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Relation of protein utilization to nitrogenous components of blood and urine in women

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RELATION OF PROTEIN UTILIZATION TO NITROGENOUS COMPONENTS OF BLOOD AND URINE IN WOMEN

by

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INTRODUCTION

Numerous reports concerning attempts to evaluate the nutritional status with regard to protein adequacy of adult human beings have appeared in the literature. However, studies on human beings living on customary diets are few. Most of these studies have been carried out by means of nitrogen balance tests (McKay et al., 1942, Roberts et al., 1948, Ohlson et al., 1952, and Swanson, 1956). In these studies, attempts were made to investigate the relation of protein intake to nitrogen retention of women living on their self-chosen diets as well as to estimate minimum requirements for this nutrient. These studies have made a contribution in providing information as to the nutritional status with respect to protein in relation to the dietary habits of adult human beings.

The use of nitrogen balance methods in the evaluation of status of protein adequacy is based on the assumption that healthy adults should be in nitrogen equilibrium and that any deviation therefrom may be expression either of the matter of dietary adequacy or of changes of body composition. Hegsted (1952), however, has raised questions as to whether balance tests as ordinarily conducted yield reliable estimates of the adequacy of a nutrient for an adult. He has commented on the significance of the metabolic balance as follows:

> It should be recognized that no person consuming freely selected diets is "in balance", except as an average

> situation. In practical terms, "in balance" means that the sum of the negative and positive balances is zero over the long run. Actually a person is in either negative or positive balance most of the time. He would be at zero balance rather rarely and this would depend entirely upon how exactly the measurements were made. In view of the dynamics of the situation we might expect that several days of positive balance would be required to compensate for one day of negative balance and that he would be in positive balance most of the time. p. 259

In the interpretation of results obtained from short-term studies, the long-term implications of any positive or negative balance that might have arisen during such studies should be considered.

Nitrogen balance studies may not always provide data that are pertinent as estimates of actual nutritional status with regard to body protein. It is known that the balance studies yield data that reflect adaptation to intake. An individual who has previously depleted his tissue mass may be able to attain equilibrium on a low protein intake. However, the amount of protein consumed, in this instance, may not be satisfactory for the support of an optimum state of protein nutrition, even though the over-all picture of nitrogen balance indicates that the individual is essentially in nitrogen equilibrium. The optimum state of protein nutrition may be described as one in which the protein reserves in the body are at a suitable level for the maintenance of metabolic normalcy so that the organism may meet various stresses of life with a minimum demand or strain on the body's protective homeostatic mechanisms.

The nitrogen balance is the sum of gains or losses of pro-

tein from all tissues of the body. Shohl (1939) has pointed out that the balance method can never give more than a bank balance; it can not state how the income was invested but only how much was spent. Animal studies have shown that depletion of the body reserves of protein may result in imbalances of tissue proteins and enzyme systems (Allison, 1955). Results of the balance test do not lend themselves to interpretation of the extent of protein storage in the body nor to the possible shifts of tissue proteins between various compartments of the body. Allison (1951), presenting experimental evidences in animals, has stated that "it is possible for an animal to be in positive nitrogen balance and yet be depleting some labile stores in protein. Nitrogen balances, like body weight or growth, is the summation of many variables and the significance of the balance becomes more meaningful as those variables are evaluated".

Mitchell (1949) has estimated that the nitrogen balance of a group of young men living on an adequate diet over a long period was consistently positive as ordinarily determined. However, dermal excretion of nitrogen in the insensible perspiration with minimal sweating may have accounted for 0.38 gram of nitrogen loss daily, and approximately 1 gram of nitrogen daily was considered as being retained for adult growth, relating chiefly to the growth of the skin and the dermal structures. The nitrogen balance test as ordinarily conducted does

not measure the nitrogen loss in sweat, growth and loss of hair. At present, sufficient data to evaluate the magnitude of losses via these routes have not been obtained. Consequently the steady state of nitrogen equilibrium may be reflected in a slightly positive balance as determined by the usual balance technique.

Methods using isotopes have been employed in the investigation of the mechanism of protein retention in adult humans. Sharp et al. (1957) have stated that the use of N^{15} presents many advantages over the balance method in the study of protein utilization in human subjects. The use of N^{15} is the most direct way in which the turnover of the protein pool may be estimated. However, the data provided by tracer methods may be more valuable if they can be interpreted in combination with those obtained from the nitrogen balance test.

Another approach in the investigation of protein metabolism in human beings has been through studies on nitrogenous components in blood and urine. The investigations of Folin (1905) on the effect of protein intakes on the levels of nitrogenous components in blood and urine led to the concept of endogenous and exogenous protein metabolism. His theory of the dichotomy of protein metabolism has been re-evaluated by the isotopic tracer studies of Schoenheimer et al. (1939). These authors demonstrated the dynamic state of body constituents, and emphasized that the rate of breakdown and resynthesis of tis-

sue protein as well as the rate of interaction between tissue constituents and the diet were for greater than had been envisaged in Folin's theory. However, the investigations of Folin have their value in presenting the close relationships between these nitrogenous components and protein metabolism. Studies on adult animals have shown that the contents of several nitrogenous components in blood and urine are correlated with the magnitude of protein reserves in tissues (Allison, et al., 1946). These nitrogenous components may possibly serve as an index of protein nutrition in adult human beings.

Since very few studies have been done on the evaluation of protein nutrition of adult human beings living on customary diets, further extension of the knowledge with regard to this aspect seemed desirable. In view of the questions that have been raised concerning the usefulness of the data provided by the balance test, the development of other methods which may yield data helpful in support of the interpretation of those obtained from nitrogen balance tests are needed. The present investigation, therefore, was initiated to study the possibility of using nitrogenous components of blood and urine as an index of the state of protein nutrition in women. The objectives were:

1. To investigate the pattern of utilization of nitrogen by women on different planes of nitrogen intakes by means of continuous daily balance studies over extended periods of

time.

2. To investigate the possible variations in selected nitrogenous components of blood and urine in relation to protein utilization on different planes of nitrogen intakes.

3. To investigate the possible relationship of the plasma cholesterol content to protein retention.

The present study was one phase of Project 1028 of the Iowa Agricultural and Home Economics Experiment Station entitled Evaluation of the state of nutrition of the older people of the population and estimation of dietary needs, which was a contributing project to the North Central Regional Project, NC-5, Nutritional status and dietary needs of population groups in the North Central Region.

REVIEW OF LITERATURE

Nutritional Status with Regard to Body Protein

Utilization of dietary nitrogen in the evaluation of nutritional status with regard to protein

Nitrogen retention It has been recognized that a continuous turnover of nitrogen takes place in the body, a turnover that is a part of the integrated catabolic and anabolic processes involving body proteins (Schoenheimer et al., 1939). The function of dietary proteins is to supply the amino acids in proper amounts and kinds to meet the demands of anabolism. The catabolic processes may also supply energy and intermediary compounds essential for anabolism. Through the intercommunication afforded by various body fluids, amino acids provided by tissues become indistinguishable from those provided by the diet (Sprinson and Rittenberg, 1949). Amino acids from both sources are withdrawn from the metabolic pool for the synthesis of new tissue proteins, for the production of metabolites, for the supply of energy, or for other purposes as need may direct. Dietary proteins must be provided if the tissues are to be properly maintained. If the diet fails to replenish the metabolic pool, the tissue proteins are called upon to step up their various contributions. When the rate of catabolism exceeds that of anabolism, depletion in tissue proteins may occur.

The investigations of Sprinson and Rittenberg (1949) and

Solomon and Tarver (1952) have shown that the rate of interchange of tissue proteins may depend upon the supply of amino acids. Thus, the nature and the amount of dietary proteins may have much influence upon the rate at which protein is metabolized by the body.

The measure of nitrogen retention in response to the utilization of dietary protein has been used in the evaluation of the nutritional status of an individual with regard to body protein. The nitrogen balance, the difference between nitrogen intake and nitrogen loss from the body, may serve as a measure of the extent of protein retained or lost from the body. The nitrogen loss is the sum of the nitrogen that leaves the body in the urine, feces, sweat, skin scales, hair, and various other possible routes. However, in the nitrogen balance tests ordinarily conducted, only urine and feces have been considered (Mitchell, 1949).

An individual is said to be in nitrogen equilibrium when the nitrogen output equals the nitrogen content of his diet. The presence of a positive nitrogen balance is usually interpretated as an indication that the individual is retaining protein, a negative balance is evidence of tissue breakdown not fully compensated for by synthesis. The healthy adult body tends to establish nitrogen equilibrium by adjusting its rate of protein metabolism to its food supply (Sprinson and Rittenberg, 1949; Solomon and Tarver, 1952). When the body is

accustomed to a certain rate of protein metabolism, it may require a certain length of time to adjust itself to a higher or lower rate. Steggerda (1956) reported that in three adult males, during the transition from a low (5 grams of nitrogen per day) to a high (12 or 19 grams) or from a high to a low nitrogen intake, there was a marked excretion lag of nitrogen in the urine lasting approximately 4 to 6 days in each subject irrespective of whether the high level of nitrogen intake was 12 grams or 19 grams daily.

Therefore, in healthy adults, a transitory period of nitrogen retention in the body may occur as the result of an increase of the protein level in the diet, but a persistent retention may occur when new tissue is being constructed, as for instance in pregnancy, or in cases where, owing to a previously insufficient intake or a wasting disease, the body protein has been diminished and consequently, the retention of protein occurs.

On the other hand, a transitory period of loss of nitrogen from the body may be due to the ingestion of less than the usual amount of protein in the diet. When the body loses nitrogen for an extended period of time, it is probable that either non-dietary factors are operating or that the diet is inadequate in the amount or quality of protein or insufficient in calories so that the usual adjustment can not take place.

Various factors have been found to influence the magni-

tude of nitrogen retention in the adult body. These factors will be discussed separately in the following sections.

Factors influencing the extent of nitrogen retention

Dietary factors

The amount and the quality of protein in the diet

Studies on animals and human subjects have shown a direct relationship between nitrogen retention and the quantity of nitrogen consumed. Balance studies with women living on self-chosen diets showed that nitrogen retention was significantly related to intake (McKay et al., 1942, Ohlson, et al., 1952). Studies with young men (Johnson et al., 1954) and young women (Wharton et al., 1953) subsisting on diets with different nutrient combinations also showed that nitrogen retention increased in direct proportion to nitrogen intake when the caloric intake was adequate. Roberts et al. (1948) reported that in a group of older women, with increased protein intake, not only the mean daily retentions, but also the number of subjects in the group retaining nitrogen increased. Works related to predicted values of nitrogen intake for nitrogen equilibrium in women living on self-chosen diets are presented in Table 1.

In animal studies, a decrease of the rate of nitrogen retention with an increase in the concentration of dietary protein has been reported. The relationship between nitrogen retention and absorbed nitrogen was curvilinear rather than

Table 1. Summary of reports on nitrogen retention of women living on self-chosen diets

Authors	Number of subjects	Age range	Nitrogen balance gm. per day	Nitrogen intake predicted for equilibrium gm. per day
McKay, et al. (1942)	124	College age	Range--1.67 to +2.89	9
Ohlson, et al. (1948)	15	50-75	Range--0.82 to +2.65	9.2
Roberts, et al. (1948)	9	52-74	Range--0.2 to +3.2	9.4
Ohlson, et al. (1952)	25	30-39	Average +0.08 ± 0.24	10.64
	34	40-49	Average -0.18 ± 0.30	10.98
	40	50-59	Average -0.47 ± 0.23	11.20
	25	60-69	Average -0.46 ± 0.24	10.76
	11	70-79	Average -0.32 ± 0.36	9.42

linear when proteins were fed to rats at different levels in the diet (Forbes et al., 1958). Allison and Anderson (1945) reported similar results in dogs. The relationship between nitrogen balance and absorbed nitrogen was linear in the region of negative balance, the linearity extended over into the positive side but became obviously curvilinear well on the positive side of nitrogen balance.

The effect of the quality of various dietary proteins in maintaining the adult organism has been recognized. Data presented by Allison (1958) showed that when protein-depleted dogs were given 0.6 gram of casein nitrogen and 80 cal. per day per kg. of body weight, nitrogen equilibrium was approached in 23 days. At that time approximately 10 grams of nitrogen per kg. of body weight had been retained. When the same amount of wheat gluten was fed at the same level of caloric intake, nitrogen retention was less, and nitrogen equilibrium was not approached in the period of the study. Therefore the rate of filling the protein reserves was shown to be a function of the nutritive value or the pattern of amino acid composition of the dietary protein. Wheat gluten is primarily deficient in lysine. The supplementation of gluten with this amino acid increased the protein retention to the same rate as that established for casein.

The adult requirements for essential amino acids have been

studied in adult males (Rose et al., 1955) and in young women (Jones et al., 1956, Leverton et al., 1956, and Swendseid et al., 1956). Data relating to the intake of amino acids of adult women living on customary diets have been reported (Wharton et al., 1953 and Reynolds et al., 1953). Since the amounts of most of the essential amino acids furnished by customary diets are likely to be greater than those recommended by Rose for young men, it is not surprising that no apparent relationship of nitrogen retention to intake of amino acids was observed in these studies.

Work related to the improvement of the nutritive quality of dietary proteins by supplementation with amino acids has also led to an awareness of the importance of amino acid balance (Harper, 1958). Immediately after the ingestion of an unbalanced diet, rats showed a marked reduction in total nitrogen retention, in food consumption, and in the capacity to retain the ingested nitrogen (Deshpande et al., 1958). Hundley et al. (1957) tested lysine, threonine and other amino acids as supplements to rice diets and obtained some evidence for an amino acid imbalance in man. These authors noted considerable individual variations in nitrogen retentions in the response to amino acid supplements.

In summary, the maintenance of nitrogen retention in the body of both adult human beings and animals depends upon the composition of amino acids in the dietary proteins and on the

amount of total protein consumed. The proportions of various essential amino acids also appear to be of importance with regard to the supplementation of a diet in which proteins from vegetable sources occupy a large part.

The caloric value of the diet The influence of the energy value of the diet on nitrogen utilization has been pointed out in the reports of Leverton and Gram (1949) and Ohlson et al. (1952) in the studies of adult women and by Oldham (1955) in the studies of pregnant women. When adequate energy was not available from the diet and from the body stores, the body seemed to draw upon protein for the supply of the needed energy (Swanson, 1951, Rosenthal and Allison, 1951). On the other hand, the addition of energy-yielding nutrients to an already adequate diet led to the retention of nitrogen (Munro and Naismith, 1953). Food energy, therefore, may conserve body protein under certain conditions, and there may be an optimum caloric intake to keep a balance between nitrogen retention and organ composition in the adult animal body (Rosenthal and Allison, 1956). The preexisting nutritional state of the organism particularly with respect to reserves of protein and fat may also influence this relationship. Animals with full reserves could resist a marked lowering of energy intake over a long period of time better than those with low reserves (Allison et al., 1946, Calloway and Spector, 1955).

The body may be able to adapt to caloric restrictions

within certain physiological limits. The decrease in nitrogen retention in the presence of a restricted caloric intake in animals and humans under various experimental conditions illustrates a minimum requirement for calories in the utilization of protein by the body. At a daily intake of 1000 calories, an intake of 12 grams of nitrogen per day did not ameliorate a negative nitrogen balance in men (Johnson et al., 1954). The nitrogen balance index of egg protein was still unity in adult dogs fed a diet that contained only 50 per cent of their caloric requirement, but it decreased rapidly when the calories were reduced to 25 per cent (Allison et al., 1946).

Physiological factors

Body stores of protein Nitrogen retention is influenced by the state of the protein stores of the body. When feeding whole egg protein to protein-depleted dogs, high positive balances resulted, indicating the increased capacity of the animals to retain protein (Allison, 1951). While feeding the same amount and kind of protein to dogs with full protein stores, only a slightly positive nitrogen balance was produced. Allison (1951) concluded that "the degree of depletion of the protein stores in an animal can be estimated by the magnitude of the positive balance which is produced by a given nitrogen intake".

In malnourished adults, a similar relationship between

nitrogen intake and nitrogen retention has also been observed. Forsythe et al. (1955), in their study with a group of soldiers who had been severely injured and malnourished, reported that nitrogen retention was directly related to intake on an adequate calorie value of the diet, with nitrogen intakes ranging from 0.16 to 0.85 gram per kg. per day. No diminished efficiency in the utilization of dietary protein was observed at the higher levels of nitrogen intake. Co Tui et al. (1954) observed that the increase in protein intake (from 2 grams per kg. of protein to 3.8 or 4.5 grams per kg. of protein) of patients with pulmonary tuberculosis resulted in high positive nitrogen balances over a long period of time.

A long-term nitrogen balance study of Holmes et al. (1954) in adults showed a continued retention of considerable amounts of nitrogen under the condition of previous malnutrition. The subjects were East African natives who lived for a long time on diets very deficient in protein. These subjects stored large amounts of protein at rates up to 10 grams of nitrogen per day even after they had been on a high protein diet for a number of months. Although they appeared to be building up tissue, their gain in weight lagged behind the estimated amounts of tissue laid down. The exceedingly high nitrogen retentions were then corrected for possible losses of nitrogen through the skin. When this was done, the calculated accretion of body tissue corresponded fairly well with the gain in body

weight. This observation confirms Mitchell's report (1949) of the retention of nitrogen in well-nourished subjects.

Age During the period of growth and reproduction, the body retains part of the ingested protein to form new tissue protein; positive nitrogen balances exist when the diet is adequate. With attainment of adulthood, it is frequently assumed that growth ceases and consequently the nitrogen balance should be close to zero over a long period. However, it is known that growth of the adult body does not cease entirely. Hair, nails, and epithelial tissues continue to grow throughout life. Of particular significance is the growth of the skin, the outer layer of which is constantly being desquamated and replaced from the lower layers.

Other morphological aspects of adult growth have been described by Hrdlicka (1936) as follows:

> "the majority of the dimensions or characters, headed by statures, progress perceptibly on the average until well into the fourth decade of life; others, including the skull and the face, increase slightly, in some groups at least, until the fifth or even the sixth decade; and still others, such as the dorsal length and breadth of the nose, the length of the ears, width of mouth, depth of the chest, keep on augmenting generally throughout most, if not all, of the adult existence. p. 896

These observations indicate that a positive nitrogen balance may be expected throughout life. This problem had been considered by Mitchell (1949) as indicated previously.

Older people appeared to have a lowered capacity to retain protein as compared to young people. Kountz and associ-

ates (1951) found negative balances in four elderly people on an adequate diet. Positive nitrogen balances were produced by increasing the protein intake to twice the requirement of young adults. The daily retention of a group of older men from 53 to 83 years of age was lower than that of a group of younger men during a similar dietary regime (Daum et al., 1952). The lowered nitrogen retention in older people might not be due to difficulties in the digestion or absorption of protein. Chinn et al. (1956) were unable to demonstrate a discrepancy between the rate of digestion and absorption of an I^{131} - labeled albumin test meal in aged and young people. Sharp et al. (1956) found that age did not appear to produce depressing effects on the capacity to absorb a protein tracer during a period of high protein intake. Protein retention in young and older subjects was studied by Sharp et al. (1957) by means of a single feeding of N^{15} tagged yeast protein. The average nitrogen retention from absorbed nitrogen was 57.6 per cent in the young subjects (24 years), but only 49.1 per cent in the older subjects (average 66 years). The half-life of N^{15} in the body averaged 61 days for the young and 86 days for the old men. This indicated that the rate of protein turnover in the young was approximately one and one half times that in the older subjects. It seems that most of the evidence points to the fact that increased physiological age may be associated with a reduction in protein retention.

Hormones Effects of several hormones on protein metabolism have been known. Sex hormones and growth hormones exhibit an anabolic effect on protein metabolism (Russell, 1953). The adrenocorticotropic hormone and adrenacortical hormones seem to bring about increased protein catabolism (Cannon et al., 1956). Insulin appears to exert a protein sparing action in man (Kountz et al., 1953a). A detailed review will not be given here.

In their investigation, Ohlson et al. (1952) have suggested that the tendency toward negativity of nitrogen balance observed among women in the fifth and sixth decade of age may be associated with hormonal imbalance during the menopause.

Stress Studies on both animals and human beings have shown that stressful conditions may cause an increase in urinary nitrogen which may be an indication of an increase in the destruction of tissue proteins. Lowering or raising the environmental temperature (Chow et al., 1954), fractures (Ingle et al., 1947), surgical operations (Ariel, 1953), thermal trauma (Moore et al., 1950) and numerous other stresses of various types have resulted in negative nitrogen balances.

The relation of nitrogen excretion to emotionally stressful conditions in human subjects has been studied by Hetzel et al. (1954). These authors have observed that worry, nervousness, depression, or excitement caused an immediate increase in

nitrogen excretion which was associated with increases in urinary 17-hydroxycorticosteroid excretion.

The relationships between stress and protein metabolism in association with hormonal functions in humans and animals have been recognized. The effect of emotion, surgery, and other types of stresses on the adrenal medulla, cortex and protein metabolism has been reviewed by Donovan and Harris (1957). A detailed review of the experimental evidence will not be given in the present study.

Relation of plasma proteins and plasma non-protein nitrogenous components to protein metabolism

It has been discussed by Peters and VanSlyke (1946) that the nitrogen partition of urine resembles that of plasma rather than of serum, since the urinary constituents are derived from the plasma. However, in the literature, studies on nitrogenous components in both plasma and serum have been reported. Therefore, the review presented here includes investigations of constituents in both plasma and serum.

Plasma proteins

The significance of plasma proteins in relation to body protein

The total protein in the plasma contains three main fractions--albumin, globulins and fibrinogin. Though variations from species to species have been reported, the proportions of the three fractions remain relatively constant between healthy individuals of the same spe-

cies. Plasma globulin can be separated further into three fractions, alpha-, beta-, and gamma-globulins, by electrophoresis. In studies using electrophoretic analysis of plasma protein fractions, the concentration of each fraction has been expressed either as the percentage distribution in the total plasma protein or as its actual concentration in the plasma. The actual concentration of each fraction in the plasma may provide a better index than the relative distribution in various types of studies. For instance, feeding of low protein rations to dogs over extended periods of time resulted in a marked fall in the total plasma protein (Zeldis et al., 1945). This was entirely due to a decrease in the albumin level while the concentrations of the globulin fractions remained unchanged. In this case, the electrophoretic areas of each globulin fraction increased. Therefore, a change in the actual concentration of one fraction may affect the relative distribution of all the other fractions, though their absolute concentration had not changed.

Madden and Whipple (1940), reviewing their experimental evidence in dogs, have shown that the plasma protein reflected tissue protein reserves. The level of total plasma protein, however, may not be as significant as that of plasma albumin in evaluating the status of body protein, since it is influenced by the globulin fraction which may vary in a number of pathological states. The investigation of Sachar et al. (1942)

with hypoproteinemic dogs indicated the usefulness of hypoalbuminemia as a quantitative measure of tissue protein depletion. There was a direct relationship between plasma albumin and the total body protein induced by dietary proteins. Based on this relationship, these authors developed a formula for estimating the amount of the total loss or gain of tissue protein from the value of the serum albumin concentration. A reduction of 1 gram in the total circulating plasma albumin might indicate a loss of 30 grams of body protein. Stare and Davidson (1945) estimated that a reduction of plasma albumin concentration from 4 to 3.5 grams per 100 ml., would mean a tissue protein loss of about 510 grams in an individual with a plasma volume of 3500 ml. It should be noted here that studies of Whipple and Bobscheit-Robbins (1949) and Allison et al. (1946) on animals have shown that the concentration of the total plasma or serum protein or albumin remained unchanged for long periods of time in the presence of a protein deficiency. This might be due in part to the masking effect of hemoconcentration. If the volume of circulating plasma had been determined, it is possible that protein depletion could have been detected at an earlier stage.

Factors influencing the levels of plasma proteins

Protein intake Experimental evidences with regard to the effect of protein intake on the level of plasma proteins in human beings have shown that changes in the con-

centration of plasma proteins may depend upon the degree of variation in the protein content of the diet and the duration of the dietary treatment.

Small fluctuations of the protein content in the diet may not affect the level of plasma protein. Addis et al. (1948), in their study of 20 young men and women, reported that increasing the protein consumption of these individuals from 0.5, to 1.5, to 2.5 grams of protein per kilogram of body weight per day did not change the serum protein concentration. Morgan et al. (1955) reported that in a group of 573 elderly persons living on self-selected diets, no relation of serum protein levels with protein intakes could be detected. Youmans et al. (1943), in a survey of the nutritional status of some 1200 persons in middle Tennessee, observed no correlation of serum concentration with protein intakes.

Hegsted et al. (1946), on the other hand, found that in adult males, total plasma protein and albumin tended to decrease when low-protein all vegetable diets were fed at a level low enough to produce negative nitrogen balances.

In the individuals or in the population groups with a prolonged history of dietary protein deficiency, low concentrations of plasma proteins have been observed. Furthermore, the concentration of plasma proteins increased after an adequate intake of protein. Krebs (1946) described hypoproteinemia in a single case of an individual with malnutrition. Low values

were found for albumin and globulin and especially for gamma-globulin. When the individual was fed a high protein diet, the values rose, gamma-globulin changing from a level of 0.15 to 0.68 gram per cent. Adult men in East Africa suffering from protein deficiency were treated with a high protein diet (approximately 180 grams per day) by Holmes et al. (1954). At the beginning of the treatment, the subjects had low levels of serum albumin and raised levels of globulin due chiefly to a raised level of gamma-globulin. During treatment with a high protein diet, the serum albumin levels rose, while gamma-globulin levels fell. The levels of plasma proteins were expressed in grams per 100 ml. of serum.

The results obtained from animal studies as reported by several groups of workers have been conflicting. Long continued restrictions of dietary protein have resulted in decreased total plasma protein levels in rats (Frisch et al., 1929) and in dogs (Zeldis et al., 1945). In contrast, other workers failed to demonstrate a significant effect of the protein level in the diet on total plasma or serum protein concentration (Bloomfield, 1933, Metcoff et al., 1945, Allison et al., 1946, and Lippman, 1948). However, all of these authors have noted that the plasma volume was markedly reduced; presumbly the deficient animals maintained the plasma protein concentration by means of hemoconcentration.

The relation between protein restriction and the concen-

tration of individual fractions of the total plasma proteins have been studied. Electrophoretic and chemical analysis of plasma proteins have shown a general trend toward alterations in plasma albumin and globulin concentrations in the protein-depleted animals. Long continuous restriction of protein intake resulted in a striking decrease in plasma albumin. The plasma globulin concentrations, on the other hand, were only slightly altered (Chow et al., 1945, Zeldis et al., 1945, and Allison et al., 1946). The alpha-globulin fraction and fibrinogin were not reduced; the reduction in globulins was apparently restricted to the beta- and gamma-fractions of globulin. A concomittant drop in plasma volume was noted as the total circulating plasma protein was reduced by the process of depletion. Replenishment of protein by feeding a dietary protein restored albumin and globulin fractions to pre-depletion values. Plasma volume was corrected rapidly concomittant by the repletion of plasma albumin.

The effect of high protein diets on plasma protein concentration of rats has been reported by Leathem (1948). When adult rats were fed a diet containing 78 per cent casein or lactalbumin, the total plasma protein, albumin or globulin was not influenced. In young rats, the total plasma protein levels were depressed by the high protein diets due to a lower globulin level when casein was used and a lower albumin level when lactalbumin was fed. This experiment gives evidence that

the various sources of protein may exert specific effect on different fractions of plasma protein.

With the use of tracer techniques, the mechanism underlying the relationship between the protein intake and plasma protein concentrations has been studied. Steinbock and Tarver (1954) found that the turnover rate of the total plasma protein depended upon the level of dietary protein. Jeffay and Winzler (1958), measuring the turnover rate of the electrophoretically separated fractions, showed that the turnover rate of serum albumin was dependent upon the level of dietary protein, whereas that of the globulin fractions was not. The total plasma protein or albumin levels of rats maintained on protein-free diets showed longer half-lives and slower replacement rates than those rats maintained on adequate diets. A shift from protein free diets to adequate diets resulted in increased replacement rates which could mean an increase in the rate of plasma protein or albumin synthesis. The plasma protein or albumin of rats maintained on high protein diets turned over more rapidly than that of rats maintained on adequate protein intake. A further increase in the protein content of the diet, therefore, would result in an increase in the rate of degradation of protein or albumin. Therefore, whether a change in the protein content of the diet would result in an increase or a decrease of plasma protein concentration would depend on the magnitude of the deviation from an

adequate level of dietary protein.

Seeley (1945) reported a correlation between nitrogen retention and the amount of plasma protein repleted in response to a dietary protein in dogs subjected to plasmapheresis. During protein repletion the plasma protein concentration showed a definite rise as a result of an increase in plasma albumin. However, this occurred only within the area of positive nitrogen balance. The extent and duration of repletion were dependent upon the magnitude of the positive balance.

To summarize, it may be stated that in human subjects a small change in protein intake does not seem to affect the concentration of plasma protein. The effect of protein intake on the level of plasma protein may be demonstrated in individuals who have been depleted previously of body proteins due to a prolonged period of dietary protein deficiency. In these individuals the repletion of body protein with a large quantity of dietary protein may result in an increase in the level of the total plasma protein.

Plasma protein concentration in animals maintained on considerably low protein diets may be low, and may reflect the state of protein depletion. However, hypoproteinemia may not develop because of the fall in plasma volume which often, but not always, occurs. Furthermore, the total circulating plasma proteins are decreased mostly as a result of a decrease in the

albumin fraction. Whether the globulin fractions are affected or not may depend upon the severity of the protein depletion.

Age That the levels of total plasma or serum protein had a tendency to decrease with age had been reported by several groups of workers (Olbrich, 1948, and Morgan et al., 1955). Rafsky et al. (1952), in an electrophoretic study of serum proteins of subjects aged from 65 to 95 years, found that the albumin fraction was significantly decreased and the beta-globulin fraction increased. This resulted in a lowered albumin-globulin ratio, as compared with similar values obtained from young adults. Chresrow et al. (1958) also found that the total serum protein and albumin levels of a group of subjects from 70 to 94 years of age were lower than those for a group of young adults between the ages of 20 to 35 years. However, none of the other electrophoretic fractions of the older group differed substantially from those of the young adults. The significance for this age difference in total serum protein and albumin concentrations was not clear.

Plasma non-protein nitrogenous components The non-protein nitrogen fractions of plasma are a heterogeneous group of nitrogen-containing substances not precipitated by the usual protein-precipitating reagents. Under physiological and nearly all pathological conditions, the greatest and most variable fraction of the non-protein nitrogen is urea. The remainder of the non-protein nitrogen fraction includes a large

number of substances of varied origin and metabolic significance, such as amino acids, creatine, creatinine, and uric acid. Other nitrogenous substances are usually referred to as undetermined nitrogen.

The non-protein nitrogenous components are intermediary products of protein metabolism in the process of transportation, or end products of metabolism en route to excretion. As such, knowledge of their concentration in the blood may serve as criteria of the state of protein metabolism and as measures of the rate of protein metabolism.

That the level of total non-protein nitrogen of plasma may be influenced by the amounts of protein in the diet has been demonstrated. Kountz et al. (1953a) noted a direct relationship between serum non-protein nitrogen and protein intake in aged men. The plasma non-protein nitrogen rose with increasing protein intakes and it remained high for a considerable time. The non-protein nitrogen levels in the blood of adult rats were 30 to 34 mg. per cent for those fed 8 and 14 per cent protein in their diet for several months but 40 to 41 mg. for those allowed a 20 per cent level of protein in the diet (McCay et al., 1941).

Serum non-protein nitrogen levels were studied in 255 men and women over 50 years of age by Morgan et al. (1955). The mean levels were about 40 per cent higher than those usually found in young adults (Krebs, 1950). A positive correlation

between the serum non-protein nitrogen and the level of dietary protein was found in men but not in women. Men appeared to have a higher average concentration of non-protein nitrogen in the serum than women up to 70 years of age. After 70 years of age, the difference was not significant because there was some decline in the level of non-protein nitrogen. Olbrich (1948) observed a similar difference in the level of plasma non-protein nitrogen between males and females in 96 subjects. The non-protein nitrogen level showed a steady increase with advancing years, but did not extend beyond the usual range.

Urea Urea is the chief end product of protein metabolism. The most important cause of variations of plasma urea in healthy individuals seems to be the rate of protein metabolism, which is frequently correlated with the amount of protein in the diet. The level of urea in plasma or serum appeared to increase with the increase in protein consumption (Addis et al., 1947 and Goldring et al., 1934). Miller et al. (1941) observed the effect of different levels of protein in the diet on the serum urea levels of one individual. The subject remained on each level of protein intake until urinary nitrogen excretion indicated equilibrium; the blood was then taken for analysis. With increasing protein content in the diet, from 19 to 57, 95 and 150 grams per day, the urea nitrogen showed a steady increase from 4.0 to 10.7, 12.7 and 17.4 mg. per cent.

Persike (1948) observed that the rate of urea excretion was increased and the serum urea concentration rose in adult rats fed a calorie-restricted diet. These rats probably had used body protein to supply calories and, as a result, the rate of deamination was increased. When the diet was supplemented with ample amount of glucose solution, the animals were able to consume enough calories for energy needs. It was no longer necessary for them to use body protein for the supply of energy. Consequently, the rate of urea excretion and the serum urea concentration fell to the pre-treatment levels.

Lewis and Alving (1938) observed that the blood urea nitrogen values of healthy men increased with age. The average value for men at 40 years was 12.03 mg. per cent while that for men at 89 years was 17.62. The increase in blood urea concentration with age was associated with declined renal functions.

Creatine and creatinine Creatine is present in the blood chiefly in the cells. In the plasma it is found only in very low concentration and the level seems to govern the extent of urinary excretion (Tierney and Peters, 1943). The author of the present study failed to find any study concering the effect of protein intake on plasma creatine in the literatures.

The level of creatinine in plasma for the individual is relatively constant. Its concentration might depend on the

rate of transformation of phosphocreatine into creatinine in the muscle and the ability of the kidneys to excrete creatinine (Borsook and Dulnoff, 1947). Creatinine in the plasma appeared to be independent of the protein content in the diet. Barrett and Addis et al. (1947) reported that the average of 286 determinations of creatinine concentration in the serum of 10 medical students was 1.02 mg. per cent. Variation in protein consumption from 0.5 to 2.5 grams of protein per kilogram of body weight had no effect on the serum creatinine concentration. The serum creatinine levels in elderly people reported by Morgan et al. (1955) were 1.27 and 1.18 for men and women, respectively, which appeared to be higher than those for young adults studied by Folin and Suedberg (1930) and Hammett (1920). Morgan et al. (1955) did not find a correlation of serum creatinine levels with protein intake in any age group.

Uric acid Uric acid is the chief end-product of purine catabolism in humans. Purine-containing food seems, however, to have little effect on blood uric acid levels, since the blood uric acid levels of healthy adults were not appreciably altered by the change from a high purine to purine-free diet or vice versa (Brochner-Mortensen, 1940). On the other hand, plasma uric acid levels appeared to be lowered by high protein diets. Folin et al. (1924) observed that in human subjects more uric acid was excreted on high protein diets

than on low protein diets, while the plasma uric acid levels were lowered. These authors suggested that high protein intakes increased the responsiveness of kidneys, thus lowering the level of circulating uric acid with a temporary increase in uric acid excretion.

Other dietary factors may also affect the concentration of plasma uric acid. Harding et al. (1925) showed that in women the level of blood uric acid rose markedly with high fat intakes. The increase in blood uric acid observed on a high fat diet was correlated with decreased excretion. However, in the study of Morgan et al. (1955), there was no correlation between the serum uric acid levels and the fat intake in a group of elderly people. In their study, a slightly positive correlation between serum uric acid levels and protein intake was observed in men but not in women.

In human subjects, a sex difference in plasma uric acid concentrations has been shown. Praetorius (1951) observed that the serum uric acid level in men was significantly higher than that in women. There was a tendency for plasma uric acid content to increase with age in women but not in men. Gertler and Oppenheimer (1953) also observed the sex difference. The subjects in their study were aged from 65 to 88 years. In both male and female groups, the highest uric acid values appeared in the age ranging from 76 to 80 years. Morgan et al. (1955) reported that mean values for uric acid in a group of

older people were 28 per cent higher than those in a group of young people. Among the elderly people, the men, in nearly all groups up to 70 years of age, had higher blood uric acid values than the women. However, after 75 years of age, the uric acid levels in both sexes decreased and the differences between sexes became less marked.

The metabolism of uric acid is in some manner associated with the symptoms of gout (Brochner-Mortensen, 1940). However the underlying mechanism is not understood. The individuals who have gout frequently experience cardiovascular degenerative diseases and plasma uric acid levels have been studied in connection with cardiovascular impairment. Gertler et al. (1951) demonstrated that the serum uric acid levels were higher in a group of males under the age of forty with coronary heart disease than in a group of healthy males of a similar age group. In another report the serum uric acid level was significantly correlated with serum total cholesterol in men but not in the women (Gertler and Oppenheimer, 1953).

Relation of various urinary nitrogenous components to protein metabolism

Urea nitrogen The chief nitrogenous end-product of protein metabolism in mammals is urea. The rate of excretion of urea in urine may be related to the rate of deamination of the proteins whether of tissue or dietary origin. Therefore, the quantity of urea excreted in the urine may be the most

significant index of the extent of protein catabolism. The rate of urea excretion depends upon a complex of conditions. Some of the factors affecting the excretion of urea are the amount and quality of protein consumed, the rate of protein metabolism, the body protein reserves, and the ability of the kidney to excrete urea.

Folin (1905), in his classic paper, showed that when an individual shifted from an adequate protein diet to a low protein diet, the protein catabolism was greatly diminished after several days. The resultant decrease in nitrogen excretion (from 16.8 to 3.60 grams per day affected entirely the urea fraction of nitrogen in urine, which changed from 14.7 grams to 2.20 grams. The output of other nitrogenous compounds changed relatively little. Urea nitrogen constituted 87.5 per cent of the total urinary nitrogen on the adequate protein diet, but lowering the protein content of the diet reduced the urinary urea so much that the percentage fell to 61.7 per cent.

Smith (1926) also presented data showing that when protein was almost entirely removed from the diet of an individual, the urinary nitrogen fell, the urea and ammonia fractions diminished most strikingly, while creatinine and uric acid were relatively little affected. Urea which made up 83 per cent of the total urinary nitrogen on the unrestricted diet, constituted only 20 per cent at the end of the experiment. Phansalkar and Patwardhan (1954) reported that in 15

Indian adult males, the urea nitrogen excretion was 4.75 grams which represented 67.4 per cent of the total urinary nitrogen. The low level of nitrogen metabolism was apparently due to a low intake of protein as well as to the low digestibility of dietary protein of vegetable origin.

The magnitude of excretion of urea was usually high when the tissue proteins of the animal were maximum and low when tissue proteins were in a depleted state. Allison et al. (1946) showed that the excretion of urea decreased to a fairly constant, minimal value in dogs depleted in certain labile tissue proteins. The excretion of urea gradually increased during feeding of a dietary protein in the repletion period. Feeding egg protein to the protein-depleted dogs at a low level in the diet decreased the excretion of urea below that found in the same animal fed a protein-free diet. The result was interpreted to indicate that most of the amino acids of this dietary protein entering the metabolic pool were used for tissue synthesis, little being catabolized to form urea. Feeding casein or wheat gluten, on the other hand, increased the excretion of urea above that observed in the same animals fed a protein-free diet. This was interpreted to mean that only a portion of the amino acids furnished by casein and wheat gluten could be utilized for anabolic purposes; those not used for tissue synthesis were catabolized, thereby increasing the excretion of urea. Feeding casein supplemented

with methionine decreased the urea output to the same extent as feeding the egg protein. However, a further increase in the quantity of these dietary proteins increased rapidly the urea excretion. The maximum sparing effect of body protein by these dietary proteins was in the region of intake where nitrogen equilibrium was maintained.

As indicated earlier, Persike (1948) found that when the food intake of adult rats was restricted, animals lost body weight progressively and exhibited higher levels of urea in urine and serum. Since energy requirements of these rats were not fulfilled, they were forced to use body protein to supply calories, and there apparently was an increased rate of tissue protein catabolism.

Another example of the effect of change in rate of catabolism of tissue protein on the excretion of urea was reported in the study of Young and Cook (1955) when adult rats were exposed to three environmental temperatures, 4°C., 23°C., and 35°C. for 50 days each. At a low protein intake, the excretion of total urinary nitrogen and urea were found to be inversely proportional to the exposure temperature. The excretion of urea rose sharply as the exposure temperature fell, due to increased rate in protein catabolism occurring at low temperatures.

Ammonia nitrogen Ammonia has been shown to be excreted in urine in amounts which tended to adjust the acid-

base balance of the body. The small amount of ammonia which was produced from the deamination of amino acids and escaped being transformed into urea failed to account for an appreciable amount of ammonia in the urine (VanSlyke et al., 1943). The experiments of Benedict and Nash (1929) furnished evidence for the renal origin of urinary ammonia. VanSlyke et al. (1943) showed that ammonia is formed chiefly from glutamine in the kidneys by the enzyme, glutaminase. It is passed into the tubular urine where it acts as a base and participates in the renal regulation of "acid-base-balance". Consequently the proportion of ammonia in total urinary nitrogen tends to vary directly with the acidity.

The daily ammonia output tended to increase somewhat with the amount of protein consumed, owing probably to the acid-producing properties of the protein (Salter et al., 1933 and Allison et al., 1946). If the acidifying effect of dietary proteins was prevented by the addition of sodium bicarbonate or citrate with the high protein diet, ammonia excretion remained low (Salter et al., 1933).

Creatine and creatinine Various factors influence creatine and creatinine excretion in adults: these include sex, diet, and protein metabolism.

Sex Creatine is present frequently in the urine of adult women. Early work in this area indicated that creatine was absent from the urine of adult males. However,

Albanese and Wangerin (1944) used improved methods for analysis and reported that creatinuria occurred in all age groups in males. They concluded that the failure to observe creatinuria in adult males previously might have been due to the lack of sufficiently sensitive instruments. In their study, a Klett-Summerson photoelectric colorimeter was used instead of the Duboscq colorimeter used by Folin (1944). Tienery and Peters (1943) studied the relationship between serum and urine levels of creatine. They observed that creatine appeared in urine only when its concentration in the serum exceeded 0.58 mg. per cent. In adult male subjects in their study, serum creatine did not exceed this limit and therefore, creatine was absent from the urine. Daum et al. (1952) reported average values ranging from 0.105 to 0.166 grams of urinary creatine per day for seven men over fifty years of age.

In human adults the daily urinary creatinine excretion was higher in men than in women. The value depended upon the muscular development of the individual (Best et al., 1953). The obese women generally excreted less creatinine than the well-proportioned ones (Tager and Kirsch, 1942). The sex variation might be due to the different relative amounts of fatty and muscular tissues of male and female bodies.

Diet Diet may alter the excretion of creatine and creatinine by saturating the organisms with creatine pre-

cursors. Roth and Allison (1949) observed that in adult rats, the excretion of these materials was increased by the addition of excess methionine to a 12 per cent casein diet, alone or in combination with glycine and arginine. Liener and Schultze (1952) reported that when a basal diet containing 24 per cent soy bean protein was supplemented with choline, methionine, or homocystine plus choline or vitamin B_{12}, the excretion of creatinine by the weanling rats was markedly enhanced.

Adult men consuming a diet low in protein and restricted in vitamins of the B-complex over several months showed decreased average creatinine coefficients (mg. of urinary creatinine per kg. of body weight per day). This change was accompanied by deterioration of physical performance and of the development of symptoms of dietary deficiency (Friedemann et al., 1948). The supplementation of protein and vitamins increased the creatinine coefficients gradually and alleviated the symptoms of the deficiency. This study indicated that changes in metabolic processes were associated with the metabolism of creatinine and were reflected in the excretion of creatinine.

Protein metabolism The daily creatine excretion was studied by McLaughlin and Blunt (1923) and by Wang et al. (1930). Wang et al. observed that creatine varied directly with the protein intake while McLaughlin and Blunt failed to find any relationship of protein intake to creatine excretion.

The daily excretion of creatinine was relatively constant for each individual (Folin, 1905). Unlike the excretion of urea, which depended largely on the protein content in the diet, the creatinine was not appreciably influenced by variations in the protein intake in human subjects. The daily excretion of creatinine was also independent of the ingestion of creatine (Hyde, 1942). The quantity of urinary creatinine has been considered an index of the magnitude of tissue metabolism especially the index of the protoplasmic activity of muscle.

Beard (1943) studied the creatine-creatinine transformation in the adult rat in relation to the change in protein intake and showed that the rate of protein metabolism greatly influenced the metabolism and excretion of both creatine and creatinine. Creatine and creatinine almost disappeared from the urine of the animals fed a 5 per cent casein diet. When the animals were changed to a 20 per cent casein diet, an immediate increase in creatine and creatinine excretion occurred. Tidwell (1946), on the other hand, reported that an increased amount of creatinine was excreted by adult rats when they were shifted from a 20 per cent casein diet to a 5 per cent casein diet. This increased excretion of creatinine appeared to be associated with the time when the animals were losing weight. When the animals on the low protein diet maintained their body weight, the excretion of creatinine was not different from

that of a group of the animals receiving the 20 per cent casein diet.

The excretion of creatinine was not affected by a decrease of protein retentions as reported by Young and Cook (1955). Adult male rats maintained on a low protein diet and exposed to a temperature of 4°C. for 50 days excreted increasing amounts of creatine with the increase in rate of tissue protein catabolism, while the excretion of creatinine bore no apparent relation to external temperatures.

To summarize, the amount of urinary creatinine of an individual appears to be relatively constant and not related to the quantity of protein consumed. Urinary creatine, on the other hand, seems to be influenced by protein intake. Studies on animals in which protein-restricted diets were used show that the urinary creatinine and creatine may be related to the rate of protein metabolism. Diets which provide creatine precursors may also vary the excretion of these two components. In addition, age, sex, and the physical development of individuals should be taken into consideration in the interpretation of the amount of creatinine or creatine excreted in urine.

Uric acid Uric acid has been considered as the end-product of the metabolism of purines (Lewis et al., 1918, Geren et al., 1950). The amount of uric acid in urine depends upon the quantity of nucleoprotein ingested and that formed

from tissue nucleic acids. On purine-free diets, the urinary uric acid in human subjects was rather constant and might have reflected the rate of nucleoprotein catabolism (Christman and Mosier, 1929). With the ingestion of isotopic yeast nucleic acid, the purines of ingested nucleic acid were rapidly converted to urinary uric acid in human subjects. (Wilson et al., 1954).

The ingestion of caffeine and theophylline have been found to cause an increase in the excretion of uric acid (Clark and deLorimier, 1926). However, several authors indicated that these substances themselves might react with most of the uric acid reagents employed in the colorimetric methods (Hanzel and Myers, 1932, Buchanan et al., 1945).

Experiments in human subjects have been reported in which the urinary excretion of uric acid has been compared with the type of diets ingested. Folin's (1905) observation that the addition of protein to the purine-free diet usually increased uric acid output has been confirmed (Rose, 1921). Lewis et al. (1918) have made a careful study of the hourly elimination of uric acid by fasting individuals following the ingestion of purine-free protein and amino acids. Definite increases in uric acid excretion were observed with a maximum effect three hours after the ingestion of protein and two to three hours after the ingestion of the amino acids; glycine, aspartic acid, and alanine. The increased uric acid elimination was consid-

ered by these authors to be due to an increased production of uric acid as a result of the stimulating action of the absorbed amino acids. Wilson et al. (1952) and Benedict et al. (1953) reported similar results. Uric acid synthesis was found to be accelerated in both normal and gouty subjects when the subjects were on high protein diets. (Bien et al., 1953).

Fats appeared to have the opposite effect, causing the urinary uric acid to diminish, while the uric acid level in blood rose (Harding et al., 1925). Adlersberg and Ellenberg (1939) have also reported a diminution in the uric acid excretion when human subjects were consuming a diet containing 250 grams of fat. No such change took place when a high carbohydrate diet, equivalent in calorie value to the high fat diet, was ingested.

In conclusion, it appears that fluctuations in protein intake accompanied by changes in the protein metabolism may not affect the level of all the nitrogenous components in the plasma and their excretion in the urine to an equal extent. Plasma and urinary creatinine of an individual may remain relatively constant under most varied conditions. Little is known concerning the significance of plasma and urinary creatine in relation to protein metabolism, but correlations have been observed between urinary creatine and protein intake. Uric acid in the plasma and urine may vary but little with the

protein intake or catabolism. Both creatinine and uric acid in plasma and urine, however, may be affected by other components in the diet as well as a number of physiological factors. The fraction of urea nitrogen in plasma and that of urea plus ammonia nitrogen in urine, represents the chief end-products of protein catabolism and exhibit the greatest fluctuations with protein intakes.

Relation of Plasma Cholesterol to Protein Utilization

Much attention has been focused in recent years on blood cholesterol levels in human subjects, particularly in aging persons because of their possible relationship to cardiovascular disorders. Though the nature of the association of high serum levels of cholesterol and atherosclerosis and related clinical conditions is not clear at the present time, there has been a great interest in dietary factors which may cause a lowering of the level of serum cholesterol. A few studies have been made on the relationship between blood cholesterol levels and protein utilization. Loewe et al. (1954) observed that when cholesterol-fed rabbits were maintained on low calorie-high protein diets, some lowering of the levels of serum cholesterol resulted. Moyer et al. (1956) found that increasing the protein content of the diet of rats, in which cholic acid was added in order to heighten cholesterolemia, caused a progressive drop in serum values, whether the protein was

casein or soybean protein.

Experimental results on human beings and rats contrary to those mentioned above have been reported. Olson et al. (1957) found that subjects with hypercholesterolemia showed a decrease in serum cholesterol during a period of low-protein feeding with appreciable amount of fat in the diet. The serum cholesterol values returned to the original level upon the resumption of the usual diet. Young adult rats developed a marked hypocholesterolemia when fed soybean protein diets low in methionine and choline (Olson et al., 1958). The addition of choline or casein to the diet raised the serum cholesterol levels. The level and the type of dietary fat did not modify the effect of choline and casein upon serum lipids. Olson et al. (1958) suggested that low choline or methionine in the diet of the young adult rats might have caused an alternation on fat transport with a decrease in the level of plasma cholesterol. Nath and Harper (1959) also found that the substitution of wheat gluten for casein as the dietary protein caused a marked reduction in the serum cholesterol concentration of rats. These authors observed that the cholesterol-lowering activity of wheat gluten was associated with a lipid material in wheat gluten. When this material was extracted, wheat gluten lost its cholesterol-lowering activity.

The study of Allison et al. (1959) demonstrated a relationship between plasma cholesterol levels and the magnitude

of protein reserves in the body. When dogs and rats were fed a protein-free diet, the levels of plasma cholesterol increased with the depletion of body proteins, and reached the highest values at maximum loss in protein reserves. The plasma cholesterol levels returned to control values upon repletion.

Studies on human beings consuming mixed diets have failed to show a definite relationship between protein utilization and blood cholesterol levels. Keys (1953) and his associates have observed that populations habitually subsisting on diets low in fats tend to have low concentrations of serum cholesterol. However, many populations subsisting on low-fat diets are also low in their consumption of calories and of total protein, or of protein of animal origin. Keys and Anderson (1957) questioned whether dietary protein had any effect on the production of low serum cholesterol values in human beings. Adult males maintained on a low-protein diet (average 8.6 per cent of calories from protein) were subsequently given a high protein intake (17.7 per cent protein calories) for 28 days, in a third period the original protein level was given. The intake of fat and calories remained constant throughout. There was no significant change in serum cholesterol levels of the subjects on any plane of protein consumption. Lutz et al. (1959) also failed to observe any relationship between protein intakes and the serum cholesterol level in adult males. A

10-week controlled diet study was undertaken by these authors with 10 adult males. All subjects were consuming a mixed diet containing 105 grams of protein daily for four weeks, then five of the subjects were fed a diet containing 160 grams of protein for 6 weeks while the others ingested 60 grams of protein. The fat intakes were constant and the diets were isocaloric. Serum cholesterol showed no consistent differences between the groups of subjects maintained on different levels of protein intakes.

In summary, these studies seemed to show that variations of plasma or serum cholesterol might be correlated with the quantity of the protein intake. The amino acid composition of dietary protein might also play a role in controlling plasma or serum cholesterol levels. However, the effect of proteins on blood cholesterol levels seems to be more easily demonstrable in animals fed experimental diets with a wide range of variations in protein levels than in human beings consuming average mixed diets.

EXPERIMENTAL PROCEDURE

Plan of Study

The subjects

All subjects participating in the study were women living in Ames, Iowa. Their ages ranged from 35 to 82 years. Medical examinations were performed by a local physician. The hematological tests and the basal metabolic rate were determined in the laboratory for each subject. These tests showed that the women were in satisfactory state of health. All subjects lived at home and maintained their customary pattern of activity. Notes were recorded daily by the investigators regarding the general state of health, the emotional status, and the reaction of the subjects to the experiment.

General plan

The study was planned in three parts:

Long-term preliminary study: Series 1 It was believed that data obtained from daily nitrogen balance studies conducted over relatively long periods of time would give a better picture of the manner in which the subjects utilized the nitrogen contained in self-chosen diets than in data obtained from short-term investigations. One part of the study was planned on this basis. The day-to-day nitrogen balance studies were determined in four subjects for two periods. All subjects were living on their self-chosen diets in the first

period; in the second period, each subject was given daily a supplement of 100 grams of lean beef. The subjects and the duration of each period of observation are listed in Table 2.

Table 2. The subjects in the long-term preliminary study, series 1

Subject	Age (yr.)	Experimental period	Number of days
NN	68	I	56
		II	14
BG	75	I	28
		II	14
MV	75	I	84
		II	28
LU	78	I	35
		II	14

An attempt was made to evaluate the nutritional status of each woman with respect to protein utilization by calculating regressions of quantities of urinary excreted on quantities of nitrogen absorbed for the two dietary regimes. Values were expressed in mg. of nitrogen per kg. of body weight per day. The slopes of the lines and their intercepts were compared.

Short-term study: Series 2 The relation of the contents of several nitrogenous components in blood and urine to protein metabolism have been reviewed. It was thought

that some of these nitrogenous components might be related to the magnitude of protein reserves in the body tissues and might serve as an index of the measure of the state of protein nutrition in adult human subjects. Nitrogen balance studies were determined on six subjects aged 43 to 82 years living on self-chosen diets for a period of ten days. One fasting blood sample was taken from each subject. Selected nitrogenous components listed in Table 3 were analyzed in plasma and in 24-hour fresh urine samples from the day preceding that on which the blood was sampled. The plasma cholesterol content was also determined. The possible relation of these substances to protein intake and retention was evaluated.

Table 3. Nitrogenous components studied in plasma and urine

Plasma	Urine
Total protein	------
Electrophoretic analysis of plasma proteins	------
NPN	------
Urea nitrogen	Urea nitrogen
------	Ammonia nitrogen
Creatinine	Creatinine
Creatine	Creatine
Uric acid	Uric acid

Long-term controlled study: Series 3 This study was planned for an extended investigation of the possible variation of nitrogenous components in blood and urine in relation to protein utilization. Three subjects, LS, MY, and LU, aged 35, 55, and 81 years, participated in this study. The whole study was divided into four periods.

Period I. Self-chosen diet period--The subjects were maintained on their customary self-chosen diets for a period of 35 days. In this period, daily dietary records of the subjects were kept and intakes of calories and protein were calculated.

Period II. Protein-supplementation period--The menu pattern of the subjects established during the first period was repeated as closely as possible during a second period of 35 days. Each subject was given a daily supplement of 100 grams of lean ground beef. The daily meat supplement was divided into three portions for the three meals. Each portion contained 33.33 grams of the meat. The menus of the self-chosen diets were adjusted with a minimum of change so that the calorie value of the diets was the same as in the first period.

Period III. Protein reduction period--During this period, the menu pattern established during the first period was again reproduced for 28 days. The protein intake of two subjects, MY and LS, was adjusted by appropriate substitu-

tions of food to achieve a reduction of 20 per cent in protein intake from that of the period of self-chosen diet. Subject LU was given a meat supplement of 50 grams of lean ground beef daily. Adjustment was made of the calorie-yielding nutrients in the diet so that the diet consumed in this period would be isocaloric to that eaten in the first period.

Period IV. Self-chosen diet period--The pattern of eating of all three subjects established during the first period was repeated for a period of 28 days.

Day to day nitrogen balance studies were determined on each subject during the four periods. Regressions of quantities of urinary nitrogen on nitrogen absorbed per kg. of body weight per day were calculated for the four periods. One fasting blood sample was drawn from each subject at the end of each period. One 24-hour urine sample was saved at the end of each seven-day period. Blood and urine were subjected to the analysis of nitrogenous components listed in Table 2.

Collection of Materials for the Metabolism Study

The subjects were instructed in the techniques of weighing food, collecting and saving food samples and excreta. The subjects weighed all portions of food to be eaten on a spring balance at the time the meal was served. Any uneaten food was weighed at the end of the meal. The weight of the food consumed was recorded on a form especially prepared for the

purpose. A portion of one-fifth of the weight of each food sample consumed was weighed and these aliquots were composited for each 24-hour period and brought to the laboratory for analysis.

The 24-hour urine samples were quantitatively collected by the subjects in a brown jar in which toluene had been added as preservative. The fecal samples collected on each day were composited in the laboratory for a period of one week. Carmine was used to mark the feces of each seven-day period. The daily samples were quick frozen until the weekly collection was complete.

Analysis of Total Nitrogen in Food, Urine, and Feces

Methods for the preparation of brown digests used in the long-term preliminary study were given by Liu (1955). The following methods were used in the long-term controlled study. Each day all foods and excreta were brought into the laboratory. The food was homogenized in a Waring blender. The mixture was seived into a graduated cylinder and made up to volume. An aliquot of blended food material was transfered into a flask to which a suitable amount of concentrated hydrochloric acid had been added. The flask was then autoclaved for 45 minutes at 15 pounds pressure. The solution was made up to a volume. The final mixture contained 20 per cent of hydrochloric acid. A portion was transferred to a stoppered

bottle, labeled, and stored until the time of analysis.

The urine was measured in a graduated cylinder and the total volume recorded. One-fifth of the day's volume of urine was measured into a beaker; a suitable aliquot of concentrated hydrochloric acid was added. The beaker was then placed on a hot plate and heated until the content was brown in appearance. A final volume was made of the content so that the concentration of hydrochloric acid was approximately 20 per cent. The sample was preserved for analysis in the same manner as the food.

The frozen fecal collections for the seven-day period were thawed and transferred quantitatively into a Waring blender in which the fecal composite was homogenized. The material was then made up to a convenient volume in a volumetric flask, thoroughly mixed, and one-half of it was prepared into a brown digest by the same procedure as that used for the food samples.

In the short-term 10-day period study, food and fecal samples were freeze dried for the determination of total nitrogen. The food was homogenized in a Waring blender. The mixture was made up to volume. An aliquot of blended food material was transferred into a VirTis freeze-drying flask and dried by a VirTis Freeze-dryer, manufactured by the VirTis Co., Yonkers, N. Y. The total fecal collections for a 5-day period were dried quantitatively. The total nitrogen

in the urine was determined in the daily fresh urine samples.

The Kjeldahl-Gunning method was used for the determination of the total nitrogen in the food, urine and fecal samples. The author had checked her skill by analyzing a standard solution of nitrogen (creatinine) and had obtained satisfactory recoveries.

Analysis of Nitrogenous Components in Blood

The subject was brought to the laboratory before breakfast on the day the blood sample was drawn. Thirty to 40 ml. of fasting blood sample was taken. The blood was transferred immediately from the syringe into a 50 ml. centrifuge tube which had sufficient potassium oxalate as anticoagulant in the form of a thin dried film. The content of the tube was mixed thoroughly and was centrifuged after standing for 20 minutes. The supernatant plasma was separated, frozen at once, and kept for analysis.

The total nitrogen in the plasma was determined by the micro-Kjeldahl method (Wong, 1923). The value of non-protein nitrogen in mg. per 100 ml. of plasma was subtracted from that of the total, yielding the nitrogen value of the total plasma protein which multiplied by 6.25 gave the value for total plasma protein.

The procedure outlined by Raymound (1955) was used for the paper electrophoresis of plasma proteins. A three com-

partment electrophoretic apparatus, serial no. 838, manufactured by E-C Apparatus Company, New York, N. Y., was employed. The three compartments of the apparatus were filled with 0.1 M barbital buffer, pH 8.6, prepared by dissolving 14 grams of barbital and 103 grams of sodium barbital in 5 liters of distilled water. Whatman 3 MM chromatography paper strips were used for the separation of plasma proteins. The strips were evenly moistened with the buffer solution before use. Plasma was applied to the moist strip by means of a melting-point capillary. Approximately .01 ml. plasma was applied in a narrow band 1.5 cm. long.

Electrophoresis was carried out at a potential of 500 volts for a period of 4.5 hours. After removal from the electrophoresis cell, the strips were hung in a vertical position and dried at 115° for thirty minutes. For quantitative determination of proteins, the strips were dyed in bromophenol blue solution for 16 hours (Block et al., 1955). After removing from the dye, the strips were rinsed three times with 2 per cent acetic acid solution followed with a 2 per cent sodium acetate-10 per cent acetic acid solution. The strips were then dried in an oven at 115° C for 10 minutes.

Optical densities of the dyed strips were measured with the aid of a photometer, Model no. 425, manufactured by Photovolt Corporation, New York, N. Y. The strips had been dipped into mineral oil before the measurement. A graphic

plot was made from the optical density measurement. The relative concentration of each protein fraction was obtained by drawing perpendicular lines at the low points of the curve and by measuring the area under the curve with a planimeter. The area under each curve was then calculated into relative percentage in the total.

Densitometeric readings from the electrophoretic patterns of the plasma indicated five major areas representing albumin, alpha-, beta-globulins, fibrinogen, and gamma-globulin in order of decreasing mobilities. Preliminary studies were carried out to test the reproducibility of the electrophoretic determinations. The relative percentages of the fractions obtained from plasma were reproduced with a difference of approximately 3 per cent.

The protein-free filtrate was prepared from plasma according to the method of Folin and Wu (1919). The filtrate was used for the analysis of non-protein nitrogen, urea nitrogen, creatine, creatinine, and uric acid. Non-protein nitrogen was determined by the method of Kock and McMeekin (1924). Satisfactory recoveries from creatinine standard solutions had been established. The method of Karr (1924) was used for urea nitrogen. This method was modified by the author of the present study. Aqueous urease solution was used instead of the alcoholic solution prepared from jack bean meal. The author had found that a 1 per cent urease solution would yield

satisfactory results. The method of Folin and Wu (1919) was used for creatine and creatinine determinations. The method for uric acid was that of Benedict and Franke (1952). The measurement of optical density was made in a Klett-Summerson photoelectric colorimeter. The selection of wavelengths for the measurement of the optical density has been given by Hawk *et al.* (1947).

Analysis of Urine

The titrable acidity was determined by method of Henderson and Palmer (1914). Direct Nesslerization method modified by Folin and Youngburg (1919) was used for urea nitrogen. The author of the present study modified the procedure as described for plasma urea nitrogen. Ammonia nitrogen was determined by the Permutit method (Folin and Bell, 1917). Folin's (1914) method was employed for creatine and creatinine analysis. The procedure for uric acid was that developed by Benedict and Franke (1922). All of these procedures had been tested for the reliability of the results prior to use and had been proved to be satisfactory. The measurement of the optical density was performed in a Klett-Summerson photoelectric colorimeter (Hawk *et al.*, 1947).

Analysis of Plasma Cholesterol

The colorimetric method of the Schoenheimer-Sperry method revised by Sperry and Webb (1950) was used for analysis of total and free cholesterol contents of the plasma. The process of measuring optical density was carried out in a Beckmann Model B spectrophotometer at a wave length of 620 mu. Cholesterol recovery experiments were performed. A known amount of cholesterol in acetone-ethanol solution was treated in the same manner as the plasma samples. An average recovery of 99 to 103 per cent had been obtained using this method.

Collection of Data

Nitrogen balance data used in Series 1 had been obtained prior to the study and were available to the author for further analysis. The collection and preparation of metabolic material for chemical analyses for the other two series were carried out by members of the research staff of the Food and Nutrition Department in Home Economics Research, the author assuming charge of the analytical work and the statistical interpretation of the data.

RESULTS AND DISCUSSION

Nitrogen Retention

Short-term study: Series 2

Data pertaining to the daily metabolism of nitrogen of the six subjects in the short-term 10-day study are recorded in Table A in the Appendix. In this table, the daily intakes of nitrogen, the quantities of nitrogen excreted in the urine and in the feces, and the amounts of nitrogen retained are recorded day by day for each subject. Table 4 summarizes the average daily intakes and retentions of nitrogen of these women.

The present recommended allowance for protein for adult women is 1 gram of protein per kg. of body weight per day (National Research Council, 1958). This value is equivalent approximately to 160 mg. of nitrogen per kg. per day. The daily diets consumed by three subjects, MC, HS, and MM provided, on the average, 274, 166, and 172 mg. of nitrogen per kg. per day, respectively. Therefore, the diets chosen by these three subjects provided liberal amounts of protein as measured by the present recommended allowance. The other three women, LE, NN, and LU consumed 143, 152, and 144 mg. of nitrogen per kg. per day, respectively. The intake of LE (age, 45 years) might not be adequate inasmuch as her daily retention of nitrogen was of the order of -0.86 gram

Table 4. Average daily nitrogen intake, excretion, and retention of six subjects in a 10-day period

Subject	Age yr.	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention
MC	43	12.95	11.61	1.61	13.22	-0.37
LE	45	7.74	7.74	0.86	8.60	-0.86
HS	55	9.68	9.47	1.69	11.15	-1.48
NN	72	8.25	7.80	0.92	8.72	-0.47
MM	73	11.88	10.16	0.96	11.12	+0.76
LU	82	7.07	5.43	0.78	6.20	+0.87

per day. However, the nitrogen provided by the diets of NN and LU might meet their needs, particularly when their intakes were considered in relation to their respective ages. Roberts et al. (1948) have observed that nitrogen equilibrium may be established in women, 52 to 74 years of age, with daily intakes between 0.7 to 1.0 gram of protein per kg. of body weight, i. e., 112 to 160 mg. of nitrogen per day.

In spite of the relatively adequate amounts of protein in the diets, MC, HS, and NN were, on the average, in negative nitrogen balance during the 10-day period (Table 4). Daily retnetions were all negative in HS. A loss of 14.8 grams of nitrogen was observed in 10 days in this subject. The nitrogen losses by MC and NN were relatively small.

Subjects MM and LU were in positive nitrogen balance during the 10-day period. Daily nitrogen retentions were all positive in LU. Negative nitrogen balances occurred only on 2 days of the 10-day period of observation in MM.

A similar study has been reported by Ohlson et al. (1952). In their study, 58 per cent of the group of subjects studied were in negative nitrogen balance for the 7- to 10-day periods of observation. The significance of results from short term studies has been questioned by Hegsted (1952). He has pointed out that data obtained from such experiments may indicate either that the subjects actually were losing nitrogen from their tissues or that this state occurred only

part of the time, the actual pattern of nitrogen retention over long intervals being influenced by periods of positive retentions occurring in the interval. In an attempt to answer this question, a series of long-term nitrogen balances were conducted. These experiments will be discussed later in this section.

The short-term studies, however, provided another approach to evaluation of the protein nutrition of the subjects. The concentration of various nitrogenous components in plasma and urine were determined. Results will be presented in separate sections.

Long-term study: Series 1

Data obtained from the daily nitrogen balance studies on the four older women, NN, BG, MV, and LU, aged from 68 to 78, subsisting on two levels of nitrogen intakes, are presented in Tables B, C, D, and E of the Appendix. The average intakes and retentions of nitrogen of the four subjects are summarized in Table 5. Figures 1 and 2 show the day to day cumulative nitrogen retentions of these subjects and daily nitrogen intakes.

In the period during which the subjects were consuming their self-chosen diets (Period I), NN was in negative nitrogen balance for 18 of the 56 days, and BG was in negative nitrogen balance for 9 of the 28 days. It was estimated that negative nitrogen balances for these two women occurred dur-

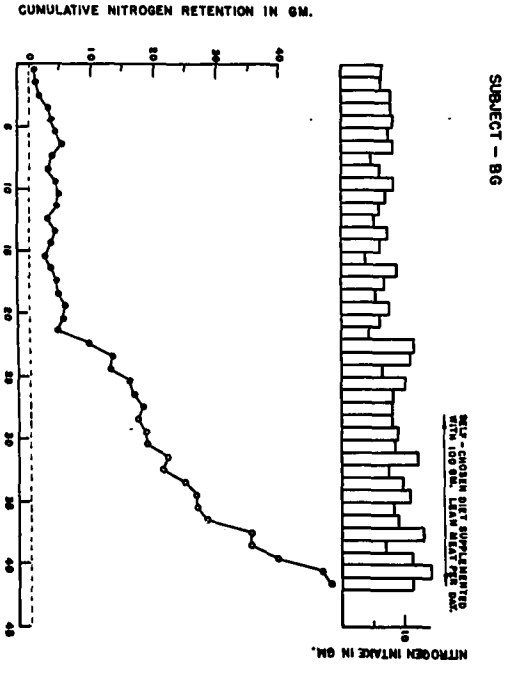
Table 5. Average daily nitrogen intake, excretion, and retention of four subjects on two levels of nitrogen intakes

Subject	Age yr.	Experimental period	No. of days	Nitrogen intake gm./day	Nitrogen excretion in urine gm./day	Nitrogen excretion in feces gm./day	Nitrogen excretion total gm./day	Nitrogen retention gm./day	Cumulative retention gm./day
NN	68	I[a]	56	6.98	5.84	0.78	6.62	+0.36	+20.32
		II[b]	14	9.58	7.69	0.75	8.44	+1.14	+15.92
BG	75	I	28	7.23	5.61	0.97	6.58	+0.65	+18.19
		II	14	9.89	6.64	1.09	7.73	+2.16	+30.21
MV	76	I	84	6.92	6.18	0.67	6.85	+0.07	+ 6.20
		II	28	9.64	8.35	0.79	9.14	+0.50	+12.90
LU	78	I	35	6.95	6.20	1.25	7.45	-0.50	-17.34
		II	14	10.33	8.01	0.99	9.00	+1.33	+18.59

[a]Period I: Self-chosen diets.

[b]Period II: Self-chosen diets plus 100 grams of lean beef per day.

Fig. 1. Cumulative nitrogen retention and daily nitrogen intakes: Subjects NN and BG (Series 1)


CUMULATIVE NITROGEN RETENTION IN GM.
DAYS
NITROGEN INTAKE IN GM.
SELF-CHOSEN DIET SUPPLEMENTED WITH 100 GM. LEAN MEAT PER DAY.
SUBJECT - BG

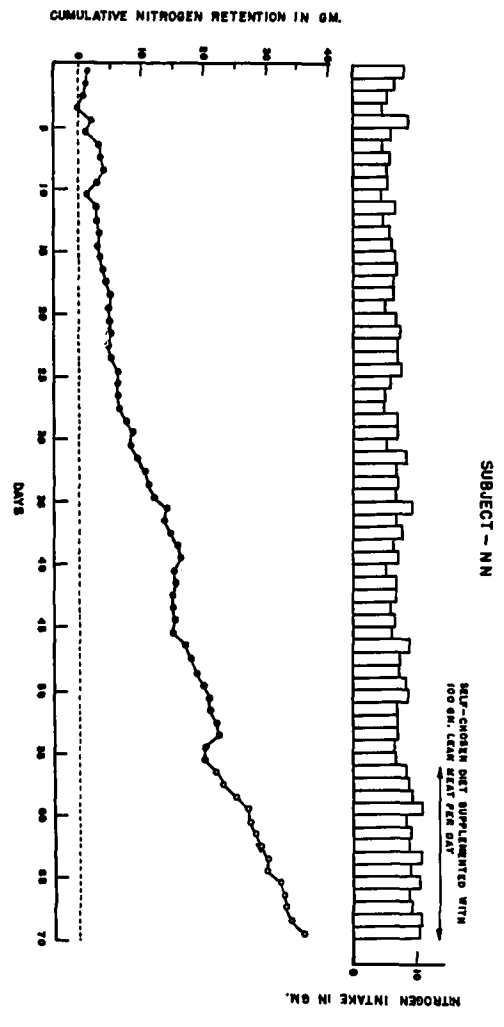
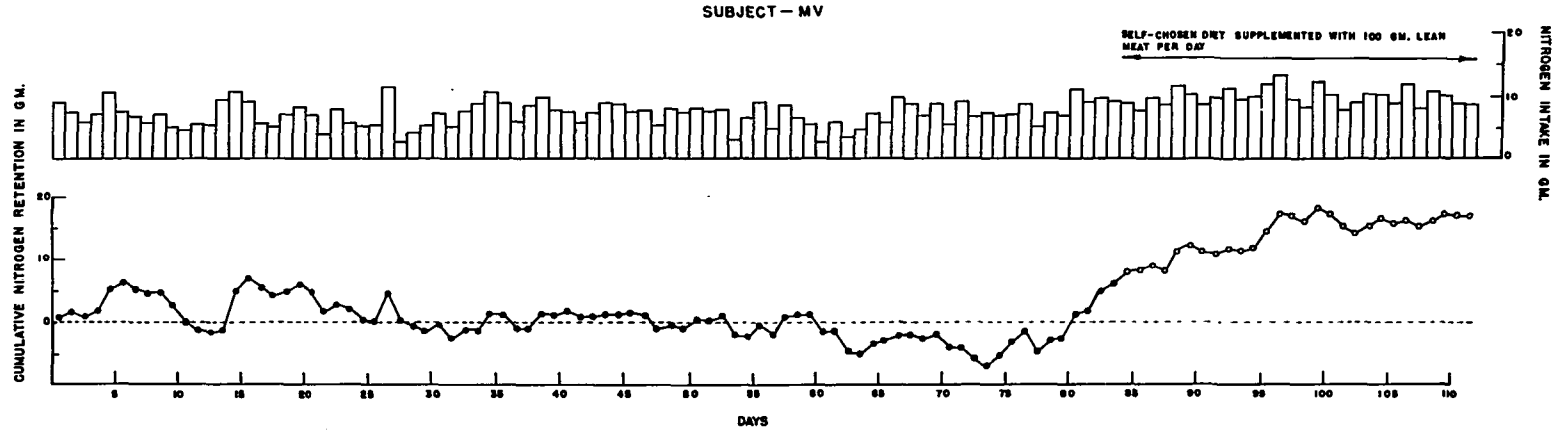
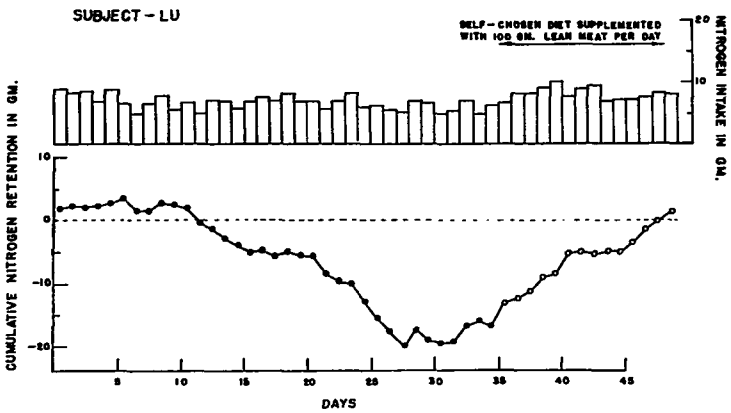

CUMULATIVE NITROGEN RETENTION IN GM.
DAYS
NITROGEN INTAKE IN GM.
SELF-CHOSEN DIET SUPPLEMENTED WITH 100 GM. LEAN MEAT PER DAY
SUBJECT - N N

Fig. 2. Cumulative nitrogen retention and daily nitrogen intakes: Subjects MV and LU (Series 1)


SUBJECT – MV
SELF-CHOSEN DIET SUPPLEMENTED WITH 100 GM. LEAN MEAT PER DAY
NITROGEN INTAKE IN GM.
CUMULATIVE NITROGEN RETENTION IN GM.
DAYS


SUBJECT – LU
SELF-CHOSEN DIET SUPPLEMENTED WITH 100 GM. LEAN MEAT PER DAY
NITROGEN INTAKE IN GM.
CUMULATIVE NITROGEN RETENTION IN GM.
DAYS

ing 32 per cent of the time of observation. Net values for the entire period of observation suggested that these two women retained considerable amounts of nitrogen. By the last day of the experimental period, NN and BG had retained 20.3 and 18.2 grams of nitrogen, respectively (Fig. 1).

MV was in negative nitrogen balance for 38 days of the 48 days, which represented 45 per cent of the time of observation. The cumulative nitrogen balances of this subject in successive days of the experimental period distinctly progressed in waves or cycles, part of the time negative and part of the time positive (Fig. 2). From the first to the last day of the experimental period, she retained 6.2 grams of nitrogen. The negative balances appeared to offset the positive balances during the period of observation with the average daily retention for 84 days close to equilibrium.

LU was in negative nitrogen balance for 22 of the 35 days. She appeared to lose nitrogen 63 per cent of the time in the period of observation. The balances accumulated on successive days gradually became more and more negative (Fig. 2). A total loss of 17 grams of nitrogen from the body was recorded from the first to the last day of the observation period.

In the period during which 100 grams of lean meat was added daily to their self-chosen diets (Period II), the increase in nitrogen intakes resulted in retentions of nitrogen

in all the women (Table 5). Positive nitrogen balances occurred 80 to 95 per cent of the time in subjects NN, BG, and LU in the 14 days of observation. In subject MV, the characteristic pattern of retention had been cyclic during Period I. In period II, positive nitrogen balances occurred only 50 per cent of the time, even though she appeared to have retained nitrogen. Her cumulative nitrogen retention was 12.9 grams in this period.

The nitrogen balance studies performed on these four women indicated the occurrence of different patterns of nitrogen retention in subjects living on self-chosen diets. A continuous nitrogen deposition may take place for long periods of time as observed in subjects BG and NN. A diminution of body protein reserves may occur as in subject LU. In addition, nitrogen retention may occur in a cyclic manner as the outcome of periods of positive or negative balances, the average retention being close to equilibrium. This pattern was observed in subject MV.

The pattern of nitrogen retention characteristic of subject MV appears to be in agreement with the assumption of Hegsted (1952) that periods of sustained equilibrium may not be representative of adults living on their self-chosen diets. Rather, they may be either in a state of positive balance or in a state of negative balance for periods of time. However, the sum of the negative and positive balances may be close to

zero over the long-run.

It should be pointed out here that a continuous positive or negative nitrogen retention for a considerable long period of time may not be compatible with a healthy state in the adult. A continuous retention barring the possibility of undetermined losses may indicate that the body has been previously depleted of protein reserves and a repletion of the reserves is in progress. Nitrogen equilibrium will be established eventually when protein reserves are replenished. On the other hand, a negative nitrogen balance persisting over a period of time suggests that the intake may be below the actual minimum requirement for this individual. Intake may be so low that adaptation does not readily occur. However, continuous loss of body nitrogen can not go on indefinitely without detrimental effects upon the organism. Therefore, the occurrence of negative balance in an individual needs careful interpretation. The ingestion of less than the needed amount of protein even for as long a period as 35 days (subject LU) may result in a temporary loss of nitrogen from the body, which does not necessarily represent poor nutrition, for nitrogen equilibrium will be established eventually at the reduced level of intake

Long-term controlled study: Series 3

In this part of the study, each subject was observed for four periods. The dietary pattern established during the first period was repeated for the fourth period and was modi-

fied for the second and third periods in regard to the amount of protein provided, the caloric value of the diet being changed as little as possible in the process. Day by day data showing the intakes of nitrogen, the quantities of nitrogen excreted in the urine and in the feces, and the amounts of nitrogen retained by the three subjects, LS, MY, and LU are presented in Tables F, G, and H in the Appendix. The nitrogen retention of each subject will be discussed separately.

Subject LS LS, aged 35 years, was the youngest of all the subjects studied. Table 6 summarizes the data pertaining to the average daily metabolism of nitrogen during successive weeks of the experiment. Daily retentions and the cumulative retentions of nitrogen of this subject are depicted in Figure 3. During the first period on her self-chosen diet, the daily intakes of nitrogen of this subject varied from 7.5 to 16.3 grams; the average was 12.3 grams or 158 mg. per kg. of body weight per day. Thus, the quantity of protein consumed was relatively adequate. Dietary records showed that approximately 60 per cent of the total protein in her diet was vegetable protein. She was fond of starches and sweets. The choice of food seemed to be restricted in variety. Daily nitrogen balances were negative for 15 days and positive for 20 days (Fig. 3-a). In the whole period of 35 days, she appeared to have retained 31.8 grams of nitrogen (Fig. 3-b).

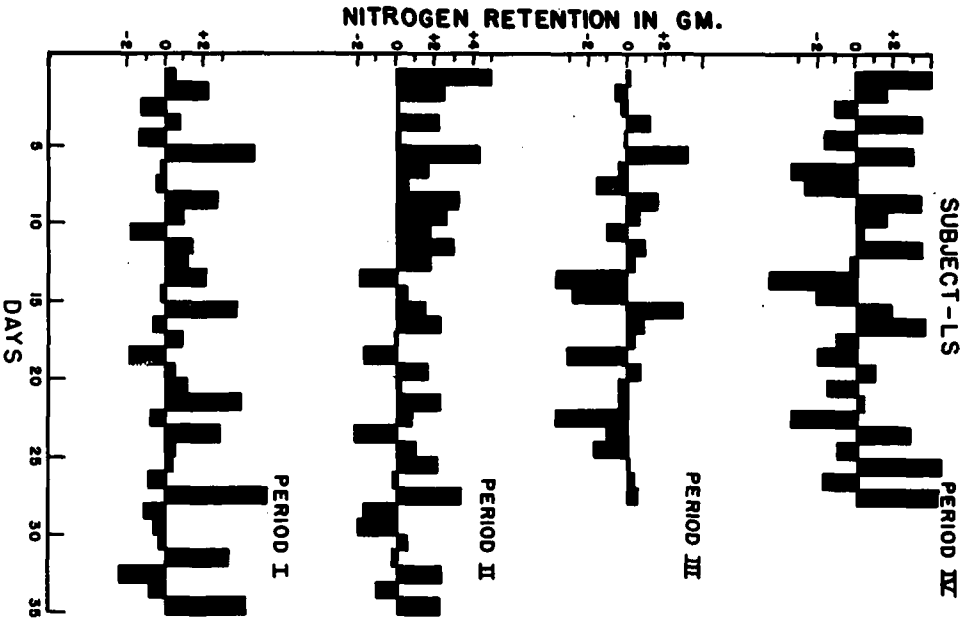
Table 6. Average daily nitrogen intake, excretion, and retention of subject LS

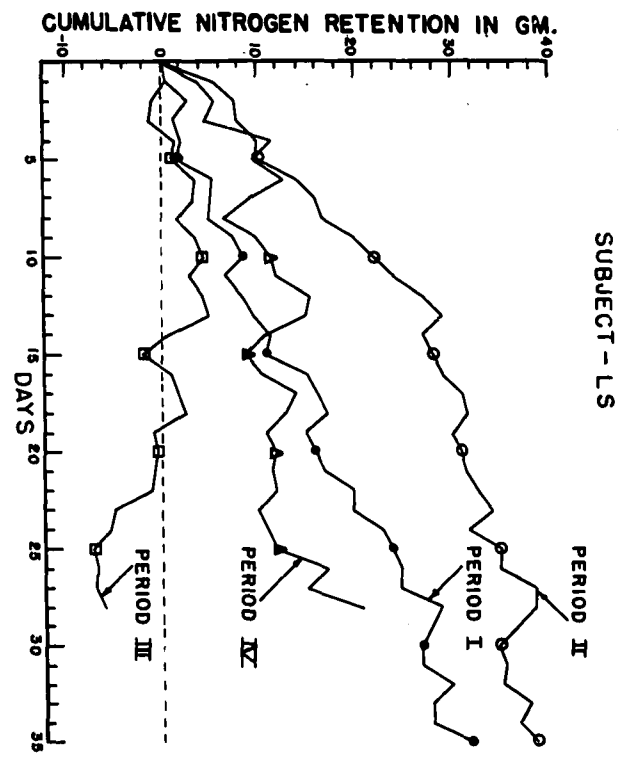
Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
I	1	11.33	9.34	1.24	10.58	+0.75	+ 5.17
	2	11.55	9.14	1.47	10.61	+0.94	+11.73
	3	12.39	10.25	1.40	11.65	+0.74	+16.95
	4	13.78	10.60	1.45	12.05	+1.73	+29.07
	5	12.23	10.14	1.70	11.84	+0.39	+31.84
	Ave.	12.26	9.90	1.45	11.35	+0.91	
II	1	15.14	11.82	1.04	12.86	+2.28	+15.97
	2	14.54	11.59	1.36	12.95	+1.59	+27.07
	3	15.78	13.58	1.57	15.15	+0.63	+31.50
	4	15.82	13.53	1.28	14.81	+1.01	+38.59
	5	15.43	13.53	1.88	15.41	+0.02	+38.74
	Ave.	15.34	12.81	1.42	14.24	+1.10	

Table 6. (Continued)

Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
III	1	9.82	7.91	1.41	9.32	+0.50	+ 3.50
	2	9.18	7.81	1.77	9.58	-0.40	+ 0.78
	3	10.32	8.80	1.70	10.50	-0.18	- 0.48
	4	9.99	8.62	2.18	10.80	-0.81	- 6.14
	Ave.	9.83	8.28	1.76	10.04	-0.21	
IV	1	11.67	8.90	1.43	10.33	+1.34	+ 9.44
	2	11.62	10.25	1.18	11.43	+0.19	+10.76
	3	12.89	11.14	1.66	12.80	+0.09	+11.40
	4	13.46	10.82	1.57	12.39	+1.07	+18.91
	Ave.	12.41	10.28	1.46	11.74	+0.67	

Fig. 3. Nitrogen retention of subject LS (Series 3)
a. Daily retentions of nitrogen
b. Cumulative nitrogen retentions


NITROGEN RETENTION IN GM.
SUBJECT-LS
PERIOD IV
PERIOD III
PERIOD II
PERIOD I
DAYS


CUMULATIVE NITROGEN RETENTION IN GM.
SUBJECT-LS
PERIOD II
PERIOD I
PERIOD IV
PERIOD III
DAYS

With a daily increase of 3.4 grams of nitrogen in the diet in the form of lean beef (Period II), she retained an average of 1.1 grams of nitrogen per day. Nitrogen retentions were consistently positive in the first two weeks of the meat-supplementation period. During the last three weeks, she retained less nitrogen than the amount retained in the corresponding weeks in Period I. Apparently, an adjustment to the higher nitrogen intake had been made after two weeks on the protein-supplemented diets in this subject. During the first 14 days, the subject seemed to retain nitrogen at an accelerated rate. Thereafter, the excretion of urinary nitrogen increased.

In the third period during which the protein content of the diet was reduced approximately by 20 per cent daily from that provided by her self-chosen diets in Period I, she was in negative balance for 14 of the 28 days. The balance cumulated from day to day became progressively negative. Six grams of nitrogen had been lost by the end of the period. During the transition from the high to the low intake of protein, the urinary excretion of nitrogen lowered immediately.

During the last period in which LS ate the same diets, day by day, as that she had chosen in Period I. Definite retention of nitrogen occurred in the first week of the period. An increased urinary excretion of nitrogen was observed in the second week. A metabolic adjustment to the

intermediate level of protein consumption seemed to occur in the second and third weeks of the period. It should be noted that there was a distinct tendency to retain nitrogen in the last week of the period. In the total period, she retained approximately 19 grams of nitrogen, a quantity less than that retained in the first period.

Subject MY MY was 56 years of age, her mean daily intake, and retention of nitrogen for each week are summarized in Table 7. Daily retentions of nitrogen and the cumulative retentions of this subject are depicted in Fig. 4. During the period when she was consuming her self-chosen diet (Period I), the mean daily nitrogen intake was 15.5 grams, with a range of 8.1 to 22.6 grams. As compared with the present daily allowance for protein for adult women (National Research Council, 1958), the daily diet consumed by MY provided a liberal amount of protein. Dietary records showed that on the average, approximately 70 per cent of the dietary proteins were from animal sources. She was in negative nitrogen balance for only 7 of the 35 days. The average daily retention of nitrogen was 1.8 grams in Period I.

Fig. 4-b shows the day-to-day cumulative nitrogen balances of subject MY. She seemed to have retained nitrogen consistently during Period I. By the last day of this period she had retained 63.7 grams of nitrogen for the total period.

During the second period, negative nitrogen balances

Table 7. Average daily nitrogen intake, excretion, and retention of subject MY

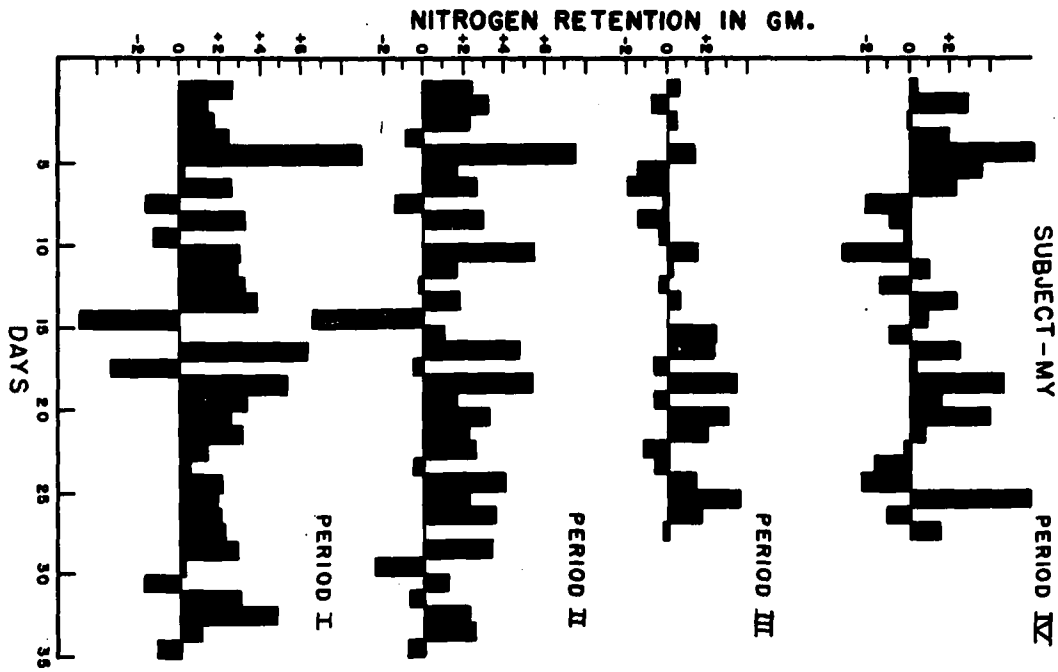
Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
I	1	16.94	13.00	1.24	14.24	+2.70	+18.86
	2	15.23	12.01	1.26	13.27	+1.96	+31.99
	3	14.25	11.76	1.17	12.93	+1.32	+41.18
	4	15.75	12.26	1.59	13.85	+1.90	+54.51
	5	15.39	12.45	1.63	14.08	+1.31	+63.66
	Ave.	15.51	12.31	1.38	13.69	+1.82	
II	1	20.63	16.46	1.46	17.92	+2.71	+18.96
	2	18.27	15.59	1.26	16.85	+1.42	+29.21
	3	18.48	15.52	1.45	16.97	+1.51	+39.58
	4	18.36	15.30	1.08	16.38	+1.98	+53.44
	5	17.93	15.68	1.45	17.13	+0.80	+59.02
	Ave.	18.73	15.71	1.33	17.04	+1.69	

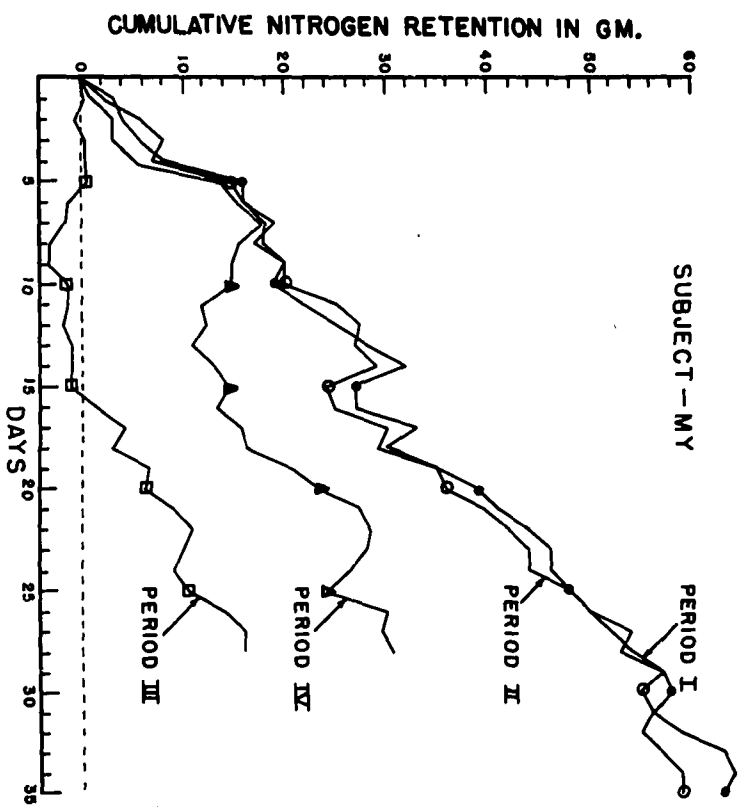
Table 7. (Continued)

Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
III	1	12.44	10.90	1.75	12.65	-0.21	- 1.48
	2	10.98	9.75	1.17	10.92	+0.06	- 1.09
	3	11.98	9.12	1.44	10.56	+1.42	+ 8.90
	4	12.30	9.83	1.36	11.19	+1.11	+16.69
	Ave.	11.93	9.90	1.43	11.33	+0.60	
IV	1	16.98	13.03	1.42	14.45	+2.53	+17.77
	2	13.89	13.23	1.29	14.52	+0.63	+13.37
	3	15.07	11.80	1.26	13.06	+2.01	+27.45
	4	14.61	12.25	1.94	14.19	+0.42	+30.35
	Ave.	15.14	12.58	1.47	14.05	+1.09	

Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
III	1	12.44	10.90	1.75	12.65	-0.21	- 1.48
	2	10.98	9.75	1.17	10.92	+0.06	- 1.09
	3	11.98	9.12	1.44	10.56	+1.42	+ 8.90
	4	12.30	9.83	1.36	11.19	+1.11	+16.69
	Ave.	11.93	9.90	1.43	11.33	+0.60	
IV	1	16.98	13.03	1.42	14.45	+2.53	+17.77
	2	13.89	13.23	1.29	14.52	+0.63	+13.37
	3	15.07	11.80	1.26	13.06	+2.01	+27.45
	4	14.61	12.25	1.94	14.19	+0.42	+30.35
	Ave.	15.14	12.58	1.47	14.05	+1.09	

Fig. 4. Nitrogen retention of subject MY (Series 3)
a. Daily retentions of nitrogen
b. Cumulative nitrogen retentions


NITROGEN RETENTION IN GM.
DAYS
SUBJECT-MY
PERIOD I
PERIOD II
PERIOD III
PERIOD IV


CUMULATIVE NITROGEN RETENTION IN GM.
DAYS
SUBJECT-MY
PERIOD III
PERIOD IV
PERIOD II
PERIOD I

occurred for 11 days in the whole period. With the increase in the protein content of the diet, she retained approximately the same amount of nitrogen as that retained in Period I. The total amount of nitrogen retained in the 35 days was 59 grams. The two lines depicting cumulative nitrogen retention for Period I and Period II are almost superimposed (Fig. 4-b).

In Period III during which the daily intakes of protein were reduced to 80 per cent of the amount consumed in Period I, negative balances were observed for 12 days, positive balances for 16 days. During the transition from high protein intakes to low protein intakes, an immediate decrease in urinary nitrogen excretion was observed. A similar result was obtained from LS. MY appeared to lose nitrogen during the first two weeks. During the last two weeks, she seemed to retain nitrogen to compensate for the nitrogen lost in the first two weeks. The cumulative nitrogen balances became increasingly positive; and in the whole period, she was able to retain 16.7 grams of nitrogen.

During the last period of 28 days (Period IV) she repeated the menu used in Period I. She suffered a severe cold in the second and the fourth weeks of the period, which might account for the lowered retention of nitrogen in this period. She retained 30.4 grams of nitrogen in this period which was approximately 54 per cent of the amount retained in the first period.

Subject LU LU was the oldest of the subjects studied in this series, being 81 years of age. She also participated in the long-term study in Series 1. The nitrogen retention in Series 1 has already been discussed.

Data relating to the metabolism of nitrogen by subject LU are summarized in Table 8. During the first period of the present study in which she took her self-chosen diets, the daily intakes of nitrogen ranged from 4.6 to 10.0 grams, with an average of 7.4 grams or 145 mg. per kg. of body weight per day. Dietary records showed that she consumed a varied, well-balanced diet, even though the quantity of each kind of food was small.

In the entire interval, the daily balances were negative for 12 days, positive for 23 days (Fig. 5-a). The cumulative nitrogen balances of LU in successive days of the first period progressed in waves only in the first week (Fig. 5-b). After the first week, the cumulative balance became increasing positive. By the last day of the period, she had retained approximately 17.7 grams of nitrogen.

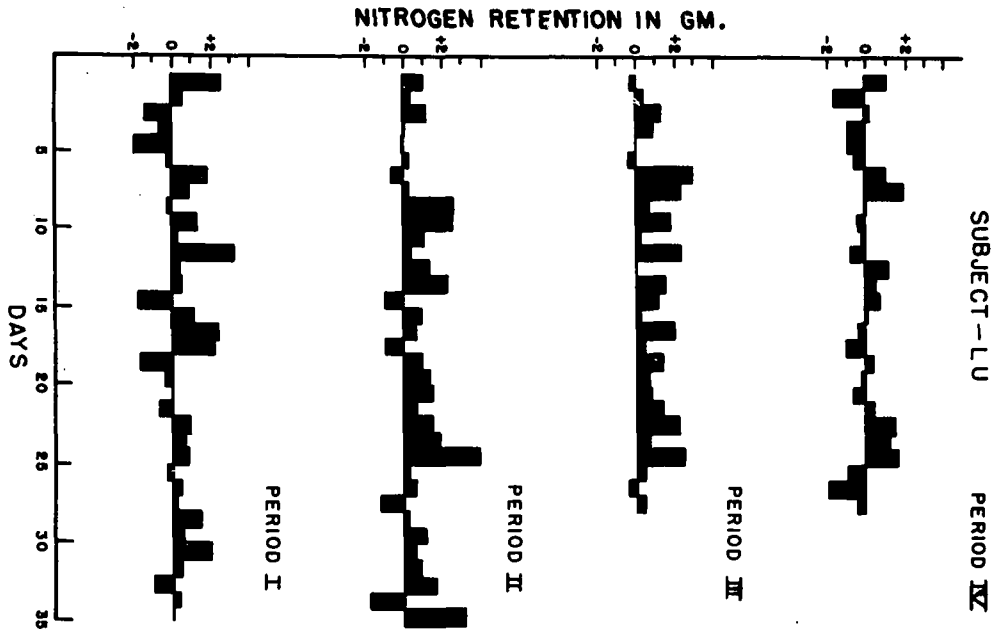
The subject appeared to have maintained somewhat the same pattern of nitrogen intake in her self-chosen diets in the two long-term metabolism experiments carried out at different intervals (Series 1 and 3). The average intake of nitrogen increased slightly from 6.9 grams in Series 1 to 7.4 grams in the observation made here. The average nitrogen

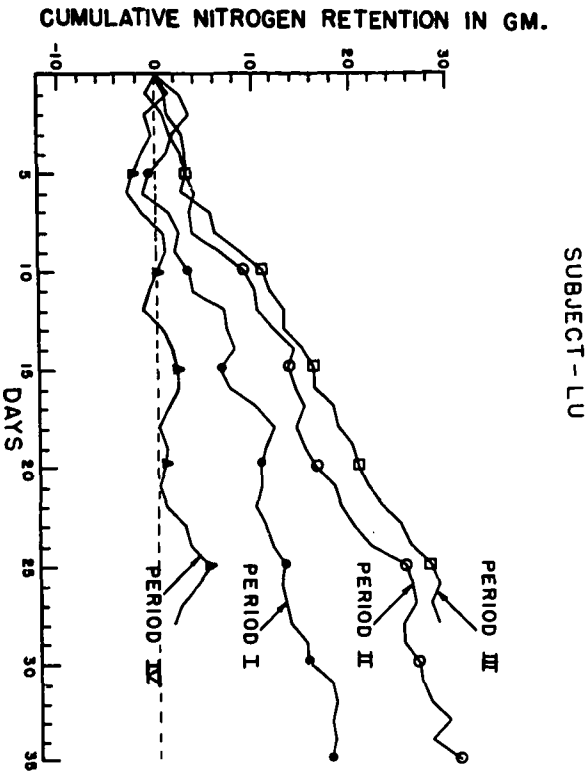
Table 8. Average daily nitrogen intake, excretion, and retention of nitrogen of subject LU

Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
I	1	6.97	6.00	0.80	6.80	+0.17	+ 1.23
	2	8.05	6.24	0.83	7.07	+0.98	+ 8.09
	3	7.46	5.95	1.15	7.10	+0.36	+10.53
	4	7.48	6.19	0.91	7.10	+0.38	+13.24
	5	7.14	5.68	0.80	6.48	+0.66	+17.86
	Ave.	7.42	6.01	0.90	6.91	+0.51	
II	1	9.74	8.29	0.95	9.24	+0.50	+ 3.47
	2	11.46	9.19	0.75	9.94	+1.52	+14.10
	3	10.71	8.84	1.36	10.20	+0.51	+17.70
	4	10.89	8.81	1.01	9.82	+1.07	+25.22
	5	10.71	9.01	0.88	9.89	+0.82	+30.96
	Ave.	10.70	8.83	0.99	9.82	+0.88	

Table 8. (Continued)

Experimental period	Week	Nitrogen intake gm. per day	Nitrogen excretion in urine gm. per day	Nitrogen excretion in feces gm. per day	Nitrogen excretion total gm. per day	Nitrogen retention gm. per day	Cumulative retention gm.
III	1	8.45	6.71	0.95	7.66	+0.79	+ 5.50
	2	9.69	7.39	1.01	8.40	+1.29	+14.50
	3	9.46	7.45	1.04	8.49	+0.97	+21.22
	4	10.04	7.71	1.28	8.99	+1.05	+28.58
	Ave.	9.41	7.32	1.07	8.39	+1.02	
IV	1	6.48	5.71	1.00	6.71	+0.23	- 1.57
	2	7.35	6.18	0.79	6.97	+0.38	+ 1.09
	3	7.41	6.48	1.08	7.56	-0.15	+ 0.04
	4	8.25	6.73	1.28	8.01	+0.24	+ 1.73
	Ave.	7.37	6.28	1.04	7.32	+0.05	


NITROGEN RETENTION IN GM.
-2
0
+2
DAYS
5
10
15
20
25
30
35
PERIOD I
PERIOD II
PERIOD III
PERIOD IV
SUBJECT - LU


CUMULATIVE NITROGEN RETENTION IN GM.
-10
0
10
20
30
DAYS
5
10
15
20
25
30
35
PERIOD I
PERIOD II
PERIOD III
PERIOD IV
SUBJECT - LU

excretion in the urine and feces, on the other hand, decreased from 7.4 to 6.9 grams. Consequently, in the 35-day observation in Series 1, she lost 17.3 grams of nitrogen and in the study described here, she retained 17.7 grams of nitrogen in 35 days.

In Period II of the present study during which she was consuming 3.4 grams of nitrogen daily in addition to that provided by her self-chosen diet, consistent retentions of nitrogen occurred. She was in positive balance for 30 of the 35 days with an average retention of 0.9 gram of nitrogen per day. The response of urinary nitrogen excretion to an increased protein intake seemed to be immediate.

In the study (Series 1), LU was in negative nitrogen balance during the 35 days. This indicated that she might have been in a state of nitrogen deficit. Therefore, no attempt was made to reduce her protein intakes in the third period as was done with the other two subjects. Instead, 50 grams of lean beef was given to her as supplementary protein to her self-chosen diets. On intakes of this order, LU was in negative nitrogen balance for only 3 of the 28 days. It may be interesting to note that in this period, she might have retained slightly more nitrogen than the amount retained in Period II. On the average, she retained 1.02 grams of nitrogen per day in Period III which was twice as much as the amount retained in Period I. This observation raises the

question as to whether this 81-year old subject had the need for or the capacity to retain the amount of protein provided in Period II.

During the last period when she returned to her self-chosen diet, negative balances occurred for 14 of the 28 days. The pattern of retention of nitrogen on an average intake of 7.4 grams of nitrogen per day was cyclic, the average daily nitrogen retention being close to equilibrium.

These three women in Series 3 showed retentions of nitrogen while living on self-chosen diets (Period I). In Period II during which the plane of protein intake was raised, two subjects, LS and LU, retained more nitrogen than they did in Period I. In subject MY, the added dietary nitrogen was reflected quantitatively in an increase in urinary nitrogen over that excreted in Period I (Table 7). With the patterns of cumulative retentions so similar in the two periods in this subject (Fig. 4), it seemed likely that nitrogen needs might have been overstepped in Period II. In the period during which the protein content of the self-chosen diets was lowered in two subjects, LS and MY (Period III), MY tended to make a metabolic adjustment to the reduced plane of protein intakes, while a nitrogen loss occurred in LS. The patterns of nitrogen retentions of these three subjects subsisting on three levels of protein intakes suggested that the self-chosen diets of LS and LU in Period I and IV might have fur-

nished marginal amounts of nitrogen for adequate protein nutrition while those of MY probably constituted optimal levels of protein intakes.

The significance of the concept of nitrogen equilibrium when applied to human studies should be noted. Hegsted (1952) has suggested that an adult adequately nourished with respect to protein may not necessarily be in a state of continuous nitrogen equilibrium. The value depends in part upon the accuracy of the metabolic measurements. Also, the quantitative definition of equilibrium is another problem. Rose et al. (1955) have considered a slightly positive nitrogen balance (approximately 0.5 gram) as a state of nitrogen equilibrium in adult human beings. Leverton et al. (1956) have defined the zone of nitrogen equilibrium as that in which the total nitrogen excretion is within 95 to 105 per cent of the nitrogen intake. When the total excretion of nitrogen was more than 105 per cent of the nitrogen intake, the individual was judged to be in negative nitrogen balance. In the present study, three subjects MC, NN, and MV were in the zone of equilibrium defined by these authors. Subjects MM, BG, LS, MY, and LU (in Series 3) could be judged accordingly to be in positive nitrogen balance whereas LE, HS, and LU (in Series 1) were in negative nitrogen balance.

The fact that retention of nitrogen occurs in adult women as observed in the present study also needs comment.

Mitchell (1949) reported nitrogen retentions of 1.38 grams daily in young adult males which he explained as representing the growth of skin, hair, nails, and the dermal excretion of nitrogen in the insensible perspiration. Sufficient data have not been available to estimate the amount of nitrogen loss through these routes in older women. The extended nitrogen retention over the long period of time in some of the subjects in the present study (BG, LS and MY) might be interpreted as an indication of a depletion of protein reserves prior to the study. Following the improvement of the diet, the replenishment of body reserves of protein appeared to take place. However, they might be actually in a state closer to nitrogen equilibrium, since the possible loss of nitrogen through the routes other than urine and feces was not measured. If the nitrogen loss through the other routes was appreciable, subject MV who was in nitrogen equilibrium during the period of observation might be actually in a state of nitrogen deficit (Table 4).

The significance of nitrogen retentions of these women in response to an increase in nitrogen intakes will be evaluated further in the following section.

Urinary Nitrogen Excretion in Relation to Absorbed Nitrogen of the Subjects on Different Planes of Nitrogen Intakes

The protein provided by the diet enters into both anabolic and catabolic processes in the body. If the protein

supply approaches inadequacy, catabolic processes are decreased; if it exceeds body needs, catabolism is increased due to deamination of protein not required by the body. Thus, the extent of the catabolism of protein is related to the level of protein in the diet.

The total nitrogen excreted in the urine is chiefly composed of nitrogenous end products of the catabolism of protein in the body. Therefore, it seemed that a correlation between urinary nitrogen and absorbed nitrogen might serve as a measure of the adequacy of the protein in the diet of an individual. This hypothesis has been tested in the present study. Regressions of urinary nitrogen on absorbed nitrogen were calculated with the use of the equation, $Y = a + bX$ (Snedecor, 1956), for the different planes of protein intakes of each subject in each of the long-term studies. In order to make comparisons in all the subjects studied, the quantities of both urinary nitrogen and absorbed nitrogen were expressed as mg. per kg. of body weight per day.

The significance of differences in the slopes of the regressions for a subject living on diets providing different amounts of protein was tested by the method described by Li (1957).

The significance of differences in the intercepts was also tested. The value of the intercept of the regression may be interpreted as the amount of urinary nitrogen excretion

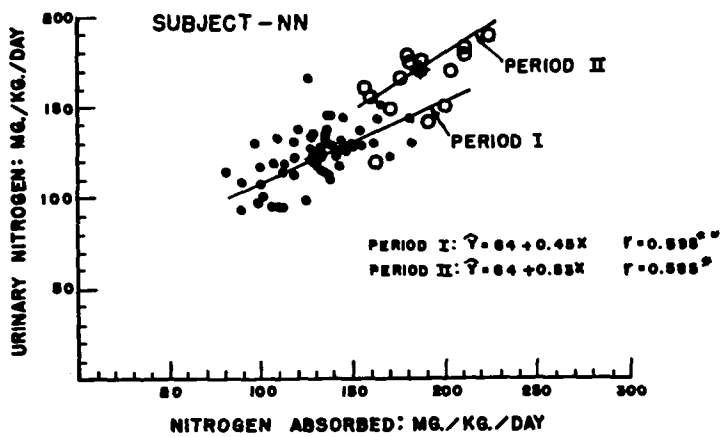
when the nitrogen absorbed becomes zero. Allison and Anderson (1945) showed that the value for the intercept was close to the actual amount of urinary nitrogen of endogenous sources for the adult animal. The measurements were made in the region of nitrogen equilibrium. Thus, the intercept may be considered as a measure of urinary nitrogen of endogenous origin. It seemed characteristic of an individual. The intercept also may serve as a measure of the level of protein metabolism on which a subject has adapted.

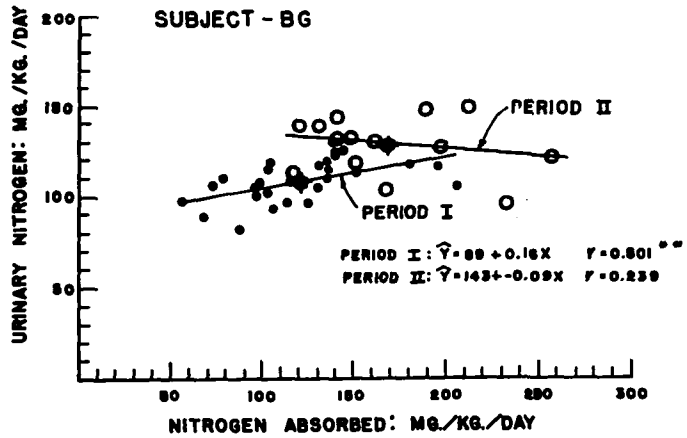
Long-term study (Series 1)

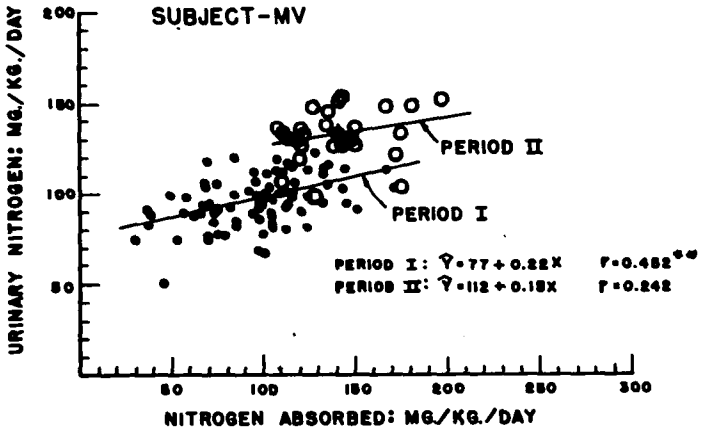
Regressions of urinary nitrogen on absorbed nitrogen for the two experimental periods were calculated separately for the four subjects in Series 1. These regressions are depicted in Fig. 6 for subjects NN,BG, MV, and LU.

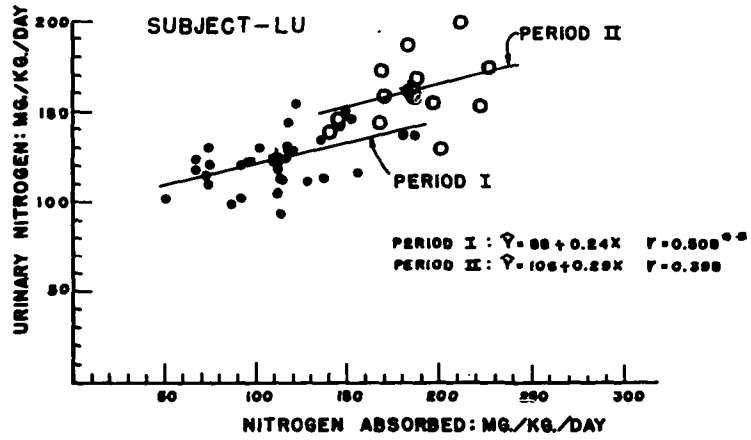
Regressions of urinary nitrogen on absorbed nitrogen for subject NN in the two experimental periods are shown in Fig. 6. The difference between the slopes was not significant. Statistically, the two regressions were identical. The excretion of nitrogen appeared to increase at the same rate with increasing quantities of absorbed nitrogen in both dietary regimes. Therefore, in Period II, there was a higher daily retention (+1.14 grams) of nitrogen than that in Period I (+0.36 grams). Apparently, the body of this subject was capable of retaining part of the extra nitrogen added to her self-chosen diets. The fact that her endogenous metab-

Fig. 6. Regressions of urinary nitrogen on absorbed nitrogen for subjects NN, BG, MV, and LU (Series 1)


SUBJECT - NN
PERIOD II
PERIOD I
PERIOD I: Ŷ = 64 + 0.45X r = 0.598**
PERIOD II: Ŷ = 64 + 0.83X r = 0.585*
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
50
100
150
200
250
300


SUBJECT - BG
PERIOD II
PERIOD I
PERIOD I: Ŷ = 89 + 0.16X r = 0.501**
PERIOD II: Ŷ = 143 + -0.09X r = 0.239
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
50
100
150
200
250
300


SUBJECT - MV
PERIOD II
PERIOD I
PERIOD I: Ŷ = 77 + 0.22X r = 0.482**
PERIOD II: Ŷ = 112 + 0.18X r = 0.242
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
50
100
150
200
250
300


SUBJECT - LU
PERIOD II
PERIOD I
PERIOD I: Ŷ = 88 + 0.24X r = 0.508**
PERIOD II: Ŷ = 106 + 0.29X r = 0.399
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
50
100
150
200
250
300

olism had not changed because the intercepts were the same suggested that possibly this subject needed more protein than that customarily provided by her diet. However, whether the same picture would prevail had more data in Period II been available is a question.

The regressions for BG showed interesting results (Fig. 6). There were significant differences between the slopes and intercepts of the regressions for the two dietary regimes. The negativity of the slope of the regression for Period II was not significant. The excretion of nitrogen in this period appeared to remain at a fairly constant level and did not seem to be affected by the amount of nitrogen absorbed. Therefore, a large fraction of the absorbed nitrogen was retained by the body. This subject was in positive nitrogen balance when she was consuming her self-chosen diets in Period I. However, a further increase in nitrogen retention in Period II might be an indication of a state of nitrogen deficit in the body. The supplementation of meat to her self-chosen diets replenished the body reserves of protein markedly.

In subject MV, the slope of the regression of Period II was not statistically significant (Fig. 6). It seemed that nitrogen excretion in this Period was not affected by the amount of protein consumed. Apparently, a higher extent of retention than that in Period I occurred. This might be an

indication that the body of this subject had the capacity to retain more nitrogen than the amount provided by her self-chosen diets. It should be noted that there was a significant difference between the intercepts of the regressions for the two dietary periods. Apparently, metabolic adjustment had been made in Period II, suggesting that the subject tended to adapt to a higher plane of protein nutrition.

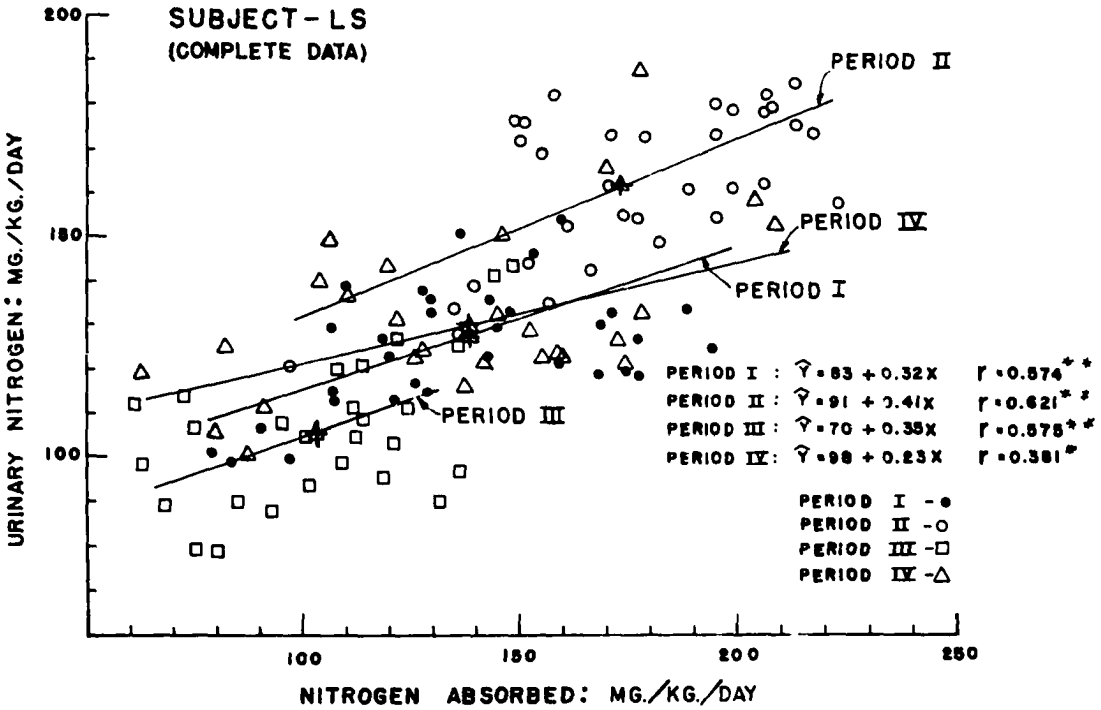
The regressions of urinary nitrogen on absorbed nitrogen for two dietary regimes in subject LU were similar to those for subject NN (Fig. 6). No significant differences between the slopes nor between the intercepts were found. The regression for Period II was not statistically significant. The excretion of nitrogen did not increase proportionally with the increase in the amount of nitrogen absorbed. Retention, therefore, must have occurred in this period. This deduction is validated by the fact that this subject retained +1.3 grams of nitrogen per day in Period II.

Long-term controlled study: Series 3

Regressions of urinary nitrogen on absorbed nitrogen were calculated for the four experimental periods of each of the three subjects cooperating in Series 3. The regressions for LS, MY, and LU are depicted in Figures 7, 8, and 9, respectively. The significance of the difference in the slopes and in the intercepts was tested (Li, 1957).

An attempt was made to eliminate the effect of the lag

Fig. 7. Regressions of urinary nitrogen on absorbed nitrogen: Subject LS (Series 3)


SUBJECT - LS
(COMPLETE DATA)
PERIOD II
PERIOD IV
PERIOD I
PERIOD III
PERIOD I : Ŷ = 83 + 0.32X r = 0.574**
PERIOD II : Ŷ = 91 + 0.41X r = 0.621**
PERIOD III : Ŷ = 70 + 0.35X r = 0.575**
PERIOD IV: Ŷ = 98 + 0.23X r = 0.381*
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
200
150
100
100
150
200
250

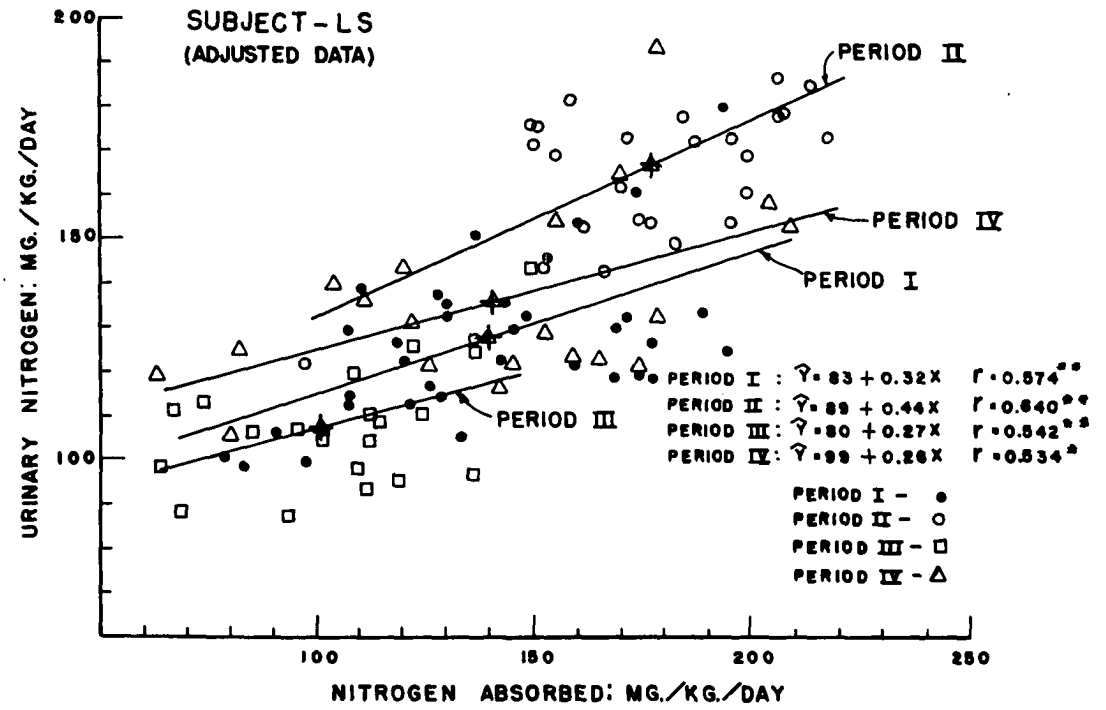
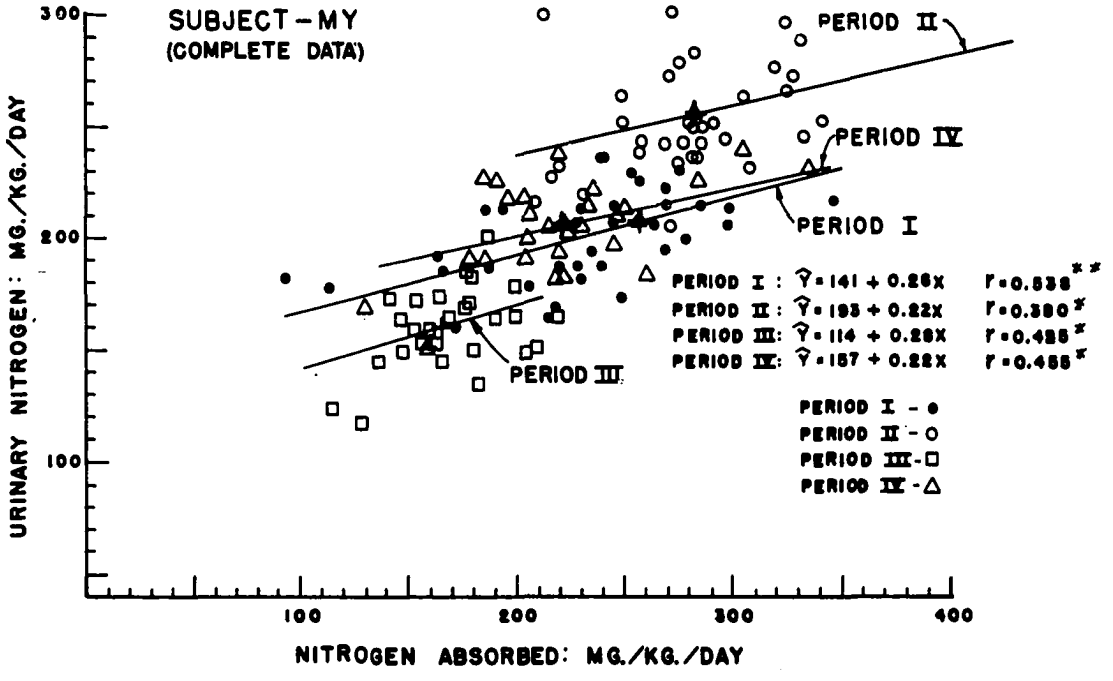

SUBJECT - LS
(ADJUSTED DATA)
PERIOD II
PERIOD IV
PERIOD I
PERIOD III
PERIOD I : Ŷ = 83 + 0.32X r = 0.574**
PERIOD II : Ŷ = 89 + 0.44X r = 0.640**
PERIOD III : Ŷ = 80 + 0.27X r = 0.542**
PERIOD IV: Ŷ = 98 + 0.26X r = 0.534*
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
200
150
100
100
150
200
250

Fig. 8. Regressions of urinary nitrogen on absorbed nitrogen: Subject MY (Series 3)


SUBJECT – MY
(COMPLETE DATA)
PERIOD II
PERIOD IV
PERIOD I
PERIOD III
PERIOD I : Ŷ = 141 + 0.26X r = 0.536**
PERIOD II : Ŷ = 193 + 0.22X r = 0.380*
PERIOD III: Ŷ = 114 + 0.28X r = 0.485*
PERIOD IV: Ŷ = 157 + 0.22X r = 0.466*
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
100
200
300
400

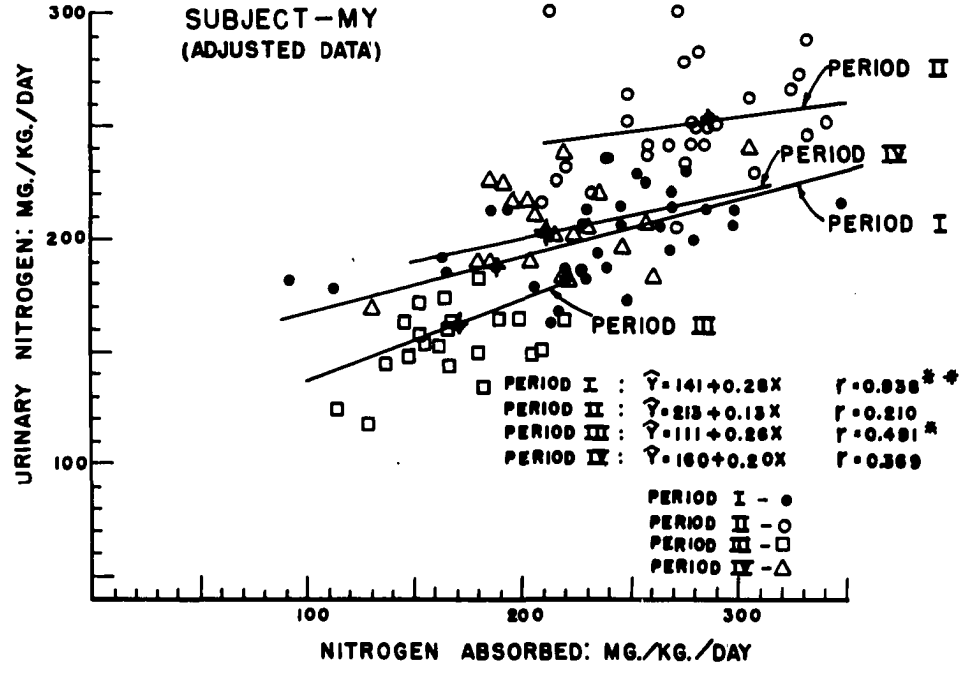
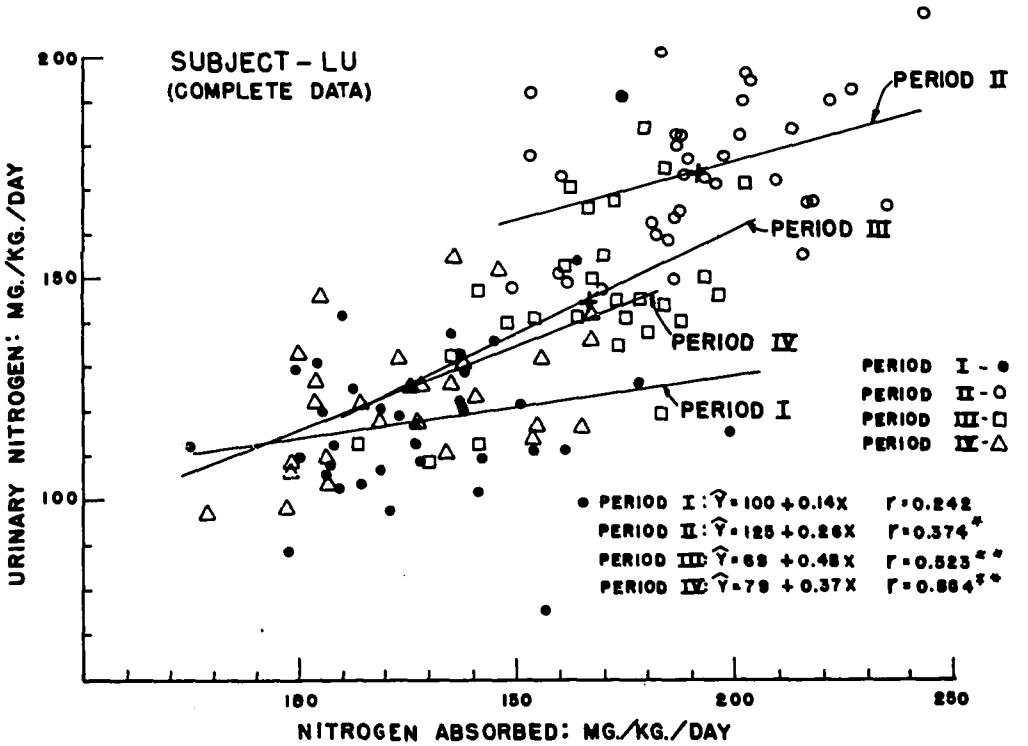
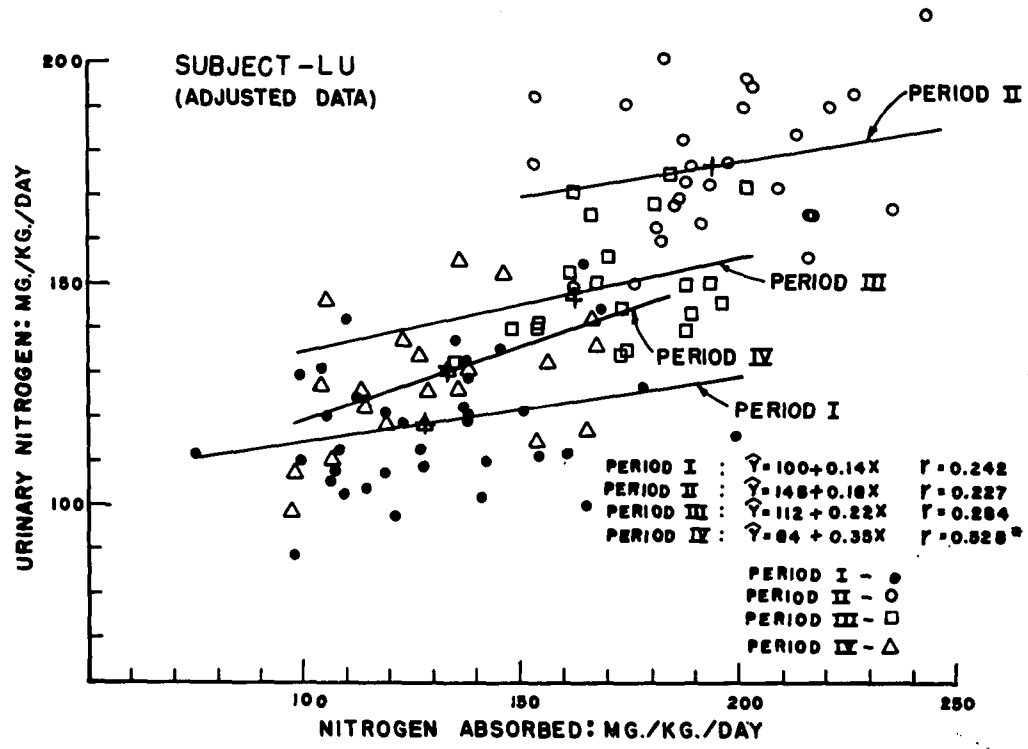

SUBJECT – MY
(ADJUSTED DATA)
PERIOD II
PERIOD IV
PERIOD I
PERIOD III
PERIOD I : Ŷ = 141 + 0.26X r = 0.536**
PERIOD II : Ŷ = 213 + 0.13X r = 0.210
PERIOD III : Ŷ = 111 + 0.26X r = 0.491*
PERIOD IV : Ŷ = 160 + 0.20X r = 0.369
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
100
200
300

Fig. 9. Regressions of urinary nitrogen on absorbed nitrogen: Subject LU (Series 3)


SUBJECT - LU
(COMPLETE DATA)
PERIOD II
PERIOD III
PERIOD IV
PERIOD I
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
● PERIOD I : Ŷ = 100 + 0.14X r = 0.242
PERIOD II : Ŷ = 125 + 0.26X r = 0.374*
PERIOD III : Ŷ = 69 + 0.48X r = 0.523**
PERIOD IV : Ŷ = 78 + 0.37X r = 0.884**
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
200
150
100
100
150
200
250


SUBJECT - LU
(ADJUSTED DATA)
PERIOD II
PERIOD III
PERIOD IV
PERIOD I
PERIOD I : Ŷ = 100 + 0.14X r = 0.242
PERIOD II : Ŷ = 148 + 0.18X r = 0.227
PERIOD III : Ŷ = 112 + 0.22X r = 0.284
PERIOD IV : Ŷ = 84 + 0.38X r = 0.528*
PERIOD I - ●
PERIOD II - ○
PERIOD III - □
PERIOD IV - △
URINARY NITROGEN: MG./KG./DAY
NITROGEN ABSORBED: MG./KG./DAY
200
150
100
100
150
200
250

in excretion of urinary nitrogen from the previous dietary period on the significance of the regressions. The original data were, therefore, adjusted by omitting the data for the first seven days for Periods II, III, and IV. The regressions were calculated using these adjusted data and are depicted in Figures 7, 8, and 9. When compared with results based on the complete data, it appears that such adjustment had little effect on the significance of the results. Therefore, discussion has been limited to the complete data pertaining to the three subjects.

Subject LS Significant differences in intercepts and in slopes of the regressions of urinary nitrogen on absorbed nitrogen for the four dietary periods of LS were not observed (Fig. 7). The urinary excretion of nitrogen did not appear to have responded to the changes in dietary protein in the four experimental regimes. This subject retained on the average +0.9 gram, +1.1 grams, -0.2 gram, and +0.7 gram of nitrogen per day, respectively in Periods I, II, III, and IV. Thus, when regressions and retentions were considered simultaneously, it seemed that, like NN and LU in Series 1, LS did not choose diets furnishing all the protein she could have retained.

Subject MY There was no significant difference in the slopes of the regressions representing the relation of urinary nitrogen to absorbed nitrogen for subject MY (Fig. 8). No difference in the intercepts of the regressions for

Periods I, III, and IV was detected. A significant difference between the intercept of the regression for Period II and those for the other periods was observed. The difference between the intercepts of the regressions for Period I and Period II was 52 mg. which was approximately identical to the average increase in the absorbed nitrogen, 53 mg. per kg. per day. The body of this subject seemed to have no use for the additional nitrogen, therefore the extra nitrogen was excreted in the urine.

Subject LU Subject LU was treated differently from the other subjects. In Periods I, II, III, and IV, she ate, respectively, her self-chosen diet, her self-chosen diet plus 100 grams of lean beef per day, her self-chosen diet plus 50 grams of lean beef per day, and a repetition of the regular diet.

The regression of urinary nitrogen on absorbed nitrogen for subject LU in Period I was not significant. This might be an indication that a large fraction of the absorbed nitrogen was retained by the body; consequently, the excretion of nitrogen was not related to the quantity of nitrogen absorbed.

There was no significant differences in slopes and in intercepts representing the four periods. The results were similar to those obtained from this same subject in Series 1. Despite the fact that differences in response between periods were not significant, the regressions illustrated in Fig. 9

suggest some interesting implications. In Periods II, III, and IV, the slopes of the regressions were significant. The subject appeared to begin to excrete nitrogen in proportion to the amount absorbed. This might indicate that initial body reserves of protein were low and that they had been repleted. For example, in Period IV when she consumed exactly day by day the same diet as one she had eaten in Period I, her nitrogen excretion might reflect the influence of the intervening 63 days on an increased protein diet. The general pattern of the regression lines, therefore, indicates that the diet as customarily chosen by this subject might not be optimal in protein for her. This deduction is supported further by the fact that this subject was losing nitrogen when she was observed in the previous study (Series 1).

Summary

The slopes and intercepts of the regressions of urinary nitrogen on absorbed nitrogen calculated for each subject subsisting on two levels of protein intakes are summarized in Table 9.

A comparison of the values for the slopes of the regressions when the diets were self-chosen (Period I in Series 1 and 3) did not seem to be related to the average amount of nitrogen absorbed. This observation is not unexpected inasmuch as it has been pointed out that some subjects (BG and LU) tended to retain a relatively high proportion of the pro-

Table 9. Summary of the regressions of urinary nitrogen on absorbed nitrogen for six subjects subsisting on two levels of protein intakes

Subject	Experimental period	Regression slope (b)	Regression intercept (a)	Nitrogen absorbed mg./kg./day	Nitrogen retained mg./kg./day
NN	I	0.45	64	130	8
	II	0.53	64	187	24
	Dif.**	0.08	0	57	16
BG	I	0.16	89	122	13
	II	-0.09	143	170	42
	Dif.**	-0.25*	64*	48	29
MV	I	0.22	77	99	1
	II	0.15	112	141	18
	Dif.**	-0.07	35*	42	17
LU (Series 1)	I	0.24	96	113	-10
	II	0.29	106	185	26
	Dif.**	+0.05	10	72	36
LS	I	0.32	83	139	11
	II	0.41	91	178	14
	Dif.**	+0.09	8	39	3
MY	I	0.26	141	230	30
	II	0.22	193	283	28
	Dif.**	-0.04	52*	53	- 2
LU (Series 3)	I	0.14	100	128	10
	II	0.26	125	191	17
	Dif.**	+0.12	25	62	7

**The difference represents the change from Period I to Period II.

*The difference is statistically significant.

tein provided by their daily diets. It has been suggested that, in these instances, protein needs had not been fully met. However, MY, whose diet seemed to meet her protein needs and who excreted nitrogen in proportion to the amount of nitrogen in the diet, had a slope for Period I which was not appreciably different from that of subject LU (0.26 versus 0.24).

The values for the intercepts, as discussed before, was characteristic of an individual and probably of her diets. The observation made in MY and MV seemed to provide an example to this point. The values for the slopes of the regressions for these two subjects were close. But MY showed higher values for intercepts which seemed to reflect her relatively high level of nitrogen intakes. MV, on the other hand, had a relatively low level of nitrogen intakes and showed low values for intercepts. These two subjects, MY and MV, appeared to show a more pronounced tendency to adjust themselves to a higher plane of protein nutrition than the other subjects studied as observed from the relative position of the regression lines.

In Table 9, data for nitrogen retentions of all the subjects studied were recorded. The results obtained from the study of regressions for each subject on two levels of protein intakes as interpreted in combination with data relating to nitrogen retentions appeared to show that:

When the slopes ran parallel and the difference between intercepts was significant and approximately identical to the difference in the nitrogen absorbed in two dietary periods, the data suggested that the self-chosen diet was adequate for the maintenance of an optimal state of body protein of the individual. The urinary excretion of nitrogen increased at the same rate with the increase in absorbed nitrogen in two dietary regimes. However, the body seemed to have no capacity to retain the additional nitrogen, therefore, the extra nitrogen was excreted in the urine. MY was an example of this case.

When the slopes ran parallel and the difference between the intercepts was significant but smaller than that between the nitrogen absorbed, the individual excreted a large fraction of the additional nitrogen. The body, however, seemed to be able to retain some of the additional nitrogen. This condition was observed in MV.

When the slopes ran parallel and there was no significant difference between intercepts, the body might have the capacity to retain at least part of the additional nitrogen since both urinary excretion and retention of nitrogen increased at the same rate with the increase in absorbed nitrogen. LU, LS, and NN showed such results.

When the slope of the regression of urinary nitrogen on absorbed nitrogen for the period of supplemented diets was significantly less steep than the regression for the period of self-chosen diets the additional nitrogen was largely re-

tained. The subject might be in a state of severe nitrogen deficit. BG was an example of this case.

Nitrogenous Components of Plasma

Plasma proteins

Electrophoretic analysis of plasma proteins were performed on plasma samples collected from subjects MC, LE, and HS in Series 2 and from the three subjects in Series 3. Since four samples were available for the four periods from each subject in Series 3, a total of 15 plasma samples were analyzed. The diagrams obtained from the densitometric measurement of the dyed paper strips were quantitatively evaluated with regard to the relative concentrations (percentage of the total plasma protein) of the albumin and the globulin fractions. From these data and from the total nitrogen value, the concentration of each fraction was calculated in grams per 100 ml. of plasma. The results thus obtained are tabulated separately in Tables 10 and 11. In addition, Table 11 lists the daily averages of intakes and retentions of nitrogen of MC, LE, and HS in the corresponding 10-day periods and of LS, MY, and LU in the corresponding 28- to 35-day periods.

Total plasma protein and albumin The mean total plasma protein level of 15 plasma samples was 6.88 grams per cent with a range of 6.27 to 7.52. These values were in the range of those reported by Albritton (1952) in healthy adult sub-

Table 10. Relative distribution of plasma protein fractions in percentage of the total plasma protein of six subjects (Series 2--Subjects MC, LE, and HS. Series 3--Subjects LS, MY and LU.)

Subject	Experimental period	Albumin (%)	Alpha-globulin (%)	Beta-globulin (%)	Gamma-globulin plus fibrinogen (%)	Albumin / globulin
LS	I	62.5	7.6	13.3	16.6	1.7
	II	61.7	10.4	13.1	14.8	1.6
	III	57.1	8.9	15.2	18.8	1.3
	IV	60.5	10.9	14.0	14.6	1.5
MC		60.7	10.4	11.3	17.6	1.6
LE		54.1	10.3	7.6	28.0	1.2
HS		59.3	11.2	9.4	20.1	1.5
MY	I	61.6	9.7	10.8	17.9	1.6
	II	60.5	10.2	10.8	18.5	1.5
	III	60.3	9.1	10.9	19.7	1.5
	IV	60.8	10.6	11.5	17.1	1.5
LU	I	53.6	10.8	14.2	21.4	1.2
	II	56.3	9.4	13.4	20.9	1.3
	III	56.3	10.0	12.4	21.3	1.3
	IV	57.5	8.8	12.7	21.0	1.4

Table 11. Plasma proteins of six subjects (Series 2-Subjects MC, LE, and HS. Series 3-Subjects LS, MY, and LU.)

Subject	Age	Experimental period	Nitrogen intake gm./day	Nitrogen retention gm./day	Total plasma protein gm. %	Albumin gm. %	Alpha-globulin gm. %	Beta-globulin gm. %	Gamma-globulin plus fibrinogen gm. %
LS	35	I	12.3	+0.9	6.83	4.27	0.52	0.91	1.13
		II	15.3	+1.1	7.16	4.42	0.74	0.94	1.06
		III	9.8	-0.2	6.71	3.83	0.60	1.02	1.26
		IV	12.4	+0.7	6.86	4.15	0.75	0.96	1.00
MC	43		13.0	-0.5	6.72	4.08	0.70	0.76	1.18
LE	45		7.7	-0.9	6.99	3.78	0.72	0.53	1.96
HS	55		9.7	-1.5	6.70	3.97	0.75	0.63	1.35
MY	56	I	15.5	+1.8	7.41	4.56	0.72	0.80	1.33
		II	18.7	+1.7	7.52	4.55	0.77	0.81	1.39
		III	11.9	+0.6	7.40	4.46	0.68	0.81	1.46
		IV	15.1	+1.1	7.47	4.54	0.79	0.86	1.28
LU	81	I	7.4	+0.5	6.27	3.36	0.68	0.89	1.34
		II	10.7	+0.9	6.40	3.60	0.60	0.86	1.34
		III	9.4	+1.0	6.43	3.62	0.64	0.80	1.37
		IV	7.4	+0.1	6.38	3.67	0.56	0.81	1.34

jects and by Rafsky et al. (1952) and Morgan et al. (1955) in healthy aged subjects.

Highly significant correlations were obtained between average daily nitrogen intakes and the levels of total plasma protein ($P = 0.01$) and albumin ($P = 0.01$) in the six subjects studied. Since it has been observed that the amount of protein required by an adult for the maintenance of nitrogen equilibrium was closely related to his body weight (Sherman, 1920, Rose and MacLeod, 1925, and Hegsted et al., 1946), it was thought that in making comparisons in different individuals, the nitrogen intake or retention expressed on the body weight basis might have more physiological significance than the total amount. When the nitrogen intake of each of the six subjects observed herein was expressed in mg. per kg. of body weight per day, the positive correlations became small ($P = 0.05$). When the statistical analysis was performed on the four samples of each individual, slightly positive correlations ($P = 0.05$) were observed only in subject LS. The relationships were not so clear in the other two subjects, MY and LU.

No correlations between the levels of total plasma protein or albumin and nitrogen retentions could be observed in the six subjects studied. However, subject MY, who showed a high extent of nitrogen retention over the whole period of study, had a higher level of total plasma protein than any other subject.

In nutritional studies on human beings, several groups of workers failed to observe a close relationship between the level of total plasma or serum protein and the protein intake. Youmans et al. (1943), in a survey of some 1200 people, found that in spite of relatively low protein intakes in this population group, there were few cases of low serum protein and albumin levels. Morgan et al. (1955) were not able to detect any correlation between serum protein levels and protein intakes. Addis et al. (1948) observed that when the protein consumption of a group of healthy human subjects was increased from 0.5 to 1.5 to 2.5 grams of protein per kg. of body weight per day, there was no change in serum protein concentration. In this study, the subjects were maintained on each plane of protein intake for 5 days. The period of observation was probably too short to expect definite results. These authors also failed to demonstrate any significant change in serum protein concentrations during the first 24 hours after a change from a high (2.5 grams per kg. of body weight per day) to an inadequate (0.1 gram per kg. of body weight per day) protein consumption in healthy adult human beings.

Hegsted et al. (1946) reported that in adult males, total plasma protein and albumin tended to fall when the subjects were consuming a low protein, all vegetable diet at a level low enough to produce a negative nitrogen balance. In their study, a direct relationship between protein intake (or reten-

tion) and the concentration of plasma protein (or albumin) was seen.

In adult human subjects, low plasma or serum protein concentrations have been observed in certain famine areas (Walters et al., 1947 and Holmes et al., 1954). Adult human beings previously depleted of protein reserves showed low levels of total plasma proteins or albumins. There were direct relations between protein intakes and retentions and plasma protein concentrations during the period of repletion with high protein intakes.

Plasma globulin fractions Apparently, there was little effect of protein intakes or retentions on the concentrations of the various globulin fractions. Variations in protein intakes were associated with a change in albumin levels while the total globulin concentrations remained essentially constant (Table 11).

Hsu et al. (1958) have found that sera of a group of people receiving approximately 70 grams of protein daily had a higher percentage of alpha-globulin and a lower percentage of gamma-globulin than the sera of those getting 85 to 90 grams of protein. In the present study, no correlation between the levels of plasma alpha-globulin and protein intakes could be detected. In contrast to the findings of Hsu et al. (1958), a significantly negative correlation ($P = 0.01$) was observed in the present study between nitrogen intakes and the percentage of gamma-globulin in the total plasma protein.

The reports in the literature reviewed above indicate that a demonstrable correlation between the level of plasma or serum proteins and the protein intake or retention should not be expected in human beings consuming adequate diets. Plasma or serum protein levels tend to fall within a narrow range which may be relatively unaffected except by rather extensive variations in the protein intake over prolonged periods of time. The observation made in the three subjects in Series 3 that small variations in protein intakes or retentions did not influence the plasma protein levels appeared to be in agreement with those cited above. However, when all data available from the six subjects were analyzed, a highly significant correlation between plasma protein levels and nitrogen intakes was found. Such relationship is illustrated by data in Table 11. MY, whose average nitrogen intake was the highest in the group of the subjects observed, had the highest levels of plasma protein. Lowest levels of plasma protein were found in LU who had the lowest average nitrogen intake of the group. LS was intermediate between the two. Each of these three individuals seemed to have a characteristic level of plasma protein which was not changed significantly by small variations in protein intakes. From these results, it is postulated that plasma protein levels may be closely associated with a characteristic long-term pattern of protein intake.

Plasma non-protein nitrogenous components

Non-protein nitrogenous components were determined in one plasma sample collected from each of the six women in Series 2 and in four samples from each of the six subjects in Series 3. A total of 18 plasma samples were analyzed for total non-protein nitrogen, urea nitrogen, creatinine, creatine, and uric acid. The results are presented in Table 12. Partitions of plasma non-protein nitrogenous components are shown in Table 13.

Plasma non-protein nitrogen and urea nitrogen The average value of plasma non-protein nitrogen for all eight subjects studied was 27.5 mg. per cent with a range of 22.9 to 35.7. The average value of urea nitrogen was 14.1 mg. per cent ranging from 7.3 to 26.8. These values were in the range of those reported in the literature for healthy adult women (Hammett, 1920, Cramer and Winnick, 1943, and Morgan et al., 1955).

The results in Table 12 show that in the three subjects of Series 3, the plasma levels of non-protein nitrogen and urea nitrogen fluctuated directly with the protein intake in the four periods of observation. Significant correlations of average daily nitrogen intake per period for each subject (in mg. per kg. of body weight) with the plasma levels of non-protein nitrogen and urea nitrogen were also observed in all eight subjects from both series 2 and 3. The distribution of

Table 12. Plasma non-protein nitrogenous components of eight subjects (Series 2 and 3)

Subject	Age yr.	Experimental period	Nitrogen intake gm./day	Nitrogen retention gm./day	Plasma NPN mg. %	Plasma urea N mg. %	Plasma creatinine mg. %	Plasma creatine mg. %	Plasma uric acid mg. %
LS	35	I	12.3	+0.9	26.6	14.1	1.16	0.18	3.86
		II	15.3	+1.1	34.1	18.5	1.16	0.39	3.64
		III	9.8	-0.2	25.1	12.1	1.04	0.20	3.57
		IV	12.4	+0.7	27.7	14.7	1.16	0.18	3.69
MC	43		13.0	-0.5	22.9	7.3	0.84	0.58	3.38
LE	45		7.7	-0.9	29.4	11.6	1.07	0.35	3.75
HS	55		9.7	-1.5	30.0	12.2	1.00	0.18	3.78
MY	56	I	15.5	+1.8	28.8	16.4	0.98	0.57	3.61
		II	18.7	+1.7	35.7	26.8	1.16	0.60	3.90
		III	11.9	+0.6	25.6	11.5	1.04	0.62	3.85
		IV	15.1	+1.1	30.9	13.2	1.10	0.45	3.69
NN	72		8.3	-0.5	24.7	12.3	0.90	0.30	2.27
MM	73		11.9	+0.8	24.7	11.5	1.44	0.51	3.53
LU	81	I	7.4	+0.5	24.5	10.4	1.10	0.56	2.59
		II	10.7	+0.9	26.6	19.1	1.16	0.81	3.14
		III	9.4	+1.0	26.6	16.8	1.21	0.76	2.49
		IV	7.4	+0.1	24.0	15.2	1.10	0.24	2.08
LU	82		7.1	+0.9	27.5	10.6	0.88	0.32	2.22

Table 13. Partitions of plasma non-protein nitrogenous components in eight subjects (Series 2 and 3)

Subject	Experimental period	NPN mg. %	Urea N mg. %	Creatinine N mg. %	Creatine N mg. %	Uric acid N mg. %	Percentage of NPN: urea N	creatinine N	creatine N	uric acid N
LS	I	26.6	14.1	0.43	0.06	1.39	53.0	1.6	0.2	5.2
	II	34.1	18.5	0.43	0.14	1.31	54.3	1.3	0.4	3.8
	III	25.1	12.0	0.39	0.07	1.28	47.8	1.6	0.3	5.1
	IV	27.7	14.7	0.43	0.06	1.32	53.1	1.6	0.2	4.8
MC		22.9	7.3	0.31	0.22	1.21	31.9	1.4	1.0	5.3
LE		29.4	11.6	0.40	0.13	1.35	39.5	1.4	0.4	4.6
HS		30.0	12.2	0.37	0.07	1.36	40.7	1.2	0.2	2.5
MY	I	28.8	16.4	0.36	0.21	1.29	56.9	1.3	0.7	4.5
	II	35.7	26.8	0.43	0.22	1.40	75.1	1.2	0.6	3.9
	III	25.6	11.5	0.39	0.23	1.38	44.9	1.5	0.9	5.4
	IV	30.9	13.2	0.41	0.17	1.32	42.7	1.3	0.6	4.3
NN		24.7	12.3	0.33	0.11	0.81	49.6	1.3	0.4	3.3
MM		24.7	11.5	0.53	0.19	1.27	46.6	2.1	0.8	5.1
LU	I	24.5	10.4	0.41	0.21	0.93	42.4	1.7	0.9	3.8
	II	26.6	19.1	0.43	0.30	1.13	71.8	1.6	1.1	4.2
	III	26.6	16.8	0.45	0.28	0.89	63.2	1.7	1.1	3.1
	IV	24.0	15.2	0.41	0.09	0.75	63.3	1.7	0.4	3.1
LU		27.5	10.6	0.33	0.12	0.80	38.4	1.2	0.4	2.2

plasma non-protein nitrogen and urea nitrogen in relation to average daily nitrogen intakes is shown in Table 14.

The observation made here that plasma levels of non-protein nitrogen and urea nitrogen varied directly with the protein consumption of adult human subjects living on self-chosen diets seemed to confirm those made in healthy adult human beings in other studies (Goldring et al. (1934), Miller et al. (1941), Addis et al. (1947), Kountz et al. (1953a), and Morgan et al. (1955).

Table 15 shows the relationship between the average daily nitrogen retention in mg. per kg. of body weight for each subject in each period of observation and plasma levels of non-protein nitrogen and urea nitrogen. Plasma non-protein nitrogen levels appeared to be relatively low when the body was in a state close to nitrogen equilibrium. The level of plasma non-protein nitrogen rose when the body was in either positive or negative nitrogen balance. In the region of positive balance, significant correlations were observed between nitrogen retentions and plasma levels of non-protein nitrogen ($P = 0.01$) or urea nitrogen ($P = 0.01$). In the region of negative balance, a slightly significant correlation was shown between nitrogen retentions and plasma levels of non-protein nitrogen ($P = 0.05$). The plasma levels of urea nitrogen did not seem to be correlated with the nitrogen retention in the negative balance region.

Table 14. Relationships between protein intake and plasma non-protein nitrogenous components (Series 2 and 3)

Nitrogen intake mg./kg./day	No. of observations	NPN mg. %	Urea N mg. %	Creatinine mg. %	Creatine mg. %	Creatinine and Creatine mg. %	Uric acid mg. %
up to 125	1	25.1	12.0	1.04	0.20	1.24	3.57
126 - 150	4	26.4	12.0	1.04	0.37	1.41	2.66
151 - 175	5	26.7	13.0	1.13	0.27	1.40	3.43
176 - 200	3	28.8	15.6	1.14	0.59	1.73	3.33
201 - 250	2	28.8	16.2	1.13	0.63	1.76	3.42
Above 250	3	29.1	16.8	1.00	0.58	1.58	3.63
Correlation		p = 0.01	p = 0.01	n.s.	p = 0.05	n.s.	n.s.

Table 15. Relationships between nitrogen retention and plasma non-protein-nitrogenous components (Series 2 and 3)

Nitrogen retention mg./kg./day	No. of observations	NPN mg. %	Urea N mg. %	Creatinine mg. %	Creatine mg. %	Creatinine + Creatine mg. %	Uric acid mg. %
Up to -20	1	30.0	12.2	1.00	0.18	1.18	3.78
-19 to -10	2	26.2	9.4	0.96	0.46	1.42	3.56
-9 to 0	2	24.9	12.2	0.97	0.25	1.22	2.92
0 to 10	4	25.4	13.0	1.14	0.36	1.50	3.05
11 to 20	7	28.1	14.8	1.16	0.49	1.65	3.22
21 to 30	2	32.2	21.6	1.07	0.59	1.66	3.76
Correlation		n.s.	n.s.	n.s.	p = 0.05	p = 0.05	n.s.

Plasma creatinine and creatine The average plasma creatinine value of all eight subjects was 1.08 mg. per cent with a range between 0.84 and 1.21. No difference due to age could be discerned in the group of subjects studied. These values were in the range reported by Morgan et al. (1955) for the corresponding age groups and were close to those found by Allinson (1945) and Barrett and Addis (1947). No correlations of plasma creatinine levels to nitrogen intakes or retentions were observed. Similar results have been reported by Barrett and Addis (1947) and Morgan et al. (1955) with respect to nitrogen intakes.

The average value for the plasma creatine was 0.43 mg. per cent for the eight subjects studied. The range was from 0.18 to 0.81. Allinson (1945) reported an average of 0.42 mg. per cent with a range of 0.28 to 0.62 mg. per cent of creatine in sera of adult humans. Significant correlations were observed between the levels of plasma creatine and nitrogen intakes or retentions (Table 14 and Table 15).

It will be discussed in the following section that the amount of urinary creatinine plus creatine might bear a more significant relationship to protein metabolism than that of either component alone. An attempt was made here to examine the possible relation of plasma levels of creatinine plus creatine to nitrogen intakes or retentions. In two of the three subjects in Series 3, LS and LU, the plasma creatinine

plus creatine appeared to fluctuate with the extent of nitrogen retentions in the corresponding dietary regimes (Table 12). A significant correlation was obtained between plasma creatinine plus creatine levels and nitrogen retentions per kg. of body weight in the total group of subjects studied. This was probably due to the relatively high correlation found for creatine.

Uric acid The average level of plasma uric acid for the 18 determinations on eight women was 3.28 with a range from 2.08 to 3.90 mg. per cent (Table 12). The plasma uric acid levels of all the subjects in the present study were in the range of those reported by Morgan et al. (1955). In their study, the mean serum uric acid of women between 50 to 80 years of age was 2.98 ranging from 1.4 to 5.5 mg. per cent. The plasma uric acid levels reported by Praetorius (1951) and Gertler and Oppenheimer (1953) for women in a similar age group were much higher than those observed in the present study.

No correlations between plasma uric acid levels and nitrogen intakes or retentions could be demonstrated. This is in agreement with Morgan et al. (1955) who failed to find a positive correlation between those two parameters in women, though a slight positive correlation between protein intakes and serum uric acid levels was seen in their male subjects.

Partition of Nitrogen in Urine

Nitrogenous components and total titratable acidity of the urine samples were determined on 24-hour collections made at the end of each seven day-period in the three subjects in Series 3. The results are presented separately in Tables 16, 17, and 18. The values recorded in each row in these tables represent data obtained on comparable days of each period.

Partition of urinary nitrogen has been calculated for this series (Tables 20, 21, and 22). Data pertaining to all the nitrogenous components studied are depicted separately for each subject in Figures 10, 11, and 12. In these figures, the amount of nitrogen absorbed in grams per day for the weekly average as well as for the seventh day of each week are included.

In addition, urinary nitrogenous components were analyzed for one 24-hour sample at the end of the 10-day observation period for the six subjects in Series 2. The findings are presented in Table 19. In this table, the values for the 24-hour urine sample collected at the end of each experimental period for the three subjects in Series 3 are included.

Ammonia nitrogen

Each of the three women in Series 3 seemed to respond to changes in the plane of protein intake by corresponding fluctuations in urinary ammonia excretions. However, the degree of fluctuation did not necessarily reflect the amount of ni-

Table 16. Urinary nitrogenous components of subject LS

Experimental period		I	II	III	IV
Nitrogen intake (average per week) (gram per day)	1	11.3	15.1	9.8	11.7
	2	11.6	14.5	9.2	11.6
	3	12.4	15.8	10.3	12.9
	4	13.8	15.8	10.0	13.5
	5	12.2	15.4		
Average		12.3	15.3	9.8	12.4
Nitrogen intake (Seventh day of each week) (gram per day)	1	10.6	13.3	8.1	9.7
	2	11.8	9.0	9.1	6.1
	3	11.4	16.0	9.7	11.3
	4	16.6	18.2	10.0	15.4
	5	15.2	18.0		
Average		13.1	14.9	9.2	10.6
Urinary nitrogen (gram per day)	1	9.5	10.6	7.1	11.7
	2	8.2	9.6	7.0	9.4
	3	8.9	14.3	8.2	10.3
	4	9.8	13.5	8.2	9.7
	5	9.3	13.9		
Average		9.2	12.4	7.6	10.3
Total titratable acidity (meq. of acid)	1	22.3	29.6	16.8	26.9
	2	30.3	29.4	34.2	41.7
	3	22.5	35.5	25.2	29.7
	4	28.6	32.7	28.4	25.2
	5	28.7	34.1		
Average		26.5	32.3	26.2	30.9
Urea nitrogen (gram per day)	1	8.2	9.5	5.6	9.3
	2	7.0	7.4	5.8	7.1
	3	7.1	10.5	6.0	7.3

Table 16. (Continued)

Experimental period	Week	I	II	III	IV
	4	7.1	11.5	5.4	7.3
	5	8.0	10.6		
Average		7.5	9.9	5.7	7.8
Ammonia nitrogen (gram per day)	1	0.46	0.48	0.30	0.34
	2	0.45	0.49	0.43	0.48
	3	0.62	0.57	0.50	0.54
	4	0.54	0.76	0.51	0.52
	5	0.47	0.68		
Average		0.51	0.60	0.44	0.47
Creatinine (gram per day)	1	1.25	1.25	1.28	1.41
	2	1.25	1.23	1.34	1.30
	3	1.22	1.24	1.27	1.24
	4	1.38	1.56	1.38	1.32
	5	1.45	1.51		
Average		1.31	1.36	1.32	1.32
Creatine (gram per day in terms of creatinine	1	0.00	0.00	0.07	0.03
	2	0.04	0.10	0.01	0.05
	3	0.02	0.14	0.00	0.00
	4	0.03	0.14	0.03	0.13
	5	0.00	0.25		
Average		0.02	0.13	0.03	0.05
Uric acid (gram per day)	1	0.52	0.44	0.41	0.46
	2	0.39	0.50	0.44	0.43
	3	0.48	0.58	0.44	0.42
	4	0.51	0.62	0.50	0.45
	5	0.46	0.45		
Average		0.47	0.52	0.45	0.44

Table 17. Urinary nitrogenous components of subject MY

Experimental period	Week	I	II	III	IV
Nitrogen intake (average per week) (gram per day)	1	16.9	20.6	12.4	17.0
	2	15.2	18.3	11.0	13.9
	3	14.2	18.5	12.0	15.1
	4	15.8	18.4	12.3	14.6
	5	15.4	17.9		
Average		15.5	18.7	11.9	15.1
Nitrogen intake (Seventh day of each week) (gram per day)	1	16.5	21.1	10.6	16.8
	2	16.5	18.4	11.1	14.7
	3	18.7	21.9	14.9	20.0
	4	16.6	18.4	12.4	16.1
	5	11.8	15.0		
Average		16.0	19.0	12.2	16.9
Urinary nitrogen (gram per day)	1	12.7	17.0	10.7	13.0
	2	11.5	15.4	9.4	11.1
	3	15.0	17.2	10.4	14.7
	4	12.8	17.4	11.1	12.6
	5	11.4	14.4		
Average		12.7	16.3	10.4	12.8
Total titratable acidity (meq. of acid)	1	21.1	28.7	17.8	14.4
	2	13.7	22.2	13.6	17.7
	3	15.0	16.9	7.5	20.9
	4	20.9	23.8	11.8	24.2
	5	22.0	15.5		
Average		18.5	21.4	12.7	19.3
Urea nitrogen (gram per day)	1	11.0	14.8	7.8	10.2
	2	10.0	13.4	7.4	10.0
	3	11.8	15.2	7.1	9.0

Table 17. (Continued)

Experimental period	Week	I	II	III	IV
	4	10.2	14.2	7.1	9.2
	5	9.7	11.9		
Average		10.6	13.9	7.3	9.6
Ammonia nitrogen (gram per day)	1	0.40	0.48	0.57	0.57
	2	0.30	0.45	0.35	0.52
	3	0.36	0.40	0.37	0.34
	4	0.35	0.45	0.34	0.41
	5	0.29	0.40		
Average		0.34	0.44	0.41	0.46
Creatinine (gram per day)	1	1.22	1.43	1.13	1.35
	2	1.13	1.26	1.15	1.09
	3	1.33	1.27	1.20	1.45
	4	1.13	1.29	1.20	1.25
	5	1.20	1.28		
Average		1.20	1.31	1.17	1.28
Creatine (gram per day in terms of creatinine)	1	0.16	0.56	0.13	0.08
	2	0.15	0.47	0.06	0.10
	3	0.24	0.53	0.07	0.11
	4	0.30	0.60	0.17	0.30
	5	0.14	0.40		
Average		0.20	0.51	0.11	0.15
Uric acid (gram per day)	1	0.56	0.84	0.56	0.52
	2	0.54	0.56	0.49	0.50
	3	0.68	0.64	0.49	0.59
	4	0.54	0.71	0.61	0.56
	5	0.47	0.65		
Average		0.56	0.68	0.54	0.54

Table 18. Urinary nitrogenous components of subject LU

Experimental period	Week	I	II	III	IV
Nitrogen intake (average per week) (gram per day)	1	7.0	9.7	8.4	6.5
	2	8.0	11.5	9.7	7.4
	3	7.5	10.7	9.5	7.4
	4	7.5	10.9	10.0	8.2
	5	7.1	10.7		
Average		7.4	10.7	9.4	7.4
Nitrogen intake (seventh day of each week) (gram per day)	1	8.0	9.1	10.1	7.7
	2	9.2	13.6	11.1	9.3
	3	6.6	11.0	9.4	6.7
	4	7.2	8.8	8.7	7.0
	5	7.8	11.8		
Average		7.8	10.9	9.8	7.7
Urinary nitrogen (gram per day)	1	5.2	8.8	6.0	5.6
	2	7.8	10.7	8.6	7.9
	3	5.4	8.3	7.5	6.3
	4	6.1	9.0	8.5	6.1
	5	7.0	9.7		
Average		6.3	9.3	7.6	6.5
Total titratable acidity (meq. of acid)	1	19.0	19.8	18.0	20.0
	2	16.8	21.4	20.1	17.2
	3	6.9	20.4	6.3	16.1
	4	4.6	16.8	19.2	12.1
	5	12.5	22.0		
Average		12.0	20.1	15.9	16.4
Urea nitrogen (gram per day)	1	4.5	7.9	5.4	4.6
	2	6.8	8.0	7.6	6.6
	3	4.9	7.2	5.9	5.2

Table 18. (Continued)

Experimental period	Week	I	II	III	IV
	4	5.5	7.6	5.9	4.9
	5	5.7	8.3		
Average		5.5	8.0	6.2	5.3
Ammonia nitrogen (gram per day)	1	0.20	0.30	0.22	0.19
	2	0.24	0.32	0.26	0.24
	3	0.15	0.34	0.13	0.20
	4	0.12	0.37	0.28	0.18
	5	0.22	0.34		
Average		0.19	0.33	0.22	0.23
Creatinine (gram per day)	1	0.79	0.84	0.72	0.75
	2	0.88	0.95	0.91	0.91
	3	0.84	0.89	0.92	0.89
	4	0.76	0.83	0.96	0.82
	5	0.84	0.81		
Average		0.82	0.86	0.88	0.84
Creatine (gram per day in terms of creatinine)	1	0.15	0.29	0.20	0.04
	2	0.30	0.56	0.41	0.06
	3	0.09	0.33	0.29	0.11
	4	0.14	0.36	0.33	0.15
	5	0.10	0.39		
Average		0.16	0.38	0.31	0.09
Uric acid (gram per day)	1	0.31	0.44	0.31	0.32
	2	0.38	0.49	0.44	0.45
	3	0.33	0.44	0.49	0.34
	4	0.32	0.40	0.44	0.31
	5	0.39	0.47		
Average		0.35	0.45	0.42	0.36

Table 19. Urinary nitrogenous components of eight subjects for one 24-hour period (Series 2 and 3)

Subject	Experimental period	Nitrogen intake* gm./day	Total titratable acidity meq. of acid per day	Total urinary nitrogen gm./day	Nitrogen ammonia gm./day	Urea nitrogen gm./day	Creatinine gm./day	Creatine in terms of creatinine gm./day	Uric acid gm./day
LS**	I	12.3	26.5	9.3	0.47	8.0	1.45	0.00	0.46
	II	15.3	32.3	13.9	0.68	10.6	1.51	0.13	0.45
	III	9.8	26.2	8.2	0.51	5.4	1.38	0.03	0.50
	IV	12.4	30.9	9.7	0.52	7.3	1.32	0.13	0.45
MC		13.0	21.8	10.6	0.40	9.3	1.01	0.18	0.55
LE		7.7	11.0	10.2	0.26	9.4	1.29	0.00	0.72
HS		9.7	26.8	10.1	0.36	9.3	1.14	0.10	0.67
MY**	I	15.5	18.5	11.4	0.29	9.7	1.20	0.14	0.47
	II	18.7	21.4	14.4	0.40	11.9	1.28	0.39	0.65
	III	11.9	12.7	11.6	0.34	7.1	1.20	0.17	0.61
	IV	15.1	19.3	12.6	0.41	9.2	1.24	0.28	0.56
NN		8.3	21.9	8.4	0.34	7.6	0.86	0.07	0.32
MM		11.9	22.0	10.0	0.51	8.8	0.98	0.22	0.52
LU**	I	7.4	12.0	7.0	0.22	5.7	0.84	0.10	0.39
	II	10.7	20.1	9.7	0.34	8.3	0.81	0.39	0.47
	III	9.4	15.9	8.5	0.28	5.9	0.96	0.33	0.49
	IV	7.4	16.4	6.1	0.28	4.9	0.82	0.15	0.31
LU		7.1	17.2	5.6	0.33	4.7	0.80	0.15	0.37

*Average for the period.

**Urinary data represent one 24-hour sample collected at the end of each period.

Table 20. Partition of urinary nitrogen in subject LS

Experimental period	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Total nitrogen	9.5		10.6		7.1		11.7	
	8.2		9.6		7.0		9.4	
	8.9		14.3		8.2		10.3	
	9.8		13.5		8.2		9.7	
	9.3		13.9					
Average	9.2		12.4		7.6		10.3	
Urea nitrogen	8.21	86.4	9.52	89.8	5.61	79.0	9.30	79.5
	6.97	85.0	7.38	76.9	5.84	83.4	7.12	75.7
	7.09	79.7	10.54	73.7	6.04	73.7	7.29	70.8
	7.06	72.0	11.52	85.3	5.40	65.9	7.28	75.1
	8.02	86.2	10.59	76.2				
Average	7.47	81.9	9.91	80.4	5.72	75.5	7.75	75.3
Ammonia nitrogen	0.46	4.8	0.48	5.0	0.30	4.2	0.34	2.9
	0.45	5.5	0.49	5.1	0.43	6.1	0.48	5.1
	0.62	7.0	0.57	4.0	0.50	6.1	0.54	5.2
	0.54	7.0	0.57	4.0	0.50	6.1	0.54	5.2
	0.47	5.1	0.68	4.9				
Average	0.51	5.6	0.60	4.9	0.44	5.6	0.47	4.6

Table 20. (Continued)

Experimental period	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Creatinine nitrogen	0.46	4.8	0.46	4.3	0.48	6.8	0.52	4.4
	0.46	5.6	0.46	4.8	0.50	7.1	0.48	5.1
	0.45	5.1	0.46	3.2	0.47	5.7	0.46	4.5
	0.51	5.2	0.58	4.3	0.51	6.2	0.49	5.1
	0.54	5.8	0.56	4.0				
Average	0.48	5.3	0.50	4.1	0.49	6.4	0.49	4.8
Creatine nitrogen	0.00	0.0	0.00	0.0	0.03	0.4	0.01	0.1
	0.01	0.1	0.04	0.4	0.00	0.0	0.02	0.2
	0.01	0.1	0.05	0.3	0.00	0.0	0.00	0.0
	0.01	0.1	0.05	0.4	0.01	0.1	0.05	0.5
	0.00	0.0	0.09	0.6				
Average	0.01	0.1	0.05	0.3	0.01	0.1	0.02	0.2
Uric acid nitrogen	0.18	1.9	0.16	1.5	0.15	2.1	0.17	1.5
	0.14	1.7	0.18	1.9	0.16	2.3	0.15	1.6
	0.17	1.9	0.21	1.5	0.16	2.0	0.15	1.5
	0.18	1.8	0.22	1.6	0.18	2.2	0.16	1.6
	0.17	1.8	0.16	1.2				
Average	0.17	1.8	0.19	1.5	0.16	2.2	0.16	1.6

Table 21. Partition of urinary nitrogen in subject MY

Experimental period	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Total nitrogen	12.7		17.0		10.7		13.0	
	11.5		15.4		9.4		11.1	
	15.0		17.2		10.4		14.7	
	12.8		17.4		11.1		12.6	
	11.4		14.4					
Average	12.7		16.3		10.4		12.8	
Urea nitrogen	11.04	86.9	14.79	87.0	7.79	72.8	10.20	78.5
	10.02	87.1	13.45	87.3	7.38	78.5	10.00	90.1
	11.80	78.7	15.20	88.4	7.07	68.0	9.00	61.2
	10.17	79.5	14.15	81.3	7.11	64.1	9.20	73.0
	9.72	85.3	11.93	82.8				
Average	10.55	83.5	13.90	85.4	7.34	70.8	9.60	75.7
Ammonia nitrogen	0.40	3.1	0.48	2.8	0.57	5.3	0.57	4.4
	0.30	2.6	0.45	2.9	0.35	3.7	0.52	4.7
	0.36	2.4	0.40	2.3	0.37	3.6	0.34	2.3
	0.35	2.7	0.45	2.6	0.34	3.1	0.41	3.3
	0.29	2.5	0.40	2.8				
Average	0.34	2.7	0.44	2.7	0.41	3.9	0.46	3.7

Table 21. (Continued)

Experimental period	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Creatinine nitrogen	0.45	3.5	0.53	3.1	0.42	3.9	0.50	3.8
	0.42	3.7	0.47	3.1	0.43	4.6	0.40	3.6
	0.49	3.3	0.47	2.7	0.45	4.3	0.54	3.9
	0.42	3.3	0.48	2.8	0.45	4.1	0.46	3.6
	0.42	3.7	0.48	3.3				
Average	0.44	3.5	0.49	3.0	0.44	4.2	0.48	3.7
Creatine nitrogen	0.06	0.4	0.21	1.2	0.05	0.5	0.03	0.2
	0.06	0.5	0.17	1.1	0.02	0.2	0.04	0.4
	0.09	0.6	0.20	1.2	0.03	0.3	0.04	0.3
	0.11	0.9	0.22	1.3	0.06	0.5	0.11	0.9
	0.05	0.4	0.15	1.0				
Average	0.07	0.6	0.19	1.2	0.04	0.4	0.06	0.4
Uric acid nitrogen	0-20	1-6	0.30	1.8	0.20	1.9	0.19	1.5
	0.19	1.5	0.20	1.3	0.18	1.9	0.18	1.6
	0.24	2.1	0.23	1.3	0.18	1.7	0.21	1.4
	0.19	1.5	0.25	1.4	0.22	2.0	0.20	1.6
	0.17	1.5	0.23	1.6				
Average	0.20	1.6	0.24	1.5	0.20	1.9	0.20	1.5

Table 22. Partition of urinary nitrogen in subject LU

Experimental period,	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Total nitrogen	5.2		8.8		6.0		5.6	
	7.8		10.7		8.6		7.9	
	5.4		8.3		7.5		6.3	
	6.1		9.0		8.5		6.1	
	7.0		9.7					
Average	6.3		9.3		7.6		6.5	
Urea nitrogen	4.53	87.1	7.90	90.0	5.43	90.5	4.63	82.7
	6.81	87.3	8.95	83.6	7.56	87.9	6.57	83.2
	4.86	90.0	7.23	87.1	5.87	78.3	5.16	81.9
	5.50	90.2	7.59	84.3	5.94	69.9	4.90	80.3
	5.69	81.3	8.30	85.6				
Average	5.48	87.2	7.99	86.1	6.20	81.6	5.32	82.0
Ammonia nitrogen	0.20	3.8	0.30	3.4	0.22	3.7	0.19	3.4
	0.24	3.1	0.32	3.0	0.26	3.0	0.24	3.0
	0.15	2.8	0.34	4.1	0.13	1.7	0.20	3.2
	0.12	2.0	0.37	4.1	0.28	3.3	0.28	4.6
	0.22	3.1	0.34	3.5				
Average	0.19	3.0	0.33	3.6	0.22	2.9	0.23	3.6

Table 22. (Continued)

Experimental period	I		II		III		IV	
	gm./day	% of total	gm./day	% of total	gm./day	% of total	gm./day	% of total
Creatinine nitrogen	0.29	5.6	0.31	3.5	0.27	4.5	0.28	5.0
	0.33	4.2	0.35	3.3	0.34	4.0	0.34	4.3
	0.31	5.7	0.33	4.0	0.34	4.5	0.33	5.2
	0.28	4.6	0.31	3.4	0.36	4.2	0.30	4.9
	0.31	4.4	0.30	3.1				
Average	0.30	4.9	0.32	3.5	0.33	4.3	0.31	4.8
Creatine nitrogen	0.06	1.2	0.10	1.1	0.07	1.2	0.01	0.2
	0.11	1.4	0.21	2.0	0.15	1.7	0.02	0.3
	0.03	0.6	0.12	1.4	0.11	1.5	0.04	0.6
	0.05	0.8	0.13	1.4	0.12	1.4	0.06	1.0
	0.04	0.6	0.14	1.4				
Average	0.06	0.9	0.14	1.5	0.11	1.4	0.03	0.5
Uric acid nitrogen	0.11	2.1	0.16	1.8	0.11	1.8	0.12	2.1
	0.14	1.8	0.18	1.7	0.16	1.9	0.16	2.0
	0.12	2.2	0.16	1.9	0.16	2.1	0.12	1.9
	0.12	2.0	0.14	1.6	0.18	2.1	0.11	1.8
	0.14	2.0	0.17	1.8				
Average	0.13	2.0	0.16	1.8	0.15	2.0	0.13	2.0

Fig. 10. Absorbed nitrogen and urinary nitrogenous components: Subject LS (Series 3)

SUBJECT - LS

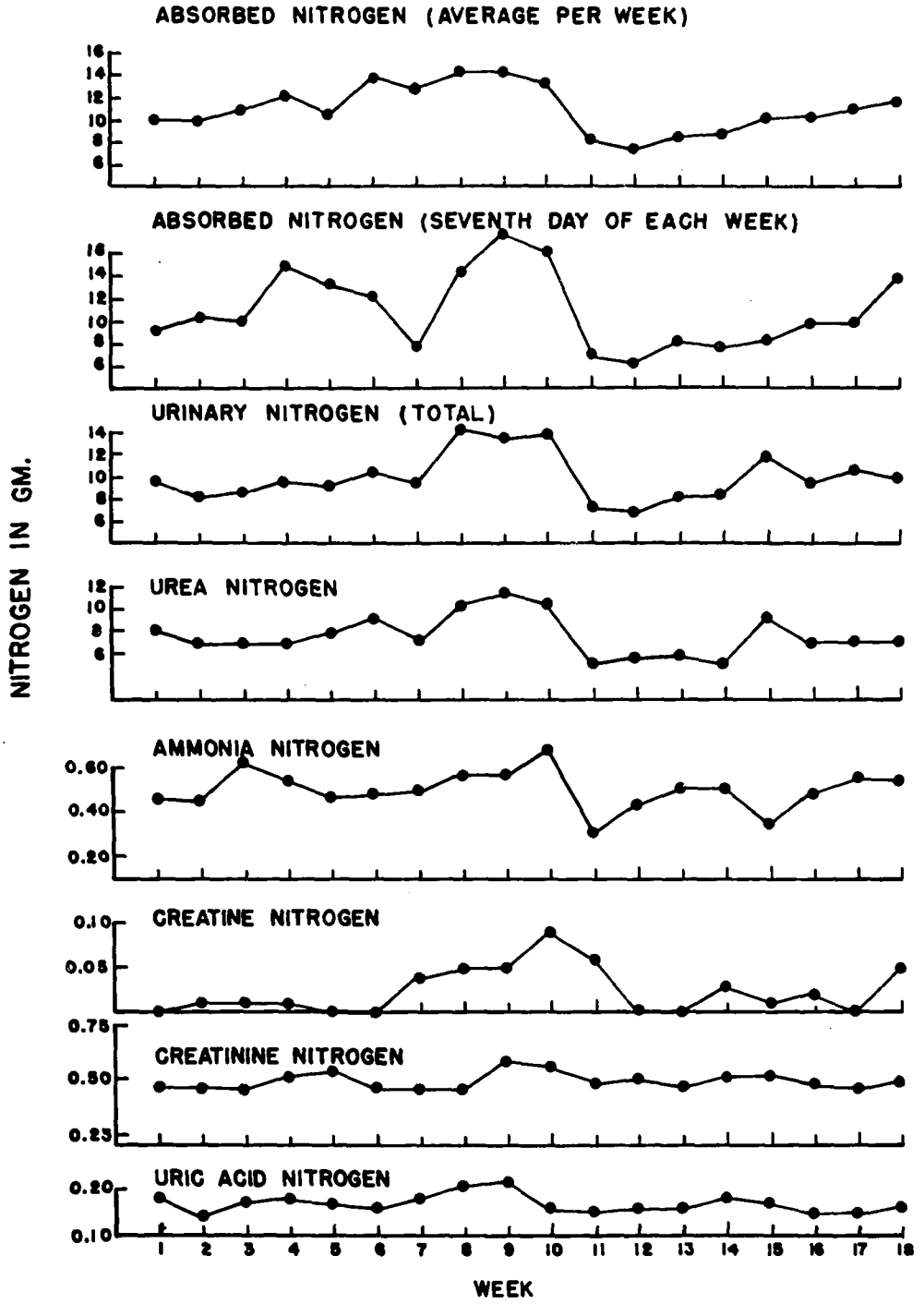

ABSORBED NITROGEN (AVERAGE PER WEEK)
ABSORBED NITROGEN (SEVENTH DAY OF EACH WEEK)
URINARY NITROGEN (TOTAL)
UREA NITROGEN
AMMONIA NITROGEN
CREATINE NITROGEN
CREATININE NITROGEN
URIC ACID NITROGEN
NITROGEN IN GM.
WEEK

Fig. 11. Absorbed nitrogen and urinary nitrogenous components: Subject MY (Series 3)

SUBJECT - MY

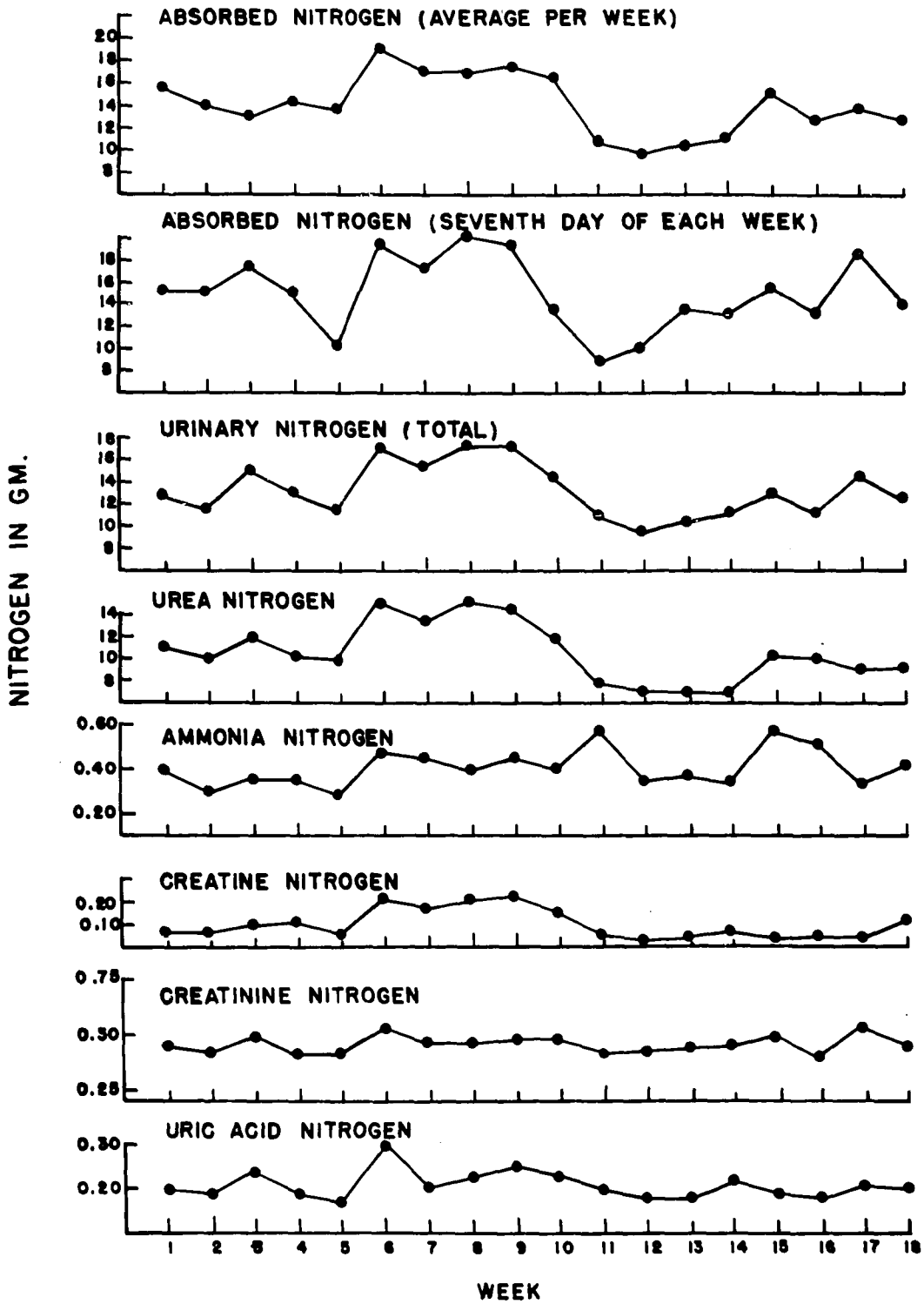
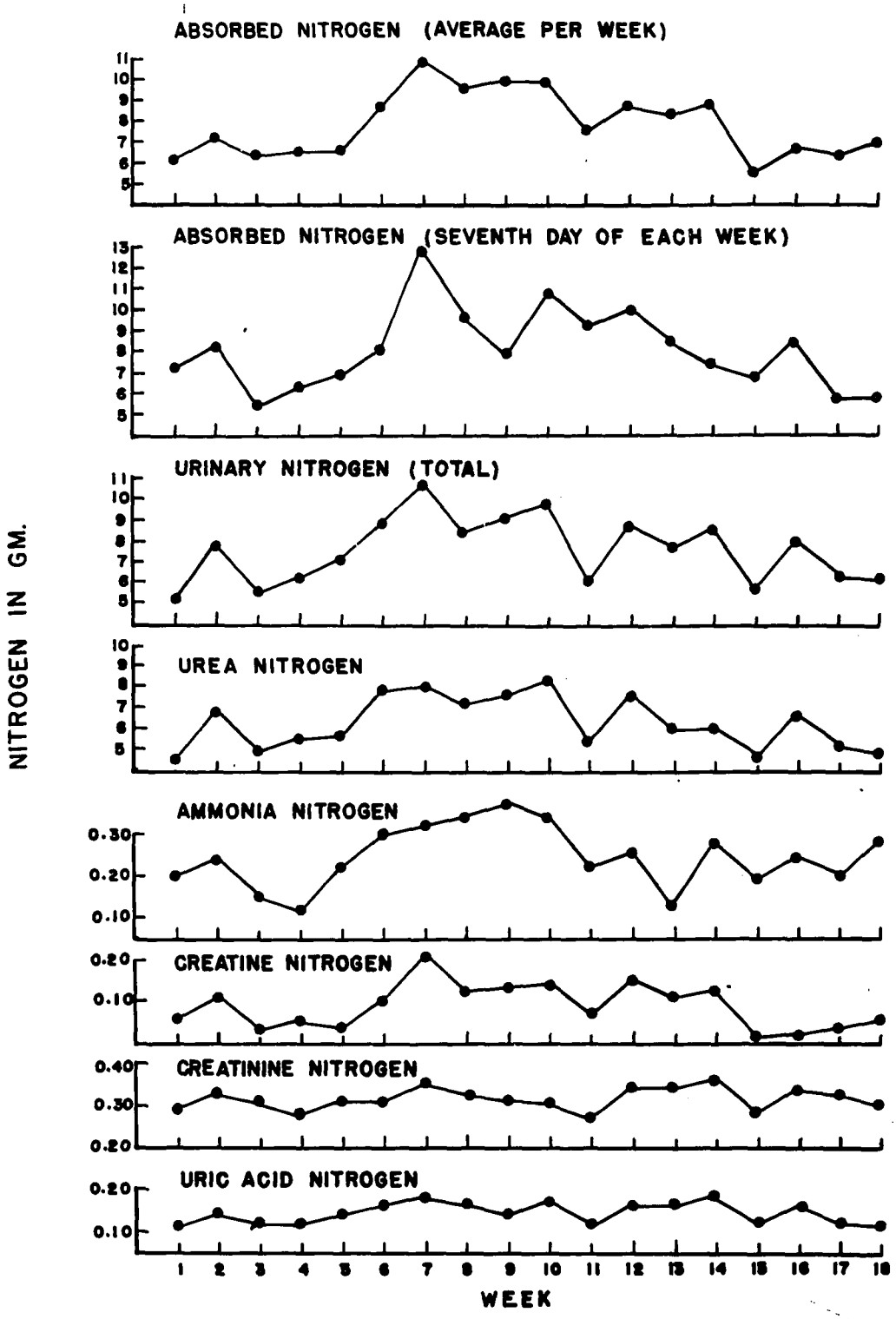

ABSORBED NITROGEN (AVERAGE PER WEEK)
ABSORBED NITROGEN (SEVENTH DAY OF EACH WEEK)
URINARY NITROGEN (TOTAL)
UREA NITROGEN
AMMONIA NITROGEN
CREATINE NITROGEN
CREATININE NITROGEN
URIC ACID NITROGEN
NITROGEN IN GM.
WEEK

Fig. 12. Absorbed nitrogen and urinary nitrogenous components: Subject LU (Series 3)

SUBJECT - LU


ABSORBED NITROGEN (AVERAGE PER WEEK)
ABSORBED NITROGEN (SEVENTH DAY OF EACH WEEK)
URINARY NITROGEN (TOTAL)
UREA NITROGEN
AMMONIA NITROGEN
CREATINE NITROGEN
CREATININE NITROGEN
URIC ACID NITROGEN
NITROGEN IN GM.
WEEK

trogen consumed proportionally. For example, in subjects MY and LS, there was rise in ammonia excretion with the increase in protein intake in Period II, a subsequent fall in Period III and again a rise in Period IV. Yet the average value for urinary ammonia nitrogen excreted by MY in Period III was higher than that in Period I in spite of a lowered protein intake (Table 17). The average value of urinary ammonia nitrogen of LU in Period III was not higher than that in Period IV. This was due to one low value in the third week (0.13 gram per day) which lowered the average for that period (Table 18). When the urinary ammonia nitrogen excretion of all eight subjects studied were compared with their average daily nitrogen intakes per period (Table 19), a slightly positive correlation was obtained ($P = 0.05$). The total titratable acidity in the urine was significantly correlated with the urinary ammonia nitrogen ($P = 0.01$) in these women (Table 19).

The fluctuation of ammonia excretion with protein intake would be expected on the basis of the fact that ammonia excretion is one of the mechanisms which regulates the acid-base balance in the body and spares fixed cations. Most of the dietary and tissue proteins yield acid ash after being catabolized because they contain sulfur and phosphorus. The excretion of the acid requires extra base and, consequently, ammonia is liberated and excreted in urine.

No relationship between urinary ammonia nitrogen and nitrogen retention could be found. Since the amount of ammonia nitrogen in urine was associated with the potential acidity of the diet, and in turn, with the protein content of the diet, any possible effect of nitrogen retention might have been obscured. This was in agreement with the findings of Salter et al. (1933), who investigated the effect of nitrogen balance on urinary ammonia excretion in human adults. These authors maintained their subjects on diets with known potential acidity and failed to establish any relationship. In their study, the urinary acidity and ammonia excretion varied with the potential acidity of the diet but not with the magnitude of the nitrogen retention.

Urea nitrogen

As would be expected, in each of the three subjects in Series 3, the output of urea nitrogen appeared to vary directly with total urinary nitrogen and with the quantity of nitrogen consumed (Tables 16, 17, and 18).

The relation of urea nitrogen in the urine to total urinary nitrogen and to nitrogen absorbed in the three subjects is shown in Figures 10, 11, and 12. It is interesting to note that the nitrogen absorbed on the seventh day of each week seemed to fluctuate in a similar pattern as the average daily nitrogen absorbed for the corresponding week. Total urinary nitrogen and urea nitrogen also appeared to follow the same pattern. Furthermore, the figures indicate that

the response of total urinary nitrogen or urea nitrogen excreiton to an increase or decrease in dietary nitrogen was relatively immediate. However, it should be remembered here that the urea nitrogen was determined on the seventh day of each week. Any adjustment that might have taken place during the first week of the transition from a high to a low (or a low to a high) protein intake (Weeks 6, 11, and 15) was not seen because determinations were made at weekly intervals only.

When a comparison was made of the daily average nitrogen intake per period to the urea nitrogen both expressed as mg. per kg. of body weight per day in subjects of Series 2 and Series 3, a highly significant correlation ($P = 0.01$) was obtained. The results are shown in Table 24.

No correlation between the urinary urea nitrogen and the nitrogen retention could be detected (Table 25). Since the correlation between nitrogen intake and the urinary urea nitrogen was highly significant, it was not surprising that there was no significant correlation between the urinary urea nitrogen and the nitrogen retention.

Data relating to the partition of nitrogen in urine of the three subjects, LS, MY, and LU, are presented in Tables 20, 21, and 22. Similar data for all the subjects studied are given in Table 23. It appeared that when the subjects were consuming their self-chosen diets, the urea nitrogen consti-

Table 23. Partition of urinary nitrogen in eight subjects (Series 2 and 3)

Subject	Experimental period	Total urinary nitrogen gm./day	Urea nitrogen in total	Ammonia nitrogen in total	Creatinine nitrogen in total	Creatine nitrogen in total	Uric acid nitrogen in total
LS	I	9.3	86.2	5.1	5.8	0.0	1.8
	II	13.9	76.2	4.9	4.0	0.6	1.2
	III	8.2	65.9	6.2	6.2	0.1	2.2
	IV	9.7	75.1	5.4	5.1	0.5	1.6
MC		10.6	87.5	3.8	3.5	0.7	1.9
LE		10.2	92.6	2.6	4.7	0.0	2.6
HS		10.1	92.0	3.6	4.2	0.4	2.4
MY	I	11.4	85.3	2.5	3.7	0.4	1.5
	II	14.4	82.8	2.8	3.3	1.0	1.6
	III	11.1	64.1	3.1	4.1	0.5	2.0
	IV	12.6	73.0	3.3	3.6	0.9	1.6
NN		8.4	90.0	4.0	3.8	0.4	1.3
MM		10.0	87.6	5.1	3.6	0.8	1.9
LU	I	7.0	81.3	3.1	4.4	0.6	2.0
	II	9.7	85.6	3.5	3.1	1.4	1.8
	III	8.5	69.6	3.3	4.2	1.4	2.1
	IV	6.1	80.3	4.6	4.9	1.0	1.8
LU		5.6	83.3	5.9	5.3	1.1	2.3

Table 24. Relationships between nitrogen intake and urinary nitrogenous components in eight subjects (Series 2 and 3)

Nitrogen intake mg./kg./day	No. of observations	Urine				
		total nitrogen mg./kg./day	urea nitrogen mg./kg./day	creatinine mg./kg./day	creatine mg./kg./day	uric acid mg./kg./day
Up to 125	1	97	69	17.6	0.4	5.7
126 - 150	4	126	120	18.2	2.0	8.7
151 - 175	5	145	124	17.0	1.4	7.5
176 - 200	3	159	124	19.3	3.2	7.9
201 - 250	2	196	156	17.9	5.0	8.8
Above 250	3	239	183	20.1	5.1	10.6
Correlation		p = 0.01	p = 0.01	n.s.	p = 0.01	p = 0.05

Table 25. Relationships between nitrogen retention and urinary nitrogenous components in eight subjects (Series 2 and 3)

Nitrogen retention mg./kg./day	No. of observations	Urine				
		total nitrogen mg./kg./day	urea nitrogen mg./kg./day	crea-tinine mg./kg./day	crea-tine mg./kg./day	uric acid mg./kg./day
Up to -20	1	143	159	14.7	1.7	11.5
-19 to -10	2	204	186	24.0	1.9	12.4
-9 to 0	2	120	104	15.8	1.7	5.8
0 to 10	4	138	104	17.2	1.8	7.1
11 to 20	7	157	127	17.7	3.5	7.6
21 to 30	2	236	171	20.3	5.8	10.0
Correlation		n.s.	n.s.	n.s.	p = 0.05	n.s.

tuted from 81.9 to 92.6 per cent of the total urinary nitrogen. Several groups of workers have reported a similar range of the percentage of urea nitrogen in terms of total urinary nitrogen in healthy human subjects subsisting on mixed diets (Folin, 1905, Smith, 1926, and Beard, 1935). This percentage was found to vary directly with the protein intake of the individual in those reports. Such a relationship might be expected in studies in which the subjects are living on controlled diets for several days. In the present study, a general trend towards a variation in the urea fraction of total urinary nitrogen with changes in the protein intake could be seen; however, a consistent relationship was not observed.

Creatinine and creatine

Data relating to urinary creatinine and creatine of the women studied have yielded interesting results. The three women in Series 3, LS, MY, and LU, responded to the variation in protein intakes in an entirely different manner. Table 26 presents the correlations examined between nitrogen intakes or retentions and urinary creatinine or creatine.

The urinary creatinine of subject LS ranged from 1.25 to 1.56 grams per day. The changes in protein intakes apparently did not influence the urinary creatinine excretion (Fig. 10). The creatine excretion in urine was low; on several occasions no significant amount of creatine could be detected (Table 16). However, it appeared that in Period II, urinary creatine was increased (Fig. 10). A slight positive correlation was ob-

Table 26. Summary of correlations between creatinine or creatine in plasma and urine and nitrogen intake or retention

Subject	Nitrogen or intake retention	No. of plasma samples	Plasma			No. of urine samples	Urine		
			creatinine	creatine	creatinine plus creatine		creatinine	creatine	creatinine plus creatine
	(ave./week)								
LS	intake	4	n.s.[a]	n.s.	0.980*	18	n.s.	0.560*	n.s.
	retention		0.957*	n.s.	n.s.		n.s.	n.s.	n.s.
MY	intake	4	n.s.	n.s.	n.s.	18	0.539*	0.788**	0.897**
	retention		n.s.	n.s.	n.s.		0.508*	n.s.	n.s.
LU	intake	4	n.s.	n.s.	n.s.		n.s.	0.791**	0.838**
	retention		n.s.	0.943*	0.991**		0.491*	0.814**	0.835**
	(ave./period)								
eight subjects	intake	18	n.s.	0.576*	n.s.	18	n.s.	0.641**	n.s.
	retention		n.s.	0.500*	0.569*		n.s.	0.532*	n.s.

[a]n.s. = non-significant.

*P = 0.05.

**P = 0.01.

***The correlation calculation was based on the four plasma samples and the corresponding 24-hour urine samples collected from each of the three subjects, LS, MY and LU, in combination with the one plasma sample and one 24-hour urine sample from subjects, MC, LE, HS, NN, MM and LU (Series 2 and 3).

served between the urinary creatine and nitrogen intakes in this subject during the whole period of the study (Table 26).

The daily excretion of creatinine for subject MY varied from 1.12 to 1.45 grams. In this subject, a fluctuation of urinary creatinine with protein intake (Table 17 and Fig. 11) could be seen and slightly positive correlations between urinary creatinine and nitrogen intake as well as nitrogen retention were found (Table 26). The creatine excreted by this subject was significantly correlated with the nitrogen intake (Table 26).

The urinary creatinine excretion of subject LU was much lower than those of LS and MY. The range was from 0.72 to 0.96 gram per day. The values of urinary creatinine of this subject were not correlated with nitrogen intakes but with nitrogen retentions (Table 26). The results of urinary creatine obtained from this subject indicated highly significant correlations of creatine excretion to nitrogen intakes and retentions (Table 26).

In all the eight subjects studied, urinary creatine but not creatinine as expressed in mg. per kg. per day was significantly correlated with the nitrogen intake and the nitrogen retention based on the same unit (Tables 24 and 25).

Several early reports have shown that the creatinine coefficient (mg. of creatinine per kg. of body weight per day) of subjects with physiological creatinuria was usually lower than that of subjects without creatine in the urine (Denis

and Minot, 1917, Rose, 1917, and McLaughlin and Blunt, 1923). Later Marples and Levine (1936) found that the total creatinine coefficient, mg. of creatine plus creatinine per kg. of body weight, for male subjects with physiological creatinuria had the same magnitude as the creatinine coefficients for those without creatinuria. These observations led to the impression that the sum of creatinine plus creatine might be more significant in relation to protein metabolism than either component alone. Following this suggestion, correlations between the daily urinary creatine plus creatinine and nitrogen intakes or retentions were examined in this study. Significant correlations were obtained in subjects MY and LU, but neither in LS nor in the subjects combined from Series 2 and 3 (Table 26).

Supports for the findings reported here may be found in the literature. In the studies of Folin (1905), McLaughlin and Blunt (1923) and Wang et al. (1930) in which human subjects were maintained on adequate diets, urinary creatinine did not appear to be affected by protein intakes, while creatine seemed to vary directly with the protein intake.

In contrast, studies with human beings and with animals have shown that a change in the level of protein in the diet could influence the excretion of creatinine and creatine. Such results were obtained when wide variations in protein levels in the diets were used in the experiments, and the results might have been due either to changes in the rate of

protein metabolism or to changes in the quantity of creatine precursors supplied by the diet (Roth and Allison, 1949, Beard, 1943, Tidwell, 1946, and Karambelkar et al., 1952). In the present study, even though the protein content of the self-chosen diet of these individuals varied from day to day, the variability was not as large as that encountered in the experimental diets used in the reports cited above. Therefore, the relationship between protein intake and urinary creatinine and creatine would not be as clear as that observed in the studies in which diets varying greatly in the level of dietary protein were used.

Uric acid

In the three subjects in Series 3 significant correlations were observed between intakes of nitrogen and the amount of uric acid excreted in the urine ($P = 0.01$). A significant correlation was also obtained from the data of all the eight subjects when both parameters were expressed as mg. per kg. per day (Table 24).

The close relationship between uric acid excretion and protein intake has been recognized. An increase in urinary uric acid excretion as a result of increased consumption of dietary protein has been reported (Lewis et al., 1918, Wilson et al., 1952, Benedict et al., 1953, and Bien et al., 1953). It was shown that the rate of uric acid synthesis was accelerated by a high protein intake. Furthermore, the in-

gestion of food rich in nucleoprotein might be followed by an increased excretion of urinary uric acid (Folin, 1905, Rose, 1921, and Wilson et al., 1954). In the present study, the change in urianry uric acid excretion might partly be due to the natures of dietary proteins in the diet. In Period II of Series 3, the supplementary protein was derived from meat while in Period III, meat was the source of dietary protein which was primarily reduced in the diets of MY and LS.

In the present study, urinary uric acid was not related to the extent of nitrogen retention. To the author's knowledge, no study on human subjects concerning such a relationship has appeared in the literature. The study of Allison et al. (1946) on dogs failed to show any effect of protein depletion on the urinary excretion of uric acid.

The findings concerning relationships of urinary nitrogen components to protein intake and retention in the group of women observed in the present study will be discussed further in relation to plasma nitrogenous components.

Relationship between Nitrogenous Components of Plasma and Urine

Data relating to the nitrogenous components in plasma and in urine have been presented in Table 12 and 19 for all subjects in Series 2 and 3. Correlations between nitrogenous components in plasma and in urine were examined. In all calculations data of the constituents of a given plasma sample

were correlated to those of a 24-hour urine sample collected on the preceding day. The urinary components were expressed in mg. per kg. per day. Results thus obtained are presented in Table 27.

Plasma non-protein nitrogen and urinary nitrogen

Urinary nitrogen was significantly correlated with the level of plasma non-protein nitrogen in the eight subjects studied. The largest and most variable fraction of urinary nitrogen and plasma non-protein nitrogen has been known to be urea nitrogen. The excretion of urea nitrogen in urine, likewise, varied with the concentration of urea nitrogen in plasma. With regard to the relationship between urinary nitrogen and plasma non-protein nitrogen to nitrogen intake and retention, it was found that the urinary values were closely related to nitrogen consumed but not to nitrogen retained. Though there was also a close correlation between urinary nitrogen and the corresponding plasma fraction, the latter was correlated not only with intake but also partially with retention, at least in the range of positive balance.

Creatinine and creatine

A highly significant correlation was observed between the plasma creatine level and the urinary creatine. A similar relationship was reported by Tierney and Peters (1943) between the serum creatine and the urinary creatine in adult female subjects. They observed that creatine was absent in the urine when its concentration in the serum was less than

Table 27. Relationship between plasma and urinary nitrogenous components

Plasma mg. per cent	Urine mg. per kg. per day	Correlation
Non-protein nitrogen	Total nitrogen	r = 0.468; P = 0.05
Urea nitrogen	Urea nitrogen	r = 0.510; P = 0.05
Creatinine	Creatinine	Not significant
Creatine	Creatine	r = 0.719; P = 0.01
Creatinine plus creatine	Creatinine plus creatine	Not significant
Uric acid	Uric acid	Not significant

0.58 mg. per cent. Above this level, the urinary creatine increased with the increase in its concentration in the serum. However, in the group of the subjects in the present study, such a limiting value for plasma creatine level was not found. The absence of creatine in urine was observed only in subjects LS and MM when their plasma creatine concentrations were 0.18 and 0.35 mg. per cent, respectively. All of the other subjects had plasma creatine concentrations exceeding 0.18 mg. per cent and excreted creatine consistently in the urine.

The plasma creatinine did not seem to correlate with the urinary creatinine.

To summarize the findings concerning the relation of creatine and creatinine in plasma and in urine to nitrogen retention (Table 26), it was observed that in LS, the plasma level of creatinine or creatine plus creatinine appeared to correlate with the magnitude of nitrogen retention while in MY, only the amount of urinary creatinine was correlated with the nitrogen retention. In subject LU, the plasma creatine or creatinine plus creatine and the urinary creatinine or creatine were correlated with nitrogen retentions. When data obtained from all eight subjects were combined, the plasma creatine or creatinine plus creatine was correlated with the extent of nitrogen retention, while in urine only creatine showed such a relationship.

Results in Table 26 show that 10 of the 24 correlations between creatinine or creatine in plasma and in urine and ni-

trogen retentions examined were significant. Even though a definite relationship of any one or the sum of these two components to nitrogen retentions could not be given from the results obtained from this group of subjects observed, a direct relationship of these two components in plasma and urine to the extent of protein retention was suggested.

The urinary creatinine has been known to be relatively constant for each individual (Folin, 1905). The creatinine coefficient (mg. of urinary creatinine per kg. of body weight) has been used as an index of the amount as well as the protoplasmic activity of muscular tissues (McLaughlin and Blunt, 1923, Hodgson and Lewis, 1928, Tager and Kirsch, 1942). The urinary creatine seemed to bear a similar significance since in the work of Hobson (1939) the output of creatine was found to increase when the proportion of muscle in the body increased. These reports showed that the variation in urinary creatinine and creatine suggested a reflection of changes in the amount of muscular tissues. However, to the author's knowledge, data are not yet available to correlate nitrogen retentions with changes in muscular tissues in adult human beings. The interpretation of the significance of the concentration of creatine or creatinine in plasma and urine in relation to nitrogen retention must await further investigation.

Uric acid

In the present study, plasma uric acid level was not correlated with the urinary uric acid in the subjects studied. Plasma uric acid appeared to be the fraction of non-protein nitrogen except creatinine that was least influenced by the amount of protein consumed by the individual. The amount of urinary uric acid excreted, on the other hand, was affected by the level of protein in the diet. Both plasma and urinary uric acid concentrations apparently were not related to nitrogen retentions in this group of women studied.

Plasma Cholesterol

The levels of total and free cholesterol were determined in one plasma sample collected from each of the six subjects in Series 2 and in four samples from each of the three women in Series 3. The results are presented in Table 28.

The average value of the 18 samples for total plasma cholesterol was 244 mg. per cent ranging from 188 to 339. The level of total plasma cholesterol for each subject was in the range reported by Swanson et al. (1955), Gillum et al. (1955) and Butler et al. (1956) for women living on self-chosen diets. No relationship between age and plasma cholesterol levels was noted in the subjects studied here. With a larger number of subjects, the observation has been made that the total serum cholesterol in women gradually increases with increasing age (Swanson et al., 1955, Gillum et al., 1955,

Table 28. Plasma cholesterol concentrations of the eight subjects (Series 2 and 3)

Subject	Age yr.	Experimental period	Nitrogen intake gm./day	Plasma cholesterol ester mg. %	Plasma cholesterol free mg. %	Plasma cholesterol total mg. %
LS	35	I	12.26	101	87	188
		II	15.34	89	104	193
		III	9.83	148	70	218
		IV	12.41	157	72	229
MC	43		12.95	212	34	246
LE	45		7.74	182	72	254
HS	55		9.68	183	45	228
MY	56	I	15.51	115	166	281
		II	18.73	109	218	327
		III	11.93	90	249	339
		IV	15.14	82	204	286
NN	72		8.25	219	66	285
MM	73		11.88	228	26	254
LU	81	I	7.42	138	104	242
		II	10.70	132	71	220
		III	9.41	131	78	209
		IV	7.37	131	64	195
LU	82		7.07	144	57	201

and Butler et al., 1956). A maximum value for serum cholesterol in women usually occurred between 60 and 70 years of age in these reports. The failure to show such a trend in the recent study might be due to the limited number of subjects participating.

The average value for free cholesterol was 99 mg. per cent with a range of 26 to 249. The values for MY which ranged from 166 to 249 mg. per cent appeared to be particularly high. By omitting these data, the average free cholesterol value for the seven subjects was 68 mg. per cent. This amount accounted for approximately 31 per cent of the total cholesterol on the average. This mean value for free cholesterol was close to those estimated by Sperry and Webb (1950) and Butler et al. (1956). In a group of healthy persons, the plasma free cholesterol was approximately 25 to 30 per cent of the total (Sperry and Webb, 1950). Butler et al. (1956) reported that the percentage of the free cholesterol in the total was 26 in a group of women between 26 to 92 years of age. Little is known concerning the factors which may influence the level of free cholesterol in plasma or serum of healthy persons. It does not seem likely that the high free cholesterol levels found in the plasma of subject MY were a result of artifacts since the plasma samples from LS and LU were treated almost identically to those of MY with respect to length of storage, handling, and method of analysis.

Hence, the significance of the high free cholesterol values observed in this subject is difficult to interpret at this point.

Comparisons of total and free cholesterol levels in plasma were made with nitrogen intakes and retentions. No significant correlations were found. In those cases where several plasma samples were available from one individual (subjects LS, MY, and LU), the plasma cholesterol levels varied in different periods during which the protein intakes were modified (Table 28). However, no consistent relation of the plasma cholesterol levels to protein intake or retention could be seen. The variability of total plasma cholesterol observed in any one individual appeared to be relatively restricted to a small range. Sperry (1937) reported that the variation in total plasma cholesterol observed at different intervals in the same individual was within 12 per cent. The variations found in LS (5 to 11 per cent), MY (6 to 11 per cent) and LU (2 to 12 per cent) were within such range.

Since it has been mentioned previously that nitrogen intakes expressed on the basis of body weight might have more physiological significance than the total, a comparison was made between nitrogen intake expressed in mg. per kg. of body weight per day and both free and total plasma cholesterol values. When this was done, a slightly positive correlation emerged (Table 29).

Table 29. Relationships between nitrogen intakes and plasma cholesterol levels (Series 2 and 3)

Nitrogen intake mg./kg./day	No. of observations	Plasma cholesterol		
		ester mg. %	free mg. %	total mg. %
Up to 125	1	148	70	218
126 - 150	4	149	74	223
151 - 175	5	178	59	237
176 - 200	3	103	144	247
201 - 250	2	114	139	253
251 - 300	2	163	100	263
Above 300	1	109	218	327
Correlation		n.s.	p = 0.05	p = 0.05

In the studies concerning the relationships between dietary proteins and blood cholesterol levels in human subjects, Swanson et al. (1955), Keys and Anderson (1957) and Lutz et al. (1959) reported that dietary intakes of protein did not seem to bear a significant relationship to the concentration of total cholesterol in plasma or serum. Gillum et al. (1955), on the other hand, have shown that serum cholesterol levels of a group of older subjects were directly correlated with protein intakes. However, in their study the close correlations between fat content and both the protein and cholesterol contents of the diets consumed by their subjects were observed. These authors suggested that the correlation between dietary protein and plasma cholesterol found in their study was secondary to the correlation between the protein and the fat or cholesterol content in the diets.

In human subjects maintained on experimental diets differing widely in protein content, Olson et al. (1957) have demonstrated that a change from a diet containing 100 grams of protein to one containing only 25 grams of protein resulted in an average drop of 52 mg. per cent of serum cholesterol, even though the diet contained a high percentage of saturated fats.

Keys and Anderson (1957) in a review of dietary habits in relation to serum cholesterol have noted that the serum cholesterol values tended to be low in undernourished persons.

On the other hand, populations habitually subsisting on large quantities of food tended to have relatively high concentrations of serum cholesterol.

In the present study, it was not possible to demonstrate a correlation between protein intakes and the plasma cholesterol levels in the three subjects in Series 3 when the protein intake in each period varied with minimum changes in caloric intakes. The slightly positive correlation found when data from all women in Series 2 and 3 were used might indicate that with a larger group of subjects with greater varying levels of protein intakes, a direct relationship between the plasma cholesterol level and protein intake might be found.

SUMMARY AND CONCLUSION

The investigation herein reported was designed to secure a picture of the pattern of utilization of nitrogen by women on different planes of nitrogen intakes by means of continuous daily nitrogen balance studies, and to investigate whether variations in selected nitrogenous components of plasma and urine could be correlated with protein intake or retention. In view of the questions that have been raised with regard to the significance of data provided by balance tests in the evaluation of protein nutrition of adult human beings living on customary diets, it was thought that the concomitant determination of nitrogenous components of plasma and urine might yield data helpful in support of the interpretation of those obtained from balance tests.

The investigation was planned in three series:

In a 10-day study (Series 2), nitrogen balance tests were carried out on six subjects, aged 43 to 82 years, living on self-chosen diets. Selected nitrogenous components were analyzed in one plasma sample and one 24-hour urine sample collected at the end of the observation period.

In a preliminary long-term study (Series 1), daily nitrogen balances were determined in four older women between the ages of 68 to 78 years for two periods. The first period lasting from 28 to 112 days on a self-chosen diet was followed by a period of 14 to 28 days in which the protein intake was

increased by adding 100 grams of lean beef daily to the customary diet.

In a long-term controlled study (Series 3), three subjects, aged 35, 55, and 81 years, were observed for four periods lasting from 28 to 35 days each. The subjects were maintained on their self-chosen diets during the first period. The dietary pattern established during this interval was repeated during the other three periods. One hundred grams of lean beef was added daily to the self-chosen diet of each subject during the second period. In the third period, the protein content of the diet was reduced by 20 per cent of that of the self-chosen diet of the first period in two subjects. Fifty grams of lean beef were added to the self-chosen diet of the other subject. The fourth period was a repeat of the first control period. An attempt had been made to keep the diets in the corresponding periods isocaloric. Analyses for nitrogen partition of urine samples were made weekly and estimations of the concentration of various nitrogenous components and of cholesterol were performed in plasma samples obtained at the end of each period.

All foods consumed and all excreta eliminated were collected daily. The nitrogen content of the food and urine was determined daily while that of the fecal samples was analyzed in a 5- or 7- day's composite. Electrophoretic fractions of protein, total and free cholesterol, and non-protein nitrogen were analyzed in plasma. Total nitrogen, urea nitrogen, crea-

tinine, creatine, and uric acid were estimated in plasma and urine. In addition, ammonia nitrogen and the titratable acidity were determined in urine.

Attempts were made to evaluate the data in several ways. The regressions of urinary nitrogen on nitrogen absorbed were calculated with the equation, $Y = a + bX$, for Series 1 and 3. From these regressions, an attempt was made to evaluate the effects of changes in protein intakes on nitrogen metabolism on the basis of the slopes and the intercepts of the regression lines.

The significance of the coefficients of correlation of individual nitrogenous components in plasma and urine with nitrogen intakes or retentions in corresponding periods of study were tested.

Data relating to the nitrogen retention in all three series confirm the observation reported in the literature that women in this age range living on their self-chosen diets are likely to be either in positive or in negative nitrogen balance. Four of the six subjects in the 10-day study were in negative nitrogen balance in spite of the relative adequacy of protein consumed based on the recommendation of the National Research Council. Where daily nitrogen balances were carried out over relatively long periods of time (Sereis 1 and 3), a continuous nitrogen retention was observed in four subjects during the period of observation. One subject, who was studied at two intervals, showed a persistent loss of body

nitrogen in the first interval and a continuous retention in the second interval. In another subject, periods of positive and negative retentions occurred. The periods seemed to balance each other so that the average retention over a 84-day period of observation approached equilibrium. With a daily addition of 100 grams of lean meat to their self-chosen diets, all subjects retained nitrogen including those who had been in negative nitrogen balance. Furthermore, the amount of nitrogen retained increased in all women except the one with the highest magnitude of nitrogen retention in the group.

The relation of urinary nitrogen to absorbed nitrogen as described by the slopes and the intercepts of the linear regressions seemed to be characteristic of an individual and probably of her dietary pattern. In three subjects no significant differences in the slopes or in the intercepts of the regressions for different planes of protein intakes were observed. The urinary excretion of nitrogen did not seem to have responded to the different amounts of nitrogen absorbed in the various dietary periods. With an increase in absorbed nitrogen, both urinary excretion and retention of nitrogen increased at the same rate. This would indicate that the self-chosen diet of these women had not furnished protein in amounts suffiecient to maintain an optimal level of body reserves with respect to this nutrient. In two subjects a significant difference in the intercepts of the two parallel re-

gressions for the two levels of protein intakes was found. These two subjects appeared to show a more pronounced tendency to adjust to a higher plane of protein nutrition than the other women. One of them apparently had no further capacity to retain the extra protein. Therefore, the extra dietary nitrogen was quantitatively reflected in an increase of urinary nitrogen excretion. The other woman, however, tended to retain at least part of the additional nitrogen. In yet another subject, the value for the slope of the regression for the dietary period with increased nitrogen intake was smaller than that for the period on the self-chosen diet. This might indicate that the body of this subject had an increased capacity to retain nitrogen during the period with additional nitrogen in the diet. The deduction made here is supported by the data pertaining to the pattern of nitrogen retention of these women.

Though the relationship of protein intake to levels of plasma and urinary nitrogenous components has been reported in the literature, the present study has extended our knowledge through the evaluation of the relationship of nitrogen retention to these substances in women living on different planes of protein intakes superimposed on the patterns of their self-chosen diets.

In contrast to the findings of most of the studies in the literature that a correlation between the levels of plasma proteins and protein intakes was not demonstrable in healthy

adults consuming relatively adequate diets, a significant correlation between total plasma protein and protein intakes was seen in the group of subjects observed here. This appeared to be due to the high correlation found for albumin. This relationship, however, was not demonstrable in a given individual with modified planes of protein intakes. The globulin fractions, unlike the albumin fraction, apparently were not associated with the levels of protein content of the diet. Like the relation of urinary nitrogen to absorbed nitrogen, it seems that the plasma protein level is characteristic for an individual and probably for the long-term pattern of her protein intake.

As shown previously by many investigators, a shift to a diet high in protein resulted in an increase of the level of plasma non-protein nitrogen, chiefly the fraction of urea nitrogen, in a given individual. Likewise, the values for these nitrogenous components decreased as the protein intake was lowered. For the group as a whole, plasma levels of these two nitrogenous components and of creatine varied with the average amount of nitrogen consumed by the individuals in each period of observation. The plasma levels of uric acid and creatinine, on the other hand, were not correlated with the protein content of the diet.

As was expected on the basis of published reports, urinary urea nitrogen, ammonia nitrogen, creatine and uric acid fluctuated directly with the protein intake. Total titra-

table acidity in the urine varied with the ammonia nitrogen and hence with the protein intake. An increase in creatinine output was observed in the three subjects in Series 3 during the period on the high protein diet. A decrease in urinary creatinine with low protein intakes was seen only in one subject in this series.

While a number of the nitrogenous metabolites measured showed a definite relationship to dietary protein, a significant correlation of these substances with nitrogen retentions was found in a few instances only.

The plasma levels of non-protein nitrogen was associated with the extent of nitrogen retention on the basis of data from all subjects in Series 2 and 3. It was relatively low when the subjects were in a state close to nitrogen equilibrium. The values increased with both positive and negative nitrogen balances. However, only in the region of positive balance did the plasma urea nitrogen vary directly with the magnitude of nitrogen retentions. This relationship did not hold in a given individual living on various planes of protein intakes. Plasma uric acid levels apparently bore no relationship to nitrogen retentions.

Furthermore, plasma and urinary levels of creatinine, creatine, or their sum appeared to fluctuate slightly with the extent of nitrogen retention, even though a statistically significant correlation of any one or the sum of these two

componets to nitrogen retentions was not obtained from the data reported in this study.

There was a slightly positive correlation between the levels of plasma total or free cholesterol and nitrogen intake in the group of subjects observed. The observation made here seemed to indicate a possible relationship between these two parameters.

In view of the correlations of several of the plasma and urinary nitrogenous components to nitrogen intake and retention demonstrated in the present study, it seems that the average concentration of these substances in plasma and urine of groups of individuals could reflect their average plane of protein nutrition. If these correlations could be verified further with data accumulated from larger groups of subjects, it might be possible that the evaluation of the concentration of these nitrogenous components could serve as indices of the nutritional status with respect to protein in the survey of population groups.

It is concluded that these components may be useful primarily in the evaluation of protein nutrition in human subjects in combination with data provided by balance tests. However, sufficient evidence is not available to indicate that any one of these substances per se may be used as an index of protein nutrition of an individual.

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APPENDIX

Table A. Daily nitrogen intake, excretion, and retention of six subjects in 10-day study

Subject	Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.
MC	1	47.3	11.64	12.45	0.88	13.33	-1.69
	2	47.3	11.64	11.69	0.88	12.57	-0.93
	3	47.3	15.62	11.00	0.88	11.88	+3.74
	4	47.3	15.62	12.61	0.88	13.49	+2.13
	5	47.3	15.62	15.20	0.88	16.08	-0.46
	6	47.3	11.00	10.51	2.31	12.82	-1.82
	7	47.3	11.00	11.61	2.31	13.92	-2.92
	8	47.3	12.46	10.63	2.31	12.94	-0.48
	9	47.3	12.46	9.98	2.31	12.29	+0.17
	10	47.3	12.46	10.38	2.31	12.69	-0.23
LE	1	54.0	8.94	4.68	0.56	5.24	+3.70
	2	54.0	8.94	8.59	0.56	9.15	-0.21
	3	54.0	6.71	6.88	0.56	7.44	-0.73
	4	54.0	6.71	7.22	0.56	7.78	-1.07
	5	54.0	6.71	5.50	0.56	6.06	+0.65
	6	54.0	7.14	6.59	1.17	7.76	-0.62
	7	54.0	7.14	9.12	1.17	10.29	-3.15
	8	54.0	7.14	9.54	1.17	10.71	-3.57
	9	54.0	9.01	10.25	1.17	11.42	-2.41
	10	54.0	9.01	9.03	1.17	10.20	-1.19

Table A. (Continued)

Subject	Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.
HS	1	58.4	11.62	12.37	1.08	13.45	-1.83
	2	58.4	11.62	12.42	1.08	13.50	-1.88
	3	58.4	8.61	9.27	1.08	10.35	-1.74
	4	58.4	9.32	9.64	1.08	10.72	-1.40
	5	58.4	8.61	10.09	1.08	11.17	-2.56
	6	58.4	9.66	8.58	2.29	10.87	-1.21
	7	58.4	9.66	8.84	2.29	11.13	-1.47
	8	58.4	9.23	7.40	2.29	9.69	-0.46
	9	58.4	9.23	7.44	2.29	9.73	-0.50
	10	58.4	9.23	8.64	2.29	10.93	-1.70
NN	1	54.2	9.30	8.41	0.90	9.31	-0.01
	2	54.2	9.30	7.59	0.90	8.49	+0.81
	3	54.2	9.30	7.15	0.90	8.05	+1.25
	4	54.2	7.31	7.57	0.90	8.47	-1.16
	5	54.2	7.31	8.25	0.90	9.15	-1.84
	6	54.2	7.38	6.18	0.95	7.13	+0.25
	7	54.2	7.38	7.34	0.95	8.29	-0.91
	8	54.2	7.38	9.02	0.95	9.97	-2.59
	9	54.2	8.90	7.81	0.95	8.76	+0.14
	10	54.2	8.90	8.64	0.95	9.59	-0.69

Table A. (Continued)

Subject	Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.
MM	1	69.1	11.04	10.36	1.32	11.68	-0.64
	2	69.1	11.04	9.33	1.32	10.65	+0.39
	3	69.1	11.04	10.68	1.32	12.00	-0.96
	4	69.1	13.89	10.81	1.32	12.13	+1.76
	5	69.1	13.89	10.35	1.32	11.67	+2.22
	6	69.1	11.58	10.22	0.60	10.82	+0.76
	7	69.1	11.58	9.49	0.60	10.09	+1.49
	8	69.1	11.58	10.64	0.60	11.24	+0.34
	9	69.1	11.59	9.72	0.60	10.32	+1.27
	10	69.1	11.59	9.99	0.60	10.59	+1.00
LU	1	49.2	7.10	5.69	0.82	6.51	+0.59
	2	49.2	7.10	4.91	0.82	5.73	+1.37
	3	49.2	7.10	5.76	0.82	6.58	+0.52
	4	49.2	7.10	5.84	0.82	6.66	+0.44
	5	49.2	7.10	5.57	0.82	6.39	+0.71
	6	49.2	7.05	5.43	0.73	6.16	+0.89
	7	49.2	7.05	4.70	0.73	5.43	+1.62
	8	49.2	7.05	5.57	0.73	6.30	+0.75
	9	49.2	7.05	5.17	0.73	5.90	+1.15
	10	49.2	7.05	5.64	0.73	6.37	+0.68

Table B. Daily nitrogen intake, excretion, and retention of subject NN

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	49.5	8.30	6.34	0.62	6.96	+1.34	Period I starts
2	48.2	6.83	6.39	0.62	7.01	-0.18	
3	48.6	5.82	5.73	0.62	6.35	-0.53	
4	48.2	4.94	5.19	0.62	5.81	-0.87	
5	48.0	8.80	5.85	0.62	6.47	+2.33	
6	47.7	6.39	6.56	0.62	7.18	-0.79	
7	47.7	4.95	3.04	0.62	3.66	+1.29	Partial urine sample
8	48.0	6.14	4.55	0.72	5.27	+0.87	
9	48.0	5.80	4.55	0.72	5.27	+0.53	
10	47.7	5.92	6.29	0.72	7.01	-1.09	
11	48.0	4.61	5.46	0.72	6.18	-1.57	
12	47.7	6.68	4.66	0.72	5.38	+1.30	Partial urine sample
13	47.7	4.97	4.42	0.72	5.14	-0.17	
14	47.7	5.99	4.51	0.72	5.23	+0.76	
15	47.7	6.35	5.76	0.65	6.41	-0.06	
16	47.7	6.69	6.00	0.65	6.65	+0.04	
17	47.7	7.01	5.85	0.65	6.50	+0.51	
18	47.7	6.90	5.73	0.65	6.38	+0.52	
19	47.7	6.94	5.47	0.65	6.12	+0.82	
20	47.7	5.42	5.12	0.65	5.77	-0.35	
21	47.7	7.02	6.26	0.65	6.91	+0.11	
22	47.7	7.65	6.80	0.72	7.52	+0.13	
23	47.7	7.33	6.90	0.72	7.62	-0.29	

Table B. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
24	47.7	7.28	6.00	0.72	6.72	+0.56	
25	47.7	7.86	6.13	0.72	6.85	+1.01	
26	47.7	6.12	5.42	0.72	6.14	-0.02	
27	47.7	5.53	4.78	0.72	5.50	+0.03	
28	47.7	5.45	4.65	0.72	5.37	+0.08	
29	47.7	7.17	5.31	0.58	5.89	+1.28	
30	47.7	7.24	5.80	0.58	6.38	+0.86	
31	47.3	5.90	5.60	0.58	6.18	-0.28	
32	47.7	8.27	6.18	0.58	6.76	+1.51	
33	47.3	7.35	5.91	0.58	6.49	+0.86	
34	47.7	7.45	6.27	0.58	6.85	+0.60	
35	47.3	7.34	5.55	0.58	6.13	+1.21	
36	47.7	9.50	6.85	0.86	7.71	+1.79	
37	47.7	7.33	6.90	0.86	7.76	-0.43	
38	47.7	8.00	6.22	0.86	7.08	+0.92	
39	47.7	7.31	5.38	0.86	6.24	+1.07	
40	47.7	7.49	6.14	0.86	7.00	+0.49	
41	47.7	5.44	6.21	0.86	7.07	-1.63	
42	47.7	6.95	5.90	0.86	6.76	+0.19	
43	47.7	7.00	6.34	0.56	6.90	+0.10	
44	47.7	6.24	5.39	0.56	5.95	+0.29	
45	47.3	6.81	6.00	0.56	6.56	+0.25	

Table B. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
46	47.7	6.22	6.22	0.56	6.78	-0.56	
47	47.7	9.23	6.20	0.56	6.76	+2.47	
48	47.3	7.82	6.46	0.56	7.02	+0.80	
49	47.3	7.46	5.93	0.56	6.49	+0.97	
50	47.3	8.39	6.73	0.69	7.42	+0.97	
51	47.7	8.57	7.22	0.69	7.91	+0.69	
52	47.3	7.15	6.11	0.69	6.80	+0.35	
53	47.7	7.35	5.34	0.69	6.03	+1.32	
54	47.3	7.16	6.48	0.69	7.17	-0.01	
55	47.7	6.72	7.88	0.69	8.57	-1.85	
56	47.7	6.81	6.29	0.69	6.98	-0.17	
57	47.3	8.28	5.61	0.60	6.21	+2.07	Period II starts
58	47.3	8.62	7.05	0.60	7.65	+0.97	
59	47.3	9.63	6.62	0.60	7.22	+2.41	
60	47.8	11.08	8.87	0.60	9.47	+1.61	
61	46.8	8.07	7.29	0.60	7.89	+0.18	
62	46.8	9.41	8.22	0.60	8.82	+0.59	
63	46.3	8.98	7.79	0.60	8.39	+0.59	
64	47.3	10.95	8.68	0.90	9.58	+1.37	
65	47.3	9.42	8.37	0.90	9.27	+0.15	
66	47.3	10.44	6.98	0.90	7.88	+2.56	Partial urine sample
67	47.7	8.40	7.62	0.90	8.52	-0.12	

Table B. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
68	47.3	9.45	8.21	0.90	9.11	+0.34	
69	47.3	10.89	8.42	0.90	9.32	+1.57	
70	47.3	10.48	8.00	0.90	8.90	+1.58	

Table C. Daily nitrogen intake, excretion, and retention of subject BG

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	51.4	6.35	4.79	0.84	5.63	+0.72	Period I starts
2	51.6	6.15	5.37	0.84	6.21	-0.06	
3	51.8	7.90	6.18	0.84	7.02	+0.88	
4	51.8	7.89	5.63	0.84	6.47	+1.42	
5	51.6	8.06	6.77	0.84	7.61	+0.45	
6	51.1	7.47	5.90	0.84	6.74	+0.73	
7	51.1	8.27	6.44	0.84	7.28	+0.99	
8	51.4	4.81	5.44	1.06	6.50	-1.69	
9	51.6	6.13	5.23	1.06	6.29	-0.16	
10	51.6	8.35	6.35	1.06	7.41	+0.94	
11	51.4	7.08	5.58	1.06	6.64	+0.44	
12	51.4	6.02	5.41	1.06	6.47	-0.45	
13	51.4	5.19	5.66	1.06	6.72	-1.53	
14	51.4	7.48	4.99	1.06	6.05	+1.43	
15	51.4	6.38	6.14	1.00	7.14	-0.76	
16	51.4	3.94	5.01	1.00	6.01	-2.07	
17	51.4	8.83	5.79	1.00	6.79	+2.04	
18	51.4	6.92	5.01	1.00	6.01	+0.91	
19	51.4	5.51	4.25	1.00	5.25	+0.26	
20	51.4	7.76	5.39	1.00	6.39	+1.37	
21	51.4	6.13	5.56	1.00	6.56	-0.43	
22	51.4	4.52	4.59	0.99	5.58	-1.06	

Table C. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
23	51.4	11.57	5.42	0.99	6.41	+5.16	
24	51.4	11.04	5.98	0.99	6.97	+4.07	
25	51.4	6.28	5.94	0.99	6.93	-0.65	
26	51.4	10.27	6.02	0.99	7.01	+3.26	
27	51.4	8.17	6.41	0.99	7.40	+0.77	
28	51.4	8.02	5.92	0.99	6.91	+1.11	
29	52.3	8.01	7.26	1.18	8.44	-0.43	Period II starts
30	51.8	8.95	6.86	1.18	8.04	+0.91	
31	51.6	8.50	6.79	1.18	7.97	+0.53	
32	51.4	12.06	7.64	1.18	8.82	+3.24	
33	51.6	7.40	7.16	1.18	8.34	-0.94	
34	51.8	9.87	5.39	1.18	6.57	+3.30	
35	51.8	10.97	7.68	1.18	8.86	+2.11	
36	51.8	8.30	7.44	1.01	8.45	-0.15	
37	52.0	8.85	6.15	1.01	7.16	+1.69	
38	51.8	13.12	4.97	1.01	5.98	+7.14	
39	51.8	7.16	5.90	1.01	6.91	+0.25	
40	51.8	11.33	6.58	1.01	7.59	+3.74	
41	52.0	14.48	6.27	1.01	7.28	+7.20	
42	52.0	9.43	6.81	1.01	7.82	+1.61	

Table D. Daily nitrogen intake, excretion, and retention of subject MV

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	64.1	9.00	7.22	0.96	8.18	+0.82	Period I starts
2	64.1	7.69	5.65	0.96	6.61	+1.08	
3	64.3	5.79	5.83	0.96	6.79	-1.00	
4	64.1	7.32	5.80	0.56	6.36	+0.96	
5	64.1	10.26	5.92	0.56	6.48	+3.78	
6	64.1	7.55	6.47	0.56	7.03	+0.52	
7	64.1	6.68	6.87	0.56	7.43	-0.75	
8	64.1	5.81	5.89	0.56	6.45	-0.64	
9	64.1	7.04	6.47	0.56	7.03	+0.01	
10	64.1	4.95	6.63	0.56	7.19	-2.24	
11	64.1	4.42	6.31	0.75	7.06	-2.64	
12	64.1	5.36	5.69	0.75	6.44	-1.08	
13	64.1	5.26	4.91	0.75	5.66	-0.40	
14	64.1	9.22	6.08	0.75	6.83	+2.39	
15	64.1	10.44	5.91	0.75	6.66	+3.78	
16	64.1	8.52	5.82	0.75	6.57	+1.95	
17	64.1	5.58	5.95	0.75	6.70	-1.12	
18	64.1	5.03	5.95	0.61	6.56	-1.53	
19	64.1	6.92	5.55	0.61	6.16	+0.76	
20	64.1	8.13	6.44	0.61	7.05	+1.08	
21	64.1	6.70	7.20	0.61	7.81	-1.11	
22	64.1	3.76	6.34	0.61	6.95	-3.19	
23	63.6	7.88	6.06	0.61	6.67	+1.21	

Table D. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
24	63.6	5.25	5.42	0.61	6.03	-0.78	
25	63.6	4.84	5.89	0.58	6.47	-1.63	
26	63.6	5.01	4.76	0.58	5.34	-0.33	
27	63.6	11.43	6.63	0.58	7.21	+4.22	
28	63.6	2.84	5.81	0.58	6.39	-3.55	
29	63.6	3.99	4.78	0.58	5.36	-1.37	
30	63.6	5.22	5.71	0.58	6.29	-1.07	
31	63.6	7.27	5.31	0.58	5.89	+1.38	
32	63.6	4.76	5.73	1.18	6.91	-2.15	
33	63.6	7.53	4.93	1.18	6.11	+1.42	
34	63.6	8.38	7.17	1.18	8.35	+0.03	
35	63.6	10.27	6.63	1.18	7.81	+2.46	
36	63.6	8.61	7.38	1.18	8.56	+0.05	
37	63.6	5.89	6.72	1.18	7.90	-2.01	
38	63.2	8.21	7.10	1.18	8.28	-0.07	
39	63.2	9.60	7.19	0.47	7.66	+1.94	
40	63.2	7.67	7.39	0.47	7.86	-0.19	
41	63.2	7.50	6.36	0.47	6.83	+0.67	
42	63.2	5.76	6.25	0.47	6.72	-0.96	
43	63.2	7.07	6.55	0.47	7.02	+0.05	
44	63.2	8.58	7.79	0.47	8.26	+0.32	
45	63.6	8.35	8.01	0.47	8.48	-0.13	

Table D. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
46	63.6	7.51	6.17	0.80	6.97	+0.54	
47	63.6	7.59	7.61	0.80	8.41	-0.82	
48	63.6	5.23	6.02	0.80	6.82	-1.59	
49	63.2	7.99	6.44	0.80	7.24	+0.75	
50	63.2	7.27	7.00	0.80	7.80	-0.53	
51	63.2	7.96	5.92	0.80	6.72	+1.24	
52	63.2	7.55	7.22	0.80	8.02	-0.47	
53	63.2	7.69	6.44	0.30	6.74	+0.95	
54	63.2	2.77	5.57	0.30	5.87	-3.10	
55	63.2	6.44	6.29	0.30	6.59	-0.15	
56	63.2	8.85	6.72	0.30	7.02	+1.83	
57	63.2	4.40	5.72	0.30	6.02	-1.62	
58	63.2	8.16	5.19	0.30	5.49	+2.67	Emotionally disturbed
59	63.2	6.21	5.71	0.30	6.01	+0.20	"
60	63.2	5.54	4.88	0.50	5.38	+0.16	"
61	63.2	2.41	4.75	0.50	5.25	-2.84	
62	63.2	5.89	5.21	0.50	5.75	+0.14	Emotionally disturbed
63	63.2	2.86	5.28	0.50	5.78	-2.92	"
64	62.5	3.29	3.18	0.50	3.68	-0.39	"
65	62.3	7.05	5.13	0.50	5.63	+1.42	"
66	62.3	5.87	5.24	0.50	5.74	+0.13	Emotionally disturbed
67	62.3	9.56	7.25	1.06	8.31	+1.25	"
68	62.5	8.43	7.23	1.06	8.29	+0.14	"

Table D. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
69	62.5	6.84	6.39	1.06	7.45	-0.61	Emotionally disturbed
70	62.5	8.41	6.70	1.06	7.76	+0.65	"
71	62.5	5.31	6.61	1.06	7.67	-2.36	Emotionally disturbed
72	62.3	8.67	7.49	1.06	8.55	+0.12	"
73	62.3	6.30	7.49	1.06	8.55	-2.25	"
74	62.3	7.18	7.47	0.54	8.01	-0.83	"
75	62.5	6.59	4.32	0.54	4.86	+1.73	"
76	62.5	6.85	4.17	0.54	4.71	+2.14	Emotionally disturbed
77	62.5	8.32	6.16	0.54	6.70	+1.62	
78	62.7	4.87	7.37	0.54	7.91	-3.04	
79	62.5	7.58	5.06	0.54	5.60	+1.98	
80	62.5	6.68	5.78	0.54	6.32	+0.36	
81	62.5	10.97	7.11	0.54	7.65	+3.32	
82	62.5	8.78	7.15	0.54	7.69	+1.09	
83	62.5	9.58	5.95	0.54	6.49	+3.09	
84	62.5	8.81	6.90	0.54	7.44	+1.37	
85	62.5	8.74	6.23	0.77	7.00	+1.74	Period II starts
86	62.7	7.66	6.72	0.77	7.49	+0.17	
87	62.7	9.48	7.94	0.77	8.71	+0.77	
88	62.7	8.27	8.11	0.77	8.88	-0.61	
89	62.7	11.50	7.64	0.77	8.41	+3.09	
90	62.7	10.13	8.21	0.77	8.98	+1.15	

Table D. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
91	62.7	8.36	8.57	0.77	9.34	-0.98	
92	62.7	9.59	9.57	0.71	10.28	-0.69	
93	62.7	11.17	9.42	0.71	10.13	+1.04	
94	62.7	9.24	9.16	0.71	9.87	-0.63	
95	62.7	9.70	8.07	0.71	8.78	+0.92	
96	62.7	11.66	8.38	0.71	9.09	+2.57	
97	62.7	13.03	9.68	0.71	10.39	+2.64	
98	62.7	9.10	8.66	0.71	9.37	-0.27	
99	62.7	7.98	8.18	0.71	8.89	-0.91	
100	62.7	12.06	9.38	0.71	10.09	+1.97	
101	62.7	9.74	9.64	0.71	10.35	-0.61	
102	62.7	7.48	8.60	0.71	9.31	-1.83	
103	63.0	8.63	9.36	0.71	10.07	-1.44	
104	63.0	10.18	8.10	0.71	8.81	+1.37	
105	63.0	10.22	8.60	0.71	9.31	+0.91	
106	63.0	8.68	8.52	0.86	9.38	-0.70	
107	63.0	11.92	6.58	0.86	7.44	+4.48	
108	63.0	7.94	8.53	0.86	9.39	-1.45	
109	63.2	10.49	8.05	0.86	8.91	+1.58	
110	63.0	10.02	8.28	0.86	9.14	+0.88	
111	63.0	8.51	8.00	0.86	8.86	+0.35	Dizzy spells, infection
112	63.0	8.41	7.49	0.86	8.35	+0.06	

Table E. Daily nitrogen intake, excretion, and retention of subject LU (1953 study)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	50.4	8.88	5.87	1.05	6.92	+1.96	Period I starts
2	50.4	8.17	6.82	1.05	7.87	+0.30	
3	50.4	8.54	7.55	1.05	8.60	-0.06	
4	50.4	6.84	5.70	1.05	6.75	+0.09	
5	50.4	8.74	7.34	1.05	8.39	+0.35	
6	50.4	7.51	5.58	1.05	6.63	+0.88	
7	50.4	4.76	5.50	1.05	6.55	-1.79	
8	50.9	6.80	5.78	0.99	6.77	+0.03	
9	50.4	7.90	5.71	0.99	6.70	+1.20	
10	50.9	5.66	5.21	0.99	6.20	-0.54	
11	50.4	6.63	5.96	0.99	6.95	-0.32	
12	50.4	4.79	6.03	0.99	7.02	-2.23	
13	50.9	7.23	7.81	0.99	8.80	-1.57	
14	50.9	7.00	7.28	0.99	8.27	-1.27	
15	50.4	5.82	6.18	0.88	7.06	-1.24	
16	50.9	6.83	6.66	0.88	7.54	-0.71	
17	50.4	7.71	6.75	0.88	7.63	+0.08	
18	50.4	6.94	6.48	0.88	7.36	-0.42	
19	50.4	8.24	7.05	0.88	7.93	+0.31	
20	50.4	6.84	6.49	0.88	7.37	-0.53	
21	50.4	6.80	6.23	0.88	7.11	-0.31	
22	50.4	5.69	5.95	2.31	8.26	-2.57	
23	50.4	6.96	6.04	2.31	8.35	-1.39	

Table E. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
24	50.4	8.06	6.18	2.31	8.49	-0.43	
25	50.4	6.05	6.53	2.31	8.84	-2.79	
26	50.4	5.72	6.18	2.31	8.49	-2.77	
27	50.4	6.00	5.74	2.31	8.05	-2.05	
28	50.0	4.88	5.07	2.31	7.38	-2.50	
29	50.4	10.40	6.87	0.98	7.85	+2.55	
30	50.4	6.14	6.56	0.98	7.54	-1.40	
31	50.4	5.36	4.94	0.98	5.92	-0.56	
32	50.4	6.63	5.27	0.98	6.25	+0.38	
33	50.4	10.07	6.89	0.98	7.87	+2.20	
34	50.4	6.71	4.68	0.98	5.66	+1.05	
35	50.4	5.87	6.12	0.98	7.10	-1.23	
36	50.4	11.10	6.53	1.00	7.53	+3.57	Period II starts
37	50.4	10.36	8.01	1.00	9.01	+1.35	
38	50.4	9.58	7.99	1.00	8.99	+0.59	
39	50.4	12.44	8.78	1.00	9.78	+2.66	
40	50.4	11.63	10.04	1.00	11.04	+0.59	
41	50.4	12.19	7.73	1.00	8.73	+3.46	
42	50.4	9.48	8.62	1.00	9.62	-0.14	
43	50.4	10.20	9.33	0.99	10.32	-0.12	
44	50.4	8.10	6.97	0.99	7.96	+0.14	
45	50.4	8.36	7.24	0.99	8.23	+0.13	

Table E. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
46	50.4	9.39	7.20	0.99	8.19	+1.20	
47	50.4	10.98	7.74	0.99	8.73	+2.25	
48	50.4	10.41	8.06	0.99	9.05	+1.36	
49	50.4	10.42	7.95	0.99	8.94	+1.48	

Table F. Daily nitrogen intake, excretion, and retention of subject LS

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	77.7	12.39	11.60	1.24	12.84	-0.45	Period I starts
2	77.3	14.48	11.04	1.24	12.28	+2.20	
3	77.3	7.65	7.68	1.24	8.92	-1.27	
4	77.3	10.96	9.01	1.24	10.25	+0.71	
5	77.3	8.20	8.30	1.24	9.54	-1.34	
6	77.7	15.03	9.24	1.24	10.48	+4.55	
7	77.7	10.58	9.55	1.24	10.79	-0.21	
8	77.3	9.76	8.70	1.47	10.17	-0.41	
9	77.3	13.73	9.42	1.47	10.89	+2.84	
10	77.3	12.82	10.25	1.47	11.72	+1.10	
11	77.7	7.50	7.82	1.47	9.29	-1.79	
12	77.3	12.48	9.48	1.47	10.95	+1.53	
13	77.7	12.77	10.11	1.47	11.58	+1.19	
14	77.7	11.82	8.22	1.47	9.69	+2.13	
15	77.7	8.97	7.80	1.40	9.20	-0.23	
16	77.3	15.10	9.85	1.40	11.25	+3.85	
17	77.3	14.42	12.42	1.40	13.82	+0.60	
18	77.3	16.32	13.90	1.40	15.30	+1.02	
19	77.7	9.72	10.12	1.40	11.52	-1.80	
20	77.7	10.77	8.75	1.40	10.15	+0.62	
21	77.5	11.43	8.88	1.40	10.28	+1.15	
22	77.7	16.04	10.41	1.45	11.86	+4.18	Fecel sample lost,
23	77.7	10.58	9.85	1.45	11.30	-0.72	average value for
24	77.7	14.56	10.12	1.45	11.57	+2.99	Period I used

Table F. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
25	77.7	13.36	11.31	1.45	12.76	+0.60	
26	77.7	13.87	11.95	1.45	13.40	+0.47	
27	77.7	11.40	10.73	1.45	12.18	-0.78	
28	78.2	16.64	9.80	1.45	11.25	+5.39	
29	77.7	12.24	11.73	1.70	13.43	-1.19	
30	77.7	11.78	10.58	1.70	12.28	-0.50	
31	77.7	11.80	10.36	1.70	12.06	-0.26	
32	77.7	14.36	9.24	1.70	10.94	+3.42	
33	77.7	10.28	10.83	1.70	12.53	-2.25	
34	77.7	9.97	8.97	1.70	10.67	-0.70	
35	77.7	15.21	9.29	1.70	10.99	+4.22	
36	77.3	18.28	12.20	1.04	13.24	+5.04	Period II starts, blood sample is taken
37	77.7	17.22	13.58	1.04	14.62	+2.60	
38	77.7	11.50	10.39	1.04	11.43	+0.07	
39	78.2	15.84	12.58	1.04	13.62	+2.22	
40	77.7	11.94	10.79	1.04	11.83	+0.11	
41	78.2	17.91	12.64	1.04	13.68	+4.23	
42	78.2	13.33	10.58	1.04	11.62	+1.71	
43	78.2	13.96	11.98	1.36	13.34	+0.62	
44	77.7	16.48	11.96	1.36	13.32	+3.16	
45	78.2	15.61	11.62	1.36	12.98	+2.63	
46	78.2	14.34	11.18	1.36	12.54	+1.80	

Table F. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
47	78.6	17.02	12.63	1.36	13.99	+3.03	
48	79.1	15.35	12.18	1.36	13.54	+1.81	
49	79.1	9.02	9.60	1.36	10.96	-1.94	
50	79.1	12.36	10.16	1.57	11.73	+0.63	
51	78.6	15.26	12.18	1.57	13.75	+1.51	
52	78.4	18.26	14.47	1.57	16.04	+2.22	
53	78.2	18.50	16.96	1.57	18.53	-0.03	
54	78.2	13.29	13.46	1.57	15.03	-1.74	
55	78.2	16.82	13.56	1.57	15.13	+1.69	
56	78.2	15.98	14.28	1.57	15.85	+0.13	
57	78.6	17.66	14.10	1.28	15.38	+2.28	
58	78.2	13.18	11.24	1.28	12.52	+0.66	
59	78.2	12.92	13.80	1.28	15.08	-2.16	
60	78.6	16.53	14.15	1.28	15.43	+1.10	
61	78.6	17.50	14.28	1.28	15.56	+1.94	
62	78.6	14.74	13.59	1.28	14.87	-0.13	
63	78.2	18.23	13.54	1.28	14.82	+3.41	
64	78.2	14.25	14.22	1.88	16.10	-1.85	
65	78.6	13.73	13.84	1.88	15.72	-1.99	
66	78.6	15.28	12.72	1.88	14.60	+0.68	
67	78.6	15.31	13.57	1.88	15.45	-0.14	
68	78.2	17.42	13.20	1.88	15.08	+2.34	
69	78.6	14.03	13.25	1.88	15.13	-1.10	

Table F. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
70	78.2	17.99	13.92	1.88	15.80	+2.19	
71	78.2	12.65	11.06	1.41	12.47	+0.18	Period III starts,
72	78.2	10.34	9.45	1.41	10.86	-0.52	blood sample is
73	78.6	7.33	6.18	1.41	7.59	-0.26	taken
74	78.6	10.95	8.16	1.41	9.57	+1.38	
75	78.6	7.59	6.28	1.41	7.69	-0.10	
76	78.6	11.77	7.14	1.41	8.55	+3.22	Has cramps
77	78.6	8.13	7.08	1.41	8.49	-0.36	
78	78.6	7.15	6.99	1.77	8.76	-1.61	
79	78.6	11.12	7.58	1.77	9.35	+1.77	
80	78.2	9.68	7.34	1.77	9.11	+0.57	
81	78.2	9.18	8.41	1.77	10.18	-1.00	
82	78.2	11.48	8.70	1.77	10.47	+1.01	
83	78.6	10.75	8.57	1.77	10.34	+0.41	
84	78.6	4.94	7.05	1.77	8.82	-3.88	
85	78.6	6.65	7.80	1.70	9.50	-2.85	
86	78.6	12.40	7.66	1.70	9.36	+3.04	
87	78.6	12.41	9.80	1.70	11.50	+0.91	
88	78.6	13.45	11.32	1.70	13.02	+0.43	
89	78.6	7.44	8.97	1.70	10.67	-3.23	
90	78.6	10.23	7.80	1.70	9.50	+0.73	
91	78.6	9.66	8.24	1.70	9.94	-0.28	

Table F. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
92	78.6	11.77	9.92	2.18	12.10	-0.33	
93	78.6	7.33	8.83	2.18	11.01	-3.68	
94	77.7	10.55	9.29	2.18	11.47	-0.92	
95	78.6	8.86	8.40	2.18	10.58	-1.72	
96	78.6	10.98	8.74	2.18	10.92	+0.06	
97	78.6	9.47	6.92	2.18	9.10	+0.37	
98	78.2	10.98	8.24	2.18	10.42	+0.56	
99	78.6	15.33	9.96	1.43	11.39	+3.94	Period IV starts, blood sample is taken
100	78.6	12.22	9.13	1.43	10.56	+1.66	
101	78.6	8.30	7.92	1.43	9.35	-1.05	
102	78.6	13.45	5.02	1.43	6.45	+7.00	
103	79.1	8.60	8.85	1.43	10.28	-1.68	
104	78.9	14.07	9.69	1.43	11.12	+2.95	
105	78.6	9.74	11.71	1.43	13.14	-3.40	
106	79.1	9.38	11.06	1.18	12.24	-2.86	
107	79.1	14.24	9.70	1.18	10.88	+3.36	
108	79.1	12.44	9.63	1.18	10.81	+1.63	
109	79.1	11.17	9.62	1.18	10.80	+0.37	
110	78.9	15.21	10.51	1.18	11.69	+3.52	
111	78.9	12.73	11.80	1.18	12.98	-0.25	
112	79.1	6.13	9.41	1.18	10.59	-4.46	
113	79.1	8.01	8.41	1.66	10.07	-2.06	
114	79.5	13.79	10.28	1.66	11.94	+1.85	

Table F. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
115	79.1	17.80	12.51	1.66	14.17	+3.63	
116	79.1	15.76	15.24	1.66	16.90	-1.14	
117	79.1	10.46	10.73	1.66	12.39	-1.93	
118	79.1	13.10	10.47	1.66	12.13	+0.97	
119	79.1	11.28	10.34	1.66	12.00	-0.72	
120	79.1	14.97	13.02	1.57	14.59	+0.38	
121	79.1	8.04	9.92	1.57	11.49	-3.45	
122	78.6	14.10	9.75	1.57	11.32	+2.78	Has an acute sore
123	79.1	12.50	9.83	1.57	11.40	+1.10	throat
124	79.1	18.08	12.08	1.57	13.65	+4.43	"
125	79.5	11.13	11.46	1.57	13.03	-1.90	"
126	79.5	15.38	9.68	1.57	11.25	+4.13	"
127							Blood sample taken

Table G. Daily nitrogen intake, excretion and retention of subject MY

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	62.7	17.36	13.47	1.24	14.71	+2.65	Period I starts
2	61.4	16.73	14.08	1.24	15.32	+1.41	
3	61.4	13.92	11.02	1.24	12.26	+1.66	
4	61.4	15.60	11.92	1.24	13.16	+2.44	
5	61.4	22.55	13.34	1.24	14.58	+7.97	
6	61.4	15.90	14.48	1.24	15.72	+0.18	
7	61.4	16.48	12.68	1.24	13.92	+2.56	
8	61.4	12.63	13.07	1.26	14.33	-1.70	
9	61.4	17.14	12.58	1.26	13.84	+3.30	
10	61.4	13.08	13.09	1.26	14.35	-1.27	
11	61.4	17.46	13.24	1.26	14.50	+2.96	
12	61.4	15.40	11.19	1.26	12.45	+2.95	
13	61.4	14.41	10.05	1.26	11.31	+3.10	
14	61.4	16.48	11.46	1.26	12.72	+3.76	
15	61.4	6.83	10.53	1.17	11.70	-4.87	
16	61.4	12.62	11.46	1.17	12.63	-0.01	
17	61.1	16.34	8.83	1.17	10.00	+6.34	
18	61.1	8.06	10.29	1.17	11.46	-3.40	
19	61.4	19.52	13.00	1.17	14.17	+5.35	
20	61.4	17.66	13.21	1.17	14.38	+3.28	
21	61.4	18.70	15.01	1.17	16.18	+2.52	
22	61.4	16.29	11.56	1.59	13.15	+3.14	
23	61.4	15.60	12.64	1.59	14.23	+1.37	

Table G. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
24	61.4	12.07	9.96	1.59	11.55	+0.52	
25	61.4	15.09	11.46	1.59	13.05	+2.04	
26	61.4	16.65	13.21	1.59	14.80	+1.85	
27	61.8	17.94	14.19	1.59	15.78	+2.16	
28	61.4	16.61	12.80	1.59	14.39	+2.22	
29	61.4	18.14	13.58	1.63	15.21	+2.93	
30	61.4	16.38	14.48	1.63	16.11	+0.27	Has a cold
31	61.4	11.68	11.76	1.63	13.39	-1.71	
32	61.8	15.10	10.45	1.63	12.08	+3.02	
33	61.8	18.84	12.38	1.63	14.01	+4.83	Has a cold
34	61.8	15.76	13.14	1.63	14.77	+0.99	
35	61.4	11.83	11.38	1.63	13.01	-1.18	
36	61.4	18.23	14.31	1.46	15.77	+2.46	Period II starts,
37	61.4	19.66	14.97	1.46	16.43	+3.23	blood sample is
38	61.4	18.78	15.10	1.46	16.56	+2.22	taken
39	61.4	17.25	17.16	1.46	18.62	-0.87	Has a stiff knee
40	61.4	27.22	18.23	1.46	19.69	+7.53	
41	62.3	21.66	18.48	1.46	19.49	+2.17	
42	61.4	21.10	17.01	1.46	18.47	+2.63	
43	61.4	16.52	16.75	1.22	17.97	-1.45	
44	61.4	18.65	14.48	1.22	15.70	+2.95	
45	61.4	17.88	16.68	1.22	17.90	-0.02	

Table G. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
46	61.4	22.18	15.49	1.22	16.71	+5.47	
47	61.4	17.69	14.82	1.22	16.04	+1.65	
48	61.4	16.51	15.45	1.22	16.67	-0.16	
49	61.4	18.43	15.43	1.22	16.65	+1.78	
50	61.1	14.51	18.30	1.48	19.78	-5.27	
51	61.1	17.28	14.80	1.48	16.28	+1.00	
52	61.4	20.40	14.16	1.48	15.64	+4.76	
53	61.4	14.28	13.28	1.48	14.76	-0.48	
54	61.4	21.94	15.04	1.48	16.52	+5.42	
55	61.4	19.08	15.92	1.48	17.40	+1.68	
56	61.4	21.88	17.16	1.48	18.64	+3.24	
57	61.4	18.06	14.80	1.08	15.88	+2.18	
58	61.4	19.80	16.17	1.08	17.25	+2.55	
59	61.4	15.27	14.69	1.08	15.77	-0.50	
60	61.4	17.75	12.62	1.08	13.70	+4.05	
61	61.8	18.25	14.97	1.08	16.05	+2.20	
62	61.8	21.03	16.46	1.08	17.54	+3.49	
63	61.4	18.38	17.40	1.08	18.48	-0.10	
64	61.4	21.58	16.68	1.45	18.13	+3.45	
65	61.6	18.21	19.11	1.45	20.56	-2.35	
66	61.8	17.31	14.62	1.45	16.07	+1.24	
67	61.8	14.82	14.00	1.45	15.45	-0.63	
68	62.3	18.94	15.38	1.45	16.83	+2.11	

Table G. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
69	62.3	19.60	15.64	1.45	17.09	+2.51	
70	61.8	15.04	14.35	1.45	15.80	-0.76	
71	61.6	12.72	10.50	1.75	12.25	+0.47	Period III starts,
72	61.6	12.68	11.40	1.75	13.15	-0.47	blood sample is
73	61.8	12.68	10.49	1.75	12.24	+0.44	taken
74	61.8	11.65	9.89	1.75	11.64	+0.01	
75	61.8	14.12	11.00	1.75	12.75	+1.37	Has flu
76	61.8	12.72	12.38	1.75	14.13	-1.41	"
77	61.8	10.55	10.66	1.75	12.41	-1.86	"
78	61.4	10.24	9.16	1.17	10.33	-0.09	"
79	61.4	10.11	10.08	1.17	11.25	-1.14	Feels well
80	61.4	10.49	9.64	1.17	10.81	-0.32	
81	61.8	12.92	10.23	1.17	11.40	+1.52	
82	61.4	11.40	10.07	1.17	11.24	+0.16	
83	61.4	10.57	9.72	1.17	10.89	-0.32	
84	61.4	11.13	9.38	1.17	10.55	+0.58	
85	61.4	9.36	7.86	1.44	9.30	+0.06	
86	61.4	13.44	9.55	1.44	10.99	+2.45	
87	61.8	12.08	8.32	1.44	9.76	+2.32	
88	61.8	8.46	7.65	1.44	9.09	-0.63	
89	61.8	14.02	9.20	1.44	10.64	+3.38	
90	61.8	11.63	10.78	1.44	12.22	-0.59	

Table G. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
91	61.6	14.87	10.43	1.44	11.87	+3.00	
92	61.6	12.47	9.22	1.36	10.58	+1.89	
93	61.4	10.82	10.62	1.36	11.98	-1.16	
94	61.4	9.75	8.92	1.36	10.28	-0.53	
95	61.4	11.53	8.82	1.36	10.18	+1.35	
96	61.4	14.24	9.25	1.36	10.61	+3.63	
97	61.8	14.92	10.82	1.36	12.18	+2.74	
98	61.4	12.39	11.15	1.36	12.51	-0.12	
99	61.4	14.03	12.20	1.42	13.62	+0.41	Period IV starts, blood sample is taken
100	61.4	16.18	11.85	1.42	13.27	+2.91	Food sample lost, nitrogen estimated
101	61.4	14.49	13.09	1.42	14.51	-0.02	
102	61.4	16.51	12.89	1.42	14.31	+2.20	
103	61.4	21.95	14.19	1.42	15.61	+6.34	
104	61.8	18.96	13.96	1.42	15.38	+3.58	
105	61.4	16.76	13.01	1.42	14.43	+2.33	
106	61.4	13.02	13.84	1.29	15.13	-2.11	Food sample lost, nitrogen estimated
107	61.4	13.74	13.31	1.29	14.60	-0.86	Sick, pain in abdomen
108	61.4	13.94	12.89	1.29	14.18	-0.24	"
109	61.8	12.68	14.56	1.29	15.85	-3.17	"
110	61.4	15.80	13.50	1.29	14.79	+1.01	
111	61.4	13.30	13.34	1.29	14.63	-1.33	

Table G. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
112	61.4	14.70	11.15	1.29	12.44	+2.26	
113	61.4	13.80	11.66	1.26	12.92	+0.88	
114	61.4	12.23	11.97	1.26	13.23	-1.00	
115	61.4	14.86	11.09	1.26	12.35	+2.51	Pain on side
116	61.4	11.00	9.45	1.26	10.71	+0.29	"
117	61.4	17.24	11.27	1.26	12.53	+4.71	
118	61.8	16.36	12.42	1.26	13.68	+2.68	
119	61.4	19.97	14.75	1.26	16.01	+3.96	
120	61.4	15.20	12.47	1.94	14.41	+0.79	
121	61.4	13.35	11.63	1.94	13.57	+0.22	
122	61.4	12.00	11.76	1.94	13.70	-1.70	Food sample lost, nitrogen estimated, has sore throat
123	61.4	9.93	10.40	1.94	12.34	-2.41	"
124	61.4	20.03	12.04	1.94	13.98	+6.05	"
125	62.3	15.65	14.85	1.94	16.79	-1.14	"
126	61.6	16.11	12.62	1.94	14.56	+1.55	"
127							Blood sample is taken

Table H. Daily nitrogen intake, excretion, and retention of subject LU (1957 study)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
1	51.4	9.95	6.55	0.80	7.35	+2.60	Period I starts
2	51.8	7.31	5.86	0.80	6.66	+0.65	
3	51.4	6.15	6.74	0.80	7.54	-1.39	
4	51.1	6.54	6.36	0.80	7.16	-0.62	Food sample lost,
5	51.4	4.64	5.75	0.80	6.55	-1.91	nitrogen estimated
6	50.9	6.25	5.51	0.80	6.31	-0.06	
7	50.9	7.97	5.19	0.80	5.99	+1.98	
8	50.9	7.87	6.08	0.83	6.91	+0.96	
9	50.9	6.87	6.18	0.83	7.01	-0.14	
10	51.1	8.54	6.25	0.83	7.08	+1.46	
11	51.1	6.37	5.28	0.83	6.11	+0.26	
12	51.1	9.28	5.11	0.83	5.94	+3.34	
13	51.1	8.24	6.95	0.83	7.78	+0.46	
14	50.9	9.16	7.82	0.83	8.65	+0.51	
15	50.9	6.74	7.20	1.15	8.35	-1.61	
16	50.9	7.31	5.00	1.15	6.15	+1.16	
17	51.1	9.40	5.74	1.15	6.89	+2.51	
18	51.1	9.29	5.95	1.15	7.10	+2.19	
19	51.1	6.19	6.62	1.15	7.77	-1.58	
20	51.1	6.69	5.76	1.15	6.91	-0.22	
21	51.1	6.56	5.40	1.15	6.55	+0.01	
22	51.1	6.03	5.60	0.91	6.51	-0.48	

Table H. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
23	51.1	7.46	5.57	0.91	6.48	+0.98	
24	51.1	7.91	6.28	0.91	7.19	+0.72	
25	51.1	7.96	6.18	0.91	7.09	+0.87	
26	51.1	7.82	7.04	0.91	7.95	+0.13	
27	51.1	7.98	6.57	0.91	7.48	+0.50	
28	51.1	7.22	6.07	0.91	6.98	+0.24	
29	51.1	8.04	5.62	0.80	6.42	+1.62	
30	51.1	6.90	5.46	0.80	6.26	+0.64	
31	51.1	8.66	5.73	0.80	6.53	+2.13	
32	51.1	6.63	5.29	0.80	6.09	+0.54	
33	51.1	6.16	6.13	0.80	6.93	-0.77	
34	51.1	5.81	4.57	0.80	5.37	+0.44	
35	51.1	7.79	6.97	0.80	7.77	+0.02	
36	51.1	10.51	8.41	0.95	9.36	+1.15	Period II starts,
37	51.1	9.12	7.73	0.95	8.68	+0.44	blood sample is
38	51.1	10.93	8.74	0.95	9.69	+1.24	taken
39	50.9	10.42	9.33	0.95	10.28	+0.14	
40	50.9	8.52	7.55	0.95	8.50	+0.02	
41	50.9	9.60	7.51	0.95	8.46	+1.14	
42	50.9	9.11	8.79	0.95	9.74	+0.63	
43	50.9	10.21	9.15	0.75	9.90	+0.31	
44	50.7	11.74	8.41	0.75	9.16	+2.58	
45	50.7	11.72	8.40	0.75	9.15	+2.57	

Table H. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
46	50.5	9.96	8.08	0.75	8.83	+1.13	
47	50.7	11.02	9.88	0.75	10.63	+0.39	
48	50.7	11.95	9.74	0.75	10.49	+1.46	
49	50.7	13.59	10.66	0.75	11.41	+2.18	
50	50.7	10.20	9.69	1.36	11.05	-0.85	
51	50.5	10.49	8.21	1.36	9.57	+0.92	
52	50.5	10.87	8.77	1.36	10.13	+0.74	
53	50.5	10.62	10.13	1.36	11.49	-0.87	
54	50.5	11.10	8.73	1.36	10.09	+0.01	
55	50.5	10.71	8.03	1.36	9.39	+1.32	
56	50.5	11.01	8.29	1.36	9.65	+1.36	
57	50.5	9.18	7.54	1.01	8.55	+0.63	
58	50.7	11.82	9.32	1.01	10.33	+1.49	
59	50.9	11.66	8.76	1.01	9.77	+1.89	
60	50.9	12.92	8.01	1.01	9.02	+3.90	
61	50.9	11.27	10.02	1.01	11.03	+0.24	
62	50.7	10.61	8.97	1.01	9.98	+0.63	
63	50.9	8.80	9.04	1.01	10.05	-1.25	
64	50.9	10.42	9.32	0.88	10.20	+0.22	
65	50.9	9.83	7.65	0.88	8.53	+1.30	
66	50.9	11.11	9.65	0.88	10.53	+0.58	
67	50.7	10.83	9.00	0.88	9.88	+0.95	
68	50.7	12.34	9.77	0.88	10.65	+1.69	

Table H. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
69	50.7	8.64	9.75	0.88	10.63	-1.99	
70	50.7	11.80	7.90	0.88	8.78	+3.02	
71	50.5	9.99	9.28	0.95	10.23	-0.24	Period III starts,
72	50.5	8.71	7.35	0.95	8.30	+0.41	blood sample is
73	50.5	8.08	5.69	0.95	6.64	+1.44	taken
74	50.5	7.51	5.52	0.95	6.47	+1.04	
75	50.5	6.68	5.71	0.95	6.66	+0.02	
76	50.5	8.06	7.42	0.95	8.37	-0.31	
77	50.2	10.13	6.03	0.95	6.98	+3.15	
78	50.2	10.69	7.30	1.01	8.31	+2.38	
79	50.2	8.74	7.04	1.01	8.05	+0.69	
80	50.0	9.67	6.77	1.01	7.78	+1.89	
81	50.7	7.87	6.73	1.01	7.74	+0.13	
82	50.2	10.44	7.03	1.01	8.04	+2.40	
83	50.0	9.34	8.30	1.01	9.31	+0.03	
84	50.0	11.09	8.59	1.01	9.60	+1.49	
85	50.0	9.46	7.24	1.04	8.28	+1.18	
86	50.5	9.68	8.47	1.04	9.51	+0.17	
87	50.2	10.08	6.94	1.04	7.98	+2.10	
88	50.2	9.14	7.70	1.04	8.74	+0.40	
89	50.0	9.68	7.23	1.04	8.27	+1.41	
90	50.2	8.79	7.08	1.04	8.12	+0.67	

Table H. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
91	50.0	9.38	7.50	1.04	8.54	+0.84	
92	50.0	10.04	7.29	1.28	8.57	+1.47	
93	50.0	10.73	7.18	1.28	8.46	+2.27	
94	50.0	9.76	7.82	1.28	9.10	+0.66	
95	50.0	11.10	7.30	1.28	8.58	+2.52	
96	50.5	10.57	8.85	1.28	10.13	+0.44	
97	50.0	9.40	8.53	1.28	9.81	-0.41	
98	50.0	8.68	7.00	1.28	8.28	+0.40	Blood sample is taken
99	50.0	8.29	6.21	1.00	7.21	+1.08	Period IV starts
100	50.0	5.99	6.64	1.00	7.64	-1.65	
101	50.0	6.35	5.22	1.00	6.22	+0.13	
102	50.0	6.22	6.10	1.00	7.10	-0.88	
103	50.0	4.94	4.83	1.00	5.83	-0.89	
104	50.0	5.92	5.38	1.00	6.38	-0.46	
105	50.0	7.68	5.56	1.00	6.56	+1.12	Food sample lost, nitrogen estimated
106	50.0	8.51	5.69	0.79	6.48	+2.03	
107	50.0	6.72	5.89	0.79	6.68	+0.04	
108	50.0	5.67	5.34	0.79	6.13	-0.46	
109	50.0	5.65	4.97	0.79	5.76	-0.11	Food sample lost,
110	50.5	7.01	6.92	0.79	7.71	-0.70	nitrogen estimated
111	50.0	8.58	6.59	0.79	7.38	+1.20	
112	50.0	9.30	7.87	0.79	8.66	+0.64	

Table H. (Continued)

Day	Body weight kg.	Intake gm.	In urine gm.	In feces gm.	Total excretion gm.	Retention gm.	Remarks
113	50.0	8.79	7.02	1.08	8.10	+0.69	
114	49.5	7.46	6.24	1.08	7.32	+0.14	
115	49.5	8.29	7.51	1.08	8.59	-0.30	
116	50.0	6.25	6.33	1.08	7.41	-1.16	
117	50.0	8.00	6.53	1.08	7.61	+0.39	
118	49.5	6.32	5.42	1.08	6.50	-0.18	
119	50.0	6.74	6.30	1.08	7.38	-0.64	
120	50.0	8.04	6.32	1.28	7.60	+0.44	
121	50.0	9.04	5.83	1.28	7.11	+1.93	
122	50.0	9.59	7.09	1.28	8.37	+1.22	
123	49.5	9.56	6.74	1.28	8.02	+1.54	
124	50.0	8.06	7.75	1.28	9.03	-0.97	
125	50.0	6.54	7.29	1.28	8.57	-2.03	
126	50.0	6.96	6.12	1.28	7.40	-0.44	Blood sample is taken