DIETARY INTAKE AND BIOCHEMICAL ANALYSES OF BLOOD OF PRESCHOOL CHILDREN

by

JUDITH LORETTA CRUMRINE

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E. Beth Tryer

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INTRODUCTION

An adequate diet is known to be important in the growth and development of children and it is believed that dietary patterns are developed during early childhood. Extensive studies have been made of the diets of school children, particularly of teenagers, but until recently little attention had been focused on the dietary habits of preschool children.

Dietary intake constitutes an essential part of any nutrition study and provides information on nutrient intake levels, sources of nutrients, and food habits. However dietary investigation is only one of several methods used in determining nutritional status. Biochemical analyses of blood and urine reveal nutrient reserves in the body. Physical examination may show any structural changes. When dietary intake, biochemical analyses and physical examination are considered together, a more complete appraisal of the nutritional status of a population is possible.

The objective of this study was to investigate the relationship of dietary intake of selected nutrients and various blood component values (hemoglobin, hematocrit, total serum protein and electrophoretic fractions of serum protein) of 40 apparently healthy preschool children.

REVIEW OF LITERATURE

Evaluation of Nutritional Status

Nutritional status can be assessed by 1) biochemical

analyses, 2) clinical observations, 3) anthropometric measurements and 4) dietary investigation. Most researchers use 2 or more methods in one study but the greater the number of methods applied the more complete will be the picture of nutritional status. Williams (1951) stated that dietary records may indicate inadequate intake; biochemical analyses may reveal low concentrations or inadequate reserves; physiologic tests may indicate imparied function; and clinical examinations may show structural changes. The Food and Nutrition Board (1964) and the Interdepartmental Committee on Nutrition for National Defense (ICNND, 1963) recommend that assessment of nutritional status be based on biochemical, clinical and dietary information.

<u>Biochemical Analyses</u>. Biochemical analysis is the most objective method of assessing nutritional status according to Williams (1951). Blood may be analyzed for packed cell volume, hemoglobin, serum ascorbic acid, serum vitamin A and carotene, serum protein fractions, total serum protein, protein bound iodine, total iodine, and cholesterol. Analysis of urine may include creatinine, thiamin, riboflavin, n'methylnicotinamide and total urinary nitrogen. The ICNND (1963) outlined procedures for these blood and urine analyses and gave suggested guides for interpreting values obtained. Biochemical values are designated as "deficient, low, acceptable and high." These ranges of values should be used as guides rather than set values. They are expressions of nutritional levels, not clinical deficiency states.

Ford (1959) stated that there was lack of agreement among investigators concerning the designation of blood nutrient

concentrations for a given level of nutrition adequacy. For some biochemical tests various methods may give conflicting values. For example, values obtained in electrophoretic separation of blood serum protein vary depending on the type of paper, temperature, duration, staining time, destaining time and evaluation technic used (Wuhrmann and Wunderly, 1960). There is a need for the adoption of a uniform method of reporting electrophoretic data on blood serum protein values in order to permit comparison of results of various laboratories (Reiner et al., 1950).

Clinical Observations. Clinical observations are often used in assessing nutritional status but should be used in combination with other methods of evaluation. These observations lack sensitivity and indicate apparent health or gross clinical deficiency symptoms. Physical examinations of children participating in the Child Research Council longitudinal study in Denver were used to assure that poor health was not a factor in their dietary intakes (Beal, 1956). These children were examined 45 times during the first 10 years of life. In a nutritional survey of Groton Township, New York (Moore and Shaw, 1951), physical examinations of eyes, lips, gums, teeth, tongue, buccal mucosa, skin, palpation of the thyroid gland, tendon reflexes. lower extremities and blood pressure from childhood to old age were done. It was concluded that the study population was wellnourished as there were few significant physical findings. Most of the abnormalities noted, except overweight, were not actually the result of dietary intake.

Anthropometric Measurements. Body measurements are useful

in several nutritional contexts; evaluation of calorie requirements, assessment of the nutriture, description of the present nutritional status and demonstration of nutritional improvement (Brozek and Keys, 1956). Not all body measurements on children are equally useful or practicable at all ages so recommended measurements are made for various age levels. Height and weight measurements are probably the most common measurements taken. Other measurements may include chest, waist, upper arm and calf of leg circumferences, bi-condular diameters of humerus and femur, bi-cristal diameter, bi-acromial diameter, and skinfold thickness of upper arm, and waist. Measurements may also include x-rays.

Bilderback (1967) reviewed previous studies and described methods of taking anthropometric measurements of preschool children. She found overall dietary adequacy was significantly correlated with height, weight, pelvic girdle and shoulder girdle of 3-6 year old boys. However, it was not significantly corrolated with any of the anthropometric measurements of girls of the same age.

<u>Dietary Investigation</u>. Dietary investigation offers a direct method of estimating dietary levels where routine laboratory or clinical tests have not been developed. The ICNND (1963) maintain that only a recent change in dietary intake would cause disagreement among biochemical, clinical and dictary data. Basic information obtained in dietary studies is essential in planning recommendations for changes in nutritional practices (ICNND, 1963).

Collection of Dietary Data. Several methods are employed in collection of dietary intake. A recall of food intake; food records in weighted, measured or estimated amounts; and dietary histories have been adapted for nutritional surveys by various workers.

Young <u>et al</u>. (1952) found that the 24-hour recall gave approximately the same results as the 7-day record in determining group means for most nutrients but concluded that the two methods should not be used interchangeably. Leitch and Aitken (1950) found food consumption was underestimated when a recall method was used as compared to recording food intake. Weighing of foods should result in the most accurate estimate of dietary intake and dietary recall the least reliable estimate. A fairly good dietary record can be obtained by recording food intake in household measurements.

Chalmers <u>et al</u>. (1952) stated that dietary records should cover a sufficient period of time to furnish an adequate picture of nutrient intake. However, if the period is too prolonged the interest and cooperation of the subjects may be lost. Three-day records (Bilderback, 1967; Eppright <u>et al</u>., 1954; and Metheny <u>et al</u>., 1962) and 7-day records (Ford, 1959; Ling, 1966; and Macy and Hunscher, 1951) are the most common.

Beal (1953) stated that nutrition histories provided the most practical method of determining nutrient intake over a long period of time for a large number of individuals. She used dietary histories to record dietary data on 121 children in a longitudinal study in the Denver, Colorado area.

Evaluation of Dietary Data. Information obtained from the dietary record should be related to a standard. Many researchers have compared nutrient values to the Recommended Dietary Allowances (RDA) proposed by the Food and Nutrition Board of the National Research Council (NRC). The first edition of the RDA was published in 1943 with revisions in 1945, 1948, 1953, 1958 and 1963.

In the opinion of the Food and Nutrition Board the RDA will maintain good nutrition in essentially all healthy persons in the United States under current living conditions. The RDA provides for a margin of sufficiency above average physiological requirements to cover individual variations in requirement, increased needs during stress and to permit full realization of growth and productive potential. The recommended allowances are in excess of need for the majority of individuals. They are not considered adequate to cover increased requirements of persons depleted by disease or traumatic stresses. The allowances should be used as goals in planning food supplies and as guides for interpretation of food consumption of groups of people. They have also been used as a guide in interpreting the adequacy of nutrient intakes of individuals in dietary surveys of population groups.

In the 1958 revision of the RDA, recommended allowances for calories were increased from 1200 to 1300 for children 1-3 years and from 1600 to 1700 for children $l_{\rm +-6}$ years with corresponding changes in thiamin (0.6 mg to 0.7 mg for children 1-3 years and 0.8 mg to 0.9 mg for children $l_{\rm +-6}$ years) and riboflavin (1.2 mg

to 1.3 mg for children 4-6 years). In addition, to correct for the conversion of tryptophan to niacin, allowances for niacin were changed from 6 mg to 8 mg equivalents for children 1-3 years and from 8 mg to 11 mg equivalents for children 4-6 years.

The latest RDA revision occurred in 1963. The age groups were changed from 1-3 and 4-6 years to 1-3 and 3-6 years. The height of the reference child age 4-6 changed from 109 cm (43 in) to 107 cm (42 in) for age 3-6. The weight remained the same. In the 1963 revision, the RDA for the older preschool age group (4-6 years, 1958 revision; and 3-6 years, 1963 revision) changed from 1700 to 1600 calories, from 50 to 40 gm protein, from 1.0 to 0.8 mg calcium, from 8 to 10 mg iron, from 0.9 to 0.6 mg thiamin, and from 1.3 to 1.0 mg riboflavin. The RDA for niacin equivalents (11 mg), ascorbic acid (50 mg), and vitamin D (400 IU) remained the same.

Even though the average intake of a nutrient by a group may be adequate according to NRC standards, the distribution of intakes usually show that some individuals consume significantly more and/or some significantly less than the average (ICNND, 1963). Beal (1953, 1954, 1955, 1956) found the distribution of nutrient intakes showed a skewing which nullified the use of mean and standard deviations. She presented her data in quartiles using the highest and lowest values observed to show the total range. Intakes of calories, carbohydrate, fat and protein were presented in actual intakes and as per kilogram of body weight.

Serum Protein Fractions and Total Serum Protein

Wuhrmann and Wunderly (1960) stated that serum proteins have essentially 3 functions; i.e., transport, maintaining colloidal osmotic stability, and nutrition. There is a reserve of plasma protein-forming-material that may be reduced by fasting, low protein diet or plasma depletion.

Electrophoretic Separation. Paper electrophoresis, because of its relative simplicity and the development of rapid methods of recording and quantitating the results, has been applied on a broad scale to the study of proteins in the blood. Serum proteins are separated into albumin, alpha 1, alpha 2, beta and gamma fractions (ICNND, 1963; Beckman Instruments, Inc., 1957). Proteins with an excess of negative charges migrate toward the anode and those with positive charges move toward the cathode. At pH 8.6, which is above the isoelectric point of the serum proteins, migration is toward the anode. The molecule having the lowest isoelectric point (albumin) migrates at the highest rate. Gamma globulin moves slightly toward the cathode because of electro-osmotic effects.

Methods for paper electrophoresis have been developed by Wieland and Fisher and Turba and Enenkel in Germany, Cremer and Tiselius in Sweden and Durrum in America (Ogryzlo <u>et al.</u>, 1959). The ICNND (1963) stated that excellent results can be obtained using the Spinco RB System (Durrum cell) with the Analytrol quantifying scanner.

Laboratory Findings. Oberman et al. (1956) studied serum

proteins of 159 well infants and children at ages ranging from birth through thirteen years and 26 mothers of newborn infants. Any infant or child who had had any evidence of infection, however mild, in the four months before the laboratory analysis of his serum was excluded from the study. They found mean values for total serum protein significantly lower in newborn infants than in their mothers at term. The level of serum protein rose slowly after birth, with average values reaching the average adult level by one to two years of age. Children six to thirteen years of age had somewhat higher total protein levels than adults.

In addition to total serum protein determinations, Oberman <u>et al</u>. (1956) studied electrophoretic fractions (albumin, alpha 1 globulin, alpha 2 globulin, beta globulin and gamma globulin) of serum proteins. Mean absolute values for serum albumin were significantly higher in newborn infants than in their mothers at term but usually fell within the adult range at about three months of age. Children six to thirteen had a higher albumin level than adults, accounting for the higher total protein level observed in children of this age group.

Mean absolute values for total serum globulin were lower in the newborn infant than in their mothers at term. After birth the level of serum globulin fell steadily, and reached the lowest value at 3 to 4 months of age. The values rose after this time and reached adult levels by 7 to 11 months of age.

Alpha and beta globulin fractions were higher in the mother than her newborn infant. After a slight fall in the alpha

globulins and a steep rise in the beta globulin during the first week, the values remained fairly stable throughout life, with a tendency to lower values with increasing age. The range of observed values for beta globulin was somewhat greater than that of alpha 1 and 2 globulins.

Mean values for serum gamma globulin was not significantly different in mothers at term, newborn infants and normal adults. At three to four months gamma globulin reached its lowest level, then rose slowly reaching adult levels by 3 to 5 years of age. The mean values for children, however, fell within the normal adult range by 7 to 11 months of age. Knapp and Routh (1949) believe these changes in the gamma fraction are related to the loss of passive immunity received by the infant from its mother and the accumulation of antibodies and the development of active immunity in the child.

Moore <u>et al</u>. (1949) found serum albumin and gamma globulin increased during the development of the fetus while alpha and beta globulins remained unchanged. Alpha and beta globulins showed a considerable increase in the serum of the mother during pregnancy with a reduction in the albumin content.

Lubschez (1948) reported no age or sex differences in average sorum protein component values after the age of 6 months in 30 children ranging in age from birth to ll years. However, Hodges and Krehl (1965) found a sex difference in students in grades 9, 10, 11 and 12 in 58 Iowa schools. Values for alpha 2 globulin, beta globulin and gamma globulin were higher in females and albumin, alpha 1 globulin and total serum protein

higher in males.

Angelopoulos and Bechrakis (1960) studied electrophoretic separation of serum proteins in newborn infants, older infants and children to age 12 in Greece. After 6 months of age, the total protein was within the adult range. Minor variations occurred in total globulins before 6 months of age. Albumin values were similar to adult values after 13 days. They compared their data with other studies and concluded that values were similar in countries having similar dietary customs. This suggests that dietary intake influences blood protein components.

Dhopeshwarkar <u>et al</u>. (1956) found that vegetarianism produced a lower than normal plasma albumin with a compensatory increase in the plasma globulin. Their subjects were Indian medical students on a vegetarian diet which included milk and milk products but no other food of animal origin and Indian medical students who consumed eggs and fish, fowl or meat daily.

Changes in the concentration of electrophoretic fractions of blood serum protein have been observed in various diseases. Many of the observed abnormalities fall into a small number of patterns, some of which characterize a whole group of pathological states. Wuhrmann and Wunderly (1960) refer to these patterns as "constellations" and list 9 of them. They are 1) acute inflammation, 2) chronic inflammatory and proliferative processes, 3) hepatitis, 4) cirrhosis, 5) biliary obstruction, 6) nephrotic syndrome, 7) malignant tumors, except myeloma, 8) gamma myeloma and 9) beta myeloma.

Within each constellation there are degrees of severity and

the plasma protein pattern often reflects the total physiology and clinical state of the patient rather than a specific disease process (Jencks <u>et al.</u>, 1956). In general, however, physical and chemical tests on serum proteins have diagnostic value only when placed in the whole clinical picture. They are of more importance in following the progress of a patient (Putman, 1960).

Relationships of Hematocrit and Hemoglobin to Nutrient Intake

Many researchers believe there is a relationship between dietary intake and the concentration of certain nutrients in the blood. The determination of hemoglobin is one of the most widely used biochemical techniques in the appraisal of nutritional status. Dietary protein and iron are expected to have a positive relationship with hemoglobin. Some workers have found a relationship while others have not. Intake of calcium and carbohydrate have also been correlated with hemoglobin. Positive correlations have been found between hematocrit or packed cell volume and hemoglobin and intakes of several nutrients.

Wilcox <u>et al</u>. (1955) studied packed cell volume of 131 children age 5 to 18 with and without rheumatic fever. Significant positive correlations were found between volume of packed cells and protein, carbohydrate, calcium, iron, thiamin, riboflavin and niacin. A significant negative correlation was found between volume of packed cells and calories.

Dibble <u>et al</u>. (1965) found hematocrit values below acceptable levels of the ICNND for a large number of males and females

ages 13 and 14 years attending 3 schools in Syracuse, New York. Significant positive correlations were found between protein and thiamin intakes at breakfast and hematocrit values taken in midmorning. Eighty percent of the hematocrit values for the females in one school were below acceptable levels. No hemoglobin determinations were done.

A positive but not statistically significant relationship between hemoglobin concentration and iron intake was found by Hodges and Krehl (1965) in 252 Iowa teenagers. Hematocrit values decreased as the intake of milk fat increased but this relationship was not significant.

Bring <u>et al</u>. (1955) studied the nutritional status of 15 and 16 year old school children in 3 Idaho communities in relation to hemoglobin and packed cell volume of whole blood and dietary iron. Seven-day dietary records were kept. A highly significant positive correlation was found for both males and females between hemoglobin and packed cell volume, and for males between iron intake and hemoglobin and between iron intake and packed cell volume.

Ford (1959) found hemoglobin was not significantly correlated with any of the nutrients in the diets of 64 school children age 8 to 13 in Riley, Kansas.

Fuhr and Steenback (1943a and 1943b) and Wilcox et al. (1955) reported that hemoglobin and dietary calcium were related. Wilcox et al. (1955) also reported a significant relationship between hemoglobin and intake of carbohydrate.

In an interregional research project 5 of the 6 states in

the northeast region analysed blood for hemoglobin (Morgan, 1959). A positive correlation was found between hemoglobin and dietary protein and dietary iron in 1500 subjects ranging in age from 4-35 years. Since the values for hemoglobin, dietary iron and protein were all high in the sixth state among male industrial workers 20 to 50 years of age, the negative correlation found between hemoglobin and protein may not represent a true nutritional relationship (Clayton <u>ot al.</u>, 1953).

In the Southern region, a positive correlation was found between dietary protein and hemoglobin but no relation between dietary iron and hemoglobin of Virginia children 8 to 11 years old. A highly significant positive correlation between dietary iron and hemoglobin but none between hemoglobin and dietary protein was found in Louisiana (Morgan, 1959). The difference may be explained by the fact that Louisiana children had diets richer in protein and analysis of blood showed higher hemoglobin values than Virginia children with about the same intake of iron.

In the North Central region, hemoglobin values for Kansas children aged 9 to 11 were much lower than for children from Iowa and Ohio. Although no correlations were made between hemoglobin and dietary protein, more children in Kansas had low protein intakes than the other two states (Patton <u>et al.</u>, 1953).

Dietary Intake

Burke <u>et al</u>. (1959) obtained diet histories on 125 children 1 to 18 years of age. A total of 2070 dietary histories were taken at 6 month intervals up to 6 years of age and at 1 year

intervals thereafter. The average intakes of calories and protein were above the 1958 RDA except for the calorie intake of the girls from 1-6 years and from 12-18 years of age.

A nutritional survey including 139 children 1-6 years old in Groton Township, New York (Young <u>et al.</u>, 1950), indicated that intake was adequate according to the 1948 RDA for all nutrients except calcium. Children age 1-10 had the best food records when compared to older children and adults.

Bilderback (1967) studied 50 children age 3-6 whose parents lived in married student housing at Kansas State University, Manhattan. She found 20% of the children had diets meeting the 1963 RDA for all nutrients, 42% had diets with one or more nutrients less than 100% of the RDA but not less than 67% of the RDA and the remaining 38% had diets with at least one nutrient less than 67% of the RDA. Iron was the nutrient supplied in the least adequate amounts, followed by ascorbic acid. Niacin was the only nutrient supplied at 100% of the RDA in diets of all the children. In general children who received vitamin and/or mineral supplements consumed diets which were more nutritionally adequate than those who did not receive such supplements.

Ling (1966) also studied preschool children in Manhattan, Kansas. The 49 children from 2 socioeconomic groups ranged in age from 1-6 years. Niacin was the most adequately supplied nutrient followed by protein, thiamin and riboflavin. Iron was the least adequately supplied nutrient. In general, the children from the lower socioeconomic group had more adequate diets than those from the higher socioeconomic group.

Beal (1953, 1954, 1955) studied diets of children from birth to 5 years in the Denver, Colorado area. Nutrient intakes were presented in quartiles and the highest and lowest values were shown. Intakes of calories, protein, carbohydrate and fat were expressed as nutrient per kilogram of body weight. Intake of calories, carbohydrate, and fat increased with age. Nutrient intake for the majority of the children equalled or exceeded the 1953 revision of the RDA. The exceptions were miacin and iron. The intake of iron rose sharply during the first year but decreased as commercially prepared cereals were replaced by regular foods. After 3 years of age, iron intake for only 25% of the children met the RDA. The RDA for niacin was not met by 75% of the children, but there was no evidence that their intake was inadequate as judged by growth rate and absence of deficiency symptoms. When this study was conducted only preformed niacin was calculated.

PROCEDURE

Selection of Subjects

The subjects of this study were 40 children between the ages of 3 years 10 months and 5 years 9 months who attended the Kansas State University Nursery School or the Presbyterian Church Nursery School in Manhattan, Kansas in May, 1967. Dietary records and blood samples were obtained during a cooperative study by the Department of Foods and Nutrition and the Department of Family and Child Development.

Collection and Recording of Dietary Intake

The first of 2 interviews with the mothers included explanation of the study, obtaining information about family characteristics and explanation of the method for keeping a 3-day dietary record. If the child was cared for by someone other than the mother, the mother instructed the person about keeping the food records. The second interview was conducted the day following the 3-day dietary record and consisted of reviewing the dietary record with the mother and recording any omitted information. The first 3-day intake records were begun on May 20, 1967 and the last record was completed on June 6, 1967.

The form developed for the North Central Cooperative Project NC-75 was used to record all food eaten and any vitamin and/or mineral supplement taken (Form I, Appendix). Written instructions for recording food eaten accompanied the form.

Each food item appearing in a child's food record was assigned a code number as outlined in the manual, Calculating the Nutritive Value of Diets (Davenport, 1964). The percentage of an average serving of each food item was determined. Master computer cards containing the nutritive values for average servings of foods which are presented in Home and Garden Bulletin 72 (Consumer and Food Economics Research Division, 1964), was used for calculation. An IBM 360-50 computer was used to determine the nutrient intake for each of the 3 days as well as an average for the 3 days. Mean daily intakes were calculated for food energy, protein, fat, carbohydrate, calcium, vitamin A, thiamin,

riboflavin, niacin and ascorbic acid. Niacin equivalents were figured by assuming that 1% of the dietary protein was tryptophan and that 60 mg of tryptophan yields 1 mg of niacin. The following formula was used:

protein (g) x 0.167 + niacin (mg) = niacin equivalents (mg)

Mean daily nutrient intakes were divided into quartiles, using the highest and lowest values observed to show the total range. In addition, the mean daily intake per kilogram of body weight was calculated for food energy, carbohydrate, fat and protein and divided into quartiles. Levels were compared to the Recommended Dietary Allowances (RDA).

Medical Examinations

Medical examinations were given by two local pediatricians from May 31 to July 24, 1967. The medical form (Form II, Appendix) was adapted from the Marking Form of the Kent Pediatric Society Research Sub-committee, Incidence of Health Among School Children. Examination of skin, eyes, ears, nose and throat, cervical glands, speech, heart and circulation, lungs and nervous system was done. Mental condition, deformities and any incidence of tuberculosis were noted. Maximum deductions for each defect were stated on the form with total deductions of 100.

Blood Analyses

Venous blood samples were drawn by a technician from the medical laboratory when the children were taken to the Medical Center for their medical examinations. A portion of the blood was oxylated for use in hematocrit and hemoglobin determinations. Another portion was allowed to clot for electrophoretic separation of the serum proteins and total serum protein. Blood samples were stored at room temperature at the medical laboratory until all examinations for each day were completed. Samples were then taken to the nutrition laboratory at Kansas State University for analyses. After clot retraction the serum was separated by centrifugation. When it was not possible to perform total serum protein determinations and electrophoretic analyses on the day the sample was drawn, the serum was stored under refrigeration, but in no case for longer than two days.

Hemoglobin determinations were made in duplicate using the cyanmethemoglobin method (ICNND, 1963).

Hematocrit determinations were done in duplicate using the Adams Autocrit Centrifuge (Clay-Adams Inc., 1963).

Total serum protein determinations were done in duplicate using a modified biuret technic-macro procedure (ICNND, 1963). A 20% solution of Diagnostic Albumin obtained from the Department of Public Health in Michigan was used as the standard.

Electrophoresis of serum protein was run in duplicate or triplicate using bromophenol blue dye in alcholic solution (procedure B), Spinco Model R Paper Electrophoresis System (Beckman, 1965). This system (Fig. 1) allows for 8 determinations with each run. The support stand is set into the cell vessel so the slit in each leg straddles the center partition. Eight filter paper strips are placed over the nylon pegs and the folding rack

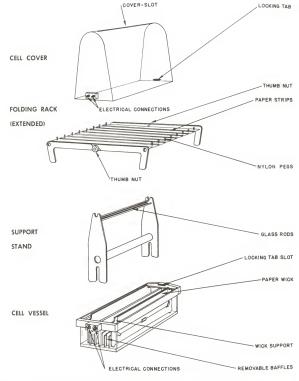


FIG. 1 PERSPECTIVE DIAGRAM OF BECKMAN MODEL R PAPER ELECTROPHORESIS CELL

placed on the support stand with the legs down as far as they will go. The paper strips are then in contact with two thick paper wicks saturated with a buffer solution in the cell vessel. The cell cover is then put in place. Paper strips are then saturated with buffer solution, and drained. The test sample is applied to the filter paper strip by removing tape over the cover slot. Direct current is applied and migration of the proteins takes place. After migration for 16 hours the strips are dried, rinsed, stained and rerinsed according to procedure B (Beckman, 1965). A model RB Analytrol with a slit width of 0.5 mm and a B-5 cam was used for scanning (Beckman, 1957). Values obtained were divided into quertiles using the highest and lowest values observed to show the total range and compared to the Interdepartmental Committee on Nutrition for National Defense (ICNND, 1963) suggested levels.

Statistical Analyses

Correlation coefficients were computed among blood values, among nutrient intakes and between blood values and nutrient intakes using an IBM 360-50 computer.

RESULTS AND DISCUSSION

Description of the Subjects and Their Families

Sex and Age of Children Studied. The 40 children included in this study consisted of 19 boys and 24 girls ranging in age from 3 years 10 months to 5 years 9 months. Previous studies

(Angelopoules and Bechrakis, 1960; Knapp and Routh, 1949; McBee et al., 1950; Morgan, 1959; ICNND, 1963; Lubschez, 1948; Oberman et al., 1956; and Williams, 1951) have shown no sex or age difference in blood data for this age range so children were not subdivided into sex or age groups.

<u>Composition of Families</u>. In 39 families both parents lived in the home. One child lived with his mother and grandmother. The number of children per family ranged from 1 to 4 with a mean of 2.38 per family. The range of ages of siblings was 3 weeks to 17 years.

Ages of Parents. Ages of parents are given in Table 1. Age data were not obtained on 2 sets of parents. Approximately one-half of the parents were 31 to 40 years of age and one-third were 21 to 30 years of age.

TABLE	

	No	21-	-30	31-	-40	41-	-50
		No	%	No	%	No	%
Mothers	38	17	45	17	45	4	10
Fathers	37	8	22	23	67	6	16
Totals	75	25	33	40	53	10	13

Ages of parents (in years)

Education of Parents. Educational level of the parents is given in Table 2. Data were not obtained on 4 mothers. All but 4 mothers had some college education and 15 had college degrees.

The majority (69%) of the fathers had more than 4 years of college.

TABLE 2

	No	Hig scho 1-3 No	ool	Hig scho 4 J No		Coll 1-3 No		Coll 4 No	ege yr %	more	llege e than 4 yr %
Mothers	35	1	3	4	8	16	46	11	35	3	8
Fathers	<u>39</u>	1	3	2	5	0		8	23	28	69
Totals	74	2	3	6	8	16	22	19	26	31	42

Education of parents

<u>Occupation of Parents</u>. Twenty-eight (72%) of the fathers had professional occupations, five were graduate students, two were managers, 3 were salesmen, and 1 was a service worker. Twelve mothers were employed outside the home on a full or parttime basis.

Dietary Intake Records

<u>Mean Nutrient Intake</u>. The mean daily nutrient intake for each child from diet plus vitamin and/or mineral supplement was calculated from the 3-day dietary record (Table 12, Appendix). In one case a 2 day record was used because dietary information was incomplete for the third day. The distribution of intakes for the whole group is presented in Table 3 as the highest and lowest values observed and the 25th, 50th, and 75th percentiles as compared to the 1963 RDA. TABLE 3

Highest and lowest values observed and 25th, 50th, and 75th percentiles of daily nutrient intake of 40 children as compared to 1963 $\rm RDA^{1}$

	Food energy	Fa.t	Carbo- hydrate	Protein Calcium Iron	Calcium	Iron	Vita- min A	Thia- min	Ribo- flavin	Nîacîn	Niacin Ascorbic acid
	cal	E.	щ	шS	шS	t0 Ei	Π	Sm	Яш	gm	8 E
Highest	2879	105.4	357.0	103.1	2.815	13.9	18760	l↓ . 66	4.17	240.8	246
75th	066T	78.7	236.6	68.5	1.080	0*6	7110	1.80	2.67	27.6	121
Soth	1568	64.2	202.8	58.0	0.730	7.5	4635	1.04	2.00	22.4	716
25th	1408	4.12	157.2	418.44	0.586	6.8	2610	0.70	l.33	18.0	70
Lowest	1028	37.8	102.2	30.6	0.362	3.9	1060	0.53	0.98	13.6	8
RDA	1600			0.04	0.8	10.0	2500	0.60	1.00	0.11	20

The RDA for iron fell between the 75th percentile and the highest value observed, the RDA for calcium and calories between the 50th and 75th percentiles, the RDA for vitamin A, protein, thiamin, riboflavin and ascorbic acid between the lowest value observed and the 25th percentile. All niacin equivalent intakes were above the RDA. The most deficient nutrient according to RDA was iron, followed by calcium.

To adjust for body size, daily intakes for each child for food energy, fat, carbohydrate and protein were calculated as intake per kilogram of body weight (Table 13, Appendix). Data for the whole group are shown in Table 4 as highest and lowest values observed and 25th, 50th and 75th percentiles as compared

TABLE 4

Highest and lowest values observed and highest 25th, 50th and 75th percentile for food energy, protein, carbohydrate and fat in nutrient per kilogram of body weight for 40 children as compared to 1963 RDA¹

		Fat	Carbohydrate	Protein
	calories per kg	gm per kg	gm per kg	gm per kg
Highest	153	5.7	18.0	5.6
75th	103	4.2	13.4	3.6
50th	90	3.5	11.0	3.0
25th	71	2.7	9.0	2.7
Lowest	60	2.0	6.0	1,6
RDA	89			2.2

1 Food and Nutrition Board, 1964.

to the 1963 RDA. When expressed as nutrient per kilogram of body weight the RDA for protein remained between the lowest value observed and the 25th percentile but the RDA for calories fell one division since it was just below the 50th percentile.

The number of children who had intakes below 100% of the RDA for 1-6 nutrients is shown in Table 5. The nutrients are subdivided to indicate those between 67% and 100% and those below 67% of the RDA. Two children were below 100% of the RDA for 6

	TABLE 5	
Number	of children with intakes below the 1963 RDA ¹ for 1-6 nutrients	Э

Fotal no. nutri- ents below 100% RDA	No. children	No. nutrients between 67%- 100% RDA	No. nutrients below 67% RDA
6	1	2	4
6	1	4	2
5	1	2	3
4	1	1	3
4	2	2	2
4	3	3	1
3	2	1	2
3	2	2	1
3	6	3	0
2	9	1	1
2	6	2	0
1	1	0	1
1	10	1	0

1 Food and Nutrition Board, 1964.

nutrients, 1 for 5 nutrients, 6 for l_1 nutrients, 10 for 3 nutrients, 15 for 2 nutrients, and 11 for 1 nutrient. Fifteen children had 1 nutrient and 8 had 2 to 4 nutrients that were below 67% of the RDA.

Iron was the most deficient nutrient in this study. Methany <u>et al</u>. (1962), Bilderback (1967), Ling (1966) and Beal (1954) also found iron to be the most deficient nutrient in the diets of preschool children. Methany <u>et al</u>. (1962) reported 51% of 87 children had diets that failed to supply the recommended allowance for iron. Five percent of their children had diets failing to supply iron at the level of 67% of the recommendation. Beal (1954) found as many as 75% of the children she studied in the Denver, Colorado area had iron intakes less than the recommendation.

In this study calcium was the second least adequately supplied nutrient compared to the 1963 RDA. The median intake was 0.73 gm (Table 3). Less than one half of the 49 children studied by Ling (1966) had intakes adequate in calcium. Forty-six percent of the children studied by Methany <u>et al</u>. (1962) had diets low in calcium. The Denver children whom Beal (1954) observed had the lowest level of calcium intake between the ages of 2 and 3 years when the median intake was 0.75 gm. However, by the age of 5 there was an acceleration so that the median intake was at the 1.0 gm level.

The RDA for calories is just above the median intake of 1568 calories in this study. Sixty percent of the children in Bilderback's (1967) study had intakes at or above 100% of the RDA and. the others had calorie intakes at or above 67% of the RDA. Calorie intake was below 100% of the RDA for 57% of the children from the lower socioeconomic group and 86% of the children from the higher socioeconomic group studied by Ling (1966). Forty percent of the children studied by Methany <u>et al</u>. (1962) had calorie intakes below 100% of the RDA. Beal (1953) found total intake of calories increased and median intake was close to the RDA throughout the period from 1-5 years.

The RDA for ascorbic acid fell below the 25th percentile in this study. Slightly more than one half of the children studied by Ling (1966) had diets supplying 100% of the RDA. Methany <u>et al</u>. (1962) found the full allowance for ascorbic acid was consumed by 85% of the 87 children studied. In contrast Bilderback (1967) found that ascorbic acid was second to iron of the nutrients supplied in inadequate amounts.

Only a few of the children's diets were below the full recommendation for protein (2), riboflavin (3) and thiamin (1). Niacin was the most adequately supplied nutrient by NRC standards with diets of all the children meeting 100% of the RDA. Ling (1966), and Bilderback (1967) also found that all of the diets included in their studies met 100% of the RDA for niacin. Only 3% of the diets included in the study conducted by Methany <u>et al</u>. (1962) failed to meet 100% of the niacin allowance.

<u>Effect of Supplementation</u>. Sixteen children received vitamin and/or mineral supplements. Of these, 13 received a multivitamin supplement, 1 a multi-vitamin plus iron and calcium supplement, 1 a multi-vitamin supplement and an ascorbic acid

supplement on alternate days and 1 a multi-vitamin supplement for two days and a multi-vitamin supplement plus iron on the third day. All of the children receiving vitamin supplements had nutrient intakes exceeding the RDA in nutrients supplied in the supplement, except iron.

Mean vitamin intake from diet alone for each child receiving supplements was calculated (Table 14, Appendix). Highest and lowest values observed and 25th, 50th, and 75th percentiles of nutrient intakes from food alone are shown for the group of children not receiving vitamin supplements (Table 6) and for the group receiving supplements and for vitamin intakes from food plus supplement for children receiving supplements (Table 7). Intakes of vitamins from food alone were similar for the two groups.

Of the 16 children receiving supplements containing vitamin A and ascorbic acid, 5 had food intakes below the RDA for both nutrients. Fourteen of the 15 largest intakes of vitamin A (food plus supplement) were consumed by children receiving a supplement containing vitamin A. The highest intake was approximately 7.5 times the RDA.

Thirteen children received supplements containing thiamin, riboflavin and niacin. Three, 5 and 1 of the children would have been deficient according to the RDA in thiamin, riboflavin and niacin, respectively, had they not taken a vitamin supplement. However, none of the children had intakes from food alone below 67% of the RDA for these 3 nutrients.

Two children were consistently in the lowest percentile

TABLE 6

Highest and lowest values observed and 25th, 50th and 75th percentiles of daily nutrient intake for 24 ohildren not reelving vitamin

Level	Food energy	Total fat	Carbo- hydrate	Protein	Calcium Iron	Iron	Vita- min A	Thia- min		Niacin	Ribo- Niacin Ascorbic flavin acid
	cal	шS	E B	шS	E 60	Вш	ΠŪ	В Ш	อน	а ш	60 E
Highest	2286	105.4	357.0	103.1	2.815	11.2	13070	1.55	μ.01	31.2	203
75th	1992	75.6	245.6	69.2	1.085	0°6	3835	1.04	2.05	23.0	46
Soth	1568	64.2	193.5	59.3	1117°°C	7.4	3085	0.84		19.7	78
25th	1408	51.0	157.2	50.8	0.626	6.6	1830	0.66	1.33	17.0	42
Lowest	1057	39.2	102.2	40.7	0.438	5.1	1060	0.53	0.98	13.6	8
ACA	1600			0.04	. 0.800	10.0	2500	0,60	1.00	11.0	20

"Food and Nutrition Board, 1964.

Highest and lowest values observed and 25th, 50th and 75th percentiles of daily nutrient intake from food alone and from food plus supplement for 16 chlidren receiving vitamin supplements as compared to 1963 RDA

TABLE 7

Level	Food energy	Fat	Carbo- hydrate		Protein Calcium Iron	Iron	Vita- min A	Thia- min		Niacin equiv.	Ribo- Miacin Ascorbic flavin equiv. acid
	cal	щ	щg	шS	щg	ଅଅ ଅ	ΠI	ВШ	ଅଳ	50 E	ы В
					Food alone	an					
Highest	2899	103.2	286.0	80 .44	1.454	13.9	13760	66.0		24.6	186
75th	1990	89.6	238.0	64.6	1.020	8.9	5335	0.82		19.2	105
Soth	1571	66.2	208.2	56.3	0.634	7.5	3155	0.66		16.9	56
25th	1328	51.4	158.4	71.74	0.522	7.0	1655	0.60	0.90	4.41	34
Lowest	1028	37.8	138.4	30.6	0.362	3.9	1490	0.50		9.3	12
				Food	Food plus supplement	plemen	ct.				
Highest							18760	4.66		40.8	246
75th							OIIOI	3.26		36.3	170
Soth							7725	2.26	3.11	30.9	112
25th							6230	1.80		25.8	96
Lowest							14610	1.59		19.3	72
RDA	1600			0.041	0.800 10.0	10.0	2500	0.60		0.11	20

^TFood and Nutrition Board, 1964.

divisions for all of the nutrients and usually one or the other had the lowest intake observed. Both of these children received vitamin supplements. Without the supplement one child would have been below the RDA in all 9 nutrients calculated.

Bilderback (1967) found that children receiving vitamin supplements had higher dietary ratings than children not receiving supplements. In general, she found that children receiving supplements already were consuming more adequate diets from food alone than the other children.

The effect of supplementation of diets is shown graphically in Figure 2. Various levels are related as percent of RDA. In every case corresponding levels are higher in the diets with supplements than without. Supplementation did little to improve the diet in comparison to RDA except for vitamin A and ascorbic acid.

<u>Correlations</u> <u>Between Nutrients</u>. Simple correlation coefficients between nutrient intakes from diet plus supplement are shown in Table 8.

Because calories are a composite of protein, fat and carbohydrate the positive correlations between these nutrients might be expected.

Most of the children consumed a fairly large amount of margarine each day. The fortification of margarine with vitamin A might account for the positive significant ($p \leq 0.05$) correlation between vitamin A and fat.

The main source of iron in the diets was enriched grain products. This fortification might account for the positive

Correlation coefficients between dietary nutrients

Miacin Ascorbic 0.66** 0.51** **74*0 0.43** 0.59** 0.50* acid 0.14 0.13 0.17 0.20 **#9*0 0.83** 0.75** 0.33* -0.01 0.13 0.20 11.0 0.41** -0.16 0.83** 0.34* flavin 0.31* 0.31* Ribo-0.19 0.28 0.25 Vitamin Thiamin 0.91** 0.52** 0.07 -0.05 0.20 0.59** -0.14 -0.02 0.84** 0.17 0.64** -0.02 -0*0⁺¹ -0.09 0.03 4 0.49** 0.53** 0.24 Iron Calcium 0.74** 0.83** 0.59** 0.84** 0.55** 0.25 hydrate 0.45** 0.54** Carbo-0.35** Fat Protein 0.70** ories Cal-Carbohydrate Riboflavin Vitemin A Calories Protein Thismin Calcium Niacin Iron Fat

*Indicates significance at the 0.05 level (0.305) with 38 degrees of freedom. **Indicates significance at the 0.01 level (0.395) with 38 degrees of freedom.

TABLE 8

significant relationship between carbohydrate and iron, protein and iron and calories and iron. If enrichment of cereal products was the reason for these correlations, correlations also might be

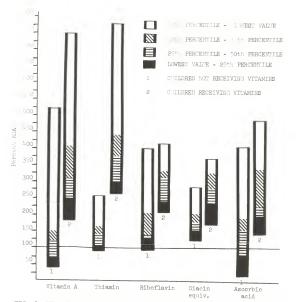


FIG. 2 HIGHEST AND LOWEST VALUE, 25th, 50th, and 75th PERCENTILES AS PERCENT RDA

35

shown in Table 15, Appendix. The lowest score received was 96 out of a possible 100. Twenty-four children (60%) received scores of 100. All the children were apparently healthy and had no obvious nutritional deficiencies.

Blood Analyses

The average blood values for each child are shown in Table 16, Appendix. Highest and lowest values observed and 25th, 50th and 75th percentiles for hemoglobin, hematocrit, total serum protein, albumin (gm/100 ml), globulin fractions (percent of total serum protein) and albumin:globulin ratio as compared to ICNND (1963) suggested levels are shown in Table 9. Simple correlation coefficients were calculated between blood values (Table 10) and between blood values and dietary intake (Table 11).

<u>Hematocrit</u> and <u>Hemoglobin</u>. The ICNND (1963) acceptable levels fell between the 25th percentile and lowest value observed for hematocrit and hemoglobin. Because 90% of the children were below 100% and 25% of these below 67% of the RDA for iron a low hemoglobin value might be expected, however, this was not shown in the hemoglobin values found. Kaucher <u>et al</u>. (1948) suggested that hemoglobin concentration is an indicator of adequate nutrition with respect to many rather than to a single nutrient. Two children with deficient hemoglobin levels according to ICNND (1963) standards had intakes in the lowest percentile for calories, fat, protein, calcium and iron. One child was also in the lowest percentile for carbohydrate. Both children received a vitamin supplement supplying thismin, riboflavin, niacin and

Highest and lowest values observed and 25th, 50th, and 75th percentiles as compared to ICNND (1963) suggested guides for blood data

						GLOBULINS	ns		Albumin:
Level	Hematocrit	Hemoglobin	Hematocrit Hemoglobin Total serum Albumin protein	nîmudlA	Alpha 1	Alpha 2	Beta	Garma.	Globulin retion
	Se Se	Em/loo ml	gm/100 mJ	8.m %	% OF	total serum protein	m proteir	d	
Highest	41.50	15.25	7.80	5.38	4.92	13.70	13.05	14.41	2.92
75th	37.75	12,80	6.70	4.70	4.22	8.42	11.20	10.71	2.40
50th	36.50	12.35	6.26	4.32	3.68	7*81	99.66	10.08	2.23
25th	35.12	11.65	6.00	4.12	3.02	7.34	8.92	8.64	2.02
Lowest	31.75	10.10	5.48	3.42	2.36	5.90	7.75	5.66	l.32
ICNND									
Deficient	<30*0	<10*0	<6.0 ¹	<2.80					
Low	30.0-33.9	30.0-33.9 10.0-10.9	6.0-6.4	2.80-3.51	51				
Acceptable	34.0-36.9	4.21-0.11	6.5-6.9	3.52-4.:	3.52-4.24 4-7	9-11		11-15 12-16	-16
High	>37.0	>12.5	>7.0	>4.25					

Total plasma protein: gm/100 ml.

Correlation coefficients between blood values

						Globulins	ins		Albumin:
	Hematocrit	Hemoglobin	Hematocrit Hemoglobin Total serum Albumin Alpha l Alpha 2 protein	Albumin	Alphe, 1	Alpha 2	Beta	Gamma	Globulin ratio
Hematocrit		0*60**	0.12	0.07	-0*0¦+	0.11	0.12	0.07	-0.05
Hemoglobin			0.06	-0.01	0.03	0.16	LL.O	0,02	-0.15
Total serum protein				0.87**	0.44** 0.37*	0.37*	0.57**	0.57** 0.31*	-0.19
Albumin					0.18	-0.01	0.23	0.23 0.04	0. ⁴ 7**
Alpha 1						0.45**	0.60**	0.60** -0.02	-0.45**
Alpha 2							0. ^{46**}	4T.0 **94.0	-0.64**
Beta								0.15	-0.58**
Gamna.									-0.46**

** Indicates significance at the 0.0 llevel (0.305) with 30 degrees of freedom.

Correlation coefficients between blood values and dietary intake

	Cal- ories	Protein	Fat	Carbo- hydrate	Calcium	Iron	Vitamin Thiamin Ribo- A flavir	Thiamin	~	Niacin	Ascorbic acid
Hematocrit	0.28	0.28	LL.0	0.20	0.24	0.19	0*02	40°0	70.07	0*0	0.04
Hemoglobin	0.26	0.33**	0.10	0.20	0.30	0.24	-0*02	-0*05	0,09	-0*02	0.12
Total serum protein	-0.18	-0*15	-0,12	-0,20	-0.26	60*0	0.22	0.20	0*0	0.33*	61.0
nîmudlA	-0.03	-0.10	0.08	0.08 -0.13	-0*2T	0.10	0.32*	0.21	60*0	0.34*	0.21
Alpha l	-0.23	-0.14	-0*37*	-0*37 ** -0*04	-0.18	-0.10	-0.18	0.14	-0.04	0.07	70.0 -
Alpha, 2	-0.13	00*00	-0.15	-0.15 -0.13	-0.08	0.03	-0,22	0.05	-0*02	10°0	-0.10
Beta	-0.24	-0.06	-0.31	-0.16	-0.16	0.03	0.13	0.14	T0.0-	0.15	-0.13
Gamme,	-0.26	-0.16	-0.23	-0.14	-0.11	-0.17	0.26	-0.11	-0*00	0.06	0.16
:ujundLA:	0.25	-0.02	0.37*	0.37** 0.06	T0*0	0.08	0.22	90*0	70,07	60*0	0.12

 $^{\rm *}{\rm Indicates}$ significance at the 0.05 level (0.305) with 38 degrees of freedom. ** Indicates significance at the 0.01 level (0.395) with 38 degrees of freedom.

ascorbic acid.

A positive significant relationship (p < 0.01) was found between hemoglobin and hematocrit. Bring <u>et al</u>. (1955) also found a positive relationship between hematocrit and hemoglobin.

Few significant correlation coefficients were found between nutrient intake and hematocrit and hemoglobin values. Because protein and iron are involved in hemoglobin formation a positive relationship might be expected between hemoglobin and protein and hemoglobin and iron. A positive significant (p < 0.01) relationship was found between hemoglobin and protein intake. The relationship between hemoglobin and iron intake was positive but not quite significant.

Total Serum Protein and Serum Protein Fractions. Total serum protein values ranged from 5.48 to 7.80 gm/100 ml. Angelopoules and Bechrakis (1960) found a mean total serum protein value of 6.39 gm/100 ml in children over 3 years. This figure would fall near the 50th percentile found in this study. Oberman <u>et al.</u> (1956) found a mean total serum protein value of 7.11 for children 3-5 years old. This value is above the 75th percentile found in this study. ICNND (1963) give suggested levels for total plasma protein instead of serum protein. Because plasma contains the fibrinogen fraction these values cannot be compared directly. The fibrinogen fraction has been estimated to be about 6-7% of the total plasma protein (Kleinor and Orten, 1966; Harrow and Muzer, 1966). Assuming that 6.5% of the plasma protein is fibrinogon, a range of 6.1-6.5 gm/100 ml for total serum protein was estimated by using tho ICNND (1963) acceptable range (6.5-6.9 gm/100 ml) for total plasma protein. This range would be between the 25th and 75th percentiles of the present study.

The lowest value for the ICNND (1963) acceptable range fell between the lowest value observed and the 25th percentile for albumin, between the 50th and 75th percentiles for alpha 1 and beta globulin and between the 75th percentile and the highest value observed for alpha 2 globulin and gamma globulin.

Mean values for globulin fractions obtained by Oberman <u>et</u> <u>al</u>. (1956) are within the ICNND. (1963) acceptable range. Both the ICNND (1963) values and mean values obtained by Oberman <u>et</u> <u>al</u>. (1956) are higher than values found in this study.

Positive significant (p < 0.05) correlations were found between total serum protein and niacin equivalent intake, albumin (gm/100 ml) and vitamin A intake, albumin and niacin equivalent intake and a positive significant (p < 0.01) correlation was found between albumin:globulin ratio and fat intake. A negative significant (p < 0.01) correlation was found between fat intake and alpha l globulin.

Because lipoproteins and glycoproteins are carried by the alpha and beta globulin fraction (Wuhrmann and Wunderly, 1960) a relation might be expected between these two globulin fractions and carbohydrate or fat rather than the negative relationship between alpha 1 globulin and fat intake and the positive relationship between albumin:globulin ratio and fat intake that was found in this study. Perhaps an analysis of total serum lipid would have shown a positive relationship with alpha and beta globulin fractions.

SUMMARY

The relationship of dietary intake and several blood values for 40 children age 3 years 10 months to 5 years 9 months was investigated. Three day dietary records were obtained for children attending the Kansas State University Nursery School and the Presbyterian Church Nursery School in Manhattan, Kansas. Blood samples were analyzed for hemoglobin, hematocrit, total serum protein, and electrophoretic fractions of serum proteins. The children were apparently healthy according to a medical examination.

Mean daily intake of the individual nutrients from diet and supplement for each child was calculated and categorized into highest and lowest values observed and 25th, 50th, and 75th percentiles and compared to the 1963 KDA. The 1963 RDA for iron fell between the 75th percentile and the highest value observed; for ascorbic acid, vitamin A, protein, thiamin and riboflavin, it fell between the lowest value observed and the 25th percentile. All dicts supplied niacin at 100% or more of the 1963 RDA. Iron was the nutrient supplied in least adequate amounts, followed by calcium and calories.

Vitamin supplements were taken by 16 of the 40 children. Except for vitamin A and ascorbic acid, supplementation did little to improve the diet. In general, the daily nutrient intakes were similar for children not receiving vitamins and from food alone for those receiving vitamins. Supplemented vitamins were higher for children receiving vitamins. Highest and lowest values observed and 25th, 50th and 75th percentiles were obtained for hemoglobin, hematocrit, total serum protein values, albumin (gm/100 ml), globulin fractions (percent of total serum) and albumin:globulin ratio. The lowest value for the ICNND (1963) acceptable range fell between the 75th percentile and the highest value observed for alpha 2 and gamma globulin, between the 50th and 75th percentiles for alpha 1 and beta globulin, and between the lowest value observed and the 25th percentile for hemoglobin, hematocrit and albumin.

A positive significant (p $\langle 0.01 \rangle$ correlation was found between hematocrit and hemoglobin and hemoglobin and protein intake. Hemoglobin was positively related with iron but this relationship was not significant. Two children deficient in hemoglobin according to IGNND (1963) standards had intakes in the lowest percentile division for calories, fat, protein, calcium and iron. Both children received vitamin supplements.

Positive significant correlations (p \langle 0.05) were found between total serum protein and niacin, albumin and vitamin A, and albumin and niacin. A positive significant (p \langle 0.01) correlation was found between fat and albumin:globulin ratio. A negative significant correlation was found between fat and alpha 1 globulin.

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50 APPENDIX

FORM I

N	0	m	0
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RECORD OF FOOD EATEN BY THE PRESCHOOL CHILD

Day of Week _____ Schedule No. _____

	Kind of Food	Amount				child		
Time	Child Eats	Cups,		lone?		Home?		a Table?
of Day	and Drinks and Descrip- tion	Tsp, Tbsp, or Size	Yes	If no, with whom	Yes	If no, where	Yes	lf no, where
							_	
-								
	Vitamin, m this chi Name of	ineral, o ld for th supplemen	is d	trient ay:	suppl	ement	given	

Amount given per dose ______ No. of times given _____

INSTRUCTIONS FOR RECORD OF FOOD EATEN BY THE PRESCHOOL CHILD

 Record all consecut: Record amou utensils (a) set o (b) l cup 	time when any food is eaten. food the child eats or drinks for three lve days. ant child eats or drinks using the following for measurements. of "standard" measuring cups o liquid measuring cup of "standard" measuring spoons r
Record only	y the amount eaten, not what is left on plate.

<u>Beverages</u>	 list all kinds of beverages as whole milk, chocolate milk, carbonated drink, fruit flavored drink or mix. record by cups (1/4, 1/3, 1/2, 2/3, 3/4, 1) or tbsp.
Bread	 list kinds of breads or rolls as whole wheat, rye, white, hamburger bun. record bread in number or parts of slices. record rolls and crackers by number.
Butter, mar- garine, Spreads	- measure and record butter and margarine, peanut butter, jam and other spreads if used.
Cereals	- list kinds of cereal as raisin bran, cornflakes. - measure added milk, cream, or sugar.
Cheese	 list kinds of cheese as cottage, Cheddar. record cottage cheese by tbsp. record Cheddar cheese in slices as 2" x 2" x 1/8" or by ounce or by slice.
<u>Desserts</u>	 list kind of dessert as apple pie, chocolate pudding, frosted angel food cake. record puddings and ice cream by cups (1/4, 1/2, 1). record cupcakes and cookies by diameter as 2 3/4" diameter. record pies in fraction as 1/8 of 8" pie.
Eggs	- record number and method of preparation as fried, scrambled, poached.

Fish, Meat and Poultry	 list kind and method of preparation as fried chicken, broiled lamb chop, roast pork. record in inches as 2" x 3" x 1/4" or 1 wing, 1 drumstick, 3" diameter hamburger patty. record number of shrimp, scallops, fish sticks, wieners, sausages.
Fruits and Fruit Juices	 list the form (fresh, frozen, or canned) and kind of fruit. if fresh fruit, specify if small, medium, large. list the form (fresh, frozen or canned) and kind of juice. if fruit flavored drink or mix, specify kind. if sugar added, indicate amount.
<u>Miscellaneous</u>	 list kind and size of candy bar. list kind and number of nuts, pieces of candy. record amount of popped corn by cups (1/4, 1/2, 1). record amount of chewing gum.
Mixed Foods (Casseroles, Salads)	 any food prepared from a combination of ingredients. record proportions of ingredients in recipes. record amount child eats.
<u>Vegetables</u>	 list kind of vegetable, as broccoli, potatoes-baked, fried, mashed. measure and record added butter and sauces. record cooked vegetables by portions of cups (1/4, 1/3, 1/2) or tbsp. record raw celery as portion of 1 stalk. record potato chips and French fries by number.
Vitamin or Other Food Supplement	- record brand name and amount.

EXAMPLES

Kind of Food Child Eats and Drinks and Description	Amount Child Eats (cup, tbsp., tsp., number)
Corn Flakes with sugar whole milk	3/4 cup 1/2 tsp 1/2 cup
Toasted cheese sandwich white bread American cheese margarine	l slice l slice 3" x 3" x 3/16" l tsp.
Raw apple	1/2 of small apple
Canned peaches	l peach half 2 tbsp. juice
Fried chicken	l leg
Roast beef	1 thin slice, 4 1/2" x 2"
Mashed potatoes with milk and butter added	1/4 cup
Gravy on potatoes	l tbsp.
Raw carrot sticks	4 - 3" long
Cherry pie	1/8 of a 9" pie
Milk chocolate bar	l ounce
Pecan halves	6
Frozen orange juice	3/4 cup
Tuna noodle casserole 1/2 lb. uncooked noodles 1-7 oz. can tuna fish 1 can cream of mushroom soup 1/2 cup crushed potato chips	3/4 cup
XYZ brand vitamin	l tablet

HEALTH EXAMINATION FORM NUTRITION STUDY

Form 2

COLLECE OF HOME ECONOMICS KANSAS STATE UNIVERSITY Manhattan, Kansas

Sex	Name			 Date	of	Birth .		
School		No. in	Family	 Place	in	Family		
Father's O	ccupation			 Height	-		Weight	

SPECIMEN MEDICAL			MARKI	NG FOR	M
RECORD CARD		Col. l	Col. 2	Code	Deduction
Skin: 11 Ringworm, Head 12 Body 13 Scabies 14 Impetigo 15 Other diseases (non-tubercular)	SKIN	5		11 12 13 14 15	3 3 1 2 according to defect
Rye: 16 Blepharitis 17 Conjuctivitis 18 Keratitis 19 Corneal Ulcer 20 Corneal Opacities. 21 Defective Vision 22 Squint 23 Other conditions	EYE	8		16 17 18 19 20 21 22 23	2 1 8 8 5 5 8 according to defect
Ear: 24 Defective Hearing. 25 Otitis Media 26 Other Ear Diseases	EAR	8		24 25 26	4 8 according to defect
Nose and Throat: 27 Chronic Tonsillitis 28 Adenoids 29 Chronic Tonsillitis and Adenoids 30 Other conditions	NOSE AND THROAT	5		27 28 29 30	2 2 5 according to defect

Form 2 (cont'd)

	CIMEN MEDICAL				IG FORM	
REC	ORD CARD		Col. 1	Col. 2	Code	Deduction
31	Enlarged Cervical Glands	ENLARGED CERVICAL GLANDS			31	5
32	Defective Speech.	DEFECTIVE SPEECH			32	5
33	Teeth: Number Decayed. Condition	TEETH	8			nus 5;4 plus 8 ding to ect
	eart and irculation: Heart Disease Organic Functional Anaemia	HEART AND CIRCULATION	11		3 ¹⁴	11 8
36	Lungs	LUNGS	11		36 accor defe	
37	uberculosis: Pulmonary: Definite. Suspected Non-pulmonary: Glands. Spine Hip Other Bones and Joints Skin Other	TUBERCULOSIS	11		37 38	ll reject ll reject
D 42 43 44	eformities: Rickets Spinal Curvature. Other Forms	DEFORMITIES	11			ll ording to Sect
45	Other Defects	OTHER DEFECTS	11			ording to
46	Mental Conditions	MENTAL CONDITIONS	11		46 det	'ect 11
		TOTAL	100			

Col. 1 of marking form shows maximum deduction possible.

Col. 2 shows actual deduction by assessor.

Form 2 (concl.)

Signed: _____ M.D.

Adapted from the Marking Form of the Kent Pediatric Society Research Sub-committee, Incidence of Health Among School Children, 14 Brampton Rd., Bexley Heath Kent, England.

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Mean daily nutrient intakes from diet plus supplements

Child	Sex	Food	Pat	Carbo- hydrate	Protein	Calcium	Iron	Vitemin A	Thiamin	Ribo- flavin	Niacin equiv.	Ascorbic acid
		calories	W2	Ę,	, WD	5	Bu	R	Bu	Bu	But	Bui
Ч	Fil	1124	9* tht	138.6	48.3	0.808	7.0	16680	1.80	3.49	27.2	72
0	Γ.,	1666	66.1	221.2	55.9	0.749	9.1	2600	0.72	1.42	19.7	59
m	Ē	1609	73.1	198.0	48.5	0.647	7.5	8270	4.66	4.10	28.9	190
4	۶ų	2173	39.2	357.0	I.IOL	2.815	7.2	1060	71.1	10*4	23.5	32
ŝ	Ē.	1395	51.6	178.4	60.3	0.709	6.8	3820	0.76	1.35	19.3	<u>8</u> 3
9	ja,	1384	68.2	152.0	47.2	0.464	7.3	1880	0.62	1.01	17.0	65
7	M	1535	50.5	228.0	51.1	0.560	7.2	1510	0.59	1.09	18.0	33
co	N	1587	73.2	181.9	4°T9	1.087	7.6	5100	0.84	1.79	19.9	77
0	M	1925	103.7	174.1	81.1	1.361	0.6	3350	0.84	2.16	24.3	28
TO	Fri	1028	37.8	145.4	34.3	0.522	2.5	6700	1.80	2,36	22.5	911
25	Ē4	1970	98.0	208.1	4*77	1.331	9.6	9870	3.41	4 .10	39.6	194
513	M	1549	59.3	190.5	69.8	0.719	 6.9	0164	1.19	1.40	25.2	121
15	M	1697	60.7	236.8	57.1	0.523	13.9	7930	2.65	3.11	31.2	TOT
JQ	ΓH	2076	92.1	271.5	63.7	1.066	8.4	18760	2.19	2.89	35.2	246
18	Гт.	1422	62.4	155.4	4.19	0.623	7.7	3560	0.61	1.23	21.2	78
19	M	2286	88.2	293.5	85.5	1.078	10.3	3460	1.04	2.09	28.0	50
딩	N	1896	78.0	235.7	68.9	1.302	8.1	1780	1.55	2.05	21.2	92
22	Ēų	1136	43.5	146.2	45.3	0.661	5.1	1220	0.62	1.11	16.6	68
23	Μ	1949	73.6	286.0	46.6	0.362	8.7	10350	2.26	2.45	30.9	87
54	Ē: 1	2879	103.2	269.6	78.8	1.454	9.7	5990	1.04	2.30	25.0	127
52	Ēų	1087	46.3	131.0	42.7	0.600	5.5	5160	0.60	1.13	13.9	130
8	Ēų	1451	71.8	138.4	65.5	0.867	7.2	8110	3.75	3.53	32.2	94
30	Μ	1689	71.8	235.6	40.7	0.535	6.3	1720	0.66	0.98	15.0	54
33	Μ	1482	69 . 6	159.1	57.6	0.739	7.0	3300	0.68	1.33	17.1	32
34	W	1506	61.7	196.5	50.6	1.019	5.5	2620	0.96	1.69	15.4	93
35	Fι	1425	56.3	184.0	55.5	0.602	7.2	4610	0.69	1.30	13.6	149
37	M	1057	4e.4	102.2	58.4	0.627	9.9	1140	0.53	1.11	18.6	¢
38	Ē	1738	79.4	207.5	55.1	0.722	8.2	2440	0.80	1.33	17.9	95

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Child	Sex	Food energy	Fat	Carbo- hydrate	Protein	Calcium	Iron	Vitemin	Thiamin	Ribo- flavin	Niacin equiv.	Ascorbic acid
		calories	mga Mga Mga Mga Mga Mga Mga Mga Mga Mga M	mg	шg	mg	Bm	PI	mg	mg	Btu	Bu
04	Ēч	1148	40*2	171.3	30.6	0.573	9°0	6260	1.70	2.43	19.3	104
5	N	2150	66.99	296.8	103.1	2.183	10.5	3590	1.4L	3.21	31.2	203
43	Г×1	2155	1.10	304.7	62.4	1.066	7.7	2870	76.0	1.83	19.8	46
44	Ēų	2010	94.0	239.2	59.2	0.974	7.5	7520	2.82	4.17	37.4	104
46	N	1429	57.1	189.0	44.2	0.438	6.7	2190	1.0h	1.03	17.0	78
47	M	1533	55.0	232.2	40.2	0.452	5.9	10740	2.01	2.37	24.5	193
48	F-1	1232	51.2	143.5	50.1	0.622	7.0	0464	1.59	2.07	23.0	TOT
49	M	2144	61.3	337.1	68.1	1.090	11.2	3850	1.06	1.85	22.9	478
50	W	2016	87.1	236.4	80.4	1.228	8.7	6200	0.88	2.13	26.2	84
57	Fu	.2058	105.4	216.7	4.69	1.015	L.0	4660	1.08	1.95	22.2	149
25	×	TTT	46.2	142.7	53.4	1.083	5.2	13070	0.66	1.82	16.3	121
53	M	1529	51.6	208.3	60.6	0.423	9.5	0649	2.74	3.48	40.8	120

m.	A.	101	r.	1.1	- 7	2
Т.	А	B	L	Ľ.		. 5

Food energy, fat, carbohydrate and protein intakes as nutrient per kilogram body weight

Child	Food energy	Fat	Carbohydrate	Protein
12345678901231568912234560334577802234678901235555	cal 67 86 90 110 69 87 79 84 103 82 99 91 126 95 60 111 153 66 63 91 100 100 100 100 100 100 100 66 90 61 116 102 122 79 91 64 104 104 109 60 76	m 6410636055115029932581970591160722615646 234224424533459432452334432422423324533245	Em 8.2 11.5 11.1 18.0 8.8 9.6 11.7 9.9 7.6 9.9 10.9 10.9 10.9 10.9 10.9 10.9 10.8 12.7 16.2 11.3 14.3 14.3 14.3 14.3 14.3 12.7 10.6 13.0 11.6 10.7 10.7 10.6 10.7 10.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.8 12.7 10.9 11.1 1.3 1.4 1.3 1.6 6.6 10.7 1.6 1.3 1.6 1.7 1.6 1.3 1.6 1.7 1.6 1.7 1.6 1.3 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.3 1.6 1.7 1.6 1.7 1.6 1.3 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.7 1.6 1.3 1.6 1.7 1.7 1.7 1.5 3.6 1.5 7.5 1.5 1.5 1.5 1.5 1.5 1.5 1.5 1	E997,1006,4530738175,47.26829,457866.06,446,42770

TABLE 14	5	ΓA	BLE	-14
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Child	Vitamin A	Thiamin	Riboflavin	Niacin equiv	Ascorbic acid
	IU	mg	mg	mg	mg
1 30 15 16 34 24 35 44 44 55 53	11,680 3,270 1,700 4,870 4,970 13,760 5,750 2,990 3,110 1,610 1,610 1,610 3,200 1,490	0.60 0.66 0.91 0.65 0.59 1.04 0.59 0.59 0.59 0.50 0.82 0.81 0.88 0.88 0.88 0.88 0.88	1.99 1.10 0.86 2.10 1.11 1.39 0.82 2.30 1.53 1.30 0.93 1.67 0.87 0.87 1.24 2.13 0.98	17.2 16.9 24.6 21.2 15.6 12.2 15.6 12.2 13.6 9.3 14.5 14.5 26.2 20.8	12 115 59 119 51 80 67 54 89 454 133 18 24 70

Mean vitamin intakes from diet alone for children receiving vitamin supplements

	Deformities	N
examinations	Heart and I circulation	
	Teeth	H H M
s on medical	Defective speech	
deducations	Enlarged cervical glands	N
score and	Nose and throat	ΩH Ω
Total	Еуe	-4
E	Skin	N N
	Total score	001 001 001 001 001 001 001 001 001 001
	Child	нималосоодиннанийийийоддоод нималосоодиннанийийийоддоод алтананийийийийоддоодоодоодооодооодооооо

on't)	
15 (c	
TABLE	

Child	Total score	Skin	Еуе	Nose and throat	Enlarged cervical glands	Dofective Teeth speech	Teeth	Heart and circulation	Deformities
113 1113	100						~		
146	001								
810	86 6		0						
120	100								
Ч N М N	001								
2 2 2	79.				m				

TABLE 16 Blood values

Child	Hemat	Hemo-	Tot.a.1			Globulins	ns				Globulins	lns		:uimudIA
	ocrit		serum	Albumin	Alpha 1	Alpha	2 Beta	Gamma	Albumin	Alpha 1	Alpha	2 Beta Gar	Gamma	Globulin ratio
	9	gm/1	100 ml		gra	8m %			0 %	of total	serum pı	protein		2
r-1		12.79	6.40	94.4	0.18	0.38	0.66	0.72	69.66	2.86	5.91		-20	2.30
сл		12.25	6.20	4.43	0.28	0.46	0.54	0.49	71.48	4.54	7.46		8	2.51
ŝ		12.75	6.60	4.74	0.29	0.57	0.63	0.37	71.88	4.37	8.60		.66	2.56
4	38.00	12.60	6.22	4.17	0.26	0.45	0.69	0.65	67.01	4.17	7.29	11.11 10	10.42	2.03
ŝ		12.80	5.68	3.67	0.20	0.51	0.74	0.56	64.73	3.57	8.93		.82	1.84
9		13.60	7.00	4.87	0.16	0.52	0.67	0.77	69.57	2.36	7.49		.98	2.29
7		11.50	6.00	3.42	0.30	0.70	0.72	0.86	56.95	4.92	17.64		14.	1.32
œ		11.50	6.18	4.37	0.17	0.46	0.59	0.59	70.70	2.82	7.40		.56	2.41
5		12.80	5.99	4.17	0.18	0.50	0.53	0.61	69.52	2.97	8.34		-53	2.28
10		12.20	6.60	4.76	0.22	0.40	0.63	0.56	72.15	3.34	6.44		64.	2.59
75		11.50	6.50	4.45	0.22	0.49	0.67	0.65	68.51	3.32	7.58		-94	2.17
13		11.80	7.80	5.38	0.28	0.60	0.97	0.57	68.96	3.63	7.66		.29	2.22
57		11.80	6.60	4.4J	0.19	0.64	0.70	0.65	66.80	2.93	9.78		.81	2.01
10		12.40	7.30	5.21	0.18	0.43	0.56	0.92	L4.L7	2.40	5.90		• 55	2.50
18		11.50	6.80	4.76	0.24	0.41	0.66	0.73	69.93	3.54	6.05		.76	2.32
19		13.50	6.10	4.18	0.21	0.62	0.58	0.51	68.50	3.47	10.12		.38	2.17
57		11.70	5.58	3.86	0.24	0.46	0.67	0.36	69.14	4.23	8.17		- 52	2.24
50		12.60	7.30	4.67	0.31	0.59	0.95	0.77	64.02	4.28	8.13		.52	.1.78
с. С		12.70	7.50	5.26	0.29	0.59	0.70	0.66	70.11	3.91	7.82		.78	2.35
57		13.75	6.50	4.78	0.18	0.51	0.58	0.46	73.57	2.71	7.80		• 00	2.78
52		14.45	6.00	3.80	0.25	0.54	0.66	0.74	63.43	4.22	10.9		• 33	1.73
26		12.30	6.80	4.39	0.29	0.57	0.80	0.74	64.58	4.32	8.41		.89	1.82
30		11.51	6.20	4.19	0.27	0.48	0.76	0*50	67.64	4.31	7.72		-0 ⁴	2.09
33		11.60	5.58	4.16	0.13	0.34	0.42	0.53	74.46	2.41	6.16		.45	2.92
34		12.20	6.30	3.72	0.21	0.86	0.69	0.78	59.14	3.72	13.70		.46	1.45
35		11.80	7.10	5.03	0.28	0.50	0.55	0.73	70.87	3.92	7.06		.30	2.43
37		12.30	7.42	5.00	0.33	0.83	0.75	0.51	67.33	444.44	11.29		. 85	5*06 5

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ABLE

	Hemat	Hemo-	Total			Globulins	ns.				Globulins	Lns		Albumin
	ocrit	globin	serum	Albumin	Alpha 1	Alpha 2	2 Beta	Garma	Albumin	Alpha 1	Alpha 2	2 Beta	Gamma	Globulir retio
	82	gm/100	Tm 00		gr	gram %			82	of total	serum pr	protein		
38	33.25	04.11	6.40	4.50	0.22	0.48	0.51	0.68	70.35	3.51	7.448	8.01	10.65	
40	31.75	10.10	5.90	11.4	0.19	0.50	0.48	0.63	69.62	3.08	8.44	8.20	10.66	
04	39.50	15.25	6.00	3.96	0.22	0.63	0.56	0.62	65.94	3.76	10.54	9.42	10.34	
43	37.50	13.40	5.80	4.16	0.16	0.37	0.48	0.63	71.69	2.80	6.39	8.23	10.89	2.53
117	36.50	12.70	6.60	4.64	0.25	0.55	0.67	0.48	70.34	3.74	8.36	10.21	7.34	
16	36.50	12.50	5.80	4.06	0.24	0.43	0.53	0.53	70.07	4.20	7.46	9.12	9.15	
47	34.25	10.75	6.00	4.13	0.24	0.44	0.54	0.65	68.78	4.09	7.43	8.92	37.01	
48	36.25	12.25	6.80	4.59	0.24	0.57	0.77	0.63	67.55	3.47	8.37	11.30	9.31	
49	38.00	13.30	6.20	4.15	0.30	0.44	0.74	0.57	66.94	4.78	7.12	11.95	9.21	
50	38.25	10.40	5.48	3.95	0.16	0.39	0.47	0.51	72.16	2.95	7.09	8.57	9.23	
51	35.00	12.80	6.00	4.06	0.22	0.47	0.63	0.62	67.65	3.59	7.86	10.58	10.32	
52	37.75	12.73	6.22	4.26	0.25	0.51	0.55	0.65	68.49	3.98	8.18	8.93	10.42	
53	39.25	12.75	6.45	4.19	0.27	0.54	0.78	0.67	64.96	4.24	8.36	12.02	10.42	

DIETARY INTAKE AND BIOCHEMICAL ANALYSES OF BLOOD OF PRESCHOOL CHILDREN

by

JUDITH LORETTA CRUMRINE

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AN ABSTRACT OF A MASTER'S THESIS

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Department of Foods and Nutrition

KANSAS STATE UNIVERSITY Manhattan, Kansas

The relationship of dietary intake and several blood values for 40 children age 3 year 10 months to 5 years 9 months was investigated. Three day dietary records were obtained for children attending the Kansas State University Nursery School and the **Presbyterian** Church Nursery School in Manhattan, Kansas. Blood samples were analyzed for hemoglobin, hematocrit, total serum protein, and electrophoretic fractions of serum proteins. The children were apparently healthy according to a medical examination.

Mean daily intake of the individual nutrients from diet and supplement for each child was calculated and categorized into highest and lowest values observed and 25th, 50th, and 75th percentiles and compared to the 1963 RDA. The 1963 RDA for iron fell between the 75th percentile and the highest value observed; for ascorbic acid, vitamin A, protein, thiamin and riboflavin, it fell between the lowest value observed and the 25th percentile. All diets supplied niacin at 100% or more of the 1963 RDA. Iron was the nutrient supplied in least adequate amounts, followed by calcium and calories.

Vitamin supplements were taken by 16 of the 40 children. Except for vitamin A and ascorbic acid supplementation did little to improve the diet. In general, the daily nutrient intakes were similar for children not receiving vitamins and from food alone for those receiving vitamins. Supplemented vitamins which were higher for children receiving vitamins.

Highest and lowest values observed, 25th, 50th and 75th percentiles were obtained for hemoglobin, hematocrit, total serum protein values, albumin (gm/100 ml), globulin fractions (percent of total serum) and albumin:globulin ratio. The lowest value for the IGNND (1963) acceptable range fell between the 75th percentile and the highest value observed for alpha 2 and gamma globulin, between the 50th and 75th percentiles for alpha 1 and beta globulin, and between the lowest value observed and the 25th percentile for hemoglobin, hematocrit and albumin.

A positive significant (p > 0.05) correlation was found between hematocrit and hemoglobin and hemoglobin and protein intake. Hemoglobin was positively related with iron but this relationship was not significant. Two children deficient in hemoglobin according to ICNND (1963) standards had intakes in the lowest percentile division for calories, fat, protein, calcium and iron. Both children received vitamin supplements.

Positive significant correlations (p > 0.01) were found between total serum protein and niacin, albumin and vitamin A, and albumin and niacin. A positive significant (p > 0.05) correlation was found between fat and albumin:globulin ratio. A negative significant correlation was found between fat and alpha 1 globulin.