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NUTRITIONAL STATUS

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EXPERT COMMITTEE ON MEDICAL ASSESSMENT OF NUTRITIONAL STATUS

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EXPERT COMMITTEE
ON MEDICAL ASSESSMENT OF
NUTRITIONAL STATUS

Report

The Expert Committee on Medical Assessment of Nutritional Status met in Geneva from 21 to 27 August 1962. Dr P. Dorolle, Deputy Director-General of the World Health Organization, in opening the meeting on behalf of the Director-General, said that WHO had convened the present Expert Committee to undertake a critical review and appraisal of the clinical and biochemical methods used in the assessment of nutritional status. He hoped that the Committee would recommend the use of reliable techniques and procedures that would serve as a guide to nutrition workers and thus ensure the comparability of results obtained in different nutrition surveys. Professor J. F. Brock was elected Chairman and Dr D. M. Hegsted Vice-Chairman.

1. INTRODUCTION

The problem of assessing the nutritional status of populations has engaged the attention of public health workers for a considerable time. The first attempt to deal with it on an international scale was in 1932, when the Health Organisation of the League of Nations convened a conference of experts in Berlin to consider which physical standards and clinical and physiological examinations could best be used to detect states of malnutrition (*Quart. Hlth Bull. L. o. N.*, 1933). The question was re-examined towards the end of 1936, and some relevant principles were agreed upon in the light of the knowledge then available.

In 1939 the Technical Commission on Nutrition of the Health Organisation published a monograph (Bigwood, 1939), the purpose of which was to suggest standard procedures for conducting nutrition surveys, so that if these were followed the results of different inquiries could be examined comparatively.

The Joint FAO/WHO Expert Committee on Nutrition which met in 1949 indicated that national nutrition policies should be based on knowledge of the nutritional status and dietary patterns of populations, food supplies and economic situations (Food and Agriculture Organization & World Health Organization, 1950). In 1951 the Joint Expert Committee

prepared a guide for nutrition workers on the assessment of nutritional status (Food and Agriculture Organization & World Health Organization, 1951). This described in broad terms the application of anthropometric, clinical and dietary methods. It did not discuss techniques in detail nor did it recommend standard procedures. Revision of the guide was advocated in the Committee's fourth and fifth reports (Food and Agriculture Organization & World Health Organization, 1955, 1958).

In 1957 the United States Interdepartmental Committee on Nutrition for National Defense (ICNND) issued a manual for the use of those engaged in nutrition surveys of armed forces in the Near and Far East. This publication describes techniques and procedures in detail and discusses the interpretations of results. Although it was designed primarily for use in surveys of military forces, it has recently been increasingly used in surveys of civilian populations (including women and children).

The FAO/WHO Seminar on Food and Nutrition Problems in Africa South of the Sahara, held in Lwiro, Congo, in 1959 (Food and Agriculture Organization, 1961), noted the urgent need in Africa for precise information on the prevalence and geographic distribution of malnutrition, and on dietary and other causative factors. The Seminar reviewed the information then available and recommended practical methods of assessing the nutritional status of populations.

The value of nutrition surveys is greatly enhanced when they are supplemented by data from dietary, socio-economic and cultural inquiries; it is important that clinical nutritionists make use of all such information. As a basis for sound conclusions, clinical observations must be considered in relation to all factors having an influence on nutrition.

After careful consideration, the Committee decided against the advisability of recommending standard schedules for use in the field. It was of the opinion that specific schedules should be prepared according to the particular objectives of the survey and other factors, including the composition of survey teams, facilities for statistical processing, the geographic and climatic features of the area and the type of population groups involved. This could be done by adapting one of many existing schedules based on the information and guide-lines indicated in Annexes 1-3. Specimen schedules could usefully be included in a manual of nutrition survey methods and techniques; the need for such a manual for international use is emphasized.

Historically, the medical study of nutritional status has been almost entirely concerned with signs of nutritional deficiency diseases. In recent years the problem of obesity and other effects of over-feeding and their association with a high incidence of degenerative diseases has received attention. There is need to assess the long-term effects of habitual dietary patterns and their correlation with infectious and degenerative disease. While the approach to assessment of nutritional status through observation

of malnutrition must continue, it is hoped that further study of the criteria of health and well-being will lead to the definition of criteria for the recognition and promotion of good nutrition.

This report is intended primarily as a guide for medically qualified workers undertaking the assessment of the nutritional status of populations. It is recognized, however, that there may be situations in which some of the observations and tests will need to be carried out by auxiliary and other non-medical personnel. Such personnel not only should have adequate preliminary training in the appropriate techniques, but should at all times work under medical supervision.

The clinical and biochemical aspects of nutritional assessment occupy most of the present report, but other approaches are also discussed because of their importance in attempts to assess comprehensively the nutritional status of populations. Many areas for research are apparent and are indicated in the appropriate sections of this report.

2. OBJECTIVES OF NUTRITION SURVEYS

In order to plan and put into effect measures not only for the control and eradication of malnutrition, but also for the subsequent maintenance of good nutrition, the epidemiology, magnitude and geographic distribution of malnutrition must be determined. Information on the ecology of populations will aid in the discovery of factors directly or indirectly involved. Such information, essential for national or international nutrition programmes, is obtained from nutrition surveys. The principal aim of these is to ascertain dietary need and to suggest corrective measures, but they may also be useful in motivating a community to take action to achieve better health through improved diet. This is especially so when members of the community participate as subjects or voluntary assistants. Surveys may also attract the attention of policy makers and administrators who are ultimately responsible for the development of practical nutrition programmes.

The survey gives an opportunity to test the value of methods proposed for specific programmes, and also provides information on the basis of which the effect of measures to improve nutritional status can be assessed. Information gained in periodic nutrition surveys should also assist in determining the effect on nutrition of other measures designed to improve socio-economic and health conditions in a community.

The Committee noted the lack of well-trained nutrition workers, without whom the development of nutrition programmes and the training of auxiliary workers to participate in them are impossible. The nutrition survey has great value as a teaching device at both the professional and the non-professional level. Where the training of public health personnel

such as nutritionists, health educators and nurses is concerned, participation in a nutrition survey is of value principally if it occurs within the region in which the individual is to be responsible for corrective measures. Such first-hand experience can also prepare others, such as agriculture extension workers, home economists and dietitians, for a better understanding of existing problems and the remedial measures needed.

The importance of according to nutrition its proper place in the curricula of schools of medicine and public health was emphasized. The Committee recommended that adequate instruction in nutrition be given not only to medical personnel, but to all other categories of workers associated with programmes to improve the health status of the population. Nutrition surveys provide students with an opportunity to observe clinical deficiency states—an opportunity often denied in schools in developed areas. Simultaneously, they can help to evolve an understanding of the importance of nutrition as a community health problem.

The survey affords a valuable opportunity to the biochemist for critical scrutiny and standardization of laboratory methods, for the training of technicians and for the application of the many laboratory methods used in the estimation of nutritional levels. Equally important, however, is the incentive that the nutrition survey has provided for research scientists and public health workers to develop methods which may be applicable in field studies, and the opportunity to test these methods in practice.

Nutrition surveys provide information as to the impact of the food industry and commerce on public opinion, family budgeting and changes in dietary patterns. Information derived from nutrition surveys should provide the basis for ethical advertising and production policies adopted by the food industry.

The results of nutrition surveys contribute towards the recognition of the need for legislation, with particular reference to (*a*) taxation and subsidy as they affect the production and distribution of food and the provision of protective foodstuffs to vulnerable groups, (*b*) food and water hygiene, and (*c*) group feeding programmes.

3. METHODS USED IN THE ASSESSMENT
OF NUTRITIONAL STATUS

The assessment of the nutritional status of an individual member of a community is accomplished by carrying out clinical, biochemical, anthropometric and biophysical examinations. To determine the nutritional status of any given community or section of a community, it is necessary to apply such techniques to all its various members, or to a representative sample including persons of all ages and both sexes in the different socio-economic groups comprising the whole.

The methods to be followed are described in subsequent sections. However, such a medical assessment of nutritional status is, by itself, of limited practical value without an assessment also of the different factors from which it results. It is essential that background information be acquired in order to interpret the clinical picture and to propose corrective measures when the clinical nutritional status is found to be unsatisfactory.

TABLE 1. INFORMATION NEEDED FOR ASSESSMENT OF NUTRITIONAL STATUS

Sources of information	Nature of information obtained	Nutritional implications
(1) Agricultural data Food balance sheets	Gross estimates of agricultural production Agricultural methods Soil fertility Predominance of cash crops Overproduction of staples Food imports and exports	Approximate availability of food supplies to a population
(2) Socio-economic data Information on marketing, distribution and storage	Purchasing power Distribution and storage of foodstuffs	Unequal distribution of available foods between the socio-economic groups in the community and within the family
(3) Food consumption patterns Cultural-anthropological data	Lack of knowledge, erroneous beliefs and prejudices, indifference	
(4) Dietary surveys	Food consumption	Low, excessive or unbalanced nutrient intake
(5) Special studies on foods	Biological value of diets Presence of interfering factors (e.g., goitrogens) Effects of food processing	Special problems related to nutrient utilization
(6) Vital and health statistics	Morbidity and mortality data	Extent of risk to community Identification of high-risk groups
(7) Anthropometric studies	Physical development	Effect of nutrition on physical development
(8) Clinical nutritional surveys	Physical signs	Deviation from health due to malnutrition
(9) Biochemical studies	Levels of nutrients, metabolites and other components of body tissues and fluids	Nutrient supplies in the body Impairment of biochemical function
(10) Additional medical information	Prevalent disease patterns, including infections and infestations	Interrelationships of state of nutrition and disease

Table 1 describes sources of information that may be available for assessing the nutritional status of a community, the nature of the information obtained by various methods and its nutritional implications. The background information necessary for a full appreciation of any nutritional situation

embraces studies of environmental and social factors, some knowledge of food consumption, and a consideration of such vital statistics as morbidity rates in different age groups and mortality rates. Therefore, when circumstances call for a survey of nutritional status, careful advance planning is necessary if all the relevant information is to be obtained.

3.1 Collection of background data

The epidemiological approach to the assessment of the nutritional status of populations is founded on the recognition that nutrition problems originate from complex interrelationships between environmental and social factors and the individuals in a community.

3.1.1 Comprehensive information

3.1.1.1 Preliminary inquiry

Environmental and cultural information obtained previous to the survey from a study of relevant literature and from discussion with persons having experience of the local cultural pattern is essential for the planning, selection, application, and acceptance by the population of the methods of study. The selection of the regions or groups to be surveyed will be influenced to a large extent by environmental, social and cultural factors. " Typical " groups are often selected accordingly. Knowledge of the degree of economic, geographic, social and ethnic heterogeneity of the population will be the basis for deciding the extent of stratification.

Successful application of nutrition survey methods involves the co-operation of the subjects (Wellin, 1955). The approaches to be used are determined by the extent of their willingness to permit a probe into their customs, beliefs and ways of life. Some groups may refuse to have their bodies subjected to the close examination required by the clinician. Particular difficulties may be encountered in the collection of blood and urine specimens for biochemical studies. However, previous knowledge of the reaction patterns of the population will allow the investigators to arrive at the most suitable plan of action.

3.1.1.2 Nutrition survey

The collection of information about the environmental, socio-economic and cultural-anthropological background of the group under study must be considered an intrinsic part of a nutrition survey. With appropriate sampling, the work involved can be reduced considerably in amount and complexity. The information should refer principally to those characteristics of the population considered to influence nutrition more directly, such as economic factors, marketing, and other circumstances determining food availability, food habits, infant-feeding practices, feeding of pregnant

and lactating women and cooking practices. Particular attention should be given to beliefs and taboos, which often determine dietary patterns independently of other causes. In contrast to more intensive surveys that require the services of a qualified anthropologist, collection of information of this sort can be carried out by other types of personnel, such as social workers, after suitable training. It is, however, desirable that the actual methods and techniques be decided in consultation with a cultural anthropologist.

3.1.2 *Detailed information*

Detailed information can only be obtained by prolonged intensive investigations by a trained cultural anthropologist, as exemplified by studies in Malaya carried out by WHO in 1958-59. Studies of this sort do not fall within the scope of a cross-sectional nutrition survey, but the results of such intensive investigations " in depth " are of great value in the selection of the areas or population groups for a nutrition survey, and for the planning of programmes of improvement designed to solve the nutrition problems that it may reveal.

3.2 Planning

The success of a community survey depends considerably on the thoroughness of preliminary planning, which will be guided by available information concerning the local environment and culture pattern and by statistical considerations. Approaches must be made through local leaders, who may be traditional, political or religious, or all of these. It is only by furnishing them with a careful explanation that a survey team is likely to be accepted and co-operation and attendance of the public assured. In addition, the arrangement of the survey programme will depend on advice from local leaders, and on seasonal events, such as the rains or widespread community activity related to agriculture, religion or ritual. Motivation of populations to ensure attendance is important, and is usually best achieved by making it known that the team will treat any sickness found. Careful thought must be given to details of transport, accommodation and survey equipment, including the handling of biochemical samples and supplies.

Team training should form an indispensable feature of the planning phase. It is essential that all members of the team understand every aspect of the procedures to be employed, such as the precise definition of the physical signs to be used in clinical assessment. It is often valuable to have a mock demonstration of the expected " line-of-flow " and of the methods to be used, as well as a demonstration of clinical signs by the use of appropriate visuals aids, prior to leaving for the actual field survey.

A preliminary pilot study to test the methods that are to be used would be desirable.

3.2.1 *Statistical considerations*

A detailed discussion of sampling methods is beyond the scope of this report. Survey plans should be made according to accepted statistical principles, preferably by consultation between nutritionist and expert statistician. The type of sampling to be employed will depend on the specific objectives of the survey and the geographic areas to be covered.

The size of samples is determined by the expected prevalence of the characteristic to be measured and the desired degree of precision in the estimates; while specifying the degree of precision required, attention should be paid to the experimental errors inherent in the measurement of the characteristics. In a multi-phase survey such as a nutrition survey, different approaches (anthropometric, clinical, biochemical) will require different sample sizes and may involve different sampling techniques. For the more general and easier measurements, such as those of height and weight, a large sample may be taken, while for the more complicated biochemical determinations a smaller sample will have to suffice. Main samples and sub-samples must be carefully chosen on a statistical basis such as by the application of multi-phase sampling methods. It is desirable that the investigation be statistically designed so that variations, such as those due to inter-observer differences and experimental errors, are reduced to a minimum.

The design of survey schedules is most important and they should be constructed by the nutritionist and statistician together. Only information which is pertinent to the survey and which is to be used in the final analysis should be included. The classification and terminology employed should be precise and unambiguous; each schedule should be accompanied by detailed instructions for its completion.

3.3 Food consumption studies

Information about predominant dietary patterns, specific foods consumed and estimated nutrient intakes serves the following purposes : (a) it indicates relative dietary inadequacies, as judged by standards available at present; (b) it is essential for studying the relationship between nutrient intake and the state of health as judged by clinical and biochemical criteria ; and (c) it defines the base-line for assessing improvement in the customary diet.

Useful accounts of this aspect of nutrition surveys are given in two issues in the *FAO Nutritional Studies Series* (Norris, 1949 ; Reh, 1961). These publications are valuable guides for planning dietary surveys covering entire populations, family groups or individuals within the family. They consider the effect of seasonal variation of food supplies upon consumption

and call attention to the importance of determining the distribution of food between socio-economic classes, as well as between individuals within the family, since in some societies small children often do not receive a fair share of the available ration.

4. VITAL STATISTICS

4.1 Implications

Crude mortality rates, age-specific and some cause-specific mortality rates, stillbirth and prematurity rates, morbidity rates of diseases such as tuberculosis and diabetes mellitus and some menstrual disorders differ according to whether the populations concerned are malnourished or well-nourished (Bengoa, Jelliffe & Perez, 1959; Platt, 1947, 1958; Sebrell & Hundley, 1954; Sinclair, 1948; Swaminathan, Apte & Someswara Rao, 1960). Mortality and morbidity statistics may thus be indices of nutritional status. This is of special significance in public health practice because the observations involved usually lend themselves to precise recording on a communal scale.

4.2 Age-specific mortality

Deaths of children between 1 and 4 years of age seem particularly related to malnutrition. It has therefore been proposed that this age-specific mortality rate be used as an index of the nutritional status of a population (Bengoa, 1957). It is recognized, however, that the relative importance of malnutrition and disease in causing mortality varies widely in different circumstances. Because of this, it has been suggested that, when the mortality among children aged 1-4 years is considered in relation to the infant mortality rate in the same community, a more specific index of nutritional status is obtained (Wills & Waterlow, 1958). This depends upon the assumption that complicating factors, such as disease and incomplete birth registration, are common to both age groups, but that malnutrition mainly affects the age group 1-4 years. Whether the latter refinement or the age-specific mortality rate for the age group 1-4 years itself is used, it must be remembered that either can be quite inaccurate if an appreciable number of infants below 1 year of age are dying because of malnutrition. It is now known that such circumstances exist, and in some instances indeed the main incidence of protein-calorie malnutrition is found in infants less than 1 year old.

There is much to be said for analysing mortality statistics according to age intervals smaller than those usually employed. In areas where thiamine deficiency is a potential danger, a relatively high mortality rate among infants aged 2-5 months is significant evidence of its occurrence (Aykroyd & Krishnan, 1941). In this connexion, the great difficulty of

assessing the problem of infantile beriberi by any other means should be recalled. In much of the world, grouping deaths of children 1-2 years old would probably be found most useful in assessing mortality from protein-calorie malnutrition. Further inquiries may reveal other age-specific mortality rates significant in relation to malnutrition.

4.3 Morbidity and cause-specific mortality

The association between nutrition and infection has a number of implications and is to be the subject of a special discussion organized by WHO in the near future. It seems clear, however, that the course of some specific diseases is adversely affected by malnutrition, and in so far as this is true, disease-specific mortality provides a good index of nutritional status.

The Joint FAO/WHO Expert Committee on Nutrition which met in April 1961 discussed revision of the listings of nutritional diseases in the *International Classification of Diseases* (Food and Agriculture Organization & World Health Organization, 1962). The Committee drew attention to the fact that malnutrition is an important contributory cause of morbidity and mortality in such diseases as tropical ulcer, diarrhoeas of infectious origin, tuberculosis, measles and bronchopneumonia, and recommended that death certificates include mention of severe malnutrition whenever it occurs concurrently with the immediate cause of death or primary disease.

Diarrhoea is one of the principal causes of death among infants and children. Malnutrition is often an important contributory, and may even be a primary, cause of diarrhoea. Thus, statistics of morbidity and mortality attributable to diarrhoea of early childhood may be valuable indices of malnutrition.

Measles is an almost universal disease. The attack rate remains high even in countries where mortality due to the condition is now very low (Wilson, 1962). Measles therefore represents a stress affecting populations all over the world. Under this stress, mortality rates and the severity of the disease seem to be related to the nutritional state of the group involved. In some instances an epidemic of measles may unmask the existence of malnutrition. Measles is a condition easily recognizable and well known to lay people so that information about it can readily be obtained by field workers.

There is evidence that, under certain circumstances, inadequacy of total food supplies influences the incidence of and mortality from tuberculosis in a community.

4.4 Sources and reliability of vital statistics

In many parts of the world, especially those where malnutrition is an important problem, no vital and population statistics are available.

In other instances available information cannot be accepted as reliable and may only apply to urban groups.

Such is the importance of accurate information about mortality and morbidity in relation to nutrition that attempts should always be made, probably by the nutrition survey worker himself, to collect it first-hand in the locality of the survey. This should be done even when official records are available, because such records usually apply to a whole country and local statistics may deviate from the over-all averages.

Information can be collected in a number of ways—for example, by questioning mothers in their homes and when they bring children to clinics (Gongora & McFie, 1959, 1960), and best of all by direct observation of the number of people ill or dying in a given area over a period of time. Attention should be given to the " early ", " intermediate " and " late " foetal death rates (or " stillbirth " and " abortion " rates, if statistics are published in these terms), the seasonal and annual incidence of disease in relation to temporary food shortage, and the age composition of the population.

Hospital records should give an indication of the state of nutrition of the patient in addition to the immediate cause of admission to hospital. Anaemia, for example, often exists in patients admitted for other reasons without its appearing in the morbidity record. It is important, however, that hospital statistics should not be confused with incidences of disease and malnutrition in the total population.

4.5 Recommendations

On present evidence, it is recommended that, for the purpose of assessing the nutritional status of a community and as a base-line from which to judge changes, nutrition workers should :

(1) Collect first-hand information about mortality, including infant mortality in the population which they are investigating. This should form an integral part of nutrition surveys.

(2) Collect information about the incidence of and mortality from relevant diseases, particularly measles and diarrhoea.

5. ANTHROPOMETRIC MEASUREMENTS

The pattern of growth and the physical state of the body, though genetically determined, are strongly influenced by nutrition, so that anthropometric measurements are useful criteria for assessing nutritional status. It may be difficult, however, to differentiate deviations of physical measurements due to factors other than nutrition. Techniques of obtaining anthro-

pometric measurements have been discussed in the literature and have been extensively reviewed (Brožek, 1956).

5.1 Standards

The results of anthropometric nutrition surveys should preferably be expressed in relation to local standards that have been constructed from measurements of apparently healthy subjects of the same ethnic group. If, as is often the case, local standards are not available, it is justifiable for comparative purposes to use a widely recognized standard, such as that based on the height and weight of children in Iowa City (Meredith, 1959), while realizing the limitations of this arbitrary type of procedure.

Whether standards of comparison are available or not, it is of the greatest importance that measurements be accurately made with good quality, frequently checked instruments. Use should be made of lever balances rather than of spring-type balances. Measuring tapes must be inexpansible. Results should be reported in a way that allows proper statistical comparison to be made ; in particular, full details of numbers, age distribution, mean results and standard deviations should be recorded.

The common absence of local standards emphasizes the need to stimulate research into patterns of growth in all parts of the world in relation to genetic and environmental influence.

5.2 Age assessment

Anthropometric measurements in children can best be interpreted if related to the standard for the particular age. This presents great difficulties in many developing regions where ages of children are not known with accuracy. Age assessment can be attempted by means of documentary evidence possessed by the parents (e.g., birth or baptism certificates, or horoscopes), but this is rarely practicable. Often it may be necessary to construct a locally relevant calendar of events, which can include agricultural, meteorological and political occurrences, and natural or man-made disasters (Jelliffe & Jelliffe, in press). In some communities there may be a lunar calendar already recognized in the local culture. Age assessment calendars of this type are time-consuming to prepare, and need to be pre-tested in a segment of the community whose ages are known (e.g., children attending a child welfare clinic from birth onwards). As supporting evidence, the child's dental eruption, other milestones of development and the presence of younger or older siblings should be considered.

The difficulty of age assessment becomes progressively greater in later childhood, and the calendar method is probably not usually applicable to school-age children. In comparing an individual or a group average

to a standard, it is desirable to know the age or average age to the nearest month.

In presenting results, the weights of infants less than six months old should be grouped together for age intervals of one month; those of infants 6 months to 2 years old for age intervals of 3 months; and those of children 2-5 years old for age intervals of 6 months. For subjects over 5 years of age, grouping in yearly age intervals is satisfactory.

During early life, weight may be appreciably influenced by birth weight. It is therefore highly desirable to include, whenever possible, a record of birth weight in the analysis of present weight, especially of infants.

5.3 Basic methods

The relation between growth and nutrition can best be examined by longitudinal studies, especially in early childhood. Healthy children 5-10 years of age increase their weight by about 10 % and their height by about 2 inches (5 cm) each year. These rates are lower in children suffering from malnutrition. Thus it is possible in longitudinal studies of growth to make an assessment of nutritional status with a knowledge of approximate age only. Opportunities for this type of investigation are not usually available in nutrition surveys, except from results collected at child welfare clinics and in schools.

Usually only cross-sectional, single-measurement studies can be achieved. However, the value of these isolated measurements of height and weight —that is to say, measures of present size rather than of growth—should not be ignored. The change in mean weight of large groups of adults can reflect quite sensitively changes in total food consumption or energy expenditure. Such changes can sometimes be assessed by comparison of present with previous observations in the same community. An appreciable number of underweight children and adults in a population may often indicate widespread malnutrition, especially when this has persisted over many years.

5.4 Anthropometric measurements

Large numbers of anthropometric measurements can be undertaken and have varying degrees of usefulness at different age periods, depending upon different relative rates of increase of body components (Table 2). Some have a specially wide practical application.

5.4.1 *Weight*

Weight is the simplest anthropometric measurement of growth and nutrition. Presentation of weight levels of a community can be expressed

TABLE 2. RECOMMENDED MEASUREMENTS IN NUTRITION SURVEYS

Age group (years)	Practical field observations	More-detailed observations

0-1	Weight
	Length
		Stem length
		Circumferences:
		  head
		  chest
		Bicristal diameter
		Skin-fold:
		  triceps
		  subscapula
		  chest

1-5	Weight
	Length (up to 3 years)
	Height (over 3 years)
	Skin-fold over biceps and
	  triceps
	Arm circumference
		Stem length (up to 3 years)
		Sitting height (over 3 years)
		Circumferences:
		  head
		  chest (mid-inspiration)
		Bicristal diameter
		Skin-fold
		  subscapula
		  chest
		Calf circumference
		Posterior-anterior X-ray film of
		  hand and wrist *

5-20	Weight Height Skin-fold over triceps	Sitting height Bicristal diameter Biacromial diameter Skin-fold—in other sites Posterior-anterior X-ray film of hand and wrist* Arm and calf circumferences
Over 20	Weight Height Skin-fold over triceps	Skin-fold—in other sites Arm and calf circumferences

* Interpreted by comparison with atlas of Greulich & Pyle (1950).

for children in terms of percentages in various percentiles of standards, or as percentages in the three degrees of malnutrition proposed by Gomez et al. (1956). As there is considerable dispersion of individual body-weights even within presumably well-nourished communities, it may be better to express weight in relation to some fixed parameter rather than in absolute terms (Garn, 1962).

The head grows at its maximum rate very early in life. At this time the infant's diet is good in most places, consisting mainly of the mother's milk, and growth is satisfactory. Subsequent growth failure may therefore be judged by relating body-weight to head circumference. This is not always satisfactory in practice owing to the difficulty of obtaining the latter measurement accurately.

Weight correlated with height (or length) can be employed to detect both under- and overnutrition. The disadvantage is that height is also

affected by malnutrition during growth. Nevertheless, the correlation can be useful in assessing protein-calorie deficiency of early childhood, because the growth retardation that occurs affects weight markedly and height only to a limited extent.

Owing to variations in rate of growth at different ages, weight increments at different periods of observation will be significant at various stages of growth. For example, in the first 6 months of life, a minimal period between successive weighings is 4 weeks; in school-age children, an interval of 12 months is required before failure to gain weight can be fully appreciated.

Weight change in pregnancy is a useful indicator of maternal nutritional status.

5.4.2 Height

The value of measurements of length for younger children and height for older children and adults is well appreciated, although in adulthood, since height is already fixed, it loses its value as an indicator of current nutritional status. It may sometimes be preferable, if ages are known, to demonstrate the physique of individuals in one graphic presentation, combining age, weight and height, methods for which have been described (Tremolières, 1957; Wadsworth, 1961). The femur normally grows relatively faster than other parts of the skeleton (Maresh, 1959), so that change in leg length, or cristal height, may provide a sensitive index of inadequate nutrition in children.

5.4.3 Skin-folds

Despite practical difficulties in obtaining consistent results with skin-fold calipers, these provide the only simple means of measuring subcutaneous fat and therefore body composition. The importance of measuring body composition in relation to problems of nutrition is now becoming apparent (Garn, 1962). Skin-fold calipers and the technique employed should be standardized (Brožek, 1956). The best sites for skin-fold measurements are debatable and may vary with the age group under consideration (Garn, 1962). The balance of evidence at present favours measurement over the triceps as an index of nutritional status.

5.4.4 Arm circumference

Measurement of the circumference of the mid-upper arm may prove to be a useful and practical means of assessing protein-calorie deficiency of early childhood. In addition, if the biceps and triceps skin-folds have been measured, an estimate of the arm muscle size can be made (Jelliffe & Welbourne, in press; McFie & Welbourne, 1962).

5.5 Recommendation

A detailed guide describing measurement techniques and ways of recording and analysing the results of anthropometric studies in relation to assessment of nutritional status would be of great value. This must form part of a more extensive manual for nutrition surveys, the preparation of which is one of the recommendations of the present report. Until such a manual becomes available, the techniques and approaches recommended by the Committee on Nutritional Anthropometry of the Food and Nutrition Board of the National Research Council (USA) may be adopted (Brožek, 1956).

6. CLINICAL ASSESSMENT OF NUTRITIONAL STATUS

6.1 General comments

Clinical examination is the most essential part of all nutritional surveys, since the ultimate objective is to assess levels of health of individuals or of population groups in relation to the food they consume. Numerous functional or structural manifestations are known to be associated with states of malnutrition. They are assessed in the course of clinical examination with or without instrumental aid. Like any other clinical examination, however, it has its own limitations, which must be known if full advantage is to be obtained from the application of this valuable method. Discussions on the difficulties involved and the shortcoming of this procedure have formed part of extensive reviews by individual scientists or expert committees during the last fifteen years (Darby et al., 1953 ; Gopalan & Rao, 1961 ; Jolliffe, 1954, 1962 ; Jolliffe et al., 1958 ; National Research Council, 1949 ; Rao, 1961 ; Sandstead et al., 1956 ; Sinclair, 1948 ; United States, Interdepartmental Committee on Nutrition for National Defense, 1957 ; Food and Agriculture Organization & World Health Organization, 1951). The more common sources of error and the pitfalls to be guarded against in clinical surveys are briefly mentioned below.

6.1.1 Non-specificity of signs

The fact that some early signs of malnutrition lack specificity is well recognized. Common signs such as glossitis, angular stomatitis, cheilosis, corneal vascularization, follicular and other lesions of the skin, on which much reliance is placed in nutrition surveys, are probably caused not only by a deficiency of more than one nutrient, but also by various non-nutritional factors. Even many advanced signs associated with such specific deficiency diseases as kwashiorkor, rickets, scurvy, beriberi and pellagra

are not necessarily pathognomonic when they are detected singly. Bitot's spots, conventionally interpreted by some investigators as signs of vitamin A deficiency, may be due to other factors as well (Darby et al., 1960). The true significance of signs encountered in surveys should be interpreted by physicians experienced in nutrition. Even with the aid of information obtained from biochemical, biophysical or dietary studies, their interpretation may be difficult. In any case they should be interpreted in the context of the local environment.

6.1.2 *Overlapping of deficiency states*

By and large, dietary deficiencies are not restricted to an isolated nutrient. This state of affairs leads to difficulties in the interpretation of results, particularly in view of the fact that a single lesion may be caused by the deficiency of more than one nutrient. To illustrate this point, glossitis may be seen in niacin, folic acid, vitamin B_{12} or riboflavin deficiency. Similarly, angular stomatitis may be encountered where there is riboflavin deficiency or iron deficiency. Follicular hyperkeratosis may be due to lack of essential fatty acids or of vitamin A or of both ; another variety is found in vitamin C deficiency. " Associating " signs with biochemical or other tests may help to decide the lack of which nutrient or nutrients is really responsible for a given lesion, but as already mentioned, the simultaneous lack of many nutrients in diets renders the final diagnosis more difficult. It is in such instances that therapeutic trials on a small scale may be useful if carried out under strictly controlled conditions.

6.1.3 *Bias of the observer*

Lack of consistency in the observations of two or more examiners, however experienced they may be, and in those of the same examiner at different times, has been well recognized as a constant source of error in all kinds of clinical assessment. The less defined the criteria adopted, the more divergent are the differences of opinion in their assessment. The grading of subjects into " good ", " fair " or " poor " by a general physical inspection, based on a number of factors such as body-build, amount of muscle, subcutaneous fat, colour of the mucous membranes, posture and behaviour, is still in use ; but such grading is influenced by so many factors—for example, the examiners' own body build or his own recent experiences—that the wisdom of including such appraisals in survey schedules can rightly be questioned. A more serious difficulty is the proneness of the observer to change his criteria from time to time, depending on the characteristics of the group he is observing. That this observer variation can be experienced even with such objective signs as dry and scaly skin and pigmentary changes is well documented (Gopalan & Rao, 1961 ; McGuinty & Darby, 1954 ; Rao, Taskar & Ramanathan, 1954 ;

Sinclair, 1948 ; United States, Interdepartmental Committee on Nutrition for National Defense, 1957). It must be realized, however, that observer errors can be considerably reduced by adopting uniform and well-defined criteria.

6.1.4 Bias of the observed

The bias of the subjects being examined is likely to enter into the picture when medical histories are obtained. Interrogation to elicit complaints and past medical history is likely to influence the answers, unless tactfully carried out. Greater reliance may be placed on complaints volunteered by the subjects, but such complaints may depend on what the complainant himself thinks of as normal or abnormal. Great caution is necessary in their interpretation. Medical histories help to indicate areas where clinical examination or biochemical tests may have to be concentrated; they elucidate the evaluation of the disease process and draw attention to conditioning factors, if any.

The difficulties discussed in the preceding paragraphs are inherent in the method; they are " non-sampling errors ". By appropriate precautions, many of them can be reduced considerably. The fact that some signs are non-specific does not exclude their use as indices of malnutrition in population studies. The frequent occurrence of a particular sign gives a lead for further investigation, while its association with other related signs may attain nutritional significance. Thus, clinical examination serves as a very useful tool in nutrition surveys since it provides visible evidence of a basic or corroborative nature that reflects the nutritional status of the communities under observation.

Considerable attention has been paid to the techniques of clinical nutrition surveys with a view to avoiding or minimizing some of the flaws discussed above. In this connexion, standardization of survey methods and adoption of uniform definitions and diagnostic criteria have been particularly emphasized (Food and Agriculture Organization, 1961 ; Gopalan & Rao, 1961 ; McGuinty & Darby, 1954 ; Muñoz & Perez Avendano, 1956 ; National Research Council, 1949 ; Rao et al., 1954 ; Sinclair, 1948 ; United States, Interdepartmental Committee on Nutrition for National Defense, 1957). Some major agencies interested in this field, for example, the Committee on Nutrition Surveys of the United States National Research Council, 1949 (NRC), the United States Interdepartmental Committee on Nutrition for National Defense (1957) (ICNND), the Institute of Nutrition of Central America and Panama (INCAP) (Muñoz & Perez Avendano, 1956), the Nutrition Survey Sub-Committee of the Indian Council of Medical Research (Rao et al., 1954) (ICMR) have evolved standard schedules and guides for the detection of signs and clinical assessment of nutritional status. This practice is followed by individual workers in many other countries. The Joint FAO/WHO Expert Committee on Nutrition, in

reviewing this problem in 1951, discussed in some detail the techniques of clinical assessment and their application and interpretation, but decided that it would not be desirable to recommend any standard schedules for this purpose. The opinion of this Committee was that schedules should be drawn up as necessary by local workers and based on experience gained through pilot surveys (Food and Agriculture Organization & World Health Organization, 1951).

6.2 Review of present practices

For general nutritional assessment, a number of schedules are now in use in different parts of the world. They are broadly of two types : an abbreviated form for rapid assessment (Food and Agriculture Organization, 1961), and a more elaborate form for detailed assessment. The latter is more commonly used and consists of medical history, nutritional rating of subjects by general inspection, some body measurements and a number of clinical signs depending on the preferences of the investigators. The number of clinical signs included ranges from about a dozen to nearly one hundred in different schedules. The variety of clinical signs mentioned is also large, covering specific nutritional signs, non-specific signs which nevertheless imply the presence of malnutrition, signs of undetermined nature, and signs unrelated to malnutrition. In certain instances, these various signs have been graded as mild, moderate or severe. While some of the agencies (ICNND, INCAP) which have designed schedules have given diagnostic criteria for different signs, others (NRC, ICMR) have left them to the choice of the investigators, with the hope that such criteria would be selected by reference to standard textbooks. This has led to the adoption of schedules made up of different sets of signs, a considerable variation in the terminology used and the adoption of different diagnostic criteria. For example, some investigators are satisfied with the term " glossitis ", meant to convey " oedema and fissuring ", while others have included changes in the papillae, surface and colour of the tongue (Rao, 1961 ; United States, Interdepartmental Committee on Nutrition for National Defense, 1957).

Again, there is variation in the pattern of reporting the results. An investigator sometimes does not refer to any pro forma schedule but merely mentions that the usual signs accepted as having nutritional significance have been taken into account. The signs actually looked for may have been the correct ones, but the diagnostic criteria are left to the guess of the reader.

The need for a common terminology and general agreement of definitions for the various signs is obvious. It can be effectively fulfilled by an international organization such as WHO, which, with its wide interest in nutrition and other health problems of the world, exerts a unifying influence

on the programmes of different nations. It is for this reason that a comprehensive list of physical signs is presented in Annex 1. As desired by the Joint FAO/WHO Expert Committee on Nutrition which met in 1951 (Food and Agriculture Organization & World Health Organization, 1951), the actual proforma schedule can still be left to the choice of the investigator, provided the known nutritional signs and techniques included in such a proforma schedule are drawn from the common framework of signs on which experts in the field agree. This precaution, it is hoped, will help to reduce difficulties in interpretation and will enable one observer to understand the language of another in the field of nutrition surveys. If any new signs are included, care must be taken to provide a clear description of them, supported by illustrations wherever possible.

6.3 Classification of signs

As mentioned earlier, most of the proforma schedules now in use include a number of signs unrelated to nutrition. Gradually enough knowledge has been gathered to throw doubt on the relation to malnutrition of some of the signs recorded in the past. The Joint FAO/WHO Expert Committee on Nutrition which met in 1951 pointed out that the use of such signs may only be misleading in nutritional assessment (Food and Agriculture Organization & World Health Organization, 1951). In Annex 2, therefore, the various signs commonly included in assessment schedules are tentatively classified into the three groups defined by the above Committee, viz. : (a) signs known to be of value in the assessment of nutritional status; (b) those which need to be investigated more fully before their value can be determined; and (c) those considered to be unrelated to nutrition (Adamson et al., 1945; Goldsmith, 1959; Muñoz & Perez Avendano, 1956; Sinclair, 1948; United States, Interdepartmental Committee on Nutrition for National Defense, 1957). The classification of signs into the three categories, and their description, are based on the experience of various specialists working in this field. The main purpose in grouping the signs is to help a worker in his selection, depending on the immediate objectives, the personnel and other facilities available. It is not implied that signs listed under Group 1 in Annex 2 are necessarily specific. They are, however, the signs most commonly associated with deficiency states. With minor adjustments, a selection of signs from Group 1 should serve well if the main objective of the survey is a rapid clinical appraisal of nutritional status. If one of the objectives of the survey is research, then a full and appropriate selection must be made from Groups 1 and 2. Elimination of the signs listed in Group 3 will avoid confusion in interpretation and save considerable time and effort.

From time to time, newly recognized manifestations of malnutrition are described and should be considered, especially in so far as they can

be easily defined. For example, such signs as easily demonstrable changes in the cells of the buccal mucosa and changes in the amino-acid composition and the tensile strength of hair may well prove to be useful, but there is not yet enough experience of them in nutrition surveys for this report to recommend them for general use.

6.4 Standardization of techniques for clinical appraisal

The central theme of this critical review on methods of nutritional appraisal has been the need for adopting standardized techniques to eliminate, as far as possible, discrepancies in the findings of different workers that cause confusion in interpretation. The need for such standardization is felt most intensely in the field of clinical appraisal, into which are likely to enter a number of subjective errors. The foremost need, therefore, is to reach an agreement about the means of assessing nutritional status.

Definition of the nomenclature and diagnostic criteria to be adopted unfortunately is not the sole answer. Even with some experience and a clear understanding of the various signs to be looked for in surveys, mistakes in their assessment are common. While good agreement exists on the interpretation of some characteristic lesions, diverse opinions are expressed with regard to others. With a view to minimizing such differences, certain practices have been adopted. These are : (a) the familiarization of workers with various signs and with the pitfalls in their assessment through a number of lectures before starting the survey (Jolliffe et al., 1958) ; (b) the carrying out of a pilot study in which the same group is investigated by more than one observer (comparison of the results may help in narrowing down the variations between the observations of different workers) ; and (c) the preparation of coloured slides to aid observers in the conduct of surveys. An illustrated guide as a standard manual for nutrition surveys is most necessary, and a recommendation has been made by the Committee that such a guide, depicting the various lesions listed in Annex 2, should be prepared by WHO.

6.5 Interpretation of clinical signs

Difficulties in the interpretation of clinical signs elicited in nutrition surveys have long been realized, and suggestions as to how to ensure a more uniform interpretation of data have been made from time to time. Many reports dealing with these signs describe them as attributable to deficiency of a specific nutrient and discuss their diagnostic significance singly or in combination. On the basis of his experiences in the field, Jolliffe (1954, 1958) developed the concept of a " key sign " to signify

deficiency of a given nutrient. For example, angular stomatitis is taken as the key sign for riboflavin deficiency, follicular keratosis on the back of the arm for vitamin A deficiency, absence of ankle jerks for thiamine deficiency, and so on. The significance of key signs is claimed to be enhanced if other signs associated with the deficiency of the same nutrient occur with some frequency in a population. The Committee, after careful consideration, found itself unable to support the concept of key signs and has preferred an alternative grouping of signs according to common patterns associated with a most-limiting nutrient (see Annex 3).

6.6 Additional clinical information

For a proper interpretation of the results of nutritional appraisal, it is necessary to determine the extent to which other pathological conditions, known to interfere with good nutrition, are prevalent among the groups under study. This category includes malaria, parasitic infestations, tuberculosis, chronic diarrhoeas and dysenteries. Clinical examination, therefore, should also aim at eliciting signs attributable to such diseases and include, at least for a suitable proportion of the sample, microscopic examination of stool specimens (collected if need be in the field by the anal tube technique (Jelliffe et al., 1961)) and of blood smears. Radiology may be required for selected cases. The haemoglobin level (expressed as g/100 ml) is a useful non-specific index of the over-all state of nutrition irrespective of its significance in anaemia. This report considers the nutritional implications of the B complex of vitamins only in respect to thiamine, niacin and riboflavin. The haematological significance of folic acid and vitamin B_{12} has been considered by a WHO Scientific Group on Megaloblastic Anaemias.

6.7 Classification and interpretation of clinical signs

A good deal of variation is apparent with regard to ways of expressing results. While some workers report the frequencies of subjects affected in terms of clinical signs, others do so in terms of individual nutrient deficiencies, and still others merely give the percentage of subjects nutritionally affected on the basis of pooled data on various signs.

As pointed out by the Joint FAO/WHO Expert Committee on Nutrition in 1951, it is unjustifiable to record the number of subjects exhibiting a certain sign and to conclude that this corresponds to the number of persons suffering from the specific nutrient deficiency commonly associated with the sign (Food and Agriculture Organization & World Health Organization, 1951).

In reporting the results of clinical appraisal, little attention seems to have been paid to an association of signs in the same individual. It is

well known that the combination of Bitot's spots with keratotic lesions in one and the same subject has greater diagnostic significance than either sign alone. On the other hand, angular stomatitis is known to occur in a variety of deficiency states, such as ariboflavinosis, iron deficiency and niacin deficiency. If, in a given case, this lesion is associated with corneal vascularization or naso-labial dyssebacea, it suggests riboflavin deficiency; its combination with koilonychia signifies iron deficiency; and with hypertrophic papillae of the tongue, niacin deficiency. But in some cases a combination of signs may indicate deficiencies of more than one nutrient. In Annex 3, a method is proposed for interpreting clinical findings based on the above considerations. This guide presents groups of signs which together constitute clinical patterns of malnutrition frequently encountered. Genetic influences may also operate, and clinical patterns cannot therefore be standardized for all areas.

6.8 Method of reporting results

The results of examinations for clinical signs must be recorded only if positive, with no grading of alleged degree of severity. The effects of external irritants, such as friction and harsh climates, on lesions of the skin, mucous membranes and their appendages must be allowed for. In all types of inquiry the presentation of results should be accompanied by a clear description of the procedures and criteria used in obtaining the information, including details of standardization and accuracy of probable observations.

As syndromes and signs of nutritional deficiency occur, or have characteristic features, at different ages, it is essential to present the results according to a widely recognized system of age grouping. An example is that described by Adamson et al. (1945) for the reporting of mortality statistics, as follows: under 1 year (infants), 1-4 years (pre-school-age children), 5-14 years (school-age children), 15-24 years (young adults), over 25 years (adults). If age groups are given in greater detail than this, they should be so arranged that they can be condensed into the above groups.

Results must not be expressed in terms of specific deficiency diseases, but in terms of individual clinical signs observed. They should be expressed in relation to the total population examined, according to age, sex, socioeconomic background and other appropriate classifications. Combinations of two or more signs in an individual must also be clearly enumerated.

On examination of the results, certain combinations of signs in individuals may suggest the presence of one or more specific deficiency diseases (see Annex 3), although in stating an opinion all other evidence, such as biochemical, epidemiological and dietary data, must be described to support the diagnosis and to indicate the possible etiology.

6.9 Recommendations

(1) The committee recommends the use of Annexes 1, 2 and 3 for drawing up schedules for nutrition surveys and for recording and interpreting physical signs. The three annexes attempt:

- (a) to standardize the description of signs;
- (b) to evaluate the role of malnutrition in their production;
- (c) to interpret groups of signs in regard to specific nutrient deficiency.

It is recognized that the information set out is tentative and will require modification in the light of further experience and intensive research.

(2) The Committee is impressed by the important relationships involved in the vicious circle of malnutrition and infection. WHO proposes to set up a special committee to study the subject of nutrition and infection. The relative importance and practicability of breaking the cycle of events, especially in the case of diarrhoea of early childhood, by improved diets on the one hand and the application of environmental hygiene on the other, should be the subject of further inquiries in the field.

7. PHYSICAL METHODS COMPLEMENTARY
TO CLINICAL EXAMINATION
IN NUTRITION SURVEYS

7.1 Radiographic examination

While routine radiographic studies of population groups are rarely possible, or indeed required, it may sometimes be valuable to carry out radiographic examinations of a sample of a population if the physical signs and other circumstances suggest that rickets, osteomalacia, infantile scurvy, beriberi, fluorosis or protein-calorie malnutrition may be present. This type of survey may sometimes be of value in the retrospective assessment of malnutrition, as with rickets and possibly protein-calorie malnutrition. Under these circumstances, the following are the principal signs sought for:

(1) *Rickets*

   (a) *Active*. Widened concave (cupped), rarified, frayed distal ends of long bones, usually the radius and ulna.

   (b) *Healed*. Concave line of increased density at distal ends of the long bones, usually the radius and ulna.

(2) *Osteomalacia*

Deformity and loss of density of bones, especially the pelvis.

(3) *Infantile scurvy*

(a) loss of density, ground-glass appearance;

(b) line of increased density, sometimes with lateral spur formation, due to increased calcification of metaphysis, with underlying zone of rarefaction, usually best seen at the knee.

(4) *Beriberi*

Increased cardiac size.

(5) *Fluorosis*

Increased density of bones, with calcification of ligaments, usually of the spine. Osteophytic outgrowths at tendinous insertions.

(6) *Protein-calorie malnutrition*

Recent evidence suggests that transverse " trabeculation " at the growing ends of long bones in infants and children may be a result of protein-calorie undernutrition (Platt & Stewart, 1962). The interpretation of this sign requires confirmation.

7.2 Biophysical tests and tests of physical function

The main purpose of biophysical tests is to assess alterations in function associated with inadequate nutrition. A large number of tests have been devised to determine deviations in visual acuity, dark adaptation of the eye, capillary fragility, nerve accommodation, physical performance, muscle co-ordination, and so on, in different deficiency states. Their nutritional significance has been critically discussed (Sinclair, 1948) and the value of most of them is uncertain.

Of these tests dark adaptation is the most widely used. Although measurement of dark adaptation is useful in the objective evaluation of the complaint of night blindness, of which one of the causes is vitamin A deficiency, it has several limitations (Kinney & Follis, 1958). The main drawbacks are : (a) tests of dark adaptation are not a specific measure of vitamin A deficiency and there are often other factors responsible for its impairment that are difficult to eliminate ; (b) it is not easy to conduct them in certain age and population groups ; and (c) responses to the tests are not entirely free from subjectivity. In spite of these difficulties, dark adaptation measurements may be of some value under special circumstances—viz., serious epidemics of night blindness where the authenticity of the complaint itself may have to be ascertained.

8. BIOCHEMICAL ASSESSMENT

8.1 Basis and scope

Variations in the quantity and composition of the diet are reflected by changes in the concentration of chemical substances in tissues and body fluids, and/or by the appearance or disappearance of specific metabolites. The nutritional spectrum varies from frank deficiency at one extreme, through optimal nutrition, to overnutrition at the other (Mickelsen, Caster & Keys, 1947). Biochemical methods should be oriented towards determination of specific nutritional states within this spectrum. Although the interpretation of results obtained by methods now in use is subject to limitations, the methods themselves are valuable procedures for the evaluation of nutritional status.

Most available biochemical tests can be divided into those which measure changes that directly reflect the supply of nutrient and those which detect biochemical changes that reflect metabolic alterations brought about by nutrient deficiencies or imbalances.

Levels of essential dietary constituents in the body fluids are indicators of nutrient supply. The concentration of essential nutrients in body fluids may be reduced as a result of dietary deficiency, poor absorption, impaired transport or abnormal utilization. Measurement of nutrient concentration is helpful in evaluating the possibility of malnutrition, but does not ascertain the existence, nor define the magnitude, of nutritional disease.

Biochemical alterations due to nutritional deficiency appear when the concentration of an essential nutrient in the tissues decreases to a point where there is interference with metabolism. The detection of such metabolic changes is of value in the assessment of nutritional status; in many instances they may indicate a state of deficiency with greater certainty than does a mere lowering of the tissue concentration of essential nutrients. Furthermore, in some instances these changes may precede the appearance of clinical manifestations of malnutrition.

8.2 Guide for the practical application of biochemical methods in nutrition surveys

An attempt is made in this section to relate biochemical studies to the detection of specific nutritional deficiencies. The studies are arranged into two categories as described in sections 8.2.1 and 8.2.2 and Table 3. The arrangement in this table should not be taken to mean that the tests are specific for the nutritional deficiency indicated. Test should be selected on the basis of their practicability—for example, some of the suggested investigations on urine are usually difficult in infants and very young children.

TABLE 3. BIOCHEMICAL STUDIES APPLICABLE TO NUTRITION SURVEYS

Nutritional deficiency	First category *	Second category
(1) Protein	Total serum protein Serum albumin Urinary urea (F) **	Serum protein fractions by electro- phoresis Urinary creatinine per unit of time (T)
(2) Vitamin A	Serum vitamin A Serum carotene	
(3) Vitamin D	Serum alkaline phosphates in young children	Serum inorganic phosphorus
(4) Ascorbic acid	Serum ascorbic acid	White blood cell ascorbic acid Urinary ascorbic acid Load test
(5) Thiamine	Urinary thiamine (F)**	Load test Blood pyruvate Blood lactate Red blood cell haemolysate transketo- lase
(6) Riboflavin	Urinary riboflavin (F)**	Red blood cell riboflavin Load test
(7) Niacin	Urinary N-methyl- nicotinamide (F)**	Load test Urinary pyridone (N-methyl-2-pyridone-5-carbonamide)
(8) Iron	Haemoglobin Thin blood Haematocrit smear	Serum iron % saturation of transferrin
(9) Iodine		Urinary iodine (F) Tests for thyroid function

* Urinary creatinine used as reference for expressing other urine measurements in first category.

** Expressed per gram of creatinine.

(F) In a single urine specimen, preferably fasting.

(T) In timed urine specimens.

Serum cholesterol levels vary widely in population groups with different dietary habits. The determination may often be included in nutrition surveys because of the reported association of serum cholesterol levels with atherosclerosis.

Furthermore, previous knowledge of nutritional situations in a given area may also help in the choice of appropriate tests. The significance and interpretation of the biochemical measurements are discussed in section 8.3.

8.2.1 First category

Tests included in the first category are those which have been most extensively applied in nutrition surveys and have had their usefulness demonstrated. They are relatively simple and their use is feasible in general nutrition surveys. The urine studies included are those which require only a single urine specimen. Samples of blood and urine should preferably be collected from subjects in the fasting state. Since this is not always possible,

the investigator may have to resort to random specimens. It is recognized that the previous meal may influence the levels of some of the constituents in blood and urine. However, under ordinary circumstances in nutrition surveys this would not seriously bias the results.

The haemoglobin concentration affords useful, albeit non-specific, information on the state of health. Hence it is desirable that it should be determined in all general nutrition surveys. If anaemia is encountered, other supporting investigations are required to determine its cause.

8.2.2 Second category

Application of the methods in the second category usually involves relatively more complicated procedures than that of methods in the first category. These methods are designed in most instances to gain more accurate and specific knowledge of particular nutritional inadequacies suggested by the application of methods in the first category, as well as by the other survey data. For example, high total serum protein concentrations accompanied by either low or normal albumin levels may be found to be prevalent. In this case electrophoretic studies of serum protein fractions may be considered necessary to confirm the albumin values by a different technique and to investigate contributions of globulin fractions to the abnormal total protein levels.

8.3 Guides for the interpretation of biochemical data

The Committee critically examined existing information on biochemical measurements in relation to nutrition and diet. It concluded that it would be premature to recommend a standard guide for the interpretation of the results of such measurements made on population groups. This omission is unfortunate, but it is unavoidable owing to the fact that only a limited amount of research has been done on the relationship between nutrient intake and biochemical change. Some excellent and classic studies in this area of research have been made, but unfortunately they have been limited to specific experimental circumstances, and in most instances have dealt only with adults. Information relating to children at different ages is particularly inadequate. It must be emphasized that standards for adults are in many instances not applicable to children.

The United States Interdepartmental Committee on Nutrition for National Defense (ICNND) (1957) has made a worthy attempt to formulate a guide for the interpretation of the urinary excretion of riboflavin, thiamine and N-methylnicotinamide, as well as for haematocrit determinations, and concentrations of haemoglobin, total serum proteins, serum vitamin A, carotene and ascorbic acid in blood. The ICNND limited the application of this guide to " physically active young men ". The authors correctly

pointed out that there is a need to investigate the applicability of the bio-chemical values given to populations living under different environmental and dietary conditions before they can be applied with confidence generally.

Accurate standards for nutritional-biochemical characteristics must await more extensive investigations. In the meantime, data obtained from studies of populations considered to be in good health aid in the interpretation of biochemical findings made in nutrition surveys. This emphasizes the need to obtain information on the nutritional-biochemical characteristics of apparently healthy people of different ages in different parts of the world. Within a particular population group the distribution of values will be of use in the interpretation of information obtained in clinical and dietary studies.

8.4 Laboratory facilities and techniques

The Joint FAO/WHO Expert Committee on Nutrition, in its sixth report, recommended making " the fullest use of existing institutions, including universities, and colleges of medicine, public health, science and agriculture " (Food and Agriculture Organization & World Health Organization, 1962). Laboratory facilities for biochemical work in these institutions should be utilized as fully as possible in nutrition surveys. If necessary, these facilities should be augmented by additional technical staff, equipment and training opportunities. Under special circumstances, however, it may be desirable to set up a special laboratory to assist in the work carried out by a national nutrition department.

Particular techniques will be selected by the biochemist according to their accuracy, sensitivity and reproducibility; and by factors such as laboratory equipment and reagents available, and facilities for trans-portation and storage of specimens. Certain relevant recommendations and suggestions are, however, within the scope of the present report :

(1) If a choice is to be made between several methods of similar merit, it is desirable to adopt those generally used by workers in other regions in order to increase the validity of direct comparisons of results.

(2) Procedures employed in obtaining specimens, in transporting them from the field to the laboratory, as well as the conditions under which they are stored before analysis, must be described in detail in reports of nutrition surveys. Such details must include : (a) time at which samples were obtained ; (b) time of the subject's last meal ; (c) timing and physical conditions of transportation such as temperature, exposure to light and oxygen ; (d) time elapsed, preservative used and storage conditions before analysis.

(3) Reference should be made to the method adopted and any deviations from the original technique, as published, should be noted.

(4) Micro-techniques have a number of advantages for biochemical determinations in nutrition surveys.

(5) Systematic standardization of the biochemical methods and procedures used in nutrition surveys is essential. It must be recognized that the utilization of the same technique by different workers does not necessarily ensure comparability of results.

8.5 Recommendations

Research in nutritional biochemistry should be encouraged, with the following aims : (a) to test the usefulness of current biochemical methods and techniques in survey work ; (b) to apply these methods to the investigation of the interrelationship among nutrients in multiple deficiencies ; (c) to investigate relationships between levels of nutrient intake and biochemical findings ; (d) to investigate the extent to which the nutritional origin of biochemical alterations can be isolated from the influence of pathological and environmental factors other than nutrition ; and (e) to develop new or improved biochemical methods and techniques for the assessment of nutritional status.

9. CRITICAL REVIEW
OF EXISTING BIOCHEMICAL METHODS
OF NUTRITIONAL ASSESSMENT AND SUGGESTIONS
FOR FUTURE RESEARCH

The main purpose of this section is to review the most common methods that have been proposed for the biochemical assessment of nutrient intake and nutritional status. Special reference is made to procedures which are more applicable in general nutrition surveys, but attention is also called to other methods, whose usefulness requires further testing. Several new fields of research are indicated. Nutrient deficiencies not of proved public health importance are not discussed, nor are folic acid and vitamin B_{12}, because these two vitamins have been considered recently in detail in relation to megaloblastic anaemia by a Scientific Group appointed by WHO.

9.1 Proteins

Some biochemical measurements related to protein nutrition indicate only relative adequacy of dietary intake ; others determine the extent of protein depletion. For adults consuming a mixed diet and in nitrogen equilibrium, the amount of urinary nitrogen is a good estimate of protein intake. This approach was recommended in 1932 at the International Conference in Berlin (Quart. Bull. Hlth Org. L. o. N., 1933) and sub-

stantiated by De Venanzi (1947) in Venezuela. However, there are two main disadvantages : the extreme difficulty of obtaining accurately timed urine specimens under field conditions; and in growing children who are in positive nitrogen balance the relationship between nitrogen intake and nitrogen excretion varies with the degree of nitrogen retention. The latter depends largely on the biological value of the dietary protein. However, the ratio of total nitrogen to creatinine in urine specimens collected for periods as short as four hours correlates reasonably well with the level of dietary nitrogen (Powell, Baker & Plough, 1960). Since the amount of urinary excretion of creatinine is more or less characteristic of the individual, it can serve as a reference for expressing the excretion of other urinary constituents in random urine specimens. However, there is a variation in creatinine excretion during the day and from day to day, and this must be borne in mind in interpreting results.

There are numerous reports of somewhat elevated total plasma protein concentrations in population groups living in tropical and subtropical regions with inadequate protein intakes. In most instances, these high plasma protein values are caused by an increase in the gamma-globulin fraction. When total plasma proteins in the normal range are found in these populations, they are sometimes the result of a compensatory increase in gamma-globulin with an actual fall in albumin (Arroyave, in press). It is quite obvious, therefore, that the measurement of total protein concentration alone may not detect early stages of protein deficiency. When deficiency is severe, there is a marked drop in total protein concentration due mainly to a lowering of that of the albumin fraction; this fall serves only as confirmation of a condition already clinically evident. It is claimed, however, that the plasma albumin levels of children predict degrees of protein depletion (Schendel, Hansen & Brock, 1960). This is of such practical importance that further testing should be done.

The electrophoretic pattern of the plasma proteins should be determined whenever possible, not only to evaluate the state of protein nutrition, but also to interpret plasma levels of other nutrients, which normally circulate bound to a plasma protein carrier.

In severe protein depletion, not only plasma albumin but also some plasma enzymes are much reduced ; this discovery stimulated investigations to determine plasma enzyme levels in populations with suboptimal protein intakes. In the case of pseudo-cholinesterase activity, however, it was found that children, even from very different socio-economic groups, had no differences in serum levels when these were measured under field conditions (Arroyave, Feldman & Scrimshaw, 1958). Direct measurements of liver protein and enzymes show changes in severe protein malnutrition (Waterlow, 1959). In humans, the effect of mild degrees of deficiency on such measurements is not known and, furthermore, liver biopsy cannot be done as a matter of routine.

When a subject is placed on a low protein diet or on a protein-free diet, the rate of loss of nitrogen from the body is, for a variable period of time, determined by the previous level of protein intake (Patwardhan, 1961), after which nitrogen excretion decreases. This change is mainly due to a fall in urea excretion ; the levels of other urinary nitrogen constituents remain stable for all practical purposes under the above conditions. This means that the catabolism of body proteins continues at its established level for some time after an abrupt change in protein intake. Thus, fasting urea excretion provides an indicator of protein intake.

The ratio of urea nitrogen to total nitrogen was found to be markedly lower in children (Platt, 1954) and lactating women (Platt, 1958) from poorly nourished populations, but may still be mostly related to diet. This has been confirmed in children when a single fasting urine specimen was used. Changes in this ratio essentially reflect variations in urea excretion, since the relative amounts of other urinary nitrogenous compounds are not altered significantly. Therefore, the ratio of urea nitrogen to creatinine in fasting urine samples should also bear a direct relationship to the level of protein in the diet. A recent study found the urea-creatinine ratio to be from 8 to 9 in pre-school children of low socio-economic status, and around 15 in children of the same age group belonging to the upper socio-economic group (Duran & Arroyave, in press). Further studies should be conducted to test the feasibility and reliability of this estimation in single fasting urine samples under field conditions.

The fasting level of plasma non-protein nitrogen (NPN), of which urea is the most important fraction, is also influenced by the amount of protein in the diet, being reduced in people subsisting on a low protein diet (Peters & Van Slyke, 1946). The concentration of plasma NPN in labourers from Caracas, Venezuela, was found on the average to be 23.9 mg/100 ml, which is lower than accepted normal values, and this was attributed to the poor intake of protein (De Venanzi, 1950). However, in starvation, as distinct from a low protein intake as such, the plasma levels of NPN tend to increase.

The level of plasma amino-acid nitrogen has also been suggested as an indicator of human protein nutrition (Man et al., 1946). There is good correlation between plasma amino-acid levels and proportionate weight deficit, if this is not greater than 30 % (Albanese, Orto & Zavattoro, 1958). As body protein deficits are made good, fasting plasma amino-acid nitrogen increases. Children with severe protein malnutrition (Arroyave et al., 1962b ; Cravioto et al., 1960) or on nitrogen-free diets (Arroyave et al., 1962a) show a relative decrease in plasma levels of essential amino acids, tyrosine, and cystine. Further investigations should be made to find out if measurements of one or several of the plasma amino acids are more informative of subclinical protein deficiency than levels of total plasma alpha-amino nitrogen.

It is well known that skeletal muscle is sacrificed during protein deprivation (Stearns et al., 1958). Deficits in muscle mass in malnutrition are proportionately larger and their estimation more sensitive than deficits in body-weight or -height. Muscle mass may be estimated from 24-hour urinary excretion of creatinine. In expressing the results, " mg of creatinine in 24 hours per cm of body-height " is to be preferred to " per kg of body-weight ", since variations in fat deposits affect the latter. As the collection of 24-hour urine specimens is very difficult in the field, the use of shorter collection periods deserves consideration. A period of even 2-3 hours during the morning is apparently suitable on which to estimate the characteristic 24-hour creatinine excretion of groups of children or adults (Arroyave & Arroyave, in press). Examples of studies of creatinine excretion are those of Stearns et al. (1958) in North American children aged 1-10 years, Standard, Wills & Waterlow (1959) in cases of kwashiorkor, and Arroyave, Sandstead & Schumacher (1958) in Central American children of different socio-economic levels.

Referring to the need for further research in the biochemical assessment of protein deficiency, Bengoa, Jelliffe & Perez, stated as recently as 1959 that " in spite of the enormous advances in this field of nutritional biochemistry, there is no new test available which can be used in field surveys as an indicator of [early] protein-malnutrition ".

9.2 Vitamin A

Inadequate dietary intake of preformed vitamin A or active carotenoids results in a decrease of the vitamin in tissues. A direct assessment of body reserves of this nutrient may be made by measuring its concentration in the liver, since this organ is the main site of vitamin A storage.

Serum levels of carotene and vitamin A have been used extensively to corroborate the diagnosis of vitamin A deficiency (Goldsmith, 1949). The levels of carotene directly reflect changes in dietary intake but are not *per se* indicative of vitamin A nutrition. Subjects receiving only preformed vitamin A in their diets may have adequate vitamin A reserves with practically no carotene in their plasma. However, in populations living on a mixed diet, a low carotene level is very informative, particularly if accompanied by a low plasma vitamin A concentration.

Plasma vitamin A levels do not necessarily reflect recent intake. Large stores of vitamin A in the livers of well-nourished human adults tend to maintain the plasma level for about one year in the absence of dietary sources of the vitamin (Great Britain, Medical Research Council, 1949). Children, because they require an extra amount of this vitamin for growth, exhaust liver reserves in a shorter time under such circumstances. In infants this seems to occur in three to four months.

While low average plasma vitamin A levels are compatible with a diagnosis of vitamin A deficiency in a population, in individual cases inter-

pretation should be cautious, since febrile conditions depress the plasma vitamin A concentration (Goldsmith, 1959). Low levels of serum vitamin A may be found in children with kwashiorkor, but they do not necessarily mean vitamin A depletion and may merely reflect an impairment in the blood transport of the vitamin (Arroyave et al., 1959, 1961 ; Friend et al., 1961).

The ICNND *Manual for Nutrition Surveys* indicates that serum levels of vitamin A between 20 and 50 µg are acceptable, 10-19 µg are low and less than 10 µg clearly indicate deficiency in young adult men. The relationship of plasma vitamin A levels in children to dietary intake and clinical disease needs investigation. Vitamin A values per 100 ml of serum for pre-school children from a low-income population in Guatemala were 11 µg for the first quartile, 17 µg for the second, and 22 µg for the third (Béhar et al., 1960). These biochemical results coincided with very low intakes, although clear-cut clinical signs of deficiency were found. Had dark adaptation tests been possible, they might well have shown a positive correlation with the biochemical findings. A study of adults in India showed that plasma levels below 18 µg per 100 ml were often accompanied by impaired dark adaptation (Hassan & Khanna, 1947).

There is no ready means of measuring metabolic derangement due to vitamin A deficiency. Although regeneration of rhodopsin is known to decrease in rate as a result of severe deficiency of the vitamin (*Nutr. Rev.*, 1959), its measurement is obviously impracticable for survey purposes. Incomplete knowledge of the mechanism of action of vitamin A in metabolism has prevented further advances in biochemical methods for the detection of its deficiency. The work of Dowling & Wald (1958) on the action of vitamin A suggests new research in this area. For example, the protein moiety opsin needs to be bound to vitamin A to preserve its integrity, and these authors speculate that this may apply to other protein moieties in the body, such as those of the epithelial cells. Plasma vitamin A alcohol is bound to the albumin fraction, and therefore the decrease in concentration of this plasma protein observed in vitamin A deficiency in cattle (Erwin, Varnell & Page, 1959) could be the result of an accelerated catabolism of the albumin moiety when insufficient vitamin is bound to it. Among other functions of vitamin A recently discovered are : (*a*) release of proteolytic enzymes from intracellular particles ; (*b*) synthesis of mucopolysaccharides ; and (*c*) synthesis of corticosterone. These developments should offer a lead for research into metabolic derangements resulting from vitamin A deficiency (Wolf, 1962).

9.3 Vitamin D

Biochemical changes due to deficiency of vitamin D have been known for some time. Adequacy of vitamin D nutriture cannot be correlated with dietary intake since body synthesis replaces, partially or totally, the

dietary need for this vitamin when the individuals are exposed to sufficient sunlight. The serum alkaline phosphatase level rises during deficiency and tends to be proportional to the severity of the disease. Measurement of this enzyme level is the most specific means of confirming the presence of rickets. Normal enzyme activity in the serum of young children is between 5 and 15 Bodansky units. Values of 20 are very suggestive of rickets. Decrease in the phosphorus concentration of the blood serum almost always occurs (Howland & Kramer, 1921 ; Kramer & Kanof, 1954), which is helpful in diagnosis. Serum calcium levels decrease inconsistently and are unreliable as a sole index of rickets (Kramer & Kanof, 1954).

The problem of detecting subclinical deficiency of vitamin D in populations is more complicated. The serum alkaline phosphatase level in groups of children is not a generally accepted criterion. Its measurement may be of value " in determining adequacy of antirachitic agents " (Bessey, 1954) and has been included in many biochemical-nutritional surveys, but the relationship of the level of this enzyme in serum with lesser degrees of vitamin D deficiency has not been established. Since the levels of serum alkaline phosphatase activity decrease in children with severe protein deficiency (Dean & Schwarz, 1953 ; Scrimshaw et al., 1956), a rise due to vitamin D deficiency might be masked when the two conditions coexist. This question warrants further investigation. A new lead for research in this field has recently been suggested (Jonxis, 1961) involving a phosphorus load test which may be sensitive enough to detect mild vitamin D deficiency. A marked decrease in plasma calcium was observed (in such deficiency states) 24 hours after oral administration of standard doses of sodium phosphate.

In adults, osteomalacia may occur as the result of a deficiency of vitamin D and is manifested biochemically by an increase in the serum alkaline phosphatase above the usual adult level of 3-5 Bodansky units, and a decrease in the concentration of serum calcium. The increase is not specific, since it is also seen in a number of other pathological conditions (Goldsmith, 1959).

9.4 Ascorbic acid

The concentration of plasma ascorbic acid decreases rapidly with dietary deprivation of this vitamin. There is strong evidence that the concentration of ascorbic acid in the white blood cells (WBC) is a good estimate of its concentration in other tissues. With a sufficiently high intake complete tissue saturation is attained. In the adult this intake is about 100 mg daily with a corresponding plasma level of 1.0-1.4 mg/100 ml and a WBC level of 20-30 mg/100 ml. Daily intakes of 10-25 mg are found associated with WBC values of 2-12 mg and plasma values of 0.1-0.3 mg/ 100 ml.

Plasma ascorbic acid completely disappears in about 40 days when none of the vitamin is taken in the diet (Grandon, Lund & Dill, 1940) but scurvy is not evident until the 143rd day of deprivation. Under the same conditions the WBC ascorbic acid content of a well-nourished adult decreases to nearly zero in 3-5 months and scorbutic lesions soon appear (Grandon et al., 1940; Great Britain, Medical Research Council, 1953). From these results, confirmed by several workers, the following practical conclusions may be drawn : (a) plasma ascorbic acid concentration mainly reflects recent intake of the vitamin ; (b) disappearance of ascorbic acid from the plasma is not diagnostic of scurvy although it is compatible with it ; and (c) the WBC level is a good index of ascorbic acid nutritional status in the lower ranges of intake and tissue concentrations (Lowry, 1952). The extent to which determination of ascorbic acid in WBC is practicable for survey work depends on what laboratory facilities are available, because the assay is much more complicated than the measurements of serum ascorbic acid.

In populations the average urinary excretion of ascorbic acid in 24 hours gives an estimate of dietary intake. The ratio of the amounts of ascorbic acid to creatinine in a random urine specimen, preferably fasting, gives information of similar value and is obtained much more easily.

Administration of large amounts (500-2000 mg) of ascorbic acid in divided doses throughout the day has been proposed as a method of estimating tissue saturation, but this is more applicable in field work. Load tests have also been employed in which the amount of ascorbic acid excreted in a given time is measured after the administration of the vitamin, either orally or parenterally. These methods indicate whether or not the tissues are saturated, but are poor indicators of the degree of unsaturation (Vilter, 1954).

The results of the intradermal injection of dichlorophenolindophenol have been studied in relation to serum levels of ascorbic acid (Cheraskin, Dunbar & Flynn, 1958). Although the presence of other reducing substances in the skin interfere with the test, it may show some degree of correlation with ascorbic acid nutrition (Banerjee, 1944 ; Banerjee & Guha, 1939 ; Reddy & Sastry, 1941).

Scorbutic guinea-pigs excrete abnormal tyrosine metabolites after administration of the amino acid (Sealock & Silverstein, 1940). The phenomenon has been studied in premature infants (Levine, Marples & Gordon, 1941) and adults (Rogers & Gardner, 1949) with severe ascorbic acid deficiency. Human adults maintained for as long as 78 days on a diet containing 7 mg or less of ascorbic acid daily excrete urine with a relatively high reducing power but with no increase in tyrosil compounds (Steele et al., 1952). Further study of this question may lead to a useful metabolic test for ascorbic acid deficiency.

9.5 Riboflavin

The original description of a micro-fluorometric method for the estimation of free riboflavin, flavine adenine mononucleotide (FMN) and flavine adenine dinucleotide (FAD) in blood (Burch, Bessey & Lowry, 1948) aroused interest in these measurements as indicators of riboflavin nutritional status. Despite a large number of studies on blood riboflavin levels in humans, few have reported the relative usefulness of measuring the different forms in which this vitamin occurs. It has been concluded that the amount of free riboflavin in blood is too variable to serve as a useful criterion for assessing the state of riboflavin nutriture; FAD has more diagnostic value in detecting riboflavin deficiency in view of its greater stability (Suvarnakich, Mann & Stare, 1952).

In a well-controlled study (Bessey, Horwitt & Love, 1956), 10 men were maintained on a diet supplying only 0.55 mg of riboflavin per day for 16 months and 6 men on intakes of 2.55-3.55 mg per day. The most consistent and significant difference was in the red blood cell (RBC) riboflavin content, which was 10.0-13.1 µg/100 ml in the deficient group and 20.2-27.6 µg in the supplemented group. All subjects on the restricted intake showed clinical manifestations of ariboflavinosis, which in three of them were very severe. Although plasma levels of free riboflavin and FMN decreased in the deficient group, the variability was very largely accounted for by striking decreases in a few cases. Plasma FAD and WBC riboflavin did not decrease significantly. In a second experiment the levels of RBC riboflavin of 8 men on 0.5 mg/day of riboflavin intake, compared with those of 4 control groups on 1.6 and 2.4 mg intakes, were determined periodically during 9 months. The RBC riboflavin of the restricted group decreased significantly within 7-8 weeks. It was concluded that RBC riboflavin concentration " is a reasonably sensitive and practical index " of riboflavin nutritional status. This work is especially important in that it related biochemical findings in the blood to a definite state of deficiency.

Much study has been made of the 24-hour urinary excretion of riboflavin in relation to daily intake and, in some instances, to the appearance of clinical manifestations of deficiency. From the information available, it appears that with intakes ranging from 0.5 mg to around 1 mg per day, approximately 9 %-14 % of the ingested riboflavin is excreted daily (Goldsmith, 1949). Discrepancies with these values may be explained by the influence of diet on the biosynthesis of riboflavin by intestinal flora (Mickelsen, 1956), which may add to the amount of the vitamin absorbed from the diet.

In well-controlled experiments in human adults, it was observed that when the daily intake of riboflavin was increased from 0.55 mg to 1.1 mg, there was a slow proportional increase in urinary excretion (Horwitt et al., 1950). When the intake reached 1.6 mg daily the urinary excretion increased

sharply and disproportionately. Subjects receiving 1.6 mg/day excreted four times as much riboflavin as did those receiving 1.1 mg daily. It was concluded that an adult male, on a diet supplying 2200 calories a day, required between 1.1 and 1.6 mg/day of riboflavin. This is supported by other studies (Williams et al., 1943), although the results are not applicable under all conditions (Pollack & Bookman, 1951).

Load tests have been extensively used for evaluating riboflavin nutrition. Of the various techniques available, the four-hour urinary excretion following parenteral administration of 1 mg of the vitamin is promising (Unglaub & Goldsmith, 1954) and deserves further study. The relationship between daily intake and retention, as judged by the above test, has been confirmed in a study of pregnant women (Arroyave, Valenzuela & Faillace, 1958).

For field surveys, the determination of riboflavin in a single random urine specimen, preferably fasting, may be used. An excretion of 150 µg/g creatinine is indicative of tissue saturation (Lowry, 1952). The ICNND *Manual for Nutrition Surveys* has set excretions of 80-270 µg/g of creatinine as acceptable for young male adults. Expressing urinary excretion per gram of creatinine has the advantage of tending to correct variations due to body size (Lowry, 1952). This may only be true in a limited sense, and cannot be used to compare adult and childhood requirements; there is evidence that children excrete more riboflavin in relation to creatinine than do adults (Aykroyd et al., 1949). This is not surprising, since children need and usually consume more riboflavin than adults per unit of muscle mass. Therefore, relationships between riboflavin excretion in the urine and adequacy of intake or deficiency in children must be established *de novo*. This is an open field for research. Urinary excretion of riboflavin increases when nitrogen balance is negative, as in starvation (Unglaub & Goldsmith, 1954). Such conditions are rarely encountered in nutrition surveys. The possible influence of the fat content of the diet on the requirement for riboflavin (Reiser & Pearson, 1949) may be of practical importance, since populations living on an apparently short supply of this vitamin usually have a very low fat intake. Perhaps research in this field may help to explain the existence of relatively normal blood riboflavin that can occur with a low dietary supply of riboflavin.

9.6 Thiamine

Thiamine concentrations in the plasma, red blood cells and white blood cells of man cannot be determined using very small quantities of material (Burch et al., 1952), because available methods are not sufficiently sensitive. Red blood cells contain 6-9 µg/100 ml, but although this is higher than the concentration in plasma, the cells do not lose their thiamine even when intake of the vitamin in reduced to a point conducive to clinical beriberi (Burch et al., 1950).

The relationship between urinary excretion and intake has been more extensively studied for thiamine than for any other vitamin. The 24-hour output, the one-hour excretion on fasting, and thiamine content of the random specimen per gram of creatinine have proved useful measurements. They help to estimate relative intake, particularly when comparisons of populations are made or when the effectiveness of dietary supplementation with thiamine is evaluated.

Since the requirement for thiamine is partly dependent on the carbohydrate content of the diet, increase in the latter without a proportional increase in thiamine will reduce the relative thiamine adequacy of the diet. This relationship between composition of the diet and thiamine intake should be kept in mind when interpreting data on urinary thiamine excretion. A probable minimum thiamine requirement is 0.23 mg/1000 calories (Sebrell, 1954). Subjects consuming less than this quantity excrete very little thiamine in the urine.

A variety of load tests have been devised in attempts to measure tissue deficit (Unglaub & Goldsmith, 1954); they may not necessarily indicate clinical deficiency. Among the tests which have been proposed, that in which parenteral administration of 350 µg of the vitamin per square metre of body-surface is followed by the determination of thiamine excretion in the subsequent four hours (Melnick & Field, 1942) offers good possibilities for standardization. Load tests are, however, quite impracticable for field studies. Measurement of the excretion of pyrimidine compounds has also been suggested (Mickelsen, Caster & Keys, 1947; Pollack, Ellenberg & Dolger, 1941) but practical methods have not been developed.

Metabolic alterations due to thiamine deficiency are well known. Lack of thiamine interferes with the entrance of pyruvate into the tricarboxylic acid cycle and the keto-acid tends to accumulate in the body fluids. An elevation of pyruvic acid in the blood is observed in thiamine deficiency, but the change lacks consistency and specificity (Horwitt et al., 1948). It is claimed that the normal ratio of lactate to pyruvate is sufficiently altered in thiamine deficiency to be of diagnostic value. Basal blood levels of these compounds have been found of limited value for estimating mild degrees of this deficiency, and therefore a test based on the effect of glucose and exercise on the lactic and pyruvic acid blood levels has been proposed (Horwitt et al., 1948). Results are expressed in terms of the relative amounts of blood glucose, lactic and pyruvic acids, one hour after the dose of 1.8 g of glucose/kg body-weight, and five minutes after completion of a standardized exercise. This is called the " carbohydrate index ". Derangement in carbohydrate metabolism due to thiamine deficiency gives a high carbohydrate index. Figures below 15 are found in non-deficient individuals. This interesting approach to the diagnosis of thiamine deficiency has the disadvantage of being too elaborate for widespread application in field surveys. For the latter, thiamine excretion per gram of creatinine in single

random urine specimens is a practical biochemical test which aids in the evaluation of thiamine nutritional status.

Recently, it has been found that the transketolase activity of RBC haemolyzates, both from rats and from humans, decreases progressively on thiamine restriction (Brin, 1962). The marked specificity of the test was shown by the fact that addition of thiamine pyrophosphate to the *in vitro* system resulted in a significant enhancement of activity ; this was not observed with control RBC haemolyzates. Studies in rats also showed a good relationship between the level of dietary thiamine and the RBC transketolase activity. In volunteers, RBC transketolase activity differed markedly at 17 days between " deficient " and control groups ; this difference gradually became larger as the deficiency progressed. This specific test for thiamine should have an advantage over urinary thiamine excretion, since it should indicate early biochemical deficiency rather than recent thiamine intake. It is necessary to extend this research to the systematic testing of the applicability and sensitivity of the method in nutrition survey work.

9.7 Niacin

This water-soluble vitamin differs from the others of the B complex because an amino acid, tryptophan, serves as its precursor in some animals and man. Approximately 60 mg of dietary tryptophan appear to be equivalent to 1 mg of niacin (Horwitt et al., 1956). Another characteristic of niacin is that it is not excreted in the urine as such, but is metabolized to at least two major methylated derivatives, N'-methylnicotinamide and N-methyl pyridones (Goldsmith et al., 1952 ; Walter et al., 1955).

Values for the concentration of the niacin-containing co-enzyme, diphosphopyridine nucleotide (DPN), for whole blood, serum and red and white blood cells of well-nourished adults have been published (Burch et al., 1955) and in general confirm the results of earlier work (Mueller & Vilter, 1950). The concentration of nicotinic acid in the blood cells of human subjects suffering from pellagra and of dogs with " black tongue " does not seem to be lowered (Klein, Perlzweig & Handler, 1942). Women on controlled niacin and tryptophan intakes have been studied (Vivian, Chaloupka & Reynolds, 1957). The lowest intake was 2.5 mg of niacin and 30 mg of tryptophan daily. The tryptophan intake was raised stepwise and each level maintained for six-day periods. On daily intakes of 2.5 mg of niacin and 30-190 mg of tryptophan the blood pyridine nucleotides (PN) were 20%-40% below control levels. Increases in blood PN started at 270 mg of tryptophan intake and reached the values observed in the control period at an intake of 670 mg. Similar studies are required to establish the extent to which blood levels of niacin or its derivatives are useful for the appraisal of niacin nutriture in humans.

The urinary output of N'-Me and pyridone decreases when the intake of niacin is restricted (Rosenthal, Goldsmith & Sarret, 1953), an effect which is more marked and rapid in the case of pyridone (Goldsmith et al., 1952). Low urinary excretion of niacin metabolites is not necessarily indicative of clinical pellagra.

Excretion levels in persons with supposedly " adequate body stores of niacin " have been reported (Goldsmith et al., 1955). The results indicate that, on a " corn diet " supplying 200 mg daily of tryptophan, an intake of 8-10 mg of niacin ensures adequate body niacin stores in human adults. Below this level of niacin supplementation, excretion of niacin metabolites is very low, but increases sharply when the intake of 8-10 mg is reached.

The need for continuing such investigations is obvious. To interpret results the " niacin equivalent " of experimental diets (Horwitt et al., 1956) should be calculated on the basis of both the niacin and the tryptophan content. For the purpose of nutrition surveys, determinations of niacin metabolites in urine may be made in timed specimens or in single random specimens in relation to creatinine. The ICNND *Manual for Nutrition Surveys* recommends the determination of N'-Me only, because of the relative complexity of the assay for pyridone. The fact that normal persons may occasionally show a very low urinary excretion of niacin metabolites and that under certain pathological conditions an excess is excreted (Goldsmith, 1949) makes them unsatisfactory as measures of niacin nutrition in individuals.

The effect of ingesting niacinamide on the urinary output of N'-Me and pyridone has been explored. The assumption that a high retention of the dose means a state of tissue depletion must be accepted with reserve, because impairment of methylation would also influence the excretion of these niacin derivatives (Ellinger & Coulson, 1944). This is particularly relevant to tests in which a large dose of niacinamide is administered. Various procedures have been proposed for applying niacin load tests. The oral administration of 500 mg of the amide followed by quantitative estimation of the extra " trigonelline " plus nicotinic acid derivatives has been suggested (Briggs, Singal & Sydenstricker, 1945). Better knowledge of niacin metabolism has since resulted in more refined procedures—for example, the use of a relatively small dose of niacinamide (10 mg) followed by urinary collections at periods of at least 12 hours (Unglaub & Goldsmith, 1954).

In field surveys the simple estimation of niacin metabolites per gram of creatinine in random urine specimens, preferably in fasting, gives useful information which may corroborate dietary data.

9.8 Vitamin B₆

Requirements for vitamin B_6 have not been definitely established, but about 0.03 mg/kg body-weight/day for adults (Williams, 1942) and between

0.01 and 0.02 mg/kg body-weight/day for infants under six months (György, 1954) have been proposed.

The fact that deficiency of vitamin B_6 is apparently not widespread has been attributed to the relatively low requirements and to the uniform distribution of vitamin B_6 among foodstuffs (Snell & Keevil, 1954). However, the methods used to determine the amount in foods may not be indicative of utilizable vitamin B_6. Interest in this factor has been aroused by observations suggesting an increased demand during pregnancy, and by the accidental occurrence of a serious outbreak of vitamin B_6 deficiency in infants fed a commercial milk preparation. There is also evidence that administration of the antituberculosis drug isoniazid increases the need for vitamin B_6. This fact may be of importance in underdeveloped areas where both malnutrition and tuberculosis are prevalent and where drugs of this type are being used extensively. Experimental vitamin B_6 deficiency in man has been attempted either by the administration of diets deficient in the factor or by the addition of a pyridoxine antimetabolite. In one such study (Snyderman, Carretera & Holt, 1950), two infants were fed low vitamin B_6 diets for 76-130 days. Three biochemical features were observed : (a) disappearance of pyridoxic acid from urine ; (b) marked reduction of urinary pyridoxine excretion ; and (c) loss of ability to convert tryptophan to niacin.

Observations on adults in whom vitamin B_6 deficiency was induced, either by the administration of desoxypyridoxine (Mueller & Vilter, 1950) or by a deficient diet (Greenberg et al., 1949), led to the conclusion that urinary excretion of xanthurenic acid without a tryptophan load is not a reliable index of early deficiency, but adults after only about two weeks on a synthetic diet devoid of vitamin B_6 show a tenfold increase in xanthurenic acid excretion in 24 hours after a 10-g dose of DL-tryptophan. This is claimed to be " one of the earliest detectable manifestations of pyridoxine deficiency " (Greenberg et al., 1949).

A tryptophan load test which can be used as a biochemical index of vitamin B_6 deficiency at an early stage has been described (Chiancone, 1950). Tryptophan in a single dose of 100 mg/kg body-weight is administered to the subject and the amount of xanthurenic acid excretion during the following 24-hour period, expressed as a percentage of the dose of tryptophan, is called the " xanthurenic acid index ". Indices not exceeding the value of 1 are regarded as normal (Verga, 1951). It is important to investigate changes in excretion of xanthurenic acid in detail after test doses of tryptophan, in order to see if shorter collection periods would give information as useful as that obtained with the 24-hour collections. This is of obvious practical importance.

Changes in blood urea levels after a load dose of alanine might be related to vitamin B_6 nutritional status (McGanity et al., 1949). An abnormal blood urea concentration curve was found in patients with hyperemesis

gravidarum after a dose of 30 g of DL-alanine; the administration of pyridoxine to these patients corrected the abnormal response to the test. The same type of abnormal response in two of three subjects receiving desoxypyridoxine to induce pyridoxine deficiency has been shown (Vilter et al., 1953). Abnormal tests coincided with the presence of certain clinical manifestations. One patient who showed no clinical signs, although receiving the vitamin antagonist, responded normally to the test at all times. The test therefore seems to reflect well-advanced deficiency, but is apparently of no value for detecting it at an early stage.

The concentration of plasma pyridoxal phosphate 24 hours after an oral dose of 100 mg of pyridoxine was found to be lower in pregnant women during the last trimester than in normal men and normal non-pregnant women (Wachstein, Kellner & Ortiz, 1960), and this suggests an altered metabolism of vitamin B_6 in pregnancy. The test seems to measure relative tissue saturation. Since only one sample of blood is necessary 24 hours after the pyridoxine dose, this test may have a practical advantage over the tryptophan load test, which requires the collection of 24-hour urine specimens.

Recently it has been shown that subclinical deficiency of vitamin B_6 in adults reduces the serum glutamic-oxalacetic transaminase (Babcock, Brush & Sostman, 1960), but this change is less sensitive an index than the tryptophan load test. The relationship between urinary oxalate excretion and vitamin B_6 deserves study (Gershoff, Mayer & Kulczycki, 1959).

Research is needed on laboratory methods to measure vitamin B_6 as a complex and to determine its three biologically active forms—pyridoxine, pyridoxal and pyridoxamine (Sherman, 1954; Snell, 1954). More practical procedures, particularly in microscale, applied to blood and other biological materials would facilitate further studies on the nutritional biochemistry of this vitamin.

9.9 Calcium

In spite of great variation of intake, there is no evidence of calcium deficiency as such occurring in population groups (Food and Agriculture Organization & World Health Organization, 1962). Among the biochemical abnormalities that appear in experimentally induced severe calcium deficiency in animals is a decrease in the concentration of calcium in serum (Goldsmith, 1953). A decrease in the ionized calcium is responsible for the low values. When this occurs, tetany and other clinical signs become obvious, so that the biochemical index has only confirmatory diagnostic value. The maintenance of plasma calcium concentration is accomplished through a mechanism in which several factors, each more important than moderate variations in dietary intake, are operating (Kirkpatrick & Robert-

son, 1953). Investigation of the relationship between dietary inadequacy of calcium and its possible biochemical indices is further hindered by the fact that the optimal intake of this mineral is unknown (Hegsted, 1957).

9.10 Iron

When iron is not available to the body in sufficient quantities, because of inadequate intake, poor absorption or excessive loss, several biochemical alterations occur, of which the best known is a decrease in the biosynthesis of haemoglobin. The clinical entity is, of course, iron deficiency anaemia, which is easily detected by determining the concentration of haemoglobin in whole blood, aided by the haematocrit and simple microscopical examination of stained blood smears.

Anaemia, however, is a fairly advanced manifestation of lack of iron. In view of the great importance of iron in other fundamental biochemical mechanisms such as cellular electron-transport, attention is being paid to possible alterations in their efficiency in iron deficiency states. Evidence from animal studies (Beutler, 1957) supported by clinical observations (Beutler, Larsh & Gurney, 1959) suggests that some of these mechanisms suffer even before haemoglobin levels become subnormal. Although no practical biochemical method for evaluating iron deficiency based on such changes yet exists, the studies are important contributions to the definition of " adequacy of intake " and " requirement " for this mineral in terms of biochemical abnormalities that occur earlier than blood changes.

Early depletion of body iron reserves may be detected directly by the estimation of haemosiderin in the bone-marrow (Rath & Finch, 1948). The relative difficulty of obtaining bone-marrow specimens under field conditions, however, limits the usefulness of this method in the evaluation of iron nutrition in population groups.

Low plasma iron levels usually indicate lack of available iron, although in some instances they may be due to pathological conditions such as infection. They may usually be accepted as presumptive evidence of latent deficiency. Increased binding capacity is a characteristic of uncomplicated iron deficiency (Beutler, 1957 ; Cartwright, Gubler & Wintrobe, 1954). In field surveys, tests for iron-binding capacity of plasma (Rath & Finch, 1949) can be made in selected sub-samples.

Hypoproteinaemia, due to protein malnutrition, may confuse signs of iron deficiency. Protein deficiency may produce a decrease in the specific iron-binding beta-globulin of plasma, and consequently lead to a reduced iron-binding capacity and a low plasma iron level (Lahey et al., 1958). In these cases, signs characteristic of typical iron deficiency anaemia may appear when the condition is treated without adequate attention to iron intake (Scrimshaw et al., 1957).

9.11 Iodine

When insufficient iodine is available to the thyroid gland, it undergoes hyperplasia in an attempt to supply thyroid hormone to the body. Decreased availability may result directly from insufficient iodine intake or indirectly from the presence of gcitrogenic factors, usually of food origin (Clements & Wishart, 1956; Curtis & Fertman, 1951; Underwood, 1953).

The 24-hour urinary excretion of iodine has been used to estimate the dietary intake ; amounts excreted by persons in goitrous regions are usually markedly lower than those in non-goitrous areas (Curtis & Fertman, 1951). An inverse correlation between the uptake of labelled iodine and urinary iodine excreted, in euthyroid patients in an endemic area, has been clearly demonstrated (Stanbury, Brownell & Riggs, 1954).

Although the amount of iodine bound to serum proteins reflects the functional state of the thyroid gland, its measurement is of very limited value because goitre patients in endemic areas are generally euthyroid. It would be logical to expect that, in the initial stages of the development of goitre, an individual might develop a relative hypothyroidism before compensation occurred. This is supported by the observation that relatively low values for protein-bound iodine in a group of children increased with the administration of potassium iodine or iodate simultaneously with a decrease in the prevalence of goitre (Scrimshaw et al., 1953). To elucidate the value of measuring protein-bound iodine more studies should be carried out in young children living in endemic goitre areas.

Quantitative relationships between iodine intake and excretion and the presence of goitre are influenced by several physiological, environmental and dietary factors. Study of these relationships should be conducted in different populations with and without endemic goitre. Methods are, however, needed to assess subclinical iodine deficiency.

10. GENERAL RECOMMENDATIONS

(1) So that the results of different surveys can be usefully compared, it is recommended that nutrition survey workers attempt to standardize their observations and interpretations by following the suggestions contained in this report. It is recommended that WHO assess from time to time the extent to which these suggestions are being adopted and proving effective, and if necessary, in the future, undertake revision by a committee of experts convened for the purpose.

(2) There is an urgent need for a standard guide which would give detailed instructions about the planning and conducting of nutrition surveys, and the reporting of results, and which would contain information of assistance to the correct interpretation of results.

The Committee strongly recommends that WHO undertake the preparation of a manual to meet this need. Such a manual should contain clear descriptions, accompanied by photographic illustrations, of the known clinical signs of malnutrition ; detailed descriptions of relevant laboratory methods and the collection, transport and storage of specimens ; information on the preparation of reagents and on useful field and laboratory instruments ; guides to relevant anthropometric techniques ; and other information of use in assessing the nutritional status of individuals and populations.

(3) The Committee emphasized the importance of poor nutritional status as a factor in the etiology and mortality pertaining to infectious diseases. It is recommended that whenever WHO is involved in projects concerned with the control of infectious conditions, especially diarrhoeal diseases and tuberculosis, plans of operation should be composed to permit adequate study of the nutritional status of the populations involved.

(4) The development of much-needed nutrition programmes is being inhibited by a lack of trained personnel. To help remedy this situation, the inclusion of nutrition as a subject in the curricula of medical schools and schools of public health should be encouraged and, where possible, assisted by WHO.

(5) It is recommended that WHO, through public health projects in which it is involved, encourage inquiries into the practicability and usefulness of applying new methods of recording and classifying mortality and morbidity data. These inquiries might be based on the revised classification of nutritional diseases recommended in the sixth report of the Joint FAO/WHO Expert Committee on Nutrition (Food and Agriculture Organization & World Health Organization, 1962).

(6) The Committee is of the opinion that WHO should assist governments to promote and initiate surveys of the growth of children. The Organization might collect and compare the results and analyses of such surveys in order to establish more clearly patterns of growth in relation to genetic and environmental influences.

(7) WHO might promote and assist research into the interpretation and use of newly recognized clinical signs, laboratory, biophysical and biochemical tests, and methods of classifying and analysing observations made in nutrition surveys.

(8) It is recommended that WHO initiate, promote and assist the compilation of bibliographies on nutrition surveys that have been made in various parts of the world.

Annex 1

DESCRIPTION OF SIGNS OF MALNUTRITION

The following descriptive list of physical signs is offered in the interest of uniformity of terminology. Many of the signs are of value in the diagnosis of malnutrition. Others probably have no value in this context, but need to be differentiated from the first group. An intermediate group may or may not have significance in the assessment of nutritional status.

In this annex the Committee has avoided any attempt to attach nutrient specificity to these signs. Descriptions are made as brief as possible and the list is intended to be supplemented by fuller descriptions in manuals and by demonstration in the field. The order of arrangement in the list is pragmatic rather than systematic and follows the usual pattern of clinical examination from head to foot.

1. Hair

Lack of lustre

Dull dry hair.

Thin and sparse hair

In certain races changes in texture can easily be distinguished. Coarse, thick and even kinky hair may lose its curl and become thin and fine in texture, with a somewhat sparse distribution. Colour changes of some degree are usually present. Allowance must be made for local practices in the treatment of hair.

Dyspigmentation of the proximal part of the hair

A distinct lightening of the hair from its normal colour which may vary in different populations. Various tinges may be seen.

Flag sign

Characterized by alternating zones of lightness and darkness along the length of the hair.

Easy pluckability

A tuft of hair can easily be pulled out with moderate force and without pain.

2. Face

Pallor

Of skin and mucous membranes.

Diffuse depigmentation

Naso-labial dyssebacea

The lesion consists of greasy filiform excrescences, greyish, yellowish or pale in colour, most commonly located in the naso-labial folds and nasi. They may be seen also on the bridge of the nose, eyebrows and the back of the ears. When extensive the condition is called sharkskin.

Malar and supraorbital hyperpigmentation

Hyperpigmented areas with or without cracking.

Moon-face

A peculiar rounded prominence of the cheeks, which protrude over the general level of the naso-labial folds. The mouth presents a pursed-in appearance. The condition is encountered mostly in the pre-school child.

3. Eyes

Xerosis conjunctivae

This condition is characterized by cloudiness, lack of lustre and dryness confined to the conjunctivae. A few seconds' exposure by drawing back the lids will aid in its identification.

Xerophthalmia (keratomalacia)

Owing to the extension of the process of xerosis and keratinization of the corneal epithelium, the cornea becomes opaque, soft and even necrotic. The term " keratomalacia " is usually applied to the advanced condition.

Conjunctival thickening

A heaping and prominence of the conjunctiva, which is moist and often pigmented, on either side of the cornea. Indirect pressure through the lower lid causes wrinkling.

Bitot's spots

Well-demarcated, superficial, dry, greyish or chalky-white, foamy lesions, triangular or irregular in shape, more often confined to the regions lateral to the cornea and never overlying it.

Corneal vascularization

Invasion of the cornea with capillaries and fine blood-vessels, which can be more clearly defined by biomicroscopic examination.

Conjunctival injection, circumcorneal injection

The terms are self-explanatory.

Superficial corneal opacities and corneal scars

Their nature should be described.

Circumcorneal and scleral pigmentation

Nature and location should be described.

Angular palpebritis

The lesion is characterized by excoriation and fissuring of external canthi.

4. Lips

Angular stomatitis

The term is used to describe sodden and excoriated lesions associated with fissuring of the angles of the mouth. The fissures may be shallow or deep and may be confined to a small area at the angles or may extend into the mouth inside and a few millimetres on to the skin outside. Milder lesions are made out more easily with the mouth half open. The lesion should be noted only if both angles of the mouth are involved.

Angular scars

Healed lesions of angular stomatitis may appear pink or blanched depending on the time elapsed.

Cheilosis

This lesion is characterized by vertical fissuring, later complicated by redness, swelling, and ulceration of areas of lips other than the angles.

Chronic depigmentation of lower lip

Usually central.

5. Tongue

Oedema of tongue

Indentations along edge of tongue.

Scarlet and raw tongue

The tongue is bright red in colour, usually of normal size or slightly atrophic, denuded and very painful.

Magenta tongue

The tongue is purplish red in colour; numerous morphological changes may coexist.

Hyperaemic and hypertrophic papillae

The papillae are hypertrophic and red or pink and give the tongue a granular or pebbly appearance (red strawberry).

Atrophic papillae

The papillae have disappeared giving the tongue an extremely smooth appearance. The distribution may be central or marginal.

Fissures

Cracks on the surface of the tongue with no papillae on their sides or floors.

Geographic tongue

Tongue with irregularly distributed patchy areas of denudation and atrophy.

Pigmented tongue

Punctate or patchy areas of mucosal pigmentation.

6. Teeth

Mottled enamel

Mottled tooth with chalky white and brownish areas with or without erosion of the enamel.

Caries

The number of cavities and of decayed or filled teeth may be taken as an index of caries.

Attrition

The cutting borders of incisors and the cusp of the molars may be flattened.

Enamel hypoplasia

Defective formation of the enamel usually generalized over tooth surface. To be distinguished from enamel abrasion due to mechanical causes.

Enamel erosion

Sharply defined areas usually around the gum margin.

7. Gums

Spongy, bleeding gums

Purplish, spongy swelling of the interdental papillae and/or the gum margins. May bleed easily on slight pressure.

Pyorrhoea

Suppuration of alveolar margins.

Recession of gum

Gum tissue atrophies, sometimes exposing the roots of the teeth; often secondary to pyorrhoea.

8. Glands

Thyroid enlargement

The gland is visibly and palpably enlarged. The enlargement may be diffuse or nodular. Inspection and palpation while the subject swallows may be helpful in the diagnosis.

Parotid enlargement

Chronic, visible, non-inflammatory swelling of the parotids.

Gynaecomastia

Bilateral enlargement of the nipple and glandular breast tissue in men.

9. Skin

Xerosis

Generalized dryness with branny desquamation.

Folliculosis

Prominences of hair follicles on thighs and extensor surfaces of forearms and over pressure points. The surrounding skin is healthy. Commonly observed in adolescents.

Follicular hyperkeratosis

(1) The lesion consists of hyperkeratosis surrounding the mouth of the hair follicle and forming a plaque that resembles a spine. It is readily detected by the spiky feeling it gives when the palm is passed over the lesion and has a characteristic distribution frequently confined to the buttocks, thighs and, in general, extensor aspects of the extremities. The surrounding skin is dry and lacks the usual amount of moisture and oiliness.

In early literature on the subject the condition was termed " toad skin " or " phrynoderma."

(2) The follicular lesion is morphologically similar, but the mouths of hair follicles contain blood or pigment. The intervening skin is *not* dry. The condition is usually seen in adults. The distribution is usually over the abdomen and extensor aspects of the thighs.

Petechiae

Small haemorrhagic spots on the skin or mucous membranes. Application of the blood-pressure tourniquet may sometimes bring about petechiae.

Ecchymoses

Relatively large areas of haemorrhage under the skin.

Pellagrous dermatosis

Typical pellagrous skin lesions are symmetrical, clearly demarcated, hyperpigmented areas with or without exfoliation. The latter, when present, starts in the centre of the patch. The lesions are common in the exposed parts ; when they appear round the neck the condition is called Casal's necklace.

Flaky paint dermatosis (resembling cracked enamel paint)

Extensive, often bilateral hyperpigmented patches of skin which desquamate to leave hypopigmented skin or superficial ulceration. It can occur anywhere, but is characteristically present on the buttocks, backs of thighs and perineum.

Mosaic dermatosis

Seen on the skin over the shins ; always bilateral. The large mosaic plaques are firmly adherent in the centre, but show a tendency to peel off at the periphery.

Thickening and pigmentation of pressure points

A diffuse thickening, with pigmentation, of the pressure points, such as the knees, elbows, and front and back of the ankles. The knuckles may also be involved. The affected areas may be wrinkled, with or without fissuring.

Intertriginous lesions

Raw, red and macerated lesions in skin flexors prone to constant friction, such as groins, buttocks, and axillary folds, frequently secondarily infected.

Scrotal and vulval dermatosis

A desquamating lesion of the skin, often highly irritant. Secondary infection may supervene.

10. Nails

Koilonychia

Bilateral spoon-shaped deformity of the nails in older children and adults.

Transverse ridging or grooving of nails

Recorded if present in nails of more than one extremity.

11. Subcutaneous Tissue

Oedema

Usually apparent over ankles and feet and may extend to other areas of extremities; it may involve genitals, face and serous cavities. In early stages detected by firm pressure for a few seconds on the lower portion of the medial surface of the tibia. The sign is taken as positive if there is a visible and palpable pit that persists after the pressure is removed. Recorded only if bilaterally present.

Fat

Increase or decrease determined by palpation of skin-fold.

12. Muscular and Skeletal Systems

Muscular wasting

In the early stages of protein-calorie deficiency, the condition is most obvious in the area of the shoulder girdle and upper arm.

Muscular hypotonia

This results in pot-belly and faulty postures, such as lordosis.

Intramuscular haematoma

Usually of calf or thigh.

Winged scapula

May be a sign of loss of muscle tone and wasting.

Craniotabes

The lesion consists of areas of softening of the skull, usually involving the occipital and parietal bones. The bones dent on pressure and recoil after the pressure is removed. The sign is positive only in infancy.

Frontal and parietal bossing

Thickening of the frontal and parietal eminences of the skull.

Epiphyseal enlargement

Enlargement of the epiphyseal ends of long bones, particularly of radius and ulna at the level of the wrist, and tibia and fibula at the level of the ankle.

Beading of ribs

A symmetrical nodular enlargement of the costochondral junctions.

Knock-knees or bow-legs

Self-explanatory.

Diffuse or local (pelvic) skeletal deformities

Deformities of thorax

Harrison's sulcus ; pigeon breast.

Subperiosteal haemorrhage

Haemarthrosis

13. Internal System

Gastro-intestinal

Hepatomelagy
Splenomegaly
Ascites

Nervous

Psychomotor change (listless, miserable, apathy in early childhood)
Mental confusion which may include psychosis
Sensory loss
Motor weakness
Loss of position sense
Loss of vibratory sense
Loss of ankle and knee jerks
Calf tenderness
Condition of ocular fundus

Cardiovascular

Cardiac and peripheral vascular dysfunction
Pulse rate
Blood pressure

Annex 2

EVALUATION OF SIGNS USED IN NUTRITION SURVEYS

In this annex an attempt is made to evaluate the signs described in Annex 1. Any such evaluation must at present be tentative and incomplete. Three categories are suggested:

(1) Signs which indicate with considerable probability deficiency of one or more nutrients in the tissues in the present or in the recent past.

(2) Signs which are found more commonly in people with low standards of living than among privileged groups. In their causation malnutrition probably plays some part, together with other factors. Malnutrition may have been operative over long periods.

(3) Some signs which, in the present state of knowledge, have little or no nutritional significance.

CLASSIFIED LIST OF SIGNS USED IN NUTRITION SURVEYS

Area of examination	Group 1: Signs known to be of value in nutrition surveys	Group 2: Signs that need further investigation	Group 3: Some signs not related to nutrition
(1) Hair	Lack of lustre Thinness and sparseness Dyspigmentation of proximal part of hair Flag sign Easy pluckability		Alopecia Artificial discoloration
(2) Face	Diffuse depigmentation Naso-labial dyssebacea Moon-face	Malar and supraorbital pigmentation	Acne vulgaris Acne rosacea Chloasma
(3) Eyes	Xerosis conjunctivae Xerophthalmia (including keratomalacia) Bitot's spots Angular palpebritis	Conjunctival injection Circumcorneal injection Circumcorneal and scleral pigmentation Corneal vascularization Corneal opacities and scars	Follicular conjunctivitis Blepharitis Pingueculae Pterygium Pannus
(4) Lips	Angular stomatitis Angular scars Cheilosis	Chronic depigmentation of lower lip	Chapping from exposure to harsh climates
(5) Tongue	Oedema Scarlet and raw tongue Magenta tongue Atrophic papillae	Hyperaemic and hypertrophic papillae Fissures Geographic tongue Pigmented tongue	Aphthous ulcer Leucoplakia

CLASSIFIED LIST OF SIGNS USED IN NUTRITION SURVEYS (continued)

Area of examination	Group 1: Signs known to be of value in nutrition surveys	Group 2: Signs that need further investigation	Group 3: Some signs not related to nutrition
(6) Teeth	Mottled enamel	Caries Attrition Enamel hypoplasia Enamel erosion	Malocclusion
(7) Gums	Spongy, bleeding gums	Recession of gum	Pyorrhoea
(8) Glands	Thyroid enlargement Parotid enlargement	Gynaecomastia	Allergic or inflammatory enlargement of thyroid or parotid
(9) Skin	Xerosis Follicular hyperkeratosis, types 1 and 2 Petechiae Ecchymoses Pellagrous dermatosis Flaky paint dermatosis Scrotal and vulval dermatosis	Mosaic dermatosis Intertriginous lesions Thickening and pigmentation of pressure points	Folliculosis Ichthyosis Acneiform eruptions Miliaria Epidermophytoses Sunburn Onchocercal dermatosis
(10) Nails	Koilonychia	Brittle, ridged nails	
(11) Sub-cutaneous tissue	Oedema Amount of fat		
(12) Muscular and skel-etal systems	Intramuscular or sub-periosteal haematomas Craniotabes Frontal and parietal bossing Epiphyseal enlargement (tender or painless) Beading of ribs Knock-knees or bow-legs Diffuse or local skeletal deformities	Winged scapula Deformities of thorax	Funnel chest Kyphoscoliosis
(13) Internal systems:			
(a) Gastro-intestinal	Hepatomegaly Ascites		Splenomegaly
(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	Condition of ocular fundus	
(c) Cardio-vascular	Cardiac and peripheral vascular dysfunction Pulse rate	Blood pressure	

Annex 3

SUGGESTED GUIDE FOR INTERPRETATION
OF CLINICAL SIGNS

In this annex an attempt is made to group clinical signs according to patterns associated with deficient diets commonly encountered in different parts of the world. These clinical patterns vary according to one or more most-limiting nutrients, but vary also in relation to staple foods in prevailing dietary patterns, and genetic influences may operate. Clinical patterns cannot therefore be standardized for all areas.

Dietary Obesity

Excessive weight in relation to height or other skeletal indices
Excessive skin-folds
Excessive abdominal girth in relation to chest girth

Undernutrition

Lethargy, mental and physical (starvation)
Low weight in relation to height or other skeletal indices
Diminished skin-folds
Exaggerated skeletal prominences
Loss of elasticity of skin

Protein-Calorie Deficiency Diseases [1]

Oedema
Muscle wasting
Low body-weight
Psychomotor change
Dyspigmentation of the hair

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[1] In protein-calorie deficiency disease in early childhood, it is advisable to estimate the prevalence of the two major syndromes, kwashiorkor and nutritional marasmus. The cardinal features of kwashiorkor are oedema, muscle wasting (in the presence of subcutaneous fat) and low body-weight (Gomez second or third degree); the essential features of nutritional marasmus are wasting of muscle and subcutaneous fat and very low body-weight (Gomez third degree). Intermediate types are common. It may also be possible to assess the commonness of less severe manifestations by weight analysis according to the Gomez method (Gomez et al., 1956), or possibly by the use of a score system, according to the clinical signs present (Jelliffe & Welbourne, in press).

Protein-Calorie Deficiency Diseases (*continued*)

Easy pluckability of the hair
Thin, sparse hair
Moon-face
Flaky paint dermatosis
Diffuse depigmentation of the skin

Vitamin A Deficiency

Xerosis of skin
Follicular hyperkeratosis, type 1
Xerosis conjunctivae
Keratomalacia
Bitot's spots

Riboflavin Deficiency

Angular stomatitis; angular scars
Cheilosis
Magenta tongue
Central atrophy of lingual papillae
Naso-labial dyssebacea
Angular palpebritis
Scrotal and vulval dermatosis
Corneal vascularization

Thiamine Deficiency

Loss of ankle jerks
Loss of knee jerks
Sensory loss and motor weakness
Calf-muscle tenderness
Cardiovascular dysfunction
Oedema

Niacin Deficiency

Pellagrous dermatosis
Scarlet and raw tongue
Tongue fissuring
Atrophic lingual papillae
Malar and supraorbital pigmentation

Vitamin C Deficiency

Spongy and bleeding gums
Follicular hyperkeratosis, type 2
Petechiae
Ecchymoses
Intramuscular or subperiosteal haematoma
Epiphyseal enlargement (painful)

Vitamin D Deficiency

(1) *Active rickets* (in children)

Epiphyseal enlargement (over 6 months of age), painless
Beading of ribs
Craniotabes (under 1 year of age)
Muscular hypotonia

(2) *Healed rickets* (in children or adults)

Frontal and parietal bossing
Knock-knees or bow-legs
Deformities of thorax

(3) *Osteomalacia* (in adults)

Local or generalized skeletal deformities

Iron Deficiency

Pallor of mucous membranes
Koilonychia
Atrophic lingual papillae

Iodine Deficiency

Enlargement of thyroid

Excess of Fluorine (Fluorosis)

Mottled dental enamel, difficult to distinguish in early stages from enamel hypoplasia.

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