NITROGEN BALANCE OF SIX 13- TO 14-YEAR-OLD GIRLS

by

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INTRODUCTION

In this country supplying an adequate quantity of protein in the diet is not a major nutritional problem. However, in some instances such as the period of adolescence, certain groups of individuals may ingest an inadequate supply of protein despite the high average figures for protein consumption for the population as a whole. Furthermore, individuals have been found to vary in their utilization of protein. Therefore, it is not only important to study the intake of protein, but also to measure the retention or loss of nitrogen by the body.

The simplest and oldest chemical method used to evaluate the nutritional properties of proteins in a biological organism is to determine the difference between nitrogen intake and nitrogen excretion (Albanese, 1959, p. 312). Nitrogen equilibrium has been defined as the zone in which the difference between intake and excretion does not exceed ± 5 percent (Leverton et al., 1956). Nitrogen equilibrium is the usual condition in adults when body proteins are being maintained and replenished as needed and when protein is neither being stored in the tissues nor withdrawn from them (Leverton, 1959, p. 60). In an adolescent, however, part of the food material is utilized in the synthesis of body proteins that are necessary for the rapid rate of muscle growth during this period. Thus, nitrogen must be retained by the adolescent and the nitrogen intake must be greater than nitrogen excretion.

Only a limited number of nitrogen balance studies of adolescent girls have been reported in the literature. Because of the apparent importance of adequate protein in the diets of adolescent girls for tissue growth, additional studies should be of value in determining nitrogen needed for good health and normal growth. The purpose of this study was to supplement present information and to provide nitrogen balance data on six 13- to 14-year-old girls.

REVIEW OF LITERATURE

Protein Metabolism

It has been affirmed that proteins are the most complex organic compounds found in nature, and that they range in molecular weight from about 35,000 up to several million (Schoenheimer, 1949, p. 25). Block (1956) stated that proteins are synthesized by the living organism from 19 or so amino acids. Dietary protein is the main source of these amino acids. After ingestion, these proteins are broken down into their constituent amino acids by the enzymes of the gastrointestinal tract (Block, 1956). In addition, it was pointed out that the hydrolytic enzymes and other tissue constituents involved in the digestion of food are proteins and are hydrolyzed also in the gastrointestinal tract to their constituent amino acids. Amino acids thus liberated are absorbed by the body.

Currently, the concept of Schoenheimer (1949, p. 25) regarding the dynamic state of body constituents is accepted.

In a study using N^{15} he found that living tissues were not metabolically inert, but that the tissue proteins were being constantly broken down to their constituent amino acids. It is believed that these amino acids then enter the body "amino acid pool" where they are mixed with amino acids coming from the digestive tract (Block, 1956). From the findings in an isotopic investigation, the size of the metabolic pool has been estimated as 0.514 g. nitrogen per kg. body weight (Sprinson and Rittenberg, 1949). According to Allison (1959, p. 98) the "pool" should not be considered as a disorganized fluid component of the body, but rather as a mechanism for transfer of amino acids from one tissue to another. He stated that data have demonstrated that some tissues are highly labile, entering into and drawing from the pool continually; others are less active in the dynamic state; whereas still others are "one-way streets," that for the most part are irreversibly formed, thereby taking from but contributing little to the "pool". Data presented by Wainio et al. (1959) demonstrated that proteins of the brain, heart, and kidney were resistant to depletion in the order, brain > heart > kidney, whereas a number of cellular proteins in the liver were easily depleted.

Block (1956) cited that the rate of amino acid turnover is rapid in such tissues as the liver and intestinal wall and slow in tissues as muscle and brain. In a study using N^{15} the average half-life of human tissue protein was calculated to be 80 days (Sprinson and Rittenberg, 1949). The rate of turnover

in the organs varied. Liver and blood plasma proteins had an average half-life of 10 days. Spleen, heart, kidneys, small intestines, pancreas, and endocrine glands had a half-life of 20 days, whereas muscle tissue proteins had a half-life of 158 days.

It generally has been accepted that no protein can be synthesized unless every amino acid of which it is composed is available in an adequate quantity at the site of synthesis. According to Simkin (1959, p. 145) there are three main ways in which amino acid residues may be joined together to form the polypeptide chain of a protein: (1) amino acid residues may be joined together to form peptides that are later linked together with other peptides or amino acids to form the complete polypeptide chain, (2) all of the amino acid residues may be linked together simultaneously after adsorption on a preformed catalytic site, or (3) the amino acids may be linked together in a definite sequential manner, beginning at one end of the polypeptide chain and proceeding to the other.

Indications from findings of various studies are that protein biosynthesis may occur in the microsomal part of the cytoplasm. A soluble ribonucleic acid (S-RNA) is thought to pick up activated amino acid molecules to put them in proper sequence upon nucleic acid templates in particulate matter as microsomes (Hoaglund et al., 1958). The explanation was given that each type of cellular protein is characterized by the arrangements of amino acids along the peptide chains that emerge

or roll off the templates to be coiled and bound into specific structures. Allison (1960) suggested that there is a specific microsomal ribonucleic acid protein for each type of cellular protein synthesized. He further indicated that it may be possible for one microsomal machine to "shift its gears" to produce a number of patterns of polypeptide chains. These shifts could be correlated with internal environmental changes. Recent studies have shown that mitochondria also can synthesize protein (Simkin, 1959, p. 148). However, it was suggested that one must recognize the danger of artifacts inherent in such investigations.

According to Hill and co-workers (1959, p. 97) studies of the amino acid sequence of proteins have as their ultimate aim the correlation of these structural features with biological activity. They indicated that this field of investigation is expanding rapidly, partly because of the availability of methods that permit unequivocal determination of structure, and partly because knowledge of structure permits precise studies of the effects of chemical alterations upon biological activity of proteins. A word of caution was issued by these workers regarding the establishment of protein structure. They stated that the knowledge of protein structure thus far has been obtained only by degradative methods and as in the case of simpler molecules, proof of the structure must be demonstrated by unequivocal synthesis, a possibility, that they believe, is remote at the present time. In addition, it has been observed

from the study of enzymes that a "folded" structure is essential for the biological activity of these protein materials. Therefore, it is apparent that more than amino acid sequence is involved in the active site of the molecule, and an understanding of the three-dimensional structure is necessary before structure-activity relationships can be established. Hill and co-workers (1959, p. 97) emphasized that much effort still must be devoted to methodology at various levels of protein investigations.

Biological Value

Not only is the mixture of amino acids important in relation to the total protein intake, but also the biological value of protein requires consideration (Hegsted, 1957). Biological value is the percentage of nitrogen absorbed that is retained by the body. Thus, a protein with a biological value of 100 percent is able to replace gram for gram those body losses that occur from day to day.

Protein Requirements of Adolescent Girls

Recommended Allowances. As a result of data obtained from previous nitrogen balance studies as reviewed by J. Johnston (1958), the Food and Nutrition Board of the National Research Council[1] (1958) recommended a daily dietary protein allowance of 80 g. for 13- to 15-year-old adolescent girls. Based on

[1]Hereafter abbreviated as NRC

the 1958 NRC recommended 2600 calorie intake for these subjects this amount of protein accounts for 12.3 percent of the total calories. The allowance of 80 g. of protein provides less than 15 percent of the total calories that J. Johnston (1958) believed necessary for circumpubertal growth and special stresses such as tuberculosis infection. Fifteen percent of the total calories would be available in 97.5 g. protein. J. Johnston (1958) stated that negative nitrogen balances or uncompensated previous protein depletion from any cause were associated with spread of disease processes. In particular, Johnston, repeatedly found that sexual maturation in girls, which is not necessarily co-incidental with the onset of menstruation, carried with it a depression in nitrogen retention. Therefore, failure to increase the dietary protein as indicated by this fall in nitrogen storage favored the spread of tuberculosis in girls with minimal reinfection type of lesion. The suggested percentage of 15 by J. Johnston (1958) is in agreement with the findings of Wang et al. (1936) and Holt and Fales (1921).

In a review, Hegsted (1957) suggested that because of the limited data available, the establishment of minimum or optimum protein allowances for children is on insecure ground. Platt and Miller (1958) in an Food and Agricultural Organization report emphasized that difficulties and uncertainties about the subject of protein allowance are evident, and therefore skill, caution, and more knowledge is necessary before defining protein requirements. They listed some of the inherent dietary

difficulties when human beings rather than laboratory- or commercially-produced animals are used in studies as: (1) complexity of the mixtures eaten by man, (2) the nature and timing of snacks and meals, (3) the effect on food values of a wide variety of methods of processing, including cooking, (4) the level in the diet, not only of protein, but also of other sources of energy - fats and carbohydrates - and of various vitamins and minerals, and (5) the balance between the proteins and several other dietary factors.

Protein Intake and Pregnancy. Some workers have expressed concern about the protein intake of adolescent girls and its effect upon child-bearing. There appears to be a tendency toward frequent complications during pregnancy among teen-age mothers. In a study of subjects on alternating self-selected diets (of which 60 percent reported an intake of 70 g. protein or less and 23 percent reported an intake of 55 g. of protein or less) and recommended 85 g. protein diets, Dieckmann et al. (1951) found that the incidence of abortion increased significantly in those patients with a low protein intake. At the same time, the highest level of anemia was evident. The percentage of excellent babies increased steadily with increased protein intake by the mother. These workers (Dieckmann et al., 1951) reported that the analysis of their data on nitrogen balance as a function of gestation period indicated a maximum protein requirement per kg. of body weight for maintenance of positive balance early in pregnancy. They believed that this

requirement decreases as pregnancy approaches term.

Distribution of Protein. In regard to the diets of teenagers, Everson (1960) stated that adequate amino acid intake, may not be as much a matter of protein intake per 24 hours as it is irregular eating habits, omitting breakfast, and poor distribution of protein throughout the day. She pointed out that the teenager enjoys meat, but does not always do a good job of providing a regular mixture of amino acids for advantageous body use.

Levels of Protein Intake. It generally is accepted that the level of protein nutrition cannot be characterized merely by establishing that the subject is in nitrogen equilibrium. Nitrogen equilibrium may be achieved on a low protein intake in an undernourished subject and at the physiological minimum with a higher intake in the normal individual. In their review Platt and Miller (1958) raised the question of what constitutes the upper level of protein nutrition. They asked if the upper level is a state of eutrophy and if so, what is the nature and purpose of the protein stores or reserves. They also suggested the possibility that the pathology of protein malnutrition may be a part of the pathology of many diseases.

Dietary Intake Surveys

From 1947 to 1958 over 200 nutritionists investigated the nutritional status, including the amount of nutrients in the food eaten, of population groups within the United States. The

state Agricultural Experiment Stations, the Institute of Home Economics of the U. S. Department of Agriculture, and several state departments of public health sponsored the studies. The country was divided into four regions: Northeast, North Central, South, and West. Eleven groups of 13- to 15-year-old girls were studied--seven in the West (Wilcox et al., undated), three in the Northeast (Tucker et al., 1952), and one in the North Central Region (Eppright et al., 1954). The mean daily intakes of protein for 13- to 15-year-old girls by state are summarized in Table 1. The state mean intakes ranged from 61

Table 1. Mean daily intakes of protein by girls 13 to 15 years old by state.

State	No. of subjects	Protein Mean	Protein Std. error	Protein Std. dev.
		g.	g.	
Western Region[1]				
Colorado	63	65	2.1	
Idaho	70	73	2.0	
Montana	111	68	1.7	
New Mexico				
Anglo American	19	61	6.3	
Spanish American	33	69	3.8	
Utah	25	76	4.5	
Washington	107	74	2.0	
North Central Region[2]				
Iowa				
13 yrs	44	74	3.0	20.0
14 yrs	37	75	3.0	17.0
15 yrs	39	75	2.0	14.0
Northeast Region[3]				
Maine	123	77		21.0
New York	113	85		24.2
Rhode Island	45	71		12.9

[1]Wilcox et al. (undated)
[2]Eppright et al. (1954)
[3]Tucker et al. (1952)

to 85 g. per day. The mean daily protein intakes in the various states, with the exception of New York, were less than the NRC recommended allowance of 80 g.

Western Region. Wilcox et al. (undated) compiled data on 428 adolescent girls in seven groups located in six western states. Seven-day food intake records and dietary histories were evaluated. Although average intakes of most nutrients were adequate the cumulative frequency curves indicated many individuals were not well fed. All groups of adolescent girls (13-20 years of age) were lower in protein than the NRC recommended allowance. However, all of the groups of 13- to 15-year-old girls in this region had mean protein intakes of 67 to 100 percent of the NRC recommended allowance.

North Central Region. In Iowa, the nutrient intakes were studied from seven-day dietary records (Eppright et al., 1954). Among the 120 13- to 15-year-old subjects the mean protein intakes conformed to the NRC allowance within about five g. Mean protein intakes tended to be slightly higher before 12 years of age and lower after 12. Negative deviation of mean daily protein intake from allowances was most marked at 16 years of age.

Northeast Region. In the Northeast region, the dietary intakes of 281 adolescent girls were evaluated from seven-day dietary records, dietary histories, and four-day dietary records (Tucker et al., 1952). The mean protein intake of Rhode Island subjects tended to be low with 71 g., whereas

New York reported an intake of 85 g. which is five g. above the 1958 NRC recommended allowance. It was found in this region that six percent of the subjects had a protein intake of 66 percent or less of the 1958 allowance; 49 percent had an intake between 67 and 100 percent of the NRC recommended allowance; and 45 percent had a protein intake of 100 percent or above.

All Regions. Morgan (1959) summarized the nutritional status surveys. Over-all nutrient intakes of adolescents 13 to 20 years old were more variable and less favorable, especially those of the girls, than were those of either the younger children or the adults. The protein intakes, for boys and men of all ages, however, were greater than the 1958 NRC recommended allowance. This was true also for girls up to the age of 12 or 13, but after that the average daily intake was significantly lower than recommended up to age 20. From 20 to 70 years of age the women's intake of protein was adequate, but it dropped significantly after age 70.

Balance Studies

Meaning of Nitrogen Balance Data. It generally is believed that the dietary and body nitrogen meet in a common metabolic pool of blood and tissue proteins---a pool that can be drawn on for exogenous and endogenous purposes (Allison, 1951). Holt and Fales (1921) stated that the proportion of amino acids in body protein are not the same as in some food protein. As a consequence, they suggested that not all amino acids of food

are utilized in building up or repair of body structure. These workers theorized that the amino acids that are not utilized as building materials are used largely for energy, being broken down into urea and excreted in the urine. Also, they pointed out that animal proteins as compared to vegetable proteins approximate more nearly human body proteins, and therefore are preferable. Allison (1951) postulated that the depletion of body protein stores is reflected by decrease in excretion of urinary nitrogen. He indicated that an initial rapid loss of nitrogen represents the catabolism, in part, of labile protein stores. After the stores are depleted, the loss of nitrogen is less rapid with the urinary nitrogen excretion approaching a low and constant value. Allison (1951) proposed that during regeneration (feeding protein), the stores depleted last probably are refilled first. He remarked that the alternate feeding of protein-free and protein diets results in a positive balance. Therefore, nitrogen balance is variable, changing with time as protein stores are increased or decreased.

Johnston and Schlaphoff (1951) suggested that the amount of nitrogen that adolescent girls need to retain for good health and normal growth is not known. However, it was pointed out that these adolescent girls need to retain enough for tissue increase and enough to cover the nitrogen lost from the body, which is not measured in the usual balance experiments. According to these workers, the amount of nitrogen needed for tissue increase may be estimated from nitrogen content of the body and

amount of weight gained by girls who are growing at a normal rate.

Harper (1959) explained that there are limitations in methods used for determining amino acid and protein requirements. When growth is an indicator, the minimum amount for maximum growth or in the case of adults, the minimum amounts for nitrogen equilibrium is sought. With nitrogen balance, previous nutritional status appears to be influential. As previously stated, given a sufficient time, an individual can come into balance even on a low protein intake. According to Harper (1959), this may require weeks if the previous protein consumption was in excessively large quantities. Frost (1959, p. 225) stated that for a nitrogen balance study to have a reasonable degree of accuracy it must extend over a period of at least three weeks. Because the body contains certain labile protein fractions that are lost during periods of low protein intake, the significance of the minimum amount versus the optimum amount apparently is not realized. Individual variation appears to be an important factor in estimating optimum or ideal amino acid intakes (Harper, 1959). Harper (1959) has maintained that the physiological state of the organism influences the requirement. During the period of rapid growth, protein is being deposited and the requirement far exceeds the maintenance requirement. This worker believed that the requirement is constantly changing during the growth stage. No single value can be given as the requirement unless it is based on some criterion

as body weight, muscle mass, surface area, or caloric requirement. Caloric requirement takes into account the factors responsible for change in protein and amino acid requirement of a subject. As a result of starvation or pathological conditions, an additional allowance is necessary for repletion of stores.

It generally is recognized that when carbohydrate and fat levels are low in the diet, protein is diverted from its normal use in protein synthesis to serve as a source of calories. Harper (1958; 1959) discussed amino acid balance and imbalance and the effect upon protein requirement. The indispensable amino acids must be present in ideal proportions, because the further the proportions of amino acids in a protein deviate from the ideal, the more of the protein is required to satisfy amino acid requirements. Harper (1959) emphasized that amino acids of some proteins are not released readily in the gastrointestinal tract, so allowance must be made for differences in protein digestibility. Also, it was noted that proteins are not of equal biologic value. All of these factors have been found to influence the establishment of the protein requirement.

It generally is accepted that the filling of the protein reserves in an organism must be described in three dimensions (Allison, 1958). These dimensions are the nutritive value of dietary proteins, the nitrogen intake, and the calorie intake.

Protein Intake. In balance studies that have been published, protein intake by adolescent girls varied (Table 2).

Table 2. Nitrogen intake and retention from results of studies by other investigators.

Investigator	Diet	Length of study	Subjects		Protein intake		Nitrogen intake	Nitrogen retention			
			No.	Age	Total	Per kg.	Total	Total	% of intake	Per kg.	Per m^2
					g.	g.	g.	g.	%	g.	g.
Holt and Fales, 1921	--	--	3	12-14	73-74	1.6-1.7	11.68-11.84	-	-	-	-
Wang et al., 1936	Self-selected	6-day periods	24	12-15	-	2.5	15.2 (11.1-21.8)	2	12.6 (2.8-28.2)	-	-
Koehne and Morrell, 1934	Self-selected	56 days	28	12-13	74	2.5	11.84	-	-	-	-
Johnston and Schlaphoff, 1951	Controlled	9-week	6	13-14	76.2 87.3	-	12.20 13.97	1.42 2.17	11.6 15.5	0.0299 0.0437	0.97 1.45
Johnston, J., 1958	Self-selected	variable	3	13			14.6	2.64	18	0.05	-
Patrick et al., 1955	Controlled	56 day	5	8-9	-	-	9.98	1.93	19.4	0.060-0.089	-
			1	11	-	-	-	-	-	0.049	-
Johnston and McMillan, 1952	Controlled	12-week	5 1	20-23 31	70.2		11.23	0.64	5.7	0.012	
Wharton et al., 1953	Controlled	99 7-day periods	18	College	47.6	0.85	136 mg		Equilibrium		

Holt and Fales (1921) reported a daily intake of 1.6 to 1.7 g. protein per kg. body weight (a total of 73 to 74 g. of protein) for three 12- to 14-year-old girls. In a protein metabolic study of six-day periods on 24 12- to 15-year-old girls on self-selected diets (Wang et al., 1936), the average protein intake was reported as 2.5 g. per kg. body weight or in terms of nitrogen, the intake ranged from 11.1 to 21.8 g. with an average of 15.2 g. per 24 hours. This was 15.2 to 23 percent of the total caloric intake. Koehne and Morrell (1934) in a 56-day study of 28 girls on self-selected diets found that the intake for 12- to 13-year-old girls averaged 2.46 g. of protein per kg. body weight. These workers pointed out that there was a decreased caloric and protein intake per kg. body weight with increased age from 10 to 18 years.

Nitrogen Retention. As reported in the literature, nitrogen retention has been found to vary among adolescent girls (Table 2). On self-selected diets consumed by 24 12- to 15-year-old girls, Wang and co-workers (1936) found that a mean of 2.0 g. of nitrogen or 12.6 percent of the total intake (15.2 g. of nitrogen) was retained.

Johnston and Schlaphoff (1951) reported the findings of a study with six healthy girls between 13 and 14 years of age on a nine-week study of which the first two weeks were considered as an adjustment period. The remaining seven weeks were divided into two periods--one three-week and one four-week. The controlled diet was made up of a wide variety of common

foods. The results were stated as a mean retention of 1.42 g. or 11.6 percent on an intake of 12.20 g. and a retention of 2.17 g. or 15.5 percent on an intake of 13.97 g. nitrogen. When reduced to units of body size, the mean daily retention was 0.97 g. nitrogen per m^2 body surface or 0.0299 g. nitrogen per kg. of body weight during the first period and 1.45 g. per m^2 body surface or 0.0437 g. nitrogen per kg. of body weight during the second period. In terms of protein, during the first period, the mean retention was reported as 0.19 g. protein per kg. body weight whereas in the second period, there was a mean retention of 0.27 g. protein per kg. body weight.

In a study of three 13-year-old girls having minimal reinfection type tuberculosis, a mean retention of 2.64 g. nitrogen or 18 percent of the 14.6 g. intake of nitrogen was reported during the "year of maximum growth" (J. Johnston, 1958). Per kg. of body weight these girls had a mean retention of 0.05 g. nitrogen. The total mean protein retention was reported as 16.5 g. or 0.31 g. per kg. body weight. By the third postmenarcheal year the total mean retention declined to 1.4 g. of nitrogen.

On a 56-day study with six healthy pre-adolescents under normal conditions the mean nitrogen retention was 1.93 g. or 19.4 percent of the 9.98 g. intake of nitrogen representing 62.4 g. of protein (Patrick et al., 1955). In this study the five 8- to 9-year old subjects had a nitrogen retention ranging from 0.060 g. to 0.089 g. per kg. body weight or in terms

of protein, 0.375 g. to 0.556 g. per kg. body weight. The one 11-year-old subject had a nitrogen retention of 0.049 g. per kg. body weight or 0.306 g. protein per kg. These findings indicated a decreased nitrogen retention with increasing age during the pre-adolescent period. Johnston (1940) found that the amount of nitrogen retained before the menarche is greater than that retained after it.

It was found in a 12-week study of six young college women (five of whom were 20-23 years of age and the other 31 years of age) on a diet of 70.2 g. of protein or 11.23 g. of nitrogen that a mean of 0.64 g. or 5.7 percent nitrogen was retained (Johnston and McMillan, 1952). The mean body weight of these subjects was 53.1 kg. with the mean retention reported as 0.075 g. protein or 0.012 g. nitrogen per kg. body weight.

As a result of an investigation of 18 college women, Wharton et al., (1953) stated that nitrogen equilibrium was established on an intake of 136 mg. of nitrogen per kg. body weight which is equivalent to 0.85 g. protein per kg. of body weight or 47.6 g. protein for a 56 kg. woman. Retention of nitrogen by these women increased in direct proportion to the intake within limits of the intake studied.

Evidence from the studies reviewed suggests that the mean retention of nitrogen decreased with age from the pre-adolescent period through post adolescence until nitrogen equilibrium is attained in the normal adult. The highest retention seemed to be in the year of maximum growth during the

adolescent period. Variations among individuals in the utilization of protein are indicated.

Fecal Nitrogen. There is a lag in change of nitrogen excretion with decrease in dietary nitrogen (Hegsted et al., 1946). Therefore, short periods on decreased dietary nitrogen intake will tend to show lowered retention values. Fecal nitrogen, however, has been found to be more or less constant. In adults fecal nitrogen has been reported as 1.28 g. per day (Hegsted et al., 1946). In a study on 24 12- to 15-year-old girls, fecal nitrogen was reported as 1.2 g. per day (Wang et al., 1936). Johnston and McMillan (1952) found an average fecal nitrogen content of 1.02 g. per day in the 12-week study with six young women on an intake of 70 g. protein. In a metabolic study with pre-adolescent girls the fecal nitrogen ranged from 0.79 to 0.81 g. per day (Patrick et al., 1955).

Hegsted et al., (1946), believed that different samples of the same foodstuff may give rise to different values of fecal nitrogen. In their study variations were found to occur because of the amount of indigestible carbohydrate. This was attributed not only to the bulk thus provided, but also because such constituents may protect in varying degrees against enzyme action in the gut. Hegsted et al. (1946) asserted that the degree of mastication is important. In at least one of their adult subjects, the high fecal nitrogen figure was attributed to poor mastication because of malocclusion. They also observed that the shorter the expulsion time, the higher the fecal

nitrogen values. In addition these workers suggested that the method of food preparation was an important factor in the fecal nitrogen value. Likewise, several investigators have demonstrated a decrease in the biological value of protein following treatment with severe heat. In 1934, Morgan and Kern reported a progressive decrease of five to 30 percent in the utilization by experimental animals of beef and horsemeat when boiled or autoclaved. In 1935, Seegers and Mattill reported similar effects from heating or hot alcohol extraction of beef liver, kidney, heart, or round. Poling, Schultz, and Robinson in 1944 found the nutritive value of proteins of cured pork shoulder slightly, but significantly, lowered by a commercial canning process. Wheeler and Morgan (1958) have suggested that there was decreased absorption of amino acids from the gut after heating the proteins. These workers theorized that the digestive enzymes apparently are incapable of hydrolyzing properly the overheated proteins so as to make available to the animal simultaneously an assortment of amino acids favorable to growth and maintenance.

It should be pointed out that the difference between nitrogen intake and fecal nitrogen is considered to be absorbed nitrogen (Patrick et al., 1955). In a study by Patrick and coworkers (1955) with pre-adolescent girls, the mean protein absorbed was 92.3 percent. In a study by Wang et al., (1936) on 24 12- to 15-year-old girls on self-selected diets, the mean absorption was found to be 92.2 percent of the intake.

Losses not Measured by Nitrogen Balances. Mitchell and Hamilton (1949) suggested that accurate balance experiments cannot be carried out unless dermal losses are measured. In their four-week study with a five-day preliminary period on six male conscientious objectors, they found losses of nitrogen through the skin on a 90 g. protein intake to average 152 mg. per hour in a hot, humid environment of 37-39°C. and 65-73 percent humidity. This constituted an average of 22.5 percent of the total output. Under more comfortable conditions of 27-28°C. and 43-45 percent humidity, the hourly dermal loss of nitrogen averaged 15 mg. or 2.7 percent of the total output. Under the latter conditions, the daily dermal loss was 360 mg. It was observed that the nitrogen concentration in sweat showed no consistent downward trend with time of sweating. The data indicated that there is an increase in nitrogen catabolism with sweating. Therefore, it was believed that under such conditions an increase in protein requirement is necessary.

Darke (1960) stated that unless cutaneous losses are measured, nitrogen balance is invalid. She attempted to measure total nitrogen loss occurring in 24 hours from cutaneous structures---the epithelial debris as well as cutaneous secretions. Fourteen determinations were made on 12 African adult males. Two determinations were done on each of two individuals and were at intervals of five days. In this study the total nitrogen cutaneous loss ranged from 147 to 479.7 mg. with a mean of 254 mg. per 24 hours. Therefore, Darke (1960) asserted that the

loss of nitrogenous compounds from the skin resulting from the secretory activity of sweat and other cutaneous glands and from the shedding of epithelial structures introduces significant errors in nitrogen balance studies if not considered.

F. A. Johnston (1958) determined the nitrogen loss from hair of the scalp. This study was done for four six-day weeks on 12 18- to 22-year-old women and for 12 six-day weeks on a 54-year-old woman. The mean annual loss of nitrogen was calculated as 11.1 g. per year. It was postulated that each day 0.043 g. would be lost. In Johnston's laboratory, it had been shown previously that young women retained approximately 0.6 g. of nitrogen a day on intakes of 60 g. of protein. About 1/40 of this would be required to cover the loss of nitrogen in hair from the scalp. F. A. Johnston (1958) firmly believed that this stated amount is a reasonable figure if the assumption is made that the adult women are in nitrogen equilibrium when all small losses are included. The additional losses considered as small are losses in hair from other parts of the body as well as losses in nails, skin, perspiration, saliva, tears, nasal secretions, and menses.

Variations in Dietary Analysis

In balance studies aliquot portions of dietary food are chemically analyzed for nutrient content. It is recognized that there are wide variations in nutrients in the same foods

at different times and under varying conditions (Hunscher and Macy, 1951). For fruits and vegetables these factors may be the degree of ripeness, species, type of soil, mode of cultivation, amount of water, geographic origin, and marketing conditions. According to Thomas et al. (1950) the nutrient content of meat differs among animals and from the anterior to the posterior sections in the animals. These workers suggested that contaminants from equipment, environment, and reagents also may influence the results in chemical analysis. They proposed that variables in tables of food composition are caused by sampling and analytical errors. Thomas et al. (1950) and Hunscher and Macy (1951) agreed that geographic origin, species, maturity, storage, method of processing, and analytical techniques are partially responsible for the variable standard values in tables of food composition.

In the fall, Thomas et al. (1950) found no significant difference between analyzed and estimated values of protein in diets, but in the spring the analyzed values were lower than those estimated from tables of food composition. This was attributed to the varying protein content of milk. It was pointed out that tables of food composition contain average values (Hunscher and Macy, 1951).

Bransby and co-workers (1948) mentioned that differences between average nutrient values found by calculation and analysis are because of differences among methods adopted for chemical analysis of diets and those used to obtain food values of tables.

They observed that differences between values found by chemical analysis and by calculation from food tables are found frequently. Reliability of values calculated from food tables was questioned seriously by these workers.

MATERIALS AND METHODS

During a metabolic study in 1958 that was primarily concerned with iron utilization by six adolescent girls, samples of the diet and body excretions were collected and preserved. In the present investigation, all samples of the diet and samples of the body excretions were analyzed for nitrogen to determine the nitrogen balance of the subjects.

Subjects

The subjects were six 13- to 14-year-old girls who had reached puberty. They lived in a home management house on the campus and continued their usual activities during the 50-day experiment. They were supervised by a faculty member of the Department of Foods and Nutrition.

Age to the nearest birthday, weight, height, body surface area at the beginning of the study, and age of the menarche of the subjects are shown in Table 3. A physician, who examined the girls, found them healthy.

Diet

A five-day adjustment period preceded the main study. The main study was divided into three parts, each part having

three five-day periods. The three periods in each part had the same basal menus (Appendix, pp. 61).

Table 3. Description of subjects in the present experiment.

Subject	Age[1]	Weight	Height	Body surface area[2]	Age of menarche
	years	kg.	cm.	sq. m	years
A	13	60.4	163.7	1.66	12
B	14	53.2	152.0	1.48	12
C	13	64.0	171.4	1.75	11
D	13	61.3	161.5	1.64	12
E	13	52.7	165.0	1.56	12
F	13	54.1	161.5	1.56	11

[1]Age to nearest birthday.
[2]Surface area was calculated from height and weight using the DuBois Body Surface Chart, prepared by Boothby and Sandiford of the Mayo Clinic.

Dietary food energy, protein, carbohydrate, fat, calcium, phosphorus, vitamin A, thiamine, riboflavin, niacin, iron, and ascorbic acid were estimated from tables of food composition. Food composition tables used were USDA Handbook No. 8 (Watt and Merrill, 1950) supplemented by Bowes and Church (1951).

The basal diet was planned to contain two levels of iron, 11 and 14 mg., and 1.0 g. calcium. Otherwise, the NRC recommended dietary allowances (1953) for nutrients were met. The most recent NRC allowances (1958) differed from the 1953 revision only in calories and niacin. The protein intake was planned to be 82 to 84 g. daily as compared to the recommended

allowance of 80 g. for adolescent girls.

The original plan was to provide 11 mg. iron per day in Part I and 14 mg. iron per day in Parts II and III. A pilot study revealed that analyzed values for iron were higher than calculated values from tables of food composition. Therefore, the diets actually were calculated for approximately nine and 12 mg. iron per day to obtain the desired amounts.

In the three parts of the study the serving sizes of various foods were adjusted to maintain approximately the same level of nutrient intake with the exception of iron. Adjustments also were made in the source of iron. Part III differed from Part II in that it contained a large quantity of milk chocolate candy. Unenriched flour products were used in Parts I and III, and enriched products in Part II. Additional meat was served in Part II. Skim milk replaced whole milk in Part III.

Distilled, demineralized water was used for cooking and drinking. Sodium chloride, C. P., served as a dentifrice.

At the beginning of the experiment, sufficient quantities of the non-perishable and frozen foods were obtained for the duration of the study. All baked products were made in the laboratory. All subjects were served the basal diet, but were allowed butter cookies ad libitum and a maximum of two soft drinks per day for between-meal snacks. Edible portions of all foods were weighed to the nearest 0.1 g.

Collection, Preservation, and Analysis of Samples

Methods used for collection and preservation of food and excretory products were described by Leichsenring et al. (1958). Aliquot portions (one-fifth) of the daily diet were collected and combined for each five-day period. The liquid food, solid food, butter cookies, chocolate, and soft drinks were placed each in separate containers. Urine and feces were collected separately in waxed paper cartons. Pads and tampons with the menstrual excretion were retained for each cycle of each subject. From each new lot, unused pads and tampons were processed for use in determining the nitrogen available in the materials. Brown acid digests of all the foods, excretory products, and pads and tampons were made and placed in paraffin-sealed glass bottles. Further details about sampling and preservation are found in the Appendix, pp. 61.

Triplicate samples of each brown acid digest of the foods and excretions of the six girls were chemically analyzed for nitrogen using the macro-Kjeldahl method with boric acid modification (Scales and Harrison, 1920). Details of the analytical procedures are given in the Appendix, pp. 61.

Analysis of Data

Nitrogen absorption was calculated as the difference between dietary intake and fecal loss.

$$\begin{matrix}\text{Absorbed} \\ \text{Food Nitrogen}\end{matrix} = \begin{matrix}\text{Food} \\ \text{Nitrogen}\end{matrix} - \begin{matrix}\text{Fecal} \\ \text{Nitrogen}\end{matrix}$$

Retention of nitrogen was defined as the difference between dietary intake and fecal and urinary excretion.

$$\begin{matrix}\text{Retained}\\ \text{Food Nitrogen}\end{matrix} = \begin{matrix}\text{Absorbed}\\ \text{Food Nitrogen}\end{matrix} - \begin{matrix}\text{Excreted}\\ \text{Food Nitrogen}\end{matrix}$$

Data were tabulated by period and by individual girl. Retention of nitrogen was calculated for each girl for each period and for the entire study. Nitrogen retentions of each subject were compared with those of the other subjects and with retentions reported in the literature.

Correlation coefficients were determined for dietary nitrogen intake and nitrogen excretion of urinary and fecal products on each subject (Snedecor, 1956).

RESULTS AND DISCUSSION

Calculated Nutrient Content of Basal Diet

The mean nutrient content of the basal diet as estimated from tables of food composition for each part of the study is shown in Table 4. Daily menus and the calculated mean nutrient content of the basal diet for each day are included in the Appendix, pp. 61.

The calculated nutrient content of the basal diet varied somewhat among the three parts of the study because of manipulation of the diet to increase the iron content from 11 mg. in Part I to 14 mg. during Parts II and III and to include chocolate during Part III. Use of enriched flour products and

Table 4. Mean daily nutrient content of basal diet calculated from food composition tables.[1]

Part	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus
	Cal.	g.	g.	g.	mg.	mg.
I	2249	82.4	105.0	213.9	1011	1354
II	2281	84.1	107.3	255.8	996	1376
III	2328	84.3	109.5	261.7	1079	1412

	Iron	Vitamin A	Thiamine	Riboflavin	Niacin	Ascorbic acid
	mg.	I.U.	mg.	mg.	mg.	mg.
I	9.6	6498	1.22	1.80	13.4	112
II	12.5	6512	2.94	1.94	15.8	118
III	12.4	5445	1.16	2.17	13.2	109

[1] Based on Watt and Merrill (1950) supplemented by Bowes and Church (1951).

additional meat during Part II resulted in increases in dietary intake of thiamine, riboflavin and niacin. Chocolate was responsible for increased riboflavin and phosphorus in Part III. Use of skim milk and smaller amounts of butter reduced the vitamin A content in Part III.

Analyzed and Calculated Values for Nitrogen Content of Basal Diet

Chemical analysis showed that the basal diet contained considerably less nitrogen than expected from the amounts of protein calculated from food composition tables (Table 5). Since tables of food composition list the protein content instead of the nitrogen content of a food, the values for protein

Table 5. Mean daily nitrogen content of the basal diet.

Period	Calculated[1] g.	Analyzed[2] g.	Difference %
Part I	13.18		
1		7.63	-42.1
2		7.54	-42.8
3		10.41	-21.0
Part II	13.46		
4		8.08	-40.0
5		7.79	-42.1
6		10.70	-20.5
Part III	13.49		
7		10.95	-18.8
8		8.33	-38.2
9		7.60	-43.7

[1]Estimated from USDA Handbook No. 8 (Watt and Merrill, 1950) and Bowes and Church (1951).

$$N = \frac{\text{g. Protein}}{6.25}$$

[2]Analyzed by method of Scales and Harrison (1920).

were divided by the factor 6.25 to estimate the amount of nitrogen in the diet. The calculated basal diet was planned to include 13.18 to 13.49 g. nitrogen (82 to 84 g. protein) daily during the entire study, but the analyzed values ranged from 7.54 to 10.95 g. nitrogen during the nine periods (Table 5). These analyzed values were from 18.8 to 43.7 (mean 34.4) percent less than the calculated values. No explanation can be offered to account for the low analyzed values for nitrogen found in the diet. The method and chemicals used in the analysis were checked by analyzing a soluble calcium caseinate material of a

known composition. Also, samples of ground beef were chemically analyzed and the analytical values for samples of ground beef analyzed in the nutrition laboratory compared favorably with those obtained for the same ground beef by the Chemical Services Laboratory.

Although the same menus were used throughout the three periods of each part, there were great variations in the analyzed nitrogen values (Table 5). Therefore, each period is reported separately. It was noted that two of the periods in each part showed lower values for the analyzed nitrogen than the third (Table 5). The low values ranged from 7.54 g. in Period 2 to 8.33 g. in Period 8. One period in each part had a much higher analyzed value for the same diet. The higher analyzed values ranged from 10.41 g. in Period 3 to 10.95 g. in Period 7. None of the analyzed nitrogen values approximated the calculated values (13.18 g., 13.46., 13.49 g. for Parts I, II, and III, respectively) during any period of the study.

In contrast to the present findings of low analyzed values, Patrick et al. (1955) found that the analyzed nitrogen content of diets they studied was 16.7 percent higher than the calculated values. They believed that this difference was reasonable because of normal variations in meats and other foods. Forsyth et al. (1954) found that the analyzed nitrogen content of solid diets averaged 8.7 percent more than the values calculated from standard food tables. Thomas and co-workers (1950) stated that wide variations in analyzed and calculated values may be

attributed to methodology, geographic origin, species, maturity, storage, method of processing, and analytical techniques. In addition, they suggested that variables in results of analyses may be attributed to sampling techniques, preservation, measuring, errors in analytical methods, variation in nutrient content within and among food products, and contaminant from equipment, environment, or reagents.

Nitrogen Intake

Mean daily nitrogen intakes of the six adolescent girls averaged 10.02 g. with a range of 9.48 g. for subject E to 10.92 g. for subject C during the 45-day study (Table 6). Intakes of the subjects varied because butter cookies and soft drinks were allowed in addition to the basal diet. In terms of protein the total mean intake fell below the NRC allowance of 80 g. (12.8 g. nitrogen) for all six subjects.

When considering the intakes of the individual subjects during the nine periods (Table 7), it was found that the lowest daily nitrogen intake was 7.60 g. (47.50 g. protein) by subjects B, E, and F in Period 9. Only in two instances (subject B in Period 3 and subject C in Period 6) did the period intake equal or exceed the recommended allowance of 12.8 g. nitrogen. The intakes for subject A in Period 7, subject C in Period 3, and subject F in Period 1 were slightly below the recommended allowance. During Period 9, five of the six subjects had an intake below two-thirds of the NRC recommendations. This also was true for subject A in Period 2 and subject F in Period 5.

Table 6. Mean daily intake and excretion with standard deviations, absorption, and retention of nitrogen by six adolescent girls during 45 days.

Subject	Intake			Excretion		Absorption		Retention			
	Total	Per m^2	Per kg.	Urine	Fecal	Total		Total		Per m^2	Per kg.
	g.	g.	g.	g.	g.	g.	%	g.	%	g.	g.
A	9.68 ±1.63	5.83	0.160	10.43 ±0.35	1.54 ±0.22	8.14	83.8	-2.28	-23.6	-1.37	-0.038
B	10.77 ±1.84	7.28	0.202	8.52 ±1.05	1.02 ±0.56	9.75	90.2	1.23	11.4	0.83	0.023
C	10.92 ±1.44	6.24	0.171	10.38 ±1.71	1.28 ±0.20	9.64	88.0	-0.75	-6.9	-0.43	-0.012
D	9.62 ±1.29	5.86	0.157	11.35 ±0.52	0.85 ±0.16	8.77	90.9	-2.59	-26.9	-1.58	-0.042
E	9.48 ±1.29	6.08	0.180	11.13 ±0.56	1.18 ±0.14	8.30	87.2	-2.83	-29.8	-1.81	-0.054
F	9.65 ±1.68	6.18	0.178	11.15 ±0.76	1.09 ±0.38	8.56	88.2	-2.59	-26.8	-1.66	-0.048
Av.	10.02	6.24	0.175	10.49	1.16		88.1		-17.1		-0.028

Table 7. Mean daily intake, excretion, absorption and retention of nitrogen per period.[1]

Period	Intake			Excretion		Absorption		Retention			
	Total	Per m^2	Per kg.	Urine	Fecal	Total		Total		Per m^2	Per kg.
	g.	g.	g.	g.	g.	g.	%	g.	%	g.	g.
					Subject A						
Part I											
1	8.83	5.32	0.146	10.83	1.28	7.55	85.5	-3.28	-37.1	-1.98	-0.054
2	7.84	4.72	0.130	10.35	1.37	6.47	82.5	-3.88	-49.5	-2.34	-0.064
3	10.71	6.45	0.177	10.69	1.33	9.38	87.6	-1.31	-12.2	-0.79	-0.022
Part II											
4	9.58	5.77	0.159	10.57	1.56	8.02	83.7	-2.55	-26.6	-1.54	-0.042
5	9.29	5.81	0.154	10.36	1.50	7.79	83.9	-2.57	-27.7	-1.55	-0.042
6	11.30	6.81	0.187	9.73	1.38	9.92	87.8	0.19	1.7	0.11	0.003
Part III											
7	12.75	7.68	0.211	10.28	1.78	10.97	86.0	0.69	5.4	0.42	0.011
8	8.93	5.38	0.148	10.21	1.95	6.98	78.2	-3.23	-36.2	-1.94	-0.054
9	7.90	4.76	0.131	10.84	1.67	6.23	78.9	-4.61	-58.3	-2.78	-0.076
					Subject B						
Part I											
1	11.53	7.79	0.217	8.45	0.36	11.16	96.8	2.71	23.5	1.83	0.051
2	11.64	7.86	0.219	9.82	0.37	11.28	96.9	1.46	12.5	0.99	0.027
3	14.01	9.47	0.263	10.05	0.99	13.02	92.9	2.97	21.2	2.01	0.056
Part II											
4	9.58	6.47	0.180	7.49	0.58	9.00	93.9	1.51	15.8	1.02	0.028
5	9.89	6.68	0.186	7.95	0.58	9.31	94.1	1.36	13.7	0.92	0.026
6	11.30	7.63	0.212	7.80	1.61	9.69	85.8	1.89	16.7	1.28	0.036
Part III											
7	11.85	8.01	0.223	9.71	1.57	10.28	86.8	0.57	4.8	0.38	0.011
8	9.53	6.44	0.179	8.01	1.36	8.17	85.7	0.16	1.7	0.11	0.003
9	7.60	5.14	0.143	7.42	1.75	5.85	77.0	-1.57	- 20.6	-1.06	-0.030

Table 7. (continued)

Period	Intake			Excretion		Absorption		Retention			
	Total	Per m^2	Per kg.	Urine	Fecal	Total		Total		Per m^2	Per kg.
	g.	g.	g.	g.	g.	g.	%	g.	%	g.	g.
					Subject C						
Part I											
1	9.73	5.56	0.152	11.53	1.23	8.50	87.4	-3.03	-31.1	-1.73	-0.047
2	10.24	5.85	0.160	10.82	1.09	9.15	89.4	-1.67	-16.3	-0.95	-0.026
3	12.51	7.15	0.195	11.41	1.06	11.45	91.5	0.04	0.3	0.02	0.001
Part II											
4	10.48	5.99	0.164	12.14	1.16	9.32	88.9	-2.82	-26.9	-1.61	-0.044
5	11.69	6.68	0.183	9.64	1.40	10.29	88.0	0.65	5.6	0.37	0.010
6	13.10	7.48	0.205	11.54	1.18	11.92	91.0	0.38	2.9	0.22	0.006
Part III											
7	11.85	6.77	0.185	10.26	1.44	10.41	87.8	0.15	1.3	0.08	0.002
8	9.83	5.62	0.154	9.63	1.25	8.58	87.3	-1.05	-10.7	-0.60	-0.016
9	8.80	5.03	0.138	6.47	1.69	7.11	80.8	0.64	7.3	0.37	-0.010
					Subject D						
Part I											
1	8.23	5.02	0.134	11.84	0.84	7.39	89.8	-4.45	-54.1	-2.71	-0.073
2	9.34	5.69	0.152	12.07	0.69	8.65	92.6	-3.42	-36.6	-2.08	-0.056
3	11.31	6.90	0.184	11.19	0.65	10.66	94.3	-0.53	- 4.7	-0.32	-0.009
Part II											
4	9.58	5.84	0.156	11.67	0.79	8.79	91.8	-2.88	-30.1	-1.76	-0.047
5	9.89	6.03	0.161	11.00	0.87	9.02	91.2	-1.98	-20.0	-1.21	-0.032
6	11.00	6.71	0.179	10.99	0.81	10.19	92.6	-0.80	- 7.3	-0.49	-0.013
Part III											
7	10.95	6.67	0.179	11.86	0.82	10.13	92.5	-1.73	-15.8	-1.05	-0.028
8	8.33	5.08	0.136	11.01	1.20	7.13	85.6	-3.88	-46.6	-2.36	-0.063
9	7.90	4.82	0.129	10.56	0.97	6.93	87.7	-3.63	-45.9	-2.21	-0.059

Table 7. (concluded)

Period	Intake			Excretion		Absorption		Retention			
	Total	Per m^2	Per kg.	Urine	Fecal	Total		Total		Per m^2	Per kg.
	g.	g.	g.	g.	g.	g.	%	g.	%	g.	g.
					Subject E						
Part I											
1	9.73	6.24	0.185	10.24	1.20	8.53	87.7	-1.71	-17.6	-1.10	-0.032
2	9.34	5.99	0.177	11.53	1.09	8.25	88.3	-3.28	-35.1	-2.10	-0.062
3	11.01	7.06	0.209	11.31	1.10	9.91	90.0	-1.40	-12.7	-0.90	-0.027
Part II											
4	8.38	5.37	0.159	10.99	1.04	7.34	87.6	-3.65	-43.6	-2.34	-0.069
5	8.99	5.76	0.171	11.82	1.04	7.95	88.4	-3.87	-43.0	-2.48	-0.073
6	10.70	6.86	0.203	11.18	1.17	9.53	89.1	-1.65	-15.4	-1.06	-0.031
Part III											
7	11.25	7.21	0.213	10.37	1.16	10.09	89.7	-0.28	- 2.5	-0.18	-0.005
8	8.33	5.34	0.158	10.98	1.41	6.92	83.1	-4.06	-48.7	-2.60	-0.077
9	7.60	4.87	0.144	11.75	1.41	6.19	81.4	-5.56	-73.2	-3.56	-0.106
					Subject F						
Part I											
1	9.43	6.04	0.174	10.32	1.03	8.40	89.1	-1.92	-20.4	-1.23	-0.036
2	9.04	5.79	0.112	11.14	0.79	8.25	91.3	-2.89	-32.0	-1.85	-0.053
3	12.51	8.02	0.231	12.66	1.06	11.45	91.5	-1.21	- 9.7	-0.77	-0.022
Part II											
4	9.88	6.33	0.183	11.14	0.61	9.27	93.8	-1.87	-18.9	-1.20	-0.035
5	7.79	4.99	0.144	10.27	0.86	6.93	89.0	-3.34	-42.9	-2.14	-0.062
6	11.30	7.24	0.209	10.80	1.34	9.96	88.1	-0.84	- 7.4	-0.54	-0.016
Part III											
7	10.95	7.02	0.202	10.74	0.83	10.12	92.4	-0.62	- 5.7	-0.33	-0.012
8	8.33	5.34	0.154	11.42	1.75	6.58	79.0	-4.84	-58.1	-2.46	-0.090
9	7.60	4.87	0.140	11.84	1.55	6.05	79.6	-5.79	-76.2	-3.71	-0.107

[1]Five-day sample collected; one-fifth used as mean value per day for the period.

In other studies that were reviewed (Table 2) analyzed values for dietary nitrogen intake were found to be higher than analyzed values for intake in the present study with the exception of Patrick and co-workers (1955) who reported an average intake of 9.98 g. for six pre-adolescents. In the studies on adolescent girls, some of the mean group intakes were below the recommended allowance (12.8 g. nitrogen), whereas others were above the recommended amount. None of the lower mean intakes reported were below 11.1 g. nitrogen, however. Holt and Fales (1921) reported an average intake of 11.68 to 11.84 g. nitrogen in a study with three girls 12 to 14 years of age. The 56-day study by Koehne and Morrell (1934) of 28 12- to 13-year-old girls on self-selected diets established similar nitrogen intake of 11.84 g. Wang et al. (1936) found the dietary nitrogen intakes to range from 11.1 to 21.8 g. (mean 15.2 g.) in their investigation with 24 12- to 15-year-old girls on self-selected diets. In a nine-week controlled investigation with six 13- to 14-year-old adolescent girls, the dietary intake was 12.20 g. during the first period and 13.97 g. nitrogen in the second period (Johnston and Schlaphoff, 1951). J. Johnston (1958) reported an intake of 14.6 g. nitrogen by three 13-year-old tuberculosis patients. In the 12-week study by Johnston and McMillan (1952) on six college-age women, the nitrogen intake was stated as 11.23 g.

On the basis of surface area, the grand mean daily nitrogen intake per square meter for all six subjects in the present

investigation was 6.24 g. and ranged from 5.83 to 7.28 g. (Table 6). The variation was caused by differences in body size of the individual subjects as well as protein intake. The mean nitrogen intakes per day ranged from 4.76 g. per square meter for subject A in Period 9 to 9.47 g. per square meter for subject B in Period 3 (Table 7).

When considering body weight, the mean nitrogen intake ranged from 0.160 g. per kg. for subject A to 0.202 g. per kg. for subject B with a grand mean nitrogen intake of 0.175 g. per kg. for all six girls (Table 6). The range for the individual subjects during the nine periods was from 0.112 g. for subject F in Period 2 to 0.263 g. for subject B in Period 3 (Table 7). In a controlled study on six 13- to 14-year-old girls Johnston and Schlaphoff (1951) reported that on a total nitrogen intake of 12.20 g. the mean daily intake of nitrogen was 8.24 g. per square meter body surface of 0.26 g. per kg. body weight. On a total nitrogen intake of 13.97 g. the mean intake was 9.34 g. per square meter or 0.28 g. per kg. body weight. These values were somewhat higher than those found in the present study. The analyzed food values of Johnston and Schlaphoff (1951) also were higher than those of the present investigation. Wang and coworkers (1936) reported an average nitrogen intake of 0.401 g. per kg. with a range of 0.285 to 0.561 g. for 24 12- to 15-year-old girls. These values also are higher than those found in the present study. However, it may be pointed out that the average body weight of the girls in the study of Wang et al. was 38.39 kg.

as compared to the average body weight of 57.62 kg. for the subjects in the present investigation.

Nitrogen Excretion

Urinary. The grand mean daily urinary nitrogen loss for all six girls for the entire study was 10.49 g. (Table 6). The mean values for each subject during the entire investigation ranged from 8.52 ± 1.05 (subject B) to 11.35 ± 0.52 g. (subject D). When considering urinary losses on the per period basis, a low value of 6.47 g. in Period 9 for subject C to a high value of 12.66 g. in Period 3 for subject F was found (Table 7). Throughout all periods, urinary nitrogen losses were found to be lower for subject B than for the other subjects. The greatest variability in urinary nitrogen losses was found among the periods for subjects B and C. They ranged from a low of 7.42 g. in Period 9 to a high of 10.05 g. in Period 3 for subject B and from 6.47 g. in Period 9 to 12.14 g. in Period 4 for subject C. The urinary nitrogen values for the remaining four girls were quite consistent from one period to the next.

In other investigations that have been reported in the literature, similar total urinary nitrogen losses were noted. Johnston and Schlaphoff (1951) reported a mean urinary nitrogen excretion of 9.70 g. and 10.59 g. on 12.20 g. and 13.97 g. dietary nitrogen intakes, respectively. From the results of their study, Wang et al. (1936) noted that the mean urinary nitrogen excretion was 12 g. on an average dietary intake of

15.2 g. nitrogen. At the same time the dietary nitrogen intake did not compare favorably with the intakes of the studies of Johnston and Schlaphoff (1951) and Wang et al. (1936).

Wang and co-workers (1936) reported a urinary nitrogen excretion of 0.32 g. per kg. body weight. In the present study, however, the mean urinary excretion for all subjects during the entire investigation was 0.183 g. nitrogen with a mean range of 0.160 g. for subject B to 0.211 g. for subject E. In terms of body weight urinary nitrogen excretion is lower in the present study than it was in the investigation of Wang et al. However, the 24 12- to 15-year-old girls studied by Wang et al. had a mean body weight of 38.39 kg., whereas the six 13- to 14-year-old girls in the present study had a mean body weight of 57.62 kg.

According to Allison (1951), urinary nitrogen has been found to vary depending upon the body protein stores and the amount of dietary nitrogen ingested. At the beginning of the present investigation, the six girls were examined by a physician and termed healthy. The diets in the study were planned to be similar in content and source of food to the diets ingested by the teenagers previous to serving as subjects on the balance experiment. In this study the urinary nitrogen excretion was consistent with findings reported in the literature, whereas the dietary nitrogen intake varied a great deal.

Fecal. For the entire study the mean fecal loss for all six girls was 1.16 g. nitrogen (Table 6). The range for the individuals was from 0.85 ± 0.16 (subject D) to 1.54 ± 0.22 g.

(subject A). During the nine periods, the average fecal losses ranged from 0.36 g. for subject B in Period 2 to 1.95 g. for subject A in Period 8 (Table 7). The stools for subject A, who had high fecal nitrogen values, were of a soft and liquid consistency. Fecal nitrogen for subject B was low, especially during the first half of the study. In an investigation by Hegsted et al. (1946) it was observed that the shorter the expulsion time, the higher the fecal nitrogen values. It was observed that the stools of subject B were few in number whereas the number of stools for subject A ranged from six to nine during any five-day period throughout the study. These findings, therefore, are in agreement with those of Hegsted et al. (1946) in that the shorter the expulsion time, the higher the fecal nitrogen values. For subject D there was a tendency for the stools to be large, hard, and few in number. The fecal nitrogen values for this individual also were low as compared to the values of some of the other subjects.

The fecal nitrogen values of the present study are approximately the same as those reported by other investigators. The mean fecal nitrogen content of six young college women was 1.02 g. on an intake of 11.20 g. nitrogen (Johnston and McMillan, 1952). In a metabolic study with pre-adolescent girls, Patrick et al. (1955) found the fecal nitrogen to range from 0.79 to 0.81 g. per day on a 9.98 g. nitrogen dietary intake. Wang et al. (1936) reported a mean of 1.2 g. per day from the study on 24 12- to 15-year-old girls. Johnston and Schlaphoff (1951)

reported fecal nitrogen losses of 1.07 g. and 1.21 g. on 12.20 g. and 13.97 g. nitrogen intakes.

Hegsted et al. (1946) suggested that fecal nitrogen is more or less constant. For adults he estimated the mean fecal nitrogen to be 1.28 g. per day. In the present investigation it was observed that there was a tendency for the fecal nitrogen to vary among the periods for the same subject (Table 7). Therefore, the findings in this study do not comply with the statement of Hegsted.

Correlation of Intake and Excretion

To determine whether or not excretion of nitrogen was dependent on intake, correlation coefficients were determined. In this study excretion was not dependent upon intake. The r values for the subjects were as follows: A, -0.36; B, 0.49; C, 0.52; D, -0.06; E, -0.53; F, 0.15.

Menstrual Losses

The mean nitrogen content of the menses for all menstrual periods during the study were determined for each subject (Table 8). Two cycles of each subject were observed except subject E where three cycles were observed. The total mean nitrogen loss for all six subjects was 1.59 g. nitrogen per menstrual period. The range was from 0.93 g. for subject A to 2.62 g. nitrogen for subject D. It was noted that subject D had an excessive flow of menstrual blood during her periods.

Table 8. Retention of nitrogen required by six adolescent girls for replacement of menstrual losses.

Subject	Cycles observed	Length of cycle: Av.	Length of cycle: Range	Av. nitrogen loss per period	Daily retention of nitrogen needed for replacement
		day	days	g.	g.
A	2	38	22-68	0.93	.024
B	2	29	28-30	0.99	.034
C	2	34	33-35	1.10	.032
D	2	30	29-30	2.62	.087
E	3	18	17-19	1.84	.102
F	2	26	25-27	2.07	.080
Av.				1.59	.060

Other workers reported a mean loss of 0.79 g. nitrogen per menstrual period for college-age women (Johnston and McMillan, 1952). However, these workers believed that the loss in their study was below the average for all women because the loss of iron (which is associated with protein) in the study was below the average. Barer and Fowler (1936) found the mean loss of blood per menstrual period was 50.5 ml. This amount would contain 1.7 g. nitrogen if the blood is considered as containing the same percentage as venous blood (3.4 percent). However, it was stated by Johnston and McMillan (1952) that Rona and Waldbauer, 1928, believed that the amount of nitrogen in menstrual blood probably would be somewhat higher than 1.7 g. because they found that most nitrogenous fractions of menstrual blood contained slightly more nitrogen than venous blood. Thus, the average

menstrual losses per menstrual period in the present study are similar to the findings of other investigators.

The mean daily retention of nitrogen needed for replacement of these losses was calculated to be 0.06 g. daily with a range of 0.024 to 0.102 g. (Table 8). Because the menstrual losses of nitrogen were small as compared to urinary and fecal losses, they were not used when calculating retention values in the study.

Nitrogen Absorption

Nitrogen absorption is calculated as the difference between nitrogen intake and fecal nitrogen. In the present study the over-all mean absorption was 88.1 percent for all six subjects with a range of 83.8 percent for subject A to 90.9 percent for subject D (Table 6). Subject B had the greatest variability in absorption among the girls in the study (Table 7). In Period 9, 77 percent of her intake was absorbed, whereas in Period 1 the absorption was 96.8 percent. Hegsted et al. (1946) have suggested that the shorter the expulsion time of feces, the higher the fecal nitrogen values found. The results of the analysis on subject B indicated that this was true in her case. Her stools were few in number throughout each period, particularly during the first half of the study where absorption values were greatest. The absorption values for subjects D and B tended to be in the highest part of the range and values for subject A were in the lowest (Table 7). Stools were frequent

for subject A who was found to have the greatest fecal nitrogen values.

It was stated by Patrick et al. (1955) that in children the average absorption is 90 percent of the intake. In their study on pre-adolescents, the mean percentage absorbed was reported as 92.3 percent for five eight- to nine-year-olds and 95.3 percent for the 11-year-old subject. Wang et al. (1936) reported an absorption of 92.2 percent of the intake with a range of 85.9 to 95.0 percent by the 24 adolescent girls in their study. In the present investigation, the mean absorption of the six subjects was slightly lower than that of the adolescents and children reported by other workers.

Nitrogen Retention

The nitrogen retention values were found to be negative in five of the six subjects (Table 6). Four of the six girls showed similar total losses ranging from 2.28 to 2.83 g. nitrogen. One of the subjects (C) had only a slight loss (0.75 g.) of nitrogen. The individual with a positive retention had a value of 1.23 g. nitrogen for the entire investigation. In terms of percentages, four of the six girls had negative losses of -23.6 to -29.8 percent whereas one subject (C) showed a smaller loss (-6.9 percent). Subject B had a positive retention of 11.4 percent. The grand mean was a loss of -17.1 percent for all six subjects. Except for the results of one individual these findings are not in direct agreement with

other similar investigations reported in the literature. Wang et al. (1936) reported nitrogen retention to be from 28.2 to 2.8 (Mean 12.6) percent of dietary intake. Johnston and Schlaphoff (1951) stated that the mean retention was 11.6 percent on the 12.20 g. nitrogen diet and 15.5 percent on the 13.97 g. nitrogen diet. On the 14.6 g. nitrogen intake the mean retention was 18 percent (J. Johnston, 1958). In a study with pre-adolescents the mean nitrogen retention was reported as 19.4 percent (Patrick et al., 1955), whereas in an investigation with college-age women the mean nitrogen retention was 5.7 percent of the dietary intake (Johnston and McMillan, 1952).

There is a difference of opinion among workers as to the unit of body size to be used when determining nitrogen retention. Hegsted et al. (1946) suggested that surface area is better than body weight. On the other hand, Patrick et al. (1955) demonstrated that surface area was not a better unit of body size than body weight for evaluating nitrogen retention and absorption. In the present investigation, nitrogen retention values are reported in three units. These are total g. of nitrogen, g. per kg. body weight, and g. per m^2 of body surface.

Values found for nitrogen retention per square meter of body surface varied among the nine periods and among the six individual girls (Tables 6 and 7). There does not appear to be any consistent pattern of retention for the individual subjects

throughout the entire study. The greatest loss of nitrogen per square meter of body surface was 3.71 g. by subject F during Period 9 (Table 7). During the same period subject E, who had the same body surface area as subject F, had lost 3.56 g. nitrogen per m^2. During Period 9 the dietary nitrogen intake by these two girls was lowest of any period throughout all parts of the study. Subjects D, E, and F were in negative nitrogen balance in all periods. This was true also for subject A except during Periods 6 and 7 where she retained amounts of 0.11 and 0.42 g. per m^2, respectively. The losses in the remaining periods for subject A were greater and more severe. Subject C was in positive retention for five of the nine periods. The positive values ranged from 0.02 to 0.37 g. per m^2 (Table 7). There was a tendency for the negative values of subject C that ranged from -0.60 to -1.73 g. nitrogen per m^2 to be less than those of the other subjects. Most of the positive nitrogen retention values were found for subject B. This individual had a loss during Period 9 only (Table 7). Her greatest retentions were found to be 1.83 g. nitrogen per m^2 in Period 1 and 2.01 g. nitrogen per m^2 in Period 3. In terms of the total dietary nitrogen for subject B this corresponded to 23.4 percent retention in Period 1 and 21.2 percent in Period 3. The negative retention values ranged from -2.5 percent for subject E in Period 7 to -76.2 percent for subject F in Period 9. As in the total retentions reported in g., there appears to be no definite trend in the percentages of negative or positive retentions for each individual girl.

The percentage retentions were found to vary greatly among the periods.

In the present study in terms of body weight, the mean nitrogen retentions were negative for five of the six subjects (Table 6). Subject B had a mean nitrogen retention of 0.023 g. per kg. whereas the greatest average loss for subject E was -0.054 g. per kg. The average for subject F was close with a mean of -0.048 g. per kg. The grand mean for all six subjects was -0.028 g. per kg. body weight.

When considering each period (Table 7), it was noted that subject B, who had a positive nitrogen retention, had the greatest mean retention during Period 3 of 0.056 g. per kg. body weight. This gradually decreased throughout the study to -0.03 g. per kg. except for the rise in Period 6. For the other subjects during the entire study, the mean nitrogen retentions ranged from 0.011 g. per kg. for subject A in Period 7 to -0.107 g. per kg. body weight for subject F in Period 9. The greatest nitrogen losses were found during Period 9 with values of -0.106 g. and -0.107 g. per kg. body weight for subjects E and F, respectively.

The nitrogen retention values per kg. body weight do not approximate the values found by other investigators. The mean values given in the studies reviewed were positive, whereas the grand mean nitrogen retentions in the present investigation were negative. Patrick and co-workers (1955) reported a retention of 0.060 to 0.089 g. per kg. body weight for the five

eight- to nine-year-old subjects. A mean retention value of 0.049 g. nitrogen per kg. body weight was reported for an 11-year-old subject. Johnston and Schlaphoff (1951) reported an average of 0.0299 g. per kg. retention on a 12.20 g. nitrogen diet and a retention of 0.0437 g. nitrogen per kg. body weight on a 13.97 g. nitrogen diet for 13- to 14-year-old girls. J. Johnston (1958) reported a mean retention of 0.050 g. per kg. body weight for the three 13-year-old tuberculosis patients. With college-age women the mean nitrogen retention was found to be 0.012 g. per kg. body weight (Johnston and McMillan, 1952).

The amount of nitrogen that girls of this age need to retain for good health and normal growth is not known. They need to retain enough for tissue increase and also enough to cover the nitrogen lost from the body that is not measured in the usual balance experiment. After reviewing studies by other investigators, positive retentions during the adolescent age were expected. At least, such great deviations as were found in the present investigation were not foreseen or anticipated. The negative retention values are believed to be the result of the low nitrogen intakes of the diet and not because of the increased catabolism by the individual girls who appeared to be healthy. During the entire study, the weight variability of each individual subject was 2.1 pounds or less (approximately 1 kg.). Subjects B and E each lost about 2 pounds, whereas subjects C, F, A, and D gained 1.3, 1.2, 1.0 and 0.8 pounds, respectively, during the course of the study.

SUMMARY

Nitrogen metabolism was studied in six healthy 13- to 14-year-old girls. The daily basal diet was calculated to include 82 to 84 g. protein (13.18 to 13.49 g. nitrogen) and adequate amounts of all other nutrients. To provide additional food energy for the subjects whose requirements exceeded the amount included in the basal diet, certain other foods were allowed and a record kept of the consumption. These additional foods brought about some variation in the total dietary nitrogen intake of the various subjects.

The study was comprised of a five-day adjustment period and nine five-day experimental periods. One set of five daily diet plans was used during Periods 1, 2, and 3, another set during Periods 4, 5, and 6, and a third set during Periods 7, 8, and 9.

Composites of the basal diet and fecal and urinary excretions for each five-day period were analyzed for nitrogen using the macro-Kjeldahl method with the boric acid modification. The menstrual excretions and samples of the ad libitum foods were analyzed using the same method.

For all nine periods, the analyzed dietary nitrogen values were lower than those calculated for the basal diet. Differences ranging from 18.8 to 42.8 percent between the analyzed and calculated values for dietary nitrogen occurred. Also, great variations in nitrogen were found among the periods when the same dietary plan was used. Analyzed values for the basal

diet ranged from 7.54 to 10.95 g. nitrogen.

As a result of the analysis of the basal diet plus the additional foods, each of the six subjects had a lower mean daily nitrogen intake for the entire study than the 12.8 g. nitrogen (80 g. protein) recommended by the NRC for adolescent girls. The range was 9.48 ± 1.29 to 10.92 ± 1.44 g. nitrogen daily. The mean intake per m^2 body surface area ranged from 5.83 to 7.28 g. nitrogen for the six subjects. The mean intake per kg. body weight ranged from 0.157 to 0.202 g. nitrogen.

Mean urinary excretion varied from 8.52 ± 1.05 to 11.35 ± 0.52 g. nitrogen with an average of 10.49 g. for the six girls. Daily fecal nitrogen losses ranged from 0.85 ± 0.16 to 1.54 ± 0.22 g. during the entire study. It was observed that the shorter the fecal expulsion time, the higher the fecal nitrogen. Menstrual nitrogen losses were determined, but values obtained were not used in calculating total nitrogen retention.

In the present study nitrogen excretion was not dependent upon nitrogen intake when data were analyzed statistically.

The mean nitrogen absorption was 88.1 percent (83.8 to 90.9 percent) for the six subjects. Five of the subjects were in negative nitrogen balance. Four of the subjects had similar total mean negative retentions ranging from -2.28 to -2.83 g. (-23.6 to -29.8 percent) nitrogen, whereas the fifth subject lost -0.75 g. (-6.9 percent) nitrogen. The sixth subject had a mean positive retention of 1.23 g. (11.4 percent) nitrogen.

Nitrogen retentions also were computed for the subjects both on the basis of body surface area and body weight. Four subjects had losses ranging from -1.37 to -1.81 g. nitrogen per m^2 body surface, whereas the subject in slight negative retention had a loss of -0.43 g. per m^2. The sixth subject had a positive retention of 0.83 g. nitrogen per m^2. By body weight, four subjects had nitrogen losses of -0.038 to -0.054 g. per kg. A slight loss of -0.012 g. nitrogen per kg. was found for the fifth subject and a positive mean retention of 0.023 g. per kg. for the sixth subject.

Five of the six subjects were in negative nitrogen balance. The negative retentions are believed to be the result of the low nitrogen intakes, and not because of increased catabolism by the individual girls who appeared to be healthy and had a weight variability within 2.1 pounds during the entire investigation.

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APPENDIX

Collection and Preservation of Samples

Unless otherwise stated, all reagents conformed in purity to Recommended Specifications for Analytical Reagent Chemicals of the American Chemical Society, and acids were concentrated. Water was distilled and further purified in a demineralizer (Crystalab Deeminizer Model C1-5, Crystal Research Laboratories, Inc., Hartford, Connecticut). Samples were stored in narrow mouth, 300 cc. glass bottles with plastic screw lids and sealed with paraffin. A paraffin coating prevented gummed labels from drying and being released from the bottles.

Food. Food was weighed in the form in which it was served, e.g., boiled, baked, broiled, steamed, or raw. Inedible parts (bones, rinds, or egg shells) were removed before weighing. Five-day composites of several foods with similar physical and chemical properties were prepared and converted into digests. Food samples were divided into liquid, fatty, and solid composites. A brown (acid) digest was used to preserve the composites for analysis. A Harvard trip balance (Ohaus Scale Corp., Union, New Jersey) with a sensitivity of 0.1 g. was used for weighing.

Preparation of Liquid Food Composite. Aliquot portions (one-fifth) of all milk, fruit and vegetable juices, ice cream, sherbets, and soft custard consumed in one day were weighed. Water and tea were omitted. Soft drinks were analyzed separately. A record was kept of the weight of each food added to the composite.

Preparation of Fatty Food Composite. Aliquots (one-fifth) of all butter, cream, mayonnaise, and French dressing were weighed and weights recorded. Butter cookies and chocolate each formed a separate digest. Since fat is difficult to handle in a digest, it was separated from the fatty foods. The foods were heated with 50 to 100 ml. water until the fat melted. Upon cooling, the solidified layer of fat was removed. The liquid was added quantitatively to the liquid food composite. Solid particles that appeared in this composite were separated by straining and then added to the solid food composite.

Preparation of Solid Food Composite. This composite included all foods not covered in the other composites. The composites were stored in beakers at refrigerator temperature until a five-day period was completed.

Preparation of Brown (Acid) Digest. Brown digests of each food composite were made with HCl. The food composite was transferred quantitatively to an electric blender (Waring). If the composite was too thick to permit thorough blending, water was added. A known weight of the total composite was transferred to an Erlenmeyer flask. From 100 to 150 ml. HCl per one liter of blended solid food was added. The flask was heated on a hot plate set at low heat. To prevent charring and to mix the contents, the flask was swirled occasionally. The heat treatment continued until the mixture was uniform in consistency, brown in color, and evaporated to an amount that could be diluted to the desired volume. The contents and water rinsings from the

Erlenmeyer flask were added to a weighed volumetric flask before diluting to volume with water. The volumetric flask plus its contents were weighed. Contents of the flask were mixed by inverting and rotating 50 times. Two 300 cc. glass bottles were filled. The flask was inverted and rotated 25 times before filling the second bottle. The digests were stored at room temperature in paraffin-sealed glass bottles.

Feces. On the first day and on the morning following the day of the balance period, a carmine capsule was taken before breakfast. To allow for any fecal lag at the end of the experiment, the subjects continued on the weighed diet until the carmine was obtained. This additional time was not considered part of the balance period but was used to avoid drastic diet changes that might alter gastrointestinal action.

Preparation of Fecal Composite. The composite contained all feces from the first appearance of the carmine, up to, but not including, those colored by the next capsule. From the first fecal collection, that part which preceded the carmine was discarded. During the collection period, the feces were rinsed with water from the cartons into a two liter wide-mouth Erlenmeyer flask containing enough HCl so the final concentration of acid in the brown digest was approximately ten percent. The covered flask was kept in a refrigerator until the collection was complete.

Preservation for Analysis. The fecal composite was preserved for analysis by preparing a brown digest. Directions

previously given for food were followed omitting the steps using an electric blender.

Urine. On the first day of the collection period, the bladder was emptied immediately after arising. The urine collected upon rising was considered a part of the urine for the preceding day.

Preparation of Urine Composite. The 24-hour sample was mixed by inverting and rotating the bottle 20 times. A graduated cylinder was used to measure volume and to obtain an aliquot (one-fifth) of each day's sample. Volume was recorded. The daily aliquots were combined in a stoppered container and stored at refrigerator temperature. A composite covered a five-day period.

Preservation for Analysis. The urine composite was transferred to an Erlenmeyer flask and 100 ml. HCl added. The composite was preserved for analysis by preparing a brown digest. Directions previously given for food were followed omitting the steps using an electric blender.

Procedure for Nitrogen Determination by the Macro-Kjeldahl Method

Digestion

Food and metabolic digests in sample bottles were inverted 50 times before removal of first sample. Thereafter, each sample bottle was inverted an additional 25 times for removal of each succeeding sample. The samples were pipetted into

weighed glass beakers and weighed. Ten ml. of urine, ten ml. of feces, 25 ml. of menses, and 15 ml. of food slurry constituted an aliquot portion of each material.

The weighed samples were placed into 800 ml. Kjeldahl flasks. Beakers and necks of flasks were washed down with warm distilled water using approximately 25 ml. water. A small portion of salt mixture composed of 100 g. anhydrous sodium sulfate and 10 g. copper sulfate was added to each flask. From 25 to 30 ml. of concentrated H_2SO_4 was added gradually. The flasks were heated slowly on digestion racks until the mixture turned a clear, light green. Then heating was continued for at least one hour more. If, in the process, specks of organic material became lodged in the neck of the flask, the flask was cooled and rinsed down with distilled water.

When the digestion was complete, the flasks were stoppered and cooled. Approximately 175 ml. of distilled water was added to each sample.

Distillation

Special Reagents. Mixed Indicator. In 100 ml. of 95 percent alchol 0.125 g. of methyl red and 0.083 g. methylene blue were dissolved.

Concentrated Sodium Hydroxide. 1090 g. of commercial NaOH flasks were used with 1525 ml. distilled water. This was a solution of approximately 1.36 in specific gravity. Preparation was in an earthenware crock. Solution was allowed to cool

before decanting off into bottles with ground glass stoppers. Several days lapsed prior to use.

Four percent Boric Acid Solution. A four percent solution of boric acid was prepared.

Procedure. Three drops of mixed indicator was added to 50 ml. of 4 percent boric acid in a 500 ml. Erlenmeyer flask and placed as a receiving flask in the distillation rack so that the tip of the glass tube was just below the surface of the acid. To the Kjeldahl flask about 125 ml. of concentrated sodium hydroxide was layered. Several pieces of mossy zinc were added and the flask was connected immediately to the distillation rack. The distillation tubes were equipped with jackets to hold water to cool the distillate and so prevent loss of ammonia. Erlenmeyer flasks were removed when distillate reached the 225 ml. mark and then stoppered.

Titration

Contents of receiving flasks were titrated with 0.1 N. HCl. The end point was observed with a reference solution prepared by placing 50 ml. of 4 percent boric acid, 175 ml. distilled water, and three drops mixed indicator in a 500 ml. Erlenmeyer flask.

Menus for Part I

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	100	Frankfurter	60	Ham	100	Coca Cola	199
	Egg	54	Bun, unenriched	40	Potatoes, boiled	84		
	Biscuits, unenriched	40	Catsup	17	Beets, canned	42		
	Butter	7	Celery, raw	20	Bread, unenriched	25		
	Strawberry Jelly	10	Carrots, raw	50	Butter	5		
	Milk, whole	183	Potato Chips	25	Angel Cake	45		
			Orange Sections	97	Raspberries, frozen	80		
			Coconut	3.9	Sugar	10		
			Milk, whole	244	Milk, whole	244		
Day 2	Apricots, canned, sirup	125	Tuna	60	Roast Beef, rolled rib	75	Coca Cola	199
	Oatmeal, cooked	177	Noodles, cooked	100	Green Beans, frozen	85	Popcorn	28
	Cream, 20%	60	Green Pepper, raw	10	Butter	10	Butter	10
	Sugar	4	Ritz Cracker	9.3	Minted Pear, canned	75	Iced Tea	
	Toast, unenriched	25	Milk, whole	30	Lettuce	25		
	Butter	7	French Bread, unenriched	40	Bread, unenriched	25		
	Milk, whole	244	Butter	7	Butter	7		
			Orange Slices	150	Blanc Mange	125		
			Coconut	3.9				
			Milk, whole	244				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Tenderloin Steak	60	Coca Cola	199
	Ground Pork Shoulder	40	Bun, unenriched	40	Corn, frozen	100	Potato Chips	20
	Toast, unenriched	25	Catsup	17	Lettuce	50	Butter	5
	Raspberry Jelly	10	Potato Chips	20	French Dressing	10	Sugar	10
	Milk, whole	244	Carrots, raw	25	Bread, unenriched	25	Iced Tea	
			Celery, raw	40	Butter	7		
			Apple, A P	150	Orange Sherbet	192		
			Milk, whole	244	Milk, whole	100		

Menus for Part I (concluded)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned sweetened	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Coca Cola	199
	Biscuits, unenriched	40	Cheddar Cheese	33	Oyster Crackers	10	Brazil Nuts	36
	Butter	10	Sweet Pickle	5	Tomato Juice, canned	25	Iced Tea	
	Grape Jelly	20	Bread, unenriched	50	Peas, frozen	100		
	Milk, whole	100	Butter	5	Potato, boiled	75		
			Mayonnaise	10	Carrots, raw	25		
			Cabbage, Slaw	50	Raisins	3.3		
			Dressing	18	Bread, unenriched	25		
			Pears, canned	100	Butter	10		
			Sugar Cookies	26	Jello, dry	21.7		
			Milk, whole	244	Sugar	20		
Day 5	Grapefruit Sections, raw	100	Bologna	80	Tomato Juice, canned	125	Coca Cola	199
	Egg	54	Bread, unenriched	50	Pork Chop	90	Hershey Goodbar	18
	Toast, unenriched	25	Butter	7	Rice	84	Sugar	10
	Butter	5	Lettuce	50	Broccoli, frozen	70		
	Honey	20	French Dressing	15	Cranberry Jelly	30		
	Milk, whole	244	Cherries, water packed	100	Lettuce	25		
			Milk, whole	229	French Bread, unenriched	40		
					Butter	5		
					Vanilla Ice Cream	124		

Menus for Part II

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	100	Frankfurter	60	Ham	125	Coca Cola	199
	Egg	54	Bun, enriched	40	Potatoes, boiled	84		
	Biscuit, enriched	40	Catsup	17	Beets, canned	42		
	Butter	2	Celery, raw	20	Green Olives	39		
	Strawberry Jelly	10	Carrots, raw	50	Bread, enriched	25		
	Milk, whole	83	Potato Chips	25	Angel Cake	45		
			Orange Sections	97	Strawberries, frozen	100		
			Coconut	11.7	Sugar	10		
			Milk, whole	244	Milk, whole	244		
Day 2	Apricots, dried	75	Tuna	70	Roast Beef, rolled rib	75	Coca Cola	199
	Oatmeal, cooked	115	Noodles, cooked	100	Green Beans, frozen	85	Popcorn	28
	Cream, 20%	60	Green Pepper, raw	30	Butter	10	Butter	10
	Sugar	4	Ritz Crackers	18.6	Minted Pear, canned	75	Iced Tea	
	Toast, enriched	25	Milk, whole	30	Lettuce	25		
	Butter	7	French Bread, enriched	40	Raisins	13		
	Milk, whole	244	Butter	7	Bread, enriched	25		
			Orange Slices	150	Butter	7		
			Coconut	7.8	Blanc Mange	100		
			Milk, whole	244				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Apple Juice, canned	187	Coca Cola	199
	Ground Pork Shoulder	40	Bun, enriched	40	Tenderloin Steak	70	Potato Chips	20
	Toast, enriched	25	Catsup	17	Corn, frozen	100	Butter	5
	Raspberry Jelly	10	Potato Chips	25	Lettuce	50	Iced Tea	
	Milk, whole	244	Carrots, raw	25	French Dressing	10		
			Celery, raw	40	Sweet Pickle	30		
			Apple, A P	150	Bread, enriched	25		
			Milk, whole	244	Butter	7		
					Orange Sherbet	96		
					Milk, whole	100		

Menus for Part II (concluded)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned unsweetened	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Coca Cola	199
	Biscuits, enriched	40	Cheddar Cheese	33	Oyster Crackers	10	Brazil Nuts	36
	Grape Jelly	10	Sweet Pickle	5	Tomato Juice, canned	25	Iced Tea	
	Milk, whole	100	Bread, enriched	50	Egg Yolk	17		
			Butter	5	Peas, frozen	100		
			Mayonnaise	10	Potato, boiled	75		
			Cabbage Slaw	50	Carrots, raw	25		
			Dressing	18	Raisins	13.3		
			Pears, canned	100	Bread, enriched	25		
			Sugar Cookies	26	Butter	10		
			Milk, whole	244	Jello, dry	21.7		
					Sugar	20		
Day 5	Grapefruit Sections, raw	100	Bologna	80	Tomato Juice, canned	125	Hershey Goodbar	18
	Egg	54	Bread, enriched	50	Pork Chop	90		
	Toast, enriched	25	Butter	7	Rice	84		
	Butter	5	Lettuce	50	Broccoli, frozen	70		
	Honey	20	French Dressing	15	Cranberry Jelly	30		
	Milk, whole	244	Cherries, water packed	100	Lettuce	25		
			Milk, whole	229	French Bread, enriched	40		
					Vanilla Ice Cream	124		
					Butterscotch Sauce	37		
					Sugar	5		

Menus for Part III

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 1	Peaches, frozen	80	Frankfurter	60	Ham	85	Hershey Bar	56.7
	Egg	54	Bun, unenriched	40	Potatoes, boiled	84	Hershey Kisses	60
	Biscuits, unenriched	40	Catsup	17	Beets, canned	42		
	Butter	5	Celery, raw	40	Bread, unenriched	25		
	Milk, skim	256	Carrots, raw	50	Butter	2		
			Green Olives	39	Angel Cake	45		
			Potato Chips	20	Strawberries, frozen	50		
			Coconut	11.7	Milk, skim	244		
			Orange Sections	97				
Day 2	Apricots, canned sirup	75	Tuna	70	Roast Beef, rolled rib	75	Popcorn	14
	Oatmeal, cooked	84	Noodles, cooked	100	Green Beans, frozen	85	Hershey Bar	56.7
	Cream, 20%	60	Green Pepper, raw	10	Minted Pear, canned	75	Hershey Kisses	60
	Sugar	4	Ritz Crackers	18.6	Lettuce	25		
	Toast, unenriched	25	Milk, whole	30	Bread, unenriched	25		
	Butter	7	French Bread, unenriched	40	Butter	7		
	Milk, skim	200	Butter	7	Blanc Mange	50		
			Orange Slices	150				
			Coconut	3.9				
			Milk, skim	200				
Day 3	Orange Juice, frozen	308	Ground Round Steak	70	Tenderloin Steak	70	Hershey Bar	56.7
	Ground Pork Shoulder	40	Bun, unenriched	40	Corn, frozen	100	Hershey Kisses	60
	Toast, unenriched	25	Catsup	17	Lettuce	50	Iced Tea	
	Milk, skim	244	Carrots, raw	25	French Dressing	10		
			Celery, raw	40	Bread, unenriched	25		
			Potato Chips	25	Butter	10		
			Apple, A P	150	Orange Sherbet	96		
			Milk, skim	244	Sugar	5		

Menus for Part III (concluded)

	Breakfast	g.	Lunch	g.	Dinner	g.	Snack	g.
Day 4	Applesauce, canned, unsweetened	100	Salmon, red, drained	50	Ground Round Steak, raw	125	Hershey Goodbar	9
	Biscuits, unenriched	40	Cheddar Cheese	16	Oyster Crackers	10	Hershey Bar	56.7
	Milk, skim	100	Sweet Pickle	5	Tomato Juice, canned	25	Hershey Kisses	60
			Bread, unenriched	50	Egg Yolk	17		
			Mayonnaise	10	Potato, boiled	75		
			Cabbage Slaw	50	Peas, frozen	80		
			Dressing	18	Carrots, raw	25		
			Pears, canned	100	Raisins	13.3		
			Milk, skim	244	Butter	15		
					Bread, unenriched	25		
					Jello, dry	21.7		
					Sugar	20		
Day 5	Grapefruit Sections, canned	80	Bologna	80	Tomato Juice, canned	125	Hershey Goodbar	9
	Sugar	5	Bread, unenriched	50	Pork Chop	90	Hershey Bar	56.7
	Egg	54	Butter	2	Rice	84	Hershey Kisses	60
	Toast, unenriched	25	Lettuce	50	Broccoli, frozen	70		
	Milk, skim	100	French Dressing	5	Cranberry Jelly	6.5		
			Cherries, water packed	100	Lettuce	25		
			Milk, skim	200	French Bread, unenriched	40		
					Butter	5		
					Vanilla Ice Cream	124		
					Butterscotch Sauce	19		

Table 9. Calculated nutrient intake of daily menus.[1]

Day	Food energy	Protein	Fat	Carbohydrate	Calcium	Phosphorus	Iron	Vitamin A	Thiamine	Riboflavin	Niacin	Ascorbic acid
	Cal.	g.	g.	g.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Part I												
1	2256	79.8	106.9	250.6	1023	1226	9.6	9211	1.27	2.03	10.8	94
2	2257	82.6	104.5	256.5	1028	1437	9.4	5532	1.01	1.87	15.9	112
3	2224	87.8	95.9	269.3	1022	1370	9.4	5623	1.25	1.90	14.1	158
4	2259	82.0	111.4	242.0	1017	1467	10.0	6077	1.13	1.36	14.1	66
5	2250	80.0	106.3	250.9	966	1272	9.7	6046	1.46	1.84	12.2	128
Mean	2249	82.4	105.0	213.9	1011	1354	9.6	6498	1.22	1.80	13.4	112
Part II												
1	2337	83.0	111.6	258.3	942	1196	12.5	8817	2.26	2.04	13.3	124
2	2275	84.9	107.7	251.5	1017	1456	12.1	5634	2.52	1.91	19.2	109
3	2255	87.2	100.6	266.7	962	1362	12.2	5678	2.79	2.04	16.1	160
4	2250	85.0	108.6	242.8	1057	1577	13.0	6298	3.40	1.56	16.2	66
5	2288	80.4	108.2	259.6	1000	1290	12.6	6131	3.74	2.17	14.3	129
Mean	2281	84.1	107.3	255.8	996	1376	12.5	6512	2.94	1.94	15.8	118
Part III												
1	2392	80.0	117.2	264.1	1110	1308	12.6	8138	1.23	2.32	9.9	102
2	2291	85.4	106.8	256.7	1079	1475	11.5	3503	0.91	2.13	16.2	107
3	2318	91.2	107.4	261.8	1082	1471	12.1	4750	1.25	2.30	14.2	154
4	2275	82.9	101.3	265.4	1111	1466	13.0	5566	0.91	1.96	14.2	63
5	2365	81.9	114.7	260.3	1011	1339	13.0	5267	1.48	2.14	11.3	118
Mean	2328	84.3	109.5	261.7	1079	1412	12.4	5445	1.16	2.17	13.2	109

[1] Based on Watt and Merrill (1950) supplemented by Bowes and Church (1951).

Table 10. Nitrogen content of solid and liquid food composites.[1]

	Part I Period			Part II Period			Part III Period		
	1	2	3	4	5	6	7	8	9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
				Solid Food Composite					
	4.86	4.32	7.32	5.30	5.13	9.42	8.74	4.66	4.04
	4.27	4.45	7.37	5.16	5.11	9.42	8.34	4.60	4.02
	4.80	4.49	7.33	5.23	5.17	9.30	8.69	4.63	4.13
Mean	4.64	4.42	7.34	5.23	5.14	9.38	8.59	4.63	4.06
				Liquid Food Composite					
	2.98	3.07	3.20	2.85	2.66	1.36	1.12	2.33	2.17
	2.98	3.22	2.99	2.87	2.63	1.31	1.17	2.30	2.11
	3.01	3.08	3.02	2.82	2.66	1.30	1.19	2.30	2.18
Mean	2.99	3.12	3.07	2.85	2.65	1.32	1.16	2.31	2.15

[1] Macro-Kjeldahl Boric Acid Method (Scales and Harrison, 1920).

Table 11. Nitrogen content of chocolate candy and butter cookies.[1]

	Chocolate[2]	Chocolate[3]	Chocolate[4]	Butter cookies
	g.	g.	g.	g.
	.53	.72	.68	.294
	.55	.73	.64	.318
	.53	.73	.65	.286
Mean	.54	.73	.66	.299

[1] Macro-Kjeldahl Boric Acid Method (Scales and Harrison, 1920).
[2] Chocolate kisses, period 7 (60 grams).
[3] Chocolate kisses, periods 8 and 9 (60 grams).
[4] Chocolate bars, periods 7, 8, and 9 (56.7 grams).

Table 12. Nitrogen[1] excreted in the urine by subjects and by periods.

	Part I Period			Part II Period			Part III Period		
	1	2	3	4	5	6	7	8	9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
Subject A									
	10.71	10.64	10.69	10.67	10.39	9.89	10.28	10.25	10.84
	10.70	10.64	10.79	10.67	10.39	9.49	10.32	10.29	11.09
	11.09	9.78	10.59	10.37	10.30	9.80	10.24	10.10	10.58
Mean	10.83	10.35	10.69	10.57	10.36	9.73	10.28	10.21	10.84
Subject B									
	8.43	9.89	10.06	7.34	8.06	7.75	9.78	8.05	7.26
	8.58	9.71	9.91	7.61	8.05	7.96	9.65	7.97	7.56
	8.33	9.87	10.18	7.51	7.74	7.70	9.69	8.02	7.45
Mean	8.45	9.82	10.05	7.49	7.95	7.80	9.71	8.01	7.42
Subject C									
	11.35	11.92	11.31	12.25	9.73	11.57	10.17	9.71	6.42
	11.71	11.75	11.56	12.21	9.74	11.54	10.46	9.64	6.51
	11.52	11.80	11.35	11.97	9.45	11.50	10.16	9.54	6.48
Mean	11.53	11.82	11.41	12.14	9.64	11.54	10.26	9.63	6.47
Subject D									
	11.86	11.87	11.13	11.64	11.19	11.04	11.77	11.04	10.40
	11.73	12.13	11.15	11.79	10.86	11.06	12.03	11.05	10.77
	11.94	12.20	11.29	11.58	10.96	10.88	11.78	10.94	10.50
Mean	11.84	12.07	11.19	11.67	11.00	10.99	11.86	11.01	10.56

Table 12. (concluded)

	Part I Period			Part II Period			Part III Period		
	1	2	3	4	5	6	7	8	9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
				Subject E					
	10.24	10.97	11.36	11.06	12.03	11.19	10.34	10.98	11.71
	10.24	11.77	11.25	11.38	11.37	11.41	10.63	10.96	11.77
	10.24	11.85	11.31	10.54	12.06	10.95	10.14	10.99	11.78
Mean	10.24	11.53	11.31	10.99	11.82	11.18	10.37	10.98	11.75
				Subject F					
	10.38	11.15	12.76	11.15	10.25	10.83	10.95	11.45	12.11
	10.40	11.38	12.90	11.13	10.26	10.86	10.49	11.45	11.56
	10.18	10.88	12.31	11.13	10.29	10.71	10.77	11.36	11.86
Mean	10.32	11.14	12.66	11.14	10.27	10.80	10.74	11.42	11.84

[1] Macro-Kjeldahl Boric Acid Method (Scales and Harrison, 1920).

Table 13. Nitrogen[1] excreted in the feces by subjects and by periods.

	Part I Period			Part II Period			Part III Period		
	1	2	3	4	5	6	7	8	9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
Subject A									
	1.28	1.36	1.32	1.55	1.50	1.38	1.77	1.92	1.66
	1.28	1.36	1.34	1.54	1.47	1.38	1.77	1.92	1.67
	1.27	1.38	1.32	1.58	1.52	1.37	1.79	2.00	1.67
Mean	1.28	1.37	1.33	1.56	1.50	1.38	1.78	1.95	1.67
Subject B									
	0.37	0.36	0.99	1.15		1.61	1.58	1.54	1.74
	0.37	0.36	1.00	1.14		1.63	1.57	1.52	1.77
	0.36	0.35	0.99	1.19		1.59	1.56	1.01	1.74
Mean	0.37	0.36	0.99	1.16		1.61	1.57	1.36	1.75
Subject C									
	1.24	1.11	1.05	1.16	1.41	1.22	1.51	1.28	1.70
	1.22	1.09	1.05	1.17	1.40	1.16	1.40	1.26	1.68
	1.22	1.07	1.09	1.15	1.38	1.16	1.40	1.22	1.68
Mean	1.23	1.09	1.06	1.16	1.40	1.18	1.44	1.25	1.69
Subject D									
	0.83	0.69	0.64	0.80	0.88	0.80	0.83	1.22	0.97
	0.87	0.70	0.66	0.80	0.87	0.82	0.82	1.21	0.96
	0.83	0.68	0.66	0.78	0.87	0.81	0.82	1.17	0.98
Mean	0.84	0.69	0.65	0.79	0.87	0.81	0.82	1.20	0.97

Table 13. (concluded)

	Part I Period			Part II Period			Part III Period		
	1	2	3	4	5	6	7	8	9
	g.	g.	g.	g.	g.	g.	g.	g.	g.
				Subject E					
	1.20	1.10	1.13	1.04	1.03	1.16	1.18	1.41	1.40
	1.22	1.11	1.10	1.06	1.04	1.17	1.15	1.42	1.41
	1.18	1.07	1.08	1.01	1.04	1.18	1.14	1.40	1.41
Mean	1.20	1.09	1.10	1.04	1.04	1.17	1.16	1.41	1.41
				Subject F					
	1.02	0.81	1.06	0.62	0.86	1.35	0.83	1.73	1.55
	1.03	0.77	1.05	0.61	0.85	1.33	0.83	1.72	1.53
	1.05	0.78	1.06	0.60	0.86	1.35	0.84	1.80	1.58
Mean	1.03	0.79	1.06	0.61	0.86	1.34	0.83	1.75	1.55

[1] Macro-Kjeldahl Boric Acid Method (Scales and Harrison, 1920).

Table 14. Nitrogen excreted in the menses by subjects and by cycles.

Length of menstruation : days	Nitrogen loss per period : g.			Mean nitrogen loss : g.
		Subject A		
8/4 - 8/7	0.42	0.44	0.40	0.42
8/26 - 8/29	0.35	0.35	0.35	0.35
8/26 - 8/29	1.09	1.06	1.11	1.09
		Subject B		
6/10 - 6/16	1.07	1.06	1.11	1.08
7/9 - 7/14	0.90	0.89	0.92	0.90
		Subject C		
6/9 - 6/15	1.21	1.20	1.20	1.20
7/13 - 7/17	1.02	1.01	1.00	1.01
		Subject D		
6/8 - 6/13	0.82	0.81	0.83	0.82
6/8 - 6/13	1.18	1.21	1.22	1.20
6/8 - 6/13	0.93	0.88	0.91	0.91
6/8 - 6/13	1.02	1.02	1.02	1.02
6/8 - 6/13	0.81	0.81	0.81	0.81
7/17 - 7/22	0.09	0.07	0.08	0.08
7/17 - 7/22	0.41	0.40	0.42	0.41
		Subject E		
6/11 - 6/15	1.43	1.40	1.36	1.40
6/28 - 7/2	0.33	0.34	0.44	0.37
6/28 - 7/2	0.54	0.59	0.48	0.54
6/28 - 7/2	1.07	1.09	1.03	1.06
7/16 - 7/20	2.09	2.19	2.13	2.14
		Subject F		
6/13 - 6/15	0.34	0.32	0.33	0.33
6/13 - 6/15	1.57	1.62	1.57	1.59
7/8 - 7/12	0.15	0.18	0.15	0.16
7/8 - 7/12	0.67	0.71	0.70	0.69
7/8 - 7/12	1.00	1.05	1.02	1.02
7/8 - 7/12	0.34	0.37	0.34	0.35

NITROGEN BALANCE OF SIX 13- TO 14-YEAR-OLD GIRLS

by

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Nitrogen metabolism was studied in six healthy 13- to 14-year-old girls. The daily basal diet was calculated to include 82 to 84 g. protein (13.18 to 13.49 g. nitrogen). Certain other foods were allowed ad libitum.

The investigation was comprised of a five-day adjustment period and nine five-day experimental periods. One set of five daily diet plans was used during Periods 1, 2, and 3, another set during Periods 4, 5, and 6, and a third set during Periods 7, 8, and 9.

The macro-Kjeldahl method with a boric acid modification was used in the analysis of the food and excretory products.

Analyzed values for basal dietary nitrogen were from 18.8 to 42.8 percent lower than the calculated values. Also, great variations in nitrogen occurred among the periods when the same dietary plan was used. Analyzed values for the basal diet ranged from 7.54 g. to 10.95 g. nitrogen.

For the entire investigation the six subjects had total mean nitrogen intakes of 9.48 g., 9.62 g., 9.65 g., 9.68 g., 10.77 g., and 10.92 g. Considering body surface area the mean intakes ranged from 5.83 g. to 7.28 g. nitrogen per m^2 with an average of 6.24 g. nitrogen. By body weight, the intakes ranged from 0.157 g. to 0.202 g. nitrogen per kg.

Mean daily urinary excretion of nitrogen varied from 8.52 to 11.35 g. nitrogen for the individual subjects with an average of 10.49 g. for six girls. Mean daily fecal excretion of nitrogen varied from 0.85 to 1.54 g. with an average of 1.16 g.

Nitrogen excretion was not dependent upon intake when the data were analyzed statistically.

A mean nitrogen absorption of 88.1 (83.8 to 90.9) percent was calculated for the six subjects. Four of the subjects had negative retentions ranging from -2.28 g. to -2.83 g. (-23.6 to -29.8 percent) nitrogen, whereas one lost -0.75 g. (-6.9 percent) nitrogen. The sixth subject had a total mean positive retention of 1.23 g. (11.4 percent) nitrogen. The nitrogen retentions on the basis of body surface area were -1.81 g., -1.66 g., -1.58 g., -1.37 g., -0.43 g., and +0.83 g. By body weight the retentions were -0.054 g., -0.048 g., -0.042 g., -0.038 g., -0.012 g., and +0.023 g.