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MANUAL FOR NUTRITION SURVEYS

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1963

Interdepartmental Committee on Nutrition for National Defense
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[1] Paul L. Pavcek, 1911–64, was an active member of the advisory panel for this second edition of the ICNND Manual for Nutrition Surveys. Dr. Pavcek has made a major contribution to international nutrition, having participated as nutritionist in ICNND surveys in Spain, Chile, and Venezuela and as an FAO–ICNND consultant in training the nutrition workers of the recent comprehensive survey of East Pakistan jointly carried out by ICNND and FAO and the Government of Pakistan. During World War II as Executive Secretary of the Food and Nutrition Board of the National Research Council, he helped in the development of technics of nutrition surveys and played an active role in the nutrition activities of the quartermaster and surgeon general groups of the military forces.

Although Dr. Pavcek will be sorely missed by his colleagues, his lasting memorial will be the host of friends that he has made throughout the world by virtue of his openness and friendliness and love of people.

Foreword

Dr. Harold R. Sandstead, one of the founders and first Executive Director of the Interdepartmental Committee on Nutrition for National Defense, lost his life in an airplane crash November 1, 1955, while on a mission to speak on nutrition problems in the Near and Far East. He had been a member of the U.S. Public Health Service since 1934. His experience in conducting nutrition surveys and teaching nutrition extended to all parts of the world. Dr. Sandstead did much pioneering work in developing standardized methods and procedures for nutrition surveys of population groups. The ideals and objectives of this Manual were originated by him. He labored on the outline and many sections of the first edition of this Manual. It was his hope, and it is the hope of the Interdepartmental Committee on Nutrition for National Defense, that this Manual will not only result in the collection of nutrition survey data in a uniform manner, but also facilitate the formulation of practical measures to improve the nutritional status of undernourished people.

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Best Available Document

Preface to the Second Edition

The first edition of this Manual was published in May 1957. Originally intended as a guide for conducting nutrition surveys of military personnel, the Manual since late 1957 has also served as a guide for surveys of civilian population groups. In the intervening years Interdepartmental Committee on Nutrition for National Defense (ICNND) teams have studied civilians in 18 countries. Other workers have also used the Manual as a guide. From these experiences additional methods and guidelines for assessment have been evolved.

For those who are not familiar with the activities of the ICNND, a brief description may be in order. The Committee was organized in 1955, with the primary objective of assisting developing countries to assess their nutritional status, to define existing problems of malnutrition, and to identify means for solving critical nutrition problems by making maximum utilization of the country's own resources. The Committee is presently composed of representatives from the (U.S.) Agency for International Development, the (U.S.) Departments of State, Health, Education, and Welfare, Defense, Army, Navy, Air Force, Agriculture, Interior, and the Atomic Energy Commission; and has liaison members from Food for Peace, the Pan American Health Organization (PAHO), United Nations Children's Fund, Food and Agriculture Organization, and World Health Organization. The Committee is served by a Secretariat, through which it acts as a clearing center for collection and appraisal of nutrition data, initiates field studies, and recommends corrective actions and other programs. The Secretariat is located at the National Institutes of Health, Bethesda, Md.

Technical advice is obtained from a group of consultants from universities and research institutions who have expert knowledge in various aspects of nutrition science and experience in international work. These consultants have served as members of survey teams and have helped in the preparation of sections of this Manual. To them and all others who contributed their experience and knowledge to this Manual, the Editorial Committee is indebted. The "all others" include the numerous members of the cooperating teams from the countries thus far surveyed as well as American team members who have helped in the development and appraisal of the methods used by the ICNND.

The ICNND has now conducted medical nutrition surveys in 24 countries in response to official requests submitted through State Department channels. Nations observing the outcome of the first military surveys requested that their civilian populations also be studied, thus enlarging the activities of the ICNND, to the extent that civilian populations have constituted the majority of the individuals studied in most surveys made since 1957.

The ICNND has had a continuing interest in assisting the collaborating countries in follow-up studies and action programs. A few examples indicate the scope and magnitude of some of these continuing activities:

1. Following the initial survey in Pakistan in 1956 (*1*) which was restricted to military troops, the Committee, through collaboration with the National Institutes of Health, established a nutrition program jointly with the Government of Pakistan, the Food and Agriculture Organization (FAO), United Nations Children's Fund (UNICEF), and the World Health Organization (WHO) in 1961. Pakistan has established a Directorate for Nutrition. The Ministry of Public Health during the past 2 years has been actively engaged in assessing the nutritional status of the civilian population of East Pakistan. During this period, over 15,000 individuals have been studied in 17 different areas of East Pakistan. Numerous nutrition problems have been identified which require additional research, such as the nutritional influence of consuming mustard oil, identification and classification of the types of anemia that occur, and development of practical therapeutic and preventive measures. These studies in East Pakistan are being made by recently trained Pakistani scientists and technicians. It is envisioned that the majority of these trained personnel will be utilized in community development and public health programs throughout the country.

2. Following the survey in Ethiopia (*2*) the Government of Sweden has initiated a pediatric nutrition program jointly with the Ministry of Health, UNICEF has assisted in establishing a salt iodization plant, FAO has assisted in the training of home economists, and the U.S. Agency for International Development (AID) has collaborated in establishing a professorship of nutrition at the School of Agriculture which is supported by a contract with Oklahoma State University.

3. The results of the Lebanon survey were used by Columbia University as a basis for planning a portion of its teaching program in Beirut. The survey results also helped the direction of research at the American University of Beirut.

4. The Committee has maintained liaison with the nutrition laboratories it has established and in a small measure has assisted in maintenance of equipment and key chemicals and supplies.

These are only a small sample to illustrate the types of activities which have followed the stimulus of the surveys themselves. By and large, the major progress and programs that have developed are those initiated by the host countries themselves. As might be expected, progress is often slow. The improvement of the nutritional status of population groups requires dedicated, continued education, interest and work. The programs cross all barriers of government activities, industry, research, and education. Interested and able individuals have been given the opportunity to receive further training in technical fields in other countries, either through personal contacts with team members or through efforts made by the Secretariat or other individuals or organizations.

A manual such as this is never completed, nor can it be considered the last word on every subject discussed. It is hoped that in its present form it will be helpful to workers planning and conducting studies of nutrition among population groups.

The Manual has been designed to—

a. Establish uniformity in methods, technics, procedures, and guidelines for conducting surveys in order to make meaningful comparisons of results within and between countries. It is recognized, as reiterated in later chapters, that in some instances more refined (and in some cases more precise) methods are known, but those included have the advantages of practicality of application, simplicity, and reproducibility as demonstrated by field use. In addition, the interpretative guidelines presented were developed for use with these methods; therefore, the guidelines may not be valid if applied to data obtained by other procedures.

b. Outline and define responsibilities and duties of team members.

c. Provide a guide to interpretation of dietary, biochemical, and clinical data collected.

d. Serve as a working reference of major facts essential to proper appraisal of nutritional status, and to aid in interpreting findings in order to draft practical, effective recommendations to alleviate nutritional problems discovered.

e. Assist in training personnel in nutritional appraisal technics and to stimulate continued nutrition work.

It should be remembered that the initial surveys conducted through collaboration with the ICNND by the very nature of the limited time involved, usually 2 to 3 months, furnish data on the nutritional status and problems occurring during the period of the survey. To obtain a more accurate picture of the overall nutrition problems, it is obvious that additional surveys should be conducted during the various seasons of the year, and in many countries special population groups will

require further study. One of the continuing objectives of the ICNND program has been to assist the host countries to make additional needed studies and to initiate action programs.

REFERENCES

1. Interdepartmental Committee on Nutrition for National Defense, Pakistan--Nutrition Survey of the Armed Forces, Bethesda, Md., September 1956.
2. Interdepartmental Committee on Nutrition for National Defense, Ethiopia--Nutrition Survey, Bethesda, Md., September 1959.

ABBREVIATIONS USED IN THIS MANUAL

AC	alternating current
ACS	American Chemical Society
°C	degrees Centigrade
ca	approximately
cc	cubic centimeter(s)
cm	centimeter(s)
DC	direct current
doz	dozen
°F	degrees Fahrenheit
ft	foot (feet)
′	foot (feet)
gal	gallon
gm	gram(s)
>	greater than
hr	hour(s)
in	inch(es)
″	inch(es)
IU	International Units
kg	kilogram(s)
lb	pound
<	less than
M	molar (chemical)
mg	milligram(s)
μg	microgram(s)
μl	microliter
min	minute(s)
ml	milliliter(s)
mm	millimeter(s)
mm Hg	millimeters of mercury
mp	melting point
mμ	millimicron(s)
N	normal (chemical)
OD	optical density
oz	ounce
pkg	package
ppm	parts per million
qt	quart
rpm	revolutions per minute
S.D.	standard deviation
S.E.	standard error
sec	second
sp gr	specific gravity
sq	square
USP	United States Pharmacopoeia
vib	vibrations
yd	yard(s)
yr	year(s)

I

Organization of Survey Team

Nutrition survey teams, as developed by the ICNND, include clinical nutritionists, dentists, and on occasion pediatricians to assess the lesions associated with nutritional deficiency; biochemists to analyze blood and urine samples; nutrition specialists and dietitians to collect data on food and nutrient intake, food patterns, taboos, customs, and food preparation; and food technologists and agricultural specialists to evaluate existing agricultural and food-processing practices and determine ways and means for improvement. Occasionally the team may have specialists in ophthalmology and dermatology. Other workers in related fields can readily be included on the team, such as specialists interested in determining the incidence of infectious and parasitic disease, and specialists in public health and sanitation. The number of associated studies is obviously dependent upon personnel. In general, the survey team consists of a U.S. component working with two to three times its number of counterpart personnel from the host country.

In order to supervise and coordinate the activities of a nutrition survey team, a director is selected. His main duties are set forth below:

Briefing of Interested Agencies

During a presurvey visit the objectives, scope, and need for collaboration and assistance are discussed with those governmental, educational, and research organizations of the country concerned that are vitally involved in food and health. These include the Ministries of Agriculture, Health, Education, Defense (since the armed forces are usually the largest single food-consuming group in the country); industry, governmental and private research organizations; organizations assisting the country through bilateral agreements or in collaboration with the United Nations agencies, such as FAO, WHO, and UNICEF; and welfare and relief organizations. In this initial briefing, guidance and advice should be obtained from all these agencies regarding data and information that will be required for pre-

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paring the background report and for the development of preliminary plans for the survey. This includes information concerning the various ethnic and population groups that either have similar or unique nutrition and feeding problems.

Determining the Laboratory Site

Deciding on the site for the laboratory headquarters should take into account the potential and possibility for continued activity. In some instances, due to limited professional personnel and funds, the location may be temporary. However, it is usually possible to obtain agreement and collaboration with either existing laboratories in universities or medical schools, or in the Ministries of Health, Agriculture, or Defense. The important feature is not so much where the laboratory is located, but the potential it shows for future continuation of work in the field of food and nutrition.

Selection of Survey Sites and Logistical Support

The first major problem to be resolved is the sample selection as discussed in chapter IV. The selection of survey sites and sample size will of necessity be dictated by the availability of transportation for the team. Thus the details of logistics and transportation of team members throughout the survey and shipment of biological samples to the headquarters laboratory must be given careful attention at the onset of planning. Upon the development of the final sample size and locations, it becomes the team director's responsibility to complete arrangements with local authorities at the various sites as rapidly as possible, with precise definition of the facilities and locations where the team will work. Arrangements must also be completed for housing of the field team, local publicity, and collaboration of subjects to be studied. Time schedules must be set for shipment of biological samples back to the laboratory. In all cases the schedule should recognize the need for flexibility and alternate possibilities in the itinerary due to delays caused by weather, travel problems, or local holidays of which the group may not have prior knowledge.

The preliminary plans for the survey should be circulated to all collaborating and interested organizations, and every effort made to enlist not only their moral support but also their active cooperation.

Briefing of Team Members

Before the start of fieldwork, the director will find it essential to hold numerous briefing sessions with all team members so that they are aware of the overall plan and objectives. In addition, such sessions

serve as general question-and-answer periods, wherein the duties of each group are more clearly defined. Throughout the survey the director must discuss at intervals the progress and preliminary findings with the entire team. Generally, it will be valuable to have the field and laboratory groups meet jointly about halfway through the survey to determine how nearly the original plan is being followed. It is of primary importance that the team realize that, although they are specialists in various fields, they must operate and function as a team, often doing additional ancillary work and studies, such as observing and obtaining data from hospitals, universities, and commercial establishments that are so vital for an overall appraisal of nutritional status of the population.

Recordkeeping

The team director is responsible for maintaining a daily log or diary, which, in addition to recording daily activities of the team, will record names and addresses of officials and establishments visited. All team members are responsible for informing the team leader of institutions visited, their observations, and such other information as would be of general value to the entire project. The director's logbook should serve as a master list of the day-to-day workload and location of each team group. This should include ancillary data obtained, such as health facilities, schools and other educational institutions, local industrial activities, school or special group supplementary feeding programs, description of housing, climate, temperature variations, altitude. The log should also record any difficulties which have hindered progress of the survey plans, such as travel difficulties, and alternative locations and studies which may differ from the original plan.

Special and Follow-up Studies

The team director, in consultation with other members of the team, will make assignments to appropriate team members for special studies, which may include performance of functional ("load") and therapeutic tests, special metabolic or biochemical studies, anthropometric measurements, etc. Some of these will, of course, have been planned before the start of the survey; others will be determined as a result of unpredictable observations. For follow-up studies, it is important to note and record either individuals or institutions that are considered cooperative, capable, and interested in or peculiarly suitable for establishing pilot corrective measures.

Preparation of Reports

There are three steps in reporting:

1. An initial brief report which outlines the activities carried out during the survey, the sites visited, contacts made, and identifies the studies undertaken. This report will incorporate any preliminary data which seem informative and give broadly interpretive impressions and recommendations for immediate continuing studies or actions, including an approximate schedule for developing further reports.

2. A preliminary report based upon carefully considered analysis of the data obtained. This draft is usually prepared by the U.S. team leader and the biostatisticians and editorial group of the ICNND, in correspondence with key host-country personnel. A draft of this preliminary report is reviewed by the ICNND Committee. The report is then jointly considered by the team director and one or two selected key U.S. members of the team, the U.S. mission in the host country, and the key host-country team members and representatives of the collaborating ministries. It is discussed with the respective ministries of the host country or their designated representatives.

3. A final report which is prepared and edited by the editorial group designated by the ICNND and which takes into consideration points raised in the extensive reviews of the preliminary draft report (2 above).

It has been the experience of the ICNND that tabulation and interpretation of the clinical, biochemical, and dietary data obtained during the usual ICNND survey require approximately 6 months, and that completion of the final report occurs some 8 to 12 months after the survey is finished. It is often possible to identify major problems which require additional followup in the field of research or action programs without waiting for complete tabulation of the data. In general, however, large-scale action programs are usually not attempted until all the data have been adequately analyzed.

The reports of nutrition surveys conducted by the ICNND are available from the Secretariat. These reports contain no specific authorship since they represent the combined collaborative efforts of U.S. and host country team members, suggestions and revisions submitted by the host country governmental agencies, the ICNND's committee members and consultant staff and its liaison members with the United Nations agencies.

II

General Background Information

Introduction

General background information about a country or region is essential for the planning and execution of a nutrition survey. Frequently there exist sources such as monographs, reports of technical expert consultants of international agencies (Food and Agriculture Organization, World Health Organization, and others), bilateral agencies (especially U.S. Agency for International Development), and of institutions or foundations such as the Rockefeller Foundation, the Stanford University Institute of Food and Nutrition, national or regional institutes of nutrition, or agricultural research groups, as well as scientific reports and observations recorded in the scientific literature, articles in encyclopedias, and in such publications as the National Geographic Magazine or other sources.

A summary statement specifically pertaining to the given area should be prepared for use in briefing team members in their preparation for the survey. The briefing paper should summarize pertinent information concerning health, agriculture, food habits, movement of foodstuffs (imports, exports), relevant general cultural, social and religious information, manners, languages, ethnic groups, and history. In addition, it should include facts needed by the visiting team member for his travel planning, clothing requirements, currency exchange, mail services, hotel facilities, and the like. The summary should contain a well-chosen list of appropriate references which will permit the reader to develop further knowledge. It is especially valuable to team members to have an understanding and appreciation of the culture and history, as well as some knowledge of the language of the country.

The following types of information are helpful in planning and carrying out a survey with satisfactory understanding:

Background of the Country

Information on the country's recent history, government, culture, ethnic composition, languages, geography, climate, and its international position currently and in the recent past should be included in the briefing statement. Further details about the country may be

available from books, reviews, and reliable news sources such as news weeklies. This material should be reviewed by team members before the mission, since some understanding of the country or region, even if based on information that is not complete, helps in the day-to-day contact with local counterpart personnel and the population segment surveyed.

Technical Information Pertaining to Food, Nutrition, and Health

Information can be obtained from the technical literature (especially scientific journals), and published reports of various United States and United Nations technical agencies (ICNND, Agency for International Development (AID), U.S. Departments of Agriculture, Health, Education, and Welfare, and Commerce; Food and Agriculture Organization (FAO), World Health Organization (WHO), United Nations Educational, Scientific, and Cultural Organization (UNESCO), etc.). If at all possible, such information should be collected, read, and discussed by the team members before they depart for the survey mission. Reports published by private agencies (foundations such as Rockefeller, Ford Foundation, China Medical Board, religious organizations like the Unitarian Service Committee, etc.) engaged in medical and educational activities and by universities and colleges working in the host country should not be overlooked.

It becomes obvious that there should be a logical subject matter distribution of the reading load in this category among the team members responsible for different aspects of the nutrition survey. Thus the clinicians will study the reports dealing with health and diseases, the nutrition specialists will acquaint themselves with the results of past food intake surveys, and the food technologist will scrutinize reports dealing with agriculture, food production and economics, food technology, etc. However, each member should be familiar with the overall food and nutrition status of the country to be surveyed insofar as data are available.

Current Information Pertaining to Food, Nutrition, and Health

Published information inevitably represents conditions as they existed some time in the past and therefore usually needs to be revised or brought up to date. The need for revision will depend on how recent the published reports are and how rapidly the local situation changes. Current information sought will fall into two categories, namely:

Information which can be requested from the local government and other sources before the start of the survey; and

Information which must be assembled by team members in the

country during the nutrition survey. In addition, statistical and other data obtained earlier should be verified in the host country.

The more reliable information the team members have before starting the mission, the more efficient and effective they can be during the limited period of the nutrition survey. During preparation for the mission, team members should carefully record what supplemental information will be needed. In the field, periodic discussions among the team members may reveal additional areas where information is required.

Suggested checklists for general background information and for background information on nutritional health are given below. The list of topics to be investigated concerning the diet pattern, food intake, etc., is given in chapter VII, whereas the information to be collected concerning food production and technology is dealt with in chapter III.

Checklist for General Background Information

Brief history of the country and its government.

Major geographic features. Geographic districts.

Climate. Regional and seasonal variations in temperature and rainfall. It should be clearly established at the outset during what phase of the annual climate cycle the survey is to be conducted.

General population characteristics:

Total population by last census and other similar estimates. Annual population growth. Regional distribution of the population.

Ethnic composition.

Languages and their relative importance.

Principal religious faiths. Their relation to ethnic and language groups.

Age distribution by groups.

Occupational composition. Information on the proportions of the population engaged in agricultural pursuits, fishing, mining, manufacturing, commerce, transportation, government service, professional services, etc.

Educational institutions and literacy level. Advanced trainees recently returned to country.

Custodial institutions, especially orphanages, hospitals, schools for the handicapped.

Mass feeding responsibilities, school feeding programs, rationing systems for total population or special groups (such as refugee groups, military).

Major cultural features. Team members should have an understanding of the host country's culture. People of any country are proud of their culture which includes literature, architecture, painting and sculpture, music, theater and the dance, etc.

Major economic features:

National income and income distribution.

Indices of production or other indications of development trends in agriculture, mining, and manufacture.

Indices of prices and living costs in recent years.

Exports and imports. Quantity and value of various items in recent years.

Checklist for Information on Health

Record source of data, how collected, when, source of statistics and indicate lack of data.

Civilian health statistics. (Indicate trends, if data are available.)

Crude birth and death rates by months and years of age if obtainable. Infant death rates. Maternal death rates. Principal causes of death. Death rate by month of age during first 12 months and by year of age during first 6 years.

Kinds of cases in hospitals.

Average life expectancy of males and females.

Birth weights and lengths. Growth rates of children. Information on age-weight-height relationships.

Incidence of disease: Tuberculosis, diarrhea and dysentery, malaria, parasitic infections, goiter, beriberi, dental caries, metabolic disorders, or genetic diseases of unusual occurrence, etc.

Medical care. Number and training of physicians, nutritionists, nurses, midwives. Medical training facilities.

Number, size and description of hospital and medical laboratory facilities.

Nutritional services, educational and research facilities. Hospital dietetics. Welfare and institutional nutritional services:

Major laboratories. Knowledge of the names and titles of directors will facilitate contacts during followup activities.

Personnel and training facilities.

Programs and their success.

List of local personnel trained in nutrition. Type of training, where and when obtained (list schools, colleges), experience, etc.

Preventive medicine and public health:

Administrative placement in government.

Personnel and training facilities.

Programs and their success.

Sanitation practices and problems.

Food and drug surveillance.

Excreta disposal and reuse.

Military health:

Recruitment practices and career status.

History of medical services.

Medical standards for military acceptance. Height-weight data.

Induction screening. Proportion of and reasons for rejections.

Proportion of and reasons for medical discharges.

Medical, hospital, dietetic practices and laboratory services.

Nutrition and dietetic services.

Food procurement practices.

Sanitation problems (general problems not covered by dietary studies).

III

Appraisal of Food Production and Technology

Observations on the state of agriculture, food production, preservation and utilization, imports and exports of foods, and many other related subjects are essential. Together with the background information discussed in chapter II, an understanding of the local agricultural and food situation is needed for the interpretation of dietary, clinical, and biochemical observations collected by the other team members. Without such information and a knowledge of the status of food technology within the country, it is impossible to draft realistic recommendations for the correction of nutritional problems and for proper recognition of nutrition in national planning for development. Economic factors often influence both the composition of the diet and the overall food intake. Therefore, obtaining some information on costs, prices, purchasing power, etc., is essential.

It is the food and agricultural specialist who is responsible for collecting this information. Since this assignment is so broad, it is important that the counterpart personnel from the country in which the survey is made have competence in this broad field. A quartermaster corps officer who is a specialist in food services and has some understanding of modern food technology is frequently an excellent counterpart person. In other cases the assignment of a quartermaster corps officer and a civilian food technologist (or food production specialist) has been necessary. Provision will have to be made for the transportation of the food and agriculture specialist and his counterparts, since their work often takes them to places other than team survey sites.

The assignment of the food and agriculture specialist is somewhat more difficult to circumscribe than are those of other team members whose work follows a fairly uniform pattern in the nutrition surveys conducted in various countries. The type of information which the food and agriculture specialist must collect will depend on the local situation and differs from country to country. Many of the questions concerning food and agriculture are apparent only during the

course of the team's work in the field. For this reason there is need for constant consultation between the food and agriculture specialist and other team members.

Early consultations with the U.S. agricultural attaché and various officials of the Agency for International Development and the United Nations technical agencies are important and helpful. Contacts with Ministries of Agriculture, Economics, Trade, Planning and Development Boards, and similar host country groups are essential. The availability of reliable statistics can be learned in this way. In making initial contacts, it is important that the food and agriculture technologist has a clear understanding of the purposes and procedures to be followed by the team. Too much importance cannot be placed on the public relations aspects of contacts within the country.

The food and agriculture specialist's activities will cover a wide spectrum, depending largely upon the extent of technical development of the host country and the availability and reliability of statistical and other government-supplied information. At times statistical information has to be assembled piecemeal in order to allow overall assessment, whereas in other instances well-established statistical bureaus provide most of the needed information. The latter is usually the case in countries well on the way toward economic and technological development.

Statistical information should be tabulated as soon as possible, since this is likely to reveal the need for more data and to point up any existing discrepancies.

An important part of this work is to correlate census and other statistical data with observations made in the host country. A further activity of major importance is the assessment of the food availability cycle with as precise pinpointing of the time of the survey in this cycle as possible. In this connection the current agricultural situation must be considered also, since in countries of limited food supply a good harvest or a bad one will greatly influence the diet both quantitatively and qualitatively.

Visits to educational institutions, especially schools of agriculture and home economics (household sciences), and contacts with professionals of the host country need special emphasis. Usually in any country good liaison easily can be established between professionals interested in the same discipline. Such contacts during the survey provide much information not clear from statistics or not available from governmental ministries. Furthermore, these contacts provide excellent opportunities for cross-checking technical data and for the later acquisition of information for which the need was not recognized at the time of the survey. The team director should coordinate such contacts of all the team in order to avoid unnecessary duplication and the appearance of team members working at cross-purposes. The food

and agriculture specialist may make an important contribution by bringing together certain specialists within the country who may contribute cooperatively to the solution of problems not previously recognized. For example, there may be food technologists and nutritionists in civilian agencies who could be of assistance to the national defense department in the development of a supplemental food or a ration item.

The food and agriculture specialist provides information that will contribute to the long-term followup activities of ICNND. It is important to know what institutions and laboratories are available for food analyses, dietary studies, nutrition appraisals, food processing development, etc. The names and addresses of the available specialists in these fields will assist the ICNND in making contacts. An appraisal of personnel and facilities will make possible further assistance in supplying laboratory and pilot plant equipment, and in making recommendations for fellowships for professional training and in assisting with postsurvey therapeutic trials or pilot correction programs.

The food and agriculture specialist can be of considerable assistance to the dietary group in checking the accuracy of food composition tables available within the country, in identifying unusual foods, and in determining recipes used in preparation of foods. For help in the identification of foods, the food and agriculture specialist should be supplied with a copy of "Selected Edible Plants and Plant Products," prepared by the ICNND Subcommittee on Food and Agriculture. In turn, he should, upon return to the United States, supply the Subcommittee on Food and Agriculture with a list of names of any unusual plant or animal food sources found within the country with their English, local, and scientific names whenever possible. He should obtain, with the aid of the team photographer, colored photographs of unusual foods important in the country.

The food and agriculture specialist should, in cooperation with the dietary specialists, collect samples of foods and plants for shipment to the United States for identification and analysis. Unnecessary duplication should be avoided and, therefore, frequent consultation between the food and agriculture specialist and dietary personnel is desirable regarding samples to be collected. The food and agriculture specialist is in an excellent position to collect samples from the manufacturers as he visits the various food processors, and should have funds available to cover the cost of purchasing such samples. Samples should be collected, identified, and handled as described in detail in chapter VII. In general, all food samples collected should be given to the specialist in charge of the food intake survey who will be responsible for shipment.

The nutrition survey teams and ICNND are often called upon for aid in the development of packaged emergency rations. The ICNND Secretariat can supply a mimeographed discussion of the basic considerations in emergency ration design. The Secretariat and various ICNND subcommittees have in some instances also provided specific assistance on the technical aspects of the problem. Whereas team personnel should give all possible aid on this subject, caution should be exercised to avoid any implication of commitments other than advice. Any promise of material assistance for ration development should come from the Secretariat through the survey team director. An important function of the food and agriculture specialist here is to pinpoint the exact aspects on which assistance is most needed and will do the most good, and in giving recommendations for followup missions on this and other subjects.

One purpose of this chapter is to provide a checklist for the food and agriculture specialist of the nutrition survey team. It would require much longer time than is usually available for the team in the field to collect information on all the points listed. However, much of the basic economic, agricultural, and food production information is usually available from publications of the host country and of the U.S. Government and United Nations technical agencies. These should be studied before departing for the field and assessed for reliability by observations in the host country. Statistical information, sought but not found before or during team briefings, should be requested from the host government, preferably early enough so that the material can be assembled by the time the team arrives. The preliminary visits by the team director and by members of the ICNND Secretariat are good occasions for transmitting such requests.

It will be evident that certain items in the checklist are more important than others in their relation to nutritional problems. The food and agriculture specialist will, therefore, concentrate on these and obtain much more detailed information. For example, in most developing countries a single staple food provides the bulk of the Calories. This may be rice, wheat, corn, cassava, or some other food. It is of primary importance to obtain exact information on the milling of rice and flour and the extent to which undermilled or highly refined cereals are used. If the principal cereal is handled in central mills, the possibilities and need for enrichment, as well as the detailed methods that may be used, should be thoroughly investigated.

Depending on the principal basic food used, the need for other foods to provide better supplies of specific nutrients such as proteins and vitamins will vary. In almost every case emphasis should be placed upon the existing, as well as the desirable, uses of pulses and of certain important vegetables and fruits.

Due to the widespread occurrence of goiter, attention should be given to the production and consumption of salt. The form in which the salt is purchased and the per capita consumption, as well as the type of plants for the production and refining of salt, will all have an important bearing on any recommendations for possible iodization.

Concerning flour enrichment and salt iodization, the food and agriculture specialist can obtain much guidance from reports and publications on these subjects that are available from the ICNND Secretariat.

During the early part of a nutrition survey, the principal nutritional problems usually become apparent. Before the midpoint of the survey, the food and agriculture specialist will have discussed these problems with the team director and other members of the team and will be in a position to pinpoint his efforts during the latter period of the survey to obtain detailed information that is especially germane to the formulation of specific recommendations. Examples given in the preceding paragraph with respect to enrichment and iodization are pertinent. To cite another example, it may be important to assess the suitability of some plants for the manufacture of products needed for infant or military feeding.

Checklist for Information on Agriculture and Food and Related Subjects

Agriculture

Production:

Land and land utilization:

(1) Estimates of total arable land and meadows and pastures.

(2) Land which could be brought under production.

(3) Group distribution by size of farm and farm families.

(4) Countrywide average of agricultural land per capita.

(*a*) Currently in use.

(*b*) Including potentially productive land.

(5) Land tenure system and land rents. Their effect on production.

Annual production:

(1) Yield per acre of important food crops.

(2) Population of various types of livestock.

(3) Production of meat, poultry, eggs, milk, cheese, fish and other products.

Importance of plant and animal diseases. Control measures and their effectiveness.

Source of farmpower. Extent of mechanization, use of tractors, types of implements used.

Use of fertilizers. Sources of fertilizers. Imports.

Irrigation. Potential for expansion.

Types of production:

(1) Extent of large-scale production.

(2) Home food production, practice of home gardening. Extent of subsistence farming.

(3) Use of uncultivated crops in the food supply.

Agricultural credit and taxation.

Government policy on agriculture:

(1) Objectives and assistance given to attain these. National and local plans to increase and improve food production.

(2) Extension and demonstrations.

(3) Aid provided by outside agencies: Technical assistance.

Education and research in agriculture:

(1) Literacy and proportion of population with grade school, high school and higher education.

(2) Current efforts and plans for education.

(3) Agricultural schools and colleges.

(4) Experiment stations and research institutions.

(5) Agricultural extension programs.

(6) Student and teacher exchange programs.

Outlook for Food Production and International Trade in Foodstuffs in an Emergency or Disaster

Data on imports and exports.

Dependence of the food supplies on imports. Prospects.

Vulnerability of the food supply in local and nationwide disasters and emergencies.

Possibilities of adjusting the food supply under emergency conditions.

Marketing Procedures

Marketing system in urban and rural areas:

Cooperatives in production and marketing associations.

Community-owned and private markets.

Relation of transportation facilities to marketing problems.

Efforts made to improve marketing procedures and to reduce food losses, spoilage, and waste:

Storage and refrigeration facilities:

(1) Facilities for grain storage by the government.

(2) Private and on-the-farm storage facilities for grains and other food products.

(3) Natural and ice-cooled cold stores.

(4) Refrigerated storage.

Food laws and food inspection facilities.

Governmental price control and rationing activities.

Food Preservation and Technology

Preserved foods are of importance in eliminating seasonal availability of many items, in making full use of crops, and in feeding certain special groups such as infants and in military feeding, and in the preparation of emergency food packets. These points should be kept in mind in collecting the information on the items listed below.

Cereal milling:

Extent of home milling and methods used.

Community and commercial milling facilities. Extraction rates, utilization of byproducts. Storage facilities in mills. Sanitation.

Where and how rice or flour could be enriched.

Baking:

Home baking.

Commercial bakeries. Source of yeast, if used.

Where and how cereal products could be enriched.

Indigenous and other methods of food preservation used in the home:

Preservation by heating (sterilization or pasteurization). Methoc containers, losses during storage due to improper preservation. Pop larity and acceptability.

Home drying methods.

Food preserved in the home by fermentation.

Other methods of home preservation.

Popularity of home-preserved foods. Their contribution to the diet.

Indigenous methods of food preservation used in commercial productio

Meat:

Slaughterhouses and meatpacking plants.

Meat storage and transportation facilities.

Extent and efficiency of sanitary and health inspection.

Fisheries: Large-scale (commercial) fish processing by drying, picklin: fermentation, canning, etc.

Commercial canning:

Can manufacture or other sources of cans and tinplate.

Glass jar manufacture or sources of glass containers.

Location, products, capacities, availability of raw materials, methods markets and over-all technical proficiency.

Sanitary and quality control.

Local acceptance of canned foods. Markets for products.

The dairy industries:

Methods of preparing milk products.

Milk processing plants.

Commercial manufacture of other dairy products.

The poultry industry.

Fermentation:

Commercial production of sauerkraut, pickles and other fermented products.

Wine and brandy production.

Malt-hop beer.

Other fermented drinks (palm juice, etc.).

The salt industries:

Storage of salt, technics of production.

Storage and distribution methods.

Is iodized salt made and used? Where and how could iodization be done?

Vegetable oil production:

Margarine manufacture.

Utilization of oil meal and cake.

Sugar manufacture:

Raw products, manufacturing methods.

Quality of sugar marketed.

Other processed foods produced commercially.

Food Consumption

Food consumption information for any country is usually assembled by the Food and Agriculture Organization of the United Nations, by the U.S. Department of Agriculture, as well as by local authorities.

Such data, customarily in the form of "food balance sheets," are often available to the team before departure for the survey. The food and agriculture specialist should be familiar with the meaning and limitations of such information, since in most instances he and the diet survey specialists will have to lean heavily on the information which it contains. However, even when food balance sheets are on hand, the food and agriculture specialist should try to assess the following points:

The reliability of the production population statistics used in the preparation of the food balance sheets.

The differences in the availability of various foods in various districts of the country.

The exact meaning in the host country of the various food groupings. For instance, what do the categories "oil and fats" or "roots, tubers and starches" include? If possible, the original data from which the balance sheet was computed should be traced.

The form in which prepared foods (dishes) are consumed. This subject is predominantly within the scope of the dietitians' work, but cooperation on this point (and many others) among the specialists on the team is imperative. It is in conjunction with work on this subject that many questions to be answered by the food and agriculture specialists are unearthed.

List of foods observed on the markets (compiled in cooperation with diet specialists).

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IV

Population Sampling and Design of Special Studies

This chapter describes basic principles involved in the use of samples for estimating certain nutritional characteristics of populations. Methods of sampling which are pertinent to making clinical and biochemical evaluations of civilian population groups and of the armed forces surveyed are discussed. Further details of the plans for dental and dietary subsamples are given in chapters V and VII, respectively.

Suggestions are advanced as to how the sample should be selected by the survey team, but no detailed catalog of procedures is given. A plan which is appropriate for one country may not be so for another due to differences in size and importance of ethnic, religious, and language groups; dietary characteristics; community organization; transportation and communication facilities; governmental and military organization; location and size of troop bases; and the like. If, however, the team adheres to the basic principles outlined below, a sufficiently representative sample of the population may be obtained.

Objectives of Sampling

Neither time nor money exists for examining the whole of every population about which information is desired. For instance, a farmer wishing to know the quality of his seed for planting will test only a small portion of his seed supply as a sample by which germination rate for the whole supply can be estimated. Sampling investigations can provide *accurate* and *precise* information about a large population by examination of only a portion of that population.

Accuracy of an estimate depends upon unbiased sampling of the true or population universe or definable portions thereof. The methods of measurement and classification must be correct. For example, determinations of hemoglobin are made with instruments standardized so that a value of, say 12.2 gm per 100 ml of blood would be reproducible by any other investigator. The recording of physical findings such as edema in an individual should be based on character-

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istics which would be recognized by any qualified examiner. The problems of accuracy in an investigation are general ones, whether or not sampling is involved. Errors due to methodology are usually called *nonsampling* errors, to distinguish them from the *sampling errors* due to the inherent variation of samples taken from the population universe. The nonsampling error is unaltered by change in the sample size, whereas the sampling error may be reduced by enlarging the sample, but only in proportion to $\sqrt{n}$ where n=sample size, so that quadrupling the sample size halves the sampling error.

Precision of an estimate refers to the reproducibility or the closeness of estimates obtained if similar samples are taken. The true values for the nutritional characteristics studied in an investigation can be approximated as closely as desired by taking a sample sufficiently large to meet the specifications of precision needed. For example, if the finding of any condition in the population studied is to be compared with a "normally expected" 1 percent rate of findings and the actual rate of occurrence of this condition in the studied population is 3 percent, a properly drawn sample of 500 individuals would be more than adequate to establish the difference. This statement holds no matter how large the studied population is, be it 50,000 or 50 million.

The Population and Its Stratification Among Civilian and Military Groups

The universe to be surveyed is the total population of a given country—civilian and military—at the time of study. The objective is to obtain, by the examination of a representative sample of individuals, estimates of nutritional status of the several population groups in order to provide an overall assessment of the nutrition of the nation concerned for the time (i.e., year and season) of study.

It may be that the nutritional status is very different for some portions of the population compared with others (e.g., preschool versus school children, armed forces compared to civilian males), and hence it may be desirable to define certain subpopulations, or population strata from which samples of sufficient size will be drawn to allow adequate estimation of their nutriture. The major strata of the civilian population (families, pregnant and lactating women, infants, preschool and school children and adolescents) and each branch of service of the armed forces (army, navy, marines, air force) should be considered as separate subpopulations. Additional subgroups may also deserve attention, such as industrial or agricultural workers, recruits as compared to seasoned troops and the elderly.

Among civilian populations, the basic unit is the family. Appropriate subdivisions might be infants (up to 2 years of age), preschool

children, schoolchildren, laborers, agricultural workers, artisans, professional and administrative workers, housewives, pregnant and lactating women, and geriatric groups.

In the armed forces the basic unit is the soldier, sailor, and airman. Appropriate subdivisions might be new inductees, trainees at completion of basic training, trained troops on active duty. The trained troops might be further divided according to type of duty or activity; e.g., tank, artillery, infantry, rifle company, quartermaster, etc. Occasionally noncommissioned and commissioned officers should also be considered.

Other subdivisions might be made according to environmental characteristics of communities or bases where civilian or military groups are located. The availability, the cost of food, and the area of production of food may be very different at locations in coastal, desert, and mountainous regions.

An alternative subdivision by civilian institutions or mass feeding programs or military bases is appropriate if there are regional food purchase centers each supplying food to locations in its vicinity, but with differences in type and quality of food supplied from the distribution centers. Locations supplied by each center would be considered as a stratum of the population, from which samples would be drawn for study.

Also relevant in the assessment of nutriture are such characteristics as age, sex, length of residence or service, and prior residence or occupation. These, however, are examples of characteristics that may not be convenient bases for stratifying the population preparatory to sampling, as it is unlikely, for example, that military men will be grouped together in units on any one of these characteristics. It will be sufficient to record such information on the individual records of sampled individuals, for later analysis of their relevance.

If feasible in the country surveyed, economic status might be indirectly estimated by recording occupation (preservice or parental of the soldier) on a broad basis such as farmowner, farm laborer, miner, urban small business owner, industrial unskilled worker, etc., and annual family income, if it can be determined. Specific categories tailored to fit the more usual occupations of the country would be set up in advance of the survey and might identify groups differing in nutritional status.

Other ancillary data such as sunlight, temperature, altitude above sea level, and other climatic characteristics of bases or communities should be recorded at the time of the survey. These factors can then be taken into account at the time of analysis of survey data.

It is *essential to record daily in a log* pertinent physical, climatic, and population characteristics of each group sampled. In addition, information and identification of the examining team and the work

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schedule of the day should be recorded in this log. Information to be included in the daily log is detailed in chapter I, page 3.

Probability Sampling

To obtain true representation of the population, any sample of individuals or families from the population must be selected *at random*. Every individual or family must have a known chance of inclusion in the sample. These chances do not necessarily have to be equal. For instance, if the navy is small, but an estimate of nutritional status of the country's sailors is desired, 10 percent of all navy men might be examined, while only 5 percent of all army men and 0.1 percent of the civilian population might be examined. This *disproportionate sampling* in different strata can be allowed for in calculating estimates for the total population.

The essential point in *probability sampling* is that individuals or families selected must be chosen at random with known probability from all those in the stratum being sampled. As a simplified illustration, suppose that from a group of 10 men, 5 were to be chosen for examination. Their names (or corresponding numbers) could be written on slips or tags, put in a hat, thoroughly mixed, and then five of the slips removed by a blindfolded person to give the randomly selected sample. If, however, the total group consisted of 1,000 men, and again 5 were to be chosen for examination, this system would be cumbersome. For such cases, tables of "random numbers" are commonly used. Each man is assigned a number, and the men for the sample are chosen by picking five of their numbers at random, using the tables. For illustration, the following row of digits is taken from one such table (*1*):

64978 17343 77305 91360 22086 38928 65507 18258 17321

If one enters the table at random and chooses this line to be the basis for the above sample selection, then, using the first three digits in each sequence of five, men numbered 649, 173, 773, 913, and 220 would be chosen.[1]

As a general procedure, the use of this random number method of selection is safest in assuring randomness of choice, even when drawing from a hat seems easier, as it is often doubtful if mixing of slips of paper in hats is sufficiently thorough to assure randomness.

Minor departures from the principles of random selection of the sample from the population may be tolerated, but major departures may well lead to evaluation of subjects atypical of the total population. Studies of specially selected unusual individuals and groups (see "Studies of Groups of Special Interest," p. 29 of this chapter) may be

[1] Tables of random numbers, with further example of how to use them, are given in A. B. Hill, pp. 341–359(*2*).

of interest, but for generalization to the whole or subpopulation, the choice of sample from each group must be randomly made.

The discussion thus far has considered the individual as the basic "element" (*or unit*) of the sampling. For large populations such as civilian groups or the armed forces of a country, *direct selection* of individuals at random is neither feasible nor necessary.

To obtain the most information for given amount of time available for surveying among the military, the most efficient choice of size of sampling unit depends on the variability in the nutritional status of individuals in different organizational units of the forces. As an example of an extreme situation, line-duty troops might be predominantly long-service men, with messes of battalion size and large differences in nutritional adequacy of diets by battalion. In such a case, it would be desirable to sample as many battalions as possible, examining only a few men from each, since the greater variability in nutriture will be found *between* battalions rather than between men *within* a battalion. In advance of the findings of the survey, since knowledge of the actual situation is incomplete, it will be desirable to "spread" the sampling as broadly as possible over all the forces, consonant with possible difficulties of travel between locations.

In contrast to the sampling of a somewhat homogeneous military population the characteristics of which may be reasonably well defined, sampling of the civilian population is more complex. This is due to the diversity of groups within the population and the variation in accessibility of the several groups coupled with the necessity for studying a sufficient number of individuals within one group to assure interpretable data. For these reasons the simpler sampling procedures which work well for the military are presented before those for the civilian population.

Sampling Procedures

Before any sample selection of subjects for examination can be made, as much census-type information as is possible must be obtained to enable appropriate stratification. The following example describes in outline a satisfactory approach for the relatively simple situation in the military.

Military

Suppose the armed forces are found to be composed approximately as follows:

Army	120,000
Marines	10,000
Navy	5,000
Air Force	5,000
Total	140,000

Additional subgroups can be defined for each service, with approximately the following numbers in each:

Army.—Twenty thousand in training, 40,000 at coastal bases, 30,000 at mountain bases, 20,000 at desert bases, 10,000 at isolated border posts. Training is for 6 months after induction, at 2 large central bases, each with about 1,500 new men accepted monthly. The two bases draw men from different sections of the country; both are readily accessible. There are five divisions of men at the various coastal bases, four divisions at the various mountain bases, and three at the various desert bases. Some of the divisions are understrength in their numbers, but differences are not marked. Each base consists of an army division, except for two divisions which have men stationed at several neighboring bases. The men at isolated border posts are stationed in groups of 5 to 500, but usually there are 20 to 30 men at each such installation.

Marines.—Ten thousand men, stationed at two similar seacoast bases of approximately equal size.

Navy.—Five thousand men, 2,000 located at 3 shore stations (approximately 1,000 men at one, 500 at each of the other 2). The other 3,000 are on some 20 ships of varying sizes.

Air Force.—Five thousand men, stationed at some 10 bases scattered around the country, 2 bases being large, the others small.

To sample from these strata, one might proceed under ideal conditions as outlined below:

Army.—For the men in training, visit both bases. At each, select at random one-fourth of the companies of men in their first month and one-fourth of men in their last month of training (375 men from each of these 2 groups at each base; total of 1,500 men).

From the five coastal divisions, select two divisions to be sampled at random.

From the four mountain divisions, select one division to be sampled at random.

From the three desert divisions, select one division to be sampled at random.

For the border posts, allot one week of the survey for sampling from these locations. Obtain further detailed information as to locations, and determine, on a *judgment* basis, a schedule for visiting enough of these posts to examine 250 to 500 subjects and to investigate their eating patterns. Posts visited should be sufficiently scattered to give a representative picture of all posts, but chosen to fit a reasonable travel schedule.

Marines.—From the two bases, select one base from which individuals will be sampled at random.

Navy and Air Force.—Allot 1 week of the survey for sampling these two groups. For the Navy, select 1 shore station at random,

[illegible] by [illegible] ships in accessible ports, make random choices of enough to provide 875 men for examination. For the Air Force, select at random one of the two large bases and two of the eight small bases. From these bases choose 750 men for examination.

From each Army division, and from the Marines, 1,500 men will be selected for the "main sample." The basic unit of sampling will be the company. The companies, or equivalent groups of men of approximately the same number as in a company, can be listed for each division, and a sufficient number selected at random. Alternatively, companies may be chosen, randomly, from a further stratification of the divisions according to its table of organization, which gives the number of each type of company it contains: e.g., engineer, quartermaster, etc.

An important point to be noted is that whenever a given company is chosen, *every man* in the company must be included in the "main sample." *This includes men on sick call and men in the hospital.* Special arrangements may have to be made for the examination of some of these men. It may also become impossible to examine others, but every effort should be made to include all men in the company, since serious bias may result from examination of only those well enough to get to the examination area.

The processing of the men selected for examination is described in later sections.

Civilian

Before any sample selection can be designed, as much census-type information as is possible must be obtained. In some countries with well-developed census and vital statistics bureaus, considerable reliable detailed information is available. In others one must rely on incomplete and sometimes recognizably inaccurate estimates or, at times, generalized appraisals by persons acquainted with conditions in the country.

As an example of a population pattern within a country, one may depict country X with a total population of 1,500,000 divided among 2 ethnic or religious groups A and B, each of which embraces 500,000 individuals and a refugee group also of 500,000.

It is further known that the refugee group is concentrated in five centers, two on mountainous plateaus, one in a desert, and two in a coastal plain.

Group A is estimated to be 20 percent urban and 80 percent rural dwellers, approximately equally divided among coastal, mountain, and desert regions. The best estimates of family size indicate four persons per family. It is assumed that group A is primarily an agri-

culturally employed population. It is further estimated that of the school age group, only 20 percent attend school.

Of group B it is estimated that 70 percent are urban dwellers and 30 percent rural, and that these are divided equally between coastal and mountain locations. The family size is estimated to be eight, the workers primarily laborers, and some 50 percent of the school age children among this group attend school.

It is evident that health and educational facilities, agricultural organization and community organization of these major groups and their subdivisions will differ. Furthermore, the accessibility for study, and the cooperativeness and availability of the individuals concerned for examination will vary. If the refugee camps are long established and well organized, the organization permits use of essentially the same approach as would be employed for military study. Such technics are also directly applicable to institutionalized individuals such as are encountered in orphanages, prisons, sanitariums, hospitals, and schools, particularly boarding schools, or to those attending organized health clinics or other community centers.

A general picture is obtained by study of family units from the major segments of the population and the appropriate subgroups. In practice the numbers of certain specified subgroups obtained through this family unit study are insufficient to afford as useful assessment as is desired of problems or conditions among potentially nutritionally vulnerable groups, such, for example, as infants of less than 2 years, pregnant and lactating women, the preschool child, the nonschool adolescent, elderly widows, the absentee laborer living in a labor or mine camp, and so on. Accordingly, concentrated studies of groups of these civilians must be made in addition to study of the family unit. *Finally, since examinees in a survey are usually ambulatory, it is imperative that the nonambulatory patient whether at home or in hospitals be sought and appraised.* Only by diligently seeking out such individuals will one obtain a picture of the severity of nutritionally based or related diseases within the population. On the other hand, prevalence of deficiency states among medically impaired groups within the population must not be interpreted as indicative of the prevalence of these conditions in the population at large. Only by a combination of study of these several types is it possible to obtain a valid assessment of the nutritional condition among the total population of the country.

The sampling methods for civilian population groups in countries studied have utilized several approaches. In some, true randomization based on family groups and their geographic location of residence has been possible. In others, a representative selection such as family groups has been made. In others, concentration has been on certain segments of the population expected to be subject to nutritional stress

that is, infants, children, pregnant and lactating women, and hospitalized patients. Some examples are shown below.

Civilian Sampling Procedure, Ecuador

Twelve sites were selected for study, to provide a sample from the varied geographic regions of the country with differing types of food crops (Northern Sierra, corn, beans, potatoes, cattle; Central Sierra, corn, beans, wheat, oats, fruits; Southern Sierra, corn, bananas; Oriente, yuca, sugar cane, fruits; Coastal Plain, rice, grains, peanuts, sunflower, yuca, bananas, fish, cacao), and to permit study of nonurban groups. The Statistical Section of the National Planning Board selected by use of a table of random numbers a sample of villages and towns representative of those with different total populations within reasonable access limits of available transportation. Alternate sites were chosen for each location, and, insofar as possible, the villages studied were limited to those samples so selected. The Statistical Section also selected districts for study in two major cities, Quito and Guayaquil, on the basis of economic level and major occupation of the population.

Civilian Sampling Procedure, West Indies

In each of the areas studied, the enumeration districts, as determined by the 1960 Census of Population conducted by the Central Statistical Office, were grouped geographically. In Trinidad and Tobago 8 out of the 40 areas were chosen to give a basic geographic distribution, and 14 of the remainder were selected by lot. From each of the 22 resulting areas, 4 enumeration districts were selected randomly, and within each of these 88 enumeration districts, 15 dwelling units were chosen. All inhabitants were encouraged to attend the survey centers. The island of St. Lucia was similarly divided into 10 geographic areas, and the enumeration districts from each of these were classified in five categories relating to income level and rural or urban location. For each of these categories 4 enumeration districts were selected and 20 dwelling units from each of these enumeration districts were chosen by lot. St. Kitts, Nevis, and Anguilla were divided into six, two, and two areas, respectively, from which three, one, and one were drawn by lot. As before, 4 enumeration districts were selected randomly from each area, and 15 dwelling units in each district were chosen for study.

Pregnant women may be examined in maternity clinics of local health departments or in hospitals in the larger communities. Infants and lactating women also can be examined in well-baby and child clinics. Preschool children often are obtainable through maternal or prenatal clinics or well-baby centers by having the mothers bring all children less than 6 years of age into the center on the day of study.

During the school terms, through contact with the local health officer and with the cooperation of the school administrations, it is usually possible to examine various age groups of schoolchildren in the range of 6 to 14+ years.

Several methods can be used to obtain samples of adults in the civilian population other than the pregnant and lactating women mentioned above. In some instances the families of schoolchildren are obtained for survey examination through the simple expedient of examining children in the schools in the forenoon, and sending them

home with a note to their parents requesting their cooperation in appearing at the school in the afternoon for the survey examination. A second method of obtaining population samples of adults in small towns is through the cooperation of local administrative and religious authorities, who, by house-to-house visits, urge families to present themselves at the place of examination. Upon other occasions the public health officers have carried the major responsibility for obtaining samples of the adult population, working either through civilian or police officials. They have randomly selected families in a given block and requested their cooperation and participation. If a family refused, they selected the family on one or the other side for examination. Upon occasion a sound truck can be used for 2 or 3 days before the date of the proposed examination by the health officer or town official to announce to the people of the small towns the time of the examination and its purpose. On the day of the examination a repetitive announcement of similar nature may be made.

Sizes of Samples

As the preceding sections imply, the total size of sample needed to accomplish the objectives of the survey cannot be specified *exactly* in advance. There must be a balancing of the facilities of the team and its problems of travel to the locations where persons for sampling are available against estimations of sample sizes needed to evaluate nutriture for both the total population and for appropriate strata within the population.

Biochemical facilities are not usually sufficient to allow study of blood and urine specimens from all individuals examined clinically. On the other hand, it is desirable to obtain sufficient height-weight-age data to enable the preparation of "internal standards" of weight status, and detailed evaluation of different units of the sample according to differences in body weight, adjusted for age and height.

For some of the clinical signs of possible nutritional inadequacy, especially those which may be expected to be infrequent, a much larger total sample of individuals is needed to obtain sufficiently precise estimates of prevalence of the signs in the population than would be needed for findings of higher frequency. For example, if a certain nutritional deficiency sign prevails in 0.5 percent of the people, the examination of 10,000 individuals will reveal about 60 cases, whereas with 2,000 persons examined, only about 10 cases would be found. The estimate of prevalence, given 50 cases found in 10,000, would be 0.50 ± 0.07 percent, whereas with 10 cases in 2,000 it would be 0.50 ± 0.16 percent. The former estimate should be adequately precise; the latter is questionably so.

The "total" clinical sampling will be in two phases. The first is the "primary sample." Here the subjects selected will all receive an

abbreviated clinical examination and every third to fifth person, chosen systematically, will *in addition* be sent for a *detailed* clinical examination. From those subjects receiving a detailed clinical examination, a systematic selection of every third to fifth subject will again be made for a blood sample and a urine or feces sample. Thus every 9th to 25th examinee from the "main sample" is selected for the biochemical sample. With families, detailed and biochemical appraisals may be made of all members of every other family.

The "secondary sample" of subjects will encompass all those individuals available at a community or base who are not entered in the "primary" group. Whenever it is necessary to obtain increased numbers to provide validity or significance for one's clinical findings, this "secondary sample" should be screened. Frequently this can be done quickly and simply by restricting one's examination to a few physical lesions such as those to be found in the eyes, lips, nose, mouth, throat, and the like. If such a screening is carried out, a minimum of basic identification data must be recorded; i.e., the total number of individuals examined, and the occurrence rate of the clinical lesion calculated. (See ch. V, p. 38, logbook data.)

The method of systematic "primary" sampling with a random selection is used in allocating persons or families to the abbreviated and the detailed clinical examinations. Start with a complete list of the people in the "primary samples." In the military this list will include those on sick call and in the hospital. Those on sick call or in the hospital should be listed in their normal position on the roster, not at the end. The persons or family on the list are then numbered consecutively. Now, a number between 1 and 5 inclusive is drawn at random. This number determines the number of the person or family to receive the first detailed clinical examination. Every third to fifth thereafter will receive a detailed clinical examination in addition to the abbreviated examination. Thus, if the number drawn were 3, those whose numbers were 3, 8, 13, 18, etc., would receive the detailed examination.

The person or family who is to receive the biochemical examination is chosen in the same way from among those receiving the detailed examination. Here a second number between 1 and 5 inclusive is drawn. If the number drawn were 4, this would mean that the fourth individual or family who receives the detailed clinical examination would also receive a biochemical examination. This would also apply to every fifth person or family thereafter from those receiving the detailed examination. In the example with the first number 3 and the second number 4, individuals numbered 3, 8, 13, 18, 23, 28, 33, 38, 43, 48, 53, 58, 63, 68, 73, 78, etc., would have a detailed examination, while those numbered 18, 43, 68, etc., would also have a biochemical examination.

The following total sample sizes are not targets for each team in each country, but they are presented as an illustration. They are, however, suggested as reasonable to attain with the given time and facilities, and probably sufficient in size for evaluations of nutriture. The assumption is made that the examination facilities described in chapter V will process 300 to 400 individuals of the "primary sample" in a full day of examinations, and that the equivalent of 25 full days of examinations is attained.

Subjects in the "total sample"	7,000 to 15,000.
Subjects in "primary sample" given abbreviated clinical examination	7,000 to 10,000.
Subjects in "primary sample" given detailed clinical examination	1,500 to 2,000.
Subjects in "primary sample" given dental examination	1,500 to 2,000.
Subjects in "primary sample" giving biochemical samples	300 to 900.
Subjects in "secondary sample" given screening clinical examination	400 to 500.

Systematic Subsampling Within the "Main Sample"

The preceding section indicates the plan for selecting one-fifth of the subjects in the "main sample" for a more detailed clinical and dental examination than the other four-fifths receive, and selecting one twenty-fifth of the subjects for biochemical sampling. These proportions are suggested as probably satisfactory, but can be modified in the field any time to procure a smoothly flowing examination "production line," or to meet changes in number of troops available for survey. Thus, in planning a survey, it might be thought that 400 to 600 persons could be given abbreviated examinations, 80 to 120 could be given more detailed clinical and dental examinations, and 20 to 25 biochemical samples could be run. This would give the sampling ratios of 1 to 5 and 1 to 25 as previously discussed.

If, on the other hand, only 375 to 450 persons can be handled by the group giving abbreviated physical examinations, it will be necessary to use a ratio of 1 to 4 for detailed and dental examinations and 1 to 20 for biochemical examinations. Another change may occur if a base chosen for survey contains only 250 men. Here, the "primary sample" will be all of the men at the base. One out of every two men will be given detailed examinations, and 1 out of every 10 will be selected for biochemical tests.

It may be necessary to change the sampling ratio fairly often to meet the needs occasioned by field conditions. It is important, however, to record in the daily log the exact procedure and ratios used each time so that changes in these ratios can be taken into consideration when the time comes for analyses of the data. Further details are given in chapter V.

Studies of Groups of Special Interest

Among both civilian and military population strata there may be groups deserving special study or therapeutic trials. Such studies add essential data of nutritional significance to those obtained from the random population sample. Plans to assess such groups should be included in the initial survey design. However, it must be recognized that data from these groups may not be representative of the total population sample of the country. These data should be kept separate from the random data throughout the survey and during the analysis of results.

Several such special groups are:

(1) Inductees—prior to and after training.
(2) Infants and preschool children.
(3) Pregnant and lactating women.
(4) Hospitalized patients and those attending hospital clinics.
(5) Groups receiving therapeutic trials of any nutrient, e.g., vitamins A and C.

In such groups the abbreviated-to-detailed-to-biochemical ratio of the individuals examined may be expected to approach 1 : 1 : 1 rather than the 1 : 5 : 25 of the random population sample.

Special Biochemical Evaluations

A portion of the biochemical laboratory's capacity should be reserved for *special studies* of individuals whose clinical status indicates they may be of special interest. Such individuals might be persons with frank deficiency disease; persons with certain clinical signs, the interpretation of which would be aided by biochemical evidence on a series of similar cases; groups who reveal "low" or "deficient" values for nutrients on biochemical assessment. The latter should be given load tests. These individuals or groups could be chosen from any of the subjects receiving clinical examinations. If chosen from the individuals given the screening or abbreviated clinical examination, a detailed clinical examination should be made. Records of such examination of "nonrandom" subjects should, of course, be kept separate from the records of individuals in the "random" sample.

The resultant biochemical data based on the routine laboratory determinations as used in the random sample, or special biochemical determinations, load tests, or results of controlled therapeutic trials, should not be combined with data obtained from the random sample.

Up to 25 percent of the biochemical facilities of the team might profitably be engaged in such special-study analyses. Preliminary analyses of the biochemical data (such as ascorbic acid levels) during the survey may reveal clear evidence of the absence of a nutritional prob-

lem from the biochemical status of the sample with respect to measurement of specific nutrients. In such a case it may be permissible to do future determinations of this measurement on only every second or third blood sample taken thereafter. The time so saved may be used for special-study work.

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V

Clinical and Dental Appraisal of Nutriture

Clinical Examination Procedures

The space and personnel requirements given in this chapter (see p. 45) are designed to allow the clinical examination of a usual sample of individuals. The total sample, either civilian or military, chosen for examination at each location is processed through a "main line," each subject being given an *abbreviated examination*. Every second to fifth individual (depending on the numbers of persons available for examination) in this line will in addition receive a second or *detailed examination*. It should be possible in the main line to accommodate up to 400 adults or school age children per working day for a full clinical team, with 60 to 80 of the examinees receiving detailed examinations.

Subjects should be brought to the examination area in groups of not more than 100 whenever possible. In military or institutional groups, units of company or classroom size will be appropriate. To facilitate an even flow of individuals, it may be useful before the examinations to set up a schedule of arrival of groups of men, women, and children or to arrange for separate lines for men and women. A convenient schedule is for 100 or so to arrive at 8, 9:30, and 10:30 in the morning, and at 1:30 and 2:30 in the afternoon. Among civilian family, clinic, or community center groups, such regimentation is almost impossible to achieve and one is frequently compelled to examine as many individuals as are available or seek examination. Care must be exercised to obtain at least reasonable representation of all segments of the civilian population. (See ch. IV.)

Civilian examinees separate for purposes of examination and analysis into categories such as family groups, schoolchildren, preschool children, clinic groups (well-baby, prenatal), orphanages and other institutional inmates, etc. Different colored cards or numbers are conveniently used for certain of these, especially to identify those "especially selected subjects" on whom data are obtained beyond that of the normal sample. For all infants and children to 5 years of age,

the "pediatric" card is used (fig. 3). The physical examination is completed on all examinees of this age, and the remainder of the card (diet history, laboratory data, etc.) is completed on each child of this age who would by the sampling sequence adopted be in order for a detailed examination.

Each individual in the main sample is given an abbreviated clinical examination which is limited to basic identification, location, activity data, age, height-weight information, and selected major signs of nutritional import, as listed on the abbreviated card. Any addition to these signs noted upon inspection can be made for a country according to prior knowledge of deficiency lesions which are more likely to be encountered.

Record cards may be prepared using a small card (either McBee edge-punched cards or simple file cards as illustrated in fig. 1) for the abbreviated examination containing the basic identification and anthropologic data. Variations appropriate for the country may be added by using unassigned punchcard spaces to supplement the clinical data. (Alternatively, stenciled cards or bound notebooks may be adopted to provide a record.) Identification items, location, area of origin, activity and, for military personnel, unit and time in service, should *all* be entered on the card by recording a predetermined number code rather than by writing the details of the item.

Survey identification numbers are assigned the subjects in a nonoverlapping manner. The numbering system should begin with the McBee edge-punched cards (see fig. 2) for the recording of the detailed clinical examination, continue to specially colored McBee edge-punched cards for individuals and groups of special interest described in the next paragraph, and end with cards for the abbreviated examination. All cards should be prenumbered before the beginning of the survey.

Thus, if the total sample size to be attained is 10,000, with 7,000 abbreviated clinical examinations, 2,000 clinical examinations, 100 special individual and 900 special group examinations, the regular buff McBee cards (for detailed clinical examinations) would be numbered consecutively from 0001 to 2000, while the pink (special) McBee cards would follow from 2001 to 2100 and the green McBee cards from 2101 to 3000. The cards for the abbreviated examination would be numbered consecutively 3001 to 10000.

Each examinee in the main sample is assigned a small card and thereby a number. In addition, every second to fifth individual, who is also to receive the detailed examination, is given a large buff McBee edge-punched detailed card. He proceeds to the appropriate examination area after his abbreviated examination. (See chart on p. 44.) Where both examinations are carried out, the abbreviated card number is recorded on the detailed card, and vice versa.

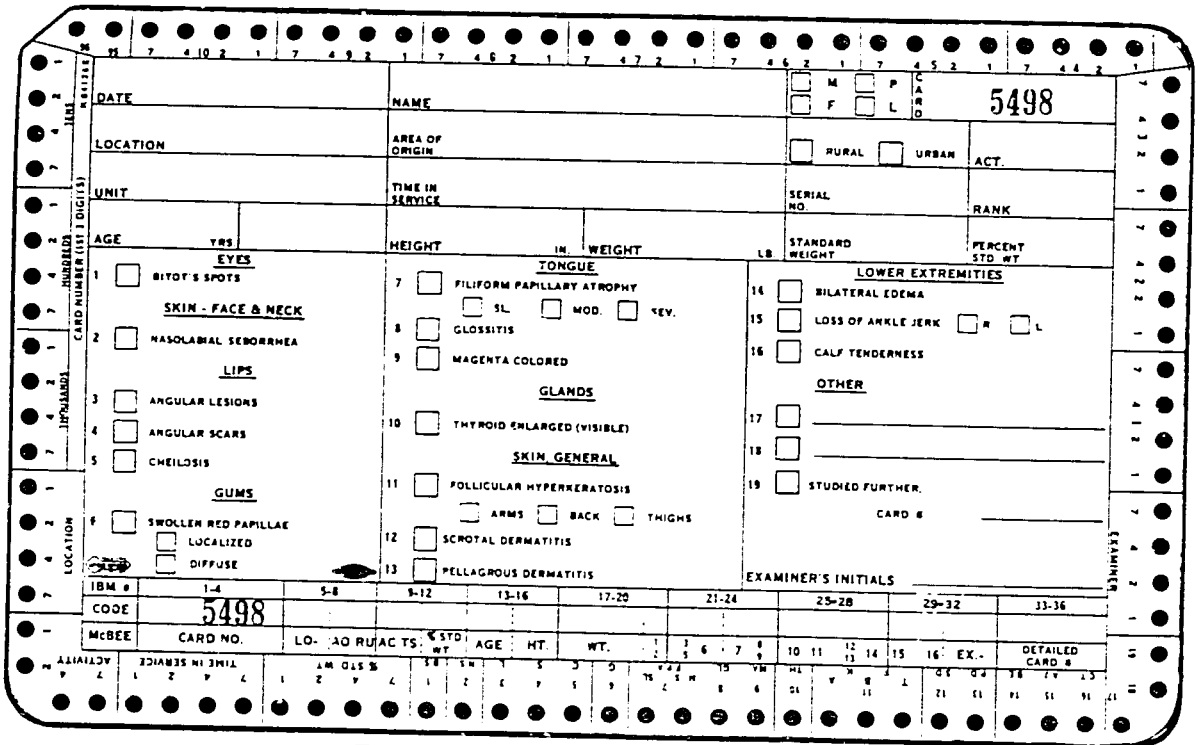


FIGURE 1.—Abbreviated Examination Card.

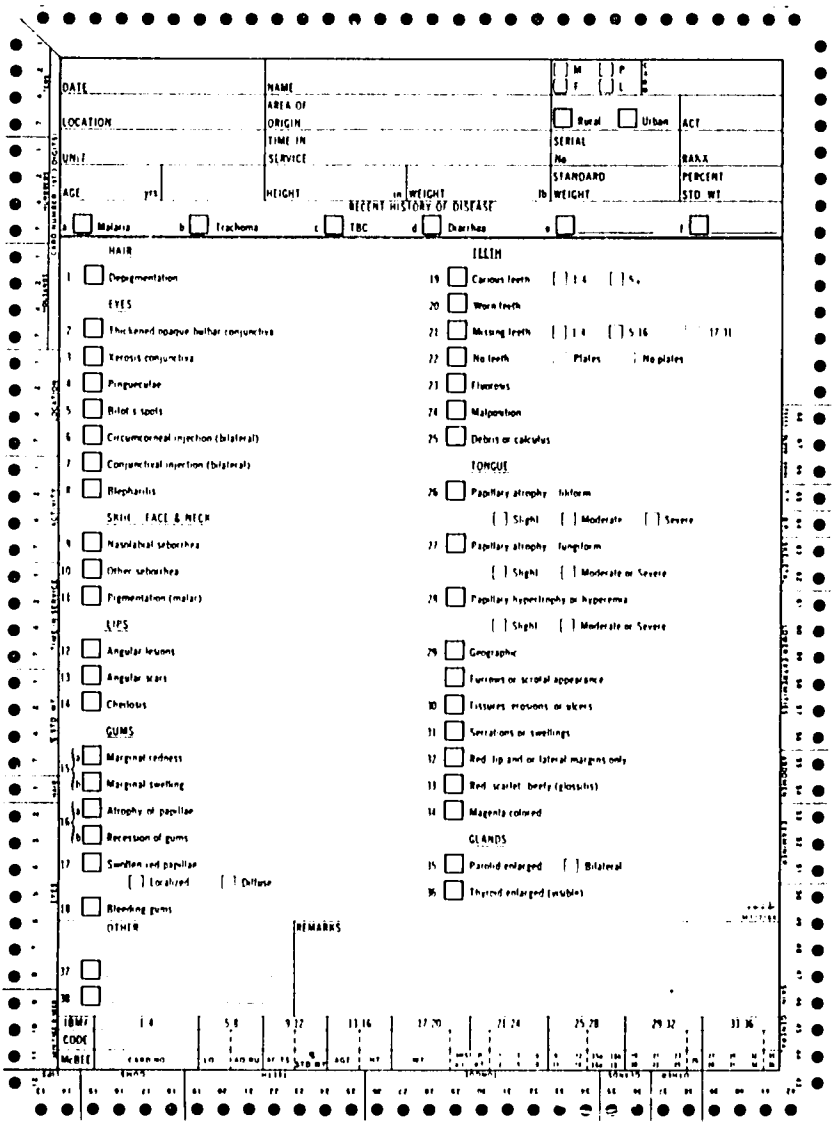


FIGURE 2.—Detailed Examination Card (front).

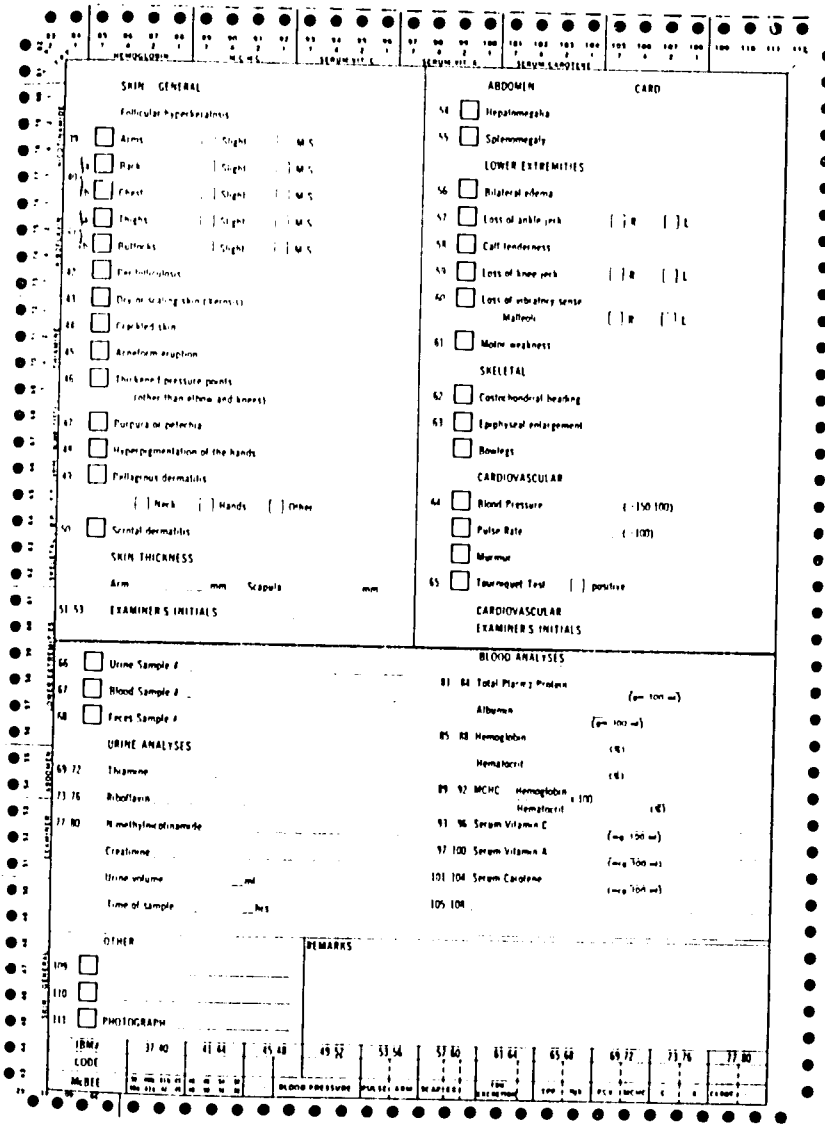


FIGURE 2 (continued).--Detailed Examination Card (back).

Pediatric Card for Children Ages 0-4 years — READ INSTRUCTIONS BEFORE USING

DATE	NAME	[ ] Boy [ ] Girl	AGE _yrs _mo	CARD NO.
LOCATION	AREA OF ORIGIN	[ ] Rural [ ] Urban	[ ] Other siblings examined	[ ] Informant not mother

LENGTH in cm	STD	%	WEIGHT lb kg	STD	%	1	2	3	4	5	6

MOTHER AGE yrs	If now pregnant mos	Number of PAST PREGNANCIES		Of the liveborn:	Of those dying:	Mother's age at:
U M R		_ liveborn _ stillborn _ abortions	MULTIPLE BIRTHS	_ now living _ now dead	_ died at <1 yr _ died at 1-4 yr _ died at 5+ yrs	term. 1st preg. yrs term. last preg. yrs

THIS CHILD:	Age	Birth order	Premature?	Walked	DIARRHEA	RESPIRATORY	
	U R M		YES NO	mos	[ ] Current [ ] Recent [ ] Recurrent	[ ] Current [ ] Recent [ ] Recurrent	[ ] Pica [ ] Parasites [ ] Malaria [ ] Other

GENERAL IMPRESSIONS
- [ ] Apathy
- [ ] Pallor
- [ ] Irritability

HAIR
- [ ] Dry staring
- [ ] Dyspigmentation
- [ ] Thin
- [ ] Easily pluckable

EYES
- [ ] Conjunctival dryness
- [ ] Conjunctivitis
- [ ] Bitot's spots
- [ ] Xerophthalmia

LIPS
- [ ] Angular lesions
- [ ] Cheilosis

GUMS
- [ ] Swollen red papillae (diffuse)
- [ ] Bleeding

TONGUE
- [ ] Filiform papillary atrophy
  - [ ] Mild [ ] Mod.
  - [ ] Severe
- [ ] Glossitis

GLANDS
- [ ] Thyroid enlarged (visible)

SKIN, GENERALLY
- [ ] Inelastic
- [ ] Petechiae
- [ ] Dermatitis, with desquamation or crazy pavement type
- [ ] _____

ABDOMEN
- [ ] Hepatomegaly
- [ ] Splenomegaly
- [ ] Potbelly

LOWER EXTREMITIES
- [ ] Bilateral edema
- [ ] Calf tenderness

SKELETAL
- [ ] Cranial bossing
  - [ ] Frontal
  - [ ] Parietal
- [ ] Craniotabes
- [ ] Costochondral beading
- [ ] Enlarged joints
- [ ] Bone tenderness
- [ ] Muscle wasting
- [ ] _____
- [ ] _____

OTHER
- [ ] 1. _____
- [ ] 2. _____

CLINICAL IMPRESSION
- [ ] Marasmus
- [ ] Prekwashiorkor
- [ ] Kwashiorkor

EXAMINER'S INITIALS _____

REMARKS:

G F P

PHOTOGRAPH # _____ of _____

[ ] URINE sample # _____ [ ] BLOOD sample # _____ [ ] FECES sample # _____

URINE DATA
- Urine volume _ ml, during _ hours
- Creatinine _ mg/ml or _ mg/hrs
- Thiamine _____
- Riboflavin _____
- N'methylnicotinamide _____
- Other, #1 _____
- Other, #2 _____
- Other, #3 _____

PARASITOLOGIC FINDINGS
1 ___, 2 ___, 3 ___, 4 ___, 5 ___, 6 ___, 7 ___

BLOOD DATA
- Total plasma protein _____
- Albumin _____
- Hemoglobin _____
- Hematocrit (PCV) _____
- M.C.H.C. = 100 Hgb/PCV _____
- Serum vitamin C (mg/100 ml) _____
- Serum vitamin A (mcg/100 ml) _____
- Serum carotene (mcg/100 ml) _____
- Alkaline phosphatase _____
- Other, #1 _____
- Other, #2 _____

FIGURE 3.—Pediatric Examination Card (front).

DIETARY Information obtained by:

CHILD BREAST FEEDING	CHILD NOW FED: (one or more) [ ] Breast milk [ ] Other milk [ ] Other foods	IF CHILD NOW NURSES: __ feedings/day [ ] Self-demand OR [ ] Schedule __ hrs	IF NURSING TERMINATED: [ ] Abruptly at __ months [ ] Gradually __ to __ mos [ ] NEVER breast fed	FACTORS IN TIME OF WEANING: *Maternal* *Infant* [ ] Illness [ ] Illness [ ] Work [ ] _____ [ ] Pregnancy [ ] _____

CHILD INITIAL NONMILK FOODS	1 _____ 2 _____ 3 _____ 4 _____ 5 _____ 6 _____

CHILD PHARMACEUTICAL SUPPLEMENTS	[ ] Iron from __ months to __ months or [ ] PRESENT [ ] Regularly [ ] Occasionally [ ] Vit. A & D from __ months to __ months or [ ] PRESENT [ ] Regularly [ ] Occasionally [ ] Vitamin C from __ months to __ months or [ ] PRESENT [ ] Regularly [ ] Occasionally [ ] _____ from __ months to __ months or [ ] PRESENT [ ] Regularly [ ] Occasionally

CHILD MILK FEEDING (not breast)	Fresh milk: [ ] Cow [ ] Water buffalo [ ] Goat [ ] _____ [ ] Sheep [ ] Camel	Processed milk: [ ] Dry skim [ ] Dry whole [ ] Evaporated [ ] _____	PRESENT intake: ____ (vol)/day ____ (wt)/day ____ (times)/day	Is formula water boiled? YES NO Is drinking water boiled? YES NO

CHILD'S PRESENT DIET

Do you give this child any special foods or juices other than the family diet? YES NO
If YES, what kinds? _____
Usual present diet _____

Food type	Food	Quantity/Day	Times/day	OR times/wk	Age this food first started
MILK					
GRUELS, CEREALS					
VEGETABLES					
FRUITS					
MEAT, FISH, FOWL					
EGGS					
OTHER					

MOTHER'S DIET during LATEST PREGNANCY						MOTHER'S DIET during LATEST LACTATION					
Foods avoided	Why?	Special foods	Why?	Supplements	Why?	Foods avoided	Why?	Special foods	Why?	Supplements	Why?
1 Salt	____	1 Milk	____	1 Iron	____	1 Salt	____	1 Milk	____	1 Iron	____
2	____	2	____	2 Calcium	____	2	____	2	____	2 Calcium	____
3	____	3	____	3 Vitamins	____	3	____	3	____	3 Vitamins	____
4	____	4	____	4	____	4	____	4	____	4	____
5	____	5	____	5	____	5	____	5	____	5	____
6	____	6	____	6	____	6	____	6	____	6	____

At what age do your children begin to drink Coffee? ____ yrs Tea? ____ yrs Wine? ____ yrs Beer? ____ yrs Soft drinks? ____ yrs (kind ____) Other? ____ yrs (kind ____)

FIGURE 3 (continued)—Pediatric Examination Card (back).

Some individuals scheduled for only the abbreviated examination and not for either the detailed examination or the biochemical assessment will, because of some special interest indicated by the clinical findings of the abbreviated examination, be considered appropriate for special individual study. These are then given one of the large green McBee cards and sent to receive the detailed clinical examination, biochemical sampling, or whatever special tests or evaluation may be indicated. The pink McBee cards are also used for special group studies among populations, such as extra observations on pregnant and lactating women, hospital patients, and the like. The reason for the several colors of the large McBee cards is to facilitate the separation of records of individuals or groups of special interest from those of individuals in the random sample as noted above (p. 29). Data on infants and preschool children to 5 years of age are recorded on the special pediatric card (fig. 3).

From the individuals receiving the detailed examination, every second to fifth person is selected systematically to give samples of blood, urine, and feces for biochemical and parasitologic examination. The number of samples collected per day is dependent on the capacity of the laboratory to perform, process, or store a given number of samples. The ratio of persons contributing these samples to the total number of examinees is dependent on the number to be examined on that working day. The laboratory should be provided 20–25 samples (exclusive of special individual studies) per working day. Therefore, if a total of 100 individuals is given the abbreviated examination, 1 in 2 should have the detailed examination and 1 in 2 of the latter should give blood, urine, and fecal samples. On the other hand if 400 examinees are available, only 1 in 4 might be given the detailed examination, and from 1 in 4 of these, samples will be taken for laboratory study.

It is imperative that the clinical group maintain an accurate and up-to-date logbook or diary which should contain, among other items and observations:

Brief description of location of examination, altitude, topography, population size, identification of the examiners by name (or number) and individual role for the day, and other attributes.

Numbers of various cards used on that day.

Sampling ratios of the day, for abbreviated-to-detailed-to-biochemical-to-dental examination sample (where the latter is applicable).

Clothing weight.

Brief description of general level of public health, dispensary and medical facilities, nursing and medical personnel, and disease patterns seen at clinics and hospitals, impression of the sample examined, names and identity of persons met during the day, and

any other relevant observations or information (such as whether water samples were obtained, source of water supply, etc.).

Dental Examination Procedures

Dental examinations are made on all persons who receive detailed medical examinations, plus a predetermined ratio of those receiving abbreviated medical examinations. The latter ratio will depend on the number of dentists on the survey team. (The flow of individuals through the dental examination section is indicated in the chart on p. 41.)

The dental examination card is numbered according to the survey identification number (abbreviated card number) assigned the subject for the medical examination (fig. 4). Certain identification data must be transferred to the dental card from the medical card. This includes name, examination site, date, serial number, age, sex, area of origin, and, if military, type of activity and branch of service. The examiner's initials or code number will be entered in the appropriate place on the record. It may be necessary to obtain additional information depending on local situations.

The subject to receive a dental examination is seated in a portable dental chair and examined in good natural light. The examination, using mouth mirror and explorer, includes inspection for dental caries, attrition of molar teeth, gingival and periodontal pathology, fluorosis and enamel hypoplasias, recession, oral debris, and calculus. The findings are dictated in code by the examiner to a recorder.

Ideal requirements of space and physical equipment for the examination areas, with diagram of usable layout, are given on pages 44–49. However, if circumstances dictate, all phases of the clinical, dental, and biochemical examinations can be conducted under blue skies, on boxes, backs of trucks, porches, and the like.

Clinical and dental findings are recorded in the field directly on edge-punched cards (figs. 1–4 already described) on which the biochemical results are recorded later. In early surveys all the findings were coded by punching the cards and basic sorts of the data were actually tabulated in the field. This phase of work has since been reduced to an amount sufficient to train host nation personnel in the use of such a puncheard system.

The clinical and dental cards include a coding system for transcription of data to IBM (International Business Machine) machine-sorted puncheards. IBM equipment is now so well distributed in most major cities throughout the world that this coding, machine punching, and even some basic data sorts have been accomplished in the host nation. However, the final tabulations of the clinical and biochemical data are all made in the United States.

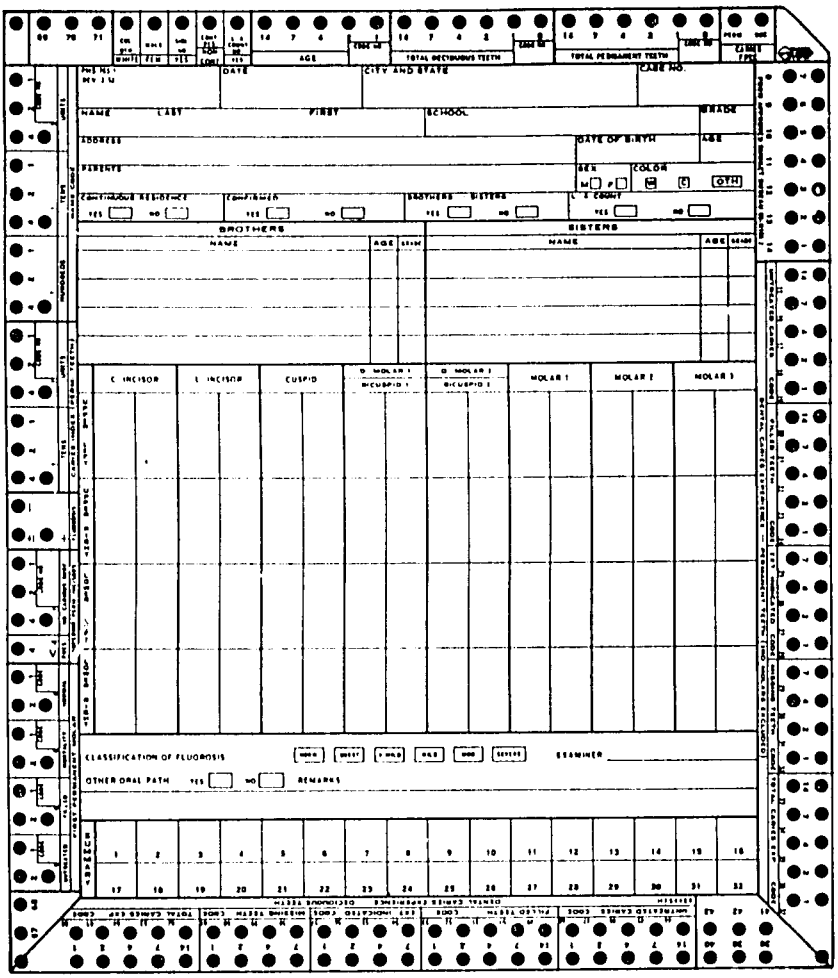
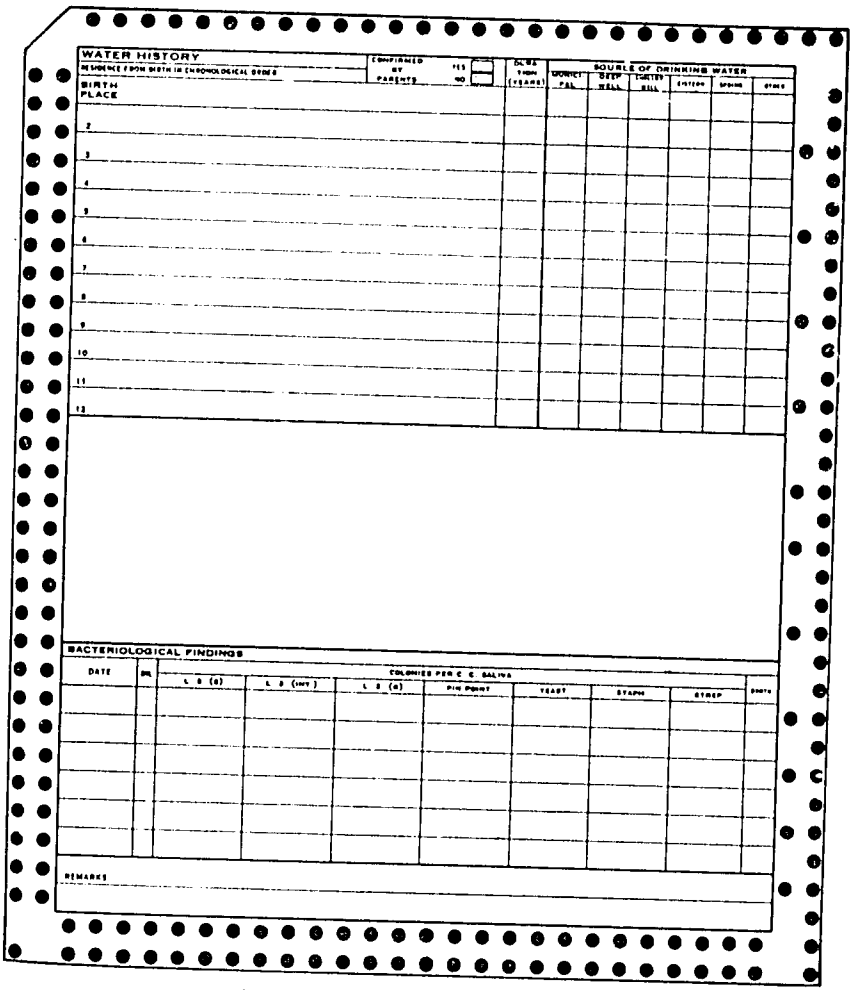


FIGURE 4.—Dental Examination Card (front).



WATER HISTORY

RESIDENCE FROM BIRTH IN CHRONOLOGICAL ORDER

CONFIRMED BY PARENTS YES NO

BIRTH PLACE	DATES FROM (YEARS)	SOURCE OF DRINKING WATER: DISTRICT PUB.	DEEP WELL	SHALLOW WELL	CISTERN	SPRING	OTHER
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							

BACTERIOLOGICAL FINDINGS

DATE	PH	COLONIES PER C.C. SALIVA: L. B. (a)	L. B. (int.)	L. B. (a)	PIN POINT	YEAST	STAPH.	STREP.	OTHER

REMARKS

FIGURE 4 (continued).—Dental Examination Card (back).

Necessity for Standardization of Diagnostic Criteria

One of the most important sources of confusion and differences in data on physical findings is the individual observer's idiosyncrasy of definition and recognition of clinical and dental signs. Spurious differences between groups lead to confusion of interpretation and may lead to misinterpretation. Every effort should be made to minimize the differences. Hence, the necessity for clear definition and standardization of observations cannot be overemphasized. Recognizing that they cannot always be eliminated, it is essential to provide some continuing measure of them and permit thereby proper interpretation of the observations.

The definition of clinical and dental signs to be recorded is a matter for joint decision by the nutritional examiners who will direct and perform the examinations. Once this decision is made, it *must* be adhered to by all groups throughout the period of the study. In order that these defined signs shall be comparably recognized, there must be planned standardization sessions at which each examiner independently examines the same sample of some 25–100 persons and records his observations. Preferably these should be individuals who will demonstrate the physical signs of nutritional interest likely to be encountered. The identical group of examinees must be examined by all examiners. At the conclusion of the session the results of the several examiners are compared and joint re-examination made of those subjects upon whom inconsistencies are obtained, with discussion in order to formulate and agree on specifications for recording.

At least two such sessions should be held prior to initiation of the study and others should take place at various intervals throughout the survey in order to detect any divergence from agreed definitions which may occur during the course of the survey. A minimum of such standardization sessions is: (1) at the initiation of the study; (2) during the mid portion of the survey; (3) at the conclusion. In addition, a daily tabulation should be made and recorded of all lesions noted by each examiner. (See app. A, last table, p. 91.)

Experience has demonstrated that there is less likelihood of divergence of opinion or of standards by examiners in the instances of physical evidence of advanced deficiency disease, such as goiter and pellagra. However, those characteristics of nutrient deficits which are dependent upon slight differences in color, texture, dryness, pigmentation, vascularity, and the like are susceptible to pronounced differences of opinion in recording.

Similar sources of error exist for observations which are more objective. These should be amenable to correction by standardization of technics such as blood pressure, pulse rate, and skinfold thickness. If short cuts are taken in these measurements due to "lack of time" or

if there is failure to standardize instruments, the resulting data will be useless for statistical analysis.

Another frequently occurring source of error is the tendency to attempt to interpret findings at the time of observation, and thereby to decide whether a particular sign should or should not be recorded. It is well established that an individual examiner's criterion for recording a specific observation changes depending upon his immediately recent background of experience. Thus, in examining a group in which a particular sign is frequently seen, one may be less impressed by the mild lesions after examining the group for a period than was the case at the beginning of the examinations. For example, physicians are inclined to change their standards of judgment of normal weight rather rapidly if they are constantly studying a lean or an obese population. Many other examples of this problem may be cited, but the accounts in the references cited give evidence of the importance of the problem in making surveys.

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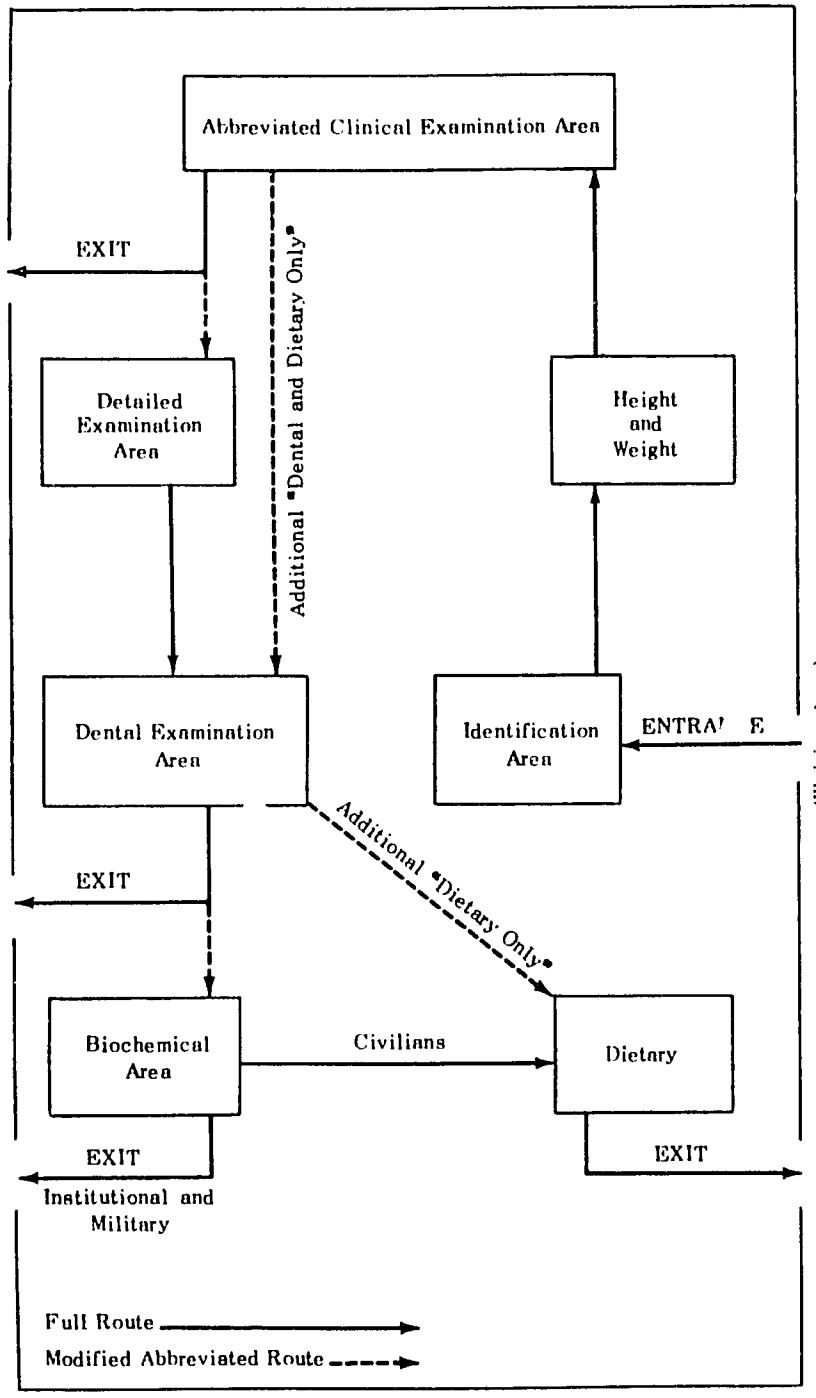
FLOW CHART FOR FIELD EXAMINATIONS
(See text for further details of personnel and equipment needs)



Field Survey Team Operations Based on Study of 300–400 Individuals Per Day

Facilities

A well-lighted (daylight and artificial light) structure is required, either permanent or under canvas, with at least 800 square feet of floorspace and an adequate ceiling height.

Identification and Height-Weight Area (see chart on preceding page)

Personnel. Minimum of five as follows:

(1) *Secretary-interpreter:* two; intelligent bilingual (both spoken and written) persons to record identification and age data on proper card(s). Must be continuously with survey.

(2) *Clerk-technician:* two; to be trained to measure and record height and weight.

(3) *Orderly or aide:* One; to facilitate movement of examinees and to serve as a messenger between examination areas.

Equipment and Supplies

Table 6′ x 3′ (1)
Table 4′ x 3′ (1)
Wastepaper baskets (2)
Chairs (5)
Weight and height scale
Height tape measure
Record books (3)
400 abbreviated, 100 detailed examination cards and 150 dental cards
Ballpoint pens and pencils (12)
Slide rules (2)
Typewriters (2)
McBee card repair kit
Punches and sorting needles for McBee cards (6 each)
Carbon paper
Pencil sharpener
Paper, typewriter (8½″ x 11″); scratch pads, pads (8½″ x 11″); envelopes, paper clips
Erasers
Postage stamps
Cleansing tissue
Twine
Masking tape and cellulose tape
Manila folders or small boxes for completed McBee examination cards
Date stamps and ink pads (2 each)
Rope, ½ inch, 250 feet
Flashlights and extra batteries

Abbreviated Examination Area

Personnel. Minimum of four as follows:

(1) *Clinicians:* one physician experienced in nutritional examinations, plus one or more physicians who may be without extensive training. If only one nutritionally trained clinician is available for both the examination areas, a preliminary training period for the other physicians, of at least 1 week's duration, may permit the latter to sort out the interesting nutritional cases. If so, a 10-minute-every-hour consultation with the experienced clinician can help confirm the findings.

(2) *Clerk-technicians:* two; as scribes for the clinicians.

Equipment and Supplies

Table 6' to 8' x 3' (1)
Stethoscope (1)
Reflex hammers (2)
Vibratory tuning fork, 128/min (1)
Flashlights (2)
Tongue blades, 500 per working day
Wash basin (1), soap, towels
Ballpoint pens (4)
Metal or wood clipboards (2)
Wastepaper basket
Chairs (4)

Detailed Examination Area, Including Field Biochemistry

Every fifth subject of the abbreviated sample is also sent to the detailed clinical examination area. Each individual has had identification data recorded on a large McBee card, and has had an abbreviated physical (clinical nutritional) examination. He is now given the detailed examination and, in addition, one examinee in five (i.e., 1/25 of the total sample) has blood, urine, and fecal samples taken for biochemical assessment and parasitologic examination.

Facilities

A well-lighted (daylight and artificial light) structure is required, either of a permanent type or a tent, with at least 800 square feet of floorspace and an adequate ceiling height.

Personnel required. Minimum of six as follows:

(1) *Clinician:* one; physician trained in nutritional examinations. This clinician's main responsibility is the detailed clinical examination phase, including the skinfold thickness measurements. If necessary he can oversee the abbreviated clinical examinations for selected signs of nutritional insufficiencies.

(2) *Physicians:* two; to examine and record suspected or current disease, and examination of the cardiovascular system, abdomen, and lower extremities.

(3) *Field laboratory personnel:* two technicians competent in taking blood samples.

(4) *Clerk-interpreter:* one; intelligent and bilingual (both spoken and written) person as a scribe for the clinicians.

(5) *Orderly:* one; to facilitate movement of examinees, to procure supplies, and serve as messenger.

Equipment and Supplies

Blood pressure sphygmomanometers (2)
Watches with second hand (2)
Stethoscopes (2)
Reflex hammers (2)
Tuning forks, 128/min (2)
Tongue blades, 100 per working day
Flashlights (3) and extra batteries
Otoscope with ocular attachments (1)
Skinfold caliper and 70 gm wt for standardization (1)
Kodak Startech box camera with flash accessories and bulbs (latter cannot be shipped by air) (1)
Table 4′ x 3′ (1)
Tables 6′ x 3′ (2)
Steel wastepaper baskets (3)
Sheets (4)
Wash basin (1), soap, towels
Chairs (12 to 20)
Clinical daily logbook (1)
Field biochemical team needs per working day:[1]

- Gasoline stove (1)
- Pressure cooker (1)
- Vacutainers with needles and adapters, 20 cc
- Vacutainers with needles and adapters, 7 cc
- Syringe and needles, variety of sizes
- Microscope slides and cover slips—labels
- Parasitologic kit
- Alcohol, 70 percent
- Gasoline
- Cotton swabs
- Microcentrifuge and equipment for blood chemistry study of infant groups
- Collecting cups
- Brown bottles
- Concentrated HCl for urine acidification, and dropper
- pH paper
- Beakers, plastic, 50 ml size
- Masking tape, adhesive tape
- Wash basins (2)
- Wastepaper basket
- Test tube racks (2)
- Tourniquets
- Hemolets
- Wooden swab sticks
- Pencils, paper, logbook

[1] A more detailed, descriptive list of the laboratory equipment may be found in ch. VI.

Dental Examination Area

Facilities

A portion of the clinical examination area approximately 80–100 square feet, either facing a window or on an open porch where sufficient natural light is available. Preferably the dental examination area should be located between the detailed and biochemical examination areas.

Personnel

(1) *Dentists:* one or two; trained in epidemiologic methods of oral examinations.

(2) *Recorders:* one or two; to transcribe the dental findings to the examination card as they are dictated by the examiner.

(3) *Clerk-interpreter:* one bilingual person to record identification and age data from the medical examination cards to the dental cards.

Equipment and Supplies

Portable dental chairs, with head rests, S. S. White #3
Bard-Parker sterilizers (2)
16 oz bottles Zephiran chloride (3)
Antirust tablets, 100
Paper towels, ½ case
Mouth mirrors, 6 doz
Mouth mirror handles, chrome-plated (5)
Explorers #3, double-ended, 6 doz
Log or record books (2)
Clipboards (2)
McBee dental cards, 5000, 25C140B
Red pencils, ballpoint, 2 doz
Blue pencils, noureplaceable, 2 doz
#3 med. hard pencils, 2 doz
Rubber bands, 1 box #32, 3" x 1½"
Paper hand keysort punch #5227 (1)
Needle for card sorting (1)
Kodak Startech camera (1)
Film (127), Type F
Flash bulbs, blue
Tablet, writing, 8½" x 11"
Plastic bottles for water samples, 8 oz polyethylene, 3 doz
Tables, 4' x 3' (3)
Wash basins, stainless steel, 11½" x 9" x 5" (2)
Chairs (4)
Date stamp and ink pad, 2¼" x 6¼" (1)
Dental photographic plastic cheek retractors, 3 pr

Dietary Assessment Area (Civilian Population)

Facilities

Adjacent to the clinical and dental examination space adequate for two interviewing areas.

Personnel. Two to four dietary interviewers, to obtain dietary histories.

Equipment and Supplies

Tables 4' x 3' (2)
Steel wastepaper baskets (2)
Chairs (6)
Record book for log and daily diary (1)
Wood or steel clipboards (3)
Carbon paper, pencils, pens, erasers, clips, pads, pads (scratch)
Dietary history forms
Manila folders (6)

Sample

Some dietary histories will be obtained on all persons among civilian population groups who receive a biochemical examination, plus a predetermined ratio of those receiving detailed medical examination. The ratio will depend on the number of nutritional interviewers on the survey team. The flow of examinees through the dietary section can be seen on the diagram, flow chart for field examinations, on page 44.

Treatment Area (To Be Staffed by Host Country Medical Personnel)

Facilities. As required.

Equipment and supplies. As available.

Team composition

The team composition described above is planned for maximal production during a single examining operation. Frequently it will be desirable to divide this group into two smaller teams to permit examinations to proceed simultaneously in two separate locations or groups.

It is desirable to have subteams of three or four in number selected from among the regular teams or composed in part of supplementary personnel, but chosen because of their special competence and interests. These subteams are responsible for one or another special clinic or other study category (e.g., prenatal, infants in well-baby clinics, preschool children, lactating mothers). The exact number and composition of these subteams will depend upon the design of the study and personnel available at the site where they work, but a minimum subteam should be composed of—

1 physician with special interest.
1 technician to take laboratory samples.
1 nutritionist to obtain dietary information.
1 nurse or clerk.

Definitions and Instructions for Recording Data From Examinations

All data obtained in the abbreviated clinical examination of each individual are to be recorded on a small McBee record card (fig. 1). The detailed examination data are to be recorded on the large McBee card (fig. 2). Additional instructions for the coding and punching of the cards for use in the analysis of the data are given beginning on page 51 of this chapter. The cards for both abbreviated and detailed clinical examinations have space at the bottom for additional coding of data, for detailed analysis using IBM (International Business Machine) equipment; this coding is explained beginning on page 81 of this chapter.

The identification data at the top of the front of both cards (all entries through age) can be obtained and recorded by a bilingual secretary, after instruction in definition of terms and methods for recording. There should be periodic verification that the secretary is adhering to the instructions. (A date stamp may be used for the date entry. For any unnumbered cards, a number stamp may be used for entering numbers in the space for card number. The other identification data should be typed if possible.)

Height and weight can be measured and recorded by clerk-technicians instructed in the methods of measurement and procedures for recording. Their work should be subjected to periodic checking to verify that instructions are being followed. Numerals must be written so as to be unambiguously legible.

Standard weight, for given age and height, is calculated from the "standards" (table 6 in ch. IX is an example) by the statistical group during final analysis of the data. It should not be entered on the cards in the field.

Positive history or direct evidence of suspected malaria, trachoma, or tuberculosis, and current presence of diarrhea should be inquired for by physicians. Space is provided on the McBee card for recording of other diseases seen, which, if thought relevant to the evaluation of nutritional status, should be recorded. Pertinent details concerning any positive findings in this section may be recorded in the space labeled "*Remarks*" at the bottom of the card. The physicians responsible for this portion of the examination may also perform the examinations of the cardiovascular system and the lower extremities.

During the physical examination a mark should be made for each sign or finding for which examination has been made. If a sign is present, an "X" should be placed in the square box immediately to the left of its listing on the card. If the sign is absent (negative), a check (√) should be placed *after* its listing to indicate that the examination was made. On completion of the examination, the clinician should

verify the completeness of the record before initialing the card (with his code number).

Some of the items on the punchcard are self-explanatory. Others require comment or special instruction if the recording is to be uniform.

Recording and Coding Clinical Examination Cards

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				Date: Record month, day, and year, in that order.
				Name, sex, whether *pregnant* or *lactating* (in women), *serial number, unit, rank:* These items are to be recorded on the card, but not punched. They are designed to identify the individual by family name or serial number and unit (such as division, regiment, battalion, company, etc.).
		1-4	1-4	*Card No.:* If not prestamped, record consecutive numbers to identify card and individual clearly. Use 4-digit numbers (e.g., 0001, 0013, 0135, 1357) on the detailed card and 5-digit numbers on the abbreviated clinical card (e.g., 04000, 09999, 10000).
		5-6	5-6	*Location:* Set up code for this item and enter code number directly on the card. (Refers to place or area where examination was performed.)
		7	7	*Area of origin:* Determine code for this item and enter code number directly on card. This item is to be used to group individuals according to origin at time of birth (civilians) or induction (military). It may indicate geographic (by province) or ethnic origin, whichever has greater significance in terms of food practices, food availability, or other characteristics which might influence nutritional status at the time of examination or entry into service.
		8	8	*Rural-urban:* Rural refers to residence in an area where home-produced foods constitute the major portion of the diet. Urban refers to living in a village, town, or city where foods are purchased at a market and where there is little dependence on home-produced foods.

[1] Abb. = abbreviated examination; Det. = detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
		9	9	*Activity:* Set up code for this item and enter code number directly on the card. This permits grouping by type of physical activity, environmental factors, or organizational units within the armed forces, which may influence nutritional requirements or nutritional status.
		10	10	*Time in service:* Enter on card in years and months. Code according to plan provided on p. 81 and enter code number on card.
		13-14	13-14	*Age:* If known, enter age at last birthday. If unknown, estimate at less than 21 or greater than 21 years. It should be noted that age is calculated in different ways in some countries. In such regions it may be useful to construct a calendar to transpose local expressions of age or dates into corresponding ones in the United States. Record all values in equivalent U.S. age.
		15-16	15-16	*Height:*[2] Record in cm to the nearest whole cm in space provided at top of McBee card; measure without shoes, using height bar on scales or a right-angle triangle against a vertical (plumb) wall. In cases where the measurement is exactly ½ centimeter, record to the nearest *even* centimeter; e.g., 167.5 cm is recorded as 168 cm, 166.5 cm as 166 cm.
		17-19	17-19	*Weight:*[2] Record in kg to the nearest one-tenth kg in space provided at top of McBee card. The weight is taken without clothing, except for shorts, in the military. For civilians, typical average clothing is weighed separately at each location where possible, and the figure is recorded in the logbook for subsequent correction of weights by the statistical group.

[1] Abb.=abbreviated examination; Det.=detailed examination.

[2] Because cards were originally designed for use with English measuring units, only 2 spaces are available in current IBM code for height, whereas 3 are set up for weight. In coding and punching IBM cards, it is necessary to code height in cm in cols. 17–19 and weight in kg in cols. 15–16 (see also p. 81).

Mellee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
		11-12	11-12	*Percentage of standard weight:* Percent standard weight for adults may be read directly from tables 6-7 in ch. IX. Do not enter on card, as this will be calculated and entered later by the statistical group.
				RECENT HISTORY OF DISEASE
	a, b, c	.....	20	Record if there is a positive history or definite evidence of the presence of the diseases listed on card or other significant diseases. Inquiry concerning this should be made by local physician, nurse, or medical aide.
	d, e, f	.....	21	*Diarrhea:* Generally defined as 4+ watery stools per day. 2 blanks are available for recording other diseases. If noted in important frequency, they are to be coded at the discretion of the director and the statistical group. (Note on clinical signs: A set of colored lantern slides is provided which illustrate most, if not all, of the lesions. The following notes concern special instructions regarding each item. Further information may be obtained from standard references.) (See also Fig. 5.)
				HAIR
	1	.....	22	*Depigmentation of the hair:* Significant type is rare in adult or military groups. Depigmentation (dyspigmentation) of the hair in infants may result in distinctive white bands, or in the lightening of normally black hair to a brownish, bleached, or reddish (especially in Negroid groups) color. Typical "flag-sign" can be revealed by combing hair upward and viewing in good light. Must distinguish purposefully bleached or dyed hair. There may be associated texture changes to fine, thin, easily pluckable hair.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				EYES
	2	.....	22	*Thickened opaque bulbar conjunctivae:* All degrees of thickening may occur. The blueness of the sclera may disappear and the bulbar conjunctivae develop a wrinkled appearance with increase in vascularity. The thickened conjunctivae may result in a glazed, porcelain-like appearance, obscuring the vascularity. Do not confuse with pterygium.
	3	.....	23	*Xerosis conjunctivae:* The conjunctivae, upon exposure by holding the lids open and having the subject rotate the eyes, appear dull, lusterless, and exhibit a striated or roughened surface.
	4	.....	23	*Pinguecula:* Small circumscribed, slightly raised spots, yellow or pigmented in color, in the exposed area of a moist conjunctiva.
1	5	20	23	*Bitot's spots:* Small circumscribed, grayish or yellowish-gray, dull, dry, foamy superficial lesions of the conjunctivae. Seen most often on the lateral aspect of the bulbar conjunctivae and in children. Usually bilateral. Not to be confused with pterygium.
	6	.....	24	*Circumcorneal injection (bilateral):* Increase in vascularity by new ingrowth of capillary loops, with particular concentration around the cornea in the absence of obvious infection, etc.
	7	.....	24	*Conjunctival injection (bilateral):* Generalized increase in the vascularity of the bulbar conjunctivae in the absence of obvious infection.
	8	.....	24	*Blepharitis:* Inflammation of eyelids.
				SKIN, FACE AND NECK
?	9	20	25	*Nasolabial seborrhea:* A definite greasy yellowish scaling or filiform excrescences in the nasolabial area which become more pronounced on slight scratching with the fingernail or a tongue blade.
	10	.....	25	*Other seborrhea:* Examine especially behind ears, at outer canthus of eyes, forehead. Do not confuse with fungus infections. Seborrhea of the scalp is not to be recorded.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				SKIN, FACE AND NECK—continued
	11	.....	25	*Pigmentation:* Areas of darkened, brown pigmentation over the malar eminences, forehead, hands, or elsewhere over the face. Note whether sharply delineated as results from exposure to sun.
				LIPS
3	12	21	26	*Angular lesions:* Record only if definitely present bilaterally when mouth is held half open. May appear as pink or moist whitish macerated angular lesions which blur the mucocutaneous junction. Angular fissures are recorded only when there is definite break in continuity of epithelium at the angles of the mouth.
4	13	21	26	*Angular scars:* Scars at the angles, which, if recent, may be pink; if old, may appear blanched.
5	14	21	26	*Cheilosis:* Different from ordinary chapping. The lips are swollen or puffy and appear as if the buccal mucosa extends out onto the lip. There may be desquamation. This category may be used to include vertical fissuring of the lips. If associated with herpes labialis, note under "*Remarks.*"
				GUMS
	15a	.....	27	*Marginal redness:* Definite red border along the dental margin of the gum.
	15b	.....	27	*Marginal swelling:* Swollen border of gum, may be spongy or firm.
	16a	.....	27	*Atrophy of papillae:* Loss of substance of interdental papillae resulting in unfilled space between teeth at gingival line.
	16b	.....	28	*Recession of gums:* Record whether with or without debris.
6	17	22	28	*Swollen red papillae:* Red, congested, spongy, swollen interdental papillae. Record whether localized to small area or diffuse throughout whole dental area.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				GUMS—continued
	18	--------	28	*Bleeding gums:* Abnormal gums which either bleed spontaneously or bleed upon slight pressure with a swab stick.
				TEETH
	19	--------	29	*Carious teeth:* Record total (filled or unfilled) caries noted, using groupings on punchcard.
	20	--------	29	*Worn teeth:* Abnormal flattening of biting surface of teeth.
	21	--------	30	*Missing teeth:* Use grading on punchcard.
	22	--------	30	*No teeth:* Record either partial or complete dental bridges or plates if present.
	23	--------	31	*Fluorosis:* Opaque paper-white areas in the enamel of the tooth, ranging in size from a few flecks to entire enamel surface. In the latter case brown stain is a frequent accompaniment as is attrition of opposing surfaces. The most severe forms of fluorosis include discrete or confluent pitting, with widespread brown staining and a general, corroded appearance.
	24	--------	31	*Malposition:* Record if abnormality is sufficient to interfere with bite.
	25	--------	31	*Debris or Calculus:* Record if such deposits are visible along papillary gum margins.
				TONGUE
7	26	23	32	*Filiform papillary atrophy:* The filiform papillae are exceedingly low or absent, giving the tongue a smooth or "slick" appearance which remains after scraping slightly with an applicator stick. "Slight" involves less than one-fourth of the tongue (tip and lateral margins only); "moderate" involves one-fourth to three-fourths of the tongue; "severe" involves over three-fourths of the tongue.
	27	--------	33	*Fungiform papillary atrophy:* This is a positive finding if the fungiform papillae cannot be readily seen. Attention must be given, however, to the variation in prominence of the fungiform papillae due to difference in height of filiform papillae. Record as moderate or severe if an estimated 50 percent or more of papillae are atrophied.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				TONGUE—continued
	28	.....	33	*Papillary hypertrophy or hyperemia:* Can be seen and is felt when a tongue blade is drawn lightly over the anterior two thirds of the tongue. Hyperemia refers to definite red papillae which give the tongue a berrylike appearance. Record as moderate or severe if over 50 percent of papillae are hypertrophied or hyperemic.
	29	.....	34	*Geographic tongue:* Irregularly outlined and distributed areas of atrophy, with irregular white patches resembling leukoplakia.
	29	.....	34	*Furrows or scrotal appearance:* Furrows are depressions in surface without break in epithelium. Multiple linear depressions may give overall appearance of contracted skin of scrotum.
	30	.....	34	*Fissures, erosions and ulcers:* Fissures are linear lesions or cracks, with definite break in continuity of epithelium. Erosions and ulcers are areas with no papillae on sides or bottoms, with definite break in epithelial continuity.
	31	.....	35	*Serrations or swellings:* Tooth impressions at sides of tip, often noted when tongue is swollen.
	32	.....	35	*Red, tip and/or lateral margins only*
8	33	24	35	*Red, scarlet, beefy (glossitis):* Entire tongue is red, angry in appearance, with or without denudation or fissures. Not just a modification of the natural color due to the loss of papillae. Associated almost always with subjective symptoms of hypersensitivity, burning, even taste changes.
9	34	24	36	*Magenta colored:* The color of alkaline phenolphthalein.
				GLANDS
	35	.....	36	*Parotid enlarged:* Because of various types of facial configuration, parotid enlargement may be easily missed in certain populations. Check by palpation, moving the gland with fingers upward and backward toward the ear. Check if bilateral.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				GLANDS—continued
10	36	25	36	*Thyroid enlarged:* The World Health Organization (WHO) criteria used for grading goiter are: Grade 0: Persons without goiter. By definition these are persons whose thyroid glands are less than 4 to 5 times enlarged. Grade I: Persons with palpable goiters. The thyroid is considered to be more than 4 to 5 times enlarged although not visible with head in normal position. Most of these will be readily visible with the head thrown back and the neck fully extended. Grade II: Persons with visible goiters. Persons with goiters which are easily visible with the head in normal position but which are smaller than those in Grade III. Palpation may be helpful in determining the mass of the gland, but is not needed for diagnosis. Grade III: Persons with very large goiters. The goiters of persons in this category can be recognized at a considerable distance. They are grossly disfiguring and may be of such size as to cause mechanical difficulties with respiration and in the wearing of clothes. (Pérez, C., Scrimshaw, N. S., and Muñoz, J. A., Technique of endemic goitre surveys. *In* Endemic Goitre; p. 369, see esp. p. 376, World Health Organization Monograph Series No. 44, Geneva, 1960.)
				SKIN GENERAL
11	39–41b	26	37–39	*Follicular hyperkeratosis:* This lesion has been likened to "gooseflesh" which is seen on chilling, but it is not generalized and does not disappear with brisk rubbing of the skin. Readily felt, as it presents a "nutmeg grater" feel. Follicular hyperkeratosis is more readily detected by the sense of touch than by the eye. The skin is rough, with

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				SKIN, GENERAL—continued
				papillae formed by keratotic plugs which project from the hair follicles. The surrounding skin is dry and lacks the usual amount of moisture or oiliness. Differentiation from adolescent folliculosis can usually be made through recognition of the normal skin between the follicles in the adolescent disorder. It is distinguished from perifolliculosis by the ring of capillary congestion which occurs about each follicle in scorbutic perifolliculosis. Record as moderate/severe if there is a degree of keratotic plugging. Record area of hyperkeratosis as indicated on punch-card.
	42	.....	39	*Perifolliculosis:* Congestion around the follicles which does not blanch upon pressure. (See discussion of follicular hyperkeratosis above.) Early ring of capillary engorgement around some hair follicles which does not disappear on pressure. It is more frequently encountered on the dependent parts such as the legs. Swelling and hypertrophy of the follicles may occur, at which time the skin becomes rough. Follicular hyperkeratosis and perifolliculosis may coexist.
	43	.....	40	*Dry or scaling skin (xerosis):* Xerosis is a clinical term used to describe a dry and crinkled skin which is accentuated by pushing the skin parallel to its surface. In more pronounced cases it is often mottled and pigmented, and may appear as scaly or alligatorlike pseudoplaques, usually not greater than 0.5 cm in diameter. Nutritional significance is not established. Differential diagnosis must be made from changes due to dirt and exposure and ichthyosis.
	44	.....	40	*Crackled skin:* Definite scales larger in size than those seen in xerosis. It is often congenital and is most prominent in cool weather. It is nonnutritional in origin.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	t.	
				SKIN, GENERAL—continued
	45	--------	40	*Acneform eruption:* The presence of acneform lesions in an active stage should be recorded. Inactive scars should not be recorded as an acneform eruption.
	46	--------	41	*Thickened pressure points (other than elbows and knees):* Look especially at belt area, ischial tuberosities, sacrum and over greater trochanters. Other sites may occur as a result of a given occupation. Do not record when found only on elbows and knees.
	47	--------	41	*Purpura or petechia:* Small localized extravasations of blood, red or purplish in color, depending on time elapsed since formation. Usually distributed at sites of pressure, and may be perifollicular.
	48	--------	41	*Hyperpigmentation of the hands:* Asymptomatic, with no inflammatory component. It is seen most frequently on the dorsum of the hands and lower forearms, particularly where skin hygiene is poor. The skin is rough, dry, and often has a grayish cyanotic base. There is not the sharp line of demarcation at the border of the lesion such as one sees in pellagra.
13	49	27	42	*Pellagrous dermatitis:* Record when symmetrical lesions typical of acute or chronic, mild or severe pellagra are observed. Record location.
12	50	27	42	*Scrotal dermatitis:* The scrotum usually must be rotated to see the lesions. Differentiate from fungus infections which usually extend onto the skin of the groin adjacent to the scrotum.
	50	--------	55–58	SKINFOLD THICKNESS (Not to be measured on children under 5 years of age.) Two sites are listed, the posterior arm and scapula. Calipers are to be calibrated and checked daily so that the spring tension gives a pressure of 10 gm per sq mm of jaw surface. The skinfolds are grasped between the

[1] Abb.=abbreviated examination; Det.=detailed examination.

McDee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				SKINFOLD THICKNESS—continued
				thumb and index finger, the fold parallel to lines of cleavage (lines of Linn) of the skin. The span of the grasp is dependent on the thickness of the skinfold. The size of the fold should be enough to include 2 thicknesses of skin and subcutaneous fat but no muscle or fascia. To insure against including such structures when in doubt, have subject perform an act which would contract the underlying muscles. The application of the calipers is about 1 cm from the fingers, at a depth approximately equal to the thickness of the fold, and at right angles to the length of the fold. Make 3 readings and record measurements in mm.
				Arm: Taken at the midposterior midpoint between the tip of the acromion and the tip of the olecranon with the elbow in 90° flexion.
				Scapula: Taken at the tip of the scapula with the subject in a relaxed standing position. Here the lines of Linn usually require the fold to be taken in the diagonal plane parallel to the medial border of the scapula.
				ABDOMEN
	54	--------	43	*Hepatomegaly:* Record if liver is palpable more than 2 cm below the costal margin.
	55	--------	43	*Splenomegaly:* Record if spleen is palpable.
				LOWER EXTREMITIES
14	56	28	43	*Bilateral edema:* Record only if bilateral. Examine pretibial area especially.
15	57	29	44	*Loss of ankle jerk:* Record only if absent after reinforcement. Record whether right, left, or both.
16	58	30	44	*Calf tenderness:* Record when definite bilateral evidence of painful sensation occurs upon squeezing the calf muscles firmly between the thumb and finger.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				LOWER EXTREMITIES—continued
				Distinguish, if possible, effects of recent severe exercise; e.g., marching. Record only if moderate or severe.
	59	--------	44	*Loss of knee jerk:* Record only if absent after reinforcement. Record whether right, left, or both.
	60	--------	45	*Loss of vibratory sense:* Test with tuning fork at 128 vibrations per minute over the lateral malleoli of both ankles. Record whether loss is right, left, or both.
	61	--------	45	*Motor weakness:* Subject's inability to squat and rise 3 or 4 times.
				SKELETAL
	62	--------	45	*Costochondral beading* ... Record in age groups 0–15 years only.
	63	--------	45	*Epiphyseal enlargement* ...
			45	*Bowlegs* ...
				CARDIOVASCULAR
	64	--------	47–52	*Blood pressure:* Take on right arm, with individual in sitting position. Make reading upon disappearance of sound. Record data to nearest even unit. Do not punch McBee card unless values exceed 150/100.
	64	--------	53–54	*Pulse rate:* Count for a minimum of 30 seconds, multiply by 2 and record. (Record in logbook any variations from this procedure.)
				Murmur
	65	--------	--------	*Tourniquet test:* Check both boxes if positive.
	51–53	31	59	*Examiner's initials:* Note signature is required for both phases of physical examination. Set up examiner code with number for each examiner and enter code number directly on card.
	66	--------	--------	*Urine sample:* Record number and mark box if sample obtained.
	67	--------	--------	*Blood sample:* Record number and mark box if sample obtained.
	68	--------	--------	*Feces sample:* Record number and mark box if sample obtained.

[1] Abb.=abbreviated examination; Det.=detailed examination.

McBee		IBM		
Abb.[1]	Det.[1]	Abb.	Det.	
				BIOCHEMICAL DATA
	69-108	...	61-80	The recording of the biochemical data may be done in the field, insofar as results are available. The coding of the biochemical data will be done by the statistical group. (See p. 85.)
17-18	109-110	...	...	*Other:* Use this space to record lesions or observations of possible interest or significance not provided for otherwise on the form.
19	...	...	...	*Studied further:* Give card number (4 digits) for specially studied subject.
	111	...	...	*Photograph:* Check if a photograph is taken. (NOTE: Photographic log should record number, location, lesion and other relevant data.)
				Remarks: Record here any information or questions which may be important in further analysis and study.

[1] Abb.=abbreviated examination; Det.=detailed examination.

Instructions for Use of Pediatric Record Form for Children Ages 0–4 Years

This form is for use in recording information about children under 5 years of age:

(1) In regular survey examination of children of families selected from the population;

(2) For special examination situations, such as—

(*a*) clinic groups;

(*b*) hospitalized children;

(*c*) orphanages.

NOTE: In regular survey examinations the card should be used instead of the regular examination card for all children under 5 years. In special examinations of groups of preschool children including some 5 or 6 years of age, the card may be used for all.

Logbook records should be made of pertinent facts, such as type of group examined; definitions used for items such as respiratory illness (e.g., how was the question put to the informant?); method of measuring length of infants; assessment of reliability of information on age.

Reasons for omission of sections of the form should be noted; e.g., whether due to —

Lack of time;

Lack of personnel;

Probable gross unreliability of information obtainable;

Inappropriate questions for the group studied, etc.

Such notation is important because of:

(1) Relevance to interpretation of such data as are obtained;

(2) Usefulness for further revision and improvement of the form.

At the end of the clinical examination section, and at the top of the dietary section, *initialing* space is provided. Indicate in the logbook who did each section for which information was recorded; e.g., that "obstetric history was taken by the clinical examiner via an interpreter," etc. The logbook should always include the identity of the initials of everyone doing examinations. Code numbers may be used for examiners to whom they have been assigned.

Obstetric History

A rubber stamp has been prepared, giving the questions relating to *Obstetric History* contained on this pediatric form. When the form is used in examination of children in the regular survey, it will be preferable to apply the stamp to the back of the abbreviated examination card used for the mother and record the obstetric information there.

It is desirable in the regular survey to use this obstetric stamp for *all women* 15+ years of age, or for *all women* 15-44 years of age. If obstetric histories are obtained only from women who present themselves with young children for examination, biased estimates of child mortality and of fertility will result. Record 0 for women never pregnant. Do not leave blank. The stamp also provides space which may be used for recording of blood pressure and "dip test" results on urine sugar and albumin—for possible use with pregnant women.

Identification Information (first two lines at top of front of card)

Location and area of origin to be recorded in some manner consistent with the plan used for the overall survey. In use with special groups, a code identification for each of the special locations may be set up separate from the coding of the regular survey locations; e.g., II-2 for second hospital where subjects are examined. The blank space after area of origin may be used for other items of identification as appropriate to the situation; e.g., ethnic category.

Record age in attained years and months, e.g., a 10-week-old baby should be recorded as 2 months of age.

Card number, in situations where the mother or other members of the child's family are examined, may be assigned as mother's number

with an added number or letter; e.g., mother has No. 2154 as basic identification number, so record the mother as No. 2154-1, first child as No. 2154-2, etc.

Space is given for indicating if other siblings of the examinee are also examined. In such cases the information about the mother's obstetric history and diet may be transcribed onto all the cards for her children subsequent to the examination session, and the records of siblings matched if need be in interpretation of findings.

If the informant is not the mother, the relationship to the child should be noted. It may be appropriate not to attempt to get historical information in cases where the mother is not available.

Physical Measurements (third line at top of front of card)

Length or height: write in an "H" if standing height is recorded, or adhere consistently to an age division, such as 24 months, between length and height measurements. *Record in logbook* relevant details about such procedures as method of measurement of weights and length/height for each group; e.g., type of scale used. Measurements should be made on the child nude; if not, indicate whether a deduction has or has not been taken from the body weight for estimated weight of clothing and give the basis for such deduction (which should be the recorded data from the weighing of the clothing of a number of children of specified age-height-weight, sex, etc.) Overall length of infants and young children should be measured using a simple board, not merely a flexible tape.

Height should be taken to the nearest 1 inch or nearest 1 cm—attempts to measure more closely are not of practical use, and the data are much easier to handle if this procedure is *consistently applied* at all locations in the survey. Weight should be taken to the nearest 1 lb or nearest 0.5 kg. The metric system is preferable.

Space is provided for entering height standard and weight standard on the card, and computing percent of standard for the actual heights and weights. As appropriate to the situation, these can be done in the field or later in the process of analysis of the data.

Six spaces are numbered but not otherwise labeled for use if appropriate in recording other physical measurements. If used, record in logbook the details as to what and how done, and by whom.

Mother's Obstetric History (fourth section at top of front of card)

Record age in attained (completed) years.

If subject is currently pregnant, indicate months of gestation, in Arabic figures, *or* indicate trimester in Roman numerals (I, II, III).

Liveborn + stillborn + abortions = number of past pregnancies, unless multiple births have occurred. If the numbers do not balance, enter the twins, triplets, etc., which explain the difference. Logbook entries should explain the definitions used for "stillborn" and for

"abortions" or miscarriages. If these are "by mother's statement," some probing may establish what the local customary definitions are. An international definition for livebirth is: "The complete expulsion or extraction from its mother of a product of conception, irrespective of the duration of pregnancy, which, after such separation, breathes or shows any other evidence of life, such as beating of the heart, pulsation of the umbilical cord, or definite movement of voluntary muscles, whether or not the umbilical cord has been cut or the placenta is attached." Various definitions for distinction between stillbirth and abortion are in use, approximations to distinguish nonviability; early fetal death at less than 20-week gestation is commonly used for abortion.

Liveborn now living + now dead = total liveborn. Explain any discrepancies.

Died at various ages should = total now dead. Explain any discrepancies.

Mother's age at termination of first pregnancy—whatever the outcome. Mother's age at termination of latest completed pregnancy—whatever the outcome. These will give information as to when reproduction starts and the average interval between pregnancies.

The letters U M R are given for overall evaluation of the obstetric history taken, as U = unreliable, R = reliable, with M for "middling" or "maybe." This is, of course, a highly subjective rating scale, but it may be useful to circle one of these to identify grossly the probable quality of the information, from the extreme of apparently clear recollection by the mother to the other extreme of apparently very hazy recall. Some general recording of probable accuracy for the whole group questioned may more appropriately be made in the logbook, instead of this individual assessment, with explanation of basis for the evaluation. Such recording may later avoid attribution of a spurious accuracy to the data.

Child, History (fifth section at top of front of card)

Age of child is recorded at the top of the card. Another space is given in this section, with notation U M R, one of which is circled to indicate reliability of the age statement, in the same fashion as for the obstetric history rating: U = unreliable, M = middling, R = reliable. It may be more appropriate, instead of using this space, to make general logbook recordings for each location of the probable accuracy of the age statements of subjects, especially in regions where exact age is not precisely known by most individuals. If age is elicited by correlation with recollections of known historical events, this should be recorded in the logbook when appropriate. Age statements should not be taken as unreliable merely because the subject does not appear to the examiner to be the stated age. Accuracy of data on age is most important in interpreting physical measurements and some

written evaluation of the reliability of the data recorded is essential to avoid errors of misinterpretation.

Birth order of child: Record order of birth, among the total live-born + stillborn of the mother.

Premature versus full-term birth: This question may best be omitted in situations where it is unlikely that some "moderately uniform" definition can be applied and "moderately reliable" answers obtained, or when time limitations require shortening of the questioning. If used, a uniform fashion of questioning should be chosen and recorded.

Age of first walking: Limit this question to children below 3 years of age, and note "*not*" for those who are not yet walking. Haphazard application of the question to some and not to others should be avoided—it is too likely that some biases will creep in and those from whom information is obtained will not be typical of the whole group.

Diarrhea=4+ loose watery stools per day: *Or*, some other definition should be recorded in the logbook and consistently used, if more appropriate in a given survey.

Respiratory illness=all or three of the following: fever, difficulty in breathing, nasal discharge, cough.

Current=last 2 days. *Recent*=last 2 weeks. *Recurrent*=four or more episodes within the last year.

Pica: If this is asked about in a survey, the wording of the questioning should be recorded in the logbook. Space is allotted for writing in clarifying statements; consistent notation should be chosen and used.

Parasites or malaria: Explain in logbook the basis for classification of a child as having parasites or malaria; e.g., clinical records, stool or blood smear examination, symptoms (specify), etc.

Other: The physicians making the survey should agree as to what, if anything, is to be recorded here, and note this in their logbook.

In general, questions on the child's illnesses should be consistently asked, or else omitted completely in areas where reliable information cannot be obtained. It may be appropriate, for instance, to limit the classification on respiratory disease to a "current" classification, and note this in the logbook. Where questions *are* being used and the answer is negative, a checkmark should be placed over the several sets of questions to indicate that the questions were asked.

Child, Clinical Examination

General impressions

Apathy: Unreactive, unresponsive, and inattentive to surroundings.

Pallor: Paleness and loss of color of skin, nail beds, mucosa and lips.

Irritability: Hyperresponsive, excessive or overreaction to minor stimuli, particularly manifest through crying or unusual indication of fear as a result of minor or relatively insignificant happenings.

Hair

Dry staring: Dry wirelike, unkempt, stiff hair, ofttimes brittle, sometimes may exhibit some bleaching of the normal color.

Dyspigmentation: Definite change from normal pigment of the hair, most usually evident distally and best seen by carefully combing hair strands upward and viewing the orderly array of hair in good light. Dyspigmentation includes both change of pigment (usually lightening of color) and depigmentation. Not to be confused with dyed or tinted hair. Dyspigmentation is always bandlike in character and usually is associated with some change in texture of hair in the depigmented band. In some ethnic groups, particularly Negroid groups, the pigment may be slightly reddish in color. In others, especially among straight black-haired peoples, the bandlike depigmentation ("flag sign") is common.

Thin: Thin hair indicates a decrease in the number of hair shafts per unit area and is usually associated with changes in texture of the hair, to a soft, silklike hair—and sometimes with dyspigmentation.

Easily pluckable: Easily pluckable hair is that in which the shafts are readily removed with minimum tug when a few strands are grasped between the finger and thumb and gently pulled. In such cases there is a lack of reaction of the child, indicating a lack of pain associated with removing of the hair.

Eyes

Conjunctival dryness: Conjunctival dryness is recorded when the conjunctiva, exposed by holding open the lids and having the subject rotate the eyes, appears dull, lusterless, and exhibits a striated or roughened surface. This is the condition sometimes referred to as "xerosis conjunctivae."

Conjunctivitis: Conjunctivitis is recorded when there is evidence of diffuse injection with discharge (sometimes purulent) of the bulbar or palpebral conjunctiva.

Bitot's spots: Bitot's spots are small circumscribed grayish or yellowish gray, dull, dry, foamy superficial lesions of the conjunctiva. They most often occur on the lateral aspect of the bulbar conjunctiva in the interpalpebral area.

Xerophthalmia: Xerophthalmia is recorded when the bulbar conjunctiva and cornea are dry and lusterless with a decrease in lacrimation. It is often associated with evidence of infection or, in extreme cases, keratomalacia. (Corneal softening with deformity, either local-

ized, usually central part of lower half of cornea, or total). If the latter is observed, it should be recorded in the blank space provided below xerophthalmia.

Lips

Angular lesions: Angular lesions are to be recorded only if they are definitely present bilaterally and noted when the mouth is held half open. These lesions may be either macerated areas with ill-defined margins, fissures which show a clear evidence of linelike break in the epithelium, or the classic perlêche with moist macerated pink base, and fissures with somewhat raised edges. Do not record healed scars.

Cheilosis: Cheilosis is recorded when the lips are swollen, tense, or puffy, and where it appears that the buccal mucosa extends out onto the lip. This category may be used to record vertical fissuring of the lips, but not for lesions of the angles of the mouth only.

Gums

Swollen red papillae (diffuse): Record only swollen red interdental papillae, with more than one papilla involved.

Bleeding gums: Abnormal gums which either spontaneously bleed or bleed upon slight pressure with a swab stick.

Tongue

Filiform papillary atrophy: Filiform papillae exceedingly low or absent, giving the tongue a smooth appearance which remains after scraping slightly with an applicator stick. "Mild" involves less than ¼ of the tongue (tip and lateral margins only); "moderate" involves ¼ to ¾ of the tongue; "severe" involves over ¾.

Glossitis: Any degree of injection or fissuring (break in lingual mucosa) or diffuse involvement of mucosa should be recorded as glossitis. Solitary delimited ulceration is not to be recorded. Geographic tongue should be so recorded even though it is the typical irregularly shaped and distributed areas of atrophy with irregular white patches resembling leukoplakia. Glossitis is usually associated with some sensation of pain or burning, particularly upon eating.

Glands

Thyroid enlarged (visible): Thyroid enlargement is to be recorded when a visually perceptible enlargement definitely palpable with or without swallowing is noted. It is preferable to examine the subject with his head slightly extended in order to detect thyroid enlargement. In this age group no effort is made to grade the degree of enlargement.

Skin, generally

Not resilient: Inelastic appearance which when pinched and raised between the thumb and forefinger slowly reassumes its contour upon release. Generally associated with emaciation or dehydration.

Petechiae: Small localized extravasations of blood, red or purplish in color depending upon the time elapsing since formation. Usually distributed at sites of pressure and may be perifollicular.

Dermatitis, with desquamation, or crazy-pavement type: Under this heading should be recorded those desquamating changes of the skin, usually with increased pigmentation, which occur on the extremities, especially legs, thighs and buttocks, but may occur over the trunk in association with kwashiorkor. (These have been termed "flaky-paint" dermatoses.) Also record the small circumscribed bleblike lesions sometimes seen in association with kwashiorkor and which on occasion may precede the desquamation. In addition, record any crazy-pavement type of lesions observed. These are characterized by a thin-appearing epithelium marked by striations usually resembling in outline the microscopic picture of epithelial cells. Not to be confused, however, with ichthyosis.

The blank space under this category may be assigned for use in recording other skin lesions.

Abdomen

Hepatomegaly: Record liver edges more than 2 cm below the coastal margin.

Splenomegaly: Record if spleen is palpable.

Pothelly: Record if abdomen appears abnormally distended and enlarged, with due recognition of the usual contour of the young child.

Lower extremities

Bilateral edema: Record only if bilateral and pitting. Examine pretibial area especially.

Calf tenderness: Record when definite bilateral evidence of painful sensation occurs upon firmly squeezing the calf muscles between the thumb and finger.

Skeletal

Cranial bossing: Record when there is abnormal prominence or protrusion of frontal or parietal areas.

Craniotabes: Record when definitely palpable areas of softening are encountered. Do not mistake open fontanels for craniotabes.

Costochondral beading: Record when there is definitely palpable and visible enlargement of the costochondral junctions.

Enlarged joints: Record when it is judged that the more obvious ends of long bones are enlarged; i.e., the wrist, ankles, knees.

Bone tenderness: Record when there is evidence of tenderness of thigh or leg bones, usually associated with assumption of typical "frog-like" position on the part of the infant, and frequently with palpable enlargements of the bones without increased heat.

Muscle wasting: Record when appearance indicates abnormal loss of muscle substance, as exhibited by unusual prominence of bony skeleton, undue degree of folding of the skin of the buttocks, or the abnormal flabby feel (sometimes described as jellylike) of the child with poor muscle tone.

The two blank spaces under this category may be assigned for use in recording other noteworthy skeletal signs, such as the presence of Harrison's groove, bowlegs, winged scapula.

Other

These spaces may be assigned for the recording of other clinical signs of possible interest or significance not provided for otherwise on the form. For these and the other unnamed spaces, decide if possible before the survey starts any signs which will be regularly recorded in these spaces, and then use the spaces exclusively for them. It will then not be necessary to write in the name of sign when the box is checked for its presence, provided all the clinicians are aware of the usage and the logbook clearly gives the details. For "occasional" findings not regularly recorded, which the clinician wishes to note, use the "*Remarks*" section.

Clinical impression

After an examination of a young infant or child the clinician has frequently arrived at a clinical impression based on the observations made. If he has arrived at either of the three impressions listed here, they should be checked. However, these impressions should be based on the following criteria:

Marasmus: Evidence of pronounced wasting of subcutaneous fat without edema or significant apathy. Frequently the face and eyes of the child may appear unusually bright due to the combination of wasting and prominence of the eyes. The child is usually considerably underdeveloped in relation to age and there may or may not be associated hair changes such as dyspigmentation, thin, easily pluckable hair, or signs of avitaminosis.

Prekwashiorkor: An underweight, undersized, underdeveloped child, without the evident pronounced wasting present in marasmus. Child is thin and undersized, but has relatively normal body proportions, has rather poor muscle tone, and hair changes may be present. Not apathetic, though would not be described as alert.

Kwashiorkor: Pitting edema at least on the pretibial region, underweight, undersize, underdeveloped for age. Muscular wasting may be present but masked by edema. Apathy of some degree is present.

Changes in the hair are usually noted, such as thin, easily pluckable hair with dyspigmentation or flag sign and change in texture to silken, sparse hair. Dermatosis with desquamation of the so-called flaky-paint type, with or without hyperpigmentation. In severe cases the dermatosis may resemble a relatively severe burn but lacks erythema.

Remarks

Record here any information which may be important in the analysis and interpretation of the clinical examination.

The letters G F P standing for good, fair, poor, are for use in making a "general appearance" classification, at the option of the clinicians on the survey.

Photographs taken of subjects having significant lesions should be identified by some system with the film rolls, and notation made of the lesion photographed.

Child, urine, blood, and fecal samples

If samples are obtained, check the appropriate boxes. If sample identification numbers are other than the child's card number, put in spaces provided.

The amount of sampling and the analyses done on each sample will depend on the laboratory capacity, which may be taxed by the samples obtained from other age groups. It may be preferable, rather than obtaining only 5 or 6 biochemical samples from children under 5 years at several successive locations, to allot laboratory facilities for 15 or so from one location and "sacrifice" getting this information at some other locations by omitting this sampling. Assuming more children than facilities for analysis, systematic subsampling selection of children for biochemical sampling should be made; e.g., every fifth child. If a "special" sample is obtained on a child, because of indication for sampling due to interesting clinical findings, write "*Special*" at top of biochemical recording section.

If urine sample is a casual one, cross out the space "during — hours." It is very desirable, and in some locations may be feasible (i.e. orphanage), to collect some timed samples. If these are obtained on a group, logbook notations should be made of the circumstances; e.g., the times of start and end of collection period, and time of preceding and concurrent meals.

Plastic disposable urine collecting bags can be used before the age of toilet training for infants of either sex. They are most successfully used if the bag is put on before the physical examination and blood drawing are done. The physical stimulation of the abdominal examination or the crying during venipuncture often leads to urination. In infants about 9–12 months of age, who are developing some degree of bladder control, the presence of the bag may somewhat inhibit uri-

nation. In this age group it may be difficult to get a urine sample in a short period of time.

Under conditions of family group studies, it is generally not practical to draw venous blood from young infants from sites other than the arm. The site can be used successfully in the majority of cases if the arm is stabilized by a helper and if a size 20 or 22 needle with a syringe (not a vacutainer) is used. Up to 20 ml of blood at a time safely can be drawn from a healthy infant over a month of age. All the current macro laboratory procedures can be made on a 5-ml sample; the volume drawn will depend on the analyses planned. In some locations it may be feasible to take only capillary samples from the heel, fingertips, or ear lobe for microdeterminations.

Child, Dietary Record

Breast feeding

This section queries concerning child's present status and history of breast feeding.

Child now fed: At present any one, pair, or all of the three categories—breast milk, other milk, or other foods. Query on this point for all subjects, now breast fed or not.

If child is now breast fed, record the frequency of feedings/24 hours, and whether self demand or schedule.

If nursing has been terminated, indicate whether this occurred abruptly or gradually. If abruptly, indicate the age in months when terminated. If termination was gradual, indicate age when other feeding was *started* and age at *final* weaning. If gradual weaning is now in process, record age when other feeding *started*. (A space is provided in this section for indicating that a subject was never breast fed.)

Factors in time of weaning: If child is recorded as weaned *abruptly*, indicate what maternal and/or infant factors entered. Ask mother for information relating to *this child*, not her "usual" weaning practices.

Initial nonmilk foods

Space is provided for up to six entries. Indicate the foods or food types, in order of introduction, recording age when started. Preliminary planning and trial runs with the dietary interview may produce some concise abbreviations for use here, and decision as to grouping of foods in the areas of fruits, vegetables, etc.

Pharmaceutical supplements

Inquire concerning vitamin and mineral supplements which were or are being given the child, and record when started and whether presently given or when stopped, and the regularity of administration.

The mother may not know the specific mineral or vitamin by name, but identification may be made from common or commercial names of the preparations used, or the source (such as UNICEF-supplied vitamins A and D). Space is provided for recording information on iron, vitamins A and D, vitamin C, and one unlabeled *other*.

Milk feeding (not breast)

Record the types of fresh and/or processed milks *now* being used in feeding the child. Enter an estimate of quantity, either volume or weight measure, and how often/24 hours such milk is taken. Questioning includes practice concerning boiling of water.

Child's present diet

The question on special foods given the child pertains to foods which are obtained especially for the child, including special preparations of foods available on the market for infant or child feeding, or foods prepared in a special way for the child.

The present diet for the infants and children consuming only milk will have been obtained before this section is reached, so the whole section can be omitted for them. Insofar as the initial nonmilk foods are still being eaten by the child, there will be some overlap with questions asked earlier. Whether or not repetition is desirable as a check on reliability of answers will be the decision of the interviewer.

The questions on present diet refer to "usual" and will not give a basis for precise quantitative estimation of nutrients in current dietary. The mother's focus might best be fixed on "what the child ate last week, insofar as it was typical," with foods eaten as often as once/week to be recorded. Broad food types are indicated; the detail with which individual foods are listed will depend on the dietaries in the area surveyed. In the milk group, cheeses, ice cream, custards, or beverages with milk would be included. Frequencies may be expressed as times/day *or* times/week. (For later handling of the data, the times/day might be multiplied by 7, to give all the foods in times/week.) Quantity/day should be expressed in weight or volume measures—selected as appropriate to the survey and each food (measures easily obtainable with fair accuracy in questioning the mother). Some operational intermediate use may need to be made of "servings"/day or per week (which may differ from times/day or week), with estimations of quantity/day or quantity/week reserved for later calculation.

Numerous qualifying statements can be made concerning use and limitations of this record. A number of ground rules will be required for its use in a survey, such as the measures for quantity of intakes which will be used, with compromises made between the desires to obtain detailed and precise information, and the time-per-interviewer

limitation on the numbers from whom information can be obtained. Pretrials of the form and consultations with nutritionists and dietitians familiar with the area's dietary can clarify details of its use and the way to get the maximum useful information in minimum amount of questioning. The interviewers should be persons with demonstrated ability in dietetic interviewing, and should be adequately instructed in use of this form. Time and personnel may not allow completion of the whole dietary record on all children under 5 years of age who are examined. Decisions in this case might be for the clinician to complete for all children the questions about breast feeding and age of weaning, with the dietary interviewer then completing the rest of the dietary questions on a systematic subsample of the total child group (say, every other child, or all children with basic identification number ending with 0, 2, 4, 6, or 8). Or, some sections of questioning might be omitted for all subjects and the others asked of all.

Operational definitions will need to be agreed on for distinction between "regular" and "occasional" use of vitamin and mineral supplements.

Questions should be reviewed as to whether "routine" answers might lead to misleading results, and modifications made as needed. For example, the question on boiling of formula water would need slight change in an area where terminal sterilization is commonly practiced in formula preparation.

Logbook records should explain the usage of the dietary form in the survey, detailing the local definitions, proposed ways to handle the data in the analysis of findings, etc.

Mother, Dietary Information

Dietary modification during pregnancy and during lactation

These are general questions, specific to the mother and inquiring about her *most recent* pregnancy and *most recent* lactation, *not* necessarily relating to the child being examined. Omit the lactation questions if the mother didn't breast-feed and omit the questions entirely if the informant is not the mother of the child being examined. If questions are appropriate and asked, with no dietary modifications indicated, place a large checkmark across the whole section to indicate the subject was asked and answered negatively.

Space is given for six items under *foods avoided*, *special foods*, and *supplements*, with salt specifically listed as a food avoided; milk as a special food; and iron, calcium, and vitamins as supplements. These items to be circled if *yes* for the woman questioned. Other items to be written in, preferably, for common answers of certain foods stated in a survey, in a set order; e.g., if women frequently state they eat more fruits during pregnancy, this might be assigned the number one

position under special foods. The *why?* spaces are for reasons, which may be vague. With the assistance of nutritionists familiar with the survey area, a code may be worked out, such as—

1. Doctor's orders.
2. Clinic advice.
3. Public health nurse advice.
4. Family or neighbor advice.
5. "Customary" here.
6. "I just do."

Beverage consumption habits

Question as to age of children when they usually begin to drink various beverages.

Recording and Coding Dental Examination Procedures

Dental caries: A tooth is recorded as carious only when definite positive lesions which readily admit the explorer point are present. When the crown of the tooth is so destroyed by caries that a restoration is impracticable, the tooth is recorded as indicated for extraction and tabulated as a missing tooth. A tooth with both a filling and a separate carious lesion is counted once only as a filled tooth. A tooth is considered to be in eruption if any portion protrudes through the gums.

The dental examination card has space provided for each of the 32 teeth (fig. 4). The code for the left-hand column of the tooth space is—

D—deciduous tooth present.
S—permanent tooth present.
O—no tooth present, deciduous tooth missing and permanent tooth not erupted.
I—no tooth present because permanent tooth has been extracted for caries.

Whenever D or S is coded in the left-hand column, one or more of the following must accompany it:

3 —tooth is normal, free of disease.
4 —tooth has active caries (decayed).
5 —tooth has been filled.
9 —tooth has caries involving the pulp and extraction is indicated.
9v—tooth with crown completely broken down by caries and roots only remain

Any abnormalities of the teeth, congenitally missing teeth, malpositioned or malalined tooth, partial or full dentures, soft tissue lesions, or any finding which should be recorded and no space is provided for such are noted under "*Remarks*" (*1*).

Attrition: Attrition is defined as the wearing away of tooth substance under the stress of mastication. The method described by McCombie (*2*) is used for scoring the attrition of the permanent molar teeth. These entries are made in the bottom area of the tooth cell (fig. 4):

0—no sign of attrition.
1—shiny facets on enamel.
2—dentine exposed on occlusal surface.
3—cusps eliminated, occlusal surface flattened.
4—maximum height of crown above cemento-enamel junction on buccal less than 3 mm.

A minus (—) is used to indicate a missing tooth space.

Periodontal condition: The condition of the tissues investing each tooth is estimated and scored according to a progressive scale developed by Russell (*3*) which gives little weight to soft tissue inflammation and relatively great weight to bone destruction.

Criteria for the periodontal score

Score	*Criteria*
0	*Negative:* Neither overt inflammation in the investing tissues nor loss of function due to destruction of supporting tissues.
1	*Mild gingivitis:* There is an overt area of inflammation in the free gingivae, but this area does not circumscribe the tooth.
2	*Gingivitis:* Inflammation completely circumscribes the tooth, but there is no apparent break in the epithelial attachment.
6	*Gingivitis with pocket formation:* The epithelial attachment has been broken and there is a pocket (not merely a deepened gingival crevice due to swelling in the free gingivae). There is no interference with normal masticating function, the tooth is firm in its socket, and has not drifted.
8	*Advanced destruction with loss of mastication function:* The tooth may be loose; may have drifted; may sound dull on percussion with a metallic instrument; may be depressible in its socket.

The periodontal scoring may be done following the scoring of dental caries and attrition. The gingival tissues are examined in the same order as in the dental caries examination.

These entries should be made in red pencil in the right-hand column of the tooth cell. Teeth which have roots only remaining (9v) should not be scored and the tooth cell should be marked with a dash or minus (—).

Fluorosis: Enamel hypoplasias caused by excessive fluoride intake are classified according to Dean's criteria (*4*).

Normal: The enamel presents the usual translucent semivitriform type of structure. The surface is smooth, glossy, and usually of a pale creamy white color.

Questionable: The enamel discloses slight aberrations from the translucency of normal enamel, ranging from a few white flecks to occasional white spots. This classification is utilized in those instances where a definite diagnosis of the mildest form of fluorosis is not warranted and a classification of "normal" not justified.

Very mild: Small, opaque, paper-white areas scattered irregularly over the tooth, but not involving as much as 25 percent of the tooth surface. Frequently included in this classification are teeth showing no more than 1–2 mm of white opacity at the summit of the cusps of the bicuspids or second molars.

Mild: The white opaque areas in the enamel of the teeth are more exclusive, but do not involve as much as 50 percent of the teeth.

Moderate: All enamel surfaces of the teeth are affected and surfaces subject to attrition show marked wear. Brown stain is frequently a disfiguring factor.

Severe: All enamel surfaces are affected and hypoplasia is so marked that the general form of the tooth may be affected. The major diagnostic sign of this classification is the discrete or confluent pitting. Brown stains are widespread and teeth often present a corroded-like appearance.

The degree of fluorosis or the normal state is marked in the appropriate box on the dental card. All other enamel hypoplasias are described in narrative form under "*Remarks.*"

Recession: Gingival recession is recorded according to the method described by Stahl and Morris (*5*). The recession score is the total number of teeth with the cemento-enamel junction visible occlusal to the gingival crest and is entered in box 29 on the dental examination card.

Oral hygiene examination: The status of oral hygiene of the subject is estimated by a modification (*10*) of the Oral Hygiene Index (OHI) developed by Greene and Vermillion (*6*). In this method the condition of the whole mouth is estimated by examining the surfaces of six teeth. These are two incisors and four molars (the first fully erupted tooth distal to the second bicuspid in each quadrant). The buccal surfaces of the selected upper molars, the lingual surfaces of the lower molars, and the labial surfaces of the upper right central and the lower left central incisors are included in the survey.

These surfaces are examined for the presence of oral debris and calculus. Oral debris is defined as the soft foreign matter loosely attached to the teeth, consisting of mucin, bacteria, and food, and varies in color from grayish white to green or orange. Oral debris is detected by running the side of an explorer across the surface being examined. Each surface examined is given a score based on the fraction of tooth surface covered The surface is scored 0 if no debris or stain is present; 1 if soft debris is present but covers not more than the gingival third, or if extrinsic stain is present with no debris; 2 if soft debris covers from one-third to two-thirds of the tooth surface; or 3 if more than two-thirds of the surface is covered.

Oral calculus is defined as a deposit of inorganic salts composed primarily of calcium carbonate and phosphate mixed with food, debris, bacteria, and desquamated epithelial cells. Dental calculus is divided into two types, differentiated primarily by location on the tooth in relation to the free gingival margin (1) "supragingival

calculus" denotes deposits located occlusal to the free gingival margin, and usually white to yellowish brown in color; (2) "subgingival calculus" denotes deposits located apically to the free gingival margin, and which are usually light brown to black in color due to inclusion of blood pigments. Calculus is scored on the same scale as used for debris. Zero is recorded if no calculus is present. A score of 1 is assigned for supragingival calculus covering not more than one-third of the tooth surface; a score of 2 for supragingival calculus covering between one-third and two-thirds of the surface, or for the presence of individual flecks of the subgingival calculus; and a score of 3 for supragingival calculus covering more than two-thirds of the tooth surface, or for a continuous, heavy band of subgingival calculus.

The spaces on the reverse side of the dental examination card under "bacteriological findings" are used to record the oral hygiene findings. Any two columns may be used. First the debris score and then the calculus score for the upper right molar, upper right central, upper left molar, lower left molar, lower left central, and lower right molar are dictated to the recorder. A dash (—) is used to indicate that the tooth to be scored is missing.

Summary of dental findings: Summary boxes 1–32 are located at the bottom of each dental examination card.

Box 1: Total number of normal deciduous teeth (D3).
Box 2: Total number of decayed deciduous teeth (D4).
Box 3: Total number of deciduous teeth with caries involving the pulp (D9).
Box 4: Total number of filled deciduous teeth (D5).
Box 5: Sum of boxes 2, 3, and 4 (def).
Box 6: Sum of boxes 1 and 5 (number of deciduous teeth present).
Box 8: Sum of the attrition scores of the permanent molars.
Box 9: Sum of attrition scores divided by number of molar teeth present (mean attrition score for the individual).
Box 13: Total number of normal permanent teeth (S3).
Box 14: Total number of decayed permanent teeth (S4).
Box 15: Total number of missing permanent teeth (I).
Box 17: Total number of permanent teeth with caries involving the pulp (S9 and S9v).
Box 18: Total number of filled permanent teeth (S5).
Box 19: Sum of boxes 14, 15, 17, and 18 (DMF).
Box 20: Sum of boxes 13 and 19 (total number of permanent teeth erupted).

In determining the number of DMF teeth and normal teeth, the third molars usually are not included; therefore the total number of permanent teeth is considered as 28. In counting a tooth for the DMF score, a tooth is counted only once. Teeth 9>5>4.

Box 29: Total number of teeth with cemento-enamel junction visible occlusal to gingival crest.
Box 30: Total number of teeth with recession divided by total number of teeth present in the mouth (percent of teeth with recession for the individual).

Box 31: Sum of the periodontal scores is written in the upper left portion of box and the total number of teeth scored in the lower right portion.

Box 32: Sum of the periodontal scores divided by number of teeth scored (periodontal score for the individual).

The debris and calculus scores for the individual are determined by calculating the average scores for the tooth surfaces examined. The Oral Hygiene Index (OHI) is determined by combining the average debris and calculus scores.

From each civilian study location within the host country, samples of water and urine are collected for fluoride determinations (see also chs. VII and VIII). The water sample can be obtained from any available tap if a communal water supply is used in the examination location. If the water from the towns or villages comes from wells in the area, it is advisable to collect samples at each location from several different wells. The water samples should be sent to the laboratory with directions as to analyses to be performed.

The location urine sample is made up of 10 ml of urine from each of the first 15 persons submitting specimens to the laboratory for analysis. To the 150 ml pooled sample of urine is added 2 ml of toluene for storage purposes. Analysis of the water samples for fluoride can then be done by one of the methods described in Standard Methods for Examination of Water and Sewage (*7*). (See also ch. VIII, p. 217.) Reference can be made to Kinser (*8*) for the method of analysis for fluoride in urine.

In order that correlations can be made among the medical, biochemical, and dental examination findings, appropriate data obtained from the medical examination cards should be transferred to the dental records. A rubber stamp can be made for this purpose and the dental cards stamped on the reverse side. Such information as vitamin levels, skinfold measurements, and "standard weights" may be secured from the medical examination cards and entered in the spaces provided by the stamp.

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IBM Code for Abbreviated Examination Card

Space is provided at the bottom of the McBee card for the coding of data for subsequent detailed analysis of the results, using International Business Machine (IBM) equipment. IT IS ESSENTIAL THAT THE NUMBERS ENTERED IN THESE SPACES BE LEGIBLE.

The IBM code for the abbreviated physical examination is outlined below:

IBM	*Instruction*
1–4	Enter 4-digit card number if card is not prestamped.
5	Location, in code, of not more than 11 categories.
6	Blank (may be used for sublocation or any other additional item).
7	Area of origin, in code of not more than 11 categories.
8	Enter rural-urban as: 0—Rural, 1—Urban.
9	Activity, in code of not more than 11 categories.
10	Time-in-service in code of not more than 11 categories.
11–12	% Std. Wt. Enter last 2 digits only. If there is an overlap of values, e.g., 150 percent and 53 percent, a double punch in 1 of the 2 columns is necessary.
13–14	Enter age.
15–16	Enter height.[1]
17–19	Enter weight.[1]
20	Bitot's spots and nasolabial seborrhea in 0–3 coding system:
	0 No finding
	1 Bitot's spots (McBee No. 1)
	2 Nasolabial seborrhea (McBee No. 2)
	3 Both
21	Lips (in 0–7 code):
	0 None of these
	1 Angular lesions (McBee No. 3)
	2 Angular scars (McBee No. 4)
	3 Cheilosis, general (McBee No. 5)
	4 Angular lesions + Angular scars

[1] Code numbers for height and weight boxes are given as they appear on the abbreviated card that was designed to record height in inches and weight in pounds. In situations where height is recorded in centimeters and weight is recorded in kilograms, the use of these boxes is reversed; i.e., weight is recorded in kilograms in boxes 15 and 16 and height is recorded in centimeters in cols. 17–19. A notation that this was done should be recorded in the logbook.

IBM Code for Abbreviated Examination Card—Continued

IBM *Instruction*

21 Lips (in 0-7 code)—Continued
- 5 Angular lesions + Cheilosis
- 6 Angular scars + Cheilosis
- 7 All 3

22 Gums, swollen red papillae, in 0-2 code (McBee No. 6):
- 0 No finding
- 1 Localized
- 2 Diffuse

23 Tongue, filiform papillary atrophy (McBee No. 7):
- 0 No finding
- 1 Slight
- 2 Moderate
- 3 Severe

24 Tongue, glossitis, magenta colored, in 0-3 code:
- 0 No finding
- 1 Glossitis (McBee No. 8)
- 2 Magenta colored (McBee No. 9)
- 3 Both

25 Glands, thyroid enlarged (visible) (McBee No. 10):
- 0 No finding
- 1 Positive finding

26 Skin, general, follicular hyperkeratosis (McBee No. 11):
- 0 No finding
- 1 Arms
- 2 Back
- 3 Thighs
- 4 Arms+back
- 5 Arms+thighs
- 6 Back+thighs
- 7 All 3

27 Skin, general, scrotal dermatitis and pellagrous dermatitis, in 0-3 coding system:
- 0 No finding
- 1 Scrotal dermatitis (McBee No. 12)
- 2 Pellagrous dermatitis (McBee No. 18)
- 3 Both

28 Lower extremities, bilateral edema (McBee No. 14):
- 0 No finding
- 1 Positive finding

29 Lower extremities, loss of ankle jerk (McBee No. 15):
- 0 No finding
- 1 Unilateral
- 2 Bilateral

30 Lower extremities, calf tenderness (McBee No. 16):
- 0 No finding
- 1 Positive finding

31 Examiner, in code as used for McBee punching.

32 Blank (may be used as judged appropriate).

33-38 Detailed card number, for subjects receiving also a detailed examination.

IBM Code for Detailed Examination Card

Space is provided at the bottom of each side of the McBee card for the coding of data for subsequent detailed analysis of results, using International Business Machine (IBM) equipment. IT IS ESSENTIAL THAT THE NUMBERS ENTERED IN THESE SPACES BE LEGIBLE.

The IBM punchcard provides 80 columns for the recording of information. Each column provides 12 spaces for coding, numbered 0-9, plus 2 other spaces usually designated X and Y.

IBM	*Instruction*
1-19	Same as abbreviated examination card.
20	Recent history of disease malaria, trachoma, and TBC (tuberculosis) in 0-7 coding system:
	0 No finding 1 Malaria 2 Trachoma 3 TBC 4 Malaria + Trachoma 5 Malaria + TBC 6 Trachoma + TBC 7 All three
21	Recent history of disease—diarrhea, and other items if consistently recorded throughout the survey.
22	Hair, depigmentation, and eyes, thickened opaque bulbar conjunctivae in 0-3 coding system:
	0 No finding 1 Depigmentation (McBee No. 1) 2 Thickened opaque bulbar conjunctivae (McBee No. 2) 3 Both
23	Eyes, xerosis conjunctivae, pingueculae, and Bitot's spots: Code McBee items Nos. 3, 4, and 5 in 0-7 coding system as used in IBM col. 20.
24	Eyes, circumcorneal injection, conjunctival injection and blepharitis: Code McBee items Nos. 6, 7, and 8 in 0-7 coding system as used in IBM col. 20.
25	Skin, face and neck, nasolabial seborrhea, other seborrhea and pigmentation: Code McBee items Nos. 9, 10, and 11 in 0-7 coding system as used in IBM col. 20.
26	Lips, angular lesions, angular scars, and cheilosis: Code McBee items Nos. 12, 13, and 14 in 0-7 coding system as used in IBM col. 20.
27	Gums, marginal redness, marginal swelling and atrophy of papillae: Code McBee items Nos. 15a, 15b, and 16a in 0-7 coding system as used in IBM col. 20.
28	Gums, recession of gums, swollen red papillae, bleeding gums: Code McBee items Nos. 16b, 17, and 18 in 0-7 coding system as used in IBM col. 20. (If this is done, a list of all diffuse swollen red papillae should be kept. In the event that these are very numerous, the code should be modified to suit each survey.)
29	Teeth, carious and worn teeth:
	0 No finding 1 Carious teeth, 1-4 2 Carious teeth, 5+ 3 Worn teeth 4 Carious teeth, 1-4 + Worn teeth 5 Carious teeth, 5+ + Worn teeth

IBM *Instruction*

30 Teeth, missing teeth and no teeth:

0 No finding
1 Missing teeth, 1-4
2 Missing teeth, 5-16
3 Missing teeth, 17-31
4 No teeth, plates
5 No teeth, no plates

(Combinations of these should be made according to data from each survey.)

31 Teeth, fluorosis, malposition and debris or calculus: Code McBee items Nos. 23, 24, and 25 in 0-7 coding system as used in IBM col. 20.

32 Tongue, filiform papillary atrophy (McBee item No. 26):

0 No finding
1 Slight
2 Moderate
3 Severe

33 Tongue, fungiform papillary atrophy and papillary hypertrophy or hyperemia: Code McBee items Nos. 27 and 28 in 0-8 coding system.

0 No finding
1 Fungiform papillary atrophy, slight
2 Fungiform papillary atrophy, moderate or severe
3 Papillary hypertrophy, slight
4 Papillary hypertrophy, moderate or severe
5 Code 1 + 3
6 Code 1 + 4
7 Code 2 + 3
8 Code 2 + 4

34 Tongue, geographic, furrows or scrotal appearance, and fissures, erosions or ulcers: Code McBee items Nos. 29, Blank, and 30 in 0-7 coding system as used in IBM col. 20.

35 Tongue, serrations or swellings, red, tip and/or lateral margins only and glossitis: Code McBee items Nos. 31, 32, and 33 in 0-7 coding system as used in IBM col. 20.

36 Tongue, magenta colored, Glands, parotid enlarged and thyroid enlarged: Code McBee items Nos. 34, 35, and 36 in 0-7 coding system as used in IBM col. 20. (This should be modified if the degree of thyroid enlargement is recorded; see p. 58.)

37 Skin, general, follicular hyperkeratosis: Code McBee items Nos. 39 and 40a in 0-8 coding system as used in IBM col. 33.

38 Skin, general, follicular hyperkeratosis: Code McBee items Nos. 40b and 41a in 0-8 coding system as used in IBM col. 33.

39 Skin, general, follicular hyperkeratosis and perifolliculosis: Code McBee items Nos. 41b and 42 in 0-5 coding system as used in IBM col. 29.

40 Skin, general, xerosis, crackled skin, and acneform eruption: Code McBee items Nos. 43, 44, and 45 in 0-7 coding system as used in IBM col. 20.

41 Skin, general, thickened pressure points, purpura or petechiae, hyperpigmentation of the hands: Code McBee items Nos. 46, 47, and 48 in 0-7 coding system as used in IBM col. 20.

42 Skin, general, pellagrous dermatitis and scrotal dermatitis: Code McBee items Nos. 49 and 50 in 0-7 coding system.

0 No finding
1 Neck
2 Hands
3 Other
4 Scrotal dermatitis
5 Neck + Scrotal dermatitis
6 Hands + Scrotal dermatitis
7 Other + Scrotal dermatitis

IBM	*Instruction*
43	Abdomen, hepatomegaly and splenomegaly, Lower extremities, bilateral edema: Code McBee items 54, 55, and 56 in 0-7 coding system as used in IBM col. 20.
44	Lower extremities, loss of ankle jerk, calf tenderness, loss of knee jerk: Code McBee items 57, 58, and 59 in 0-7 coding system as used in IBM col. 20. (Code only cases of bilateral loss of ankle jerk and loss of knee jerk and list all unilateral cases.)
45-46	Blank. These two columns may be used for coding other of the clinical findings or for coding additional biochemical determinations such as iodine excretions for which this card does not make provision.
47-52	Enter blood pressure in full, systolic followed by diastolic, each as 3-digit numbers; e.g., 150110, 120080, 090060.
53-54	Enter pulse rate as the last 2 digits of the count; e.g., pulse 84 is entered as 84, pulse 60 is entered as 60, but pulse 120 is entered as 20.
55-56	Enter arm skinfold measurement in mm, preceded by 0 if less than 10; e.g., 9 is entered as 09.
57-58	Enter scapula skinfold measurement in mm, preceded by 0 if less than 10.
59	Enter examiner code number, according to numeric code used for the abbreviated examination card. If only one clinician does all the examinations of the survey, this column can be left blank, for possible use in recording other data.
60	Blank. For use as judged appropriate in each survey.

Column 60 completes the recording of the clinical examination. Columns 61-78 are for recording biochemical data; these results can be recorded in the field only insofar as the laboratory results are completed at the time. This recording will involve only the fewer numbers of individuals sampled for biochemical measurements.

For the blood measurements, two IBM columns are provided for each item. Coding systems suggested below are contingent upon their appropriateness according to the general level of values found in any survey area.

61 Thiamine excretion, in numeric code as follows:

62 Riboflavin excretion, in numeric code as follows:

63 N'-methylnicotinamide excretion, in numeric code as follows:

Code No.	Thiamine	Riboflavin	N'-Methylnicotinamide
	μg/gm creatinine	μg/gm creatinine	mg/gm creatinine
1	<27	<27	<0.50
2	27-65	27-49	0.50-1.59
3	66-129	50-79	1.60-2.99
4	130-199	80-99	3.00-4.29
5	200-249	100-149	4.30-5.99
6	250-299	150-199	6.00-7.99
7	300-399	200-269	8.00-9.99
8	400-499	270-399	10.00-14.99
9	500-599	400-599	15.00-19.99
0	≥600	≥600	≥20.00
Y	(1)	(1)	(1)

[1] No data.

IBM	*Instruction*
64	Blank. For use as judged appropriate in each survey.
65-66	Total plasma protein: Code units and tenths. Round recorded value to nearest tenth of gm; e.g., 6.81=68; 6.88=69.
67-68	Hemoglobin: If few or no determinations are below 10.0, then code the grams and tenths of grams by which a measurement exceeds 10.0; e.g., for 13.6, code 3 in col. 67 and 6 in col. 68. For a value of, say, 9.1, code X in col. 67 and 1 in col. 68. The 9 should be written in by hand on the IBM card. This distinguishes the coded value from 19.1.
69-70	Hematocrit: Code the percent value of the determination, discarding fractions; e.g., code 46.5 with 4 in col. 69 and 6 in col. 70.
71-72	Mean cell hemoglobin concentration: In calculation, record to 0.1 percent, discarding remainders; e.g., record 32.86 as 32.8. Code the percent value of the determination; e.g., 32.8 with 3 in col. 71 and 2 in col. 72.
73-74	Serum vitamin C: Code milligrams and tenths, discarding hundredths; e.g., code 1.27 with 1 in col. 73 and 2 in col. 74. Code 0.21 with 0 in col. 73 and 2 in col. 74.
75-76	Serum vitamin A: Code micrograms, in tens and units, discarding fractions; e.g., code 28.7 with 2 in col. 75 and 8 in col. 76. Code 08.3 with 0 in col. 75 and 8 in col. 76.
77-78	Serum carotene: Code micrograms, in hundreds and tens, discarding units and fractions; e.g., code 128.3 with 1 in col. 77 and 2 in col. 78. Code 61.9 with 0 in col. 77 and 6 in col. 78.
79-80	Blank. To be used as judged appropriate in each survey.

Appendix A. Examples of a Daily Survey Log

Daily Entries Are of the Utmost Importance

Typical military day: Monday, October 24, 19—. 0730-1300 hrs. Location 3, —— Regimental Combat Team and Military Province

Today, we examined men of the —— Regimental Combat Team and Military Province—a combination of Army units based at the Army base about 4 km from ————, 26 km from ————. Although the combat team is composed of 3 infantry batallions and 1 artillery battalion, the men at this base are only 1 of 4 battalions. Today we examined the headquarters company, 31 men; heavy mortar company, 40 men; service company, 40 men; engineer company, 43 men; headquarters company of the Military Province, 64 men; prisoners from the stockade, 30 men; medical company, 34 men, total=282. Laboratory samples total blood 18, urine 16, stools 13. Ratio for sampling: abbreviated: detailed=5:1; detailed: laboratory=4:1. Cards nos. 3553-3834 and 0110-0163. The officers interviewed were Col. ——, the Chief of Staff of the Military Province, and Lt. Col. ——, the Regimental acting CO. Two hundred eighteen men were presented for examination. It is our understanding that today and tomorrow we will examine all the privates on this base, comprising about 690 men; therefore, no sample selection is involved. The overall commander of the entire area was not at the camp.

There are 3 messes, an infantry battalion (about 350-400 men), a medical company (<50 men), and a "circle mess," feeding the others. About 70-80 percent are Islamic, the remainder are mainly Buddhist (the latter about 10 percent). They all come from the 5 southernmost provinces. The heaviest-

working men are the prisoners, mostly in the stockade for 3-6 months for being AWOL; their food allowance is smaller, and they live by low standards. They work about an 8-hour day at heavy labor such as road repairing. The second heaviest working group is infantrymen, because their training is more intensive. The third most active group is the engineers, the fourth the service company, and the least active are the headquarters and medical company men. Most of the men work about 7 hours a day with 1 hour for lunch, 5 days a week. In several days, they will all be going for intensive combat training. All men seen are draftees, theoretically in for 2 years.

The examining building is a large open auditorium, quite adequate, with 4 screened booths. The flow system used is the same as on the 21st. Today, we started rotation of examining personnel 1 by 1. Examiners are No. 1 and 3, abbreviated; 5 and 9, detailed. The men were weighed in [outer] shorts only, with the help of 2 medical company NCO's. Heights and weights were taken by 2 medical company NCO's who did a good careful job. Scrotal exams were omitted except for the detailed subjects. *Vibratory sense results are probably unreliable because of interpreter difficulties.*

Typical civilian day: Wednesday, November 2, 19 . 0815-1330 hrs. Location 8, ——— Village, ——— Commune, ——— District, ——— Province

The village is 15½ km from ——— (11 km along the road and 4½ km down a narrow lane to the west of the main road). There are 122 families in the village, with a total population of 570. Of these, we have selected 40 families in one clearly delineated section (by road) of the village containing 40 families, with a population of 172, all of whom are expected to come for examination. The socioeconomic position of the sample is not recognizably different from that of the other villages—all are farmers. Although this particular district has had a beriberi problem over the years, no cases have been recognized in the village for about 4 years. However, about 4 km further up the road and in villages off the highway, some beriberi is thought to exist currently. This village was deliberately selected at the meeting with the senior provincial health officers in the capital, whereas the villages planned for tomorrow and the next day were arbitrarily selected.

Although all families are farmers, productivity is stated to be very little more than the family needs for rice and vegetables. Some tobacco is raised, and there is a tobacco curing station nearby where some of the men work parttime. For the 122 families, there are 60 cattle and 13 buffaloes, all used for plowing (none for meat). Hogs are raised, but slaughtering is prohibited. About 100 are sold a year at the local market. Each family has about 5 chickens for eggs and meat—they used to have many more, but an epidemic seems to kill their broods every time they get a good stock. Consumption of meat, eggs, and poultry seems to vary between 1 day a week and daily, depending on week-to-week economic status. The village itself sits in the midst of beautiful large ricefields, close to the base of a mountain, with lush vegetation of bamboo and bananas. Neighboring temples are in good shape.

Interviews were obtained from ——— ———, the District Officer, ——— ———, the Commune Headman, and ——— ——— ———, the Village Headman.

We are examining in the school house, smaller but otherwise similar to those used previously. The flow system is 3 abbreviated→dentists→2 first part of detailed→1 second part detailed→laboratory. Heights and weights are being determined fully clothed, without shoes, in light clothing, by our nurse and 2 NCO's from the Army medical company. Scrotal and buttocks examinations are limited again to the detailed examinations. Examiners No. 1 and 9,

detailed; 5 and 6, abbreviated. Laboratory samples total 22 bloods, 20 urines, 10 stools. Sampling ratios: abbreviated: detailed is 2:1; detailed: laboratory 4:1. Card Nos. (abbreviated) 5231 5401; (detailed) 0568–0652.

We ended up with the 40 expected families, plus 4 persons who apparently volunteered. All 171 examined are accounted for in the following table. One hundred seventy-one out of a total of 176 were examined (i.e., 97.2 percent—pretty good). Characteristically, those missing are the young adults and teenagers. Thirty-six of the 40 families were examined in their entirety. Average size of family: 4.4 persons.

Family No.	Number persons in family	Number persons seen	Number persons not seen	Age and sex not seen	Family No.	Number persons in family	Number persons seen	Number persons not seen	Age and sex not seen
1	8	8	0		22	2	2	0	
2	3	3	0		23	4	4	0	
3	1	1	0		24	1	1	0	
4	7	7	0		25	5	5	0	
5	4	3	1	23M	26	3	3	0	
6	6	6	0		27	5	5	0	
7	5	4	1	18M	28	6	6	0	
8	7	7	0		29	3	3	0	
9	3	3	0		30	7	7	0	
10	3	3	0		31	4	4	0	
11	6	6	0		33	4	4	0	
12	4	4	0		43	7	7	0	
13	2	2	0		44	9	9	0	
14	2	2	0		47	5	3	2	20M 18F
15	8	8	0						
16	4	4	0		48	3	2	1	29F
17	6	6	0		57	3	3	0	
18	6	6	0		60	3	3	0	
19	1	1	0		61	3	3	0	
20	2	2	0		62	2	2	0	
21	5	5	0			4	4	0	

Date	Location	Location code	Number briefs	Brief card sequence	B:D ratio [1]	Number detailed	Detailed card sequence	D:LAB ratio [2]	Number blood	Number urines	Number swabs and stools
Oct. 20	Cavalry Regiment	1	151	3000–3150	5:1	31	0001–0031	1:1 (−12)	12	12	12
Oct. 21	Infantry Regiment	2	402	3151–3552	5:1	78	0032–0109	4:9	20	20	18
Oct. 24	Regimental Combat Team and Military Province	3	282	3553–3834	5:1	54	0110–0163	4:1	14+1 [3] SP	14+1 SP	13
Oct. 25	Same as Oct. 24	3	278	3835–4112	4:1	69	0164–0232	2:1(PM) 4:1(AM)	20	20	17
Oct. 26	Village	4	239	4113–4351	3:1	79	0233–0311	3:1	26	24	11
Oct. 27	do	5	143	4352–4494	2:1	72	0312–0383	3:1	24	24	14
Oct. 31	Regimental Combat Team and Military Province	6	420	4495–4914	4:1	105	0384–0488	5:1	21	21	20
Nov. 1	Field Artillery Battalion Camp	7	316	4915–5230	4:1	79	0489–0567	4:1	20	20	17
Nov. 2	Village	8	171	5231–5401	2:1	85	0568–0652	4:1	22	20	10
Nov. 3	do	9	143	5402–5544	2:1	69	0653–0721	4:1	17	15	----------

[1] Brief: Detailed ratio.
[2] Detailed: Lab ratio.
[3] Special (see green card).

Example of daily summary of clinical findings

[Percent prevalence]

	Daily summary of brief clinical findings													
Number examined	151	402	282	278	239	143	420	316	171	143	210	406	166	378
Bitot's spots		<1	<1			4	<1							
Nasolabial seborrhea	18	5	10	6	3	5	9	5	2	4	2	3		2
Angular lesions	1	4	<1	<1	3	3	12	5	12	6	10	2	4	0
Angular scars	1	2	<1	2			1			<1		1		
Cheilosis	<1													
Papillae, red-localized	1	2	4	3	7	5	5	2	8	4	3	<1	2	<1
Papillae, red-diffuse			<1			<1								
Filiform papillary atrophy—slight	3	3	2	<1	2	5	<1	1	4	4	3	1	5	<1
Filiform papillary atrophy—moderate	<1	1	<1		<1	2	<1			1			2	<1
Filiform papillary atrophy—severe					<1	<1							1	
Glossitis														
Magenta tongue														
Thyroid enlarged	2	1			2	1	2	3	63	10	24	12	19	5
Follicular hyperkeratosis arms														
Follicular hyperkeratosis back						<1								
Follicular hyperkeratosis thighs					<1									
Scrotal dermatitis [1]														
Pellagrous dermatitis														
Bilateral edema														
Bilateral loss of ankle jerk	<1		<1	1		<1	<1	<1	<1	1	5	<1	4	<1
Calf tenderness					<1	<1								
Location code No.	1	2	3	3	4	5	6	7	8	9	10	11	12	13

[1] Not examined for.

Number of cases seen

	Daily summary of brief clinical findings													
Number examined	151	402	282	278	239	143	420	316	171	143	210	406	166	378
Bitot's spots		2	1			6	1							
Nasolabial seborrhea	28	19	27	18	6	7	38	15	4	6	4	11	1	6
Angular lesions	2	17	2	1	6	4	51	15	21	8	20	7	6	
Angular scars	2	10	1	5			5			1		3		
Cheilosis	1													
Papillae, red localized	2	9	11	7	16	7	20	5	13	6	6	1	4	3
Papillae, red—diffuse			1			1								
Filiform papillary atrophy slight	5	12	5	1	4	7	2	4	7	6	6	6	8	1
Filiform papillary atrophy—moderate	1	6	1		1	3	1			2			3	1
Filiform papillary atrophy severe					2	1							2	
Glossitis														
Magenta tongue														
Thyroid enlarged	3	4			4	2	10	9	107	27	54	50	32	20
Follicular hyperkeratosis—arms														
Follicular hyperkeratosis—back						1								
Follicular hyperkeratosis—thighs					1									
Scrotal dermatitis [1]														
Pellagrous dermatitis														
Bilateral edema														
Bilateral loss of ankle jerk	1		2	3		1	2	3	1	2	10	1	7	1
Calf tenderness					1	1								
Location Code No.	1	2	3	3	4	5	6	7	8	9	10	11	12	13

[1] Not examined for.

CLOTHING WEIGHTS, 2D SPOT CHECK, MILITARY, NOVEMBER 21, ——— RGT.

Weight of khaki shorts, undershorts, and belt (variable)

Weight with clothes (kg):	Nude weight	Difference
65.7	64.9	0.8
51.4	50.6	.8
50.0	49.0	1.0
56.6	56.0	.6
56.6	55.4	1.2
54.7	53.7	1.0
57.3	56.4	.9
53.3	52.6	.7
56.0	55.4	.6
51.5	50.8	.7
Mean		8.3
		0.83

Weight of long cotton trousers and undershorts

Weight with clothes (kg):	Nude weight	Difference
58.3	57.5	0.8
44.3	43.5	.8
55.5	54.9	.6
63.8	63.0	.8
73.9	73.1	.8
59.4	58.8	.6
51.6	51.0	.6
56.0	55.4	.6
Mean		5.6
		0.7

Daily determination of gross examiner differences on abbreviated examinations

Date _____

Location _____

Physicians	1	2	3	4	5	6	7	8	9	Total
Bitot's spots										
Nasolabial seborrhea										
Angular lesions										
Angular scars										
Cheilosis										
Papillae, red, swollen localized										
Papillae, red, swollen diffuse										
Filiform papillary atrophy—slight										
Filiform papillary atrophy—moderate										
Filiform papillary atrophy—severe										
Glossitis										
Magenta colored										
Thyroid enlarged										
Follicular hyperkeratosis—arms										
Follicular hyperkeratosis—back										
Follicular hyperkeratosis—thighs										
Scrotal dermatitis										
Pellagrous dermatitis										
Bilateral edema										
Bilateral loss of ankle jerk										
Calf tenderness										
Total number examined										

PLATES

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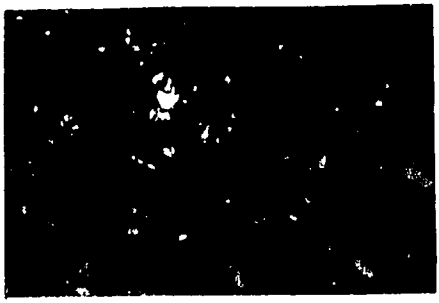


Bitot spots

Bitot spots

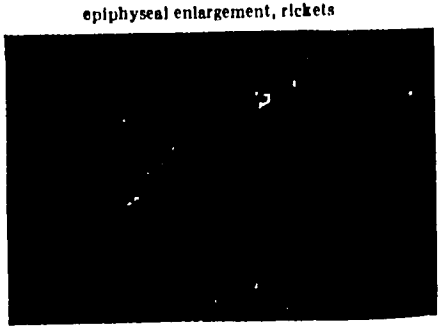
Keratomalacia (courtesy Dr. H. A. P. C. Oomen)

Depigmentation of hair, flag sign

Edema and skin changes, kwashiorkor

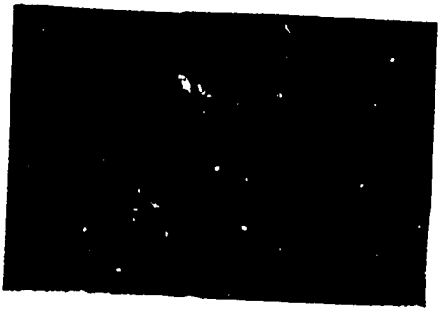
Skeletal deformities, costochondral beading and epiphyseal enlargement, rickets

Angular lesions



Angular scars

FIGURE 5. Illustrations of some clinical signs of nutritional diseases.



Cheilosis, severe

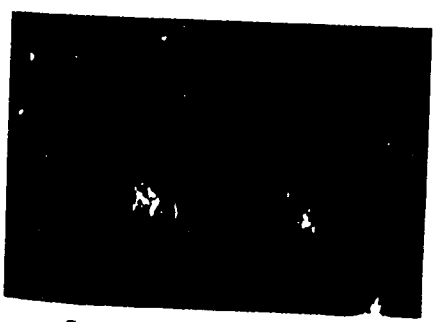
Swollen, red interdental papillae, scurvy

Marginal redness of gums

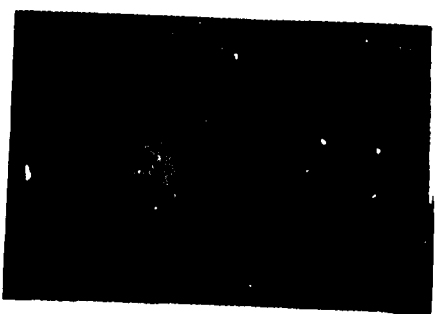
Fluorosis, moderate

Filiform papillary atrophy, moderate

Filiform papillary atrophy, patchy, severe and angular scars



Geographic tongue with glossitis



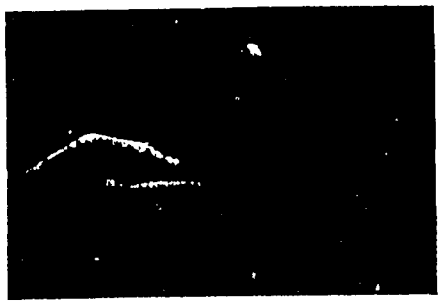
Beefy red marginal glossitis with central fissuring

FIGURE 5.—Continued

Goiter, grade I

Goiter, grade II

Goiter, grade III



Parotid enlargement

Pellagrous dermatitis

Pellagrous dermatitis, hands

Follicular hyperkeratosis, arm

Scrotal dermatitis

FIGURE 5.—Continued

VI

Biochemical Methods

Results of the biochemical studies together with the data from the physical examinations provide a means for estimating the proportion of the population in various broad zones of nutriture. When considered together with the physical examinations and dietary data, the biochemical studies make possible a more definitive appraisal of the nutritional status of a population.

The equipment and supply list in chapter X has been compiled to supply all the essentials for the prescribed and elective biochemical methods for blood and urine analysis. Sufficient equipment and supplies have been included to allow completion of the prescribed analyses on approximately 150 blood and 150 urine samples per week. Sufficient quantities of chemicals have been listed to permit analysis of approximately 4,000 samples. For most surveys a total of from 500 to 1,000 blood and urine samples should be analyzed; however, the actual number of determinations will vary with the overall sampling of the the population and personnel resources. An excess supply of chemicals and expendable equipment has been purposely included so as to enable the host country to continue to operate a clinical biochemical laboratory.

The biochemical tests are to be performed on both blood and urine specimens from a sample of the subjects who are given clinical examinations. Once these persons have entered the clinical detailed examination phase, individuals are selected systematically for biochemical testing as predetermined according to the expected sample size. (See chs. IV and V.) The selected individuals are then routed through the biochemical line where blood is drawn and a urine sample is collected. In most cases practical considerations dictate that "casual" urine specimens are collected, even though fasting 6-hour urine samples are to be preferred.

The number of biochemical samples to be collected depends (*a*) upon the number of individuals examined clinically, (*b*) the fulfillment of statistical requirements for a sufficient sample size, and (*c*) the capacity of the laboratory to complete a given number of analyses either on receipt or within the period of time that stored samples may be kept without deterioration. In military populations the sample size can

usually be predetermined so that systematic selection of the biochemical subjects is a simple matter. In civilian populations the sample size at a given location is rarely known in advance. Under such circumstances it may be better to obtain more than a full day's laboratory load. From this a systematic selection of samples can be made and, in case of a gross laboratory overload, the field director can decide which samples are to be discarded. All blood samples are mixed and the well-stoppered labeled containers are placed in an ice-filled thermos. The urine specimens are acidified to a pH less than 3 prior to transport to the central laboratory for analysis. The standard determinations usually made are hematocrit, hemoglobin, total plasma protein, vitamin C, vitamin A, and carotene of the blood; riboflavin, thiamine, N′-methylnicotinamide, and creatinine in the urine. Certain other analyses may be required as the situation demands.

Equipment for Blood and Urine Collection

The following list of equipment to be taken into the field is sufficient to obtain approximately 80 to 100 samples. This equipment should fit conveniently into three or four small boxes in such a manner as to facilitate its use in the field. The method of packing should be standardized; the containers should be such that they can be used over again routinely, and of course they must be convenient to handle.

Urine sampling equipment ("casual" method) [1]

Polyethylene bottles, 1 liter	50
Bottles, brown, 60 ml, screw cap	288
Hydrochloric acid, 1.0 N, 1 liter	1
Serological pipet, 10 ml	6
Masking tape, 2-inch roll	4

Blood sampling equipment (vacutainer technic)

B.D. vacutainer 3200Q [2] with EDTA (ethylene diamine tetra-acetic acid)	110
Needles, disposable sterile, 20-gage, double-ended vacutainer	60
Plastic holder for vacutainer needles	12
Swab (2″ x 2″) gauge, package of 100	3
Test tubes, 13 x 10 mm	40
Basins, plastic, 8-quart size	3
Brush, test tube	1
Detergent, 100 gm bottle	1
Evaporating dish, 200 ml (for alcohol swabs)	3
Cotton, nonabsorbent, 1 lb	2
Alcohol, 95 percent, 500 ml	1
Alcohol, 70 percent, 500 ml	3
Wire test tube rack, 40-hole	2
Wire basket, 6″ x 6″ (partitioned)	2

[1] See p. 111 for alternative equipment lists and procedures for drawing blood samples using the syringe technic and for collecting timed urine specimens.

[2] Becton Dickinson; see p. 299 for supply sources for equipment.

Syringe, serological, 20 ml	
Insulated food containers, 4 gal (for refrigeration)	
Wide-mouth thermos bottle, 2-quart (for shipping blood)	
Masking tape, 2-inch roll	
Rubber tubing for tourniquet	
Lantern, large—2 spare batteries	
Honing stone, and light oil, 2 oz can (for sharpening needles)	
Saline, physiological, 1 liter bottle	
Stove, gasoline, 2-burner	
Tongs	
Forceps	
Cooker, pressure, 4-quart	
Cleansing tissue, small size box	
Towels, laboratory	1
Pencils, No. 2	
Tablets, writing, 8½" x 11"	
Carbon paper, 8½" x 11" sheets	
Laboratory book, for recording raw data	
Twine, ball	
Pencil, red wax	
Laboratory coats	as require
Ice	as require

Blood for Macro Analyses

The samples needed for the analysis are (*a*) 3 to 5 ml of unclotted blood to be stored at icebox temperature (not frozen) for the determination of hemoglobin, hematocrit, and plasma protein; and (*b*) 8 to 10 ml of blood to be stored at icebox temperature (not frozen) for the determination of vitamin C, vitamin A, and carotene.

Either serum or plasma may be used interchangeably for any determination where one or the other is specified, without any change in results except for protein.

Sterilization of Needles

By Autoclaving or Hot Air Oven

Pack a small amount of cotton in the bottom of as many test tubes as there are needles to be sterilized. Place a vacutainer needle in each tube with the blood-drawing tip down. Plug the tubes with cotton and sterilize in an autoclave for 20 minutes at 121° C. If a pressure cooker is used, sterilize for 20 minutes at the maximum possible pressure. If a hot air oven is available, sterilize at 170° C for one hour. Avoid overheating to prevent charring and the release of oils from the cotton.

For use, remove cotton plug, place the plastic vacutainer holder in mouth of test tube and invert the tube. The needle should fall more or less into place so that it can be readily screwed into the holder. If vacutainer needle adapters are used, they need not be sterilized. They

can be attached to the plastic holders and the needles (sterilized as above) can be seated as described.

As needles are used they should be cleaned and returned to the test tubes *with the blood-drawing tip up.* If this procedure is followed, there will be no danger of confusing sterile and nonsterile needles in the field.

By Boiling

(Sterilization by boiling is not recommended, although widely practiced.)

Prior to use, boil needles for at least 20 minutes in an electric sterilizer or in a metal pan. Be certain that the water actually boils—in many instances there will be insufficient amperage to operate the electric sterilizer properly. After sterilization, needles can be stored in a covered receptacle containing 70–80 percent ethyl alcohol.

For use, grasp a needle by its shank with clean forceps, screw into plastic holder, and shake off excess alcohol.

Drawing of Blood

A table (6′ x 3′ is a convenient size) is arranged so that the subject sits on a chair at one end with his right or left arm straightened over the table conveniently for blood to be drawn from the antecubital vein. Racks containing the vacutainer tubes are placed near the subject's arm. Each tube should be labeled with masking tape. Dishes containing the sponges (dry and in alcohol) and needles are placed in a convenient position. At the other end of the table is a basin containing clean water with a thin layer of cotton on the bottom. The middle of the table is reserved for the plastic holders and extra vacutainer tubes.

Two men are engaged in these activities. The subjects are lined up in single file. Each comes up in turn, sits in the chair, and straightens out his arm over the table. The assistant cleans the antecubital arm area and applies the tourniquet. Meanwhile, the operator screws the needle into the plastic holder, taking care to keep the sharp end sterile. He next takes the vacutainer tube and inserts it into the plastic holder allowing the short end of the needle to pierce the rubber stopper until the end of the needle is level with the mark on the adapter. The unit—tube, holder, and needle—is now one. The excess alcohol is wiped from the arm, the needle is inserted into the vein, and the tube is pushed the rest of the way into the holder. This breaks the vacuum and blood will be sucked into the tube until the pressure is equalized. The tourniquet is released and the tube is removed, leaving the plastic holder and needle in place. Slight pressure with cotton gauze over the vein will control the flow of blood.

When filled the vacutainer tube is removed, then the needle and holder. A dry cotton gauze is placed in the antecubital space, and the

subject is released with instructions to maintain firm pressure by hand for 5 minutes to prevent formation of a hematoma. Mere flexing of the elbow is insufficient to prevent hematomas and may even increase bleeding.

The assistant removes the needle from the holder, attaches it to the special cleaning bulb, and rinses it several times with 0.9 percent NaCl solution. This is an extremely important step. If carried out immediately after the blood is drawn, it will save the considerable time that is necessary to clean a needle in which blood has been allowed to clot. The holder is then rinsed in cold water.

The assistant numbers the waterproof labels on the tubes with a ballpoint pen or indelible pencil. Immediately after its removal from the holder, the tube containing oxalate is inverted *gently* 20 times to mix the blood thoroughly. It is then placed on ice. Do not remove the stoppers from these tubes. Make certain that the labels being used *will withstand water immersion* and retain the identifying numbers.

The blood specimens are packed upright in an iced thermos jug and readied for shipment to the main laboratory.

After the day's blood samples have been taken, each needle should be washed in detergent, rinsed in water, and then checked for cleanliness. Those needles that have been used with vacutainer needle adapters should be removed from the adapter and each cleaned separately. Otherwise, they tend to accumulate blood at the needle-adapter joint and "freeze." Check each needle for burs and sharpness. If they require sharpening, use an oiled (mineral oil) stone hone. After sharpening, wash thoroughly, rinse, and replace the needles in test tubes for resterilization.

If no new vacutainers are available, it may be necessary to "regenerate" old ones. This can be accomplished simply in the field by replacing the rubber stopper of a clean dry vacutainer containing appropriate quantity of mixed oxalate or heparin, inserting a 22-gage needle through the stopper and sucking out the air with a 20-ml syringe. The needle is snapped out of the rubber stopper when the syringe barrel has reached its full upward excursion. Vacutainers should be regenerated on the morning of use because they tend to lose vacuum if made up several days in advance.

Fingertip Blood for Micro Analyses

Equipment

Blood collecting tubes

a. Heparinized tubes 1.5–1.75 mm diameter, 75 mm long
b. 150 mm^3 capacity melting point capillary tubes 1.5–2.0 mm in diameter by 100 mm long

Serological tubes 6 x 50 mm
Cork stoppers to fit 6 x 50 mm tubes

"Pyseal" cement for sealing capillaries
Burner—automatic precision for alcohol
Hemolet—disposable blood lancets
Ethyl alcohol, 70 percent
Cotton swabs
Sahli hemoglobin pipet, 20 mm^3, and rubber tubing
Hand centrifuge
Levy-Lang constriction pipets, 20 μl, 8 μl and rubber tubing
Trichloroacetic acid, 5 percent
Refrigeration facilities

Procedure

The fingertip is first washed with 70 percent alcohol, dried and lanced with a hemolet. The application of a film of sterile silicon grease to the fingertip after the alcohol washing will considerably increase the flow of blood. The grease may be conveniently spread with a sterile hemolet. After the first drop of blood has been blotted off, a Sahli hemoglobin pipet is filled. The blood thus obtained is expelled into 5 ml of Drabkin's reagent contained in a Coleman 19 x 150 mm cuvette. The pipet is rinsed five times with the solution in the cuvette. The cuvette is then stoppered with a clean rubber stopper, inverted to complete the mixing, and stored for transport to the main laboratory.

Two of the smaller heparinized capillary tubes are then filled about two-thirds full of blood from the fingertip, care being taken to keep one end of the capillary dry. The column of blood is "centered" to leave unfilled space at each end of the tube. The ends are then sealed in the alcohol flame. Alternately, "Pyseal" may be used for sealing by heating the latter in a flame and applying it to the dry end of the capillary which has also been warmed. A tight seal in this procedure requires that the glass be warm enough for the wax to "wet" it. Critocaps, plastic stoppers, which are available commercially, may be used. Two, preferably three, of the larger tubes (150 mm^3) are also filled and sealed in the same manner.

All the capillary tubes of blood from a given subject are wrapped together by rolling them in a small square of paper. The end of the paper is folded over one sealed end of the tubes and fastened with scotch tape. The whole package is then inserted into an ordinary test tube. If possible the samples should be stored at or near 4° C while awaiting transportation to the base laboratory.

Serum

The heparinized tubes are placed in the microhematocrit centrifuge for determination of the hematocrit. After the hematocrit is read, the plasma can be removed from the tube for plasma protein determinations by scratching the tube just above the red cell layer with a diamond-point pencil and breaking. The serum is removed readily

by a Levy-Lang constriction pipet. The larger capillary tubes may be centrifuged in an ordinary centrifuge in the tubes in which they are transported. The serum is removed from centrifuged capillary tubes as described above for the heparinized tubes.

If samples are not to be transported to the base laboratory until the next day, the serum samples for vitamin C analysis should be aliquoted and precipitated with 5 percent trichloroacetic acid. In the field, serum may be separated conveniently by use of a small hand centrifuge. Twenty μl amounts of serum are removed and placed in 6 x 30 mm test tubes and 80 μl of 5 percent trichloroacetic acid are added to each tube. The tubes are corked and refrigerated or frozen if possible.

Urine

The sample taken for the determination of thiamine, riboflavin, N′-methylnicotinamide and creatinine is an acidified 50 ml aliquot of the casual urine sample collected in a 1-liter bottle. This sample also furnishes 10-ml subsamples as needed in the preparation of a composite urine sample for fluoride analysis (see dental section). If urinary iodine is to be determined, a 30-ml aliquot should be prepared from the casual urine sample collected at the field laboratory.

Preparation of Aliquot Bottles

At the base laboratory, 5.0 ml of 1.0 N HCl is added to each of the plastic collection bottles. This should adjust the specimen to a pH of 2–3. It is best to check the pH of each sample with pH paper after transfer to the 60-ml brown aliquot bottles and acidify if necessary.

Collection

In the field, a suitably shielded area is selected for urine collection. As each subject presents himself, his full name and McBee card number (detailed card) are recorded in a notebook. *A separate notebook page should be used for each location.* It is imperative that handwriting be legible and that the date and locations be entered. He is then given a numbered polyethylene bottle (female subjects are given an appropriate wide-mouthed container) and is asked to urinate into it. An aliquot of about 50 ml (a second aliquot of 30 ml if iodine analyses are to be performed) is transferred to a brown 60 ml bottle containing HCl and the excess urine is discarded. After mixing, the pH of the specimen is checked with pH paper. If the pH is greater than 3.0, then more HCl should be added. The bottle is clearly labeled with the subject's McBee card number (detailed card), using masking tape and a lead pencil or other water-resistant marking device.

The numbered bottles are protected from light and are packed, preferably in iced containers (4–10° C), for immediate shipment to the central laboratory. Refrigeration of the urine is less critical than

in the case of blood; if the samples are properly acidified and protected from light, no loss of urinary B vitamins will occur for at least 3 days, even at ambient temperatures of 25-30° C.

At the end of the day's work the plastic bottles are well rinsed with ordinary tap water in preparation for use during the next sampling period.

Laboratory Facilities and Organization

The laboratory space should be equipped with running water (preferably hot and cold) and a large sink (at least 2′ x 3′ if possible) for washing glassware.

A constant source of electricity capable of a 100-ampere load is necessary to insure the proper functioning of the laboratory electrical equipment. During preliminary planning for the survey, the exact nature of the electrical supply at the prospective laboratory site should be determined so that proper electrical equipment, transformers, and adapters can be purchased. A telephone is essential. Refrigeration facilities (about 10 cubic feet) are required, and at least 2 cubic feet of freezing space is highly desirable.

A minimum of 80 linear feet of conventional laboratory bench space is required—more is desirable. Bench storage facilities for glassware and a separate storage area for chemicals are essential. A small semidarkened laboratory area (separate if possible) is necessary for carrying out light-sensitive (riboflavin) determinations. Other housing needs at the laboratory headquarters include a separate room for storage of the clinical and dietary supplies, a survey office for the director which is of sufficient size to accommodate the secretarial work, and a storage area for field supplies. It is highly desirable to have a conference room of a size adequate to accommodate the whole team.

Six local biochemists, chemists, or technicians are needed. These individuals should be trained in methods of laboratory analysis. It is important that one or more of the group speak English. They should be personnel who will continue in nutritional research and/or biochemistry after completion of the survey.

In addition, the following local personnel are needed:

Dishwashers or helpers—2 (experience not necessary but helpful).

Secretary-interpreter—1. This position requires an intelligent bilingual (both spoken and written) typist-secretary competent to deal with minor administrative details that arise.

Clerk-driver. This man should have a good knowledge of the city in which the laboratory is located since he will be called upon frequently to procure samples, make purchases for the laboratory, deliver official messages, and the like.

The first assignment faced by the laboratory director upon his arrival is to locate the survey equipment and have it delivered to the laboratory site. This may have been done, but frequently the equipment is stored in a warehouse for release only upon arrival of the

survey team. The shipment is a bulky one—a 2½-ton truck and three or four men will be required for its transportation.

A large open area adjacent to the laboratory is desirable for unpacking. Tools for uncrating large pieces of equipment are packed together in an appropriately labeled box. The large wooden shipping boxes with padlocked hasps are to be used by the clinical and dietary teams and must be saved. It is also necessary to save a number of sturdy cardboard cartons and an ample supply of insulating material for the use of team members, especially for the shipment of food and other samples to the United States at the end of the survey. The metal cans in which corrosive chemicals are packed should also be saved since they are useful in packing food composites for shipment.

As the equipment is unpacked it should be checked against the master shipping list and inspected for damage. All packing material should be examined carefully to make certain nothing is discarded unintentionally. Glassware is best left in its original packing until it is washed. As it is unpacked, it is convenient to place the equipment in designated areas marked for chemicals, glassware, electrical equipment, hardware, secretarial, clinical, and dietary. If possible, these marked areas should coincide with the areas of intended use.

Once unpacked, certain priorities should be observed for efficient operation. The oven should be installed to expedite the drying of glassware. Since the initial need for distilled water is great, a distillation apparatus or deionizer should be installed, or other arrangements should be made in advance for initial supplies of distilled water. All electrical equipment should be checked immediately. In the case of the Coleman spectrophotometer, the storage battery will need to be filled with the proper strength sulfuric acid (sp gr=1.28) and brought to full charge.

Generally speaking, one person should be available for each of the following determinations: thiamine, riboflavin, vitamin C, vitamin A (and carotene). One worker can usually be responsible for the hematocrit, hemoglobin, and plasma protein estimations and another can do both the N′-methylnicotinamide and creatinine methods. If more dual responsibility for methods is necessary, then thiamine-creatinine, riboflavin-N′-methylnicotinamide, and vitamin A-vitamin C are the most convenient working pairs. Excessive workloads should be avoided, however, since it leads inevitably to a loss of accuracy.

In the interests of training, it is beneficial for each of the laboratory workers to acquire experience with a number of the methods. Whether this can be accomplished depends upon a variety of factors, and its feasibility must be determined by the laboratory director and his counterpart.

Personnel should be instructed in the laboratory procedures to be used. Particular emphasis should be placed upon the need for keeping

neat, legible, and complete laboratory notes and upon the laboratory control program. The necessity of completing all calculations at the end of a day's work should also be stressed.

Recording of Laboratory Data

Each procedure will be recorded in a separate book. In the front of the book the method will be described with attention to the details of preparation of standards, aliquoting technic, and the conditions of crucial measurements.

All raw data for the standard curves will be recorded and plotted, and the method of calculation of results will be described. All factors, tables of equivalence, or plot readings used will also be recorded.

A separate page will be used for each day's samples. All results should be signed by the worker who made the determination. McBee card subject identification numbers will be entered in sequence from lowest to highest. Laboratory controls will be clearly identified. *The laboratory data must be legible.*

For each day's calculations the factor, curve, or formula used will be identified. The operator should clearly record mistakes, complications, or uncertainties as footnotes. The initials of the operator will be recorded daily along with those of the person checking the calculations. The laboratory director should check the recording of data at frequent intervals.

A separate notebook should be available for the recording of suggestions for use in future surveys by laboratory personnel.

Replication of Determinations

Because of the limited amount of blood that is drawn, it is not possible routinely to do serum vitamin determinations in duplicate. With urine, the methods are rather time consuming and duplicate determinations may not be possible. However, some duplicate determinations should be made on samples as normally handled or discussed under laboratory control. (See under "Laboratory Control" below.)

Sensitivity of Methods

Spectrophotometric Methods

It is to be expected that in sampling large populations, some individual values will be found that fall below the limits of sensitivity of the methods employed. Although values may be calculated for such determinations, the analyst should understand at what point values lose significance because of limitations of the method.

Plasma or Serum Ascorbic Acid

It is entirely possible to have serum ascorbic acid values of 0. Therefore, extremely low readings may be obtained and these usually are not appreciably influenced by increasing the sample size.

Plasma or Serum Vitamin A

Samples containing around 10 μg per 100 ml will have a corrected optical density reading of about 0.03. Values less than this should not be calculated but should be recorded as $<10\ \mu g$.

Plasma or Serum Carotene

Samples containing 30 μg per 100 ml will give an optical density reading of about 0.03. Values less than this should not be calculated but should be recorded as $<30\ \mu g$.

Creatinine in Urine

This is an extremely critical determination since urinary vitamin excretions are usually related to it. For this reason any sample having an optical density of less than *0.03* should *be repeated*, with the sample size increased threefold.

Fluorometric Methods

In general, the errors encountered in fluorometry are greater than those in colorimetry. In the analysis of urine, considerable variation will occur from sample to sample with respect to interfering substances. Very concentrated urines (less than 100 ml/6 hrs) will frequently give abnormally high readings, and in such cases the sample should be diluted and then reanalyzed. When samples read out of range, either high or low, it is a simple matter to reset the instrument with the fluorescent standard (quinine or fluorescein).

Laboratory Control

Although the methods described in this Manual have been selected for their relative reliability, the experienced chemist knows that any analytical procedure is subject to numerous possible errors. Consequently, the laboratory supervisor should routinely check all methods to be certain of valid results. The setting up of a method should include recovery experiments and tests on duplicate samples, including blind duplicates. All of these data should be recorded in the notebook. It is frequently stated that once a method has been established, the continuation of results of similar magnitude will usually suffice to indicate validity. However, in nutrition surveys where wide variations, or even consistent abnormal values, may result, it is not possible to judge a method merely by the "appearance" of results.

It is essential that a laboratory control program be included in order to obtain a measure of precision (reproducibility) and accuracy (conformity to absolute value). Under field conditions, it is most

practical to arrive at an estimate of the precision of a procedure by repeated analysis of a control urine or serum sample.

In preparing these control samples, it is necessary to have a sufficient amount of homogeneous urine or serum sample stored under such conditions that the concentration of the nutrient in question is stable over a long period of time. It is then dispensed in small aliquots into a large number of small test tubes which are well stoppered for storage. Storage in the frozen state is recommended, but may not always be possible. Specific instructions as to the preparation and storage of controls are included in the actual description of each method. Ideally, three controls (of low, average, and high content of the nutrient in question) should be analyzed for each procedure. However, practicality may dictate that only one control be prepared. Each control should be subjected to a series of five consecutive daily determinations in quadruplicate during the first week of laboratory operation. From these 20 determinations a mean and a standard deviation can be calculated for each control.

The standard deviation is calculated by:

$$\text{S.D.} = \sqrt{\frac{\sum X^2}{n-1}}$$

when n is the number of determinations, Σ is the sum of, and X is the deviation of each determination from the mean.

Once the mean values and standard deviations have been determined for the control samples, an allowable error can be calculated. This is conveniently set at ± 2 standard deviations, since some 95 percent of an infinite number of determinations should fall within these limits. These control samples are then introduced into the daily laboratory runs. When the result of a daily determination falls within the ± 2 standard deviation limits, it may be inferred that the method is "in control" on that day. If it does not, the method is "out of control" and the analyst must determine the reason for the erroneous results.

Quality control charts should be prepared with the value of the control plotted as the ordinate, and the chronologic order in which the analyses were performed as the abscissa. The mean value of the control is drawn as a line parallel to the X axis and lines designating the ± 2 standard deviations (allowable error) are also inserted. This permits the laboratory director to determine at a glance whether or not a procedure is in control.

Laboratory Reproducibility Data Using ICNND Methods

The results of a reproducibility study carried out in one ICNND survey are presented in table 1. The control samples for analysis were prepared as described on the previous page and each was analyzed for each constituent 10 to 20 times during the first week of the survey

to determine a "true" value and its standard deviation. Subsamples were frozen and one was then introduced daily into the laboratory load. Methods were considered to be in control on a given day if the daily standard value fell within 2 standard deviations of the true mean as determined by the initial analysis. Since each operator knew which sample was the control, the reproducibility results shown here are probably somewhat biased. It is preferable that the controls be included in the daily run without identification to the technician; i.e., they should be "blind" controls.

TABLE 1.—*Biochemical laboratory reproducibility studies under survey conditions*

BLOOD DETERMINATIONS

Determination	Standardization				Daily analyses			
	No.	Mean	S.D.[2]	Coef.[3] var. percent	No.	Mean	S.D.[2]	Coef.[3] var. percent
Hemoglobin:[1] gm/100 ml					36	14.36	0.10	0.7
Vitamin A: μg/100 ml	10	54.8	2.2	4.0	30	54.2	2.6	4.7
Carotene: μg/100 ml	10	64.0	1.3	2.1	31	60.8	3.7	6.4
Serum protein: gm/100 ml (microbiuret)	20	5.75	.12	2.1	36	5.92	.17	2.9
Vitamin C: mg/100 ml (macro method)	20	.93	.07	7.5	25			
Vitamin C: mg/100 ml (micro method)	5	1.73	.12	6.9				

[1] Instrument variability only.
[2] S.D. = standard deviation.
[3] Coefficient of variation.

Certain of these data demand comment. The data on hemoglobin differ from the others in that it was not possible to run the same blood sample each day. Instead, the standard hemoglobin solution was read each day. The standard deviation in this case thus represents instrument variability and the ability of the operator to read the scale in a reproducible manner. The serum protein procedure, which is very simple, had a coefficient of variation of only 2.9 percent, whereas the more complex vitamin A and carotene determinations had coefficients of variations at 4.7 and 6.4 percent, respectively. The ascorbic acid control procedure (macro method) was not satisfactory, since the ascorbic acid deteriorated during storage and no data are available regarding the day-to-day reproducibility of this method. It will be observed, however, that in the initial analysis it showed a coefficient of variation of 7.5 percent which was relatively high. The method used was the dichlorophenol indophenol method. The micro procedure for ascorbic acid was slightly more reproducible than the macro technic. A more satisfactory control procedure for the ascorbic acid methods requires the preparation daily of a fresh standard.

Reproducibility data for urinary methods are shown in table 2. The data compare the reproducibilities found in a study at a university school of medicine in the United States with those found under actual survey conditions. It is not too surprising that the analyses run during the survey proved to be more variable than those found at the U.S. lab-

oratory. This is in part the result of the pressure to complete work during the survey. It is interesting, however, to note that the degrees of reproducibility of the vitamin methods fell in the same order in both laboratories. Thiamine was most reproducible and N′-methylnicotinamide was the least reproducible. The reproducibility of the creatinine method is reassuring, since all urinary results are calculated in terms of creatinine. It should be mentioned that a creatinine curve was run daily and a factor determined which was used for calculation of the day's samples.

TABLE 2.—*Biochemical reproducibility studies in 2 laboratories*

URINE DETERMINATIONS

Determination	U.S. Laboratory				Survey Laboratory							
	Standardization No.	Mean	S.D.[1]	Coef.[2] var. per cent	Standardization No.	Mean	S.D.[1]	Coef.[2] var. per cent	Daily analyses No.	Mean	S.D.[1]	Coef.[2] var. per cent
Thiamine, μg/ml	20	0.182	0.004	2.2	20	0.204	0.021	7.9	31	0.255	0.019	7.5
Riboflavin, μg/ml	20	.530	.035	6.7	29	.223	.029	13.0	37	.183	.019	10.4
N′ Methylnicotinamide, μg/ml	20	2.17	.20	9.2	20	2.80	.67	24.0	32	4.80	2.80	58.4
Creatinine, mg/ml						.962	.043	4.3		.948	.038	4.0

[1] S.D., standard deviation.
[2] Coefficient of variation.

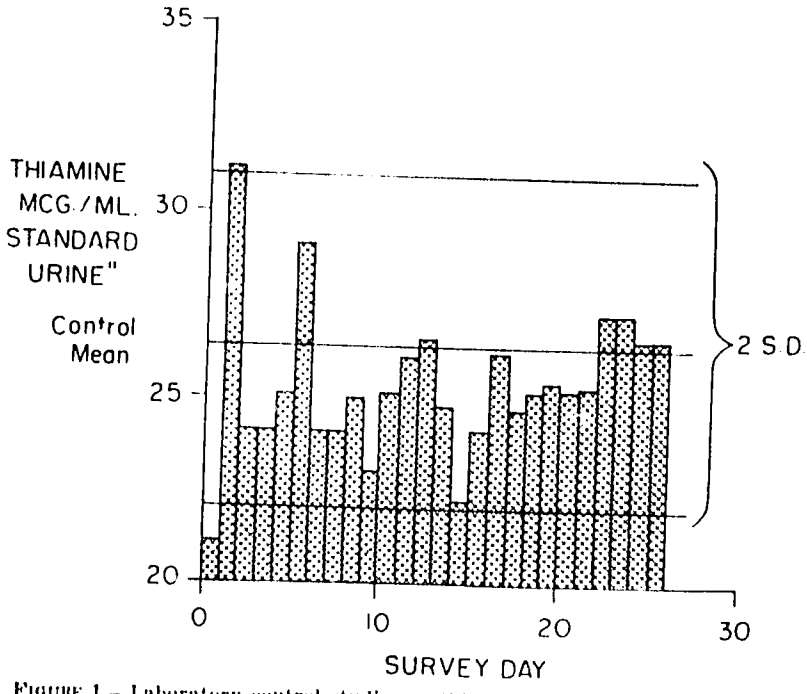


FIGURE 1.—Laboratory control studies on thiamine under survey conditions.

A typical method control chart for thiamine is seen in figure 1. The reproducibility was such that this method stayed "in control" throughout the survey, except for a single day.

The data seen in tables 1 and 2 give some idea of the reproducibility of the methods described in this survey Manual. The range of values found is considered to be entirely adequate for the type of work involved. It should be emphasized, however, that this procedure measures reproducibility and accuracy (absolute values). For this reason, the necessity for the use of pure, carefully prepared standard solutions cannot be overemphasized.

If there are situations where the control program described above cannot be employed, the laboratory director should carry out a control program of his own design.

Ranges of values found in healthy U.S. adult males[1]

Total plasma protein	6.5–8.2	gm/100 ml
Hemoglobin	13.5–17.0	gm/100 ml
Hematocrit	44–50	percent
Serum vitamin C	0.4–1.0	mg/100 ml
Serum vitamin A	25–70	μg/100 ml
Serum carotene	40–150	μg/100 ml
Urinary thiamine	60–130	μg/gm creatinine
Urinary riboflavin	80–270	μg/gm creatinine
Urinary N'-methylnicotinamide	1.6–4.3	mg/gm creatinine
Urinary creatinine	1.00–1.60	gm/day

[1] Ranges are average of values reported in the literature. Advisory Board on Quartermaster Research and Development, Committee on Foods. Methods for Evaluation of Nutritional Adequacy and Status—a Symposium. National Academy of Sciences National Research Council, Washington, D.C., December 1954.

Alternate Equipment and Procedures for Blood and Urine Collection

Although it is usually more convenient to collect blood specimens by the vacutainer technic, the syringe method may be used. Also, instead of collecting "casual" urine specimens, 6 hour timed urine samples may be obtained. Such specimens are to be preferred to "casual" samples and can best be collected from military groups in areas where the survey team spends several days. However, it is usually impractical to collect such samples from civilians or when the survey team is at a site only one day.

The fractions of blood and urine to be collected are those already described on pages 97 and 103. If these alternate procedures are elected, the following equipment should be taken into the field:

Urine sampling equipment ("timed" method)

Polyethylene bottles, 1 liter	50
Bottles, brown, 60 ml, screw cap	288
Hydrochloric acid, 1.0 N, 1 liter	1
Serological pipet, 10 ml	6
Masking tape, 2" roll	2

Urine sampling equipment ("timed" method)—Continued

Cylinder, graduated:	
100 ml	1
500 ml	1
1,000 ml	1

Blood sampling equipment (syringe technic)

Vials, screw cap, with labels:	
9 ml containing anticoagulant (for whole blood)	110
9 ml (for storage of plasma)	110
23 ml to receive 12 ml HPO_3 solution (for vitamin C)	110
Centrifuge tubes, 15 ml, containing anticoagulant, with labels and corks (or test tubes, 125 x 16mm)	160
Syringes, 20 ml	130
Needles, 20 gage, sterile	150
Gauze sponges (2" x 2"), package of 100	3
Basins, 8-quart size	3
Brushes, test tube	2
Detergent, 100 gm bottle	1
Evaporating dish, 200 ml (for alcohol swabs)	3
Alcohol, 95 percent, 1 pint	1
Alcohol, 70 percent, 1 pint	3
Wooden applicators, box	1
Syringes:	
1 ml	12
5 ml	4
10 ml	6
Racks (for whole blood vials)	6
Wire test tube racks, 40-hole	6
Wire basket, 6" x 6" (partitioned to hold centrifuge tubes in insulated cans)	4
Needles:	
No. 15, 4"	6
No. 22, 1"	12
No. 22, 6" (for hematocrit tubes)	4
Insulated food container, 4 gal	4
Wide-mouth thermos bottle	4
Masking tape, 2" roll	2
Rubber tubing for tourniquet, 2 ft lengths	4
Lantern, large—2 spare batteries	1
Saline, physiological, 250 ml bottle	1
Towels, laboratory	10
Pencil sharpener	1
Cleansing tissue, box	1
Pencils, No. 2	6
Tablets, writing, 8½" x 11"	3
Carbon paper, 8½" x 11" sheets	6
Twine, ball	1
Pencil, red wax	3
Stove, gasoline, 2-burner	1
Tongs	1
Forceps	2
Ice	As required
Laboratory coats	As required

Collection Procedures

Blood

Sterilization of Needles and Syringes

The sterilization and care of needles are described on page 99. Syringes should be washed thoroughly in soapy water and well rinsed. A syringe and its barrel can be wrapped in paper and sterilized in a hot air oven at 170° C for 1 hour. They can likewise be rolled in gauze and sterilized in an autoclave or presser cooker for 20 minutes at 121° C. If neither autoclave, pressure cooker, nor hot air oven is available, a reasonable assurance of sterility can be obtained by boiling them for 20 minutes. Syringes and needles *must* be completely dry before use. Otherwise hemolysis will occur.

Anticoagulant—Oxalate Mixture (Heller and Paul)

Dissolve 6 gm ammonium oxalate and 4 gm potassium oxalate in 250 ml of water. Pipet 0.25 ml of the oxalate solution into 125 x 16 mm Pyrex test tubes. This amount of oxalate mixture (10 mg) is sufficient for 5 ml of blood. Spread the oxalate solution in a film over the lower walls of the test tube and dry in an incubator at not over 50° C. (Fifteen ml centrifuge tubes may be used instead.)

Procedure

Needles and syringes are to be checked and sterilized as described above. This should be accomplished the previous day.

The table is arranged so that the subject sits on a chair at one end with his right arm straightened over the table so that one man may draw blood from an antecubital vein. Racks containing centrifuge tubes and vials with anticoagulant are placed near the subject's arm. Dishes containing sponges and needles in alcohol are placed conveniently within reach of the man drawing blood. A basin containing clean water is at the other end of the table. The space in the middle of the table is reserved for syringes.

A crew of three men engages in these activities. The subjects are asked to line up single file. Each individual comes up in turn, sits in the chair, and straightens out his arm over the table. One man cleans and dries the arm area. The operator applies a tourniquet and draws the blood. The second man on the side opposite the chair labels a centrifuge tube and a vial with the subject's McBee card number. When about 15 ml of blood have been drawn, the needle is removed from the arm, a dry cotton gauze is placed in the antecubital space and the subject is released with instructions to maintain firm pressure by hand for at least 5 minutes. Mere flexing of the elbow in some instances is not enough to prevent formation of hematomas and, in fact, may actually increase bleeding.

The needle is removed from the syringe.

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The man who has labeled the vial and centrifuge tube holds them in such a position that the operator can easily introduce about 2 ml of blood into the vial and the remainder of the blood into the centrifuge tube. *Do not shake.* The man who held the vial and tube caps the vial and stoppers the tube. He then mixes the blood in the vial thoroughly but gently by inverting at least 20 times. He then packs the blood specimens upright in an iced thermos jug and readies them for shipment to the main laboratory.

The third member of the crew washes used syringes and needles immediately after receiving them from the operator. A sterile needle is fitted on a clean syringe and the operator prepares to draw blood from a new subject. The man washing syringes must be sure that needles do not become plugged. Chipped syringes must be discarded. Sterilization of needles and syringes is *mandatory* to prevent the spread of infection, such as hepatitis. Be sure that the syringes and the oxalated vials are completely dry before use.

Urine

Preparation of Aliquot Bottles

At the base laboratory, 5.0 ml of 1.0 N HCl is added to each of the plastic collection bottles. This should adjust the acidity of the added specimens to a pH of 2–3. It is best to check the pH of each sample with pH paper after transfer to the 60-ml brown aliquot bottles and acidify if necessary.

Procedure

Collect fasting specimens in the morning over a 6-hour period of time. This is best accomplished in military groups by billeting the subjects together and arousing them at 12 midnight to empty their bladders completely. They are each given a numbered plastic bottle, and a half pint (200 ml) of water to drink to insure diuresis. They are then permitted to return to bed after being told that any urine passed during the night should be collected. At 6 a.m. the subjects are aroused and asked to urinate into the plastic collection bottles. The actual time of urination is recorded for each subject.

It is convenient to have a crew of three men for measuring and storing the urine. The first man measures the volume of each specimen, reports it to the recorder, and pours an aliquot of about 50 ml into the 60-ml bottle (a second 30-ml aliquot must be prepared if iodine analyses are to be performed). The excess urine is poured into a waste jar. The second man records the volumes, names, and McBee card numbers for each subject in a notebook. The third member of the crew is responsible for putting McBee card identification numbers on the 60-ml bottles, to correspond with the sample from the collection bottle.

The numbered specimens are placed in racks and stored, if possible, at 4°–10° C.

Hemoglobin by the Cyanmethemoglobin Method

REFERENCES

Hainline, A., Hemoglobin, *in* Standard Methods of Clinical Chemistry, Vol. II, 49-60, see esp. p. 52, Academic Press, New York, 1958.

Canman, R. K., Hemoglobin standard. Science *127*, 1376-1378, 1958.

Principle

Whole blood is diluted with a potassium ferricyanide-cyanide solution which oxidizes hemoglobin to methemoglobin, which is in turn converted to cyanmethemoglobin. The intensity of the color is measured photometrically. The procedure is standardized with blood which has been analyzed for its iron content or for oxygen with a Van Slyke gasometric apparatus.

Reagents

Potassium ferricyanide-cyanide solution: transfer the following reagent-grade chemicals to a 1-liter volumetric flask:

1 gm $NaHCO_3$
50 mg KCN
200 mg $K_3Fe(CN)_6$

Dilute to 1 liter with distilled water. Store in a brown bottle away from direct sunlight. Do not use for longer than 1 month.

Procedure

Measure 5.0 ml of the mixed cyanide solution into a cuvette. Add exactly 0.02 ml of well-mixed blood that contains an anticoagulant, using a Sahli hemoglobin pipet. Only carefully standardized pipets should be used. Rinse the pipet five times with the solution in the cuvette. Stopper the tube with a clean rubber stopper and invert five times. Let stand for 10 minutes and read in the spectrophotometer at 540 mμ using the mixed cyanide solution as the blank.

Under normal survey conditions the blood is from 12 to 24 hours old before the procedure can be carried out. It is therefore important that the tube be inverted gently (not shaken) at least 30 times before an aliquot is taken. After filling to the mark, the tip of the pipet should be carefully wiped clean with cleansing tissue. Failure to do this will result in the introduction of a serious error.

Standardization

Use the Walter Reed Army Institute of Research cyanmethemoglobin standards to prepare the standard curve as specified in their instructions. Calculate a factor from the curve and use it in the calculations.

Day-by-day quality control of hemoglobin determinations is difficult because of the lack of a stable biological standard. Some estimate of precision can be obtained by measuring every fifth sample in duplicate.

Precaution

The cyanide in the reagent is *poisonous*. Care should be given in pipeting, and particularly in discarding old solutions. Always flush a sink well with water *before* and *after* discarding a solution. The liter of solution contains only about one-fourth of a lethal dose, but this is no invitation for careless handling.

Hematocrit

REFERENCES

Wintrobe, M. M., Macroscopic examination of the blood. Am. J. Med. Sc. *185*, 58–71, 1933.

Gradwohl, R. B. H., Clinical Laboratory Methods and Diagnosis. C. V. Mosby Co., St. Louis, 1935.

Kolmer, J. A., Approved Laboratory Technic, 3d edition, Appleton-Century, New York, 1941.

Todd, J. C., and Sanford, A. H., Clinical Diagnosis by Laboratory Methods, 12th edition, W. B. Saunders Co., Philadelphia, 1953.

Principle

A sample of the blood is introduced into a Wintrobe hematocrit tube approximately to the 100 mark (not above). It is centrifuged at 3,000 rpm until no further packing of the cells occurs. If the tube has been filled exactly to the 100 mark, the red cell volume percent is read directly on the scale on the tube from the height of the column of red cells. If the tube has not been filled exactly to the 100 mark, the red cell volume percent is calculated.

Apparatus

Centrifuge
Hematocrit tubes, Wintrobe, with rubber caps
Transfer needles, 5", No. 18
Two-ml syringe

Procedure

Venous blood is drawn into a 7-ml vacutainer containing EDTA (ethylene diamine tetra-acetic acid). After the sample has been well mixed by inversion (30 times), about 1 ml is drawn into a syringe fitted with a long transfer needle. The tip is then passed to the bottom of the hematocrit tube and the blood is slowly expelled to fill the tube to the 100 mark. There should be no bubbles. The tube is capped and centrifuged at 3,000 rpm (checked at intervals with the tachometer) for 30 minutes and the height of the red cell column is read from the graduations on the tube. The reading should be made of the height of the red cells only and should not include the "buffy coat" above them. Centrifugation is continued for another 15 minutes in order to be sure that complete packing of the cells has been obtained.

Precaution

It is important that the centrifugal force, and not necessarily the rpm, be uniform from one run to the next. This is dependent upon the radius of the centrifugal head and the speed.

Calculation

If the tube is filled exactly to the 100 mark:

$$\text{Height of packed cells} \times 100 = \text{percent red cell volume}$$

If the tube is not filled exactly to the 100 mark:

$$\frac{\text{Height of packed cells}}{\text{Height of total blood column}} \times 100 = \text{percent red cell volume}$$

Serum Vitamin C (Ascorbic Acid)—Dinitrophenylhydrazine Method

Two satisfactory methods for determining vitamin C are available—the dye method using 2,6-dichlorobenzenone indophenol which measures the reduced vitamin and the dinitrophenylhydrazine procedure which measures the total ascorbic acid. Inasmuch as the dinitrophenylhydrazine procedure may be applied at both the macro and micro levels, this latter method is recommended. It represents a combination of the technics described in the following references.

REFERENCES

Roe, J. H., and Kuether, C. A., The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. *147*, 399-407, 1943.

Lowry, O. H., Lopez, J. A., and Bessey, O. A., The determination of ascorbic acid in small amounts of blood serum. J. Biol. Chem. *160*, 609-615, 1945.

Bessey, O. A., Lowry, O. H., and Brock, M. J., The quantitative determination of ascorbic acid in small amounts of white blood cells and platelets. J. Biol. Chem. *168*, 197-205, 1947.

Schaffert, R. R., and Kingsley, G. R., A rapid, simple method for the determination of reduced, dehydro-, and total ascorbic acid in biological material. J. Biol. Chem. *212*, 59-68, 1955.

Principle

Ascorbic acid is oxidized to dehydroascorbic acid by copper ion. The 2,4-dinitrophenylhydrazine derivative is dissolved in concentrated sulfuric acid and the red-orange product is measured spectrophotometrically.

Apparatus

Coleman Jr. spectrophotometer, Model 6
Test tubes, 13 x 100 mm
Cuvettes, 19 x 150 mm
A water bath set at 60° C
An electric centrifuge
An ice bath

Syringe pipet, 10 ml
Pipets, transfer, 1, 2 and 3 ml
Interval timer

Reagents

2,4-dinitrophenylhydrazine reagent: dissolve 2.2 gm of the reagent in 100 ml of 9 N sulfuric acid (3 parts of water and 1 part of concentrated sulfuric acid). Allow to stand overnight and filter.

A 5-percent trichloroacetic acid solution in distilled water: dissolve 50 gm of trichloroacetic acid in a small amount of water and dilute to 1 liter. Store in the refrigerator.

A 65-percent sulfuric acid solution: to 300 ml of distilled water, add *with caution* 700 ml of concentrated sulfuric acid. Do this mixing carefully in the sink.

A 5-percent thiourea solution in distilled water: dissolve 25 gm of thiourea in distilled water and dilute to a final volume of 500 ml. Store in the refrigerator. This solution is stable for about 2 months.

A 0.6-percent copper sulfate solution in water: dissolve 0.6 gm of copper sulfate in distilled water and dilute to a final volume of 100 ml.

Dinitrophenylhydrazine-thiourea-copper sulfate reagent (DTC): mix 1 volume of 5 percent thiourea + 1 volume of 0.6 percent copper sulfate + 20 volumes of 2.2 percent dinitrophenylhydrazine. This reagent is stable for at least 1 week at 4° C, but should be centrifuged before use unless crystal clear. Under ordinary conditions 200 ml will be used each week in the macro method.

A 5-percent metaphosphoric acid-10 percent acetic acid solution: dissolve 10 gm of metaphosphoric acid pellets in 10 percent acetic acid and dilute to 200 ml with the acid.

Stock vitamin C standard: dry 1 gm of vitamin C for 1 hour at 105° C. Place in desiccator overnight. Dissolve exactly 100 mg of this in a solution of 10 percent acetic acid containing 5 percent metaphosphoric acid and dilute to a final volume of 100 ml. This solution, even when frozen, is stable for only a few days.

Working vitamin C standard: dilute the stock standard 1 : 100 with 5 percent trichloroacetic acid solution.

Procedure

Standard Curve

To a series of ordinary test tubes, add 0.1, 0.5, 1.0, 2.0, and 4.0 ml of the working standard, respectively.

Using a 10-ml serological pipet, bring the volume in each tube to a final volume of 10.0 ml with 5 percent trichloroacetic acid. Mix well.

Remove duplicate 3-ml aliquots from each tube and place in a series of Coleman tubes (19 x 150 mm). Use 3.0 ml of 5 percent trichloroacetic acid as the blank.

Add 1 ml of the DTC reagent to each tube, mix well, and incubate in a 60° C water bath for exactly 1 hour.

Chill the tubes in an ice bath and slowly add 5 ml of 65 percent H_2SO_4 to each tube with vigorous mixing. This is readily added from a syringe pipet by releasing the plunger of the syringe and letting it deliver the solution by its own weight.

Allow the tubes to stand at room temperature for a least 20 minutes. Set the Coleman Jr. spectrophotometer at zero optical density with the blank and read the unknowns at 515 mμ.

Since the standard solutions have been subjected to the same procedure as the serum samples, the above concentrations are equivalent to the following values (in mg per 100 ml) of vitamin C: 0.1, 0.5, 1.0, 2.0, and 4.0. Plot the optical densities against these values and calculate a factor for the direct conversion of optical density values to mg per 100 ml of vitamin C.

Plasma or Serum

Place exactly 8 ml of 5 percent trichloroacetic acid in an ordinary test tube.

Add slowly 2.0 ml of serum.

Mix well and centrifuge for 10 minutes at 3,000 rpm.

Remove two 3-ml aliquots of the clear supernatant fluid and place in separate Coleman cuvettes.

To each cuvette add 1 ml of the DTC reagent and incubate both tubes at 60° C for 1 hour.

Chill the tubes in an ice bath and slowly add 5 ml of 65 percent sulfuric acid as described for the standard curve.

Remove the tubes from the ice bath and allow to stand at room temperature for 20 minutes.

Read the tubes at 515 mμ in the Coleman Jr. spectrophotometer, using a blank prepared by carrying 3 ml of 5 percent trichloroacetic acid through the above procedure.

By means of the factor calculated from the standard curve, estimate the milligrams per 100 ml of ascorbic acid in the sample.

Serum Vitamin C (Ascorbic Acid)—Micro Procedure

REFERENCES

Bessey, O. A., Lowry, O. H., and Brock, M. J., A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. *164*, 321-329, 1946.

Bessey, O. A., Lowry, O. H., and Brock, M. J., The quantitative determination of ascorbic acid in small amounts of white blood cells and platelets. J. Biol. Chem. *168*, 197-205, 1947.

Principle

This procedure is, with slight modifications, that of Bessey, Lowry, and Brock. A Beckman DU spectrophotometer with the appropriate micro attachments is the instrument of choice for the spectrophotometric measurements but a Beckman Model 151 microspectro-

photometer can also be used. The same solutions are used as those described for the macro procedure.

Apparatus

A Beckman DU spectrophotometer equipped for micro determinations or a Beckman Model 151 microspectrophotometer
Transfer pipets, 1, 3, 4, 5 ml
Pasteur and Levy-Lang constriction micropipets, 25, 60, 80, 100 μl, to be used with a hemacytometer, rubber tubing, and mouthpiece. (See Bessey, Lowry, and Brock, 1946.)
Test tubes, 6 x 50 mm
Corks, #000 to fit above tubes
An electric centrifuge with cups designed to hold 6 x 50 mm tubes
Racks, metal, micro test tube, to hold 6 x 50 mm tubes
Water bath set at 60° C

Standard Curve (Micro Procedure)

Prepare the same standard solutions as those used for the macro procedure. (See p. 118.)

After mixing, remove duplicate 20-μl aliquots and place in clean 6 x 50 mm test tubes.

Add to each of these tubes 80 μl of 5 percent trichloroacetic acid solution. Mix well.

Remove a 60-μl aliquot from each tube and place in a clean 6 x 50 mm test tube.

Add to each tube 25 μl of the DTC reagent. Cork the tube and incubate at 60° C for 1 hour.

After incubation, chill the tubes in a mixture of ice and water. Then add 100 μl of cold 65 percent sulfuric acid, chill and mix well by rotating or swirling.

After letting the samples stand at room temperature for at least 20 minutes, place them in the Beckman microcells or in the flowthrough cell of the Beckman Model 151 instrument for reading at 515 mμ.

The blank is made by treating 60 μl of the trichloroacetic acid in the same manner as the standards.

Calculation of Standard

Since the standards have been treated in exactly the same fashion as the serum sample, the optical densities can be plotted against the following values in milligrams per 100 ml of ascorbic acid: 0.1, 0.5, 1.0, 2.0, and 4.0. Calculate a factor for use in determining the ascorbic acid content of the serum samples.

Measurement of Serum Ascorbic Acid

Pipet 20 μl of serum into a 6 x 50 mm test tube.

Add 80 μl of 5 percent trichloroacetic acid and mix by tapping with the finger.

Cork the tube and centrifuge at 3,000 rpm for 10 minutes.

Remove a 60 μl aliquot of the supernatant liquid and place in a clean 6 x 50 mm test tube.

Add 20 μl of the DTC reagent. Cork the tube and incubate at 60° C for 1 hour.

After incubation, chill the tubes in a mixture of ice and water. Then add 100 μl of cold 65 percent sulfuric acid, chill and mix by rotating or swirling in the tube.

The reagent blank is made by treating 60 μl of the trichloroacetic acid in the same manner as the unknown.

Colorimetry

Read the samples in the Beckman DU at 515 mμ using the microcuvettes (2.5 x 10 x 25 mm). Alternately, the samples may be read in the Beckman Model 151 microspectrophotometer using its flow-through microcell.

Calculations

If the same pipets have been used throughout, the results as expressed in milligrams per 100 ml can be calculated by using the factor estimated from the standard curve.

It is desirable to make a new standard curve whenever a new pipet is introduced into the procedure. However, a correction factor can be calculated from the volume change and applied to the original standard curve as follows:

$$\frac{\text{serum vol. (1)}}{\text{serum vol. (2)}} \times \frac{\text{vol. serum} + \text{vol. TCA (2)}}{\text{vol. serum} + \text{vol. TCA (1)}} \times \frac{\text{vol. TCA aliquot (1)}}{\text{vol. TCA aliquot (2)}} \times \frac{\text{final vol. measured (2)}}{\text{final vol. measured (1)}} = \text{correction factor}$$

The value of the correction factor usually falls in the range of 1.0 to 1.50.

Storage of Samples and Comments

Samples are best stored after precipitation of the protein and separation of the acid extract. The samples should be analyzed as soon as possible, even though it has been reported that losses are minimal if the extracts are kept frozen at −20° C. The rate of addition of the sulfuric acid is particularly critical since too rapid addition may result in charring of the extract which would produce a positive error in the analyses. The volumes of all the reagents may be adjusted proportionately upward without detriment if sufficient serum is available.

ALTERNATE METHOD

Serum Vitamin C (Ascorbic Acid)—Dye Method

REFERENCES

Bessey, O. A., A method for the determination of small quantities of ascorbic acid and dehydroascorbic acid in turbid and colored solutions in the presence of other reducing substances. J. Biol. Chem. *126*, 771–784, 1938.

Mindlin, R. L., and Butler, A. M., The determination of ascorbic acid in plasma; a macromethod and micromethod. J. Biol. Chem. *122*, 673-686, 1938.
Association of Vitamin Chemists, Inc., Methods of Vitamin Assay, p. 148. Interscience Publishers, New York, 1947.

Principle

This method is based on the measurement of the extent to which the indophenol dye is decolorized by ascorbic acid in biological fluids. Since reduction of the dye is instantaneous but reduction of dye with interfering reducing substance is slow, the decrease in color intensity with time is determined. This permits correction for reduction of the dye by substances other than ascorbic acid. This method estimates the reduced form of vitamin C.

Storage

Since this method estimates reduced vitamin C, clotted whole blood collected in a vacutainer and held at refrigerator temperature may be safely stored for up to 48 hours prior to separation of serum and completion of the estimation. Once the serum is separated, however, it cannot be stored for more than a few hours, even though precipitated, without serious loss of ascorbic acid.

Apparatus

A quantity of 100-ml volumetric flasks
A quantity of 10 ml volumetric flasks
Volumetric flasks, 200 ml
Filter paper, Whatman No. 1, No. 40
Paraffin or a polyethylene bottle
Bottles, polyethylene, 500 ml
An electric centrifuge
Stirring rods
Coleman Jr. spectrophotometer
Cuvettes, 19 x 150 mm
Centrifuge tubes, 15 ml
Syringe pipets 1, 2, 3, 4 and 5 ml

Reagents

All reagents should meet American Chemical Society specifications or be of reagent grade.

Redistilled water (distilled in glass and free from copper) or deionized water must be used for all solutions. The latter has been found to be generally satisfactory.

Metaphosphoric Acid Solutions

Metaphosphoric Acid, 6 Percent Solution

Dissolve 6-gm metaphosphoric acid pellets (34-36 percent HPO_3 by assay) without heating in redistilled water and make up to a volume of 100 ml. Store in the refrigerator. A fresh solution should be made every 10 days.

Metaphosphoric Acid, 3 Percent Solution

Add 5-ml 6 percent metaphosphoric acid to a 10-ml volumetric flask. Make up to volume with redistilled water. Prepare this solution daily.

Vitamin C Standard

Dissolve 100-mg ascorbic acid (see p. 118 for specifications on standard) in 3 percent HPO_3 in a 100-ml volumetric flask and dilute to 100 ml. Dilute 1 ml to 100 ml with 3 percent HPO_3 (10 μg per milliliter); dilute 20 ml of the latter with 20 ml of 3 percent HPO_3 (5 μg per milliliter). Use 1, 2, 3, and 4 ml of this solution for preparation of standard curve. Bring each tube to 4 ml with 3 percent HPO_3.

Solution of 2,6-Dichlorobenzenone Indophenol (Sodium Salt)

Stock Dye Solution, 50 mg per 100 ml

Dissolve 100 mg of 2,6-dichlorobenzenone indophenol sodium salt in about 150 ml of boiling redistilled water which contains 42 mg of $NaHCO_3$. Cool to room temperature and make up to a volume of 200 ml with redistilled water. Filter through No. 1 Whatman filter paper and store solution in a brown bottle in the refrigerator. Renew solution every 4 weeks.

Working Solution, 2 mg per 100 ml

Dilute 4 ml of stock solution to 100 ml with redistilled water. Prepare this solution daily.

Sodium Hydroxide Solutions

Saturated NaOH

Dissolve 200 gm of sodium hydroxide in 200 ml of redistilled, boiled water in a Pyrex flask. Stopper and allow to stand several days to permit the precipitation of sodium carbonate. Decant the supernatant solution and store the carbonate-free NaOH in a paraffin-lined or a polyethylene bottle. If desired, instead of waiting several days for the precipitate to settle, one may centrifuge the solution.

2 N NaOH Solution

Weigh out 32.0 gm of the saturated NaOH into an erlenmeyer flask. Transfer to a 200-ml volumetric flask and make up to the mark with boiled, cooled, redistilled water. Do not expose the solution to air any longer than necessary.

Citrate Buffer

Dissolve 29.4 gm citric acid (monohydrate) in 140 ml of 2 N NaOH (carbonate-free) and make up to a volume of 250 ml with redistilled water. Small amounts of the solution may be stored in the refrigerator for several days, but, before use, the solution should be checked carefully to see that there is no mold growth. If a freezer is available, it can be stored in the frozen state.

Procedure

Place 12 ml of 6 percent metaphosphoric acid in a 16 x 150 mm glass-stoppered test tube.

Add exactly 2 ml of plasma or serum and mix well with a stirring rod. Allow to stand at least 10 minutes.

Filter the supernatant fluid through No. 40 Whatman filter paper.

Pipet exactly 4 ml of the filtrate into a 19 x 150 mm cuvette, and, for the blank, pipet 4 ml of 3 percent metaphosphoric acid into another cuvette.

To all the tubes add 1 ml of the citrate buffer.

Set the spectrophotometer at 515 mμ and zero optical density with distilled water as the blank.

Add 4 ml of "working" dye solution to the blank quickly with a syringe pipet and shake quickly. Exactly 15 seconds after the addition of the dye (start counting time after last drop of dye has been added), obtain a reading (D_1).

Add a crystal of ascorbic acid to decolorize the dye completely and obtain a second reading (D_2). Repeat this procedure for the unknown samples.

Calculations

Subtract the density of the solution after decolorization (D_2) from the value obtained before decolorization (D_1). This (D_3) represents the density value corrected for turbidity.

Subtract this value for the sample from the corresponding value for the reagent blank. This value will be referred to as "density difference."

Determine the ascorbic acid concentration of the plasma or serum by referring to the chart prepared from the ascorbic acid standard curve.

Checks on the Method

After the ascorbic acid is added to the blank tube, the reading should be 0.015 or lower. If it is not, a new dye solution should be made. Sometimes a particular batch of dye is at fault. If this is so, discard dye and use another batch.

The blank reading at 15 seconds should be about 0.350. If the reading falls below this value, either some error occurred in the preparation of the dye solution, or the solution decomposed on standing.

Plasma or Serum Vitamin A and Carotene—Carr-Price Method

REFERENCES

Consolazio, C. F., Johnson, R. E., and Marck, E., Metabolic Methods. C. V. Mosby Co., St. Louis, 1951.

Dann, W. J., and Evelyn, K. A., The determination of vitamin A with the photoelectric colorimeter. Biochem. J. 32, 1008–1017, 1938.

Holmes, H. N., and Corbet, R. E., The isolation of crystalline vitamin A. J. Am. Chem. Soc. 59, 2042–2047, 1937.

Kaser, M., and Stekol, J. A., A critical study of the Carr-Price reaction for the determination of β-carotene and vitamin A in biological materials. J. Lab. Clin. Med. 28, 904–909, 1943.

Kimble, M. S., The photocolorimetric determination of vitamin A and carotene in human plasma. J. Lab. Clin. Med 24, 1055–1065, 1939.

Koehn, C. J., and Sherman, W. C., The determination of vitamin A and carotene with the photoelectric colorimeter. J. Biol. Chem. *132*, 527–538, 1940.

Lewis, J. M., Bodansky, O., and Haig, C., Level of vitamin A in the blood as an index of vitamin A deficiency in infants and in children. Am. J. Dis. Child. *62*, 1129–1148, 1941.

Neeld, J. B., Jr., and Pearson, W. N., Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. J. Nutrition *79*, 454–462, 1963.

Principle

The proteins of the plasma or serum are precipitated with alcohol, and the carotene and vitamin A are extracted with petroleum ether. The carotene concentration is determined by measuring the absorption of the extract at 450 mμ.

The petroleum ether is evaporated under nitrogen, and vitamin A is determined by reading the intensity of the blue color produced by the addition of either an antimony trichloride-chloroform or trifluoroacetic acid-chloroform chromogen. A correction is made for the amount of carotene present, since carotene contributes to the total color.

Apparatus

Coleman Jr. spectrophotometer, cuvettes, 10 x 77 mm, and adapter
Special, rapid delivery, 1.0 ml pipets
Glass-stoppered test tubes, 16 x 150 mm or 14 x 125 mm
An electric centrifuge
A tank of nitrogen (optional)
Water bath, 40°–50° C constant temperature
Pipets, 0.1, 2.0 and 3.0 ml

Reagents

95 percent ethyl alcohol
Petroleum ether (20°–40° C)
Chloroform
Chromogen solution

20 percent antimony trichloride in chloroform: dissolve one-quarter pound (113.4 gm) $SbCl_3$ in 455 ml of chloroform. Filter through fluted funnel containing Na_2SO_4 (anhydrous) into brown bottle. If solution is turbid, continue to filter through Na_2SO_4 until clear. Store in brown bottle containing Na_2SO_4. Do not expose to room air for any length of time or it will pick up moisture.

An alternate chromogen that may be used is a mixture of one part of trifluoroacetic acid and two parts of chloroform. This is prepared just prior to use. This chromogen is not sensitive to moisture and is therefore recommended.

Acetic anhydride
Anhydrous sodium sulfate, granular
A 6 N HCl solution ($SbCl_3$ method only)

Procedure (on single samples, only)

Place 2.0 ml plasma or serum in a 16 x 150 mm glass-stoppered test tube.

Add 2.0 ml 95 percent alcohol; mix.

Add 3.0 ml petroleum ether; shake vigorously for 2 minutes.

Centrifuge at low speed for 3 minutes.

Carefully pipet off 2.0 ml of the petroleum ether, using a 4- or 5-inch needle of as small diameter as possible (No. 20 or 18) on a syringe pipet, and place in a 10 x 75 mm cuvette.

Read carotene at 450 mμ. Use a blank of petroleum ether.

Evaporate to dryness in 40°–50° C water bath, under a stream of nitrogen.

Take up the residue immediately in 0.1 ml chloroform; add 1 drop of acetic anhydride from a No. 25 needle; cork the tube to prevent evaporation.

Set the Coleman Jr. spectrophotometer at 620 mμ at zero optical density with a blank tube containing 0.1 ml chloroform and 1.0 ml of the chromogen solution. Remove the blank tube and place sample tube in the instrument. Add 1.0 ml of the chromogen solution from a rapid-delivery pipet, and record the density reading at the pause point (3 to 5 seconds after the addition of the reagent). Immediately after noting the density reading, remove the tube and observe the color. The solution must be clear, and a blue color should be detectable.

Precautions

$SbCl_3$ must contain no moisture. (Keep stoppered when not in use.)

Sample must be dry before addition of $SbCl_3$.

Cleaning glassware: soak glassware containing $SbCl_3$ in a 6 N solution of HCl, rinse and wash with detergent as usual. It is imperative that the stopcocks be removed from the special $SbCl_3$ pipets before soaking the pipet in 6 N HCl. Otherwise, the stopcocks may "freeze." Glassware contaminated with trifluoroacetic acid can be washed in the usual fashion.

If no blue color is observed in the unknown tube (density less than 0.04), then the sample contains less than 14 μg per 100 ml of vitamin A. If more than 25 percent of the group of samples gives readings of less than 0.04, the size of the plasma sample should be increased to 3.0 ml.

When pipeting off the petroleum ether extract, be careful to avoid picking up protein particles from the wall of the tube.

Standard Curves

Since the Coleman spectrophotometer vitamin A factors vary somewhat from instrument to instrument, standard carotene and vitamin A curves should be made and factors calculated for each instrument.

Vitamin A Reference Standard

A U.S.P. reference standard solution of vitamin A acetate in cottonseed oil can be purchased from the United States Pharmacopoeial Convention, 46 Park Avenue, New York 16, N.Y. This standard, distributed in 250-mg capsules, contains 344 mg of trans vitamin A acetate per gram of solution. The $E^{1\%}_{1cm}$ for vitamin A acetate in ethanol at 328 mμ is 1,730 (expressed as vitamin A alcohol).

Carotene Reference Standard

Ampules containing pure β-carotene or a 90-percent β-, 10 percent γ-carotene mixture can be obtained from Nutritional Biochemicals Co., Cleveland 28, Ohio. Either is suitable. The ampule should be opened immediately prior to use and once opened should be stored in a desiccator under nitrogen. The $E^{1\%}_{1cm}$ for crystalline carotene in hexane at 452 mμ is 2,550. Carotene is notoriously unstable and it is not unusual to find that the standard furnished has deteriorated.

Preparation of Carotene Standard Curve

Weigh out exactly 50 mg of β-carotene standard, dissolve in a few milliliters of reagent grade chloroform, and bring to a final volume of 100 ml in a volumetric flask with petroleum ether. These solutions should be prepared at the time of calibration since they deteriorate if stored. Dilute this solution 1 to 100 with petroleum ether to prepare the intermediate standard. Prepare working standards by diluting the intermediate standard with petroleum ether as follows:

Dilution	β-carotene, μg/ml
1 to 10	0.5
2 to 10	1.0
4 to 10	2.0
8 to 10	4.0

Deliver duplicate 2-ml aliquots of these standards into Coleman 10 x 75 mm cuvettes and read their optical densities against petroleum ether at 450 mμ. Plot the standard curve and calculate the factor.

Preparation of Vitamin A Standard Curve

Carefully weigh out exactly 100 mg of the U.S.P. vitamin A reference standard (trans vitamin A acetate in cottonseed oil). This amount contains 3.44 mg of vitamin A acetate. Dissolve in $CHCl_3$ and

dilute to 50 ml in a volumetric flask. To prepare working standards, dilute this stock with $CHCl_3$ as follows in 10 ml volumetric flasks:

Dilution	*Vitamin A acetate, μg/ml*
1 to 10	6.88
2 to 10	13.76
3 to 10	20.64
4 to 10	27.52
5 to 10	34.40

To prepare the standard curve, place duplicate 0.1 ml aliquots of these standards in Coleman 10 x 75 mm cuvettes and add the chromogen solution as indicated in paragraph 13, page 126. Plot the optical density values on the ordinate and the micrograms (per tube) of vitamin A acetate on the abscissa of rectangular coordinate paper and calculate the factor.

Constant Factors

With the reagents, volumes, and cuvettes given in the procedure outlined on pages 125–126, the constants for carotene and vitamin A as measured with the Coleman Jr. spectrophotometer are reasonably uniform from one instrument to another. Although these should be determined in each laboratory, the values below are typical:

Carotene standard curve:

$D450 \times 7.28 = \mu g$ of carotene per milliliter of petroleum ether.

Carotene correction:

One microgram of carotene is equivalent to 0.017 density units at 620 mμ.

Vitamin A standard curve:

$D620 \times 4.81 = \mu g$ of vitamin A per tube.

Carotene in serum:

$D450 \times 1092 = \mu g$ per 100 ml of carotene.

Vitamin A in serum:

$(D620 = (D450 \times 0.248)) \times 361 = \mu g$ per 100 ml of vitamin A.

The above factors for vitamin A are typical for the antimony trichloride chromogen only. The trifluoroacetic acid factors, although similar, are not identical.

Quality Control

Frozen aliquots of a pooled serum sample are satisfactory for quality control of the vitamin A and carotene method. The vitamin A and carotene contents of the pooled sample are determined by analyzing it in quadruplicate on 5 consecutive days during the first week of laboratory operation. A tube is thawed and run with the workload each day. A quality control chart is prepared as described in the section of this chapter on laboratory control. (See p. 107.)

Equivalents [1]

One International Unit (IU) or United States Pharmacopoeia Unit (USP) of vitamin A is equivalent to 0.3 μg of vitamin A alcohol.

[1] Hume, E. M., Standardization and requirement of vitamin A. Brit. J. Nutrition 5, 104–113, 1951.

Thus, International Units of vitamin A×0.3=μg of vitamin A.

One International Unit (IU) or United States Pharmacopoeia Unit (USP) of provitamin A is equivalent to 0.6 μg of β-carotene. Thus, International Units of provitamin A activity×0.6=μg of β-carotene.

Plasma or Serum Vitamin A and Carotene—Micro Procedure

REFERENCE

Neeld, J. B., Jr., and Pearson, W. N., Macro- and micromethods for the determination of serum vitamin A using trifluoroacetic acid. J. Nutrition 79, 454-462, 1963.

Principle

This method is essentially an adaptation of the macro trifluoroacetic acid method that has already been described. (See p. 124.)

Apparatus

Beckman DU spectrophotometer with micro attachments and 2.5×10×25 mm microcells or the Beckman Model 151 microspectrophotometer

A Vortex Jr. mixer, or high-speed hand drill for use as a mixing device

Levy-Lang constriction pipets of 10, 20, 75, and 100 μl capacities and some uncalibrated constriction pipets of about 200 μl capacity

Two 0.2 ml serological pipets drawn out to a long slender tip

A quantity of 6×50 mm test tubes and #000 cork stoppers

A stopwatch

Reagents

Ethanolic KOH: 90 percent ethanol is made 1.0 N by the addition of an approximate weight of KOH pellets.

Petroleum ether (boiling range 38°-60° C) or n-hexane

Trifluoroacetic acid reagent: mix 1 volume of trifluoroacetic acid with 2 volumes of reagent grade chloroform just prior to use.

The standard carotene and vitamin A solutions are those described in the macro procedure.

Procedure

Place 50 μl of serum in a 6 x 50 mm test tube.

Add an equal volume of the alcoholic KOH saponification mixture.

Add 150 μl of petroleum ether.

Stopper and agitate on the Vortex mixer for 45 seconds.

Centrifuge for 10 minutes at 3,000 rpm.

Rapidly transfer 100 μl of the supernatant layer to the microcuvette and read at 450 mμ against a petroleum ether blank.

Transfer as much as possible of the sample to a clean 6 x 50 mm test tube. Rinse the cuvette with ca. 50 μl of petroleum ether and add this to the sample in the test tube.

Evaporate the sample to dryness in a 40° C water bath (about 5 minutes).

Take up the residue in 10 μl of $CHCl_3$ and add 100 μl of the trifluoroacetic acid reagent with vigorous shaking.

Transfer the solution rapidly to the microcuvette and read at 620 mμ against a trifluoroacetic acid reagent blank exactly 30 seconds after addition of the chromogen.

Standards and Calculations

Standard curves are prepared using appropriate aliquots of the same standards used in the macro procedure. Factors are derived as previously described to permit calculation of the carotene and vitamin A content of the sera.

Comments

n-Hexane may be substituted for petroleum ether if so desired. This reagent is less volatile than the latter and is thus handled more easily. Conversely, it cannot be easily evaporated to dryness in a short period of time unless an impinging jet of nitrogen is used.

Saponification is not absolutely necessary although it gives somewhat higher values. Precipitation of the proteins with 95 percent ethanol will result in the extraction of more than 90 percent of the vitamin A.

ALTERNATE METHOD

Plasma or Serum Vitamin A and Carotene—Micro Procedure

REFERENCES

Bessey, O. A., Lowry, O. H., Brock, M. J., and Lopez, J. A., The determination of vitamin A and carotene in small quantities of blood serum. J. Biol. Chem. *166*, 177–188, 1946.

Utley, M. H., Brodovsky, E. R., and Pearson, W. N., Hemolysis and reagent purity as factors causing erratic results in the estimation of vitamin A and carotene in serum by the Bessey-Lowry method. J. Nutrition *66*, 205–215, 1958.

Principle

Serum protein is precipitated with ethanol, and saponification of carotene and vitamin A is accomplished by heating with KOH. Carotene and vitamin A are extracted with a mixture of kerosene-xylene and carotene is determined by measurement of the optical density of the extract at 460 mμ. Vitamin A is measured at 328 mμ. The sample is irradiated with ultraviolet light of wavelength 310 to 400 mμ, thus destroying the vitamin A present. After irradiation the optical density of the sample is again measured at 328 mμ, the difference between the two readings at 328 mμ being due to the concentration of vitamin A originally present in the solution.

Apparatus

Beckman DU spectrophotometer with micro attachments and quartz microcells

Soft-glass tubes 4 cm long and 3 mm inside diameter: glass tubing is cut into 4-cm lengths, cleaned by soaking overnight in cleaning solution, rinsed,

dried, and sealed at one end in a blast lamp flame. The tubes should be cleaned before each use by soaking overnight in cleaning solution or half-concentrated nitric acid, thoroughly rinsing and drying. If desirable, new tubes can be made rather than cleaning old ones.

Levy-Lang constriction pipets, 60 and 120 μl volume (accurately calibrated), and pipets without constrictions with long slender tips that will reach to the bottom of the narrow soft-glass tubes.

General Electric H100 B138 4 mercury discharge lamp with purple envelope and with its special transformer No. 9T6413518. A special socket (medium) is required for this lamp.

Arrangement for irradiating samples in the soft-glass tubes:

The mercury lamp is inserted vertically, base down, in a large block of wood which serves as a base. Circular racks are provided for holding the glass tubes in a circle equidistant from the lamp (6 cm from the center of the lamp). Twenty holes are drilled in the rack to accommodate the soft-glass tubes. The rack should be placed so that the brightest part of the light source is opposite the lower half of the tubes and this portion of the tubes receives full illumination. A moderate air current from a fan or open window must be used to keep the tubes cool.

Reagents

11 N KOH solution in distilled water. Store in a rubber-stoppered bottle.

95 percent ethyl alcohol.

One volume of 11 N KOH in water (stored in a rubber-stoppered bottle), plus 10 volumes of 95 percent ethyl alcohol.

Kerosene-Xylene Mixture

Xylene

Redistill xylene in an all-glass still and collect the 134°–137° C fraction. Store in a dark place.

Kerosene

Shake 100 ml of odorless, white reagent kerosene[1] with an equal volume of a 5-percent solution of Na_2CO_3; discard the carbonate layer and wash the kerosene three times with an equal volume of distilled water. Store kerosene overnight in the presence of $CaCl_2$ granules. Shake the kerosene with activated charcoal (3 gm per 100 ml) and filter through Whatman No. 1 filter paper. Store in a dark place.

Mix one part of xylene with one part of kerosene and store in the dark. Do not store for more than 1 week.

Procedure

Pipet 60 μl of serum and 60 μl of alcoholic KOH into the bottom of a 6 x 50 mm tube. Mix by tapping with the finger.

Place in a water bath at 60° C for 20 minutes.

Cool to room temperature, add 120 μl of kerosene-xylene mixture.

Extract by holding tip of the tube at a 45° angle against the motor drill for 45 seconds.

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[1] Obtainable from Fisher Scientific Co., Fisher # K-10.

Stopper tube, cool in ice water and centrifuge for 10 minutes at 3,000 rpm.

Transfer as large a quantity as possible of the kerosene-xylene layer to a Beckman microcuvette. (Avoid the aqueous layer since this causes turbidity.)

Make readings at 460 and 328 mμ using kerosene-xylene as a blank.

Transfer the sample to a narrow soft-glass tube and irradiate for 40 minutes with the mercury discharge lamp. (If the optical density at 328 mμ is greater than 0.100, irradiate for an hour.)

Transfer back to the cuvette and read again at 328 mμ.

Calculations

Factors for converting optical density readings to concentration of carotene and vitamin A in serum or plasma are obtained by measuring the optical densities of solutions of carotene and vitamin A of known concentration.

The factor for a solution of pure β-carotene in kerosene-xylene expressed as

$$\frac{\mu g \text{ percent of carotene}}{E_{460}}$$

is about 493. Concentration of carotene in the serum sample is calculated as follows:

$$E_{460} \times 493 \times \frac{\text{vol. kerosene-xylene}}{\text{vol. serum}} = \mu g \text{ of carotene per 100 ml of serum}$$

The factor for a solution of vitamin A acetate in kerosene-xylene as determined as

$$\frac{\text{IU percent of vitamin A acetate}}{E_{328} - \text{Irradiated } E_{328}}$$

is usually about 2,200.

The volumes of serum and of alcoholic KOH must be equal, but the volume of kerosene-xylene may be varied independently.

Storage of Samples

Serum may be stored frozen in tightly stoppered tubes for several months without prejudice to the results. If desired, samples may be stored overnight in the refrigerator after extraction with kerosene-xylene and centrifugation.

Comments

Hemolysis causes a false increase in serum vitamin A values when the micro method of Lowry and Bessey is used. For this reason, all sera should be inspected against a white background and any that show more than a trace of hemolysis should be discarded. An especially purified and freshly prepared kerosene-xylene mixture minimizes the false increase in vitamin A caused by hemolysis and avoids

errors in the carotene measurements. Permitting an unpurified kerosene-xylene mixture to stand at room temperature unprotected from light may cause up to a tenfold increase in the apparent vitamin A content of a hemolyzed blood sample and a definite diminution in the apparent carotene. Even in the absence of hemolysis, faulty reagents can cause a false increase in vitamin A. Changes in the optical properties of these reagents can be detected by frequent comparison of their densities with that of water at 328 and 460 mμ.

Total Serum Protein, Albumin, and Globulin by a Modified Biuret Technic

REFERENCE

Wolfson, W. Q., Cohn, C., Calvary, E., and Ichiba, F., Studies in serum proteins. V. A rapid procedure for the estimation of total protein, true albumin, total globulin, alpha globulin, beta globulin and gamma globulin in 1.0 ml of serum. Am. J. Clin. Path. *18*, 723–730, 1948.

Principle

Total serum protein is determined by a conventional biuret technic. Another aliquot of the same serum is added to a solution of sodium sulfite which precipitates the globulin. The clear centrifugate which contains "true" albumin is then withdrawn and analyzed by the biuret method. A micro procedure for total serum protein is also described that is particularly useful when dealing with fingertip blood samples.

Reagents

Biuret Reagent—Macro Procedure

Completely dissolve 90 gm of Rochelle salt (NaK tartrate·4 H_2O) in about 400 ml of standard 0.2 N NaOH solution. Now dissolve 10 gm of $CuSO_4 \cdot 5H_2O$. Finally, add 10 gm of KI and make up to 2 liters with 0.2 N NaOH. Store in a polyethylene bottle.

Biuret Reagent—Micro Procedure

To 45 ml of the biuret reagent described above, add 50 ml of 0.85 percent NaCl and 5.0 ml of 6 N NaOH.

28 Percent Sodium Sulfite Solution

Dissolve exactly 28.0 gm of anhydrous sodium sulfite in 75 ml of distilled water at 28° C. It may be difficult to dissolve, but with much continuous shaking it will go into solution. Dilute to 100 ml with distilled water. It may be necessary to store this solution at 28°–30° C to prevent crystallization.

Span-Ether Reagent

Mix 1 ml of Span-20 (Atlas Chemical Co., Wilmington, Del.) with 99 ml of ethyl ether, USP. Filter through a moderately fast filter

paper into a graduated cylinder and make up to 100 ml with ethyl ether. Store in a tightly corked bottle.

Procedure

The standard protein solution used may be either pooled normal human serum, the protein content of which has been accurately determined by a Kjeldahl method, or human albumin of known composition. Standards ("Versatol") for the former are now available commercially. The albumin solution has the advantage of permitting protein concentrations well above the normal range. Using 0.9 percent saline as the diluent, prepare exact standards in the vicinity of 1.0, 2.0, 4.0, 6.0, and 8.0 gm per 100 ml. Using the serum total protein technic described below, construct a standard curve.

Serum Total Protein—Macro Procedure

Pipet exactly 0.2 ml of serum into a test tube and add 4.8 ml of distilled water. Mix well by inversion.

Transfer 3 ml to a cuvette, add 3.0 ml of biuret reagent and mix well by shaking.

Prepare a blank with 3 ml of distilled water and 3.0 ml of biuret reagent.

Let the solution stand for at least 30 minutes. (Color is stable for 24 hours.) Read in a spectrophotometer at 540 mμ.

Serum Total Protein—Micro Procedure

Pipet exactly 0.01 ml (10 mm³) of serum into a 10 x 75 mm Coleman cuvette. A Levy-Lang constriction pipet is to be preferred for this procedure.

Add exactly 1.0 ml of the micro procedure biuret reagent and mix well by inversion.

Let the solution stand for at least 30 minutes and read in the spectrophotometer at 540 mμ.

A standard curve for this procedure can be constructed by appropriate modification of the macro procedure described above. Volumes can be adjusted proportionately upward if so desired.

Serum Albumin—Macro Procedure

Place 4.8 ml of 28 percent sodium sulfite solution in a test tube. This should not be pipeted because of a tendency to crystallize, but be added rapidly from a large burette. Prepare sufficient tubes for the daily workload at the same time.

Pipet in exactly 0.2 ml of serum and mix well by inversion.

Add 1 ml of Span ether reagent and invert gently about 5 to 10 times. Centrifuge for 5 to 10 minutes at 1,500 to 2,000 rpm.

After centrifugation, carefully introduce a pipet through the Span-ether layer and beneath the packed globulin, slanting the tube to separate the precipitate from the wall. Withdraw 3.0 ml of the clear

liquid and place in a cuvette. Add 3.0 ml of biuret reagent and mix well by shaking.

Prepare a blank with 3.0 ml of sodium sulfite solution and 3.0 ml of biuret solution.

Let stand for 30 minutes and then read in the Coleman at 540 mμ.

Quality Control

Frozen aliquots of a pooled serum sample may be used in quality control of this method. The entire pooled specimen should first be frozen and thawed so that any protein precipitation can occur. This is removed by centrifugation and the supernatant is dispensed in 2-ml quantities into 50 small test tubes which are then frozen. The protein content of the supernatant and its standard deviation are determined by analyzing it in quadruplicate on 5 consecutive days during the first week of laboratory operation. Each day one tube is thawed, brought to room temperature and introduced into the daily workload. A quality control chart is prepared as described in the section on laboratory control.

Calculations

Determine the total protein content and the albumin content of the sample by extrapolation from the standard curve. Subtract the albumin value from the total protein value to obtain the globulin value. The albumin-globulin ratio is calculated by dividing the amount of albumin by the amount of globulin in the sample.

Creatinine in Urine—Picrate Method

REFERENCE

Folin, O., and Wu, H., A system of blood analysis—determination of creatinine and creatine. J. Biol. Chem. 38, 98–100, 1919.

Principle

Creatinine reacts with picrate in alkaline solutions at room temperature, and in a few minutes a stable, intense orange color is produced which can be measured spectrophotometrically.

Apparatus

Coleman Jr. spectrophotometer, Model 6
Cuvettes, 19 x 150 mm
100-ml volumetric flasks
Calibrated syringe pipets, 1, 2, 4, and 10 ml
Volumetric pipets, 20-ml capacity

Reagents

Sodium hydroxide, a 10-percent solution in water.

Picric acid, a 1-percent solution in water (gentle heating may be required to dissolve the acid): mix 100 ml NaOH and 100 ml picric acid and dilute to 1,000 ml. This solution is called the "alkaline picrate" solution.

Crystalline creatinine zinc chloride: dissolve and dilute 1.6106 gm to 1,000 ml in 0.1 N hydrochloric acid. This solution contains 1.0 mg of creatinine per milliliter.

Procedure

Into a 100-ml volumetric flask measure accurately 0.1 ml of urine with a 0.1-ml serological pipet.

Dilute to approximately 20 ml with distilled water and add exactly 20 ml of the alkaline picrate solution.

Allow to stand for exactly 15 minutes, after gentle mixing, and then dilute to 100-ml mark.

Set up a blank using exactly 20 ml of the alkaline picrate mixture and dilute to 100 ml.

Pipet an aliquot (10 ml) of the blank into a cuvette and set at zero density at a wavelength of 520 mμ.

Then read aliquots of the unknowns.

Calculation

Prepare a standard curve from creatinine zinc chloride solution.

Dilute exactly 5 ml of standard to 100 ml. This solution is equivalent to 0.05 mg creatinine per milliliter.

Into 100 ml volumetric flasks pipet 0, 1, 2, 3, 4, and 5 ml of the dilute standard.

Add 20, 19, 18, 17, 16, and 15 ml of water, respectively.

Add exactly 20 ml of the same alkaline picrate used for analysis of the unknowns.

Allow to stand for exactly 15 minutes and then dilute to 100 ml.

Set blank at zero density and read all standards at 520 mμ.

Plot the concentration-density curve on semilog paper.

Precautions

Many variables affect the intensity of the creatinine picrate. Important among these are time of standing, temperature, concentration of alkali, and presence of noncreatinine color-producing substances. Therefore, it is essential to establish standard conditions and to adhere strictly to them. It is preferable to run at least two points of the standard curve each day and use the factor so obtained for calculation of the results on that day.

Thiamine in Urine

REFERENCES

Modified from method described by Consolazio, C. F., Johnson, R. E., and Marek, E., Metabolic Methods. C. V. Mosby Co., St. Louis, 1951.

Other pertinent references:

Hennessey, D. J., and Cerecedo, L. R., The determination of free and phos-

phorylated thiamin by a modified thiochrome assay. J. Am. Chem. Soc. *61*, 179–183, 1939.

Conner, R. T., and Straub, G. J., Combined determination of riboflavin and thiamine in food products. Indust. Eng. Chem., Anal Ed. *13*, 385–388, 1941.

Mickelsen, O., Condiff H., and Keys, A., The determination of thiamine in urine by means of the thiochrome technique. J. Biol. Chem. *160*, 361–370, 1945.

Principle

Thiamine is adsorbed from urine in acid solution by means of activated zeolite. It is eluted with concentrated potassium chloride, and is converted to thiochrome by ferricyanide in alkaline solution. Thiochrome is extracted into isobutyl alcohol and measured fluorometrically.

Apparatus

Coleman Model 12C photofluorometer and filters
Hennessey adsorption tubes (capillary tip not to exceed 5 mm in length or be less than 0.5 mm internal diameter)
Fine glass wool
Reaction vessels, 40–50 ml glass-stoppered (interchangeable stoppers) vessels with a conical bottom
Cuvettes, Coleman, 19 x 105 mm
Syringe pipets, 1.0, 5.0 and 10.0 ml
Wooden applicators for inserting glass wool into columns
Waste pans
Beakers, 30 or more, 50 ml
Erlenmeyer flasks, 30 or more, 50 ml
Special rack for Hennessey tubes
Special rack for reaction vessels

Reagents

Potassium chloride, 25 percent solution: dissolve 500 gm KCl in 1,500 ml of 0.1 N HCl with the aid of heat. Dilute to 2,000 ml with 0.1 N HCl and filter.

Bromcresol green indicator, 0.1 percent in 70 percent ethyl alcohol.

Sodium acetate, 2.5 M: dissolve 205 gm anhydrous $NaC_2H_3O_2$ or 345 gm of $NaC_2H_3O_2 \cdot 3H_2O$ in water and dilute to 1 liter.

Decalso: wash 60- to 80-mesh Decalso three times with 3 percent acetic acid, and once with 25 percent KCl. Wash again with 3 percent acetic acid and then several times with distilled water. Each washing consists of stirring the Decalso 15 minutes in the solution, allowing it to settle and then decanting. Store in a wet state in a stoppered bottle.

Decalso labeled "Thiochrome Decalso" which has already been activated may be purchased from Fisher Scientific Co. Used Decalso may also be reactivated.

Sodium hydroxide, 1 N solution: dissolve 40 gm of sodium hydroxide in distilled water and dilute to 1,000 ml.

Sodium sulfate, anhydrous, granular.

Acetic acid, 0.5 percent solution in water: dilute 2.5 ml of glacial acetic acid to 500 ml with distilled water.

Isobutyl alcohol, redistilled in an all-glass still: collect the fraction between 105° and 108° C.

Potassium ferricyanide, 1 percent: dissolve 1 gm potassium ferricyanide in distilled water and dilute to 100 ml. This solution is stable for 6 months if kept in a dark bottle.

Potassium hydroxide, 15 percent: dissolve 15 gm potassium hydroxide in distilled water and dilute to 100 ml.

Oxidizing reagent: one part of 1 percent ferricyanide solution is mixed with 9 parts of 15 percent potassium hydroxide. Prepare fresh daily.

Quinine Sulfate Standard

Stock standard

Dissolve about 10 mg of quinine sulfate in 100 ml of 0.1 N H_2SO_4. This solution is stable for 1 year.

Working standard

Dilute exactly 1.25 ml of the stock standard to 500 ml with 0.1 N H_2SO_4. It is stable indefinitely in the refrigerator.

Stock Thiamine Solution

Dry thiamine hydrochloride (USP reference standard obtained from U.S. Pharmacopoeial Convention, Inc., 46 Park Ave., New York) for 2 hours at 105° C and store in a desiccator over P_2O_5 for at least 24 hours before use. In a 500-ml volumetric flask dissolve 50 mg of thiamine hydrochloride in 200 ml of 95 percent ethyl alcohol. Add 5 ml 0.1 N HCl and bring to volume with distilled water. Store in refrigerator; it is stable for 6 months.

Intermediate Thiamine Solution

Dilute exactly 5.0 ml of stock thiamine solution to 100 ml with distilled water.

Working Thiamine Solution

Transfer 4.0 ml of the intermediate solution to a flask containing 75 ml of 0.1 N H_2SO_4 and 5 ml of 2.5 N sodium acetate. Adjust to 100 ml. Prepare daily. The final concentration is equivalent to 0.2 μg of thiamine per milliliter.

Procedure

Place a small piece of glass wool in the tip of the adsorption columns; fill the column with activated Decalso so that the bottom of the bell is just covered. Uniform filling is easily accomplished by first filling the tube with water, then adding the Decalso and allowing it to settle by gravity.

Wash the column with 3 ml of 0.5 percent acetic acid and allow to drain in a waste pan.

Place a 10-ml aliquot of urine in a 50-ml beaker, add 2 drops of bromcresol green, and neutralize to a light greenish-yellow color with 1 N NaOH or 1 N H_2SO_4 (pH±3.5).

Pour the sample onto the column and allow it to drain by gravity into the waste pan.

Rinse the beakers once with 10 ml of hot distilled water and pour these washings through the column. Then wash the columns twice more with 10 ml aliquots of hot distilled water. Each washing should be permitted to drain into the column before another is added.

Place a 50-ml erlenmeyer flask under each column and elute successively with 10, 10, and 5 ml of the acidic 25-percent KCl solution. Each aliquot should be permitted to drain into the column before another is added. This eluate is stable in the refrigerator for at least a week, if it is necessary to interrupt the procedure at this point.

Remove two 5-ml aliquots from each flask and pipet into two reaction vessels. One aliquot is the unknown and the other the blank. To the "unknown" tube add 0.7 ml of oxidizing agent and mix. Do not pipet more than three or four samples at a time, as they must be shaken with isobutanol within 1 minute of the addition of the oxidizing agent. To the blank tube add 0.7 ml of a mixture consisting of 1 part of distilled water mixed with 9 parts of 15 percent KOH. The timing here is also critical; the blanks should also be shaken with isobutanol within 1 minute of the addition of the KOH.

Add 10 ml isobutanol, stopper with a glass stopper and shake vigorously for 45 seconds. After the layers have settled, the bottom layer is drawn off and discarded. This is most conveniently carried out by using a long needle (5″, #16 or #18 is suitable) attached to a trap which in turn is connected to a water pump, by means of a readily compressible rubber tubing.

A small amount of anhydrous granular Na_2SO_4 is added and the vessel is tilted gently. This can be added rapidly by using a funnel to which a 3-inch length of rubber tubing is connected, controlling the flow by means of a pinch clamp. The isobutanol layer must be clear after the sulfate has settled. If not, repeat this step. (A slightly turbid solution may frequently be clarified by placing the tube in a boiling water bath for a short time.)

Repeat steps above, beginning with placement of urine in beaker, using 10 ml of working thiamine standard. Be sure to adjust the pH as in paragraph one, above. A reagent blank using 10 ml of 0.5 percent acetic acid should also be run with each new batch of reagents.

The sample is then decanted into cuvettes to be read on the photofluorometer.

The thiamine filters (B_1 and PC–1) are used. After the instrument has "warmed up" and been balanced, the working quinine sulfate standard is used to set the instrument at 60.

Calculation

$$\frac{\text{Unknown} - \text{Unknown Blank}}{\text{Standard} - \text{Standard Blank}} \times \frac{\mu\text{g thiamine in standard}}{2} \mu\text{g thiamine/ml urine}$$

$$\frac{U - UB}{S - SB} \times \frac{0.4}{2} \mu\text{g thiamine/ml urine}$$

Comments

Columns should drip at a rate of at least 1 ml per minute. Those that run more slowly than this should be discarded.

Laboratory Control

Obtain a large sample of pooled urine (2 liters), acidify to a pH of about 3.0 and filter. Dispense in 10-ml quantities into test tubes and freeze. The thiamine content and its standard deviation are ascertained during the first week of laboratory operation by assaying the content in quadruplicate for 5 consecutive days. Thereafter, one tube is thawed each day and analyzed with the daily workload. A quality control chart is prepared as described in the section of this chapter on laboratory control.

Urinary Riboflavin by a Modification of the Method of Slater and Morell

REFERENCE

Slater, E. C., and Morell, D. B., A modification of the fluorimetric method of determining riboflavin in biological materials. Biochem. J. 40, 644–652, 1946.

Principle

Riboflavin is measured fluorometrically after some interfering substances have been destroyed by oxidation and others have been eliminated by extraction of the riboflavin into a butanol-pyridine solution. An internal standard is used and the blank is determined by destruction of the riboflavin by irradiation.

Apparatus

Coleman Model 12C photofluorometer and B_2 and PC–2 filters
Cuvettes, Coleman, 19 x 150 mm
Short-stemmed funnel
Irradiation rack
Pyrex test tubes, 16 x 150 mm, with standard-taper glass stoppers

Reagents

Acetic acid, glacial

Riboflavin, dry, USP reference standard: dissolve 20 mg pure riboflavin in 1 liter of distilled water containing a few drops of acetic acid. Store in the dark under toluene. For use, dilute 1 to 20 with distilled water.

5 percent aqueous $KMnO_4$: prepare daily.

3 percent aqueous H_2O_2 solution.

Na_2SO_4, granular anhydrous, reagent grade.

Butanol-pyridine mixture: to 8 volumes of redistilled pyridine, add 92 volumes of redistilled n-butanol. To recover this after use, make the mixture alkaline with 30 percent NaOH and filter off the brown precipitate. Then distill and collect the 93°–118° C fraction. Add enough pyridine to make it 8 percent by volume.

Fluorescein standard: dissolve about 10 mg of sodium fluorescein in 1 liter of distilled water. For use, dilute 1 to 200 or to another appropriate volume.

Procedure

Pipet exactly 0.5 ml of glacial acetic acid into 16 x 150 mm glass-stoppered test tubes. Each sample requires two tubes, A and B.

To tube A and tube B add 1.0 ml of urine (if urine contains a small quantity of riboflavin use 2.0 ml). Prepare a reagent blank by substituting distilled water for the urine. Only one need be prepared for a series of analyses.

Add 0.5 ml of distilled water to tube A and 0.5 ml of the riboflavin standard to tube B. Treat the reagent blank similarly.

Add 0.5 ml of 5 percent $KMnO_4$ to each tube, mix and allow to stand for exactly 1 minute.

Add 0.5 ml of 3 percent H_2O_2 to each tube and agitate gently.

Add 10 ml of the butanol-pyridine solution.

Shake vigorously for 30 seconds. Allow to stand in the dark for 10 minutes.

Remove the bottom (aqueous) layer by using a long needle attached to a water pump.

Add anhydrous granular Na_2SO_4 (ca. 1.5 gm) with the aid of a small funnel.

Shake gently and place in the dark for 10 minutes. Decant the crystal-clear supernatant into *Coleman* cuvettes (*19* x *150* mm).

Set the photofluorometer at 70 or 80 units with the fluorescein standard and read each sample.

Place the cuvettes in the irradiation rack and irradiate until 80 to 90 percent of the riboflavin is destroyed. This may take 2 hours if a 100-watt bulb is employed. Check the reagent blank containing riboflavin at 30-minute intervals to determine the rate of destruction.

Reread all tubes.

Providing these directions are followed exactly, the average urine sample usually will read around 20 to 30 units, with a recovery increment of from 15 to 20 additional units.

Calculations

A–A¹ (after irradiation)—deflection due to riboflavin destruction in unknown

B–B¹ (after irradiation)—deflection due to riboflavin destruction in recovery

C–C¹ (after irradiation)—change in reagent fluorescence induced by irradiation

$$\frac{(A-A^1)-(C-C^1)}{[(B-B^1)-(C-C^1)]-[(A-A^1)-(C-C^1)]} \times 0.5 = \mu g \text{ of riboflavin per tube}$$

Example

A sample gave the following readings:

	Unknown	Recovery	Blank
Before irradiation	30 (A)	50 (B)	1.0 (C)
After irradiation	8 (A¹)	10 (B¹)	.0 (C¹)
Difference	22	40	1.0

By application of the above formula we have:

$$\frac{22-1.0}{(40-1)-(22-1)} \times 0.5 = \frac{21}{18} \times 0.5 = 0.58 \ \mu g \text{ of riboflavin per tube.}$$

If 1.0 ml was the original volume of urine used, then this amount of riboflavin was present in 1.0 ml.

NOTE.—The reagent blank (c) usually has no change in fluorescence. In most instances it can be completely dropped from the calculations.

N′-Methylnicotinamide in Urine

REFERENCES

Huff, J. W., and Perlzweig, W. A., The fluorescent condensation product of N′-methylnicotinamide and acetone. II. A sensitive method for the determination of N′-methylnicotinamide in urine. J. Biol. Chem. *167*, 157–167, 1947.

Carpenter, K. J., and Kodicek, E., The fluorimetric estimation of N′-methylnicotinamide and its differentiation from coenzyme I. Biochem. J. *46*, 421–426, 1950.

Principle

N′-methylnicotinamide reacts with ketones in an alkaline aqueous solution to produce a green fluorescent material. An excess of acid converts this compound into another more stable substance with a blue fluorescence, which may be measured with a photofluorometer.

Apparatus

Coleman Model 12C photofluorometer
Filters, same as for thiamine, B_1 and PC-1
Test tubes, 10-ml graduated or 15-ml graduated centrifuge tubes
Pipets, 1-ml and 5-ml graduated and 0.2-ml serological
Water bath, boiling
Erlenmeyer flasks, 50 ml
Volumetric flasks, 25 ml

Reagents

Methyl-ethyl ketone-$MnCl_2$ solution or acetone-$MnCl_2$ solution: to remove residual fluorescence, these solvents should be distilled in an all-glass still or shaken with activated charcoal and filtered. To prepare the working solution add 1 ml of aqueous M/10 $MnCl_2$ to 500 ml of the purified ketone.

Sodium hydroxide, 6 N solution.

Hydrochloric acid, 6 N solution.

Potassium dihydrogen phosphate, 20 percent solution.

Quinine sulfate, USP reference standard. (Same as thiamine standard.)

N′-methylnicotinamide stock standard: Ten mg of N′-methylnicotinamide are dissolved in 100 ml of water. This solution contains 100 μg per milliliter and may be used for several months if stored in the refrigerator. If the iodide salt of N′-methylnicotinamide is used, calculations are made on N′-methylnicotinamide ion.

Dilute solution: Two ml of the stock standard are diluted to 100 ml. This solution contains 2 μg per milliliter and is made fresh each day.

Procedure

Every tenth sample should be done in duplicate to check the reliability of the method.

Into graduated 10-ml or 15-ml test tubes (centrifuge tubes), add the following solutions:

[In milliliters]

	Blank	Unknown	Recovery
Filtered urine	0.2	0.2	0.2
N′-methylnicotinamide diluted standard	0	0	.4
Water	1.0	.8	.4
Methyl-ethyl ketone or acetone	.5	.5	.5
6 N NaOH	0	.2	.2
Mix, wait 5 minutes and add 6 N HCl	.3	.3	.3

Add porcelain boiling chip to each tube.

Mix all the tubes thoroughly and heat for 5 minutes in a boiling water bath and cool in cold water.

Add 1 ml of the potassium dihydrogen phosphate solution and make up to 10 ml with distilled water.

Readings are made using filters B_1 and PC-1, with the same quinine standard as for thiamine to adjust the instruments set at 60–80 units.

All the tubes should be read at the same time and temperature (26°–30° C is the steadiest range), and readings should be made quickly in order to avoid heating the tubes in the photofluorometer.

Calculations

$$\frac{\text{Unknown} - \text{Blank}}{\text{Recovery} - \text{Unknown}} \times 0.8 \times \frac{5}{1{,}000} \times 6 \text{ hr volume} = \text{mg per 6 hours}$$

Comments

Some workers[1] have decolorized highly pigmented urines and those with high blanks with charcoal, but this procedure may adsorb some N′-methylnicotinamide and is of doubtful value.

Although either may be used, methyl-ethyl ketone is less volatile than acetone and also gives higher fluorometer readings. The ketone should always be added before the sodium hydroxide, never after it. If N′-methylnicotinamide solutions are made alkaline in the absence of ketone, significant destruction occurs.

Manganese ion catalyzes the production of the fluorescent condensation product. Therefore, this is added in trace amount since Mn^{++} may or may not be provided as a contaminant in the NaOH.

Cholesterol in Plasma or Serum

REFERENCES

Mann, G. V., A method for measurement of cholesterol in blood serum. Clin. Chem. 7, 275–284, 1961.

Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. *195*, 357–366, 1952.

Morris, T. G., A comparison of methods for the estimation of serum cholesterol and values in random samples of populations in the 55–64 age group. J. Clin. Path. *12*, 518–523, 1959.

Anderson, J. T., and Keys, A., Cholesterol in serum and lipoprotein fractions. Clin. Chem. 2, 145–159, 1956.

Principle

The serum is digested with alkaline alcohol and the cholesterol is extracted with petroleum ether. The extract is treated with a solution of iron in sulfuric and acetic acids and the resulting violet color is measured photometrically.

Apparatus

Coleman Jr. spectrophotometer
Water bath set at 65° C
Test tubes, Pyrex, with glass stoppers, 125 x 200 mm
Cuvettes, Coleman, 19 x 150 mm

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[1] Huff, J. W., Perlzweig, W. A., and Tilden, M. W., A simple method for the determination of N′-methylnicotinamide in urine. Fed. Proc. 4, 92–93, 1945.

Reagents

Absolute alcohol

Potassium hydroxide, 33 percent solution: dissolve 330 gm of KOH in about 750 ml of distilled water, cool, and dilute to 1 liter.

Alcohol—KOH mixture: prepare each day as needed a mixture of 6 parts of the 33-percent KOH with 94 parts of absolute ethyl alcohol.

Petroleum ether: redistill reagent grade solvent in a glass still. Collect the 30°–60° C fraction and store in a brown bottle.

Iron Stock Solution

Dissolve 2.50 gm $FeCl_3 \cdot 6\ H_2O$ in 25 ml of glacial acetic acid. This is stable in the refrigerator for many months.

Iron-Sulfuric Acid Reagent

Place 1 ml of the iron stock solution in a 100-ml volumetric flask and dilute cautiously to volume with reagent grade concentrate H_2SO_4. Discard if and when a precipitate appears.

Cholesterol Standards

Stock standard

Dissolve 100 mg of repeatedly recrystallized cholesterol (MP>148.0° C) in glacial acetic acid in a 100-ml volumetric flask. Bring to volume with glacial acetic acid. This contains 1,000 μg cholesterol per milliliter.

Working standard

Place 10 ml of the stock standard in a 100-ml volumetric flask and dilute to volume with glacial acetic acid. This contains 100 μg per milliliter. Both standards are stable in the refrigerator for 2 to 3 months.

Procedure

Transfer 0.1 ml of well-mixed serum or plasma to the bottom of a digestion tube. The amount is critical; a Levy-Lang constriction pipet is used. Between samples rinse the pipet with water and blow out.

Add 5 ml of freshly prepared alcoholic KOH; mix by swirling.

Place the tube in a 65° C bath for 60 minutes with stopper loose.

Remove the tube and cool to room temperature. Add exactly 10 ml of petroleum ether with a pipet, replace the stopper, and mix vigorously by repeatedly inverting for 1 minute. Add 5 ml of distilled water to each tube, stopper, and mix vigorously (by inverting) for 1 minute.

When the petroleum ether layer separates—and if it does not—centrifuge 10 minutes at 1,500 rpm, remove exactly 2 ml of the ether layer with a pipet. This is a convenient place to take duplicate samples. Transfer this to the bottom of a 19 x 150 mm cuvette. The cuvette must be wide enough for subsequent mixing. Place the cuvette in the 65° C bath in a well-ventilated place until all the solvent evaporates. This requires about a half hour. The evaporation can be hastened by passing nitrogen gas over the tube. *Smell*

the cuvette for the last traces of solvent. Cool the cuvette to room temperature.

Add 4 ml of glacial acetic acid to each cuvette. Prepare four additional cuvettes, one for a blank and three for standard solutions, containing 20, 50, and 100 μg of cholesterol, respectively. Add 3.8, 3.5, and 3.0 ml of glacial acetic acid to each standard as appropriate.

Add exactly 2 ml of iron-sulfuric acid reagent to each cuvette with a 5-ml pipet. Do two cuvettes at a time and mix the contents well by swirling. The cuvettes will heat and a transitory brown color will change to violet. Gas bubbles will form. If a petroleum ether residue is present, an emulsion will result. Discard such a tube.

After 20 to 30 minutes at room temperature, the tubes are cool. Read at 560 mμ with the instrument set at OD zero with the reagent blank.

If the optical density of the sample is more than 0.600, a smaller amount of the petroleum ether extract should be used. If the optical density is less than 0.100, a larger amount should be used.

Calculations

Calculate a multiplication factor from the standard curve each day by dividing the milligrams of each standard tube by the OD observed. Average these for the multiplication factor, F. The OD$\times F\times$dilution factor = mg percent.

Procedure With Filter Paper

When the samples are stored and transported on filter paper, the procedure is almost the same. Either 0.1 or 0.2 ml of serum is distributed on 5-cm of Whatman No. 1 filter paper. It is convenient to apply this directly from a Levy-Lang constriction pipet. The paper is dried for an hour at room temperature, with care taken to handle only the edges bearing the penciled identity numbers. The papers are then stored and transported in envelopes.

The portion of the paper stained with serum is cut with scissors into 5 x 10 mm sections that are dropped into the digestion tube. The saponification fluid is added as with serum. Care is taken that all the paper fragments are submerged. The procedure continues as above, except that 6 ml of water is added instead of 5 during the hexane extraction stage. This accounts for the little water absorbed by the paper and prevents an otherwise slightly low recovery, apparently due to a disturbance of the solvent distribution equilibrium during extraction.

Comments

Table 3 below shows how this method compares with four others in common use. Table 4 presents reproducibility data obtained with this method.

TABLE 3.—*Precision of 4 methods for measurement of cholesteremia*

[mg/100 ml]

Level	No.	Abell		Present method		Pearson [2]		Sackett [3]	
		Mean	S_E [1]	Mean	S_E	Mean	S_E	Mean	S_E
<210	15	187.8	6.40	179.9	5.55	179.1	7.68	237.3	3.16
210-274	15	231.8	4.08	228.3	5.67	225.2	8.80	288.0	3.18
275-499	14	368.0	5.22	383.5	7.55	340.0	15.07	451.6	12.85
>499	15	671.7	7.73	667.1	10.39	643.0	26.86	837.0	23.02
All levels	59	364.8	6.01	364.7	7.55	346.8	17.05	453.5	14.19

[1] S_E = technical error: $\sqrt{\frac{\Sigma \text{ diff}^2}{2\,Pr}}$

[2] Pearson, S., Stern, S., and McGavack, T. H., A rapid, accurate method for the determination of total cholesterol in serum. Anal. Chem. 25, 813-814, 1953.

[3] Sackett, G. E., Modification of Bloor's method for the determination of cholesterol in whole blood or blood serum. J. Biol. Chem. 64, 203-205, 1925.

TABLE 4. *Performance of the iron-sulfuric acid method with blind duplicates under normal working conditions*

	Serum duplicates	Solvent duplicates [1]
Number of pairs	18	36
Mean level (mg/100 ml)	207.2	206.9
Technical error	11.0	5.45

[1] Duplicate hexane extracts of the same samples (not blind).

Electrophoretic Separation of Serum Proteins

REFERENCES

Block, R. J., Durrum, E. L., and Zweig, G., A Manual of Paper Chromatography and Paper Electrophoresis, 2d ed. Academic Press, New York, 1958.

Hoerman, K. C., and Woodruff, C. W., Blood proteins in residents of Egypt. Arch. Environ. Health 2, 673-678, 1961.

Paper Electrophoresis System, Instruction Manual RIM-5. Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.

Principle

Proteins manifest a number of properties which may be utilized to effect a resolution of mixtures such as are found in blood serum. Thus, each of the serum proteins has distinctive size and shape, carries a net charge, and demonstrates amphoteric behavior.

Paper electrophoresis has proved to be a most useful method for determining the various protein components of serum. Rapid resolution of albumin, alpha$_1$-, alpha$_2$-, beta-, and gamma-globulins can be obtained. It should be borne in mind that in paper electrophoresis, proteins with an excess of negative charges migrate toward the anode and those with positive charges move toward the cathode. For example, at pH 8.6, which is above the isoelectric points of the various serum proteins, migration will be toward the anode. The molecule having the lowest isoelectric point and thus the greatest excess of negative charges (albumin) will migrate at the highest rate. Gamma-

globulin exhibits slight migration toward the cathode because of electro-osmotic effects. Since many factors will affect the rate of migration of proteins on paper, the environmental conditions must be carefully controlled, e.g., electrolyte concentration, ionic strength, dielectric properties, pH, temperature, and the character of the electric field which is applied. A detailed description of the general theory and application of filter paper electrophoresis is given by Block, Durrum, and Zweig.

Many systems have been described for the separation of serum proteins by paper electrophoresis. Excellent results can be obtained employing the Spinco RB System with the Analytrol quantifying scanner.[1] Detailed instructions for operation of the equipment in the RB system are given in the Spinco Manual RIM 5.

The method by which blood samples are taken and handled is important to assure valid results. Ideally 5-ml samples should be taken by venipuncture and gently expelled into a paraffin-lined centrifuge tube, allowed to clot, then centrifuged. Hemolyzed samples should be discarded since hemoglobin and other red blood cell components adversely affect electrophoretic resolution of serum proteins. The serum samples should be frozen if the analyses are delayed, but they should not be thawed and refrozen more than three times. If facilities for freezing are not available, the serum samples probably will be stable at room temperatures not above 35° C for 24 hours and at refrigerator temperatures (4° C) for 48 hours.

Usually blood samples are taken by the examining physicians by use of vacutainers or by finger puncture. The vacutainers have proven satisfactory on a number of surveys. If samples are taken by the finger-puncture method, pressure should not be applied to the finger. Blood is drawn by capillary action into a 5-cm length of 1-mm glass tubing, the ends of the tube are sealed with soft wax[2] and the sample is stored just as are the venous blood samples. The serum separates without centrifugation and may be withdrawn for freezing, etc. If electrophoresis of serum proteins is to be done using finger-puncture samples, a number of preliminary determinations should be performed on finger and venous blood obtained simultaneously, since these two modes of obtaining samples have not given comparable results with some populations. (See Hoerman and Woodruff.)

Apparatus

Spinco Model R paper electrophoresis cell
Spinco Model RD 2 Duostat regulated power supply
Spinco Auxiliary Equipment set
Drying oven

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[1] Spinco Division of Beckman Instruments, Inc., Palo Alto, Calif.
[2] Utility wax, Type 1, from J. Birc' Moyer Co., Inc., 117 North 5th St., Philadelphia, Pa.

Reagents

All of the necessary reagents except methanol and acetic acid are supplied in the Spinco Auxiliary Equipment set. However, the other items can be prepared from commercial chemicals.

Veronal buffer, pH 8.6, 0.075 ionic strength: dissolve 2.76 gm diethyl barbituric acid and 15.40 gm sodium diethyl barbiturate in water and dilute to 1 liter.

Methanol, redistilled.

Acetic acid, 5 percent solution in water: dilute 150 ml glacial acetic acid to 3 liters with distilled water.

Bromphenol blue dye in redistilled methanol: dissolve 1.0 gm bromphenol blue in methanol and dilute to 1 liter with methanol.

Sodium carbonate, 0.5 percent: dissolve 5 gm of sodium carbonate in distilled water and make to 1 liter.

Procedure

The procedure outlined below is essentially that described in the Spinco Instruction Manual RIM 5, which should be consulted for more explicit directions.

Setting Up the Electrophoresis Cell

Insert the four removable baffles in the narrow inner grooves of the cell vessel, then insert the two wick supports in the outside grooves with fresh paper wicks in place. The wicks should face the outside.

Open folding rack into fully extended position and tighten both thumb nuts. Place eight paper strips on rubber pegs. Always use eight strips. Number and date the strips in pencil for identification.

Put the three glass rods into their holes in the support stand and fasten the metal bar into position. Put the support stand on the cell so that the slit in each leg straddles center partition, sliding into grooves.

Place extended folding rack on support stand, loosen the two thumb nuts and swing legs of rack down as far as they will go, then tighten thumb nuts.

Pour about 800 ml of the veronal buffer solution at room temperature into the cell vessel, put on cell cover engaging locking tab, then pour remaining buffer onto tops of paper strips using a funnel to do so. Put black tape over cover slit.

Make certain that each end of each paper strip is in contact with the paper wick below it. Strips should be brought in contact with wicks by means of a glass rod.

Raise the end of the cell which is opposite to the electrical plugs to a height of about 1 to 2 inches for a period of about 15 seconds. The port in the center partition near the end on which the plugs are located permits equalization of fluid levels. Make sure that

power to the cell is off before fluid levels are equalized. Check that the fluid level is at least one-sixteenth inch above the tops of the fluid baffles and one-sixteenth inch below the tops of the removable baffles.

Allow 15 minutes for excess liquid to drain from strips and for the closed cell to become saturated with water vapor.

Fill micropipet with 0.006 ml of serum and transfer to sample applicator. Place tip of pipet between the wires at one end of the applicator and deliver one-half to three-quarters of the sample while moving pipet along wires. Carefully expel the remainder of the sample and remove pipet from wires.

Apply sample to paper strip by inserting sample applicator through cover slot, resting applicator on middle glass support rod. Depress the red button briskly to obtain an even distribution of the sample across the paper strip. Apply samples from both ends of the cell so that cover tape is off a minimum of time.

Connect Duostat

Be sure that the Output Adjust switch on Duostat is in "Off" position, then plug Duostat into cell or cells. Each cell should be out of drafts or sunlight and at least 8 inches from the Duostat. Plug Duostat into power outlet.

Set Output Selector knob to 2-30 MA position and Meter Range knob to 0–15 MA position. Turn on Output Adjust, allow a few seconds for instrument to warm up, then turn Output Adjust switch until needle on meter reads 2.5 (ma) on bottom scale if one electrophoresis cell is connected and 5 (ma) on bottom scale if two cells are connected.

After 16 hours, turn off current, remove cell cover, open folding rack to its extended position and put rack into preheated oven at 120–130° C for 30 minutes.

Staining and Rinsing

Place dried strips in dyeing rack, prerinse in methanol, then insert into tray filled with fresh bromphenol dye in methanol (may be used up to 10 times) and place cover on tray. After 30 minutes transfer rack with strips to rinse bath of 5 percent acetic acid.

After 6 minutes, transfer rack to second rinse bath with 5 percent acetic acid. After 6 minutes, transfer rack to third rinse bath of 5 percent acetic acid. After 6 minutes remove strips, place on blotter and transfer to drying rack.

Place drying rack in preheated oven at 120–130° C; remove after 15 minutes.

Evaluation

The determination of the concentration of the various protein fractions is best done by use of the Spinco Model RB Analytrol. If an

Analytrol is available to the laboratory group, the strips to be scanned should be placed in a closed vessel which contains ammonium hydroxide vapors for at least 15 minutes before scanning and then the analysis should be conducted according to the procedure recommended by the manufacturer. If the strips are to be shipped to another laboratory for analysis, precautions should be taken to keep the strips dry during shipment to avoid the possibilty of migration of the stained zones in a humid atmosphere.

A more laborious method of evaluation involves the elution of areas from the strips and measurement of the optical density of the eluate in a spectrophotometer. The following outlines a procedure that may be used if an evaluation of some samples is desired at the base laboratory.

Cut across lightest region between each pair of bands, following any curvature. Also cut just before first band and just beyond last band. Cut a section where there is no obvious staining, of a width intermediate between sections already cut, to be used as a blank. Beginning with the leading component, four segments should be obtained: albumin, alpha$_1$-globulin, alpha$_2$-globulin, beta-globulin, and gamma-globulin.

Elute each section by gentle agitation with 0.5 percent sodium carbonate (anhydrous) in water. Use 20 ml for the leading albumin segment and 10 ml of eluate for the other segments. Extract 40 minutes with occasional agitation, or 20 minutes with shaking. Centrifuge or let stand for a few minutes to allow particles to settle, then read optical densities in Coleman spectrophotometer at 590 mμ against the blank described in the preceding paragraph.

Set up a standard curve relating optical densities versus known concentrations of bromphenol blue in 0.5 percent sodium carbonate using the carbonate solution as a blank.

Convert colorimeter readings of the various eluates to concentration by means of the calibration curve. Multiply the concentrations thus found by the eluant volumes required, as described above, to obtain the amount of bromphenol blue eluted from each segment. The sum of the values for each segment gives the total dye bound.

Divide the amount of bromphenol blue bound by each component by the total dye bound. This gives the fraction of the total dye bound by each component. This figure provides a measure of the distribution of electrophoretically resolved protein components of a serum.

The fraction of each component multiplied by the total concentration of serum protein as determined by the biuret or other methods will give a measure of the concentration of each component in serum.

The Preservation, Staining, and Concentration of Fecal Specimens by the "M.I.F.C." Technic for Protozoa and Helminth Egg Examination

This technic was developed in the Protozoology Laboratory of the Naval Medical Research Unit No. 3, Cairo, Egypt (NAMRU-3). The preparation of a fecal specimen takes approximately 4 minutes. The examination of the specimen can be made at a later period by laboratory personnel. Specimens preserved by this method have been used for study and reference material more than 2 years after preparation.

M.I.F. Preservative and Stain

Stock Solutions

Stock No. 1
Tincture of merthiolate, 200 ml
Formalin (37 percent formaldehyde solution), 25 ml
Glycerine, 5 ml
Water, distilled, 250 ml
Stock No. 2
Iodine crystals, 5 gm
Potassium iodide, 10 gm
Water, distilled, 100 ml

Working Solutions

Stock No. 1: 9.4 ml placed in a screw-cap vial
Stock No. 2: 0.6 ml placed in a screw-cap vial

Preparation for Use

Pour working stock no. 1 into working stock no. 2; mix thoroughly.

Place approximately 1 gm (size of two peas) of the fecal specimen to be examined into the mixed solutions and shake the vial vigorously to mix. The prepared specimen can now be examined at leisure, or sent to a laboratory for examination.

Concentration Technic

This procedure employs the M.I.F. solution as a preservative and a stain, with the addition of ether to dissolve fats and to float fecal detritus. The preserved fecal specimen is prepared as follows:

Mix the M.I.F. preserved specimen by shaking vigorously for 5 seconds.

Strain this mixture through two layers of wet surgical gauze into a 15-ml centrifuge tube.

Add 4 ml of ether to the centrifuge tube, insert a rubber stopper, and shake vigorously. (If ether remains on top after shaking add 1 ml of tap water and reshake.) The ether used should be refrigerated to reduce volatilization.

Remove stopper and let stand for 2 minutes.

Centrifuge for 1 minute at 1,600 rpm. Four layers will appear in the tube: an ether layer on top, a plug of fecal detritus, an M.I.F. layer, the sediment containing protozoa and helminth eggs on the bottom.

Loosen the fecal plug by ringing with an applicator stick.

Quickly but carefully pour off all but the bottom layer of sediment.

Thoroughly mix this sediment, pour a drop on a slide, mount with a cover glass, and examine.

Total Urinary Nitrogen by a Rapid Micro Method

References

Johnson, M. J., Isolation and properties of a pure yeast polypeptidase. J. Biol. Chem. *137*, 575-586, 1941.

Short, E. L., The estimation of total nitrogen using the Conway micro-diffusion cell. J. Clin. Path. 7, 81-83, 1954.

Toal, J. N., and Daniel, E. P., Simple digestion unit with notes on the micro-determination of nitrogen by direct Nesslerization. J. Lab. Clin. Med. *36*, 950-952, 1950.

Principle

The urine sample is diluted and an aliquot is digested with H_2SO_4. The ammonium sulfate formed is determined by a direct Nesslerization procedure. Twenty samples in duplicate can be easily handled by a single operator.

Apparatus

Two or three small digestion racks. These may be made conveniently from an empty 7-ml vacutainer can by punching or drilling 8 to 10 18-mm holes around the periphery of the bottom of the can. Five or six triangular-shaped wedges should be cut from the sides of the can top so that the samples can be seen during digestion. This rack is set directly on the top of an ordinary 7-inch hotplate and the test tubes, which should be slanted toward the center of the plate, rest directly on the hotplate surface.

Hotplate, electric, 3-speed (low, medium, high), 660 watts, 115-120 volts.

Test tubes, Pyrex, 150 mm long. Any diameter from 15 to 20 mm is suitable. (Vacutainer tubes are soft glass and should not be used.)

Boiling chips, porcelain.

Coleman 19 x 150 mm matched cuvettes.

Reagents

2 N H_2SO_4 containing 0.2 gm per liter of $CuSeO_3$ or $CuSeO_4 \cdot 5H_2O$. Add 55 ml concentrated H_2SO_4 cautiously to 500 ml of distilled water and dilute to 1 liter. Add the selenium compound.

NH_3-free distilled water. Deionized water is perfectly acceptable.

Modified Nessler's reagent: weigh out 1 gm of KI and 1 gm of HgI_2 and dissolve them in about 50 ml of distilled water. Select some light-colored pieces of gum ghatti and grind them to a powder in a mortar.

Weigh out 1.75 gm of the powder and drop into 750 ml of rapidly boiling distilled water. Boil until most of the gum ghatti is dissolved. Add the $KI\text{-}HgI_2$ solution, dilute to 1 liter, and filter through Whatman No. 1 filter paper. It will be necessary to replace the filter paper very frequently to speed filtration. Store in a brown bottle in a refrigerator. This is stable indefinitely, apparently even at room temperature.

2 N NaOH: dissolve 80 gm of NaOH in 500 ml of distilled water and dilute to a final volume of 1 liter.

Ammonium sulfate standard solution: dissolve exactly 0.2357 gm of pyridine-free ammonium sulfate in a little distilled water, add 5–6 drops of concentrated H_2SO_4, and dilute to 500 ml. This solution contains 1 mg of nitrogen in 10 ml of solution. It is stable indefinitely in a refrigerator.

Procedure

Place 0.1 ml of urine in a test tube using 0.1-ml blowout pipet. Add 9.9 ml of distilled water with a 10-ml blowout pipet. Mix well by inversion.

Transfer a 0.2-ml aliquot to the bottom of a 15 x 150 mm Pyrex test tube.

Add 1.0 ml of the 2 N sulfuric acid digestion mixture and a porcelain boiling chip.

Place the digestion tubes in the hotplate rack. Turn on the hotplate at the medium setting. The water will evaporate (30 minutes) and Kjeldahl fumes will soon appear. Permit digestion to take place for 2 hours after the fumes appear. Alternately, the water may be evaporated from each tube in a gas flame. The sample should be agitated constantly to avoid splattering. The digestion is then completed on the hotplate.

Another procedure is as follows:

Dilute 2 ml urine to 100 ml (volumetric flask).

Pipet 0.1 ml of above dilution into the tube for digestion. (Rinse pipet three times between samples.)

Place in a sand bath (ca. 290° C) until water evaporates off (about 2 hours).

Remove to a hotplate set at medium and digest for 2 hours.

Place the tube in a test tube rack and permit to cool for 5 to 10 minutes.

Add in order:

3 ml of distilled water
2 ml of Nessler's reagent
3 ml of 2 N NaOH

Permit the tube to stand for 15 minutes, transfer to a 19 x 150 mm Coleman cuvette, and read at 490 mμ.

Preparation of Standard Curve

Place the following duplicate aliquots of the $(NH_4)_2SO_4$ standard in 15 x 150 mm Pyrex test tubes:

ml	*μgN/tube*
0.1	10
0.2	20
0.4	40
0.6	60
0.2 ml distilled water	0 (blank)

Bring all volumes to 2.0 ml with distilled water.

Add 1 ml of the sulfuric acid digestion mixture.

Add 2 ml distilled water (*Caution:* avoid splattering!) and Nesslerize as above. "Digestion" of the standards before Nesslerizing has no effect on the readings.

A blank and the 20 and 40 μg standards should be run with each series of samples.

Comments

Under these conditions, the factor for the standard curve has been found to be ±75.0:

$$F = \frac{\mu g\ N/tube}{\text{Optical Density (OD)}}$$

It is necessary to run a curve daily since this factor will vary slightly from day to day.

Using the above factor and the amounts of urine specified, the nitrogen content of a sample is calculated as follows:

$$N_{(mg)}/ml\ urine = \frac{F \times 500}{1{,}000} \times OD$$

A 1-hour digestion is not normally sufficient to convert all of the nitrogenous compounds into ammonium sulfate. In two reproducibility studies, coefficients of variation of 6.1 and 7.8 percent were obtained.

Protein-Bound Iodine and Total Iodine—Zak Method Modified by Benotti and Benotti [1]

REFERENCE

Zak, B., Willard, H. H., Myers, G. B., and Boyle, A. J., Chloric acid method for determination of protein-bound iodine. Anal. Chem. 24, 1345–1348, 1952.

Principle

Iodine is oxidized to iodate by chloric acid digestion in the presence of sodium chromate. After digestion, iodate is estimated by its catalytic action on the ceric-arsenite system.

[1] Special acknowledgment is made to Joseph Benotti, director, Boston Medical Laboratory, Boston, Mass., for permission to print his adaptation of the method of Zak et al. for determination of iodine in blood and urine specimens.

Apparatus and Equipment

P.B.I. digestion tubes—Pyrex
Pipets, 0.5 ml Folin (48), 1.0 ml volumetric (24), 5.0 volumetric (6), 1.0 ml serological (6), 10.0 serological (6)
Separatory funnel, 500 ml
Volumetric G.S. flasks, 500 ml (2)
Reagent bottles, 1 liter, amber (12)
Florence flasks, 2 liter (3), 3 liter (3)
Graph paper, Dietzgen 340, 10 x 10 inch
Water bath adjustable between 27-37°C and thermometer
Racks, 8 holes, No. 68765 (Scientific Products, 101 Third Ave., Boston 54, Mass.) (3); 24 holes, No. 98922 (Standard Scientific Supply Corp., 808 Broadway, New York 3, N.Y.) (3)
Refrigerator with freezer compartment
Filter flask, 1 liter
Büchner funnel, size 4
Pipet washer and dryer, Technicon
Bunsen burner (Fisher)
Tripod
Wire gauze asbestos pads (2)
Centrifuge No. 1 SBR
Coleman Jr. spectrophotometer
Coleman cuvettes, 12 x 75 mm, No. 6-308 (12)
Adapter for above, tube No. 6-106
Special Tenco Hotplate (Benotti)
Hood

Reagents

Chloric acid: weigh into a 3-liter florence flask 500 gm of potassium chlorate (Merck reagent). Add 910 ml of distilled water and heat the mixture until solution is effected. With constant stirring, slowly add 375 ml of 70 percent perchloric acid (Merck reagent) by means of a separatory funnel. When the addition is complete, the solution will be greenish yellow. Potassium perchlorate will be largely present as a precipitate at this time. Cool and place the flask in the freezing compartment of a refrigerator for 12 hours. Filter the supernatant liquid through Whatman No. 1 paper in a büchner funnel by suction. This represents approximately a 28-percent solution of chloric acid.

33.3 percent sodium chromate: weigh out 33.3 gm of sodium chromate $4 \cdot H_2O$ and dilute to 100 ml. Add 1 ml of this to a liter of chloric acid. (Three ml of chloric acid will then contain 1 mg of sodium chromate.)

Ceric ammonium sulfate: add 24 gm to 1 liter of 3·5N H_2SO_4. (96.5 ml concentrated H_2SO_4 made up to 1 liter.)

Arsenious acid: dissolve 1 gm NaOH in 100 ml H_2O. Add 1.5 gm As_2O_3 and 44 gm NaCl. Add cautiously 382 ml H_2SO_4 to 500 ml

water. When cool, add the acid to the above mixture. Make up to 1 liter with water.

Standard iodate: weigh out 168.5 mg of dried analytical reagent potassium iodate and make up to 1 liter. Dilute 10 ml of this stock solution to 1 liter to make a standard solution containing 1 μg of iodine per ml.

Working Standards

Take 10 ml of dilute standard containing 1μg per ml and dilute to 500 ml. This gives 0.02 μg per ml.

Take 20 ml of dilute standard and dilute to 500 ml. This gives 0.04 μg per ml. 30 ml to 500 gives 0.06 μg per ml.

Sea sand washed and ignited (Merck)

Procedure

Serum Protein-Bound Iodine

To 0.5 ml of serum in a 40-ml round bottom centrifuge tube (Pyrex No. 8260), add 10 ml of 5 percent trichloroacetic acid. This should be done by blowing from a 10-ml serological pipet which has a wide orifice. The force of the stream will finely disperse the precipitated protein, resulting in better extraction of extraneous inorganic iodide from the protein. The precipitate is allowed to stand for 20 minutes to coagulate the proteins, then the tubes are centrifuged for 10 minutes. The supernatant fluid containing inorganic iodide is decanted and discarded. The protein is washed again with 10 ml of 5 percent trichloroacetic acid, recentrifuged, and the supernatant fluid discarded as before. The packed protein is now ready for digestion.

Serum Total Iodine

Take 0.5 ml of serum and 3 ml of chloric acid; digest as described under digestion below.

Urine Total Iodine

Take 0.5 ml of urine and 3 ml of chloric acid; digest as described below.

Digestion

To the material to be analyzed, containing less than 0.1 μg of iodine, is added 3 ml of chloric acid containing 1 mg of sodium chromate. The tubes are placed in an electrically heated sand bath.[1] The temperature is set between 105° C and 110° C and the tubes are allowed to digest for 1 hour unattended. At the end of this time the tubes have to be watched rather closely for color changes such as orange-yellow to light green, and finally to colorless. It is important to add chloric acid drop by drop whenever necessary in order to prevent the above color changes and thus avoid the loss of iodine. This procedure

[1] Obtainable from E. F. Mahady Co., 224 Msgr. O'Brien Highway, Cambridge, Mass.

is continued for 1 hour and the [illegible]
allowed to cool. Red crystals of chromium trioxide should appear at this point. If not, the digestion must be continued for several more minutes, or until red crystals appear on cooling.

Colorimetric Determination of Iodine

Add 10 ml H_2O.

Add 2 ml of arsenious acid.

Incubate at 27° C for one-half hour.

Add 0.5 ml ceric ammonium sulfate serially to each tube at 2-minute intervals. Continue incubation for 24 minutes and transfer to 12 mm Coleman tube. Read in colorimeter every 2 minutes at 420 millimicrons.

Prepare a linear graph showing iodine concentration against transmission in percent.

$$\mu g \text{ iodine found} \times \frac{100}{\text{ml used}} = \mu g \text{ percent}$$

VII

Dietary Studies

Objectives

Dietary studies constitute an essential part of any complete nutrition survey, providing essential information on nutrient intake levels, sources of nutrients, food habits, and preparation practices and attitudes. Under conditions where clinical observations are insensitive for detecting basic nutritional problems, nutrient intake studies are essential. Nutrient intake data offer direct means of estimating dietary levels for several nutrients for which routine laboratory or clinical tests have not yet been developed. The solution of problems thus uncovered depends upon knowledge of eating habits and the availability of food in conjunction with a sound program of food technology.

Although both clinical examinations and biochemical tests provide direct means of studying the nutritional status of the individual, the basic information collected in dietary studies is necessary for planning desirable changes in nutritional practices. Dietary studies give information which is needed for modifying existing economic, agricultural, and food management policy programs.

For these reasons dietary studies include the collection of information concerning food habits and customs, food supply and seasonal variations, food procurement and distribution, methods of food preservation and preparation procedures. Information is collected also on infant and child feeding, school and institutional feeding practices, economy of food use, unique nutrient contributions of special food items and nutrient content of unusual foods of significance. In addition, the amount of specific foods ordinarily consumed, as well as the adequacy of the nutrient content of food actually consumed, is also obtained.

Dietary studies are made to obtain information needed to plan recommendations for improved use of food. In military organizations the basis for the ration system and specific practices concerning food procurement, distribution, and use are studied. The study in-

cludes ration allowances in terms of food items or monetary value, menu preparation, food waste, and occasionally food production. An additional objective is the training of local personnel in methods used in conducting a nutrition survey.

Personnel and Equipment

The minimum personnel requirements for conducting dietary surveys in military units or institutions where group feeding is practiced are:

A nutritionist (in ICNND surveys a U.S. team member) with experience in field survey procedures, assisted by local personnel. The latter should include a nutritionist with training in biochemistry and foods, a food service specialist (familiar with food procurement practices in the military), two additional assistants, and an interpreter, if needed. A biochemist or veterinarian may be substituted for the food service specialist. In addition, a medical officer or sanitarian at the unit level should participate if available. These requirements are less than those encountered in surveys of civilian populations.

In dietary surveys of civilian population groups it is necessary to obtain data through questionnaires and limited home or institutional surveys. Consequently, the success is dependent largely upon the availability of capable and interested individuals from the host country. For this type of survey, when information must be collected within a relatively short time, the minimum personnel needs include a nutritionist (U.S. team member) with experience in civilian dietary survey procedures, a senior dietitian from the host country, and five or more women from the host country familiar with foods and food preparation procedures who are capable of interrogating housewives and conducting home studies, and an interpreter if required.

The senior dietitian coordinates the work of the other local personnel and assists in the processing of data. One of the remaining five is available for collecting dietary data from special groups examined, including infants, pregnant women, and hospital patients. The others are used to interrogate housewives in the home or at clinical examination sites, and individuals in school, as well as to collect actual food intake data in a limited number of homes.

The equipment and supplies needed for a dietary survey team are presented in table 1.

TABLE 1.—*Dietary equipment and supplies*

Scales, platform-type, capacity 300 lbs, ¼-lb interval. Scales should be in metric units if metric system is used in the country, 1 each
Triple beam balance, 600 gm capacity, 1 each
Balance, dietetic scale, spring, 500-gm capacity movable dial, 5 each

TABLE 1.—*Dietary equipment and supplies*—Continued

Scale, Triner, model ME-8DB, capacity 8 kg x 1 gm, special metric beam, 5 each
Hand-tally counter, 2 each
Dietary record forms, pencils (24), paper pads (12), ballpoint pens (12), manila folders or envelopes for records (12), record books, etc.
Clipboards for record sheets, 10 each
Waring blendor:
  1-gallon capacity, 1 each
  1-quart capacity, 2 each
Heat-resisting ink or marking crayons for marking tare weights
Kitchen spatulas (rubber plate scrapers), 4 each
Plastic sample containers, wide mouth (about 8-10 dozen 500-1,000-ml sample containers)
Plastic reagent bottles with screwcaps, small mouth (1 dozen 500 ml and ½ dozen 1 liter)
Oxalic acid, reagent grade, 3 lb
Potassium hydroxide, reagent grade, 1 lb
Alcohol, ethyl, 95 percent (the amount of ethanol required will vary with the number of food composites prepared), 2 qt
Chloroform, reagent grade, 2 lb
Hydrochloric acid, reagent grade (for acidifying large food composites), 2 lb
Cylinder, graduated, 100 ml (plastic preferred), 2 each
pH paper (range of 4.5 to 8.5 pH and range of 2.0 to 5.0), 2 rolls
Labels for sample containers, 4 packets or rolls
Automatic or hand-operated calculator, 1 each
Slide rules (10″), 2 each
Masking tape, 1″ x 60 yd, 6 rolls
Camera, film, and flash bulbs. (If an official photographer is present as a team member, he will supply this equipment.)
Marker, felt, 6 each
Thermometer, pocket, metal case, 20°–105°, 2 each
U.S. Department of Agriculture Handbook No. 8, 1963, 4 copies
Food and Agriculture Organization of the United Nations, 1959, Food composition tables—minerals and vitamins—for international use (FAO nutritional studies No. 11), 4 copies
INCAP-ICNND Food composition tables for use in Latin America, 1961, 4 copies
U.S. Department of Defense, composition of foods used by the armed forces, 1964, No. DSAH 1338.1, 4 copies
Food and Agriculture Organization of the United Nations, 1958, Calorie requirements (FAO nutritional studies No. 15), 4 copies
Food and Agriculture Organization of the United Nations, 1960, Protein requirements (FAO nutritional studies No. 16), 4 copies
Food and Agriculture Organization of the United Nations, 1962, Calcium requirements (FAO nutrition meetings report series No. 30), 2 copies
British Medical Research Council, 1962. Tables of representative values of foods commonly used in tropical countries, Special report series No. 302, 4 copies

Procedures for Military or Other Institutional Dietary Surveys

In appraising dietary adequacy in the military or other institutional messes, studies should be made of the official ration allowances, system of menu planning, method of food procurement and distribu-

tion, food preparation practices, and quantitative measurements of food consumed in operating messes. These should include observations on food habits, overall availability and costs of different foods, seasonal differences in availability of foods, economy of food use, and sanitary practices involved in procurement, handling, storage, preparation, and service of food. Form 1 provides a guide for obtaining information on food services available to the armed forces. (See p. 190.) Some of the items are discussed below.

Ration Allowances and Menu Planning

Most military forces have a monetary allowance which cannot be exceeded in the procurement of the ration. In most cases this is based on specified quantities of individual food items authorized per man per day, while in others no specified allowance of food exists. Unless the monetary ration allowance or portion thereof is given directly to unit commanders for the direct procurement of food items, menus are prepared which determine to a large extent the food items procured and issued to the unit kitchens. In some instances a master menu is prepared and subsequent area menus are made which embody only those changes considered necessary to render them most appropriate for each location, consistent with availability of local food items and local food habits. Complete information should be obtained concerning the basic food and monetary allowance, master menus, and several area menus during the year preceding, as well as during the period of the survey in order to evaluate seasonal differences in nutritional adequacy.

If monthly or quarterly menus are available, the nutritional adequacy of these menus should be calculated over a sufficient period of time to include all major seasonal variations. Similar calculations of nutrient intake per man per day should be made if a master menu is available or if a basic food allowance is prescribed. These calculations will reveal the amount of various foods available and the contribution of each critical nutrient from the various foodstuffs. In addition, estimates of possible nutrient losses during preparation and cooking should be made with the assumed losses clearly indicated.

Food Procurement and Distribution

Where centralized procurement and distribution of food items are practiced, detailed information concerning the system should be obtained. In many cases, certain items of food will be procured centrally for the entire military force, while such food items as perishable vegetables will be obtained locally through field buying offices. In other instances, unit commanders will be provided a portion of the monetary allowance for the ration and be charged with procuring certain perishable commodities.

Determination of nutritional adequacy of food issues to divisions or subordinate units provides exceedingly important information. These data usually can be calculated from the supply records for different seasons of the year. This is done by selecting the appropriate units which are receiving the full ration and determining the average amount of the foods issued per man per day on the basis of unit strength reports for those periods. From such data, the average nutrient composition of the ration as issued may be calculated. This method permits an appraisal of the nutritional adequacy of the ration for units in seasons of the year other than that in which the survey is being conducted when the available food supply might be quite different. Hence, one can obtain information for a longer period than is feasible with mess survey technics.

Prior to starting a dietary intake study in the unit kitchen, the dietary team members should become familiar with the procedure of food procurement, food storage and issue, menu planning, and monetary value of the ration. Throughout the survey, the procedure of food service should be further investigated. The report should contain a completed form (form No. 1) of the general food procurement and issue system in operation. Special attention should be given to the various types of rations; e.g., supplementary, hospital, emergency, and field.

Dietary Intake Studies

The military or institutional mess provides an ideal opportunity to determine with accuracy the average amounts of various food items consumed per day by the individual. From this quantitative intake information the nutrient intakes may be readily computed using tables of food composition (*1-6*). Two procedures described below for collecting this information are the recipe method (food preparation and consumption method) and the food inventory method. The direct sampling of food served in the mess is readily accomplished with subsequent laboratory analysis of a suitable composite mixture representing the average diet as consumed. This latter approach, termed the "food composite analysis method," provides certain information not obtainable by the other procedure.

Whenever possible, it is desirable to conduct the detailed dietary intake studies in units from which individuals are drawn for the detailed clinical and laboratory surveys. In any case, a typical normal mess is selected. A period of approximately 2 days is generally sufficient unless the variety of foods used is considerable. Sundays and holidays should be included in proportion as they occur in the calendar of feeding and should be weighted in relation to the strength fed on those days.

A general description of primary food likes and dislikes of the individuals and a record of the number taking various foods should be made. These data are to be obtained by observation during the time meals are served. Of the three methods used to determine the nutrient intake, the recipe method and food composite analysis method are usually done at the same time in military messes. Under some conditions where a large variety of food items is included during the study and where a well-organized food handling system exists, the food inventory method may be used together with the food composite analysis method. These methods are discussed below.

Recipe Method (Food Preparation and Consumption Method)

This procedure consists of determining the amount of each food item consumed in the mess during the survey period by weighing each edible food used in the preparation of each meal and correcting this amount for any food which is not consumed. From the number of individuals served (head count), the average amount of each edible food item used in the preparation of each meal is determined for the survey period. From these data, nutrient intake calculations are made using food composition tables (*16*). It is desirable for the nutritionists to prepare a table of food composition data to be used in both military and civilian work, even though the values for certain foods may be tentative. Forms 2 and 3 are used for this method and for the food composite analysis method. (Where unusual foods are encountered, they should be identified and described. If they contribute a significant portion of the ration, arrangements should be made to have samples forwarded to ICNND or to another laboratory for chemical analysis. (See app. A and ch. VIII.))

This procedure is suitable for any type of kitchen operation, but is particularly well suited where little variety and relatively simple food preparation practices are involved. It also eliminates the need for personnel to record the food brought into and removed from the kitchen during the study period. The procedure is described as follows:

1. Prior to or at the start of the study, weigh and mark all cooking vessels to be used in food preparation.

2. During the period of the survey, an accurate count must be made of all men eating at each meal, including regulars, casuals, cooks, etc.

3. A measure must be obtained of the fat lost as waste grease and fat drippings not subsequently consumed. Unless this is an appreciable amount, several determinations may be made of the small quantity of fat drippings and fat in plate washings and this value may then be used in all subsequent computations.

4. During the preparation of each meal, the amount of edible food items used in the preparation of each menu component served at the meal is recorded. All weights are expressed in terms of edible portion as contrasted to "as purchased" weight. This includes amount of fat, seasonings, salt, etc., as well as the major components of the recipe (form No. 2, p. 191). The method of preparation, cooking time approximate temperature, pH of cooking water, amount of water used, and method of service should be recorded.

5. After food preparation is completed, the weight of each prepared menu item is recorded on form No. 3. (See p. 192.) After the meal is served, similar weights are obtained to determine the amount of prepared food not served. Additional weighings are required to determine the amount of each prepared food served but not consumed. This is accomplished by collecting each food item in separate containers and weighing the plate scrapings at the end of the meal. Where plate scrapings include inedible items such as bone or seed, or where the plate scrapings represent a selected portion of the food as served, such as fat from meat, it is necessary to determine the approximate amount of edible food which is not consumed. In most messes the amount of edible food appearing as plate scrapings is quite small and does not constitute a serious problem, except in messes where excessive amounts of food are available. In these instances the plate scrapings must be clearly identified quantitatively in order to make proper corrections for the edible food discarded.

6. From the data recorded on form No. 3, the total amount of food consumed per person per meal is determined following each meal. This is then expressed as percent of prepared food actually consumed, and this percentage value for each menu item is multiplied by the edible weight of each food used in its preparation to determine the average amount of each food item consumed per person per meal. (See form No. 2.)

7. At the end of the survey the average amount of food items consumed per man per meal is combined to give the average amount consumed per man per day during the survey period. Form No. 4 is used to calculate the average nutrient intake per man per day from these data. (See p. 193.)

8. After the total gross nutrient content of food consumed is evaluated, corrections for losses of heat-labile or water-soluble vitamins which are deemed to have occurred during the study may be applied. In some instances it is possible to determine the losses of critical nutrients which occur during the study. In other instances the loss must be estimated from knowledge of the cooking methods and preparation and storage practices. Estimated cooking losses which may

occur are given in table 5 as a guide. (See p. 185.) Deviations from these values, however, should be made where knowledge makes better estimates possible. Local data on nutrient cooking losses should be used when available. References and corrections applied should be recorded and documented in the final report.

Food Composite Analysis Method

The food composite analysis method is complementary to the other methods of determining dietary intake data. It provides a means of determining more nearly the actual intake of critical nutrients after losses in preparation and variability in nutrient content of foods. This approach is particularly valuable in determining intakes of those nutrients for which little or no reliable food composition data are available. This includes nutrients such as pantothenic acid, pyridoxine, folacin, cobalamine (vitamin B_{12}), biotin, iodine, and other trace minerals of critical importance which might be expected to vary appreciably. This procedure also reflects in part nutrients (such as calcium or iron) which may be provided by water used in food preparation.

This method embodies the sampling of each menu item served during the meal with subsequent blending of representative samples of these foods in the actual proportion consumed to give a representative composite of the meal. At the end of the survey period, composite samples which present the entire survey period are prepared for analyses.

The amount of each prepared food served at each meal is obtained from the data derived, using the recipe method described above and recorded on form No. 3. This is based on the weight of the prepared food item less the amount not served, corrected for plate scrapings, and expressed on the basis of average amount per person at the meal.

As the food is being served during the meal, aliquots of each prepared food item are taken directly from the serving container. Two to four average servings of each item are desirable. Where sampling is difficult as, for example, with soup, a minimum of four aliquots should be taken at the start of the meal.

Preparation of the food composite should be made following each meal in order to minimize losses of labile nutrients. The exact approach for the preparation of the food composites will vary in accordance with the number of days the survey is to be conducted in a particular mess. The following example illustrates a procedure which is suitable for a 2-day survey period. This procedure as described involves the blending of the components of each meal immediately following the meal and mixing with preservatives to minimize nu-

trient losses. (See app. B.) Assume the following food items were served for the six meals of a 2-day survey as follows:

Date	Meal	Number of men fed	Food item	Average weight eaten per person	5 percent of total of average weight eaten
1962				*gm*	*gm*
Aug. 24	Breakfast [1]	158	Coffee	313.2	
			Banana	121.5	
			Bread	39.5	
			Total	474.2	23.7
Do.	Lunch	174	Soup	443.3	
			Meat	105.6	
			Rice	209.2	
			Beverage	335.1	
			Baked plantain	84.7	
			Bread	69.1	
			Banana	109.5	
			Water	150.0	
			Total	1,506.5	75.3
Do.	Dinner	156	Soup	680.5	
			Beans	160.9	
			Rice	213.7	
			Coffee	354.8	
			Bread	31.7	
			Total	1,441.6	72.1
Aug. 25	Breakfast	149	Coffee	313.3	
			Bread	39.7	
			Banana	117.7	
			Total	470.7	23.6
Do.	Lunch	157	Soup	497.1	
			Pudding	86.0	
			Banana	80.9	
			Rice	239.9	
			Beverage	361.3	
			Bread	27.5	
			Total	1,292.7	64.7
Do.	Dinner	152	Soup	500.7	
			Peas	176.2	
			Rice	142.4	
			Coffee	305.3	
			Bread	33.8	
			Total	1,158.4	58.0

[1] At this meal, for example, the coffee, banana, and bread were homogenized in a Waring blendor before the 5-percent aliquot was weighed. The weights of each food item blended equal the "average weight eaten."

It is desirable to utilize the beverage with its high moisture content to reduce the viscosity of the food for a particular meal with the minimal addition of water in order to facilitate homogenization in the blender. Sometimes additional water must be added for satisfactory blending; in this case the amount of water used is weighed and recorded as part of the aliquot. In all cases samples of the water used in making up the aliquot should be collected. The water normally used for routine food preparation is recommended for this purpose.

Immediately following the preparation of a homogenized meal, the sample of homogenized food representing 5 percent of the average amount eaten is weighed and introduced into sample containers that

already have the various preservatives (app. B) added to them. Before any food is introduced into a sample bottle, the bottles are made ready by weighing the empty bottle with its screwcap and then introducing *weighed amounts* of oxalic acid or alcoholic potassium hydroxide, water, and chloroform so that after all food has been added to make the composite, the concentration of preservative will be at the desired level. A concentration of approximately 5 percent of oxalic acid is recommended in the final food slurry, although it may be reduced to one-half of this level without concern. For a composite where the final weight will be about 500 gm, 25 gm of oxalic acid crystals are introduced. The recommended concentration of alcoholic potassium hydroxide for samples on which carotene and vitamin A are to be determined is 50 gm of 1 percent alcoholic potassium hydroxide (KOH in ethanol, 95 percent) in each 250 ml of total food slurry (7). In addition, 7.5 gm (approximately 5 ml) of chloroform is added per 250 gm of final food slurry. The following scheme will indicate how samples preserved with oxalic acid and alcoholic potassium hydroxide and chloroform are prepared from the six meals shown above.

Preparation of food composite

	Oxalic acid-preserved sample	KOH preserved sample
	gm	*gm*
Tare weight of sample container	122.5	120.4
Oxalic acid	25.0	
H_2O	90.0	100.0
Alcoholic KOH (1%)		15.0
$CHCl_3$		
Total weight of tare and preservatives	237.5	235.4
Breakfast: Aug. 24, 1962	23.7	23.7
Lunch: Aug. 24, 1962	75.3	75.3
Dinner: Aug. 24, 1962	72.1	72.1
Breakfast: Aug. 25, 1962	23.6	23.6
Lunch: Aug. 25, 1962	64.7	64.7
Dinner: Aug. 25, 1962	58.0	58.0
Additional H_2O added	67.6	67.6
Total weight	622.5	620.4

In the sample shown above it will be noted that when the tare weight is subtracted from the total weight, the contents of the bottle will weigh exactly 500 gm. Therefore one full ration would have weighed 5,000 gm. (This 500 gm represents 5 percent of the ration for each of 2 days.)

The amount of water added is just sufficient to give a rounded figure for the sample weight. The same source of water should be used for preparing the food composites that is used for drinking and for preparation of foods. Samples of this water should be taken and sent to the laboratory for analysis for calcium, iodine, fluorine, and sometimes iron. Corrections may then be made for these inorganic elements added with the water.

If a survey period other than 2 days is employed, the amount of an average meal added to the food composite sample will differ from 5 percent. For example, in a 1-day survey, 10 percent of an average meal would be needed. Following the addition of each 5 percent of an average meal, the sample bottle is closed and shaken or swirled sufficiently to mix the contents well with the preservative. Bottles should be filled to only two-thirds of capacity, especially if they are to be frozen. The bottle cap should be screwed on firmly and sealed with masking tape.

At the conclusion of the survey period the samples are placed under refrigeration or are frozen as soon as practicable. They should be clearly labeled giving total weight, tare weight, sample numbers, dates of collection, unit and location, and weight required for one ration. The amounts of preservative used also should be recorded (see example below). They may be shipped at once to the laboratory for analysis or may be stored in the frozen state until all samples from different military units are ready for a single shipment.

Example of label for oxalic-acid preservative sample

Sample No.	0-12.
Dates of collection	May 1 and 2, 1962.
Unit and location	113 Inf. Bn. (location)
Total weight	622.5 gm.
Tare weight	122.5 gm.
Amount required for average ration	5,000 gm.
Amount oxalic acid included	25 gm.
Amount of 1% alcoholic KOH	None.

The homogenized food remaining after weighing four different 5 percent portions will equal about 80 percent of an average meal. This remaining food may be discarded if not needed or, if additional studies are desired, it may be combined in a suitable container with the remaining food from the other meals of a particular day to give a quantity of food representing about 80 percent of a single ration for 1 day. It is best to acidify this food to a pH of about 2 to 2.5 with concentrated hydrochloric acid to prevent fermentation or bacterial spoilage, unless the samples can be refrigerated or frozen.

The food composite analysis method is particularly desirable where unusual food items are eaten in large quantities. It also provides an excellent check on the dietary intake data obtained by either of the other methods described previously. The analysis of foods after cooking gives a more accurate assessment of the true dietary intake of those nutrients, such as vitamins and minerals, which are partially destroyed or lost in the preparation and cooking of foods. It is not necessary to apply corrections (estimates) for cooking losses in this method. Furthermore, samples may be subjected to assay for nutrients for which analytical values are not available, such as amino acids, certain vitamins, and minerals.

Food Inventory Method

Although the food inventory method is more difficult to perform and more complicated than the recipe method described above, it may be the method of choice where a great variety or considerable plate waste occurs or where extremely large kitchens are involved. This procedure together with a definition of terms employed is given below:

Definition of Terms Used in Food Inventory Method

a. "As purchased." The form in which the food is issued to the mess, such as carcass beef (raw), untrimmed fruits and vegetables, etc.

b. "Nonedible refuse." The portion of food that is not usually eaten, such as bones, fruit pits, rind and peel, egg shells, and outer leaves of vegetables. In practice, it is necessary to consider actual procedures in use in the mess being surveyed for estimating "nonedible refuse" rather than rely on "nonedible refuse" values found in food consumption tables.

c. "Edible portion." As purchased weight minus refuse weight.

d. "Plate waste." Weighed quantities of edible plate scrapings that are recorded by food item.

e. "Kitchen waste." Weighed quantities of food prepared but not served, *only* if discarded. Also food partially prepared but discarded, including cooking fats.

f. "Preparation loss." All nonedible portions of food that are discarded during preparation of the food.

Procedure

1. The evening before the study is to start, a complete inventory is made of all food items in the mess (kitchen, storage tent, etc.,) and tare weights are marked on the cooking utensils.

2. During the period of the survey, all foods coming into the mess, including the daily issues of perishable foods and periodic issues of nonperishable items, are weighed and recorded. The source of foods also should be recorded.

3. Head count at each meal is determined. The number of men eating varies from meal to meal; hence the total number fed at the three meals is divided by three to give the average number of rations for the day. Where more than three meals are served, such as midmorning and afternoon teas, it is necessary to weight the meals according to their relative importance in obtaining the total number of rations for the period.

4. A measure of fat lost as fat drippings during cooking which are not subsequently consumed should be made. This is sometimes ignored and may account for considerable difference in estimated caloric intake. Nonedible kitchen waste, such as vegetable peelings, bones, cores, and pits from fruit, etc., is weighed and recorded. When

this contains proportions of potentially edible food, it should be separated and recorded as edible waste.

5. During the hours of mess operation, one or more members of the survey team must be present to record recipes, method of preparation, cooking time, temperature, pH of cooking water, amount of water and fat used, seasoning, etc., as well as the weights of all issued foods utilized by the cooks.

6. All plate and kitchen edible food which is discarded is collected, weighed, and recorded by food item as described in paragraph 5, page 165. The weight of edible food waste is converted to the "as purchased" weight by use of the recipe and correcting for moisture gain or loss, fat loss, etc. (See form 7 and example below.) In order to convert cooked foods to the "raw" or "as purchased" basis and to determine the quantities of the ingredients in various cooked, mixed foods, the recipe giving the quantity (weight) of each item used and the final cooked weight must be obtained.

The following example illustrates the type of calculation which may be required:

Potatoes, hashed brown	Grams	Pounds	Percent in cooked product
Weight of unpeeled potatoes	36,350	80.07	
Weight of peeled potatoes	27,310	60.15	91.
Weight of fat drippings	2,550	5.61	8.
Total weight (uncooked)	29,860	65.76	
Total weight (cooked)	25,630	56.50	

Sample calculation

Total amount cooked (25,630 gm) was served.
Plate waste and leftovers discarded = 5,000 gm, or 11.21 pounds.
Raw equivalent of discards is obtained by:

$$\frac{\text{Total weight (uncooked), } 29,860}{\text{Total weight (cooked), } 25,630} \times \text{wt of cooked discards, } 5,000 = 5,930 \text{ gm, or } 13.1 \text{ lbs.}$$

Weight of raw, peeled potatoes discarded = 5,930×.915 = 5,426 gm, or 11.9 lbs.
Weight of raw fat drippings discarded = 5,930×.085 = 504 gm, or 1.1 lbs.
Factor for converting raw peeled potatoes to "as purchased" is $\frac{36,350}{27,310}$, or 1.33.
Weight of potatoes (as purchased, discarded) is 11.9×1.33, or 15.8 lbs.
Prepared dishes containing more ingredients are calculated in the same manner.

7. During the collection of the dietary intake data, the data on forms Nos. 5 through 7 are recorded as required. (See page 194–196.)

8. After the last meal (supper) of the last day of the study, another complete food inventory is made.

9. At the end of the study the difference between the initial inventory plus subsequent purchases and the final inventory, corrected for losses, provides a means to calculate the amount of foods consumed during the survey. Cooking losses are corrected for as described under the recipe method above.

It should be borne in mind that the accuracy of results with the inventory method requires a complete knowledge of the amount of foods going into and out of the mess during the entire period of study

One person must be responsible for maintaining constant observation and noting all movements of food during the survey period.

Analysis of Food Purchases Over an Extended Period

It is usually desirable to obtain data on food purchases or food issues to military or other institutional feeding units during a period prior to that of the dietary survey. The nutritional adequacy of the ration, as purchased, can be calculated from such data. Correctness and accuracy of such records must be considered in their use, as well as whether they are representative of actual food supplies for the time under consideration.

This analysis serves as an additional check on the dietary intake studies by other methods. Also it will indicate if the unit being surveyed has suddenly increased its food purchases for the period during which the survey is being conducted. Such increases in food purchases have been suspected for certain food items in a few military units during some surveys. In addition, soldiers have been heard to make the remark that they "are eating better" while the survey was underway.

By analyzing the food purchase data for nutritional adequacy, based on a 7- to 15-day period during the month previous to the time of the survey, the extent to which additional foods have been provided can be appraised. Most military units keep some form of ration records for at least a 30-day period. The records may show only the money spent, but usually show also the quantities of food items purchased.

Supplemental Sources of Food

In many instances some supplemental foods and beverages are available to soldiers eating in military messes or to inmates of some institutions (hospitals, for example). The quantities may be relatively small amounts obtained through post exchanges and food establishments located on and off the post but at times they may be very significant, especially as sources of one or two nutrients. Sometimes this supplemental food will consist only of fruits, snacks, candy, and beverages which are consumed between meals. In other situations, some men may eat their evening and morning meals off the post with their families. The method of determining the amount of food consumed outside the mess and its nutritional value varies with the specific circumstance.

Food consumed by soldiers in post exchange or similar on-post installations selling food or beverages usually can best be determined by obtaining records of purchases over a period of time or actually measuring the flow of food through such an installation by use of the food inventory approach. This information with the number of

troops normally serviced by the post exchange permits one to estimate the mean intake per man per day from such sources.

Another approach involves the use of questionnaire interviews where forms appropriate to the situation are completed by the soldier under supervision of a member of the dietary group. This questionnaire is designed to determine the actual amounts of each food item or beverage consumed by the soldier outside the mess during the period of the survey. This need not be done for all members of the mess as long as a sufficiently representative sample of individuals is obtained. From these data an approximation of nutrient intakes from sources other than the mess can be obtained.

In cases where individuals consume one or more meals outside the mess, home surveys of a few families are desirable in order to obtain good information. This can be done by using either the recipe method or the 24-hour recall questionnaire method as described below. Such is not required, however, unless a large proportion of the mess consumes at least one meal outside the mess. If only a few individuals are involved, then the approximate food intake of soldiers consuming all their meals in the mess can be determined by calculating the actual food consumption per person by meal and then combining this for the entire day as is done with the recipe method. If a considerable number of subjects eat one or more meals outside of the regular mess, it may be necessary to weight the average number of persons fed per day in the mess by appropriate consideration of the head count per meal and the content of the particular meals. This is not so readily accomplished, however, when the inventory method is used.

Unit Military Food Production

In many instances military units may produce some of their own food supply in unit gardens. In some units small animals, including pigs and poultry, may be raised for food. It is important to obtain as much information as possible about such activities since they provide a means of training individuals for subsequent roles in civilian life as well as producing, at times, badly needed foodstuffs. Such gardens may be important in supplying fresh vegetables to units. When these gardens or other means of producing food exist, it is important to make certain that foods from these sources are considered with other foods coming into the mess when the food inventory method is used.

Procedures for Civilian Dietary Studies

In any civilian study the same rules of courtesy and protocol apply that are required for work in the armed forces. The population must be approached through the local government and public health authorities. It is necessary to enlist the cooperation and assistance of all

civilian agencies related to social welfare, food rationing, and distribution.

Obtaining reliable food and nutrient intake data in civilian populations is generally more difficult than in armed forces or institutional feeding situations. This is mainly because many small family units rather than a single large mess are involved. In general, a much longer survey period is required to obtain equally reliable data for small family units than for a mess that was feeding a large number of people, if the same methods and procedures were employed in both situations. Another difficulty arises from the fact that different members of the family (adults, children, infants, and pregnant women) have different nutrient requirements; i.e., the family is not a homogeneous population like a military unit. It would be desirable, of course, to measure the average nutrient intake for each category separately (*8–15*).

Because of such factors it is usually possible to do only a limited number of direct studies, which give very precise information on a few individuals, and adopt procedures that are inherently less accurate but which include large numbers of family groups. The latter approach includes the use of questionnaires to obtain food consumption data based on 24-hour recall and other information concerning food uses and customs.

Both the recipe method and the food composite analysis method as described above should be used in direct collection of dietary intake data in a limited number of families within certain population groups in order to supplement the data from the questionnaire method.

Due to the limitations of time and trained personnel normally available during the conduct of a nutrition survey, it becomes extremely important that the selection of families for dietary study, regardless of method used, be done so that the results have meaning. Since it is virtually impossible to study a sufficient number of families to sample adequately an entire country, the families used should be selected from within specific important subgroups which can be properly described and evaluated. Experience indicates that a minimum of 15 families is required to give satisfactory data by means of the 24-hour recall questionnaire method within a population sample of approximately 1,500 people. When direct nutrient intake data are obtained by means of the recipe method, a minimum of five families should be studied in a specific population group. Since the direct study approach, even for 1 day's food consumption, involves considerable time and effort of well-trained people, this approach should be used primarily in those population groups which are most likely to have nutritional problems. In this way it is possible to obtain more reliable information pertaining to groups with existing nutritional problems and, at the same time, to check the results of the 24-hour recall questionnaire method.

In some instances, it is possible to select families to be interviewed and homes for collection of three-meal intake data on a random statistical basis. If possible, it is desirable to obtain such data from families which have been or will be examined by the clinical team. In some instances, families have been selected for detailed food intake studies immediately following their clinical examination. Where this is not possible, it is necessary to select families judged as representative of given categories from within a specified population. This may be done without appreciable prior information about the families, excluding only those who will not cooperate or who are not considered reasonably representative as to composition. Frequently the local public health nurse, home demonstration agent, or other community worker or leader may be helpful in providing information about the families of the area. Extreme caution must be taken in all cases, however, to avoid selecting families on a basis which would permit the introduction of unrecognized bias. Since the composition of families varies within civilian populations, it is necessary to obtain certain specific data for each population group studied, including age, sex, weight of the family members, whether or not the women are pregnant or lactating, and temperature at the location. Height and weight data are normally collected by the clinical team. From these data a table showing the percent distribution of each category is formulated with which the appropriate dietary standards for the specific population subgroup may be computed.

From the percentage distribution of any specific population (see example in table 2), the mean dietary requirements or allowances for certain nutrients (*16*) may be calculated as shown for protein in table 3. The other mean nutrient allowances may be obtained by multiplying the percentage distribution of the specific population for each subcategory (for example, percent from age 1–3) times the respective allowance. A simplified procedure is that shown in table 4. Here the allowances for various age categories are expressed as the fractional percentage of the allowance for the reference man. These fractional percentages can be viewed as the basis for arriving at the total number of nutrition units in a family or population. An example is given following table 4.

As indicated above, it is obvious that dietary surveys of civilian populations cannot be done satisfactorily without the assistance of well-trained, highly motivated individuals who are readily accepted by the housewives during interviews or direct food intake studies. Such an individual must be alert to the many problems which may be encountered during an interview and able to understand the person being interviewed. In order for data to be accurately collected, each individual who conducts interviews or home studies must be aware of the special nutritional value of available foods, must be

accurate in recording, and must have a great sense of responsibility, since small details may be extremely important in estimating accurately the amounts of various foods consumed. In direct intake home studies of 1 day's duration, the interviewer must be able to impress the housewife with the importance of following the same food preparation and menu practices that she would do normally. Only local individuals with adequate training can serve satisfactorily in such dietary surveys.

TABLE 2.—*Composition of families surveyed by location*

Age group (years) from to	Location A				Location B			
	Male	Female	Pregnant	Lactating	Male	Female	Pregnant	Lactating
	NUMBER OF INDIVIDUALS							
0-1	5				1			
1-3	10				6			
3-6	15				4			
6-9	11				14			
9-12	7	2			12	9		
12-15	1	5			2	4		
15-18	5	4			1	4		
18-35	11	7	1	1	16	8		1
35-55	26	28	1	1	32	34		
55-75	13	26			22	29		
Total number of persons	180				199			
	PERCENTAGE DISTRIBUTION							
0-1	2.77				0.50			
1-3	5.56				3.02			
3-6	8.33				2.01			
6-9	6.11				7.05			
9-12	3.88	1.11			6.03	4.52		
12-15	0.56	2.77			1.00	2.01		
15-18	2.77	2.22			.50	2.01		
18-35	6.11	3.89	0.56	0.56	8.04	4.02		0.50
35-55	14.44	15.56	.56	.56	16.08	17.08		
55-75	7.22	14.44			11.06	14.57		
Percent	100				100			

TABLE 3.—*Calculation of the per caput protein allowance for the population distribution shown in table 2, location A*

Age group (years) from to	Protein per kg [1] body weight (gm)				Average body weight [2] of sample studied (kg)				Protein allowance (gm) (col. 2 × col. 3)				Population distribution [2] (percent)				Average per caput protein [3] allowance (col. 4 × col. 5) / 100			
	Male	Female	Pregnant	Lactating	Male	Female	Pregnant	Lactating	Male	Female	Pregnant	Lactating	Male	Female	Pregnant	Lactating	Male	Female	Pregnant	Lactating
0–1	2.5				7.3				18.25				2.77				0.51			
1–3	2.5				12.9				32.25				5.56				1.79			
3–6	2.2				18.8				41.36				8.33				3.44			
6–9	2.2				26.2				57.64				6.11				3.52			
9–12	1.8	1.7	------	--------	35.0	36.6	------	--------	63.00	62.22	--------	--------	3.88	1.11	------	--------	2.44	0.69	------	--------
12–15	1.7	1.3	+20	+40	50.0	50.4	------	--------	85.00	65.52	--------	--------	.56	2.77	------	--------	.47	1.81	------	--------
15–18	1.4	1.1	+20	+40	63.7	55.4	------	--------	89.18	60.94	--------	--------	2.77	2.22	------	--------	2.47	1.35	------	--------
18–35	1.0	1.0	+20	+40	69.3	58.9	62.0	57.2	69.30	58.90	82.00	97.20	5.11	3.89	0.56	0.56	4.23	2.29	0.46	.54
35–55	1.0	1.0	+20	+40	74.6	64.3	69.5	60.7	74.60	64.30	89.50	100.70	14.44	15.56	.56	.56	10.77	10.01	.50	.56
55–75	1.0	1.0	------	--------	72.0	66.8	------	--------	72.00	66.80	--------	--------	7.22	14.44	------	--------	5.20	9.65	------	--------
																		Sum = 62.72		

[1] NRC, Recommended Dietary Allowances, 1963.
[2] Location A, table 2.
[3] The sums of this column yield the average daily per caput protein allowance for the population surveyed at location A, table 2

TABLE 4.—*Factors suggested for estimating nutrient allowances for population samples of mixed sex and ages. Reference man is unity* [6 7]

Age group from to	Protein [1]		Calories [2]		Calcium [3]		Iron [4]		Vitamin A [5]		Ascorbic acid	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0-1	0.29		0.29		1.22		0.80		0.30		0.43	
1-3	.40		.41		1.00		.80		.40		.57	
3-6	.57		.50		1.00		1.00		.50		.71	
6-9	.74		.66		1.00		1.20		.70		.86	
9-12	0.86	0.79	0.75	0.75	1.44	1.44	1.50	1.50	0.90	0.90	1.00	1.14
12-15	1.07	.89	.97	.81	1.44	1.44	1.50	1.50	1.00	1.00	1.14	1.14
15-18	1.21	.83	1.12	.75	1.22	1.22	1.50	1.50	1.00	1.00	1.14	1.00
18-35	1.00	.83	1.00	.72	1.00	1.00	1.00	1.50	1.00	1.00	1.00	1.00
35-55	1.00	.83	.95	.69	1.00	1.00	1.00	1.50	1.00	1.00	1.00	1.00
55-75	1.00	.83	.84	.60	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[1] Recommended allowance, reference man, /gm/kg body weight, NRC.
[2] Calorie requirements, FAO, Rome, reference man, 3,200 calories, 65 kg body weight, 10° C temperature, activity defined, age 25 years.
[3] Calcium requirement, FAO, Rome, reference adult 400-500 mg/day.
[4] NRC 1963, reference man, 10 mg/day.
[5] Suggested practical allowance for reference man, 3,500 I.U. This is 2.5 times the estimated minimum requirement of 20 I.U. of vitamin A/kg body weight. This allowance is not excessive because of the high proportion of carotene as vitamin A in many areas of the world.
[6] Suggested practical allowance for reference man, ascorbic acid, 30 mg; thiamine, 0.3 mg; riboflavin, 0.4 mg; niacin, 6.6 mg.
[7] It is suggested that the factors for women for the various nutrients be increased by an amount equal to ⅓ the factors for infants to allow for increased requirements during pregnancy, and that the factor for the infant be added to that of the mother if the infant is nursing.

Further Explanation of Table 4

The suggested factors can be viewed as nutrition units in estimating the dietary requirements for a given family. For example, a family might have the following composition: 1 child, 4 years; 1 child, 6 years; 1 child, 9 years; 1 child (girl), 11 years; father, 40 years; mother, 37 years; and grandmother, 60 years. The number of protein and calcium nutrition units would be calculated as follows:

	Protein	*Calcium*
Child, 4 years	0.57	1.00
Child, 6 years	.74	1.00
Child, 9 years	.74	1.00
Child, 11 years	.79	1.44
Father, 40 years	1.00	1.00
Mother, 37 years	.83	1.00
Grandmother, 60 years	.83	1.00
	5.50	7.44

Thus, this family of seven would require 5.50 nutrition units of protein (equivalent to 5.50 times the protein allowances for the adult reference man) and 7.44 nutrition units of calcium. Applying the factors in table 4 to the population distribution shown in location A, table 2 yields 146 protein nutrition units for the 180 individuals, or an average of 0.81 nutrition unit per individual. This contrasts with an average of 0.89 nutrition unit per individual (62.7 gm of protein divided by 70 gm, the allowance for the reference 70 kg man) derived from the calculations shown in table 3 where actual body weights were used. This discrepancy emphasizes that when body weights are available, the derivation of a nutrition unit by the procedure given in table 3 will yield more accurate estimates, especially when allowances for nutrients are based on a unit of body weight.

Family Food Questionnaire

The use of dietary questionnaires for family surveys has yielded information of essentially the same character as that obtained in the detailed direct measurements of food intake by the recipe method in the same families (*19*). This applies to the information concerning nutrient intake based on 24-hour recall as well as frequency of use of food items. The dietary questionnaire form for family surveys shown

in appendix C provides a means of recording such data as: the com position of the family, 24-hour food intakes for the previous day frequency of use of various food items, amount of money spent fo food, method of cooking, practices used in feeding babies, and othe information concerning food customs. With minimal modification t make the questionnaire appropriate to the country concerned, it ha been used for several surveys with excellent success.

This questionnaire approach should be made with the aid of th host country personnel, since it requires competent personnel com pletely informed on dietary customs, habits, cooking practices, lan guage, local food names, and relative price and availability of vari ous foods. By use of actual food portions of different sizes or simu lated food portions as visual aids, it is possible to obtain by question ing very reliable data on the kinds and amounts of foods eaten durin the previous day. Such interviews are best done in the home, but ma be conducted in conjunction with the clinical examination site if neces sary. Such interviews require approximately 30 to 45 minutes pe interview. When direct food intake studies are made in the home similar information for comparative purposes should be obtained b questionnaire for the food consumed during the previous day. Ad equate transportation must be provided for workers who make th home visits.

Recipe Method Conducted in Home

For the recipe method of survey as described above (p. 164), data a obtained for three consecutive meals. The dietitians weigh the foo portions that are included in a particular recipe item and then weig the prepared food when it is ready to serve. Any food not serve also is weighed. From the weights of the ingredients that were use in a particular menu item and the weight of that food consumed b the members of the family, it is possible to determine the averag weight of each food item or ingredient consumed per person per day (See p. 165.) From these data the average intake of essential foo nutrients may be calculated, using appropriate food compositio tables. Each dietitian should be able to collect data for the recipe an food composite analysis methods in two families during 1 day. Thi requires considerable cooperation from the family being surveyed but such cooperation is usually not difficult to obtain wherever th dietitians can converse freely with the housewife. The example (p 180) illustrates a convenient method of recording data for severa families.

Example of recording[1]

[Amount of each food item consumed per family per day at 1 location]

[Figures in parentheses indicate number of persons in each family]

Food item	Amount per family (grams)					Average per person for 5 families
	A(7)	B(4)	C(5)	D(6)	E(8)	
Milk	2,018	3,232	1,526	1,666	737	306
Cheddar cheese	.....	27	66	55	174	11
Bread	630	.....	268	546	1,562	100
Rice	230	.....	151	.....	.....	13
Macaroni	140	118	.....	128	728	37
Bananas	350	359	310	.....	.....	34
Apples	.....	.....	114	560	1,240	64
Pears	2,005	.....	.....	93	868	99
Tomatoes	1,334	331	.....	314	1,524	117
Carrots	.....	260	332	330	.....	31
Potatoes	1,205	.....	830	202	.....	80
Onions	189	.....	.....	.....	140	11
Lettuce	794	302	590	220	1,172	103
Butter	.....	132	59	46	140	13
Corn oil	126	106	112	418	341	37
Beef	.....	1,680	418	1,302	667	138
Beef liver	3,087	.....	.....	.....	.....	103
Eggs	314	49	326	127	241	35
Wine	875	.....	66	.....	800	58
Coca-Cola	.....	.....	.....	150	.....	5
Sugar	116	131	77	340	732	46
Apricot jam	.....	368	102	.....	.....	16
Total grams for food composite	.....	.....	.....	.....	.....	1,457

[1] These data are handled in this manner to facilitate the calculation of the reference standards for the total number sampled in this location. Using these averages and tables of food composition, one can calculate the average nutrient intake. These averages may be used also to prepare food composites for chemical analyses.

Food Composite Analysis Method

The food composite analysis method should be used in conjunction with the recipe method conducted in homes within a specific population group. This is done in the same way described before for military messes, except that the food samples normally are not obtained from each home studied. Although this can be done, it is recommended that at the end of a series of home surveys involving the recipe method, various food items used in all of the homes studied be purchased from the local marketplace. Then the average amount of each of these is used to prepare homogenized composite samples, representing the average ration as consumed by all individuals included within the homes studied in that particular locality. Where important foods constitute a large part of the diet (example: rice in rice-eating areas), several samples should be obtained from the market and some of each should be used in making up the composite. The foods purchased to simulate the food actually eaten should be cooked prior to making up the sample in a manner considered representative for the families studied. Losses of nutrients incurred during cooking are considered to be equivalent to that which would take place in the home. Both oxalic acid-stabilized and alcoholic KOH-stabilized samples for subsequent laboratory analysis are prepared as described above (p. 166). Where special foods are used in significant amounts, and reliable food composition data are unavailable, special samples should be taken

and stabilized for subsequent laboratory analysis. (See apps A and B.)

In thus making up a composite sample, it is important to include all items such as condiments, salt, etc. Furthermore, the water used should be that used locally in food preparation and a separate sample of this water should be obtained for analysis. The actual cooking conditions should be recorded and the number of samples of each food item used in making up such a composite. Any other information of relevance concerning ingredient source should be included such as the kind and source of flour.

Longer Period Direct Food Intake Studies

In obtaining information about a particular family rather than an average for several families within a population, it is necessary to conduct the nutrition survey over a longer period. For this it is desirable to include 7 days, since this represents a normal family food intake cycle. The recipe method as described may be used for such studies and is probably the method of choice where simplified methods of food preparation are involved. Another method which may be used is the food inventory method, as described for use in military messes. In this method the person conducting the study weighs and records the quantity of all foods in the house by kind or item. As additional food is acquired, this is also weighed and recorded. At the end of the study period the amount of food left in the house is determined. During each meal the amounts of food used and weights of those foods not consumed are determined and converted in terms of the food items consumed. Thus at the end of the study, the total food used less the food prepared but not consumed represents the total food intake of the family. By obtaining accurate information as to number of individuals, including guests, eating at each meal and the sex and age of each, the average nutrient intake per person per day may be computed. As in other methods where families vary in composition, it is necessary to calculate corresponding nutritional standards to compare with the data obtained.

This longer survey period applied to individual families is one of the most accurate methods which may be used, but requires considerable time and personnel if many individuals are to be studied. Such a procedure is appropriate in followup studies of families within particular population groups where specific nutritional problems are being studied. Experience with the 1-day studies, however, as well as with the 24-hour recall method, indicates that these are satisfactory for early identification of nutritional problems.

Food Intake Record by Housewife

In many countries where women's homemaker clubs or other groups are organized, housewives may be approached and trained to maintain detailed food records on appropriate forms for weekly periods.

Forms used for such studies should be designed to obtain data on age, sex, and approximate body weight of each family member, as well as information concerning food purchases, food preparation, and general food habits. In some instances this procedure can be improved by providing kitchen scales for weighing of food by the housewife. This method is of value for use in educational activities, particularly when corrective measures are being implemented in a problem area.

Individual Food Intakes

One of the most difficult problems is to obtain sufficient information on enough individuals of the same physiologic category to permit a nutritional evaluation of their diet. This is needed most for the infant and child up to 4 years of age and for pregnant and lactating women.

This may be done as a part of direct food intake studies in homes by weighing and recording the proportions of prepared food served to and consumed by each family member. From data thus obtained, the nutritional intake of each family member may be obtained. Even though it may not be possible to make such a study for all members of a family, it is possible to do this for the infants and young children. Even if this study is done in only a limited number of family studies, it provides a basis for comparison with data obtained by less direct procedures.

A second and commonly used approach for obtaining individual intake data for infants and children is the interview of the mother by the 24-hour recall method. Figure 3 in chapter V provides a form for recording such information with other pertinent data concerning breast feeding and specific supplements used in infant feeding. These forms should be completed for infants and children under 4 in all home interviews involving the regular questionnaire method.

In addition to obtaining individual data for infants and children as indicated above, specific questionnaires may be used for schoolchildren (age, approximately 9 to 12 years) and for pregnant or lactating women. These questionnaires would be primarily a means of obtaining, by interview, 24-hour recall food intake data from schoolchildren in schools or from mothers in clinics attended by pregnant or lactating women. In most surveys a special team composed of a clinician, a dietitian, and a biochemical aide is used to obtain information in well-baby and prenatal clinics, as well as in hospitals.

Diets in Hospitals and Other Institutions

Important dietary information may be obtained through dietary surveys of boarding schools, orphan homes, prisons, asylums, child welfare centers, hospitals, rest homes, police barracks, or similar institutions, using the methods described for studying military messes. Such surveys give information pertaining to a larger number of people

in a short time. Studies of hospitals and institutions also provide a means of obtaining group samples of food for laboratory analysis which reflect conditions encountered in other areas of civilian feeding. In addition, they provide information of particular value in planning corrective measures, such as in hospital food programs, school feeding, and the like.

In civilian dietary work, as in military studies, food that is consumed outside the home also must be determined and included in assessing the total nutritional intakes. Such information should be obtained by questionnaire.

It is recognized that the dietary survey procedures which may be used during a short-term nutrition survey covering a large population are fraught with difficulties. In addition to the problems already discussed relevant to the study of large numbers of people in a short period of time, no opportunity is presented to determine differences in nutritional intakes at other seasons of the year. Despite this drawback, these procedures provide, in general, a satisfactory means of identifying nutritional problems and a basis for collecting sufficient information to permit an intelligent solution of the problems. One of the major objectives of civilian nutrition surveys in other countries, by personnel from the United States and the host country, is to identify nutritional problems and stimulate work on them which will be continued after the initial survey. It is hoped that during such a survey an improvement in the training of local personnel will result and that through their followup efforts, information can be obtained over a longer period of time and at different seasons of the year in areas where nutritional problems are present. Unless such followup studies are made so that the findings of the nutrition survey are extended and implemented in practice, the results of one survey are of limited value.

Recordkeeping

In both military and civilian nutrition studies, it is imperative that detailed records be kept. These should include such information as date, location, altitude, temperature, unit or group studied, and other details as discussed elsewhere (p. 3). For the food composite analysis method, it is essential to record all the data used in the calculations in a bound notebook for later referral. This is important since the amount of water added to a sample, check of errors made in calculations, etc., may frequently be derived from the log for later evaluation of values. In civilian dietary studies, where a large number of questionnaire forms are completed each day, it is necessary to transcribe promptly the information from these questionnaires. This permits one to maintain records up to date, to avoid the excessive accumulation which is difficult to process at the end of the survey,

and allows recording of details while accurate recall is possible. Such practice also permits careful appraisal of the records being obtained in order to modify instructions as required to collect the data properly. Data from the questionnaire sheets are recorded on large summary sheets by sections, with approximately four summary sheets required to summarize all of the information from the questionnaire forms for a particular area studied. Transferring the information from the questionnaires to these summary sheets requires the efforts of the individuals who were involved in filling out the questionnaires.

Losses of Nutrients During Cooking

Corrections for losses of the various nutrients which take place during preparation of foods for consumption are difficult to make. It is obvious that methods of food preparation under the variety of existing conditions will range from well-controlled cooking practices in large-scale army messes and hospitals to inefficient practices in the field or in primitive homes. Optimum retention of nutrients requires that certain procedures, such as short cooking time, minimum addition of water, optimum pH, and brief holding time before serving the food, be carefully followed. In general, large-scale cooking tends to minimize cooking losses, but it is also possible to prepare food at the household level with minimum losses of nutrients. In recommending corrective measures the use of pressure cookers which keep the amount of water added and time of cooking to a minimum is to be encouraged wherever feasible. Under local conditions, minimum addition of water and brief cooking and holding time should be stressed. Since leaching of soluble nutrients takes place during the cooking operation in addition to the recognized thermal destruction, it is important to advocate the use of both the liquid and solid components of the prepared food. Additional reheating and holding of the food prior to serving imposes further losses of vitamins and is to be discouraged.

From the above discussion, it is evident that it would be difficult to adopt one set of values to apply in all cases of cooking practice. Ideally the determination of cooking losses would be an integral part of the laboratory work under the survey distinct from, but as important as, the clinical studies of vitamin sufficiency. This is seldom if ever possible. Although there are numerous reports in the literature describing losses of nutrients in large-scale and family-scale cooking of foods, the many variables involved make it difficult to choose single values to cover cooking practices under the local conditions. Krehl and Winters (*20*); Hewston et al. (*21*); Oser, Melnick, and Oser (*22*); and Teply and Derse (*23*) have studied various vegetables and other foods under small-scale methods of preparation.

The Army Technical Manual TM 8-501 (*24*) lists nutrient losses in certain Army ration components as prepared under conditions for large-scale feeding, and the National Research Council (*25*) and Crosby et al. (*26*) have tabulated vitamin losses for various categories of food prepared for institutional feeding on a large scale.

It has been thought useful to prepare, from the above studies, an abbreviated table (table 5) listing average (to the nearest 5 percent) losses for the main categories of foods. The values in the table are based on studies under large-scale cooking conditions, but, as pointed out earlier, small-scale practices can yield similar retention values when suitable attention is given to the various factors responsible for excessive losses of nutrients. It is to be noted that no value for retention of mineral nutrients is given; however, losses of the latter have been shown to be negligible under the variety of conditions generally encountered.

An indication of how realistic the above cooking loss factors are can be obtained by comparing the calculated dietary values with those obtained by actual assay of the daily dietary composite samples representing the food as eaten during the survey. In many ICNND nutrition surveys, the chemically determined nutrient intakes were appreciably different from those obtained by use of food composition tables corrected for cooking losses, indicating that perhaps the latter corrections were not correct in many instances. Where preparation losses may be critical with respect to potentially limiting nutrients (such as vitamin C might be), attempts should be made to determine the actual losses obtained during preparation. This can be done in military messes or in special studies designed to provide the same cooking conditions. This is most important where other factors such as environmental temperature and humidity, pH of the water, variation within the cooking container, and mineral content of cooking water as well as heat and cooking time may greatly influence the rate of nutrient losses.

TABLE 5.—*Average percentage of nutrients lost during cooking*[1]

	Thiamine	Riboflavin	Niacin	Ascorbic acid
Meats	35	20	25	.....
Meats plus drippings	25	5	10	.....
Eggs	25	10	0	.....
Cereals	10	0	10	.....
Legumes	20	0	0	.....
Vegetables, leafy green and yellow	40	25	25	60
Tomatoes	5	5	5	15
Vegetables, other	25	15	25	60
Potatoes	40	25	25	60

[1] Based on good cooking practices with U.S. Army ration components. For individual foods in some of the food classes the loss may be considerably greater or less. These estimated average cooking losses are useful as guides, but may not necessarily apply when cooking practices and other conditions (e.g., pH) differ greatly. It is preferable to use local values where available and reliable (*25*).

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Appendix A. Desirable Information on Food Samples

Sample No. ____ Date obtained: ____ Source: ____

English name: ____ Common name: ____ Botanical name (incl. variety): ____

Growth conditions:
- Locality: ____
- Soil: ____
- Fertilizer: ____
- Elevation: ____
- Other: ____

Price per unit:
- Local currency: ____
- U.S. currency: ____

Photograph taken: ____

Description of sample:[1]
- Appearance: ____
- Handling, storage, processing: ____
- Edible portion (E.P.) data: ____
- Dry weight determination: ____
- Other information: ____

Sampling procedure:
- Method of selecting sample: ____
- Type and amount of preservative used: ____
- Dilution of sample, if any: ____

Customary procedures used in preparation of food for eating: ____

Relative significance in diet: ____

Appendix B. Handling of Food and Water Samples for Analysis

Water Samples for Fluorine Determination

Collect 100 to 300 cc samples in polyethylene bottles. Add one pellet of reagent grade sodium hydroxide to each sample. Submit for analysis a few grams of the batch of sodium hydroxide used along with the water sample.

General Comments on Food Sampling

Oxalic acid-preserved samples are suitable for determination of ascorbic acid and most other nutrients usually measured except carotene and vitamin A. Certain other nutrients which are sometimes assayed (e.g., folic acid) are also unstable in the oxalic acid preparation.

[1] For mixed food preparations, describe as completely as feasible.

An alternate satisfactory preparation for carotene studies is made by adjusting the pH of a slurry to approximately 8.0 and adding 2 cc of chloroform ($CHCl_3$) and 2 cc of toluene for each 100 cc of slurry.

Under these conditions the ascorbic acid (*1*) and carotene and vitamin A (*2*) are fairly stable (at least in "typical food mixtures") for at least 2 weeks at room temperature if protected from light. However, for maximum stability, the colder the temperature, the better the vitamins will be preserved. Except for short periods, refrigeration (preferably freezing) is desired. This is especially true of the slurries for carotene determination.

The procedure commonly used for preserving foods for carotene and vitamin A determination is addition of 50 gm of alcoholic potassium hydroxide (KOH) (95 percent ethyl alcohol containing 1 percent KOH) to 250 gm of final food slurry, including 7.5 gm of chloroform (approximately 5 ml) (*7*).

Samples of salt used should be obtained for iodine and fluoride analysis.

Under most conditions it is desirable to add local drinking water to the food samples in their preparation. At the same time, water samples are taken for other analyses. Where special foods are composited for subsequent chemical analysis, distilled or demineralized (deionized) water should be used in making up the sample.

In any case, the exact method of making up the food samples should be recorded, including the exact amount of oxalic acid added. (See identification of dietary food composites, p. 169, and the form for recording information on dietary special samples (app. A).)

Adequate sample will be provided if one-tenth of a day's ration is supplied, oxalic acid-preserved, and one-tenth of a day's ration is preserved for vitamin A and carotene determination.

Food samples may be taken back to the United States expeditiously by a team member as excess baggage. If they start the trip in the frozen state, they will remain cold for a good part of the trip. If refrigerated storage space is not available, they should be shipped periodically via air express.

Appendix C. Dietary Questionnaire for Family Survey

Card No. _____ Date _____

Investigator No. _____

Family name _____ Town _____ Province _____

Ethnic group _____ Religion _____

Father's occupation _____

Number in household: Total _____ Adults _____ Children _____

Males over 16 years: No. _____ Ages _____

Number pregnant women: _____ Ages _____

Number lactating women: _____ Ages _____

Number other women (over 16 years): _____ Ages _____

Number children (under 16 years):

Babies under 1 year: _____ Breast fed: Yes _____ No _____

Number other boys: _____ Ages _____

Number other girls: _____ Ages _____

Questions:

1. What is the total cash income for the family per week? _____
2. How much is spent on food per week? _____
3. How many times per week do you buy food? _____
4. Do you prepare your food on an open fire _____ or stove _____?
   Type fuel used _____
5. How long does it take to cook a meal? _____ hours.
6. Do you breast-feed your babies? Yes _____ No _____

Questions—Continued

7. If there is no mother's milk, what is the young baby fed?

_____

8. At what ages are your babies weaned? _____ months.
9. At what age are other foods given to the baby? _____ months.
10. What foods would a breast-fed baby receive at—
    6 months of age? _____
    12 months of age? _____
11. What do you feed a weaned child with diarrhea? _____
12. Are any of your children in school at any time during the year?
    Yes ____ No ____
    If yes, do they receive milk at school? Yes ____ No ____ Any other foods? _____
13. Do you or others in your household take vitamin pills or other medication? _____ How often? _____
14. Do you use any chemicals in the cooking water? _____
    lime? _____ other? _____
15. Do you chew special leaves or nuts? ____ When did you begin? ____
16. What foods did your family eat yesterday?

(Day of week _____)

Meals	Menu items	Food items, kinds	Amounts, if known	P, H, F[1]	Comments
Breakfast					
Lunch					
Supper					
At other times					

	Breakfast	*Dinner*	*Supper*
No guests present			
No family members absent			

[1] P = purchased; H = home grown or gathered; F = free gifts or supplements.

17. How often does your family eat the following foods?

Food	Number of meals per—			Remarks
	Day	Week	Month	
Cereals and cereal products:				
Corn				
Rice				
Wheat				
Barley				
Oats				
Bread				
Other				
Milk products:				
Milk, fluid				
Milk, powdered				
Milk, evaporated				
Cheese				
Other				
Eggs				
Meats				
Beef, etc				
Fats and oils				
Legumes				
Vegetables, leafy, green, and yellow				
Other vegetables				
Citrus fruits				
Other fruits				
Miscellaneous:				
Sugar				
Cocoa				
Coffee, etc				

FORM No. 1.—*Survey of food and nutrition services in the armed forces*

Date: _____ Unit: _____

General—Describe or answer the following:

1. Food procurement system
2. Finances of food procurement
3. Ration distribution and issue system
4. Food storage system
5. Are troops employed in food production, especially on a local basis? Extent?
6. Are troops employed in food processing, locally?
7. Food service supervision:
   a. Schools for cooks and bakers? Manuals or training aids?
   b. Mess hall—kitchen, preparation, serving
   c. Rations—extent to which scales are met
   d. Ration supplements and frequency
   e. Monetary allowance for ration? How used?
   f. Welfare funds? Red Cross, etc.
8. Do troops receive vitamin pills or other medication?

Hospital and Medical

1. Dietetic service in military hospitals. Describe the following:
   a. Type
   b. Adequacy of service
   c. Training of personnel
2. Is a manual used in feeding of sick and wounded?
3. Status of nutrition training of medical staffs
4. Summarize reports and collect copies when available (include source) of:
   a. Heights, weights, and other anthropometric data
   b. Surveys of nutrition
   c. Casualties and disabilities from malnutrition
   d. Special reports on nutrition problems

Status of Civilian Nutrition (as it would influence military nutrition—include source and data):

1. Are dependents of soldiers consuming army rations?
2. Is food from civilian sources coming into troops' hands?

Locally Determined Nutritional Requirements or Standards

List report and summary data available on the following:

1. Trainees
2. Combat
3. Bivouac or detention camps
4. Civilian

Sanitation (check and describe the following):

1. Cleansing of utensils and messing facilities.
2. Water supply—source, treatment, etc.
3. Disposal of wastage
4. Inspection of mess

FORM No. 2.—*Recipe method*

Meal: _____ Date: _____ Unit: _____

Number of persons: _____

Menu items	Ingredients	Gross weight	Tare weight	Net weight	Percent of food consumed	Total grams consumed	Grams consumed per person

FORM No. 3.—*Recipe method with correction for food not consumed*

Meal: _____ Date: _____ Unit: _____

Number of persons: _____

Menu items	Total food prepared			Prepared food not served			Plate scrapings			Total amount of food consumed		Percent of prepared food actually consumed
	Gross weight	Tare weight	Net weight	Gross weight	Tare weight	Net weight	Gross weight	Tare weight	Net weight	Total grams	Grams per person	

FORM No. 4.—*Summary of nutritional evaluation*

Unit: _____ Physical activity: _____

Date: _____

Food	Quantity per man	Calories	Protein (gm)	Carbohydrate (gm)	Fat (gm)	Calcium (gm)	Iron (mg)	Vitamin A (IU)	Thiamine (mg)		Riboflavin (mg)		Niacin (mg)		Vitamin C (mg)	
									(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Total																

[1] No cooking losses considered.
[2] Corrected for estimated cooking losses. (See table 5.)

FORM No. 5.—*Food inventory record*

Unit surveyed: _____ Dates: _____ Average number men per day fed: _____

Food item	Unit of food	Unit weight (lb or kg)	Initial inventory units	Units issued during period	Total issue units	Final inventory units	Total units used	Total used (lb or kg)	Average per man per day "as issued" (lb or kg)	Average edible waste per man per day	Average "as consumed" per man per day

Form No. 6.—*Recipe—food preparation* [1]

Unit: _____

Day: _____ Food: _____ _____

Meal: _____ (Native name) (English name)

Ingredients	Weight as purchased	Refuse weight (percent)	Raw weight	Edible weight	Cooked weight	Conversion factor [2] cooked weight to "as purchased"

[1] Description of preparation: Give actual recipe, including cooking time, method of cooking. Time prepared—time served, number of men for whom prepared, etc.

[2] See p. 171.

FORM No. 7.—*Food waste*

Unit: _____ Number served: _____

Day: _____

Meal: _____

EDIBLE FOOD

Food	Plate scrapings total pounds (kg) food	Prepared food not used total pounds (kg) food	Ingredient list (from recipe)	Conversion factor to "as purchased"	Total food; wasted pounds (kg) "as purchased"

Total fat and grease discarded = _____

VIII

Food Analysis Procedures

Local Facilities for Analysis of Food Samples

Although it is feasible to ship food samples to the United States for analysis—and in any case this may be done to some extent for purposes of cross-checking of results—it is desirable that resources be available to each country for making food analyses. In many cases there will be an existing national or regional laboratory conducting or equipped for food analysis. For analysis of food, equipment and supplies in addition to those for the survey laboratory are needed. They are not automatically included in shipment of ICNND survey supplies. In the preliminary visits to a country it should be ascertained whether local analysis of food samples should be done and whether requisite local facilities are available. Such facilities may or may not be housed in the quarters selected for housing the biochemical laboratory for the nutrition survey, although there are certain advantages if proximity exists.

Methodology

For most food constituents (even the proximate components for which analytical methods have been under development for decades) a single method will not necessarily give accurate results with all of a variety of foods. The methods given in this Manual have been selected as being the most widely useful.[1] "Official Methods of Analysis of the Association of Official Agricultural Chemists" (9th edition, 1960) has been relied on heavily.[2] In this book are listed many specific modifications applicable to particular food items; it is included in the library shipped with the ICNND survey supplies.

Methods of preparing food and water samples for analysis are presented in appendix B, p. 187.

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[1] The advice of the laboratories of the Wisconsin Alumni Research Foundation is gratefully acknowledged.

[2] Permission granted by the Association of Official Agricultural Chemists to reprint many of these methods from their "Official Methods of Analysis" is hereby acknowledged with gratitude.

Proximate Analysis

Moisture

Vacuum Oven Method

A sample representing approximately 2 gm dry material is dried to constant weight at 95°–100° C under pressure not greater than 100 mm Hg. Loss in weight is reported as moisture. Aluminum dishes are used to hold the samples and they are kept covered except during the drying step.

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 283.

Materials

Vacuum oven
Aluminum sample dishes
Analytical balance

NOTE: If a vacuum oven is not available, samples may be dried to constant weight in an ordinary oven at 135° C. In this case there is a greater chance of spurious results due to decomposition of the sample and loss of components other than moisture. This method should not be used if the dried sample is to be subjected to fat determination.

Another alternative is to dry over sulfuric acid without heat: Weigh 2–5 gm sample into metal dish 5–10 cm in diameter with tight-fitting cover. (If subsequent fat determinations are to be made, fat extraction cones may be used.) Mix substances that dry down to hornlike material with fat-free cotton (previously weighed) or other suitable material. Place 200 ml of the fresh sulfuric acid in strong, tight vacuum desiccator. Place uncovered dish in desiccator and exhaust with vacuum pump to pressure of not more than 10 mm Hg.

If vacuum pump is not available, place 10 ml ether in small beaker in desiccator and exhaust with water filter pump. Between pump and desiccator interpose empty bottle next to desiccator and bottle of water next to pump. Draw air from desiccator through the water and turn desiccator stopcock the instant water begins to rise in tube leading from empty bottle.

Gently rotate desiccator four or five times during first 12 hours. After 24 hours, open desiccator, causing incoming air to bubble through sulfuric acid; place cover on dish and make first weighing. After weighing, place sample in desiccator containing fresh sulfuric acid and exhaust as before. Rotate desiccator several times during interval and weigh again after suitable drying period. Repeat process to constant weight.

Preparation of sulfuric acid. Boil sulfuric acid in large Kjeldahl flask with stopper carrying a $CaCl_2$ tube and cool (Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 281).

Ash

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 284.

Materials

Muffle furnace
Porcelain crucibles, 50 ml
Desiccator
Analytical balance

Method

Weigh 2 gm sample into a tared porcelain crucible and place in a muffle furnace which has been preheated to 600° C and hold at this temperature for 2 hours. The crucible is transferred to a desiccator, allowed to cool and weighed.

When dealing with slurries, excess moisture is driven off at lower temperature before the sample is placed in the furnace.

Crude Fat (in Cereal-Containing Foods)

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 287.

Materials

Mojonnier fat-extraction tubes
100° C oven
HCl
Ethyl ether:
  Ethyl alcohol
  Petroleum ether (redistilled)
Steam bath

Method

Place 2 gm ground, well-mixed sample in Mojonnier fat-extraction tube, add 2 ml alcohol to prevent lumping on addition of acid, and shake to moisten all particles. Add 10 ml HCl (25+11), mix well, and set tube in water bath at 70°–80° C for 30–40 minutes, shaking frequently. Fill to within 1–2 ml of mark with alcohol and cool. (Level of liquid should be in neck of Mojonnier tube just below pouring-off level.)

Add 25 ml ether, stopper with glass, cork, Neoprene, or good quality rubber stopper thoroughly cleaned with alcohol, and shake vigorously 1 minute. Release pressure carefully so that no solvent is lost. Wash adhering solvent and fat from stopper back into extraction tube with a few milliliters of redistilled petroleum ether (boiling point <60° C). Add 25-ml redistilled petroleum ether, stopper, and shake vigorously 1 minute. Let stand until upper liquid is practically clear or centrifuge 20 minutes at approximately 600 rpm. Pour as much as possible of ether-fat solution through filter consisting of cotton pledget packed just firmly enough in funnel stem to let ether pass freely into 150-ml beaker containing several glass beads. Rinse lip of tube with few milliliters of petroleum ether. Re-extract liquid remaining in tube twice, each time with only 15 ml of each ether, shaking 1 minute after addition of each ether. Draw off clear ether solution through filter into same beaker as before, and wash tip of tube, stopper, funnel and end of funnel stem with few milliliters of mixture of the 2 ethers (1+1). Evaporate slowly on steam bath.

Redissolve dried fat residue in four 10-ml portions of ethyl ether, filtering each portion through small, fat-free paper into 100-ml beaker containing a few glass beads that has been predried at 100° C, cooled in air, and then weighed against counterpoise treated similarly. Use fifth 10-ml portion of ether for rinsing paper and funnel. Evaporate ether on steam bath, dry in 100° C oven 90 minutes, cool in air, and as soon as room temperature is reached weigh against counterpoise treated similarly. Correct this weight by blank determination on reagents used.

Crude Fat (Ether Extractable)

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 287.

Materials

Fat extraction apparatus
Fat extraction thimble

Anhydrous ether. To prepare, wash commercial ether with two or three portions of distilled water, add solid NaOH or KOH and let stand until most of the water is abstracted from the ether. Decant into dry bottle, add small pieces of carefully cleaned metallic sodium, and let stand until hydrogen evolution ceases. Keep the ether over metallic sodium in loosely stoppered bottles.

The sample which has been dried in a vacuum oven or over sulfuric acid for moisture determination may be subjected to either direct or indirect fat determination:

Direct Method

Approximately 2 gm dried sample is placed in a porous fat extraction thimble for extraction with anhydrous ether for a period of from 4 hours (at a condensation rate of 5–6 drops per second) to 16 hours (at a condensation rate of 5–6 drops per second). The extract is dried 30 minutes at 100° C, cooled and weighed.

Indirect Method

The dried sample is extracted with ether as above; the extraction thimble and contents are dried and reweighed. The loss in weight is reported as ether extract.

If there is any doubt as to presence of any fat not readily extractable with ether (and such fat is likely to occur in any cooked or processed foods), a preliminary hydrolysis of the food is necessary. A suitable method is the A.O.A.C. procedure described above (p. 199).

Crude Fiber

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 288.

Materials

Digestion apparatus. Condenser and digestion flasks of such dimensions that solution is from 1 to 1½" in depth; a 700–750 ml erlenmeyer flask is suitable.

Filtering cloth, linen with approximately 45 threads per inch.
Sulfuric acid, 0.255 N, 1.25 gm H_2SO_4 per 100 ml.
Sodium hydroxide solution, 0.313 N, 1.25 gm NaOH per 100 ml; free, or nearly so, from Na_2CO_3.
Asbestos. Gooch grade, medium fiber asbetos is digested at steam bath temperature for at least 8 hours with approximately 5 percent NaOH and washed thoroughly with hot distilled water; then it is digested for 8 hours with HCl (1 part HCl to 3 parts H_2O) and again washed with hot distilled water, dried and ignited at bright red heat.
Dow-Corning Antifoam A emulsion (1+3).
Alundum crude fiber crucibles or Gooch crucibles.
K_2SO_4, 10 percent solution.
Alcohol.
Desiccator.

Method

Extract 2 gm dry material with ether, or use residue from ether extract determination, and transfer residue, together with approximately 0.5 gm asbestos, to digestion flask. (If residue from the ether extract is used and proper quantity of asbestos has already been added, further addition is unnecessary.) If material is difficult to wet, add 3 drops diluted Dow Corning Antifoam A emulsion (1+3). Add 200 ml of the boiling H_2SO_4 solution, immediately connect digestion flask with condenser, and heat. Contents of flask must come to boiling within 1 minute and boiling must continue briskly exactly 30 minutes. Rotate flask frequently until sample is thoroughly wetted. Take care to keep material from remaining on sides of flask out of contact with solution. (A blast of air conducted into flask serves to reduce frothing of liquid.)

After 30 minutes remove flask, immediately filter through linen in fluted funnel, and wash with boiling distilled water until washings are no longer acid. Bring quantity of the NaOH solution to boiling and keep at this temperature under reflux condenser until used. Wash charge and asbestos back into flask with 200 ml of the boiling NaOH solution, using wash bottle marked to deliver 200 ml. (Boiling NaOH solution is conveniently transferred to wash bottle by bent tube through which liquid is forced by blowing into tube connected with top of reflux condenser attached to NaOH flask.) Connect flask with reflux condenser and boil exactly 30 minutes, timing boiling with alkali so that contents of different flasks reach boiling point about 3 minutes apart, which permits enough time for filtration.

After 30 minutes remove flask and immediately filter through gooch prepared with asbestos mat, through Alundum crucible, or through filtering cloth in fluted funnel. If filtering cloth is used, thoroughly wash residue with boiling distilled water and transfer to gooch prepared with thin but close layer of ignited asbestos. For materials difficult to filter, after 30 minutes boiling remove flask and immediately

filter through filtering cloth in fluted funnel using vacuum and wash with hot 10 percent K_2SO_4 solution. The K_2SO_4 solution may be added during filtering whenever filtration becomes difficult. Return residue to digestion flask, thoroughly washing all residue from cloth with the hot K_2SO_4 solution. Filter into gooch prepared with thin but close layer of ignited asbestos.

After thorough washing with boiling distilled water, wash with about 15 ml of alcohol. Dry crucible and contents at 110° C to constant weight. Cool in efficient desiccator and weigh. Ignite contents of crucible in electric muffle or over Meeker burner at dull red heat until carbonaceous matter is consumed (approximately 20 minutes). Cool in desiccator and weigh. Report loss in weight as crude fiber.

Crude Protein (Total Nitrogen)

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 12.

Reagents

Sulfuric acid, 93-98 percent H_2SO_4, N-free.

Mercuric oxide (HgO) or metallic mercury (Hg), reagent grade, N-free.

Potassium sulfate (or anhydrous sodium sulfate), reagent grade, N-free.

Sulfide or thiosulfate solution: dissolve 40 gm commercial K_2S in 1 liter distilled water. (Solution of 40 gm Na_2S or 90 gm $Na_2S_2O_3 \cdot 5H_2O$ in 1 liter may be used.)

Sodium hydroxide. Pellets or solution nitrate-free. For solution, dissolve approximately 450 gm solid NaOH in distilled water and dilute to 1 liter (specific gravity of solution should be 1.36 or higher).

Zinc granules, reagent grade.

Methyl red indicator: dissolve 1 gm methyl red in 200 ml alcohol.

Hydrochloric or sulfuric acid standard solution, 0.5 N, or 0.1 N when amount of nitrogen is small.

Alkali standard solution, 0.1 N.

Standardize each standard solution with primary standard, and check one against the other. Test reagents before using by blank determination with 2 gm sugar, which insures partial reduction of any nitrates present.

Apparatus

Kjeldahl digestion and distillation unit.

Flasks, for digestion. Use Kjeldahl flasks of hard, moderately thick, well annealed glass with total capacity of approximately 500-800 ml. Conduct digestion over heating device adjusted to bring 250 ml of water at 25° C to rolling boil in about 5 minutes. To test heaters, preheat 10 minutes if gas or 30 minutes if electric. Add 3-4 boiling chips to prevent superheating.

Flasks, for distillation. Use Kjeldahl or other suitable flask of 500-800 ml capacity, fitted with rubber stopper through which passes lower end of efficient scrubber bulb or trap to prevent mechanical carryover of sodium hydroxide during distillation. Connect upper end of bulb tube to condenser tube by rubber tubing. Trap outlet of condenser in such a way as to insure complete absorption of ammonia distilled over into acid in receiver.

Method

Place weighed sample (0.7–2.2 gm) in digestion flask. Add 0.7 gm HgO (mercuric oxide) or 0.65 gm metallic Hg, 15 gm powdered K_2SO_4 or anhydrous sodium sulfite (Na_2SO_4), and 25 ml H_2SO_4. If sample larger than 2.2 gm is used, increase H_2SO_4 by 10 ml for each gram of sample. Place flask in inclined position and heat gently until frothing ceases (if necessary, add small amount of paraffin to reduce frothing); boil briskly until solution clears and then for at least 30 minutes longer (2 hours for samples containing organic materials).

Cool, add approximately 200 ml H_2O, cool below 25° C, add 25 ml of the sulfide or thiosulfate solution, and mix to precipitate mercury. Add a few zinc granules to prevent bumping, tilt flask, and add a layer of NaOH (25 gm solid reagent or enough solution to make contents strongly alkaline) without agitation. (Thiosulfate or sulfide solution may be mixed with the NaOH solution before addition to flask.) Immediately connect flask to distilling bulb on condenser, and, with tip of condenser immersed in standard acid in receiver, rotate flask to mix contents thoroughly; then heat until all ammonia has distilled (at least 150 ml distillate). Titrate excess standard acid in distillate with standard alkali solution, using methyl red. Correct for blank determination on reagents.

Calories

"Carbohydrate by difference" is determined by subtracting the percentages of ash, water, fat, and protein from 100.

Caloric value may be calculated by multiplying the percentages of protein carbohydrate ×4 and percentages of fat ×9. If desired, more refined values for various products may be applied and the available information on digestibility and physiological energy of various foods may be taken into consideration (Food and Agriculture Organization, Rome, "Energy-yielding Components of Food and Computation of Calorie Values," 1947).

Total Calories may also be determined by combustion in a bomb calorimeter (Tech. Manual No. 130, Parr Instrument Co., 211 53d St., Moline, Ill.).

Minerals

Iron

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 159.

Reagents

Orthophenanthroline solution: dissolve 0.1 gm orthophenanthroline in about 80 ml distilled water at 80° C, cool and dilute to 100 ml.

Alpha, alpha-dipyridyl solution: dissolve 0.1 gm α,α-dipyridyl in distilled water and dilute to 100 ml.

(Keep above two reagents in cool, dark place and they will remain stable several weeks.)

Hydroxylamine hydrochloride solution: dissolve 10 gm $NH_2OH \cdot HCl$ in distilled water and dilute to 100 ml.

Magnesium nitrate solution: dissolve 50 gm $Mg(NO_3)_2 \cdot 6H_2O$ in distilled water and dilute to 100 ml.

Acetate buffer solution: dissolve 8.3 gm anhydrous Na acetate (previously dried at 100° C) in distilled water, add 12 ml acetic acid, and dilute to 100 ml. (It may be necessary to redistill the acetic acid and purify the sodium acetate by recrystallization from distilled water, depending on the quantity of iron present.)

2 molar acetate buffer solution, containing 272 gm $NaOAc \cdot 3H_2O$ per liter.

Buffer solution, pH 3.5: dilute 6.4 ml of the 2 M acetate buffer solution (immediately above) and 93.6 ml 2 M acetic acid (120 gm per liter) to 1 liter with distilled water.

Bromphenol blue indicator: grind 0.1 gm of the powder with 1.5 ml 0.1 N NaOH and dilute to 25 ml.

Preparation of Standard Curve

Dissolve 0.1 gm analytical grade iron wire in 20 ml HCl and 50 ml distilled water, and dilute to 1 liter. Dilute 100 ml of this solution to 1 liter. (1 ml = 0.01 mg Fe.) Or,

Dissolve 3.512 gm $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water, add 2 drops HCl, and dilute to 500 ml. Dilute 10 ml of this solution to 1 liter (1 ml = 0.01 mg Fe).

Prepare 10 solutions containing 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, and 45.0 ml, respectively, of the final diluted stock solution, plus 2.0 ml HCl, in 100 ml. Also prepare a blank solution containing only the HCl. Using 10 ml of each of these solutions proceed as in line 3, page 205, beginning "add 1 ml of the $NH_2O \cdot HCl$ . . ." Plot concentration against scale reading.

Determination

By dry ashing. Ash 10.0 gm flour in platinum, SiO_2, or porcelain dish (about 60 mm in diameter, 35 ml capacity) as in determination of ash. (Porcelain evaporating dishes of approximately 25 ml capacity are satisfactory. Do not use flat-bottom dishes of diameter greater than 60 mm.) Cool, and weigh if percent of ash is desired. Continue ashing until sample is practically carbon-free, using one of following ash aids:

Moisten ash with 0.5-1.0 ml of the $Mg(NO_3)_2$ solution or with redistilled HNO_3. Dry contents and carefully ignite in muffle to prevent spattering. (White ash with no carbon results in most cases.) Do not add these ash aids to self-rising flour (products containing NaCl) in platinum dish because of vigorous action on dish. Cool, add 5 ml HCl, letting acid rinse upper portion of dish, evaporate to dryness on steam bath, dissolve residue by adding 2.0 ml HCl, accurately measured, heat 5 minutes on steam bath with watch glass on dish, rinse

watch glass with distilled water, filter into 100 ml volumetric flask, cool and dilute to volume.

Pipet 10-ml aliquot into 25-ml volumetric flask and add 1 ml of the $NH_2OH \cdot HCl$ solution; in a few minutes add 5 ml of the acetate buffer solution and 1 ml of the orthophenanthroline or 2 ml of the dipyridyl solution and dilute to volume. Read intensity of color in 2-inch cell in neutral wedge photometer, using No. 51 filter (wavelength about 510 mμ). (Other suitable instruments of equivalent precision may be used.) From reading determine iron concentration from equation of line representing standard points or by reference to standard curve for known iron concentration. Determine blank on reagents and make correction. Calculate quantity of iron in flour as milligrams per pound. Rinse all flasks, beakers, funnels, etc., with distilled water before use, and filter all reagents to remove suspended matter.

By wet digestion. Transfer 10.00 gm flour to 800-ml Kjeldahl flask, previously rinsed with dilute acid, then with distilled water; add 20 ml distilled water and mix; pipet 5 ml H_2SO_4 into flask and mix; add 25 ml HNO_3 and mix well. After a few minutes heat flask very gently at brief intervals (to avoid foaming out of flask) until heavy evolution of NO_2 fumes ceases. Continue to heat gently until material begins to char; then add a few milliliters of HNO_3 cautiously at intervals until SO_3 fumes evolve and colorless or very pale yellow liquid is obtained (60–65 ml HNO_3 in all in about 2 hours). Cool, add 50 ml distilled water and 1 Pyrex glass bead, and heat until SO_3 fumes appear; cool, add 25 ml distilled water, and filter through 11-cm paper into 100-ml volumetric flask; rinse out flask, cool, and dilute to mark.

Pipet 10 ml into 25-ml volumetric flask, add 1 ml of the $NH_2OH \cdot HCl$ solution, rotate flask, and let stand a few minutes. Add 9.5 ml of the 2-molar sodium acetate solution, and 1 ml of the orthophenanthroline solution, dilute to mark and mix. Let stand at least 5 minutes and read in 2-inch cell in neutral wedge photometer or other instrument of similar precision.

With self-rising flour the 9.5 ml of the 2-molar sodium acetate solution may be reduced to 8.0 ml. To determine the exact amount of this buffer solution needed to adjust each digest to the most desirable pH range, mix a 10-ml aliquot of the sample with a measured amount of the buffer solution, dilute with distilled water to 25 ml, and determine pH either electrometrically or colorimetrically.

For colorimetric determination, add 5 drops bromphenol blue indicator to the solution and compare color with that of an equal volume of the pH 3.5 buffer solution, also treated with 5 drops of the indicator. Although color develops from pH 2–9, avoid a pH of less than 3.0 and preferably work at pH 3.5–4.5. With cereal products the 9.5 ml of the 2 M sodium acetate buffer solution is satisfactory.

With samples high in iron, an aliquot of 5 ml instead of 10 ml may be used with 4.8 ml of the 2 M sodium acetate buffer solution. Conduct digestion so as to avoid contamination with iron, and determine blank. After correction for blank, calculate as milligrams of iron per pound.

Determination of Iodine in Food Composites

Apparatus

Constant temperature water bath, 30±0.5° C.

Any suitable colorimetric photometer or spectrophotometer, wave length 420 mμ.

1 ml hypodermic syringe without needle.

NOTE.—An Evelyn photometric colorimeter with 19-mm test tubes was used with this method. Therefore, other instruments and cells with differing cell lengths and volumes will necessitate changes in procedure.

Reagents

Double distilled water.

Purified sulfuric acid: add 10 ml of concentrated HCl to 2 liters of concentrated H_2SO_4; boil for 1–2 hours.

0.15 N arsenious acid in 1.5 N H_2SO_4: dissolve 3.71 gm of As_2O_3 in 10 ml of water containing 2.5 gm of NaOH. Dilute to about 300 ml and neutralize with the purified H_2SO_4. Add an additional 21 ml and make to 500 ml with water.

Ceric ammonium sulfate or ceric sulfate: 0.1 N solution in 3.5 N H_2SO_4.

Standard iodine solution: 0.02 μg of iodine per milliliter. Prepare fresh every few weeks from a known KI stock solution.

Ash aid solution: 2 gm NaOH + 1 gm KNO_3 yields 100 ml with water.

Alkaline water: 1 ml 1 percent NaOH in 300 ml redistilled water.

Sample Preparation

Weigh 5.0 gm of thoroughly mixed sample into 22 x 200 mm Pyrex combustion tube. Add redistilled water to bring the volume to approximately 10 ml. Add 1.0 ml ash aid solution. Place tubes in forced air drying oven at 105°–110° C until samples are completely dry. Transfer tubes to muffle furnace and ash at 525°–550° C for 6–8 hours. Remove tubes from furnace, cool and add 5 ml alkaline water. Just bring tube contents to a boil using a bunsen or similar type burner. Filter with suction through 6 ml frit (Selas No. 01) into 50-ml volumetric flask. Rinse tube with three additional 5-ml portions alkaline water and pour through filter.

Make to volume and mix. Take 2.0 ml of mixture through colorimetric iodine procedure.

Method

Pipet an aliquot of the sample solution into a colorimeter tube. At the same time prepare a series of standard tubes by pipeting 0.5, 1.0, 2.0, 3.0, and 4.0 ml of the standard solution into six tubes, leaving one tube as a blank. To each of the standard tubes, add the same quantity of blank solution as was taken for the sample. To all tubes, add 1 drop of purified sulfuric acid and 0.8 ml arsenious acid solution. Make all tubes to exactly 10 ml with distilled water. Place tubes in a suitable rack in the 30° C water bath for approximately 1 hour.

The following steps are done with the bath adjacent to the colorimeter: At 30-second intervals, rapidly add 0.6 ml of the ceric sulfate solution using the hypodermic syringe. The tube is briefly removed from the rack, swirled and replaced. At approximately 20 seconds before 5 minutes, read the percent transmittance. At 30-second intervals, read the remaining tubes, returning each tube to the water bath immediately after reading. At exactly 20 minutes, repeat the reading of all tubes with 30-second intervals.

Convert all percent transmittance readings to optical density (OD). Plot OD (5 minutes) minus OD (20 minutes) versus concentration on straight-line paper. From the OD (5 minutes) minus OD (20 minutes) of the sample, determine the concentration from the standard curve.

Since six standard tubes are used, only five sample tubes can be used per set. However, by starting a second set immediately after the last 5-minute reading, an additional 11 samples may be analyzed using the same standard curve. If more than this is desired, a second standard curve should be run. In any case, each time the assay is run a new standard curve should be prepared.

NOTE. For a simpler procedure for determination of iodine in iodized salt, see Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 461.

Calcium

REFERENCES

Ingols, R. S., and Murray, P. E., Urea hydrolysis for precipitating calcium oxalate. Anal. Chem. *21*, 525–527, 1949.

Official Methods of Analysis of the A.O.A.C., 9th ed, 1960, p. 160.

Apparatus

Porcelain evaporating dishes	Muffle furnace
Hotplate	Fritted glass funnels

Reagents

HNO_3	Urea
HCl	Ammonium oxalate
H_2SO_4	$KMnO_4$

Weigh out sample containing at least 5 mg calcium into porcelain evaporating dish.

Heat on hotplate to complete dryness.

Place in cool muffle furnace and heat to 550° C, holding for 3-4 hours. Then cool and wet residue with redistilled nitric acid. Dry on hotplate and remuffle at 550° C for one-half hour. Repeat nitration if necessary to obtain white ash.

Add 10 ml 50 percent hydrochloric acid and heat to dissolve ash. Transfer to 250 ml beaker with redistilled water.

Neutralize with concentrated ammonium hydroxide to yellow color of methyl red.

Add 1.5 ml 6 N hydrochloric acid.

Add 5 gm urea.

Add 5 ml 4 percent ammonium oxalate. (If precipitate forms, a smaller sample should be taken.)

Cover with watch glass and heat to slow boil until solution turns orange.

Cool and filter through fine glass-fritted funnel using ammonia water (1 ml concentrated ammonium hydroxide in 300-ml water) to wash beaker and precipitate.

Place fritted funnel back in original beaker and cover with redistilled water. Add 2.5 ml concentrated sulfuric acid.

Warm on hotplate to at least 85° C and titrate with 0.05 potassium permanganate until faint pink color lasts for at least 30 seconds.

Calculation

$$\frac{\text{ml} \times \text{N of } KMnO_4 \times 20 \times 100}{\text{sample weight}}$$

NOTES.—Avoid heating samples past orange color to yellow after addition of hydrochloric acid since this may cause magnesium to precipitate also.

Keep solution above 70° C while titrating to get a sharp end point.

Fluorine in Foods

Principles

The general method specifies ashing of the sample with $Ca(OH)_2$ as fluorine fixative, isolation of fluorine by Willard-Winter distillation (*1*) from $HClO_4$, and estimation in distillate by $Th(NO_3)_4$ back-titration procedure (*2*). Technic and reagent concentrations are designed to handle conveniently not more than 10.0 mg fluorine (F). Modifications of this general procedure, applicable to specific products, are described.

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1. Willard, H. H., and Winter, O. B., Volumetric method for determination of fluorine. Indust. Eng. Chem., Anal. Ed. 5, 7–10, 1933.
2. Clifford, P. A., Report on fluorine. J. Assn. Offic. Agr. Chemists 27, 246–256, 1944.
3. Clark, E. P., Concerning the Dyer method for the identification and determination of volatile fatty acids. J. Assn. Offic. Agr. Chemists *21*, 684–688, 1938.
4. Clifford, P. A., Report on fluorine. J. Assn. Offic. Agr. Chemists *24*, 350–363, 1941.
5. Largent, E. J., Aliquant samples in the determination of fluorides in mixed foods. Indust. Eng. Chem., Anal. Ed. *13*, 93, 1941.
6. Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, see esp. p. 311.

Apparatus

Fluorine still: A Claisen 100–125 ml distilling flask is most practical for general work. It must be of Pyrex glass with auxiliary neck sealed off immediately above side arm to prevent pocketing and refluxing of distillate. The still

should be as small and simply designed as practicable; in fact, ordinary distilling flasks can be used for some work. They are slightly more efficient than the Claisen type, except that there is more danger of spraying over of the distilling acid.

Equip still with a dropping funnel and 0°–150° C thermometer, the latter extending to within one-fourth inch of bottom of flask, so that bulb is immersed in boiling acid mixture. Acid-alkali washed beads, preferably Pyrex, should be on hand. Clean rubber stoppers by boiling in 10 percent NaOH solution. All-glass apparatus with standard taper accessories is convenient, especially in routine work, and eliminates need for rubber stoppers.

While not entirely necessary for heating still, use of a Wood metal (50 Bi, 25 Pb, 12.5 Sn, 12.5 Cd) bath, adequately shielded, will prevent undue decomposition of $HClO_4$ and aid materially in securing low blank and low acid distillate; hence its use is strongly urged. If a metal bath is used, do not immerse flask so deeply that bath level is above that of liquid in flask; if bath is not used, transite or asbestos shielding boards are essential, and the flask should be heated through a small hole in the shield by a low "clean" flame. (The purpose of the bath and shielding boards is to prevent overheating of upper still walls.)

At the analyst's option, distilling water may be added as steam instead of through a dropping funnel; an electric boiler (*3; 6*, p. 236) is a convenient steam generator. If steam is used, the inlet tube should dip below the surface of the liquid in the still. One advantage in adding distilling water through a funnel is that the last portions of the rinse water used in transferring an ash can be used in distillation. If the funnel plug is thinly notched with a sharp file on either side of the bore, the dropping rate can be more easily controlled, and the end of the funnel stem need not extend into the liquid in the still. The still is used in conjunction with a clean, straight-tube condenser no longer than necessary for adequate cooling. (Vertical arrangement of the condenser will conserve bench space.)

Nessler tubes, tall form, 100 and 50 ml, glass-stoppered type preferred. They should be matched in sets of at least 6. (100-ml size will be used more frequently in the general method.)

Additional apparatus: There will also be required carefully cleaned and tested platinum or well-glazed porcelain dishes of at least 100-ml size; 150- or 200-ml volumetric flasks; and 10-ml burettes (conveniently automatic) to deliver various solutions required in distillation and titration. An overhead radiant heater will be found invaluable for drying and preliminary charring of samples, especially those of high-sugar type.

Reagents

Lime suspension: carefully shake about 56 gm 1 mole low-fluoride CaO (approximately 2 ppm F) with about 250 ml distilled water, and add 250 ml 60 percent $HClO_4$ slowly and with stirring. Add a few glass beads and boil down to copious fumes of acid, then cool, add 200 ml distilled water, and boil down again. Repeat dilution and boiling down once more; cool, dilute considerably, and filter through a fritted glass filter, if precipitated SiO_2 appears. Pour

the clear solution, with stirring, into 1 liter of NaOH solution (10 gm per 100 ml), let precipitate settle, and siphon off the supernatant. Remove Na salts from precipitate by washing 5 times in large centrifuge bottles, shaking up mass thoroughly each time. Finally, shake precipitate into suspension and dilute to 2 liters. Store in paraffined bottles. (100 ml of this suspension should give no appreciable F blank when evaporated, distilled and carried through the titration procedure described below.) Always shake suspension well before use.

Perchloric acid solution, 60 percent: dilute $HClO_4$ with 3-4 volumes of distilled water and boil down to original volume. Do not fume strongly. Repeat, and store in Pyrex. (Prepared acid should be Cl-free by test.)

Sulfuric acid solution: carefully mix equal volumes H_2SO_4 and distilled water, boil down to fumes, cool, dilute carefully, boil down once more, and dilute to 1+1 volume.

Silver perchlorate solution: 50 gm per 100 ml.

p-Nitrophenol indicator: 0.5 percent alcohol solution.

Potassium hydroxide solution: exactly 0.05 N.

Potassium chloride solution: 0.5 N, 3.727 gm per liter.

Hydroxylamine hydrochloride solution: 1.0 percent.

Hydrochloric acid solution: exactly 0.05 N.

Alizarin indicator: 0.01 percent aqueous solution of sodium alizarin sulfonate (alizarin red S).

Potassium fluorosilicate: if pure K_2SiF_6 is not obtainable, prepare as follows: Add, through a dropping funnel, a saturated solution of NaF, or a suspension of crude K_2SiF_6, into a 500 ml Claisen distilling apparatus containing 60 ml H_2SO_4 (1+1), some glass beads, and 10-20 gm powdered SiO_2 (or glass) kept at a boiling temperature of 120°-125° C. Conduct the distillate into a solution of about 25 gm pure KCl in distilled water, held at simmering temperature on a hotplate so that the volume of distillate does not become excessive. If necessary, add more distilled water to mixture from the dropping funnel in the side neck of the still. Regulate rate of addition of fluoride to still and temperature of condensing water so that the sidearm and condenser do not become clogged with evolved H_2SiF_6, which tends to lodge as gelatinous mass. K_2SiF_6 is formed in the receiver and, although entirely crystalline, it assumes the appearance of gelatinous mass.

When a substantial quantity collects, pour contents of the receiver into a large centrifuge bottle and wash repeatedly by centrifuging (shaking up precipitate thoroughly each time), until washings are Cl-free by test. Collect on büchner and either air-dry or bring to constant weight *in vacuo* at 50°-70° C.

Determine purity by Travers titration (*6*, see p. 32) at boiling temperature with 0.2 N NaOH (1 ml=0.01102 gm K_2SiF_6); also by conversion to K_2SO_4 by treating 0.3-0.4 gm in a deep platinum dish with a little distilled water, then H_2SO_4 plus a little HF, fuming off excess acid carefully (if overheated, mixture has a tendency to spatter), and heating to constant weight of K_2SO_4 at 650° C. With use of glass apparatus an entirely pure product is not usually obtained, as some contamination with SiO_2 results from the leaching effect of vapors on the condenser. A pure product can be obtained by use of a platinum still. Diluting 0.9662 gm pure K_2SiF_6 to 1 liter gives a stock solution containing 0.5 mg F per milliliter. Much more will not dissolve. Prepare such a solution, correcting this weight of 0.9662 by purity factor of the K_2SiF_6 (figure for purity obtained from average of 2 above methods of assay). The solution keeps indefinitely in paraffined bottle.

Prepare solution used in "titration" (see below) by diluting 20 ml of this stock solution to 1 liter (1 ml=10 μg F). It will retain its strength several weeks in ordinary volumetric ware.

Thorium nitrate solution: 0.25 gm $Th(NO_3)_4 \cdot 12\ H_2O$ or 0.20 gm $Th(NO_3)_4 \cdot 4\ H_2O$ per liter. Check titer against standard (10 μg per milliliter) F solution as follows: Measure 10, 20, 30, etc., up to 80 μg F into 100 ml Nessler tubes and add 4.00 ml of the 0.05 N HCl (2.00 ml if 50 ml Nessler tubes are used, and carrying range to only 50 μg F for this size tube) (5). Dilute mixture to about 80 (or 40) ml mark and add 1.00 ml 1.0 percent $NH_4OH \cdot HCl$ to solution. Mix; then add exactly 2.00 ml alizarin indicator (or 1.00 ml for smaller tube) and measure the thorium solution from the burette, mixing frequently until, when sighting down the tube toward a white reflecting surface, an incipient pink or salmon pink color is observed. Add a little distilled water occasionally so that solution is nearly to the mark as the end point is approached. Finally, make exactly to the mark and mix thoroughly before checking final end point. Do not shake tubes violently (5–6 gentle inversions are enough).

Make an effort to secure an endpoint shade intermediate between the yellowish-green of the acid indicator and the reddish purple of the fully developed thorium lake. Complete series and plot milliliters of thorium solution against milliliters of standard fluoride to obtain a rough equivalence curve for two solutions. Depending upon the quantity of fluorine known to be present, add the thorium solution in 1–2 ml portions at first, with final additions of 0.25 ml.

Precautions and Interferences

Control magnitude of determination blank by careful choice and purification of reagents. With care, blank will be low (1–3 μg F), but with low-fluoride foods it may represent a considerable part of the total fluorine determined. Hence it must be stable. A large part of it will be "distillation blank," apparently resulting from fluorine leached from glassware of the still during distillation. This blank can be minimized by preliminary treatment of still (see "final distillation" below), and it should be possible to deduce and correct for an average distillation blank if stills of the same material and design are routinely used; otherwise, each still must bear its special blank. New, unused stills will usually be found to exhibit a high blank, which will diminish to a constant low figure after several runs. They should not be used until the analyst has assured himself, by means of several consecutive blank runs, that the still is yielding not more than a constant, low quantity of fluorine.

Check ashing utensils by blank determinations with fixative solution to ascertain whether they contribute appreciable fluorine. Even platinum vessels may become contaminated (owing presumably to a slight calcium content) if they have been used recently for HF volatilization of SiO_2. In addition, such blank determinations are useful for testing reagents and apparatus used in the method as well as evaporators, hoods, muffles, and laboratory atmosphere for presence of fluorine fumes and dust. If HF bottles are permitted in the same laboratory, seal immediately after use; avoid contamination from roach powders.

Ordinary tap water may be a source of fluorine contamination, since 1 ml of water containing 2 ppm fluorine will contribute 2 μg fluorine if allowed to remain or to dry in the still. Therefore, routinely rinse all glassware (stills, flasks, burettes, etc.) with distilled water, preferably redistilled from alkaline $KMnO_4$. Filter papers may contribute small (μg) quantities of fluorine, and glass filters are preferred if filtration is required in micro determinations.

Interferences are gelatinous SiO_2; aluminum and boron compounds, which repress evolution of fluorine as H_2SiF_6 in distillation; materials such as nitrates, nitrites, peroxides, Cl, SO_2, and H_2S, which act upon the indicator in titration or otherwise interfere; halides (Cl), which distill to give excessive acidity in the distillate; and phosphates and sulfates, which react with thorium in titration to give high results. The method is so designed that most of these interferences are automatically eliminated, but the analyst should be on guard against their possible occurrence under unusual circumstances.

Preparation of Sample

Methods of sample preparation are designed to furnish a representative sample in a workable quantity of material and to obtain the sample in condition for final distillation. Mineralization by ashing is usually involved. Some mineral food products can be dissolved in and distilled from $HClO_4$, provided no interferences appear in the final distillate.

In general, 20 gm or more of dry material, 50–100 ml liquid samples, and 50–100 gm undried food products or plant material can be taken for analysis, depending upon the expected content of fluorine and interferences, such as excessive chlorine, which use of large samples may introduce. For reasonable precision in analysis of low-fluorine foods, the sample should be sufficient to yield a titer of at least 0.5 ml for the aliquot taken in final titration. However, it may not always be possible to handle this quantity of material. If adequate grinding and mixing equipment is available, it is often feasible to prepare large quantities of material (vegetables, mixed foods) and to take aliquant portions for analysis (*5*).

Dry plant materials, feeds, bone meal, etc., can be ground to convenient size in a Wiley mill and thoroughly mixed before the sample is taken. The following special methods for certain products are indicated:

Direct ashing: Applicable to fibrous (not highly fatty) food materials, liquid samples, and in general to all foods that can be thoroughly wet with aqueous fixative solution. (This procedure will apply to the majority of food products.)

Weigh a suitable portion of prepared sample into a clean platinum dish and add 25 ml $Ca(OH)_2$ suspension. (Porcelain casseroles or

dishes are second choice because they may contribute small quantities of fluorine and Al_2O_3 to the sample.) Mix in the $Ca(OH)_2$ suspension with glass rod, adding more distilled water if necessary; rinse and remove rod. Dry thoroughly on steam bath or in hot air oven, then slowly char the sample by heating over a low flame or electric plate with a thermostat. An overhead radiant heater is convenient for both drying and charring the sample. Control excessive swelling of high-sugar foods by playing a small flame over the surface of the sample from time to time, and char these products slowly so that excessive acidity is not generated. When the sample is charred past danger of catching fire, ash in muffle furnace at 600° C. (For very small samples and minute blanks, it may be advisable to cover the ashing vessel with an inverted Pyrex petri dish while ashing.)

When clean ash is obtained, cool the dish and wet the ash with about 10 ml of distilled water. (A small quantity of unburned carbon does not interfere, but if much is apparent, dry down and repeat ashing.) Cover the dish with a watch glass and cautiously introduce under the cover just enough of the $HClO_4$ solution to dissolve the ash. Rinse down the cover with a little distilled water and transfer the solution to a freshly prepared fluorine still (see "final distillation") through a long-stem funnel. Rinse the dish with the remainder of the distilling acid, using about 20 ml in all, and adding and transferring in several small portions. Do not prolong the transferring operation. Finally rinse the funnel and stirring rod into the dish, assemble the still, and complete rinsing of the dish with several small portions of distilled water, pouring these into the dropping funnel of the still. If the distilling water is added as steam (see "fluorine still" under apparatus, above), rinse the dish with a little additional distilled water and add directly to the acid mixture in still, but avoid an excessive initial volume. Add about 6 Pyrex beads and enough $AgClO_4$ solution (see reagents, above) to precipitate all Cl. (A reasonable excess of $AgClO_4$ does no harm; enough solid Ag_2SO_4 may also be used.) Proceed as in "final distillation" (below).

Preliminary distillation: (Necessary with certain products high in phosphate, such as calcium phosphate and bone meal, in order to eliminate distilled H_3PO_4 that may be present in appreciable quantities in first distillates. Also advisable with certain excessively fatty materials that may not be thoroughly wet with the $Ca(OH)_2$ fixative, thus causing fluorine loss in direct ashing method.)

For inorganic phosphatic materials, such as calcium phosphate: Weigh sample, usually 10 gm, into still, add a few glass beads, enough $AgClO_4$ to precipitate possible Cl, and about 20 ml of the $HClO_4$. (If the inorganic phosphatic material does not contain excessive calcium (enough to cause heavy precipitation of $CaSO_4$ in the still), use a similar quantity of the 1+1 H_2SO_4.) Distill at 135°–140° C, col-

lecting approximately 200 ml of distillate. (For this preliminary distillation, extreme care in securing low-acid distillate is not essential.) Evaporate distillate to dryness in platinum dish after addition of excess $Ca(OH)_2$ suspension, assuring alkaline conditions by testing with a drop of phenolphthalein. (If H_2SO_4 is used in this preliminary distillation, add to distillate few drops of F-free 30 percent H_2O_2 to oxidize possible sulfites.) Heat dried residue at 600° C for a few minutes to destroy indicator residues and possible Cl-containing compounds. Transfer contents of dish to freshly prepared still (see "final distillation" below), with the 20 ml distilling $HClO_4$ as in "direct ashing" above, and proceed with final distillation.

Take 20 ml samples of sirupy H_3PO_4 and collect at least 300 ml of the first distillate at 135° C, letting the H_3PO_4 function as its own distilling acid. (More distillate is necessary because the H_3PO_4 is less effective as fluorine distilling acid.) Neutralize with $Ca(OH)_2$ suspension, evaporate to dryness, transfer to prepared still as above, and proceed with "final distillation."

Final Distillation

Always make final distillation from $HClO_4$, and take precautions to secure low acid distillate. (See "Apparatus.") Since interferences, such as organic matter, phosphate, sulfate, etc., must be absent from distillate, make distillation with careful temperature control in the presence of enough silver salt to repress HCl evolution. It is well to check distillates for presence of possible phosphate as in "preparation of sample, preliminary distillation," and where advisable to test for sulfate with a little dilute $BaCl_2$ solution (*6*, see p. 315, b 4). $HClO_4$ used in final distillation is usually used in transferring ash to still ("direct ashing," above). A few acid-alkali washed beads are used to control bumping. (Use of powdered SiO_2 does not appear necessary for microdetermination.)

To promote better recoveries, and to minimize and render constant the distillation blank discussed in "Precautions and Interferences" (above) and "Titration" (below), prepare the still by a special cleaning process before this transfer by treating it with hot 10 percent NaOH solution after each run, flushing out with tap water, and then rinsing with distilled water. Occasionally (at least once a day, and especially after it has stood idle for any length of time), give the still additional treatment by boiling down 15–20 ml of the 1+1 H_2SO_4 until the still is filled with fumes. Cool, pour off the acid, treat with the 10 percent NaOH solution and thoroughly rinse out. (Cleaning should be especially meticulous after high-F or high-SiO_2 samples have been distilled and in such cases the condenser should also be cleaned.)

At this stage the prepared sample has been transferred to a specially treated still, as directed above, for final isolation of F. Begin dis-

tillation, and when the temperature reaches 137° C, keep at this point (±2°) by adding distilled water from a dropping funnel ("fluorine still" under apparatus, above). Heat the still at such a rate that all distillations require about the same time. (This promotes uniformity in blank correction.) Catch the distillate in a 150 or 200 ml volumetric flask. After a few milliliters of distillate collect, add 1–2 drops p-nitrophenol indicator (see "reagents" above), and keep distillate alkaline to this indicator (faintest perceptible yellow) by adding a drop or two of the 0.05 N KOH from a 10-ml burette from time to time during distillation while swirling the receiver. So regulate this addition of alkali that distillate is neutralized (within 1 drop of alkali) as it approaches the mark. Note carefully the volume of alkali used. Dilute distillate to mark and mix thoroughly. Do not let F distillate stand more than a few minutes before neutralizing.

If the sample contains such large quantities of Cl that bumping in still cannot be controlled, dissolve ash of another sample, and acidify slightly with $HClO_4$. Dilute considerably and precipitate Cl in dish with the $AgClO_4$ solution, avoiding a large excess. Filter through a glass filter, wash precipitate thoroughly with hot distilled water, and evaporate filtrate and washings to dryness after adding excess (to alkalinity) of the $Ca(OH)_2$ suspension. Transfer residue to the still with the $HClO_4$ and repeat distillation as above.

Titration

Place an aliquot of final distillate in Nessler tube and mark "S" (sample). (Optimum F content for titration is 60–70 μg for 100 ml Nessler tubes and 30–40 μg for 50 ml size, and it is well to make an exploratory titration on a small aliquot to check approximate F content of the distillate. Larger tubes are necessary for precise results on low-fluoride foods.)

Add the 0.05 N HCl, 4.00 ml for 100 ml tubes and 2.00 ml for 50 ml size, and 1.00 ml of the $NH_2OH \cdot HCl$ solution. (For routine work with 100 ml tubes the acid and $NH_2OH \cdot HCl$ can be blended as 0.04 N HCl made to 0.2 percent concentration with $NH_2OH \cdot HCl$, and the proper quantity of both reagents added to tubes in a single operation with a 5 ml pipet.) Dilute to about 90 (or 40) ml, mix well, then add the proper quantity of the alizarin indicator (2.00 or 1.00 ml), and mix again. Always add and mix the $NH_2OH \cdot HCl$ before adding the indicator.

Prepare a blank tube "B" by adding the proper quantity of HCl and $NH_2OH \cdot HCl$, and a quantity of the 0.05 N KCl solution representing the same proportion of the total volume of 0.05 N KOH used to neutralize distillate as the aliquot volume taken for the sample tube represents of total distillate volume. (Thus, if 1.50 ml 0.05 N KOH was used to neutralize a distillate of 150 ml and the aliquot taken for tube

"S" was 75 ml, add 0.75 ml of the 0.05 N KCl to tube "B.") Dilute and mix, allowing slightly more headspace than in the sample tube. Then add the proper volume of alizarin indicator and mix.

Measure thorium solution into tube "S," mixing between additions, until an end point of about proper shade is reached. Dilute to the mark, mix, and check this endpoint shade. Note from curve ("Reagents," thorium nitrate solution) the approximate volume of standard F solution corresponding to this volume of thorium solution, and add about 0.5 ml less than this quantity of standard F solution to "B." Mix; then add exactly the same volume thorium solution as was added to "S," duplicating the approximate increments in which it was added and the number of mixings. Dilute nearly to the mark and compare colors of "S" and "B." (If the volume of standard F solution added to "B" was properly chosen, this tube should be only slightly pinker in shade than the sample tube.)

Bleach "B" tube to an exact match with tube "S" by adding more standard F solution to the former in increments of 1–2 drops, mixing gently between additions. Dilute to the mark for final comparison and observe the usual precautions of letting bubbles subside and of transposing tubes when final comparisons are made. (At match-point, the F content of tube "S" equals the quantity added to tube "B.") Check this end point by adding 1–2 drops excess of standard F solution to tube "B." A distinct overbleach should develop.

Repeat titration on aliquots of different size to obtain the total quantity of F distilled. If time is available, repeat the entire determination with a different weight sample.

For precise work, evaluation of reagent and of distillation blank is necessary. (See "Precautions and Interferences".) Determine the distillation blank by making several distillations with prescribed quantities of $HClO_4$ and $AgClO_4$ solutions from a freshly cleaned still, titrating distillate as above with as large an aliquot as practicable. The average of values found should not be greater than 2–3 μg F. If quantities found by individual blank determinations are too small to be determined accurately, make five or more separate distillations and evaporate distillates, 150 ml each time, successively in same platinum dish for final distillation and average blank figure. Distillation and total determination blanks can usually be combined by carrying the run (with the same quantities of reagents and similar evaporation and ashing treatment) through the entire procedure. Reagents and manipulations should increase the distillation blank but little.

Calculate the total quantity of F distilled from the quantity found in the aliquot titrated, subtract the proper blank, and refer the net figure to the weight of sample taken. If a double distillation procedure was used, make an appropriate blank correction.

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REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 452.

Apparatus

Claisen flask, 250 ml.

Nessler tubes, 6 long-form 50-ml tubes with double optically plane disks fused to tubes. Match tubes for length and test for optical similarity as follows: Add approximately 40 ml distilled water, 1 ml of the indicator, 2 ml of the HCl, and distilled water to the mark on tube. Then to one tube add such quantity of the $Th(NO_3)_4$ solution that, after making to the mark and mixing, the color is barely changed to faint pink. Note quantity of $Th(NO_3)_4$ solution used. Add the same quantity of $Th(NO_3)_4$ solution to each of 5 remaining tubes. Reject tubes showing detectable differences in shade or intensity.

See also method for fluorine in foods (p. 208).

Reagents

Sodium fluoride stock solution: dissolve 2.22 gm NaF (minimum purity 98 percent) in 1 liter distilled water. (1 ml=1 mg F.)

Sodium fluoride standard solution: dilute 10 ml stock solution to 1 liter. (1 ml=0.01 mg F.)

Thorium nitrate solution: dissolve 0.25 gm $Th(NO_3)_4 \cdot 12H_2O$ or 0.2 gm $Th(NO_3)_4 \cdot 4H_2O$ in 1 liter distilled water.

Alizarin red indicator: 0.01 percent aqueous solution Na alizarin sulfonate (alizarin red S).

Hydrochloric acid: exactly 0.05 N.

Sodium hydroxide solution: exactly 0.05 N.

Hydroxylamine hydrochloride solution: 1.0 gm per 100 ml.

Preparation of Sample

If sample has an odor of H_2S, oxidize with 0.1 ml 30 percent H_2O_2 solution before evaporation.

Place 100-ml sample in porcelain or platinum dish, make alkaline to phenolphthalein with 10 percent NaOH solution (avoid excess), and evaporate to 20 ml over burner at a temperature just below the boiling point. During evaporation keep the sample alkaline by adding small quantities of the NaOH solution from time to time. Transfer the 20-ml evaporated sample to a Claisen flask containing glass beads or boiling tube previously rinsed with boiling 10 percent NaOH solution to eliminate all traces of gelatinous SiO_2 accumulating in the flask.

Place flask containing sample on asbestos board (6″ x 6″ x ¼″ with 1″ center hole) over burner adjusted for medium flame. Close straight neck of flask with 2-hole rubber stopper through which pass thermometer and stem of small separator with outlet constricted to 2-mm diameter. (Adjust thermometer and outlet tube of separator to extend almost to bottom of flask.) Close other neck of flask with solid rubber stopper. (Alternatively, an all-glass distillation assembly may be used.)

Connect the flask with water condenser, add 20 ml 60 percent $HClO_4$ to flask via evaporating dish and separator; then add a quantity of saturated $AgClO_4$ solution that will precipitate chlorides (determined previously by titration with standard $AgNO_3$ solution), and distill at 132±3° C, adding distilled water dropwise through separator to maintain temperature during distillation. Collect nearly 200 ml distillate. Dilute to volume (200 ml) and mix well. To determine acidity use 40-ml distillate, add 1 ml of the indicator, mix thoroughly, and note milliliters of the 0.05 N NaOH required for neutralization.

Repeat preparation and distillation, using 100 ml distilled water in place of sample, to determine blank.

Determination

Prepare one standard, one color comparison tube, and one or more sample tubes as follows:

Color comparison tube: To 40 ml distilled water, add 2 ml of the 0.05 N HCl, 1 ml of the alizarin red indicator, 1 ml of the $NH_2OH \cdot HCl$ solution, and enough $Th(NO_3)_4$ solution to give a faint but definite pink end point. Compare all endpoint colors with this color.

Sample tube: To sample tube containing 40 ml distillate add 1 ml of the indicator, 1 ml of the $NH_2OH \cdot HCl$ solution, and such quantity of 0.05 N HCl that the total quantity of acid in the tube (acidity previously determined plus quantity of 0.05 N HCl added) equals 2 ml 0.05 N HCl. Dilute to volume and mix. If in the preliminary acidity determination it is found that the 40-ml distillate requires more than 2 ml of the NaOH solution for neutralization, do not add the HCl solution to the sample tube, but add to the standard tube the same quantity of acid as was found in the sample tube. If 40 ml of distillate requires more than 5 ml 0.05 N NaOH, repeat distillation under conditions favorable to low acidity. From 10 ml burette, graduated to 0.05 ml, add the $Th(NO_3)_4$ solution with frequent mixing until faint pink appears, comparable to comparison tube (see immediately above). Note the volume of $Th(NO_3)_4$ solution used.

Standard tube: To standard tube containing 40 ml of distilled water, add 1 ml of the indicator, 1 ml of the $NH_2OH \cdot HCl$ solution, and 2 ml or more of the 0.05 N HCl, as was required in sample tube immediately above. If the aliquot chosen for determination already contains 2.5 ml 0.05 N acid, add exactly the same quantity to the standard tube. Add exactly the same quantity of the $Th(NO_3)_4$ solution as was added to the sample tube. To the standard tube (now more highly colored than the sample tube) add the standard NaF solution from a 10 ml burette, mixing until the color matches that of the sample tube. Dilute contents of both standard and sample tubes to the same volume. Mix the solution in each tube and let all air bubbles escape before making color comparisons. Check the end point by adding 1-2 drops of

the NaF solution to the standard tube. A distinct color change should develop.

Calculation

Subtract ml NaF solution required by the blank from ml NaF solution required by the sample.

$$\frac{\text{ml NaF solution} \times \text{ml total distillate} \times 10}{\text{ml aliquot titrated} \times \text{weight sample taken}} = \text{F (ppm)}$$

Example: 100 ml sample, evaporated and distilled to 200 ml, of which 40 ml aliquot corresponds to 5 ml of the NaF solution, gives:

$$\frac{5 \times 200 \times 10}{40 \times 100} = 2.5 \text{ F (ppm)}$$

Vitamins

Vitamin A and Carotenes

REFERENCES

1. Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 652.
2. Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 645.
3. Parrish, D. B., and Smith, H. A., Report on vitamin A in mixed feed. J. Assn. Offic. Agr. Chemists *30*, 126–138, 1956.
4. Beadle, B. W., and Zscheile, F. P., Studies on the carotenoids. II. The isomerization of β-carotene and its relation to carotene analysis. J. Biol. Chem. *144*, 21–33, 1942, see esp. p. 26.

Apparatus

Photoelectric colorimeter: Evelyn or similar colorimeter or spectrophotometer with direct-reading deflection type galvanometer. Optical mechanism or filters to transmit light at 620 mμ. (Instrument which provides for linearity between absorbance and concentration is preferable but not essential.) Use matched absorption tubes. For carotene determination, use 440 mμ filter or wavelength setting.

Carr-Price reagent dispenser, 9 or 10 ml, delivering volume of reagent rapidly through 3–4 mm diameter opening. Use all-glass apparatus. Automatic pipet, hypodermic syringe, glass cylinder, or other types of apparatus may be used. Apparatus must be clean and moisture-free.

Chromatographic tubes, 18 x 200 mm (about 12 mm internal diameter), sealed to tube 5 x 100 mm.

Eluate receiver: Corning, special equipment, fraction collector No. 91200, or equivalent, or simple receiver consisting of 100 ml lipless graduated cylinder or 100 ml regular graduated cylinder with top cut off below lip and fitted with two-hole stopper. Pass stem of chromatographic tube through one hole and bent glass tube connected to source of vacuum through the other. Ordinary water aspirator may be used for vacuum.

Saponification apparatus: Any suitable standard taper joint, water-cooled refluxing apparatus with 300–500 ml flask. Use boiling water or steam for heat, or use special saponification and extraction apparatus (Corning, special apparatus XA-4108, total capacity about 500 ml).

Extraction apparatus: As above, or 500 ml separator, stopcock 4 mm, with device to push feed through stopcock, if needed. To make device, use two-hole

stopper; through one hole insert bent glass tube attached to rubber tube with glass mouthpiece. Through the other hole insert iron or stainless-steel wire with metal bead attached at about midpoint so that when wire is raised, bead will plug second hole and permit application of pressure; when wire is lowered, it will pass through opened stopcock bore (3).

Ultraviolet light, long wavelength, such as Mineralight model SL 5660 (Fisher Scientific Co., Cat. No. 11-984-1).

Evaporation assembly: To concentrate extracts after extraction, if needed. Use water aspirator and 50-65° C water bath (do not expose evaporating solution and dry residue to atmosphere). Suitable assembly consists of Corning flask 4100 or 4320 of suitable size, connecting tube 8820 (standard taper 19/38-24/40), and male standard taper 19/38 joint sealed to single arm of 2 mm bore, three-way stopcock. One arm of the stopcock is bent at 135° angle and attached to the source of vacuum through a trap; the other arm is sealed to a small reservoir made from a test tube for adding solvent to the dry residue as the vacuum is broken (3). For samples of high vitamin A potency, the aliquot may be evaporated directly in colorimetric tubes attached to the source of vacuum through the stopper and Y-tube. Optionally, evaporation may be under partial vacuum and nitrogen.

Reagents—Vitamin A

Adsorbent: use Woelm Alumina, nonalkaline (Alupharm Chemicals, Box 755, 322-28 Lafayette, New Orleans, La.). Add 5 percent distilled water by placing a measured quantity of distilled water in a small glass-stoppered bottle and distributing it over the walls, then add alumina and mix by shaking the bottle until no lumps are observed. Let stand and cool at least 2 hours before use. Store in a tightly closed bottle. (Woelm Alumina is heat-treated by the manufacturer. Do not expose original or prepared alumina to the air, since the moisture content must be controlled carefully.)

Chloroform, reagent grade: Purify if necessary by distillation, discarding first and last 10 percent.

Hexane: Skellysolve B or other good quality commercial hexane. Redistill from all-glass apparatus, using only 64°-68° C fraction. Solvent must be free from alcohols, esters, etc.

Alcohol: 95 percent. Aldehyde-free by Schiff's test.

Acetone in hexane solution, 4 percent and 15 percent. Dilute reagent-grade acetone with hexane.

Solution for removing antimony trichloride from tubes: wash tubes with HCl, or let stand in 10 percent Rochelle salt solution to which detergent is added. Wash thoroughly in hot detergent solution.

Potassium hydroxide solution: dissolve 50 gm reagent-grade KOH in distilled water and dilute to 100 ml with distilled water. Mix thoroughly.

Antimony trichloride (Carr-Price) reagent: to 200 gm $SbCl_3$ crystals add enough $CHCl_3$ to make 1 liter. Warm and shake to dissolve. Cool, and add 30 ml acetic anhydride. If solution is not clear, filter, centrifuge, or let settle and decant. Solution will keep in tightly stoppered brown bottle for several months. (Use fresh unopened bottle of reagent grade $SbCl_3$ crystals. Crystals should be translucent; no fluids or colored decomposition products should be present. Crystals stored too long or in previously opened bottle often fail to meet conditions. $SbCl_3$ contained in ¼- or ½-pound glass-stoppered bottles, with stopper sealed in, is recommended.)

Vitamin A reference solution: solution of crystalline vitamin A acetate in cottonseed oil, encapsulated in gelatin. Potency, 30 mg vitamin A per gram of oil or as stated at time of purchase. (30 mg vitamin A = 100,000 units.)

Carotene reference crystals: 10 percent α-90 percent β-carotene in sealed 100-mg or 200-mg vials, obtainable from General Biochemicals, 677 Laboratory Park, Chagrin Falls, Ohio. Crystals should dissolve in hexane without residue and have characteristic spectrophotometric curve. Determine concentration, using spectrophotometer. (See determination, p. 225.)

Reagents—Carotenes

Acetone: dry, alcohol-free.

Commercial hexane, boiling point 60°–70° C.

Adsorbent: activated magnesia (Micron brand, No. 2642; Westvaco Chlorine Products Co., Newark, Calif.) or SeaSorb 43.

Diatomaceous earth, Hyflo Super-Cel, Johns-Manville.

(Work in subdued light. Avoid high laboratory temperatures. Complete all steps of method as rapidly as consistent with careful following of directions.)

Preparation of Adsorption Column

Place small pledget of cotton at bottom of chromatographic tube and pack with adsorbent mixture added in several portions, tamping each lightly with a blunt rod, to a depth of 7 cm. Keep column under vacuum during packing. Add 0.5 cm layer of powdered anhydrous Na_2SO_4 on top of column, level, and pack lightly.

Column performance test. Check for recovery of vitamin A as follows: Saponify about 0.1 gm USP vitamin A reference solution plus 2 gm fresh cottonseed oil. Extract with hexane. Mix solution of about 50–100 μg carotene and 30 μg of the saponified vitamin A. Dilute to a volume of 15 ml. Wash column with 20 ml hexane and adjust elution rate to about 2 drops per second. Before top of column runs dry, add vitamin A-carotene mixture. Elute carotene with 4 percent acetone in hexane. (Approximately 20–30 ml will be required.) Check for vitamin A band by brief inspection with ultraviolet light. Band should be not more than 2 cm below top of alumina column.

Elute vitamin A with 15 percent acetone in hexane. (Approximately 30 ml should be enough.) Inspect last few milliliters of this eluate for vitamin A fluorescence, and if found, elute with a few more milliliters of solvent, until fluorescence no longer is observed in eluate. Evaporate a suitable aliquot of eluate to dryness under vacuum, add 1 ml $CHCl_3$ and determine vitamin A. Compare result with an aliquot of saponified vitamin A in hexane not chromatographed.

Preparation of sample. Store sample in tightly closed glass containers, at 0° C or below. Grind to pass No. 20 sieve immediately before analyses. Mix and sample carefully.

Determination

Determine a blank on all reagents, including cottonseed oil. Absorbance of this blank should be almost 0.

Preparation of standard vitamin A curve. Cut the tip from a capsule of Standard Reference Solution and express an appropriate

quantity of the oil into a small weighed beaker or watch glass. Weigh accurately. Transfer the oil to a volumetric flask and dilute to volume with reagent grade $CHCl_3$. Use the solution as soon as possible, but never after 8 hours. Work in subdued light or use low-actinic glassware. Make a series of dilutions of vitamin A solution (5 or more) with $CHCl_3$ so that 1-ml aliquots treated as for the sample give transmissions of 20–85 percent. Plot absorbance against μg of vitamin A. On most photometers the plot will be a straight line in this transmission range and a factor may be calculated for determining μg vitamin A. (See "Colorimetry," below.)

Preparation of standard carotene curve. Prepare a series of dilutions of α-β carotene in hexane. Plot absorbance against μg carotene. (See "Carotene, determination," p. 225.) Use curve or factor for determining carotene content.

Determination of correction factor for yellow pigment in vitamin A eluate. Cryptoxanthin and similar pigments may elute with vitamin A alcohol. Correct for this pigment if it is present in more than mere traces. A suitable correction may be obtained by saponification and extraction of a sample of yellow corn as is done for the sample. Chromatograph and save the 15 percent acetone in hexane fraction. Determine the concentration of yellow pigment in this fraction by comparison with carotene calibration. Evaporate the solvents and dissolve the residue in $CHCl_3$. Make a series of dilutions covering the range of concentrations of yellow pigment of the sample solutions in the 1 ml $CHCl_3$ from which the Carr-Price vitamin A color is read. Obtain a factor for correcting for reaction of this pigment in the Carr-Price determination of vitamin A by plotting the concentration of yellow pigment in the solution determined at 440 mμ against the concentration of vitamin A determined from the standard vitamin A curve.

Saponification and extraction. (1) For products containing less than 4,000 units per pound, weigh a 40-gm sample into a 500-ml boiling flask; (2) for products of 4,000–20,000 units per pound, use 20–40 gm samples; (3) for products containing more than 20,000 units per pound, use a 10-gm sample. When analyzing premixes or concentrates of low fat content, add 1 gm fresh cottonseed oil to the sample in the boiling flask.

Add a volume (ml) of alcohol three times the weight (gm) of the sample. Swirl until all particles are thoroughly wetted. Add a volume of KOH solution (see reagents, above) equal to the weight of the sample. Swirl again for thorough mixing. Reflux for 30 minutes at a rate of two drops per second. Swirl and shake flasks at least three times during digestion until all clumps are broken and particles are well dispersed. Repeat once prior to extraction.

Cool to room temperature under running water. Add a volume of distilled water two times the weight of the sample. Extract three times with hexane, the first time using a volume of hexane two to three times the weight of the sample, and about two-thirds as much for subsequent extractions. On high potency samples it may be necessary to use a larger volume of hexane or additional extractions. (If considerable quantities of carotenoid pigments are present, all are not removed in three extractions, but those remaining in the alcohol-alkali layer are largely polyhydroxy and oxidized carotenoids.)

Combine all hexane extracts in one separator. Pour 100 ml cool distilled water into separator and drain it when the layers separate, retaining any emulsion in the hexane layer. Repeat the washing with successive 100-ml portions of distilled water, with shaking, until the washing is colorless to phenolphthalein. If emulsions cause difficulty, use 10 percent alcohol-distilled water wash containing 0.1 percent HCl on the third washing. At least two distilled water washings should follow the alcohol-distilled water wash. Separate the final distilled water wash as completely as possible. Swirl the separator, let the solution stand 5 minutes, and drain any water collecting in the bottom. Pour the solution carefully from the top of the separator through a small pledget of cotton into an appropriate volumetric flask. Rinse the separator and cotton with small portions of hexane, and dilute to volume with hexane.

Chromatography. If possible, chromatograph an aliquot containing about 30 μg vitamin A, but preferably not less than 20, in 10-15 ml hexane extract. If necessary, concentrate a portion of the hexane extract under vacuum to obtain enough concentration of vitamin A. In no case should more than 25 ml of solution be chromatographed.

Pack the column as directed, wash with 20 ml of hexane, and add extract containing vitamin A just before the top of the column runs dry. Elute at a rate of 2 drops per second. Elute carotene and then vitamin A separately as above. Cryptoxanthin and similar pigments elute with vitamin A. Dilute carotene and also vitamin A eluate to volume for colorimetry. A volume of 50 ml of vitamin A eluate is convenient; a 10 ml aliquot may be evaporated for vitamin A determination.

Colorimetry. Determine concentration of carotene in carotene eluate. (See "Carotene, determination," p. 225.)

Transfer 10 ml vitamin A eluate to colorimeter tube and read yellow color as carotene. If this is more than a trace, especially on low-potency samples, determine the correction for yellow pigment as directed above.

Evaporate solvents under vacuum in hot (60-65° C) water bath. Dissolve the residue in 1 ml $CHCl_3$. With 620 mμ setting, adjust colorimeter to 100 percent transmission, using 1 ml $CHCl_3$ and a

measured volume (9 or 10 ml) of $SbCl_3$ reagent. Place tube containing 1 ml $CHCl_3$ solution in instrument and add $SbCl_3$ reagent rapidly. Take maximum reading of galvanometer (work rapidly; color begins to fade in 3-5 seconds). Examine the tube within a few seconds. The solution should be blue and without turbidity; the color should fade rapidly. Calculate the weight of vitamin A by reference to the standard vitamin A curve. Determine the recovery factor, R, for vitamin A in analysis by additions of known quantities of vitamin A to duplicate samples or blank feeds in each series of similar samples analyzed.

Calculate the vitamin A content of the sample from the formula:

Vitamin A (μg/lb) $= (C_u - C_k) \times 454/C_s \times R$, where C_u = uncorrected concentration of vitamin A in μg per milliliter of final dilution, C_k = correction for yellow pigment expressed as μg vitamin A per milliliter of final dilution, C_s = weight of sample in gm per milliliter of final dilution, and R = vitamin A recovery factor.

Carotenes

Chromatographic Method

Extraction. Dried plants: Grind sample to pass No. 40 sieve. Weigh accurately 2 gm sample (1 gm if carotene content is high, 4 gm if low) and place in flask of extractor (Goldfisch, Bailey-Walker, or American Society for Testing Materials is suitable if no thimble is used). Add 30 ml acetone-commercial hexane mixture (3+7) to flask and (1) reflux 1 hour at a rate of 1-3 drops per second, and cool to room temperature, or (2) stopper and place in the dark at room temperature overnight (at least 15 hours). Decant or filter extract into 100 ml volumetric flask, wash residue, and dilute solution to volume with hexane. (This solution now contains 9 percent acetone.)

Fresh plant materials: Cut material finely with scissors or knife, or grind in food chopper to assure representative sample. If analysis cannot be performed immediately, blanch in boiling distilled water 5-10 minutes and store in frozen condition. Place weighed sample, 2-5 gm, in high-speed blender; add 40 ml acetone, 60 ml hexane, and 0.1 gm $MgCO_3$, and blend 5 minutes. Filter with suction or let residue settle and decant into separator, wash residue with two 25-ml portions of acetone, then with 25 ml hexane, and combine extracts. Wash acetone from extract with five 100-ml portions of distilled water, transfer upper layer to 100 ml volumetric flask containing 9 ml of acetone, and dilute to volume with hexane. If desired, alcohol may be used instead of acetone for extraction. Use 80 ml of alcohol and 60 ml of hexane in blender; the other quantities are the same as for acetone.

Separation of Pigments

Prepare chromatographic column with 1+1 mixture of activated magnesia and diatomaceous earth. (A suitable chromatographic tube

can be made from a Pyrex test tube 22 mm in outside diameter and 175 mm long by sealing a smaller tube (about 10 mm outside diameter) to the bottom.) To prepare the column, place a small glass wool or cotton plug inside the tube, add loose adsorbent to 15 cm depth, attach the tube to the suction flask, and apply full vacuum of water pump. Use flat instrument (such as inverted cork mounted on rod) to press adsorbent gently and flatten surface (packed column should be about 10 cm deep). Place a 1-cm layer of anhydrous Na_2SO_4 above the adsorbent.

With vacuum continuously applied to the flask, pour the extract into the column. Use 50 ml acetone-hexane (1+9), or slightly more, if necessary, to develop chromatogram and wash visible carotenes through the adsorbent. Keep the top of the column covered with a layer of solvent during the entire operation (conveniently done by clamping an inverted volumetric flask full of solvent above the column with the neck 1-2 cm above the surface of the adsorbent).

Collect the entire eluate. (Carotenes pass rapidly through the column; bands of xanthophylls, carotene oxidation products, and chlorophylls should be present in the column when the operation is complete.) Transfer the eluate, which has been reduced in volume by loss of vapor through a water pump, to a 100-ml volumetric flask, and dilute to volume with acetone-hexane (1+9). Determine the carotene content of this solution photometrically.

Determination

Determine the absorbance of the solution as soon as possible with the spectrophotometer at 436 mμ or with some other instrument provided with a suitable filter system, such as a Klett photometer with a No. 44 filter, or an Evelyn photoelectric colorimeter with a 440 filter. Calibrate these instruments first with solutions of high purity β-carotene as shown by characteristic absorption curve (4). Prepare the calibration chart and convert the absorbance of solution to be determined to carotene concentration from the chart.

When determinations are made with a properly calibrated spectrophotometer at 436 mμ, calculate from the formula

$$C = \frac{\text{Absorbance} \times 454}{196 \times L \times W}$$

where C = concentration of carotene (mg/lb) in the original sample, L = cell length in cm, and W = gm sample per milliliter final dilution. Report results as mg β-carotene per pound. Multiply by 2.2 to give ppm or by 1,667 to give International Units (IU) per pound.

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 655.

NOTE.—For enriched flour or products in which bound thiamine or thiamine pyrophosphate is not significant, a rapid fluorometric method has been described, *ibid.*, p. 657.

(Methods not applicable in the presence of materials that adsorb thiamine or which contain extraneous materials which affect thiochrome fluorescence.)

Fluorometric Method

Reagents and apparatus.

Double-normal sodium acetate: dissolve 275 gm $NaOAc \cdot 3H_2O$ in enough distilled water to make 1 liter.

Bromcresol green pH indicator: dissolve 0.1 gm indicator by triturating in an agate mortar with 2.8 ml 0.05 N NaOH, and dilute to 200 ml with distilled water.

Thymol blue pH indicator: dissolve 0.1 gm indicator by triturating in an agate mortar with 4.3 ml 0.05 N NaOH, and dilute to 200 ml with distilled water.

Enzyme solution: prepare, on day on which it is to be used, 10 percent aqueous solution of enzyme preparation potent in diastatic and phosphorolytic activity. (Among enzymes available for this purpose are Polidase-S (Schwarz Laboratories, 230 Washington St., Mount Vernon, N.Y.); Mylase P (Wallerstein Laboratories, 125 Lake Ave., Staten Island, N.Y.); Clarase (Takamine Laboratories, Clifton, N.J.); and Takadiastase (Parke, Davis & Co., Joseph Campau Ave. at the River, Detroit, Mich.).)

Base-exchange silicate: purify artificially prepared silicate of base-exchange type, in the form of granular powder of "50-80 mesh" size, as follows: Place a convenient quantity (100-500 gm) base-exchange silicate in a suitable beaker, add enough hot 3 percent acetic acid to cover material, and boil 10-15 minutes, stirring continuously. Let mixture settle and decant the supernatant. Repeat washing three times, then wash similarly three times with hot KCl solution (1 part by weight KCl per 4 volumes of solution), and finally wash with boiling water (distilled water must be used) until the last washing gives no reaction for chlorine. Dry the material at about 100° C and store in a well-closed container. (Purified base-exchange silicate may be purchased as "Special Decalso for Thiochrome Determination" from Fisher Scientific Co., 717 Forbes St., Pittsburgh 19, Pa.)

Chromatographic tubes: use glass chromatographic tubes (approximately 275 mm overall length, with reservoir capacity of about 60 ml) consisting of three parts fused together with the following approximate internal diameter: reservoir at top, 95 mm long, 30 mm diameter, converging into adsorption tube, 145 mm long, 6 mm diameter, and at the lower end the tube is drawn into a capillary 35 mm long and of such diameter that when the tube is charged, the rate of flow will be not more than 1 ml per minute. Prepare the tubes for use as follows: Place over the upper end of the capillary, with the aid of a glass rod, a pledget of fine glass wool. Add to the adsorption tube a distilled water suspension of 1.0-2.0 gm purified base-exchange silicate, taking care to wash down all the silicate from the walls of the reservoir. To keep air out of the adsorption column, keep a layer of liquid above the surface of the silicate during the adsorption process. (Prevent the tube from draining by placing a rubber cap, filled with water to avoid inclusion of air, over the lower end of the capillary.)

Neutral potassium chloride solution: dissolve 250 gm KCl in distilled water to make 1 liter.

Acid potassium chloride solution: add 8.5 ml HCl to 1 liter of the neutral KCl solution.

Sodium hydroxide solution, 15 percent: dissolve 15 gm NaOH in distilled water to make 100 ml.

Potassium ferricyanide solution, 1 percent: dissolve 1 gm $K_3Fe(CN)_6$ in distilled water to make 100 ml. Prepare the solution on the day it is used.

Oxidizing reagent: mix 4.0 ml of the 1 percent $K_3Fe(CN)_6$ solution with the 15 percent NaOH solution to make 100 ml. Use solution within 4 hours.

Isobutyl alcohol, redistilled in all-glass apparatus. Use the redistilled product as anhydrous or water-saturated.

Quinine sulfate stock solution: use quinine sulfate solution to govern reproducibility of fluorometer. Prepare a stock solution of this reagent by dissolving 10 mg quinine sulfate in 0.1 N H_2SO_4 to make 1 liter. Store in light-resistant containers.

Quinine sulfate standard solution: dilute 1 volume of the quinine sulfate stock solution with 39 volumes 0.1 N H_2SO_4. (The solution fluoresces to about same degree as does the isobutyl alcohol extract of thiochrome obtained from 1 μg thiamine hydrochloride.) Store the solution in light-resistant containers.

Thiamine hydrochloride stock solution I: weigh accurately 50-60 mg USP Thiamine Hydrochloride Reference Standard that has been dried to constant weight over P_2O_5 in a desiccator. Since the reference standard is hygroscopic, take precautions to avoid absorption of moisture. Dissolve in 20 percent alcohol adjusted to pH 3.5-4.3 with HCl, and dilute to 500 ml with the acidified alcohol. Add enough additional acidified alcohol to make concentration exactly 100 μg per milliliter. Store at about 10° C in glass-stoppered, light-resistant bottle.

Thiamine hydrochloride stock solution II: dilute 100 ml thiamine hydrochloride stock solution I to 1 liter with 20 percent alcohol adjusted to pH 3.5-4.3 with HCl. Store at about 10° C in glass-stoppered, light-resistant bottle (1 ml=10 μg thiamine hydrochloride).

Thiamine hydrochloride standard solution: to 10 ml thiamine hydrochloride stock solution II, add about 50 ml approximately 0.1 N HCl, digest or autoclave as in "Extraction," paragraph (1) below, cool, and dilute to 100 ml with the 0.1 N acid (1 ml equivalent to 1 μg thiamine hydrochloride). Prepare a fresh solution for each assay.

NOTE.—Proceed to "enzyme hydrolysis" for materials containing thiamine pyrophosphate or to "purification" for materials containing free thiamine using a 20-ml aliquot of this solution. Designate the final 25-ml volume eluate (equivalent to 5 μg USP Thiamine Hydrochloride Reference Standard) so obtained as Standard Solution.

Extraction.

For materials that contain free thiamine (not applicable in the presence of thiamine pyrophosphate): Place a measured quantity of the sample in a flask of suitable size and prepare sample by method (1), (2), or (3), and proceed directly to "oxidation."

(1) For dry or semidry materials that contain no appreciable quantity of basic substances: Add a volume of 0.1 N HCl equal in milliliters to not less than 10 times the dry weight sample in grams. Comminute and evenly disperse the material in liquid if it is not readily soluble. If lumping occurs, agitate vigorously so that all particles come in contact with the liquid; then wash down

the sides of the flask with 0.1 N HCl. Digest 30 minutes at 95–100° C in a steam bath, or in boiling water, with frequent mixing, or autoclave the mixture 30 minutes at 121–123° C. Cool, and if lumping occurs, agitate mixture until particles are evenly dispersed. Dilute with 0.1 N HCl to a measured volume containing about 0.2–5.0 μg thiamine per milliliter.

(2) For dry or semidry materials that contain appreciable quantities of basic substances: Add dilute HCl to adjust the mixture to about pH 4.0. Add such a quantity of distilled water that the total volume of liquid is equal in milliliters to not less than 10 times the dry weight of the sample in grams. Add the equivalent of 1 ml 10 N HCl per 100 ml liquid and proceed as in (1), beginning with the second sentence.

(3) For liquid materials: Adjust the material to approximately pH 4.0 with dilute HCl, or, with vigorous agitation, NaOH solution, and proceed as in (2), beginning with the second sentence.

For materials that contain thiamine pyrophosphate: Proceed as in (1), followed by enzyme hydrolysis and purification, as described below.

Enzyme Hydrolysis

Take an aliquot containing approximately 10–25 μg thiamine, dilute to about 65 ml with 0.1 N HCl, and adjust pH to 4.0–4.5 with about 5 ml of the 2 N NaOAc, using bromcresol green indicator on a spot plate. Add 5 ml of the enzyme solution, mix, and incubate 3 hours at 45°–50° C. Cool, dilute to 100 ml with 0.1 N HCl, and filter through paper known not to adsorb thiamine (ash-free papers have been found satisfactory).

Purification

Pass through a prepared chromatographic tube an aliquot of the filtered solution containing about 5 μg of thiamine, and wash the column with three 5-ml portions of almost boiling distilled water, taking care to prevent the surface of the liquid from falling below the surface of the base-exchange silicate.

Elute thiamine from the base-exchange silicate by passing through the column five 4.0–4.5-ml portions of almost boiling acid–KCl solution, taking care to prevent the surface of the liquid from falling below the surface of the silicate until the final portion of the acid-KCl solution has been added. Collect the eluate in a 25-ml volumetric flask, cool, and dilute to volume with the acid-KCl solution. Designate this as Assay Solution.

Oxidation of Thiamine to Thiochrome

To each of four or more tubes (or reaction vessels) of approximately 40 ml capacity, add about 1.5 gm NaCl or KCl and 5 ml of the

standard solution. (Precision and accuracy of results depend upon uniform technic in conducting the following oxidation procedure. Protect the solution from light which destroys thiochrome. Use pipet that delivers 3 ml in 1-2 seconds for addition of the oxidizing reagent.) Place the tip of the pipet containing the oxidizing reagent in the neck of the tube and hold so that the stream of the solution does not hit the side of the tube. Swirl the tube gently to produce a rotary motion in the liquid and immediately add 3 ml of the oxidizing reagent. Remove the pipet and swirl tube again to insure adequate mixing. Immediately thereafter, add 13 ml isobutyl alcohol, stopper, and shake the tube vigorously at least 15 seconds. Treat one or more of the tubes similarly, and treat each of two or more of the remaining tubes (standard blanks) in the same manner, except to replace the oxidizing reagent with the 15 percent NaOH solution.

To each of four or more similar tubes, add 5 ml of the assay solution. Treat these tubes in same manner as directed for tubes containing the standard solution.

After isobutyl alcohol has been added to all the tubes, shake again about 2 minutes. (Tubes may be placed in a shaker box for this additional shaking.) Centrifuge the tubes at low speed until a clear supernatant extract can be obtained from each. Pipet or decant about 10 ml of the isobutyl alcohol extract (upper layer) from each tube into the cell for measurement of thiochrome fluorescence.

Thiochrome Fluorescence Measurement

(Thiamine content of the oxidized assay solution is determined by comparing the intensity of fluorescence of an extract of this solution with that from the oxidized standard solution. Intensity of fluorescence is proportional to quantity of thiamine present and may be measured with a suitable electronic fluorometer. An input filter of narrow transmittance range with a maximum of about 365 mμ and an output filter of narrow transmittance range with a maximum of about 435 mμ have been found satisfactory. Use quinine sulfate standard solution to govern reproducibility of the fluorometer.)

Measure fluorescence of the isobutyl alcohol extract from the oxidized assay solution and call this reading "A." Next measure fluorescence of the extract from the assay solution which has been treated with 3 ml of the 15 percent NaOH solution and call this reading (assay blank) "b." Then measure fluorescence of the extract from the oxidized standard solution and call this reading "S." Finally, measure fluorescence of the extract from the standard solution which has been treated with 3 ml of the 15 percent NaOH solution and call this reading (standard blank) "d."

Calculation

Calculate as follows:

$$\mu g \text{ thiamine hydrochloride in 5 ml assay solution} = (A-b)/(S-d)$$

Vitamin C—Dye Method

REFERENCE

Official Methods of Analysis of the A.O.A.C., 9th ed., 1960, p. 661.

This method measures reduced vitamin C.

NOTE.- Products containing ferrous iron, stannous tin, and cuprous copper give values in excess of their actual ascorbic acid content by this method. The following are simple tests to determine whether these reducing ions are present in such quantities as to invalidate the test: Add 2 drops 0.05 percent aqueous solution of methylene blue to 10 ml of a freshly prepared mixture (1+1) of juice and the metaphosphoric acid-acetic acid reagent and mix. Disappearance of methylene blue color in 5-10 seconds indicates the presence of interfering substances. Stannous tin does not give this test and may be tested for as follows: To another 10 ml sample solution to which 10 ml HCl (1+3) has been added, add 5 drops *0.05 percent aqueous solution of indigo carmine* and mix. Disappearance of color in 5-10 seconds indicates the presence of stannous tin or other interfering substances.

The apparatus, reagents, and procedure for this method are described in chapter VI, pages 121-124.

Vitamin C—Total

REFERENCE

Roe, J. H., and Kuether, C. A., The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid. J. Biol. Chem. *147*, 399-407, 1943.

Principle

Ascorbic acid is oxidized to dehydroascorbic acid in the presence of norite. The 2,4-dinitrophenylhydrazine derivative is treated with strong sulfuric acid producing a reddish colored product which is measured photometrically.

Apparatus

Coleman Jr. spectrophotometer, Model 6
Cuvettes, 19 x 150 mm
Centrifuge tubes, 50 ml capacity
A quantity of 50 ml erlenmeyer flasks
A boiling water bath
An electric centrifuge
An ice bath
Quantitative filter paper, Whatman No. 42
Syringe pipets, 1 and 12 ml
Pipet, transfer, 4 ml
Pipet, serological, 5 and 10 ml
Interval timer
Funnels, 65 mm

Reagents

2,4-dinitrophenylhydrazine reagent: dissolve 2 gm of the reagent in 100 ml of 9 N sulfuric acid (3 parts of water and 1 part of concentrated sulfuric acid). Allow to stand overnight and filter.

Acid-washed norite (charcoal): place 200 gm norite in a large flask. Add 1,000 ml of 10 percent HCl and heat to boiling. Filter with suction. Remove norite cake to a large beaker and add 1 liter distilled water. Stir thoroughly and filter. Repeat until washings give a negative or very faint test for ferric ion. (Test filtrate with 1

percent potassium ferrocyanide.) Dry norite cake in oven overnight at 110°–120° C.

Metaphosphoric acid, 6 percent solution: dissolve 60 gm HPO_3 and dilute to 1 liter with redistilled water. Store in the cold.

Metaphosphoric acid, 4 percent: dissolve 40 gm HPO_3 and dilute to 1 liter with redistilled water. Store in the cold.

Sulfuric acid, 85 percent solution: to 100 ml of distilled water, add 900 ml of concentrated sulfuric acid (specific gravity 1.84). Do this mixing very carefully in a sink.

Thiourea, 10 percent solution: dissolve 10 gm thiourea in 100 ml of 50 percent (by volume) aqueous ethyl alcohol. This reagent keeps satisfactorily for 2 months, but to check, see that it readily reduces $HgCl_2$ or $KMnO_4$.

Stock vitamin C standard (USP reference standard if available): dissolve exactly 100 mg L-ascorbic acid in 100 ml 4 percent HPO_3 solution (1 mg per milliliter).

Working standard: dilute 2 ml of stock standard to 100 ml with 4 percent HPO_3 solution, adding 1 ml of thiourea solution prior to diluting to volume (20 μg per milliliter).

Standard Curve

To 25 ml of working standard L-ascorbic acid (20 μg per milliliter) in a 50 ml erlenmeyer flask, add one-half teaspoon of norite. Shake for 1 minute and filter. Set up a standard curve using 0 to 3.0 ml of this filtrate at 0.5 ml intervals and dilute each to 4 ml with 4 percent HPO_3 solution (each 0.5 ml contains 10 μg). Continue as under "Development of color," below.

Procedure

Dilute 2 gm of food in a 100-ml volumetric flask to volume with 0.5 percent oxalic acid.

Mix in a blender for the minimum time necessary.

To 20 ml of the homogenate in a 50-ml centrifuge tube, add one-half teaspoon of norite, shake vigorously for 1 minute, and filter through Whatman No. 42 filter paper.

Development of Color

Add exactly 4 ml of the filtrate (food) to each of two 19 x 150 mm cuvettes.

To each, add 1 drop of 10 percent thiourea solution.

Reserve one tube as a blank.

To the other tube add 1 ml of 2,4-dinitrophenylhydrazine solution and mix.

Place both tubes in a boiling water bath for exactly 10 minutes.

At the end of this time, immediately place the tubes in a beaker containing crushed ice.

Now add slowly, *drop by drop*, during a 1–1½-minute period, 5 ml of 85 percent sulfuric acid to both tubes with vigorous mixing. Keep an abundant supply of crushed ice in the beaker.

To the blank tube, add 1 ml of the 2,4-dinitrophenylhydrazine solution.

Remove the tubes from the ice bath and allow to stand at room temperature for 10 minutes.

Set the instrument at 100 percent transmittance at 515 mμ with a tube of distilled water and read the unknown and blank.

Subtract the density of the blank from that of the unknown and calculate, using the standard curve. It is advisable to include a working standard in the range of values expected each time the method is run.

Determination of Dehydroascorbic Acid

Dehydroascorbic acid is measured by the above procedure with the omission of the norite.

Calculations

$$\text{mg vitamin C per 100 ml sample} = \frac{\mu\text{g in cuvette} \times \text{total dilution} \times 100}{1{,}000 \times \text{ml aliquot} \times \text{ml sample}}$$

Example

A reading of 0.110 optical density was equivalent to 10 μg of ascorbic acid in the cuvette. Four ml of sample were diluted to 16 ml, of which 4 ml were taken for analysis.

mg total ascorbic acid/100 ml sample

$$= 10 \times \frac{16}{4} \times \frac{1}{1{,}000} \times \frac{100}{4} = 1.0 \text{ mg/100 ml}$$

Total ascorbic acid = reduced ascorbic acid + dehydroascorbic acid.

NOTE.—The laboratories of the Wisconsin Alumni Research Foundation have found it advisable to make the following modification in the above procedure:

Extraction Solution

6 percent trichloroacetic acid: Baker iron-free acid is suitable. Otherwise remove the traces of copper found in most reagent grade trichloroacetic acid with dithizone.

Oxidizing Agent

Saturated bromine water (1–2 ml): remove excess bromine by aeration.

Incubation Time

3 hours at 37° C.

Coupling Agent

2 percent 2,4-dinitrophenylhydrazine in 9 N H_2SO_4 with 4 percent thiourea added before using.

Colorimetric Reading

515 to 540 mμ.

Range

0 to 2.5 mg per 100 ml.

IX

Suggested Interpretative Guides

Introduction

This chapter presents suggested guides for interpretation of biochemical, dietary, and clinical data, including height-weight standards. The primary purpose of these guides is to permit within the limits of knowledge and methods the realistic interpretation of data collected in nutrition surveys.

The general objectives of a nutrition survey are to determine dietary intake, assess the evidence concerning prevalence of clinically (physical and biochemical) measurable nutriture, and to identify means of improvement. This combined appraisal gives more meaningful information than any single type of study. Dietary data represent the situation at a given, limited time. Unusual intakes may be recorded which are not reflected in nutritional status. Seasonal foods and other variations in dietary intake may alter significantly the nutritional status of an individual. Physical signs evaluated together with biochemical findings reflect dietary habits of longer duration, and provide additional evidence concerning nutrition of the population. When these different types of information are at variance, an explanation should be sought. Where evidence indicates a nutritional problem, further study is warranted. This should include therapeutic studies, load tests or other functional measurements.

In interpreting the significance of physical findings, several factors should be kept in mind. Almost any population group, even a well-nourished one, may show a low prevalence of some of the physical signs. Even though the *average* intake of a nutrient by a group may be adequate, the distribution of intakes is usually such that some individuals consume significantly less than average amounts and may be deficient or borderline with respect to the nutrient. Few if any of these physical signs are "specific" or "diagnostic" of a particular mild nutrient deficiency. They may be produced by lack of more than one nutrient or they may be nonnutritional in origin. Physical stigmata of nutrient deficiencies come and go, often unpredictably, in mild deficiency states. In spite of these limitations, the prevalence and severity of physical signs *in population groups* have value in assessing the nutritional state of the group, especially when considered

collectively with dietary and biochemical data. Certain of the physical signs are looked for not because they have specific importance in predicting nutrient deficiency, but because they have some relationship to general health status, with possible secondary nutritional implications.

These suggested guides for interpretation which apply to a reference man, namely a physically active 25-year-old adult male 170 centimeters (67 inches) in height, weighing 65 kilograms (143 pounds), living in a temperate climate and consuming a varied diet (*1*), are best established. Guides for other groups are more tentative than those for the reference man.

In interpretation of the data, cognizance should be given to differences in sex, age, size, and activity and physiologic state. Groups of special concern are pregnant and lactating women, infants and preschool (1–5 years) children, and children 5–15 years of age.

Most of the substantiating data for these provisional guides have been obtained in Western countries, often in controlled experiments. The effects of other environments and the many possibilities of interrelations among nutrients are comparatively unexplored. In preparing the present revision of interpretative guides, the experiences gained from nutrition surveys conducted by the ICNND in 24 countries have been taken into account.

At present, no nutrition standards can be considered as definitive, but they should rather be taken as working guides to be modified as better evidence justifies.

The appraisal of nutrition survey data according to uniform guidelines allows comparisons to be made which may not be apparent otherwise (*1*). In the setting of these guides the categories are arbitrary, but reflect the best judgment which can be reached on the basis of data available at the time of writing. The limits set by the categories for corresponding assessments are not independent. For example, the correspondence between the blood level of a given nutrient and dietary intake may be well documented in one study, while evidence on the correspondence between occurrence of clinical signs and dietary or biochemical levels may be much less complete, or the reverse may pertain. In applying categorized levels such as these, one must recognize that they are guides which reflect the best interpretation of data by a group experienced in nutritional appraisal—they are working guides which will be modified as additional evidence justifies.

The use of the designations "deficient," "low," "acceptable," and "high" seems self-explanatory in most instances. These are expressions of nutritional levels, not of varying quantities of clinical deficiency states. In a few instances the terms may seem inappropriate, but for the sake of comparability and simplicity they are retained throughout for levels considered to correspond.

REFERENCE

1. Interdepartmental Committee on Nutrition for National Defense, Suggested guide for interpreting dietary and biochemical data. Pub. Health Reports 75, 687–698, 1960.

Biochemical Data

TABLE 1.—*Suggested guide to interpretation of blood data* [1]

	Deficient	Low	Acceptable	High
Total plasma protein: gm/100 ml [2]	<6.0	6.0–6.4	6.5–6.9	≥7.0
Serum albumin (electrophoretic method): gm/100 ml	<2.80	2.80–3.51	3.52–4.24	≥4.25
Serum globulin (percent of serum protein):				
Alpha			4–7	
Alpha₂			9–11	
Beta			11–15	
Gamma			12–16	
Hemoglobin, gm/100 ml:				
Men	<12.0	12.0–13.9	14.0–14.9	≥15.0
Women (nonpregnant, nonlactating; >13 years)	<10.0	10.0–10.9	11.0–14.4	≥14.5
Children (3–12 years)	<10.0	10.0–10.9	11.0–12.4	≥12.5
Hematocrit (PCV), percent				
Men	<36	36–41	42–44	≥45
Women (nonpregnant, nonlactating; >13 years)	<30	30–37	38–42	≥43
Children (3–12 years)	<30.0	30.0–33.9	34.0–36.9	≥37.0
Plasma ascorbic acid: mg/100 ml	<0.10	0.10–0.19	0.20–0.39	≥0.40
Plasma vitamin A: μg/100 ml	<10	10–19	20–49	≥50
Plasma carotene: μg/100 ml	(3)	20–39	40–99	≥100
Red cell riboflavin: μg/100 ml red blood cells	<10.0	10.0–14.9	15.0–19.9	≥20

[1] Except for the particulates in blood, serum levels of nutrients in children do not differ appreciably beyond infancy from those of adults. Similarly, with the exception of hemoglobin and hematocrit, the serum levels of blood constituents in women of child-bearing age are comparable to those of males. Data on pregnant women are presented separately by trimester in table 2.

[2] See notes on total plasma protein.

[3] See notes on carotene.

Total Plasma Proteins

REFERENCES

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The values suggested for plasma protein are based upon distributions of plasma protein found in healthy persons. Age and sex make relatively little difference in total plasma protein values; there are differences associated with race and with environmental factors. No effort is made to include all of these variants. Many factors other than the plasma protein level may be associated with nutritional edema. Since a protein-deficiency edema is seen only after severe depletion, close correlation between plasma protein levels and occurrence of edema should not be expected.

Nutrition surveys of some malnourished population groups have shown normal values of total plasma protein with low or acceptable albumin and high globulin. Thus total serum protein levels are a relatively insensitive criterion. Albumin concentrations are less insensitive measures. The levels specified in the suggested standards assume a normal A/G ratio. If protein malnutrition is suspected in a population group in which total plasma protein levels are found to be in the normal range, measurement of plasma albumin is indicated.

Values for pregnant women are presented in table 2.

TABLE 2.—*Suggested guide to interpretation of blood data in pregnant women* [1]

Constituent	Trimester	Deficient	Low	Acceptable	High
Total serum protein: gm/100 ml	1	<6.0	6.0-6.4	6.5-6.9	≥7.0
	2	<5.5	5.5-5.9	6.0-6.9	≥7.0
	3	<5.5	5.5-5.9	6.0-6.9	≥7.0
Serum albumin: gm/100 ml	1	<3.0	3.0-3.9	4.0-4.9	≥5.0
	2	<3.0	3.0-3.4	3.5-4.4	≥4.5
	3	<3.0	3.0-3.4	3.5-4.4	≥4.5
Serum globulin: gm/100 ml	1	<1.4	1.4-1.9	2.0-2.9	≥3.0
	2	<1.4	1.4-1.9	2.0-2.9	≥3.0
	3	<1.7	1.7-2.1	2.2-2.9	≥3.0
Hemoglobin: gm/100 ml	1	<10.0	10.0-10.9	11.0-14.4	≥14.5
	2	<9.5	9.5-10.4	10.5-12.9	≥13.0
	3	<9.0	9.0-10.4	10.5-12.9	≥13.0
Hematocrit (PCV): percent	1	<30	30-37	38-42	≥43
	2	<30	30-34	35-37	≥38
	3	<30	30-32	33-34	≥35
Mean corpuscular hemoglobin concentration percent	1, 2, 3	<28.0	28.0-30.4	30.5-34.9	≥35.0
Plasma ascorbic acid		Same as values given in table 1.			
Plasma vitamin A		Same as values given in table 1.			
Plasma carotene: μg/100 ml	1	(2)	20-39	40-99	≥100
	2	(2)	30-79	80-199	≥200
	3	(2)	40-79	80-249	≥250

[1] Based on observations in: The Vanderbilt cooperative study of maternal and infant nutrition IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565-598, 1953.
Values for pregnant women in the first trimester do not differ appreciably from those of nonpregnant women in the same age group.
[2] See notes on carotene.

Serum Albumin

REFERENCES

Brock, J. R., Dietary proteins in relation to man's health. Fed. Proc. *20*, 61-65, suppl. 7, 1961.

Darby, W. J., et al., The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565-598, 1963.

In the absence of abnormal protein losses or impaired liver function, low serum albumin levels are presumed to indicate dietary protein deficiency.

Provisional guides for low serum albumin levels have been adapted from results of studies by Brock in protein-deficient children. In these studies clinical evidence of protein depletion was noted at albumin levels corresponding rather closely to the lowest levels of serum albumin found in cured cases of kwashiorkor and the highest levels found in acute kwashiorkor.

Values found in studies on pregnancy may be seen in table 2.

Serum Protein Fractions (Electrophoretic Studies)

REFERENCES

Sterling, K., and Ricketts, W. E., Electrophoretic studies of the serum proteins in biliary cirrhosis. J. Clin. Invest. *28*, 1469-1476, 1949.

Keys, A., et al., The Biology of Human Starvation. Minneapolis, University of Minnesota Press, 1950.

Armstrong, S. H., Budka, M. J. E., and Morrison, K. C., Preparation and properties of serum and plasma proteins. XI. Quantitative interpretation of electrophoretic schlieren diagrams of normal human plasma proteins. J. Am. Chem. Soc. *69*, 416-429, 1947.

Dhopeshwarkar, G. A., et al., The effect of vegetarianism and antibiotics upon proteins and vitamin B_{12} in the blood. Brit. J. Nutrition *10*, 105-110, 1956.

Putnam, F. W., ed., The Plasma Proteins, Vol. II. New York, Academic Press, 1960.

Bakker, A. W., Blick, A., and Luyken, R., The serum proteins of malaria-free inhabitants of Central Netherlands, New Guinea. Documenta de Medicina Geographica et Tropica *9*, 1-8, 1957.

Schofield, F. D., The serum protein pattern of West Africans in Britain. Tr. Roy. Soc. Trop. Med. Hyg. *51*, 332-337, 1957.

Concentrations of the serum protein fractions remain fairly constant in the well-fed, healthy adult. However, interpretation of serum electrophoresis data requires consideration of the interactions of many factors. Dietary customs also may influence the pattern of serum proteins. Dhopeshwarkar et al. have suggested that vegetable proteins tend to increase the gamma-globulin fraction. Certainly infections and immune responses alter the picture. Concentrations of the various components may reflect the overall physiologic state of an individual rather than a specific disease or deficiency syndrome. Some quantitative changes which may occur in association with clinical conditions are presented below. Putnam should be consulted for more complete information.

Albumins increase: acute starvation. *Albumins decrease:* hereditary analbuminemia, low protein diet, liver disease, burns, hemorrhage, nephritis and nephrosis, chronic infection and parasitism. *Alpha-globulins increase:* trauma, infection, fever, rheumatic arthritis. *Alpha-globulins decrease:* cirrhosis, viral hepatitis, myelomas, hy-

pothyroidism. *Beta-globulins increase:* nephrosis, viral or toxic hepatitis, obstructive jaundice, cirrhosis, malaria, multiple sclerosis. *Beta-globulins decrease:* rarely seen. *Gamma-globulins increase:* portal cirrhosis, viral and toxic hepatitis, starvation, kwashiorkor, all types of infection and parasitism. *Gamma-globulins decrease:* congenital or acquired agammaglobulinemia, pregnancy and nephrosis.

The values presented in table 1 are normal levels for healthy Americans and may not be found in other populations. An approximation of values to be expected in an area may be obtained from the reports of ICNND surveys for nearby countries or in Bakker et al., which tabulates studies on blood proteins of people throughout the world. Africans, Asians, and many other groups usually have higher gamma-globulin levels than Western populations. These elevated gamma-globulin concentrations may persist for some time after a change in environmental conditions. Thus, Schofield noted that as long as 6 years was necessary before African men who moved from Nigeria to England approached European values.

Hemoglobin and Hematocrit

REFERENCES

Sinclair, H. M., The assessment of human nutriture. Vitamins and Hormones *VI*, 101–162, see esp. p. 154, 1948.

Wintrobe, M. M., Clinical Hematology. 5th ed., Philadelphia, Lea & Febiger, 1961.

Milam, D. F., and Muench, H., Hemoglobin levels in specific race, age and sex groups of a normal North Carolina population. J. Lab. Clin. Med. *31*, 878–885, 1946.

Cannan, R. K., Proposal for distribution of a hemoglobin standard. Science *122*, 59–60, 1955.

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The proposed guides for hematologic findings are derived entirely from studies with Occidental races. Impaired nutriture should be suspected if more than 3 percent of an adult male population has hemoglobin levels of 12 gm per 100 ml or less at sea level. Low hemoglobin concentrations alone should not be the criterion for diagnosis of iron deficiency without further characterization of the anemia. Much iron deficiency in tropical regions is induced by blood loss associated with intestinal parasitism, and the possibility of conditioned deficiency must always be considered. Other factors such as race, climate, and customary altitude may also play a role.

According to Wintrobe, the average hemoglobin level for normal young men should be 16.0 gm per 100 ml (range 14–18) with a corresponding hematocrit of 47 percent (range 40-54). However, in most surveys the average hemoglobin levels of adult men have been found to be lower.

Adult women normally have hemoglobin levels lower than those of men. Children under 14 years of age have hemoglobin levels below those of men. Variation with age is most marked under 3 years. In this age group boys and girls are the same. (See fig. 1.) Hemoglobin concentrations in pregnant women normally show variations which change with the different phases of pregnancy. (See table 2).

Plasma or Serum Ascorbic Acid

REFERENCES

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Meiklejohn, A. P., The physiology and biochemistry of ascorbic acid. Vitamins and Hormones *XI*, 61–96, 1953.

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Ralli, E. P., and Sherry, S., Adult scurvy and the metabolism of vitamin C. Medicine *20*, 251–340, 1941.

Johnson, R. E., et al., Effects of variations in dietary vitamin C on the physical well being of manual workers. J. Nutrition *29*, 155–165, 1945.

A plasma or serum ascorbic acid level of less than 0.1 mg per 100 ml of serum is consistent with but not diagnostic of scurvy. In clinical scurvy there is usually no measurable serum ascorbic acid. No clear evidence of malnutrition or ill health due to lack of ascorbic acid has been obtained with serum levels above 0.2 mg per 100 ml. Serum concentration stabilizes at different levels of intake. Since the amount of ascorbic acid in different samples of the same food may vary considerably and variable losses occur during food storage and preparation, serum levels are considered better indicators of ascorbic acid status than dietary data.

Surgery, trauma, illness, parturition, and other forms of stress may reduce the serum ascorbic acid concentration. This effect may be due

VARIATIONS IN HEMOGLOBIN THROUGHOUT LIFE
CONSTRUCTED FROM DATA OF MRC SPECIAL REPORT NO. 252

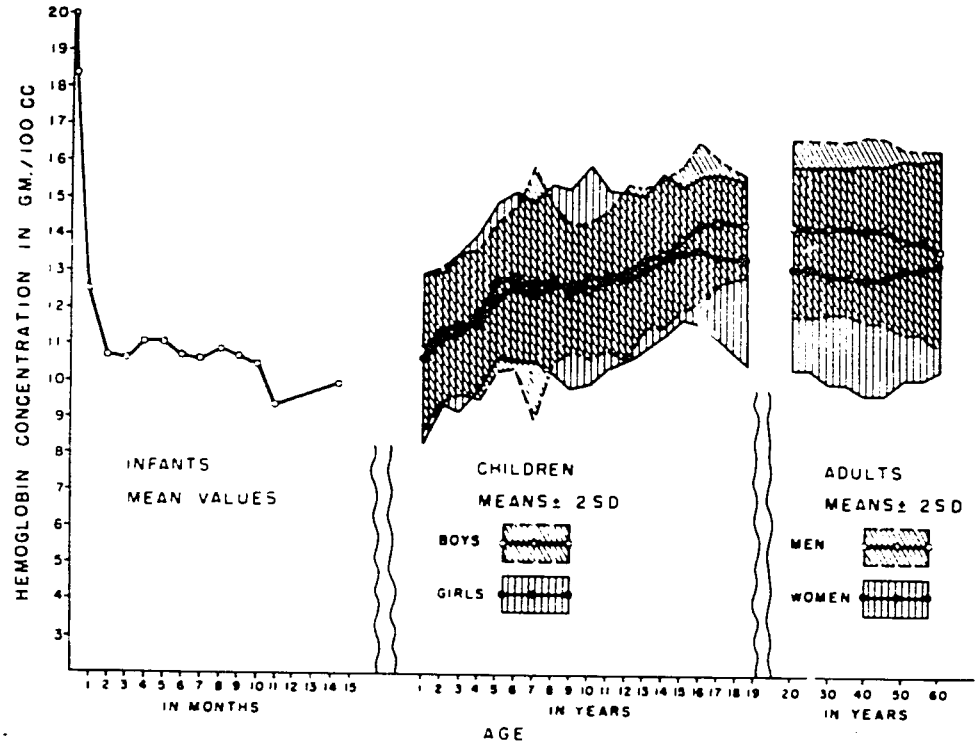

FIGURE 1.—From Darby, W. J., Iron and Copper. Chapter V *in* Handbook of Nutrition, 2d ed., published for the American Medical Association by Blakiston Co., New York, 1951, pp. 89–110.

in part to a concomitant reduction in intake, but may be largely metabolic.

The levels presented in table 1 apply to both sexes and all age groups. No major physiologic changes in serum ascorbic acid levels take place as a result of pregnancy *per se*.

Plasma or Serum Vitamin A

REFERENCE

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Aykroyd, W. R., et al., Medical resurvey of nutrition in Newfoundland, 1948. Canad. Med Assn. J. *60*, 329–352, 1949.

Williams, H. H., et al., Nutritional status survey, Groton Township, New York. VI. Chemical findings. J. Am. Dietet. Assn. *27*, 215–221, 1951.

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Kagan, B. M., Thomas, E. M., Jordan, D. A., and Abt, A. F., Serum vitamin A and total plasma lipid concentrations as influenced by the oral administration of vitamin A to children with the nephrotic syndrome. J. Clin. Invest. *29*, 141–145, 1950.

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Serum vitamin A concentrations in well-nourished individuals usually range from 30 to 50 μg per 100 ml, with a few values between 20 and 30 μg. In controlled feeding studies in adults the serum concentration on a vitamin A-free diet gradually decreased over many months to about 3 to 12 μg per 100 ml. Although evidence of night blindness has usually been reported to appear at serum vitamin A concentrations between 15 and 20 μg, several workers have failed to find

clinical deficiency signs until the serum levels were about 2–3 μg per 100 ml. In dark adaptation studies of individuals with low serum vitamin A levels (2–30 μg per 100 ml), 96.7 percent were found to have normal vision.

It is generally agreed that 15 μg per 100 ml represents no serious depletion. In population studies it is suggested that 5 percent or more of the sample with values below 10 μg or 15 percent below 20 μg per 100 ml constitutes evidence of vitamin A deficiency within the population.

Considerable amounts of vitamin A may be stored and it requires a long period for depletion. Seasonal fluctuations in the supply of foods containing vitamin A often occur. Correlations of serum vitamin A levels and dietary intakes are often poor.

Apart from the association with nutrient intake, a variety of conditions or diseases may result in reduced or elevated serum vitamin A values. For an optimum absorption of vitamin A to take place, conditions favorable to fat absorption are required. Chronic diarrhea, celiac disease, sprue, or biliary or pancreatic dysfunction may result in reduced absorption of carotene, vitamin A and other fat-soluble factors. Also, vitamin A levels are frequently significantly decreased in infections and in hepatitis and cirrhosis. Conversely, vitamin A levels and carotene levels may be elevated in nephrosis. Carotene levels may be depressed in hyperthyroidism and elevated in hypothyroidism.

Levels indicative of depletion in pregnancy do not differ from those found in the rest of the population, although there is a tendency for carotene values to rise and for vitamin A levels in the serum to be depressed somewhat during the later part of pregnancy.

Plasma or Serum Carotene

References

Sinclair, H. M., The assessment of human nutriture. Vitamins and Hormones *VI*, 101–162, see esp. p. 154, 1948.

Hume, E. M., and Krebs, H. A., Vitamin A requirement of human adults. An experimental study of vitamin A deprivation in man. Medical Research Council, Great Britain, Special Report Series No. 264, London, 1949.

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Stockell, A. K., et al., A study of the dietary background and nutriture of the Navajo Indian. IV. Biochemical findings. J. Nutrition *60*, suppl. 2, 63–73, 1956.

Darby, W. J., et al., The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565–598, 1953.

The serum concentration of carotene usually reflects recent dietary intake. Beta-carotene as such is not stored for long periods in large amounts as is evidenced by the decrease of serum levels within eight weeks on a carotene-free diet. Serum levels of this nutrient may not necessarily correlate with the over-all vitamin A status. Persons consuming adequate intakes of preformed vitamin A may have little or no carotene in their serum, but this is not the usual situation. Most populations derive the major fraction of their vitamin A activity from vegetables, hence carotene is the main source of vitamin A activity. In such groups the finding of low carotene levels is informative as to the type of diet consumed (low in green and yellow vegetables and fruits).

Serum carotene in well-nourished men varies widely. Gillum and coworkers found values in older men ranging from 25 to 405 μg per 100 ml of serum with a mean of 111–130. The guides suggested must be regarded as a compromise, being considerably lower than levels recommended by Sinclair, but fitting fairly well the data of Williams et al. and Clayton et al.

Serum carotene levels show an elevation in the second and third trimesters of pregnancy. (See table 2.)

Cholesterol

REFERENCE

Am. J. Cardiol., vol. 10, no. 3, September 1962.

The measurement of cholesterol in nutrition surveys has two purposes. The information may be of some use in interpreting the prevalence and extent of certain metabolic disorders, especially thyroid, liver disease, and semistarvation, and it may contribute to the research effort to understand the relation of sterol metabolism to arteriosclerotic cardiovascular disease.

It is not possible to state a single set of criteria for the interpretation of cholesterol levels in blood serum. The levels observed in apparently well people vary widely from place to place and according to cultural characteristics. In general, mean levels under 200 mg per 100 ml of serum are found in underdeveloped societies and mean levels of about 230 mg percent are found in technically advanced societies. It is likely that way of living, amount of exercise, and dietary habits, rather than place, are important in determining serum cholesterol content. It is commonly observed that those living in urban areas show higher levels than rural dwellers.

There are remarkable sex and age effects in technically advanced societies. Men show higher levels than women until about the fifth decade of life. Thereafter both sexes are equal or the women are slightly higher. The increase with age is pronounced, but occurs later in women than in men. In technically advanced populations a notable

hypercholesteremia occurs in the third trimester of pregnancy. In underdeveloped areas there is often no age increment and the levels in women are often slightly higher than in men.

The levels of cholesterol are regularly diminished in thyrotoxicosis and increased in myxedema or hypothyroidism. The serum level may be greatly increased with biliary obstruction. The levels are generally decreased with inanition and with the physiologic stress of infection or trauma.

There is a need to collect additional comparable data on cholesterol levels and associated attributes, including diet, from widely varying places and people. It would be especially desirable to know more about the prevalence of heritable hypercholesteremia in different societies. Instances of hypercholesteremia should be followed up by investigations of the cholesterol levels in members of the family of such subjects.

The technical requirements for measuring the level of cholesterol are not demanding. Blood may be drawn without regard to feeding or time of day and the separated serum or plasma may be transferred to a filter paper—in measured amount—dried and stored for transport to the laboratory. A single, carefully measured observation is likely to be a good characterization of an individual, especially if his level is below 230 mg percent. Persons with higher levels show larger unexplained variability.

TABLE 3.—*Suggested guide to interpretation of urinary vitamin excretion data*

	Deficient	Low	Acceptable	High
	ADULTS (MALES AND NONPREGNANT, NONLACTATING FEMALES)[1]			
Thiamine:				
μg/6 hours	<10	10-24	25-49	≥50
μg/gm creatinine	<27	27-65	66-129	≥130
Riboflavin:				
μg/6 hours	<10	10-29	30-99	≥100
μg/gm creatinine	<27	27-79	80-269	≥270
N'-Methylnicotinamide:				
mg/6 hours	<0.2	0.2-0.59	0.6-1.59	≥1.6
mg/gm creatinine	<0.5	0.5-1.59	1.6-4.29	≥4.3
	PROVISIONAL GUIDE FOR URINARY EXCRETIONS IN CHILDREN[2]			
Thiamine: μg/gm creatinine:				
Age (years):				
1-3	<120	120-175	176-600	>600
4-6	<85	85-120	121-400	>400
7-9	<70	70-180	181-350	>350
10-12	<60	60-180	181-300	>300
13-15	<50	50-150	151-250	>250
Riboflavin: μg/gm creatinine:				
Age (years):				
1-3	<150	150-499	500-900	>900
4-6	<100	100-299	300-600	>600
7-9	<85	85-269	270-500	>500
10-15	<70	70-199	200-400	>400

[1] The urinary values indicated above for adults are based on an expected creatinine excretion of 1.5 gm daily for a reference man weighing 65 kg, for creatinine coefficient of 23. Values for pregnant women are presented in table 4.

[2] The guides offered here for children are based on considerably less extensive data than are the guides for adults.

Thiamine

Adults

References

Unglaub, W. G., and Goldsmith, G. A., Evaluation of vitamin adequacy: Urinary excretion tests. *In* Methods for Evaluation of Nutritional Adequacy and Status—A Symposium. Advisory Board on Quartermaster Research and Development, Committee on Foods. Washington, D.C., National Academy of Sciences-National Research Council, 1954, pp. 69-81, see esp. pp. 69-71.

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Elsom, K. O., et al., Studies of the B-vitamins in the human subject. V. The normal requirement for thiamine; some factors influencing its utilization and excretion. Am. J. Med. Sci. *203*, 569-577, 1942.

Plough, I. C., and Bridgforth, E. B., Relations of clinical and dietary findings in nutrition surveys. Pub. Health Reports *75*, 699-706, 1960.

Darby, W. J., et al., The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565-598, 1953.

Thiamine excretion appears to be linearly related to intake except at very low levels. The relationship to clinical symptoms is less clear. Thiamine excretion may also be characteristic of the individual. In controlled experiments excretions of 5 to 20 μg or less per day have been associated with daily intakes of 0.2 mg per 1,000 Calories and an excretion of 40 μg per day with intakes of 0.35 mg per 1,000 Calories. On the basis of these data the guides presented for urinary thiamine excretion seem somewhat generous in relation to the proposed dietary guides for thiamine intake, but are supported by results from surveys. Excretions above 100 μg per day are considered incompatible with deficiency.

Thiamine excretions are lowered during pregnancy. (See table 4.)

TABLE 4.—*Suggested guide to interpretation of urinary vitamin excretion in pregnant women*[1]

Constituent	Trimester	Deficient	Low	Acceptable	High
Thiamine: μg/gm creatinine	1	<27	27-65	66-129	>130
	2	<23	23-54	55-109	>110
	3	<21	21-49	50-99	>100
Riboflavin: μg/gm creatinine	1	<27	27-79	80-269	>270
	2	<30	30-119	120-399	>400
	3	<30	30-89	90-299	>300
N'-Methylnicotinamide: mg/gm creatinine	1	<0.5	0.5-1.59	1.6-4.29	>4.3
	2	<0.6	0.6-1.99	2.0-4.99	>5.0
	3	<0.8	0.8-2.49	2.5-6.49	>6.5

[1] Based on observations in: The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565-598, 1953.
Values for pregnant women in the first trimester do not differ appreciably from those of nonpregnant women in the same age group.

Children

REFERENCES

Stearns, G., et al., Excretion of thiamine and riboflavin by children. Am. J. Diseases Children *95*, 185-201, 1958.

Stearns, G., et al., The protein requirements of children from one to ten years of age. Ann. N.Y. Acad. Sci. *69*, 857-880, 1958.

The urinary interpretative guides for thiamine excretion in adults cannot be applied to children because the latter excrete lower levels of creatinine. There are few data regarding thiamine excretion in children and no interpretative guides have hitherto been proposed. Except for the work of Stearns et al., no systematic study seems to have been made of thiamine excretions in children by age groups. These workers concluded that children excreted approximately 10 percent of their daily intake when the latter was 0.5 mg of thiamine or less. Above these intake levels urinary thiamine excretion varied considerably although the mean approximated 20 percent. A series of predicted excretions has been calculated for children at different ages and different levels of intake from these results and Stearns' previously published data on creatinine excretions of children. These data are presented in table 3. The deficient, low, acceptable, and high values were calculated from dietary intake standards (see table 5 on p. 250) with the assumption that excretion would be 10 percent of intakes of 0.2 mg per 1,000 Calories or less and 20 percent at intakes greater than this.

This proposed interpretative guide is based on limited data, but it is hoped that it will serve as a useful stimulus for the development of a more appropriate standard.

Riboflavin

Adults

REFERENCES

Horwitt, M. K., et al., Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. J. Nutrition *41*, 247-264, 1950.

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Extensive studies have been made of excretion of riboflavin in the urine at different intake levels. Excretions in the range of 50 μg a day have been associated with clinical symptoms of deficiency (Horwitt et al.). Up to an intake of about 1.1 mg daily, excretion is 10 percent of the intake. Above these intake levels excretion rises rapidly, and may be as much as 30 percent of the amount ingested. Excretions between 50 and 120 μg per day (10 to 30 μg per 6 hours) thus appear to represent unsaturation. Negative nitrogen balance, physical activity, and other factors, such as disease states and administration of certain antibiotics, may be associated with variations in excretion. Level of excretion varies relatively greatly from individual to individual and from time to time in the same individual. The applicability of the values cited above to conditions of diet and environment other than those under which they have been obtained needs to be determined (Unglaub and Goldsmith; Lowry).

It should be noted that these levels of excretion were derived from a study using a method with high specificity for measurement of riboflavin, and cannot be applied to data obtained by other less specific procedures. These guides are not applicable to children. (See below.)

Riboflavin levels rise during the second trimester of pregnancy and drop again in the third trimester. (See table 4.)

Children

REFERENCES

Stearns, G., et al., Excretion of thiamine and riboflavin by children. Am. J. Diseases Children *95*, 185-201, 1958.

Stearns, G., et al., The protein requirements of children from one to ten years of age. Ann. N.Y. Acad. Sci. *69*, 857-880, 1958.

Holman, W. I. M., Biochemical investigations into the B-vitamin metabolism of children having the experimental diets. *In* Studies on the Nutritive Value of Bread and on the Effect of Variations in the Extraction Rate of Flour on the Growth of Undernourished Children, Widdowson, E. M., and McCance, R. A., ed. Medical Research Council, Great Britain, Special Report Series No. 287, London, 1954, pp. 92-118.

Oldham, H. et al., A study of the riboflavin and thiamine requirements of children of preschool age. J. Nutrition *27*, 435-446, 1944.

Snyderman, S. E., et al., The minimum riboflavin requirement of the infant. J. Nutrition *39*, 219-232, 1949.

Pearson, W. N., Biochemical appraisal of nutritional status in man. Am. J. Clin. Nutrition *11*, 462-476, 1962.

Reference guides for riboflavin excretions for adults are not applicable to children because the latter excrete less creatinine and consume more nutrients per kilogram of body weight than do adults (Stearns et al.). Thus they tend to excrete considerably more nutrient on a creatinine basis than adults. Children 11–15 years of age excrete riboflavin at levels compatible with saturation on intakes of 0.3–0.4 mg per 1,000 Calories (Holman). In two 5-year-olds saturation excretions were not seen on intakes of 0.25–0.35 mg per 1,000 Calories (Oldham). From these few data it would appear that a minimum requirement is 0.25 mg per 1,000 Calories per day. (See below, under "Dietary Guide.") By using such data in the literature pertaining to riboflavin intake and excretion, and to creatinine excretion in children, it is possible to predict within limits the riboflavin excretions of children of various ages on different riboflavin intakes. Such a tentative guide is presented in table 3. Although these data require further validation, it is not likely they are in gross error.

N'-Methylnicotinamide

REFERENCES

Goldsmith, G. A., et al., Studies on niacin requirement in man. II. Requirement on wheat and corn diets low in tryptophan. J. Nutrition *56*, 371-386, 1955.

Unglaub, W. G., and Goldsmith, G. A., Evaluation of vitamin adequacy: Urinary excretion tests. *In* Methods for Evaluation of Nutritional Adequacy and Status—A Symposium. Advisory Board on Quartermaster Research and Development, Committee on Foods. Washington, D.C., National Academy of Sciences-National Research Council, 1954, pp. 69-81, see esp. pp. 73-75.

Horwitt, M. K., et al., Tryptophan-niacin relationships in man; studies with diets deficient in riboflavin and niacin, together with observations on the excretion of nitrogen and niacin metabolites. J. Nutrition *60*, suppl. 1, 1-43, 1956.

Frazier, E. I., Prather, M. E., and Hoene, E., Nicotinic acid metabolism in humans. I. The urinary excretion of nicotinic acid and its metabolic derivatives on four levels of dietary intake. J. Nutrition *56*, 501-516, 1955.

Wachstein, M., and Lobel, S., Abnormal tryptophan metabolites in human pregnancy and their relation to deranged vitamin B_6 metabolism. Proc. Soc. Exp. Biol. Med. *86*, 624-628, 1954.

Pearson, W. N., Biochemical appraisal of nutritional status in man. Am. J. Clin. Nutrition *11*, 462-476, 1962.

Darby, W. J., et al., The Vanderbilt cooperative study of maternal and infant nutrition. IV. Dietary, laboratory and physical findings in 2,129 delivered pregnancies. J. Nutrition *51*, 565-598, 1953.

The deficiency range of excretion of 0.2 mg per 6 hours or 0.5 mg per gram of creatinine has been derived principally as the result of the work of Goldsmith and coworkers. These levels were found in subjects on daily intakes of about 5 mg of niacin and 200 of tryptophan when they began to show clinical evidence of pellagra. Similar excretion levels have been noted in subjects on slightly higher trypto-

phan intakes (250–300 mg) who had not developed clinical symptoms (Horwitt et al.).

With daily niacin intakes above 8 to 10 mg per day, the excretion of niacin metabolites increases rapidly. Excretions in the acceptable range are associated with niacin intakes of 12 to 16 mg per day. Early in pregnancy there is a detectable increase in the urinary excretion of N′-methylnicotinamide.

Occasionally a normal individual may fail to excrete appreciable amounts of N′-methylnicotinamide in the postabsorptive state. Elevated excretions are found in prolonged fasting, muscle wasting, and pregnancy, particularly in the last trimester. (See table 4.) The latter is believed to be related to an alteration in pyridoxine metabolism. The well-known interplay of tryptophan and niacin metabolism as well as many other nonnutritional interrelations of niacin with other compounds produces such a multiplicity of end products that excretions of N′-methylnicotinamide are not very satisfactory for assessing niacin nutriture, but at present it is the most practical index for studying population groups.

The principal urinary metabolites of niacin, N′-methylnicotinamide and the 6-pyridone of N′-methylnicotinamide, are usually excreted in approximately equal amounts, although there may be slightly more 6-pyridone. Since the assay procedure for 6-pyridone is considerably more cumbersome than that for N′-methylnicotinamide, only the latter method is recommended for large-scale survey studies.

No data are available on the excretion of niacin metabolites in small children. Observations to date indicate that children do not differ so markedly from adults in their excretions of N′-methylnicotinamide as is the case with thiamine and riboflavin.

Iodine

REFERENCES

Follis, R. H., Jr., Vanprapa, K., and Damrongsakdi, D., Studies on iodine nutrition in Thailand. J. Nutrition *76*, 159–173, 1962.

Stanbury, J. B., Iodine metabolism and physiological aspects of endemic goitre. Bull. World Health Org. *18*, 201–215, 1958.

Iodine excretions less than 50 μg per gm of creatinine are believed to be an indication of a deficiency of this nutrient.

Dietary Data

These guides apply to 25-year-old physically active males. The quantities specified should never be considered inflexible "requirements." Suggested practical allowances or guides for other segments of the population that may be useful in assessing adequacy of nutrient intakes in a heterogeneous population have been given in Chapter VII, "Dietary Surveys." In nutrition surveys of population groups, average values falling in one or another of the above categories con-

TABLE 5.—*Suggested guide to interpretation of nutrient intake data for reference man*

	Deficient	Low	Acceptable	High
Calories		(See discussion below)		
Protein, gm/kg body weight	<0.5	0.5–0.9	1.0–1.4	≥1.5
Calcium, gm/day	<.3	0.30–0.39	0.4–0.7	≥0.8
Iron, mg/day	<6	6–8	9–11	≥12
Vitamin A, IU/day	<2,000	2,000–3,499	3,500–4,999	≥5,000
Ascorbic acid, mg/day	<10	10–29	30–49	≥50
Thiamine, mg/1,000 Calories	<.2	0.20–0.29	0.3–0.4	≥.5
Riboflavin, mg/day	<.7	0.7–1.1	1.2–1.4	≥1.5
Niacin, mg/day	<5	5–9	10–14	≥15

ceal the fact that some individuals will receive more and others less than average. In addition, there may be variability among individuals in the requirements for various nutrients. Variations in body size, activity, climate, types of food available, and other factors such as parasitic infections modify requirements and, consequently, interpretation of survey data (see also discussion under each nutrient).

The nutrient content of food may be altered materially during preparation, a fact which must always be considered in evaluating dietary intakes.

Calories

REFERENCES

Calorie Requirements, Report of the Second Committee on Calorie Requirements. FAO Nutritional Studies No. 15, Food and Agriculture Organization, Rome, 1957.

Recommended Dietary Allowances, Revised 1964, A Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 3–4.

Konishi, F., et al., The dietary composition and adequacy of the food consumed by young men on an ad libitum regimen. J. Nutrition 63, 41–56, 1957.

Keys, A., et al., The Biology of Human Starvation, Minneapolis, University of Minnesota Press, 1950.

Consolazio, C. F., et al., Food consumption of military personnel performing light activities in a hot desert environment. Metabolism 9, 435–442, 1960.

Pollack, H., French, C. E., and Berryman, G. H., Calories expended in military activities. Bull. U.S. Army Med. Dept., No. 74, pp. 110–114, March 1944.

Evaluation of the adequacy of caloric intake uses the guides of the Food and Agriculture Organization as modified by the National Research Council (NRC), Food and Nutrition Board. Various factors influence Calorie requirements. Adjustments may be calculated for those discussed below.

Body Size

Larger allowances must be derived for individuals of body size greater than that indicated for the reference man and woman and smaller allowances for those of smaller size. Weight may be used as a basis for adapting allowances for size provided the subjects are not overweight. In the United States the recommended Calorie allow-

ances for adults pertain to desirable body weight (the average weight for given sex and height at age 25). The following table may be used as a guide for this adjustment:

DESIRABLE WEIGHTS FOR HEIGHTS

(Home Economics Research Report No. 10, ARS, USDA)

	Weight [1] *in pounds*	
Height,[1] *inches*	*Men*	*Women*
60		109 ± 9
62		115 ± 9
64	133 ± 11	122 ± 10
66	142 ± 12	129 ± 10
68	151 ± 14	136 ± 10
70	159 ± 14	144 ± 11
72	167 ± 15	152 ± 12
74	175 ± 15	

[1] Heights and weights are "without shoes and other clothing." Conversion factors: Inches×2.54=centimeters; pounds×0.454=kilograms. Adjustment of Calorie allowances for individuals whose weight and height differ from those of the reference man and woman is illustrated in the table below. A mean environmental temperature of 20° C and average physical activity are assumed. Simplified formulas for the age-ange periods are included.

Desirable weight		*Calorie allowance* [1]		
Kilograms	*Pounds*	*25 years*	*45 years*	*65 years*
		Men		
		(1)	(2)	(3)
50	110	2, 300	2, 050	1, 750
55	121	2, 450	2, 200	1, 850
60	132	2, 600	2, 350	1, 950
65	143	2, 750	2, 500	2, 100
[2] 70	154	2, 900	2, 600	2, 200
75	165	3, 050	2, 750	2, 300
80	176	3, 200	2, 900	2, 450
85	187	3, 350	3, 050	2, 550
		Women		
		(4)	(5)	(6)
40	88	1, 600	1, 450	1, 200
45	99	1, 750	1, 600	1, 300
50	110	1, 900	1, 700	1, 450
55	121	2, 000	1, 800	1, 550
[2] 58	128	2, 100	1, 900	1, 600
60	132	2, 150	1, 950	1, 650
65	143	2, 300	2, 050	1, 750
70	154	2, 400	2, 200	1, 850

[1] Rounded to nearest 50 calories.
[2] Reference man and woman (NRC).

Formulas

(1) 725 + 31W (2) 650 + 28W (3) 550 + 23.5W
(4) 525 + 27W (5) 475 + 24.5W (6) 400 + 20.5W
W = weight in kilograms.

Climate

The guidelines used have presumed a mean environmental temperature of 20° C. This applies to most persons in the United States. When there is exposure to cold or heat, Calorie allowances should be adjusted.

There appears to be no reason to increase Calorie allowances for activity in the cold, except to compensate for the relatively small (2–5 percent) increase in energy expenditure associated with carrying the extra weight of clothing required and the "hobbling" effect of such clothing. Consolazio, et al., at the U.S. Army Medical Research and Nutrition Laboratory (J. Nutrition 73: 126, 1961), have adequately demonstrated that energy requirement of men living in extreme heat (approximately 40° C.) is increased, not decreased. Under such conditions the body temperature and metabolic rate increase and the body expends extra energy in its efforts to maintain temperature homeostasis. Thus while little adjustment appears to be necessary for a change in environmental temperature between 20° C and 30° C, it would seem desirable under conditions of increased activity to increase Calorie allowances by at least 0.5 percent for every degree of temperature rise between 30 and 40° C.

At best, the suggestions concerning climatic effects are approximations due to the variability in air movement, degree of exposure, type of shelter, and activity.

Age

Allowances have been estimated to meet average needs in a particular decade. For example, the allowance for an individual 25 years of age applies between 20 and 30 years of age. Due to lessened activity and a decrease in basal metabolic rate Calorie needs decrease with advancing age. A reduction of Calories by 5 percent for each decade between 35 and 55 years of age and by 8 percent of the decades between 55 and 75 years has been proposed. A further decrease of 10 percent is recommended for age 75 and beyond.

For infants, allowances recommended are based on body weight as follows:

Age (months):	*Calories per kilogram body weight*
1 to 3	130
4 to 9	115
10 to 12	100

Recommendations for breast-fed children are included in those of lactating mothers (see below). For older children and adolescents, requirements are classified into age groups, and are averages for the

group. Above 9 years of age, boys and girls are considered separately, due to differences in activity, size, growth, and basal metabolism.

Age (years):	Calorie allowance	
Under 1	kg×115±15	
1–3	1,300	
3–6	1,600	
6–9	2,100	
	Boys	*Girls*
9–12	2,400	2,200
12–15	3,000	2,500
15–18	3,400	2,300

Physical Activity

This is the principal factor causing variation in caloric needs, and the one most difficult to assess. The physical activity of the reference man and woman is considered to be midway between that of sedentary and very active individuals. It is defined as employment in light industry, saleswork, housework and the like. Heavy labor may increase caloric requirements up to 25 percent, but seldom above this level. A relatively sedentary life will, conversely, decrease requirements. Physically active individuals frequently adjust their caloric intake to energy expenditure while inactive persons do not usually have so efficient a control.

Expected increases in Calorie requirements during pregnancy may be partly offset by reduction in physical activity in many cultures and in developed countries. The often proposed increase of 300 Calories daily in the latter half of pregnancy may apply where activity is not curtailed during pregnancy. For lactation, an additional allowance of 1,000 Calories per day is suggested.

Other Factors

Many factors characteristic of a particular culture, such as the use of labor-saving devices and types of physical recreation, and differences in physical efficiency in performing certain tasks, must be considered in estimating requirements. It should be noted, however, that race as such has no influence on caloric requirements.

It is emphasized that these guides are designed to apply to groups of people, and cannot be used for individuals, nor are they so intended. They may over-estimate the needs of certain groups. Furthermore, it is difficult to determine whether populations upon which guides have been based were actually at an ideal weight. Also, individuals will adapt to Calorie restriction by lowered metabolism and decreased activity. For these reasons evaluation of adequacy of Calorie intake in nutrition surveys must consider physical appearance and physical measurements as well as intake data.

Protein

REFERENCES

Protein Requirements, FAO Nutritional Studies No. 16, Food and Agriculture Organization, Rome, 1957.

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 10–15.

Holt, L. E., et al., Protein and Amino Acid Requirements in Early Life, New York, New York University Press, 1960.

It is well known that nitrogen balance can be readily attained by normal adults with diets providing as little as 0.3 gm of high-quality protein daily per kilogram of body weight, and with many diets of vegetable origin providing 0.5 gm of protein daily per kilogram of body weight. However, nitrogen balance does not necessarily reflect all the functions of protein in maintaining normal metabolism and health. Also, signs typical of protein deficiency are rarely seen in adults in areas where protein intakes are low by U.S. standards. This can be explained by the fact that a cereal diet of 3,000 Calories can provide some 60 gm or more of protein. Thus an intake of 1 gm of protein per kilogram of body weight may be regarded practically and realistically as minimal when dealing with populations where a high proportion of the dietary protein comes from nonanimal sources.

Cereal proteins are often deficient in certain essential amino acids, especially lysine, and perhaps methionine, threonine, and tryptophan. Nitrogen balance can be maintained on vegetarian diets if the quantity and variety of protein are sufficient. The level of protein in diets should provide some margin to allow for variations in individual requirements and to facilitate repletion of protein stores after nitrogen loss due to injury, disease, or temporary diet inadequacies. (Protein levels specified here assume that caloric needs have been met.)

Calcium

REFERENCES

Calcium Requirements, Report of an FAO/WHO Expert Group. World Health Organization Technical Report Series No. 230; FAO Nutrition Meetings Series No. 30, Geneva, 1962.

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 15–17.

Hegsted, D. M., Calcium requirements. Nutrition Reviews *15*, 257–258, 1957.

Hegsted, D. M., The estimation of relative nutrient intake and nutritional status by biochemical methods: Minerals. Am. J. Clin. Nutrition *11*, 477–483, 1962.

Recommended intakes of calcium have in the past been based on balance studies. It is now believed that these do not necessarily measure calcium need, but usually reflect the level of intake of calcium in the

recent past. No clear-cut signs of calcium deficiency have ever been reported in adults, other than occurrence of osteomalacia in women following pregnancy and prolonged lactation in certain limited areas. However, this condition is recognized to be due primarily to vitamin D deficiency, to which a low calcium intake is a concomitant. It is well recognized that rickets and dental caries are largely independent of the calcium intake level. The role of calcium intake in the etiology of osteoporosis is debatable. No disorders or diseases which could be attributed primarily to a dietary deficiency of calcium have been seen in populations on a habitual intake of 0.3 to 0.4 gm per day. While an adequate supply of vitamin D, either dietary or from sunlight, is required for calcium metabolism, adaptive mechanisms readily adjust to substances which affect calcium absorption adversely, such as phytates, oxalates, saturated fatty acids, citric and other acids.

The FAO/WHO group, recognizing the lack of evidence of a minimal requirement, has recommended a range or "suggested practical allowance," defined as an intake at which the needs of the great majority of persons in a defined population group may be expected to be met. There should be an awareness of possible undesirable effects on health at an average intake habitually below this level, although the onset of such symptoms cannot be predicted with certainty in man. Accordingly, intakes between 0.4 and 0.5 gm per day are suggested as practical for adults, and raising the daily intake above 1 gm was not considered to serve any useful physiologic purpose.

It is assumed that the breast-fed infant receives sufficient calcium for its needs. Recommendations for other children take into account needs for skeletal growth as well as for maintenance. The ranges recommended are as follows:

Age	*Suggested practical allowance gm per day*
0–12 months (not breast fed)	0.5–0.6
1–9 years	0.4–0.5
10–15 years	0.6–0.7
16–19 years	0.5–0.6

Due to the demands of the fetus and of lactation on the maternal organism, the FAO/WHO group recommends a suggested practical intake of 1.0 to 1.2 gm per day for the third trimester of pregnancy and the duration of lactation.

In evaluating calcium intakes in diet studies, sources of calcium such as drinking water, salt, and calcium compounds used in processing foods should, of course, also be taken into consideration.

Iron

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 17–19.

Moore, C. V., The importance of nutritional factors in the pathogenesis of iron-deficiency anemia. Am. J. Clin. Nutrition *3*, 3–10, 1955.

Hegsted, D. M., The estimation of relative nutrient intake and nutritional status by biochemical methods: Minerals. Am. J. Clin. Nutrition *11*, 477–483, 1962.

Normal males have a daily loss of iron from all sources (urine, feces, sweat, hair, and nails) of approximately 1 mg. Absorption of dietary iron is usually approximately 5–10 percent of intake, so that a daily intake of 9–12 mg for adult males should be realistic (Moore). It should be evident that these figures may simply reflect the mechanics required to maintain balance on a daily intake of about 10 mg. There is evidence that, as body stores fall, iron is conserved by decreased excretion as well as by increased absorption. Thus, there appear to be adequate mechanisms to protect the normal male against iron deficiency at much lower intakes than those recommended.

The stresses imposed by growth, pregnancy, and periodic loss of iron during menstruation may increase the needs of children and women during their reproductive years. For infants in the first year of life an intake of 0.8 mg per kilogram of body weight has been recommended by the NRC. Studies in preschool children suggest that 0.3 to 0.4 mg per kilogram per day is necessary. Allowances for preadolescent children as set forth by the NRC are 10 to 12 mg per day. An additional allowance of 3 mg per day has been proposed for those 13 to 15 years of age to take care of the additional iron requirements of this age group.

The demands of pregnancy have been suggested to require an additional 5 mg per day above the normal daily requirement for nonpregnant, nonlactating women of 12 mg. Since iron secreted in milk may amount to 1 mg per day, an additional 5 mg per day has also been recommended during lactation.

It should be emphasized that iron intakes may need to be increased where certain parasitic infestation is high. The figures cited in this section thus may apply only to nonparasitized individuals.

In a number of surveys the figures for chemical analysis of food samples have been considerably higher than those obtained by calculation from food tables. It is not known whether this additional iron from the cooking utensils is biologically available and utilized.

Vitamin A

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 19–20.

Hume, E. M., and Krebs, H. A., Vitamin A requirement of human adults. An experimental study of vitamin A deprivation in man. Medical Research Council, Great Britain, Special Report Series No. 264, London, 1949.

Vitamin A requirement appears to be proportional to body weight, and quantitatively similar in all animals studied, man included. An intake which provides 20 International Units (IU; 6 μg) of preformed vitamin A · o IU (24 μg) of β-carotene (one IU equals 0.6 μg of β-carotene) p kilogram of body weight per day will meet minimal requirements. This represents an average daily individual intake of 1,300 IU of vitamin A or 2,600 IU of β-carotene. Hume and Krebs have estimated that approximately 1,300 IU of preformed vitamin A is required daily, and that 2,500 IU provides a reasonable margin of safety. They also concluded that 3,000 IU of β-carotene is the minimal requirement, but that 7,500 IU is a more desirable intake because of individual variability in carotene absorption.

Although by definition the ratio of activity of vitamin A to carotene is 1 to 2, studies on requirements indicate a ratio of 1 to 4. As intake of carotene is increased, its absorption and utilization probably fall. Furthermore, the availability of carotene in different foods varies considerably, and its utilization may be influenced by other dietary constituents such as fat and antioxidants, and methods of food preparation. Thus, no single value for meeting vitamin A requirements in terms of carotene can be specified accurately.

Present recommended allowances for vitamin A assume that one-third will be supplied by preformed vitamin A and its remainder as carotene. This situation is often not met, especially where wide variations in local and national dietary patterns occur. Thus a fairly wide margin between minimal and acceptable intake levels seems justified, since a high intake is readily achieved where good sources are available.

Dietary sources of vitamin A are often seasonal. Since a long period is needed for depletion of body stores, an intermittent intake is dangerous only in prolonged intervals of serious shortages within population groups (such as infants and small children) with limited stores. Indeed, intermittent or seasonal intakes may be an economical method of meeting needs. However, in areas where seasonal variations do exist, studies of vitamin A status are definitely indicated.

Ascorbic Acid

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 23-24.

Peters, R. A., et al., Vitamin C requirement of human adults. Experimental study of vitamin C deprivation in man. Lancet *1*, 853-858, 1948.

Bartley, W., Krebs, H. A., and O'Brien, J. R. P., Vitamin C requirement of human adults. A Report by the Vitamin C Subcommittee of the Accessory Food Factors Committee. Medical Research Council, Great Britain, Special Report Series No. 280, London, 1953.

Meiklejohn, A. P., The physiology and biochemistry of ascorbic acid. Vitamins and Hormones *XI*, 61-96, 1953.

Berry, F. B., and Schaefer, A., Nutrition surveys in the Near and Far East. Report of the Interdepartmental Committee on Nutrition for National Defense. Am. J. Clin. Nutrition *6*, 342-353, 1958.

Thirty milligrams of ascorbic acid a day is considered an adequate intake to prevent any evidence of deficiency. Whether higher intake levels are of benefit is an unsettled question, since they result in higher tissue concentrations. The level cited above represents an amount which permits tissue storage in the range found in animals which synthesize the vitamin. Since ascorbic acid is particularly susceptible to losses in cooking and food storage, an allowance somewhat more above the minimum need than that recommended for other nutrients seems justified.

Daily intakes of 10 mg per day will prevent frank scurvy in most adults. It was found in early ICNND surveys (Berry and Schaefer) that a daily intake in the range of 15 to 23 mg resulted in reduced serum ascorbic acid levels, and these levels appeared to be associated with an increase in gingival pathology.

Thiamine

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 20-21.

Holt, L. S., Jr., The thiamine requirement of man. Fed. Proc. *3*, 171-178, 1944.

Jansen, B. C. P., Thiamine requirements of animals and factors influencing them. *In* The Vitamins, Sebrell, W. H., and Harris, R. S., ed., New York, Academic Press, Vol. 3, 1954, pp. 472-478.

Sebrell, W. H., Thiamine requirements of man and factors influencing them. *In* The Vitamins, Sebrell, W. H., and Harris, R. S., ed., New York, Academic Press, Vol. 3, 1954, pp. 478-480.

Cruickshank, E. K., Dietary neuropathies. Vitamins and Hormones *X*, 1-45, 1952.

Horwitt, M. K., Liebert, E., Kreisler, O., and Wittman, P., Investigations of human requirements for B-complex vitamins. National Academy of Sciences-National Research Council Bull. No. 116, Washington, 1948.

The minimum need for thiamine has been reasonably well established, and studies in animals and in man have given essentially similar results. Clear-cut evidence of deficiencies has been produced with intakes less than 0.2 mg per 1,000 Calories. Epidemiologic evidence bears out this figure. Evidence of tissue unsaturation occurs at intakes of about 0.3 mg per 1,000 Calories. Although a relationship between thiamine need and Calorie expenditure is evident, it is not proved that the relation is uniform at all levels of Calories ingested. That dietary fat spares thiamine is accepted, but data on man do not yet allow a quantitative relationship to be set. Thus a modi-

fication of this effect upon the amount of thiamine needed has not been attempted, but the relationship should be kept in mind in interpreting dietary intakes.

Body stores of thiamine are never large, and quite labile to disease and other stresses. Thus stores should be maintained.

Daily intakes of 0.3–0.5 mg per 1,000 Calories can be regarded as acceptable, provided Calorie intake does not fall below 2,000. The few data available suggest the need for thiamine may be increased during pregnancy. Because of the increased needs for lactation, the NRC recommended allowance is 1.2 mg. Thiamine requirements are elevated in hyperthyroidism and perhaps in other conditions associated with elevated metabolic rates.

Riboflavin

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 21–22.

Horwitt, M. K., Harvey, C. C., Hills, O. W., and Liebert, E., Correlation of urinary excretion of riboflavin with dietary intake and symptoms of ariboflavinosis. J. Nutrition *41*, 247–264, 1950.

Parsons, H. T., Further studies on human requirements for riboflavin. Fed. Proc. *3*, 162–171, 1944.

Pollack, H., Studies on nutrition in the Far East. III. Clinical indicator signs of nutritional insufficiencies before and after enrichment of rice with synthetic vitamins. Metabolism *5*, 231–244, 1956.

Mannering, G. J., Lipton, M. A., and Elvehjem, C. A., Relation of dietary fat to riboflavin requirement of growing rats. Proc. Soc. Exp. Biol. Med. *46*, 100–104, 1941.

Widdowson, E. M., and McCance, R. A., ed., Studies on the Nutritive Value of Bread and on the Effect of Variations in the Extraction Rate of Flour on the Growth of Undernourished Children. Medical Research Council, Great Britain, Special Report Series No. 287, London, 1954.

Riboflavin requirements are not so clearly related to caloric intake or physical activity as is the case with thiamine, although on the basis of its known functions it might be expected to be so related. Indeed, there is some evidence to the contrary.

Clear-cut signs of deficiency have been produced experimentally in man with dietary intakes below 0.6 mg per day. There has been clinical evidence of deficiency with intakes less than 0.9 mg daily, and progressive tissue unsaturation has been noted at levels below 1.1 to 1.3 mg per day. The composition of the diet does have some effect on riboflavin requirements. As is the case with protein, requirements for riboflavin are increased during growth, pregnancy, and lactation. In growing animals the requirement appears to be about one and one-half times that of thiamine. It has been hypothesized that the requirement may be affected by factors such as exposure to sunlight, availability of riboflavin in foods, and intestinal synthesis. The evi-

dence relative to an effect of sunlight is meager. More studies under varying conditions are needed.

Despite the above-outlined uncertainties the suggested acceptable daily intake of 1.2 to 1.4 mg serves as a useful guide for practical appraisal of the significance of survey findings. (See table 5.)

Niacin

References

Recommended Dietary Allowances, Revised 1964, a Report of the Food and Nutrition Board. National Academy of Sciences-National Research Council Publication 1146, Washington, pp. 22–23.

Frazier, E. I., and Friedemann, T. E., Pellagra, a study in human nutrition. The multiple-factor principle of the determination of minimum vitamin requirements. Quart. Bull. Northwestern Univ. Medical School *20*, 24–48, Spring 1946.

Goldsmith, G. A., Rosenthal, H. L., Gibbens, J., and Unglaub, W. G., Studies on niacin requirement in man. II. Requirement on wheat and corn diets low in tryptophan. J. Nutrition *56*, 371–386, 1955.

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The requirement for niacin by both man and animals is about 10 times that for thiamine. Frazier and Friedemann estimated, from work with pellagragenic diets, that 7.5 mg of niacin is a minimum daily requirement. Pellagra has been produced experimentally with diets very low in tryptophan (200 mg) containing less than 7.5 mg of niacin, but it was not observed with diets supplying about 5 mg of niacin per day when relatively good sources of tryptophan were present (Horwitt et al.; Dann). Evidence of tissue unsaturation has been found with diets low in tryptophan supplying up to 8–10 mg of niacin. It thus seems clear that the level and type of protein in the diet as well as the niacin and tryptophan intakes must be considered in estimating the adequacy of niacin intake. Sixty milligrams of tryptophan may be considered roughly equivalent to 1 mg of niacin. It is possible that the availability of niacin in various foods varies, which would contribute to the epidemiologic relationship between corn diets and pellagra.

Other Nutrients

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Neither the ICNND nor the Food and Nutrition Board have presently established guidelines for intakes of a number of other nutrients of demonstrated importance to man. (A vitamin D requirement for children and pregnant and lactating women is given in the NRC Recommended Dietary Allowances, but ICNND does not have suggested guidelines for this vitamin. Moderate exposure to sunlight will provide for the needs for this nutrient in most individuals.)

Although carbohydrates are, together with fat, the important sources of food energy, metabolic interconversions along with differences in digestibility influence the need to the extent that no recommendations can be made for intakes. It should be noted here that alcoholic beverages can contribute importantly to energy intake. Although it is possible that certain fatty acids are essential for man, and the role of fat as a carrier for other nutrients, such as vitamins A, E,

D, and K, is recognized, it is not yet possible to state safely a reasonable fat intake.

Water is a requirement of man. Intake is usually controlled by physiologic mechanisms producing thirst, except in extreme heat.

Most of the minerals for which a need can be proved, including the trace elements are generally available in a varied, mixed diet. However, the diets of population groups in developing countries are often greatly restricted as regards variety. In these instances especially, cognizance should be given to the possibility of deficiencies of such trace minerals as copper, zinc, magnesium, and others. For example, recent evidence has implicated zinc deficiency in the syndrome of dwarfism and hypogonadism found in Egypt and Iran. Where the intake of seafoods is negligible and the water supply is low in iodine, thyroid enlargement is frequently seen. Although it is known that the need for iodine is increased in adolescence and pregnancy, exact level of requirement has not been established. There is substantial evidence that controlled intake of fluoride results in protection against dental caries.

As with the minerals, usual mixed diets provide sufficient amounts of vitamins B_6, B_{12}, K, E, folacin, pantothenic acid, and biotin. However, recent evidence that deficiency of some of these exists has been forthcoming. There is presumptive evidence of suboptimal intakes of vitamin B_6 in some areas. Vitamin E has been found effective in the treatment of a macrocytic, megaloblastic anemia of malnourished infants in the Middle East. Individuals on exclusively vegetable diets may develop vitamin B_{12} deficiency. The order of magnitude of minimal satisfactory intakes for adults of these nutrients may be 0.5-0.75 μg per day for vitamin B_{12}; 25-100 μg per day (as pteroylglutamic acid) for folacin; and 1.0 mg per day for vitamin B_6. The evidence does not yet permit a similar indication of an approximate reference intake for pantothenic acid, nor for detailed reference intakes for the others.

There is need for additional research in this field.

APPENDIX I.—*Food and Nutrition Board, National Academy of Sciences-National Research Council*

Recommended Daily Dietary Allowances,[1] Revised 1963

Designed for the Maintenance of Good Nutrition of Practically All Healthy Persons in the U.S.A.

[Allowances are intended for persons normally active in a temperate climate]

	Age[2] years from to	Weight kg. (lbs.)	Height cm. (in.)	Calories[3]	Protein gm.	Calcium gm.	Iron mg.	Vitamin A value IU	Thiamine mg.	Riboflavin mg.	Niacin equiv.[4] mg.	Ascorbic acid mg.	Vitamin D IU
Men	18-35	70 (154)	175 (69)	2,900	70	.8	10	*5,000	1.2	1.7	19	70	.....
	35-55	70 (154)	175 (69)	2,600	70	.8	10	5,000	1.0	1.6	17	70	.....
	55-75	70 (154)	175 (69)	2,200	70	.8	10	5,000	.9	1.3	15	70	.....
Women	18-35	58 (128)	163 (64)	2,100	58	.8	15	5,000	.8	1.3	14	70	.....
	35-55	58 (128)	163 (64)	1,900	58	.8	15	5,000	.8	1.2	13	70	.....
	55-75	58 (128)	163 (64)	1,600	58	.8	10	5,000	.8	1.2	13	70	.....
	Pregnant (2d and 3d trimester)			+200	+20	+.5	+5	+1,000	+.2	+.3	+3	+30	400
	Lactating			+1,000	+40	+.5	+5	+3,000	+.4	+.6	+7	+30	400
Infants[5]	0-1	8 (18)	.....	kg.x115	kg.x2.5	.7	kg.x1.0	1,500	.4	.6	6	30	400
				+15	+0.5								
Children	1-3	13 (29)	87 (34)	1,300	32	.8	8	2,000	.5	.8	9	40	400
	3-6	18 (40)	107 (42)	1,600	40	.8	10	2,500	.6	1.0	11	50	400
	6-9	24 (53)	124 (49)	2,100	52	.8	12	3,500	.8	1.3	14	60	400
Boys	9-12	33 (72)	140 (55)	2,400	60	1.1	15	4,500	1.0	1.4	16	70	400
	12-15	45 (98)	156 (61)	3,000	75	1.4	15	5,000	1.2	1.8	20	80	400
	15-18	61 (134)	172 (68)	3,400	85	1.4	15	5,000	1.4	2.0	22	80	400
Girls	9-12	33 (72)	140 (55)	2,200	55	1.1	15	4,500	.9	1.3	15	80	400
	12-15	47 (103)	158 (62)	2,500	62	1.3	15	5,000	1.0	1.5	17	80	400
	15-18	55 (117)	163 (64)	2,300	58	1.3	15	5,000	.9	1.3	15	70	400

[1] The allowance levels are intended to cover individual variations among most normal persons as they live in the United States under usual environmental stresses. The recommended allowances can be attained with a variety of common foods, providing other nutrients for which human requirements have been less well defined. See text for more detailed discussion of allowances and of nutrients not tabulated.

[2] Entries on lines for age range 18–35 years represent the 25-year age. All other entries represent allowances for the midpoint of the specified age periods, i.e., line for children 1–3 is for age 2 years (24 months); 3–6 is for age 4½ years (54 months), etc.

[3] Tables 1 and 2 and figs. 1 and 2 in text show calorie adjustments for weight and age.

[4] Niacin equivalents include dietary sources of the preformed vitamin and the precursor, tryptophan. 60 mg tryptophan represents 1 mg niacin.

[5] The calorie and protein allowances per kg for infants are considered to decrease progressively from birth. Allowances for calcium, thiamine, riboflavin, and niacin increase proportionately with calories to the maximum values shown.

*1,000 I.U. from preformed vitamin A and 4,000 I.U. from beta-carotene.

From NAS-NRC Publ. 1146.

APPENDIX II.—*Comparative dietary standards for adults in selected countries and FAO with explanations as to their meaning*

[The purpose for establishing a national dietary standard is not the same in all countries. Therefore, some variation in nutrient allowances from country to country is to be expected. At the same time, it must be recognized that the "reference" individual will vary from country to country. Furthermore, even in instances when there are presumed similar objectives among countries as to the purpose and usefulness of proposed standards, it can be seen that there is by no means uniform agreement as to the nutrient allowances considered desirable as national guides. Standards are also subject to revision as newer knowledge becomes available. Particular attention should be paid to the footnotes, which explain, in brief form, the basis for nutrient allowances in the various countries and those of FAO. The original publications should be consulted for detailed explanations. The Board is indebted to Dr. L. A. Maynard for the preparation of this table.]

Country	Sex	Age years	Weight kg.	Activity	Calories	Protein gm.	Calcium gm.	Iron mg.	Vitamin A activity I U	Thiamine mg.	Riboflavin mg.	Niacin equiv.[3] mg.	Ascorbic acid mg.
U.S.A.[1]	M	18–35	70	Footnote [2]	2,900	70	0.8	10	5,000	1.2	1.7	19	70
	F	18–35	58	Footnote [2]	2,100	58	.8	15	5,000	.8	1.3	14	70
FAO[1]	M	25	65	Footnote [2]	3,200	[3] 43	[4] .4–.5						
	F	25	55	Footnote [2]	2,300	[3] 36	[4] .4–.5						
Australia[1]	M	25	65	Footnote [2]	2,700	65	.7	10	[3] 2,500	1.1	1.6	[4] 15	30
	F	25	55	Footnote [2]	2,300	55	.6	12	[3] 2,000	.9	1.4	[4] 15	30
Canada[1]	M	25	72	Footnote [2]	2,550	[3] 50	.5	6	[4] 3,700	.9	1.4	9	30
	F	25	57	Footnote [2]	2,400	[3] 39	.5	10	[4] 3,700	.7	1.2	7	30
Central America and Panama.[1]	M	25	55	Moderate work	2,700	55	.7	10	[2] 4,333	1.4	1.4	14	50
	F	25	50	Moderate work	2,000	50	.7	10	[2] 4,333	1.0	1.2	10	45
India[1]	M	25.4	55	Moderate work [2]	2,800	[3] 55							
	F	21.5	45	Moderate work [2]	2,300	[3] 45							
Japan[1]	M	Footnote [2]	56	Moderate work [2]	3,000	[4] 70	.6	10	[3] 2,000	1.5	1.5	15	65
	F	Footnote [2]	48.5	Moderate work [2]	2,400	[4] 60	.6	10	[3] 2,000	1.2	1.2	12	60
Netherlands[1]	M	20–29	70	Light work	3,000	[3] 70	1.0	10	[3] 5,500	1.2	1.8	12	50
	F	20–29	60	Light work	2,400	[3] 60	1.0	12	[3] 5,500	1.0	1.5	10	50
Norway[1]	M	25	70	None	3,400	70	.8	12	[3] 2,500	1.7	1.8	17	30
	F	25	60	Given	2,500	60	.8	12	[3] 2,500	1.3	1.5	13	30
The Philippines[1]	M	None specified	53	Moderate work	2,600	55	.7	6	[3] 4,000	1.6	[3] 1.4	16	75
	F	None specified	45	Moderate work	2,300	45	.7	10	[3] 4,000	1.4	[3] 1.1	14	70
South Africa[1]	M	None specified	73	Moderate work	3,000	65	.7	9	[3] 4,000	1.0	1.6	15	40
	F	None specified	60	Moderate work	2,300	55	.6	12	[3] 4,000	.8	1.4	12	40
United Kingdom[1]	M	20 up	65	Medium work [2]	3,000	[3] 87	.8	12	[4] 5,000	1.2	1.8	12	20
	F	20 up	56	Medium work [2]	2,500	[3] 73	.8	12	[4] 5,000	1.0	1.5	10	20
U.S.S.R.[1]	M and F			Moderate work					[3] 5,000	[3] 2.0	2.5	15	[3] 70

U.S.A.:
[1] Source: Recommended Dietary Allowances, Revised 1963. NAS-NRC Publ. 1146. Washington (1964).
[2] Allowances are intended for persons normally active in a temperate climate.
[3] Niacin equivalents include dietary sources of the preformed vitamin and the precursor, tryptophan. 60 mg tryptophan represents 1 mg niacin.

FAO:
[4] Source: Calorie Requirements, FAO. Nutritional Studies, No. 15, Rome (1957).
Protein Requirements, FAO. Nutritional Studies, No. 16, Rome (1957).
Calcium Requirements, FAO. Nutrition Meetings Report Series No. 30, Rome (1962).
Mean annual temperature, 10° C.

[2] The activity for the reference man is described as "on each working day he is employed 8 hours in an occupation which is not sedentary, but does not involve more than occasional periods of *hard* physical labor. When not at work, he is sedentary for about 4 hours daily and may walk for up to 1½ hours. He spends about 1½ hours on active recreations and household work." The activity of the reference woman is described as "she may be engaged either in general household duties or in light industry. Her daily activities include walking for about 1 hour and 1 hour of active recreation, such as gardening, playing with children, or non-strenuous sport."

[3] The protein value is defined as a safe practical allowance and is based on an average minimum requirement for a reference protein: increased by 50% to allow for individual variability, and by a further percentage in accordance with the estimated protein score of the protein of the diet. The values given in the table are for a diet similar to that of the USA, using a coefficient of 1.25 to allow for differences in protein quality, thus arriving at an allowance of 0.66 gm. per kilogram body weight.

[4] The value is considered a safe practical allowance. A range is given to emphasize that present knowledge does not permit any greater accuracy as to a safe allowance.

Australia:

[1] Dietary allowances for Australia, 1961 revision, Med. Journal Australia, Dec. 30, 1961. The allowances are designed to be used as a basis for planning food supplies for persons or groups.

[2] The activities specified are similar to those of the reference man and woman (Calorie Requirements, FAO Nutritional Studies No. 15, Rome). Mean annual external temperature, 18° C.

[3] Three I.U. of carotene equivalent to one I.U. of vitamin A activity.

[4] Preformed niacin plus (gms. of protein x 0.16).

Canada:

[1] "Recommended daily intakes of nutrients adequate for the maintenance of health among the majority of Canadians." Issued 1963.

[2] Five categories of activity are listed and described. The values for calories and nutrients here given apply to "most household chores," "office work," "laboratory work," "shop and mill work," "mechanical trades or crafts," various sports.

[3] Based on normal mixed Canadian diets.

[4] Based on mixed Canadian diet supplying both vitamin A and carotene. As preformed vitamin A the suggested intake would be two-thirds of amounts indicated.

Central America and Panama:

[1] Institute of Nutrition of Central America and Panama (INCAP), Boletin de la Oficina Sanitaria Panamericana, Supplemento No. 2, Noviembre, 1955, page 225. The figures for nutrients are designed to meet the needs of all individuals. Average annual temperature, 20°C., activity not defined.

[2] This figure is given in the INCAP table as 1.3 mg. of vitamin A and assumes that two-thirds of the activity is supplied from vegetable sources. International units shown were derived by converting as follows: one I.U. = 0.0003 mg. vitamin A alcohol.

India:

[1] Patwardhan, V. N., Dietary allowances for India. Calories and Protein. Indian Council of Medical Research, Special Report Series No. 35, New Delhi, 1960. The data are 1958 revisions of earlier figures.

[2] The activities corresponding to the calorie recommendations are detailed in the above publication. "Moderate" refers to activity in a "light industrial occupation."

[3] "An allowance of one gm. of protein per kilogram body weight of vegetable proteins in properly balanced diets."

Japan:

[1] Nutrition in Japan, 1962, Ministry of Health and Welfare, Tokyo, 1962. Data adopted by the Council on Nutrition in 1960. The allowances are believed to be sufficient to establish and maintain a good nutritional state in typical individuals.

[2] Age not specified.

[3] Five categories of activity are specified for men and four for women, with corresponding intakes for calories and in B-vitamins.

[4] Higher intakes are specified for heavy and very hard work.

[5] Requirement for both sexes specified as 2000 I.U. of preformed vitamin A or 6000 I.U. of carotene.

Netherlands:

[1] Recommended quantities of nutrients, Committee on Nutritional Standards of the Nutrition Council Voeding 22, 210–214, 1961. The figures for nutrients are set to cover individuals having high requirements. The figures for calories are average requirements.

[2] Assumes one-third is from animal sources. Figures are increased for heavy and very heavy work.

[3] Assumes 1500 I.U. as preformed vitamin A and 4000 I.U. of activity as carotene.

Norway:

[1] Evaluation of nutrition requirements, State Nutrition Council, 1958. Figures are "somewhat higher than average requirements."

[2] Vitamin A as present in animal foods.

The Philippines:

[1] Recommended daily allowances for specific nutrients, Food and Nutrition Research Center, 1960, "Objectives toward which to aim in planning practical diets."

[2] Assumes two-thirds contributed by carotene.

[3] (Grams protein x .025.

South Africa:

[1] Recommended minimum daily dietary standards, National Research Council, S. A. Med. J., 30: 105, 1956.

[2] Assumes two-thirds contributed by carotene.

United Kingdom:

[1] Report of the Committee on Nutrition, British Medical Association, 1950. The levels of nutrients recommended are believed to be sufficient to establish and maintain a good nutritional state in representative individuals or groups concerned.

[2] Values are given for 6 levels of activity for males and 5 for females. Medium work is described as 8 hours at 100 Calories per hour and traveling (130 Calories per hour).

[3] The protein allowance is increased with calories on the basis that the protein in the diet should provide not less than 11 per cent of the energy for adults not engaged in hard work.

[4] A mixed diet containing one-third vitamin A and two-thirds carotene.

U.S.S.R.:

[1] New daily vitamin supply standards in man, 1961, Yarusova, N.S., Vop. Pitan., 20: 3, 1961.

[2] I.U. is equivalent to 0.3 μg. of natural vitamin.

[3] To be increased up to 50% in far north.

From NAS–NRC Publ. 1146.

Height-Weight Standards

REFERENCES

Association of Life Insurance Medical Directors and Actuarial Society of America. Medico-Actuarial Mortality Investigation, Vol. 1, 1912.

Society of Actuaries, Build and Blood Pressure Study, Vol. 1, Chicago, The Society, 1959.

Hathaway, M. L., Heights and weights of children and youth in the United States. Home Economics Research Report No. 2, U.S. Department of Agriculture, Washington, 1957.

Hathaway, M. L., and Foard, E. D., Heights and weights of adults in the United States. Home Economics Research Report No. 10, U.S. Department of Agriculture, Washington, 1960.

Garn, S. M., Anthropometry in clinical appraisal of nutritional status. Am. J. Clin. Nutrition *11*, 418–432, 1962.

Jackson, R. L., and Kelly, H. G., Growth charts for use in pediatric practice. J. Pediat. 27, 215–229, 1945.

Heights are measured without shoes, to the nearest centimeter. Weights are recorded in kilograms, to the nearest tenth. When clothed individuals are weighed, the average weights of clothing are determined at intervals, and corrections made. The percent of "standard weight" is determined by reference to a table of standards for height, age, and sex. Until recently the standards used for adults were the American medicoactuarial tables published in 1912. In order to have an age independent standard that would eliminate the bias which enters with the tendency to increasing body fatness in older individuals, the standards for age 25 have been adopted for all individuals above that age. The data for those 20–24 years of age and 25–29 years of age have been averaged in order to obtain a mean figure for the decade so selected. The figures used in these calculations were published by the Society of Actuaries in 1959 (tables 6 and 7).

Due to the lack of any universally appropriate standards for increase in height and weight with age, three policies have been followed for evaluating the height and weight status of children: (1) use of the old Baldwin-Wood tables giving weight standards by height for a given age for ages 6–16 years (see tables 8 and 9); (2) comparisons of measures of central tenancy (mean height and median weight) with a widely used growth chart (Jackson and Kelly; the use of a single growth chart permits comparisons between groups in the same country and between countries that have been surveyed without accepting it as an absolute standard); (3) considered interpretation of the above approaches in relation to each other and to the general trend of the data under study.

TABLE 6.—*Conversion factors for percent "standard weight," adult males, 17+ years (corrected for shoe height and clothing weight)*

Standard weight group	Code	Height (cm)										
		145	146	147	148	149	150	151	152	153	154	155
50–54	00	26.0–28.5	26.2–28.7	26.5–29.0	26.8–29.4	27.0–29.6	27.3–29.9	27.5–30.2	27.8–30.5	28.1–30.8	28.3–31.1	28.6–31.4
55–59	01	28.6–31.1	28.8–31.4	29.1–31.7	29.5–32.0	29.7–32.3	30.0–32.6	30.3–32.9	30.6–33.3	30.9–33.6	31.2–33.9	31.5–34.3
60–64	02	31.2–33.7	31.5–34.0	31.8–34.3	32.1–34.7	32.4–35.0	32.7–35.4	33.0–35.7	33.4–36.1	33.7–36.4	34.0–36.7	34.4–37.1
65–69	03	33.8–36.3	34.1–36.6	34.4–37.0	34.8–37.4	35.1–37.7	35.5–38.1	35.8–38.4	36.2–38.9	36.5–39.2	36.8–39.6	37.2–40.0
70–74	04	36.4–38.9	36.7–39.2	37.1–39.6	37.5–40.1	37.8–40.4	38.2–40.8	38.5–41.2	39.0–41.6	39.3–42.0	39.7–42.4	40.1–42.8
75–79	05	39.0–41.5	39.3–41.9	39.7–42.3	40.2–42.7	40.5–43.1	40.9–43.5	41.3–43.9	41.7–44.4	42.1–44.8	42.5–45.2	42.9–45.7
80–84	06	41.6–44.1	42.0–44.5	42.4–44.9	42.8–45.4	43.2–45.8	43.6–46.3	44.0–46.7	44.5–47.2	44.9–47.6	45.3–48.1	45.8–48.6
85–89	07	44.2–46.7	44.6–47.1	45.0–47.6	45.5–48.1	45.9–48.5	46.4–49.0	46.8–49.4	47.3–50.0	47.7–50.4	48.2–50.9	48.7–51.4
90–94	08	46.8–49.3	47.2–49.7	47.7–50.2	48.2–50.8	48.6–51.2	49.1–51.7	49.5–52.2	50.1–52.8	50.5–53.2	51.0–53.7	51.5–54.3
95–99	09	49.4–51.8	49.8–52.3	50.3–52.8	50.9–53.4	51.3–53.9	51.8–54.4	52.3–54.9	52.9–55.5	53.3–56.0	53.8–56.5	54.4–57.1
100–104	10	51.9–54.4	52.4–55.0	52.9–55.5	53.5–56.1	54.0–56.7	54.5–57.2	55.0–57.7	55.6–58.3	56.1–58.9	56.6–59.4	57.2–60.0
105–109	11	54.5–57.0	55.1–57.6	55.6–58.1	56.2–58.8	56.8–59.3	57.3–59.9	57.8–60.5	58.4–61.1	59.0–61.7	59.5–62.2	60.1–62.9
110–114	12	57.1–59.6	57.7–60.2	58.2–60.8	58.9–61.5	59.4–62.1	60.0–62.6	60.6–63.2	61.2–63.9	61.8–64.5	62.3–65.0	63.0–65.7
115–119	13	59.7–62.2	60.3–62.8	60.9–63.4	61.6–64.2	62.2–64.8	62.7–65.4	63.3–66.0	64.0–66.7	64.6–67.3	65.1–67.9	65.8–68.6
120–124	14	62.3–64.8	62.9–65.5	63.5–66.1	64.3–66.8	64.9–67.5	65.5–68.1	66.1–68.7	66.8–69.5	67.4–70.1	68.0–70.7	68.7–71.5
125–129	15	64.9–67.4	65.6–68.1	66.2–68.7	66.9–69.5	67.6–70.2	68.2–70.8	68.8–71.5	69.6–72.2	70.2–72.9	70.8–73.5	71.6–74.3
130–134	16	67.5–70.0	68.2–70.7	68.8–71.4	69.6–72.2	70.3–72.9	70.9–73.5	71.6–74.2	72.3–75.0	73.0–75.7	73.6–76.4	74.4–77.2
135–139	17	70.1–72.6	70.8–73.3	71.5–74.0	72.3–74.9	73.0–75.6	73.6–76.3	74.3–77.0	75.1–77.8	75.8–78.5	76.5–79.2	77.3–80.1
140–144	18	72.7–75.2	73.4–75.9	74.1–76.7	75.0–77.5	75.7–78.3	76.4–79.0	77.1–79.7	77.9–80.6	78.6–81.3	79.3–82.0	80.2–82.9
145–149	19	75.3–77.8	76.0–78.6	76.8–79.3	77.6–80.2	78.4–81.0	79.1–81.7	79.8–82.5	80.7–83.4	81.4–84.1	82.1–84.9	83.0–85.8
150–154	20	77.9–80.4	78.7–81.2	79.4–82.0	80.3–83.0	81.1–83.8	81.8–84.5	82.6–85.4	83.5–86.3	84.2–87.0	85.0–87.9	85.9–88.8
		156	157	158	159	160	161	162	163	164	165	166
50–54	00	29.0–31.8	29.3–32.2	29.7–32.5	30.0–32.9	30.3–33.2	30.6–33.5	30.9–33.9	31.2–34.2	31.5–34.5	31.8–34.9	32.0–35.1
55–59	01	31.9–34.7	32.3–35.1	32.6–35.5	33.0–35.9	33.3–36.2	33.6–36.6	34.0–36.9	34.3–37.3	34.6–37.7	35.0–38.0	35.2–38.3
60–64	02	34.8–37.6	35.2–38.0	35.6–38.5	36.0–38.9	36.3–39.3	36.7–39.6	37.0–40.0	37.4–40.4	37.8–40.8	38.1–41.2	38.4–41.5
65–69	03	37.7–40.5	38.1–41.0	38.6–41.4	39.0–41.9	39.4–42.3	39.7–42.7	40.1–43.1	40.5–43.5	40.9–44.0	41.3–44.4	41.6–44.7
70–74	04	40.6–43.4	41.1–43.9	41.5–44.4	42.0–44.9	42.4–45.3	42.8–45.8	43.2–46.2	43.6–46.7	44.1–47.1	44.5–47.6	44.8–47.9
75–79	05	43.5–46.3	44.0–46.8	44.5–47.4	45.0–47.9	45.4–48.3	45.9–48.8	46.3–49.3	46.8–49.8	47.2–50.3	47.7–50.7	48.0–51.1
80–84	06	46.4–49.1	46.9–49.7	47.5–50.3	48.0–50.9	48.4–51.4	48.9–51.9	49.4–52.4	49.9–52.9	50.4–53.4	50.8–53.9	51.2–54.3
85–89	07	49.2–52.1	49.8–52.7	50.4–53.3	51.0–53.9	51.5–54.4	52.0–54.9	52.5–55.5	53.0–56.0	53.5–56.5	54.0–57.1	54.4–57.5
90–94	08	52.2–54.9	52.8–55.6	53.4–56.3	54.0–56.9	54.5–57.4	55.0–58.0	55.6–58.8	56.1–59.1	56.6–59.7	57.2–60.3	57.6–60.7
95–99	09	55.0–57.8	55.7–58.5	56.4–59.2	57.0–59.8	57.5–60.4	58.1–61.0	58.9–61.6	59.2–62.2	59.8–62.8	60.4–63.4	60.8–63.9
100–104	10	57.9–60.7	58.6–61.5	59.3–62.2	59.9–62.8	60.5–63.5	61.1–64.1	61.7–64.7	62.3–65.4	62.9–66.0	63.5–66.6	64.0–67.1

TABLE 6.—*Conversion factors for percent "standard weight," adult males, 17+ years (corrected for shoe height and clothing weight)*—Continued

Standard weight group	Code	Height (cm)										
		156	157	158	159	160	161	162	163	164	165	166
105–109	11	60.8–63.6	61.6–64.4	62.3–65.2	62.9–65.8	63.6–66.5	64.2–67.2	64.8–67.8	65.5–68.5	66.1–69.1	66.7–69.8	67.2–70.3
110–114	12	63.7–66.5	64.5–67.3	65.3–68.1	65.9–68.8	66.6–69.5	67.3–70.2	67.9–70.9	68.6–71.6	69.2–72.3	69.9–73.0	70.4–73.6
115–119	13	66.6–69.4	67.4–70.3	68.2–71.1	68.9–71.8	69.6–72.6	70.3–73.3	71.0–74.0	71.7–74.7	72.4–75.4	73.1–76.2	73.7–76.8
120–124	14	69.5–72.3	70.4–73.2	71.2–74.1	71.9–74.8	72.7–75.6	73.4–76.3	74.1–77.1	74.8–77.8	75.5–78.6	76.3–79.3	76.9–80.0
125–129	15	72.4–75.2	73.3–76.1	74.2–77.1	74.9–77.8	75.7–78.6	76.4–79.4	77.2–80.2	77.9–80.9	78.7–81.7	79.4–82.5	80.1–83.2
130–134	16	75.3–78.1	76.2–79.1	77.2–80.0	77.9–80.8	78.7–81.6	79.5–82.5	80.3–83.3	81.0–84.1	81.8–84.9	82.6–85.7	83.3–86.4
135–139	17	78.2–81.0	79.2–82.0	80.1–83.0	80.9–83.8	81.7–84.7	82.6–85.5	83.4–86.3	84.2–87.2	85.0–88.0	85.8–88.9	86.5–89.6
140–144	18	81.1–83.9	82.1–84.9	83.1–85.9	83.9–86.8	84.8–87.7	85.6–88.6	86.4–89.4	87.3–90.3	88.1–91.2	89.0–92.0	89.7–92.8
145–149	19	84.0–86.8	85.0–87.9	86.0–88.9	86.9–89.8	87.8–90.7	88.7–91.6	89.5–92.5	90.4–93.4	91.3–94.3	92.1–95.2	92.9–96.0
150–154	20	86.9–89.8	88.0–91.0	89.0–92.0	89.9–92.9	90.8–93.8	91.7–94.7	92.6–95.7	93.5–96.6	94.4–97.5	95.3–98.5	96.1–99.3
		167	168	169	170	171	172	173	174	175	176	177
50–54	00	32.3–35.5	32.6–35.8	33.0–36.2	33.3–36.5	33.7–36.9	34.0–37.3	34.4–37.7	34.7–38.1	35.1–38.5	35.4–38.9	35.8–39.3
55–59	01	35.6–38.7	35.9–39.1	36.3–39.5	36.6–39.9	37.0–40.3	37.4–40.7	37.8–41.1	38.2–41.6	38.6–42.0	39.0–42.4	39.4–42.9
60–64	02	38.8–41.9	39.2–42.3	39.6–42.8	40.0–43.2	40.4–43.7	40.8–44.1	41.2–44.6	41.7–45.0	[illegible].1–45.5	42.5–45.9	43.0–46.5
65–69	03	42.0–45.2	42.4–45.6	42.9–46.1	43.3–46.5	43.8–47.0	44.2–47.5	44.7–48.0	45.1–48.5	[illegible].6–49.0	46.0–49.5	46.6–50.1
70–74	04	45.3–48.4	45.7–48.8	46.2–49.4	46.6–49.9	47.1–50.4	47.6–50.9	48.1–51.5	48.6–52.0	49.1–52.5	49.6–53.0	50.2–53.6
75–79	05	48.5–51.6	48.9–52.1	49.5–52.7	50.0–53.2	50.5–53.8	51.0–54.3	51.6–54.9	52.1–55.5	52.6–56.0	53.1–56.6	53.7–57.2
80–84	06	51.7–54.9	52.2–55.4	52.8–56.0	53.3–56.5	53.9–57.1	54.4–57.7	55.0–58.3	55.6–58.9	56.1–59.5	56.7–60.1	57.3–60.8
85–89	07	55.0–58.1	55.5–58.6	56.1–59.3	56.6–59.9	57.2–60.5	57.8–61.1	58.4–61.8	59.0–62.4	59.6–63.0	60.2–63.7	60.9–64.4
90–94	08	58.2–61.3	58.7–61.9	59.4–62.5	60.0–63.2	60.6–63.9	61.2–64.5	61.9–65.2	62.5–65.9	63.1–66.5	63.8–67.2	64.5–68.0
95–99	09	61.4–64.5	62.0–65.1	62.6–65.8	63.3–66.5	64.0–67.2	64.6–67.9	65.3–68.6	66.0–69.3	66.6–70.0	67.3–70.7	68.1–71.5
100–104	10	64.6–67.8	65.2–68.4	65.9–69.1	66.6–69.9	67.3–70.6	68.0–71.3	68.7–72.1	69.4–72.8	70.1–73.6	70.8–74.3	71.6–74.1
105–109	11	67.9–71.0	68.5–71.7	69.2–72.4	70.0–73.2	70.7–74.0	71.4–74.7	72.2–75.5	72.9–76.3	73.7–77.1	74.4–77.8	75.2–78.7
110–114	12	71.1–74.2	71.8–74.9	72.5–75.7	73.3–76.5	74.1–77.3	74.8–78.2	75.6–79.0	76.4–79.8	77.2–80.6	77.9–81.4	78.8–82.3
115–119	13	74.3–77.5	75.0–78.2	75.8–79.0	76.6–79.9	77.4–80.7	78.3–81.6	79.1–82.4	79.9–83.2	80.7–84.1	81.5–84.9	82.4–85.9
120–124	14	77.6–80.7	78.3–81.5	79.1–82.3	80.0–83.2	80.8–84.1	81.7–85.0	82.5–85.8	83.3–81.7	84.2–87.6	85.0–88.5	86.0–89.5
125–129	15	80.8–83.9	81.6–84.7	82.4–85.6	83.3–86.5	84.2–87.5	85.1–88.4	85.9–89.3	81.8–90.2	87.7–91.1	88.6–92.0	89.6–93.0
130–134	16	84.0–87.2	84.8–88.0	85.7–88.9	86.6–89.9	87.6–90.8	88.5–91.8	89.4–92.7	90.3–93.7	91.2–94.6	92.1–95.5	93.1–96.6
135–139	17	87.3–90.4	88.1–91.3	89.0–92.2	90.0–93.2	90.9–94.2	91.9–95.2	92.8–96.1	93.8–97.1	94.7–98.1	95.6–99.1	96.7–100.2
140–144	18	90.5–93.6	91.4–94.5	92.3–95.5	93.3–96.5	94.3–97.6	95.3–98.6	96.2–99.6	97.2–100.6	98.2–101.6	99.2–102.6	100.3–103.8
145–149	19	93.7–96.9	94.6–97.8	95.6–98.8	96.6–99.9	97.7–100.9	98.7–102.0	99.7–103.0	100.7–104.1	101.7–105.1	102.7–106.2	103.9–107.4
150–154	20	97.0–100.3	97.9–101.2	98.9–102.2	100.0–103.4	101.0–104.3	102.1–105.5	103.1–106.5	104.2–107.7	105.2–108.7	106.3–109.9	107.5–111.1

Standard weight group	Code	Height (cm)								
		178	179	180	181	182	183	184	185	186
50–54	00	36.2–39.7	36.7–40.2	37.1–40.7	37.5–41.2	37.9–41.6	38.3–42.0	38.9–42.4	39.1–42.9	39.5–43.3
55–59	01	39.8–43.4	40.3–43.9	40.8–44.5	41.3–44.9	41.7–45.4	42.1–45.8	42.5–46.3	43.0–46.8	43.4–47.3
60–64	02	43.5–47.0	44.0–47.6	44.6–48.2	45.0–48.7	45.5–49.2	45.9–49.7	46.4–50.2	46.9–50.7	47.4–51.2
65–69	03	47.1–50.6	47.7–51.2	48.3–51.9	48.8–52.4	49.3–53.0	49.8–53.5	50.3–54.0	50.8–54.6	51.3–55.2
70–74	04	50.7–54.2	51.3–54.9	52.0–55.6	52.5–56.2	53.1–56.8	53.6–57.3	54.1–57.9	54.7–58.5	55.3–59.1
75–79	05	54.3–57.9	55.0–58.6	55.7–59.3	56.3–59.9	56.9–60.6	57.4–61.1	58.0–61.8	58.6–62.4	59.2–63.1
80–84	06	58.0–61.5	58.7–62.2	59.4–63.0	60.0–63.7	60.7–64.4	61.2–65.0	61.9–65.6	62.5–66.3	63.2–67.0
85–89	07	61.6–65.1	62.3–65.9	63.1–66.7	63.8–67.4	64.5–68.2	65.1–68.8	65.7–69.5	66.4–70.2	67.1–70.9
90–94	08	65.2–68.7	66.0–69.6	66.8–70.4	67.5–71.2	68.3–71.9	68.9–72.6	69.6–73.4	70.3–74.1	71.0–74.9
95–99	09	68.8–72.3	69.7–73.2	70.5–74.1	71.3–74.9	72.0–75.7	72.7–76.4	73.5–77.2	74.2–78.0	75.0–78.8
100–104	10	72.4–76.0	73.3–76.9	74.2–77.9	75.0–78.7	75.8–79.5	76.5–80.3	77.3–81.1	78.1–81.9	78.9–82.8
105–109	11	76.1–79.6	77.0–80.6	78.0–81.6	78.8–82.5	79.6–83.3	80.4–84.1	81.2–84.9	82.0–85.9	82.9–86.7
110–114	12	79.7–83.2	80.7–84.3	81.7–85.3	82.6–86.2	83.4–87.1	84.2–87.9	85.0–88.9	86.0–89.8	86.8–90.7
115–119	13	83.3–86.8	84.4–87.9	85.4–89.0	86.3–90.0	87.2–90.9	88.0–91.8	89.0–92.7	89.9–93.7	90.8–94.6
120–124	14	86.9–90.5	88.0–91.6	89.1–92.7	90.1–93.7	91.0–94.7	91.9–95.6	92.8–96.6	93.8–97.6	94.7–98.6
125–129	15	90.6–94.1	91.7–95.3	92.8–96.4	93.8–97.5	94.8–98.5	95.7–99.4	96.7–100.5	97.7–101.5	98.7–102.5
130–134	16	94.2–97.7	95.4–98.9	96.5–100.1	97.6–101.2	98.6–102.3	99.5–103.2	100.6–104.3	101.6–105.4	102.6–106.5
135–139	17	97.8–101.3	99.0–102.6	100.2–103.9	101.3–105.0	102.4–106.1	103.3–107.1	104.4–108.2	105.5–109.3	106.6–110.4
[illegible]	18	101.4–105.0	102.7–106.3	104.0–107.6	105.1–108.7	106.2–109.9	107.2–110.9	108.3–112.1	109.4–113.2	110.5–114.4
145–149	19	105.1–108.6	106.4–109.9	107.7–111.3	108.8–112.5	110.0–113.7	111.0–114.7	112.2–115.9	113.3–117.1	114.5–118.3
150–154	20	108.7–112.3	110.0–113.6	111.4–115.1	112.6–116.4	113.8–117.6	114.8–118.6	116.0–119.8	117.2–121.1	118.4–122.3

TABLE 7.—*Conversion factors for percent "standard weight," adult females, 17+ years (corrected for shoe height and clothing weight)*

Standard weight group	Code	Height (cm)										
		140	141	142	143	144	145	146	147	148	149	150
50–54	00	22.5–24.6	22.7–24.9	23.0–25.2	23.2–25.4	23.5–25.8	23.8–26.1	24.0–26.3	24.3–26.7	24.6–27.0	24.9–27.3	25.2–27.6
55–59	01	24.7–26.9	25.0–27.2	25.3–27.5	25.5–27.8	25.9–28.1	26.2–28.4	26.4–28.7	26.8–29.1	27.1–29.5	27.4–29.8	27.7–30.2
60–64	02	27.0–29.1	27.3–29.4	27.6–29.8	27.9–30.1	28.2–30.5	28.5–30.8	28.8–31.1	29.2–31.5	29.6–31.9	29.9–32.3	30.3–32.7
65–69	03	29.2–31.4	29.5–31.7	29.9–32.1	30.2–32.4	30.6–32.8	30.9–33.2	31.2–33.5	31.6–33.9	32.0–34.4	32.4–34.8	32.8–35.2
70–74	04	31.5–33.6	31.8–34.0	32.2–34.4	32.5–34.7	32.9–35.2	33.3–35.6	33.6–35.9	34.0–36.4	34.5–36.8	34.9–37.3	35.3–37.7
75–79	05	33.7–35.9	34.1–36.3	34.5–36.7	34.8–37.1	35.3–37.5	35.7–37.9	36.0–38.3	36.5–38.8	36.9–39.3	37.4–39.8	37.8–40.3
80–84	06	36.0–38.1	36.4–38.5	36.8–39.0	37.2–39.4	37.6–39.9	38.0–40.3	38.4–40.7	38.9–41.3	39.4–41.8	39.9–42.3	40.4–42.8
85–89	07	38.2–40.3	38.6–40.8	39.1–41.3	39.5–41.7	40.0–42.2	40.4–42.7	40.8–43.1	41.4–43.7	41.9–44.2	42.4–44.8	42.9–45.3
90–94	08	40.4–42.6	40.9–43.1	41.4–43.5	41.8–44.0	42.3–44.6	42.8–45.1	43.2–45.5	43.8–46.1	44.3–46.7	44.9–47.3	45.4–47.8
95–99	09	42.7–44.8	43.2–45.3	43.6–45.8	44.1–46.3	44.7–46.9	45.2–47.4	45.6–47.9	46.2–48.5	46.8–49.1	47.4–49.7	47.9–50.3
100–104	10	44.9–47.1	45.4–47.6	45.9–48.1	46.4–48.7	47.0–49.3	47.5–49.8	48.0–50.3	48.6–51.0	49.2–51.6	49.8–52.2	50.4–52.9
105–109	11	47.2–49.3	47.7–49.9	48.2–50.	48.8–51.0	49.4–51.7	49.9–52.2	50.4–52.7	51.1–53.4	51.7–54.1	52.3–54.7	53.0–55.4
110–114	12	49.4–51.6	50.0–52.2	50.5–52.7	51.1–53.3	51.8–54.0	52.3–54.6	52.8–55.2	53.5–55.8	54.2–56.5	54.8–57.2	55.5–57.9
115–119	13	51.7–53.8	52.3–54.4	52.8–55.0	53.4–55.6	54.1–56.4	54.7–57.0	55.3–57.6	55.9–58.3	56.6–59.0	57.3–59.7	58.0–60.4
120–124	14	53.9–56.1	54.5–56.7	55.1–57.3	55.7–58.0	56.5–58.7	57.1–59.3	57.7–60.0	58.4–60.7	59.1–61.5	59.8–62.2	60.5–63.0
125–129	15	56.2–58.3	56.8–59.0	57.4–59.6	58.1–60.3	58.8–61.1	59.4–61.7	60.1–62.4	60.8–63.1	61.6–63.9	62.3–64.7	63.1–65.5
130–134	16	58.4–60.6	59.1–61.3	59.7–61.9	60.4–62.6	61.2–63.4	61.8–64.1	62.5–64.8	63.2–65.6	64.0–63.4	64.8–67.2	65.6–68.0
135–139	17	60.7–62.8	61.4–63.5	62.0–64.2	62.7–64.9	63.5–65.8	64.2–66.5	64.9–67.2	65.7–68.0	66.5–68.9	67.3–69.7	68.1–70.5
140–144	18	62.9–65.1	63.6–65.8	64.3–66.5	65.0–67.3	65.9–68.1	66.6–68.8	67.3–69.6	68.1–70.4	69.0–71.3	69.8–72.2	70.6–73.1
145–149	19	65.2–67.3	65.9–68.1	66.6–68.8	67.4–69.6	68.2–70.5	68.9–71.2	69.7–72.0	70.5–72.9	71.4–73.8	72.3–74.7	73.2–75.5
150–154	20	67.4–69.5	68.2–70.5	68.9–71.2	69.7–72.0	70.6–73.0	71.3–73.7	72.1–74.5	73.0–75.5	73.9–76.4	74.8–77.3	75.6–78.0

Standard weight group	Code	151	152	153	154	155	156	157	158	159	160	161
50–54	00	25.5–28.0	25.8–28.3	26.0–28.5	26.3–28.8	26.6–29.1	26.9–29.5	27.2–29.8	27.5–30.1	27.8–30.5	28.1–30.8	28.5–31.2
55–59	01	28.1–30.5	28.4–30.8	28.6–31.1	28.9–31.4	29.2–31.8	29.6–32.1	29.9–32.5	30.2–32.9	30.6–33.2	30.9–33.7	31.3–34.1
60–64	02	30.6–33.1	30.9–33.4	31.2–33.7	31.5–34.1	31.9–34.4	32.2–34.8	32.6–35.2	33.0–35.6	33.3–36.0	33.8–36.5	34.2–36.9
65–69	03	33.2–35.6	33.5–36.0	33.8–36.3	34.2–36.7	34.5–37.1	34.9–37.5	35.3–37.9	35.7–38.4	36.1–38.8	36.6–39.3	37.0–39.8
70–74	04	35.7–38.2	36.1–38.8	36.4–38.9	36.8–39.3	37.2–39.8	37.6–40.2	38.0–40.7	38.5–41.1	38.9–41.6	39.4–42.1	39.9–42.6
75–79	05	38.3–40.7	38.9–41.1	39.0–41.5	39.4–41.9	39.9–42.4	40.3–42.9	40.8–43.4	41.2–43.9	41.7–44.3	42.2–44.9	42.7–45.5
80–84	06	40.8–43.3	41.2–43.7	41.6–44.1	42.0–44.6	42.5–45.1	43.0–45.6	43.5–46.1	44.0–46.6	44.4–47.1	45.0–47.7	45.6–48.3
85–89	07	43.4–45.8	43.8–46.3	44.2–46.7	44.7–47.2	45.2–47.7	45.7–48.3	46.2–48.8	46.7–49.3	47.2–49.9	47.8–50.5	48.4–51.1
90–94	08	45.9–48.4	46.4–48.9	46.8–49.3	47.3–49.8	47.8–50.4	48.4–51.4	48.9–51.5	49.4–52.1	50.0–52.7	50.6–53.3	51.2–54.0
95–99	09	48.5–50.9	49.0–51.4	49.4–51.9	49.9–52.4	50.5–53.0	51.5–53.6	51.6–54.2	52.2–54.8	52.8–55.4	53.4–56.1	54.1–56.8
100–104	10	51.0–53.5	51.5–54.0	52.0–54.5	52.5–55.1	53.1–55.7	53.7–56.3	54.3–57.0	54.9–57.6	55.5–58.2	56.2–59.0	56.9–59.7
105–109	11	53.6–56.1	54.1–56.6	54.6–57.1	55.2–57.7	55.8–58.4	56.4–59.0	57.1–59.7	57.7–60.3	58.3–61.0	59.1–61.8	59.8–62.5
110–114	12	56.2–58.6	56.7–59.2	57.2–59.8	57.8–60.3	58.5–61.0	59.1–61.7	59.8–62.4	60.4–63.1	61.1–63.8	61.9–64.6	62.6–65.4
115–119	13	58.7–61.2	59.3–61.8	59.9–62.4	60.4–63.0	61.1–63.7	61.8–64.4	62.5–65.1	63.2–65.8	63.9–66.6	64.7–67.4	65.5–68.2
120–124	14	61.3–63.7	61.9–64.3	62.5–65.0	63.1–65.6	63.8–66.3	64.5–67.1	65.2–67.8	65.9–68.6	66.7–69.3	67.5–70.2	68.3–71.1
125–129	15	63.8–66.3	64.4–66.9	65.1–67.6	65.7–68.2	66.4–69.0	67.2–69.8	67.9–70.5	68.7–71.3	69.4–72.1	70.3–73.0	71.2–73.9
130–134	16	66.4–68.8	67.0–69.5	67.7–70.2	68.3–70.8	69.1–71.7	69.9–72.5	70.6–73.3	71.4–74.1	72.2–74.9	73.1–75.8	74.0–76.8
135–139	17	68.9–71.4	69.6–72.1	70.3–72.8	70.9–73.5	71.8–74.3	72.6–75.1	73.4–76.0	74.2–76.8	75.0–77.7	75.9–78.7	76.9–79.6
140–144	18	71.5–73.9	72.2–74.6	72.9–75.4	73.6–76.1	74.4–77.0	75.2–77.8	76.1–78.7	76.9–79.6	77.8–80.4	78.8–81.5	79.7–82.5
145–149	19	74.0–76.5	74.7–77.2	75.5–78.0	76.2–78.7	77.1–79.6	77.9–80.5	78.8–81.4	79.7–82.3	80.5–83.2	81.6–84.3	82.6–85.3
150–154	20	76.6–79.2	77.3–79.9	78.1–80.7	78.8–81.4	79.7–82.3	80.6–83.3	81.5–84.2	82.4–85.1	83.3–86.1	84.4–87.2	85.4–88.2

Standard weight group	Code	Height (cm)							
		162	163	164	165	166	167	168	169
50–54	00	28.8–31.6	29.2–32.0	29.5–32.3	29.8–32.7	30.1–33.0	30.4–33.3	30.7–33.7	31.1–34.1
55–59	01	31.7–34.5	32.1–34.9	32.4–35.3	32.8–35.6	33.1–36.0	33.4–36.3	33.8–36.8	34.2–37.2
60–64	02	34.6–37.4	35.0–37.8	35.4–38.2	35.7–38.6	36.1–39.0	36.4–39.4	36.9–39.8	37.3–40.3
65–69	03	37.5–40.3	37.9–40.7	38.3–41.2	38.7–41.6	39.1–42.0	39.5–42.4	39.9–42.9	40.4–43.4
70–74	04	40.4–43.1	40.8–43.7	41.3–44.1	41.7–44.6	42.1–45.0	42.5–45.5	43.0–46.0	43.5–46.5
75–79	05	43.2–46.0	43.8–46.6	44.2–47.1	44.7–47.5	45.1–48.0	45.6–48.5	46.1–49.1	46.6–49.6
80–84	06	46.1–48.9	46.7–49.5	47.2–50.0	47.6–50.5	48.1–51.0	48.6–51.5	49.2–52.1	49.7–52.7
85–89	07	49.0–51.8	49.6–52.4	50.1–52.9	50.6–53.5	51.1–54.0	51.6–54.6	52.2–55.2	52.8–55.8
90–94	08	51.9–54.7	52.5–55.3	53.0–55.9	53.6–56.5	54.1–57.0	54.7–57.6	55.3–58.3	55.9–58.9
95–99	09	54.8–57.5	55.4–58.2	56.0–58.8	56.6–59.4	57.1–60.0	57.7–60.6	58.4–61.3	59.0–62.0
100–104	10	57.6–60.4	58.3–61.2	58.9–61.8	59.5–62.4	60.1–63.1	60.7–63.7	61.4–64.4	62.1–65.1
105–109	11	60.5–63.3	61.3–64.1	61.9–64.7	62.5–65.4	63.2–66.1	63.8–66.7	64.5–67.5	65.2–68.3
110–114	12	63.4–66.2	64.2–67.0	64.8–67.7	65.5–68.4	66.2–69.1	66.8–69.8	67.6–70.6	68.4–71.4
115–119	13	66.3–69.1	67.1–69.9	67.8–70.6	68.5–71.4	69.2–72.1	69.9–72.8	70.7–73.6	71.5–74.5
120–124	14	69.2–72.0	70.0–72.8	70.7–73.6	71.5–74.3	72.2–75.1	72.9–75.8	73.7–76.7	74.6–77.6
125–129	15	72.1–74.8	72.9–75.8	73.7–76.5	74.4–77.3	75.2–78.1	75.9–78.9	76.8–79.8	77.7–80.7
130–134	16	74.9–77.7	75.9–78.7	76.6–79.5	77.4–80.3	78.2–81.1	79.0–81.9	79.9–82.9	80.8–83.8
135–139	17	77.8–80.6	78.8–81.6	79.6–82.4	80.4–83.3	81.2–84.1	82.0–84.9	83.0–85.9	83.9–86.9
140–144	18	80.7–83.5	81.7–84.5	82.5–85.4	83.4–86.2	84.2–87.1	85.0–88.0	86.0–89.0	87.0–90.0
145–149	19	83.6–86.4	84.6–87.4	85.5–88.3	86.3–89.2	87.2–90.1	88.1–91.0	89.1–92.1	90.1–93.1
150–154	20	86.5–89.4	87.5–90.4	88.4–91.3	89.3–92.3	90.2–93.2	91.1–94.1	92.2–95.3	93.2–96.3

TABLE 8.—*Weight-height age standards for boys of school age*[1]

Height (centimeters)	6 years	7 years	8 years	9 years	10 years	11 years	12 years	13 years	14 years	15 years	16 years	17 years	18 years	19 years	Height (centimeters)
	Weight (kg)														
97	*15.0														97
98	*15.1														98
99	*15.3														99
100	*15.5														100
101	*15.7														101
102	*15.9														102
103	*16.1														103
104	16.6														104
105	16.8	*16.8													105
106	17.0	*17.0													106
107	17.2	*17.2	*17.2												107
108	17.5	*17.6	*17.4												108
109	17.8	*17.9	*17.7												109
110	18.3	*18.4	*18.2												110
111	18.9	*18.9	*18.8												111
112	19.4	19.4	*19.4												112
113	19.7	19.7	*19.7												113
114	20.0	20.0	*20.0												114
115	20.4	20.4	*20.4	*20.3											115
116	20.7	20.8	20.7	*20.6											116
117	21.1	21.8	21.1	*21.2											117
118	21.5	21.5	21.4	*21.4											118
119	21.9	21.9	21.8	*21.7											119
120	22.3	22.4	22.2	*22.2	*22.2										120
121	22.5	22.8	22.7	*22.7	*22.7										121
122	22.9	23.2	23.1	23.2	*23.2										122
123	23.4	23.5	23.6	23.6	*23.5										123
124	23.9	23.8	24.1	24.0	*23.8										124
125	24.3	24.3	24.5	24.4	24.2	*24.4									125
126	*24.6	24.9	24.9	24.9	24.8	*24.9									126
127	*25.0	25.3	25.3	25.3	25.4	*25.4	*25.4								127
128		25.9	25.9	25.7	25.9	*25.9	*25.9								128
129		26.4	26.5	26.2	26.4	*26.4	*26.4								129

130		26.9	27.0	26.7	26.8	27.0	*26.9							130
131		27.2	27.4	27.3	27.3	27.5	*27.3							131
132		27.5	27.9	27.8	27.8	28.0	27.7							132
133		*28.2	28.4	28.4	28.3	28.5	28.3	*28.5						133
134		*28.5	28.9	28.9	28.8	28.9	29.0	*29.3						134
135			29.4	29.4	29.3	29.4	29.6	*29.9						135
136			30.0	29.9	29.7	29.8	30.3	*30.3						136
137			30.6	30.4	30.2	30.2	30.9	*30.7						137
138			*30.9	30.8	30.9	30.8	31.3	31.2	*31.7					138
139			*31.2	31.1	31.5	31.5	31.9	31.8	*32.0					139
140			*31.6	31.6	32.2	32.2	32.4	32.4	*32.5					140
141			*32.1	32.3	32.9	32.8	32.9	33.2	*33.1					141
142			*32.6	33.1	33.7	33.5	33.4	34.0	*33.7					142
143				*33.6	34.1	34.2	34.1	34.7	34.5	*35.3				143
144				*34.1	34.4	35.0	34.7	35.2	35.5	*35.8				144
145				*34.6	34.9	35.7	35.4	35.8	36.3	*36.3				145
146				*35.3	35.7	36.2	36.2	36.5	36.9	*37.0				146
147				*36.0	36.5	36.7	36.9	37.1	37.4	*37.7				147
148					37.0	37.2	37.6	37.8	38.0	38.2				148
149					37.5	37.8	38.2	38.4	38.6	38.7				149
150					38.1	38.5	39.0	39.1	39.3	39.3	39.2			150
151					*38.7	39.2	39.5	39.7	40.0	40.3	40.3			151
152					*39.4	39.9	40.0	40.3	40.7	41.3	41.5			152
153						40.5	40.6	41.1	41.6	42.3	42.6			153
154						41.0	41.4	41.9	42.5	42.8	43.7			154
155						41.5	42.1	42.7	43.4	43.5	44.8	*46.3		155
156						*42.3	42.9	43.4	44.0	44.2	45.5	*47.2		156
157						*43.2	43.8	44.1	44.7	44.9	46.3	48.1		157
158						*44.0	44.6	44.9	45.5	45.8	47.3	49.2	*51.3	158
159						*44.9	45.4	45.8	46.4	46.9	48.6	50.3	*52.6	159
160						*45.8	46.2	46.7	47.4	48.0	49.8	51.5	53.9	*55.4	160
161							47.4	47.3	48.1	48.8	50.2	52.0	54.4	*55.9	161
162							48.7	48.0	48.8	49.6	50.6	52.5	54.9	*56.4	162
163							49.4	48.8	49.6	50.5	51.2	53.2	55.5	*57.0	163
164							*49.6	49.9	50.5	51.4	52.1	54.1	56.3	*57.7	164
165							*49.7	50.9	51.4	52.3	53.1	55.1	57.1	58.3	165
166								51.4	52.1	53.2	54.1	56.1	57.9	59.3	166
167								51.8	52.9	54.0	55.1	57.0	58.7	60.3	167
168								52.3	53.7	54.8	56.1	57.8	59.4	61.0	168
169								*53.1	54.7	55.7	57.1	58.5	60.0	64.4	169

See footnotes at end of table.

TABLE 8.—*Weight-height-age standards for boys of school age*[1]—Continued

Height (centimeters)	6 years	7 years	8 years	9 years	10 years	11 years	12 years	13 years	14 years	15 years	16 years	17 years	18 years	19 years	Height (centimeters)
	Weight (kg)														
170								*53.9	55.6	56.5	58.1	59.1	60.5	61.7	170
171									56.7	57.3	58.8	59.9	61.2	62.5	171
172									57.8	58.0	59.5	60.9	62.0	63.4	172
173									58.7	58.7	60.2	61.8	62.8	64.2	173
174									59.2	59.6	61.1	62.7	63.8	65.1	174
175									59.7	60.4	61.9	63.5	64.7	65.9	175
176									60.5	61.2	62.5	64.0	65.3	66.5	176
177									61.4	62.0	62.9	64.3	65.7	67.2	177
178									62.4	62.8	63.4	64.7	66.1	67.8	178
179									*63.3	63.9	64.5	65.4	66.6	68.4	179
180									*64.2	65.1	65.7	66.1	67.1	68.8	180
181										65.8	66.4	66.7	67.7	69.6	181
182										66.3	67.0	67.3	68.3	70.4	182
183										66.9	67.6	68.0	69.0	71.2	183
184										*67.5	68.6	69.1	70.1	71.8	184
185										*68.2	69.5	70.3	71.3	72.5	185
186										*68.8	70.3	71.3	72.2	73.2	186
187										*69.3	71.0	72.3	73.1	73.8	187
188										*69.8	71.7	73.3	73.9	74.4	188

[1] Starred (*) figures represent values based upon theoretical computations, rather than on exact averages. Age is taken at the nearest birthday, height at the nearest centimeter, weight at the nearest tenth of a kilogram. Translated and extended from the Baldwin-Wood tables in the English system of measurement by B. T. Baldwin, Ph. D. Issued by the Iowa Child Welfare Research Station, State University of Iowa, Iowa City, Iowa, September 1924.

TABLE 9.—*Weight-height-age standards for girls of school age*[1]

Height (centimeters)	6 years	7 years	8 years	9 years	10 years	11 years	12 years	13 years	14 years	15 years	16 years	17 years	18 years	Height (centimeters)
	Weight (kg)													
100	15.3													100
101	15.7													101
102	16.0	*16.0												102
103	16.1	*16.1												103
104	16.3	*16.3												104
105	16.6	*16.6												105
106	16.8	*16.8												106
107	17.1	17.1												107
108	17.6	17.5												108
109	18.0	17.8												109
110	18.2	18.1	*18.2											110
111	18.4	18.5	*18.4											111
112	18.6	18.8	*18.6											112
113	19.2	19.3	*19.1											113
114	19.7	19.8	*19.7											114
115	20.2	20.1	20.2											115
116	20.6	20.3	20.8											116
117	21.0	20.5	21.3	*21.2	*21.2									117
118	21.4	21.1	21.5	*21.5	*21.5									118
119	21.8	21.8	21.8	*21.9	*21.9									119
120	22.3	22.3	22.2	*22.3	*22.3									120
121	22.7	22.7	22.7	*22.7	*22.9									121
122	23.1	23.1	23.1	23.2	23.4	*23.4								122
123	23.3	23.4	23.5	23.6	23.3	*23.7								123
124	23.4	23.7	23.8	24.2	24.1	*24.0								124
125	23.7	24.1	24.3	24.7	24.6	*24.7								125
126	*24.2	24.4	24.8	25.2	25.3	*25.8								126
127	*24.7	24.8	25.4	25.8	26.0	25.9	*27.3							127
128		25.2	25.8	26.2	26.4	27.2	*27.1							128
129		25.7	26.2	26.6	26.7	27.5	26.9							129
130		*26.3	26.7	27.0	27.1	27.9	27.3							130
131		*27.1	27.4	27.5	27.7	28.2	28.4							131
132		*27.8	28.1	28.0	28.2	28.6	29.5							132
133		*28.4	28.6	28.6	28.8	29.1	29.9							133
134		*28.9	29.1	29.2	29.5	29.6	30.3							134

See footnotes at end of table.

TABLE 9.—*Weight-height-age standards for girls of school age*[1]—Continued

Height (centimeters)	6 years	7 years	8 years	9 years	10 years	11 years	12 years	13 years	14 years	15 years	16 years	17 years	18 years	Height (centimeters)
	Weight (kg)													
135			29.6	29.8	30.1	30.1	30.7	*31.5						135
136			30.0	30.4	30.5	30.7	31.0	*31.9						136
137			30.4	31.0	31.0	31.3	31.4	*32.2						137
138			*30.9	31.6	31.6	31.9	32.0	*32.8						138
139			*31.4	32.3	32.3	32.5	32.6	*33.4						139
140				32.9	32.9	33.1	33.2	34.1	*34.8					140
141				33.3	33.6	33.7	34.0	34.9	*35.6					141
142				33.6	34.4	34.3	34.8	35.8	*36.5					142
143				*34.2	35.1	35.0	35.5	36.3	*37.4					143
144				*34.9	35.9	35.6	36.0	36.7	*38.4					144
145					36.6	36.4	36.6	37.2	39.3	*41.1				145
146					36.8	37.3	37.3	38.0	40.3	*42.0				146
147					37.1	38.2	37.9	38.8	41.4	*42.8				147
148					37.6	38.9	38.6	39.5	42.0	*43.5	*45.1			148
149					38.2	39.5	39.2	40.3	42.5	*44.0	*45.5			149
150					38.8	40.2	39.9	41.1	43.0	44.6	*45.9	*46.4		150
151					*39.5	41.0	40.8	41.9	43.8	45.5	*46.8	*47.3		151
152					*40.2	41.8	41.7	42.7	44.6	46.4	*47.7	48.1		152
153						42.6	42.7	43.5	45.4	47.1	48.6	48.9	*49.9	153
154						43.4	43.8	44.2	46.2	47.6	49.4	49.7	*50.7	154
155						44.2	44.8	45.0	47.0	48.1	50.2	50.4	51.4	155
156						*44.1	45.5	45.7	47.5	48.9	50.7	51.1	51.7	156
157						*44.0	46.2	46.5	48.1	49.8	51.1	51.8	52.0	157
158							47.0	47.4	48.7	50.5	51.4	52.2	52.4	158
159							47.9	48.3	49.2	51.0	51.7	52.5	52.7	159
160							48.9	49.2	49.8	51.5	51.9	52.8	53.1	160
161							*49.6	49.9	50.7	52.1	52.6	53.3	53.6	161
162							*50.3	50.6	51.5	52.7	53.2	53.7	54.0	162
163							*51.0	51.4	52.3	53.3	53.8	54.2	54.6	163
164							*51.7	52.2	53.2	53.7	54.3	54.8	55.3	164
165							*52.4	53.1	54.0	54.2	54.8	55.4	55.9	165
166								54.0	54.5	54.6	55.7	56.1	56.6	166
167								54.9	54.9	55.0	56.6	56.9	57.4	167
168								*55.6	55.5	55.7	57.4	57.6	58.2	168
169								*56.2	56.6	56.9	58.2	58.2	59.2	169

								*56.8	57.6	58.0	58.9	58.9	60.1	170
170								*57.2	58.2	58.8	59.5	59.7	60.7	171
171								*57.8	58.7	59.5	60.0	60.7	61.1	172
172									59.1	60.1	60.5	61.4	61.6	173
173									*59.6	*60.5	*60.9	*61.8	*62.3	174
174									*60.0	*60.8	*61.2	*62.1	*62.9	175
175									*60.2	*61.0	*61.6	*62.5	*63.4	176
176									*60.4	*61.2	*62.0	*62.8	*63.7	177
177									*60.6	*61.5	*62.4	*63.2	*64.0	178
178									*60.9	*61.8	*62.7	*63.5	*64.2	179
179									*61.3	*62.2	*63.0	*63.9	*64.4	180
180														

[1] Starred (*) figures represent values based upon theoretical computations, rather than on exact averages. Age is taken at the nearest birthday, height at the nearest centimeter, weight at the nearest tenth of a kilogram. Translated and extended from the Baldwin-Wood tables in the English system of measurement by B. T. Baldwin, Ph. D. Issued by the Iowa Child Welfare Research Station, State University of Iowa, Iowa City, Iowa, September 1924.

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Clinically evident frank deficiency syndromes, such as pellagra, moderate rickets, beriberi, kwashiorkor, are readily recognized. The earlier or milder evidences of nutrient deficiencies are not so characteristic or specific as is a full-blown syndrome. In nutrition surveys of ambulatory populations it is these milder signs which are more frequently encountered. The survey technic demands the isolated recording of signs without permitting the examiner to formulate a diagnosis in an individual case. This role of noninterpretive observer initially is not easy for the clinician to assume. Accordingly some effort is required to assure a meaningful uniformity of recording of his observations regardless of his "clinical impression" as to pathogenesis of the sign observed. The clinician recognizes that multiple factors may give rise to a sign which he is recording in a nutrition survey, to which he is seemingly imputing nutritional significance, and concerning which, in a given case, he may be of the opinion that the particular sign is most likely not of nutritional origin. These considerations may lead him to tend to underrecord or overrecord the occurrence of the lesion.

In interpreting the significance of the clinical observations tabulated it is essential, therefore, to consider relevant data from other sources—biochemical and dietary—as well as other clinically obtained knowledge. Rarely is it rational to state that the occurrence of a single lesion within a given percentage of examinees is clear evidence of a particular deficiency. One exception is significant enlargement of the thyroid gland among 5 percent or more of the nonpregnant population. The occurrence of a *syndrome*, on the other hand, may be valid evidence of deficiency; for example, the presence of pellagra, beriberi, hypochromic microcytic anemia with its associated clinical findings, and the like.

Of the signs recorded, the following frequently may result from nutritional deficiencies or may give an index of nutritional status. In all instances the clinical interpretation must be made with recognition of the differential points which may distinguish the nutritionally caused from some nonnutritionally caused condition.

Xerosis conjunctiva
Bitot's spots
Circumcorneal injection (bilateral)
Nasolabial seborrhea
Angular lesions
Angular scars
Cheilosis
Marginal redness of gums
Marginal swelling of gums
Swollen red papillae
Bleeding gums
Carious teeth
Fluorosis
Filiform papillary atrophy of the tongue
Fissures, erosions, or ulcers of the tongue
Serrations or swellings
Red, tip, and/or lateral margins only
Red, scarlet, beefy (glossitis)
Magenta colored
Thyroid enlarged (visible)
Follicular hyperkeratosis of arms, back, chest, thighs, buttocks
Perifolliculosis
Dry or scaling skin (xerosis)
Crackled skin
Thickened pressure points (other than elbow and knees)
Purpura or petechiae
Hyperpigmentation of the hands
Pellagrous dermatitis
Scrotal dermatitis
Bilateral edema
Loss of ankle jerk
Calf tenderness
Loss of knee jerk
Loss of vibratory sense (malleoli)
Motor weakness
Costochondral beading
Epiphyseal enlargement
Tourniquet test (positive)

Among infants the most useful signs differ somewhat from those for adults. Again, it is important to consider these signs in relation to other observations in order properly to interpret the observations. The more useful signs for infants and small children (under 6 years of age) within populations with significant malnutrition of this age group are those listed on the pediatric card (fig. 3, ch. V). This card likewise affords an opportunity for the observer to record a clinical impression as to the presence of protein-Calorie malnutrition; i.e., to

indicate if the child leaves an impression of having marasmus, kwashiorkor, or prekwashiorkor. Such a decision is justifiable on the basis of the frequency with which these signs occur in many populations and the ability of the physician rapidly to arrive at a tentative diagnosis. The clinical signs of malnutrition listed on the pediatric card are generally evidences of more severe disease than many of the signs on the detailed card for adults. This distinction is due to the nature of malnutrition as seen in the field—usually a more rapidly occurring, more severe clinical condition among infants and young children than among ambulatory adults (except in epidemics of starvation or when endemic disease, such as pellagra, beriberi, or endemic goiter, is prevalent).

Height-weight data in relation to age provide an index of adequacy or abundance of caloric supply of the dietary for both adults and infants and children. The mean pattern of weight and length (height) of infants and children reveals the average caloric adequacy of the diet and many periods during which marked interference with adequate nutrition may limit growth. When such interference occurs with a resulting plateau of the growth curve during the interval from 6 months to 2–4 years of age, it frequently reflects protein-Calorie malnutrition and associated deficiency states, infectious diseases, or diarrheal episodes.

Examination of individual growth records (charts) in infant clinics (well-baby clinics of health departments or of hospitals) and in schools provides confirmation and extension of data based on averages of measurements taken at a single season during the survey. Review of such records often will quickly reveal the nature of the pediatric problems associated with the period of growth retardation.

Ideally, for any population, standards of expected or desirable growth should be developed for the particular ethnic or racial group. Unfortunately, for no population is there a wholly satisfactory set of such standards. For most areas there are no standards based on locally made observations. For purposes of comparing one population and dietary level with another, to compare *pattern* of growth of children, and in order to have a single reference against which to view data, this Manual includes a set of height-weight-age tables and charts developed for a U.S. population. While not necessarily ideal even for the American population, and certainly not necessarily applicable to all other populations, these references do allow a comparison relative to some fixed point. The comparisons, especially of the "percent of standard weight," should be interpreted with recognition of their limitations. Similar comments pertain to data on skinfold thickness. In some instances data on skinfold thickness may permit a calculation of total body fat.

Changes in the hair are of little nutritional significance except in infants and young children. Here thinning, easy pluckability, altered texture or dyspigmentation (light bands, abnormal reddish coloration) may reflect protein-Calorie malnutrition. Color changes may also occur due to application of dye, bleaches, or excessive exposure to extreme environmental conditions. The bandlike appearance is important in distinguishing those of nutritional etiology.

Among infants and young children the more common nutritionally caused signs of the eye are xerophthalmia and keratomalacia, evidences of severe avitaminosis A. Circumcorneal injection (bilateral) may result from ariboflavinosis. The presence of Bitot's spots is not a specific sign of avitaminosis A, but the spots do occur in quite young children, at times in association with a deficiency of the vitamin. The other eye signs listed are to be noted as differential diagnoses from the nutritionally related ones.

Nasolabial seborrhea may reflect a deficiency of riboflavin, of niacin, of vitamin B_6, or may not be nutritionally related. In the latter instance, it is frequently associated with acne or with seborrheic dermatitis elsewhere.

Cheilosis and/or angular lesions may result from a lack of riboflavin, nacin, vitamin B_6, or iron. Angular lesions may result from ill-fitting dentures ("pseudoariboflavinosis") in the older person; or represent what may be a chronic infection, one type of perlêche. Cheilosis sometimes is due to exposure at high altitudes, and is readily confused with ordinary wind chapping in dry climates. Unilateral angular lesions are seldom nutritionally based and may represent herpetic lesions, impetigo, or other infections.

While the peridental gingivitis (swollen, red, bleeding gums) of active scurvy is relatively readily recognized, it is not possible categorically to attribute any of the other components of gingivitis directly to a single specific deficiency state. There seems to be a mild interaction between marginal gingivitis and ascorbic acid nutriture, but this is not sufficient to attribute a causal relationship to the vitamin lack. Periodontal disease is discussed in the dental section. The important interpretative point to bear in mind concerning this condition is its role in increasing the DMF (decayed, missing, filled teeth) rate among adults in a population, often without evidence of prevalent dental caries. Dental and oral hygiene are of great importance in control of periodontal disease.

The section on dental examination outlines the preferred method of describing the teeth. The clinical cards provide but a rough assessment of their condition which is of relatively little value if the dental data are also available. In the absence of a special dental examination the data on the detailed examination card (fig. 2, ch. V), Nos. 19–25, are useful in assessing whether dental or gross periodontal dis-

cases are widespread, in determining the probable significance of some angular lesions, or other oral changes, and in detecting gross or moderate-to-severe fluorosis.

Many influences, nutritional and nonnutritional, result in similar changes in the surface of the tongue. The most significant nutritional tongue lesions are likely to be the smooth, often pale, atrophic-appearing lingual surface associated with anemias of vitamin B_{12}-folacin etiology or with iron-deficiency anemia, or the red, painful tongue with denudation of the epithelium, with or without fissures. The latter again may be associated with any of the nutritionally caused anemias due to deficiencies of vitamin B_{12}, folacin, iron, vitamin B_6 (rarely), or may reflect a lack of niacin (and tryptophan as in pellagra) or riboflavin (especially classic magenta-colored tongue). Nutritionally caused lesions may be of all degrees and stages and involve but the tip and/or margin. The listings 26-34 offer descriptive groupings. No single one can be interpreted as diagnostic of a particular deficiency state. A furrowed or scrotal appearing surface, presence of serrated margins, or geographic tongue are not held as likely to be nutritionally related. In individual cases diagnosis of a glossitis must consider many differential points (see discussion in ch. V), but the presence of moderate degrees of atrophy of the filiform papillae, fissures, redness or magenta-colored tongue in even a small percentage of a population is highly suggestive of a deficiency of some member of the B-complex (B_{12}, folacin, niacin, B_6) or of iron.

Enlargement of the parotid glands is widely encountered and has often been described as occurring among protein-deficient populations. It is not possible to attach any specific nutritional significance to this finding even when the prevalence is high.

Endemic goiter is most probably the only cause of widespread thyroid enlargement throughout a population (including men and nonpregnant, nonlactating women). When so encountered with a prevalence of 5 percent or more of grade II or greater, it is widely agreed to constitute evidence of iodine deficiency of public health significance.

Changes in the skin which may be of nutritional significance are varied in character and depend on many secondary factors, such as degree and extent of exposure to sunlight, cold, dry atmospheric conditions, repeated pressure at surface points, skin hygiene. Inherited variations in pigmentation, texture or structure, or alterations which occur with different physiologic states may be of such frequency that they must be differentiated before attributing nutritional significance to skin changes. When properly differentiated and supported by other evidence the presence of the following are meaningful: petechiae or purpura (vitamin C or K deficiency) especially at pressure points or legs; dry, inelastic skin, especially over abdomen (dehydration

with possible electrolyte deficiency in infants); nonreactive dermatitis with desquamation or "crazy-pavement" skin (protein-Calorie malnutrition); scrotal dermatitis (ariboflavinosis); thickened pressure points (pellagra), especially when associated with pellagrous dermatitis of exposed surfaces; hyperpigmentation of hands (and face) may reflect niacin lack or possibly low vitamin B_{12} or folacin nutriture (as seen in sprue), or may be seen in "simple" semistarvation. Follicular hyperkeratosis has often been related to a deficiency of vitamin A, or by some workers to lack of the essential fatty acids. Follicular hyperkeratosis or perifolliculosis may occur in vitamin C deficiency. It is often encountered in healthy persons without other evidence of nutritional disease, is easily imitated by tinea versicolor or by the overactive erector pilaris when the subject is cold or apprehensive. Its possible nutritional significance in a population must be critically assessed in each situation and it can be considered only of supportive usefulness.

Hepatomegaly may, in extreme malnutrition, reflect nutritionally related changes in the liver (fatty liver of kwashiorkor, cirrhosis) or indicate the presence of parasitic diseases of nutritional importance (bilharziasis, malaria, cirrhosis). In the latter instances there may be associated splenomegaly.

In infants and young children, exaggerated dimensions of the abdomen, "potbelly" appearance, is a sign frequently associated with general undernutrition or parasitism. Although it has no specificity as a sign, it is worthy of note as a portion of an overall picture of nutritional status.

Bilateral edema of the lower extremities with or without detectable edema elsewhere may be due to deficiency of protein, Calories, or thiamine. It is sometimes seen in pellagra and sprue. It may appear in a starved population as refeeding is instituted. On the other hand, bilateral edema of the feet and legs is common during the latter months of pregnancy in well-nourished women in many Western areas (U.S., for example), and can arise from many medical conditions such as varicose veins, cardiovascular or renal disease, etc. In infants edema is a sine qua non of kwashiorkor.

Skeletal changes are primarily of value in infants and young children except in rare instances in adult groups afflicted with osteomalacia, lathyrism, or severe fluorosis. Costochondral beading may occur either in rickets or scurvy, as may enlarged joints. Cranial bossing, craniotabes, and epiphyseal enlargement are seen in rickets of moderate to severe degree. Muscle wasting may accompany any type of malnutrition, but is especially pronounced in protein-Calorie malnutrition.

Neurologic signs reveal nutritional evidence primarily of deficiency of thiamine or vitamin B_{12}. Deficiency of the former leads to loss of

vibratory sense and of ankle and knee jerk and to motor weakness and, in severe acute cases, calf tenderness.

It is always important to consider appropriate groups of findings, clinical, biochemical, and dietary, before reaching a conclusion regarding nutritional status. In addition, properly designed therapeutic tests or a limited series of special supplementary studies (for example, bone marrows in anemia) frequently may be essential in order to reach a conclusive decision regarding a given deficiency state. Such evidence should be collected and weighed in interpreting the results of nutrition surveys.

X

Laboratory Equipment and Supplies[1]

A. Electrical Equipment and Supplies[2]

Centrifuges and Accessories

	Quantity	Unit	Unit price	Total price [1]
1. Centrifuge, International Model CS, with stainless-steel guard bowl, unitized control panel including direct reading tachometer, automatic timer, electric brake, pilot light and autotransformer controller, enclosed cabinet with casters (IEC No. 3450). Available for either 50-60 cycle, 115 or 230 volt, AC (International Equipment Co.).	1	Each		$695.00
2. Head, 8-place, IEC No. 240 for IEC Model CS centrifuge.	1	Each		43.50
3. Shield, stainless steel, 50 ml, IEC No. 320.	8	Each	$2.40	19.20
4. Shield, stainless steel, 10 ml, IEC No. 356.	24	Each	1.55	37.20
5. Cushion, rubber, IEC No. 571, for shield 320.	12	12		2.16
6. Cushion, rubber, IEC No. 570, for shield 356.	12	12		1.08
7. Ring, trunnion, 50 ml, IEC No. 325, for shield 325.	8	Each	1.75	14.00
8. Ring, trunnion, 3-place, for 10 or 15 ml tubes, IEC No. 355.	8	Each	2.95	23.60
9. Reducing cap, for supporting 10-15 ml shields in 50 ml shield, IEC No. 312.	8	Each	.50	4.00
10. Tube, centrifuge, 15 ml, conical, plain, beaded rim, heat-resistant glass, Corning No. 8060.	72	Each	.37	26.64
11. Tube, centrifuge, 15 ml, conical, graduated, Corning No. 8101.	144	Each	1.40	201.60
12. Tube, centrifuge, 45 ml, conical, plain, with flat head ⅞ stopper No. 16, heavy wall, Kontes K-41005.	72	Each	1.75	126.00
13. Support, test tube, polyethylene, large, 12 wells for 50-ml centrifuge tubes, Scientific Products No. S9250-3.	12	Each	1.81	22.08

[1] Approximate price as of 1962.

[1] In numerous instances possible sources of supply are indicated to clarify specifications. These items may be available from other sources.

[2] All electrical equipment is based on 60 cycle, 110 volt. If other types of current are to be used, specifications for equipment must be revised.

Centrifuges and Accessories—Continued

	Quantity	Unit	Unit price	Total price
14. Centrifuge, International, clinical, with 6-place head for 15-ml tubes, complete with head, 6 metal shields and 6 rubber cushions (IEC No. 458). Available for either AC or DC, 115 or 230 volts.	1	Each		$153.00
15. Centrifuge, high speed, IEC Model MB. Available for either 50 or 60 cycle, 115 or 230 volts (IEC No. 3412).	1	Each		197.50
16. Hematocrit reader, micro, circular type, IEC No. 2201.	1	Each		49.50
17. Hematocrit reader (Guest), A. H. Thomas No. 2916-V80.	1	Each		3.20
Subtotal				1,619.26

pH Meter

18. pH meter, Beckman, Zeromatic complete with glass electrode, reference electrode, electrode holder, 50-ml beaker, pint of pH 7.00 buffer solution, 100 ml KCl solution, KCl crystals and cleaning tissues. Operates on either 50 or 60 cycle, Beckman No. 9600.	1	Each		$330.00
19. Electrode, glass, general-purpose type, 0 to 11 pH, Beckman No. 41262.	1	Each		24.00
20. Electrode, reference, calomel, Beckman No. 41239.	1	Each		19.00
21. Electrode, sodium ion, for Beckman Zeromatic pH meter, Beckman No. 39278.	1	Each		35.00
22. Input can assembly, Beckman No. 45615.	1	Each		71.00
Subtotal				479.00

Photofluorometer and Accessories

23. Photofluorometer, electronic, Model 12C (Coleman No. 12-005) for 110–120 volts, 60 cycle operation. Complete with mercury vapor lamp, 12-357 "Sta-Bal" voltage-regulated lamp transformer, one dozen 12-190 selected cuvettes, 6-312 plastic light shield and 12-901 operating directions.	1	Each		$480.00
24. Photofluorometer, electronic, Model 12C (Coleman No. 12-006), for 115 volts, 50 cycle operation.	1	Each		475.00
25. Transformer, voltage compensating, 95/190-125/250 volts, 50 cycle, for use with 12-006 to stabilize line voltage (Coleman No. 14-354).	1	Each		252.00

Photofluorometer and Accessories—Continued

	Quantity	Unit	Unit price	Total price
26. Mercury vapor lamp (without leads) for Coleman photofluorometer, Model 12C, Coleman No. 12-301AH3.	1	Each		$31.50
27. Electronipak, complete, for Coleman photofluorometer, Model 12C, 60 cycle, Coleman No. 12-020.	1	Each		170.00
28. Electronipak, complete, for Coleman photofluorometer, Model 12C, 50 cycle, Coleman No. 12-021.	1	Each		170.00
29. Cover, instrument, Coleman No. 6-215.	1	Each		3.75
30. Cuvettes, 19 x 105 mm, round, Coleman No. 12-190 (all same classification).	24	Dozen	$12.00	288.00
31. Filter, primary, thiamine, B_1, Coleman No. 12-221.	2	Each	12.00	24.00
32. Filter, primary, thiamine, B_1 S, low concentration, Coleman No. 12-225.	2	Each	11.00	22.00
33. Filter, primary, riboflavin, B_2, Coleman No. 12-222.	2	Each	13.00	26.00
34. Filter, secondary, PC-1, thiamine, B_1, Coleman No. 14-211.	2	Each	13.00	26.00
35. Filter, secondary, PC-2, riboflavin, B_2, Coleman No. 14-212.	2	Each	9.00	18.00
36. Connecting cable for Model 6C, Coleman No. 12-057.	1	Each		6.00
37. Operating directions (extra) for Coleman photofluorometer, Model 12C.	1	Each		2.00
Subtotal				1,994.25

Spectrophotometer

38. Spectrophotometer, Coleman Jr., Model 6A, complete with battery charger, No. 6-009.	1	Each		$430.00
39. Adapter for cuvettes, 10 x 75 mm, round, Coleman No. 6-108.	2	Each	$10.00	20.00
40. Adapter for cuvettes, 19 x 150 mm, round, Coleman No. 6-102.	2	Each	10.00	20.00
41. Adapter, for semimicro cuvettes, Coleman No. 6-110.	1	Each		15.00
42. Battery, Willard storage, 6 volts, dry (acid *is not* to be provided for these batteries).	2	Each	15.00	30.00
43. Cuvette, 10 x 75 mm, round, selected, No. 6-310 (all same classification).	15	Dozen	11.50	172.50
44. Cuvette, 19 x 150 mm, round, selected, Coleman No. 6-304-B (all same classification).	30	Dozen	12.50	375.00
45. Filter, didymium, calibrating, standard, Coleman No. 6-400.	1	Each		19.00
46. Instrument cover for spectrophotometer, Coleman Jr., Model 6A, Coleman No. 6-215.	1	Each		3.75

Spectrophotometer—Continued

	Quantity	Unit	Unit price	Total price
47. Operating directions for spectrophotometer, Coleman Jr., Model 6A.	1	Each		$2.00
48. Manual of Clinical Methods for Coleman Jr. spectrophotometer, Coleman No. 6-912.	1	Each		20.00
49. Galvanometer assembly for spectrophotometer, Coleman Jr., Model 6A.	1	Each		70.45
50. Galvanometer lamp, 2 per set, Coleman No. 6-502.	2	Each	$3.00	6.00
51. Exciter, lamp, Coleman No. 6-500.	2	Each	6.50	13.00
Micro Attachments				
52. Bushing for cuvette well, Coleman No. J-389.	1	Each		1.70
53. General purpose procedure kit, Coleman No. 6-830.	1	Each		44.50
54. Mask, adapter, Coleman No. V-259.	1	Each		.75
55. Spare parts kit, Coleman No. 6-842.	1	Each		4.50
56. Ultra microcell, adapter and pump, Coleman No. 6-801.	1	Each		124.25
Subtotal				1,372.40

Spectrocolorimeter and Accessories

57. Spectrocolorimeter, Beckman/Spinco Model 151, 115 volt, 50-60 cycle.	1	Each		$475.00
58. Macrocuvette adapters for Beckman/Spinco Model 151 for 19 x 105 mm round cuvettes.	1	Each		9.75
59. Macrocuvette adapters for Beckman/Spinco Model 151 for 19 x 150 mm round cuvettes.	1	Each		9.75
60. Macrocuvette adapters for Beckman/Spinco Model 151 for 10 x 75 mm round cuvettes.	1	Each		9.75
61. Phototube assembly for Beckman/Spinco Model 151.	1	Each		35.00
62. Lamp for Beckman/Spinco Model 151.	2	Each	$0.25	.50
63. Fuse for Beckman/Spinco Model 151.	2	Each	.20	.40
Subtotal				540.15

Other Electrical Equipment

64. Blendor, Waring, model CB-4, 5 qt stainless-steel container, 3 variable speeds, 115 volt, 60 cycle. (Available also for 230 volt, 50 cycle.)	1	Each		$217.00

Other Electrical Equipment—Continued

	Quantity	Unit	Unit price	Total price
65. Blendor, Waring, complete with 1,000-ml Pyrex container, single speed, 115 volt, AC DC (available also for 230 volt).	2	Each	$34.95	$69.90
66. Demineralizer, portable, 5 gal/hr (Crystalab Deeminizer Model CL 5), Scientific Products No. D5020.	1	Each		39.50
67. Deeminite unit, for use with Crystalab Model CL 5 demineralizer, 6 refills/carton, ion exchange resin cartridge, Scientific Products No. D5021.	6	Each	13.00	78.00
68. Distilling apparatus, Barnstead, extra duty, for hard water, electric, 1 gal/hr, 2,600 watts, must operate on either 115 or 230 volt, 50 or 60 cycle, electric current, Scientific Products No. D4016.	1	Each		180.00
69. Low water cutoff for A68, Scientific Products No. D4150.	1	Each		131.00
70. Hotplate, electric, type 1900 Temco, for 115 volt, 50 60 cycle, Scientific Products No. H2260 1 (available also for 250 volt, 50-60 cycle).	5	Each	22.75	113.75
71. Mixer, test tube, Vortex Jr. Model for 115 volt, 60 cycle (available for 230 volt, 50 cycle).	3	Each	59.50	178.50
72. Shaking machine, electric, Universal, variable speed, with graduated rheostat dial to permit resettings at required speeds. With rack or tray with device for holding various size containers. For 115 volt, AC or DC, Aloe No. V73030.	1	Each		198.00
73. Oven, Thelco, gravity convection, single wall, constant temperature. Inside dimensions: Size No. 15, 17½" x 11" x 14", total watt 750, volt 115, Fisher No. 13-256.	2	Each	115.00	230.00
74. Transformer, 115 to 230 volts, 1,000 watt capacity, Fisher No. 9-497E.	2	Each	37.60	75.20
75. Transformer, variable, powerstat, for use on 230 volt, dial graduated 0-100 in 1% graduations, current capacity: amperes 9, voltamperage (maximum) 2,500, range, volt 0-280, Type No. 2PF236, A. H. Thomas No. 9708H.	2	Each	65.00	130.00
76. Transformer, variable, stepdown type, powerstat, identical to 9708H Type No. 216, but with mechanical stop to limit output voltage to 115 volt. For 230 volt.	2	Each	26.00	52.00

Other Electrical Equipment—Continued

	Quantity	Unit	Unit price	Total price
77. Transformer, variable, powerstat, for use on 230 volt. Dial graduated from 0 280 in 2-volt divisions, current capacity: amperes 3, volt-amperage (maximum) 840, range, volt 0 280, Type No. 216, A. H. Thomas No. 9708H.	4	Each	$26. 00	$104. 00
78. Volt - ohm - milliamp meter, Model 630, Triplett, Scientific Products No. E 2060.	1	Each		54. 50
79. Transformer, stepdown, 3,000 watts, 115 to 220 volt, Graybar 9T51Y6437.	1	Each		65. 52
80. Vacuum pump, 2-stage, Welch Duo-Seal 1400. Free air capacity of 21 liters per minute at 450 rpm and guaranteed vacuum of 0.1 micron (0.0001 mm Hg). Mounted on steel base with motor, V-belt, oil, cord, switch and plug. For 115 volt, AC 60 cycle (1/3 horsepower), Fisher No. 1 096V1.	1	Each		137. 50
81. Mercury vapor lamp, GE H100BL38 4.	2	Each	18. 00	36. 00
82. Transformer, electric, for lamp No. H100 BL38 4, GE 9T61Y3518.	1	Each		16. 50
83. Socket, Ad medium, GE 57-87 7.	2	Each	3. 75	7. 50
84. Waterbath, Precision, seroutility, stainless steel, 115 volt, 500 watt. Inside dimension 5″ x 10½″ x 7″, Precision No. 66643.	2	Each	95. 00	190. 00
85. Waterbath gable cover (for A84 above).	1	Each		12. 50
Subtotal				2, 316. 87

Electrophoresis Equipment

86. Electrophoresis apparatus, paper, Spinco Model R, complete, including 2 electrophoresis cells, 1 Duostat power unit, 1 RB Analytrol and instruction manual, 115 volt, 60 cycle, Beckman No. 320650, plus 2 auxiliary equipment sets for serum protein, Beckman No. 320649.	1	Each		$2, 714. 00
87. Blotter, 25/pkg, Beckman No. 320039.	8	Package	$1. 75	14. 00
88. Buffer, B 2, Veronal, 12 pkgs/box, Beckman No. 320024.	6	Box	15. 80	94. 80
89. Chart paper, 100/pkg, Beckman No. 319742.	6	Package	2. 95	17. 70
90. Dye, B-4, bromphenol blue, 6 pkg/box, Beckman No. 320047.	4	Box	9. 80	39. 20
91. Filter paper strips, 200/box, Beckman No. 320046.	2	Box	6. 55	13. 10
92. Folder, file, 25/pkg, Beckman No. 320020.	2	Package	4. 50	9. 00
93. Glass rods, Beckman No. 319335.	6	Each	. 35	2. 10

Electrophoresis Equipment—Continued

	Quantity	Unit	Unit price	Total price
94. Oven, drying, Bockel, large model, 1,500 watt, 115 or 230 volt (specify voltage required), inside dimensions 18″ x 17″ x 14″, Chemical Rubber Company No. 20-7275.	1	Each		$116.00
95. Rack, fixed metal, Beckman No. 320037.	2	Each	$7.60	15.20
96. Tape, cover, Beckman No. 320033.	2	Roll	.75	1.50
97. Wicks, filter paper, 50/box, Beckman No. 319329.	2	Box	3.00	6.00
Subtotal				3,042.60
Total, electrical equipment.				11,364.53

B. Balances, Scales, and Accessories

1. Balance, Christian Becker Model HB-4, dial reading chainomatic, with notched beam and magnetic damper, analytical.	1	Each		$345.00
2. Balance weights, Permas, precision, class S, without fractionals.	1	Set		35.00
3. Balance, triple beam, stainless steel, 311 gm capacity, sensitivity 0-01 gm, Ohaus Model 311, Will No. 3560.	1	Each		17.85
4. Balance, triple beam, Ohaus type with attached weights, capacity 610 gm, Ohaus Model 750-S, Will No. 3502.	2	Each	$12.30	24.60
5. Extra weight for balance, Ohaus type, 500 gm and 1,000 gm, Will No. 3506:				
Ohaus 701 (500 gm)	2	Each	1.00	2.00
Ohaus 702 (1,000 gm)	2	Each	1.00	2.00
6. Balance, Harvard trip, double beam, stainless plates, capacity 2 kg, sensitivity 1/10 gm, Ohaus Model 1450-S, Will No. 3450.	1	Each		12.35
7. Scales, platform scale, beam construction, size 11″ x 11″, capacity 136 kg in 1/10 kg intervals (overall height 13½″), Fairbanks Morse No. 12006.	3	Each	67.00	201.00
8. Scales, clinical infant, graduated in kg, 33 kg capacity, Aloe No. P5296.	1	Each		55.00
9. Scales, balance, dietetic, spring, 500 gm capacity, movable dial, Fisher No. 2-127.	2	Each	6.88	13.76
10. Scales, Triner Model ME-8DB, capacity 8 kg in 1 gm intervals, special metric beam.	4	Each	80.00	320.00
11. Weights, hook, 50 gm (for skinfold calipers), Ohaus 40050.	2	Each	.90	1.80

	Quantity	Unit	Unit price	Total price
12. Weights, hook, 20 gm (for skinfold calipers), Ohaus 40020.	2	Each	$0.70	$1.40
13. Weighing paper, S & S, No. B-2, 6" square, glazed, both sides, 100/pkg, A. H. Thomas No. 9978-P.	1	Package		.45
Total, balances, scales, and accessories.				1,032.21

C. General Laboratory Supplies

1. Beakers, Pyrex brand glass (Corning No. 1000), Griffin, low form, with spout:				
50 ml	24	Each	$0.39	$9.36
2. 100 ml	72	Each	.41	29.52
3. 250 ml	24	Each	.39	9.36
4. 400 ml	24	Each	.46	11.04
5. 600 ml	24	Each	.57	13.68
6. 1,000 ml	8	Each	1.10	8.80
7. 2,000 ml	4	Each	2.15	8.60
8. Beaker, Griffin, low form, polyethylene, 1,000 ml, Nalge No. 1201.	6	Each	2.35	14.10
9. Beaker, stainless steel, Type 302 (bainmarie), 1,800 ml.	2	Each	6.20	12.40
10. Bottle, dropping, 60 ml, with projecting lug on flat stopper.	6	Each	.78	4.68
11. Bottle, prescription, amber, with screwcap, 2 oz, 96/case.	8	Case	4.54	36.32
12. Bottle, metric, shelf, narrow mouth, flathead stopper, reagent glass, 4,000 ml.	6	Each	6.00	36.00
13. Bottle, narrow mouth, polyethylene, with polypropylene screwcaps:				
1 oz, Nalge No. 2002, 72/case	10	Case	7.28	72.80
14. 8 oz	2	Dozen	3.10	6.20
15. 16 oz	3	Dozen	4.27	12.81
16. 32 oz, 24/case	7	Case	13.92	97.44
17. 1 gal, Nalge No. 2202	6	Each	2.38	14.28
18. Bottle, narrow mouth, amber, 2 oz, Nalge No. 2206, 72/case.	2	Case	12.32	24.64
19. Bottle, wide mouth, Nalge No. 2105, 2 oz, 72/case.	6	Case	14.65	87.90
20. Bottle, wide mouth, polyethylene, with polypropylene caps:				
Nalge No. 2104, 48/case, 16 oz.	5	Dozen	6.09	30.45
21. Nalge No. 2104, 24/case, 32 oz.	1	Case		19.90
22. Bottle, wash:				
250 ml, Nalge No. 2402	6	Each	.55	3.30
23. 500 ml	6	Each	.65	3.90
24. Bottle, specific gravity, Kimax, with thermometer and standard taper joint, 25 ml.	2	Each	15.40	30.80
25. Borer, cork, hard brass, 15/set, 5 mm to 22 mm.	1	Each		8.35

	Quantity	Unit	Unit price	Total price
26. Brush, beaker, flask, 16″ long, 3/pkg.	2	Package	$1.80	$3.60
27. Brush, burette, 33″ long	2	Each	.36	.72
28. Brush, test tube, bristle end, 1½″ x 4″.	1	Dozen		2.00
29. Brush, test tube, bristle end, 7/16″ x 3″.	1	Dozen		1.20
30. Burette, straight standard taper stopcock, Kimax class A: 10 ml, subdivision, ml 1/20, 2/case.	1	Case		23.35
31. 25 ml, subdivision, ml 1/10, 2/case.	1	Case		24.59
32. 50 ml, subdivision, ml 1/10, 2/case.	1	Case		29.27
33. Burner, blast, MIT pattern, Fisher No. 4 175.	1	Each		18.00
34. Burner, Meeker, Fisher No. 3 900.	2	Each	5.00	10.00
35. Burner, micro, Fisher No. 4 067A.	2	Each	2.25	4.50
36. Capillary tubes, heparinized, 75 mm long, Fisher No. 5 040 10.	30	Vial	.90	27.00
37. Carboy, with handles, Nalge No. 2238, 5 gal.	2	Each	7.82	15.64
38. Clamp, test tube, Stoddard, without finger loops.	6	Each	.24	1.44
39. Clamp, burette, double, for micro to 100 ml size.	2	Each	4.75	9.50
40. Clamp, burette, vinylized jaws, Castaloy, small size.	12	Each	1.75	21.00
41. Clamp, versatile, vinylized jaws, Castaloy, medium size.	6	Each	1.90	11.40
42. Clamp, versatile, vinylized jaws, 3 prong, large size.	6	Each	2.15	12.90
43. Clamp, holder, regular, Castaloy, Fisher No. 5 754.	12	Each	.75	9.00
44. Clamp, hosecock, Castaloy, holds tubing to ¾″.	12	Each	.80	9.60
45. Clamp, pinchcock, Castaloy (heavy duty).	12	Each	.75	9.00
46. Clamp, day, pinchcock, Fisher No. 5867.	1	Dozen		2.64
47. Counter, hand tally, reciprocating, to 9,999.	2	Each	10.55	21.10
48. Crucible, Gooch, Coors, size 3, capacity 25 ml.	6	Each	1.14	6.84
49. Cups, paper, 16 oz (widemouth type), 100/pkg.	1	Package		1.88
50. Cup lids, 100/pkg	1	Package		1.15
51. Cylinder, graduated, with standard taper ground glass stopper, 25 ml.	6	Each	3.00	18.00
52. Cylinder, graduated, Exax, calibrated to deliver, with spout, single graduation:				
100 ml	12	Each	3.00	36.00
53. 250 ml	6	Each	4.10	24.60
54. 500 ml	4	Each	5.65	22.60
55. 1,000 ml	4	Each	7.90	31.60
56. 2,000 ml	4	Each	12.[illegible]	49.60

	Quantity	Unit	Unit price	Total price
57. Cylinder, graduated, polyethylene:				
100 ml, Nalge No. 3662	2	Each	$2.95	$5.90
58. 1,000 ml, Nalge No. 3662	2	Each	6.50	13.00
59. Desiccator, vacuum type, Pyrex glass with standard taper sleeve in cover, 200 mm inside diameter, Corning No. 3120.	2	Each	35.40	70.80
60. Desiccator plate, 190 mm diameter, Coors No. 600	2	Each	6.28	12.56
61. Dish, evaporating, Coors, porcelain, 150 ml capacity.	2	Each	1.10	2.20
62. Distilling apparatus (Graham) with condenser 19/38, 1,000 ml, Corning No. 3360.	3	Each	16.78	50.34
63. Flask, Erlenmeyer, Pyrex:				
50 ml, takes rubber stopper No. 1.	96	Each	.49	47.04
64. 125 ml, takes rubber stopper No. 4.	24	Each	.46	11.04
65. 1,000 ml, takes rubber stopper No. 9.	6	Each	.97	5.82
66. Flask, filtering, with side tube, Pyrex, 1,000 ml.	2	Each	3.10	6.20
67. Flask, volumetric, glass, stoppered, Pyrex, with standard taper stopper:				
10 ml, 10/case, with standard taper stopper No. 9.	4	Case	25.38	101.52
68. 25 ml, 12/case, with standard taper stopper No. 9.	4	Case	28.08	112.32
69. 100 ml, 12/case, with standard taper stopper No. 13.	8	Case	32.40	259.20
70. 250 ml, with standard taper stopper No. 16.	4	Each	3.65	14.60
71. 500 ml, with standard taper stopper No. 16.	4	Each	4.60	18.40
72. 1,000 ml, with standard taper stopper No. 22.	4	Each	5.65	22.60
73. Funnel, Bunsen, filtering long stem, Pyrex, fluted:				
Top diameter 50 mm, 60° angle, 150 mm stem, 48/case.	2	Case	25.49	50.98
74. Top diameter 100 mm	6	Each	.92	5.52
75. Glass wool, Pyrex brand glass, filtering.	1	Pound	--------	7.10
76. Grease, stopcock, silicone, Dow-Corning, 2 oz tube.	3	Tube	1.25	3.75
77. Grease, stopcock, silicone, high-vacuum, type 203 jar.	3	Jar	1.25	3.75
78. Grid, wire gauze, asbestos center, 5″ x 5″.	6	Each	.20	1.20
79. Hydrometer, battery tester, syringe type.	1	Each	--------	1.50
80. Hydrometer, Precision:				
Specific gravity, 1.000 to 1.070, length 150 mm.	2	Each	3.50	7.00
81. Specific gravity, 1.000 to 1.225, length 1.65 mm.	2	Each	1.96	3.92
82. Mortar, Coors, porcelain, with pestle, 10 oz size.	2	Each	3.73	7.46
83. Oil, vacuum pump, 1 qt container.	4	Each	.80	3.20

	Quantity	Unit	Unit price	Total price
84. Paper, filter, Whatman No. 1: Pkg of 100 sheets, 11 cm diameter.	24	Package	$0.47	$11.28
85. Pkg of 100 sheets, 18.5 cm diameter.	3	Package	1.14	3.42
86. Paper, filter, Whatman No. 40, 11 cm diameter.	10	Package	2.11	21.10
87. Paper, filter, Whatman No. 42, 11 cm diameter.	10	Package	3.12	31.20
88. Paper, pH, "Hydrion," range 2 to 10 in dispenser holding two 15′ rolls, with color chart.	6	Each	2.00	12.00
89. Paper, pH, "Hydrion," range 0 to 3, as above.	6	Each	2.00	12.00
90. Paper, pH, "Hydrion," range 3 to 5.5, as above.	6	Each	2.00	12.00
91. Pencil, wax, red, nonrun, for marking glassware.	3	Dozen	2.20	6.60
92. Pen, marking, Tech-pen, with extra ink, Aloe.	4	Each	5.25	21.00
93. Pipet, straight, "medicine dropper" with rubber bulb.	3	Dozen	.50	1.50
94. Pipet, microliter (lambda), Pyrex:				
10 microliter	6	Each	2.20	13.20
95. 20 microliter	6	Each	2.20	13.20
96. 50 microliter	6	Each	2.20	13.20
97. 100 microliter	6	Each	2.20	13.20
98. 200 microliter	6	Each	2.20	13.20
99. Pipet, serological, Kimble color coded "Kimax":				
1/10 ml capacity, 1/100 subdivisions, 18/case.	2	Case	21.22	42.44
100. 2/10 ml capacity, 1/100 subdivisions, 18/case.	2	Case	23.00	46.00
101. 1 ml capacity, 1/100 subdivisions, 18/case.	2	Case	23.00	46.00
102. 5 ml capacity, 1/10 subdivisions, 18/case.	3	Case	23.65	70.95
103. 10 ml capacity, 1/10 subdivisions, 18/case.	4	Case	27.86	111.44
104. Pipet, volumetric, Kimble color coded "Tekk":				
1 ml capacity	24	Each	.73	17.52
105. 2 ml capacity	24	Each	.73	17.52
106. 3 ml capacity	24	Each	1.30	31.20
107. 4 ml capacity	18	Each	1.30	23.40
108. 5 ml capacity	24	Each	.84	20.16
109. 10 ml capacity	6	Each	.97	5.82
110. 20 ml capacity	6	Each	1.70	10.20
111. Pipet, hemoglobin, Sahli 1 1%, S.P.	3	Dozen	10.80	32.40
112. Pipet, blood, Exax, with rubber tubing and mouthpiece, 20 cm x mm (0.02 ml).	12	Each	1.58	18.96
113. Pipet, constant volume, syringe type, Krogh-Keys:				
1 ml	6	Each	11.70	70.20
114. 2 ml	10	Each	11.70	117.00
115. 5 ml	12	Each	13.65	163.80
116. 10 ml	6	Each	16.25	97.50
117. 20 ml	6	Each	16.25	97.50
118. Pipet, jar, Nalge No. 5242, Size D, 6½″ x 18″.	3	Each	13.50	40.50

	Quantity	Unit	Unit price	Total price
119. Pipet, basket, Nalge No. 5241, Size D.	3	Each	$16. 50	$49. 50
120. Pump, coupling, filter, smooth, Fisher No. 9-980:				
¼″ inside diameter	1	Each		1. 50
121. ⅜″ inside diameter	1	Each		1. 50
122. Pump, coupling, filter, threaded, ⅜″ female and ¼″ male thread, Fisher No. 9-978.	2	Each	2. 00	4. 00
123. Pump, filter, Richards, 7″ long, with I.P. thread connection, ⅜″, Fisher No. 9-965.	1	Each		5. 50
124. Rod, glass, stirring, 6 x 250 mm	2	Dozen	. 70	1. 40
125. Sand bath, shallow, 8″ diameter.	4	Each	1. 20	4. 80
126. Sand bath, deep, 8″ diameter	4	Each	1. 30	5. 20
127. Skinfold thickness calipers, with 70 gm spring tension. Order from Mr. Vern Gersmehl, 3817 Warren Ave., Cheyenne, Wyo.	2	Each	35. 00	70. 00
128. Spatula, porcelain, spoon on one end, Coors, Size 1A.	6	Each	. 90	5. 40
129. Spatula, stainless steel:				
4″ blade	10	Each	. 80	8. 00
130. 6″ blade	3	Each	1. 20	3. 60
131. 8″ blade	4	Each	1. 75	7. 00
132. Stand, apparatus, support, rectangular base, 6½″ x 11″, rod 36″.	3	Each	3 95	11. 85
133. Stopper, cork, XXXX quality:				
Size 0, 100/box	2	Box	. 80	1. 60
134. Size 5, 100/box	2	Box	1. 60	3. 20
135. Size 6, 100/box	2	Box	1. 90	3. 80
136. Size 7, 100/box	2	Box	2. 15	4. 30
137. Stopper, rubber, solid:				
Size 1	3	Pound	1. 50	4. 50
138. Size 4	1	Pound		1. 50
139. Size 5	3	Pound	1. 50	4. 50
140. Size 6	3	Pound	1. 50	4. 50
141. Size 7	1	Pound		1. 50
142. Size 8	1	Pound		1. 50
143. Size 9	1	Pound		1. 50
144. Size 10	1	Pound		1. 50
145. Size 11	1	Pound		1. 50
146. Size 12	1	Pound		1. 50
147. Support, test tube, neoprene-coated, for 40 tubes up to ¾″ diameter in 4 rows of 10 tubes, Chicago Apparatus No. 54075.	16	Each	3. 60	57. 60
148. Support, test tube, neoprene-coated, for 40 tubes, ⅞″ diameter, Chicago Apparatus No. 54076.	12	Each	4. 00	48. 00
149. Support, test tube, Kahn serological, neoprene-coated, for 30 tubes, 9/16″ holes, Alee No. VP69501.	5	Each	4. 75	23. 75
150. Support, centrifuge tube, for 50 ml centrifuge tubes, Scientific Products No. S9215.	12	Each	2. 55	30. 60
151. Tape, measuring, steel, spring-wound return, graduated in inches on one side (1/16″) and millimeters on other, 2 meters long.	3	Each	3. 00	9. 00

	Quantity	Unit	Unit price	Total price
152. Test tube basket, galvanized wire, 6″ x 6″ x 6″, Fisher No. 14-971.	24	Each	$2.75	$66.00
153. Thermometer, pocket, −20 to 105° C, Chemical Rubber Co. No. 14-4915.	2	Each	3.00	6.00
154. Thermometer, mercurial, centigrade 76 mm, immersion, "Visipak," permanent markings, Precision grade:				
−10 to 110° C	2	Each	2.40	4.80
155. −5 to 200° C	2	Each	2.65	5.30
156. −5 to 360° C	2	Each	4.00	8.00
157. Timer, interval, clock type, alarm type, spring driven, range 15 seconds to 120 minutes.	4	Each	10.00	40.00
158. Timer, stopwatch, interval 0 to 66 seconds in ½ second divisions, Kodak.	1	Each	--------	9.50
159. Tongs, crucible, stainless steel, 9″ long.	3	Each	1.65	4.95
160. Tripod, iron, single ring, 5″ diameter, 9″ high.	3	Each	1.40	4.20
161. Tube, chromatographic, AOAC, 200 mm long with reservoir at top, 25 mm in diameter, absorption column 5-6 mm in diameter, terminating in a capillary tip, Fisher No. 5-721.	8	Dozen	13.20	105.60
162. Tube, connecting Y-shape, glass, outside diameter ⅜″, length of arms 1¾″.	1	Dozen	--------	3.78
163. Tube, test, bacteriological, Pyrex, without flare, 16 x 150 mm.	1	Gross	--------	10.08
164. Tube, test, ignition, Pyrex, heavy wall, without flare, 20 x 150 mm.	48	Each	.33	15.84
165. Tube, test, with solid pennyhead standard taper 13 stopper complete, 16 x 150 mm, Kimble No. 45100, Corning No. 99650.	12	Dozen	12.42	149.04
166. Tubing, glass, standard flint glass:				
Outside diameter 4 mm, 0.013 lb/ft.	1	Pound	--------	1.36
167. Outside diameter 5 mm, 0.022 lb/ft.	1	Pound	--------	.95
168. Outside diameter 6 mm, 0.028 lb/ft.	1	Pound	--------	.95
169. Outside diameter 8 mm, 0.041 lb/ft.	1	Pound	--------	.79
170. Tubing, rubber, 3/16″ bore:				
1/16″ wall	48	Foot	.12	5.76
171. 3/16″ bore, 3/32″ wall	48	Foot	.28	13.44
172. 3/8″ bore, 1/32″ wall	24	Foot	.38	9.12
173. 3/16″ bore, 3/64″ wall	24	Foot	.23	5.52
174. ¼″ bore, 1/32″ wall	24	Foot	.24	5.76
175. ¼″ bore, 3/16″ wall	12	Foot	.40	4.80
176. ½″ bore, ¼″ wall	12	Foot	.65	7.80
Total, general laboratory supplies.				4,068.32

D. Surgical Supplies

	Quantity	Unit	Unit price	Total price
1. Adhesive plaster, surgical, 3″ x 5 yds.	6	Roll	$0.50	$3.00
2. Applicator, wood, 6½″, 72 doz/box.	2	Box	.85	1.70
3. Bag, physicians, black, top-grain cowhide, 17″ long, with 1 large and 2 small compartments, Aloe No. B-2145A.	2	Each	29.93	59.86
4. Cotton, absorbent, sterile, 1 lb roll.	6	Each	1.50	9.00
5. Depressor, tongue, wood, 6″ x ⅞″, 500/box.	20	Box	1.65	33.00
6. Forceps, dressing, thumb, straight, serrated tips, 4½″.	3	Each	.90	2.70
7. Fork, tuning, 128 vib/sec (must not have weights or clips), Clay-Adams No. A-1708.	4	Each	3.00	12.00
8. Gauze, absorbent, medium mesh, 28 x 24, plain, nonsterile, 36″ wide, 100 yds to bolt.	1	Bolt		12.00
9. Gloves, rubber, autopsy, size 7½.	4	Pair	.50	2.00
10. Hammer, percussor, Taylor, Aloe No. A-129.	4	Each	1.50	6.00
11. Infantometer, slide rule for measuring infants, graduated in centimeters, Aloe S-3739.	1	Each		17.00
12. Kit, first-aid, medium size, for 25-50 persons, J & J Insurance Case No. 8-5578.	3	Each	17.80	53.40
13. Needle, hypodermic, short bevel, 20 gage, 5″, Empire.	2	Dozen	3.21	6.42
14. Needle, hypodermic, short bevel, 16 gage, 3½″, Empire.	1	Dozen		4.82
15. Needle, hypodermic, syringe, Luer slip, long bevel, 23 gage, ⅝″, Aloe A-642.	4	Box	1.50	6.00
16. Needle, vacutainer, sterile, disposable, 20 gage, 1½″, 1,000/case, Becton-Dickinson H-1004.	1	Case		60.75
17. Needle, disposable, for vacutainer tubes 22^{G} x 1½″, 250/pkg, Aloe No. RH210C.	1	Package		22.00
18. Needle cleaner, rubber, bulb type, designed to clean vacutainer needles, Fisher No. 5-958-120.	2	Each	.86	1.72
19. Ophthalmoscope - otoscope combination set, consisting of No. 110 ophthalmoscope and No. 216 otoscope with 5 specula, large handle, extra bulbs in leatherette case, Welch Allyn 983, less 10 percent.	2	Each	79.00	158.00
20. Batteries for use with D19	8	Each	.25	2.00
21. Pediatric urine collectors, PUC-10, 10/box.	50	Box	1.75	87.50
22. Sphygmomanometer, mercurial portable type with standard arm band, complete, calibrated to 260 mm (Kompak model, Lifetime Baumanometer), Aloe No. A1A.	2	Each	38.25	76.50

D. Surgical Supplies—Continued

	Quantity	Unit	Unit price	Total price
23. Sphygmomanometer, aneroid, complete with hook cuff inflation system, black leatherette case, VMA, New York City.	2	Each	$15.68	$31.36
24. Tube, specimen, vacutainer, 20 ml, 6½″ x ⅝″, 240/pkg, Becton-Dickinson 3208.	2	Package	17.16	34.32
25. Tube, specimen, vacutainer, 10 ml, with 10 mg ethylene diamine tetraacetic acid, 240/pkg (5 pkg), Becton-Dickinson 3200Q.	1	Case	--------	71.80
26. Tube, specimen, vacutainer, 10 ml, with 10 mg ethylene diamine tetraacetic acid, 240/pkg, Becton-Dickinson 3200Q.	1	Package	--------	16.03
27. Vacutainer needles, 1½″, 20 gage, Becton-Dickinson 3200N.	5	Dozen	2.00	10.00
28. Vacutainer adapter, holder to Luer needle, Becton-Dickinson 3200A.	2	Dozen	2.25	4.50
29. Vacutainer holder, plastic, Becton-Dickinson 3200H.	2	Dozen	1.87	3.74
30. Welch Allyn professional flashlight with batteries and lamp, Aloe No. WA777.	4	Each	4.95	19.80
31. Batteries for Welch Allyn flashlight.	12	Each	.30	3.60
Total, surgical supplies.				832.52

E. Equipment for Micro Vitamin A Determination

1. Burner, automatic, precision, for alcohol, A. H. Thomas No. 2660-A.	2	Each	$11.65	$23.30
2. Capillary tube, heparinized, 150 mm long, 100/vial, Aloe No. 23923.	20	Vial	1.50	30.00
3. Capillary tube, heparinized, 75 mm long, 100/vial, Aloe No. 23922.	12	Vial	1.033	12.40
4. Capillary tube, melting point, 100/vial, size B (1.5-2.0), A. H. Thomas No. 6401-P2.	10	Vial	.84	8.40
5. Catheters, depressed eye, Davol No. 8, 6/lot, Aloe No. RD-1408.	1	Lot	--------	1.99
6. Cell carriage, for microcells for Beckman DU spectrophotometer.	2	Each	40.00	80.00
7. Centrifuge, micro, hand, A. H. Thomas No. 2926-A.	2	Each	38.00	76.00
8. Corks, No. 000, XXXX quality, bags of 500, A. H. Thomas No. 4051.	4	Bag	2.59	10.36
9. Critoseal, 6 cards/box, Aloe No. 23929C.	3	Box	3.50	10.50
10. Diaphragm attachment, for Beckman DU spectrophotometer to adapt for use of microcells.	1	Each	--------	25.00
11. Microcells, quartz, size 2.5 x 10 x 25 mm, for Beckman DU spectrophotometer.	4	Each	25.00	100.00

	Quantity	Unit	Unit price	Total price
12. Microcells, quartz, size 3.0 x 10 x 25 mm, for Beckman DU spectrophotometer.	4	Each	$15.00	$60.00
13. Mouthpiece, for hemocytometer tubing only, A. H. Thomas No. 3396.	12	Each	.02	.24
14. Pencil, diamond point, A. H. Thomas No. 4466-A.	2	Each	2.00	4.00
15. Sera-sharps (hemolets), 1,000, Aloe No. 16102.	2	Lot	15.00	30.00
16. Test tube, bacteriological, 6 mm x 50 mm, Aloe No. 78140.	15	Gross	2.19	32.85
17. Tubing, rubber, hemocytometer, 1 box of 12 ft, A. H. Thomas No. 4448.	1	Each		1.32
18. Wax, sealing, "Pyseal," Fisher 4-758.	2	Box	1.25	2.50
19. Critocaps, 12 pkg/box, Aloe No. E-Y23929.	2	Box	1.35	2.70
Total, equipment for micro vitamin A determinations.				511.56

F. Dental Equipment

1. Chair, dental, portable, with headrest only.	2	Each	$141.60	$283.20
2. Explorers, dental, double end, No. 3.	6	Dozen	18.00	108.00
3. Handles, mouth mirrors, simple stem, Taino 23.	6	Dozen	9.00	54.00
4. Mirrors, dental, mouth, simple stem, plain, Size No. 5-P-S, S. S. White Dental Mfg. Co.	6	Dozen	9.80	58.80
5. Pans, dish, polyethylene	4	Each	1.00	4.00
Total, dental equipment.				508.00

G. Hardware

1. Bit:				
Size ¾"	1	Each		$1.25
2. Size 1"	1	Each		1.52
3. Size ½"	1	Each		1.10
4. Size ¼"	1	Each		1.10
5. Brace	1	Each		4.49
6. Carpenter hand saw, 20" long (cross cut).	1	Each		5.25
7. Cooker, pressure, 4 qt	2	Each	$10.95	21.90
8. Cooker, safety plugs, extra	4	Each	.40	1.60
9. Cooler, rectangular type, 1" insulated walls, no spigot, size 15" x 12½" x 14½".	8	Each	10.95	87.60
10. Cord, electric, extension, heavy duty, 15 ft.	2	Each	1.50	3.00
11. Jug, outing, wide mouth, without pouring spout, thick insulation, 1 gal size.	6	Each	3.23	19.38

	Quantity	Unit	Unit price	Total price
12. Lantern, battery operated, Delta powerlite, 800′ beam at front, floodlight at top, Noland No. A-1530.	2	Each	$5.45	$10.90
13. Lantern batteries, for use with lantern above.	2	Each	.98	1.96
14. Padlocks (ordinary Master No. 3).	5	Each	1.00	5.00
15. Pail, polyethylene, *without lid*, 10 or 12 qt size.	12	Each	.90	10.80
16. Pans, dish, polyethylene, approximately 13½″ diameter.	4	Each	.90	3.60
17. Screwdrivers, instrument type.	1	Set		1.49
18. Screwdriver, plastic handle, No. 3, Phillips, 6″ for screw No. 12-16.	1	Each		.40
19. Screwdriver, flat tip, plastic handle, common:				
3″ blade	1	Each		.30
20. 6″ blade	1	Each		.40
21. Solder, electric, solid wire, no flux type, ⅛″ diameter, for electrical appliance and radio repair work "bit," 1 lb/spool.	2	Lot	.99	1.98
22. Soldering iron, electric, to be used with solder (small gun type).	1	Each		5.95
23. Sponge, cellulose, 1½″ x 3″ x 4½″.	6	Each	.10	.60
24. Sterno (canned heat), small size.	24	Can	.19	4.56
25. Stone, sharpening, fine, 6″ x 2″ x 1″.	1	Each		3.00
26. Stove, gasoline burner, 2-burner type, heavy duty, Coleman No. 413E.	1	Each		17.95
27. Twine, jute, 4-ply, ½ lb	4	Ball	.35	1.40
28. Wrench, monkey, 10″	1	Each		2.50
Total, hardware.				220.98

H. Chemicals

1. Acetic anhydride, ACS, reagent, 1-lb bottle.[1]	7	Bottle	$1.07	$7.49
2. Acetone, reagent, ACS, 1-lb bottle.[2]	15	Bottle	.66	9.90
3. Acid, acetic, glacial, reagent, 1-lb bottle.[1]	6	Bottle	.97	5.82
4. Acid, hydrochloric, reagent, 1-lb bottle.[1]	12	Bottle	.46	5.52
5. Acid, metaphosphoric, glacial, pellets, reagent, 1-lb bottle.[3]	9	Bottle	3.48	31.32
6. Acid, potassium phthalate, 60-gm bottle, Bureau of Standards 51-L-50-100.	2	Bottle	4.75	9.50
7. Acid, sulfuric, ACS, 2-lb bottle.[1]	30	Bottle	.65	19.50
8. Ammonium hydroxide, reagent, 10 percent solution, specific gravity 0.95, 1-lb bottle.[1]	3	Bottle	2.31	6.93

See footnotes at end of section.

	Quantity	Unit	Unit price	Total price
9. Ammonium oxalate, crystals, reagent, 1-lb. bottle.	1	Bottle		$1.95
10. Ammonium sulfate, Merck, reagent.	6	Each	$0.80	4.80
11. Antimony trichloride, C.P., ¼-lb bottle.	6	Bottle	1.69	10.14
12. Alcohol, butyl (N), quarts, Fisher A-399.[2]	30	Quart	2.10	63.00
13. Alcohol, ethyl, absolute (99.9 percent), USP, 1 pint.[4]	2	Pint	.30	.60
14. Alcohol, ethyl, 95 percent, USP (in quart cans).[4]	6	Gallon	.68	4.08
15. Alcohol, isobutyl, C.P., 1-qt can.[2]	40	Can	2.25	90.00
16. Alcohol, methyl (methanol), special reagent, 1-lb bottle.[2]	8	Bottle	.66	5.28
17. L-ascorbic acid, 25-gm bottle.	4	Bottle	.70	2.80
18. Ascorbic acid tablets, 100 mg, 1,000 tablets/bottle.	1	Bottle		12.00
19. Bromcresol green, 1-gm bottle	2	Bottle	2.75	5.50
20. Charcoal, Norite A, powdered (stock, carbon, technical, decolorizing), 1 lb (Norite A).	3	Bottle	1.24	3.72
21. Chloroform, ACS, 1-lb bottle.[2]	18	Bottle	.80	14.40
22. Cholesterol, USP, 100-gm bottle.	1	Bottle		3.60
23. Citric acid, powder, reagent, monohydrate, 1-lb bottle.	2	Bottle	1.32	2.64
24. Cupric sulfate crystals, reagent (copper sulfate) ($CuSO_4 \cdot 5\ H_2O$), ACS, fine or powdered.	2	Bottle	1.35	2.70
25. Diacetyl monoxime (2,3-butanedione monoxime), Eastman No. 86, 100-gm bottle.	1	Bottle		7.05
26. 2, 6-dichlorobenzenone indophenol, sodium salt (lot), 25-gm bottle.	1	Bottle		21.00
27. 2, 4-dinitrophenylhydrazine, melting point 199°–200° C, 25-gm bottle.	2	Bottle	2.00	4.00
28. Drierite, indicating, 8 mesh, 1 lb, Fisher 7-578.	1	Pound		2.20
29. Ether, anhydrous, chemically pure, ACS, 1-lb can (must be in sealed metal cans).[2]	6	Can	1.00	6.00
30. Ether, petroleum benzene, special, chemically pure, boiling point 20°–40° C, 1 lb.[2]	16	Pound	1.27	20.32
31. Ferric ammonium sulfate ($FeNH_4(SO_4)_2 \cdot 12\ H_2O$), chemically pure (51-L-100), 1-lb bottle.	1	Bottle		1.48
32. Ferric chloride, lump, ¼ lb, Fisher 1-88.	4	¼ pound	1.35	5.40
33. Calcium chloride, anhydrous, 4 mesh, lb, 5 x 1, Fisher C-72.	5	Pound	1.15	5.75
34. Formaldehyde, 40 percent by volume, pint, Fisher F-78.[1]	6	Pint	1.05	6.30
35. Ghatti gum, No. 1, tears, soluble, Fisher G-103.	1	Pound		1.16
36. Glycerin, 1-lb bottle.	1	Pound		.75
37. Heparin, Nutritional Biochemicals, ammonium, approximately 100,000 units per gm, 1-gm bottle.	2	Bottle	6.35	12.70

See footnotes at end of section.

	Quantity	Unit	Unit price	Total price
38. Hydrogen peroxide, 30 percent, reagent hyperoxide.[1]	3	Bottle	$4.18	$12.54
39. Iodine, resublimed, ACS, ¼-lb bottle.[3]	1	Bottle		.95
40. Maleic acid, 1-lb bottle.	2	Bottle	7.85	15.70
41. Manganese (manganous) chloride, Fisher certified, 1 lb, Fisher M-87.	1	Pound		3.30
42. Mercuric iodide, Fisher certified, ¼ lb, Fisher M-166.[3]	2	¼ pound	5.40	10.80
43. Merthiolate, tincture, Gilman.	4	Quart	4.00	16.00
44. Metaphen tincture, 1:200, tinted.[1]	2	Pint	2.17	4.34
45. Nicotinamide tablets, 50 mg each, 1,000 tablets bottle.	1	Bottle		9.50
46. Oxalic acid, crystals, reagent, 1-lb bottle.[3]	4	Bottle	1.28	5.12
47. Paraffin, 50°-52° C, white, USP, 1-lb cake.	2	Cake	.45	.90
48. Petrolatum liquid, heavy, USP.	1	Quart		.47
49. Picric acid, reagent, crystals, with 10 percent water added, 4-oz bottle.[2]	2	Bottle	.63	1.26
50. Potassium chloride, reagent, 1-lb bottle.[3]	15	Bottle	1.10	16.50
51. Potassium cyanide, Fisher certified, ACS, ¼-lb bottle, Fisher P-226[3].	2	Bottle	.47	.94
52. Potassium dihydrogen phosphate (monobasic), secondary crystals, reagent, 1-lb bottle.	2	Bottle	1.34	2.68
53. Potassium ferricyanide, crystals, reagent, 1-lb bottle.	2	Bottle	1.47	2.94
54. Potassium ferrocyanide, crystals, reagent, 1-lb bottle.	2	Bottle	1.85	3.70
55. Potassium hydroxide, pellets, reagent, 1-lb bottle.[3]	3	Bottle	1.44	4.32
56. Potassium iodide, crystals, reagent, 1 lb bottle.	1	Bottle		4.00
57. Potassium oxalate, crystals, reagent, 1-lb bottle.	2	Bottle	2.14	4.28
58. Potassium permanganate, crystals, reagent, 1-lb bottle.	1	Bottle		1.11
59. Potassium persulfate, Fisher certified, ¼-lb bottle.	1	Bottle		.42
60. Potassium phosphate, dibasic, primary, crystals, reagent, 1-lb bottle.	2	Bottle	1.68	3.36
61. Potassium sodium tartrate, crystals, reagent, 1-lb bottle.	2	Bottle	1.68	3.36
62. Pyridine, Fisher certified, ACS, Fisher P-368.[2]	12	Pint	3.95	47.40
63. Quinine sulfate, 1 oz bottle.	1	Bottle		1.04
64. Riboflavin, 25-gm bottle.	2	Bottle	2.25	4.50
65. Riboflavin tablets, 5 mg, 1,000 tablets/bottle.	1	Bottle		10.00
66. Sand, sea, washed and ignited, 5-lb box, Fisher S-25.	1	Box		6.10
67. Silica gel, desiccant (activated), 5-lb can, AHT YLTZJ.	1	Can		7.50
68. Sodium acetate, crystals, 1-lb bottle.	8	Bottle	1.37	10.96

See footnotes at end of section.

	Quantity	Unit	Unit price	Total price
69. Sodium bicarbonate, $NaHCO_3$, chemically pure, 1-lb bottle.	3	Bottle	$0.70	$2.10
70. Sodium bichromate, sodium dichromate, reagent, 1-lb bottle.	4	Bottle	1.32	5.28
71. Sodium carbonate, anhydrous, Fisher certified, ACS, 1 lb.	2	Pound	2.05	4.10
72. Sodium chloride, ACS, 1-lb bottle.	2	Bottle	.75	1.50
73. Sodium hydrosulfite, powder, pure (low in iron), ¼ lb bottle.	2	Bottle	2.10	4.20
74. Sodium hydroxide, pellets, reagent, 1-lb bottle.[3]	12	Bottle	1.10	13.20
75. Sodium sulfate, anhydrous, granular, reagent, 1-lb bottle, Fisher S-421.	16	Bottle	.68	10.88
76. Sodium sulfite, anhydrous, Fisher certified, ACS, 1-lb bottle, Fisher S-430.	10	Bottle	1.20	12.00
77. Sodium tungstate, crystals, reagent, ¼ lb bottle.	4	Bottle	2.88	11.52
78. Span 20, 4 oz, Atlas Powder Co., Wilmington, Del.	1	Bottle	.....	1.00
79. Thiamine tablets, 5 mg, 1,000 tablets/bottle.	1	Bottle	.....	9.50
80. Thiamine hydrochloride, 5-gm bottle.	4	Bottle	.75	3.00
81. Thiochrome Decalso, permutit-T, for thiamine determination, 50–80 mesh, 5-lb bottle, Fisher T-97.	2	Bottle	13.85	27.70
82. Thiourea, 1 lb bottle.	2	Bottle	1.04	2.08
83. Toluene, 1 lb can.[2]	6	Can	.75	4.50
84. Trichloroacetic acid, reagent, 1 lb.	8	Pound	5.40	43.20
85. Trifluoroacetic acid, 100 gm/bottle, Baker No. 6287.	6	Bottle	5.72	34.32
86. Tris hydroxymethyl amino methane (THAM), 1 kg, Fisher T-395.	1	Bottle	.....	10.90
87. DL-tryptophan, 1-kg bottle, Nutritional Biochemicals.	1	Bottle	.....	147.50
88. Urea, Fisher certified, 1-lb bottle, Fisher U-15.	1	Bottle	.....	1.95
89. Xylene, ACS, 1-lb bottle.	4	Bottle	2.50	10.00
Subtotal				993.72
Chemical Standards				
90. Beta-carotene, pure, 100 percent beta-carotene, 1-gm vial, sealed in glass. Order from General Biochemicals Inc., Chagrin Falls, Ohio, or Z. D. Gilman.	3	Vial	3.15	9.45
91. Creatinine zinc chloride, 10-gm vial.	2	Vial	2.45	4.90
92. Hemoglobin standard (Cyano). Obtain from Walter Reed Army Institute of Research, Washington 12, D.C.	1	Each	No charge	
93. N′-methylnicotinamide chloride, 1-gm bottle. Order from W. A. Taylor Co., 7300 York Rd., Baltimore 4, Md.	5	Bottle	5.70	28.50

See footnotes at end of section.

	Quantity	Unit	Unit price	Total price
Chemical Standards—Continued				
94. Uranine (Fluorescein sodium salt), ¼ lb, Fisher A-833, Eastman No. 735.	1	Bottle		$1.86
95. Versatol (Warner-Chilcott), blood chemistry control standard, box of 3-5-ml vials, Aloe No. VX 3622Q.	1	Box		7.50
96. Vitamin A standard, 24 capsules, vitamin A acetate, reference standard; each capsule contains 8.6 mg vitamin A. Order from USP Reference Standards, 46 Park Ave., New York 16, N.Y.	2	Package	$11.00	22.00
97. Xanthurenic acid, 1-gm bottle.	2	Bottle	5.25	10.50
Subtotal				84.71
Drugs				
98. Acetylsalicylic acid tablets, 100/bottle.	3	Bottle	.27	.81
99. Aluminum hydroxide tablets, 200 tablets, 100/bottle.	2	Bottle	.75	1.50
100. Camphorated opium tincture, 1-pint bottle.	2	Bottle	.95	1.90
101. Chloroquine-Primaquine tablets; each tablet contains chloroquine phosphate, USP 500 mg (7½ gr) and primaquine phosphate USP 79 mg (1¼ gr), Winthrop Laboratories, 100/bottle.	3	Bottle	7.00	21.00
102. Compound dimethyl phthalate stick (612), 1 oz.	10	Ounce	.59	5.90
103. DDT aerosol bomb.	6	Each	1.59	9.54
104. DDT dusting powder, 1½ oz.	6	Can	.75	4.50
105. Dimenhydrinate tablets, 50 mg, 100/bottle.	2	Bottle	4.73	9.46
106. Diodoquin, 650 mg tablets, 500/bottle.	1	Bottle		25.69
107. Glyceryl trinitrate tablets (sublingual), 0.6 mg.	1	Bottle		4.73
108. Milk of bismuth, 8-oz bottle.	1	Bottle		2.00
109. Neosynephrine nose drops, 15 ml, 0.25 percent.	4	Bottle	.70	2.80
110. Probantheline bromide tablets, 15 mg, 100/bottle.	1	Bottle		4.75
111. Secobarbital sodium capsules, 100 mg.	1	Bottle		2.70
112. Sulfadiazine tablets, 0.5 gm, 100/bottle.	1	Bottle		4.00
113. Tetracycline capsules (Tetracyn), 0.25-gm capsule, 100/bottle.	3	Bottle	17.23	51.69
114. Tubex, pkg of 10, Wyeth pkg No. 1B564, Meperidine hydrochloride injection, 50 mg/ml (Demerol).	2	Package	4.00	8.00
115. Tubex syringe, Wyeth No. 99A71.	2	Each	1.75	3.50

See footnotes at end of section.

691-752—64——22

	Quantity	Unit	Unit price	Total price
Drugs—Continued				
116. Water purification tablets (tetraglycine hydroperiodide; each tablet liberates 8.0 mg iodine), 50/bottle.	20	Bottle	$0.55	$11.00
117. Zinc, undecylenate ointment, 1 oz.	4	Tube	.75	2.25
118. Zinc, undecylenate powder, 1½ oz.	4	Can	1.00	4.00
Subtotal				181.72
Total, chemicals.				1,260.15

[1] White Label—"Corrosive liquid or acid." Each to be packed in 1-quart glass bottles, maximum, plus absorbent cushioning, and put in metal container, each container to be packed in individual strong shipping box.
[2] Red Label—"Flammable liquids." Must be in 1-quart glass or 1-quart metal container, maximum, plus absorbent cushioning, each unit to be packed in individual strong shipping container.
[3] ORA—B Label—"Corrosive when wet." Individual container of not more than 5 pounds each with a total maximum of 25 pounds in 1 package, packed in absorbent material for air shipment.
[4] Price quoted is to U.S. Government, without tax.

I. Office Supplies

1. Ballpoint pen, blue-black ink, medium point.	24	Each	$0.25	$6.00
2. Book, memo, ruled, cloth bound, side opening, 10½" x 8".	24	Each	1.00	24.00
3. Calculator, contex (including $7.50 U.S. tax), Bohn Duplicator Co., 444 Park Ave. South, New York 16, N.Y., Bohn No. 50-114.	2	Each	132.50	265.00
4. Calculator carrying case.	2	Each	14.50	29.00
5. Cards, clinical record, abbreviated, white, Royal McBee No. K-43071X, numbered 3,000 to 9,999, pkg 1,000/box.	7,000	1,000	25.40	177.80
Unnumbered.	3,000	1,000	25.40	76.20
6. Cards, clinical record, detailed, buff, Royal McBee No. K43455-6X, numbered 0001 to 2,000, 500/pkg	2,000	1,000	105.00	210.00
Unnumbered.	1,000	1,000		105.00
Ditto, pink (250); ditto, green (750).	1,000	1,000		105.00
7. Cards, pediatric, blue 8" x 10½".	1,000	1,000		35.00
8. Pediatric card rubber stamp, special.	1	Each		1.50
9. Card savers, McBee, No. K48, packed, 10 pads/pkg.	1	Package		2.80
10. Clipboard file, legal size, 9" x 17".	6	Each	.50	3.00
11. Envelope, wallet, letter size, 1¾" expansion.	25	Each	.25	6.25
12. Folder set, file, 9½" x 11¾", letter size, ⅕ cut.	1	Box		1.50
13. Ink, stamp pad, black, 2 oz.	2	Bottle	.15	.30
14. Inking pad, dating stamp for Triumph No. 5 or No. 15 stamp.	2	Each	1.00	2.00
15. Inking pad for rubber stamp, 3¼" x 6¼".	1	Each		.50

	Quantity	Unit	Unit price	Total price
16. Keysorts, McBee 5003.	6	Each	$3.50	$21.00
17. Kodaslide viewer, Sawyer's Monovue.	2	Each	.80	1.60
18. Label tape, self-adhesive, red, ¾″ x 400″.	5	Each	1.25	6.25
19. Notebooks, leatherette, 6¾″ x 3¼″, flexible, Vernon Royal Line No. 606.	12	Each	.95	11.40
20. Notebook fillers, lined, for 19.	20	Package		7.11
21. Numbering machine, office type, hand operated, 6 wheel, 5 movement repeat, consecutive to quadruplicate action.	1	Each		17.50
22. Pad, writing, 8″ x 10½″, white, ruled, 12/pkg.	2	Package	1.50	3.00
23. Paper, bond, 25 percent rag, white, 8″ x 10½″, 16-pound basic weight, 500 sheets/pkg.	2	Package	1.00	2.00
24. Paper, carbon, typewriter, 8″ x 10½″, black, one box each, light weight; medium, 100/box, Grade A.	2	Box	.50	1.00
25. Paper set, white, manifold and carbon, 8″ x 10½″ (second sheet attached to a 1-time use carbon).	2	Box	1.50	3.00
26. Pencil, drawing, blue and red.	2	Dozen	1.00	2.00
27. Pencil, general writing, No. 2, medium soft.	3	Dozen	.50	1.50
28. Pencil, general writing, No. 3, medium hard.	3	Dozen	.50	1.50
29. Punch, McBee 5201.	4	Each	6.60	26.40
30. Refill, ballpoint pen, medium point, blue-black ink.	24	Each	.15	3.60
31. Rubber stamp, band type, dating, No. 1, month, day, year.	2	Each	.25	.50
32. Rulers, plastic, X-ray, 18″, B 95.	6	Each	2.00	12.00
33. Sharpener, pencil, office use, for pencil up to 7/16″ diameter.	2	Each	2.00	4.00
34. Shears, office, straight trimmers, 6″.	2	Each	1.00	2.00
35. Shears, office, straight trimmers, 9″.	2	Each	1.50	3.00
36. Slide rule, polyphase, 10″, K & E 4053-3.	6	Each	12.15	72.90
37. Stapler, paper fastening, office, lever type, standard duty, holds 210 ¼″ x ½″ staples.	1	Each		2.25
38. Staples, paper fastening, office type, for 137 above.	1	Box		.35
39. Tablet, section, regular coordinate, 50 sheets/pad, Shaw-Walker Mastercraft No. T710-C.	1	Pad		.95
40. Tape, pressure-sensitive adhesive, masking, creped, manila color:				
½″ x 60 yds.	8	Roll	.50	4.00
41. 1″ x 60 yds.	20	Roll	.60	12.00
42. Tissue, facial, 400/box.	48	Box	.25	12.00
Total, office supplies.				1,285.66

J. Miscellaneous

	Quantity	Unit	Unit price	Total price
1. Box, wood, 16″ x 16″ x 20″.	4	Each	Made locally.	
2. Box, wood, 8¼″ x 10¾″ x 20¾″, inside dimensions.	2	Each	Made locally.	
3. Box, wood, 8¼″ x 5¼″ x 14¼″, inside dimensions.	4	Each	Made locally.	
4. Irradiation rack (for riboflavin determination).	2	Each	Made locally.	
5. Knockdown tube racks, for thiamine determination.	2	Each	Made locally.	
6. Polyethylene lay-flat tubing (for use with formalin), 0.005″ thick, 500 ft roll, 3″ wide.	1	Each		$8.00
7. Ditto, 6″ wide.	1	Each		12.60
8. Sealer, bag (Teflon covered), Model No. 72, 115 or 230 volts (specify voltage required). Order from Clamco Division, Cleveland Detroit Co., 5400 Brookpark Rd., Cleveland 29, Ohio.	2	Each	$14.95	29.90
Total, miscellaneous.				50.50

K. Books (Reference)

	Estimated price
1. Association of Official Agricultural Chemists. Official methods of analysis of the Association of Official Agricultural Chemists. 9th ed., Washington, D.C., The Association, 1960	$17.50
2. Becker, B. G., Indik, B. P., and Beeuwkes, A. M. Dietary intake methodologies—a review. Ann Arbor, Mich., University of Michigan School of Public Health, 03188-2-T, 1960	
3. Burgess, R., and Dean, R. F. A. Malnutrition and food habits. N.Y., Macmillan, 1963	5.00
4. Burton, B. T., ed. The Heinz handbook of nutrition. N.Y., Blakiston, McGraw-Hill, 1959	5.75
5. Chemical Rubber Co. Handbook of chemistry and physics. Latest ed., Cleveland, Ohio, Chemical Rubber Co	12.00
6. Cochran, W. G. Sampling techniques. 2d ed., N.Y., Wiley, 1962	7.50
7. Consolazio, C. F. Biochemical and dietary procedures. Denver, Colo., U.S. Army Medical Research and Nutrition Laboratory Report 242, 1960	
8. FAO-WHO Expert Group. Calcium requirements. Geneva, Switzerland, published jointly by Food and Agriculture and World Health Organizations, 1962 (Food and Agriculture Organization Nutrition Meetings Report Series No. 30) (World Health Organization Technical Report Series No. 230)	.75
9. Follis, R. H., Jr. Deficiency disease. Springfield, Ill., C. C. Thomas, 1958	14.00
10. Food and Agriculture Organization. Calorie requirements. Rome, Food and Agriculture Organization of the United Nations, 1957 (FAO Nutritional Studies No. 15)	1.50
11. Food and Agriculture Organization. Manual on household food consumption surveys. Rome, Food and Agriculture Organization of the United Nations, 1962 (FAO Nutritional Studies No. 18)	1.00
12. Food and Agriculture Organization. Food composition tables—minerals and vitamins—for international use. Rome, Food and Agriculture Organization of the United Nations, 1954 (FAO Nutritional Studies No. 11)	1.00
13. Food and Agriculture Organization. Protein requirements. Rome, Food and Agriculture Organization of the United Nations, 1957 (FAO Nutritional Studies No. 16)	.75

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14. Frandsen, J. H., ed. Dairy handbook and dictionary. Amherst, Mass., The Editor, 92 High Street, 1958	$15.50
15. Goldsmith, G. A. Nutritional diagnosis. Springfield, Ill., C. C. Thomas, 1959	5.50
16. Harris, R. S., and von Loesecke, H. Nutritional evaluation of food processing. N.Y., Wiley, 1960	13.50
17. Harrison, T. R., et al. Principles of internal medicine. 4th ed., N.Y., Blakiston, McGraw-Hill, 1962	27.50
18. Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical physiological chemistry. 13th ed., N.Y., Blakiston, McGraw-Hill, 1954	13.50
19. Hill, A. B. Principles of medical statistics. 7th ed., N.Y., Oxford University Press, 1961	4.50
20. Holt, L. E., Jr. Pediatrics 13th ed., N.Y., Appleton-Century-Crofts, 1962	18.00
21. INCAP (CNND food composition table for use in Latin America. Bethesda, Md., Institute of Nutrition of Central America and Panama and Interdepartmental Committee on Nutrition for National Defense, National Institutes of Health, 1961 (available in English or Spanish)	
22. Interdepartmental Committee on Nutrition for National Defense. Reports: Iran, 1956; Pakistan, 1956; Korea, 1957; The Philippines, 1957; Libya, 1957; Turkey, 1958; Spain, 1958; Alaska, 1959; Ethiopia, 1959; Peru, 1959; Vietnam, 1959; Ecuador, 1960; Chile, 1961; Colombia, 1961; Republic of China, 1961; Thailand, 1962; Lebanon, 1962; West Indies, 1962; Burma, 1963; Uruguay, 1963; Jordan, 1963; Bolivia, 1964; Venezuela, 1964	
23. Jacobs, M. B. Chemistry and technology of foods and food products. 2d ed., N.Y., Interscience, Wiley, 1951	16.50
24. Jelliffe, D. B. Infant nutrition in the tropics and subtropics. Geneva, Switzerland, 1955 (World Health Organization Monograph Series No. 29)	5.00
25. Long, C., ed. Biochemists handbook. N.Y., D. Van Nostrand, 1961	25.00
26. Manson-Bahr, P. H., ed. Tropical diseases. 15th ed., Baltimore, Md., Williams & Wilkins, 1961	15.00
27. Meites, S., and Faulkner, W. R. Manual of practical micro and general procedures in clinical chemistry. Springfield, Ill., C. C. Thomas, 1962	11.50
28. Merck & Co. The Merck index of chemicals and drugs. 7th ed., Rahway, N.J., Merck & Co., 1961	12.00
29. Merck & Co. Merck manual of diagnosis and therapy. 10th ed., Rahway, N.J., Merck & Co., 1961	7.50
30. Natelson, S. Microtechniques of clinical chemistry for the routine laboratory. Springfield, Ill., C. C. Thomas, 1957	14.75
31. National Research Council. Evaluation of protein nutrition. Washington, D.C., National Academy of Sciences, 1959 (National Research Council Publ. 711)	2.00
32. National Research Council. Meeting protein needs of infants and children. Washington, D.C., National Academy of Sciences, 1961 (National Research Council Publ. 843)	5.00
33. National Research Council. Recommended dietary allowances. Washington, D.C., National Academy of Sciences, 1964 (National Research Council Publ. 1146)	1.00
34. Nicholls, L. N. Tropical nutrition and dietetics. 4th ed., Baltimore, Md., Williams & Wilkins, 1961	10.00
35. Platt, B. S. Tables of representative values of foods commonly used in tropical countries. Her Majesty's Stationery Office, F. Mildner & Sons, London, 1962	1.40
36. Rand Corp. A million random digits with 100,000 normal variations. Glencoe, Ill., Free Press, 1955	15.00
37. Toverud, K. U., Stearns, G., and Macy, I. G. Maternal nutrition and child health, an interpretative review. Washington, D.C., National Academy of Sciences, 1957 (National Research Council Publ. 123)	2.00
38. U.S. Department of Agriculture. Amino acid content of foods. Washington, D.C., U.S. Government Printing Office, 1957 (Home Economics Research Report No. 4)	.45

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39. U.S. Department of Agriculture. Composition of foods—raw, processed, prepared. 2d ed., Washington, D.C., U.S. Government Printing Office, 1963 (Agriculture Handbook No. 8)	$1.50
40. U.S. Department of Agriculture. Fatty acids in food fats. Washington, D.C., U.S. Government Printing Office, 1959 (Home Economics Research Report No. 7)	.05
41. U.S. Department of Agriculture. Folic acid content of foods. Washington, D.C., U.S. Government Printing Office, 1951 (Agriculture Handbook No. 29)	.45
42. U.S. Department of Agriculture. Food—the yearbook of agriculture. Washington, D.C., U.S. Government Printing Office, 1959	2.25
43. West, E. S., and Todd, W. Textbook of biochemistry. 3d ed., N.Y., Macmillan, 1962	16.75
44. White, A., Handler, P., Smith, E., and Stetten, D. Principles of biochemistry. 3d ed., N.Y., Blakiston, McGraw-Hill, 1962	15.00
45. Wohl, M. G., and Goodhart, R. S. Modern nutrition in health and disease. 2d ed., Philadelphia, Pa., Lea & Febiger, 1960	18.50
Total, books.	363.35

Grand total: $21,497.78.

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