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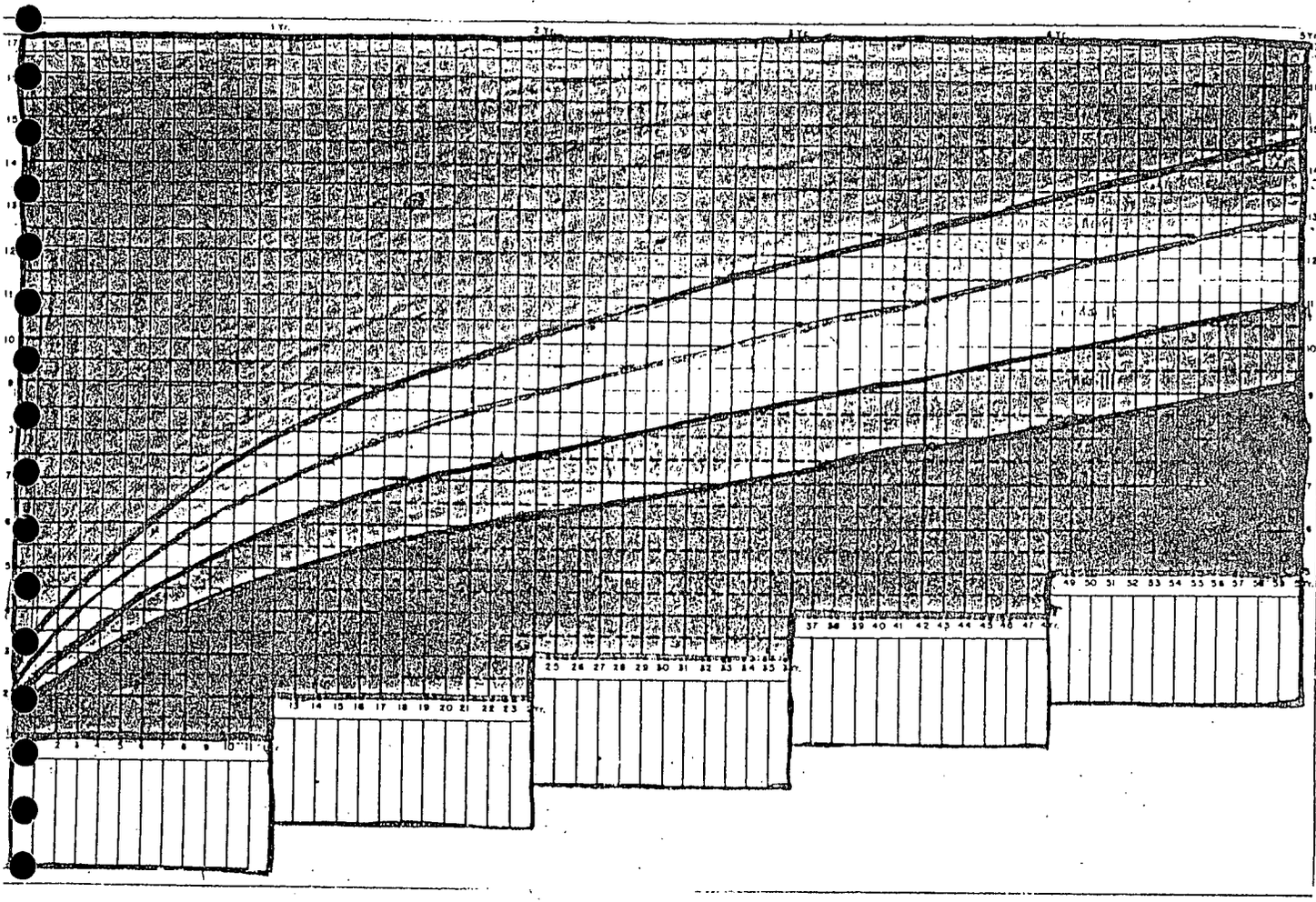
SHORT COURSE ON

ASSESSMENT OF NUTRITIONAL STATUS

8th - 27th JULY 1996



CENTRE OF ADVANCED STUDIES

P.G.& RESEARCH CENTRE - HOME SCIENCE

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PREFACE

Nutritional status assessment is gaining importance in today's world, both in examining the nutritional status of populations in developing and developed countries and in determining the nutritional status of individuals in hospitals and in the commuinity.

For Nutritionists engaged in teaching, research and field activities, the knowledge on assessment of nutritional status is essential as this forms an important step in initiating, monitoring and evaluation of any nutritional programmes. Methods of nutritional status assessment constantly undergo revision, in order to refine the existing methods and the interpretation. More and more innovative and rapid methods are being added in this context. Hence, it is essential that all nutritionists become abreast of latest developments. With this intention, the present training programme has been conceived and formulated.

The content of the training programme covers the recent trends in established methods of assessing the nutritional status such as anthropometry, bio-chemical and clinical methods, dietary assessment, vital statistics etc. Efforts have been made to

include some of the latest methods such as rapid & participatory rural assessment techniques, focussed ethinography & stake holder analysis. In all the areas, emphasis was given to the scientific principles, the advantages, limitations and applicability of the various methods and the use of appropriate reference data. Often nutritional assessment is marred by an inadequate appreciation of confounding factors that may affect a proper interpretation of results, as well as the use of in appropriate procedures and reference data. Attempts have been made to discuss these problems at appropriate points to highlight confounding factors where these are known to exist, and to provide guidance on the selection of the most suitable procedures and reference data.

It is obvious that this compilations is based on the research and reports of many investigators, but we are particularly greatful to the scientists from National Institute of Nutrition and Faculty members of Foods & Nutrition Department of APAU, Hyderabad.

We trust and hope this forms a good reference material for all the nutritionists.

Uma Reddy.M &
Yasoda devi.P

(Course Coordinators)

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Nutritional status assessment - need and importance

Dr. Uma Reddy

Nutritional status assessment is the primary step involved to initiate any intervention programmes in a community or treatment in a hospital situation in a hospital. In developing countries, where several welfare programmes are being implemented and some are being initiated it becomes all the more important to assess the nutritional status of communities before starting the programme, periodical monitoring and for evaluation of the impact of the programme. The nutritional assessment may be required to encompass nations, communities, vulnerable segment of comunities or individual. It may be done as a part of exercise to document current status as compared with past status, or as a specific attempt to evaluate the impact of an intervention programme.

Research has been carried over several decades by national and international organisation to evolve methods of evaluating the nutritional status. Assessment of nutritional status can be done using the following methods.

1. Direct methods nutritional anthropometry, biochemical and clinical method.

2. Indirect parameters - Diet surveys, morbidity and mortality rates, especially age, specific mortality rates.

The basic criteria involved in selection of the parameters to evaluate nutritional status in field situation are

1. Specificity - the extent to which the selected parameters specifically identify nutritional status.

2. Sensitivity - the capacity of responding to minor changes attributes to nutrition.

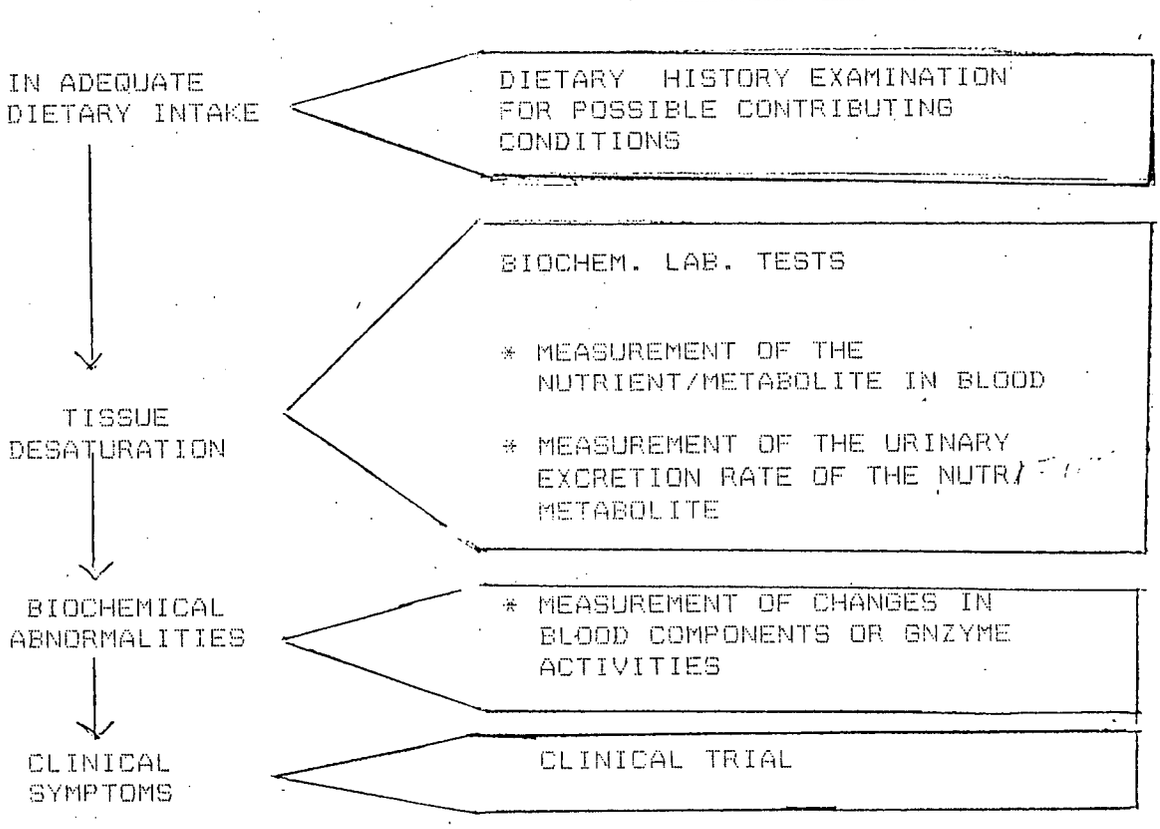
3. Simplicity - in effect the need for the minimum of skill, time and organisation as well as feasibility under the conditions of the study.

4. Economy - involving the least cost yet reliable.

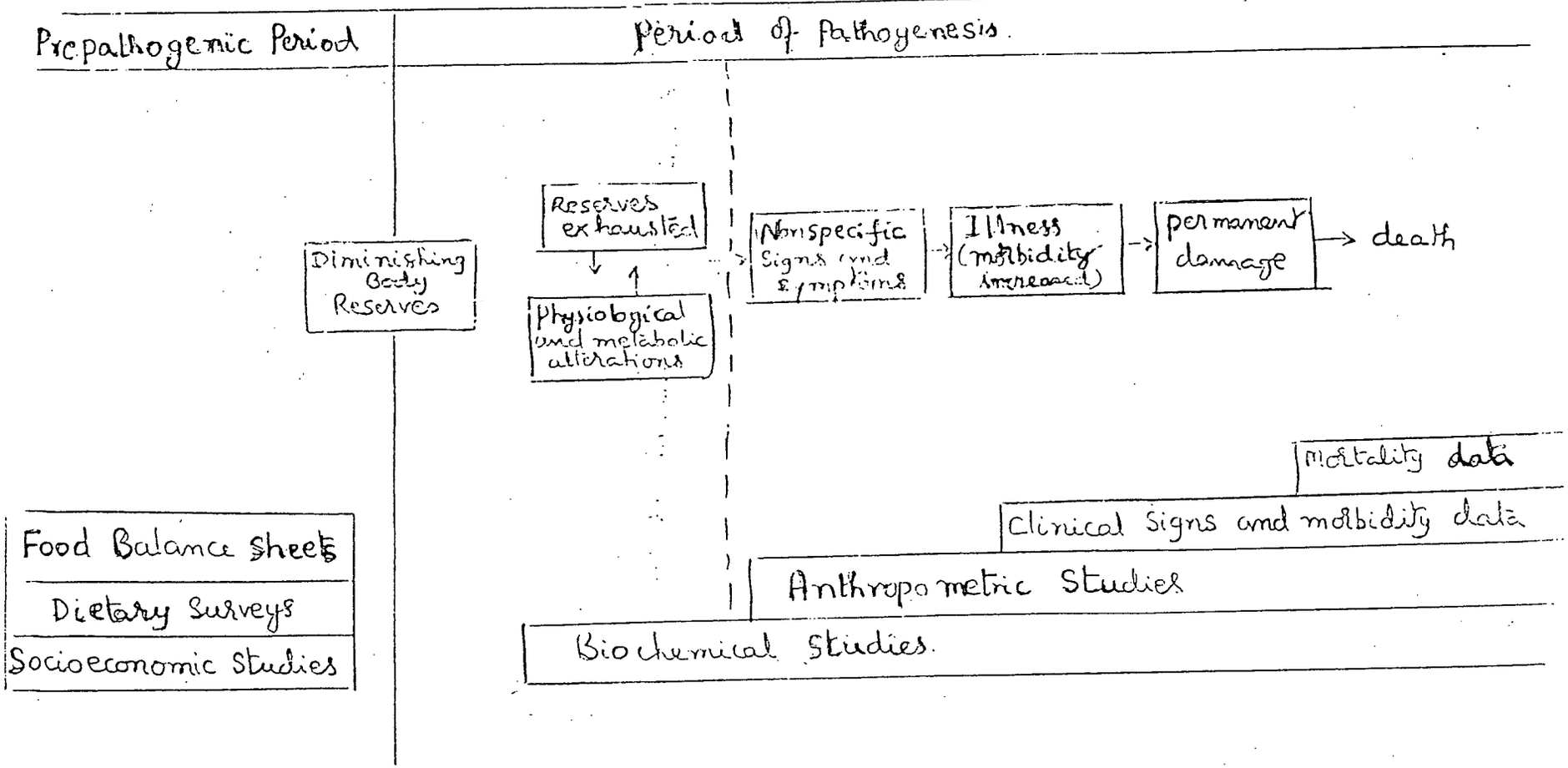
Relative values of parameters

Methods	Specificity	Sensitivity	Simplicity					
			Skill	Time	Organi-sation	Cost	Errors	Overall rank
Mortality	Poor	Poor	Less	Less	Less	Low	More	IV
Morbidity								
Non-nutrition	Poor	Poor	More	More	More	High	Less	V
Nutrition	Good	Good	More	More	More	High	Less	I
Anthropometry	Fair	Fair	More	Less	Less	Low	Low	II
Dietary intake	Fair	Fair	More	More	More	High	More	III
Social & phychological measurement	Poor	Poor	More	More	More	High	More	VI

SEQUENCE OF EVENTS LEADING TO THE DEVELOPMENT OF NUTRITIONAL DEFICIENCIES



NUTRITIONAL ASSESSMENT AND RELATIONSHIP TO THE NATURAL HISTORY OF DISEASE



ANTHROPOMETRIC ASSESSMENT OF YOUNG CHILDREN

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Nutritional anthropometry has been defined by Jelliffe (1966) as the "Measurement of the variations of the physical dimensions and the gross composition of the human body at different age levels and degrees of nutrition".

Advantages of Anthropometric assessment

1. The procedures are simple and safe
2. Equipment required is inexpensive, portable and durable and can be made or purchased locally
3. Relatively unskilled personnel can perform measurement procedures
4. The methods are precise and accurate, provided that standardized techniques are used
5. Information is generated on past long-time nutritional history, which cannot be obtained with equal confidence using other techniques.
6. The procedures can assist in the identification of mild to moderate malnutrition, as well as severe states of malnutrition (over/under nutrition)
7. The methods may be used to evaluate changes in nutritional status over time and from one generation to the next, a phenomenon known as the secular trend (Johnston, 1981).
8. Screening tests, to identify individuals at high risk to malnutrition, can be devised (Chen et al., 1980).

Disadvantages of Anthropometric assessment

Despite of the advantages, nutritional anthropometry has several disadvantages.

1. It is relatively insensitive method and it cannot detect disturbances in nutritional status over short periods of time or identify specific nutrient deficiencies.
2. Nutritional anthropometry is unable to distinguish disturbances in growth or body composition induced by

nutrient (eg. zinc) deficiencies from those caused by imbalances in protein and energy intake.

Nevertheless, nutritional anthropometry can be used to monitor periodic changes in growth and or body composition in individuals and in population groups.

Physical dimensions of the body are much infuenced by (1) biological factors (sex, intra uterine environment, birth order, birth weight, parental size etc.) (2) Genetic background (3) Environmental factors (season, climate, socio-economic level, nutrition, exposure to infection/infestation and (4) psychological factors.

Research studies suggested that environmental influences specially nutrition play important role in infants growth than genetic background or other biological factors (Habicht et al. (1974). The physical dimensions of the body are much influenced by nutrition particularly in rapidly growing period of early childhood. Well to do and well-fed Indian children upto 14 years of age in case of boys and 12 years in case of girls were found to be growing similar to western children. This suggests that up to this age, the genetic role is minimum but environmental influences like nutrition, health factors like exposure to infection is mainly responsible for the growth. However for growth during adolescence, more than nutrition and environment genetic capacity appears to be important (Gopalan and Meera Chatterjee). Because of this genetic difference among Indian and western children the final heights and weights of even in well-fed well-to-do Indian children remain slightly less than those of western children.

Assessment of growth basically involves two steps

1. Obtaining the necessary data on suitable anthropometric measurements

2. Analysis of the data to understand and interpret the growth phenomenon.

The first of these largely consists of taking the appropriate measurement with due attention to techniques and quality control.

The most commonly used Anthropometric measurements are:

1. Body mass - as judged by weight

2. Linear measurements - Height or length
   - Head/chest circumference

3. Body composition and reserves of calories and protein as judged by special soft tissues i.e., fat and muscle

AGE ASSESSMENT

Determining the correct age of a child is extremely important in evaluating anthropometric data, since reference standards for growth are broken down into age categories by month. The age of a child should be determined as the number of years/months of life completed.

Birth date of young child can be estimated by

Birth records, or talking to the mother/local dia/ANM; or by dai/ANM by using local events calender

BODY WEIGHT

Measurement of total body mass undressed. The body weight indicates the body mass and gives a rough estimate of body volume.

Indication:
* Sensitive indicator of current nutritional status.
* Deficit in weight indicates short term under nutrition which can be easily reversed.
* PEM is best identified by weight deficiency in all groups. Weight measurement should be combined with other appropriate measurements and with clinical exam.

STATURE

Height: Distance from the crown of the head to the bottom of the feet (heels) while the child is measured standing (for children 2 years of age or older) is called height.

Length: Distance from the crown of the head to the bottom of the feet (heels) while child is measured supine (for children less than 2 years of age) is called length.

Indication:
* Gives a picture of past nutritional status
* Deficit in height indicates chronic and prolonged under nutrition resulting often in permanently stunted physical status.

SOFT TISSUES

Muscle and fat constitute the soft tissues that vary most with deficiency of protein and calories. Tissue anthropometry can be carried out on both of these in the assessment of the nutritional status of a community.

SUBCUTANEOUS FAT: Human subcutaneous fat can be carried out by various methods. Only physical anthropometry using skin fold calipers is practicable in field circumstances.

MUSCLE

Poor muscle development/muscle wasting are cardinal features of all forms of protein-energy malnutrition, especially those of early childhood.

MID UPPER ARM CIRCUMFERENCE (MUAC)

The measurement of a child's arm circumference at the mid point between the tip of the shoulder and elbow. This is usually employed as a screening device.

Indication:

* Indicates poor muscle development and fat component of body
* Muscle wasting which is extreme thinness, reflects acute, current malnutrition.
* Indicates present under nutrition and over nutrition and features of all forms of PEM of early childhood used for screening children into different groups of malnutrition.

Because arm circumference changes only about 1 cm in children between the ages of 1-4, It has been used as an age independent measure in children of that age range.

HEAD CIRCUMFERENCE

The measurement of head circumference is related mainly to brain size and to a small extent to the thickness of the scalp tissues and the skull.

Indication:

* Head circumference is a standard procedure to detect pathological conditions accompanied by a large head/one of increasing size with hydrocephalus or too small a skull as with microcephaly.

* Intrauterine and childhood nutrition (chronic undernutrition)

* Brain size and both the thickness of scalp soft tissues and the skull can vary with nutritional status.

* Head circumference is slightly affected in the second year of life in PEM.

* Head circumference may also be used as a rough additional guide in age assessment.

* The chest/head circumference ratio is of value in detecting PEM in early childhood.

CHEST CIRCUMFERENCE

The circumference of the head and chest are about the same at six months of age. After this, the skull grows slowly and the chest more rapidly.

A chest/head circumference ratio less than one may be due to failure to develop or to wasting of the muscle and fat of the chest wall, and can be used as community indicator of protein energy malnutrition of early childhood.

INTERPRETATION OF ANTHROPOMETRIC DATA

Analysis of anthropometric data collected is very important for proper interpretation.

Growth standards/norms

The growth standards/norms are mainly used

* To detect whether the child is normal or abnormal

* To compare measurements made in different places/ different times by relating them to a single reference population.

Criteria followed for development of a standard

1. Measurement should be related to a well nourished population.

2. Sample should include atleast 200 individuals in each age and sex group.

3. The sample should be cross sectional since the comparisons made will be of cross sectional nature.

4. Sampling procedures should be well defined and reproduceble.

5. Measurements should be carefully made and recorded by trained people in anthropometric techniques, using equipment of well tested, designed and caliberated at frequent intervals.

TYPES OF STANDARDS AVAILABLE

1. Local standards developed by ICMR

2. International standards

1. Harvard standards:	Based on well nourished children in Boston in 1930's (Stuart et al., 1959).
2. Standards used in: Road-to-health card	Based on homogenous group of British children (Tanner et al., 1950).
3. NCHS (U.S.Natio-: nal Centre for Health Sciences)	Based on economically and ethnically heterogenous U.S. child population (WHO, 1978).

Inspite of heterogenous population used to derive NCHS data compared to homogenity of Boston and British groups NCHS are lowered only minimally.

Local/International - Which one to use?

Some argue that local standards should be used to be more realistic about growth achievement of child population (Goldstein and Tanner, 1980; Seth et al., 1979).

According to Gopalan (1985) "if our objective is to assess true magnitude of growth retardation and under nutrition in a country/community, purpose will be defeated by adopting lower standards which does not reflect full genetic potential for growth and development of children in the country"

Now that there is good evidence from studies from different parts of the world that ethinic differences in growth are minimal the case for the use of a common international reference standard appears to be strong (Habicht et al., 1974).

Limitations in use of local standards

Further more local standards is complicated by the fact that:

- Improvement in nutrition in a country changes the standard itself.
- Time consuming
- Expensive
- Difficult to derive.

The U.S. and U.K. standards were derived from data in population which were no longer subject to drastic change and so most countries continued to use these international reference standards. WHO recommends NCHS standards for use by all the countries (WHO, 1978).

Presentation of growth data

Standards of growth in use employ either the percentile division/the standard deviation.

Percentiles

Refers to the value of measurement of that particular number in a series of hundred, when they are arranged in an ascending order.

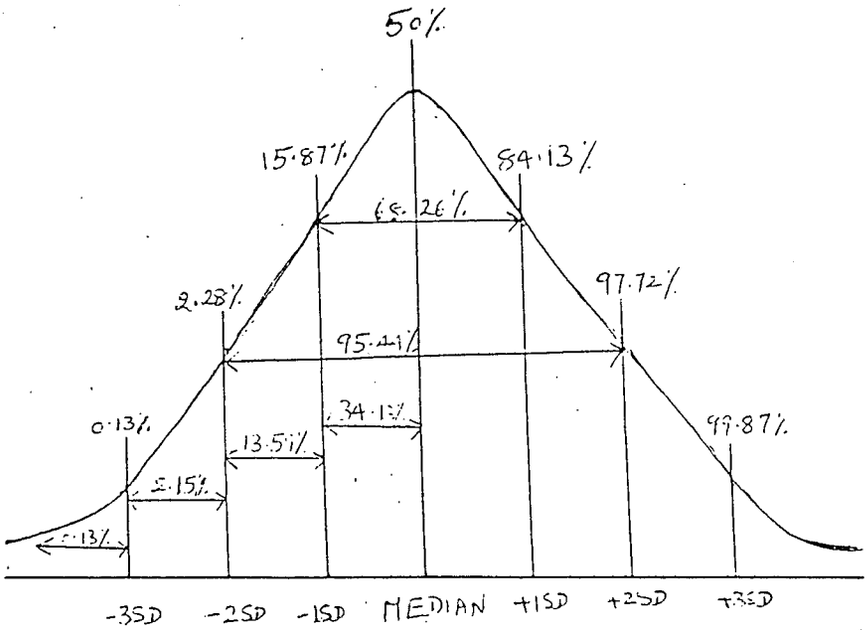
50th percentile - (Median) refers to the 50th value in a series of 100.

3rd percentile indicates that 3% of children are below the value and 97% are above that value.

Common percentiles are 3, 5, 10, 25, 50, 75, 90, 95 and 97th.

Standard deviation

The relationship of percentiles to SD in a normally distributed characteristic.



3rd and 97th percentiles include 94% of the population and coincide with values ±2 SD from the mean.

Conventionally +1-2 SD units are taken as cut offs to determine retardation in growth.

Percentage deviation from the median of standard

To classify children into Nutrition grades or grades of deficit (mild, moderate, severe) by establishing arbitrary cut-off points.

Eg. Gomez classification.

When distributions are highly skewed, the values should be expressed as multiples of the SD of reference population rather than as per cent of the median.

Velocity of growth:

Velocity is the rate of growth per unit time, usually one year and is referred to as annual increment.

It is the difference in the measurements obtained at initial point of time (T_1) and at the end of one year (T_2), in a longitudinal study.

Distance chart are prepared using data collected in cross sectional studies.

Children belonging to different ages are measured at one point of time. Means for different ages are plotted on a graph and are joined by a smoothened curve.

Anthropometric indicators/indices of nutritional status

One of the physiological parameters in children which shows tangible alterations during under-nutrition is growth. Growth retardation is therefore being extensively used to assess extent of malnutrition. The common indicators used are based on weight, height, weight/height and mid arm circumference.

Birth weight: Birth weight is considered to be an index of not only child's nutritional status but also of mothers nutritional status. Birth weight of less than 25 kg is considered as low birth weight (LBW).

Weight for age: The popularly used classification using weight for age for determining the nutritional grades is Gomez classification. This classification is based on percentage deviation from the median of the reference standard.

Using Gomez classification, one can distinguish grades of deficit (mild, moderate, severe) by establishing arbitary cut off points.

Cut off is a value that marks the boundry of acceptability for individual on an item of data.

Gomez classification

Normal : ≥ 90% standard weight for age

Grade I (mild) : 75-89% standard weight for age
Grade II (moderate) : 60-74% standard weight for age
Grade III: (severe) < 60% standard weight for age

Indian academy of pediatrics has suggested a modification of cut off points used for Gomez classification as shown below:

Normal : ≥ 80% standard weight for age
Grade I : 80-70% - standard weight for age
Grade II : 70-60% - standard weight for age
Grade III: 60-50% - standard weight for age
Grade IV : < 50% - standard weight for age

The above classification is being used by JCDS for the growth charts.

Jalliffe classification

Normal : 110-90% - standard weight for age
Grade I : 90-81% - standard weight for age
Grade II : 80-61% - standard weight for age
Grade III: < 60% - standard weight for age

Well come classification

Using the parameter wt/age and also observing the presence/absence of oedema in preschool children Wellcome classified the children as shown below:

% Wt for age	Oedema	
	Present	Absent
80-60%	Kwashiorkar	Under weight
< 60%	Marasmic Kwashiorkar	Marasmus

Height for age classification

Giant : >105% - standard height for age
Normal : 93-105% - standard height for age
Short : 80-93% - standard height for age
Dwarf : < 80% - standard height for age

Waterlow's classification

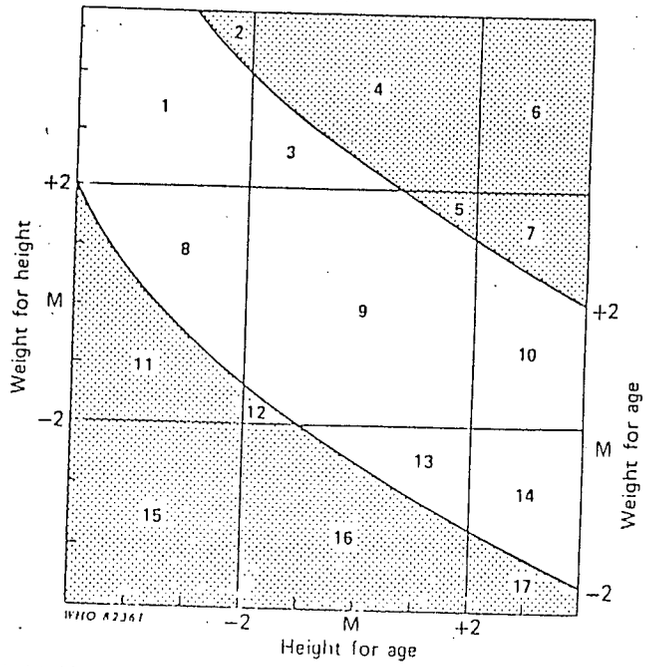
Waterlow classified children into different grades of nutrition as normal, stunted, wasted and stunted and wasted using Ht-for-age as well as Wt-for-Ht parameters. The cut off point used for Ht-for-age is 90% of standard i.e., a child with >90% of standard ht-for-age is considered as normal; and <90% standard Ht-for-age is considered as low.

Similarly for Wt-for-Ht cutoff point used is 80% of standard. The Waterlow's classification is shown below:

Ht-for-age	Wt-for-Ht	Nutrition grade
Low	Low	Current long duration mal nutrition
Low	Normal	Long duration malnutrition
Normal	Low	Short duration malnutrition
Normal	Normal	Normal

Assessing the nutritional status of children using Ht-for-age and weight-for-height, wt-for-age parameters

Relation between the classifications "low", "normal" and "high" for the indicators weight for height, height for age and weight for age with cut-offs at 2 standard deviations above and below the median*



*Calculated from the data for 18-month-old boys in the reference population

DATA ANALYSIS AND INTERPRETATION

Combinations of indicators	Interpretation of nutritional status
11. Normal wt/ht + low wt/age + low ht/age	Normally fed with past history of malnutrition
9. Normal wt/ht + normal wt/age + normal ht/age	Normal
7. Normal wt/ht + high wt/age + high ht/age	Tall, normally nourished
17. Low wt/ht + low wt/age + high ht/age	Currently underfed ++
16. Low wt/ht + low wt/age + normal ht/age	Currently underfed +
14. Low wt/ht + normal wt/age + high ht/age	Currently underfed
2. High wt/ht + high wt/age + low ht/age	Obese ++
1. High wt/ht + normal wt/age + low ht/age	Currently overfed with past history of malnutrition
4. High wt/ht + high wt/age + normal ht/age	Overfed but not necessarily obese

Wt/Ht ratios: are generally termed as body mass indices (BMI)/obesity indices, as those ratios are highly correlated with obesity.

Advantages of Wt/Ht ratios:

(1) It is an age independent parameter.

(2) Wt/Ht can be measured easily. Hence no need for the assessment of the age which is a difficult task in the field.

(3) Is a quick method.

(4) More precise than skin fold thickness measurement.

Hence widely used as indirect measures of obesity.

There are two types of wt/ht ratios.

1. Relative Wt/Ht indice - expressed as Wt of a given person as per cent of average Wt of persons of same Ht.

2. Power type indices - Wt relative to some power function of Ht.

   or

   Ht relative to some power function of Wt.

Power type indices for Wt/Ht ratios

1. Wt/Ht ratio $= \frac{\text{Wt in grams}}{\text{Ht}^2 \text{ (cm)}}$

2. Quetelet's index $= \frac{\text{Wt}}{(\text{ht})^2}$

3. Ponderal index $= \frac{\text{Wt}}{\text{Wt}}$

4. Benn's index $= \frac{\text{Wt}}{(\text{ht})^p}$

5. $\text{Wt/Ht}^2 \times 100$ = < 0.15 indicates PEM

6. Brokas index - Ht in cm - 100 = ideal Wt in kg
   Eg. 152 cm - 100 = 52 kg

7. Wt/Ht % classification (% of standard) - < 80 under nutrition
   80-120 norms
   120-130 over nutrition
   > 130 obese

Weight/height^2 x 100: If the value is < 0.15 - it indicates absence of protein energy malnutrition; > 0.15 - indicates presence of protein energy malnutrition.

Compared to all other wt/ht indices, Quetelet's index showed good co-relation with percentage of body fat estimated by densitometry and skinfold thickness.

Devices to assess nutritional status

* Growth charts ; Bubble chart
* Growth table
* Graphs for wt-for-ht and Ht-for-age based on Waterlow's classification
* Programmable calculator
* Slide rule
* Thinnes chart
* QUAC stick
* Multicoloured Mid upper arm circumference tapes
* Foot Tape measure
* Wasting stunting classifying scale are the few devices developed for rapid assessment of nutritional status of young children.

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Nutritional Anthropometry - Techniques of measurements

K.Mallikharjuna Rao

Anthropometry is one of the methods used in nutritional assessment. Nutritional Anthropometry can be defined as "measurements of the variation of the physical dimensions and the gross composition of the human body at different age levels and degrees of Nutrition".[1]

Growth retardation may be first response of the body towards nutritional deficiencies while appearance of clinical signs may be the final stage. From the public health point of view, identification of sub-clinical forms of malnutrition is very important for planning programmes of nutrition intervention so as to prevent such milder cases going into severe forms with consequent risk of high mortality. Use of anthropometric measurements depends on two factors (1) accurate age assessment (2) appropriate normal values for comparison.

AGE ASSESSMENT

Accurate age assessment in rural areas of India is often difficult since the parents do not keep any documentary evidence of birth. However, since the rural folk are used to the festivals and other local events related mainly to agriculture, age assessment can be done accurately using calender based on local events. The calendar contains the dates on which the various festivals and other events occured for the previous 5-6 years. By finding out the festival during which the child was born and the number of such festivals that occured after the child's birth and

referring to the calender approximate date of birth of a child can be assessed. This, however, is best suited for preschool children.

Anthropometric Standards

Use of body measurements like, weight, height etc. is meaningful only if the actual measurements obtained on an individual are compared with known normal values. These normal values are referred to as growth standards or norms and are obtained by surveying a large number of well-fed, healthy children who are medically and socially well protected. Generally, it is now accepted that the growth potential of well-fed children in all the countries is similar and comparable except in specific communities like pygmies etc. Studies carried out in India have clearly established that atleast until adolelscence the growth pattern of well-to-do Indian children is comparable to that of American children.[2]

Though the ultimate aim of nutritionist should be to prepare and use local standards for different ethnic groups with potentially different patterns of growth, difficulties in preparing local standards are great. Large number of careful measurements have to be made on healthy, well-fed sections of the community whose ages are known and on whom there are no constraints of health or nutrition.

It is often necessary, because of absence of local standards, to use a possibly genetically less appropriate but widely available general and more often International standards.

The observation that well nourished children in developing countries grows in much the same way as their counterparts in the developed world has lent support to the use of a single international standard for all (Graitcher, 1981).[3] The NCHS reference data are now recommended for use by the WHO.[4]

The methods and the measurements employed in anthropometry can vary greatly in number and complexity. Obviously, those chosen will depend on the purpose and objectives of the particular survey or study. The most commonly used body measurements are height, weight, mid upper arm circumference, fat fold at triceps, head circumference and chest circumference.

Measurement of Height

Height is measured using an anthropometric rod. This is usually made up of 4 pieces of metal tube of 2 meters length, which can be dismantled or reassembled. The calibrations are made in centimeters (0 to 20 0 cm) starting from lower end of the rod. Each centimetre is divided into 10 mm.

There is an assembly which can be moved along the rod. This has a window. There is provision to fix a blade on to the moving part, at right angles to the rod. The upper edge of the window in this assembly corresponds to the lower edge of the blade fixed to it. Hence, while taking the height of an individual, the reading should be recorded as seen at the upper edge of the window.

Ask the subject to stand erect on a flat surface (foot wear should be removed) with feet together and hands hung close to sides of the body. The investigator should stand left side of the

child. The rod held in the right hand, should be kept at the back of the subject, such that it touches the heels, buttocks, and back of the head. The chin of the subject should be held by left hand and occipital prominence by right hand. The head should be positioned such that an imaginary line drawn from tragus of the ear to the infra orbital margin (lower border of the eye) is parallel to ground. It is known as Franfurt Hortizontal plane. By holding the head in this position, a gentle pull is applied, taking care that the subject does not lift his heels. Then the moving part of the rod has to be bought down so as to touch the head, taking care that the blade of the rod is in the sagital plane of the body. This movement is repeated thrice and the consistant reading is recorded.

For measuring length of children who cannot stand, wooden or metal "infantometer" should be used.

Weight

Weight is the anthropometric measurement most in use. It is measured with either beam balance or lever actuated platform scales. Salter spring balances are also being extensively used (eg. ICDS). Of late, digital weighing scales are being used for measuring weights. Beam balance and salter weighing scale to weigh only preschool children with an accuracy of 50 to 100 g are available. Lever actuated balances can provide an accuracy of 20 to 100 g. As in the case of any instrument, there is a need to adjust the 'zero error' and check for accuracy with known standard weights before each session of measurements. As far as possible it is advisable to desist from using the bath room

weighing scles which are known for inaccuracy. It is to be kept in mind to allow minimum clothing on the subject before weighing.

Body weight reflects the current nutritional status. It varies greatly with conditions like heavy intake of food just before weighing or intake of fluids, episode of diarrhoea, defecation etc. Hence, it is advisable to record body weights at fixed hours especially for followup examinations.

Mid Upper arm circumference

It is measured with fibre glass tape at the mid point of left upper arm. The mid point is marked on the triceps between acromion of the shoulder girdle and olecranon process of ulna when elbow is extended and arm is lying loose by the side of the body. Care should be taken to see that the tape runs firmly around the arm, not too loose or not too tight. The tape should not be eliptical but kept horizontal. The reading on the tape corresponding to'0' mark is the arm circumference.

Fat Fold at triceps

The measurement of fat fold thickness provides the values indicating fat or adipose tissue which is a measure of energy deposit or reserve energy. This measurement is obtained at the mid point of the left upper arm where arm circumference is measured. The skin fold is pickedup 1 cm above the mid point and the caliper should be applied at the mid point, with the arm hanging in a relaxed position.

The fat fold calipers designed to exert uniform pressure of 10 g/sq.mm at the time of measurement. Various makes of calipers are available which measure the fat fold in millimeters with an accuracy of 1 mm.

Head and chest circumference

These measurements are usually included only for preschool children. At the time of birth, head circumference (HC) is greater than chest circumference (CC). By the age of 6 months, usually the chest circumfernce over take the head circumference. In undernourished, this process of overtaking gets delayed to as long as 1.5 to 2 years. Hence these measurements are useful to know the nutritional status of young children.

For the head circumference, the maximum circumference including the occiput, glabella, parietal region just above the ears is measured.

For the chest circumference, the tape should pass around the chest over both the nipples in front and just below the shoulder blades at the back. Care is taken to see that there is no clothing or any other material between the tape and the body.

Need for Standardization

The techniques employed, must be carefully carried out, standardized and thoroughly understood by all team members and given adequate preliminary practical testing to ensure uniformity of results. Particular accuracy of both techniques and equipment is important in growth studies.

Variation in measurements occuring between two consecutive measurements obtained on a subject by an investigator is termed as intra-individual variation. On training it is expected that two consecutive measurements on the same subject, under similar conditions when done by an investigator should be similar or within the permissible limits of error. The inter-individual variation denotes the error in measurements when different investigators measure different reading on the same subject for a measurement. For uniformity of reporting when more than one investigator is collecting data and for the purpose of comparison of various sets of data it is expected that all the investigators will employ same methodology and report values within permissible limits of error when they undertake the exercise of measurement. Hence, standardization is mandatory before Commissioning of any evaluation survey. The measurements of a senior person who is already standardized is considered as a reference for the purpose of comparison.

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4. Hamill,P.V.V. et al (1979). Physical growth : National Centre for Health Statistics percentiles. Am.J.Clin.Nutr. 32 : 607-629.

Addresses for Anthropometric equipment.

1) Digital weighing scale. 1) ATCO Products Ltd.

Rs 1800/-

19A, Shriram Industrial estate
13, Kalrak Road,
Wadala, Bombay - 400031

2) ATCO Products Ltd.
Topaz Complex, 5th floor
unit 6, 6/3/713
Amrutha Hills, Panjagutta.
Hyd - 500 482.
ph. 040 - 311754.

2) Anthropometer Rod
Infantometer
~~Steinf~~ Skin fold Calipers.

Military Science House.
First floor
2/35, Roop Nagar
Delhi - 110007.

ph: 2923204.

50th percentile values of Ht., Wt., AC. FFT. HC of Well-to-do Hyderabad Boys and Girls

Boys						Age	Girls					
Ht.	Wt.	AC	FFT	HC	CC	(Months)	Ht.	Wt.	AC	FFT	HC	CC
73.8	9.3	15.1	9.1	46.6	45.3	12	72.8	8.7	14.5	8.9	45.4	44.7
77.2	9.9	15.2	9.0	47.0	46.1	15	75.8	9.2	14.7	8.8	45.7	45.5
80.2	10.5	15.3	8.6	47.4	46.9	18	78.5	9.8	14.8	8.8	46.1	46.0
83.0	11.2	15.4	8.4	47.7	47.7	21	81.3	10.1	14.9	8.7	46.4	46.6
85.5	11.6	15.5	8.2	48.0	48.4	24	83.8	10.5	15.1	8.6	46.7	47.2
87.8	12.0	15.6	8.2	48.3	49.1	27	86.0	10.9	15.2	8.7	47.0	47.8
90.0	12.5	15.7	8.4	48.6	49.7	30	88.5	11.3	15.4	8.9	47.0	48.4
92.0	13.0	15.8	8.6	48.8	50.3	33	90.8	11.8	15.5	9.1	47.6	49.0
94.0	13.5	15.9	8.7	49.1	50.9	36	92.8	12.3	15.6	9.3	47.8	49.5
96.0	14.0	16.0	8.8	50.3	51.4	39	94.8	12.8	15.7	9.6	48.1	50.0
98.5	14.5	16.0	8.8	50.5	51.9	42	97.0	13.3	15.9	9.9	48.3	50.5
99.8	15.1	16.1	8.7	50.6	52.2	45	98.5	13.9	16.0	10.2	48.5	50.9
101.5	15.7	16.2	8.6	50.7	52.6	48	100.3	14.5	16.1	10.4	48.6	51.3
103.3	16.3	16.2	8.4	50.8	52.9	51	102.0	15.0	16.2	10.4	48.7	51.7
104.8	17.0	16.3	8.1	50.9	53.1	54	104.0	15.7	16.4	10.1	48.8	52.1
106.5	17.6	16.4	7.7	51.0	53.3	57	105.5	16.3	16.5	9.5	48.9	52.3
108.0	18.3	16.4	7.3	51.0	53.4	60	108.0	16.9	16.6	8.8	48.9	52.6

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50th percentile values of Ht., Wt., AC. and FFT. of well-to-do Indian Adolescents and Young Adults

Age	Ht.	Wt.	AC.	FFT.
17	169.8	56.0	24.3	8.5
18	171.5	57.5	25.0	10.6
19	171.8	58.8	25.5	10.3
20	172.0	59.5	25.5	9.6
21	172.0	59.5	26.0	9.3
22	172.0	59.5	26.2	9.7

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50th percentile values of height,* weight,* A.C.** and FFT** of school age children

Boys					Girls			
Ht.	Wt.	AC.	FFT	Age	Ht.	Wt.	AC.	FFT
112.4	19.2	16.0	6.5	5+	112.5	18.6	16.1	9.0
118.8	21.9	16.5	7.7	6+	117.8	20.5	16.5	9.7
123.2	24.3	17.2	8.1	7+	123.2	23.8	17.3	9.9
127.9	26.1	17.4	8.0	8+	127.2	26.0	17.5	10.1
133.3	29.2	18.1	8.3	9+	132.5	29.0	18.3	10.3
138.0	31.0	18.5	8.5	10+	138.2	32.6	19.1	10.6
142.7	34.0	19.2	8.8	11+	145.1	36.3	19.5	10.2
148.4	37.8	20.1	8.9	12+	151.5	42.5	20.8	12.1
155.0	42.4	20.8	8.5	13+	153.8	43.9	21.1	12.7
162.6	47.3	22.0	8.3	14+	154.5	45.0	21.8	12.8
165.5	51.1	23.0	8.1	15+	155.8	47.3	22.6	13.2
168.9	54.8	24.0	8.0	16+	155.8	49.0	23.0	14.1

* Vijayaraghavan, K., Darshan Singh and M.C.Swaminathan (1971) Height & weight of well-nourished Indian School children, Indian J.Med.Res. 59, 648-

** Vijayaraghavan, K., Darshan Singh, M.C.Swaminathan (1974). Arm circumference and Fat at Triceps in well-nourished Indian school children. Indian J.Med.Res. 62, 994-1001.

ANTHROPOMETRY FOR THE ASSESSMENT OF THE NUTRITIONAL STATUS OF ADULTS AND PREGNANT AND LACTATING WOMEN

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Pregnant and lactating women are the most important segment of the population. The health and nutritional status of the children and adolescents are dependent on them. Adults are the major working group of the population. Work efficiency and health of adults are well associated with nutrient adequacies in the dietaries and maintenance of body composition. Anthropometry has been found as a good measure of the nutritional status. The variations in height and body composition of adults are found due to variations in their nutritional status during childhood and their maternal health and nutritional status. Body composition of women is found varied with the age, duration of lactation and trimesters of pregnancy. It has been shown that healthy children are with healthy mothers.

Anthropometric measurements and indices are useful for assessing the present and the past nutritional status of the population. Many anthropometric measurements we have, of which basic are height and weight. Anthropometric indices are the combinations of measurements.

Of the many indices, Weight/Height^2 (or BMI), Weight for height (%) and Broka's index are suitable for the nutritional status of adults. In the nutrition field, low height and low weight or index relative to reference data have been used as indicators of undernutrition for individuals and groups, similarly elevated body weight for given height and thickness of subcutaneous fat have become common indicators of overnutrition

or obesity.

The major components of body weight are water, protein and minerals. Their composition vary by forms of undernutrition and overnutrition, by age and sex and by types of life styles and exposure to infections.

Anthropometric indices change rapidly during pregnancy. Gain in weight during pregnancy, gestational age, rate of weight gain, height and prepregnancy BMI (or Weight for height or Broka's index) are the good indicators of nutrition in pregnant women. Prepregnancy weight or BMI or Broka's index is an indiicator of maternal weight gain and a predictor of fetal growth. These are found helpful for studying the interrelationship between nutrition and reproduction. Total weight gain during pregnancy reflects both fetal weight and maternal tissue gain.

The nutritional status of lactating women depends on their past nutritional status, weight gain in pregnancy, immediate postpartum weight loss, duration and intensity of lactation, dietary intake and physical activity. Weight loss during lactation stabilises during 4-6 months of lactation. This depends on weight gain during pregnancy, energy intake and pattern of breast feeding. In well nourished lactating women, changes in weight are generally minor and gradual. Weight losses are highest in the first three months of lactation and are greater in women who breast fed exclusively. Among undernourished women gestational weight gain and postpartum weight loss are lower than in well nourished women.

Milk composition of women in developing countries vary a lot and lower nutrient levels have usually been found in

undernourished women. Total energy cost of producing milk is estimated to be 2930 KJ/day during the first six months of lactation and 2090 KJ/day during the next 18 months of lactation. Nutritional status and lactation performance are closely associated. BMI/Broka's index/Weight for height decline steadily throughout the first six months of lactation. The cut-off for BMI of 2.0 at one month of lactation and 1.85 during 2-6 months of lactation are found good as a cut-off for identification of women at risk.

Women with height below 150cm are found to have high risk of pregnancy wastage and low birth weight babies.

In all adults, the Broka's index and BMI are found useful for the assessment of the forms of malnutrition. Prevalence rates of overweight and coranary heart disease are positively associated. A ten percent reduction in weight is associated with a twenty percent reduction in the risk of coranary heart.

A high abdominal adiposity is associated with stroke and abdominal cancer. Overweight is also closely associated with high prevalence of hypertension, gall bladder disease, non-insulin dependent diabetes, development of gallstones, osteoarthritis and post-minopausal breast cancer. Accident pronness and prevalence of infertility are frequently observed with overweight and obesity. Mortality rates are higher in both undernourished and overweight groups. Anthropometric indices are useful for (i) improving the nutritional status, (ii) for providing nutritional intervention for the pregnant and lactating women and (iii) to minimise mortality and complications during pregnancy.

Classifications of malnutrition generated and found useful with anthropometry will be presented and discussed.

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Body Mass Index (BMI) and its application to evaluate nutritional status

- Dr. Uma Reddy

Body mass index is express as

$$BMI = \frac{Wt\ (Kg)}{Ht^2(m)}$$

This is a better index to measure the ideal body weight, as it is independent of age and sex in adulthood.

Interpretation suggested

<18.5 under weight

BMI - normal

20-25 - above normal

25-30 - over weight

>30 - Obesity

Relationship between BMI and Nutritional Status

Body mass index and energy requirement

Two significant developments over the last decade have influenced an understanding of energy requirements of humans and their implications in arriving at the number of individuals in population groups. The first recommendation is the FAO/WHO/UNU Expert Consultation on Energy and Protein requirements (1985) based on as energy expenditure and b) use of basal metabolic rate factorial approach for the assessment of total energy expenditure of individuals. The second advance, more recently made is one based on anthropometric measure more specifically the use of body mass index could be a simple, relisable and easily obtainable objective criterian for both the definition and as an estimate of under nutrition of chronic energy deficiency (CED) in adults (James et al 1988). (Shetty et al. 1994)

Body mass index - a measure the nutritional status

One of the recent methods suggested for assessing the chronic energy deficiency (CED) state of individuals is based on BMI. This method is relatively simple, easy to measure and does not suffer from estimation errors encountered in energy intake methods and energy expenditure methods (James et al 1988)

Naidu and Rao (1994) have analysed the relationship of anthropometric data (BMI) of NNMB with nutritional status of India population. Results of the study indicated the following.

Anthropometry & BMI Relationship

Though the men were taller and heavier than their women Counterparts, there was no significant differences in the average BMI values of men of women. Since there was no significant gender differences, a common BMI criterion of classification can be done for studying the relationship with other parameters.

Relationship of anthroprmetric data & BMI

Sex	n	Ht(cm)	SD	Wt(kg)	SD	BMI
M	9447	162.6	6.79	50.2	7.95	18.9
F	11914	150.4	5.95	43.1	7.41	19.0

Adult BMI and Child nutrition

Households with lower BMI value had lower proportion of under nounshed children. The households with BMI had 85% of the children under nowrished, where as households with BMI values 18.5 had 62% of malnourished children. This established that adult BMI and Children's nutritional status the associated (X^2 P)

BMI and Socio - Economic status

The relationships between BMI and Socio-economic variable, showed that the land less labourers with lowest monthly income had lower values of BMI with those who had better occupation and higher income.

Kennedy and Garcia (1994) assessed linkages below body mass index and morbidity in adults based on evidences from four developing countries. This study showed a small but statistically significant effect of low BMI on proneness for morbidity in Pakistan and Kenya but not in Philippines and Ghana.

BMI and Mortality

Relation between BMI and mortality based on the NNMB data (NIN Report 1989-90) are dipicted in the following graph. It clearly shows that mortality steadily increased from 12/1000 population with normal BMI class with BMI 18.5 to 33/100 population in grade III CED.

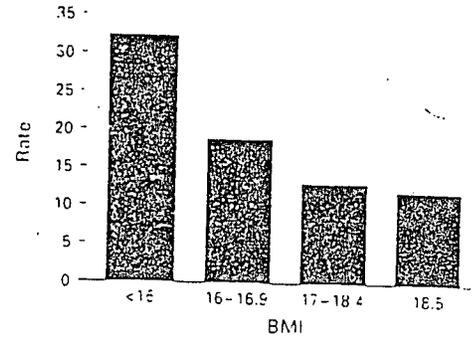


Fig. 3. Adult mortality rate by BMI (men) (Deaths per 1000 per year.)

The BMI index proved to be useful in ascertaining the change in nutritional status over a period of time. An absence of dietary survey results, adult BMI can be reasonably good substitute not only for assessing energy status of the house hold but also for evaluating the nutriture of the pre-school child in that family adult BMI was also found to be useful index for assessing socio-economic level of households interms of occupation and income status. This because adult BMI is closly associated with weight which is responsive variable to energy balance.

Maternal body mass index and pregnancy outcome

Alter et al (1994) have studied relationship between BMI pregnancy outcome in 4 countries in 9 nutrition Collaborative Research Support Programme. This study indicated that the weight gain during pregnancy was high in women who had low BMI, and these who have high BMI (pre-pregnancy) gain less weight during pregnancy.

BMI and Childhood malnutrition

The relationship between BMI and Childhood malnutrition was studied based on the results of a longitudinal study of 23 years conducted at NIN. Results of the study is shown in the following graph. The incidence of malnutrition among low BMI group compared to normal BMI group.

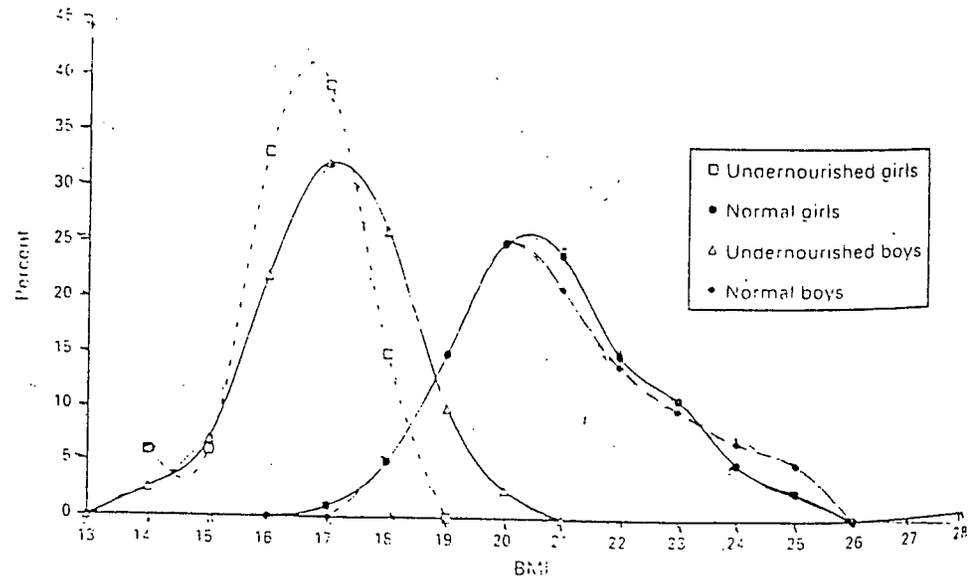


Fig. 4. BMI distributions of adults who were malnourished as 5-year-old children compared with adults who were normal-weight children.

BMI distribution of adults who were malnourished as 5 year old children compared to adults who were normal weight children.

Analysis of the data indicated that a lower BMI at Conception predicts higher weight gain during pregnancy, as well as more fat gain on the extremites and trunk. In contrast, fatter women actually lost fat from the triceps site throughout pregnancy.

It is well established that women with higher BMI gave birth to heavier infants. The correlation between maternal BMI in the first trimester and birth weight was 0.29 (P) in Mexico and 0.59 (P) in Kenya. All the low birth weight infants in kenya were born to women with a BMI 22).

BMI and Energy Consumption

Analysis of energy intake data and the BMI of adults showed a consistant relationship. Higher energy intakes were seen in households with the better BMI status.

Another study conducted by de vasconcellos (1974) in Brazil also showed that the probability of a thin person being in a family of low energy adequacy in grater than being in a family of high energy adequacy. A good correspondence between energy intake and BMI was observed. BMI values correlated well with energy intake, income, expenditure on food and occupation.

Adult BMJ and Morbidity

Same study conducted in Brazil indicated that cigarette smokers had a higher probability of being thinner than non smokers. They also found correlation between BMI and the number of days spent in bed due to various illnesses. The graph shown below indicate that thin as well as obese people have a greater probability of being in bed than normal individuals.

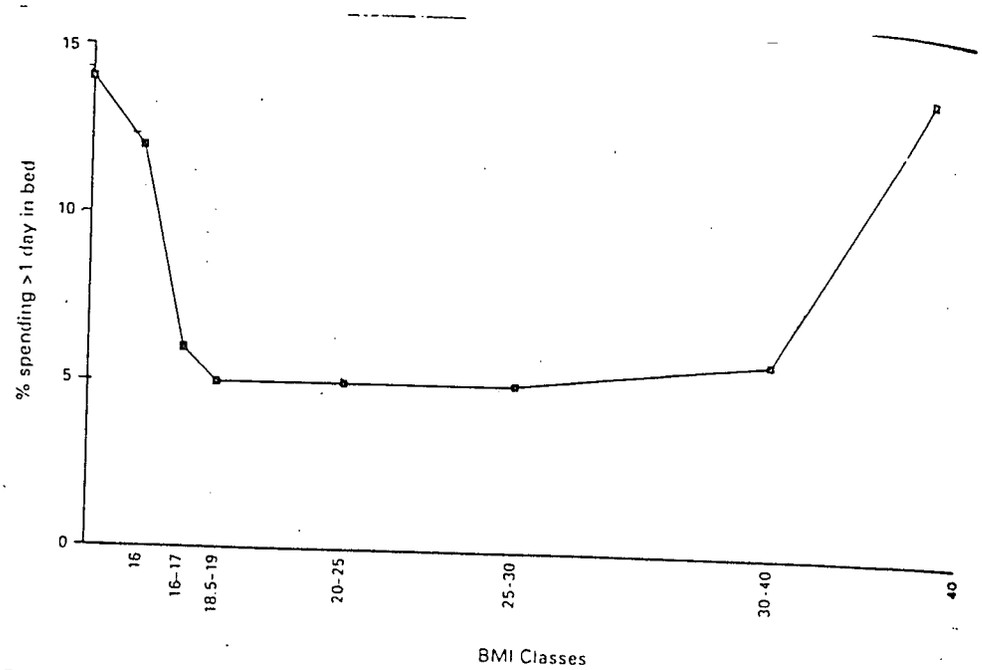
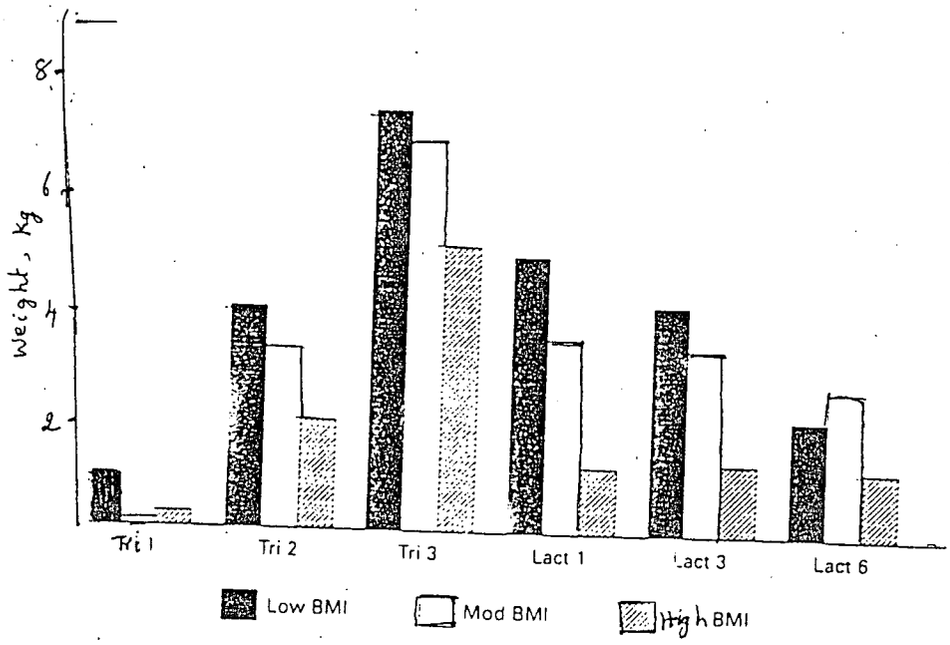


Fig. 6. The proportion of adults with different BMIs who had spent one or more days in bed over previous 2 weeks.



BMI and weight changes in women

Women with a lower BMI at Conception gain more weight and fat in pregnancy and last more weight and fat during location. In contrast, fatter women lose fat in pregnancy and regain it in lactation. These things tend to have implications to interpret energy adequacy to assess the energy adequacy.

BMI and Lacation performance

Lactation imposes a significant additional energy stress on women. Prentice et al (1994) analysed world literature to test the relation between BMI and lactation performance. They have reported that human lactation performance is extremely robust and that BMI does not provide a useful indicator of function.

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METHODS OF ASSESSING BODY COMPOSITION

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Methods for measuring specific components of body compostion are necessary to assess the effects of nutritional deprivation and intervention on body composition. Such information is essential for establishing the appropriate prognosis and treatment of hospital patients. The methods currently use a two- or four-compartment model for body composition. The two-compartment model assumes that the total body mass is composed of two major chemical compartments: body fat and the fat-free mass, whereas the four-compartment model divides the body into four chemical groups: water, protein, minerals, and fat, all of which can now be determined *in vivo*.

The amount of body fat is highly variable and the fat-free mass consists of three components: total body protein, total body water, and bone minerals. The density of the fat-free mass depends on the proportions in which these three components occur. In healthy persons, the fat-free mass has a relartively constant composition, with a water content of 72% to 74%, a potassium contnet of about 60 to 70 mmol/kg in men and 50 to 60 mmol/kg in women, and a protein content of about 20% (Garrow, 1982). By measuring total body water, total body potassium, total body nitrogen, or body density, the proportion of the body composed of fat and the proportion composed of fat-free mass can be estimated.

Selection of a method to measure body composition depends on its precision and accuracy, the objective of the study, cost, convenience to the subject, equipment and technical expertise required, and the health of the subject (Lukaski, 1987). In normal healthy individuals, all the body components except fat occur in relatively fixed proportions. In malnourished individuals and subjects with metabolic disturbances, the relative proportions of the body components may be altered. For instance, losses of protein and fat may occur, often in association with the rapid accumulation of water. Changes such as these invalidate the determination of fat and the fat-free mass in the two-compartment model. Therefore, in such circumstances, the four-compartment model must be used.

It is not possible to validate measurements of body composition with an absolute standard based on results of direct chemical analysis on the same subject.

Instead, relative validity is estimated by comparing results with those obtained on the same subject using alternative, indirect methods. The following sections describe the individual procedures used to assess body composition and the assumptions of these methods.

Chemical analysis of cadavers

Studies of body composition by direct chemical analysis of human cadavers are limited. Most of the cadavers were analyzed between 1945 and 1968 and were adults of varying ages who had died as a result of illness; hence the values obtained may not be representative of an average healthy adult. The fat-free tissues of the cadavers were of a relatively constant composition, containing about 72% water, about 20% protein, and about 69 mmol/kg potassium. In contrast, the amount of fat was very variable, ranging from 4.3% to 27.9% of body weight in the six cadavers.

Total body potassium using ^{40}K

Potassium occurs almost exclusively as an intracellular cation, primarily in the muscle and viscera. Negligible amounts occur in extracellular fluid, bone, or other noncellular sites. Measurement of total body potassium is therefore used as an index of the fat-free mass in healthy subject, on the assumption that the fat-free mass has a constant amount of potassium.

A constant fraction (0.012%) of potassium exists in the body as the isotope ^{40}K (half-life = 1.3×10^9 years). The latter emits a high-energy gamma ray of 1.46 MeV, allowing the amount of potassium in the body to be measured. Once total body potassium has been determined from the ^{40}K measurements, the fat-free mass. Thus the determination of the fat-free mass and hence the total body fat and percentage body fat from total body potassium involves:

* Measurement of ^{40}K radiation from the subject using a whole body counter;
* Calculation of total body potassium (g) from ^{40}K data;
* Conversion of t otal body potassium (g) to mmol potassium:

$$\text{mmol K} = \frac{\text{Total body potassium (g)}}{\text{Atomic weight of potassium (39.098)}}$$

* Calculation of the fat-free mass (kg) from total body potassium assuming that the average potassium content of the fat-free mass = 69.4 mmol/kg (or an alternative factor);

* Calculation of total body fat (kg);

Total body fat(kg) = Body weight (kg) - fat-free mass (kg)

* Calculation of percentage body fat.

$$\% \text{ Body fat} = \frac{\text{Total body fat (kg)} \times 100}{\text{Body weight (kg)}}$$

The 1.46 MeV gamma ray of ^{40}K is counted with a whole body gamma spectrometer using either liquid/plastic scintillation counters or sodium iodide detectors. The latter are preferred because they have a good energy resolution and a low background rate. The isotope occurs in low concentrations so that the background counts from external radiation (cosmic rays and local sources of ionizing radiation) are usually large relative to the ^{40}K counts. Hence the whole body counter must be shielded from the background radiation with lead or steel shielding. The long counting times, necessary because of the low ^{40}K counts, may be a problem for ill patients. Calibration of the whole body counter is a major difficulty with this method because the ^{40}K count detected by the whole body counter is a function of both total body potassium and the geometric configuration of the subject. As a result, the whole body counter must be calibrated to allow for differences in the body build of the subjects being measured.

Once the fat-free mass is determined, total body fat can be derived indirectly, as the difference between body weight and the fat-free mass. This indirect approach should not be used to derive total body fat for patients with a wasting disease such as cancer (Cohn *et al.*, 1981a), because total body potassium measurements are low in these patients as a result of loss of muscle mass. Hence, total body fat derived indirectly as the difference between body weight and fat-free mass will always be overestimated in these patients.

Total body water using isotope dilution technique

Body fat contains no water. Instead, all the body water is present in the fat-free mass, which is assumed to contain 73.2% water on average. Hence, by measuring total body water (TBW), the fat-free mass can be estimated:

$$\text{Fat-free mass (kg)} = \frac{\text{Total body fat (kg)} \times 100}{\text{Body weight (kg)}}$$

Total body water can be measured in both healthy and diseased persons using an isotope dilution technique. Deurterium (^{2}H) and tritium (^{3}H) and the stable isotope of oxygen (^{18}O) can be used. Standardized conditions are necessary to measure total body water because fluid and/or food intake and exercise can all affect total body water. As a result, the measurements should be taken in the morning, after an overnight fast, with restriction of fluid intake, and after the bladder has been emptied. A tracer dose of sterile water labelled with an accurately weighed amount of ^{2}H, ^{3}H, or ^{18}O is administered either orally or intravenously to the subject and allowed to equilibrate. No food or water is permitted during equilibration, which may take two to six hours, depending on the isotope used, the sample form, and the health condition of the patient. Longer equilibration periods are necessary if urine samples are used, and for obese patients, or for those with edema, ascites, and shock. At the end of the equilibration period, a sample of serum, saliva, or urine is collected and total body water is calculated from the dilution observed. Saliva samples are preferable in field studies. Alternatively, if ^{18}O is used, breath samples can be collected and carbon dioxide analyzed for ^{18}O. Table 14.2 demonstrates the relatively small variations in total body water when calculated from isotopic enrichments of different physiological fluids and at different times postdose (Schoeller *et al.*, 1985).

The calculation of total body water is based on the dilution principle (i.e., the extent to which the isotopic dose is diluted by the total body fluid).

$$\text{Total body water} = \frac{V_1 C_1}{C_2}$$

where V_1 = volume of dose; C_1 = concentration of administered isotope; and C_2 = concentration of isotope in serum/urine/breath sample. In the case of serum and

breath samples, a correction may be necessary for urinary loss of the tracer.

Substances or tracers used to measure total body water should:

* be present only in body water;
* equilibrate rapidly and thoroughly with the body water;
* not be metabolized or excreted;
* be nontoxic in the amounts used; and
* not alter normal physiological processes or homeostatic mechanisms (Pinson, 1952).

Selection of the isotopic tracer depends on several factors. Tritium (^{3}H) is easy to measure by scintillation counting but involves radiation to the subject, making the technique unsuitable for children and women of childbearing age, or when repeated measurements over a short time period are necessary. The nonradioactive isotopes ^{2}H and ^{18}O must be measured by mass spectrometry. Sample preparation for ^{2}H is time consuming and tedious because the water sample must be converted to hydrogen gas. In constrast, ^{18}O can be readily analyzed, because the carbon dioxide expired by the subject is in isotopic equilibrium with body water. Use of ^{2}H and ^{3}H produces an overestimate of total body water because the hydrogen label exchanges with some nonwater hydrogen in the body. The stable isotope of oxygen (^{18}O) appears to be the isotope tracer of choice, but its use may be limited by the high cost and need for a mass spectrometer for the analysis.

The major limitations of the total body water method for estimating fat-free mass are the assumptions that the fat-free mass of an adult contains a constant percentage of water and that the total body water content is independent of the fat content of the body. Chemical analysis of human cadavers has shown that the actual total body water content of the fat-free mass body compartment can vary from 67% to 77%. None of these cadavers was of a normal, healthy person, and the degree to which their illnesses may have affected total body water is unknown. Furthermore, the water content of the fat-free tissue has been shown to be higher in obese and pregnant subjects. Consequently , if the usual equation is applied in obese and pregnant individuals, fat-free mass will be over-

estimated, and body fat will be underestimated. In pregnant women, for example, the estimate of fat mass may be underestimated by as much as 1.0-2.0 kg (van Raaij *et al.*, 1988). New equations have been developed for estimating body fat mass from total body water, which will result in more valid estimates of maternal body fat mass during pregnancy. These equations are shown in Table 14.3. Likewise, for patients with wasting disease, the hydration constant is also increased because of loss of body tissue and the accumulation of extracellular fluid, so that estimate of fat will be too low. Hence, measurement of total body water to estimate total body fat indirectly is not an appropriate method for obese persons and patients with wasting disease.

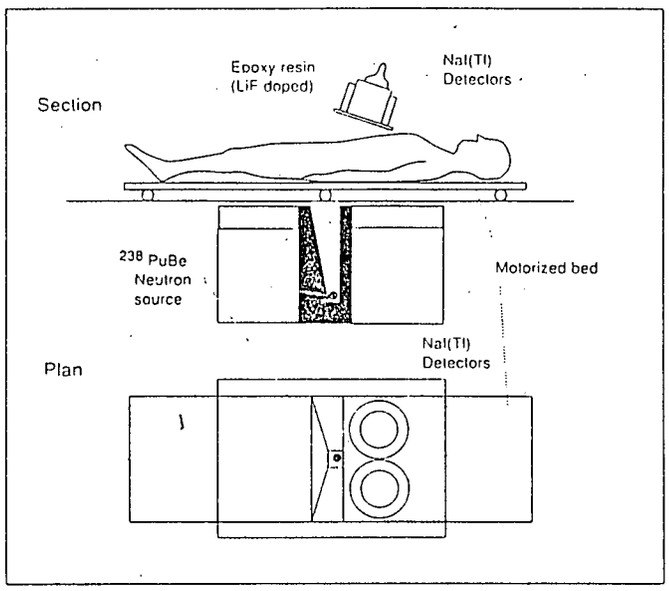

Fig. 1: *In vivo* prompt gamma neutron activation.

Total body nitrogen

Measurement of the nitrogen content of the body gives a measure of total body protein because the mass of nitrogen bears a fixed ratio to the mass of protein (1 g N : 6.25 g protein). A total-body neutron activation system has been developed to measure *in vivo* total body nitrogen. This technique is based on the conversion of a proportion of ^{14}N to an excited state of ^{15}N by bombarding the patient, in a supine position, with a low neutron flux from $^{238}PuBe$ sources or from a cyclotron or neutron generator. The resultant excited ^{15}N decays almost immediately to its ground state, emitting gamma rays at 10.83 MeV which are counted by sodium iodide detectors in a whole body counter. The detected gamma ray counts are then proportional to the absolute mass of total body nitrogen.

water. Residual air in the lungs can be measured while the subject is in the tank or separately, using nitrogen washout, helium dilution, or oxygen dilution. The residual air volume is then subtracted from the body volume;

* the volume of the air trapped in the gastrointestinal tract also contributes to the amount of water displaced. This volume is never measured, and is often taken as 100 mL. The intra-subject variability in gastrointestinal gas volume, however, can be quite large (0 to 500 mL in adults), reducing the precision of the method.

The underwater weighing method can give very reproducible results for body density provided that the examiners and the subjects are well trained. The underwater weighing method is not suitable for children younger than eight years, the elderly, obese, or unhealthy persons.

Total body fat is derived from percentage body fat by multiplying body weight by percentage fat:

$$\text{Total body fat (kg)} = \frac{\text{Body weight (kg)} \times \text{\% body fat}}{100}$$

The fat-free mass can then be calculated by subtracting the total body fat from the body weight:

$$\text{Fat-free mass (kg)} = \text{Body weight (kg)} - \text{total body fat (kg)}$$

Ultrasound or Ultra sonography

In the ultrasound technique, high-frequency sound waves emitted from an ultrasound source/meter penetrate the skin surface and pass through the adipose tissue until they reach the muscle tissue. At the adipose-muscle tissue interface, a proportion of the sound waves are reflected back as echoes that return to the ultrasound meter.

To use this technique, the measurement site is marked with a water-soluble transmission gel which provides acoustic contact without depression of the dermal surface. The high-resolution ultrasound source/meter is positioned so that the ultrasonic beam is perpendicular to the tissue inter-faces at the marked site. A transducer receives the echoes and translates them into depth readings viewed on an oscilloscope screen. Subcutaneous fat thicknesses of 100mm or more can be measured and

The accuracy of prompt gamma neutron activation for measuring total body nitrogen has been validated by comparing total body nitrogen in two human cadavers with results obtained on the same cadavers by direct chemical analysis of nitrogen. Close agreement between the two techniques was found. This study also confirmed the use of the ratio 6.25 for the relationship between total body protein and total body nitrogen.

Determination of body density by underwater weighing

The most widely used method of directly measuring whole body density is the determination of body volume according to Archimedes' principle, which allows the volume of an object submerged in water to be calculated from the apparent loss in weight. The percentage of body fat can then be calculated from the measured whole-body density using one of the empirical equations describing the relationship between fat content and body density.

To measure body density, the volume of the subject is first determined. This is done by weighing the subject first in air and then when completely submerged in water in a large tank. The subject is instructed to squeeze out any air bubbles trapped inside the bathing suit, and to expel as much air as possible from the lungs before immersion. The underwater weight is recorded at the end of the forced expiration. Three readings are usually taken and the heaviest recorded corresponding to the most complete expiration. The body volume is then calculated from the apparent loss of weight in water (i.e. the difference between the weight of the person in air and his/her corresponding weight in water). Once total body mass and body volume have been determined, body density can be readily calculated, on the basis that density is mass per unit volume and the density of water is 1000 kg/m^3 at 4^{o}C.

$$\text{Body density} = \frac{\text{Body weight in air (kg)}}{\text{Volume of water displaced at } 4^{o}\text{C (1)} \; [= \text{apparent loss in weight (kg)}]}$$

Three corrections must be applied:

* Underwater weighing is usually performed in water at 30^{o}C instead of 4^{o}C. At this temperature, 1 m^3 of water weighs 995.7 kg instead of 1000 kg, and a water temperature correction factor must be applied;

* air trapped in the lungs also contributes to the amount of water displaced by the subject under

density interfaces detected with an accuracy of 1 mm. The tissue is not compressed, eliminating errors associated with variations in compressibility of skinfolds.

The ultrasound technique can also be used to measure the thickness of muscle tissue as well as subcutaneous fat, enabling changes in body composition of hospital patients receiving nutritional support to be monitored.

Total body electrical conductivity

The total body electrical conductivity (TOBEC) method is based on the change in electrical conductivity when the subject is placed in an electromagnetic field. The technique depends upon the differences in electrical conductivity and dielectric properties of the fat-free mass and fat of the body. For example, the fat-free mass, composed largely of electrolyte-containing water, will readily conduct an applied electric current, whereas fat is a poor conductor.

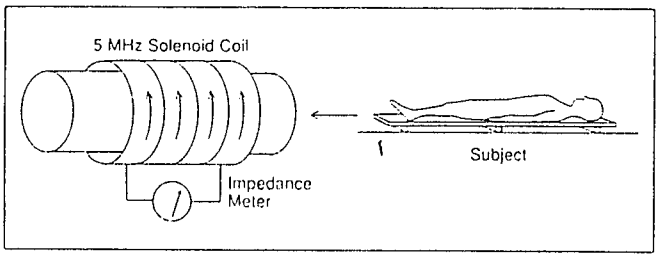

Fig.2: Measurement of body composition by total body electrical conductivity.

For the measurement, the subject lies supine on a stretcher in a long uniform solenoid coil through which a 5 MHz current is passed for a few seconds (Figure 2). This induces an electromagnetic field in the space enclosed by the coil, which, in turn, induces a current in the subject, the magnitude depending on the conductivity of the subject. A secondary magnetic field is produced, which is then measured. A second measurement is taken when the coil is empty; the difference represents the measurement.

The method is simple, safe, and fast and can be used on individuals who cannot be weighed underwater. The instrument, however, is expensive.

Bioelectrical impedance

The bioelectrical impedance (BEI) method also depends upon the differences in electrical conductivity of fat-free mass and fat. The technique measures the

impedance of a weak electrical current ($800_{u}A$; 50 KHz) passed between the right ankle and the right wrist of an individual. The impedance is proportional to the length of the conductor--a distance which is usually a function of the height of the subject--and indirectly proportional to the cross-sectional area; equivalently, the impedance is proportional to the square of the length of the conductor/subject, divided by its volume.

Subjects should avoid alcohol and vigorous exercise for twenty-four to forty-eight hours before testing, so that body fluids are not perturbed prior to the measurements. The measurements are taken on subjects approximately two hours after eating, and within thirty minutes of voiding. Subjects lie clothed, but without shoes and socks, in the supine position on a stretcher, with limbs not touching the body. Two current electrodes are placed on the dorsal surfaces of the right hand and foot, at the distal metacarpals and metatarsals respectively. Two detector electrodes are placed at the right pisiform prominence of the wrist and between the medial and lateral malleoli at the right ankle. A thin layer of electrode gel is applied to each electrode before it is placed on the skin. The resistive component of body impedance between the right wrist and right ankle is then measured to the nearest ohm. The lowest resistance (R) value for an individual is used to calculate the co nductivity (ht^2/R), and hence to predict the fat-free mass. Bioelectrical impedance is safe and convenient, and the equipment is portable and relatively inexpensive. In over-hydration for example, resistance measurements will be higher, but lower in dehydration. Predictive equations for assessing both total body water and total body fat from BEI have been developed, using total body water measured by isotope dilution and total body fat by underwater weighing as the reference methods.

Computerized tomography

Computerized tomography (CT) is based on the relationship between the degree of attenuation of an X-ray beam and the density of the tissues through which the beam has passed. From this relationship, a two-dimensional radiographic image of the underlying anatomy of the scan area can be constructed.

The CT scanner is made up to two components: a collimated X-ray source and detectors, and a computer which processes the scan data and produces an X-ray image. The subject lies on a movable platform within the scanner gantry. The designated area to be scanned is a plane through the middle of the central aperture of the gantry,

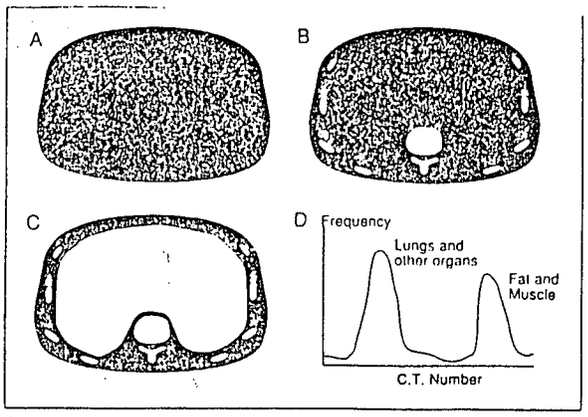

Fig. 3: Body composition from a computerized tomography scan at the thorax level, showing three images resulting from different computer processing of the same scan. A: The air/skin interface. B: major skeletal elements. C: Adjusted to show the interface between the lungs and other organs and the surrounding muscle and fat. D: Histogram of the pixel density from scan C.

and parallel to the gantry. The X-ray beam is rotated around the subject, cutting a cross-sectional 'slice' through the patient. As the X-rays pass through the tissue, the beam undergoes attenuation, the intensity of which is recorded and stored in the scanner computer. The latter then processes the stored information by using a series of complex algorithms to reconstruct cross-sectional images (Fig.3). The images consist of a matrix of picture elements, or pixels, each about 1 mm x 1 mm, arranged in rows and columns. The pixels vary in their degree of shading according to the magnitude of the X-ray beam attenuation, which in turn depends on the physical density of the scanned tissues. Tissues with a greater density cause a greater absorption of X-ray energy and consequently a higher attenuation value. The demarcation between tissues of differing density can be very good. The degree of pixel shading is scaled as the CT number, a measure of attenuation relative to that of water. Examples of the CT numbers and densities (D) are: fat: CT# = -70, D = 0.91 g/cc; muscle: CT# = 20, D = 1.05 g/cc; liver CT# = 25, D = 1.06 g/cc; and water: CT# = 0, D = 1.0 g/cc.

The cross-sectional area of each of the tissues can be determined using specialized computer programmes. The volume of tissues and organs (such as liver, kidney, and spleen) can also be assessed.

The method has several uses. It can be used to assess changes in the visceral organ mass in undernutrition and obesity; to measure regional muscle mass; to assess the distribution of subcutaneous versus internal fat; and to establish bone density in osteopenia. Computerized tomography involves exposure to ionizing radiation, and hence is not recommended for pregnant women or children, for routine whole-body scans, or for multiple scans on the same person. The method is also very expensive.

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Table : Summary of laboratory techniques used to measure body composition and their limitations

Method and Procedures	Limitations
Total Body Potassium (TBK)	
TBK measured by counting radiation from naturally occurring ^{40}K in a whole body counter. FFM derived from assumption that average potassium concentration of FFM is constant.	Required equipment is expensive. Obese and elderly subjects have lower potassium concentrations, leading to overestimates of total body fat.
Total Body Water (TBW)	
Tracer dose of water, labeled with ^{3}H, ^{2}H or ^{18}O, is given orally or intravenously, and then equilibrated. Concentration of isotope measured in serum, urine, saliva, or breath (for ^{18}O). TBW calculated from dilution observed.	Involves radiation to the subject if ^{3}H is used. Water content of fat-free tissue is increased during obesity, pregnancy, and wasting disease leading to an underestimate of fat.
Neutron Activation Analysis	
Radioactive isotopes of N, P, Na, Cl, Ca are created by irradiating the subject. The radioactivity of the element is measured using a whole body counter.	Expensive. Subjects exposed to radioactivity. Elements are not uniformly activated, and thus sensitivity varies.
Underwater weighing	
Subject is weighed in air and then when totally submerged in water. Body volume calculated from the apparent loss of weight in water. Body density calculated from body mass and body volume measurements. Correction factors for water temperature, volume of air trapped in gastrointestinal tract. Preferable to measure residual lung volume.	Requires high degree of cooperation from subject. Not suitable for young children, the elderly, sick patients. Relatively expensive.
Ultrasound	
High-frequency sound waves from an ultrasound meter pass through adipose tissue to adipose-muscle tissue interface. At interface, some sound waves reflected back as echoes, which are translated into depth readings via a transducer.	Validity of technique for subjects with wide range of body fatness unknown. Technique does not provide same degree of structure resolution possible with computerized tomography.

Total Body Electrical Conductivity (TOBEC)	
Subject lies supine in a solenoid coil through which a 5 MHz current is passed. The latter induces a current in the subject, which creates a secondary magnetic field. The conductivity value of the subject is obtained by subtracting the background value when the coil is empty. Conductivity value is proportional to body electrolyte content, and hence reflects amount of fat-free tissue.	Edema, ascites, dehydration, and electrolyte balance will alter conductivity and interfere with reading. Extent to which variation in body shape and size affects readings not yet known. Variations in bone mass may affect readings. Expensive method.
Bioelectrical Impedance (BEI)	
The impedance to a weak electrical current passed between the right ankle and right wrist of subject in supine position is measured. Impedance is proportional to the square of the length of the conductor - a function of the height of the subject - divided by the volume..	Edema, ascites, and dehydration will alter the resistance measurements and invalidate the method. Sensitivity of BEI to detect changes in body composition during nutrition intervention or physical training unknown.
Computerized Tomography (CT)	
Method measures attenuation of X-rays as they pass through tissues, the degree of attenuation being related to differences in physical density of the tissues. Image reconstructed from matrix of picture elements (pixels) which vary in their shading.	Exposure to ionizing radiation limits use of CT for whole body scans, multiple scans in same person, and scans of pregnant women or children. Expensive equipment which is not readily available. The CT does not provide information on chemical composition of the structures.

STATISTICAL TOOLS FOR THE ANALYSIS OF NUTRITIONAL ANTHROPOMETRY

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Good data has been generated through studies on nutritional anthropometry of various segments of the population. The data will provide valuable inferences with the use of appropriate statistical methods for the collection of data and analysis of data. Utilisation of random sampling procedures in the collection of data will generate good representative characteristics. Descriptive and analytical statistical procedures will help for good interpretation of data and appropriate decision making.

Descriptive procedures include measures of averages, and variation, frequency distributions, and graphic depiction of anthropometry for comparison between groups or regions or time periods. Quantiles, deciles and percentiles are frequently utilised.

Statistical techniques such as tests of inference, correlation coefficients, linear regression, multivariate regression and discrimination analysis are useful for interpretation and decision making in the studies. Factorial/principal component analytical techniques are utilised for generating composite, anthropometric and nutritional indices. These techniques help for quantification of differentials existing between groups or regions.

Statistical methods for drawing the following inferences can be visualised.

1. Variation in anthropometric measurements within communities and between communities is massive.
2. All anthropometric measurements are interrelated.

3. Nutritional status and infection are associated.
4. Muscle and fat reflective anthropometric measurements are retarded in clinical PEM cases.
5. Gains in height and weight are closely associated with grades of calorie protein adequacies.
6. Body weight composition is altered with the severity of infection and malnutrition.
7. A combinatiton of height with weight or BMI or Weight for height (%) is found useful for differentiation of malnourished children from those who are well-nourished.
8. Japanese of today are taller and heavier than the Japanese of earlier decades.
9. Children of educated parents in India are as tall and heavy as those of the children in Europe and America.
10. BMI, Broka's index and Weight for height are well correlated with each other and one can be used in place of the other.
11. Differentials of malnutrition are closely associated with the grades of educaiton and knowledge of health and nutrition concepts.
12. Longevity is higher in communities and countries where the sex ratio is higher, body built is normal and life styles are better.

Data needed and statistical tools useful for the analysis and decision making can be visualised and discussed.

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BIO-CHEMICAL ASSESSMENT

BIOCHEMICAL ASSESSMENT AS A TOOL TO EVALUATE NUTRITIONAL STATUS OF PEOPLE IN COMMUNITY

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Biochemical assessment of nutritional status is a more objective and precise approach than dietary/clinical assessment methods. However the interpretation of biochemical assessment data is often difficult and needs to be done carefully.

OBJECTIVES OF BIOCHEMICAL ASSESSMENT

The use of laboratory tests has two primary functions.

* To detect subclinical nutritional deficiencies in individuals, particularly when dietary histories are questionable or unavailable. These tests permit the initiation of appropriate remedial steps.

* To supplement other studies such as dietary/ clinical assessment among specific population groups in order to pinpoint nutritional problems that these modalities may have suggested or failed to reveal.

Laboratory investigations are of little use if it merely confirms a known clinical diagnosis. Often laboratory values will be obtained suggesting marginal or acute deficiencies where the patient appears clinically normal, since clinical signs usually occur only after prolonged inadequate intake of nutrients. The probability is that the subject may be in various stages of depletion and if this state continues will become ill.

BIOCHEMICAL METHODS

Subclinical deficiency states can be identified by two methods namely

- Static biochemical tests
- Functional tests

Static biochemical tests

Subclinical deficiency states can be identified by measuring the levels of a nutrient or its metabolite in a preselected biopsy material that reflects either the total body content of the nutrient or size of the tissue store most sensitive to depletion. These measurements are termed as "static biochemical tests". These tests are grouped into two categories i.e.

- Measurement of a nutrient in biological fluid or tissue
- Measurement of urinary excretion rate of a nutrient

Body fluids and tissues used for biochemical analysis

- Whole blood, serum and plasma
- Urine
- Hair
- Saliva
- Semen
- Amnoitic fluid
- Finger nails and toe nails
- Skin and
- buccal mucosa

Plasma and **serum** tend to reflect recent dietary intake. Near normal plasma/serum nutrient concentration may be present even in the presence of severe depletion of body stores. In ruch cases alternative biochemical indices should be selected.

Erythrocytes can reflect chronic nutrient status. The analysis of erythrocyte is difficult and more over erythrocytes contain only a small percentage of the total body nutrient content, they are unlikely to be a valid index of nutrient status.

Leukocytes or specific cell types such as lymphocytes or neutrophils can be used to monitor short term change in nutritional status. For this estimation relatively large blood sample is required and generally restricts the use of these indices to adults.

Tissue stores like liver, bone marrow, adipose tissue and bone are the storage sites for iron, vitamin E and calcium. Sampling these biopsy materials is too dificult for population studies and can only be used in research and clinical setting. **Hair** is used to assess trace element deficiencies and to excessive exposure to heavy metals (eg. zinc) **Finger nails** and **toe nails** are

biopsy material for trace element analysis (e.g. Selenium).

MEASUREMENT OF URINARY EXCRETION RATE OF THE NUTRIENT OR ITS METABOLITE

Urine specimens can be used for the biochemical assessment of some minerals, water soluble B complex vitamins, vitamin C and proteins provided the renal function is normal. Urine cannot be used to assess the vitamins A, D, E and K as metabolites are not excreted in proportion to the amount of these vitamins consumed, absorbed and metabolized.

Urinary excretion assessment methods almost always reflect recent dietary intake rather than chronic nutritional status. The methods depend on the existence of a renal conservation mechanism that reduces the urinary excretion of the nutrient and/or metabolite when body stores of the nutrient are depleted. Unfortunately, for some nutrients (eg. ascorbic acid and phosphorus) urinary excretion of the nutrient is reduced before body stores are depleted. In other circumstances such as infection, the use of antibiotics and conditions which produce negative balance, increases in urinary excretion may occur despite depletion of body nutrient stores. For measurement of a nutrient or a corresponding metabolite in urine, it is essential to collect a clear, preferably over a complete 24 hour period. To monitor the completeness of 24 hour urine collection, urinary creatinine excretion canbe measured. The approach assumes that daily urinary creatinine excretion is constant for a given individual the amount being related to muscle mass.

FUNCTIONAL TESTS

Some important examples of functional tests are

1. Measurement of abnormal metabolic products in blood or urine arising from suboptimal intakes of the nutrient.

   Eg. Increased excretion of xanthurenic acid in vitamin B_6 deficiency

2. Measurement of changes in blood components or enzyme activities. These are preferred biochemical methods of nutritional assessment, because they are generally the most sensitive and specific. The enzyme that is dependent on a given nutrient and for which specific metabolic defect has been identify is given below

Enzyme	Nutrient
Lysyl oxidase	Copper
Aspartate amino-transferase	Vitamin B_6
Glutathione reductase	Riboflavin
Transketolase	Thiamine

Sometimes not only enzymes but coenzymes are also measured. The tissue selected for the enzyme assay should be particularly sensitive to the pathological lesion. Ideally the assay selected should

- reflect the amount of nutrient available to the body
- Respond rapidly to changes in supply of the nutrient
- Relate to the pathology of deficiency or excess

Many nutrients have more than one functional role, so that the activities of several enzymes may be affected during the development of deficiency, thereby providing additional information on the severity of the deficiency state.

Instead of measuring the activity of an enzyme, changes in blood components that are related to the intake of a nutrient can be measured.

3. In vitro tests of invivo functions

These tests involve replicating in vitro a corresponding function in vivo. For these tests, tissues/cells must be isolated and maintained under physiological conditions.

eg. d-uridine suppression test for vit. B_{12} and folate

4. Induced responses and load tests invivo

These functional tests conducted on the subject invivo include many well established load and tolerance tests. Such tests are used for individuals with a suspected deficiency of a nutrient. They are not suitable for survey studies. They are generally employed to assess

the status of water soluble vitamins and minerals like magnesium, zinc and selenium.

Test	Vitamins
Tryptophan load test	Pyridoxine
Histidine load test	Folic acid
Vitamin C load test	Vitamin C
Valine load test	Vitamin B_{12}

In a load test, a loading dose of the nutrient or an associated compound is administered orally, intramuscularly or intravenously. After the load a timed sample of the urine is collected and excretion level of the nutrient or a metabolite is determined. In a deficiency state, when tissues are not saturated with the nutrient, excretion of the nutrient or metabolite will be low, because net retention is high. When calculating percentage excretion in load tests the basal intake of the nutrient must be allowed by means of a correction for the base line urinary excretion of the nutrient.

Tolerance tests sometimes referred to as plasma appearance tests are used to assess the nutritional status of nutrients such as zinc and manganese. In these tests, the concentration of the nutrient is measured both in fasting plasma and in plasma after an oral pharmocological dose of the nutrient. The response is enhanced in cases of nutrient depletion because intestinal absorption of nutrient is assessed to increase in a nutrient deficiency state.

SELECTION OF LABORATORY TESTS

Rigorous protocols must be followed for the sampling, transportation, preservation and analysis of the biological tissues and/or fluids. This is particularly important for samples collected under field conditions and for trace element analysis. Laboratory tests vary with respect to their precision, accuracy, analytical specificity, analytical sensitivity, predictive value and validity.

Precision : Repeated measurements on a single sample or individual can be used to assess precision. The coefficient of variation as determined by the ratio of the standard deviation to the mean of replicates is the best quantitative measure of the precision. The level of

precision attainable depends on the procedure, whereas precision required depends on the projected application of data. It is possible for a method to be precise but not accurate.

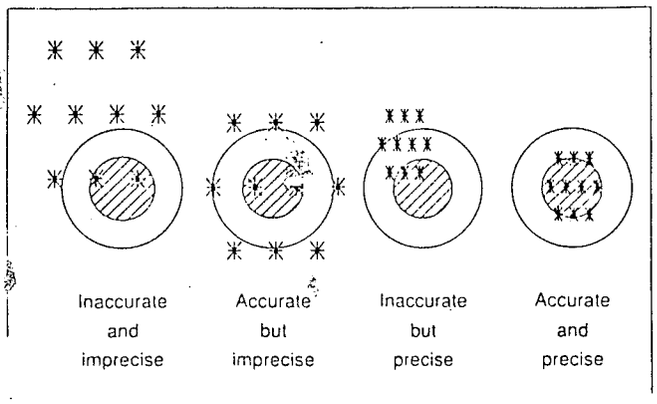

Figure 1. : Differences between precision and accuracy. Modified from Pi-Sunyer and Woo. In: Nutrition Assessment: A Comprehensive Guide for Planning intervention by M.D. Simko, C. Cowell, and J.A. Gilbride, p. 140, with permission of Aspen Publishers, Inc., (c) 1984.

Accuracy : The difference between the reported and the true amount of the nutrient/metabolite present in the sample determine the accuracy of a method. For methods involving direct analysis of nutrients in tissues or fluids a recovery test is generally preferred. This involves the addition of known amounts of nutrient to samples. The sample is then analysed.

Analytical sensitivity : For any analytical method the analytical sensitivity refers to the smallest single result which can be distinguished from zero. Routine procedures should be capable of analyzing the nutrient of interest at a level which is five times greater than the detection limit.

Analytical specificity : The ability of an analytical method to measure only the substance of interest is referred to as the analytical specificity. Analytical specificity is improved in the analysis of minerals or trace elements by removing organic material by dry ashing wet digestion.

Predictive value : The predictive value refers to the ability of a laboratory test when used as an index to predict correctly, the presence or absence of a nutrient deficiency or disease. Sensitivity, specificity and

prevalence affect the predictive value of a laboratory index. Sometimes laboratory index and anthropometric measurements are used together to form a multiparameter index to enhance the predictive value of a test.

Validity : Laboratory test when used as an index can be considered valid if it correctly reflects the nutritional parameter of interest. Thus for example if the objective is to assess the total body content of a nutrient the laboratory index correctly reflects the total body content, the index is said to be valid. Unfortunately the action of certain drugs and hormones in enzyme activity and/or nutrient metabolism may affect the validity of a laboratory index.

Planning Laboratory assessment

While laboratory assessment of nutritional status may seem formidable it can be undertaken if proper preliminary planning takes place.

Primary considerations in planning laboratory studies

1. Requires a method of coordinating the collection of samples of blood and urine from the subjects to be surveyed.

2. Appropriate laboratory with good facilities should be selected and arrangements made to be provide samples and accumulate data.

3. Medical or paramedical personnel/well trained research staff should obtain and process the blood and urine samples.

4. Sample needed for the test should be easily collectable (A finger prick sample of blood or random specimen of urine).

5. The sample should be stable during transport.

6. Simple equipment should be handled at field level.

7. All subjects should be informed about the purpose of the study and their permission obtained. Parental permission is mandatory in the case of minors.

8. The methods utilized for nutritional assessment vary in cost, degree of technical expertise required and reliability. They are also constantly being revised and improved. Appropriate method should be selected and advice on proper interpretation of the data need to be obtained.

SAMPLING PROCEDURE

- Based on the objective of the study appropriate biochemical tests should be selected.

- A decision must be made on the number of specimens that can be collected and processed. This depends on statistical consideration in relation to total number of persons examined in the field and on the limitations imposed by facilities for transport, storage and actual analysis. For biochemical tests, a sub sampling technique will normally be required (10% or 20% based on sample size).

Sample collection and preservation

Equipment needed like disposable syringes and needles, glass stoppered vials, bottles etc. must be immediately at hand when samples are collected. The technique for drawing blood must be thoroughly known and practiced.

Blood and urine samples will deteriorate rapidly unless they are properly managed and preserved for transmission to the laboratory for assay. Optimal attention must be given to specimen collection, preservation and transportation.

Time of collection

The time of day that samples are to be obtained from the subject may influence the findings particularly if this is done shortly after the individual has eaten or taken a vitamin supplement. Optimally, blood samples would be taken in the morning before breakfast or any food or drink is consumed. For urine analysis, the optimal sample is a total 24 hour collection. If this is not possible the best sample is the first upon arising. When that is not possible, best would be to obtain samples atleast 2-3 hours after the last meal. Ideally all samples are to be collected under the same circumstances.

Preservation of blood samples

Blood samples should be collected into stoppered glass vials containing an anticoagulant such as dried oxalate or heparine lithium. Each vial should be labelled properly. All blood samples must be processed and preserved under refrigerated conditions till further analysis under field conditions. Ice boxes can be used. It is necessary to make an acid filtrate of blood immediately (the acid would be trichloroacetic or

metaphorphoric) store by freezing the filtrate for analysis of most unstable vitamins like vitamin C. Similarly separated serum, plasma by centrifugation should be properly preserved and frozen immediately. Certain enzymes are unstable to freezing necessitating immediate assay. To avoid erroraneous results therefore, it is better to review each analytical technique to be used with a view toward its specific needs for sample collection and preservation.

Preservation of urine samples : Urine samples are difficult to collect under field conditions. The cooperation of the subject is required. Urine should be collected in screw capped glass or preferably plastic bottles containing hydrochloric acid or acetic acid as preservative and store in refrigerator till further analysis.

EVALUATION OF LABORATORY INDICES

Laboratory indices both static and functional are generally evaluated using two techniques namely

- Comparing the observed values with reference values which have derived from a reference sample.
- Comparing the observed values with cut off points based on data from subjects with clinical or functional manifestation of a nutrient deficiency

To summarise the following are some of the methods used to evaluate nutrient status.

EVALUATION OF PROTEIN STATUS

Somatic protein status

Urinary creatinine excretion
Creatinine height index
3-Methyl histidine excretion

Visceral protein status

Serum albumin
Serum transferrin
Serum thyroxine binding prealbumin (TBPA)
Serum retinol binding protein (RBP)

Metabolic changes as indices of protein status

Serum amino acid ratio
Urinary 3-hydroxyproline excretion
Hydroxyproline : creatinine ratio
Hydroxyproline index
Nitrogen balance studies

EVALUATION OF VITAMIN STATUS

Some of the biochemical tests used for estimation of vitamin intake and stores are given in table 1.

Table 1: Biochemical tests for vitamin intake and stores

Vitamin	Tests of intake levels	Tests of tissue stores
A	Plasma or serum retinol	Liver retinol
D	-	25 OHD, 1, 25 OH_2D
E	Plasma tocopherols	Erythrocyte fragility test
K	-	Prothrombin time
C	Serum ascorbate	Leukocyte, urinary ascorbate, load test
Thiamine	Urinary thiamine excretion	Erythrocyte transketolase
Riboflavin	Urinary riboflavin excretion	Erythrocyte glutathione reductase, erythrocyte riboflavin, pryridoxamine coxidase
Phyridoxine	Urinary pyridoxine excretion	Tryptophan load test, erythrocyte transaminase, plasma pyridoxal phosphate
B_{12}	Serum B_{12}	Serum B_{12}, methylmalonic acid excretion
Folacin	Plasma Folacin	Erythrocyte folacin, formiminoglutamate excretion test
Niacin	-	Urinary N-methyl nicotinamide,2-pyridone excretion

Evaluation of mineral status

Status with respect to the major minerals (sodium, potassium, calcium and magnesium) is commonly assessed using serum levels (Table 2). The body normally maintains serum levels of these minerals within narrow limits because they have a very important role in electrolyte balance, nerve and muscle function.

Table 2: Assay for major mineral status

Mineral	Assays for functional status	Assays for stores
Sodium	Serum sodium level, urine sodium excretion	In vivo neutron activation analysis
Potassium	Serum potassium level, urine potassium excretion	Serum potassium level
Calcium	Plasma ionized calcium, alkaline phosphatase, vit. D levels	Bone density measurement, in vivo neutron activation analysis
Magnesium	Plasma magnesium, urine magnesium excretion	-
Phosphorous	Plasma phosphate	In vivo neutron activation analysis

Assessment of iron status is closely linked with assessment of hematologic status. Hematology screening includes

* White blood cell count (WBC)
* Red blood cell count (RBC)
* hematocrit (HCT)
* hemoglobin (Hgb)
* Differential white cell count (Diff)
* Mean cell/corpusular volume (MCV)
* Mean cell/corpusular hemoglobin (HCH)
* Mean cell/corpuscular haemoglobin concentration (MCHC)

The entire hematologic examination should be considered in evaluating the significance of nutritional deficiency since dietary factors usually affect more than

one aspect of the formed elements of the blood. But under field situation test to detect anaemia is restricted to only haemoglobin estimation. Several simplified methods of haemoglobin suitable for field situation have been developed recently Ex|| Wong's method, cyanmethae moglobin method and filter paper technique. This filter paper technique has been further modified by NIN.

The number of trace minerals considered essential for human nutrition has been growing. Some of the assays for trace minerals are given in Table 3.

Table 3: Assays for trace minerals

Mineral	Intake	Function	Stores
Zinc	Hair, nail zinc	-	Plasma zinc
Copper	-	Presence of iron like anaemia	Ceruloplasmin plasma copper, hair levels
Iodine	Thyroid hormone levels Thyroid stimulating hormone level	Thyroid hormone levels, Thyroid stimulating hormone level	-
Fluoride	Urinary fluoride level	-	-
Manganese	-	Serum manganese	-
Chromium	-	Change in plasma levels following a meal	Hair levels
Selenium	Plasma selenium	Platelet glutathione peroxidase activity	Red cell selenium, platelet glutathione peroxidase activity

All the tests listed may not be feasible for field situations. But tests which are easy to carry out, where samples are simple to collect and which have been most extensively used in the past can be selected to assess nutritional status of a population.

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Assessment of protein nutritional status by Biochemical methods

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It is a very simple concept that a person is deficient in a substance if his body contains too little of it. In the field of protein status, this leads to the question of how do you obtain a reliable estimate of body protein content, then how do you establish a significant degree of deficiency, and finally how does one recognise clinically those conditions leading to a state of deficiency ? (Water low, 1978). According to Beaton and Patwardhan (1976), a series of steps can be recognized in the development of any deficiency state, beginning with depletion of stores, passing through deterioration in normal cell function and the clinical symptoms of deficiency of the acutely malnourished individual and eventially morbidity and mortality.

Protein consumed in the diet is enzymatically hydrolysed into the elementary tract, and passes into the blood stream as free amino acids which mingle with amino acids coming from the tissues. Amino acids occur in the body in the free form and in the form of body protein. (Hamish and Vernon, 1980).

Role of Skeletal muscle in protein: Skeletal muscle is the largest tissue in the body and metabolism of amino acids in this tissue is of considerable significance for general protein metabolism. Muscle is also the main site of metabolism of the branded - chain amino acids (leucine, isoleucine and valine).

For Assessment of protein status

Various methods have been suggested by Gibson (1990)

1. Assessment of somatic protein status

   A. Urinary creatinine excretion
   B. 3-Methylhistidine excretion

2. Assessment of Visceral protein status

   A. Total serum protein
   B. Serum albumin
   C. Serum transferrin
   D. Serum retinol-binding protein
   E. Serum thyroxine-binding pre-albumin
   F. Serum somatomedin-C

3. Metabolic changes as indices of protein status

   A. Serum amino-acid ratio
   B. Urinary 3-hydroxyproline excretion
   C. Nitrogen balance
   D. Uninary urea nitrogen: creatinine ratios

4. Muscle function tests

   A. Skeletal muscle function after electrical stimulation

   B. Hand grip strength

5. Immunological tests

   A. Lymphocyte count
   B. Measurement of thymus-dependent lymphocytes
   C. Lymphocyte mitogen assays
   D. Delayed cutaneous hypersensitivity
   E. Other methods.

1. Assessment of Somatic protein status:

   A) Urinary creatinine excretion: Creatinine derived from the catabolism of creatinine phosphate, a metabolite in muscle. Urinary creatinine can be used as an index of muscle mass (each gm of creatinine excreted is said to represent approximately 18-20 kg of fat-free muscle). Several methods are used to express urinary creatinine excreation.

   - Urinary creatinine excretion mg per 24 hours.

   - Dietary urinary creatinine excreation (mg) per cm body height.

   - Creatinine height Index (CHI) as percentage.

$$\text{CHI} : \frac{\text{Measured 24 hr urinary creatinine}}{\text{Ideal 24-hr urinary creatinine for height}} \times 100\%$$

CHI as a percentage deficit (mild deficit 5 to 15%, moderate 15-30% and severe above 30%)

Per cent deficit = 100-CHI %

Several factors such as day to day variation, strenous exercise emotional stress, Dietary intake of creatinine and creatinin, menstruation, age, infection, fever and chronic renal failure.

B) 3-Methylhistidine excretion

3-Methylhistidine (2-MH) is an amino acid present almost exclusively in the actin of all skeletal muscle fibres and the myosin of white fibres. It is formed by the methylation of histidine residues after the synthesis of actin and myosin. When the proteins actin and myosin are catabolized, 3-MH is released and excreted quantitatively into the urine without further metabolism.

Nevertheless, the use of 3-MH excretion as a routine index of muscle mass is not recommended at the present time. The precise effects of factors such as sex, age, maturity, nutrition, hormonal status, fitness, recent intense exercise, injury and disease on excretion of 3-MH have not been quantified. Any catabolic state, such as fever, starvation, trauma, infection, etc., will increase muscle turnover and alter the relationship between muscle mass and excretin of 3-MH, thus invalidating index. Urinary 3-MH excretion may be useful for monitoring effectiveness. Ion-exchange chromatography with ninhydrin or ninhydrin-orthophthal aldehyde whereas others use high-performance liquid chromatography for analysis of 3MH.

2. **Assessment of visceral protein status:**

Visceral protein status is frequently assessed by the measurement of one or more of the serum proteins. The main site of synthesis for most of these is the liver,one of the first organs to be affected by protein malnutrition.

Serum proteins useful for measuring short-term changes in protein status have: a small body pool, a rapid rate of synthesis, a major proportion present within the viscular space, a fairly constant catabolic rate that responds specifically to protein-energy deprivation but is not affected by extraneous factors.

a) **Total serum protein**

Total serum protein has been used as an index of visceral protein status. A marked decrease in the serum albumin concentrations which represent 50% to 60% of the total serum protein.

b) **Serum albumin:**

Serum albumin reflects changes occurring within the intravascular space and not the total visceral protein pool. Serum albumin is not very sensitive to short-term changes in protein status; it is a long half-life, of fourteen to twenty days.

Serum albumin levels are also influenced by a variety of other conditioning factors, such as

* Inadequate protein intake resulting from: low dietary intakes, anorexia, unbalanced diets, hypocaloric intravenous infusions.

* Altered metabolism generated by: trauma, stress; sepsis, and hypoxia.

* Specific deficiency of plasma proteins caused by; Protein losing enteropathy, and liver disease.

* Reduced protein synthesis resulting from inadequate energy intake, electrolyte deficiency, trace element deficiencies (e.g. iron and zinc), vitamin deficiency (e.g.vitamin A).

* Pregnancy induces changes in the amount and distribution of body fluids.

* Capillary permeability changes.

* Drugs (e.g. oral contraceptive agents).

* Strenuous exercise.

Serum albumin is assayed in most clinical laboratories via an automated dye-binding method using bromocresol green. Other methods include standard electrophoresis and salt fractionation.

A stable isotope procedure has been developed by Halliday and Mc Keran (1975) for the estimation of albumin synthesis in humans by using 15N - glycine donor of 15N to liver free arginine.

C.SERUM TRANSFERRIN

Transferrin is a serum B-globulin protein synthesized primarily in the liver but, unlike albumin, it is located almost totally intravascularly. Transferrin serves as the iron transport protein, each molecule of tranferrin binding with two molecules of iron. In general,serum transferrin is not an appropriate index of protein status where both iron deficiency anaemia and chronic protein-energy malnutrition are widespread.

Serum transferrin concentrations are assayed by a radial-immunodiffusion technique. This method is expensive time consuming, and not routinely performed in most clinical laboratories. Instead, serum transferrin is

estimated indirectly, using a prediction equation based on total iron-binding capacity. For plasma transferrin based on an antigen-antibody reaction using commerially available transferrin antibody.

D. Serum retinol-binding protein

Retinol-binding protein (REP) is the carrier protein for retinol, with a single binding site for one molecule of retinol. The complex travels together with one molecule of plasma thyroxine-binding pre-albumin (TBPA) to form a trimolecular complex of retinol, retinol-binding protein.

A fall in serum RBP levels is also observed during deficienceis of vitamin A or zinc. Serum retinol-binding protein, like transferrin, is also measured by radial-immunodiffusion techniques.

E. Serum thyroxine-binding pre-albumin

Thyroxine-binding pre-albumin (TBPA) serves as a transport protein for thyroxine, and as a carrier protein for RBP. Serum TBPA is a more sensitive index of protein status and responds more rapidly to dietary treatment than serum albumin or transferrin. Gastrointestinal diseases, renal and kidney diseases, surgical trauma, stress, inflammation, and infection, all lead to modifications in the metabolism of TBPA and reduce its specificity as an index of protein status (Farthing, 1982).Deficiencies of Vitamin A, zinc, and iron do not affect the levels of TBPA but may modify the concentrations of RBP and/or transferrin, as noted previously.

Radial-immunodiffusion techiniques are also used to determine TBPA. Thyroxine-binding pre-albumin values appear to vary according to age, and sex (Carpentier and Ingenbleek, 1983) and possible ethnic group and geographical area.

F. Serum somatomedin -C

Somatomedins are growth-hormone dependent serum growth factors produced by the liver. They have a proinsulin like structure and broad anabolic properties. Reductions in serum somatomedin-C value occur in patients with hypothyroidism, and with estrogen administration. Somatomedin-C can be assayed using a radio-immunoassay method.

3. Metabolic changes as indices of protein status:

For example, in marasmus, fasting serum insulin levels are low, whereas in kwashiorkor they are elevated. Reduced urinary hydroxyproline excretion and increased urinary nitrogen excretion.

A. Serum amino-acid ratio

Measurement of the serum ratio of nonessential amino acids (NEAA) to essential amino acid (EAA) as an index of kwashiorkor was developed by Whitehead and Dean (1964). The EAA, particularly the branched chain amino acids leucine, isoleucine, and valine, as well as methionine, fall to low concentrations in the serum of children with Kwashiorkor.

A simplified technique to determine serum amino-acid ratios using one-dimensional paper chromatography and a finger prick blood sample was developed for field survey use.

In general, serum amino-acid concentrations are not sensitive indices of protein-energy malnutrition because the regulatory systems for the maintenance of serum amino acid concentration are very effective.

B. Urinary 3-hydroxyproline excretion

Urinary 3-hydroxyproline, principally in the peptide form, is an excretory product derived from the soluble and in soluble collagens of both soft and calcified tissues. In adults levels of 3-hydroxyproline in the urine are often used to dignose certain bone and connective tissue or endocrine disorders.

Several extraneous factors influence urinary hydroxyproline excretion. These include the ignestion of collagen and hookworm and/or malarial infestations.

The hydroxyproline: creatinine ratio

This ratio corrects at least partially for differences in adult body size. Hydroxyproline decreases with age while creatinine excretion increases.

The hydroxyproline index

The hydroxyproline index as an age-independent interpretive standard for children.

$$\text{Hydroxyproline index} = \frac{\text{mg hydroxyproline per mL urine}}{\text{mg creatinine per mL urine}} \times \text{Kg body}$$

In normal children, between one and six years of age, the hydroxyproline index is relatively constant and is approximately 3.0

C. Nitrogen balance

Nitrogen balance is a measure of net changes in total body protein mass. Approximately 90% to 95% of daily nitrogen losses is excreted in the urine, the remainder being lost through the skin, stools, hair and nails. Instead, urinary urea nitrogen is more frequently determined. More than 80% to 90% of the total nitrogen in the urine is normally excreted as urea excretion of the non urea nitrogen components (e.g.creatinine nitrogen 6.4%, ammonia nitrogen 7.4% uric acid nitrogen 2% to 3% and other minor nitrogenous compounds 1% to 2%) remains fairly stable on a general diet. limitations and sources of error in balance studies are

* Balance studies are expensive to perform
* Balance procedures require substantial time of the patient and staff
* The intake and excretion of nitrogenous compounds are not measured by standard techniques (e.g nitrate) and may introduce errors.

Urea nitrogen in the urine can be assessed by an enzymatic method of Searcy et al. (1965).

D. Urinary urea nitrogen: Creatinine ratios:

This ratio has been used as an index of dietary protein intake because several studies have reported a relationship between the level of protein intake and ratio.

4. Muscle function tests: Muscle wasting characterized the marasmic form of protein-energy malnutrition. Changes in muscle function, such as muscle contractility, relaxation rate, and endurance.

A. Skeletal muscle function after electrical stimulation

Skeletal muscle function tests generally measure the function of the adductor pollicis muscle after electrical stimulation of the ulnar nerve.

B. Hand grip strength

Hand grip strength has also been used as a test of skeletal muscle function. Psychological factors,such as motivation as anxiety, may also influence hand grip strength and confound the interpretation of the results.

5. Immunological tests

During protein-energy malnutrition and deficiencies of specific nutrients suh as iron and zinc, consistent changes in immunological responses have been observed. Immunocompetence has been used as function index of nutritional status. Immunological tests, are not specific enourgh to detect individual nutrient deficiencies.

A. Lymphocyte count

Lymphocytes comprise 20% to 40% of the total white blood cells (WBC) (leukocytes). Measurement of the total lymphocytes in the peripheral circulation is usually performed routinely on almost all hospital patients in industrial countries. In healthy subjects, the average lymphocyte count in peripheral blood is generally above 2750 cells per mm^3. In malnutrition, the blood lymphocyte count is reduced. A level between 900 and 1500 cells per mm^3 is said to indicate moderate depletion, whereas below 900 per mm^3 represents severe depletion.

$$\text{Total lymphocyte count} = \frac{\%\ \text{lymphocytes} \times \text{WBC}}{100}$$

Many other factors can affect the lymphocyte count, the specificity and sensitivity of this test is low.

B. Measurement of thymus-dependent lymphocytes

Approximately 75% to 80% of the circulating lymphocytes are thymus dependent lymphocytes (T-cells). During protein-energy malnutrition, both the proportion and absolute number of T-cells in the peripheral blood may be reduced.

For the test, the lymphocytes are first isolated from a venous blood sample. After washing, the lymphocytes are mixed with sheep red blood cells and incubated briefly (Five to ten minutes) at 37°C.During which time the T-cells bind to the sheep red blood cells forming rosettes. Hence, immune dysfunctions that result from increased numbers of supressor T-cells cannot be detected by this test.

C. Lymphocyte (nitrogen assays

To assess human lymphocyte function, lymphocytes are incubated in the presence of mitogens, which stimulate carbohydrate receoptors on lymphocyte surfaces causing them to divide. In cases of immune dysfunction, decreases in the mitogen-induced proliferation occur. Mitogens commonly used include concanavalin phytohemagglutinin (PHA) which primarily stimulate division of T-cells,and the thymus dependent B-lymphocyte mitogen, pokeweed mitogen (PWM). The results must be interpreted with caution becasuse technical variations and difficulties in the assays can influence the results.

D. Delayed cutaneous hypersensitivity

When healthy persons are re-exposed to recall antigens intradermally, T-cells respond by proliferation, and release of soluble mediators of inflammation. This produces an induration (hardening) and erythema (redness). These skin reactions are often decreased in malnourished persons with marasmus and or kwashiorkor and/or nutrient deficiencies such as Vitamin A, zinc, iron, and pyridoxine, but are reversed after nutritional rehabilitation. The recall skin test antigens commonly used are; purified protein derivative (PPD), mumps, trichophyton, dinitrochlorobenzene (DNCB).

Several investigators have studied the usefulness of DCH testing for assessing risk of morbidity and mortality in hospital patients. The multi-test Cell Mediated Immunity (CNI) Delayed Hypersensitivity Skin test kit has been developed by the Institut Merieux (Kniker et al., 1979).

E. Other methods

Mixed leukocyte culture is another method used to assess immune function, which involves measureing the ability of cells to divide in response to a specific antigen challenge again using tritiated thymidine incorporation.

Determination of body nitrogen through in vivo neutron activation analysis (INAA) is a recently described Technique (Mernagh et al (1977) and Corn et al (1972).

Connected arm muscle area (CAMA) accurately reflects total muscle mass and is the simplest means to detect muscle wasting (Heymofield et al, 1982).

Summary

Laboratory indices of protein status measured by

1. Stomatic protein status; 2. Visceral protein status;
3. Metabolic changes; 4. Muscle function; and 5. immune function.

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BIO CHEMICAL ASSESSMENT OF VITAMIN STATUS

Dr. Uma Reddy

approaches

The assessment of vitamin status is best achieved by the application of biochemical methods, clinical evaluation and, to a lesser extent, dietary methods. Anthropometric evaluation can be informative regarding energy and protein status, but yields no information relevant to vitamin status. Clinical evaluation can be effective in the diagnosis of late-stage vitamin defeciencies, that is, those involving physiologic dysfunction and/or morphological changes. However, overt vitamin deficiency syndromes are relatively are compared to the incidence of sub-optimal vitamin status about which clinical evaluation is informative. Diets and food habits that are likely to provide insufficient amounts of available vitamins can be identified by dietary evaluation. However, as discussed in Chapter 19, these methods are almost always imprecise with respect to the vitamins, and the parameters they measure usually have greater inherent variability than those for other nutrients. The detection of early stage vitamin defeciencies is, therefore, best achieved using the variety of biochemical methods that are available.

requirements of useful biochemical methods

Many biochemical parameters of vitamin status can be identified. However, in order to be useful for the purposes of assessment of vitamin status, a parameter must satisfy several requirements.

To be useful in assessing vitamin status, a biochemical parameter must:

i. correlate with the rate of vitamin intake, at least within the nutritionally significant range, and respond to deprivation of the vitamin

ii. relate to a meaningful period of time

iii. relate to normal physiologic function

iv. be measurable in an accessible specimen

v. be technically feasible, reproducible and affordable

vi. have an available base of normative data

available biochemical methods for assessing vitamin status

The ideal parameter of vitamin status would be a measure of actual metabolic function of the vitamin. In some cases this is possible; however, in most cases, direct measurement of vitamin metabolic function is not possible due to the absence of a discrete functional parameter, to the existence of more than one metabolic function with different sensitivities to vitamin supply, to the function of the vitamin in a loosely bound fashion which is unstable to the methods of tissue preparation, etc. Therefore, other parameters are useful for assessing vitamin status. These include measurements in accessible tissues or urine of the vitamin, certain metabolities or other enzymes related to the metabolic function of the vitamin.

Accessible tissues for biochemical assessment of vitamin status:

tissue	**relevance**
<u>blood</u>	
plasms/serum	contain newly absorbed nutrients as well as vitamins being transported to other tissues and, therefore, tend to reflect recent nutrient intake; this effect can be reduced by collecting blood after a fast
erythrocytes	with a half life of ca, 120 days, they tend to reflect chronic nutrient status; analyses can be technically difficult
leukocytes	have relative short half-lives and, therefore, can be used to monitor short-term changes in nutrient

	status; isolation of these cells can present technical difficulties
<u>tissues</u>	
liver, adipose, bone, marrow, muscle	sampling is invasive, requiring research or clinical settings
hair, nails	easily collected and stored specimens offer advantages particularly for population studies of trace element status; not useful for assessing vitamin status.

Interpreting results of biochemical tests of vitamin status

The guidelines originally developed by the ICNND are generally used for the interpretation of the results of biochemical parameters of vitamin status. It is important to note, however, that those interpretive guidelines were originally developed for use in surveys of populations. Their relevance to the assessment of the vitamin status of individuals is not straightforward, due to issues of intra-individual variation and confounding effects which may be quantitatively more significant for individuals than for populations. For example, intra- individual (*within-person*) variation is frequently noted in serum analytes. Therefore, a measurement of a single blood sample may not be appropriate for estimating the usual circulating level of the analysts of an individual, even though it may be useful in estimating the mean level of a population. Several factors can confound the interpretation of parameters of vitamin status: those affecting the response parameters directly, drugs that can increase vitamin needs, seasonal effects related to the physical environment or food availability, use of parenteral feeding solutions, use of vitamin supplements, smoking, etc.

Limitations of some biochemical methods of assessing vitamin status

Vitamin	parameter	limitation
Vitamin A	plasma retinol	reflects body vitamin A stores only at severely depleted or excessive levels; confounding effects of protein and Zn deficiencies and renal dysfunction
Vitamin D	Plasma alk, P-ase	affected by other disease states
Vitamin E	Plasma tocopherol	affected by blood lipid transport capacity
thiamin	plasma thiamin	low sensitivity to changes in thiamin intake
riboflavin	plasma riboflavin	low sensitivity to changes in riboflavin intake
Vitamin B_6	RBC glutam-pyruv transaminase	genetic polymorphian
folate	RBC folates	also reduced in vitamin B_{12} deficiency
	urinary FIGLU	also increased in vitamin B_{12} deficiency
Vitamin B_{12}	urinary FIGLU	also increased in folate deficiency

*or serum

*formiminoglutamic acid

Biochemical methods for assessing vitamin status

vitamin	functional parameters	tissue levels	urinary excretion
Vitamin A		serum retinol change in serum retinol with oral vitamin A dose liver retinyl esters	
Vitamin D		serum $25(OH)_2$-vitamin D_2 serum vitamin D_3 serum $1,25\text{-}(OH)_2D_3$ serum alkaline phosphatase	
Vitamin E	erythrocyte hemolysis	serum tocopherols breath alkanes	

Vitamin K	clotting time prothrombin time		
Vitamin C		serum ascorbic acid leucocyte ascorbic acid	ascorbic and ascorbic ac
thiamin	erythrocyte transketo-lase stimulation	blood thiamin blood pyruvate	thiamin(thiochrome) thiamin after load
niacin		erythrocyte NAD erythrocyte NAD:NADP ratio	1- methylnicotinamide 1-methyl-6-pyridone-3 -carboxamide
riboflavin	erythrocyte glutathione reductase stimulation	blood riboflavin	riboflavin riboflavin after load
vitamin B_6	erythrocyte transaminase	plasma pyridoxal phosphate erythrocyte transaminase stimula-tion erythrocyte pyridoxal-P, plasma pyridoxal	xanthurenic acid after TRY load quinolinic acid pyridoxine 4-pyridoxic acid
biotin		blood biotin	biotin
pantothe -nic acid	erythrocyte sulfanilamide acetylase	serum pantothenic acid erythrocyte pantothenic acid blood pantothenic acid	pantothenic acid
folate		serum folates eryth rocyte folates leucocyte folates liver folates	FIGLU after HIS load urocanic acid after HIS load
vitamin B_{12}		serum vitamin B_{12} erythrocyte vitamin B_{12}	FIGLU methyimalonic acid

[a]most useful parameter
[b]relative dose-response test
[c]a single large oral dose
[d]formininoglutamic acid

Interpretive guidelines for assessing vitamin status

vitamin	parameter	age group	values, by category of status (risk)		
			deficient (high risk)	low(moderate risk)	acceptable (low risk)
Vitamin A	plasma[b] retinol, g/dl	<5mo.	<10	10-19	>20
		5-17 yrs.	<20	20-29	>30
		adult	<10	10-19	>20
Vitamin D	Plasma[b] 25-(OH)D_{3c} ng/ml plasma[b] alk, P-ase[c],u/ml	all ages	<3	3-10	>10
		infants	>390	298-390	99-298
		adults	<40	40-56	57-99
Vitamin E	Plasma[b] a-tocopherol, mg/dl	all ages	<.35	.35-80	>.80
Vitamin K	clotting time, min. prothrombin time, min.	all ages		>10	ca. 10
Vitamin C	plasma[b] ascorbic acid, mg/dl	all ages	<.20	.20-.30	>.30
	leucocyte ascorbic acid,mg/dl	all ages	<8	8-15	>15
	whole blood ascorbic ac.,mg/dl	all ages	<.30	.30-.50	>.50
thiamin	urinary thiamin, g/g creat.	1-3 yrs.	<120	120-175	>175
		4-6 yrs.	<85	85-120	>120
		7-9 yrs.	<70	70-180	>180
		10-12 yrs.	<60	60-180	>180
		13-15 yrs.	<50	50-150	>150
		adults	<27	27-65	>65
		pregnant			
		2nd trim.	<23	23-55	>55
		3rd trim.	<21	21-50	>50
	urinary thiamin, μ g/24 hrs.	adults	<40	40-100	>100

urinary thiamin, μ g/6 hrs.	adults	<10	10-25	>25
urinary thiamin after thiamin load, μg/4 hrs.	adults	<20	20-80	>80
erythrocyte transketolase stimulation by TPP[1,g],%	adults	>25	15-25	<15

*from ICNND, 1963. Manual for Nutrition Surveys, 2nd e., U.S.Gov. Printing Off., Washington, D.C., 327 pp.; Sauberich,H.E., J.H. Skala and R.P.Dowdy, 1974, Laboratory Tests for the Assessment of Nutritional Status, CRC Press,Cleveland, 136 pp.; and Gibson, R.S. 1990 Principles of Nutritional Assessment,Oxford Univ. Press, New York,. 691 pp. [b]or serum [c]subject to effects of season and gender [d]Results vary according to assay conditions;most assays are designed such that normal prothrombin times are 12-13 sec., with greater values indicating suboptimal vitamin K status. *single oral 2 mg dose thiamin pyrophosphate [9]the 'TPP effect'.

Interpretive guidelines for assessing vitamin status (part 2)

vitamin	parameter	age group	values, by category of status (risk)* deficient (high risk)	low (moderate risk)	acceptable (low risk)
riboflavin	urinary riboflavin, g/g creat.	1-3 yrs.	<150	150-500	>500
		4-6 yrs.	<100	100-300	>300
		7-9 yrs.	<85	85-270	>270
		10-15 yrs.	<70	70-200	>200
		adults		27-80	80
		pregnant			
		2nd trim.	<39	39-120	>120
		3rd trim.	<30	30-90	>90
	urinary riboflavin,μ g/24 hrs.	adults	<40	40-120	>120

	urinary riboflavin,µg/6 hrs.	adults	<10	10-30	>30
	urinary riboflavin after riboflavin load[b], µg/4 hrs.	adults	<1000	1000-1400	>1400
	erythrocyte riboflavin, µg/d	adults	<10.0	10.0-14.9	>14.9
	erythrocyte glutathione red. stimulation by FAD[6],%	adults	>40	20- 40	<20
niacin	urinary N-methylniocotinamide µg/g creatinine	adults pregnant	<.5	.5- 1.6	>1.6
		2nd trim.	<.6	.6-2.0	>2.0
		3rd trim.	<.8	.8-2.5	>2.5
	urinary N-methylnicotinamide µg/6 hrs.	adults	<.2	.2-.6	>.6
	Urinary 2-pyridone[4]/N-methyl nicotinamide ratio	all ages	-	<1.0	≥1.0
Vitamin B_6	plasma pyridoxal-P,nM	all ages	-	<60[d]	≥60[d]
	urinary pyridoxine,µg/g creat.	1-3 yrs.	-	<90[d]	≥90[d]
		4-6 yrs.	-	<75[d]	≥75[d]
		7-9 yrs.	-	<50[d]	≥50[d]
		10-12 yrs.	-	<40[d]	≥40[d]
		13-15 yrs.	-	<30[d]	≥30[d]
		adults	-	<20[d]	≥20[d]
	urin. 4-pyridoxic ac., mg/24 hrs.	adults	<.5[d]	.5-.8[d]	>.8[d]

urinary xanthurenic acid after TRY load[b],mg/24 hrs.	adults	>50	25-50[d]	<25[d]
urinary 3-OH- kynurenine after TRY load,mg/24 hrs.	adults	>50[d]	25- 50[d]	<25[d]
urinary kynurenine after TRY load,mg/24 hrs.	adults	>50[d]	10-50[d]	<10[d]
quinolinic acid after TRY load[b],mg/24 hrs.	adults	>50	25-50[d]	<25[d]
erythrocyte alkanine aminotransferase stimulation by PalP[h],%	adults-	-*	>25[d]	≤25[d]
erythrocyte aspartate aminotransferase stimulation by PalP[h],%	adults	-*	>50[d]	≤50[d]

*from ICNND, 1963, Manual for Nutrition Surveys, 2nd ed., U.S. Gov. Printing Off., Washington, D.C., 327 pp.; Sauberlich, H.E., J.H. Skala and R.P.Dowdy,1974. Laboratory Tests for the Assessment of Nutritional Status, CRC Press, Cleveland, 136pp.; and Gibson,R.S. 1990 Principles of Nutritional Assessment, Oxford Univ. Press, New York,691 pp. [b]single 2g oral dose [c]flavin adenine dinucleotide, reduced form, 1-3mM [d]N- methyl-2 pyridone-5-carboxamide.

Interpretive guidelines for assessing vitamin status (part 3)

Vitamin	Parameter	age group	values,by category of status(risk)*		
			deficient (high risk)	low (moderate risk)	acceptable (low risk)
biotin	urinary biotin,µg/24 hrs.	adults	<10[b]	10-25[b]	>25[b]
	whole blood biotin,ng/ml	adults	<.4[b]	.4-.8[b]	>.8[b]
pantothenic acid	plasma[c] pantothenic acid,µg/dl	adults	-[d]	<6[b]	≥6[b]

	blood pantothenic acid,µg/dl	adults	-[d]	<80[b]	≥80[b]
	urinary pantoth. ac.,µg/24 hrs.	adults	-[d]	<1[b]	≥1[b.1]
folate	plasma[c] folates,ng/ml	all ages	<3	3-6	>6
	erythrocyte folates,ng/ml	all ages	140	140-160	>160
	leucocyte folates,ng/ml	all ages	-[d]	<60	>60
	urinary FIGLU[g] after HIS load[h],mg/8 hrs.	adults	>50[b]	5-50	<5[m]
Vitamin B_{12}	plasma[c] vitamin B_{12},pg/ml	all ages	100	100-50	>150[n]
	urinary methylmalonic acid after VAL load[b],mg/24 hrs.	adults	≥300	2-300	≤2
	urinary excretion of a radiolabelled vitamin B_{12} dose after vitamin B_{12} flushing dose,%	adults	<3	3-8	>8

*from ICNND, 1963. Manual for Nutrition Surveys, 2nd ed., U.S.Gov.Printing Off., Washington, D.C. 327 pp.; Sauberlich, H.E., J.H. Skala and R.P.Dowdy. 1974. Laboratory Tests for the Assessment of Nutritional Status, CRC Press, Cleveland, 136 pp.; and Gibson, R.S. 1990 Principles of Nutritional Assessment. Oxford Univ. Press, New York, 691 pp. These values have only a small data base and, therefore, are tentative, or sarum data base is insufficient to support a guideline Normal values are ca. 100 mg/dl. Normal values are 2-4 mg/24 hrs. formininoglutamic acid [h]single oral 2-30 mg dose Normal adults excrete 5-20 mg/8 hrs. Most healthy persons show 200-900 pg/ml. [b]single oral 5-10 g dose [l]This is the Schilling Test; it involves measurement of labelled vitamin B_{12} excreted from a 5-2 mg tracer dose after a large flushing dose (e.g., 1 mg) given 1 hr. after the tracer.

Estimated vitamin reserves of human adults:

reserve capacity	**vitamins**
3-5 years	vitamin B_{12}
1-2 years	vitamin A

3-4 months	folate	
2-6 weeks	vitamin D	riboflavin
	vitamin E	vitamin B_6
	vitamin K	choline
	vitamin C	
4-10 days	thiamin	pantothenic acid
	biotin	

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Overview of Vitamin A Nutriture and Metabolism

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Humans are incapable of synthesizing vitamin A-active substances; therefore, vitamin A must be obtained from the diet. The most important sources of vitamin A are preformed vitamin A , found mainly in animal products such as liver and meat, and the biologically active carotenoids,obtained mostly from brightly colored fruits and vegetables, i.e., papaya, carrots and squash (1). Because of the fat-soluble nature of vitamin A, it is treated in the gut as a lipid.Dietary retinyl esters, retinol and provitamin A carotenoids (mainly β-carotene in the human diet) are dispersed and emulsified in the stomach. Then they pass into the duodenum, where the retinyl esters are hydrolyzed by a nonspecific pancreatic lipase (2,3). The retinol and β-carotene are solubilized in mixed micelles allowing them to reach the microvillus membrane. Absorption of retinol and β-carotene probably takes place by passive diffusion (4).

After absorption into the mucosal cell, the β-carotene is cleaved by dioxygenase to retinaldehyde, which is subsequently reduced to retinol. The retinol is mostly reesterified with long chain fatty acids, predominantly palmitic and stearic acid. These retinyl esters are incorporated by mucosal cells into the core lipid of chylomicra. The chylomicra are transported through the lymphatic system via the thoracic duct into the plasma. In the plasma, the chylomicra acquire specific apolipoproteins (apo C and apo E) from plasma high-density lipoproteins. Apo C activates the enzyme lipoprotein lipase to hydrolyze the chylomicron triglycerides. Ultimately, this process leaves a smaller particle called a chylomicron remnant. The retinyl esters are almost entirely retained in the chylomicron remnant.The apo E on thechylomicron remnant is responsible for its rapid hepatic uptake (5).

Hepatic uptake of the chylomicron remnants most likely occurs through receptor-mediated endocytosis. Inside the hepatic cell, lysosomal degradation of the remnant occurs along with retinyl ester hydrolysis. After ester hydrolysis, the free retinol can be reesterified (6,7), predominantly to form retinyl palmitate; bound to retinol binding protein (RBP), which is secreted into the plasma;or converted to other metabolites (8).

Assessment of Vitamin A Status

Vitamin A status can be divided into five categories: deficient, marginal, satisfactory, subtoxic and toxic.Liver retinol concentrations characteristic of the deficient, marginal and satisfactory states are <17 nmol/g (<5 ug/g), 17-66 nmol/g (5-19 ug/gl) and >70nmol/g (>20 ug/g), respectively (9).

Subtoxic and toxic states can not be appropriately characterized by liver reserves. Assessment of vitamin A status is difficult because of the manner in which the vitamin is stored, circulated and metabolized.

Vitamin A deficiency continues to be a major health concern especially among preschool children in less industrialized nations. Annually, 20,000 to 100,000 new cases of preventable blindness caused by hypovitaminosis A occur in the world today (10,11).Mortality among young children has also been reduced by programs which provide vitamin A supplementation (12, 13).

The nutritional indicator for vitamin A status has been defined as the total body content of retinol, with the minimal acceptable reserve set at 70 nmol/g (20 ug/g) of liver. Under most circumstances, the liver has been found to contain 90% or more of the total body vitamin A (14).

The general term used for vitamin A-dependent ocular involvement is "xerophthalmia", meaning "dry eye" (Greek). The world Health Organization has classified xerophthalmia into primary and secondary signs. According to this classification, the primary signs include conjunctival xerosis (X1A), Bitot's spot with conjuctival xerosis (X1B), corneal xerosis (X2), corneal ulceration with xerosis (X3A) and keratomalacia (X3B).

The secondary signs, which are derived from vitamin A deficiency but may have other causes, include nightblindness (XN), xerophthalmia fundus (XF) and corneal scars (XS) (15).

Although vitamin A deficiency can be assessed by classical eye signs of xerophthalmia, a marginal status is more difficult to detect. In the past, serum retinol concentration has been used; however, serum retinol is homeostatically controlled throughout a wide range of liver values. Serum retinol concentration is only diagnostic when it fall below 0.35 umoles/1 (10 ug/dl) or above 1.4 umol/1 (40 ug/dl). Thus, it is not a good general indicator of vitamin A status because it does not

decrease significantly until liver reserves are very low (1).

Techniques which detect marginal vitamin A status before clinical signs are manifested are of significant value. In this regard, several techniques for the assessment of vitamin A status other than clinical deficiency have been developed. The most accurate indicator is analysis of liver samples obtained at autopsy or by surgical and needle biopsies (16-18). However, the application of this method is only possible in special, justifiable situations and is not appropriate in field studies.

Other indicators that have been developed to determine the vitamin A status of an individual include the conventional relative dose response (RDR) assay, the modified relative dose response (MRDR) assay, conjunctival impression cytology (CIC), isotope dilution, and rapid dark adaptation testing.

Hatchell and Sommer have developed a technique to determine the prevalence of goblet cells on the bulbar conjuctiva (19). This method is commonly called conjunctival impression cytology (CIC).During vitamin A depletion, the number of goblet cells decreases in various tissues, including the conjuctiva (20, 21). The method involves taking a cellulose acetate filter paper impression of the bulbar conjuctiva. These impressions are examined by light microscopy to observe the number of goblet cells and the number of enlarged epithelial cells.Hatchell and sommer (19) found that the goblet cell population decreased and the number of enlarged epithelial cells increased before clinical signs of xerophthalmia appeared. Thus, by using this relatively non invasive technique, ocular surface abnormalities due to vitamin A deficiency can be detected before clinical signs of xerophthalmia appeared

Thronton developed a method to measure the rapid dark adaptation time of individuals (22). The test, done under night time lighting conditions, mesures the time that it takes for a subject to sort a pile of white, blue and red chips with 100% accuracy.Vitamin A-depleted subjects tend to take longer to sort the chips than normal subjects. This method correlates well with the classical dark adaptation test in some studies (23,24), but not with plasma retinol concentrations in another investigation.The coefficient of variation is also quite large.

Another approach to vitamin A status assessment which holds great potential is the use of an isotope-dilution assay.Several groups have studied the use of radioactive tritium (^{3}H)-labeled vitamin A in rats, sheep and cattle (25-28) with promising results. Tritium has also been used in humans (28, 29), but without verification by liver analysis.A non-radioactive deuterated analogue of vitamin A has been used to assess total body stores of vitamin A in humans (30); the serum ratios of labeled to unlabeled (D/H ratios) can be determined using capillary gas chromatography-mass spectrometry (GC-MS) (30, 31). This study has been verified with analysis of surgical liver biopsies.

Biochemical Basis for Using Dose Response Tests as Indicators of Vitamin A Status

In the liver, apo-retinol binding protein (apo-RBP) synthesis is not controlled. Therefore during vitamin A depletion, apo-RBP accumulates in the liver (33). Allthough retinol is the preferred ligand, 3,4-didehydroretinol binds to apo-RBP in the liver and the holo-RBP complex then circulatees in the plasma. Moreover, after a suitable oral dose,retinol or dehydroretinol should appear in significant amounts in the plasma only when endogenous liver retinol concentrations are indequate i.e., when liver reserves of vitamin A are <70 nmol/g <20 ug/g).

Relative Dose Response Test

The relative dose response test was developed in rats by Underwood and her coworkers (37). This technique has since been applied to human populations with encouraging results (16, 38-41).

The relative dose response (RDR) test is a good indicator of marginal vitamin A status. The RDR assay has been validated in humans using direct measures of liver vitamin A (16, 39). After a small oral dose of retinyl ester or dehydroretinyl ester is given, the holo-RBP is released into the serum from the liver and transported to target tissues. The response of the individual to this dose of vitamin A is measured in the serum 5 hours after administration of the dose.

The RDR involves giving a standard oral dose of 450 ug (1.57 umol) of retinol equivalents dissolved in oil (38). The dose has also been given intravenously as a water-dispersed suspension to children with liver disease (39). Two blood samples are taken, at time 0 and 5 hours after the dose. After lipid extraction of the serum

samples, the serum vitamin A concentration is measured by use of high-performance liquid chromatography (HPLC) or a suitable colorimetric method. The response is measured in the serum as a percentage $(A_5-A_o)/A_5 \times 100\%)$, where A_5 is the serum retinol concentration 5 hours after the dose and A_o is the concentration at time 0. Theoretically, the value can be 0 to 100%. A response of 20% or higher is indicative of inadequate liver reserves (<0.07) umol/g liver; <20 ug/g liver) (16).

If the RDR value is $\geq$ 20%, the subject is almost certainly in marginal vitamin A status. To determine if children in a particular village are at risk, marginal status can be assessed in a sub population. Thus, only a representative number of children need be studied to make suitable public health judgements about vitamin A status in a population. After analysis, supplements can be made available to residents of villages who have a high incidence of positive RDR values typically, a dose of 50,000 IU-200,000 IU every six months dependent on age, is enough to lower the morbidity and mortality associated with marginal vitamin A status in preschool children. Tentatively, a public health problem might be assumed to exist if 20% of a population of preschool children show abnormal RDR >20% values.

The RDR test has been used with success in serveral studies, including France (39), Brazil (38),Thailand (40) and Belize (55).Flores et al. (38) used the RDR test at four different times to determine the effectiveness of supplementing children with vitamin A. The RDR value in an individual reverts to normal soon after supplementation, but then becomes abnormal again when liver stores are depleted.

The Modified Relative Dose Response Assay

The modified relative dose response (MRDR) assay is similar to the relative dose response (RDR) assay. An analog of retinol (R), 3,4-didehydroretinol (DR), is used as the indicator in the MRDR test. Dehydroretinol is found naturally in fresh water fish and has also been identified in human skin. Because dehydroretinol is a biologically active, naturally occuring analog of retinol, its use as a probe of vitamin A status is more attractive than the use of synthesized derivatives, i.e., deuterated, tritiated and chlorinated (35) vitamin A.Thaitawee and Tosukhowong first suggested the use of 3,4-didehydroretinol as an indicator of vitamin status (36). They observed that the ratio of dehydroretinol to retinol in the serum, 24 hours after a dose of vitamin A_2 was inversely related to the amount of vitamin A stored in the livers of experimental rats (32, 42-46). A single dose of 3,4-didehydroretinyl

acetate in oil is given orally. Like R, DR binds to accumulated apo-RBP in the liver and is released into the serum as holo-RBP. A molar ratio of dehydroretinol/retinol (DR/R) in serum is determined 4-6 hours after dosing. MRDR values theoretically can range from 0 to∞.

Specifically, the MRDR assay involves first giving children a single oral dose (100 ug/kg body weight) of 3, 4 didehydroretinyl acetate dissolved in an oil and them taking a single venous blood sample 4-6 hours later. After the serum is extracted with ethanol/hexane, retinol (R) and dehydroretinol (DR) in an aliquot are measured by high-performance liquid chromatography (HPLC). The monitoring wavelength of the detector is set at 350 nm, which optimizes the measurement of DR. Standards of both R and DR of known concentration are used to calibrate the HPLC system. Thereby, a molar ratio of DR to R can be calculated. A DR/R molar ratio of $\geq$0.030 is generally indicative of a marginal vitamin A status (32).

Children with DR/R ratios of $\geq$0.03 are judged to be in a marginal vitmain A status. The suggested cutoff value of 0.030 requires further validation. Tentatively,a public health problem might be assumed to exist if >20% of a population of preschool children show abnormal (>0.030) MRDR ratios.

Currently, the MRDR has been validated in rats of varying vitamin A status by directly measuring liver stores of R (42-44). The MRDR has been applied to two populations of children. Americans (45) and Indonesians (32). Fewer than 10% of Americans showed DR/R ratios $\geq$ 0.030 (45). The ratio decreases to <0.030 after treatment with retinol.Although the Indonesian children (n=53) did not show any overt signs of clinical vitamin A deficiency, more than 60% tested in a general survey had DR/R ratios <0.030 (32).When a subgroup of the same children were given therapeutic doses of vitamin A (200,000 IU), the DR/R ratios dramatically decreased (Tanumihardjo, S.A and Muhilal H., unpublished observations).

Advantages and Constraints of the Dose Response Tests

The dose response tests are good indicators of the individual's vitamin A status. The effects of confounding factors, such as infectious disease and moderate protein-calorie malnutrition, on serum retinol concentrations are minimized by use of response tests. For analysis in the RDR, investigators have a choice of using either HPLC, which is a fairly widespread technique, or any suitable colorimetric or photodegradative assay. The latter methods require much simpler instrumentation.

The major drawback of the RDR is that two blood samples are required. In some cultures, it is difficult to obtain blood samples. Thus, the need to ontain two samples at a 5-hour interval can pose large logistic and cultural problems. Also, the need to analyze two blood samples to obtain a single human value can become onerous.

The MRDR offers several advantages over other procedures. It is a naturally occurring form of vitamin A found predominantly in freshwater fish, but also to a small extent in mammalian tissues, including the human. DR has approximately 40% of the biological activity of retinol. Only one blood sample is required and the serum samples can be frozen and stored for analysis. The required HPLC instrumentation, although costly and sophisticated, is fairly widespread around the world.By employing the ratio of DR/R in a single sample, the effects of storage on vitamin A stability and of sample extraction efficiency are minimized. The MRDR can be validly repeated approximately one week after a therapeutic dose of vitamin A. The other advantage is that children can be dosed in the morning at their homes and brought to the clinic or the survey site several hours later for the sampling.

On the other hand, the MRDR assay has several constraints. DR is not stable once it is extracted from the serum. Therefore, one must exercise extreme care to protect the extracts from light and to inject them as soon as possible onto the HPLC system. Because DR-acetate currently is not available commercially, it must be synthesized (42, 46) or isolated from fish liver oils.Also, blood must be analyzed using HPLC, which may pose various limitations in some institutions.

Thus, the MRDR holds considerable promise as a minimally invasive technique by which a marginal vitamin A status can be detected in individual children before clinical manifestations appear. Correlation of MRDR values in individuals with their liver vitamin A concentrations would be useful in defining the least ratio to be used as a cutoff between a marginal and satisfactory vitamin A status.

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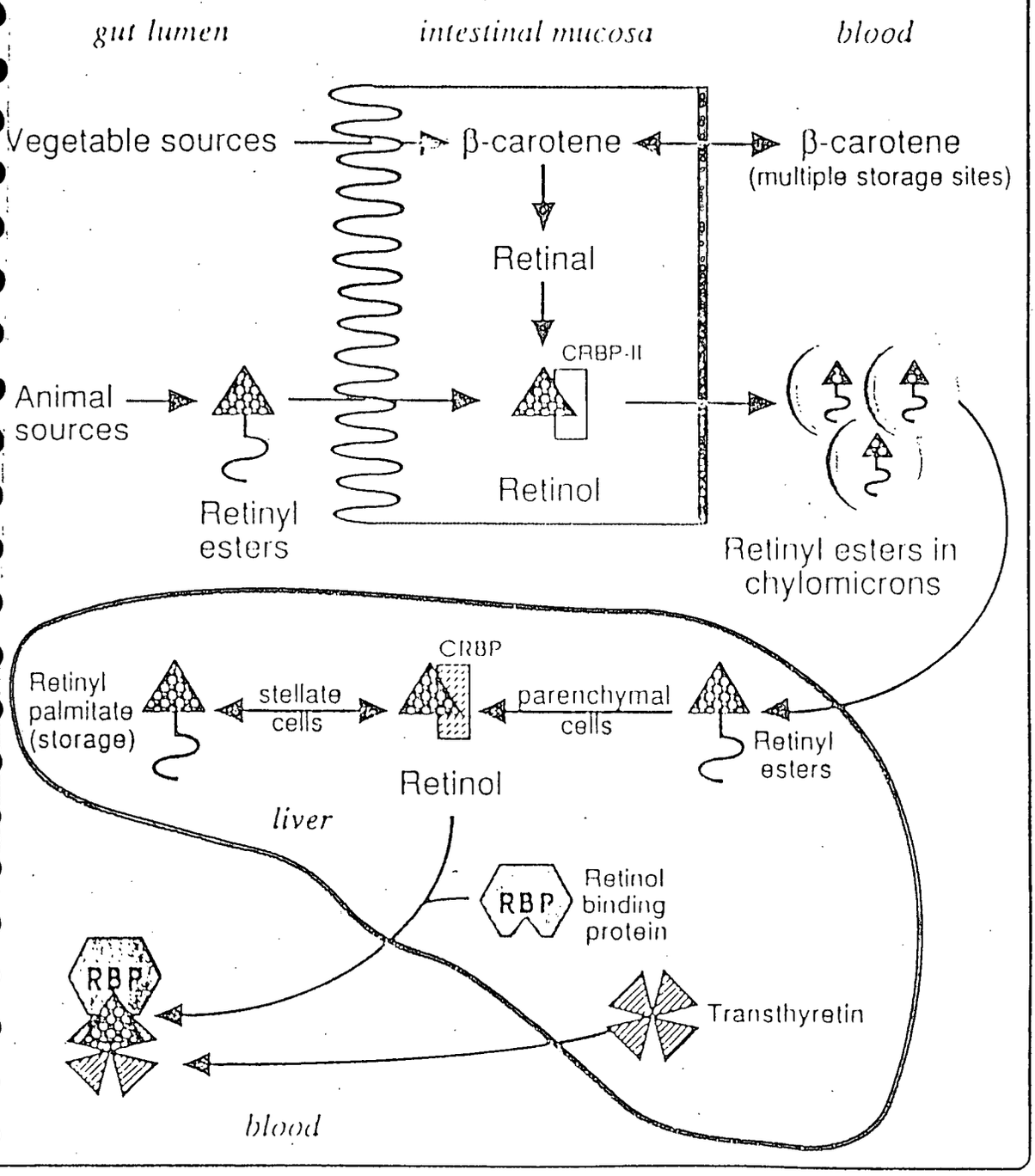
g. 31 VITAMIN A METABOLISM I



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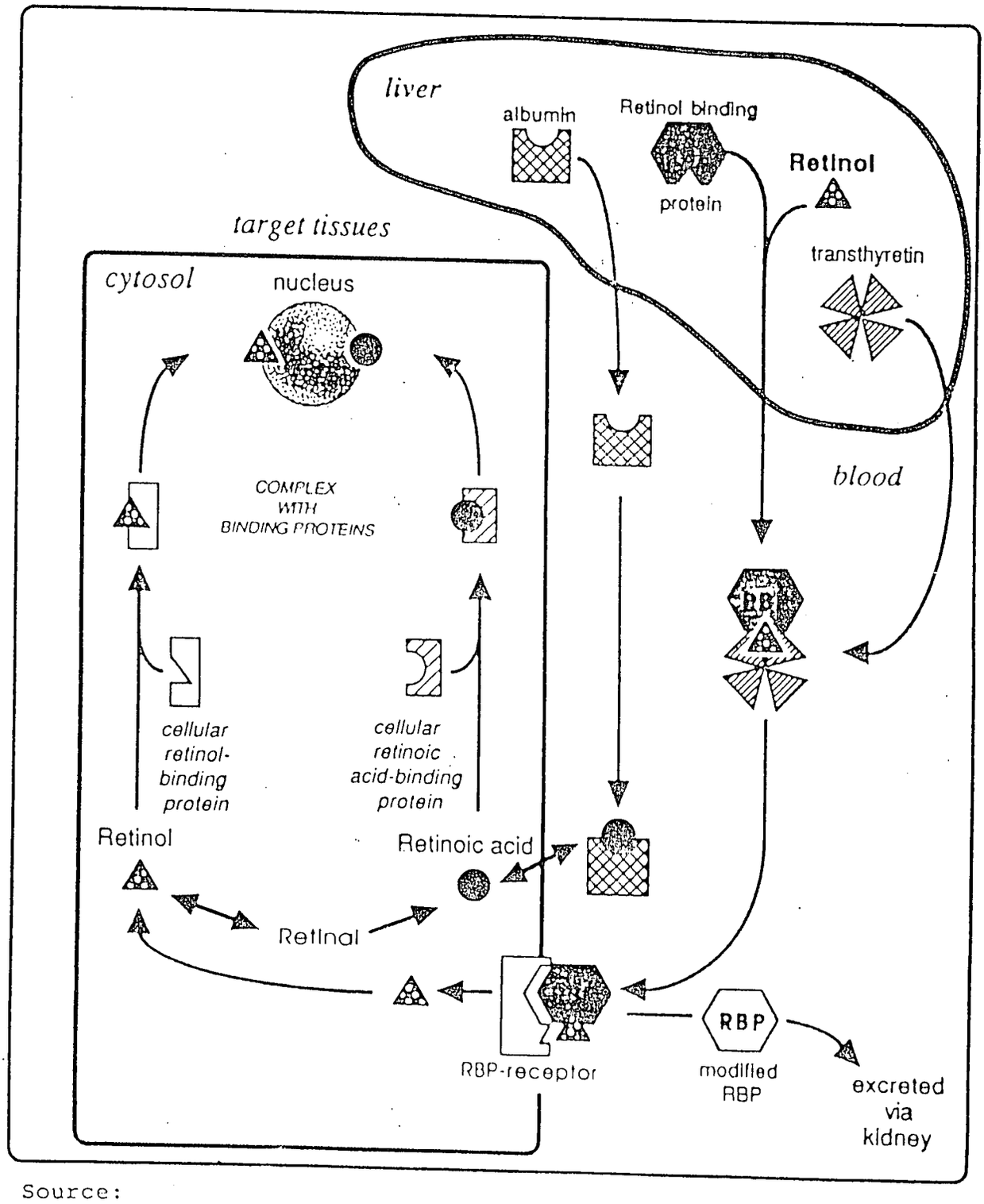
J Nutr Biochem , 1991, vol 2, September

Eig.32 VITAMIN A METABOLISM II



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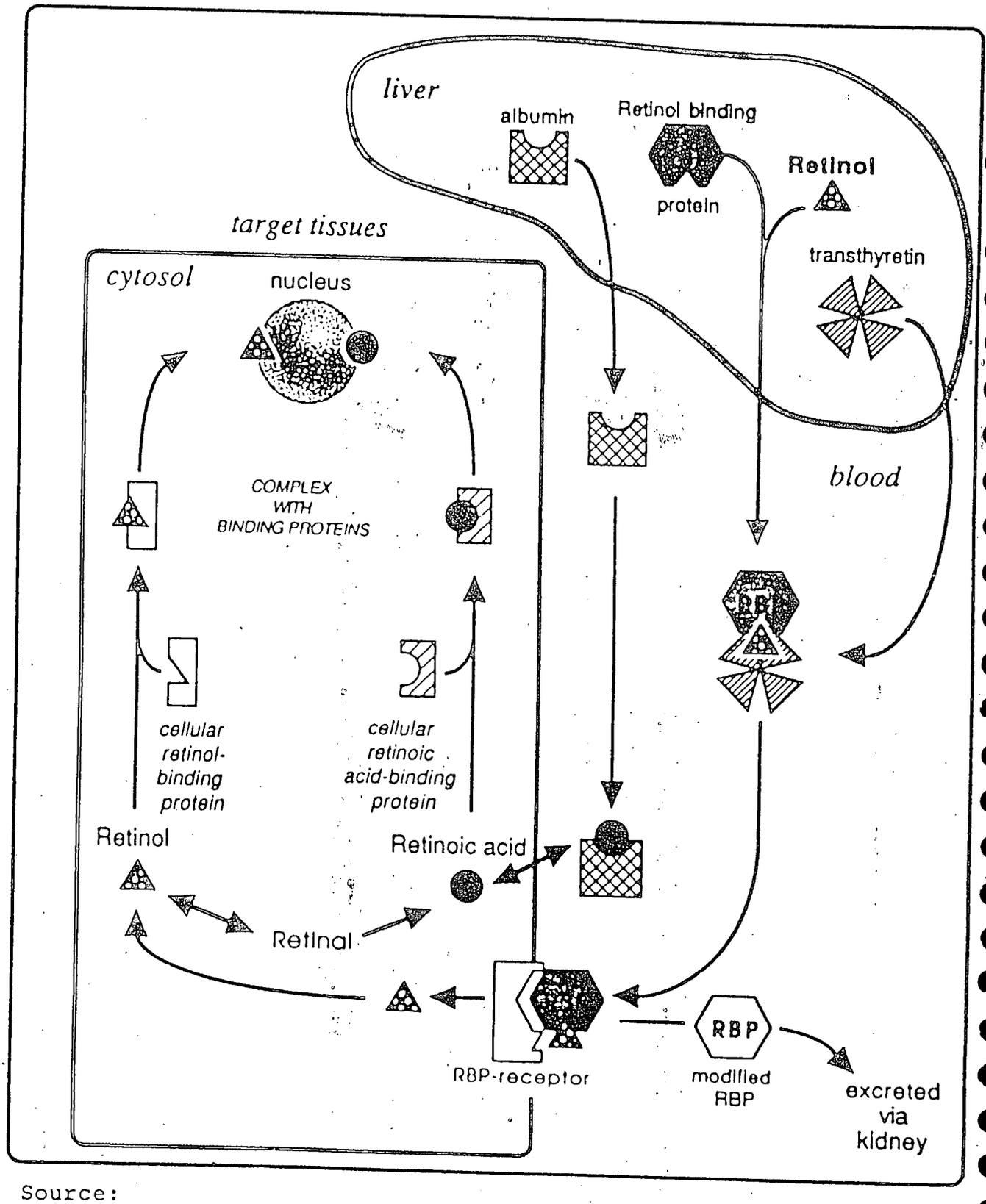

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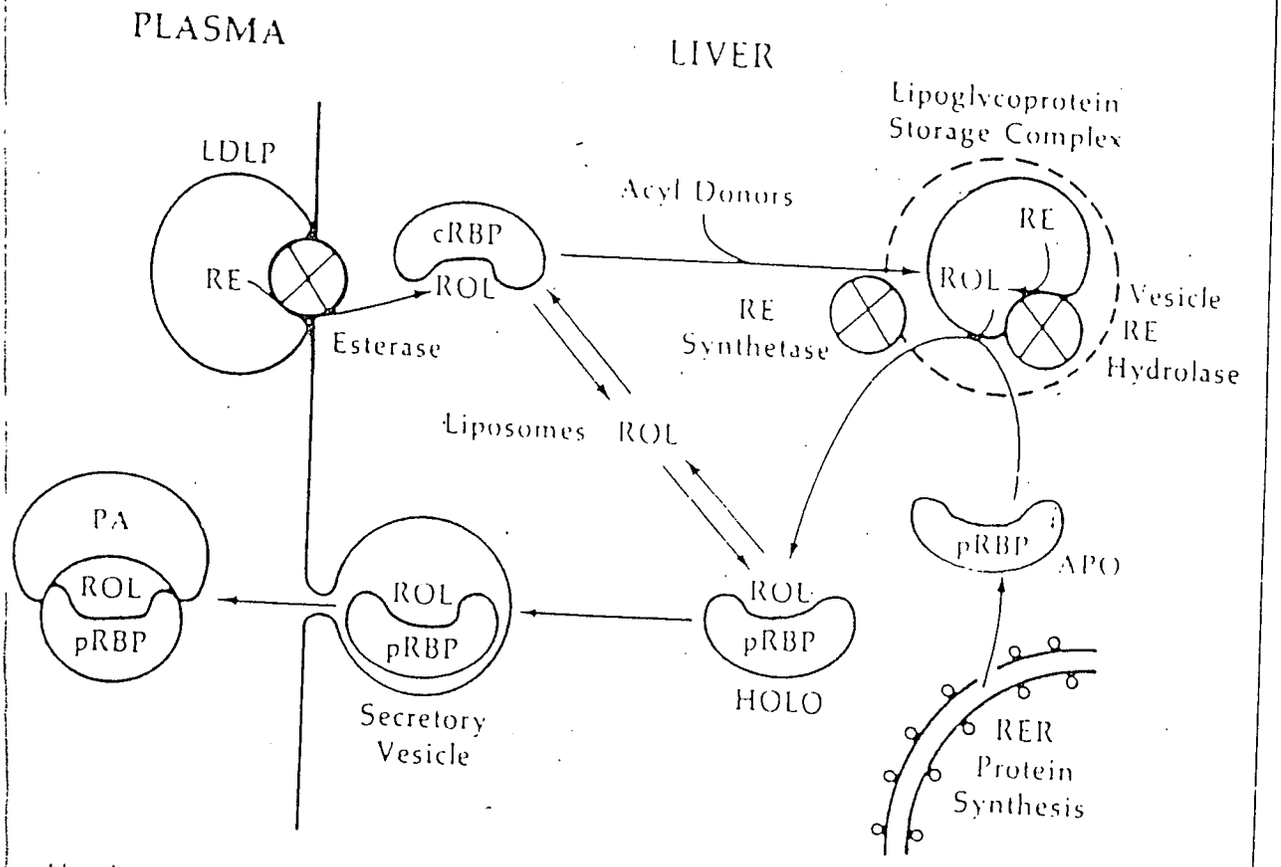
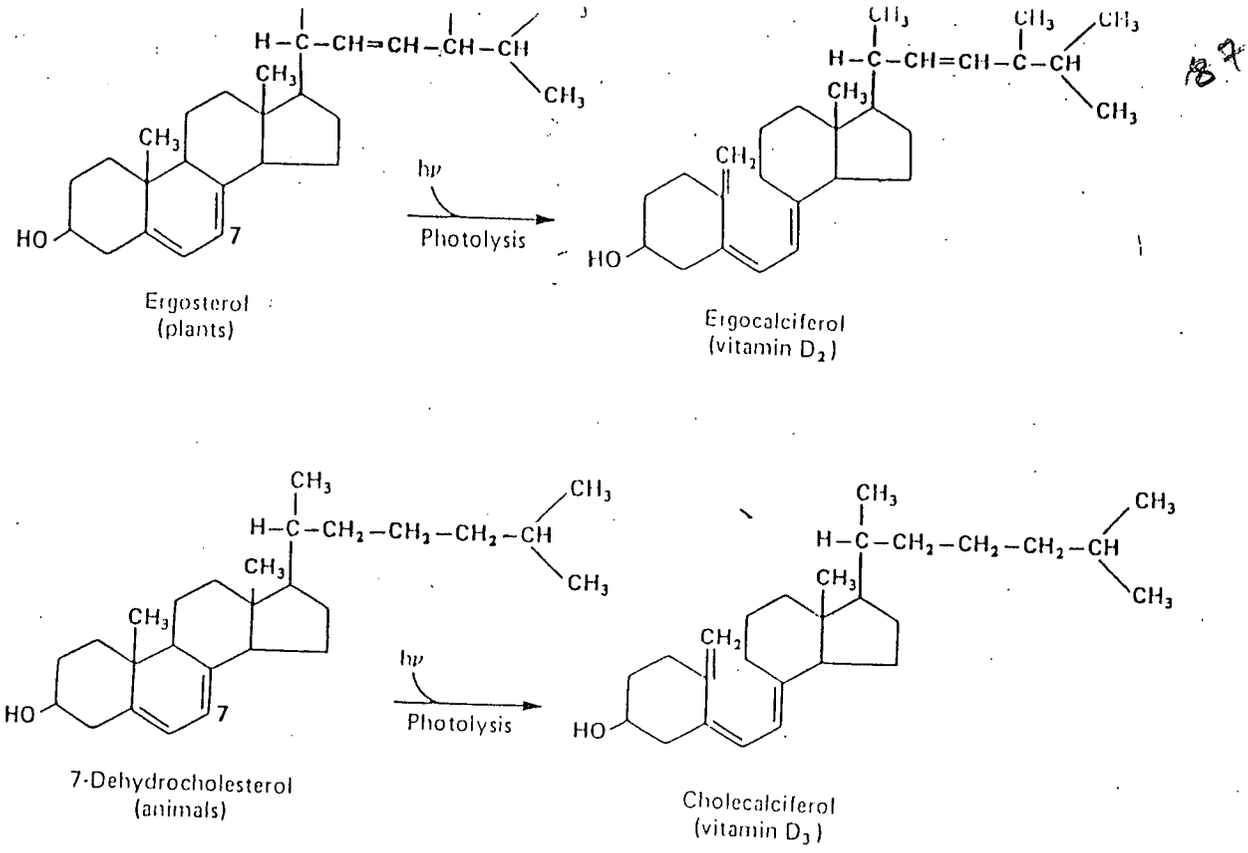
J Nutr Biochem., 1991, vol 2, October

FIGURE 33

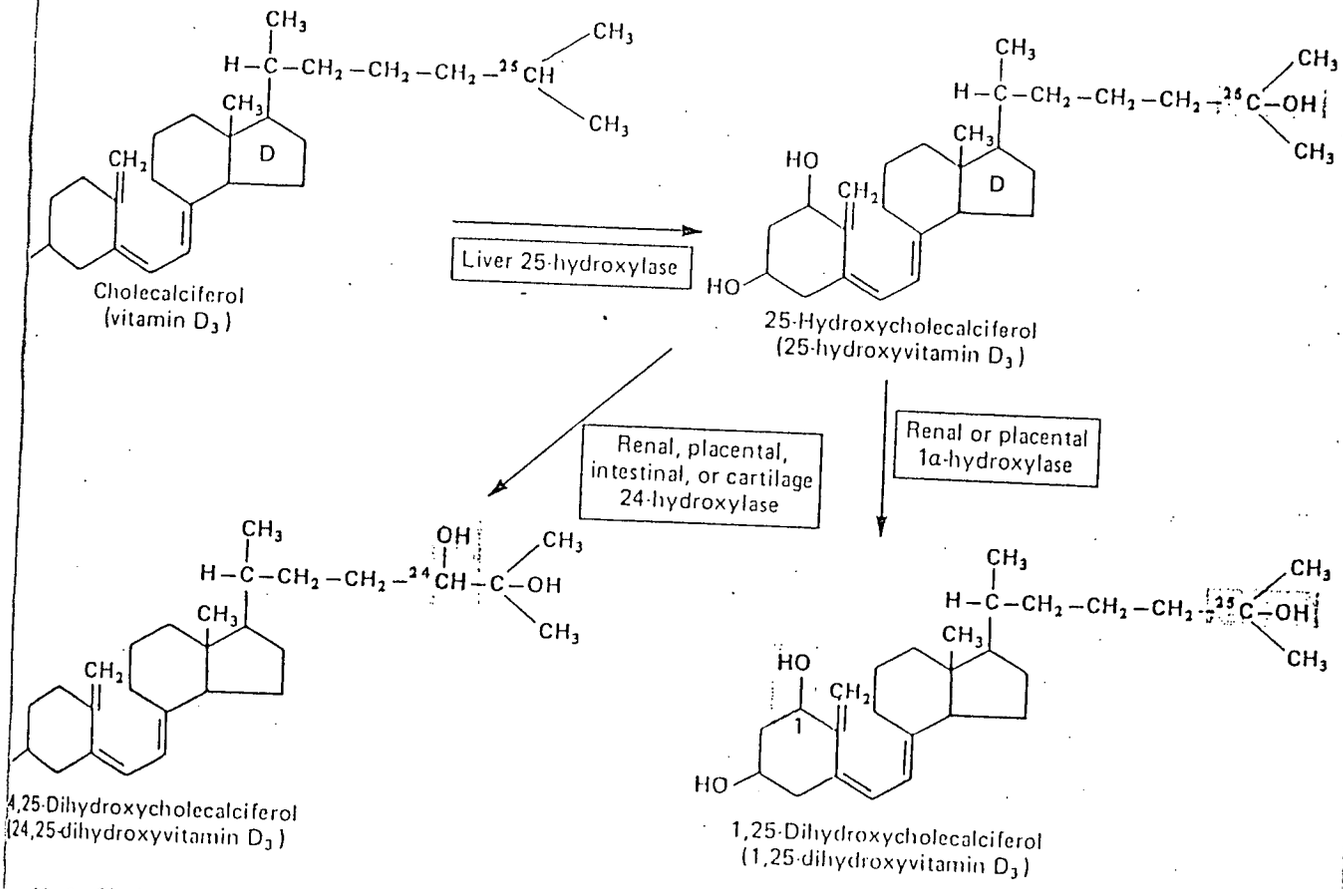


Uptake, storage and release of vitamin A from liver cells. Abbreviations are: ROL, retinol, RE, retinyl ester; cRBP, cellular retinol-binding protein, pRBP, plasma retinol-binding protein.

Source: DeLuca et al. In Recent Advances in the metabolism and function of vitamin A and their relationship to Applied Nutrition, IVACG, Washington DC, 1979



ure 11–5. Ergosterol and 7-dehydrocholesterol and their conversion by photolysis to ergocalciferol and cholecalciferol, pectively.



ure 11–6. Cholecalciferol can be hydroxylated at the C_{25} position by a liver enzyme. The 25-hydroxycholecalciferol is her metabolized to 1α,25-dihydroxycholecalciferol or to 24,25-dihydroxycholecalciferol. The levels of 24,25-dihydroxy-lecalciferol and 1,25-dihydroxycholecalciferol

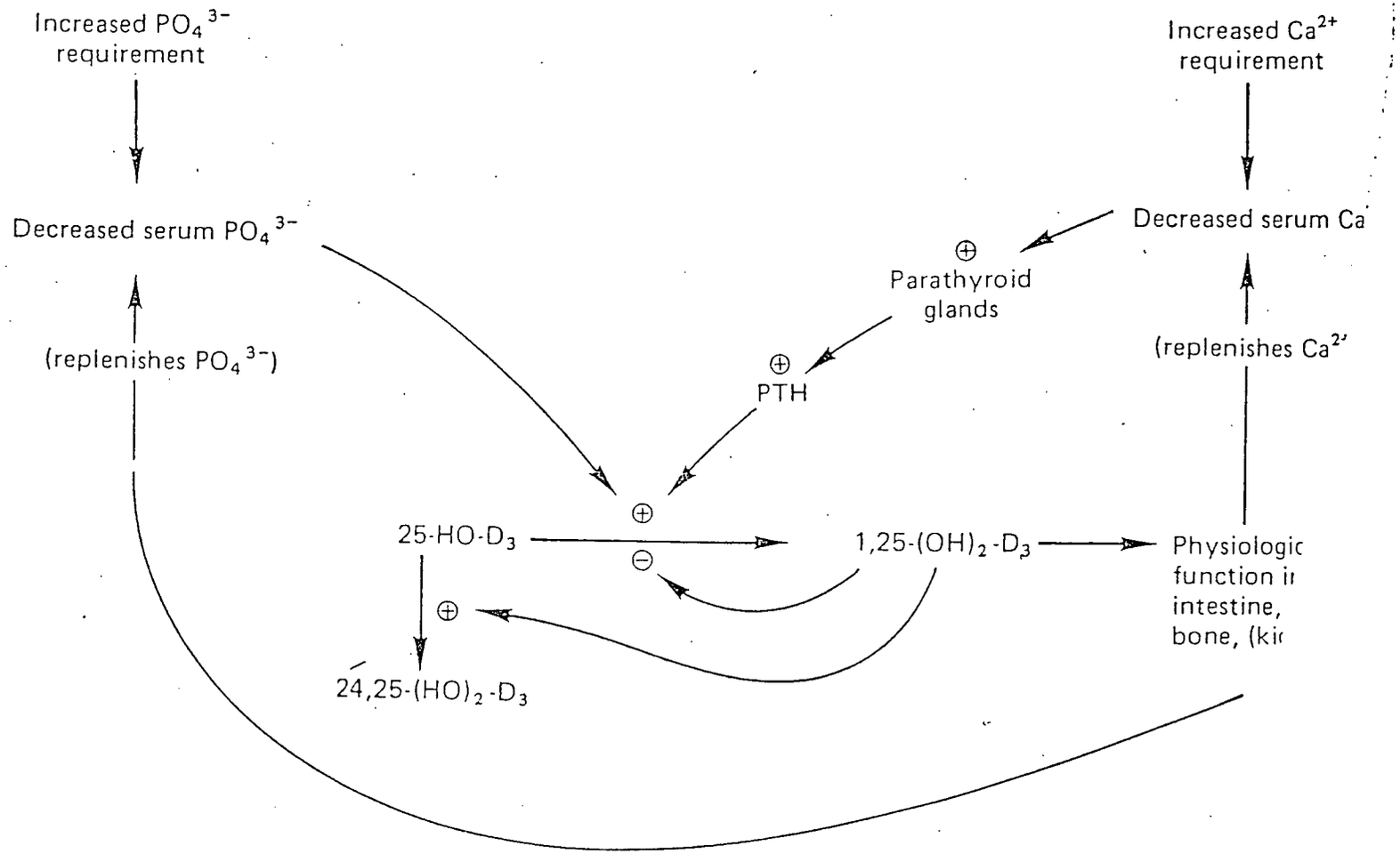

Increased PO_4^{3-} requirement
Decreased serum PO_4^{3-}
(replenishes PO_4^{3-})
Increased Ca^{2+} requirement
Decreased serum Ca
(replenishes Ca^{2+}
Parathyroid glands
PTH
25-HO-D_3
1,25-$(OH)_2$-D_3
24,25-$(HO)_2$-D_3
Physiologic function in intestine, bone, (kid

Table 41–16. Essential fat-soluble vitamins:

Vitamin/Provitamin[1]	Metabolism[2]	Active Metabolite: Physiologic Function
Vitamin A Provitamin: β-carotene Vitamin: retinol	Transported in lymph as retinyl esters in blood bound to retinol-binding protein and prealbumin.	11-*Cis* retinal: constituent of rhodopsin and other light-receptor pigments. Unknown metabolite(s) (retinoic acid?): required for growth and differentiation of epithelial, nervous, and bone tissues.
Vitamin D Provitamins: ergosterol (plants, yeast) and 7-dehydrocholesterol (skin) Vitamins D_2 (ergocalciferol) and D_3 (cholecalciferol)	Provitamins converted to vitamins by ultraviolet irradiation. Vitamins hydroxylated in liver to 25-hydroxy-vitamin D and in kidney to 1,25-dihydroxy-vitamin D and other metabolites.	1,25-Dihydroxy-vitamin D_3 is major hormonal regulator of bone mineral (calcium and phosphorus) metabolism.
Vitamin E tocopherols	Generally unknown.	Active metabolite unknown. Functions as an antioxidant.
Vitamin K K_1 (phylloquinone), K_2 (menaquinone), others	Generally undefined.	Active metabolite unknown but probably hydroquinone derivative. Activates blood clotting factors II, VII, IX, and X by γ-carboxylating glutamic acid residues; also carboxylates bone and kidney proteins.

ASSESSEMENT INDICATORS OF VITAMIN A STATUS

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Assistant Professor, PGRC, H.Sc. Hyderabad-30

Liver samples

The nutritional indicator for vitamin A status has been defined as the total body content of retinol, with the minimal acceptable reserve set at 20 ug/g of liver. Under most circumstances, the liver has been found to contain 90% or more of the total body vitamin A.

Vitamin A status can best be divided into five categories: deficient, marginal, satisfactory, subtoxic and toxic. Liver retinol concentrations characteristic of the deficient, marginal and satisfactory states are <5 ug/g, 5-19 and ≥20 ug/g, respectively.

Subtoxic and toxic states can not be appropriately characterized by liver reserves. Asssement of vitamin A status is difficult because of the manner in which the vitamin is stored, circulated and metabolized.

Xerophthalmic signs of deficiency

The general term used for vitamin A-dependent ocular involvement is "xerophthalmia", meaning "dryeye" (Greek). The world health organization has classified xerophthalmia into primary and secondary signs.

According to this classification, the primary signs include:

1. Conjuctival zerosis (XIA)
2. Bitot's spot with conjuctival xerosis (XIB)
3. Corneal zerosis (X2)
4. Corneal ulceration with xerosis (X3A)
5. keratomalacia (X3B)

The secondary signs, which are derived from vitamin A deficiency but may have other causes,include:

1. nightblindness (XN)
2. xerophthalmia fundus (XF)
3. corneal scars (XS)

Conjuctival Impression Cytology (micro xerophthalmia)

Hatchell and Sommer have developed a technique to determine the prevalence of goblet cells on the bulbar conjunctiva. This method is comonly called conjuctival

impression cytology (CIC). The number of goblet cells decreases in various tissues during vitamin A depletion, including the conjuctiva.

The method involves taking a cellulose acetate filter paper impression of the bulbar conjuctiva. These impressions are examined by light microscopy to observe the number of goblet cells and the number of enlarged epithelial cells.

The goblet cell population decreased and the number of enlarged epithelial cells increased before clinical signs of xerophthalmia appeared. Thus, by using this relatively noninvasive technique, ocular surface abnormalities due to vitamin A deficiency can be detected before xerophthalmia is evident.

Marginal Vitamin A status

Although vitamin A deficiency can be assessed by classical eye signs of xerophthalmia, a marginal status is more difficult to detect.

In the past,serum retinol concentration has been used; however, serum retinol is homeostatically controlled throughout a wide range of liver values. Retinol concentration is only diagnostic when it falls below 0.35 umoles/L (10 ug/dL) or above 1.4 umol/L (40 ug/dl).

Thus, it is not a good general indicator of vitamin A status because it does not decrease significantly until liver reserved are very low.

Techniques which detect marginal vitamin A deficiency before clinical signs are manifested are of significant value. In this regard, several techniques for the assessment of vitamin A status other than clinical deficiency have been developed.

Rapid dark adaptation

Thornton developed a method to measure the rapid dark adaptation time of individuals. The test, done under nighttime lighting conditions, measures the time that it takes for a subject to sort a pile of white, blue and red chips with 100% accuracy.Vitamin A-depleted subjects tend to take longer to sort the chips than normal subjects.

A dark adaptometer uses a regular 100 W light blub. When the light blub is on, a white sphere is illuminated. After 2 minutes of bleaching the eye, the

light goes off and a screen opens up. On the screen is a single letter. A timer starts when the light goes off.The time when the child correctly identifies the letter is noted.

This technique has been used in children 6 years and older.

The theory behind Dose Response tests

In the liver, apo-retinol binding protein (apo-RBP) synthesis is not controlled.Therefore during vitamin A depletion, apo-RBP accumulates in the liver.

Although retinol is the preferred ligand, 3, 4-didehydroretinol (dehydroretinol, vitamin A_2) is a suitable signal for RBP release.Thus, the retinol or or 3 ,4-didehydroretinol binds to apo-RBP in the liver and the holo-RBP complex then circulates in the plasma.

Moreover, after a suitable oral dose,retinol or dehydroretinol should appear in significant amounts in the plasma only when endogenous liver retinol concentrations are inadequate, i.e., when liver reserves of vitamin A are <20 ug/g.

Relative Dose Response Test

The relative dose response (RDR) test is a good indicator of marginal vitamin A status.The RDR assay has been validated in humans using direct measures of liver vitamin A.

The RDR involves giving a standard oral dose of 450 ug (1.57 umol) of retinol equivalents dissolved in oil.The dose has also been given intravenously as a water-dispersed suspension to children with liver disease.

Two blood samples are taken at time 0 and 5 hours after the dose.

After lipid extraction of the serum samples, the serum vitamin A concentration is measured by use of high-performance liquid chromatography (HPLC) or or a suitable colorimetric method.

The response is measured in the serum as a percentage $(A_5-A_o/A_5 \times 100)$,where A_5 is the serum retinol concentration 5 hours after the dose and A_0 is the concentration at time 0. Theoretically, the value can be 0 to 100%. A response of 20% or higher is indicative of inadequate liver reserves (<0.07 umol/g liver; <20 ug/g liver).

The RDR test has been used with suscess in several studies, including France, Brazil, Thailand and Belize.

The Modified Relative Dose Response Assay

An analog of retinol (R),3, 4-didehydroretinol (DR), is used as the indicator in the MRDR test. A single dose of 3, 4-didehydroretinyl acetate in oil is given orally. A molar ratio of dehydroretinol/retinol (DR/R) in serum is determined 4-6 hours after dosing. MRDR values theoretically can range from 0 to ∞.

Specifically, the MRDR assay involves first giving children a single oral dose (100 ug/kg body weight) of 3,4-didehydroretinyl acetate dissolved in an oil and then taking a single venous blood sample 4-6 hours later. After the serum is extracted with ethanol/hexane, retinol (R) and dehydroretinol (DR) in an aliquot are measured by high-performance liquid chromatography (HPLC).

Children with DR R ratios of $\geq$0.030 are judged to be in a marginal vitaminA status. The suggested cutoff value of 0.030 requires further validation.Tentatively, a public health problem might be assumed to exist if >20% of a population of preschool children show abnormal (>0.030) MRDR ratios.

30 days response test

This test is used mainly for assessment of populations. Two blood samples are also needed for this test at 0 time and 30 days after a massive dose (200,000 IU).

Typically a shift in population serum A has been observed.

Deuterated vitain A assay and/or tritiated

Isotope dilution assays using heavy vitamin A and mass spectrometry have been conducted.

Several groups have studied the use of radioactive tritium (^3H) - labeled vitamin A in rats, sheep and cattle with promising results. Tritium has also been used in humans, however, without verification by liver analysis.

Furr and coworkers have suggested the use of the non-radioactive deuterated analogs of vitamin A in place of the tritiated analogs. The serum ratios of

labeled to unlabeled (D/H ratios) can be determined using capillary gas chromatography-mass spectrometry (GC-MS). These studies have been verified with analysis of surgical liver biopsies.

HPLC ESTIMATION OF SERUM RETINOL

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A. EXTRACTION OF SERUM FOR RETINOL

The serum sample is treated with an equal volume of ethanol or methanol to denature the serum proteins.An internal standard, typically retinyl acetate, can either be added to the serum directly as a known quantity in a small amount of ethanol or can be added directly to the alcohol phase used for extraction. The serum is then mixed briefly. The serum is extracted two three times with an equal volume of hexane.After hexane is added, the sample is vortexed briefly and then centrifuged to expedite layer separation. The organic layers are pooled and evaporated to dryness under a stream of argon or nitrogen. The sample is resuspended in a known amount of solvent suitable for HPLC injection.

Supplies: Ethanol

Hexane

Internal standard (retinyl acetate solution)

Inert gas (argon or nitrogen) for sample concentration

Glass test tubes

1.5 ml microfuge tubes

1. Pipet 100-500 ul serum into glass test tube (the amount of serum used depends upon the volume of the original sample). As little as 100 ul of serum has been used. Duplicates are recommended but not always available.

2. Add 100-500 ul ethanol directly to serum (an equal volume of the serum). Internal standard may be added at this stage. Vortex 1 minute to denature proteins (referred to as aqueous phase).

3. Add 1000 ul hexanes to aqueous phase. Vortex 1 minute to begin extraction of lipids (including retinol). Centrifuge to expedite separation of phases. Carefully transfer organic layer to microcentrifuge tube.

4. Extract 1 or 2 more times with 500 ul hexanes (the number of extractions will depend upon extraction efficiencies and environmental conditions). In more tropical countries it is generally better to limit the number of extractions to two. This shortens the time of processing the samples.

5. Pool the hexane extracts and evaporate to dryness under a slow stream of inert gas, i.e. argon or nitrogen. Take care to not splash the sample.

6. Redissolve the residue in 50 ul of appropriate solvent (i.e., 4:1 isopropanol: methylene dichloride). Vortex centrifuge and keep sample cool until injection.

IV. HPLC ANALYSIS OF RETINOL AND 3,4- DIDEHYDRORETINOL

Separation of retinol and retinyl acetate depends on partitioning between the stationary phase (column packing) and mobile phase (solvent). Exact composition of the mobile phase for optimum resolution in minimum time will depend on the particular column used.

Supplies: Octadecylsilane (C^{18}) HPLC column,

with appropriate guard column
HPLC pump, injector, absorbance detector
recorder/integrator, HPLC fittings
Mobile phase (methanol: water, typically 95:5)

1. Filter the mobile phase through a 0.45 um Nylon 66 filter.

   This removes particulates and degasses the mobile phase

2. Establish flow at 1.0 ml/min, and allow the column packing to equilibrate and the detector to warmup. Confirm that detector baseline is stable.

3. Inject standard (volumes within a range of 1 to 25 ul, concentrations to cover the range of analytes expected), to confirm that detector response is linear. Confirm that all analytes of interest are well resolved from one another, the solvent front, and any other samples components.

4. Inject samples.

V. DATA ANALYSIS

Construct a standard curve for each analyte, plotting peak versus amount of standard injected. (Alternatively, peak heights may be used, if retention times are consistent. Peak areas may be estimated as (peak height) x (peak-width-at-half- height). Determine the best-fit- straight line

$$Y = mx + b$$

using a calculator or computer program, or by eye if necessary. (Y= peak area or peak height, x = mass injected, m= slope of the line, b = y-intercept of the line). Confirm that the best fit line is linear over the range of interest, and that it passes through or near the origin (0,0).

Determine mass of sample from its peak area (or peak height) either directly from the graph, or from the relationship

$$x = (y-b)/m$$

correct the analyzed mass for the fraction of sample actually injected and the volume of serum (or plasma) extracted, and calculate the concentration in serum (or plasma).

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ESTIMATION OF CREATININE

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Principle

Creatinine reacts with alkaline picric acid solution to form creatinine picrate which is yellow to orange in colour and is read colorimetrically.

Reagents

1. 1% picric acid solution
2. 10% sodium hydroxide
3. Standard creatinine: 1 gm creatine is dissolved in sufficient amount of 0.1N HCl and made upto 1 litre, to contain 1 mg/ml creatinine.

Procedure

	Standard	Experimental	Blank
Urine	-	0.25 ml	-
Distilled H_2O	-	-	0.25 ml
Standard creatinine	0.5 ml	-	-
1% picric acid	20 ml	20 ml	20 ml
10% NaOH	1.5 ml	1.5 ml	1.5 ml

Mix well and allow to stand for 15 minutes. Dilute with water and make upto 100 ml. Mix thoroughly and read the colour at 520 nm.

Calculations

$$\text{Creatinine (gm/dl)} = \frac{\text{Reading of standard}}{\text{Reading of sample}} \times \frac{\text{mg of creatine in standard}}{\text{Vol. of urine}} \times \text{Total output of urine /day}$$

Interpretation

Normal daily excretion of creatinine in normal adults ranges from 1.0 to 1.8 g, and may be considerably higher under certain conditions. The value is nearly constant from day to day, being influenced by diet, only to the extent that the diet itself contains significant amounts of creatinine, eg. a meat diet.

Its excretion is not influenced by the level of nitrogen metabolism in the body. It appears to be entirely a waste product and is excreted almost quantitatively when ingested or injected.

The relative constancy of creatinine excretion on a creatinine-free diet appears to reflect some constant metabolic process involving body creatinine. Since the bulk of the creatinine in the body is in the muscles, there is a rough correlation between creatinine excretion and that of muscle tissues. Obese individuals excrete less creatinine relative to their body weight, than lean individuals. The mg. of creatinine excreted daily per kg. of body weight is known as creatinine coefficient. It has a normal range of 19 to 30. If it is expressed as the number of mg. of creatinine nitrogen excreted daily per kg. body weight, the normal range will be 7 to 11. Exercise may cause a small increase in excretion, as a result of release from muscle stores. Excretion also varies with age.

Urinary excretion is elevated in the early stages of muscle wasting diseases, and falls to below normal levels when the musculature becomes atrophic. It is increased in fever and is little affected by other metabolic disorders. It has greatest value in determining the completeness of 24 hour collection or the rate of blood flow through the kidney.

Reference : Hawk's Physiological Chemistry (4th edition). Ed. Bernard L. Osser p. 1233-37

ESTIMATION TOTAL SERUM PROTEIN & ALBUMIN RATIO

Introduction

A major function of plasma protein is to aid in the normal distribution of water between blood and tissues.

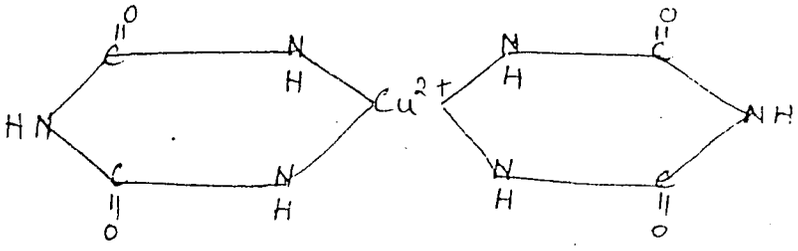
Normal values of plasma proteins are as follows:

Albumin 4.6 - 6.7 g/dl
Globulin 1.2 - 2.3 g/dl
Fibrinager 0.3 - 0.6 g/dl

Odema occurs when the total plasma proteins fall below the critical level of 5.3 g/dl. Increased plasma protein levels are noted in dehydration due to diminished fluid intake eg. in diarrhoea, vomitting, excess burns).

Principle : The principle of Biuret reaction is used in this method. The intensity of colour produced is proportional to the number of peptide bonds and hence to the concentration of protein. Cupric ions in an alkaline medium forms a violet coloured complex with peptide nitrogen. Biuret is formed by the condensation of two molecules of urea when treated at 180°C (NH_2 CO NH $CONH_2$).

The minimum requirement for a positive test is the presence of two peptide bonds in the molecule.



Copper-biuret compl

The differences in the solubility of albumin and globulins in salt solutions are used advantageously in their differential estimation.

Albumin is estimated in the supernatant after precipitating the globulins with sodium sulphite.

Reagents

1. Biuret reagent : 45 g of sodium, potassium, tartarate in dissolved in 400 ml of 0.2 N NaOH. 15 g of $CuSO_4.5$ H_2O in powder form is added to the solution by stirring. After the copper salt dissolves 5 g of Potassium Iodide is added and the solution made upto 1 litre with 0.2 N NaOH.

2. Standard protein solution

A solution of bovine albumin containing 2 mg protein/ml.

3. 28% sodium sulphate
4. 0.9% sodium chloride

Procedure

Pipette out 0.2 ml of serum into a test tube. Add 5.8 ml of 28% sodium sulphate solution. Mix by inversion and allow to stand for 5 minutes. Filter through Whatman No.44 dry filter paper for estimation of albumin. Set up fo test tubes.

	Tube 1	Tube 2	Tube 3	Tube 4
Distilled H_2O	3 ml	-	-	-
Standard protein solution	-	3 ml	-	-
Serum	-	-	0.1 ml	-
0.9% Sodium chloride	-	-	2.9 ml	-
Sodium sulphate filtrate	-	-	-	3 ml
Biuret reagent	3 ml	3 ml	3 ml	3 ml

After 10 minutes, measure the colour in a spectrophotometer at a wavelength of 540 nm.

Calculations :
(Subtract blank value (tube 1) from all values)

1. Total protein g/dl $= \frac{\text{OD of tube 3}}{\text{OD of tube 2}} \times 6 \times \frac{100}{0.1} \times \frac{1}{1000}$

2. Albumin g/dl $= \frac{\text{OD of tube 4}}{\text{OD of tube 2}} \times 6 \times \frac{6}{3} \times \frac{100}{0.2} \times \frac{1}{1000}$

3. Globulin = Total protein - Albumin

Estimation of Riboflavin status by Erythrocyte Glutathione Reductase Activity

Ref: Rayoumi R.A. and Rosalki B. 1976.
Clin.Chem. 2 : 327

Principle

Glutathione Reductase catalyses the reduction of oxidised glutathione to reduced glutathione, which can be measured by monitoring the oxidation of NADPH at 340 nm.

$$\text{Ox Glutathione (GSSG)} + \text{NADPH} + \text{H} + \xrightarrow[\text{Reductase, FAD}]{\text{Glutathione}} \text{Red. Glutathione (GSH)} + \text{NADP}^+$$

The activity is measured in the presence and absence of added FAD and expressed as percentage activity coefficient.

Reagents

1. Phosphate buffer pH 7.4

a) 2.722 g KH_2PO_4 is dissolved in distilled water and made upto 200 ml and stored at 4^oC.

b) 22.8 g of $K_2HPO_4.3\ H_2O$ is dissolved in water and made upto 1000 ml and stored at 4^oC.

The two components were allowed to reach room temperature. 100 ml of component (b) is taken in a beaker and titrated with component (a) to a pH of 7.4 and stored in a refrigerator at 4^oC.

2. Sodium bicarbonate 0.1 M

8.4 g of $NaHCO_3$ was dissolved in distilled H_2O and made upto 1 litre. It was prepared fresh every 14 days and stored at 4^oC.

3. 50 mM GSSG solution

155 mg of GSSG (sigma) is weighed into a small beaker. 5 ml of distilled water and 50 ul of 0.8 M (32 g/l) NaOH are added, mixed well and placed in an ice bath. The solution has to be prepared fresh daily.

4. FAD solution (250 u mol/L)

1.0 mg of FAD (sigma) is dissolved in 5 ml of distilled water and protected from light. It is prepared fresh daily.

5. NADPH + H^+ solution (4 m mol/L)

33.2 mg of sigma NADPH was dossolved in 10 ml of 0.1 M $NaHCO_3$ solution, mixed well and kept in an ice-bath. It is prepared fresh daily.

6. Potassium-EDTA solution (80 m mol/L)

Preparation of Haemolysate

(To 200 ul of the frozen red cells, add 3.0 ml of phosphate buffer. Mix well and centrifuge).

The capillary tubes containing erythrocytes were cut at the phase of red cells and plasma. The portion containing red cells was measured, and 200 ul of the cells were crushed with a glass rod, and 3 ml of phosphate buffer was added to it, and centrifuged for 15 minutes, to separate out the crushed glass tube.

Procedure

The tubes were set as follows :

Reagent	Tube 1	Tube 2
Phosphate buffer	2.00	2.10
Haemolysate	0.10	0.10
GSSG	0.10	0.10
FAD	0.10	-
EDTA	0.05	0.05

The tubes were incubated at 37°C for 15 min. following which 0.01 ml of NADPH + H^+ solution was added to each tube. The reaction rate was constantly monitored at 340 nm for 5 min and linear absorbance change was measured with a 3 sec, dwell time. Haemoglobin was also estimated by cyanmethhaemoglobin in 50 ul of hemolysate.

Calculation

One unit of activity is expressed as u moles of NADPH oxidised/hour/ml of red cells.

A = decrease in absorbance/min at 340 nm.

Molar Extinction coefficient of NADPH = 6.22×10^3

$$\text{EGR activity} = \frac{A \times 2.45 \times 16 \times 60}{6.22 \times 0.1} \times A \times 3781 \text{ u/ml}$$

Results should be expressed as basal and stimulated activity and activity coefficient in the ratio of stimulated to basal activity.

A = Decrease in absorbance in one minute at 340 nm. Activity coefficient (A.C.) is the ratio of stimulated to basal activity.

$$\text{A.C.} = \frac{\text{Reduction in absorbance with added FAD}}{\text{Reduction in absorbance without FAD}}$$

...

HAEMOGLOBIN

CYANMETHAEMOGLOBIN

PRINCIPLE

Hb is converted into Cyanmethaemoglobin by the addition of KCN and ferricyanide. The color of cyanmethaemoglobin is read in a photoelectric colorimeter at 540nm against a standard solution. Since cyanide has the max. affinity for Hb this method estimates the total Hb.

Reagent:

Drabkin's solution:- Dissolve 0.05g of KCN, 0.20g of potassium ferricyanide and 1.00g of sodium bicarbonate in 1lit of distilled water.

Procedure:

Blood 20 ul is transferred with the help of a Hb pipette into a test tube containing 5 ml of Drabkin's solution. The tubes are mixed and reading taken in a photoelectric colorimeter(Klett-Summerson) using 54 filter. The reagent blank (Drabkin's diluent) is adjusted to zero.

Construction of a standard curve:

(i) Since the amount of iron present in Hb per unit weight is constant, one can calculate the Hb conc. of a given blood sample by determining its iron content. This blood sample can be used as a reference standard for Hb estimation.

(ii) A standard curve can also be constructed by using the standard cyanmethaemoglobin solutions(supplied by BDH or V.P Chest Institute, Delhi).

Standard solution(ml)	Drabkin's diluent(ml)	Hb conc (%)
5	0	100
5	2.5	67
5	5.0	50
5	7.5	40
5	10.0	33

The suppliers of the standard solutions mention the conc. of the standard on the ampoule. The corresoponding blood Hb in g/100 ml can be obtained by multiplying the concentration on the ampoule by the dilution factor (251)

Note

1) Drabkin's solution should be stored in amber colored bottle. If any precipitate is formed, the reagent should be discarded.

2) Since the dilution is enormous(251 times) accurate measurement of 20 ul of blood is absolutely essential for reproducability. Hb pippettes must be checked for their accuracy by weighing pure mercury upto the mark.

Reference

Practical haematology, J.V. Dacie and S.M.Lewis(Eds),Churchill Livingston, V Edition P 32 (1975)

Filter paper technique

The CMH method has been modified to suit the needs of field investigations. 20 ul of blood are measured accurately by a Hb pippette and delivered on to a Whatman No.1 filter paper disc. The filter paper is air dried,labelled and brought to a laboratory.

In the laboratory the portion of the filter paper containing the blood is cut out and dipped in 5 ml of Drabkin's solution in a test tube. After 30 minutes, mix the contents on a vortex mixture and take the readings with 54 filter as described above. The filter paper disc containing blood can be stored upto one week.

Reference

Annual report, NIN, P 153 (1974)

CLINICAL ASSESSMENT

Clinical Assessment

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Clinical assessment consists of a routine medical history and a physical examination to detect physical signs and symptoms associated with malnutrition by a qualified examiner. These assessment procedures are normally used in a community nutrition surveys and they are useful during the advanced stages of nutritional depletion. Many of the critical physical signs are non-specific and must therefore be interpreted in conjunction with laboratory, anthropometric and dietary data before specific nutritional deficiency can be identified.

The physical examination for clinical assessment, as defined by Jelliffe (1991) examines "those changes believed to be related to inadequate nutrition, than can be seen or felt in superficial epithelial tissue, especially in skin, eye, hair and buccal mucosa or in organs near the surface of the body".

These physical signs indicate the nutrient deficiency, and when used to determine the nutritional status, one should take into account atleast two caveats.

1. The leisions appreciable on physical examination are among the very last to appear during the development of malnutrition; therefore the physical examination cannot be relied upon to reveal the early stages of malnutrition.

2. Tissues have very limited ways of responding to trauma, illness or deficiencies, thus many signs observable on physical examination are non-specific, definitive diagnoses of nutritional deficiencies should always be based on the aggregate of data available.

Limitations of the physical examination

Clinical assessment is generally done by a clinician in a hospital, whereas in community surveys of other than a clinician has to do, they should be given careful training in recognising the critical signs and ongoing supervision is necessary.

Non-specificity of the physical signs

This is a major limitation, especially in mild or moderate deficiency states. Some physical signs may be produced by more than one nutrient deficiency. Other physical signs may be caused by non-nutritional factors such as eczema, allergic manifestation and weather or in some cases heridity.

Multiple physical signs

Subjects with co-existing nutrient deficiencies eg. protein and zinc deficiency, riboflavin, niacin and vit. C deficiency may exhibit multiple physical signs, confusing the diagnosis.

Signs may be two-directional

Signs may occur during the development of a disease and during recovery also. eg. enlarged liver occurs in PEM and during its treatment.

Examiner inconsistencies

The recording of certain leisions may be subject to inconsistencies. Examiners with very limited experience may record leisions in subject with mild or border line evidence of the leisions other, more experienced examiners may record only severe forms. Generally, these inconsistencies are less when symptoms are severe. The following table indicate the inconsistencies recorded by three examiners.

The prevalence as a percentage of selected physical signs by three examiners

Number of examination	Examiner		
	1 1123	2 1127	3 589
Papillary atrophy	4.1	1.1	11.2
Follicular hyper keratosis	4.0	0.6	6.8
Swollen red gums	2.8	3.7	4.1
Angular leisions	0.4	0.4	1.2
Glossitis	0.6	0.4	0.5
Goiter	3.6	6.6	3.6

Examiners bias can be minimised by standardising the criteria used to define the physical signs and by training the examiners. Inconsistencies also arise of the physical leisions are graded during the physical examination. It is desirable, not to grade the severity of physical signs in the field and it is desirable simply to record as positive or negative, except if measured objectively using instruments.

Variation in the pattern of physical signs

There are no universal set of signs and symptoms suitable for all ages and all countries. The pattern of physical leisions associated with specific nutrient deficiencies varies according to genetic factors, activity level, environment, dietary pattern, age and the degree, duration and speed of onset of malnutrition. Examples of such variations shown in the following table. All these limitations of physical examination confound the diagnosis, making it essential to include laboratory tests to confirm the existence of specific nutrient deficiencies.

Variation in the percentage prevalence of clinical signs associated with nutritional deficiencies with age

Deficiency	Clinical signs	Age years		
		0-6	6-16	16+
PEM	Abnormal hair	2.1	0.6	0.3
Iron + B vits.	Papillary atrophy	3.3	4.4	6.2
Vit. A	Follicular hyperkeratosis	3.1	4.9	3.9
Vit. D	Enlarged wrists	2.8	-	-
Vit. C	Swollen red gums	0.6	5.9	8.5
Ribo-flavin	Angular leision	1.4	0.9	0.6
	Glossitis	0.4	2.0	8.3
Iodine	Visible enlargement of thyroid	1.1	5.4	5.5

References

Gibson R S (1990). Principles of nutritional assessment. Oxford University Press.

Jelleffe D B (1991). The assessment of the nutritional status of the community. WHO Monograph No.53, WHO, Geneva.

Mc Granity W J (1974). The clinical assessment of nutritional status. In: Hawkins W W (ed) Assessment of nutritional status. Miles Symposium II. Miles Laboratories Limited, Rexdale, Ontario pp. 47-64.

Knight M A (1987). The nutrition physical examination. CRN Quarterly 11:9-12.

World Health Organisation (1963). WHO Expert Committee on Medical Assessment of Nutritional status. WHO Technical Report No.258. WHO, Geneva.

Table Physical signs in nutrient deficiency and the possible mechanism of their formation.

Deficiency	Physical signs	Mechanisms for signs
Calories (marasmus)	Sunken temples, prominence of bone structure, loss of muscle strength, sunken eyes	Loss of muscle mass, subcutaneous and retro-orbital (=behind the eye) fat
Protein (kwashiorkor)	Hair dull and dry, easily plucked; dry, thin, scaly skin (flaky paint dermatosis); loss of muscle mass; poor wound healing	Inadequate protein for new tissue growth
	Edema	Inadequate plasma protein levels; inappropriate growth hormone and corticosteroid secretion; increased capillary permeability.
	Parotid enlargement	Inappropriate growth hormone and corticosteroid secretion.
	Enlarged liver and spleen	Poor liver protein production, transport, and release
	Apathy or irritability	Inadequate fuel supply to the brain; amino acid and electrolyte imbalance
Essential fatty acids	Scaly skin lesions; rough, dry skin; poor wound healing	Inadequate essential fatty acid supply for membrane, prostaglandin and leukotriene formation leading to poor skin growth and repair
Vitamin A	Dry skin; xerophthalmia (=dry eyes); Bitot's spots (=piling up of epithelium near cornea); keratomalacia (= softening of the cornea); follicular hyperkeratosis (=sandpaper skin);	Abnormal growth of epithelial cells with conversion from columnar to undifferentiated squamous (=flat) cells, and ? failure to develop receptors for epidermal growth factor
	Night-blindness	Failure to form rhodopsin
Folate	Atrophy of tongue papillae (tongue appears to be shiny and red)	Decreased rate of tissue renewal due to poor formation of DNA
Vitamin B12	Atrophy of tongue papillae	Decreased rate of tissue renewal due to poor formation of DNA
	Stocking-glove loss of sensation in hands and feet	? Decreased ability of nervous tissue to form neurotransmitters (e.g. epinephrine)
Thiamin	Beri-beri: heart enlargement and failure; neurological changes (sensory loss, loss of reflexes, burning sensations in hands and feet); muscle tenderness and loss of muscle strength; Wernicke-Korsakoff syndrome (see Chapter 16)	Defective delivery of energy to tissues due to defective carbohydrate metabolism (failure to metabolize pyruvate)
Biotin	Scaly skin lesions; rough dry skin	? Poor metabolism of essential fatty acids; ? poor metabolism of phenylalanine

Zinc (cont'd)	Hypogonadism	Lack of active enzyme for testosterone formation
	Growth failure; alopecia (=hair loss)	Failure of active enzyme formation for new tissue growth
	Anorexia, hypogeusia (=loss of taste)	? Inability to form active taste buds
	Pallor	Iron-deficiency type anemia, due to lack of formation of porphyrins
	Night-blindness	Poor conversion of vitamin A to active forms
Selenium	Cardiomyopathy (=disease of the heart muscle)	? Failure to maintain intracellular redox potential in tissue with high oxidative phosphorylation rates
Molybdenum	Tachycardia (=rapid heart rate), tachypnea (=rapid breathing rate), neurological disturbances	? Sulfur amino acid intolerance, due to lack of active sulfite oxidase
Iodine	Lethargy; dry skin with abnormal doughy consistency; sparse hair with coarse texture; slowness of thinking	Lack of thyroid hormone
	Enlargement of the thyroid gland;	Excess accumulation of non-iodinated thyroid hormone precursor and excess stimulus for thyroid tissue formation in the absence of feedback by thyroid hormones to the pituitary, so excess thyroid stimulating hormone is released.

Riboflavin	Abnormalities of the skin where the skin and mucous membranes meet: redness and fissuring of the corners of the eyelids the mouth, the vulva and the anus, scaling around the nostrils and scrotum; deep red beefy tongue;	? Abnormal oxidation-reduction function leading to poor tissue repair at sites of mechanical and chemical stress and sites of constant new tissue generation
Niacin	Pellagra(redness and scaling where the sun damages the skin); weakness, anorexia diarrhea, mental changes (confusion and depression);scarlet raw tongue	Inability to repair damaged tissue, especially DNA repair Note that the metabolic abnormalities in niacin deficiency have been difficult to define.
Pyridoxine	Redness and fissuring around eyes; pellagra-like picture	Impairment of intermediate metabolism; the function of other vitamins (niacin, is dependent on pyridoxine
	Depression	? Failure to decarboxylate glutamic acid; failure to transaminate amino acids
Vitamin C	Scurvy: bleeds in skin (=petechiae) and gums; bone pain due to bleeds under the periosteum,; swelling at the ends of bones, giving (among other signs) a "rosary" consisting of swellings at the points where the bony parts of the ribs join the cartilage	Impaired collagen formation with difficulty regenerating blood vessel walls (note that in daily life we are constantly having microtears in blood vessel walls that are constantly and rapidly repaired)
Vitamin D	Bone softening, swelling at the growing ends of long bones, rachitic rosary, Harrison's groove (where the diaphragm is attached to the ribs and draws the softened ribs inward), softening and bulging of the bones of the skull, and in an infant failure to close the fontanelles; knock knees and bowed legs;	Failure to absorb calcium and to form well structured new bone.
Vitamin E	Neurological changes consistent with a loss of spinal cord function; ceroid (wax-like) pigment deposition in smooth muscle; muscle destruction	Failure of anti-oxidation
Vitamin K	Bleeding	Failure to form clotting factors
Copper	Pallor	Anemia due to failure to form hemoglobin
	Skeletal deformities	Poor bone formation due to poor collagen formation and poor bone mineralization
	Kinky hair with abnormal pigmentation	Poor formation of extracellular tissue matrix
Iron	Blue sclerae (=whites of the eyes), pallor especially of the conjunctivae,	Failure to form hemoglobin
	Koilonychiae (=spoon nails)	Failure to form proper keratin
Zinc	Infections of the skin and mucous membranes; diarrhea	Depressed cell-mediated immunity

(V)

A GUIDE TO THE CLINICAL SIGNS OF MALNUTRITION*

*SOURCE: Jelliffe, D.B. The Assessment of the Nutritional Status of the Community, World Health Organization, Geneva, 1966.

CLINICAL EXAMINATION

Clinical examination is an important practical method for assessing the nutritional status of a community.

The method for clinical examination is usually based on examination for changes, believed to be related to inadequate nutrition, that can be seen or felt in superficial epithelial tissues, especially the skin, eyes, hair and buccal mucosa, or in organs near the surface of the body, such as the parotid and thyroid glands.

Clinical assessment of a community can give valuable information to the public health worker, especially in regions of the world where malnutrition is widespread.

CLASSIFIED LIST OF SIGNS USED IN NUTRITION SURVEYS

According to the report of the WHO Expert Committee on Medical Assessment of Nutritional Status, the signs and symptoms that are considered to be of value in nutritional assessment are recorded as follows:

	Signs known to be of value in nutrition surveys
(1) Hair	- Lack of lustre - Thinness and sparseness - Straightness - Dyspigmentation - Flag Sign - Easy pluckability
(2) Face	- Diffuse depigmentation - Naso-labial dyssebacea - Moon face

(3)	Eyes	- Pale conjunctiva - Bitot's spots - Conjunctival xerosis - Corneal xerosis - Keratomalacia - Angular palpebritis
(4)	Lips	- Angular stomatitis - Angular scars - Cheilosis
(5)	Tongue	- Oedema - Scarlet and raw tongue - Magenta tongue - Atrophic papillae
(6)	Teeth	- Mottled enamel
(7)	Gums	- Spongy, bleeding gums
(8)	Glands	- Thyroid enlargement - Parotid enlargement
(9)	Skin	- Xerosis - Follicular hyperkeratosis - types 1 and 2 - Petechiae - Pellagrous dermatosis - Flaky-paint dermatosis - Scrotal and vulval dermatosis
(10)	Nails	- Koilonychia
(11)	Subcutaneous tissue	- Oedema - Amount of subcutaneous fat
(12)	Muscular and skeletal systems	- Muscle wasting - Craniotabes - Frontal and parietal bossing - Epiphyseal enlargement (tender or painless) - Beading of ribs - Persistently open anterior fontanelle - Knock-knees or bow legs - Diffuse or local skeletal deformities - Deformities of thorax (selected) - Musculo-skeletal haemorrhages
(13)	Internal Systems: (a) Gastro-intestinal	- Hepatomegaly

(b) Nervous*	- Psychomotor change - Mental confusion - Sensory loss - Motor weakness - Loss of position sense - Loss of vibratory sense - Loss of ankle and knee jerks - Calf tenderness
(c) Cardio-vascular	- Cardiac enlargement - Tachycardia.

* The symptoms described under this section may be seen in lathyrism and 'tropical neuropathies'. Further confirmatory tests for these will be required in community surveys.

INTERPRETATION

The relatively easy organization and inexpensive nature of nutritional assessment by means of clinical examination may make it appear that the method is simple, quickly mastered by the beginner and yields results that are easy to interpret. This is not the case. Various non-nutritional environmental influences may be responsible for identical appearances. Further, most signs of malnutrition are not specific to lack of one nutrient and can often be produced by various non-nutritional factors.

However, the fact that most clinical signs are non-specific does not preclude their use as indices of malnutrition. The interpretation of clinical signs can be best made by using a 'grouping of signs' which have been commonly found to form a pattern as associated with the deficiency of a particular nutrient.

The following section discusses the groupings of clinical signs and their interpretation with respect to certain nutritional deficiency diseases.

GUIDE TO THE INTERPRETATION OF GROUPINGS OF CLINICAL SIGNS

(1) Protein-Calorie malnutrition (PCM)

(a) PCM in adults and school children:

The usual signs are :

diminished subcutaneous fat, and
muscle wasting.

Other associated signs may include:

Parotid enlargement (especially in school children),
oedema of the ankles, and
gynaecomastia in males.

(b) PCM in young children:

The signs suggestive of PCM in young children are:

oedema,
dyspigmentation of the hair,
thin sparse hair,
straight hair,
muscle wasting,
diffuse depigmentation of the skin,
psychomotor change,
moon face,
hepatomegaly (enlargement of liver), and
flaky-paint dermatosis.

(c) Calorie overnutrition in children and adults (obesity):

The signs of calorie overnutrition are:

increased subcutaneous fat, and
increased abdominal girth.

(2) Vitamin A deficiency

The signs suggestive of Vitamin A deficiency are:

Bitot's spots,
conjunctival xerosis,
corneal xerosis,
keratomalacia,
xerosis of skin, and
follicular hyperkeratosis (type 1).

The clinical signs of avitaminosis A vary with age. In particular, keratomalacia is principally seen in infancy and the pre-school age group, often associated with PCM; while Bitot's spots and conjunctival xerosis are more common in school children.

Recent studies have suggested that both xerosis of the skin and follicular hyperkeratosis (type 1) are more usually due not to lack of Vitamin A but to deficiency of unsaturated fatty acids such as linoleic acid.

(3) Riboflavin deficiency

The signs suggestive of riboflavin deficiency are:

angular stomatitis (or angular scars),
cheilosis,
magenta tongue,
atrophic lingual papillae(smooth, glazed tongue),
dyssebacea,
angular palpebritis (angular blepharitis),
scrotal (or vulval) dermatosis, and
corneal vascularization

Recent work has indicated the non-specificity of corneal vascularization and has also sugested that it is more commonly due to causes other than ariboflavinosis.

(4) Thiamine deficiency

The common suggestive signs are

oedema,
loss of ankle and knee jerks,
motor weakness (Squatting test),
calf muscle tenderness,
sensory loss,
cardiac enlargement, and
tachycardia (increased palpitations).

Thiamine deficiency in babies (infantile beri beri) has a completely different picture. Usually it is one of convulsions and acute cardiac failure in the early months of life. Assessment of the incidence of this condition is difficult owing to the acuteness of the illness and because of the other diseases that produce a similar clinical picture at this age. The age-specific mortality rate between two and five months is a useful guide, while indirect evidence may be gained by estimating the thiamine content of breast milk.

(5) Niacin deficiency

The suggestive signs are :

pellagrous dermatosis,
scarlet and raw tongue,
atrophic lingual papillae (smooth glazed tongue),
tongue fissuring, and
molar and supraorbital pigmentation

(6) Vitamin C deficiency

The signs suggestive of Vitamin C deficiency are:

spongy and bleeding gums,
petechiae (haemorrhages under the skin),
ecchymoses (blue stains on the skin due to petechiae),
follicular hyperkeratosis (type 2)
intramuscular or subperiosteal haematoma, and
epiphyseal enlargement (painful)

The clinical picture varies greatly with age. Infantile scurvy is characterized more by lassitude, anaemia, haematoma formation, especially subperiosteally, and painful epiphyseal enlargement, particularly at the costochondral junction. Spongy, bleeding gums do not occur in the absence of teeth.

When teeth are present, the commonest cause of bleeding gums in the absence of hypertrophy, is a varying degree of marginal gingivitis or pyorrhoea.

(7) Vitamin D deficiency

Active rickets (in young children) is typically suggested by:

epiphyseal enlargement (painless) - (over 6 months of age)
beading of ribs,
persistently open anterior fontanelle - (after 18 months of age),
craniotabes - (under 1 year of age), and
muscular hypotonia.

Healed rickets (in older children or adults) is suggested by:

frontal or parietal bossing,
knock-knees or bow-legs, and
deformities of the thorax (Harrison's sulcus and pigeon chest)

Osteomalacia (in adults) may give rise to:

local or generalized skeletal deformities, especially of the pelvis, with tender bones.

The lack of specificity of these signs has suggested that a minimum of three signs are needed for diagnosis, preferably backed by biochemical and radiographic evidence.

(8) Iron deficiency

Iron deficiency is suggested by:

pale conjunctiva,
koilonychia (in older children and adults), and
atrophic lingual papillae.

(9) Folic acid or vit. B_{12} deficiency

This condition is accompanied by pale conjuctiva due to anaemia

(10) Iodine deficiency

A deficiency of iodine produces enlargement of thyroid

(11) Fluorosis

An excess of fluorine is suggested by mottled dental enamel. Mottling of the finger nails may also found.

DIET SURVEYS

Methods of Diet survey

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Dietary studies are generally an integral part of most nutritional surveys. The main objective of any dietary assessment is to discover what the person under investigation is in the habit of eating over the long range and in the short run (Diva Sanjur, 1982). Dietary assessment has been adopted to a wide variety of emphasis and uses including appraising (a) how well fed specific segments of population are (b) providing baseline data for the development of programs on nutrition (c) fixing minimum wages & rations (d) constructing food guides and (e) formulating low-cost food plans; and providing a basis for food legislation.

Over the years, dietary survey data have been employed for a number of other purposes. Initially emphasis was on family food consumption studies, with later interest focussing on estimation of diets of individuals.

Dietary data on individuals are usually collected either to obtain information concerning average nutrient intake, food intake or to know food habits or to obtain estimates of nutrient intake of a given individual to .pa correlate with clinical or biochemical measurement obtained on that individual.

Dietary assessment is not direct measurement of nutritional status. As Maynand (1950) points out "they provide presumptive evidence as well as yield data which can assist in interpretation of the results of more direct measures of nutritional status such as laboratory and clinical tests and anthropometric measurements.

Methods of assessing food intake

Various dietary methods are used to measure and assess the food and nutrient intake of individuals and population groups, depending upon the purpose for which the data are to be used.They may be broadly classified as National level agreegate, institution, family and individual data system.

1. The agreegate data system include (a) Food balance sheets (b) Food disappearance (the net disapperance of food stuffs from the national supply for human consumption on an annual basis) and (c) Commodity

reports (estimates of several food commodities available for human consumption). They provide information on total food or specific commodities available for consumption by a country or area but are not definite in terms of individual food consumption.

Methods of diet surveys:

National level:

Food balance sheets:

To carry out the surveys at the national level, The usual method employed to know the food availability per individual is food balance sheets. The quantities of various foods available for consumption at the retail level per head of population is calculated from the total net supply figures.

Per capita food available in a country per day =

(Stocks at the biginning of the year + total food produced during the year + imports) - (Stocks at the end of the year + exports + amount used for seeds and industrial purposes + cattle & poultry feeds + wastage in harvesting/threshing/distribution/processing/storage) ÷ by

Total Population x 365

Merits:

(a) It gives a comprehensive picture of pattern of country's food supply

(b) Trends in food supply in a country is known; if compiled over different years

(c) Dietary pattern of the population can be known

(d) The availability of food supplies per person relative to other countries can be known

(e) Basing on this, administrators and planners can have a broad idea about the availability and deficit of food in the country and to take proper steps to remedy them

(f) Useful for food programme formulation/for rationing of food/for exceptional conditions

Demerits:

(a) The allowance is made for inedible portion only. No allowance is made for the wastage of edible portion between the retail level and consumption level

(b) Since the distribution and other things are not taken care of, and ony the average is calculated, the actual dietry consumption with not be known through the food balance sheets

(c) No information on seasonal variation/ different socio economic groups/ecological and geographical zones is obtained

(d) Accuracy depends on reliability of statistics of population/supply/non food supply/wastage etc.

II. Institutional level

To assess the food intake at Institutional level, two methods have been employed.

(a) Inventory method (food list method)

(b) Actual weighment method

Inventory method

In the inventory method, the food stuffs supplied as per the estimates of the warden of the institution are taken for calculation and there is no direct measurement.

The average intake per caput per day is calculated as follows.

(Stocks at the beginning of the week + stocks purchased during the week) (Stocks at the end of the week) ÷ by

Total number of inmates partaking the meal x No. of days.

This method though less time consuming, not very accurate, compared to actual weighment method.

Actual weighment method

In this method the total raw foods for cooking are weighed taking into consideration wastage of food. This procedure is repeated for at least seven days. To assess the intake per person per day, the following procedure is adopted.

$$\text{Intake in terms of raw food per person per day} = \frac{\text{Total raw amounts used for a preparation}}{\text{(Total No. of residents partaking the meal) x (No. of days of survey)}}$$

III. Household level:

Household food consumption methods measure all food and beverages available for consumption by a household, family group, during a specified time period. The following methods are used: food accounts, list-recall methods, inventory methods, food records, family food scales, and telephone surveys. All involve collecting information on demographic and socio-economic characteristics of the household, enabling data to be presented in terms of income level, family size, region of the country, etc. Per capita nutrient intake data are derived by multiplying the per capita food consumption data by the corresponding nutrient values obtained from appropriate food composition tables. Adult consumption units weighted according to age and sex and physiological status, are assigned to each family member, and used to calculate more precisely nutrient intake per person.

Food account method

A food account consists of a daily record, prepared by the householder, of all food entering the household, either purchased, received as gifts, or produced for household use during a specified period-usually seven days. Quantities of each food item are recorded in retail units (where applicable) and household measures. Generally, no account is taken of food and beverages consumed outside the home, or food discarded as plate waste, spoilage, or fed to pets. The respondent burden for the food account method is low, and it is relatively inexpensive.

List-recall method

In this method the householder is asked by a trained interviewer to recall all foods used by the household on an 'as purchased' basis, and their quantity,

price, or purchase value, over a specified period of time usually the preceding one to seven days. A structured questionnaire containing a list of major food items likely to be consumed is generally used to assist in the recall. Frequently, no account is taken of food wasted, spoiled, or fed to pets. Quantities may be estimated by weight or household measures.

Additional information on the age and sex of persons consuming the household food supply, the number of meals eaten both at home and away from home for each household member, income and other socio-economic characteristics of the household may also be obtained.

Only one interview, taking up to two and half hours, is reuired for this method, so that the field survey costs are lower than for other household methods, and the response rate is generally high.

Inventory method:

The inventory method aims at recording acquistitions and changes in the food inventory of households during the survey period. An inventory is prepared of the weight and type of all food commodities in the household at the beginning and end of the survey period, which is generally one week (Burk and Pao, 1976). In addition, the types and weights of all food items brought into the home, whether purchased, home produced, as gifts, or as payment in kind, are also recorded daily during the survey period.

The number and age of persons consuming each meal are often recorded on a daily basis during the survey, allowing detailed information on the mean food consumption per person during the survey period to be calculated.

Household food record method

Food records are usually completed over at least a one-week period, either by the householder or a fieldworker. During this time, the amount of all foods consumed at each meal is recorded separately, either by weight or household measure. Detailed descriptions of all foods (including brand names) and their method of preparation are recorded. For composite dishes the amount of each raw ingredient used in the recipe and the final weight of the prepared composite dish should also be recorded.

Generally, however, kitchen and plate waste, and food fed to pets, is not accounted for in this method.

Instead, an arbitrary wastage factor of 10% of all edible portions of the foods consumed is applied, as noted for the inventory and food accounts methods.

The amount of each food consumed by the entire household can then be divided by the corresponding total adult consumption units to provide food intakes per person. This approach produces a better estimate of the adequacy of the household food intake, particularly for families.

The weighed food record method is the most accurate of the household method.

Telephone survey

Telephone survey have been used to collect consumer information on the purchase and use of certain products. Telephone surveys cost on an average 40-50% of personal interviews.

Family food scale:

Some investigators have used scalogram techniques, such as a Guttman scale, to measure the complexity and diversity of food patterns of families (Chassay et al., 1967), particularly in less developed countries (Beaudry Darisme et al., 1972). The food scale is constructed from information on the frequency with which a range of food items or food groups is eaten.

It is cumulative in the sense that a diet which includes food items within a given scale step position (or score) will also include the items in all the preceding steps. Thus, once the position of a respondent on the scale is determined, it is possible both to predict the food items consumed and to specify the dietary complexity and diversity. Highly trained interviewers are not required, and information on a large number of subjects can be collected using this techinique (Cassidy, 1981). The method can be used for individuals (Schorr et al., 1972; Harrison and Bond, 1984) as well as households.

National food consumption surveys:

Several countries use household methods for their national food consumption surveys. Close attention must be paid to the sampling design of these surveys to ensure that a representative national sample is obtained which accounts for the influence on food intake of season, holidays, weekends, socio-economic status, and region.

Most national surveys utilize an arbitrary allowance (e. g. 10%) for food waste at the domestic level.

IV. Individual level:

Methods used for measuring food consumption of individuals can be classified into two major groups. The first group, known as quantitative daily consumption methods, consits of recalls or records, designed to measure the quantity of the individual foods consumed over a one day period.By increasing the number of measurement days for these methods, quantitative estimates of actual recent intakes, or for longer time periods, usual intakes of individuals, can be ontained. Assessment of usual intake is particularly critical when relationships between diet and biological parameters are assessed.

The second group of methods includes the dietary history and the food requency questionnaire. Both obtain retrospective information on the patterns of food use during a longer, less precisely defined time period. Such methods are most frequently used to assess usual intake of foods or specific classes of foods. With modification, they can provide data on usual nutrient intakes.

(a) Twenty-four-hour recall method:

In the twenty-four-hour recall method, subjects, their parents, or caretakers are asked by the nutritionist, who has been trained in interviewing techniques, to recall the subject's exact food intake during the previous twenty-four-hour period or preceding day. Detailed descriptions of all foods and beverages consumed, including cooking methods and brand names (if possible), are recorded by the interviewer. VItamin and mineral supplement use is also noted.Quantities of foods consumed are usually estimated in household measures and entered on the data sheet.

The flat slope syndrome may be a problem in the twenty-four-hour hour recall method (Gersovitz et al., 1978). In this syndrome, individuals appear to overestimate low intakes and underestimate high intakes.

A single twenty-four-hour recall is most appropriate for assessing average intakes of food and nutrients for large groups. When using a twenty-four-hour recall to characterize the average usual intake for a population group, the sample should be representative of the population under study, and all days of the week should be proportionately included in the survey. In this way, any day-of-the-week effects on food and/or nutrient intakes will be taken into account. The respondent burden is small for a twenty-four-hour recall, so that compliance is generally high. The method is quick, relatively inexpensive, and can be used with illiterate individuals.

The success of the twenty-four-hour recall depends on:

- Interviewer qualification, skills and training received
- Physical setting of the interview
- Presence of neighbours,accidental distractions
- Social class difference between interviewer and respondent
- Degree of interviewer's familiarity with respondent
- Rapport, the interviewer establishes with respondent
- Ability of the interviewer (a) to ask 'right' questions (b) to probe for details of consumption without using leading questions, (c) to converse freely with the respondent, without the need for interpreter and (d) to identify whether the respondent is providing reliable data or not.

Other factors are:

The respondents:

(a) Memory power to recall correctly

(b) Efforts to recall correctly

(c) Lack of eagerness or over eagerness to respond and impress the interviewer

(d) Ability to understand the questions of interviewer

(e) Ability to describe quantity cooked or consumed accurately

(f) Cooperation

(g) Knowledge of the purpose of the study being conducted

To minimize these biases, the interviewer must be trained in interviewing with warmth and an understanding attitude. The respondent must believe that he or she is understood and can trust the interviewer.

(b) Repeated twenty-four-hour recalls

Twenty-four-hour recalls can be repeated during different seasons of the year to estimate the average food intake of individuals over a longer time period (i.e usual food intake). This number of twenty-four-hour recalls required to estimate the usual nutrient intake of individuals depends on the degree of precision needed, the nutrient under study, and the population group.

In general, if an adequate sampling procedure is designed to take into account the influence of weekends, seasons and holidays on the pattern of food intake, the results can provide an estimate of national food consumption.

(c) Estimated food records

The respondent is asked to record, at the time of consumption, all foods and beverages (including snacks) eaten for a specified time period. Detailed descriptions of all foods and beverages (including brand names) and their method of preparation and cooking are recorded. Final weight of the dish, and the amount consumed by the subject should be recorded.

Food portion size can be estimated by the respondent using a variety of procedures, each differing in level of precision.Standard household measuring cups and spoons are used wherever possible, supplemented by measurement with a ruler (for meat and cake) and counts (for eggs and bread slices). Portion size measures are usually coverted into grams by the investigator before calculating nutrient intakes. Unfortunately, errors may arise as a result of the inability of the respondent to adequately quantify portion sizes consumed and as a result of difficulties associated with the conversion of volume estimates to quantities expressed in grams.

The number of days included in an estimated record varies; usually three, five, or seven days are used. Weekned days should be proportionately included in the dietary survey period for each subject, to account for

potential day-of-the-week effects on food and nutrient intakes.

(d) Weighed food records:

A weighed food record is the most precise method available for estimating usual food and/or nutrient intakes of individuals.

In a weighed record, the subject, parent, or caretaker is instructed to weigh all foods and beverages consumed by the subject during a specified time period.Details of methods of food preparation, description of foods, and brand names (if known) should also be recorded. Weights of all raw ingredients used in the recipe should be noted, as well as the weight of the portion consumed and the final volume of the dish. The method of recording is similar to that given for a household food record.

As for the estimated record, the number, spacing, and selection of days necessary to characterize the actual of usual nutrient intakes of an individual using the weighed method vary, depending on the nutrient of interest, study population, objective of the survey, etc. Again, weekend days should be proportionately included in the dietary survey period to account for any weekend effect on the nutrient intake.

Respondent must be motivated, numerate, and literate, if a dietary record method is selected. Respodents may change their usual eating pattern to simplify the measuring or weighing process, or alternatively, to impress the investigator. Respondent burden for food records is higher than for the twenty-four-hour recall, so the individual may be less willing to co0operate. Precision is greater in the weighed record compared to the estimated record method because the portion sizes are weighed.

(e) Dietary history

In general, dietary history methods provide qualitative, not quantitative, data on usual food intake over a period of several weeks or months.

Numerous modifications of the dietary history method exist. For example, portion size estimates can be made using a variety of techniques including common utensils, commercial plastic food models, standard measuring cups and spoons, photographs, or real foods.The time periods covered by the dietary history method may also vary. The maximum time period which can be used has

not been firmly established.When shorter time frames (i.e one month) are used, precision and validity are apparently higher than for longer periods.

The dietary history method is very labour intensive, and unsuitable for large surveys. Moreover, the results obtained depend on the skill of the interviewers.

(f) Food frequency questionnaire

A food frequency questionnaire is designed to obtain qualitative, descriptive information about usual food consumption patterns. It does not generally provide quantitative data on food or nutrient intakes. The questionnaire consists of two components: (a) a list of foods and (b) a set of frequency-of-use response categories. The list of foods may focus on specific groups of foods, particular foods, or foods consumed periodically in association with special events/seasons, when it is designated a focused questionnaire (Anderson, 1986). Alternatively, the food list may be extensive to enable estimates of total food intake, and hence dietary diversity, to be made.

The aim of the food frequency questionnaire is to assess the frequency with which certain food items of food groups are consumed during a specified time period (e.g. daily, weekly, monthly, early). Specific combinations of foods included in a focused questionnaire can be used as predictors for intakes of certain nutrients or non-nutrients, provided that the dietary components are concentrated in a relatively small number of foods or specific food groups. Examples include the frequency of consumption of fresh fruits and fruit juices as predictors of vitamin C intake; green leafy vegetables and carrots as predictors of carotenoid intakes.

The data for the food frequency method may be obtained by a standardized interview of self-administered questionnaire. The result generally represent usual intakes over an extended period of time and are easy to collect and process. The food frequency questionnaire imposes less burden on respondents than most of the other dietary assessment methods. It is often used by epidemiologists studying associations between dietary habits (both usual and past) and disease (Acheson and Doll, 1964; Hankin et al 1970; Hirayama, 1981), although its use for estimating food intakes in the remote past has not been clearly established.

The data from a food frequency questionnaire are often used to rank subjects into broad categories of low,

medium, and high intakes of certain foods, for example. In epidemiological studies, such rankings, are often compared with the prevalence and/or mortality statistics for a specific disease within the population studied.

Food scores can be calculated from food frequency data, based on the frequency of consumption of certain food groups.

Sometimes, the food frequency questionnaire attempt to quantify usual portion sizes of the food items. This modification produces semiquentitative procedure is used, nutrient scores of each subject can be computed by multiplying the relative frequency that each food item is consumed (with, for example, once a day equal to one), by the nutrient content of the average portion size specified. The nutrient content is obtained from appropriate food composition data.

Use of direct chemical analysis

Direct chemical analysis of representative samples of individual food items, meals, or prepared diet composites must be carried out if precise information on nutrient intakes of individuals is required for metabolic studies.This approach can also be used to validate food composition data obtained from tables or nutrient data banks (Gibson and Scythes, 1982).

Care must be taken to exclude contamination during sample collection, preparation, and analysis, particularly if trace elements are to be determined.Adequate storage and preliminary treatment of the sample are also essential to preserve the activity of certain vitamins (e.g. folic acid, thiamin, vitamin C. and riboflavin) (Cooke, 1983). The individual food items, meals, or composite diets must also be homogeneous, so that a representative aliquot can be removed for chemical analysis. Homogeneity can be checked by analyzing multiple aliquots of a single composite diet or food.

Three techniques are used for collecting meals or one-day diet composites for nutrient analysis (Pekkarinen, 1970).

Duplicate portions

This technique involves the collection of a duplicate portion of all foods and beverages consumed during a twenty-four-hour period. These are weighed, or estimated in household portions, by the respondent or a field worker. Several separate consecutive twenty-four-hour diet composites may be collected from each

respondent. Diet composites are then homogenized using a blender and later chemically analyzed.

Aliquot sampling of foods

This procedure involves weighing all foods and beverages consumed by an individual during the survey period, and collecting daily aliquot samples of each item. These samples are then combined and subsequently analyzed.Care must be taken in this method to ensure that representative aliquots are taken both during the collection and the subsequent pooling and homogenization.

Equivalent food composites

These are used less frequently than the two methods described above. In this method, weights of all foods and beverages consumed by an individual during the survey period are again recorded.Subsequently, samples of raw foods, equivalent to the mean daily amounts of foods consumed by each individual in the survey, are chemically analyzed. Thus only the nutrient content of the uncooked food is determined and consequently the method is not suitable for estimating the intake of heat labile and water-soluble vitamins.

Telephone survey

Both twenty-four-hour telephone recall and seven-day telephoned food records are feasible methods.

Photographic method

Elwood and Bird (1983) instructed subjects to photograph, at a specified distance and angle, all food items and leftovers, and to record descriptions of each foodstuff, including the method of preparation.Estimates of the weights of the food items consumed were obtained by viewing the photographs alongside previously prepared standard photographs of portions of foodstuffs of known weights.

The photographic method appears to be less demanding for the subject than the weighed record, and relatively easy and acceptable. Preliminary work suggests that nutrient intakes calculated from photographs and a weighed record are similar, provided that training for estimating weights from photographs is given (Bird and Elwood, 1983).

Electronic devices for recording food intakes

An electronic device has been developed by Stockley et al, (1986) for the quantitative collection of food intake data for individuals for periods of up to three weeeks. The device consists of a digital blance with a capacity of 1 kg interfaced to a microcomputer with a key board. The latter has an upper bank of color-coded sequency control keys which register 'start', 'waste', 'no waste', 'mixed waste' and 'done' and 55 color-coded food record keys. The keyboard is fitted with a removable transparent keyboard overlay, to assist in the correct identification of the food keys. An incorrect food key entry can be cancelled using the 'error' function.The device is fully compatible with a more powerful computer. Consequently, at the end of the study period, the accumulated data on the weights and types of food consumed and time of consumption can be transferred to a host computer for calculation of the nutrient composition of the diet.

The respondent burden is reduced when using the food-recording device because the subject does not have to read the balance or keep a written diary (Stockley et al., 1986). Moreover, the memory capacity of the device is sufficient to store dietary data from surveys lasting up to three weeks.The device also eliminates the process of coding the food records, a task considered to be the most time-consuming part of a quantitative dietary study (Black, 1982).

A portable electronic set of tape-recording scales (PETRA)

This has been developed in the United Kingdom. In this system, the respondent places an empty plate onto the PETRA digital recording scale, presses a switch in front of the machine and describes the plate. The scale simultaneously records the spoken words and the weight in digitally coded from. The respondent then adds each food item separately onto the plate and, at the same time, dictates a description of the food into the microphone. At the end of the study, tapes are retrieved and read by the investigator using the PETRA Master Console. The latter plays back the description of the food and displays the decoded weight information.The system is simple to operate,and it is difficult for the subject to modify the coded food record. Consequently, the habitual food intake is more likely to be truthfully recorded compared to the conventional weighed food record (Bingham, 1987).

All these developments aim to reduce respondent burden and hence increase compliance, reduce errors resulting from memory lapses, and in the case of

electronic devices, to eliminate the tedious process of coding the food records.

Selecting an appropriate method:

All the methods described above have its own uses and limitations. An important aspect is to consider carefully the information needed for a given purpose and which method provides the data within acceptable precision and cost parameters. All researchers would like to find a method that is simple, easy, inexpensive, valid, reliable and objective. To be valid, the method must truly mesure what the investigator wishes to measure.To be reliable, the instrument must give the same results on repeated trials of the same result as another widely accepted method.And to be objective, the method must be standardized, that is sytematically applied so that results can be compared with the work of other invetigators.

It is also important to know the intra individual variability in food or nutrient intake as well as the inter individual variability. Intra individual variability will influence the length of time an individual needs to be studied to get a true picture of intake. Inter individual variability is of particular importance in determining the size of the sample needed to meet the requirements of the study.

One of the major problem with dietary methods is that, the methods which are assumed to be most accurate are least feasible and most reactive (producing changes in the diet). While food weighing by investigator may be the most accurate means of determining nutrient intake, it is usually reserved for small samples due to high cost and excessive burden on respondents.Further, because of reactivity, weighed intake may not be a valid way to study food habits. Therefore most studies of "free-living" populations involve self-reporting by individuals, using methods that are easier to administer but less precise. Most commonly used are the 24 hour diet recall and diet record. The decision of which method to use for dietary investigation depends on (a) the type of study (b) sample size (c) feasibility (d) education of the respondent (e) funds available (f) Man power available (g) Time available and (h) other logistical consideration necessary to conduct the survey.

There is no 'deal' dietary method. There may be prefered methods for particular purposes.

Variations of dietary intakes observed by different researcher when 2 or 3 dietary methods are compared:

The review collected revealed that the validity of different methods for collecting data on dietary intakaes has been continuously criticized because in all the methods there are sources of error which are difficult to eliminate.

Although comparative studies of different dietary interview methods have been published during four decades, the results still seems to be inconclusive.The discripancies observed in different studies can be attributed to some extent on the differences is design of the studies and also due to methedological errors in some studies.

For example, to show the differences in study design to compare the weighment method with 24 hour recall is given below:

Thimmayamma and Hanumanth Rao (1969) in their study assessed the intake of individual by weighment method on 1st day and on the following day another trained researcher collected the same individuals previous day's dietary intake, by 24 hour recall. But in Teresita E. Valerio (1983), study the dietary intakes of family were weighed by a researcher on one day and the same day another researcher recalled the previous day's diet of the family. Methodology used for recall is also different in the two studies.

Though 'dietary recall' is termed as a method of diet survey and universally used, there is no standardized method of conducting it. Few researchers used visual aids Madden et al (1976) Elemas Quiogne (1970) Teresita E. Valerio (1983) used standardized volumetric measures to help the respondent to indicate the amount of used food or individual intake. Timmayamma and Hanumanth Rao (1969), Regina Sundararaj (1972), Shobha Tilve (1978) and other researchers even approached groceries or resturants where food was purchased by respondent and requested them to help in determining the food compositions and portion size. This could be one of the reasons for the discripencies observed in the results of different studies.Moreover the interview itself can be a source of human error and thus can limit the validity of the recall method. To recall food intake accurately is rather difficult and requires skill in the personal interview technique.

Assessment of nutrient intakes from food consumption data

Energy and nutrient intakes can be calculated from quantitative food consumption data by using food composition tables, or data which have been transferred to and maintained on a computer. In an attempt to provide a more regorous and accurate approach to the development of food composition databases, an international network of food data systems has been established.

Nutrient values derived from both food composition data and direct chemical analysis represent the maximum available to the body and not the amount actually absorbed and utilized.

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Focussed Ethnography As A Support To Diet Surveys

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Introduction

Diet surveys are conducted to assess the nutrient intakes of family members or individuals and to evaluate the same against the recommended daily dietary allowance figures for various nutrients - mainly, calories, proteins and micronutrients. The main purpose of diet surveys, however, is to use the information from these data for identifying the malnourished population groups especially preschool children, pregnant and lactating mothers requiring attention in terms of nutrition education with or without food supplementation.

Several reviews of programmes directed at improving the nutritional status of at risk groups have pointed out that traditional methods of nutrition education do not have the desired impact. In recent years, attempts have been made to utilize techniques and methods hitherto confined to the behavioural sciences or to other disciplines such as anthropology and ethnography. It has been realized that in order to achieve effective nutrition interventions, firstly, an extensive understanding of the existing socio-cultural matrix of the community under study is necessary. It is therefore, imperative that any technique selected for the purpose of such a study is culturally appropriate to elicit the needed information.

One technique which is fast catching on especially in the health and nutrition field is the FES (Focussed Ethnographic Studies) for which a manual has recently been developed by Dr.Pelto in Geneva. This manual especially aims at increasing the

nsumption of vitamin A rich foods in populations at risk of tamin A deficiency. However, it is important to assess whether is technique is applicable to and appropriate for the pulation being studied. The FES manual was evaluated recently a study aimed at understanding the socio-cultural patterns and actices which perpetuate vitamin A deficiency in a rural area Andhra Pradesh. This paper presents the details of the study nducted on the basis of the guidelines, strategies and ocedures detailed in the FES manual as well as the methods of a analysis. The paper also presents the finding of the study the rural areas of Andhra Pradesh.

The FES manual aims at the following objectives :

To identify significant sources of vitamin A and carotene rich foods in the study area;

To describe patterns of consumption of vitamin A rich foods particularly with respect to infants;

To identify cultural beliefs that influence consumption patterns;

To identify cultural, ecological and socio-economic factors that constrain or facilitate consumption of vitamin A foods; and

To describe the community explanations and understandings of vitamin A deficiency diseases and symptoms.

Information on the relevant aspects stated in the objectives he manual are necessary to formulate effective and meaningful rventions against vitamin A deficiency in population groups isk.

The FES manual is divided into 4 parts as follows :

Preparation of a community food source journal (Part I); Site selection, recruitment and training field assistants, as well as key informant interviewing (Part II); Description of specific research procedures (Part III); and Guidlines for preparation of report (Part IV).

I. The preparation of the community food source journal consists of : (a) Preliminary data collection on food category, local name, scientific names; (b) Food species identification of vitamin A rich foods; (c) Determining vitamin A content of foods; (d) completion of the journal with information on seasonality/ availability, and (e) Search for little used or known vitamin A rich foods. During the study, the data on the above aspects were collected by interviewing key informants and by examining the vitamin 'A' content of foods with specific reference to vitamin 'A' rich foods named by the respondents.

II. After selection of the study population, field assistants are trained especially in interviewing techniques. Key informants are individuals among the population groups who can influence target groups. They include health functionaries such as the ANM, AWW, local leaders, mahila mandal president, traditional trained mid wife and senior members of the community who have knowledge about food behaviour and consumption patterns followed in the community. In addition to interviewing key informants, cultural practices with regard to infant feeding, preparation of food and consumption patterns were observed through participant observation technique.

After the preliminary survey, the research procedures in the FES manual were used.

III. The research procedures in the FES manual consist of the following 8 modules :

Module 1 : Free listing and key informant interviewing.

The purpose of this exercise is to identify an operational list of 25-30 foods that are available and consumed in the community with primary emphasis on vitamin A rich foods. A secondary concern is with staples in order to understand how vitamin A containing foods 'fit" in the overall dietary pattern. This module is conducted with key informants (i.e. individuals from the local community who can be sources of information about the community such as TBAs. Sarpanch, an old resident who is well informed etc.). These individuals are asked recall and name all the foods they can think of. Lists of the foods collected from them were tabulated in the order of those receiving the highest to lowest mentions. From this data, 25-30 foods which include all vitamin A rich foods in addition to other foods were selected for the next exercise (Table-1).

Module 2 : Pile Sorting Task

The purpose of this exercise is to understand perceptions of the community regarding the relationships between staple foods and vitmain A rich foods and how they relate to eating patterns.

Using raw samples (in polythene bags) of the 32 key foods selected earlier, the mothers were asked to sort them into groups

that go together or "belong together". After the mother finished sorting, she was asked to explain the basis for grouping them in a particular way. Forty eight mothers representing the population groups in the community were selected as respondents to this exercise. Results indicated (Fig.1) the following criteria used by mothers as basis for sorting the foods :

1. Food groups (cereals, pulses, GLVs, foods used for seasoning etc., 50%),
2. Foods good/bad for children (10%)
3. Foods good/bad for heatlh (10%)
4. Usage (regularly used, rarely used, never used, 20%)
5. Recipes or preparations (10%)

Modules 3 : Eliciting Food Attributes

This exercise is conducted with mothers to elicit and identify in detail the attributes, qualities or characteristics that people within a community apply to foods. This adds to the information from the pile sorting exercise.

Using the list of 32 key foods identified earlier, the mothers were asked to state the qualities or attributes they attach to each of these foods. Some of the positive and negative attributes obtained from mothers especially for vitamin A rich foods are given as examples:

Carrot : Good for health, eaten raw, not available (Fig.2).

Papaya : Eaten rarely, good for eyes, hot fruit, causes abortion (Fig.3).

Pumpkin : Causes body pains, eaten during festivals only, bloats the body, not good food (Fig.4).

Egg : Good for health, regularly eaten, hot food, gives strength, nutritious (Fig.5)

Palak : Good for health, good for eyes, tasty, regularly used, gives strength (Fig.6).

Module 4 : Rating of food qualities

In this, structured rating techniques are used to explore the degree of the quality or attribute people assign to foods :

Selecting some of the attributes that mothers had stated in the previous exercise, 3 points or 5 points rating scale was administered to the mothers in order to rate the degree of the quality of each food item. For example, the "hot vs. cold" attribute was rated on a 5 point scale by the mothers for all the key foods. To state an example, palak was rated as "very cold" by nearly 6 percent mothers, "cold" by 27 percent, "neutral" by 18 percent, "hot" by 38 percent and "very hot" by nearly 12 percent mothers (Table-2).

Module 5 : Food Acquisition

This exercise is conducted to determine how households in the community acquire key foods - whether home grown, gathered or purchased. Information is also collected on the cost of the foods purchased as well as the family member responsible for acquisition of key foods. The study provided the information on the aspects such as availability/acquisition of vitamin A rich foods from cultivated fields/home gardens, local shop, weekly

shandies and town/city market place (Table-3), as well as on the family members who usually procure these foods (Table-4).

Module 6 : Food frequency check list :

This exercise ascertains the consumption of key foods by members of the household, specifically, consumption by children aged 6 months to 6 years and women of reproductive age. Data on frequency of consumption in terms of times per day and per week were collected through this exercise. For example, palak was consumed three times per week and twice per day (lunch and dinner). This gives a total 6 times per week. The vitamin A rating is then given to the list of foods scored as shown above in the example. Data on this was separately tabulated for children below 3 years (Table-5), below 6 years (Table-6) and for women of reproductive age (Table-7). This exercise is similar to the semi-quantitative diet survey technique. It does not provide information on the actual quantity of food consumed. Importance is attached only to the frequency of consumption of vitamin A rich foods.

Module 7 : Presentation of hypothetical case scenarios

In this exercise, short scenarios depicting signs and symptoms of vitamin A deficiency from mild to severe are presented to mothers in order to elicit information on (1) whether they can recognise these signs and symptoms (2) how they would respond if a child in the household had these signs and symptoms (whether care would be given at home or sought elsewhere), (3) how they would respond if they themselves had

such signs and symptoms (4) at what stage would the care be administered and (5) what would be the sequence of action in providing home care, or seeking it outside the home. The scenarios included short narrations of situations which can occur in communities relating to (a) night blindness, (b) Bitot's spots and (c) diarrhoea and respiratory infection. An example of the responses obtained on the Night-Blindness Scenario is given in Table-8.

Module 8 : Market survey - commercial food sources

The purpose of this exercise is to determine the costs involved when purchasing food items on the key foods list. Since prices vary according to season, price variation over 12 months is assessed. Since the main market in the area where the study was conducted was the weekly shandy in the town nearby, the survey was conducted there. After obtaining the price ranges of all key foods, the vitamin 'A' foods were rank ordered based on the cost, i.e. from the most expensive to the least. Checking the food sources journal for vitamin 'A' content of foods, the price per 1000 RE of foods was determined (Table-9).

IV. Data obtained through these 8 modules are analyzed using simple procedures like converting into percentages (respondents or responses). These are depicted graphically to emphasize differences qualitatively. Factor analytical technique can be applied to the data from the pile-sorting exercise for grouping similar foods into specific factors.

The data from the modules should be supplemented with observations of the style of living of the community members, macro-environmental facilities/deprivations, etc., to get a clear picture of the community.

Hence, the FES as an adjunct to diet survey methods can provide indepth information on frequency of consumption of foods rich in micronutrients especially vitamin A and iron. Additionally, it can even elicit qualitative and behavioural information on constraints, beliefs, dislikes, fears and taboos which prevent consumption of foods. This information can be utilized for selection of foods to be promoted (cost-effective; available) and for planning effective and sustainable nutrition intervention programmes.

c:sv1/modules.fes

Table - 1

Free Listing of Foods

Foods selected from free listing	Vitamin A content rating*	Foods selected from free listing	Vitamin A content rating*
1. Rice (97%)	0	17. Gogu (55%)	3
2. Sorghum (92%)	1	18. Amaranth (38%)	3
3. Wheat (40%)	1	19. Curry leaves (18%)	3
4. Redgram dhal (73%)	1	20. Colocasia leaves (7%)	3
5. Bengal gram dhal (60%)	1	21. Mint (35%)	3
6. Tomato (97%)	2	22. Coriander (45%)	3
7. Brinjal (80%)	1	23. Ponnaganti (17%)	3
8. Potato (67%)	1	24. Drumtick leaves	4
9. Colocasia ((55%)	1	25. Fenugreek leaves (8%)	3
10. Onion (53%)	1	26. Carrot (15%)	3
11. Green chillies (42%)	1	27. Pumpkin (2%)	2
12. Ladies finger (53%)	1	28. Papaya (32%)	2
13. Cucumber (55%)	1	29. Meat (82%)	1
14. Tamarind (52%)	1	30. Egg (75%)	2
15. Red chillies (40%)	1	31. Milk (67%)	2
16. Spinach (98%)	1	32. Curds (43%)	2

Figures in brackets indicate percentage mentions during free listing.

* Vitamin A content scale

0-1	ug	R.E. -0
1-99	ug	R.E. -1
100-1600	ug	R.E. -2
1601-2999	ug	R.E. -3
3000	ug	R.E. -4

Table - 2

Hot and cold (35)

	Very Cold	Cold	Neutral	Hot	Very hot	Total
Palak	2 (5.9)	9 (26.5)	6 (17.6)	13 (38.2)	4 (11.8)	34
Gogu	4 (11.8)	4 (11.8)	19 (55.9)	7 (20.6)	-	34
Amaranth	-	7 (21.9)	17 (53.1)	6 (18.8)	2 (6.3)	32
Curry leaves	-	1 (2.9)	32 (91.4)	1 (2.9)	1 (2.9)	35
Colocasia leaves	1 (3.0)	2 (6.1)	11 (33.3)	7 (21.2)	12 (36.4)	33
Mint	1 (2.9)	2 (5.9)	30 (88.2)	1 (2.9)	-	34
Coriander	1 (3.2)	4 (12.9)	25 (80.6)	1 (3.2)	-	31
Bonnaganti	1 (2.8)	6 (16.7)	28 (77.8)	1 (2.8)	-	36
Drumstick	3 (8.8)	4 (11.8)	17 (50.0)	5 (14.7)	5 (14.7)	34
Fenugreek	2 (6.1)	1 (3.0)	17 (51.5)	8 (24.2)	5 (15.2)	33
Carrot	2 (6.1)	3 (9.1)	26 (78.8)	2 (6.1)	-	33
Pumpkin	2 (6.5)	1 (3.2)	14 (45.2)	5 (16.1)	9 (29.0)	31
Papaya	-	-	3 (8.8)	2 (5.9)	29 (85.3)	34

Figures in parenthesis indicate percentage responses.

Table - 3

Food Acquisition Tabulation Form (Aggregated)

Food items	Cultivated	Local Store	Ration shop	Shandy	Others	Part of wages given in the field
Palak	7	27	-	20	1	0
Gogu	16	31	-	13	4	3
Amaranth	1	26	-	14	1	3
Curry leaves	22	21	-	4	13	4
Colocasia leaves	5	6	-	2	30	0
Mint	7	22	-	13	5	4
Coriander leaves	9	25	-	14	3	3
Ponnaganti leaves	8	10	-	5	30	2
Drumstick leaves	3	-	-	-	15	0
Fenugreek leaves	7	25	-	15	1	2
Carrot	-	2	-	24	-	6
Pumpkin	-	-	-	6	1	7
Papaya	18	1	-	4	19	2
Meat	3	34	-	14	-	6
Egg	10	34	-	8	-	1
Milk	13	33	-	-	-	1
Curds	32	25	-	-	-	0

Table - 4

Food Acquisition Tabulation Form (Aggregated)

Food items	Wife	Husband	Children	In-law	Servant	Prices* Rs/kg.
Palak	38	10	7	1	2	Re.1 - 5-7 bunches
Gogu	38	01	7	1	2	Re.1 - 4-8 bunches
Amaranth	30	7	6	2	2	Re.1 - 4-6 bunches
Curry leaves	32	3	8	1	2	Re.1 - 3-6 bunches
Colocasia leaves	33	3	6	2	2	Re.1 - 5-6 bunches
Mint	33	11	6	2	2	Re.1 - 4-7 bunches
Coriander leaves	36	4	7	1	2	Re.1 - 4-8 bunches
Ponnaganti leaves	30	6	9	1	4	Re.1 - 3-6 bunches
Drumstick leaves	6	-	2	-	-	-
Fenugreek leaves	30	8	9	1	4	Re.1 - 5-7 bunches
Carrot	16	15	6	-	2	Rs. 6 - 8 /kg
Pumpkin	9	8	4	-	1	Re. 6 / kg
Papaya	22	3	22	-	2	Rs. 4 / fruit
Meat	7	28	8	-	2	Rs. 48-52 / kg.
Egg	18	15	12	1	2	Re. 1-1.10/Egg
Milk	28	8	14	-	2	Rs. 6.00-8.00/litre
Curds	39	3	2	-	-	Rs. 7.00 - 14.00/kg.

* Study data collected in 1994

Table - 5

Frequency of food consumption (Vitamin A Rich foods)
(Aggregated Responses)

Children : 1-3 years

Most to least Food items	Frequency	Vitamin A Rating*
Milk	10	2
Tomato	6	2
Curds	5	2
Palak	3	2
Egg	1	2
Carrot	1	3
Gogu	1	3
Coriander leaves	<1	3
Fenugreek leaves	1	3
Colocasia leaves	<1	4
Mint leaves	<1	3
Drumstick leaves	<1	4
Papaya	<1	2
Amaranth	<1	3

* Vitamin A content scale

0-99	ug	R.E. -0
1-99	ug	R.E. -1
100-1600	ug	R.E. -2
1601-2999	ug	R.E. -3
>=3000	ug	R.E. -4

Table - 6

Frequency of food consumption (Vitamin A Rich foods)
(Aggregated Responses)

Age Group : 4-6 years

Food code	Most to least Food items	Frequency	Vitamin A Rating*
32	Milk	12	2
19	Amaranth	7	3
07	Tomato	7	2
20	Curry leaves	6	3
23	Coriander leaves	5	3
17	Palak	5	2
33	Curds	3	2
21	Colocasia leaves	2	3
31	Egg	2	2
25	Drumstick leaves	1	4
29	Papaya	1	2
27	Carrot	1	3
18	Gogu	<1	3
22	Mint leaves	1	3
26	Fenugreek leaves	1	3
24	Ponnaganti	<1	3
28	Pumpkin	0.0	2

* Vitamin A content scale

0-99	ug	R.E. -0
1-99	ug	R.E. -1
100-1600	ug	R.E. -2
1601-2999	ug	R.E. -3
>=3000	ug	R.E. -4

Table - 7

Frequency of food consumption (Vitamin A Rich foods)
(Reproductive Age)

Food code	Most to least Food items	Frequency	Vitamin A Rating*
32	Milk	11	2
07	Tomato	9	2
33	Curds	6	2
20	Curry leaves	4	3
17	Palak	3	2
31	Egg	3	2
19	Amaranth	2	3
23	Coriander leaves	2	3
21	Colocasia leaves	1	3
18	Gogu	1	3
24	Ponnaganti	1	3
27	Carrot	1	3
26	Fenugreek leaves	1	3
22	Mint leaves	1	3
25	Drumstick leaves	1	4
29	Papaya	<1	2
28	Pumpkin	<1	2

* Vitamin A content scale

0-99	ug	R.E. -0
1-99	ug	R.E. -1
100-1600	ug	R.E. -2
1601-2999	ug	R.E. -3
>=3000	ug	R.E. -4

Table - 8

Results on hypothetical case scenarious

For example : Night blindness in children

I.	What is wrong with the child ? (Night blindness)	24
II.	What caused it ?	
	a) Don't know	20
	b) Diet related	4
III.	Home care sought	
	a) Begging food from 7 houses for 7 days	14
	b) Through vitamin A rich diet	10
		4
IV.	How soon improvement expected ?	
	a) After completion of treatment	8
	b) After 15 days of continuous intake of vitamin A rich foods	4
	c) Don't know	2
V.	Evidence of improvement ? When child resumes play and other activities in dim light	14
VI.	What to do if no change?	
	a) Again beg for 7 days	1
	b) Left to fate	1
	c) Take to local/city doctor	11
	d) Ophthalmologist	1
VII.	Signs which inspire other steps.	
	a) If no improvement and condition deteriorates	9
	b) Don't know	5
VIII.	Household decision maker	
	a) Mother	1
	b) Father	3
	c) Parents	4
	d) Older women	3
	e) Parents and elders	3

Additionally data was collected from mothers about their perceptions regarding health care providers.

Other scenarious on measles, bitots' spots and night blindness during pregnancy were presented to mothers and their responses were recorded.

Table - 9

Rank ordering of food prices for Vitamin A foods from most to least expensive

Vitamin A food item	Price or price range 1000 R.E.
1. Curds	11.60
2. Milk	8.75
3. Egg	4.00
4. Pumpkin	1.10
5. Tomato	0.57 - 2.87
6 Curry leaves	0.40 - 0.60
7. Amaranth	0.39 - 0.79
8. Fenugreek leaves	0.39 - 0.79
9. Carrot	0.39
10. Colocasia leaves	0.39
11. Palak	0.30 - 0.80
12. Mint leaves	0.30 - 0.60
13. Gogu leaves	0.26 - 0.40
14. Coriander leaves	0.25 - 1.25
15. Papaya*	0.0 - 0.9
16. Drumstick leaves	Gathered
17. Ponnaganti leaves	Gathered

*It is usually gathered

Fig.1

PILE SORTING RESPONSES

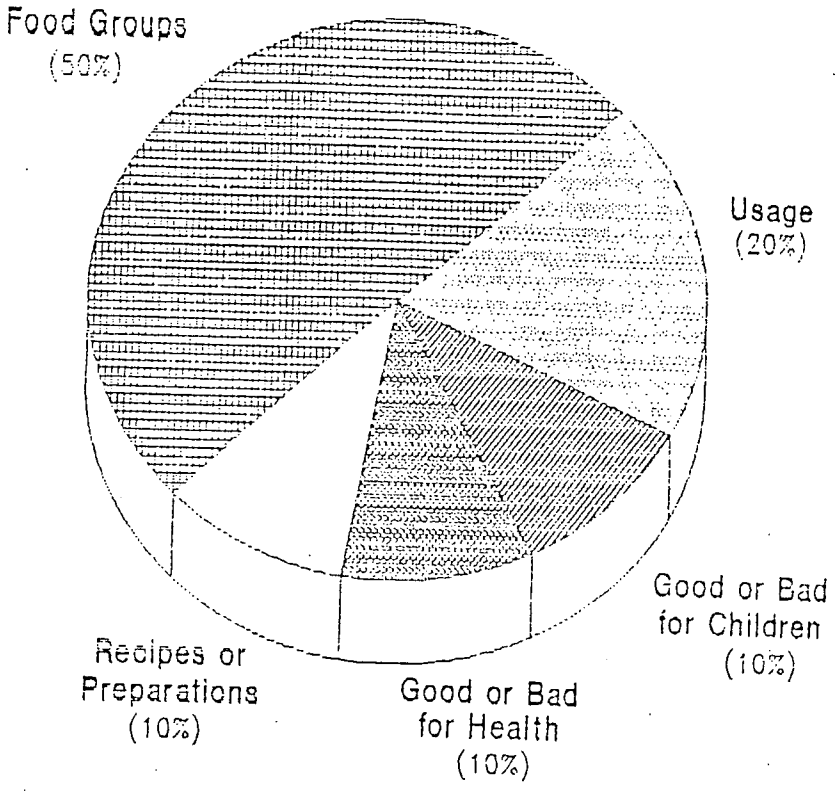


Fig.2

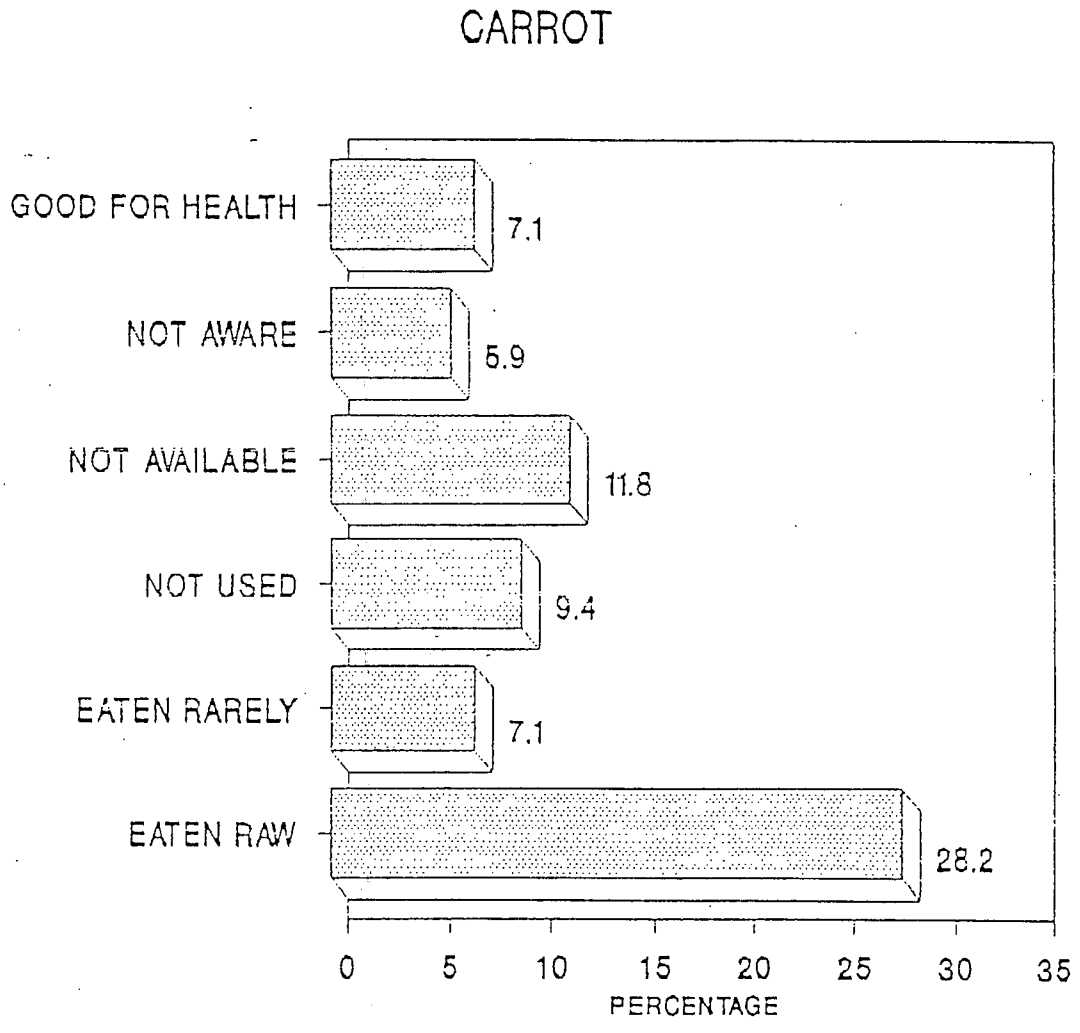

CARROT
GOOD FOR HEALTH
7.1
NOT AWARE
5.9
NOT AVAILABLE
11.8
NOT USED
9.4
EATEN RARELY
7.1
EATEN RAW
28.2
0
5
10
15
20
25
30
35
PERCENTAGE

Fig.3

PAPAYA

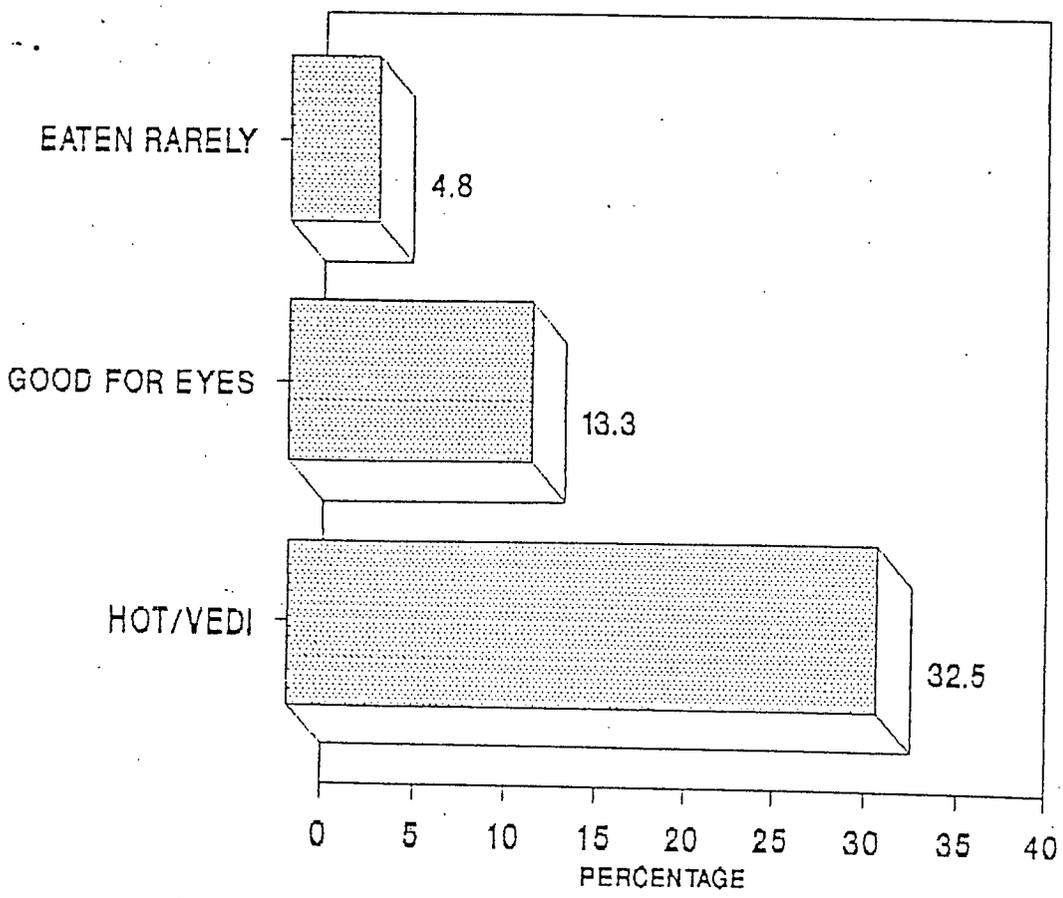

EATEN RARELY
4.8
GOOD FOR EYES
13.3
HOT/VEDI
32.5
0
5
10
15
20
25
30
35
40
PERCENTAGE

Fig.4

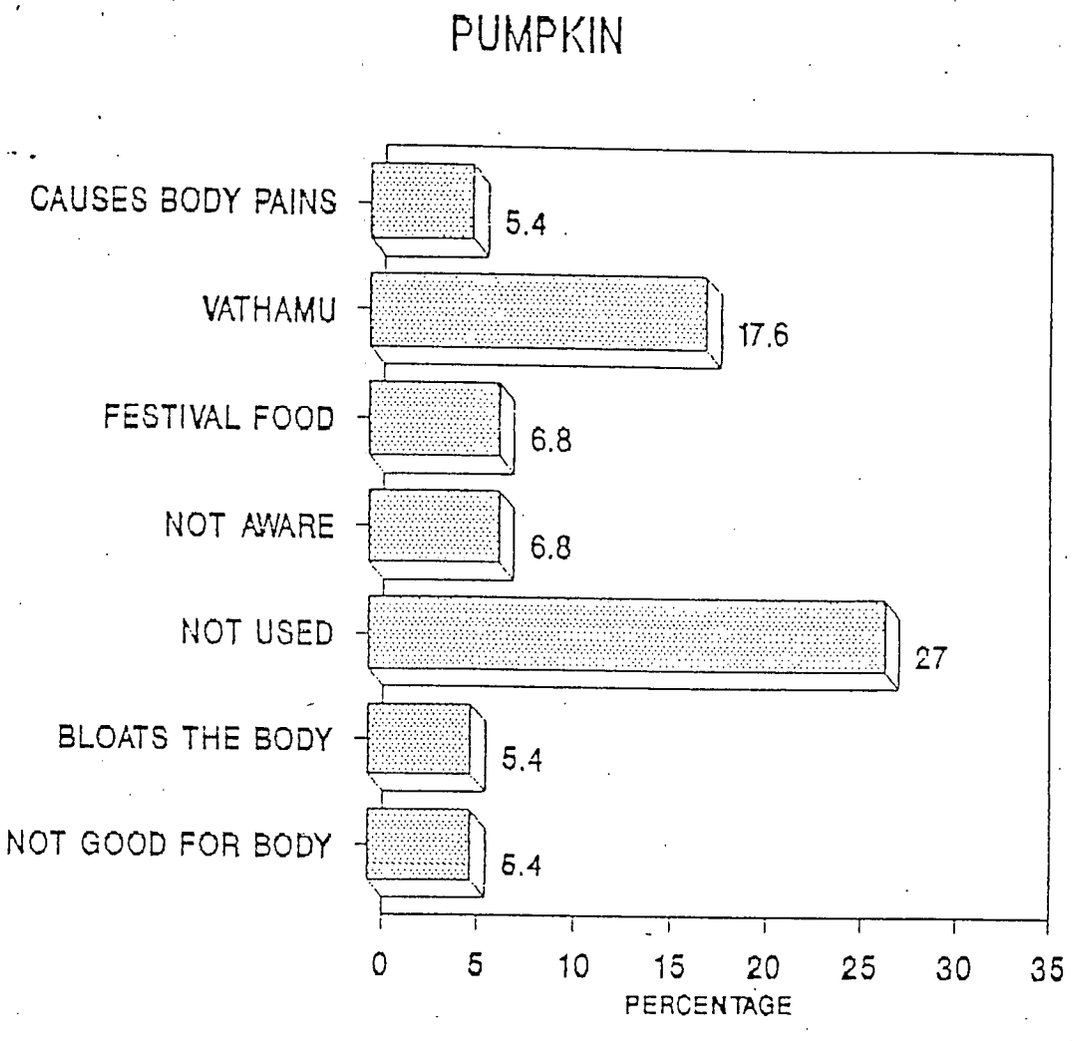

PUMPKIN
CAUSES BODY PAINS
5.4
VATHAMU
17.6
FESTIVAL FOOD
6.8
NOT AWARE
6.8
NOT USED
27
BLOATS THE BODY
5.4
NOT GOOD FOR BODY
5.4
0
5
10
15
20
25
30
35
PERCENTAGE

Fig.5

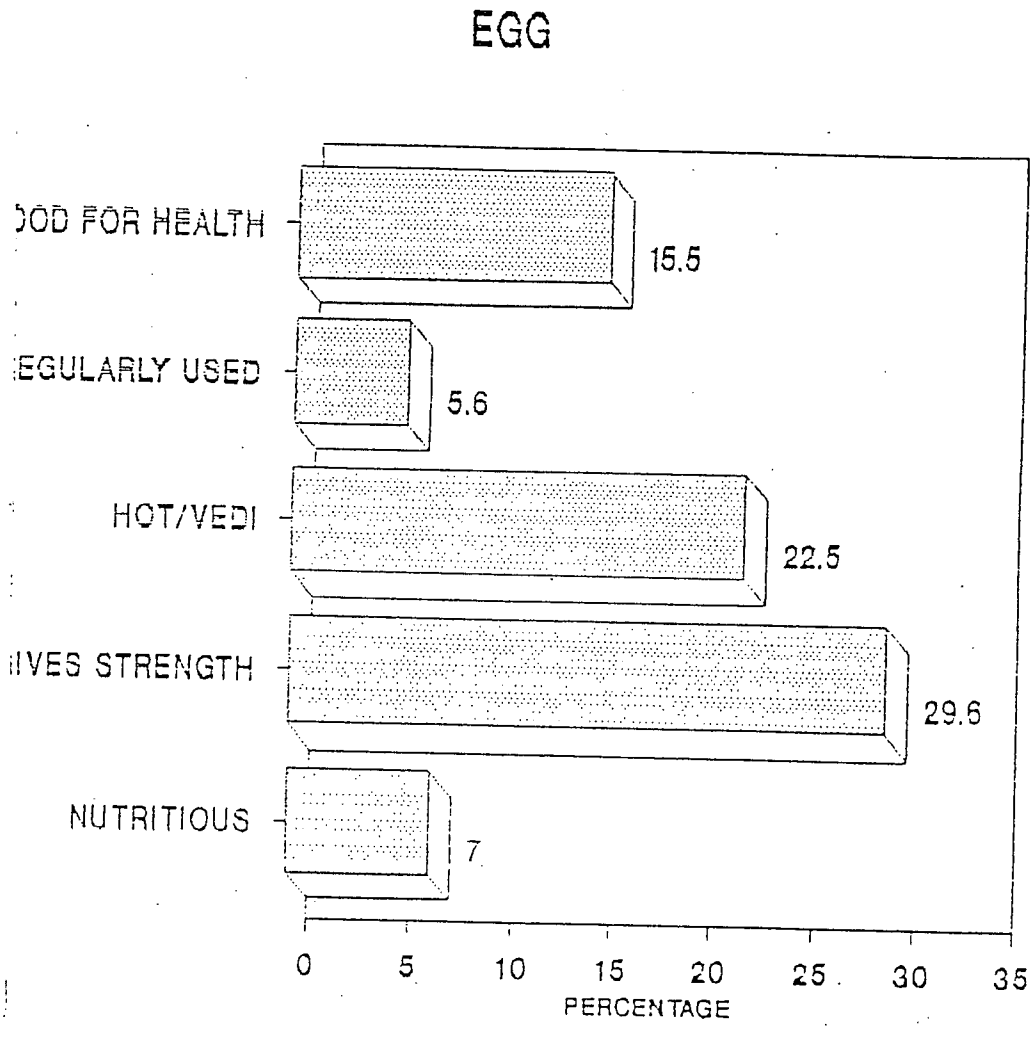
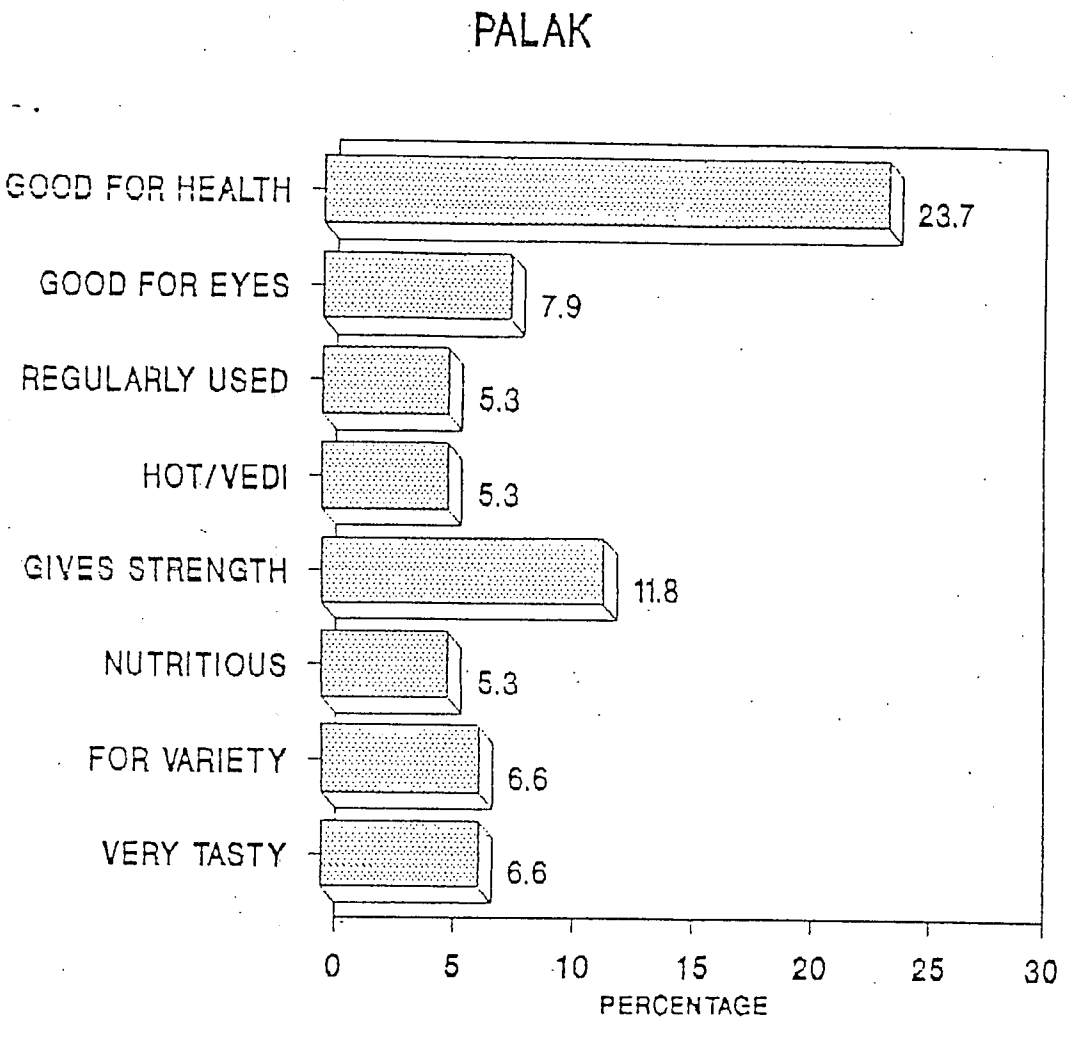

EGG
)OD FOR HEALTH
15.5
EGULARLY USED
5.6
HOT/VEDI
22.5
IVES STRENGTH
29.6
NUTRITIOUS
7
0
5
10
15
20
25
30
35
PERCENTAGE

Fig.6


PALAK
GOOD FOR HEALTH
23.7
GOOD FOR EYES
7.9
REGULARLY USED
5.3
HOT/VEDI
5.3
GIVES STRENGTH
11.8
NUTRITIOUS
5.3
FOR VARIETY
6.6
VERY TASTY
6.6
0
5
10
15
20
25
30
PERCENTAGE

VITAL STATISTICS

VITAL STATISTICS, COLLECTION OF DATA AND DATA SOURCES

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The term "VITAL STATISTICS" signifies the data and analytical methods for describing the vital events occuring in communities. Vital events are those pertaining to human life such as births, deaths, sickness, marriage, divorces, migration, health and nutrition. Statistics covering various aspects of health, nutrition and demographic picture of population are included under vital statistics.

The raw data of vital statistics are generally obtained through the population censuses, sample surveys and vital statistics registers.

There are some most useful and widely used rates, ratios and proportions. These include crude birth rate, general fertility rate, general marital fertility rate, age specific fertility rate, total fertility rate, gross reproduction rate, net reproduction rate, crude death rate, age or sex or cause specific death rates, and infant mortality rates. Ratios frequently utilised are sex ratio, foetal death ratio and proportional mortality rate by age, region or diseases.

Health and nutritional status of the communities can be assessed through various measures of morbidity. Morbidity relates to types and varieties of diseases, one experiences affecting the day to day activity. Data for the study of the morbidity of community are not available as are the data on births and deaths because of the scarcity of the facilities for registration through hospitals. Rates or ratios frequently utilised are incidence rate, prevalence rate,

period prevalence rate, case fatality ratio and proportion of cases by type of morbidity.

Life tables are generated for having the mean expectation of life at birth or any specific age by sex or region.

For hospital administration and maintenance, indices like bed occupancy ratio, average duration of illness, average cost of medicines for a patient, number of physicians or hospital beds or nurses per unit of population are found useful.

Statistics on these aspects at state or national level are generated through the work of the research organisations, Central Statistical Organisation, State bureaus of statistics, sample registration system and National sample survey organisation in India. At global level for comparison between countries on these statistics, the publications of United Nations, WHO, UNICEF, NCHS, FAO and World Bank are praise worthy. Though statistics generated have limitations of coverage, inadequate sampling designs and delayed availability, there have been impressive improvements in the generation of statistics over years in many countries like India. In countries like Japan, statistics of health, nutrition, mortality, fertility and morbidity and resource utilisation and availability are generated for each village and regions with quick publication.

The details on utility of vital statistics and interrelationships between health, nutrition, fertility and mortality will be presented and discussed with case studies and international studies. Can we confirm that females have healthier and longer life than males only in developed

countries ?

The available statistics are of use for decision making on this hypothesis.

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COMMUNITY ASSESSMENT PROCEDURES

Rapid Assessment Procedures and Ethnographic Research in Nutrition and Health

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Fundamental to any successful effort at helping communities to over come nutritional problems is understanding community conditions and cultural perspectives. Developmental planners need information to base their aproach to planning appropriate programme.

With regard to health and nutrition, the Alma-Ata Confernce defend primary health care as "essential health even made accessible to individuals and families in the community by means acceptable to them, through their full participation and at a cost that the community and the country can afford".

To achieve the above, communities have to be studied and necessary information obtained.

Two ways of obtaining such information are :

1. Large scale planned research surveys
2. Visit by the administrators to the areas - (development tourism).

The former approach takes pretty long time and the latter may not provide unbiased information so there is need to develop.

Some quicker and most cost-effective methods of obtaining information.

People are studied in either in terms of numbers or words. The first type is a quantitative approach to information collection. The second approach is qualitative in nature and rests on the collection of subjective information as given by

either the researcher or the subject or informant. This information is in the form of words rather than in numbers. "Qualitative information is like a mountain view, it is both panoramic and awe inspiring, yet seductively attractive".

"If we describe what people do, without enquiring into their subjective reasons for doing it - we are talking about their behaviour.

If we study the subjective aspects of what they do, the reasons and ideas underlying and doing it, then we are concerned with the world of meaning.

If we concern ourselves both with what people are overfly and objectively seen to do (not to do) and their reasons for doing so (or not doing) which relate to the world of meaning and understanding, we then describe Action" Reynolds (1976).

To understand and obtain information, people are studied in different ways.

Rapid Rural Appraisal (RRA), Rapid Assessment Procedures (RAP) Participating Rural Appraisal (PRA), Community Diagnosis are the new approaches to meet the growing needs of the planners to obtain accurate and appropriate information at low cost in timely manner.

The RAP/PRA procedures are to help understanding of the success and problems in the provision of primary health care to all. It "requires a close relationship between health care workers and the community. There is need to understand the

beliefs and perceptions regarding health, prevention and treatment of illness and utilisation of traditional and modern health systems".

"It provides fresh insight into the disease pattern of a population and makes it possible to make more efficient action to improve the health of the group", Sidney Kark. The PRA stresses the researcher intensity, interaction and progressive learning.

RRA is a methodology that approaches information from several intentionally different points of view - "Triangulation" to improve accuracy, three angles being :

1. Team composition
2. Units of observation
3. Research Method

Multi-disciplinary are needed to approach the same information from different angles.

Stratified sample is necessary to improve understanding of the community.

Better tools and techniques help to improve quality of information and cross-checking.

Basic principles underlying these rapid procedures :

1. Learning and understanding the community - rapidly and progressively.
2. Learning from and with the people - face to face - their problems and priority needs; perception, attitudes.
3. The process of learning intensive, iterative and interactive.

The tools/techniques are multidisciplinary - anthropological/geographical and psychological etc.

Multidisciplinary teams visit and stay with the people to establish rapport and to learn. The people express their views, attitudes and perspection in their own language.

Methods of expression by the people could be

1. Visual sharing - diagrams, maps - quantification.
2. Ranking/scoring
3. Participatory mapping/modelling :
   - Social information about the village
   - Occupation pattern
   - Health/Nutrition mapping

Main principles in approach

Emic Approach - Kenneth Pike :

"It is an attempt to discover and to describe the pattern of that particular language or culture in reference to the in which the various elements of that culture are related to each other in the functioning of the particular pattern, rather than can attempt to discuss them in reference to a generalized classification derived in advance of the study of that culture"

Emic approach seeks to :

1. to categorize 'meanings' as nearly as possible in the ways the natives define things.
2. to assess people defemlion of meaning, their idea systems as the most important "causes" or explanations of behaviour.

The above principles in mind, the following information is collected from the people.

Franz Boas a poineer American Anthropologist was the first to use the field techniques which requires intensive field work, the investigator living and participating among the people over a long period in order to get "in-depth" and "holistic" information about those people.

1. Demographic, socio-economic particulars including nutritional status etc.
2. Crop pattern, food production, marketing availability - cost, structure.
3. Health infrastructure and their utilization.
4. Morbidity - mortality pattern and underlying causes.
5. Health and nutritional status of children/mothers.
6. General food consumption pattern of various age, sex and physiolgoical groups.
7. Food, specially, given and avoided to children, pregnant/lactating women/during various sickness/ diseases.
8. Health seeking behaviour of the community.
9. Role of traditional and health system.
10. Community perception/attitude towards the present system and expectations.
11. Concepts of Health and disease - vitamin A deficiency, anaemia etc.

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The following methods are applied to collect the information from the community :

Methods Applied :

1. Formal Interview - structured
2. Informal Inlein
3. Observation
4. Participant observation
5. Focus groups

The information when collected using the above methods and approach would reflect the community's inference and diagnose of the problem, prescription of the solution, initiation of the action and evaluation of the success or failure of the programme by the community.

PRA stresses researcher intensity, interaction and progressive learning.

Ethnographic Research

Many people refer to qualitative studies as "Anthropological research" - the approach is developed within anthropology.

Ethnology/ethnography - is the study of way of life of the people including the behaviour, belief, knowledge, attitudes and values of the community. Collection of ethnographic information is some times called "Ethnographic Research".

Ethnographic Research Cycle

Identification of problems

Training of question

Data Collection

Processing of Information

Analysis

Action

Recommendations

Types of Ethnographic Research :

1. Comprehensive
2. Topic oriented
3. Hypothesis oriented

Topic oriented ethnographic Studies - Vitamin A deficiency

1. Free listing to obtain inventory of food items.
2. Pile sorting
3. Eliciting food attributes
4. Ratings of food qualities
5. Household food acquisition
6. Food frequency checklist
7. Hypothetical cases.

C : DHR : APAU96.PAP

PARTICIPATORY RURAL APPRAISAL(PRA)

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In rural development there is rarely sufficient resources i.e., personnel, finance, to deal comprehensively with the problems. In most circumstances, administrators, planners, policy makers, researchers, specialists, workers have to resort to effective and quick methods with peoples involved while collecting information and then using such data in planning and organisation of improvement programmes. Certain general aspects, key principles and alternative methods for participatory appraisal are outlined below. This technique is highly useful to closely work with and understand the minds of rural people by an outsider.

Characteristics of participatory rural appraisal methods

Participatory methods do not have to be second rate and unprofessional, indeed, if given thought, then can be effective and efficient.

- Techniques for PRA are not put forward as substitutes either for scholarly work or for long-term studies but as complements to existing methods of enquiry.

- In academic approach, it is meticulous and rigorous. In contrast, practioner wants quick insights and quick results. Brief rural visits, snatches of information here and there, and a few observations, anecdotes and impressions are put together as the basis for time-bound decisions.

Scope and function of PRA

PRA can have many purposes.

Assessment of rural condition and social relationship.
Assessment of rural condition and social relationship
Getting data for decisions then and there
Investigation of natural sources, their utilisation and changes over time.

Principles of PRA

In determining appropriate methods and intensities of investigation, judgements need to be made on the amount accuracy, relevance, timeliness and practical utility of information required.

Key features of good PRAS

Interactive

1. The process and goals of the study are not fixed before hand, but modified as the research team realises what is or is not relevant. This involves "as-you-go" learning.

Innovative:

2. There is no simple, standardised methodology. Techniques are developed for particular situation depending on the skills and knowledge available.

Interactive:

3. All team members and disciplines combine together in a way that fosters innerdisciplinary insights.

Informal

4. The emphasis is, in contrast to the formality of other approaches, on partly structured and informal interviews and discussions.

Comparison of conventional and PRA approaches

Techniques employed	Conventional	PRA
1. Statistical analysis	Often a major part	little or none, use of triangulation(Several sources of information).
2. Formal questionnaires	Often included	Avoided

3.	Sampling	Statistically acceptable	Often small sample size. Statistical requirements not always adhered to.
4.	Consulting secondary	Yes	Yes
5.	Measurements	Detailed, accurate	Qualitative or indicators used.
6.	Interview with local farmers and key informants	Through formal questionnaire if at all.	A major component using semi-structured interviewing
7.	Group discussion	Informal unstructured sessions	Via semi-structured workshops and brainstorming

The diversity of analysis is the characteristic feature of PRA. This is pursued through the process of 'triangulation' that, is, the use of several different sources and means of gathering information. The accuracy and completeness of an PRA study is maximised by investigating each aspect of the situation in a variety of ways. PRA is a semi-structured activity carried out in the field by a multi-disciplinary team and designed to acquire quickly new information on rural life.

TRANSECT

A transect is a diagram of main land use zones undertaken along with a team in an exploratory walk observing minute details.

Purpose

1. To get an idea about farming practices, cropping patterns, physical layouts
2. To know agro-eco-system of village
3. To get cross-section view of the area.
4. To know the ITK

STEPS

1. Identify community members willing to participate in the transect walk.
2. Discuss with them different factors to be observed.
3. Walk the transect
4. Observe, ask, listen, dont lecture
5. Discuss problems and opportunities
6. Identify the features and sketch the details
7. Draw and cross check

TIME LINE :

A technique of getting historical incidents/information regarding the past to understand the present.

Helps to identify points of change and probe into causes, its effects, so as to understand the present.

EVENTS INCLUDE -

* Infrastructure
* Crops/yields
* Epidemics
* Admn. changes
* Political events

Time line

1907 Floods
1925 Railwayline
1930 Drought/famine
1935 First citrus tree planted

TIME LINE

1907 - Floods
1925 - Railway line
1930 - Drought/famine
1935 - First citrus tree planted
1945 - Freedom movement - Jailed
1955 - School building
1960 - Road
1964 - Irrigation scheme
1970 - Sugar mill
1975 - Drought
1986 - Bank
1990 - P.House New Bldg.

Participatory Mapping

It is a map constructed by villagers with the help of locally available materials on the ground for understanding village layout, infrastructure and the natural resources.

Purposes:

1. To get an impression of physical layout of village
2. To understand the village social structure - who live where
3. To understand the facilities available
4. To get an impression about natural resource environment
5. To discover the mental maps of community members.

Steps in participatory mapping

1. Decide what sort of map is to be drawn
2. Find people who know the area and topic of mapping and who are willing to share their knowledge.
3. Choose a suitable place (Ground, floor), paper) and a medium (Local material/pens) for the maps.

4. Help the people to start the mapping but let them draw the map by themselves. Be patient and Dont interrupt - it is their map.

5. If they are struckup, invite others to help them

6. At times cross check the presentations with others and ask them to modify if needed.

7. Keep a permanant record (paper) including mappers name to give theem credit.

8. Thank the people for participation.

Use of mapping

1. To identify status of land holding
2. Animal resources
3. Beneficiaries of Dev. programmes
4. Residential stratification
5. Natural resources like forests, land, rivers, tanks, irrigation etc.
6. Land use
7. Mobility of people
8. Treatment plan of soil conservation

Seasonality or Seasonal Calendar

It is a technique to understand the activities, problems, opportunities throughout the year in diagramatic form.

Purpose: To identify the months/period of vulnerability or variance having an impact on peoples' lives, on which further planning can be done to overcome the same.

Can be used to:

1. Identify indigeneus seasons
2. Climate/rain fall/temperature
3. Crop operations/crop sequences

4. Crop pests and diseases
5. Livestock diseases
6. Human diseases
7. Fodder requirement
8. Labour requirement/availability
9. Fuel wood requirement
10. Food consumption
11. Migration
12. Commodity prices
13. Income generating activities

Seasonality steps

1. Set the climate for participatory discussion
2. Sit down with the informants
3. Carry the materials needed
4. Initiate discussion on particular topic
5. Ask informants to explain seasonal changes and draw on paper or ground
6. Slowly handover the materials to them and encourage them to draw.
7. Help them to show the detail's by visual representations for easy understanding.
8. Allow them to change or correct
9. Donot interfere toomuch or impose ideas
10. Prepare seperate illustrations for each item
11. Transfer the diagram on paper for record
12. Triangulate the findings

Venn Diagram

A Venn diagram shows the key institutional linkages in a community along with importance and relationships for decision making.

Steps

1. Decide the village and prepare materials
2. Make the villagers settledown
3. Initiate discussion
4. Show the villagers the indication of options in terms of access/importance
5. Ask them to use the material and prepare the diagram
6. Probe for more details or if they are confused
7. Allow them to change or modify
8. Triangulate the information
9. Record the diagram for future use
10. Thank every one for cooperation

Changing Trends

Useful mechanism to understand the pattern of changes in population, culture, technology, vegetation resources, wealth and soon, over the years.

Purpose:

1. To understand the past
2. To understand major events
3. To probe into causes of changes
4. For planning for future

STEPS

1. Set the climate for participatory discussion
2. Encourage elderly people to give information
3. Initiate discussion and ask questions
4. Ask them to write down
5. Dont insist on specifics too much

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6. Dont insist on specifics too much
7. Record information
8. Compare changes
9. Identify trends
10. Cross check the information
11. Thank all the villagers

Sustainability analysis

It is an analysis in a simple manner to identify the suitable scheme for a group or community.

Purpose:

1. For review of developmental activity
2. To identify the best prioritised scheme available
3. To develop a suitable scheme
4. To identify the feasibility of schemes.

Steps

1. Decide the village and set the frame work
2. Initiate discussion on schemes
3. Analyse each scheme on various aspects
4. Use simple scoring mechanism
5. Identify the feasibility of schemes by checking the scores (Total)
6. Cross check the information
7. Decide the scheme for the village/for groups of people.

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STAKE-HOLDER ANALYSIS

Dr.G.N.V.Ramana, Consultant, ASCI, Hyderabad

Who are stake-holders ?

Stake-holders can be defined as individuals/institutions that are involved in planning, financing, implementing and benefiting from developmental programmes and without answering their needs and requirements it is difficult to run a successful programme.

Some of the stake-holders include:

- Consumers
- Providers
- Programme managers
- Policy makers
- Donors

Why stake-holder analysis ?

- *Scientific proof Vs. people's preferecne:*

  The essential logic most programme designers use is that it is based on a scientific proof of proven efficacy as evinced by controlled experiments and community trials. Unfortunately, we often find many of these successful experiments when translated into National programmes, fail to deliver the desired impact.

- *Supply Vs. Demand:*

  The overriding emphasis on supply with little concern for demand continue to haunt the programme design. While one can not find fault with the programme managers and policy makers as they certainly are attempting to do their best to address the perceived problems, the need for developing a logical frame work to develop a programme which is sensitive to the needs of the stake holders is being very much felt.

Tools for stake-holder analysis:

Quantitative:

- Surveys
- Exit polls
- Facility Check lists

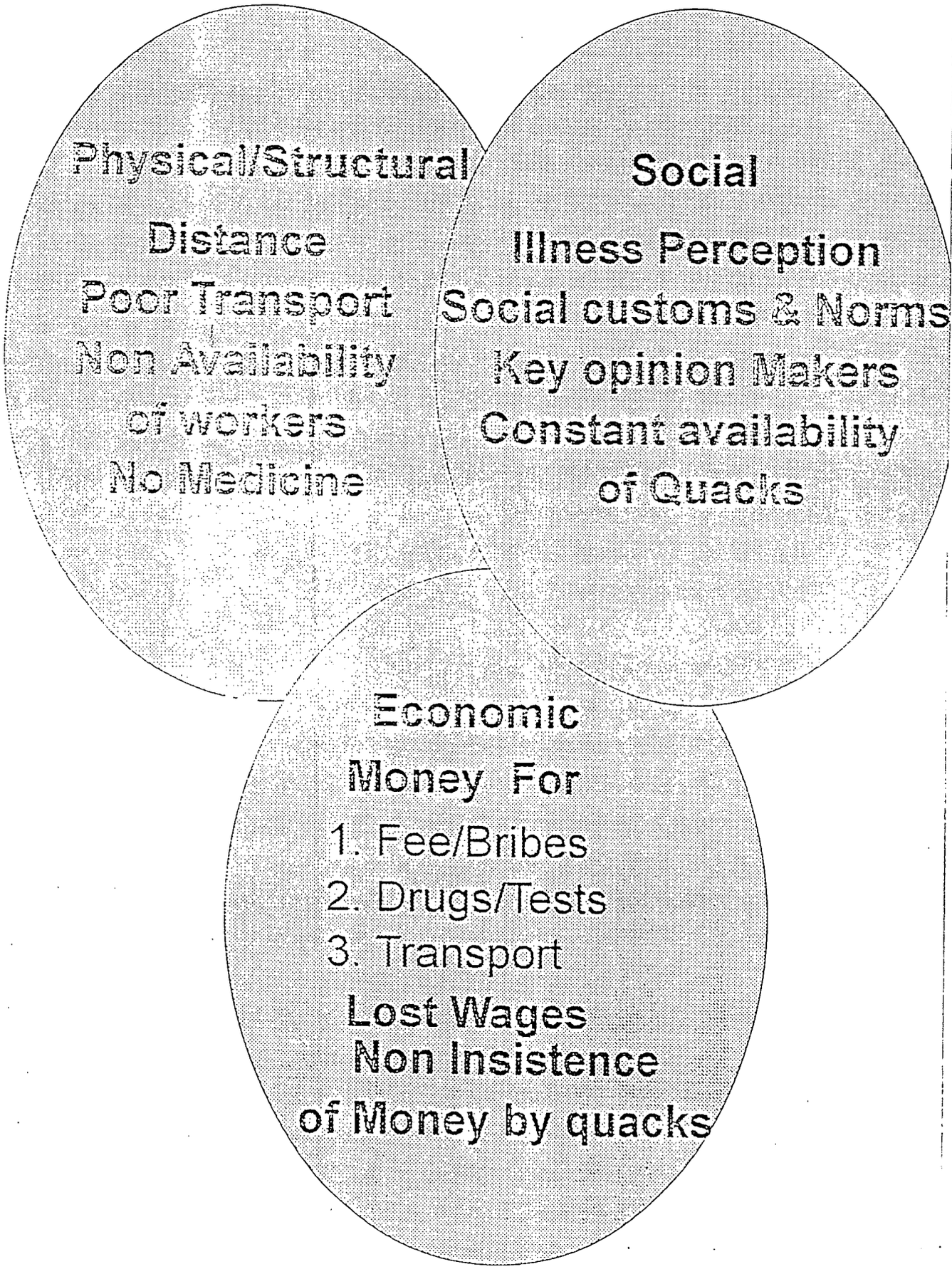
Advantages: Less complex, larger coverage in shorter time and provides statistically acceptable outputs and the skeleton

Qualitative:

- Focused interviews
- Observation studies
- Focus Groups
- Participatory Rapid/Rural Appraisals

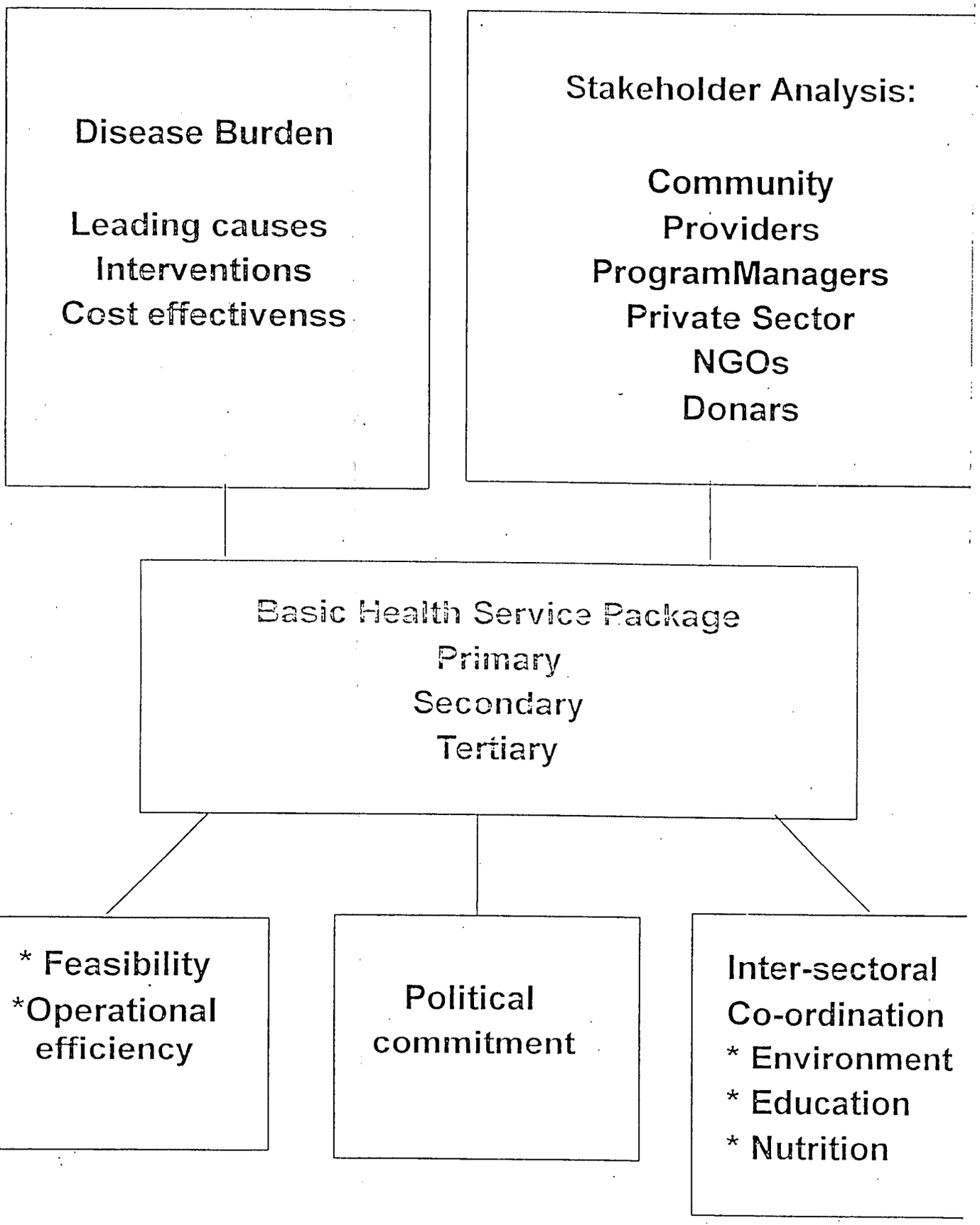
Advantages: Provides flesh and blood which give more in-depth understanding of the complexities of the problem which is being studied.

ACCESS TO HEALTH CARE



Organisation of health care delivery system in India

Level	Population covered	Type of facility	Functionaries	FP & MCH services delivered	Beds
Primary	1,000		• Anganwadi worker • Trained Birth Attendant • C H V	• Supplementation: Calorie, Vit A, Iron • Non formal Education • Growth Monitoring • Health and Nutrition Education • Treatment of minor ailments • Referral • Home deliveries by TBA	Nil
	3000-5000	Sub Centre	• MPHW (F) • MPHW(M)	• Household visits & Fixed Centre • FP: Motivation, Condoms, OP, IUD • Antenatal care, Intranatal care, Immunisation • Vitamin A & Iron folic acid supple. • IEC on MCH & FW • Treatment of Minor ailments • Referral of high risk and needy cases	Nil
	30,000	Primary Health Centre	• Gen.Doctors • Supervisors • Nurses • Lab Tech. • Support	• Supervision & Co-ordination • Permanent FP methods • Basic Laboratory Services • Intranatal care • Stabilisation of obstetric and paediatric emergencies • Referral	6
First Referral	100,000	Community Health Centre	• OBGY sp. • Paediatrician • Gen.Doctors • Lab. Tech. • Support	• All emergency obstetric procedures • Blood transfusion facilities	30
Secondary	300000-500000	Area Hospital		• Basic specialities • Curative services	50-75
	1-2 Million	District Hospital		• Most speciality services offered	200-350
Tertiary	3-5 Million	Teaching Hospitals		• All specialities and some superspeciality services	500-1000
	25-30 Million	Super speciality Hospitals		• Only superspeciality services	500-1000


Disease Burden
Leading causes
Interventions
Cost effectivenss
Stakeholder Analysis:
Community
Providers
ProgramManagers
Private Sector
NGOs
Donars
Basic Health Service Package
Primary
Secondary
Tertiary
* Feasibility
*Operational efficiency
Political commitment
Inter-sectoral Co-ordination
* Environment
* Education
* Nutrition

National Institute of Nutrition
Indian Council of Medical Research
Hyderabad

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Sr.Res.Officer

DESIGN OF SAMPLE SURVEY

Sampling is the selection of a part of an aggregate of material or population to represent the whole population. The use of sampling in making inference about an aggregate (or population) is possibly as old as civilisation itself. When one has to make an inference about a large lot and it is not practicable to examine each individual member of the lot, one invariably takes recourse to sampling. Sampling may become inevitable because we may have limited resources in terms of money and/or man-hours, or it may be preferred because of practical convenience.

Basic principles for the design of a sample survey are (i) validity and (ii) optimisation. The principle of optimisation takes into account the factors of (a) efficiency and (b) cost by validity of a sample design we mean that the sample should be so selected that the results could be inter-preted objectively in terms of probability.

Suppose it is proposed to investigate the nutritional status of the preschool children (1-5 year age) in India. At present there are nearly 130 million preschool children in India. To examine these children, it will take more time, money and require more number of skilled personnel, so the alternative is to study a sample of preschool children. Samples can be drawn from the

entire population through various procedures. They are :

i) Simple random sampling.

ii) Systematic random sampling.

iii) Stratified random sampling.

iv) Cluster sampling.

v) Multi-stage sampling.

vi) Multiphase sampling and

vii) Purposive or quota sampling procedures.

viii) Probability proportional to size (PPS)

i) Simple random sampling (SRS)

This method is well applicable when the population is small, homogeneous and readily available such as patients coming to a hospital or lying in the wards. The principle here is that every unit of the population has an equal chance of being included in the sample.

The sample may be drawn unit by unit, either by numbering the units such as persons, families or households of a particular population from the published tables of random numbers.

ii) Systematic random Sampling

This is simple procedure and utilised when a complete list of population from which a sample is to be drawn is available. It is more often applied to field studies when the population is large, scattered and heterogeneous. Systematic procedure is followed to choose a sample by taking every 'k'th house or patient where k refers .PA to the sample interval which

is calculated by the following formula :

$$K \quad \frac{\text{Total population}}{\text{Sample size desired}} = \frac{N}{n}$$

One random number less than K is chosen from random number tables. If the first random number is 03; then the subsequent numbers will be 03 +K; 03 +2K, 03 + 3K and so on.

iii) Stratified random sampling

Stratified random sampling procedure is followed when the population is not homogeneous. The population under study is first divided into homogeneous groups called strata and the sample is drawn from each stratum at random according to some rule. This procedure gives more representative sample than simple random sampling in a given large population.

iv. Cluster sampling

A cluster is a group consisting of units such as villages, wards, blocks, factories, slums of a town, workshops or children of a school. Sampling procedure is adopted for the selection of clusters. Usually, simple random sampling or systematic random sampling procedure is utilised in the selection of clusters. After the selection of clusters randomly, the complete enumeration of the subjects in the cluster is carried out.

v) Multi-stage Sampling

This method refers to the sampling procedures carried out in several stages using random sampling techniques. This procedure is employed in large scale country-wide or region-wise surveys. Suppose it is planned to carry out dietary intake pattern on 800 households in four districts of a State during one year. First, the state is divided into agro-economic regions. Atleast one

district is selected from each region in the first stage. At second stage 20 villages representing each block, are chosen. From each selected village a representative sample of 10 households are selected for diet survey at third stage.

vi) Multiphase sampling

In this method, part of the information is collected from the whole sample and part from the sub-sample. In health examination survey among school age children, all children in the selected school will be surveyed in the first phase. In this second phase for blood examination a sub-sample will be chosen in the second phase.

Different steps in a large scale sample survey

Conducting a large scale sample survey involves three main stages : (a) planning stage, (b) execution stage and (c) analysis and reporting stage.

(a) The planning stage consists of the following steps :

(i) Defining of the objectives

(ii) Defining the poulation

(iii) Determination of the data to be collected

(iv) Deciding on the methods of collection of data

(v) Choice of sample unit (S.U.)

(vi) Getting a sampling frame

(vii) Designing the survey

(viii) Drawing the sample

(ix) Training of personnel

(b) Execution stage involves the identification of the sampled individuals in the field and the filling in of the questionnnaires.

(c) The analysis and reporting stage consists of the following stages.

(i) Scrutiny of data

(ii) Tabulation of data

(iii) Statistical analysis

(iv) Reporting

(v) Storing of information for future surveys

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DESIGNS OF EXPERIMENTS

Designing an experiment means deciding how the observations or measurements should be taken to answer a particular question in a valid, efficient and economical way. The design and the analysis go together, they are inseparable in the sense that if an experiment is properly designed then there will exist an appropriate way of analysing the data.

Before explaining the principles of designs, it is proper to know the terminology used in this context. The terms commonly used are experiment, treatment, experimental unit, experimental error and precision.

(i) Experiment : Experiment is a means of getting an answer to the question that the experimenter has in mind. Example : An experiment may be planned to compare the Japan method of cultivation with the standard methods used in India.

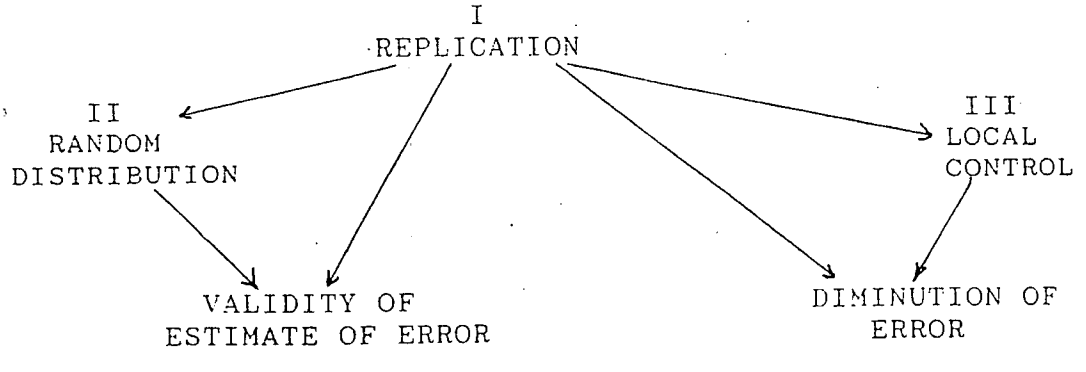
(ii) Treatment : The different procedures under comparison in an experiment are the different treatments. For example, in an agricultural experiment, the different varieties of a crop or the different measures will be the treatments.

(iii) Experimental unit : An experimental unit is the material to which the treatment is applied and on which the variable under study is measured. Examples are the plot of land, cow and individual.

iv) Experimental error : The unexplained random part of the variation is termed as the experimental error. An estimate of the experimental error can only be obained by replication, and it is controlled by the principle of local control. An estimate of the experimental error can only be applied by replication and it is controlled by the principle of local control.

The provision of an experiment is measured by the reciprocal of the variance of a mean.

The three basic principles of experimental design, as developed by R.A.Fisher, are (i) Replication (ii) Randomisation (iii) local control. Fisher illustrated the function of the principles, from which modern experimental design have been evolved in the diagram below :



There are so many experimental designs developed for use. Prominent among them are:

a) Completely randomized design(CRD), b) Randomized Block design(RBD), c) Factorial design, d) Latin square design, e) split unit design and f) Cross-over designs.

a) Completely randomized design (CRD)

A very simple form of experimental design in which the treatments are allocated to the experimental units (rats or monkeys or human beings) purely on a chance or random basis.

Examples

1) The reduction in blood sugar recorded for groups of rabbits given different doses of insulin.

2) The value of a certain lung infection test recorded for men of the same age group in a number of different occupational categories.

3) Gain in weight of albinorats of the same age and sex by varied levels of protein.

4) The volume of liquid taken up by an experiment using various pipettes to measure a standard quantity. The repeated measurements on any one pipette being grouped together.

The results may be analysed by one way analysis of variance and Fishers "F" test.

b)Randomized block design (RBD)

This is one experimental design in which each block contains a complete replication of the treatments, which are allocated to the various units within the blocks in a random manner and hence allow unbiased estimates of error to be constructed.

Suppose we can arrange the experimental units in groups (or as we shall say blocks) such that members of the same block are on the whole likely to be have more similarity than members of different blocks. For example, in some circumstances litter mates 8(in case of albinorats) may be more alike in their response than members of different litters (whether for genetic or environmental reasons) . If say, five treatments are to be compared, one could take litters of five animals and allocate treatments at random to the individual animals in each litter so that each treatment occurred once in each litter.

If the blocks are sufficiently large, it may be possible to arrange that each treatment occurs two or more times within a block. Each block then provides its own internal replication, and it becomes possible in the analysis to see whether the relative treatment effects vary appreciably from one block to another. This may be particularly useful if the blocks are really interesting in their own right - if for instance, they differentiate between animals of different strains or weights rather than of different litters.

Here two way of analysis of variance and Fisher's "F" ratio test are used.

c)The factorial design

Frequently, we may be interested in studying simultaneously, the effects of two or more variables. The variables in which we are interested are also referred as factors. The experiments in which two or more factors are investigated simultaneously are called the factorial experiments.

If the experiments are properly designed and conducted it is possible to study the effects of individual factors as well as

the interaction between the factors. Interaction is said to exist when the combination of some level of one factor with some level of another factor produces an effect different from the effect of some other combination of levels of the two factors. For example, let us suppose that we are studying the effect of age and sex on the ability to learn a skill. The variables age and sex are the factors. Sex occurs at two levels, male and female, and if we have three age groups 1,2 and 3, then age is at three levels. If males in age group three tend to have higher effects than other age male- combinations, we say that there is interaction between the factors, age and sex.

Other examples of interest are:

1) Levels of protein, levels of calories and interactions between levels of proteins and calories on gain in body weight (or gain in height).

2) Scales of percaput income, grades of maternal literacy and interactions of percaput income and maternal literacy on heights or weights or calorie intake or protein intakes of children.

3) Levels of calories, levels of proteins and levels of iron and interactions on the gain in weight of albinorats.

4) Scales of body mass, scales of body fat and their relationships to serum cholesterol or blood sugar of adult individuals.

5) Blood clotting time and the effects of periods of storage and concentrations of adrenalin.

The advantages of the factorial experiment or design are:

a) The interaction of the factors may be studied.

b) There is a saving of time and effort. In the factorial experiment all the observations may be used to study the effects of each of the factors under investigation. To achieve the level of accuracy of the factorial experiment, more experimental units would be needed if the factors were studied through two experiments. It is seen that 1 two-factor experiment is more economical than 2 one-factor experiments.

c) Since the various factors are combined in one experiment the results have wider range of application.

A factorial arrangement may be studied with the completely randomised design or randomised block design.

(D) Latin square Design

The latin square designs were first used in the agricultural experiments. They are useful also in medical and biomedical research work. Then designs were developed by Prof. R.A.Fisher.

When we wish to compare the effects of treatments numbering "a" in an experiment in which there are two other known sources of variation, each at "a" levels. A complete factorial design, with only one observation at each factor combination, would

require a^3 observations. Consider the following designs, in which a = 4. The principal treatments are denoted by A,B,C and D and the two secondary factors are represented by the rows and columns of the table as below:

		Column			
		1	2	3	4
Row	1	A	B	C	D
	2	B	C	D	A
	3	C	D	A	B
	4	D	A	B	C

Since at each combination of row and a column only one of the four treatments are used, there are only a^2 (= 16) observations. The design is balanced as only one treatment occurs precisely once in each row and precisely in each column.

In medical or biomedical research some similar experiments can be there when treatments are to be applied to a two - dimensional array of experimental units. For example, various substances may be inoculated subcutaneously over a two - dimensional grid of points on the skin of a human subjects (or an animal). Also, the rows and columns may represent in experiments two identifiable sources of variation. The latin square can be visualised as a generalisation of a randomised block design, the rows and columns representing two different systems of blocking. Some examples of interest are the following:

i) An animal experiment with animals chosen from litters and studied over different days is performed. Litters can be taken as representing rows and the days as representing columns. The individual animals with in each litter and days receive different treatments. Here three sources of variation - Litters, days and treatments. All the three must be in equal numbers.

ii) A clinical trial on several subjects (or individuals) with treatments being provided to each individual on different occasions. Three factors under study here are - subjects, treatments and occasions. All these three must be in equal numbers. Here each subject receives various treatments on different occasions.

In this type of application the investigator must satisfy that the response observed on any occasion is influenced only by the treatment currently given and not by any preceding treatments.

The interest in this design is to eliminate the influences of other factors such as rows effect and column effect for judging the treatment effect.

(d) Split - Unit designs:

Split-unit designs are frequently utilised in medical and biological experiments. This design is also named as nested design. Some examples of the distinction between main units and sub-units are as follows:

Main unit	Sub unit
Individual human subject or animal	Different occasions with the same subject or animal
Litter	Animals within a litter
Day	Periods during a day (Forenoon, Afternoon, Night)

Split - unit design might be employed to compare long term effects of drugs say D_1, D_2, D_3 and simultaneously short term effects of drugs say S_1, S_2 or S_3. Suppose there are 12 subjects, each of whom must receive one of D_1, D_2, or D_3 and each subject is observed for three periods during which S_1, S_2, and S_3 are to be given in a random order. The design, determined by randomly allocating the D's to the different subjects and the S's to the periods within subjects, might be as follows:

Patients	A Drug through out	Drug during period 1	2	3
1	D_1	S_1	S_2	S_3
2	D_3	S_1	S_3	S_2
3	D_2	S_3	S_2	S_1
4	D_1	S_3	S_1	S_2
5	D_3	S_2	S_3	S_1
6	D_2	S_2	S_1	S_3
7	D_2	S_2	S_1	S_3
8	D_1	S_2	S_1	S_3
9	D_2	S_2	S_1	S_3
10	D_3	S_1	S_3	S_2
11	D_3	S_3	S_1	S_2
12	D_1	S_1	S_2	S_3

This type of design can be utilised for survey data also rather than for an experiment. Analysis of variance is carried for the variables or parameters studied with this experimental design.

CROSS-OVER DESIGNS

i) CROSS - OVER DESIGN - GENERAL

The "Cross - over design" is also known as change - over design. In biological assays, when the number of treatments is small and the effect of treatment is not long - lasting, the cross- over design is preferred and utilised. Each subject or patient under study receives all the treatments or drugs.

This design resembles the Latin square design. The simplest case is of two treatments, T_1 and T_2. The number of replicates or subjects must be taken as a multiples of two treatments. The experimental subjects are to be grouped into pairs. It is possible that one member of a pair is likely to be superior than the other. Since each member of a pair is taken for each of the treatment, the superiority of the members existing in a specific pair will be the same for all treatments. We can classify the subjects of the pairs as good and poor or as well nourished (or normal) and undernourished based on a specific criteria using

ii. Cross-over designs for Clinical trials

The clinical trial is an investigation that utilises human subjects to evaluate methods of treatment or management of patients in hospitals.

The clinical trials may be classified as follows:

a) Controlled clinical trials - These are conducted for drug testing and for the improvement of therapeutic strategies

b) Partially controlled trials; and

c) Historical studies of patients (or patient series).

The controlled clinical trial is the testing procedure for an evaluation of new treatments or new procedures. Specifically for an evaluation of drug efficacy and for the improvement of the therapeutic strategies controlled clinical trials are utilised. There are many studies conducted to determine safety and dose of drugs in large series of patients. These studies help the physicians to choose the type and quantum of drugs to use depending on the condition of the patients.

Partially controlled clinical trials are those of investigations which fall some where between a controlled trial and a report of a personal series of patients. That is, the investigator will plan his trial and include only some of the essential design ingredients. In some cases, the nature of therapy being evaluated does not permit a controlled trial. For this reason, there are a number of trials that fall below ideal standards but yet are noteworthy contributions. Major limitations consisted of in these studies lack of appropriate controls, lack of randomization of subjects to the groups, poor coverage of needed areas etc.

Historical studies of patients (or patient series)is the classic unplanned clinical trial . In this method, the investigator reviews a series of his patients. At the time when the investigator saw a patient, he may not have a plan to use the data for research purposes. The results are usually the investigator's or clinician's experiences with the patients. These investigations involve usually tabulation of data from patients schedules. Observational biases of investigators and memory lapses of patients in provision of data are some limitations. Statisticians indicate caution in the utilisation of data and the generalisation of results based on these patient case sheets as there are no controls and no randomisation in the selection of patients.

In controlled clinical trials, patient assignment to the different drugs or treatments is usually carried out using a randomisation procedure. The general use of randomisation in these trials has resulted in naming these clinical studies randomised clinical trials. Many new drugs are always being developed and placed in the market. Institutions and physicians must assess the efficacy as well as relative efficacy of the drugs. The randomised control trials of drugs or treatments may be patterned after one of the designs given below.

Body mass index limits for malnutrition. After division of the subjects of pairs as normal and undernourished, the treatment T_1 is given to the "normal" members of the pairs selected at random from all pairs and treatment T_2 is given to the "undernourished" members of the remaining half of the pairs. After the period of treatment T_1 is completed for normal (or under nourished) subject of each pair, the second treatment is started. The subjects who are to be given second treatment can be given some time without treatment to eliminate the impact of previous treatment. Thus each treatment is exposed to the same type of units equally and frequently.

An example of the cross - over design, we may provide as below:

Group	Pair							
	1	2	3	4	5	6	7	8
Normal	T_1	T_2	T_1	T_2	T_2	T_1	T_2	T_1
Undernourished	T_2	T_1	T_2	T_1	T_1	T_2	T_1	T_2

T_1 = First treatment ; T_2 = Second treatment

Randomisation made the provision of treatment T_1 to the normal subjects of pairs 1,3,6 and 8. When random allotment is given to the normal subject, automatically, the second treatment T_2 is given to the undernourished counterpart of each pair.

The cross-over design may be used with advantage for human beings as well as animals like cows or buffaloes where each of the human beings or animals will serve as replicates. The treatments are given after some time lag so that there are no carry over effects of the application of the first treatment. This is more convenient if there are smaller number of treatments. If there are only two treatments, the first half of the subjects are selected at random and the first treatment is given and the second treatment is given after noting the result of the first treatment and after some time-lag. To the remaining half of the subjects, second treatment is given first and then the first treatment is given later.

The advantage of cross-over design is that patient or subject taken for study serves as his own control. This reduces the patient-to-patient (or subject-to-subject) variability on the parameters (or variables) studied.

Disadvantage of this cross over design is that the patient or subject may have possible carryover effect. That is, the first treatment (or drug) might still be having an effect when the second treatment (or drug) is given.

There are many modifications of the cross - over designs. Most commonly used are: Cross-over with baseline, and Double cross-over. In cross-over design with baseline, whenever possible, all therapy is stopped during the baseline period. This makes the patients or subjects control level measured. This measured level of subject will be free of any other treatment effects. For two drugs T_1 and T_2 with eight patients, the details of drug sequences with baseline period is provided below:

DEVELOPING A SCHEDULE/QUESTIONNAIRE FOR COMMUNITY ASSESSMENT

PROF. VENKATARAMAIAH
Principal and Univ. Head, E.E.I., Hyd'bad.

Questionnaire is a series of written questions on a topic about which the respondents' options are sought. It is a frequently used tool in survey research - the systematic gathering of information about people's beliefs, attitudes, values and behaviour. There are two general types of questionnaires : self-administered which respondents fill out themselves and interviewer administered, in which the interviewer asks questions and records the responses. The self administered form is more efficient in time and effort. For example copies of questionnaire can be distributed to 100 employees, filled out by them, and collected within an hour's time. Conducting 100 individual interviews would be much more difficult and time consuming. However, a self-administered questionnaire requires clear instructions and a very careful wording of items. The most difficult aspects of a questionnaire are its construction and the interpretation of the results. Distributing and scoring a well-constructed questionnaire are usually easy. Overall, it is difficult to surpass a questionnaire for economy. That is why it is such a popular and widely used research tool.

Questionnaire Construction :

There are two general aspects to every questionnaire its content and its format. The content of a questionnaire refers to the subject matter. The format pertains to its

structure and appearnce - how the items are worded, their appearance on the page, and the form used for answering the questions.

Content

In general, it is best to restrict a questionnaire to a single issue. If you want to find out what college students think about the canteen stay with that topic. Don't ask about teaching methods or the adequacy of the library.

You can save considerable time and effort if a previous survey developed suitable questions. You can always add new questions, but try to include some of the old ones. Wherever possible, retain the same wording.

Assuming you are going to construct your own questionnaire, you will start by using two other methods: casual observation and interview. The purpose of observation is to learn the range of activities about which questions must be asked. Interview should accompany the observations to learn the range of opinions students hold regarding the food, off this stage a loose open-ended interview is preferable.

The following brief list of questions would probably be appropriate for a casual interview on this topic.

1. What do you think of the food here ?
2. What do you like most about it ?
3. What do you like least about it ?
4. What do you think about the service and facilities?
5. Is the anything else you would like to say about the food or the service ?

These general questions are only to learn what questions should be asked on the questionnaire.

Format

It can either be structured or unstructured questionnaire.

Structured questionnaires are those in which there are definite, concrete, predetermined questions. The questions are presented with exactly the same wording and the same order to all respondents.

The form of the questions may be either closed or open. Structured questionnaire may also have fixed alternative questions in which responses of the respondents are limited to the stated alternatives. When these characteristics are not present in a questionnaire, it can be termed as unstructured or non-structured questionnaire.

In an unstructured questionnsire the interviewer is provided with a general guide on the type of information to be obtained, but the exact question formation is largely his own responsibility and the replies are to be taken down in the respondents own words to the extent possible.

Structured questionnaires are simple to administer, and relatively in expensive to analyse. The provision of alternative replies helps to understand the meaning of the question clearly. But such questionnaires have limitations too. For instance, wide range of data and that too in respondents' own words cannot be obtained with structured questionnaires.

Open-ended and closed questions

There are two major categories of questions : open ended and closed. With open-ended questions, the respondents write in their own answers :

Example : What do you like most about this canteen?
What do you like least about the canteen ?

Closed questions, also known as multiple - choice questions, ask the respondent to choose among alternatives provided by the researcher.

Example : What do you think of the coffee here ?
(like) (dislike) (indifferent)

What do you think of the meals here ?
(too expensive) (very reasonable) (about right)

An open-ended format is desirable

1. When the researcher does not kno all the possible answers to a question,
2. When the range of possible answers is so large that the question would become unwieldy in multiple choice form
3. When the researcher wants to avoid suggesting answers to the respondent, and
4. When the researcher wants answers in the respondent's own words.

Multiple-choice (closed) answers are desirable :

1. When there is a large number of respondents and questi...
2. When the answers are to be scored by machine, and
3. When responses from several groups of individuals are to be compared.

Number of questions

Beginning researchers often include too many items in a questionnaire. In theire desire to omit nothing, they forget that the respondents will become fatigued and lose interest as they go through an unending list of questions. For most purposes, the shortest the instrument, the better.

Wording your questions

Your questions should be clear and meaningful to the respondents. Terms should not be too difficult. Don't overestimate the vocabulary level of your respondents. Define all difficult or jargon terms. It is helpful to include synonyms. Instead of asking a question about nuclear power, you can ask about nuclear or atomic power. The synonym may reach some people who miss the primary term.

Avoid loaded terms, stare words and phrases that immediately raise a red flag.

Avoid double-barraled questions. Ask about one thing at a time. Don't ask about the cost and efficiency of municipal services. It is preferable to divide this into separate questions, one concerned with efficiency and the other with cost.

Whenever possible, avoid phrasing questions in the negative. If you must use a negative, be sure to underline it or have it printed in italics so that it will not be missed.

Example: Of all the classes that you took in high school, which was the single class that you liked least ?

Balance

Balance refers to the neutrality of questions, or providing sufficient items so that the number leaning towards one view are balanced by an equal number leaning toward the other view. The goal of balance is to counteract implicit influence from the questions themselves. The following shows imbalance in questions about canteen food.

Is the meat for salty ?
Are the vegatables overcooked ?
Is the coffee watery ?

Balance can be provided by asking "Is the coffee too weak, too strong or about right?" The second level of bias is that the implied responses for all the items are in the same direction - that the food is not very good. alternative to balancing each individual item is to balance them overall. Fro example leare the implicit bias in the question "Are the vegatables overcooked"?, but balance it with another item of the reverse implied bias such as "Is the meat properly prepared?"

Checklist for evaluating questionnaire items

1. Is the question necessary ? How useful will the answers be ?
2. Is the item clear and unambiguous ?
3. Will the respondent be competent to answer the question as asked ?
4. Will the respondent be willing to answer the questions as asked ?
5. Have double barreled questions been eliminated or revised ?

6. Is the item as short as possible, while remaining clear and precise ?

7. Do the multiple choice questions provide a comprehensive set of choices ? Do they include a "don't know" or "not applicable" category ? Is there an "other" category if appropriate ?

8. Is the answer likely to be affected by social desirability (saying the "right thing") ? If so, can the question be altered to reduce this bias ?

9. Have negatives such as "no" and "not" been eliminated in so far as possible ?

10. Are the questions balanced so that the number of favourable items equals the number of unfavourable items ?

Layout

A self-administered questionnaire must begin with an introductory statement, present the questions in an easily read and easily answered format and close with a note of thanks or appreciation. For interviewer-administered questionnaire, instructions at the bagining will help to ensure consistency from one interview to the next.

Introductory statement

At the top of the questionnaire briefly describe the purpose, identify the person or group conducting the survey, request assistance and provide general instructions. For example,

The purpose of this questionnaire is to learn about attitudes towards the canteen. The survey is being conducted by Mr/Dr/Miss/Mrs/ and from office. We would very much appreciate your assistance in answering the questions below. Please do not write your name on this form in order that the replies remain anonymous.

Question Order

Begin with factual, non-controversial questions.

General questions on a topic should precede specific questions, as in this example.

1. What do you think of this playground ?
2. Is there enough play equipment ?
3. Do you feel that any of the play equipment is dangerous ?

This sequence avoids suggesting danger to the respondent on the first two questions. If danger is mentioned in the first two answers, it is likely to be a salient issue and influence subsequent answers.

Maintain a logical order understandable to the respondent. Routine items such as age and gender can be included at the end. The questionnaire should not begin with overly personal or sensitive questions regarding illicit activities, sex or controversial religious or political opinions.

Answer Format

There are various answer formats in which a respondent inserts a checkmark, number, or letter. The response blanks are placed either to the right or left of the question usually take one of five forms : _____ , ☐ , ( ) , ☐ or ◯

With regard to neatness and appearance, the [ ] has been rated best and the [/] was the worst and the left hand side was best for presenting the response blanks. However, for typed questionnaire, the (') or _____ are easy and acceptable. Another acceptable practice is to have the respondents circle their choice.

Arrange the response blanks to permit easy tabulation. This is especially important if the answers are to be key punched and analysed by computer. Put the answers where the operator can find them. Don't scatter them around the page. Insert computer code numbers close to the answers.

Example :

What is your class in school ? 1 2 3 ( )

Are you a full-time or part time employee ? Full time 1 Part ( )

What is your sex ? Male 1 Female 2 ( )

What is your age ? years (4-5)

Closing statement

At the end of self-administered questionnaire ask for any other comments or suggestions and then thank the respondents for their time and effort.

Pretesting

The first draft of your questionnaire will need revision. No matter how carefully you phrase the original questions, there will be some words that are difficult or unclear, some topics left out. Elaboration is easy in an interview, almost impossible on a questionnaire, because

there may be no person physically present to help explain the meaning of difficult terms or confusing questions, you must anticipate all possible sources of error.

The best way to reduce ambiguity is to pretest the questions. Try them out on a group of people who are asked the items and, in addition, asked to comment on their wording and clarity. Pretesting can be done in a short period of time. There is no need for detailed sampling or statistics. Try it out on a few people who will not be in the final sample but are similar to that sample.

Distributing the Forms

Since a questionnaire is to be answered in writing, it is more easily administered to people sitting down than to those standing or on the move. The questionnaire is ideally suited for office workers, students, library readers and others who are seated at desks or tables.

There are various methods of distributing questionnaires to potential respondents questionnaires can be circulated at a group meeting, handed to people individually or mailed.

Group Meetings

When an organization or class meets regularly, you have a good opportunity to distribute a questionnaire. If the survey is brief and relevant to the groups' purpose, it may be possible to have the questionnaire filled out during the meeting and returned directly to you.

Individual Distribution

Questionnaire can be handed to people as they enter or leave a setting with the request that the questionnaire be filled out and returned through the mail in an attached postage paid return envelope. For maximum returns, the questionnaire should be given out and collected in person. Confidentiality can be assured by not requiring names and enclosing the questionnaire in an envelope.

Mail Surveys

A mail survey is efficient for covering a large geographical area quickly. The advantages of a mail survey are its low labour and travel costs and complete standardization. There is no need to train interviewers and all respondents receive the same questions posted in the same manner. A mail survey also provides more anonymity to the respondents than is possible in a personal interview.

Disadvantages of a mail survey are its impersonality, low return rate and slowness.

A mail survey results in 10 to 33 percent return rate, thus requiring the researcher to send out from three to five times as many questionnaires as are needed for the final sample. There is also a problem of potential response bias when only a small percentage of questionnaires are returned.

A mail survey is not appropriate when timely information is needed.

P.T.O

Difference between Questionnaires and Schedules

Questionnaire	Schedule
1. It is sent through mail	It is filled by the researcher through personal interview.
2. Cheap	More expensive
3. Non-response is high	Non-response is low
4. Not known who replies	Identity of the respondent is known
5. It is very slow. Many of the respondents do not return	Information is collected in time as many as you want.
6. Not possible with illiterates	Can be collected with illiterates.
7. Wider and more representative distribution of sample	Relatively less representative distribution
8. Risk of collecting incomplete and wrong information	Generally the information is complete and accurate.
9. Success lies more on the quality of the questionnaire	Depends on honesty, skills and competence of the investigator.
10. Appearence of the questionnaire must be attractive	It is not required here
11. Observation method is not possible along with this questionnaire method.	Observation method can also be used with schedules.

DEVELOPMENT OF SUITABLE SCALES FOR NUTRITION SURVEYS AND TESTING ITS VALIDITY

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Measuring requires instruments. Measurement is the link between mathematics and science. In a clear form measurement concers the assignment of numbers to objects to represent amounts or degrees of a property possessed by all of the objects. Hence, in its broadest sense, measurement is the assignment, according to rules of numerals to events or objects.

Scales & Tests:- Tests are scales but scales are not necessarily tests because scales do not ordinarily have the meanings of completion and success or failure that tests do. This is the reason of calling achievement testing but not achievement scaling, intelligence testing and not intelligence scaling. Scale is a measuring instrument.

A test is a systemetic procedure in which the individual tested is presented with a set of constructed stimli to which he/she responds, the responses enabling the tester to assign the testee a numeral or set of numerals from which inferences can be made about the tester's possession of whatever the test is supposed to measure.

Procedure for developing standard test:- Following are the steps involved for development of test.

- identification of test areas and collection of test items
- selection of items by determining the item difficulty index and item descrimination index.
- point biserial correlation.
- co-efficient(rpbis) and its estimation.
- establishing validity and reliability of the test.

(i) Identification of test areas:- It is important to identify the areas that should be included in the test and also the most relevant items under each area for example :diet during pregnancy and lactation, weaning etc.

(ii) Selection of items:- The following criteria should be considered in selection of items.

(a) Item difficulty index: The index of item difficulty indicated the extent to which an item is difficult.

(b) Descrimination idex:- This is the second criteria for item selection and is indicated by E 1/3 value for an item. This is mainly to find out whether an item really discriminates a well informed respondent from a poorly informed respondent.

(c) **Point-Biserial correlation coefficient(rpbis):-** This indicates interval validity of the test.

The formula for the point biserial 'r' is $rpbis = \frac{M_p - M_q}{a_r} \sqrt{p \cdot q}$

where

rpbis = Point biserial correlation coefficient

M_p = mean score on continuous variable of successful group on dichotomous variable.

M_g = mean score on continuous variable of unsuccessful group on dichotomous variable

a = standard deviation in continuous variable for total group

p = proportion of persons falling in successful group on dichotomous variable

g = 1 - p (or) the second group.

(d) **representativeness of the test:-** Care should be taken to cover relevant items that represent the test. Validity and Reliability of the test should be established.

ATTITUDE SCALES

Attitudes play an important role in determining behaviour with respect to a particular psychological object. Attitudes may be measured by a variety of methods and techniques.

Construction and Standardisation of attitude Scale:- This involved collection and editing of statements or items, pre-testing and item analysis.

(a) **Collection of items :-** Collection of items should be done in such a way that the acceptance or rejection of each one would imply a different degree of favourable or unvavourable attitude towards that particular statement.

(b) **Editing of items:-** Screening of items(or) statements should be done for content accuracy, ambiguity and repetitions.

Item analysis:- Respondents with higher score and respondents with lower score are the criterian groups for calculating the critical ratio for each item. Responses of these two groups are subjected to item analysis.

Critical Ratio:- This is a measure of the extent to which a given statement differentiated between the high and low groups of the respondents.

$$t = X_H - X_L / \sqrt{E(X_H - X_H)^2 + E(X_L - X_L)^2 / n(x-1)}$$

where

X_H = the mean score on a given statement for the high group

X_L = the mean score on a given statement for the low group

n = member of respondents in the high and low groups

$$E(X_H - X_H)^2 = X_H^2 - \frac{(X_H)^2}{n}$$

and

$$E(X_h - X_L)^2 = X_L^2 - E(X_L)^2/n$$

As suggested by Edward the items with 't' value of less than 1.75 should be rejected and items having 't' values equal or greater than 1.75 should be included in the final scale.

The final scale should be tested for its validity and reliability.

Validity and Reliability:-

Nutrition educators require valid and reliable instruments for evaluation of educational efforts and for research purposes.

Validity - A valid instrument measures what it purposes to measure. In order to be valid, an instrument must match the depth and scope of its intended topic, must be reasonable and understandable to its intended audience, and must bear a measureable relationship to the characteristic or quality that it is intended to assess. Validity is mainly of four types i.e., content,face,criterian and construct validity.

Content Validity - This considers the representativeness or appropriateness of the items of an instrument.

Face Validity - relates to the apprearance of reasonableness of the test from the perspective of the test taken.

Criterian validity - refers to the capacity to infer something about a variable from the scores obtained on the measurement instrument.

Construct Validity requires that the instrument be based upon a sound theory or conceptual rationale.

Reliability - A reliable instrument is one that is relatively free of measurment errors, that is an instrument that would yield a similar score upon repeated administrations all other factors being the same.

Concepts of reliability - Each concept of reliability represents a different approach to measurement of error.

Stability - represents the consistency with which the instrument yields the same or similar scores across time, all other sources of inconsistency held constant.

Split-half reliability - In split-half reliability, on constructs parallel forms within a single test and computes th correlation between the two parts.

Internal consistancy reliability - This refers to the degree t which each item or luster of items relates to the total tes score and is closely related to the split-half reliability.

Variety of instruments are available for research purposes in th field of nutrition to collect date by oral questioning, bu suitable and relevant tests and scales developed are very few t measure nutrition and health knowledge,attitude and practices. Hence efforts should be made to develop different tests an scales by nutritionists for use in nutrition surveys.

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NATIONAL NUTRITION MONITORING BUREAU

NATIONAL NUTRITION MONITORING BUREAU

Dr.Gowrinath Sasthry,Asst.Director,NIN.,Hyderabad.

INTRODUCTION

One of the major activities of the National Institute of Nutrition (NIN), Hyderabad since its inception has been collection of data on diet and nutritional situation of different population groups through specific surveys. In addition, NIN also used to collate the diet and nutrition data collected by different State Nutrition Departments. However, some of these databases, collected by the State Departments, suffered from methodological flaws such as lack of uniform procedure of survey, inadequate sample size, lack of representativeness of the population, etc. Realising this weakness, the Indian Council of Medical Research (ICMR), established National Nutrition Monitoring Bureau (NNMB) in 1972, with the Central Reference Laboratory at NIN with the following objectives:

a) To collect data on diet and nutrition status from representative segments of population, and

b) To conduct evaluation of ongoing nutrition programmes.

Area of Operation

At present, the Bureau is operating in the States of :

Andhra Pradesh
Gujarat
Karnataka
Kerala
Madhya Pradesh
Maharashtra
Orissa
Tamil Nadu
Uttar Pradesh
West Bengal

Every year, each State unit has been conducting diet and nutrition surveys covering 500 households in rural areas and 250 households from five selected groups of urban population (viz. 50 households each from High Income Group, Middle Income Group, Low

Income Group, Industrial Labourers and Slum Dwellers). Currently in each State, about 800 HH are surveyed in rural areas per year.

In each of the States, NNMB adopts NSSO sampling frame of villages to conduct diet and nutrition surveys in a sub-sample of them. Evaluation of ongoing nutrition intervention programmes such as Supplementary Feeding Programme (SFP), Vitamin A, Anaemia prophylaxis programme etc. was undertaken by the Bureau.

Personnel at State Units

Each State Unit of NNMB comprises of the following staff :

1. Medical Officer - One
2. Nutritionist - One
3. ANM - One
4. Driver - One
5. Helper - One

Each unit will be provided with a vehicle for field tour. Equipment like personal weighing scale, anthropometric rod, infantometer, skin-fold calipers, measuring tape, diet survey cups etc. are also provided to conduct the survey work.

Administrative Control of State Units

Currently the State Units function under the Administrative Control of the State Nutrition Officer of the State Department of Health and Medical Services. The Senior Officer of the concerned Department (viz. Deputy Director/Assistant Director) is designated as Officer-in-Charge and vested with the necessary administrative powers to take decisions at the local level and is responsible for the progress of survey work according to time schedule suggested by the Central Reference Laboratory of NNMB.

CENTRAL REFERENCE LABORATORY

The Central Reference Laboratory (CRL) of NNMB, located at NIN, is headed by an Officer-in-Charge with executive powers to administer and co-ordinate the activities of the CRL and State Units. The Director of NIN will be the over-all in-charge of the Bureau. The CRL will have the following responsibilities :

a) Providing survey protocol to State Units - areas (villages) to be surveyed.
b) Training of field investigators.
c) Overall supervision of survey activity.
d) Data processing and analysis.
e) Periodic reporting of data.
f) Organising review meetings and implementing Steering Committee recommendations.

Data base generated :

The following data is collected on the selected households in the village.

a. Socio-economic data of the household;
b. Demographic particulars of family members;
c. Dietary consumption of the family - one day weighment method;
d. Dietary consumption of different members in the family - 24 Hour Recall Method;
e. Assessment of nutritional status of family members
   - Clinical
   - Anthropometric measurements
     (Height, Weight, Arm Circumference, Fat fold at triceps)

NATIONAL AND INTERNATIONAL SOURCES OF INFORMATION

National and International Sources of Information with special reference to Foods and Nutrition.

Dr.E.B.Eswar Reddy, Librarian, A.P.A.U. Hyderabad.

The primary goal of agriculture is the production of food and fiber and in developing countries the production and preparation of food is a major activity. Plant and animal "Crops" constitute raw material for food and food products. Plant and animal "Crops" begin to deteriorate shortly after harvest, gathering or slaughter; and this deterioration very quickly results in the loss of nutritional values and occasionally in the formation of dangerous toxins. The successful application of technology to conserve food nutrients is one of the basic aims of modern food processing.

While more and more worldwide concerns are noticed for an adequate food quantity, food safety, food quality. Greater emphasis is also given for maintaining types and amount of essential nutrients in the foods consumed. Food and Nutrition, therefore, represent two sides of the same basic human need. One is visible and the other is invisible. Hunger and malnutrition go together and their toll on human life and health will be glaring.

INTER-DISCIPLINARY NATURE OF FOODS AND NUTRITION

In recent years nutrition is being given more attention in medical curriculum and there is widespread interest in nutrient labeling, vitamins, minerals and trace elements in the diet, problems associated with obesity, food allergies and possible relationships with between diet and carcinogenesis. Recent special interests in food technology center on new types of food processing, the use of microwaves, convenience foods, synthetic foods and potential dangers from the use of food additives and food substitutes. As such the study of Foods and Nutrition involves not only biology, chemistry, engineering, microbiology, biochemistry, medicine, toxicology, but also food habits and economic and social factors related to food consumption and diet. The Psychology and sociology of food are important, in an affluent society where there is a wide choice in the selection of purchased foods and also in the less fortunate

areas where customs and taboos often are responsible for malnutrition although there may be no shortage of essential nutrients.

Therefore when we discuss about the sources of information we cannot just confine to Food and Nutrition perse.

INFORMATION EXPLOSION

Innumerable estimates have been made of the size of scientific literature, which has grown at an exponential rate in recent years. The number of Scientific Journals, for instance increased from 2 in the seventeenth century to between 30,000 and 100,000 at the present time in Science and Technology alone. It is estimated that 2,000,000 research papers a year are published. Food Science and Technology Abstracts contain over 2000 abstracts every month (IFIS), compiling from 1300 scientific and technical periodicals. Nutrition abstracts and Reviews, series A - Human and experimental covers equal number of periodicals (CAB).

In 17th century information was disseminated via personal comments. In 19th century a scientist could keep up-to-date by persuing 10-20 Journals by spending 30 minutes per day. Today one has to spend 12 years at 13 hours per each day to cover one years literature. For every 10-15 years articles are doubling.

CHANNELS OF COMMUNICATION

Research is an instrument of establishing effective communication for productive use. The ability to preserve and transfer the experiences in arts and crafts and sciences, the ability to use symbols or language in short the ability to communicate with each other differentiates human beings from other animals. So the function of communication has the survival value. Transference of ideas is the transference of culture. Communication system consists four important components, Communicator, Receiver, Media and Message. Communication channels are of two types - Formal and informal channels of communication.

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Why Do We Cite Others ?

1. Paying homage to pioneers
2. Giving credit for related to work; and it is moral obligation
3. Identifying methodology, Equipment etc.
4. Providing back ground reading
5. Correcting one's own work
6. Correcting the work of others
7. Criticising the work of others
8. Substantiating claims
9. Alerting researchers to forthcoming work
10. Providing leads to poorly disseminated poorly indexed or uncited work
11. Authenticating data
12. Identifying original publications
13. Disclaiming the work of others
14. Disputing priority claims of others
15. Identifying original publications desproving a concept or team such as Hodkins disease Raman's effect etc.
16. Making easy for your readers to research your subject further

Documents

The whole of civilization has been built on the basis of man's capacity to send messages over space and time. The human communication network would be incomplete without some agency to store the messages of past and present. They are libraries or archives.

Documents can be classified broadly as Conventional documents, Neoconventional documents, non conventional documents and Meta documents. Each of these categories can be further classified and each of them serve a different purpose or constitute a different format.

Bibliographic control

Delivery of books Act, 1953
National Libraries
Indian National Bibliography
Accession List South Asia
Indian National Scientific Documentation Center
Library of Congres
National Library of Medicine
National Agricultural Library
Institute For Scientific Information
Mix University Microfilms International (UMI)
Online Computerised Library Catalog
British Library Lending Division
INFLIBNET

Information Systems

National Information System for Science and Technology (NISSAT)
National Information Center - NICNET
AGRIS - International Information System for Agricultural Sciences and Technology - AGRINDEX

AGRICOLA, CARIS

CGIAR Institutes

International Food Information Service - FSTA

MEDLARS

BIOSIS

CABI

Online access - Dialog, BRS

Digital Libraries

INTERNET

E-Mail
News groups
Listservs
FTP
Electronic Journals
Tele Conferencing
Library Catalogues
WWW - Hyper Text based system for exploring the Internet resources
WAIS - Wide Area Information Service used document location from Databases
Gopher - Manubased system for exploring Internet resources
Chats/Talks