A STUDY OF THE PROTEIN REQUIREMENTS OF NORMAL FOUR YEAR OLD CHILDREN

THESIS FOR THE DEGREE OF M. S.

Margaret J. Nunn

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A Thesis Submitted
to the Faculty of
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by

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THESIS

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A STUDY OF THE PROTEIN REQUIREMENTS

OF

NORMAL FOUR YEAR OLD CHILDREN

Introduction:

In the field of metabolism studies through dietary experiments upon human beings, considerable work has been done with adult subjects and some with infants, but very little with individuals of the early growth period or pre-school age. Standards have been set up showing the optimum proportions of protein, calories, and minerals for adults, then these values have been reduced proportionately and allowances made to meet the needs of younger people.

The object of this study was to determine the level on which normal four year old children maintained the most efficient nitrogen retention.

Review of Literature:

A study of the literature shows that different methods have been used.(1) The earliest investigators considered the needs of two children equivalent to the needs of one adult. In 1866, Atwater (1) proposed a scale with the adult male as the standard, and calculated the needs of the other individuals in proportion. Engel, a Saxon statistician, nine years later took an infant during the first year of age as the unit, and calculated on the basis of height-weight statistics that an increase of one-tenth over the demands for the first year was needed for each additional year. The plan, however, was not extensively accepted. Atwater used the energy standards of Voit and interpolated an assumed value for children of two to six years, arranging his table in terms of a man's need.

A review of the literature shows that little thoroughly controlled research was done before the latter part of the nineteenth century. Most of the early work was done in Germany, and was of a purely clinical type. The conclusions were not warranted by either the number of children studied or the conditions under which they lived. Many of the subjects were abnormal. Variations are great in normal children, but are greater in abnormal. Therefore many misleading deductions were made, but they were used because they were the only ones available. Among the German authors whose conclusions we find much quoted in spite of their inaccuracies are Hasse, Herbst, Muller, Uflemann, Camerer, Baginsky and Starling. These investigators studied the caloric requirement of children as well as the protein, yet many of the subjects under observation proved to be under-fed. In their summary of caloric schedules proposed by various authors, Holt and Fales (2) show by comparison the great variations among data that have previously been accepted as stand-ards. This summary is as follows:

Author	Calories/Kilogram					
Boys	1st yr.	2nd yr.	3rd yr.	4th yr.	5th yr.	6th yr.
Camerer	89	80	78	75	84	77
Ufelmann	88	80	73	71	68	--
Steffen	113	99	105	125	114	---
Holt & Fales	100	93	88	84	82	80
Gillett	105	90	83	79	77	75
Lusk-quiet child	79	97	90	88	79	78
" -active "	--	129	117	116	105	105
" -very " "	--	193	177	175	158	158
Girls						
Camerer	89	80	78	75	70	67
Holt & Fales	101	94	87	82	78	76
Gillett	105	93	83	79	73	71

Holt and Fales (2) recommend that, in calculating the total caloric requirement of children, the component parts of that total be considered. These are: basal metabolism, growth, muscular activity, and the food values lost in the excreta. The basal requirements used by them are the ones determined by Benedict and Talbot. The latter studied a large number of children in Boston and furnished most of the data on which all the modern metabolism curves are based (3). They have found basal needs highest per kilogram of body weight at 9 months and steadily falling from then to adult life. The excreta loss is about 10 per cent for all ages after infancy. The growth requirement is greatest when growth is most active, or during the first year and adolescence. It is nearly uniform from four to ten years. Activity is the only factor that varies widely with the individual, the other three being nearly uniform for children of the same weight under similar conditions. Holt and Fales give as the total caloric requirement of children: 100 calories per kilogram at 1 year (9.5 kilograms). For boys, this falls to 80 calories per kilogram at 6 years (20 kilograms) and remains constant to 15 years. The activity increase is met by basal reduction. After 50 kilograms are reached, the energy value is reduced to the adult standard of 48 calories per kilogram. For girls, the requirement falls to 76 calories per kilogram at 6 years (20 kilograms) and continues to fall until 10 years, At this age it rises, and remains at 80 calories per kilogram until growth is complete; when it falls to the adult standard of 48 calories per kilogram.

Various dietary scales and standards have been used to measure the nutritive needs of the members of the family. Edith Hawley reviews these in her development of the scale used by the U. S. Department of Agriculture. (1) Her description of the scales of Atwater and Engel has already been reviewed. From 1886 to 1915, the increase in nutrition studies gave new light on metabolic needs. In view of this, and because of the inconsistent use of dietary scales for the requirements of the various members of the family, Sherman felt it necessary to study the energy requirements of children and express them in calories instead of the percentage of a man's need. In his introduction to Gillett's study (4), he says, "In our opinion, the food requirement for each member of the family should be determined on his own merits rather than in terms of the man's requirement." This was the first real step towards attention to the child's individual needs, and as a result of it, Gillett (4) brought together all the dietary studies and metabolism experiments that contained data on the energy requirements of healthy children. On the evidence of 564 studies, she made the following table of standards:

Age (years)	Calories/day (Boys)	Calories/day (Girls)
Under 2	900 - 1200	900 - 1200
2 - 3	1000 - 1300	980 - 1280
3 - 4	1100 - 1400	1060 - 1360
4 - 5	1200 - 1500	1140 - 1440
5 - 6	1300 - 1600	1220 - 1520
11 - 12	2100 - 2400	1750 - 2050
12 - 13	2300 - 2700	1850 - 2150
16 - 17	2800 - 4000	2250 - 1600

The energy allowances for children of 3 - 5 years may be summarized in the following table. (This is the specific age period of the present study and is therefore used in this table).

Author	Boys		Girls	
	Cal/kilo/day	Total cal/day	cal/kilo/day	Total cal/day
Camerer	78 - 84	---	75 - 70	---
Ufelmann	73 - 68	---	---	---
Steffen	105 - 114	---	---	---
Holt & Fales	88 - 82	1135 - 1380	82 - 72	1150 - 1350
Gillett	83 - 77	1150 - 1350	79 - 73	1130 1290
Lusk	90 - 77	145 - 1650	---	1400 - 1650
Rose	90 - 80	---	90 - 80	---
Atwater	---	1225 - 1425	---	1225 - 1425

The total protein requirement during the growth period depends upon the character of the protein and the total caloric intake.

In estimating a child's protein requirement, two equally important factors must be considered; first, tissue maintenance; and second, growth.

Sherman (5) states that, on the basis of adult needs, a child's requirement for maintenance is about 1 to 1.5 grams per kilogram per day, from either animal or vegetable source. In addition to this, growth requirements must be met. This should be done largely with animal protein. It is difficult to estimate the amount required.

Little work has been reported on the protein need and the effect of protein upon the growth of children. Osborne and Mendel's (6) experiments on rats and the work of Newburgh and his associates (7) on rats and rabbits indicate that growth is affected by the kind and amount of protein. One of the best ways to determine the protein need of normal children is through a metabolism study, whereby the nitrogen balance will indicate the efficiency with which the body is using its protein supply.

There is little scientific data on the composition of the diet of children over one year of age. Lucy Gillett (4) summarizes studies made from 146 cases of 18 authors whose work was large observational, and includes the values of protein intake as indicated by various authors up to 1917.

Age (years)	No. cases	Total protein gms/day	Protein intake av. gm/kilo
2 - 5	51	53	3.3
6 - 9	44	67	2.6
10 - 13	33	73	2.0
14 - 17	18	94	2.2

The total range in all of the cases over one year, as studied by Miss Gillett, was from 1.37 to 4.86 gms. of protein per kilogram of body weight.

Camerer (8), in 1896, recommended over 4 grams of protein per kilogram of body weight for the first year, 3 to 4 grams per kilogram up to 8 years of age, then over 2 grams per kilogram until 14 years, and 1.7 grams per kilogram at the end of the growth period.

J. P. Parsons, in his report on the nitrogen metabolism of children (9), gives a comprehensive survey of the work on protein requirements done by foreign workers since 1900. Several of his comments are interesting.

Siegert, 1916 (9), showed that nursing children require one gram of protein per kilogram of body weight per day, while children of three to six years require three to four grams per kilogram per day.

Muller (9) made a metabolism study of 32 children in an orphan asylum. This was done during the cool part of the year, using from 5 to 7 preliminary days with the ordinary diet and 6 days with the special diet. He found the average of protein calories to be 13.8 per cent of the total. The average nitrogen intake was 0.55 grams per kilogram or about 3.4 grams per day. The daily nitrogen output was about 3.18 grams. Holt and Fales (8) criticize this work because much of it was done on underweight, undernourished children.

Schlossman and Murschhauser, in 1910, (9) worked on a study of the oxygen exchange of individuals of various ages. They decided that the difference between the metabolism of children and adults is due to the added growth needs of children.

A study of food and protein requirements of three children was made by Stargardter in 1912. (9) His results are not of great value, however, because he varied the caloric intake from day to day.

Ruotsalainen (9), in 1921, made a study of protein requirements, keeping the total caloric value constant from day to day and varying only one type of food. On a diet with sufficient basal protein intake, he added isodynamic quantities of fat, protein and carbohydrate. He found a nitrogen balance maintained on two to three grams of protein per kilogram per day. The nitrogen retention was greatest when protein was added to the constant diet. He found that the nitrogen retention seemed to be the expression of an actual protein addition. More recent workers have found a greater nitrogen retention when carbohydrate is added to the diet. Practical use is made of this fact in feeding during disease where there is a toxic destruction of protein.

Gephart (10), in 1917, made an observational dietary study in a more reliable way than much of the work reviewed by Parsons. He studied the diet of 350 boys through adolescence at St. Paul's boarding school, Concord, N. H. He found the protein intake very high. The daily average was 160 grams or 2.6 to 3.8 grams of protein per kilogram of body weight. His children were beyond pre-school age, however, and were very active.

Holt and Fales (8), in 1921, collected the food consumption data for more than 100 health normal children from 1 to 16 years of age and found that their figures agreed rather well with the average for the various age groups. The urinary excretion was 4 to 6 grams of nitrogen per day for all ages. This figure is higher than was found by either Muller or Rubner. The average total protein intake during the second year was 44 grams; in the sixth and seventh year, 60 grams; in the twelfth year, 80 grams; in the fourteenth to sixteenth years, 130 grams; and at eighteen years, 115 grams. The protein intake of children under 6 years averaged over 3 grams per kilogram. For older children, the average was 2 grams per kilogram. This indicates a reduction with age. These children received about two thirds of their protein from animal sources and one third from vegetable sources.

Fleming and Hutchinson (11), in 1924, found the normal absorption of nitrogen to be 80 - 95 per cent of the intake.

From 1926 to 1929, Chi Che Wang and her associates (12) made a series of studies on the metabolism of undernourished children. They used normal children as controls. Their results indicate that the per cent of absorption of nitrogen is about the same in both normal and underweight children (90 - 92 per cent). The nitrogen retention, however, increases with the degree of undernutrition. Dr. Wang has also found that high protein diets do not appear to have any harmful effects on children. She found that the nitrogen retention for all children is directly proportioned to the nitrogen intake.

Other investigators who have differed in the per cent allowance of protein for adults or children are (13):

Voit, Playfair and Gautier - 16 per cent of total fuel value (high)

Atwater - 15 per cent of total fuel value

Landworthy - 12 per cent of total fuel value

Chittenden - 8.5 per cent of total fuel value - advocates low protein intake

Benedict - liberal allowance

Meltzer - allow for storage of protein as a factor of safety.

The dietary standards of the past tend to indicate a liberal intake of protein.

Sherman (13) claims that it is inadvisable to use as a standard the least amount to which the body can adjust itself, but rather to regard as the normal requirement an amount which will enable the body to maintain not only its equilibrium, but also some such reserve store of protein as we are accumstomed to carry.

Parsons, in 1930 (9), studied protein maintenance levels for the pre-school child, finding a nitrogen balance possible on 1.1 grams of protein per kilogram of body weight.

An allowance of one gram of protein daily per kilogram of body weight, or 50 per cent above the present estimate of actual requirements seems a reasonable standard to use as a general guide for adults.

Little can be said with confidence regarding the optimum amount of protein for children after the nursing period (13). Well planned dietaries generally contain 12 to 15 per cent of the total energy in the form of protein calories. During the period of rapid growth, much of the protein of the food is used to synthesize body protein. The amount of protein needed to produce one gram of body protein varies with the amino acid content of the food. Therefore, the specific amino acids supplied are of great importance.

Hoobler, B. R. (9), in 1915, found that infants utilize their protein most efficiently when the calories from this source represent 10 per cent of the total caloric intake. Sherman placed this figure at 9 per cent to meet the needs of an adult.

To allow for varying conditions and for individual preferences, as well as to provide a liberal margin of safety, it is customary to consider from 10 to 15 per cent of the total calories in the form of protein calories. Mary Swartz Rose (14) says that it is apparent that an allowance of 15 per cent of the total calories in the form of protein calories gives the growing child at all ages a surplus of 0.75 to 1.33 grams of protein per kilogram of body weight above the probable requirement. Any intermediate value should be adequate when the proteins are of good quality and the diet as a whole meet all other nutritive requirements.

Procedure:

The purpose of this study was to determine the level on which normal four year old children maintained the most efficient nitrogen retention. Three separate periods were planned, with about six or eight weeks time elapsing between them. It was expected that the optimum level would be between 2 and 4 grams of protein per kilogram of body weight. During the first period, therefore, 2 grams per kilogram were taken as the lower level. The intermediate level during the second period was on the basis of 3 grams per kilogram, and on the third period, 4 grams per kilogram formed the higher level.

A quantitative metabolism study involves the planning of a diet adequate for experimental purposes, the feeding of this diet to the subject for a given preparatory period, followed by a period of collection of urine and fecal samples. Then, by a chemical analysis of an accurately measured sample of the food intake as well as analysis of the excreta, the amount used by the body for metabolic purposes may be determined. One or more of the food constituents may be varied in amount or quality, and the results thus determined. In general this was the plan followed in this study. The

Table I

Food Requirements and Standards. I

Subj.	Age	Wt. Kg.	Ht. in.	Calories Per Kg.	Calories Daily Total	Protein % total calories	Protein Low (2gm/kg)	Protein Med. (3gm/kg)	Protein High (4gm/kg)
Av.Boy	48 mo	16.3	40-41	#79 *84	X av.1368 #1264 *1344	X 10-15 *15 +10-15	+1-1.5 (mainte nance) 32.6	X 2-3 *3 #3.3 48.9	4 65.2
B. L.	50 mo	18.9	43	80	*1596	15	38	*57	76
J. B.	43 mo	19.0	41 1/8	80	*1577	15	37.8	*56.7	75.6
Av.Girl	54 mo	17.0	40-41	*80	X av.1445 #1584 *1760	15	34	X 32-43 *43 #52.3	68
E. J.	55 mo	20.0	41½	80	*1600	15	40	*60	80

Gillett

X Rose

* Holt and Fales

+ Sherman

caloric intake remained constant throughout the series of studies at the value best suited to each child according to accepted standards. The protein intake was varied in each study, first on an estimated low level, supposed to be just above maintenance, then on the expected normal level, and lastly, on a high level. Table No. I summarizes these requirements and variations for the children studied. From the data secured from the analysis of the food and excreta, the optimum intake for nitrogen balance and adequate metabolism was determined.

The difficulties met in such a study are great, and explain to a certain degree the absence of extensive data. Care is necessary to prevent contamination of samples. In order to keep the child's daily routine just as normal as possible during the study, the attention of at least one person is constantly required to be sure food habits are properly observed and no excreta lost.

The subjects for this experiment were three normal, healthy, active, nursery school children, two boys and one girl. Their ages, weights, and heights are given in Table II. The children spent each day according to usual schedule in the nursery school, where the splendid cooperation of the instructor and

Table II.

Height-Weight-Age Table and Calculated Caloric Intake

Subj.	Exp.	Sex	Age	Height inches Sitting	Height inches Standing.	Weight Kg.	Weight Var. from norm acc. to ht. #	Weight Var. from norm acc. to age ‡	Caloric Intake Exp.	Total 72 hr	Total 24 hr	Total kg/24 hr.
B.L.	I	♂	52 mo	24 1/2"	43"	19.3	+3.6%	+11.9%	I	4755	1585	82
	II		53 mo			20.8		+18.3%	II	4815	1605	77
	III		55 mo	24 5/8"	44 1/4"	20.5	+1.5%	+15.1%	III	4950	1651	80
J.B.	I	♂	49 mo	24 1/2"	43.0"	19.5	+4.6%	+15.4%	I	4803	1601	82
	II		50 mo			19.8		+16.2%	II	4815	1605	81
	III		52 mo	24 3/4"	43 1/4"	20.2	+5.9%	+15.8%	III	4869	1623	80
E.J.	I	♀	56 mo	23 1/2"	41 1/2"	20.7	+16.4%	+15.9%	I	4815	1605	78
	II		57 mo			21.4		+17.8%	II	4860	1620	76
	III		59 mo	25.0"	43.0"	22.2	+16.2%	+18.5%	III	5169	1723	77

‡ Holt and Fales tables "Science of Nutrition in Infancy and Childhood" McLean & Fales

Baldwin - Wood tables, American Child Health Association

her assistants made it possible for the children to follow their usual life. Equally splendid cooperation was obtained from the mothers of the children who took charge of collection of samples over night.

The general health of the subjects during the experimental periods was satisfactory. The prevalence of colds during the early part of the first and second stuies interfered somewhat. The children were urged to drink all the water they could in order to prevent constipation which occurred to some extent during the second period, but not seriously enough to interfere with the completion of the metabolism study. Special precautions were taken to prevent this before the subjects began the high-protein diet. Since constipation seemed to be so easily caused, each child was given a small amount of mineral oil on the first and second days of the preliminary period. The children were also kept out of doors in the fresh air and sunlight as much as possible. The meals were prepared and served in a separate room at the college. The menu and calculated value of the diet (Rose Handbook of Dietetics) are given in Tables III and IV.

A three day's preliminary period on each level was followed by a three day collection period. During the last three days, duplicate samples of the daily

Table III

Daily Menus

	Exp. I Approx. 2gm. Pro/kg.	Exp. II Approx. 3gm. Pro/kg.	Exp. III Approx. 4gm. Pro/kg.
Breakfast	Orange juice Farina Cream Sugar Buttered toast Milk	Orange juice Cream of Wheat Cream sugar Buttered toast Milk	Orange juice Cream of Wheat Cream Sugar Buttered toast Milk
10:30 A.M.	Orange juice Cod liver oil	Orange juice Cod liver oil	Orange juice Cod liver oil
Lunch	Lettuce sandwich Buttered potatoes Scrambled egg Milk Peaches	Lettuce sandwich Buttered potatoes Scrambled egg Milk Peaches	Potato } potato baked Meat } with meat loaf Tomato juice Lettuce & cottage cheese sandwich Milk Baked custard
3 P. M.	Milk	Milk	Milk Peanut butter sandwich
Supper	Creamed carrots Thickened tomato juice (liver extract) Baked custard	Buttered carrots Tomato juice Spinach puree Baked custard Milk	Scrambled egg Buttered carrots Spinach Milk Peaches
Variations in Preparation	Creamed potatoes & buttered carrots Iced tomato juice instead of soup. Ice cream or boiled custard instead of baked custard. Peaches in custard instead of plain	Creamed carrots & buttered potatoes. Tomato & spinach soup. Ice cream Scrambled eggs with tomato sauce.	Tomato sauce with meat loaf. Creamed carrots. Lettuce as vegetable and plain cheese sandwiches Spinach sandwiches Ice cream

diet were kept for analysis. Pure fat and pure carbohydrate were eliminated from the sample for analysis. These analysis portions were dried in the oven at a low temperature, ground and pulverized and reserved for chemical analysis.

The addition of liver extract to the low protein diet brought the very low iron value up to the required amount. Spinach was used for this purpose on the medium and higher protein level dietaries, since less supplementary iron was needed and an extra vegetable was of added value. Since the filtered butter, cod liver oil, sugar, and corn starch were considered pure fat and carbohydrate, furnishing only calories, they were not included in the sample to be used for analysis. The content of the low and medium protein diets was changed very little except in the amounts of the protein-rich foods, but several changes were made in the high protein diet. One half the milk used consisted of skimmed milk and the other half whole milk in order to keep the fat content at its normal level of thirty-five per cent of the total calories. Lean beef, finely ground, cottage cheese, and peanut butter were added to raise the protein value. In the first dietary, a whole grain cereal, Ralstons, was used because it would aid materially in increasing the iron content. It was found, however, that the bran particles were not digested and appeared in the feces, interfering considerably with analysis. Cream of Wheat was used, therefore, in the medium and high protein dietaries. The additional protein needed to meet individual requirements above the basal allowance was in the form of egg white. Additional calories were made up with sugar.

The urine and feces were collected separately. A daily record was kept of the volume, specific gravity, acidity, (by the Folin method), and creatinine, (by the Folin micro chemical method) content of the urine. The creatinine value indicated the accuracy in collecting 24 hour samples (15). The daily samples were then combined, and further analyses were made on the three day portion. The nitrogen content of the urine was determined by the Folin-Dennis micro Kjeldahl method and the amount of protein calculated. The feces were marked for collection by charcoal, given to the subjects in capsule form on the morning when the collection period began and the evening when it closed. The feces were washed into a flask with 10 per cent H_2SO_4 and gently boiled in this solution until completely disintegrated. This solution was made up to volume and aliquot portions used for determining nitrogen according to the standard Kjeldahl method.

The caloric value of the food was determined by the oxycalorimeter (16).

Discussion:

Dr. Amy Daniels and her associate workers (17) have made studies on the relation of the rate of growth of infants to diet. One of her conclusions is that the accepted standards of growth for infants are too low. In Table II the weight of each child used in this experiment has been compared with the accepted standards of the Baldwin-Wood Tables (18) and the Holt and Fales tables (19). The variations from the normal weights according to age ranges from 11.9 to 18.5 per cent above these standards, indicating that these children are quite overweight according to their ages. When the weights are compared with the heights, the range for the boys is 1.5% to 5.9 per cent and for the girl , E. J. is 16.3 per cent above the accepted value. E. J., however, was about average height, and was very solidly built. She was a very active child, but not of a nervous temperament. B. L. was a tall, slender, rapidly growing boy. He was very active with a great deal of nervous energy. J. B. was chubby, fairly solidly built child, very deliberate in all of his actions. All three of the children were of average height or taller, therefore these figures coincide with Dr. Daniels results with younger children.

J.B. E.J. B.L.

Therefore, since these children were above the normal weight and height for their age group, the daily allowance of protein and calories was increased accordingly. The variations thus made are shown in Table I. That the children were growing rapidly is shown in the differences in their heights and weights at the first period and at the last.

A typical, adequate, daily diet was selected for each experiment containing amounts to meet the needs of the lowest individual protein level and caloric requirement. Throughout the three experiments, the energy intake was kept at approximately 80 calories per kilogram (2). The children were increasing in weight, therefore the total calories increased from experiment to experiment, but the proportion was kept constant. The analysis sample is referred to in section (1) of Table IV as the minimum individual need, and section (2) of the same table gives the additions to this basal menu which were used to raise the caloric intake to the minimum amount needed by any one of the children. If these additions did not meet the requirements, further additions of carbohydrate or protein were made as shown in section (3) of Table IV.

It was a bit difficult for the children to eat all of the food given them, especially at the evening

Table IV

Results of Calculation of Daily Diet (Rose Handbook)

	Experiment I (2gm/kilo)	Experiment II (3gm/kilo)	Experiment III (4gm/kilo)
(1) Total weight of fresh food *(minimum individual need)	1250 grams	1555	1617
Amt. Protein	38.17 grams	57.44	80.58
Am't. Fat	89.50 grams	46.77	44.09
Am't. Carbohydrate	155.19 grams	142.29	143.29
Calories in minimum need	957.98 calories	1219.85	1292.29
(2) Additions to Minimum need			
Sugar	35 grams	50	40
Butter	42 grams	12	14
Cod Liver Oil	5 grams	5	5
Corn Starch	16 grams	8	-
Extra Calories	627 calories	385	331
Total calories in minimum portion	1584.58 calories	1604.85	1623.29
(3) Individual additions			
B. L.	No extras above minimum need Protein 38 grams Calories 1585	No extras above minimum Protein-57.44 Calories-1605	15 gms.egg white and 5 grams sugar Protein-82.44 extra Calories 1651
J. B.	4 grams sugar extra Protein-38 grams Calories-1601	No extras Protein 57.44 grams Calories 1605	No extras Protein 80.58 Calories 1623
E. J.	15 grams egg white & 2 gm.sugar extra Protein 40 grams Calories 1605	30 gms.egg white extra Protein 60.0 gm. Calories 1620	40 gm.egg white & 20 gm.sugar extra Protein 85.5 Calories 1723

* (~~Copy from~~ Refer to analysis sheet) Table V.

meal, for the amounts did seem larger than those to which they were accustomed. Many ways were devised to make the meals interesting and full of fun. Small tables, chairs, and some of the dishes were brought to the apartment from the nursery school. A colored oil cloth cover was used on the table, adding both attractiveness and ease of recovering any spilled particles. The spirit of competition was great. Even though E. J. was a girl, she "wasn't going to let a pair of boys beat her getting done". When individual preferences were learned, attempts were made to meet them. For example, J. B. much preferred his spinach combined with the tomato juice and served in a cup to be drunk as a bouillon, whereas B. L. ate his much more willingly if it were served on his plate as a separate vegetable, and his tomato juice sweetened and served in his orange juice cup. Different colored glass cups were borrowed from the nursery school, but it was a delightful treat if one might drink his milk from a measuring cup. Ice cream, of course, was a constant request and delight. Any meal was a "party" if "pink" ice cream were the dessert. If some special naughtiness arose, one of the children was moved to another table to eat alone, but when conversation was interesting, a meal would go quite rapidly. A story or account of some exciting adventure told by one of the adults who ate with the children would often mean that the children were finished eating before they realized it. They did not seem to tire especially of eating the same type of food day after day, and only once did J. B. ask if "maybe by tomorrow won't we have all the tomatoes eaten up". The best of table manners could not be emphasized, but one mother told us, after the experiments were finished, that her only objection to what we had taught her son was that he still insisted on scraping the flowers off the dishes.

After the food for each dietary was analyzed, the total intake for each child was computed, and the calculated and analysis values were compared. This comparison is shown in Table V. Analysis proved the calculated values to vary considerably from the levels planned for them. For example, the total daily protein content of the diet for J. B. in the first period was, according to the analysis figures, three grams over the daily protein according to the calculated figures. During the second and third periods, however, the analysis figure was five grams short of the amount

Table V.

Results of Analysis of Daily Diet

	Exp. I				Exp. II				Exp. III			
	1st day	2nd day	3rd day	av. for 1 day	1st day	2nd day	3rd day	av. for 1 day	1st day	2nd day	3rd day	av. for 1 day
I Protein (In minimum individual need*)												
gms.fresh food	1250	1250	1250	1250	1555	1555	1555	1555	1617	1617	1617	1617
% n. (av. of 2 samples)	.5318	.5294	.5321		.5460	.5376	.5295		.7548	.7537	.7288	
grams N.	6.648	6.618	6.651		8.490	8.360	8.234		12.21	12.19	11.79	
gms N/day				6.64				8.36				12.06
gms.Prot/day				41.49				52.24				75.39
gms.N/3 days				19.92				25.08				36.18
gms.Prot/3 days				124.48				156.81				226.11
Calories (in minimum individual need*)												
Total cal.(av. of 2 samples)	1034	1025	988		1217	1270	1224		1334	1341	1315	
Total cal/ Av. day				1016				1237				1300
Total cal/ Av. 3 days				3047				3811				3990

* The minimum individual need was the amount saved daily in duplicate samples for analysis. It correesponds to the amount given the child who has the smallest requirement of protein.

Table VI

Comparison of Calculated and Analysis Figures

Exp.	Gms. Prot.		Calories	
I	calc.	Anal.	calc.	Anal
B.L.	38	41.5	1585	1643
J.B.	38	41.5	1601	1659
E.J.	40	44.8	1605	1651
II				
B.L.	57.4	52.2	1605	1622
J.B.	57.4	52.2	1605	1622
E.J.	60.0	60.8	1620	1635
III				
B.L.	82.4	78.8	1651	1668
J.B.	80.6	75.4	1623	1661
E.J.	85.5	84.4	1723	1679

planned by calculation from Rose's (20) Handbook. Dr. Wang (12) reports this same situation during a study on high and low protein levels. She says, "this variation was due partly to the discrepancy between the published figures and the actual nitrogen content of the food as analyzed, and partly to the fact that some of the children refused to eat all of the amount planned". In this case, however, it may be said with confidence that each child ate all of the amount planned for him. A possible explanation of the variation is indicated in the analysis figures on egg white. Egg white was the only food in the diet to be analyzed separately for protein. It was done because it was used to supplement the basal or minimum individual needs (Table IV) in the case of E. J. and B. L. According to the analysis, the protein content of the egg white was 0.10694, 0.10430, 0.10778 gm. per gram of material. Rose gives the protein content as 0.1230 gm. per gram of material. On the first experiment, there were only 15 grams of egg white used, while on the second and third experiments, 30 grams and 40 grams respectively were required. By comparing the two values of egg white, the second and third periods would be lower proportionately in protein than the first, thus giving a possible explanation for the drop in the last two periods. Variations in the analyzed and calculated figures may have occurred in other foods of the diet as well as the egg white.

In the determination of the caloric content of the diet by means of the oxycalorimeter, no difficulties were met in the analysis of the mixed sample of food. When the dried egg white representing the supplementary protein was run, however, it proved to be impossible to obtain complete burning of the sample. Even when 0.5 gram of egg white was mixed with 2 grams of sugar, the mixture charred rather than burned. This was due, it seems, to the excess of nitrogen gas formed when the high nitrogen material began to burn. Nitrogen gas does not support combustion and prevented the complete burning of the sample. Since the use of a smaller amount of egg white would allow for so great a percentage error, it seemed advisable, in order to obtain complete analysis results for the caloric value of the diets in which supplementary egg white had been used, to calculate the calories from protein and fat determinations. The protein value of the egg white used in the three respective periods had varied so greatly from the figures given by Rose that it was advisable to do fat analysis of the samples, because in most foods that contain large amounts of protein, when the protein runs lower the fat tends to run higher. This was found to hold true, although the samples of egg white used did not have as high a fat content as Rose gives. The percentages of fat in the egg white were found to be 0.071, 0.086, and 0.065 respectively, as compared with Rose's figure of 0.2 per cent. This value made the caloric value of egg white to be 0.434, 0.424, and 0.438 calories per gram for the three respective periods, as compared with Rose's caloric value of 0.510 calories per gram.

The shortage of protein and the fact that J. B. and B. L. had colds during the first and second periods would account in part for the irregular continuity in the nitrogen balance that they have shown in Tables VII and VIII. B. L. and J. B. had rather slight colds at the beginning of the first experiment, but they were quite well by the end of the three day preliminary period. During the second experiment, however, B. L. had had trouble with constipation and cold just before the preliminary period began. An enema during the preliminary period was necessary, but during the collection period, he seemed quite all right. J. B. showed signs of a severe cold on the second day of the collection period during the second experiment. During the third experiment, however, all three of the subjects

Table VII

Nitrogen Balance - I 2 gm. Protein/Kg body wt./24 hrs.calc.(Analysis fig.2.11 gm/kg)

Subj.	Wt. kg.	Age	N. Intake gm/72 hr	N. Intake gm/24 hr	N. Intake gm/kg/24 hr	N. Output Urine gm/72 hr	N. Output Feces gm./72 hr	N. Output Total gm./72 hr	N. Output Total gm./24 hr	N. Output Total gm/kg/24 hr	Retention gm/72 hr	Retention gm/24 hr	Retention gm/kg/24 hr	Retention % intake/24 hr
B.L.	19.3	4 yr 4 mo	19.92	6.64	0.344	16.10	2.31	18.41	6.14	0.318	1.51	0.50	0.026	7.53
J.B.	19.5	4 yr 1 mo	19.92	6.64	0.341	14.27	2.76	17.03	5.68	0.291	2.89	0.96	0.050	14.46
E.J.	20.7	4 yr 8 mo	21.52	7.17	0.346	15.27	2.99	18.26	6.09	0.294	3.26	1.08	0.052	15.06
			N. Balance. II. 3 gms.Pro/kg/24 hrs.calc.(Analysis fig.2.65)											
B.L.	20.8	4 yr 5 mo	25.08	8.36	0.402	19.95	3.41	23.36	7.79	0.375	1.72	0.57	0.027	6.82
J.B.	19.8	4 yr 2 mo	25.08	8.36	0.422	21.17	3.65	24.82	8.27	0.418	0.26	0.09	0.004	1.08
E.J.	21.4	4 yr 9 mo	28.21	9.40	0.439	19.79	3.70	23.49	7.83	0.366	4.72	1.57	0.073	16.70
			N. Balance. III. 4 gms.Pro/kg/24 hrs.calc.(Analysis fig.3.79)											
B.L.	20.5	4 yr 7 mo	37.80	12.60	0.615	29.16	3.01	32.17	10.72	0.523	5.63	1.88	0.092	14.92
J.B.	20.2	4 yr 4 mo	36.18	12.06	0.597	28.66	3.52	32.18	10.72	0.531	4.00	1.34	0.066	11.11
E.J.	22.2	4 yr 11mo	40.49	13.50	0.608	31.08	3.72	34.80	11.61	0.523	5.69	1.89	0.085	14.00

Table VIII

Summary of Nitrogen Balance for Each Child

Subj.	Period	N. Intake			N. Output			Retention		
		gm/72 hr	gm/24 hr	gm/kg/24 hr	gm/72 hr.	gm/24 hr	gm/kg/24 hr.	gm/72 hr.	gm/24 hr	gm/kg/24 hr
B.L.	I	19.92	6.64	0.344	18.41	6.14	0.318	1.51	0.50	0.026
	II	25.08	8.36	0.402	23.36	7.79	0.375	1.72	0.57	0.027
	III	37.80	12.60	0.615	32.17	10.72	0.523	5.63	1.88	0.092
J.B.	I	19.92	6.64	0.341	17.03	5.68	0.291	2.89	0.96	0.050
	II	25.08	8.36	0.422	24.82	8.27	0.418	0.26	0.09	0.004
	III	36.18	12.06	0.597	32.18	10.72	0.531	4.00	1.34	0.066
E.J.	I	21.52	7.17	0.346	18.26	6.09	0.294	3.26	1.08	0.052
	II	28.21	9.40	0.439	23.49	7.83	0.366	4.72	1.57	0.073
	III	40.49	13.50	0.608	34.80	11.61	0.523	5.69	1.89	0.085

were in excellent condition. E. J. had been in good health during all of the experimental periods.

It was expected that, if the caloric intake were adequate, the nitrogen retention would be in direct proportion to the nitrogen intake (12). This proved true in the case of E. J., but with B. L. and J. B., the second period does not show a consistent increase over the first in the amount of nitrogen retained. Parsons (9) states that it is doubtful if a negative balance always means tissue destruction. He found that one of his subjects went into a negative balance during the time she had an infection of the upper respiratory tract. During the acute phase of such an infection, a patient is more or less toxic. Considerable water may be bound in the tissues. Protein, unlike carbohydrate and fat, requires ample "free" water for complete metabolism. If the free water is not available, the required protein cannot be utilized and the stored nitrogen may be spilled. Thus a negative balance may serve as a protective mechanism, just as refusal of food by a patient may be a protective mechanism. The situation is different from a chronic condition where the patient becomes accustomed to functioning on less free water. DuBois (3) discussed this point from a somewhat different angle than Parsons.

He states that "toxic destruction of protein" occurs in infectious diseases with toxemia, cancer, and other wasting illnesses. This condition may be prevented if a sufficient increase is made in the caloric intake, but that increase must be about double the number of calories actually produced by the patient's metabolism. Protein metabolism is usually elevated in severe infections and nitrogen balance can be more easily obtained when the caloric intake is greatly increased. It is quite possible that the colds that the boys had were not severe enough to cause any great variation in protein metabolism, but since their caloric intake was not raised above their normal requirements, it is a possible explanation for their irregular nitrogen balances.

The general indication from the results in Tables VII and VIII are that nitrogen retention is in direct proportion to the nitrogen intake when the calories are adequate; that is, on a higher level of protein such as 4 grams per kilogram of body weight daily a greater amount of nitrogen will be stored or retained by the body than there would be on 3 grams or 2 grams of protein per kilogram per day.

Dr. Wang and her associates (18) used children of

4 - 12 years of age. The relation of her findings to those of the present study is seen in the following table. The results cited are those of a child whom Dr. Wang places in her normal, vigorous class. It is

Child	N.Intake (gm/kg)		N.Output (gm/kg)		Retention (gm/kg/24hr)				Cal.Intake (kg/24 hr)	
	Low	High	Low	High	Low	High	% Intake		Low	High
							Low	High		
Dr. Wang's	.343	.682	.317	.509	.026	.172	7.5	25.2	97.2	122.4
Present Study	.344 to .346	.597 to .615	.291 to .318	.523 to .531	.026 to .052	.066 to .092	6.82 to 14.92	14.0 to 14.9	77-82	77-80

interesting to note, however, that this child at 5 years of age is 2 kilograms less in weight than the children of 4 to 5 years used in this study.

Children of the pre-school age are facing a long period of rapid growth, and need as much reserve nitrogen as possible to make the process efficient and successful. Results of this study confirm the view of Dr. Wang (12) that protein diets as high as the ones used do not appear to have any harmful effects on children when the criteria used are appearances, behavior, and relation between intake and output, for the children seemed in the best condition both mentally and physically during the last period when the diet contained the largest amount of protein. It would seem logical therefore to recommend a higher level of protein intake for children of pre-school age, rather than a lower level.

Summary and Conclusions:

1. The accepted standards of growth for children of the pre-school age are too low.

2. Nitrogen retention varies in direct proportion to the nitrogen intake when the calories are adequate.

3. An indication of a lower nitrogen retention on a higher protein level may be due to the discrepancy between the calculated values according to published figures and the actual analyzed values of the diet. This variation is also found in the caloric content of the diet.

4. A condition interfering with general metabolic processes, such as a cold, will affect the nitrogen retention, tending to reduce it.

5. As high a level of protein as 4 grams per kilogram of body weight per day does not appear to have any harmful effects on rapidly growing children of 4 - 5 years of age.

6. From 3 - 4 grams of protein per kilogram of body weight are to be recommended for children of the pre-school age rather than lower levels.

7. At least 80 calories per kilogram of body weight per day are to be recommended for active, normal children of 4 - 5 years of age.

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