROTEINS

S.No.	Total Protein gm/100ml.	Albumin gm/100ml	Globulin gm/100ml
1.	7.31	4.29	3.02
2.	6.97	4.11	2.86
3.	7.14	4.17	2.97
4.	6.8	4.06	2.74
5.	6.63	3.67	2.96
6.	6.97	4.165	2.905
7.	7.43	4.49	2.99
8.	7.65	4.99	2.66
9.	7.14	4.33	2.81
10.	7.82	4.83	2.99
11.	6.38	3.75	2.63
12.	7.06	4.83	2.23
13.	6.38	4.08	2.3
14.	7.06	4.99	2.7
15.	6.33	3.75	2.45
16.	6.46	3.75	2.71
17.	6.545	4.17	2.33
18.	6.89	3.99	2.9
19.	6.72	4.66	2.06
20.	6.38	3.75	2.45
21.	7.74	5.25	2.49
22.	7.43	5.03	2.4
23.	6.46	3.99	2.47
24.	6.72	4.08	2.64
25.	6.89	4.83	2.06

S.No.	Total Protein gm/100ml.	Albumin gm/100ml.	Globulin gm/100 ml.
26.	6.65	4.41	2.14
27.	6.65	4.17	2.33
28.	6.33	4.03	2.3
29.	6.89	4.75	2.14
30.	7.06	4.83	2.23
31.	6.80	4.75	2.05
32.	6.39	4.66	2.89
33.	7.65	4.99	2.66
34.	6.30	3.92	2.37
35.	6.715	3.92	2.9
36.	6.630	3.93	2.7
37.	7.65	4.99	2.66
38.	6.80	3.92	2.97
39.	7.14	4.25	2.89
40.	7.57	4.99	2.58
41.	6.90	3.93	2.97
42.	7.23	4.91	2.32
43.	7.06	4.99	2.03
44.	9.31	5.08	2.23
45.	7.06	4.17	2.89
46.	7.23	4.75	2.42
47.	7.43	4.93	2.65
48.	6.80	3.92	2.88
49.	7.74	5.25	2.49
50.	7.14	5.08	2.06

S.No.	Total Protein gm/100 ml.	Albumin gm/100 ml	Globulin gm/100 ml.
51.	7.57	4.99	2.58
52.	6.89	3.94	2.9
53.	6.715	3.83	2.8
54.	6.39	4.08	2.63
55.	6.375	3.74	2.68
56.	6.89	4.75	2.14
57.	6.38	4.17	2.38
58.	6.89	4.83	2.06
59.	6.205	3.99	2.31
60.	7.74	5.25	2.49
61.	6.38	4.08	2.3
62.	7.43	4.83	2.65
63.	6.55	4.17	2.38
64.	6.21	3.75	2.46
65.	6.46	3.75	2.71
66.	6.89	4.08	2.81
67.	7.74	5.3	2.44
68.	7.06	4.83	2.23
69.	6.38	3.83	2.55
70.	6.89	4.08	2.3
71.	7.43	5.03	2.4
72.	7.74	5.25	2.49
73.	6.85	4.08	2.77
74.	6.72	4.66	2.06
75.	6.80	4.75	2.05

S.No.	Total Protein gm/100 ml.	Albumin gm/100 ml.	Globulin gm/100 ml.
76.	6.89	3.99	2.9
77.	7.06	4.83	2.23
78.	6.89	3.76	3.14
79.	6.715	4.08	2.635
80.	6.80	4.76	2.05
81.	6.715	4.66	2.053
82.	8.715	3.76	2.965
83.	6.89	4.83	2.06
84.	7.65	4.99	2.66
85.	7.06	4.99	2.97
86.	7.14	4.66	2.48
87.	6.630	4.08	2.55
88.	7.06	4.76	2.31
89.	7.23	4.08	3.15
90.	6.80	4.76	2.06
91.	7.06	4.08	2.98
92.	8.630	4.08	2.55
93.	7.14	3.99	3.15
94.	6.630	3.99	2.58
95.	7.74	4.08	2.99
96.	6.72	4.08	2.64
97.	6.89	3.79	3.14
98.	7.14	4.66	2.48
99.	6.63	3.75	2.93
100.	6.80	4.08	2.72