

METHODS FOR DETERMINING THE
NUTRITIONAL STATUS OF POPULATIONS

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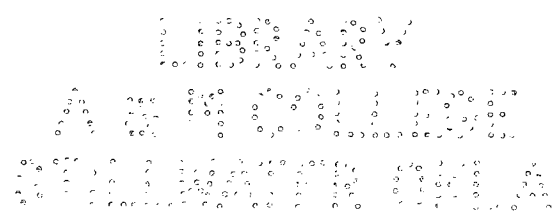
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INTRODUCTION

The question of nutritional status is one of the most important problems now confronting the United States. Due to the progress that has been made in the field of nutrition and to the acute importance of physical fitness at the present time the condition of undernutrition of the American people has become very apparent.

The failure to recognize the existing degree of undernutrition in the past, except by scientists, has shown the need for reliable measurements for determining the nutritional condition of individuals and of populations. It was from this need that the present study of methods for the determination of the nutritional status of populations arose.

The present study might well be divided into three parts:

1. To review, in general, the existing methods for determining the nutritional status of individuals and of populations.

2. To formulate procedures suitable for making a study of the nutritional condition of the State of Oklahoma.

3. To apply as much of the plan formulated as is feasible to a small group of subjects.

The methods for determining the adequacy of food intake for optimal nutritional condition of individuals and of populations fall into three general groups:

1. The comparison of the food consumption with accepted recommendations for nutritional adequacy.

2. Anthropometric, physiological and biochemical measurements, made on the individuals, in addition to physical and dental examinations.

3. Combination of the first and second methods in which food consumption and physiological studies are both made.

Food consumption is generally measured by the use of various kinds of dietary surveys. The different ways of conducting dietary surveys will be discussed in the Literature Review of this paper.

The physical examination has been one of the means by which physicians have judged nutritional condition for many years. This method is being used at the present time by the Army and Navy in examining men for Selective Service (46).

The anthropometric measurements which have been used in the past as being indicative of nutritional status are varied and numerous. Not all of the measurements will be discussed in this paper, only those believed to be directly related to nutrition.

The physiological measurements that have been used to assist in ascertaining nutritional condition are almost as numerous as the anthropometric measurements. The physiological and biochemical tests which are considered in detail in this study include: erythrocyte counts, hemoglobin determinations, determinations of the volume of the packed cells and the vitamin C content of the blood plasma. The Literature Review also includes studies of vitamin B₁ and of vitamin A.

As can readily be seen it would not be feasible to apply any procedure to every individual in Oklahoma to determine the nutritional status of the people of the State. Therefore, it is necessary to study the State from the standpoint of choosing regions and portions of populations in such a manner as would assure a representative sampling.

It is the problem at hand to formulate a plan for selecting a representative sampling and a plan for applying to these samples measurements which are reliable, practical, and possible to use in determining the nutritional status of the people of the State.

In the present study, it was not possible to apply the plan for the whole State, therefore, two groups of pre-school children were chosen as subjects for application of the procedures formulated that were possible.

The two groups that cooperated in the study consisted of the children from the Nursery School of the Oklahoma Agricultural and Mechanical College and the children from the Nursery School of the Works Projects Administration. Both schools were located in Stillwater. The children studied were four and five years of age and represented economic levels which would be expected anywhere in the State.

REVIEW OF LITERATURE

Dietary Surveys and Sampling of Populations

According to the 1937 report of the Technical Commission on Nutrition of the League of Nations Health Organisation (6), there are four types of dietary surveys. The Commission stated the following:

"These types are differentiated not so much by the extension of the group of persons under examination as the nature and the size of the social unit to which they relate."

Thus, the units selected for investigation may consist of an entire nation; a particular social group such as the Army, a school, a prison, or more frequently, a family or a single person.

The first type of dietary survey described consists of statistical studies of the food resources. However, the records of the resources cannot be regarded as equivalent to actual consumption records during the period studied, due to the fact that part of the supplies are intended for purposes other than for local consumption. An attempt may be made to assess the amount so used and this amount can be subtracted from the figure for the total food resources. The result gives, roughly, a figure for the quantity of food used for human consumption.

The second type of dietary survey, set up by the Commission, is concerned with inventory studies covering

particular social groups and institutions. Dietary studies of this type have been carried out in the Army and Navy and in institutions with a large number of inmates, such as children's homes, boarding houses, and prisons. The technique of the survey is simplified by the fact that the food is prepared and eaten in large common kitchens and dining halls. The total amount of food served to the groups divided by the number of persons comprising the groups gives, with a minimum of error, an idea of the diet of each individual.

The third type of dietary considered is the family-enquiry type, and is that most frequently used. There are certain difficulties which arise in making this type of study. The investigator has to live with the family under investigation, or at least to visit it frequently to procure and check the information desired. He may, therefore, seriously disturb the habits of the family, especially the dietary habits, either because the housewife tries to conceal the real circumstances of her family or because, during the survey, the cooking is simplified in order to facilitate the work of the enquiry. According to the Commission, there are several factors which can be used in classifying the families to be studied into homogeneous and representative groups. The most important factors listed were: 1. Physiological, which includes the differences due to age, sex, race, size and state of health of the group considered.

2. Social, which could be divided into geographic and pecuniary factors.

The fourth type of dietary survey is the exclusively individual enquiry. It is by far the most difficult kind of enquiry to carry out. The procedure, as a rule, consists of weighing the foods actually consumed by the persons concerned.

In summarizing the four types of dietary surveys considered, the Technical Commission on Nutrition stated:

"The more elaborate the technique employed for the nutritional survey, the more likelihood there is of introducing this fundamental cause of error, that the investigation tends to modify the dietary habits to be analyzed."

The United States Departments of Agriculture and Labor, 1936-37, sponsored the analysis of the food supplies of families of the United States which was reported by Hazel K. Steibling (48). Each family that cooperated in the study consisted of a husband and wife, both native born Americans. The families lived in various parts of the country, some on farms, some in villages and others in cities. The method of collecting the information concerning diets was as follows:

" A trained worker helped the homemaker make a record of the kinds and quantities of food on hand at the beginning of the study. Each day they weighed the foods brought into the house for family meals and listed the name, age, and work of every person eating from the family larder."

"After seven days another inventory was taken of the food on hand."

"From these data the quantities of each kind of food that the family had during the one-week period was

determined, and the nutritive value of the diet was computed from average figures on food consumption. Each family's record was then compared with standards of what would constitute an adequate diet for persons included in the group. Each diet was classified as good, fair, or poor."

The classifications were made according to the standards set forth by the author (43) in 1941. Excellent diets met in all respects the specifications of the liberal standards. Good diets exceeded the minimum standard by at least a fifty per cent, but by less than a hundred per cent margin in the case of the vitamins. Fair diets met the minimum standard in all respects but exceeded it by less than a fifty per cent margin. Poor diets failed to meet the minimum standard in one or more respects.

The standards set up were as follows:

<u>Food Values Considered</u>	<u>Liberal Standards</u>	<u>Minimum Standard</u>
Protein, in grams	75.00	50.00
Calcium, in grams	0.69	0.45
Phosphorus, in grams	1.32	0.88
Iron, in milligrams	15.00	10.00
Vitamin A, in International Units	6,000.00	3,000.00
Thiamin, in milligrams	2.00	1.00
Ascorbic Acid, in milligrams	60.00	30.00
Riboflavin, in milligrams	1.80	0.90

According to Dr. Stiebling, there are four points to consider in interpreting the facts from the study made.

These facts summarized as follows:

" 1. Information on the vitamin and mineral recommendations was tentative and subject to change with the progression of science. 2. The potential nutritive value of food brought into the house for family meals may have been higher than the value of

the food actually eaten, due to the household waste of edible food. 3. The dietary studies covered only a single week. Diets vary in nutritive quality from week to week and from season to season. 4. Knowledge of human food needs, especially for minerals and vitamins, was far from complete at the time the study was made."

Despite these factors the survey was significant because the diets of each group were analyzed in the same manner.

At the present time only a few studies have been made to determine the nutritional status of the people in the State of Oklahoma.

In 1937, a survey (8) was conducted to determine the habits of food consumption of the farm people in the different type-of-farming areas in Oklahoma and to determine the portion of the amount of food consumed that was bought and the portion that was produced on the farm.

According to the bulletin (8), this project was initiated as an Agricultural Adjustment Planning Project. A state-directing committee and a state-working committee were organized to carry out the work and a staff member of the Extension Division was designated as the leader. The original organization was made in 1935, at which time the Extension Service of the United States Department of Agriculture and the Agricultural Adjustment Administration launched cooperatively the County Agricultural Planning Project. However, before 1937, the activities carried on under the project related to adjustment in the farm plant and there was little or no consideration of the needs of the family or possible adjustment in the home that might be significant in connection with

the general welfare of the family.

In 1937, information forms were prepared and distributed to county home demonstration agents together with instructions for their use. The home demonstration agents conducted a school for representatives from each of the home demonstration clubs in the county to instruct them in the use of the schedules. These representatives from the various clubs assumed the responsibility of getting a certain number of the schedules completed. A total of 3,845 usable schedules were obtained from the seventy-seven counties of the State.

One schedule was made out for each family represented in all of the home demonstration clubs. There were recorded on the schedule the number of adults in the family, the number of children under twelve and the number of children over twelve. The annual dietary requirements as established by Hazel K. Stiebling (49) for various foods and for classes of foods for men, women, children over twelve and children under twelve, were set up on the basis of moderately active labor. Records of food consumption were recorded on the schedules and were compared with the standards set up by Stiebling.

There are in Oklahoma sixteen different type-of-farming areas (12). The term "type-of-farming" indicates a definite system of agricultural operation, the kind, the amount, and the proportion of crops and livestock found on an individual farm. A single farming area is considered a region in which

exists a fairly high degree of uniformity in the type of farming prevailing as well as in the soil and climatic condition. The type of farming of any section is largely determined and limited by certain physical factors most of which are beyond the control of the farmer. The natural agencies that influence the type of farming include the five general divisions of location, topography, soil, water supply and weather.

In making the preceding study the different type-of-farming areas were used as a guide for the sampling of the State. The counties included in each are as follows:

<u>Area 1</u>	<u>Area 2</u>	<u>Area 3</u>	<u>Area 4</u>
Beaver Cimarron Texas	Ellis Harper Woods Woodward	Alfalfa Canadian Garfield Grant Kay Kingfisher Major Noble	Osage
<u>Area 5</u>	<u>Area 6</u>	<u>Area 7</u>	<u>Area 8</u>
Graig Mayes Nowata Ottawa Rogers Tulsa Washington	Blaine Custer Dewey Roger Mills	Cleveland Lincoln Logan Oklahoma Pawnee Payne Pottawatomie	Creek Hughes Okfuska Pontotoc Seminole
<u>Area 9</u>	<u>Area 10</u>	<u>Area 11</u>	<u>Area 12</u>
Haskell Wagoner Leflore McIntosh Muskogee Okmulgee	Adair Cherokee Deleware	Beckham Greer Harmon Jackson Tillman	Caddo Comanche Cotton Grady Kiowa Stephens Washita

<u>Area 13</u>	<u>Area 14</u>	<u>Area 15</u>	<u>Area 16</u>
Garvin	Atoka	Carter	Bryan
McClain	Coal	Jefferson	Choctaw
	Latimer	Johnston	McCurtaim
	Pittsburg	Love	Marshall
	Pushmataha	Murray	

The conclusions, which were drawn as a result of the study, are given in Table 1.

Another report in which the sampling of the State's population was studied was that of W. H. Sewell (47), of the Department of Sociology and Rural Life, Oklahoma Agricultural and Mechanical College. He stated that in setting up requirements for the selection of an area to be studied in measuring the socio-economic status of the State, there were two general requirements to be met.

"First, it should be representative of the state as a whole in as many characteristics as possible. Second, it should contain within its boundaries as many levels of farm family socio-economic status as possible so that the final scale constructed would have wide usefulness in other rural sections of the United States."

Since no one county in the State of Oklahoma was found, according to Dr. Sewell, either to possess characteristics sufficiently representative of the State as a whole or to have sufficient families in the various economic levels to be considered, it was decided that the sample area would have to include more than one county.

In choosing the counties for study the following specific criteria were employed, pre-dominant type of farming, tenure distribution of farm operators, and rural farm plane of

Table 1.

FOODS IN WHICH THE VARIOUS TYPE-OF-FARMING AREAS WERE FOUND TO BE DEFICIENT. (" indicates a deficiency)

Area	Potatoes	Dried Beans Peas & Nuts	Tomatoes	Green Vegetables
1	"	"	"	"
2		"	"	"
3		"	"	"
4		"	"	"
5				"
6			"	"
7			"	"
8			"	"
9			"	"
10				
11			"	"
12			"	"
13			"	"
14			"	
15			"	"
16			"	"

Area	Other Vegetables	Fruits	Grain Products	Milk	Eggs	Meat
1	"	"		"	"	
2	"	"		"	"	
3	"	"		"	"	
4	"	"	"	"		"
5	"			"		
6	"	"		"		"
7	"	"		"		
8	"	"		"		
9	"	"		"		
10	"			"		"
11	"	"		"		
12	"	"		"		
13	"			"		
14		"		"		
15	"	"		"		
16	"	"		"		

Oklahoma as a whole showed a deficit in the consumption of green vegetables, other vegetables, fruits and milk.

living. After taking these criteria into consideration, three counties were chosen as the sample area from which to collect the data desired. These counties were Haskell, Cotton and Major. Haskell was chosen to represent the southeastern small scale cotton and self-sufficing farming area. Cotton county was chosen to represent the southwestern large-scale cotton and wheat farming area. Major county was selected to represent the northwestern large scale wheat and livestock area.

In the actual sampling of the population of these three counties random sampling of 13.0 per cent of the unbroken farm families was employed. The farm families were interviewed by trained "schedule takers", each of whom was a college graduate.

At the present time there is being carried out in the State of Pennsylvania a long-time study on the nutritional condition of families representing various socio-economic groups. This plan was inaugurated by the Division of Home Economics Research of the Pennsylvania State College (30) in 1935. In this study the nutritional status, as judged by physiological and biochemical tests made on the people themselves, members of families that are also being studied in relation to the family income, money spent for food, types of food selected and quantities eaten and the education of the adult members of the family. In investigating the nutritional status in relation to their dietary habits,

family dietary intakes in the form of daily records kept by the housewife and supplemented by grocery slips, were kept for three months for the first families. An effort was made to have records kept of the food consumed rather than the food purchased. Analyses of these dietary data showed that a two-week period of record keeping gave average weekly intakes varying but little from those obtained from the three-month records, and the shorter time was used for the remaining families.

Anthropometric Measurements

The chief difficulty encountered in judging nutritional status has been in the determination of reliable measures for normal nutritional condition. One measure which has been used the most in the past has been weight in relation to height and age. The Baldwin-Wood (3) scale of height, weight and age has been universally used. In preparing these scales, in 1924, 74,000 boys and 55,000 girls were observed. These subjects were all healthy, normal individuals and ninety-five per cent of them were American born. Although the curves of growth, in relation to age, for boys and girls show the same trends, they are not identical. According to these standards boys are usually heavier and taller than girls at birth and their growth curves remain above those of girls throughout middle childhood.

There have been many different methods used in measuring height and weight during the past thirty years. The method

adopted by the Society of Research in Child Development of the National Research Council (39) in 1940, will be described in the Procedure of this paper.

Objections to the use of the height and weight relationship have been based partly on the fact that it fails to take into consideration the differences in other skeletal dimensions or the body build. According to a 1940 report by Zayaz, Mack, Sprague and Bauman (57) there are three objections to the commonly used Baldwin-Wood height-weight scale and these authors think it is doubtful that such a measure is valuable for research purposes, because:

" (1) No objective criterion of normality was available to those preparing the scale; (2) body width is not taken into consideration and (3) the tables represent average and not optimum height and weight relations."

Lucas and Pryor (29), believing that weight should be in proportion to skeletal size, tested various indices derived from skeletal measurements on 1,000 children from two to seventeen years of age, and chose a simple width-length index as the most satisfactory measurement to show normal growth. They chose the pelvis diameter because it was easily taken and was a constant measurement and not subject to change with respiration as were chest measurements. These authors reported that such anthropometric measurements are advantageous in that it is possible with them to record and analyze the changes in dimensions of the body and to determine the rate of growth.

The total number of physical measurements which have been devised (39) is very large, but only a few will be discussed here, as all do not relate directly to nutritional condition.

According to Hrdlicka (23) "the most important qualities in anthropometry are the need of precision and the value of simplicity." The measurements recommended by Hrdlicka (10) for the purpose of indicating the nutritional status include the following: weight, height, chest breadth and depth, circumference of each arm and of the left leg, and the pressure measurement of each hand.

Some of the anthropometric measurements recommended by the National Research Council (39) in 1938, as being informative on growth processes were: 1. body weight 2. stature 3. sitting height 4. width of chest 5. depth of chest 6. waist girth 7. chest girth 8. circumference of hips 9. circumference of right arm and 10. circumference of right leg.

In recent years there has been much controversy regarding the reliability of anthropometric measurements even as measurements of dimensions of bony structure. Mack and Smith (30) in 1939, Maresh and Deming (31) in the same year, and Lucas and Pryor (29) in 1931. Some authorities contend that they are not reliable or accurate enough to be used as a measurement of nutritional status.

By comparing anthropometric measurements with roentgenograms of living subjects, Maresh and Deming (31) found that

there was apparently so much error inherent in the anthropometric measurements that data on individual cases could not be subjected to precise statistical analysis. These conclusions were drawn as a result of a study made on the growth of the long bones of eighty newborn infants at the Florence Crittendon Home in Denver. Measurements and roentgenograms were taken on the children on the same day. Serial examinations were made at approximately six-weeks intervals, and no data on a child were used unless the child had had at least three examinations. All measurements, anthropometric and x-ray, were made by the same person. In contrast to the inaccuracy of the anthropometric measurements it was found that roentgenograms were accurate enough for detailed statistical analysis.

Physiological and Biochemical Measurements

During the past decade a consideration of the vitamins has become an important part of the study of nutrition and of nutritional status. The deficiency diseases due to the lack of certain vitamins have for a long time offered means of detecting extreme cases of vitamin deficiency. The prevalence of marked deficiencies has decreased since their cause has been known but that of mild cases, which are much more difficult to detect, is still great.

In recent years the vitamin C content of body fluids has been given a prominent place as a possible measure of nutritional status with regard to this vitamin. According

to McLester (32) in 1940, and Everson and Daniels (11) in 1936, the values of cevitamic acid in the urine and in the plasma of the blood have been shown to have a definite correlation with the degree of tissue saturation.

Wolbach (54) in 1937 stated that:

"The intercellular substances concerned with vitamin C deficiency are the collagen of all fibrous tissue structures, the matrice of bone, dentin and cartilage, and all non-epithelial cement substances including that of the vascular endothelium."

Wolbach also stated that other pathological conditions resulting from vitamin C deficiency were changes of a hemorrhagic nature in the soft tissues. These could be observed by the response of the blood capillaries to mechanical stresses and trauma. This test is commonly known as the capillary fragility test and was used as a measure of vitamin C nutrition until it was found to be a non-specific test.

Everson and Daniels (11), in 1936, made a study of pre-school children to determine how much vitamin C was needed for normal growth. The study was made with three normal boys aged 31, 57 and 59 months. During the investigation the children lived under controlled conditions. Each study period consisted of fifteen days, a five-day preliminary period for physical adjustment and two five-day balance periods. During four periods a part of the vitamin C was supplied by a synthetic commercial preparation. Under the condition of the investigation, urinary excretions of ascorbic acid were found to parallel the intake of the acid.

Retentions of ascorbic acid paralleled the ingestion only up to 7.5 mg. per kg. of body weight per day. Higher ingestions (10-15 mg. per kg. of body weight) were without influence on the retentions of ascorbic acid of the children studied. The highest retentions, estimated either on the basis of weight or creatinine elimination, were obtained with the younger child. This observation suggested that there was a greater demand in younger tissue for vitamin C. Equal amounts of commercial ascorbic acid and ascorbic acid from foods resulted in similar retentions.

Yavorsky, Almaden and King (56), in 1934, found by chemical analysis of the vitamin C content of human tissues from 67 hospital autopsies that the vitamin C content of human tissues corresponded closely to the vitamin C content of tissues of guinea pigs. The tissues listed in decreasing order with respect to vitamin C concentration, were: adrenals, brain, pancreas, liver, spleen, kidney, lung, heart, and muscle. In the subjects in the younger age group the content of the thymus was about as high as that of the pancreas. The average values ranged from about 0.55 mg. per gm. for adrenal tissue to 0.04 mg. for heart tissue. Approximately twenty per cent of the human cases studied gave evidence of a condition of latent scurvy.

Studies of the intake and urinary excretions of vitamin C of four normal adults were reported by O'Hara and Hauck (41) in 1936. The basal diet used in the study was adequate

except that it contained only 5 mg. of vitamin C per day. The diet was ingested for approximately thirty days, following which, it was supplemented daily with 200 mg. of cevitamic acid in the form of orange juice. This high intake was continued until the subjects were saturated, as evidenced by their failure to show further increases in urinary output of vitamin C on a constant high intake. In addition to quantitative analysis of the urine for vitamin C, capillary resistance studies were made. These yielded variable results and could not be correlated with the urinary excretions. The estimated storage of vitamin C for these subjects, was from about 2,500 to 3,000 mg.

According to McLester (32), in 1940, the determination of ascorbic acid in the urine or blood plasma by titration was the most dependable measure for determining the condition of the body with respect to vitamin C. As applied to urine the following procedure determined the subjects' reserves of this substance. A measured quantity of ascorbic acid was given intravenously and the nature of the response was taken as indicative of the adequacy with which the subject had been previously supplied with the vitamin. If the body was in a saturated state there would be an immediate increase in the excretion of the ascorbic acid in the urine. If the body was in a state of unsaturation little or no rise in excretion would occur.

According to Bessey, Boyle and Wolbach (5), the measure-

ment of the ascorbic acid in the blood plasma, by titration, was more dependable than the measurement of the excretion in the urine. The author also stated that this test

"provided means for evaluating the reserve of vitamin C by a single determination for persons in that wide zone between scurvy and saturation."

According to King (26), normal body reserves of vitamin C resulted in 1.2 mg. of vitamin C per 100 cc. of blood, but depletion reduced this value to about 0.8 mg. as the "pre-scurvitic state" was reached, and reduced it to approximately 0.5 mg. with the appearance of "clinical scurvy."

Farmer and Abt (14) determined, in 1935, the reduced ascorbic acid content of blood plasma of living subjects and reported values of from 0.69 to 2.36 mg. per cent.

Mirsky, Swadesh and Soskin (23) reported, in 1935, ascorbic acid values in blood plasma, of apparently normal individuals, from 1.19 to 2.66 mg. per cent. The figures for this report were based on values obtained from blood samples taken from subjects in the post-absorptive state and represented observations on about 100 different individuals.

Farmer and Abt (13), in 1936, devised a method in which the reduced ascorbic acid could be determined in small amounts of blood. This method consisted of first collecting about 0.3 ml. of blood from a lancet wound and centrifuging it for a few minutes. Then the plasma was deproteinized by adding metaphosphoric acid and the mixture was centrifuged again. The vitamin C content of the deproteinized plasma

was determined by titration with a standard solution of sodium 2:6- dichlorobenzeneindophenol. The details of the method will be described in the Procedure of this paper.

Wortis, Liebman and Wortis (55), in 1938, studied the vitamin C content of the blood, the spinal fluid, and the urine of 133 patients. The study was made after a test dose of one gram of cevitamic acid had been given intravenously. It was found that a value for vitamin C above 0.7 mg. per 100 cc. of blood plasma was almost invariable associated with a normal spinal fluid content of vitamin C and a normal urinary excretion level for vitamin C. A blood content below 0.4 mg. per 100 cc. of blood plasma was almost invariably associated with a sub-normal urinary excretion and sub-normal spinal fluid content. In these ranges (0.4-0.7 mg. per 100 cc. of blood plasma) the vitamin C content of the blood was said to be an adequate and accurate index of the state of vitamin C nutrition. The authors stated that in the intermediate, sub-normal range (from 0.4 to 0.69 mg. per 100 cc. of blood plasma) all available tests should be used, including clinical evaluation to determine the health of a patient.

It is difficult to evaluate the physiological and pathological significance of the milder types of malnutrition and deficiency diseases, but it is becoming evident that these are closely related to other diseased conditions previously believed to be unrelated to nutrition.

King and Monton (37), in 1935, determined the influence of various levels of vitamin C on the resistance of guinea pigs to diphtheria toxin. The main purpose of the experiment was to find out whether or not there was a significant lowering of resistance to the toxin in the zone of vitamin C deficiency where the typical symptoms of scurvy were not well developed. Guinea pigs receiving daily amounts of vitamin C at abundant, protective, and sub-protective levels were given subcutaneous injections of standard diphtheria toxin in 0.1, 0.3 and 1.0 minimum lethal doses. When the animals had been partially depleted of their vitamin C reserve but did not show signs of scurvy the injections of the toxin shortened the survival time about fifty per cent and the loss in body weight was severe. Hemorrhage and necrosis at the site of the toxin injection were marked when the animals were in the condition of "latent scurvy". The decrease in the oxygen consumption of the kidney tissue after the injection of toxin was in the range of 5 to 15 per cent. It was evident from this study that there was a wide zone of vitamin C deficiency without the appearance of scurvy.

Tests for vitamin A status have been used widely as a measure of determining the nutritional condition of individuals with regard to vitamin A. The types of experimental methods that have been used include the optical tests for

dark adaptation and vitamin A content of the blood (30).

The optical tests for vitamin A status are based upon the generally conceded fact that retarded dark adaptation in a human subject is the result of a distinct avitaminosis, and that nyctalopia is one of the first indications of vitamin A deficiency. The explanation of this method lies in the relationship between the vitamin A available in the body and the functioning of the visual purple and violet in the retina, or in other words, the ability to regenerate these pigments after bleaching by light is associated with the amount of vitamin A supplied to the eye by the blood stream.

Jeans and Zentwre (24), in 1934, introduced the Birch-Hirschfeld photometer as an instrument for ascertaining the condition of nyctalopia. This instrument consisted of a metal tube mounted horizontally on a standard, enclosing a small light bulb, and containing in the order stated: an iris diaphragm, a five point disc and a Goldberg wedge in front of an opening in the end of the tube which housed the light bulb. The intensity of the light, which operated on a 110-volt circuit was regulated by a rheostat. In using this test instrument two tests were made with each subject: first, after the subject had looked for five minutes at a white screen placed at a standard distance in front of him and illuminated by a 200-watt Mazda lamp, and second, after a 10-minute subsequent period in the darkness.

Jeans, Blanchard and Zentmire (25) used a new photometer for measuring the ability to adapt to darkness. The intensity of the light with which the photometric readings were made was controlled by a rheostat calibrated in terms of millifoot candles. This calibration permitted the results of one investigator to be compared with those of another, a procedure generally difficult in the case of dark adaptation tests. In one study by these authors 23 children of an orphanage were tested and 35 per cent were found to give abnormal results. A survey of a group of 35 children showed 19 per cent to be sub-normal and 5 per cent were found to be on the border line. Observations on two boys eleven years of age indicated that vitamin A in the amount of 3,000 units daily met the daily requirement as judged by the photometer test.

Hecht and Schlaer (21) designed and built an adaptometer in 1937, which was described in 1938. The Hecht adaptometer provided for a pre-adaptation light of standard intensity; a measuring light from the violet end of the spectrum, below 460 mm., in which area the retinal location, the color, and the duration of the flicker were under control; and a fixation point at which the subject looked while taking the test. This apparatus is being used at the present time in determining vitamin A deficiency in the mass studies in human nutrition, which are being carried out by the Pennsylvania State College (30).

The status tests for vitamin A and carotene in the blood

has been reported by the Food and Nutrition section of the American Public Health Association (50) in 1939, as follows:

"Carr and Price (7) in 1926, developed a quantitative method of measuring vitamin A. potency from its color reaction with antimony trichloride. Since the blue plates of the Lovibond Tintometer were used in matching the intensity of the color produced the Carr-Price method results are usually expressed in "Lovibond Blue Units." Correlating such tests with spectrophotometric tests and using a highly concentrated A vitamin preparation British workers reported that they got an "E" value of 1600 and a blue unit value of 80,000; a ratio of 1/50. Holmes and Corbet (22) with their pure crystalline vitamin A got an "E" value of 2,000 and a blue unit value of 100,000, the same ratio as the British workers. The Holmes-Corbett product bioassayed at 4,000,000 International Units per gram. On that basis a blue unit value of 1 would correspond to a unitage of 40 I. U. per gm."

"A method of applying the Carr-Price reaction to blood plasma to detect its vitamin A content has been worked out and used for estimating vitamin A content per 100 cc. Unfortunately, when one uses this test with vitamin A sources other than the pure or highly concentrated A materials, there are substances that interfere with the test. The color reaction is also transitory and it is difficult to detect the highest intensity attained. For that reason it is probably impossible as yet to use a conversion factor such as 40 I. U. per 1 blue unit of A found in a natural or biological source and be sure that it gives the absolute quantity of A in units."

Measurement of carotene and vitamin A in the blood plasma promises to be of clinical value, but, according to Munsell (35), the antimony trichloride method now used for this purpose is not a dependable quantitative method. This author believes that "spectroscopic assay holds greatest promise." Perhaps, one reason that the blood vitamin A test has not been extensively used in the vitamin A determinations of populations is the fact that the equipment generally used in spectrophotometric work is expensive and requires a skilled operator to obtain accurate results.

Tests for deficiencies of vitamin B₁ have not yet reached the point at which they are adaptable for general use in studies on the nutritional status of populations. The Food and Nutrition Section of the American Public Health Association (50) reported on this subject in February, 1939, as follows:

"In this field the most widely studied test is that based on a conversion of thiamin into thiochrome, the latter having a blue fluorescence whose intensity is measurable by colorimetric methods. . . ."

"This compound is producible by the action of potassium ferrocyanide on thiamin or on the co-carboxylase, thiamin-pyrophosphate."

"The principles involved in making this test are: the extraction of the B₁ from the urine by an adsorbent, the removal of the thiamin from the adsorbent by a suitable reagent. Then the conversion of the thiamin into thiochrome, production of fluorescence by ultra-violet irradiation and measurement of the intensity in contrast to standard solutions of thiochrome."

Harris and Leong (20) studied the excretion of vitamin B₁ through the kidney to determine the amount of the vitamin required for optimal health. A study was made of adults from 13 to 37 years of age subsisting on normal diets. The subjects were found to excrete daily from 12 to 35 international units, 30 to 90 micrograms, of the pure vitamin hydrochloride; this excretion was estimated to be approximately from 5 to 8 per cent of the total daily intake of the vitamin. These authors concluded that a urinary output of vitamin B₁ below 12 international units daily, approximately one unit per 100 cc. of urine, should be regarded as evidence for strongly questioning the adequacy of the vitamin B₁ in the diet. On the basis of these observations the minimum daily requirement

for a man weighing 140 pounds was set at 200 international units.

Knott (28), in 1936, reported vitamin B₁ balance studies made with eight children from 4 to 7 years of age. As a result of this study it was found that the intakes which resulted in the highest retention were found to be from six to seven times greater than the minimum requirement for preventing beriberi as determined by means of the formula suggested by Cowgill (9). Knott made the remark that

"If the intakes resulting in the highest retentions may be considered optimum, this wide range between minimum and optimum requirement would seem to explain both the existence of vitamin B₁ deficiency among children and the beneficial results obtained by additions of vitamin B₁ to the diets."

The suggested optimum for young children was expressed by Knott as "About 40 Chase-Sherman units per kilogram per day." This would be equal to about 20 international units daily per kilogram of body weight. On the basis of the body weight and caloric intake, according to Knott, this would mean about 20 to 25 international units per 100 calories of food eaten.

Iron Metabolism

In addition to the vitamin C content of the blood, other constituents of the blood are used as criteria in the determination of the nutritional status of an individual. The tests which are used for judging iron content include: erythrocyte counts, hemoglobin content and volume of the packed cells of the blood. The hemoglobin content and the

red cell counts have been used for some time as routine clinical procedures in the differentiation of the various types of anemia, and in the minds of a great many people this has been the most important use of hematology. However, according to Pepper and Farley (44)

"Hematology does not necessarily find its field of greatest usefulness in the identification and study of the diseases of the hemopoietic system."

Hematology is not only important in the field of medicine but is recognized to be of fundamental importance in the science of nutrition. Since it is believed that the hemoglobin molecule contains four units that are formed of an ironpyrrol compound known as hematin, the estimation of the hemoglobin content of the blood may be used as an indirect measure of the iron content.

There have been many reports of studies made of the erythrocytes of subjects of various ages. Murgage and Andresen (36) studied the red cell counts, the quantity of hemoglobin and the volume of packed cells of 80 boys and girls from the ages of 13 to 21 years. Averages for the boys were almost identical with those for the girls at 13 years of age. The mean values gradually increased with age and at 17 years reached the adult levels for men. Averages for the girls during the adolescent period showed little variation from the levels for women. In the adolescent as well as in the adult subjects, the average volume of the individual red blood cell of the male was found to be smaller

than that of the female. The corpuscular hemoglobin concentration remained at the same level throughout adolescence as it was in other age periods.

Andresen and Mudge (2) in 1936, studied 40 white men and 40 white women of various nationalities, between the ages of 20 to 45 years, to determine the red cell values. These subjects were all healthy normal individuals living at an altitude of 5,000 feet above sea level. Determinations of the number of red cells, the volume of packed cells and the amount of hemoglobin, calculated from the oxygen capacity values, were reported for 240 samples of blood. The mean values for hemoglobin of 16.54 gm. per 100 cc. of blood for men and 14.45 gm. for women and also the mean packed cell volume of 48.25 cc. per 100 cc. of blood for men and 43.44 cc. for women were reported. Mean red blood cell counts of 5,420,000 per cu. mm. for men and 4,630,000 for women were reported.

McMamara and Senn (34) made a study of the blood of 45 new born infants, 146 older infants and 38 children between the ages of 3 and 11 years. The values for hemoglobin, red cell counts and red cell volume showed a rapid decrease during the first two months of life. The average hemoglobin content was 19.1 gm. per 100 cc. of blood on the day of birth, and fell to 17.4 gm. at the end of the first week, and reached its lowest value of 11.2 gm. around the second month. Between the third month and the end of the second year it remained

between 11.6 gm. and 12.3 gm. and thereafter it rose to 13.1 gm. The erythrocyte counts averaged 5,290,000 per cu. mm. of blood on the day of birth, and 4,970,000 at the end of the first week and decreased to 3,720,000 during the second month. It rose to levels between 4,500,000 and 4,810,000 after the fourth month.

In setting up standards, there have been a great many more studies made on the blood of the adult than on the infant and child. A number of standards for the blood of the adult have been reported in various parts of this country. Haden (19) has reported standards for Kansas City, Cleveland and Detroit. Osgood (43) reported standards for Portland, and Wintrobe (53) reported standards for New Orleans.

Most of the hematological studies, with the exception of those done by Murgage and Andresen (37) in Colorado, have been carried out at altitudes of less than 1000 feet above sea level. Residence at high altitudes will, it is generally believed, produce a definite temporary and probably a permanent increase in the hemoglobin content and the number of red cells of the blood. Osgood (43) stated that the red cell count rises 50,000 to 100,000 cells per cu. mm. for each increase of 1000 feet in altitude, and that the values for the volume of packed cells rise correspondingly, but the hemoglobin values do not increase as rapidly.

Andresen and Murgage (2) have prepared a table which shows the values of venous blood reported by workers in

various parts of the country. These values are shown in Table 2.

It has been shown by Andresen and Murgage (1) that in the red cell counts, hemoglobin content and volume of packed cells, that venous blood and peripheral blood are in close agreement and can be used interchangeably. These conclusions were drawn after averages from blood samples of 120 subjects were taken. The subjects ranged in ages from 19 months to adulthood.

In 1939 Barber (4) studied the formed elements of the blood of 101 women enrolled as freshmen in the Oklahoma Agricultural and Mechanical College. The subjects ranged in age from 17 to 23 years. The mean average red cell count reported was 4,510,000 per cu. mm. of blood. A mean value for hemoglobin of 13.31 gm. per 100 ml. of blood was reported, and a mean cell volume of 40.35 per 100 ml.

In the above Literature Review of this paper an attempt has been to review in general the methods for determining, directly or indirectly, status of individuals and of populations.

The methods used in the present study for determining the nutritional status of a limited number of individuals are described in detail in the Procedure of this paper.

Table 2

VENOUS BLOOD VALUES IN THE UNITED STATES					
Authority	Location	Number of subjects in series	Hemoglobin gms. per 100 cc. blood	Volume packed cells percentage	Red Blood Cells millions per cu. mm.
Osgood, Haskins, Trotman	Portland, Oregon	196 men	15.8	46.3	5.4
		100 women	13.7	42.4	4.8
Wintrobe and Miller	New Orleans	100 men	17.0	49.6	5.8
		50 women	13.8	41.5	4.9
Wintrobe	Baltimore	86 men	18.0	47.0	5.5
		101 women	14.1	42.0	4.8
Haden	Kansas City,	70 men	15.3	45.5	4.9
	Cleveland	30 women	13.4	39.8	4.4
	Detroit				
Magrage and Andresen	Denver	40 men	16.5	48.5	5.4
		40 women	14.5	43.0	4.6

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SELECTION OF METHODS

In selecting the methods to be used in studies on nutrition, the Home Economics Research Division of the Pennsylvania State College (30) made a critical examination of each test that seemed likely to assist in ascertaining the nutritional status of human subjects as follows:

"(1) Is the test possible with the funds available for the equipment and personnel which may be required for the study under consideration?"

"(2) Is the test practicable from the point of view of the time which the subjects can conveniently spend in the laboratory?"

"(3) Is the test one to which the subject will submit willingly?"

"(4) Does the test require participation of the subject in such a way that errors may occur through this participation?"

These four criteria were employed in the selection of the tests which were employed in the present study on the children of the nursery schools.

Anthropometric and physiological measurements were employed. The anthropometric measurements consisted of standing height, chest width, chest depth, circumference of each arm and circumference of the left leg. The physiological measurements which were made were erythrocyte counts, hemoglobin content, volume of packed cells and the vitamin C content of the blood plasma.

EXPERIMENTAL PROCEDURES

The subjects of this study consisted of children from the Nursery School of the Oklahoma Agricultural and Mechanical

College and of children from the Nursery School of the Works Projects Administration, both of which were located in Stillwater.

The group from the College Nursery School was made up of 6 girls and 1 boy ranging in age from 52 to 71 months. The group from the Federal Nursery School was made up of 3 girls and 2 boys ranging in age from 49 to 60 months.

All measurements, anthropometric and physiological were made in the same manner and by the same person on the two groups of subjects except in the case of the weight and heights. The weights of the College Nursery School group were taken on a set of Fairbanks scales, while those of the Federal Nursery School group were taken on a set of portable, spring type scales that had been calibrated accurately and in the latter case the heights were measured by means of a portable apparatus.

The height was determined with the subject standing straight, with heels, shoulders and buttocks touching a flat vertical wall. The head was held in the position of the Frankfort (39) plane so that a straight line connecting the lower point on the inferior border of the orbit and the superior border of the tragus was horizontal.

Each subject was weighed with practically all clothing removed and the weight was recorded to the nearest quarter of a pound.

Chest measurements were made with the large metal caliper

after the method of Hrdlicka (39), with the subject's arms partly elevated and held limp. The width of the chest was measured by placing the caliper on the sternum at the level of the fourth chondrosternal articulation and holding it firmly against the thorax. For the depth measurement the caliper was placed in the same horizontal plane as for the width determination. The chest measurements recorded were the means of readings noted during the excursions made on inspiration and expiration.

The maximum circumference of each upper arm below the insertion of the deltoid muscle was obtained with the subject inclining slightly to the side that was being measured in order that the arm was away from the body and relaxed. The maximum circumference of the calf of the left leg was taken while the leg rested on an elevation and the weight was placed on the right leg. All circumference measurements were made with a steel tape.

Freely flowing blood from a finger puncture, made with a spring lancet, was used in all the blood determinations. The first drop of blood was discarded because of its possible dilution with the tissue fluid. The blood was allowed to drop into a phial that had been treated with heparin as an anti-coagulant.

For the determination of the red cell counts, three pipets were prepared from each sample of blood. A 1:200 dilution was made with Hayem's (17) diluting fluid in pipets

certified by the National Bureau of Standards. Six counts were made on each subject, two from each pipet. A certified Levy-Hausser counting chamber was used throughout the study. In counting the cells the following procedure was used:

1. The pipets were shaken for at least ten minutes.
2. The fluid in the capillary of the pipet was discarded before the counting chamber was filled.
3. The cells in five large squares, each of which contained sixteen small squares, were counted and totaled.
4. The total was multiplied by the factor 10,000 to obtain the number of cells in one cubic millimeter of blood.

The mathematical derivation of the factor 10,000 is as follows (4):

"Each small square of the chamber is $1/400$ square millimeter in area; therefore, one large square is $1/25$ square millimeter; and five large squares, the area actually counted, are $1/5$ square millimeters in area. The correction factor for area is 5. Since the blood was originally diluted 1:200, the necessary correction for dilution is 200. The counting chamber is $1/10$ millimeter in depth, therefore, this correction factor for volume is 10. The total correction which must be made to the cells counted in five large squares, in order to obtain the number of cells in one cubic millimeter of blood, is $5 \times 200 \times 10$ or 10,000."

Hemoglobin determinations were made by the acid hematin method which was described by Newcomer (33) in 1919. A one per cent solution of hydrochloric acid was used as the diluent, and two pipets were prepared from each sample of blood. Each dilution was compared colorimetrically with a brown glass standard, which had been prepared by the Bausch and Lomb Company to match the acid hematin color. All readings were

made in a darkened room. A blue filter was used in the eyepiece of the colorimeter to eliminate the matching of the yellow color. A daylight base lamp provided the constant source of light. The average of the colorimeter readings for each subject was converted into grams of hemoglobin per 100 cc. of blood by reference to the conversion table supplied by the company from whom the standard was purchased. The table had also been checked in this laboratory by the oxygen capacity method.

The Van Allen (52) hematocrit method was used in all determinations of the packed redcell volume. Hematocrit tubes were filled to the 100 per cent mark with the heparinized blood. The blood was drawn slightly above the mark to prevent leakage when the tubes were sealed and suspended on the rubber cushions of the special spring-type holder. The tubes were placed in the centrifuge in such a manner that their axes were perpendicular to the axis of rotation while centrifuging. The tubes were centrifuged at 2,750 revolutions per minute for a period of about thirty minutes or until constant readings were obtained. Two determinations were made on each subject.

The Farmer and Abt (15) micro titration method for ascorbic acid analysis in plasma was used in the vitamin C determinations. The reagents which were used consisted of the following: 1. the dye, sodium-2,6-dichlorobenzene indophenol, obtained in powder form from the Eastman Kodak

Company, was used in titrating the vitamin C. 2. Meta-phosphoric acid (5%) was used as the deproteinizing agent for the plasma. 3. Lithium oxalate was used as an anti-coagulant. Special apparatus which were required consisted of a micro-burette, reading directly to 0.002 cc., and micro-blood bottles and micro pipetes. The micro-burette consisted of an accurately graduated capillary pipet held horizontally on a cast fixture. Over the blunt end was slipped a piece of medium-walled rubber tubing whose other end was sealed with a glass plug. This tubing was so placed that it could be compressed by a clamp having a finely threaded screw. When the whole burette was filled with mercury and all of the air expelled, the turning of the screw accurately controlled the position of the mercury meniscus in the burette. The mercury acted as a fluid piston and the dye was drawn in and pushed out by its action. This special apparatus was purchased from E. H. Sargent & Co.

In preparing the dye, 100 mg. of sodium 2, 6- dichloro-benzenone indophenol were placed in a folded filter paper and 80 cc. of boiling water were poured over it. Practically all of the dye was dissolved. After the solution had cooled it was made to volume in a 100 ml. volumetric flask. Five cc. of this solution were then transferred to a 50 ml. volumetric flask and a 1:10 dilution was made. This dilution was standardized against standard solutions of sodium thiosulphate and crystalline ascorbic acid.

Seven or eight drops of blood were collected from the finger of the subject in a micro-blood bottle which contained a small amount of lithium oxalate, and the blood was immediately stirred rapidly with a tooth pick. The micro-blood bottles were placed in ordinary centrifuge cups in which a number of corks were placed in order to decrease the depth, and the blood was centrifuged for ten minutes. At the end of the centrifugation period exactly 0.1 ml. of clear plasma was pipetted into a centrifuge tube. Then 0.1 ml. of distilled water and 0.2 ml. of 5 per cent metaphosphoric acid solution were added, and the mixture was centrifuged for a period of ten minutes. The 1:10 stock dye which had been previously standardized was diluted with an equal amount of distilled water to obtain a 1:20 dilution. The conversion of the amount of dye used to the amount of ascorbic acid in the blood plasma was done as suggested by Farmer and Abt (15) as follows:

" cc. of dye used x (mgs. of ascorbic acid equivalent to 1 cc. of dye) x 1000 = mgs. ascorbic acid per 100 ml. of plasma."

The dye was taken into the micro-burette by the following procedure: all of the air was expelled from the burette by turning the screw and forcing the mercury to the tip. The tip of the burette was then submerged in the dye and the latter made to fill the burette by reversing the motion of the screw.

Exactly 0.2 ml. of deproteinized plasma was measured

into the end depression of a spot-plate. In the center depression was placed 0.2 ml. of 2.5 per cent metaphosphoric acid to serve as a blank, that is, to measure the amount of dye required to give the phosphoric in the deproteinized plasma the first faint pink color. Dye was run into the depression of the plate from the burette. The mixture was stirred continuously with a fine glass rod. The first faint pink color that lasted for thirty seconds was taken as the end point. The amount of dye required to give the blank the same pink color was deducted from the readings on the blood samples. A daylight lamp was used as the constant source of light. All titrations were made in duplicate.

RESULTS AND DISCUSSION

The results of the anthropometric measurements are shown in Table 3.

Table 3

ANTHROPOMETRIC MEASUREMENTS OF SEVEN A. & M. AND FIVE FEDERAL NURSERY SCHOOL CHILDREN.

Subject A. & M.	Age Mo.	Weight		Height		Chest Width	
		lbs.	kg.	cm.	in.	cm.	in.
V.L.B.	67	39.75	18.03	110.0	44	17.1	6.8
D.J.W.*	69	46.75	21.2	113.2	45.2	19.0	7.6
J.L.M.	58	47.25	21.4	112.4	44.9	18.7	8.4
R.W.	56	34.50	15.6	104.9	41.9	16.8	6.7
V.S.	57	35.75	16.2	103.6	41.4	15.8	6.7
C.D.	52	39.25	17.8	107.2	42.8	19.1	7.6
B.L.N.	71	44.50	20.1	117.8	47.1	18.0	7.4
Federal							
B.A.R.	54	32.00	14.2	105.0	42.0	16.2	6.8
C.I.	58	33.00	14.4	102.5	41.0	16.8	6.7
G.H.*	59	45.00	20.4	104.5	41.8	18.5	7.4
B.M.*	49	38.00	17.2	104.5	41.8	18.0	7.4
V.M.J.	60	31.00	14.0	102.5	41.0	16.7	6.6

Table 3 (continued)

Subject A. & M.	Chest Depth		Cir. Right Arm		Cir. Left Arm		Cir. Left Leg	
	cm.	in.	cm.	in.	cm.	in.	cm.	in.
V.L.B.	13.8	5.5	17.2	6.8	17.0	6.8	23.3	9.3
D.J.W.*	13.3	5.2	16.8	6.7	16.8	6.7	24.0	9.6
J.L.M.	13.4	5.3	17.9	7.1	17.9	7.1	25.2	10.08
R.W.	11.7	4.2	15.5	6.2	15.4	6.1	20.7	8.2
V.S.	12.2	4.8	17.0	6.1	16.8	6.7	22.9	9.1
C.D.	13.9	5.5	16.9	6.7	16.9	6.7	22.7	9.08
B.L.N.	13.2	5.2	17.5	6.0	17.4	5.9	23.5	9.4

* Boys

Table 3 (continued)

Subject	Chest Depth		Cir. Right Arm		Cir. Left Arm		Cir. Left Leg	
	cm.	in.	cm.	in.	cm.	in.	cm.	in.
B.A.R.	13.0	5.6	15.0	6.0	15.0	6.0	21.4	8.5
C.I.	12.7	5.0	15.3	6.1	15.2	6.0	20.9	8.3
G.H.*	13.7	5.4	15.3	6.1	15.5	6.2	21.1	8.4
B.M.*	14.7	5.8	16.7	6.6	16.6	6.6	22.0	8.8
V.M.J.	12.7	5.0	14.2	5.6	14.2	5.6	20.5	8.2

The mean of the ages of the girls from the College Nursery School was 61 months and the mean of the ages of the girls from the Federal Nursery School was 56 months. There were not enough boys in either group to warrant the calculation of the mean of their ages.

When compared with the Grandprey (18) range of variability curves in weight and height of children under six years of age, the children from the College Nursery School fell into the following percentiles: 75, 90, 50, 25, 90, and 90 for height for age. The children from the Federal Nursery School ranked in the 90, 50, 50, 50, 90, percentiles in height for age. In weight for age the children from the College Nursery School were in the 75, 90, 50, 25, 90, 90, percentiles. The children from the Federal Nursery School were much lower in comparison to weight for age than the College group, their percentile rankings were as follows: 25, 25, 10, 90, and 75. As judged by weight in comparison to

height the College Nursery School children were well developed. The percentile ratings of the group were 50, 90, 50, 75, and 90. The Federal Nursery School children were not as well developed as judged by weight in comparison to height as were the College group. The Federal Nursery School group in a comparison of weight for age fell into the following percentiles: 25, 25, 10, 90, and 75. These values are shown in Table 4.

Table 4

COMPARISON WITH THE GRANDPREY RANGE (OF VARIABILITY) IN WEIGHT AND HEIGHT OF CHILDREN UNDER SIX YEARS OF AGE, IN PERCENTILES.

A. & M.	Weight for age	Height for age	Weight for Height
V.L.B.	75	75	50
J.L.M.	90	90	90
R.W.	50	50	50
V.S.	25	25	75
C.D.	90	90	90
B.L.N.	90	90	75
D.J.W*	90	90	90
<u>Federal</u>			
B.A.R.	25	90	10
C.I.	25	50	25
V.M.J.	10	50	10
G.H.*	90	50	90
B.M.*	75	90	50

The means of the weight, height, chest width, chest depth, circumference of each arm, and circumference of the left leg of the girls in the groups are shown in Table 5.

* Boys

Table 5

MEAN VALUES FOR AGE, WEIGHT, HEIGHT, CHEST WIDTH, CHEST DEPTH, CIRCUMFERENCE OF EACH ARM, AND CIRCUMFERENCE OF LEFT LEG OF SIX A. & M. AND THREE FEDERAL NURSERY SCHOOL GIRLS.

	Age no.	Weight		Height		Chest Width	
		lbs.	kg.	cm.	in.	cm.	in.
A. & M.	31	40.15	18.2	109.6	43.8	17.7	7.08
FEDERAL	56	32.00	14.8	103.3	41.2	16.5	6.6

Table 5 (continued)

	Chest Depth		Cir. Right Arm		Cir. Left Arm		Cir. Left Leg	
	cm.	in.	cm.	in.	cm.	in.	cm.	in.
A. & M.	13.0	5.2	17.0	6.8	16.7	5.7	23.0	9.2
FEDERAL	12.8	5.1	11.5	4.6	11.4	4.5	20.9	8.3

The measurements of the boys were not included in the averages for the two schools due to the limited number of boys in the groups.

According to the Grandprey (16) range of variability in weight and height the five months difference in the mean of the ages of the two groups would account for a difference of 1.35 pounds in the weights of the two groups, and a difference of 0.82 inches in the mean of the heights. The mean of the weights of the College Nursery School group was 10.35 pounds greater than that of the Federal Nursery School. The mean of the heights of the College Nursery School was 2.6 inches greater than that of the Federal Nursery School group.

The data obtained from the study of the red cell counts, hemoglobin content, packed cell volume, and vitamin C content

of the blood plasma of the blood of 12 nursery school children are presented in Table 6. The three corpuscular constants, mean corpuscular hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin concentration, calculated from the absolute values are also given.

The corpuscular constants were calculated from the following formulas:

1. MEAN CORPUSCULAR HEMOGLOBIN, IN MICROMICROGRAMS (μ)

$$\frac{\text{Hemoglobin, in grams per 1000 cc. of blood}}{\text{Red blood cells, in millions per c.m. of blood}}$$

2. MEAN CORPUSCULAR VOLUME, IN CUBIC MICRONS (μ^3)

$$\frac{\text{Packed cell volume, in cc. per 1000 cc. of blood}}{\text{Red cells, in millions per c.m. of blood}}$$

3. MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION, IN PER CENT

$$\frac{\text{Hemoglobin, in grams per 100 cc. of blood}}{\text{Packed cell volume, in cc. per 100 cc. of blood}} \times 100$$

The average of the red cell counts for the College Nursery School children was 170,000 higher than that of the Federal Nursery School group, while the grams of hemoglobin per 100 ml. of blood was the same for the College group as for the Federal Nursery School group. In volume of the packed cells, measured in mls. per 100 mls. of blood, the College Nursery School group measured 0.80 higher than the Federal Nursery School group.

McManara and Senn (34) in 1940 studied the bloods of fourteen children, in New York, ranging in age from 3 to 7 years and obtained the following results which are compared

Table 6

RED CELL COUNTS, HEMOGLOBIN CONTENT, PACKED CELL VOLUME, CORPUSCULAR CONSTANTS AND VITAMIN C CONTENT OF THE BLOOD OF SEVEN A. & M. AND FIVE FEDERAL NURSERY SCHOOL CHILDREN.

Subject	Age	Red blood cells,	Hemoglobin,	Volume,	Vitamin C,	Mean Corpuscular		
		millions per cu. ml. of blood	gms. per 100 cc. of blood	cc. per 100 cc. of blood	mg. per 100 cc. of blood plasma	Hemo- globin	Vol.	Hemo- globin concen- tration
<u>A. & M</u>								
V.L.B.	67	4.77	10.45	41.15	-----	21.9	84.1	25.4
D.J.W.	69	4.66	11.41	38.38	.862	24.4	82.4	29.7
J.L.M.	58	4.50	10.11	37.70	1.274	22.4	83.7	26.7
R.W.	56	4.80	12.28	35.45	.6051	24.0	75.4	34.6
V.S.	57	4.58	13.47	33.95	.8332	29.4	74.1	36.4
C.D.	52	4.53	11.82	35.80	1.165	27.2	82.6	33.0
B.L.N.	71	4.42	12.28	33.95	.7836	27.7	81.3	36.1
<u>FEDERAL</u>								
B.A.	54	4.52	10.62	35.60	-----	23.4	78.5	29.7
C.I.	58	4.49	12.17	35.55	.8283	24.8	79.1	34.2
G.H.	59	4.35	12.05	35.00	-----	27.4	79.7	34.4
B.M.	49	4.35	-----	38.95	-----	-----	89.3	-----
V.M.J.	60	4.28	12.17	34.0	-----	28.4	79.4	35.8

Table 7

MEAN RED CELL COUNTS, HEMOGLOBIN CONTENT, PACKED CELL VOLUME AND CORPUSCULAR CONCENTRATION OF THE BLOOD OF SEVEN A. & M. AND FIVE FEDERAL NURSERY SCHOOL CHILDREN.

School	Age no.	Red blood cells, millions per cu. ml. of blood	Hemoglobin, gms. per 100 cc. of blood	Volume, cc. per 100 cc. of blood	Mean Corpuscular		
					Hemo- globin	Vol.	Hemo- globin concentration
A. & M.	61	4.6	11.7	36.8	23.4	83.3	29.2
FEDERAL	56	4.4	11.7	35.8	24.3	81.2	29.8

with those of the present study in Table 8.

The mean hemoglobin and mean volume of packed cells of the Oklahoma group were lower than in the New York group.

In the vitamin C determination only the College Nursery School children were used. The results reported here were obtained after the group had received its regular morning orange juice and, therefore, cannot be compared with results obtained with subjects in the fasting condition.

Table 8

COMPARISON OF THE OKLAHOMA STUDY WITH THE NEW YORK STUDY MADE BY MCMANARA AND SENN
IN 1940.

No. of Subj.	Ages	Mean Hemo- globin, gms. per 100 cc. of blood	Range	Red blood cells, million per cu. mm. of blood	Range	Packed cell volume, % of whole blood	Range
<u>Okla.</u>							
12	4-5	11.71	10.11-13.47	4.51	4.28-4.77	36.22	33.95-41.15
<u>N.Y.</u>							
14	3-7	13.1	11.6-15.1	4.52	3.55-5.05	37.0	33.2-45.3

CONCLUSIONS AND SUMMARY

From a review of the literature pertaining to methods for determining the nutritional status of individuals and of populations, it was found that methods for determining the adequacy of food intake fell into three general groups:

1. The foods consumed, as measured by dietary studies, were compared with accepted recommendations for nutritional essentials necessary for optimal health.
2. Anthropometric measurements and specific tests for each nutritional essential were made on the individual subjects.
3. A combination of the first and second methods in which food consumption and physiological studies were both made.

The third method, although time consuming and exacting, would give much more information than either of the first and second methods taken separately.

The indirect method, number 1, in the above paragraph, of measuring nutritional status has been more commonly used than the direct method. number 2 in the above paragraph, due to the fact that it is more readily adapted to field work than is the direct method. The indirect method, however, gives no authentic information on the condition of the subjects studied except that a high correlation between the quality and the quantity of food eaten and the health of the subjects has been proved.

In view of the fact that the important factor is the

health of the individuals, any study of nutritional condition is not complete unless the persons themselves have been studied as well as their food intake.

There are two main problems to consider in making a plan for determining the nutritional condition of the people of the State of Oklahoma, that is, the people with whom the studies are to be made and the specific tests to be used as criteria for judging nutritional condition.

A possible plan for procuring a sampling of the State of Oklahoma would be to use the natural divisions of the different type of farming areas of the State. It would be necessary to sample various areas since, according to Dr. Sewell, there is no part of the State that is representative of the State as a whole. The different type of farming areas have been set up with regard to predominant type of farming, plane of living, soil, climatic condition and various other factors. It is believed that a representative sample of the population of the State could be obtained by selecting 0.05 per cent of the families of different economic levels and from rural and urban communities in each of the 16 different type of farming areas of the State.

A study of this type would, of course, require many trained workers and adequate financial support. Stiebling stated that one trained worker was needed for every five cases studied.

The tests that are suggested as criteria for judging

nutritional condition and are possible to carry out, are the following: 1. A comparison of the food consumption of chosen subjects with accepted recommendations for optimal nutritional condition. 2. Anthropometric measurements on each person which include weight, height, chest width, chest depth, circumference of each arm and the circumference of the left leg. 3. Physiological measurements which include erythrocyte counts, hemoglobin content, determination of the volume of the packed cells and the vitamin C content of the blood plasma.

A survey of this type, to be of the greatest value, should be done in connection with the other social and economic studies including housing, food production, education of the family members, etc.

The measurements chosen for judging the nutritional condition of the children of the two nursery schools include: 1. anthropometric measurements and 2. physiological and biochemical measurements.

The results of these showed that the children of the College Nursery School were better developed with regard to weight and height in comparison to age than the Federal Nursery School children. The difference in the mean of the weights of the girls in the two groups was 10.85 pounds and the difference in the heights was 2.6 inches. The difference in the means of the ages of the two groups was 5 months. This difference in age, according to the Grandprey scale, would

account for only 1.35 pounds difference in weight and 0.82 inch in height. The mean of the erythrocyte counts of the College Nursery School group was 170,000 higher than the Federal Nursery School group, and the volume of the packed cells was 0.80 cc. per 100 cc. of blood higher, although the means of the hemoglobin values of the blood of both groups were the same. From the results of the study it was shown that the College Nursery School children were probably well nourished with regard to vitamin C since the values for the vitamin C content of blood plasma were still high several hours after taking orange juice.

One of the greatest difficulties encountered in the field of nutrition today is the lack of reliable standards for good nutritional condition. This may be due to the facts that ideas of good or optimal nutrition are changing with the progression of the science of nutrition; that there is a wide variation in individuals' abilities to utilize foods and that normal variation, previously accepted as physiological differences, may be evidence of differences in nutritional condition of the various members of the experimental groups.

As more information is obtained on these problems, no doubt, simpler methods of determining nutritional status will develop.

REFERENCES CITED

1. Andresen, Marjory I. and Murgage, Edward R. Venous and peripheral red blood cell values. *Am. J. Clin. Path.*, 8; 1, 1938.
2. Andresen, Marjory I. and Murgage, Edward R. Red blood cell values for normal men and women. *Arch. Int. Med.*, 58; 136, 1936.
3. Baldwin, Bird T. The use and abuse of weight-height tables as indexes of health and nutrition. *J. Am. Med. Assoc.*, 82; 1-4, 1924.
4. Barber, Anna Lee Pardew The formed elements of the blood of Oklahoma women. Unpublished Masters Thesis, Stillwater, Oklahoma, Oklahoma Agricultural and Mechanical College, 1939.
5. Bessey, O. A., Boyle, P. E. and Wolbach, S. B. Vitamin C: method of assay and dietary sources. *J. Am. Med. Assoc.*, 111; 1290, 1938.
6. Bigwood, E. J. Guiding principles for studies on the nutrition of populations. League of Nations Health Organizations, Technical Commission on Nutrition, Geneva, 1939.
7. Carr, F. H. and Price, E. A. Colour reactions attributed to vitamin A. *Biochem. J.*, 20; 497, 1926.
8. Cooperative Extension Work in Agriculture and Home Economics Consuming habits of the farm people of Oklahoma. Oklahoma Agricultural and Mechanical College and United States Department of Agriculture 1935.
9. Cowgill, George R. Human requirements for vitamin B₁. *The Vitamins, A Symposium, Chicago, Ill.* *Am. J. Med. Assoc.*, 1939.
10. Donelson, Eva G., Ohlson, Margaret A., Kunerth, Bernice, Patton, Mary Brown, and Kinsman, Gladys M. Anthropometric data on college women of the middle states. *Am. J. Phys. Anthropol.*, 27; No. 3, 1940.
11. Everson, Gladys J. and Daniels, Amy L. Vitamin C studies of children of pre-school age. *J. Nut.*, 12; 15, 1936.

12. Ellsworth, J. O. and Elliot, F. F. Type-of-farming in Oklahoma. Oklahoma Agricultural and Mechanical College, Agricultural Experiment Station Bulletin No. 181; June, 1929.
13. Farmer, Chester, and Abt, Arthur F. Determination of reduced ascorbic acid in small amounts of blood. Proc. Soc. Exper. Biol. and Med., 34; 146, 1936.
14. Farmer, Chester, and Abt, A. F. Ascorbic acid content of blood. Proc. Soc. Exper. Biol. and Med., 32; 1625, 1935.
15. Farmer, Chester, and Abt, A. F. Micro test for ascorbic acid in plasma. What's New, Chicago, Ill. Abbot Laboratories, Sept. 1940.
16. Franzen, Raymond Selection of malnourished school children. Am. J. Dis. Child., 47; 789, 1934.
17. Gradwohl, R. B. H. Clinical laboratory methods and diagnosis. St. Louis, C. V. Mosby Co., 1935.
18. Grandprey, Medora B. Range of variability in weight and height of children under six years of age. Child Development, 4; 26, 1935.
19. Haden, R. L. Clinical significance of volume and hemoglobin content of red blood cells. Arch. Int. Med., 49; 1032, 1932.
20. Harris, L. J. and Leong, P. C. Vitamins in human nutrition; the excretion of vitamin B₁ in human urine and its dependence on dietary intake. Lancet, 1; 886, 1936.
21. Hecht, S. and Schlaer, S. An adaptometer for measuring human dark adaptation. J. Opt. Soc. Am., 28; 269, 1938. Cited by Mack and Smith (30).
22. Holmes, H. N. and Corbet, R. E. A crystalline vitamin A concentrate. Sci., 85; 103, 1937. Cited by Mack and Smith (30).
23. Hrdlicka, Ales Anthropometry. Philadelphia, The Wistar Institute of Anatomy and Biology, 1920.
24. Jeans, P. C. and Zentmire, Zelma A clinical method for determining moderate degree of vitamin A deficiency. J. Am. Med. Assoc., 102; 892, 1934.

25. Jeans, P. C., Blanchard, Evelyn and Zentmire, Zelma
Dark adaptation and vitamin A. J. Am. Med. Assoc.,
108; 451, 1937.
26. King, C. G. The physiology of vitamin C., J. Am. Med.
Assoc., 111; 1098, 1938.
27. King, C. G. and Menten, M. L. The influence of vitamin
C levels upon resistance to diphtheria toxin. J.
Nut., 10; 129, 1935.
28. Knott, Elizabeth M. A quantitative study of the utiliza-
tion and retention of vitamin B by young children.
J. Nut., 12; 597-611, 1936.
29. Lucas, W. P. and Pryor, H. B. Physical measurements and
physiological processes in young children. J. Am.
Med. Assoc., 97, 1127, 1931.
30. Mack, Pauline Berry and Smith, Janice M. Methods of
conducting mass studies in human nutrition. Penn.
State College Studies, Study No. 4; 1939.
31. Maresh, Marion H. and Deming, Jean Roentgenograms versus
anthropometry. Child Development, 10; 91, 1939.
32. McLester, James B. Nutrition and diet in relation to
health and disease. Philadelphia, W. B. Saunders
Co., 1940.
33. Mirsky, A., Swadesh, S. and Soskin, S. Total ascorbic
acid content of human blood. Proc. Soc. Exper.
Biol. and Med., 32; 1130, 1935.
34. McMamara, Helen and Senn, M. J. E. Glutathione and red
cells in blood in infancy and childhood. Am. J.
Dis. Child., 59; 97, 1938.
35. Munsell, Hazel E. Vitamin A: methods of assay and food
sources. J. Am. Med. Assoc., 111; 245, 1938.
36. Mudge, Edward and Andresen, Marjory I. Red blood cells
in adolescence. Am. J. Dis. Child., 56; 997, 1938.
37. Mudge, Edward and Andresen, Marjory I. Normal standards
for red blood cell values in Colorado. The Child
Research Council, Denver, Colorado, University of
Colorado School of Medicine.
38. Murphy, Elizabeth Vitamin C in childrens' diets. J.
Nut., 21; 527, 1941.

39. National Research Council Handbook for the study of adolescent children. Vol. 111; no. 2, 1940.
40. Newcomer, H. S. Absorption spectra of acid hematin, oxyhemoglobin, and carbon monoxide, a new hemoglobinometer. J. Biol. Chem., 37; 465, 1919.
41. O'Hara, Patricia and Hauck, Hazel M. Storage of vitamin C by normal adults following a period of low intake. J. Nut., 12; 413, 1936.
42. Osgood, E. E. Laboratory diagnosis. Philadelphia, P. Blakiston's Son and Co., 1935.
Cited by Mudge and Andresen (34).
43. Osgood, E. E. Haskins, H. D. and Trotman, F. E. The value of accurately determined color, volume, and saturation indexes in anemia. J. Lab. and Clin. Med., 17; 859, 1931.
44. Pepper, O. H. Perry and Farley, David L. Practical Hematological Diagnosis. Philadelphia, W. B. Saunders Co., 1934.
45. Roberts, Lydia Nutrition work with children. Chicago, University of Chicago Press, 1935.
46. Selective Service Regulation Physical Standards. Vol. 6, U. S. Gov. Printing Office, 1941.
47. Sewell, William H. The construction and standardization of a scale of Oklahoma farm families. Oklahoma Agricultural and Mechanical College, Agricultural Experiment Station, Technical Bulletin No. 9; April, 1940.
48. Stiebling, Hazel K. Are we well fed? U. S. Dept. Agric., Misc. Pub. No. 430, 1941.
49. Stiebling, Hazel K. and Ward, Medora M. Diets at four levels of nutritive content and cost. U. S. Dept. of Agric. Cir. No. 296, Nov. 1933.
50. Supplement of American Journal of Public Health, 29; No. 2, P;. 42-53, 1939. Cited by Mack and Smith (30).
51. Unpublished Report Oklahoma nutrition conference, Oklahoma City, Oklahoma. Feb. 1941.
52. Van Allen, C. M. An hematocrit method. J. Lab. and Clin. Med., 10; 1027, 1925.

53. Wintrobe, M. M. Blood of normal men and women. Bulletin, Johns Hopkins Hosp., 53; 118, 1933.
Cited by Andresen and Mudge (2).
54. Wolbach, S. B. The pathological changes resulting from vitamin deficiency. J. Am. Med. Assoc., 108; 7, 1937.
55. Wortis, H., Liebman, J. and Wortis, E. Vitamin C in blood, spinal fluid, and urine. J. Am. Med. Assoc., 110; 1896, 1938.
56. Yavorsky, Martin, Almaden, Phillip and King C. G. The vitamin C content of human tissue. J. Biochem., 106; 525, 1934.
57. Zayaz, Stella, Mack, Pauline B., Sprague, Phillis K. and Bauman, Arthur Nutritional status of school children. Child Development, 11; 1, 1940.

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