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Utilization of egg protein by well nourished and undernourished rats

Bibha Mukhopadhay
Iowa State College

NOTE TO USERS

UTILIZATION OF EGG PROTEIN BY WELL NOURISHED AND UNDERNOURISHED RATS

by

Bibha Mukhopadhay

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Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

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BACKGROUND AND PURPOSE OF THE EXPERIMENT

Today, perhaps as never before, there is urgent need of more comprehensive knowledge concerning the basic principles of nutrition. Speedy rehabilitation of underfed persons in many nations of the world with only limited quantities of food available necessitates the most judicious utilization of the food supply. To accomplish this purpose, we must know clearly the role played by the major components of the diet. Within the last ten years, there has been a renewed interest in regard to the importance of the protein constituents of the diet.

Dietary proteins have varied roles in maintaining the nutrition of the animal body. They are preeminently important in the support of a normal rate of growth and in the maintenance of processes essential to life. They are needed for the synthesis of specific functional proteins like plasma proteins, hemoglobins, etc. They maintain important labile reserves of protein in the form of liver proteins and plasma proteins. These reserves may act like bulwarks in helping the organism combat the effects of shock, hemorrhage, fracture, etc. Certain proteins are used in the fabrication of antibodies that serve as barriers against infection. The role of the problems in the synthesis of metabolites like glutathionine, creatine and choline has been recognized of late. Proteins also represent important parts of tissue enzyme systems.

The proteins of the diet differ in regard to their ability to support these various functions. A protein extremely efficient in one respect may be of little value in another (Robscheit-Robbins, Miller and Whipple, 1943). There is a great need for the re-evaluation of the nutritive value of the proteins in terms of function. Many investigations in the nutrition field are directed toward this goal.

The most important problem in India today is how to secure an adequate nutrition for her people. There is practically a world-shortage of food at this moment. The two world wars in this century and the political changes and disturbances in India have added to the complications of securing a food supply adequate for proper health, growth, maintenance,and the continuance of the race from generation to generation.

The present task in India is to end poverty and raise the living standard of the masses. The purchasing power of the individual is, on the average, extremely low. He is largely dependent upon the grain crops for his food. These furnish the most food value available, either from his own fields or through exchange of labor. It is expected that as the economic condition improves, the adequacy of the food supply will improve. It is folly to suggest foods to the Indian people that are nutritionally superior but beyond the reach of common people.

The average person in India receives 1500 calories daily against normal requirements of 2000 to 2500 calories. The nutrients that in the main are apt to be deficient in our food are proteins, minerals and vitamins. Probably with educational progress, the vitamin and

mineral problems can be met. These nutrients can be obtained from available fresh vegetables such as cabbage, tomato, spinach, carrots, potatoes, beans, peas, and some varieties of cheap fruits like mangoes, guavas and pineapples, etc. The fulfillment of the protein requirement, however, presents rather a great problem.

Excellent sources of protein like meat and milk are not available due to low production, inadequate distribution and prohibitive cost. Areas near the sea get occasional supplies of fish. But due to lack of preservation outfits and cold storage systems, fish is not available throughout the year.

Also, the ability of the body to secure full benefit of protein ingested when the energy value of the food is low, as is often the case in Indian diets, must be considered.

It was demonstrated by Benditt, Woolridge and Stepto in 1948 that restriction of the caloric intake below a certain critical level restricts the utilization of ingested protein for the fabrication of tissue. Workers in the nutrition laboratory of the Home Economics Division at the Iowa State College have shown that this critical level is represented by a fifty per cent reduction in caloric intake (Willman, et al., 1947).

When one considers what foods might be used in meeting the protein requirement of the people of India, poultry products perhaps have a bright future as a protein source. Poultry is the only class of livestock which has increased in number faster than human population during this century. Our government particularly is encouraging production

and commercialization of egg and egg products. It is highly probable that egg powder will become a common commodity in the near future.

There are indications in the literature that protein-depleted and well nourished animals utilize their supply of dietary protein in different manners. Allison and his coworkers (1946) have shown that the retention of protein is greater in depleted than in well nourished animals. They suggest that there is a different type of internal supplementation in the depleted than in well nourished animals.

What the requirements of these undernourished people for protein may be constitutes a vital problem. It becomes a matter of some practical importance as well of scientific and national interest to the Indian people to determine whether different proportions of protein with an adequate calorie intake are associated with differences in the distribution of total protein within the body of well nourished and depleted animals.

These were the considerations which inspired me to undertake the present problem. The problem deals with the utilization of protein by albino rats representing two different planes of nutrition. Defatted dehydrated egg powder has furnished the protein in rations that have been fed to well-nourished and under nourished rats. The response of the rats to daily doses of egg proteins ranging from very small to high amounts has been observed. It is hoped that these experiments may throw some light on the nutrition problems of the protein-deficient population of our country.

REVIEW OF LITERATURE

Concepts of Protein Metabolism

A review of protein metabolism is the story of amino acids, singly and in various combinations, as they enter into, and become a part of, the chemical matrix of living tissue (Allison, 1948).

Before the early twentieth century, few attempts had been made to develop a comprehensive theory of protein metabolism. Then certain facts were established largely through the efforts of Folin. He developed the analytical methods necessary for testing theories of protein metabolism and saw experimental possibilities that proved helpful in developing new concepts. He drew certain deductions as to the nature of protein metabolism. He concluded that there were two kinds of catabolism, one variable or exogenous, which was dependent upon the protein ingested, and the other constant or endogenous, the result of the daily "wear and tear" of the protein structures of the organism. The metabolic processes resulting in these constant end products he assumed to be indispensable for the continuation of life, and, therefore, "to represent the lowest level of nitrogen metabolism achievable" (Folin, 1905). Food protein was drawn upon to replace these losses, and any excess was either discarded or stored. Beyond this, no synthesis of protein in the body was assumed, the structural units of the body being considered static. Although at first Folin did not recognize the possibility of

storage, later he explained the lag in establishment of a constant level of nitrogen excretion after a marked change in diet by a depletion of storehouses of amino acids that existed in muscle and other tissues (Folin and Denis, 1912).

The concept of a constant "endogenous" nitrogen metabolism was challenged soon after Folin presented it. Osborne and Mendel were among the first to question Folin. They suggested that the "endogenous" metabolism postulated by Folin might represent a requirement for specific amino acids for the synthesis of enzymes or homones or for other protein or non-protein metabolites. In their opinion, the entire protein molecule might undergo degradation only to liberate specific amino acids. Osborne and Mendel's proposal (1915a-b) that the minimum requirement for protein might best be met not by a protein most like body protein, but by one rich in the specific amino acids needed for the synthesis of new tissue or of a specific hormone, enzyme, etc., took on significance in the light of later discoveries. Mitchell and Hamilton (1929), in commenting on this theory, said that if it was true, theoretically, the endogenous metabolism could be depressed below that characteristic of a period of low nitrogen feeding by feeding complete proteins.

Henry C. Sherman, one of the leading nutritionists of the early twentieth century, too, believed that the catabolism of tissue could be decreased by dietary means. He was aware that the nitrogen of mixtures of amino acids incomplete in regard to maintenance requirements may have a favorable effect on nitrogen balance. He proposed (1920) that hydrolysis of proteins constituted the initial step in protein

metabolism, and that hydrolysis might be checked or even reversed by the increased concentration of amino acids that follows the digestion and absorption of protein. A mixture of amino acids would check hydrolysis in a measure commensurate with its completeness because any one amino acid could be effective only at the point at which it was liberated from the "catabolizing protein molecule". Sherman's concept eliminated the possibility of a constant tissue metabolism, and made it dependent on the quantity, as well as on the quality, of the protein in the diet.

As time went on, more and more evidence accumulated that a dynamic rather than a static state characterized protein metabolism. In 1935, Borsook and Keighley presented a theory of "a continuing nitrogen metabolism" which they defined as "the nitrogen metabolized on any one day that was already present in the tissue" (1935). It was thus distinct from exogenous nitrogen, and bore no relation to the endogenous or "wear and tear" metabolism postulated by Folin. In a man in nitrogen equilibrium, the continuing metabolism constitutes more than one-half the total urinary nitrogen and is a function of previous dietary history. Borsook and Keighley interpreted the extent of the continuing metabolism, together with the constancy of amino acids in the tissues, as indicating that, in the adult, synthetic processes involving amino acids constantly occur. Their theory was mainly based upon experimental work, which showed that the excretion of sulfur in a period when a constant diet was fed was far in excess of the so-called "endogenous" sulfur excretion resulting from the administration of a low sulfur diet during the nitrogen balance period.

Whipple and his group, working at the University of Rochester, early made a definite contribution to the understanding of protein metabolism. Their work started in 1919. Madden and Whipple made an interesting report in 1940. By means of a special technique, known as plasmaphoresis, it was possible for them to produce acute hypoproteinemia in the dog in two to six weeks.

Madden and Whipple demonstrated the existence of the reserve stores of protein in the body sufficient in quantity to reform at least 40 to 60 per cent of the original circulating mass of plasma. With its stores of protein depleted, the hypoproteinemic dog exhibited considerable ability in regenerating plasma proteins and hemoglobins from proteins derived from various dietary sources. Madden and Whipple found that the greatest stimulation in the production of plasma protein and of hemoglobin occurred when the concentration of plasma protein was just above that typical of edema.

Whipple and his co-workers evaluated the plasma-protein rebuilding capacity of various foods by determining the amount of plasma protein over and above the basal output that was produced by the feeding of the test food. The work of this group (Robscheit-Robbins, Miller, and Whipple, 1943) showed also that proteins valuable for plasma protein regeneration were not necessarily effective in the building of hemoglobin. Reserve protein stores, however, were drawn upon for the production of the latter as well as for the former. This observation supports the earlier idea of Osborne and Mendel that body tissues are broken down not for the whole protein molecule but for specific amino

acids, in this case, different amino acids being needed for the formation of plasma protein than for hemoglobin. The work of Whipple and his colleagues shows clearly the dynamic relationship that exists between food proteins, plasma proteins, reserve proteins in the liver and tissue proteins, and demonstrates the fluidity of body processes. Whether such fluidity could be traced in evaluating the role of egg protein seemed worthy of trial.

Direct proof of the dynamic nature of protein metabolism rests largely on studies reported by Schoenheimer and his associates (1942). The course of nitrogen metabolism was followed with the use of isotopic elements. They showed (1942) that dietary amino acids may enter directly without transformation into tissue structure, or that by transamination reactions their nitrogen may be transferred to deaminated molecules to form new amino acids. For example, the feeding of one labelled amino acid resulted in the presence of isotopic nitrogen in all amino acids isolated from the tissues, except lysine. Thus, Schoenheimer and his co-workers concluded that automatic and non-interruptible processes of synthesis and of degradation occurred, and that amino acids liberated by the opening of peptide bonds mixed freely with others to form a metabolic pool of constituents whose origin is indistinguishable.

Measurement of the Nutritive Value of Protein

Thomas (1909) was first to use the nitrogen balance technique for the estimation of the nutritional value of proteins. He based his method

upon the fact that protein is the only considerable source of dietary nitrogen. The food nitrogen in the body can be followed by the determination of the nitrogen in the food, urine and feces of the animal under observation. Thomas introduced the term "biological value" which he defined as "the number of parts of body nitrogen replaceable by 100 parts of nitrogen of the food stuff."[1]

The method of Thomas for determining biological value was developed and improved by Mitchell (1924b), working at the University of Illinois. He introduced two assumptions in the development of his method.

The first had to do with the postulation that the excretion of nitrogen in the feces in the nitrogen-feeding period was equivalent to that in the previous metabolism period when no protein food was fed plus the nitrogen derived from any undigested food.

His second assumption in his interpretation of the significance of the urinary nitrogen in the two metabolism periods of the test is based on the idea that the catabolism of nitrogenous body substances that occurs during the feeding of nitrogen-free diets continues at a constant level when protein feeding is resumed. The use of this assumption in the calculation of biological value originated in the concept of endogenous metabolism proposed by Folin in 1905.

Data questioning the existence of a constant independent endogenous metabolism have been obtained by workers at the Iowa State College lab-

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[1] As translated by Martin and Robinson (1922).

oratory when balance studies were used for the evaluation of food proteins by Mitchell's method. Contrary to expectations, a decrease in the excretion of urinary nitrogen was observed when egg proteins were added to a nitrogen-free diet fed to animals partially depleted of their body reserves of protein. This finding, indicating a suppression of the catabolism of body tissue, seemed to deny the existence of an endogenous metabolism that continued at a constant rate in periods of protein and non-protein feeding (Marshall, 1943). Later work (Willman)[1] demonstrated that when either hepatic or muscle tissue extirpated from nitrogen-depleted rats was added to the diet, there was an increase rather than a decrease in the urinary excretion of nitrogen. This increment, in contrast to the decrement observed when egg proteins were fed, was interpreted to mean that the animal's own tissue proteins were used less efficiently than egg proteins in meeting the physiological requirements for life.

Allison and Anderson (1945), working at Rutgers University, also objected to Mitchell's method for the calculations of biological values on the basis that the existence of a constant endogenous nitrogen excretion was assumed. They believed that Schoenheimer's dramatic exposition of the dynamic relationship existing between the tissues and surrounding media made a separation of food and body protein impossible.

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[1] Iowa Agricultural Experiment Station Project 799.

They stated, however, that the elimination of the assumption of an endogenous metabolism did not prohibit the expression of the utilization of food nitrogen in terms of two fundamental variables, nitrogen balance and food intake.

Allison and Anderson developed from a mathematical standpoint an expression of the relationship between these two measurable factors. They began with a simple equation based on the definition of nitrogen balance. First, determining the quantity of food nitrogen absorbed, they found that a linear relationship existed between nitrogen balance and absorbed nitrogen in the region of negative and low positive balance. The slope of this line was indicative of the rate of change of nitrogen balance with respect to absorbed nitrogen, and was a function of the retention of nitrogen. The empirical equation for the linear portion of this relationship could be expressed as follows:

$$NB = k\ (AN) - NE_o$$

where NB is nitrogen balance, AN, absorbed nitrogen, NE_o, the excretion of nitrogen on the protein-free diet, and k, the slope of the line. If certain assumptions are made, k is the fraction of absorbed food nitrogen that is retained in the body of an animal and which, according to definition, is biological value.

In their first experiment, Allison and Anderson used casein as the source of protein and found that the value for k was identical with the biological value calculated from metabolic data. In commenting upon this finding they stated, however, that if the endogenous excretion did not

remain constant during the two experimental periods, the two values would not be identical. They predicted further that if such were to occur there would be an inverse relationship between the quantity of urinary nitrogen excreted and the quantity of food nitrogen absorbed in the protein feeding period. It is interesting, in view of this prediction, that in experiments at the Iowa State College laboratory (Willman et al. 1945) the excretion of urinary nitrogen was depressed in the second experimental period when egg proteins were fed after an interval of nitrogen deprivation. Allison, Anderson and Seeley (1945) later demonstrated the same phenomenon in metabolic studies with depleted dogs when egg proteins served as the test proteins. On the basis of these results, they suggested that k be called "nitrogen balance index" rather than biological value.

A new method has been developed also at the Iowa State College for the evaluation of the nutritional value of proteins. Marshall (1943) first showed that ingestion of the egg-containing diet by the rat gave rise to an unexpected decrease in the excretion of urinary nitrogen as compared to that characteristic of the excretion in the low-nitrogen feeding period. To determine whether or not nitrogen balance was directly proportional to the quantity of dietary protein, egg was incorporated into the protein-free diet at different levels. It was found that the relationship between the quantities of nitrogen fed and the nitrogen balances was linear.[1] Therefore, the difference in the nitrogen balances

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[1]Unpublished data: Files- Food and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

in the two test periods was used as the basis of an index to express the nutritional value. The difference in the two balances was designated as "body nitrogen spared". This value can be related to either food nitrogen ingested or to food nitrogen absorbed. The latter ratio was taken for the expression of nutritional value at the Iowa State College laboratory and was designated as "biological efficiency". One theoretical assumption was introduced in the calculation of food nitrogen absorbed. It is based on the idea that the excretion of fecal nitrogen of metabolic or physiological origin remains constant during the two experimental periods. The relationship between the body nitrogen spared and food nitrogen ingested was stated as the "utilization ration".

Mitchell and Block (1946) suggest that the nutritive value of a protein may be measured by means of chemical assay. These workers compared the amino acid contents of certain food-products, as determined by modern methods, with the results of rat feeding experiments designed to determine the biological value of these products in the nutrition of growth. Amino acid analyses assembled by Block and Bolling (1944) were used in making these comparisons. Egg was chosen as the reference substance because its protein moiety is highly digestible and almost perfectly utilizable in rodent metabolism (Mitchell and Carman, 1925; Summer, 1938). Murlin et al. (1944) also proved that for the adult human subject egg proteins were better utilized than whole milk proteins.

The amino acid deficiencies of other proteins were compared in relation to the composition of egg. The percentage deviation of the quantity of each essential amino acid in the specified protein mixture

from that present in the protein of dried whole egg was calculated. They considered that the amino acid limiting the nutritional value of a specific food protein would be the amino acid present in the least amount with reference to its occurrence in the whole egg protein. For example, beef muscle proteins were found to contain 46 per cent less cystine than whole egg protein. This amino acid, therefore, represents the factor limiting the nutritional value of beef muscle protein. On the other hand, the limiting amino acid in blood serum is iso-leucine and in wheat proteins, lysine.

This method for the correlation of chemical and biological data has not been perfected. Though the predicted and actual biological values approximated each other very closely for a number of proteins, there were several cases in which disagreement between these values existed. One factor limiting the accuracy of the method was the lack of reliable amino acid analysis for many food proteins. The picture was further complicated by the fact that biological value took into account the total nitrogen content of the food material, which was not always true of the amino acid analyses. Such a discrepancy may mean that a protein will rank higher on the chemical scale than on the biological scale.

Nevertheless, this scheme of analysis may become very useful in the future in the estimation of nutritive value, perhaps even to the extent of replacing the laborious methods of biological assay which are now largely relied upon for the evaluation of protein foods.

Utilization of Protein

The attainment of optimal nutrition remains a major problem of medicine and surgery despite accumulating knowledge concerning the importance of vitamins, amino acids and other essential factors in the dietary. Until certain relationships are more clearly elucidated, however, we still cannot utilize this knowledge with full effectiveness. Important among these are the interrelationships that exist between the respective quantities of protein, fat, and energy producing foods in the diet and the utilization of dietary protein. These relationships will be discussed briefly. It should be noted, however, that other relationships among dietary constituents are reflected in nitrogen metabolism. Some that may be mentioned are those demonstrated between ascorbic acid and phenyl alanine and tyrosine; between pyridoxine and tryptophane; between niacin and tryptophane, etc.

Benditt, et al. (1948) studied the rate of utilization of protein for tissue fabrication by the protein-depleted animal when the calorie intake was varied and the protein intake was held constant. They also reversed the situation and varied the protein intake, while they held the calorie intake constant. Finally they varied simultaneously both the calorie and protein intakes of the animals. They found that at the lowest level of calorie intake, protein utilization was very poor. With the increasing calorie intake protein utilization improved until a maximum was reached at an intake level of 1240 calories per square meter per day. Beyond this point there was no additional utilization

of protein despite the fact that the calorie intake was increased up to 1840 calories per square meter per day.

Utilization of nitrogenous food increased as the protein intake was increased when the energy value of the diet was held constant. The rise was almost linear with daily intakes ranging from 3 to 15 grams per kilogram of body weight. Beyond this point the rate of protein utilization became progressively less. When the levels of protein and calorie intakes were varied simultaneously, the rate of protein utilization increased with increased diet intake. In other words, when the quantity of the diet was restricted, the rate of protein utilization was restricted. The quantity of fat deposited at a constant protein intake and increasing calorie intake was approximately proportional to the calorie intake. On the other hand, when the calorie intake was maintained constant and the protein intake varied, gains in fat increased with increasing protein intake up to levels of 8 grams per kilogram per day. Further increase in protein intake resulted in moderate decrease in the quantity of fat deposited since the protein calories were increased at the expense of the non-protein calories of the diet. All these above relationships were found to be similar in the growing rat and in the adult depleted rat during repletion.

Benditt, Woolridge and Stepto (1948) also found with protein-depleted human subjects that at a constant protein intake, increasing the calorie intake from 1350 to 2650 Calories per square meter per day had no influence upon protein utilization. On the other hand, protein

utilization was proportional to protein intake over the ranges of protein intake studied.

Similar results with rats as the test animals have been reported from the Nutrition laboratory of the Iowa State College (Swanson 1946, Swanson et al. 1947). These workers showed that a 50 per cent reduction in the energy value of the diet increased nitrogen catabolism in nitrogen-depleted animals, and decreased the utilization of egg proteins when they were added to the diet. Another aspect of protein utilization is of extreme importance in feeding undernourished groups of people living on limited food supplies. This problem relates to the effect that varying quantities of fat in the diet may have on protein metabolism. Swanson and co-workers (1947) fed protein-free diets to rats that contained 0, 5, 10, 15 and 20 per cent of fat. These diets were fed in isocaloric quantities each day. When the rations containing 20 per cent of fat and no fat, respectively, were fed in quantities that met the calorie requirements of the animals, the rate of nitrogen catabolism was nearly identical in both groups. Excretion of nitrogen in the urine was the basis of evaluation. When, however, the calorie intakes of the animals were reduced to one-fourth of the normal ingestion, the rats fed the low-fat diet broke down nearly twice as much body tissue as the rats given the 20 per cent fat diet. It was further demonstrated that methionine may replace fat in the diet. These investigators added 40 milligrams of methionine daily to the low-fat diet and again reduced the calorie intake to 25 per cent of the normal. Instead of excreting

818 mg. of nitrogen in the urine as the rats did in the first experiment, they now excreted only 577 mg., nearly the exact quantity (544 mg.) excreted when the high-fat diet was fed at one-fourth the normal calorie intake.

Schwimmer in 1947 reported studies on the response of human beings which the work of Swanson had spearheaded. He found that subjects fed a diet supplying 900 calories and 6.0 grams nitrogen and 180 grams of fat (30 per cent by weight) excreted decreased quantities of nitrogen in the urine and maintained nitrogen balance. Schwimmer writes "it becomes evident that the nitrogen-sparing effect of 30 per cent fat was not due to increased calories, but rather to something intrinsic in the higher fat intake per se."

THE SYNTHESIS OF THE COMPONENTS OF THE BLOOD STREAM

Blood is one of the important fluids of the body. It has been recognized for a long time that dietary protein plays an important role in the synthesis of the formed elements and hemoglobin. In order to evaluate the influence of variations in quality and quantity of ingested protein on the various blood components of an animal, it is necessary to measure total quantities of circulating blood, plasma, hemoglobin, erythrocytes, etc.

Hahn and Whipple in 1938 demonstrated that a standard anemic dog on a limited protein intake was unable to produce the usual amount of globin and, therefore of hemoglobin, even in the presence of a large excess of iron. Orten and Orten (1943) clearly showed that the administration of a diet low in protein (lactalbumin) but adequate in all other respects produced a mild chronic anemia in rats. Increasing the intake of either calories or iron has no beneficial effect on inducing the formation of new hemoglobin in the animals. These experiments clearly indicate that the iron content of liver is not wholly responsible for its potency in anemia. But recently Whipple et al. (1947) have shown that under conditions of protein-fasting with abundant iron intake the body can given up large amounts of proteins from its organ and tissues to produce new hemoglobin and plasma protein when experimental anemia and hypoproteinemia are maintained by bleeding and plasmaphoresis.

By means of a standardized anemic dog, Madden et al. (1940) evaluated the relative potencies of the proteins and amino acids of various foods for plasma protein production. The addition of casein to the ration caused the formation of large quantities of plasma protein, equal to 33 per cent of the protein fed. This equaled the potency of liver protein (17 to 33 per cent) and approached values for plasma protein itself when given by mouth (40 per cent). Zein had no effect upon plasma protein regeneration but when it was supplemented with cystine, tryptophane, lysine, and glycine, there was a doubling of the basal plasma protein production and a retention of the fed-protein nitrogen. Supplementary threonine did not modify the above reaction. Liver protein supplemented with cystine, leucine, glutamic acid, and glycine in the basal diet yielded double the amount of new-formed plasma protein produced by the feeding of liver alone. Whipple (1938) showed that liver, kidney and beef heart furnished valuable building stones for the construction of hemoglobin, whereas beef serum, egg white and casein were only moderately effective. The potency of certain amino acids, for example, phenylalanine and histidine in producing a regeneration of hemoglobin was high when used as the sole supplement of the basal diet.

Robscheit-Robbins, Miller and Whipple (1943) showed that invariably more hemoglobin was produced than plasma protein when dogs were rendered both hypoproteinemic and anemic. They mentioned that under all circumstances on all diets tested this was found to be true. A possible

explanation lies in the fact that the normal hemoglobin concentration is about three times as much as the concentration of plasma protein. Then when there is a deficiency in both proteins the production flow of protein-building factors favors hemoglobin, much of the globin for hemoglobin comes from plasma. It seems obvious that plasma protein can contribute to the building of hemoglobin and vice versa whenever needed.

It has been shown by Holman and co-workers (1933) that dogs rendered anemic and receiving sugar and iron by mouth and plasma protein by vein can supply all body protein requirements and maintain nitrogen equilibrium under the stress of emergency. When the plasma protein was fed by mouth under identical conditions, the same general reactions occurred only the excretion of urinary nitrogen was a little higher. They thought that these differences might be due to deaminization of the protein fed by mouth. The same thing was observed when hemoglobin was injected into the anemic dog. All these observations add up to the concept that a remarkable fluidity must exist between blood protein and tissue protein or organ proteins without any wastage of nitrogen.

Fink, et al. (1944) used heavy nitrogen to label protein to study the protein exchange in the body. They found that in normal dogs there was at first a rapid disappearance of the labelled plasma from the circulation, then a slower disappearance as time went on. This outflow was balanced by a simultaneous inflow of plasma proteins from the tissues. This may indicate that the plasma proteins are normally in constant and rapid exchange with a mobile pool of body protein.

Madden, Anderson, Donovan and Whipple studied (1945) the normal and abnormal physiology of the utilization of the essential amino acids, in particular as it was related to the production of new plasma proteins. They thought that the depletion of the normal levels of plasma proteins in the circulating blood by means of plasmapherosis put a strain upon the body and stimulated the normal demands for plasma protein production. The vigorous new production of plasma protein may be for the diversion of protein-building materials from the tissues.

Weech, et al. (1937) investigated the influence of a protein-deficient diet on the number of erythrocytes in the blood of dogs. They found after a certain interval of time that the number of erythrocytes and the concentration of hemoglobin were gradually reduced. The volume occupied by the red cells decreased also.

In 1944 Cannon and his group working at the University of Chicago published an important paper on protein deficiency and the incidence of surgical infection. They called attention to the fact that in surgical cases, patients often lost sufficient serum proteins to lead to severe depletion of the reserve body protein. Such a loss could occur if the diet were deficient in protein or if the body was unable to absorb or utilize protein.

EXPERIMENTAL PROCEDURE

General Plan

The present experiment was planned with the hope that it might describe, at least partially, the response of well-nourished and under-nourished adult rats to the feeding of defatted, dehydrated whole egg protein. The study included a series of experimental units in which the problem was considered from several angles.

The respective abilities of under-nourished and well-nourished rats to utilize their dietary nitrogen were evaluated by means of studies of the retention of nitrogen, studies of the components of the blood, studies of the nitrogen, fat and water contents of the liver, and studies of weights of liver and adrenal glands.

Dehydrated defatted egg powder was chosen as the dietary source of nitrogen because their high biological efficiency of egg proteins had been demonstrated previously in this laboratory by means of the nitrogen balance technique.

In order to produce under-nourished animals, rats were transferred from the stock diet to a low-protein diet for a period of three weeks before the test proteins were offered. In the last seven days of this period, nitrogen balances were determined. In this interval, the animals usually lost approximately one-sixth of their body weight. Such animals represented standardized under-nourished animals, and

were ready to receive the test protein. Then after an adjustment period of four days on the egg-containing diet the animals were ready for the final balance test.

The well-nourished animals were given the diets containing different percentages of the egg-proteins for 25 days. Then a balance test was made.

Since proteins are utilized differently when fed in varying proportions in both tests the dehydrated egg powder was incorporated in the diet in quantities that provided between 1.5 and 25.0 per cent of protein.

For adequate comparison, a smaller group of normal rats raised on the stock ration were maintained as positive controls; another group receiving the protein-free diet throughout the experiment served as negative control animals. The experimental groups are indicated in Table I together with total number of rats used in the various phases of the experiment.

Table 1. Experimental groups

Series	Diet	Group	Approximate quantity of protein	No. of rats in each group
Undernourished	N-low plus egg	A-1	1.5	18
		A-2	2.5	6
		A-3	3.0	16
		A-4	3.5	6
		A-5	4.5	6
		A-6	6.5	12
		A-7	8.5	11
		A-8	10.0	19
		A-9	12.0	19
		A-10	14.0	15
		A-11	18.0	19
		A-12	25.0	15
Well nourished	N-low plus egg	B-1	1.5	10
		B-2	3.0	10
		B-3	3.5	10
		B-4	6.5	10
		B-5	8.5	10
		B-6	10.0	10
		B-7	12.0	10
		B-8	18.0	10
		B-9	25.0	11
Control	Steenbock XVII	C-1	23.6	21
	Nitrogen-low	C-2	0	15

EXPERIMENTAL DIETS

The Basal Nitrogen Low Diet

The basal nitrogen-low diet fed throughout the experiment was a synthetic ration consisting of dextrin, butter fat, lard, Osborne and Mendel salts, sodium chloride, and ruffex. Its composition is shown in Table 2.

This ration contained approximately 0.07 per cent of nitrogen. It was supplemented with a mixture of vitamins made up from materials which were synthetic except for Rice Bran Polish, factor II which was used as a source of unrecognized factors. In addition, cod liver oil and alpha tocopherol were supplied.

The components of the mixture and the quantity of each vitamin supplied daily by the mixture are shown below:

Thiamin[1]	40 mcg.
Riboflavin[1]	60 mcg.
Pyridoxine[1]	40 mcg.
Nicotinic acid[1]	500 mcg.
Calcium pantothenate[1]	100 mcg.
Biotin[2]	1.9 mcg.
Inositol[1]	10 mg.
p-amino benzoic acid[1]	10 mg.
Ascorbic acid[1]	1 mg.
Choline[1]	5 mg.
Rice bran polish, Factor II[3]	100 mg.

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[1]Nutritional Biochemicals Co., Cleveland.
[2]Merck and Co., Rahway, N.J.
[3]Borden Co., Prescription Products Div., New York.

Table 2. Composition of low-nitrogen and egg diets

Approximate per cent of protein	Dextrin[a]	Egg powder[b]	Butter fat[c]	Lard[d]	Salts[e]	Ruffex[f]	Sodium chloride[g]	Total
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0	73	0	10	10	4	2	1	100
1.5	71	20	10	10	4	2	1	100
2.5	69.7	3.3	10	10	4	2	1	100
3.0	69	4.0	10	10	4	2	1	100
3.5	68.4	4.6	10	10	4	2	1	100
4.5	67.1	5.9	10	10	4	2	1	100
6.5	64.4	8.6	10	10	4	2	1	100
8.5	61.8	11.2	10	10	4	2	1	100
10.0	59.8	13.2	10	10	4	2	1	100
12.0	57.0	15.9	10	10	4	2	1	100
14.0	54.6	18.4	10	10	4	2	1	100
16.0	51.9	21.1	10	10	4	2	1	100
18.0	49.1	23.9	10	10	4	2	1	100
25.0	39.8	33.2	10	10	4	2	1	100

[a] Purchased from Fisher Scientific Co.

[b] Dehydrated defatted whole egg powder given by the National Poultry Association to the Bureau of Biological Research at Rutgers University for use in a collaborative assay.

[c] Purchased on the local market as butter and prepared in the Nutrition Laboratory.

[d] Armour or Swift's Lard, purchased on the local market.

[e] Osborne and Mendel (1919).

[f] Purchased from Eimer and Amend, New York; also termed Fisher Celluration.

[g] Baker Chemical Co.

The rice bran polish, Factor II, choline and biotin were dissolved in 50 per cent alcohol, dried on dextrin, then mixed with additional dextrin so that 500 mg. of the mixture contributed the above quantities of the respective vitamins (Kuehl, 1949). Alpha-tocopherol[1] was dissolved in Wesson oil in such proportions that 50 mg. of the solution provided daily for each rat 0.75 mg. of alpha-tocopherol.

The vitamin mixtures were offered to the animals in a separate cup. Fifty mg. of Squibbs medicinal cod liver oil[2] was dropped on the top of the vitamin mixture. These vitamin mixtures were, in general, immediately eaten. If this was not the case, no food was given until the supplement was completely taken.

Egg-containing Diets

The egg-containing diets given to the test rats were prepared so as to be isocaloric with the nitrogen-low diet. The defatted dried egg powder was substituted for dextrin in the basal nitrogen-low diet in the proportions required by the analyzed nitrogen content of the egg powder. Since it was impossible to investigate the response of both the undernourished and the well nourished animals to the egg-containing diets in a single year, a sufficient quantity of all diets for series A was prepared and fed during 1947, and for series B in 1948. The diets were stored in a deep freeze unit until needed, and then refrigerated during the ex-

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[1] Nutritional Biochemicals Co., Cleveland.

[2] Judisch Drug Store, Ames, Iowa.

perimental period. The diets were formulated according to the same plan in both series. The vitamin supplement previously described was given to these groups also.

Samples of the egg powder were analyzed in 1948 and 1949. The egg powder on the basis of the 1948 and 1949 analyses contained 75.1 and 75.6 per cent of protein, respectively.

The composition of all diets is indicated in Table 2.

Positive Control Ration

The positive control animals were fed a ration known in the laboratory as the Steenbock XVII diet. This ration is used for the maintenance of the stock colony. The proportions of the various ingredients present is as follows:

	gm.
Yellow cornmeal[1]	560
Casein, technical[2]	50
Linseed meal[3]	160
Alfalfa leaf meal[4]	20
Sodium chloride[5]	5
Calcium carbonate[6]	5
Yeast, brewers' (Pabst)[7]	95
Yeast, brewers' (Pabst irradiated)[8]	5
Wheat germ[9]	100
Klim[10]	163
	1163

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[1]From Animal Husbandry Dept., Iowa State College.
[2]B₃F — Casein Co. of America, Div. of Borden Co., Bainbridge, N.Y.
[3]Ames Grain and Coal Co.
[4]"Jack Rabbit" brand, Denver alfalfa M and P Co., Lexington, Nebraska.
[5]Ames Service Store
[6]Bakers Chemical Co.
[7]Pabst, Code R_1, Pabst Sales Co., General Offices, 221 N. LaSalle St., Chicago.
[9]Oat Type A, Gold Medal, General Mills, Inc.
[10]Borden Co., Prescription Products Div., New York.

The trace elements were mixed with calcium carbonate in proportions to supply to each rat approximately 20 mcg. of potassium iodide, 70 mcg. of manganese sulfate, 26 mcg. of potassium aluminum sulfate, 102 mcg. of copper sulfate per day. Fifty mg. Squibb's cod liver oil was offered in a supplement cup daily. In addition, 5 gm. of fresh ground beef and 10 gm. of fresh carrot were fed three times each week.

ANALYSIS OF DIETS

The Kjeldahl-Gunning procedure was used in all determinations of nitrogen. Weighed portions of the nitrogen-low, the egg-containing diets, and of the vitamin mixture were transferred quantitatively to Kjeldahl flasks. The diets were digested for two hours with 35 ml. of concentrated sulfuric acid, 15 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide. The samples were allowed to cool, and diluted with 200 ml. of tap water. Experimentation by the present investigator had shown that period of time to be adequate for complete digestion of carbonaceous particles of the diet. An excess of concentrated sodium hydroxide above the amount needed to neutralize the sulfuric acid to prevent bumping was added with a small quantity of granular zinc during the distillation process. The ammonia thus released was distilled into a known amount of 0.1N hydrochloric acid. The hydrochloric acid that was not neutralized by ammonia was titrated with standard sodium hydroxide solution, approximately 0.1N, with the use of a methylene blue-methyl red indicator.

The Steenbock XVII diet contains larger proportions of cellulose and carbonaceous particles. To save time and to secure complete digestion, the diet was hydrolized partially before analysis. In this procedure, five grams of Steenbock XVII diet was mixed with 50 ml. of 20 per cent hydro-chloric acid, diluted with 200 ml. of distilled water, and autoclaved under 15 lb. pressure for one hour. After hydrolysis 20 ml.

aliquots were taken for the analysis of nitrogen. Five grams of meat and 10 grams of carrot were also analyzed by the same procedure as was the Steenbock XVII ration.

The diets were also analyzed for moisture, according to the method to be described below, in order to calculate results of analysis on a moisture-free basis.

In analysis of moisture, weighed samples of the diets were dried in an air oven at 105°C. for seven days or until their weight was constant. The percentages of protein and moisture in the Steenbock XVII diet were 23.61 and 7.00 respectively. Five grams meat and 10 grams carrots contained 192 mg. nitrogen and 12.32 per cent of moisture. The nitrogen content of the seven days intake of the vitamin mixture was 19.31 mg.

The percentages of protein and moisture present in the egg-containing diets are shown in the following table.

Table 3. The percentages of protein and moisture present in the experimental diets

Approximate per cent protein[a,b]	Series A		Series B	
	Protein %	Moisture %	Protein %	Moisture %
0	0	5.0	0	5.0
1.5	1.7	5.3	1.6	5.3
2.5	2.2	5.3	-	-
3.0	3.1	5.3	3.2	5.3
3.5	3.6	5.2	3.5	5.2
4.5	4.6	5.1	-	-
6.5	6.7	5.1	6.9	5.1
8.5	8.9	5.1	8.3	5.1
10.0	10.3	5.1	9.8	5.1
12.0	12.3	5.0	12.1	5.0
14.0	14.0	-	-	-
18.0	18.3	5.0	18.2	4.8
25.0	25.7	4.4	25.4	4.4

[a]Factor 6.25 x N.

[b]Protein results are on wet basis.

EXPERIMENTAL ANIMALS

Description

Approximately six-month old male albino rats of Wistar stock, strain A, inbred by brother and sister mating for about 97 generations were used for all units of the experiment. They were fed the stock diet from the time of weaning until they were used for experiment. The adult rats were chosen as test animals so that the utilization of the proteins would be limited to body maintenance only.

All animals were in good physical condition at the beginning of the experiment as judged by their general appearance, alertness, muscle tone, and freedom from respiratory infection. Particular care was taken to distribute the rats among the experimental groups so that age, weight, and litter representation were as uniform as possible. At the initiation of the experiment, the weights of the rats were approximately 300-350 gm.

The composition of the stock diet used in the Animal Research Laboratory of the Foods and Nutrition Section has remained nearly the same since 1932 and the quality of the components of the ration has been kept as uniform as possible. It is designated "Steenbock Va" diet, and is similar in composition to the Steenbock XVII diet used for the positive control rats, except that the Klim was reconstituted and fortified with trace elements and cod liver oil. It was offered separately in the

proportion of 12.5 ml. per rat per day instead of being incorporated in the basal ration. To one quart of distilled water, 130 gm. of Klim, 2 ml. of a solution of trace elements[1], and 1 tsp. of cod liver oil[2] are added, and mixed for five minutes in the Hobart mixer at high speed.

Preparation of Animals for Experiment

The rats were housed individually for two or three days before taking them from the regular stock ration.

The data obtained in this laboratory in 1947 suggested that the animals possessed larger protein reserves than was expected. Therefore, in 1948 to make the animals as uniform as possible, they were starved for 24 hours immediately prior to the initiation of the experiment.

Series A: undernourished

Preliminary study showed that the food consumption of animals ingesting a low nitrogen diet tended to decline gradually. Therefore, the low-nitrogen diet was offered ad libitum to the members of all groups for three weeks. During the last seven days of this interval, the daily food intake was carefully recorded. In the subsequent period, the food intake was limited daily to one-seventh of the total quantity consumed in the preceding week.

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[1]Prepared by dissolving 0.08 gm. of potassium aluminum sulfate and 0.4075 gm. of anhydrous copper sulfate in 100 ml. of distilled water, and added to milk in the proportion of 1:500.

[2]A and D feeding oil, from Pearson Ferguson Co., St. Louis, Mo.

Series B: well nourished

It had been shown in Table 4 that the ingestion of egg-containing diets exceeded that of the nitrogen-low diet if both were offered ad libitum. This difference in food consumption might fluctuate the result of the experiment to a great extent. It was necessary to offer the certain amount of food which gave the same calories to all the rats. Thirteen gm. was the average food consumption of nitrogen-low diet during the seven-day ad libitum period. After the seven-day ad libitum period the well-nourished rats were fed 13 gm. of the egg-containing diet for 34 days.

Series C: control

All the members of this series were fed ad libitum for seven days to provide some preliminary information regarding appetite and food consumption habits. Subsequently the diet was offered daily, was one-seventh of the total amount consumed during the ad libitum feeding.

All the animals in each series were weighed and fed at a regular hour throughout the experiment. Food jars were weighed on a Torsion balance and the food intake recorded daily except on Sunday.

Table 4. Average food intakes during the experimental period

Series	Group	Analyzed per cent of egg protein	7-day average food intake during ad libitum feeding	Food intake during balance period
			gm.	gm.
Under-nourished	A- 1	1.7	12.1	11.7
	A- 2	2.2	12.7	12.7
	A- 3	3.0	13.2	12.9
	A- 4	3.6	11.7	11.7
	A- 5	4.6	13.0	12.3
	A- 6	6.7	12.7	12.2
	A- 7	8.9	14.2	14.2
	A- 8	10.3	16.0	16.0
	A- 9	12.3	13.0	12.7
	A-10	14.0	13.8	13.7
	A-11	18.3	15.4	15.1
	A-12	25.7	13.6	13.1
Well-nourished	B- 1	1.6	13.7	11.8
	B- 2	3.2	15.2	12.2
	B- 3	3.5	16.2	12.5
	B- 4	6.9	15.3	11.6
	B- 5	8.3	15.6	11.8
	B- 6	9.9	16.7	12.2
	B- 7	12.1	16.9	12.0
	B- 8	18.2	15.3	11.6
	B- 9	25.4	15.5	11.4
Control				
Positive	C- 1	23.6	14.5	13.7
Negative	C- 2	0.7	13.4	11.6

NITROGEN BALANCE TECHNIQUE

The classic method developed by Mitchell (1924) for studying the nutritional value of proteins was modified in this investigation for the determination of protein utilization. The duration of the entire experiment was 34 days for the well nourished, under nourished, and negative control groups. This included for the undernourished animals, an interval of twenty-one days in which they consumed the low-nitrogen diet; and 11 days in which they ingested the respective protein diets. During the first seven days of these periods, collections of urine and feces were made. A quantitative record was kept of the amount of food consumed by each rat.

The well nourished animals received the egg-containing diet from the very beginning to the end of the experimental period. The first 25 days of this interval was termed the preliminary period and no collections were made during this time. They were made, however, from the 26th to the 33rd days.

The negative control group animals ingested the nitrogen-low diet throughout the experimental period, and balance tests were made from the 26th to the 33rd days. The positive control group was fed the test diet only for 15 days because it was only slightly different from the regular stock ration, and the metabolism tests were run from the 8th through the 15th day.

Collections of metabolic materials were made for seven days. The animals were starved at 10:00 p.m. on the night before the balance study started. At 8:00 a.m. on the first day of the collection period, each animal was changed into an individual wide-meshed cage which rested on a pyrex plate containing nine acid-treated nitrogen-free filter papers. At the end of each test, the rats were transferred into regular cages at 8:00 a.m. In order to differentiate clearly between the fecal material excreted as a result of the ingestion of the different test diets, the diets were colored red with ferric oxide (0.1 gm. per 100 gm. of diet). The red food was offered on the first day of the balance period, and collection started at the first appearance of red feces. Red food also was fed the day after the balance test ended, and fecal collections were terminated as soon as the red feces were excreted.

After the balance test was completed, blood analyses were made. Then the experiment was concluded with the extirpation of tissues for analyses of moisture, fat and nitrogen.

COLLECTION OF SAMPLES FOR ANALYSIS

Urine

High-quality filter papers containing only traces of nitrogen were soaked over night in a 10 per cent solution of glacial acetic acid in 95 per cent ethyl-alcohol and air-dried. During the collection period one filter paper was removed each day. The papers were placed in wide Erlenmeyer flasks containing 200 ml. of 20 per cent hydrochloric acid and covered with two layers of cellophane impervious to moisture. At the end of a collection period, each cage with its pyrex plate was quantitatively washed with hot, distilled water applied under pressure and the washings transferred to the Erlenmeyer flask containing the filter papers corresponding to the particular cage.

The acid extract from the filter papers was poured quantitatively through a Buchner funnel fitted into a two-liter suction flask. The somewhat disintegrated papers were then transferred to the funnel and washed with hot water until all of an urine was extracted, and rinsed into the suction flask below, after which the Erlenmeyer flask was thoroughly washed. The contents of the suction flask were transferred quantitatively to a two-liter volumetric flask, and made up to volume, after cooling to room temperature. Twelve-ounce pharmacy bottles were filled with the adequately mixed urine sample, and the excess discarded.

Recovery experiments in which a known amount of standard ammonium sulfate was sprinkled on cages at regular intervals over a 7-day period and carried through the entire cage-washing procedure, upheld the validity of this method of collection as shown in Table 5.

Feces

Collection of feces was initiated when the first red feces appeared in the beginning days of the experiment and continued until they re-appeared after the administration of a new diet, the red feces now being discarded. The fecal material was picked up each day, brushed free of food and hair, and placed in Erlenmeyer flasks (125 ml.) containing 50 ml. of 20 per cent HCl. When the collection period was completed, the total suspension was autoclaved at 15 lb. pressure for two hours. The contents of each flask were then rubbed through a fine sieve into a volumetric flask and made up to a volume of 250 ml.

Organs

The organs were removed from the anesthetized animal as soon as blood for serum protein determinations was withdrawn. The organ was blotted on filter paper to remove excess blood and any attached tissues trimmed away. A part of the liver was placed in a small previously weighed weighing bottle at constant weight; the remaining portion of the liver was placed in a 250 ml. previously weighed Erlenmeyer and stoppered. Samples were weighed as soon as possible. This procedure

provided one sample for the moisture and fat determinations and one for the nitrogen analysis. The adrenal glands from each animal were placed in a small bottle and weighed.

Table 5. Nitrogen recovered from cages sprinkled with aliquots of standard ammonium sulfate solution

Cage number	Total nitrogen in aliquot (mg.)	Theoretical quantity of nitrogen present (mg.)	Recovery of nitrogen (per cent)
1	375.76 375.76	376.32	99.9
2	378.56 381.36	376.32	101.0
3	378.56 375.76	376.32	100.2
			Average 100.3

The same procedure that was used in preparing fecal material for analysis was used in preparing the livers for nitrogen analysis. The larger weighed portion of the liver was autoclaved in 50 ml. of 20 per cent hydrochloric acid for two hours at 15 lb. pressure and then made up to a volume of 100 ml. A 10 ml. sample was used for analysis.

Blood

The rats were starved for 10 hours preceding the time of bleeding, for the purpose of eliminating the variable effect of food consumption on the composition of blood.

Samples of blood to be used in routine determinations in this study were obtained from the tail vein of the rat. The rat was firmly, but carefully wrapped in several soft towels, with the nose and the tail free. The tail was immersed in moderately warm water (40-50° C.) for about one minute to make their veins prominent and increase the rate of blood circulation. The tails were dried and coated with vaseline to keep the blood as a drop. Then a diagonal incision was made with a sharp lancet and four or five drops of the blood were allowed to flow freely onto a wax spot plate containing a very small amount of heparin. The blood was stirred with a small glass rod to prevent clotting. Pressure was applied to the incision to stop bleeding and the tails were bandaged with cotton and collodion to prevent further loss of blood.

For the blood volume determination, the rats were anaesthetized in an "ether chamber", a large dessicator equipped with cotton padding on the under side of the cover which was soaked with ether. The animal remained in this chamber until the only visible sign of life was a very faint heart beat or until the animal appeared to be in a deep sleep. This procedure requires approximately two or three minutes.

On removal from the chamber, the tail of the animal was immersed in a vessel of warm water, and a piece of cotton saturated with a solution of 50 per cent alcohol and 50 per cent xylene was rubbed on the tail to dilate the tail vein where the dye was injected. Samples of blood were withdrawn as before, and the intensity of the color in the plasma determined.

ANALYTICAL PROCEDURES

Nitrogen in Urine, Feces and Liver

The Kjeldahl-Gunning procedure was used in all determinations of nitrogen.

Fifty ml. aliquots of the urine extracts were digested in a Kjeldahl flask with 20 ml. concentrated sulfuric acid, 10 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide for one and one-fourth hours.

Aliquots, 25 ml. in size, of the thoroughly mixed fecal digest were measured with a pipette of large bore and digested with 20 ml. of sulfuric acid, 15 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide for a period of one and one-half hours. The remaining procedure was the same as for the analysis of the diet.

Concentration of Hemoglobin

The concentration of hemoglobin in the blood of animals in the various experimental groups was determined by the acid-hematin method of Cohen and Smith (1919) as modified by Exton (1937). The principle of the technique involved the conversion of hemoglobin to acid-hematin by hydrochloric acid, and the comparison of colorimetric readings of the blood with those of a standard.

From a freely flowing drop, 0.02 ml. of blood was measured with a U.S.B.S. blood pipette and mixed with 10 ml. of 0.1N hydrochloric

acid. After an interval of one-half hour, the color of the acid-hematin solution read in a Klett-Summerson photoelectric colorimeter was compared against the Klett acid-hematin standard, a solution with a color equivalent to that of 14.4 gm. hemoglobin in this dilution. The concentration of hemoglobin of the blood was computed from a hemoglobin factor determined from the reading of the standard as follows:

$$\frac{14.4}{\text{colorimeter reading of standard}} = \text{factor.}$$

The readings observed for the samples of blood were then multiplied by the factor. As the standard was set up on the basis of 5 ml. of hydrochloric acid and 0.02 ml. of blood, hence, the results were multiplied by two to indicate the gm. per cent of hemoglobin present.

Enumeration of the Red Blood Cells

A rapid method for the routine enumeration of the number of erythrocytes present in the blood has been devised in which the estimation of the number of cells present is based on the determination of the turbidity of a carefully diluted sample of blood (Blum, 1945). This method was chosen for use in the present study. Blum recommends the use of Gower's solutions as the diluting fluid. He has found that the red cell suspensions prepared with it are uniform and change very slowly on standing.

The composition of Gower's solution is as follows:

Sodium sulfate	12.5 gm.
Acetic acid	33.3 ml.
Distilled water	200.0 ml.

The blood was drawn from a freely-flowing drop obtained from the tail vein of the unanesthetized rat into a 20 cm. blood pipette. The blood was transferred into a tube containing 15 ml. of Gower's solution and the pipette rinsed by sucking the liquid back and forth several times. The tube was stoppered and shaken well. The solution was transferred to an appropriate tube and the turbidity determined in the Klett-Summerson photocolorimeter. The green filter (540 m/u) was used. The samples were read within an hour after the preparation of the sample.

The turbidity measurement was translated to its equivalence in number of red cells by means of a calibration curve. In the formulation of this curve, five dilutions of several samples of rat's blood were prepared. The turbidity of each dilution was determined. The number of red cells in the original samples of blood was determined by the regular technique, and the number in each diluted sample thereby counted. The turbidity of each sample was correlated with the actual number of erythrocytes present and the reference curve drawn, as shown in Figure 1.

Blood Volume

Evans (T-1824) blue dye was used in the determination of blood volume. A calibrated tuberculin syringe fitted with a 3/4 inch, 24-gauge needle was used to inject one mg. (0.30 ml. of a 0.333 per cent solution) of the dye into the caudal vein of the tail of an anesthetized

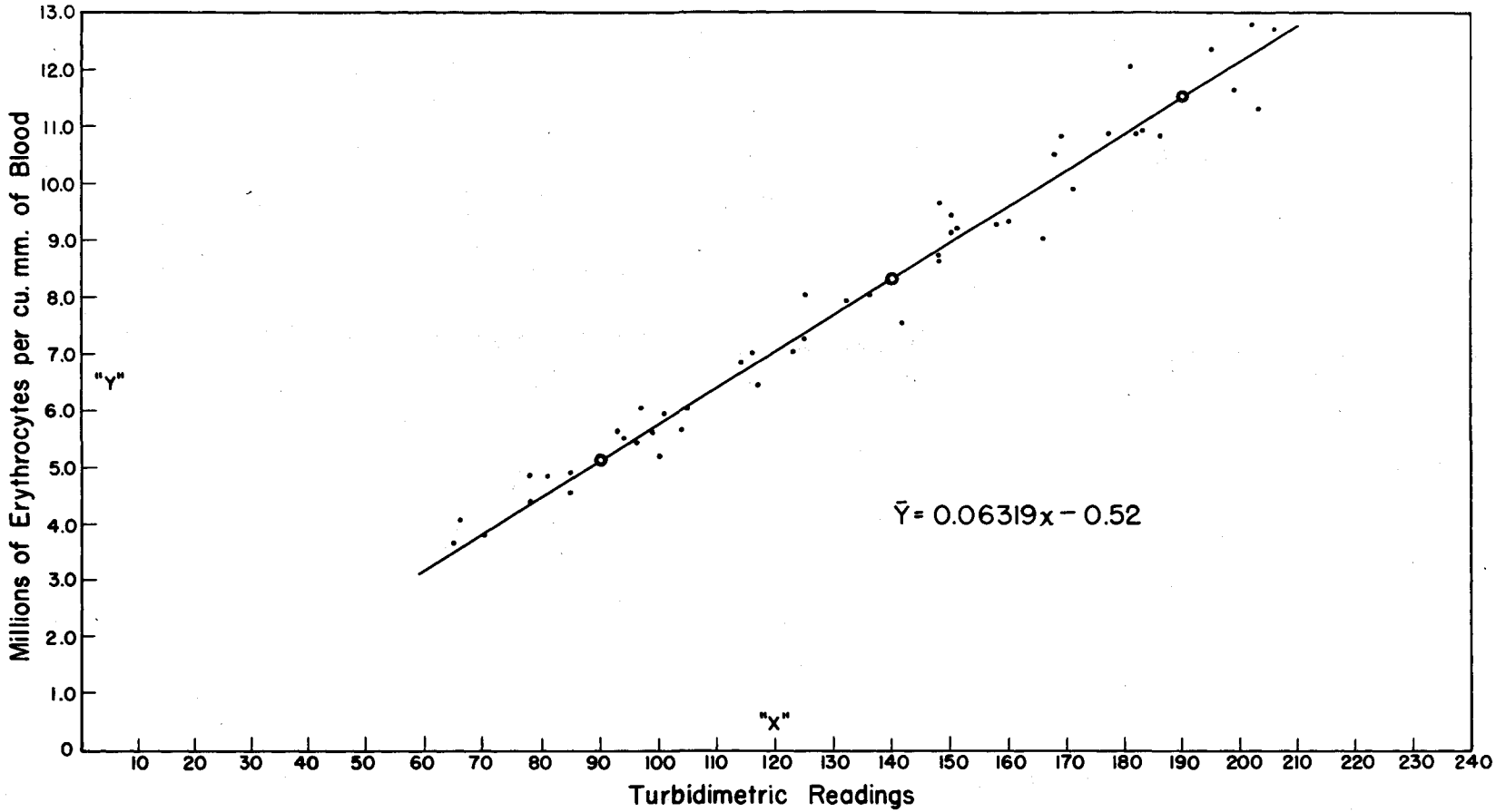


Figure 1. Regression of the number of the erythrocytes per cmm. of rat blood on turbidimetric readings.

animal. The needle was held in place for 15 seconds with slight pressure of the fingers and then withdrawn. Pressure was maintained for about one minute or until the bleeding ceased. Four minutes later the animal was again anesthetized and then one minute ± 15 seconds later the animal was bled from a different tail vein. The blood was collected directly into a 0.1 ml. blood sugar pipette. This blood sample was introduced immediately into 2.0 ml. of a special diluting fluid, mixed thoroughly, and centrifuged for 10 minutes at 1500 r.p.m. in the standard centrifuge.

The special diluting fluid was made as follows: it was composed of 2.0 gm. of purified beef plasma albumin dissolved in 100 ml. of 0.86 per cent sodium chloride solution containing 0.06 per cent of ammonium oxalate and 0.04 per cent of potassium oxalate. This solution was centrifuged for 15 minutes at 4000 r.p.m. in the standard centifuge. The decanted supernate was pale yellow and clear, and could be kept for 24 hours in the refrigerator.

A standard solution for the colorimetric comparison was prepared. Three-tenths ml. of the T-1824 solution was injected from the tuberculin syringe with needle attached, into 10.0 ml. of the albumin diluent. One-tenth ml. of this solution was then introduced into 2.0 ml. of diluent in a colorimeter tube. This and the blank, (the control blood from rats treated in the same way as the dye-stained sample) were read in the Klett-Summerson photoelectric colorimeter with a 620 m/u filter along with the unknown samples.

Concentration of Serum Protein

Blood was taken from the hearts of the rats for the estimation of the protein content of the serum. The concentration of the protein in the undiluted serum was measured with a La Motte densiometer according to the falling drop method of Barbour and Hamilton (1926).

The technique as developed by these workers has as its basis the finding that the specific gravity of plasma is almost directly proportional to its protein content, the relation between plasma protein and specific gravity being expressed as the equation of a straight line.

$$p = 343\ (G - 1.0070)$$

where p = gm. of total protein/100 ml. plasma

and G = the specific gravity.

The method depends on the fact that the specific gravity of the plasma determines the rate at which a drop of plasma will fall in a solution non-miscible with it.

In the present investigation, xBB mixture No. 3' was used as the non-miscible fluid. The falling time of a carefully measured drop of serum was compared with that of a drop of standard potassium sulfate of a known specific gravity. Standard no. 1 with a specific gravity of 1.0149 at 20°C. was used. A precision timer recorded the number of seconds elapsing as the drop fell between two gradations on the tube containing the non-miscible fluid, and a thermometer revealed the temperature of the waterbath surrounding the fluid. The apparent density difference between the standard, or the serum, and the non-

miscible liquid was calculated from the falling time of the drop and the temperature of the water-bath by means of an alignment chart. From the apparent density difference thus measured, the true density difference between the standard and the serum was computed, the apparent density difference of the standard being subtracted algebraically from that of the serum. The addition of the specific gravity of the standard to the true density difference between serum and standard yielded the density of the serum. The protein content per 100 ml. serum was then calculated from the formula mentioned above.

The falling time of at least two drops from each sample was observed and the average time recorded. Determinations were repeated until the falling time of two drops of serum checked within 0.2 seconds. Care was taken to exclude air bubbles from the special pipette used for measuring the fluid.

Alcohol-ether Soluble Substances

The estimation of the concentration of alcohol-ether soluble substances in liver was based on the method developed by Bloor in 1929. Moisture-free samples were ground in a mortar with one-half teaspoon of acid-washed sand and transferred quantitatively to 125 ml. Erlenmeyer flasks, mortar and weighing bottle being washed with three 1-ml. portions of absolute ethyl alcohol. Approximately 35 ml. of a 3:1 mixture of absolute ethyl alcohol and anhydrous ether were added to the ground samples. The mixture was heated to boiling on a steam bath, and boiled for five minutes with continuous shaking.

The solution was cooled at room temperature. Then it was filtered quantitatively through Whatman no. 45 fat-free filter paper into a 200 ml. volumetric flask. The filter paper was washed with small portions of the alcohol-ether mixture, wrapped with its residue in two additional filter papers, and extracted for 5 hours with anhydrous ethyl ether in a Soxhlet apparatus.

The ether extract was filtered into the 200 ml. volumetric flask containing the first filtrate. The Soxhlet flask was washed quantitatively thrice with small portions of ether. The solution was then made to volume with anhydrous ethyl-ether. From this solution, triplicate 50 ml. aliquots were measured into large weighing bottles, evaporated to dryness on a steam-bath at a temperature of 80 to 90 degrees, and brought to constant weight in a vacuum oven held at 80 degrees under 30 pounds pressure.

RESULTS

This thesis is particularly concerned with a comparison of the manner in which standardized under-nourished and well-nourished rats utilize the protein of their ration when it is incorporated therein in quantities ranging from approximately 1.5 to 25 per cent of the ration by weight. Response has been evaluated in terms of changes in body weight induced by feeding the test rations, by retention of dietary nitrogen, by the blood picture, and by the size and composition of certain organs, particularly the liver.

BODY WEIGHT

The balance test in the experiment was seven days long. The under-nourished rats reached this experimental interval with body stores of protein more or less depleted after living on the nitrogen-free diet for 21 days; the well-nourished with theoretically normal stores depleted only by a 24-hour starvation period. For example, analysis in later sections will show that the total nitrogen in liver per 300 gm. rat in the well-nourished group was 319 mg. in contrast to 218 mg. in the negative control group that had lived on the nitrogen-low diet for 34 days. The grams per cent of protein in the serum in the blood of the two groups were 7.2 and 6.7 respectively.

Analysis of changes in average body weights during the balance test of the animals in the various subgroups in each series gives the first clue in respect to the way under-nourished and well-nourished animals utilize the supply of egg protein in their diets. In Tables 6 and 7 are shown the data describing the average response of the animals in each series to graded protein intakes. It may be recalled that food consumption was held at as constant a level as possible in the various experimental units.

In the under-nourished group, 1.5 per cent of dietary protein apparently was not quite enough to avert the loss in body weight; 2.6 per cent stopped catabolism and permitted maintenance of body weight.

Table 6. Average body weights at various experimental intervals of rats in Series A, the undernourished animals

Number of rats	Group	Protein in diet	Weight of food consumed	At beginning of experiment	At end of depletion period	At end of balance period	Change in wt. in balance period
		%	gm.	gm.	gm.	gm.	gm.
18	A-1	1.5	70.5	307	257	250	-7
6	A-2	2.6	76.4	299	248	248	0
16	A-3	3.0	77.7	302	251	253	+2
6	A-4	3.6	70.6	291	242	245	+3
6	A-5	4.6	73.8	275	239	242	+3
12	A-6	6.7	73.8	302	257	270	+13
11	A-7	8.6	76.8	307	261	281	+20
19	A-8	10.3	71.1	307	262	287	+25
19	A-9	12.3	78.8	311	260	280	+20
15	A-10	14.8	76.9	312	266	291	+25
19	A-11	18.0	75.3	318	269	303	+34
15	A-12	25.7	78.4	302	256	270	+14

Table 7. Average body weights at various experimental intervals of rats in Series B and C, the well nourished and the control animals

Number of rats	Group	Protein in diet	Weight of food consumed	At beginning of experiment	After 24 hours of starvation	At end of balance period	Change in wt. in balance period
		%	gm.	gm.	gm.	gm.	gm.
10	B-1	1.6	82.2	318	305	274	-31
10	B-2	3.2	85.3	347	328	314	-14
10	B-3	3.5	88.2	329	329	308	-21
10	B-4	6.9	81.7	321	313	328	+15
10	B-5	8.3	84.8	325	315	330	+15
10	B-6	10.0	84.6	355	334	343	+ 9
10	B-7	12.1	84.0	322	303	325	+22
10	B-8	18.2	80.9	324	315	328	+13
11	B-9	25.4	79.4	308	297	305	+ 8
21	C-1 Steenbock XVII:	23.6	94.4	339	330	328	- 2
15	C-2 Nitrogen-low:	0.7	82.8	364	356	291	-65

Diets containing protein ranging from 3.0 to 4.6 per cent of the ration caused slight gains in the average weights of the groups. Thereafter, additional dietary protein was reflected in very good gains during the 7-day period, the maximum increment being made when the diet contained 18 per cent of egg protein. However, gains resulting from the ingestion of diets containing 8.6 to 18 per cent of protein were of nearly the same order. It would appear that the nitrogen needs for maintenance of body weight after partial depletion of body stores of nitrogen are met at the lower levels of this range in protein intake.

It is interesting, however, to note that gains dropped from 34 to 14 gm. when the protein in the diet was increased from 18 to 25.7 per cent.

The picture was different when well-nourished rats received graded doses of dietary protein. Reference to Table 7 shows that the rat placed on a diet containing much less protein (1.6, 3.2, and 3.5 per cent) than it had been accustomed to receiving apparently squandered his body nitrogen. Metabolism went on at the same rate as when the ration provided adequate amounts of protein. As a result, body tissue was broken down. Other experiments in the laboratory have demonstrated that after a time, the animal will adjust to a low protein intake of this kind and will not expend his body tissue as lavishly as it did before. As a result, losses in weight either will stop or will be curtailed. However, in the present experiment, 6.9 per cent of protein in the diet seemed to prevent such catabolism and more than made good the losses

sustained in the previous 24-hour-starvation period. Gains in general thereafter were somewhat less than those made by the animals partially depleted of bodily stores of nitrogen. The lesser need for dietary protein by the well-nourished animals is indicated in this analysis.

The inclusion of egg proteins in the ration seemed to lead to somewhat larger increments in body weight in the 7-day test period than did continued feeding of the Steenbock XVII diet.

NITROGEN BALANCE

The two series of animals, the undernourished and the well nourished, utilized the nitrogen furnished in their rations differently. Nitrogen balance curves showed nitrogen retention at two distinct levels. The experimental data are shown in Tables 8 and 9; the nitrogen balance curves in Figure 2.

The under-nourished animal seemed to retain nitrogen in direct proportion to the quantity present in its diet until the ration contained 6.7 per cent of egg protein by weight (intake of nitrogen in the 7-day period, 775 mg.). Then the rate of retention dropped. When the ration provided 3181 mg. of nitrogen (25.7 per cent of protein), 820 mg. were retained by these animals on the average. This was about 189 mg. more than the well nourished rats retained at approximately the same level of protein intake. This difference in favor of the under-nourished rats showed up when all experimental diets were fed.

It is also very interesting that the rats whose tissues had been depleted partially of their body stores of nitrogen attained nitrogen equilibrium at an intake of nitrogen considerably lower than that required by the well-nourished rats, i.e., 325 vs. 575 mg. of nitrogen. The author believes that this shows how eagerly the body utilizes dietary nitrogen for repletion of body stores. Again, the well nourished rats had not learned how to be economical of their tissue stores when food nitrogen is limited and they metabolize nitrogen quite lavishly.

Table 8. Nitrogen balances of rats fed varying amounts of egg protein in Series A, the under-nourished rats

Number of rats	Protein in diet	Group	Av. body weight	Wt. of food consumed	Total nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance per 300 gm. body weight
	%		gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
6	1.5	A-1	250	70.5	163	142	208	350	-187	-224.4
6	2.6	A-2	248	76.4	298	172	299	471	-173	-209.1
6	3.0	A-3	255	77.7	414	209	302	511	- 97	-114.0
6	3.6	A-4	245	70.6	385	164	209	373	+ 12	+ 14.7
6	4.6	A-5	242	73.8	521	167	208	375	+146	+180.9
6	6.7	A-6	268	73.8	773	208	298	506	+267	+298.5
5	8.6	A-7	268	76.8	922	189	341	530	+392	+438.5
5	10.3	A-8	262	71.1	990	145	383	528	+462	+528.6
11	12.3	A-9	284	78.8	1410	229	630	859	+551	+582.0
11	14.8	A-10	278	76.9	1666	285	841	1126	+540	+583.8
11	18.0	A-11	266	75.3	2148	255	1116	1371	+777	+876.3
6	25.7	A-12	266	78.4	3181	396	1965	2361	+820	+924.6

Table 9. Nitrogen balances of rats fed varying amounts of egg protein in Series B and C, the well-nourished and control rats

Number of rats	Protein in diet	Group	Av. body weight	Wt. of food consumed	Total nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance per 300 gm. body weight
	%		gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
10	1.6	B-1	274	82.2	227	190	257	447	-219	-240
10	3.2	B-2	314	85.3	450	315	301	512	- 61	- 58.5
10	5.5	B-3	308	88.2	510	218	275	493	+ 18	+ 17.1
10	6.9	B-4	328	81.7	918	260	572	821	+ 97	+ 89.1
10	8.3	B-5	330	84.8	1147	274	726	999	+149	+135.0
10	10.0	B-6	343	84.6	1353	279	898	1176	+177	+155.1
10	12.1	B-7	325	84.0	1641	271	1005	1277	+368	+339.6
10	18.2	B-8	328	80.9	2378	328	1610	1938	+440	+402.3
11	25.0	B-9	305	79.4	3239	354	2254	2608	+631	+621.0
21	Steenbock XVII: 23.6	C-1	293	94.4	4152	643	3118	3761	390	+399.9
15	Nitrogen low: 0.7	C-2	295	82.8	79	212	292	505	-425	-432.4

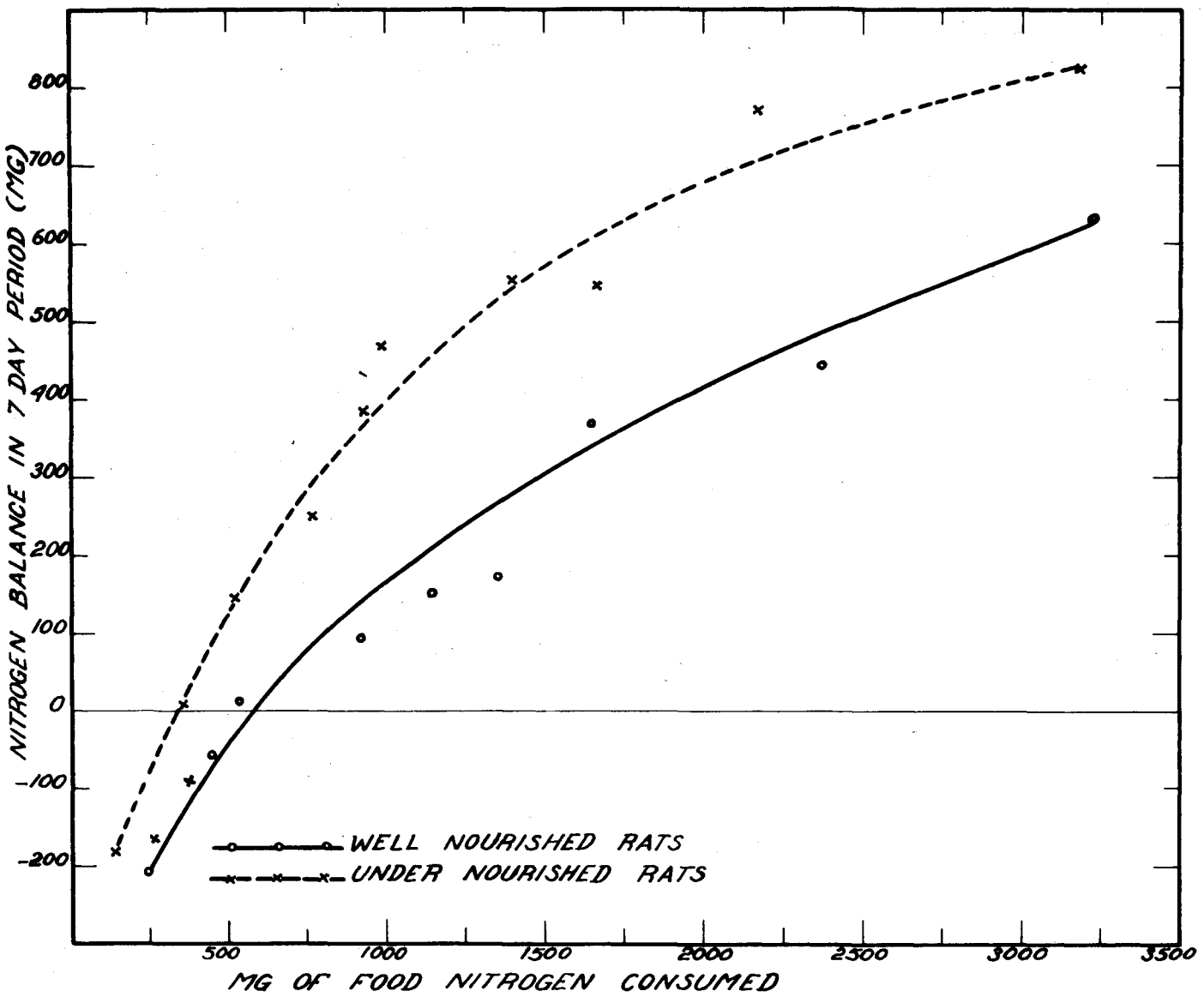


Figure 2. Nitrogen balance curve of rats fed varying quantities of nitrogen in their diets.

They, therefore, required more food nitrogen than the depleted animals to establish nitrogen equilibrium.

It is interesting to speculate on the reason for the differences observed in the retention of nitrogen by the two groups of rats.

Comparison of the data relating to the excretion of nitrogen in the feces in Tables 8 and 9 showed that the under-nourished rats excreted considerably less N in their feces than did the well nourished rats. For example, when the diets contained about 6.7 per cent of protein, the normal rats excreted 259 mg., the under-nourished 208 mg. When diet contained about 10.2 per cent of protein, the respective figures were 278 mg. and 145 mg. It may be that the situation in protein metabolism is similar to that in iron metabolism. Cantarow and Trumper (1945) write:

> There is evidence that, in experimental animals at least, the normal organism absorbs iron only in proportion to its needs, the quantity absorbed being determined by the magnitude of the body reserves of this element. It would appear that the intestinal mucosa is the tissue responsible for its acceptance or rejection, being perhaps conditioned by the iron content of the blood, anemic animals absorbing and utilizing iron very efficiently.

Then also it should be recalled that as early as 1943, Marshall, working in this laboratory, showed that the inclusion of small quantities of egg proteins in the diets of depleted rats were body-sparing and reduced the quantity of urinary nitrogen to two-thirds that excreted in the previous balance period when the rats lived on a protein-free ration. Therefore, the quantities of nitrogen excreted by the various

groups fed the graded doses of protein in each series were plotted against the quantities of nitrogen absorbed (Figure 3).

In order to secure data in respect to the quantity of food nitrogen absorbed by the well nourished rats, an estimation of metabolic fecal nitrogen was obtained by extrapolating to the zero point the curve showing the relation of the quantity of fecal nitrogen excreted to the quantity of food absorbed (Figure 4).

It may be seen again that the well nourished rats expended nitrogen much more freely than the under-nourished as judged by urinary excretion of the element. Also, over a certain interval the under-nourished rats excreted nitrogen in nearly constant quantities even though the protein in the diet was increased.

The data make possible a comparison of the relative depressing power of egg proteins on the excretion of urinary nitrogen when fed to rats representing different states of nutrition. They are recorded in Table 10. In this analysis, data regarding the quantity of nitrogen the well-nourished rat would have excreted had it lived on the nitrogen-low ration for a period equivalent to the time (34 days) the other groups of rats received the egg diets were obtainable from Table 9, i.e., 292 mg. Also data were obtainable as to the nitrogen excretion of the depleted rats just prior to repletion.

Inspection of the data in Table 10 show that when the protein in the diets fed the under-nourished rats varied from 1.5 to 6.8 per cent, the excretion of nitrogen in the urine was depressed in every

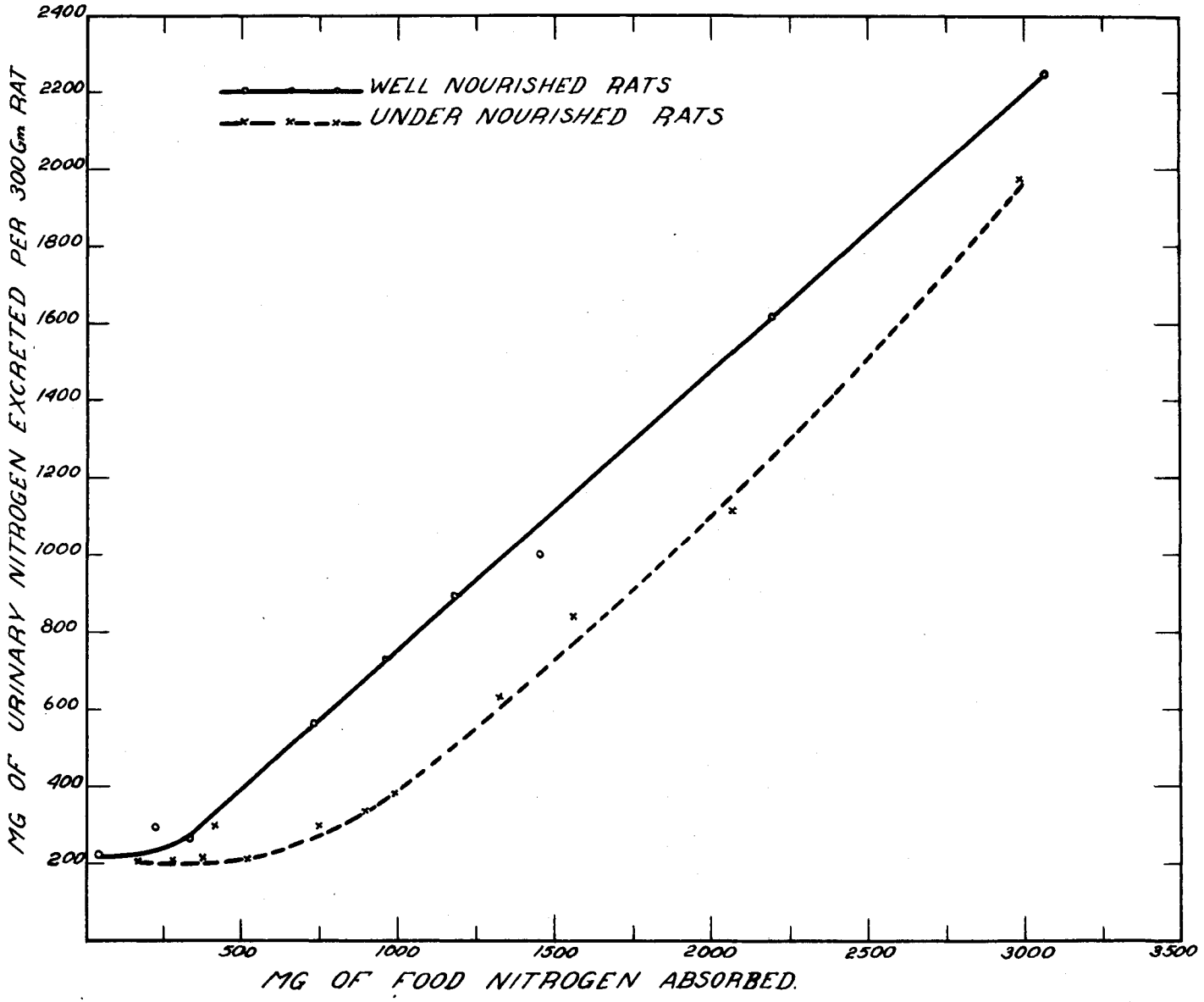


Figure 3. Ratio of quantity of nitrogen excreted in the urine of rats to the quantity of food nitrogen absorbed.

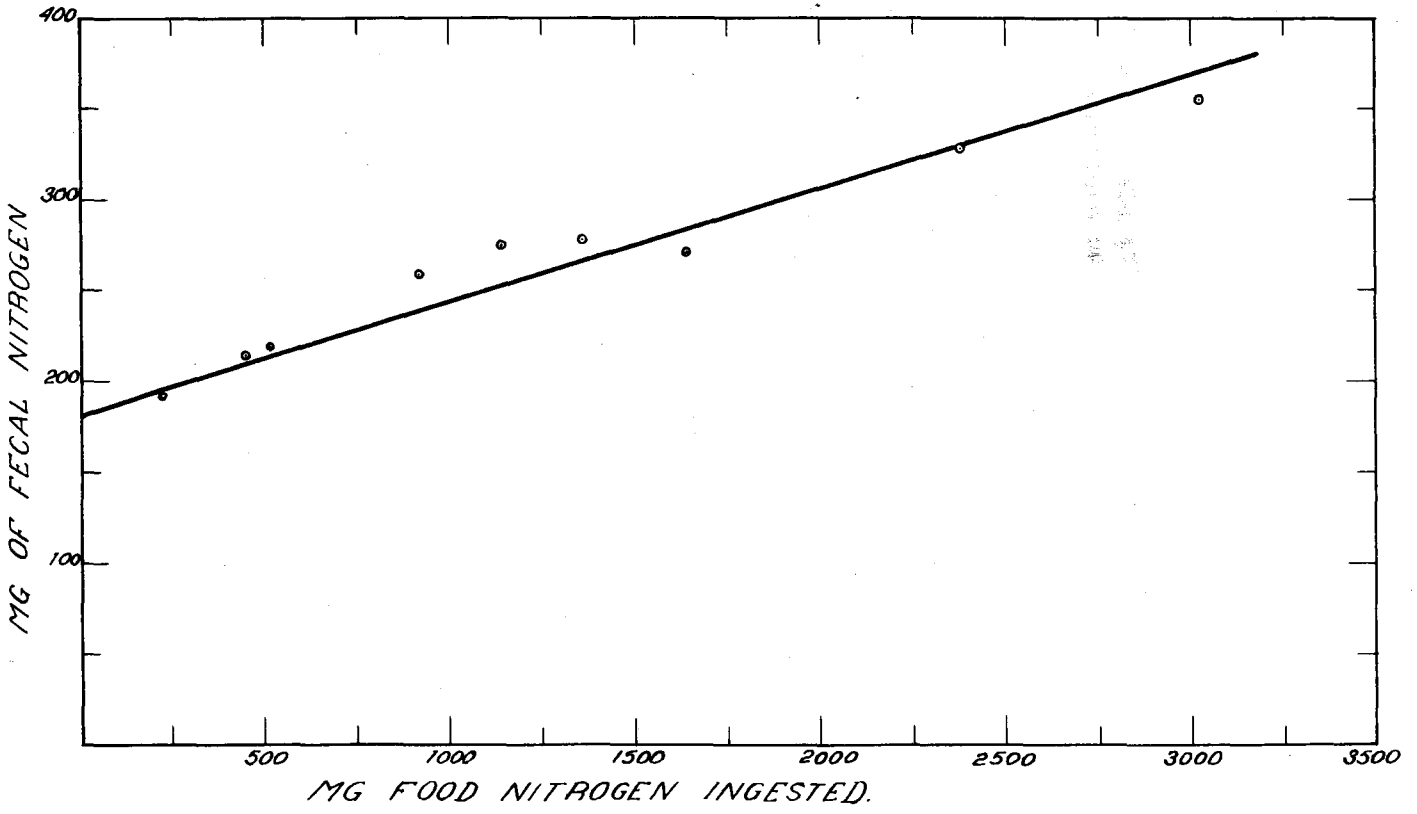


Figure 4. Relationship between quantity of fecal nitrogen excreted by well nourished rats and quantity of food nitrogen ingested (Bosshardt and Barnes 1946).

Table 10. Excretion of nitrogen in urine by undernourished and well nourished rats

Approximate quantity of protein in diet	Undernourished rats		Well nourished rats		Change in urine nitrogen when proteins are added to the diet	
	Urine N at 0% intake of protein	Urine N with protein in diet	Urine N at 0% intake of protein	Urine N with protein in diet	Undernourished rats	Well nourished rats
%	mg.	mg.	mg.	mg.	mg.	mg.
1.5	273	208	292	257	- 69	- 35
2.6	295	204	-	-	- 91	-
3.0	323	302	-	-	- 21	-
3.2	-	-	292	301	-	+ 9
3.5	272	209	292	275	- 63	- 17
4.6	275	208	-	-	- 67	-
6.8	329	298	292	572	- 31	+ 280
8.6	275	341	292	726	+ 65	+ 434
10.3	273	383	292	898	+ 100	+ 606
12.2	314	630	292	1005	+ 316	+ 813
15.0	319	842	-	-	+ 523	-
18.0	282	1116	292	1610	+ 834	+1318
25.5	305	1965	292	2254	+1660	+2162

instance when compared to the excretion by the rats in the previous period when they were given the protein-free diet. These data confirm Marshall's findings as well as those of other investigators in the laboratory.

The ability of egg proteins to spare body tissues was not as evident when the animals were well nourished, although the trend was there at the lower levels of protein intake. Actually, the addition of these amounts of egg proteins to the diets of the well nourished rats changed the excretion only a very little until the diet contained 6.8 per cent of protein. Then the quantity of urinary nitrogen excreted jumped to 572 mg.; in contrast, the undernourished rats were only excreting 298 mg. of urinary nitrogen. Thereafter with each increment in the quantity of protein present in the diet, a large proportion of the nitrogen ingested was excreted. When the undernourished rats were absorbing 990 mg. of nitrogen, they excreted 383 mg., or nearly 40 per cent. On the other hand, the well nourished animals excreted 898 mg. of the 1173 mg. absorbed, or nearly 80 per cent. When the diet contained 18 per cent of protein, the respective percentage values were about 50 and 80.

The picture in general depicts the physiological adjustments animals make to rations of varying protein value and how state of nutrition determines the use an animal will make of its dietary protein. It either picks it up and deposits it in the body or throws it away as waste material, depending on whether it is undernourished or well nourished in respect to protein. It must be kept in mind that response will vary according to the nutritional value of the protein fed.

CHANGES IN ORGANS

The question as to whether nitrogen equilibrium represents an adequate state of nutrition interested the author very much. Does it measure optimal stores of nitrogen in the liver and the blood? In the next section, the quantity of nitrogen deposited as stores of labile liver nitrogen was studied in the animals making the two experimental series.

Liver

The weights and the respective moisture contents of the livers of the rats in the various experimental groups are shown in Tables 11 and 12.

The average moisture contents of the organ in the animals showed some variation between experimental groups and between series. Reducing the protein in the diets of the well nourished rat both in respect to kind and quantity, caused an increase in the amount of water present in the liver (71.1 per cent in the rats fed 1.5 per cent of egg protein; 68.8 per cent in the normal controls fed Steenbock XVII diet). Increasing the amount of protein in the ration induced dehydration so that rats fed diets containing 8.3 per cent or more of protein seemed to have livers of normal water content.

Feeding egg protein to the undernourished rats seemed conducive to the maintenance of a high moisture content of the liver. The

Table 11. Average weights of fresh, moisture-free, and water free-fat free liver in rats of Series A, the under-nourished animals, expressed in terms of a 300-gram rat

Number of rats	Diet	Group	Fresh liver	Moisture-free liver	Moisture free-fat free liver	Total moisture	Moisture
			gm.	gm.	gm.	gm.	%
8	1.5	A-1	7.6	2.3	1.70	5.4	71.0
4	2.6	A-2	7.5	2.2	1.710	5.3	71.3
77	3.0	A-3	7.3	2.1	1.63	5.2	71.9
5	3.6	A-4	8.7	2.5	1.97	6.2	71.3
5	4.6	A-5	8.4	2.4	1.94	6.0	70.6
7	6.7	A-6	7.6	2.3	1.84	5.3	70.1
6	8.6	A-7	7.4	2.3	1.98	5.1	70.3
7	10.3	A-8	7.9	2.9	2.47	5.1	71.0
6	12.3	A-9	10.2	2.8	2.40	7.4	72.3
8	14.8	A-10	8.9	2.6	2.25	6.3	70.8
8	18.0	A-11	8.9	2.5	2.20	5.6	71.0
8	25.7	A-12	7.9	2.3	1.90	5.6	71.0

Table 12. Average weights of fresh, moisture-free and moisture free-fat free livers in rats of series B and C, the well nourished and control animals, expressed in terms of a 300-gram rat

Number of rats	Diet	Group	Fresh liver	Moisture-free liver	Moisture free-fat free liver	Total moisture	Moisture
			gm.	gm.	gm.	gm.	%
10	1.5	B-1	7.9	2.5	2.00	5.4	71.1
10	3.2	B-2	7.7	2.3	1.78	5.4	69.7
10	3.5	B-3	7.5	2.1	1.72	5.4	70.8
10	6.9	B-4	7.4	2.3	1.84	5.1	69.6
10	8.3	B-5	7.2	2.1	1.62	5.1	68.7
10	10.0	B-6	7.4	2.3	1.80	5.1	68.7
10	12.1	B-7	7.5	2.4	1.90	5.1	68.7
10	18.2	B-8	7.3	2.3	1.87	5.0	68.9
11	25.4	B-9	7.5	2.3	1.91	5.2	68.8
21	Steenbock XVII	C-1	8.0	2.6	2.20	5.4	68.8
15	Nitrogen-low	C-2	7.3	2.2	1.65	5.1	69.7

average value for all groups was 71.5 per cent. The values for the individual groups in the series fluctuated around this value. It is possible that continued maintenance on the various egg diets would lead to a reduction in the moisture contents of the livers of the rats in this series.

The livers of the undernourished animals fed the various protein diets increased in size as the quantity of nitrogen in the ration increased. The dry fat-free tissue weighed on the average 1.65 gm. in the negative control rat; in the positive control 2.20 gm. When the diet fed the depleted rats contained 1.5 per cent of protein, the tissue weighed 1.7 gm. The data showed a definite trend toward an increase in weight with the dietary increments of protein so that a normal weight (2.5 gm.) was characteristic when the diet contained 18 per cent of protein.

On the other hand, for the most part the dry fat-free weights of the organ in the rats of the various groups of well nourished rats were close to 1.80 gm. in weight. The livers of these groups, therefore, were small in relation to those of the Steenbock XVII controls. The data make one wonder whether smaller stores of hepatic nitrogen suffice when the dietary protein is of high value. Also the data may indicate that building hepatic tissue is not a physiological necessity in these rats.

The balance curves in Figure 2 showed that retentions of nitrogen were much higher in the undernourished than in the well nourished groups.

That these higher retentions were due in part to increments in liver size are indicated in Figure 5. The need of the depleted animal to rebuild its hepatic tissue is clearcut; the lack of need in the well nourished rat is just as evident.

Whether or not this increment in liver size is due to actual deposition of nitrogen was explored next. It may be seen in Figure 6 that the quantity of nitrogen in the liver increased in both groups as the nitrogen balance increased. The change was more marked in the undernourished than in the well nourished rats. The data in the figure that show values translated in terms of a 300 gram rat probably represent a better picture than when the data are expressed as mg. of total nitrogen. This figure shows the concentration of nitrogen in the livers of the well nourished rats plateaued as nitrogen balances increased. Then further enrichment of the diet resulted in simultaneous increments in nitrogen content of the livers and nitrogen balance. It seems as though the Steenbock-fed rat was able to improve its hepatic stores when ample egg protein was present in the ration.

Thus, nitrogen equilibrium in the undernourished rat does not represent optimal tissue storage of the element. It appeared that dietary protein that will assure a balance of about 300 mg. of nitrogen was necessary to provide maximal hepatic stores. To do this, a diet containing 6.8 per cent of protein was required. It should be recalled that it was at this point also that egg proteins no longer exerted body sparing action (Figure 3) as shown by urinary excretion of nitrogen.

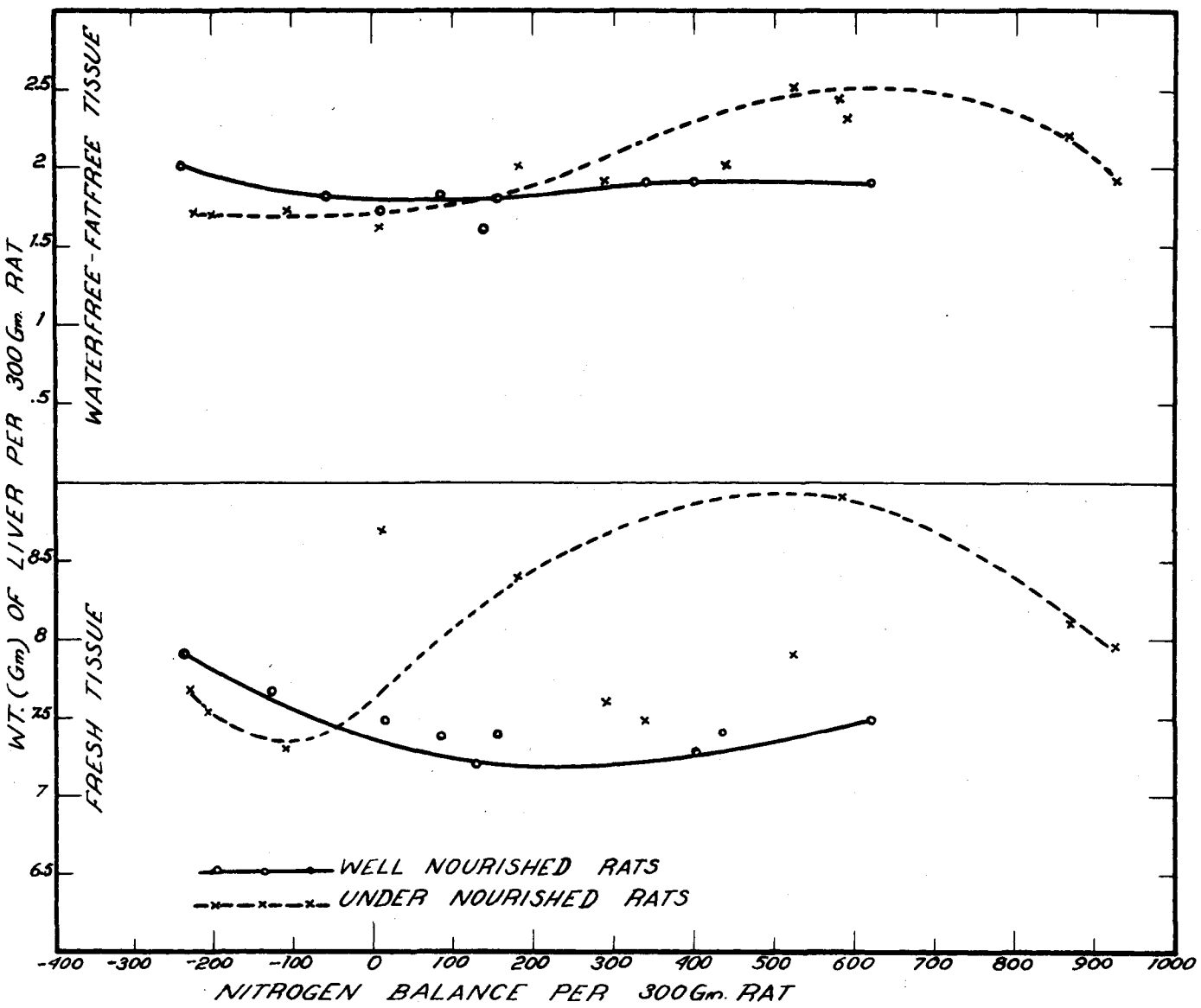


Figure 5. Relationship between weight of livers of rats and the quantity of food nitrogen retained.

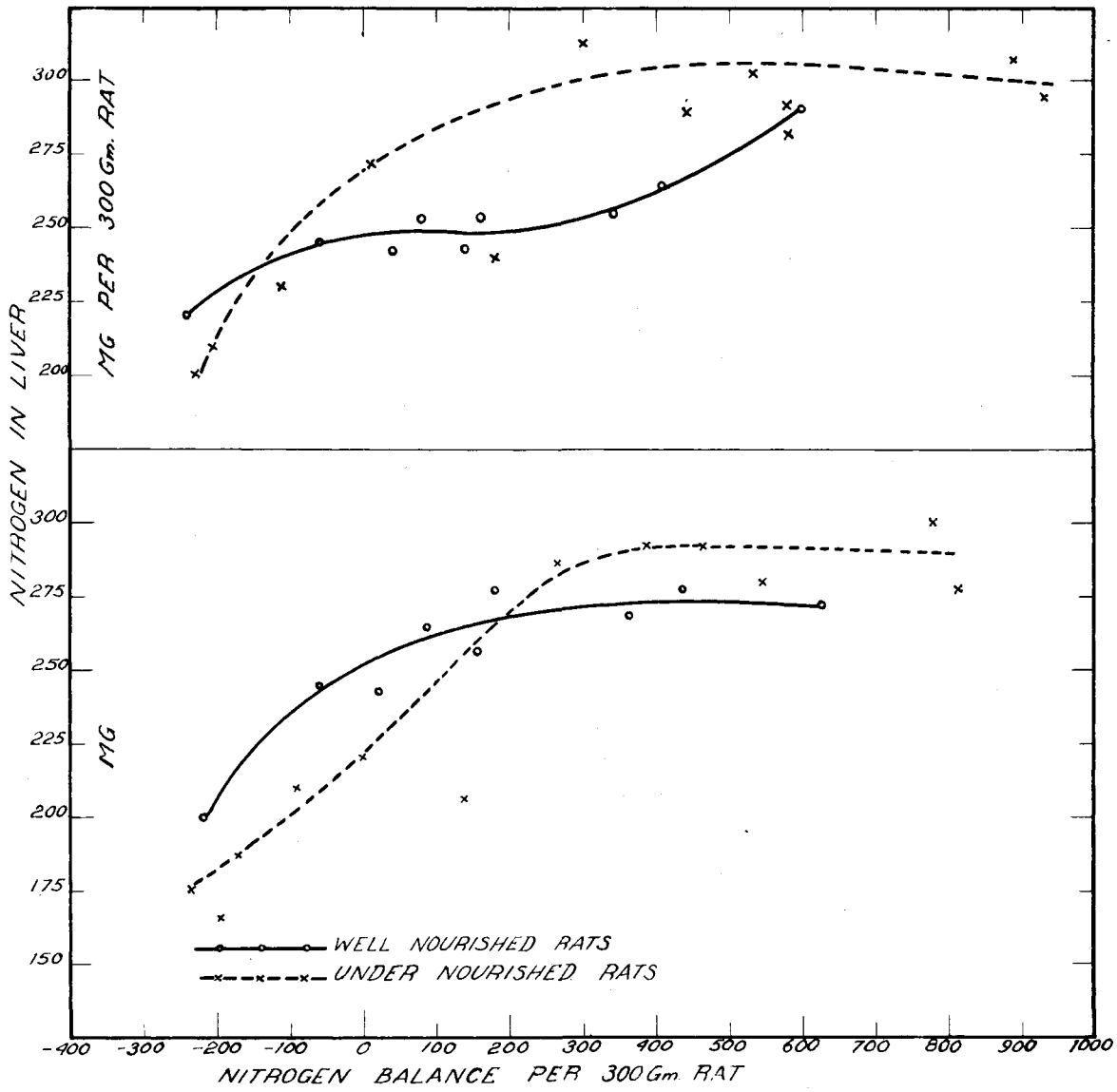


Figure 6. Relationships between the quantity of nitrogen deposited in livers of rats and the quantity of food nitrogen retained.

In the well nourished rats, balances of over 400 mg. per seven days were necessary in order to increase the content of nitrogen that was characteristic of the livers of rats in nitrogen equilibrium.

The relation of protein metabolism and fat metabolism have been mentioned previously. The beneficial effects of the inclusion of fat in low-calorie diets has been discussed.

As the nitrogen in the diet was increased in the present experiment, the livers in the groups of rats in each series showed less fat (Tables 13 and 14). Increasing the quantity of protein in the diets given the rats in each series eventually brought the quantity of hepatic fat to about the same value as that in the normal control rat. The depleted rat (negative control) had more fat in its liver than did the rats in any other group.

The ratios of fat to liver nitrogen were nearly constant in all groups except the last in the well-nourished series. In the undernourished, the ratios decreased as nitrogen was deposited in the liver.

The relation of the quantity of fat in the liver to the quantity of nitrogen retained are shown in Figure 7. A rather abrupt decrease in liver fat is associated with more favorable nitrogen balances in both series. The figure shows that by decreasing the dietary protein and thereby reducing nitrogen retention, livers decidedly richer in fat were produced, even though the fat intake had not been varied. Protein seemed directly involved in controlling the deposition of fat in the liver. Perhaps here is another experimental finding supporting Swanson's theory (1947) that the metabolism of fat and of protein are related intimately.

Table 13. Average quantities of fat and nitrogen in livers of rats in series A, the undernourished animals

Number of rats	Protein in diet	Group	Total fat	Total fat per 300 gm. body wt.	Total nitrogen	Total nitrogen per 300 gm. body wt.	Ratio: total fat to total nitrogen
			mg.	mg.	mg.		
8	1.5	A-1	430	504	167	198	2.5
4	2.6	A-2	425	490	189	218	2.2
7	3.0	A-3	399	474	211	251	2.1
5	3.6	A-4	431	534	220	273	1.9
5	4.6	A-5	383	458	206	246	1.9
7	6.7	A-6	421	460	286	314	1.5
6	8.6	A-7	317	316	291	290	1.1
7	10.3	A-8	410	430	290	303	1.4
6	12.3	A-9	391	399	277	282	1.4
8	14.8	A-10	334	349	285	291	1.2
8	18.0	A-11	343	355	304	308	1.1
8	25.7	A-12	382	405	277	294	1.4

Table 14. Average quantities of fat and nitrogen in livers of rats in series B and C, the well nourished and the control animals

Number of rats	Protein in diet	Group	Total fat	Total fat per 300 gm. body wt.	Total nitrogen	Total nitrogen per 300 gm. body wt.	Ratio: total fat to total nitrogen
			mg.	mg.	mg.	mg.	
10	1.6	B-1	442	500	201	222	2.0
10	3.2	B-2	526	519	248	246	2.1
10	3.5	B-3	486	480	263	262	2.0
9	6.9	B-4	503	456	265	254	1.8
10	8.3	B-5	501	483	258	244	2.0
9	10.0	B-6	564	501	279	253	2.0
10	12.1	B-7	524	498	267	253	2.0
10	18.2	B-8	463	432	276	259	1.7
11	25.4	B-9	385	390	271	291	1.3
20	Steenbock XVII	C-1	453	399	342	319	1.3
13	Nitrogen-low	C-2	510	543	206	218	2.5

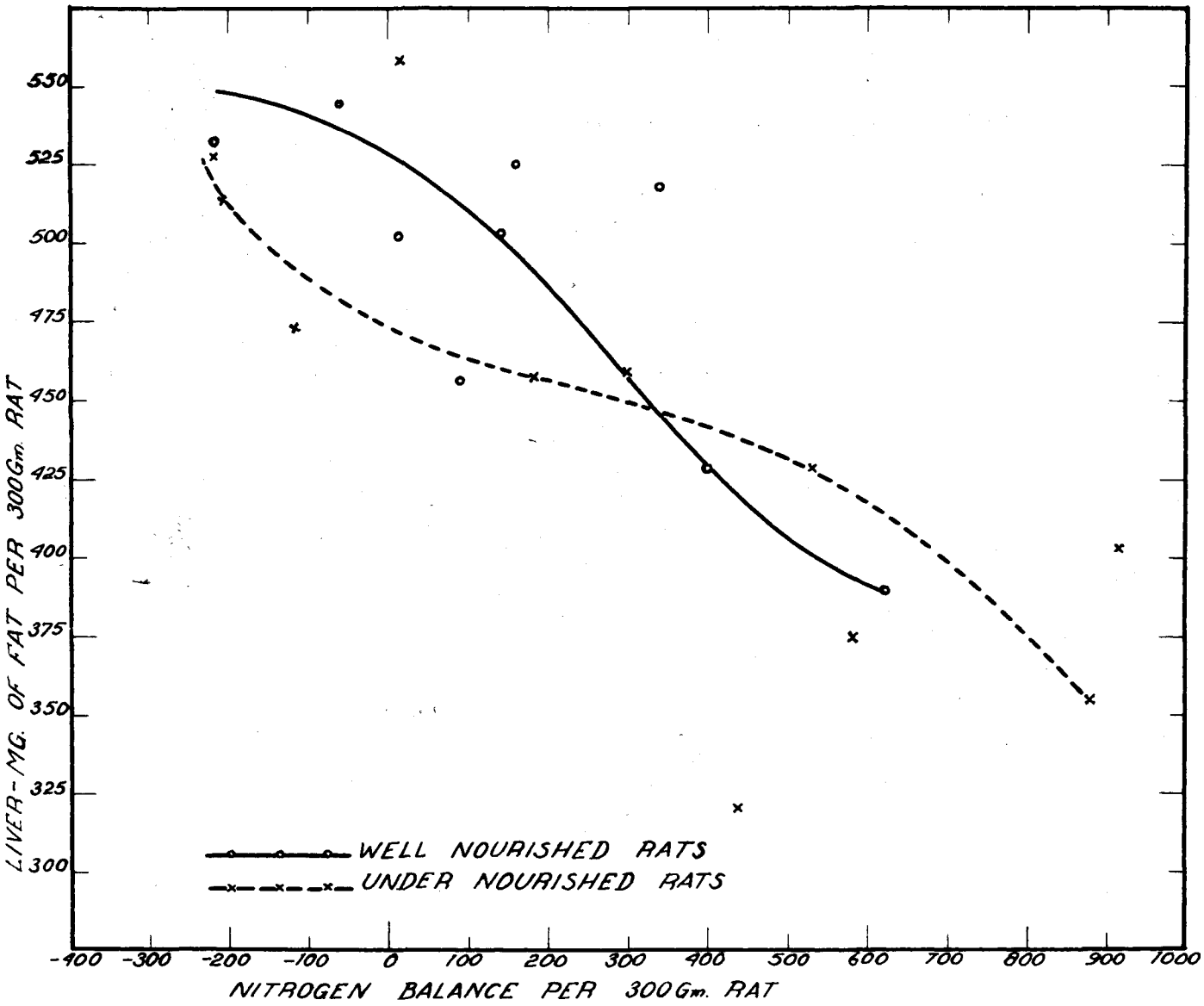


Figure 7. Relationship between quantity of fat in the livers of rats and quantity of food nitrogen retained.

Adrenal Glands

The direct relation of the intensity of nitrogen metabolism to the size of the adrenal gland has been discussed by Long (1942).

The average size of the gland in the rats of the normal control series (C-1) was 43.5 mg. per 300 gm. rat; that of the severely depleted negative control rat, 33.6 mg. The data recorded in Table 15 show that the adrenal gland increased in weight as the protein content of the diets increased. The data were very comparable in both series.

Table 15. Average weights of the adrenal glands of rats comprising the three experimental series

Series	Number of rats	Protein in diet %	Group	Weight of adrenal glands mg.	Weight of adrenal glands per 300 gm. body weight mg.
Under-nourished rats	5	1.5	A-1	32.3	36.0
	-	2.6	A-2	-	-
	5	3.0	A-3	30.5	36.9
	-	3.6	A-4	-	-
	-	4.6	A-5	-	-
	5	6.7	A-6	34.4	38.4
	6	8.6	A-7	37.6	36.9
	4	10.3	A-8	37.6	40.2
	9	12.3	A-9	44.4	42.6
	6	14.8	A-10	39.0	39.9
	5	18.0	A-11	42.7	42.9
	6	25.7	A-12	41.7	46.5
Well nourished	10	1.5	B-1	31.8	36.0
	10	3.2	B-2	35.2	35.7
	10	3.5	B-3	36.7	36.3
	9	6.9	B-4	38.9	36.6
	9	8.3	B-5	41.3	39.0
	10	10.0	B-6	44.0	39.9
	10	12.1	B-7	41.7	39.6
	10	18.2	B-8	43.8	41.1
	11	25.4	B-9	42.2	47.4
Control rats	20	Steenbock XVII	C-1	46.6	43.5
	8	Nitrogen-low	C-2	30.9	33.6

Table 16. Characteristics of blood in rats of series A, the undernourished animals

Diet	Group	Av. body weight	Serum protein concentration	Blood volume		Hemoglobin			Erythrocytes	
				Total	Total/ 300 gm.	Relative concen-	Total amt.	Total amt./ 300 gm. body wt.	Millions/ cmm.	Millions x 10^4
		gm.	gm. %	ml.	ml.	gm. %	gm.	gm.		
1.5	A-1	247	6.8	17.8	21.5	15.3	2.8	3.3	8.1	14.4
2.6	A-2	260	6.7	-	-	16.3	-	-	-	-
3.0	A-3	260	6.8	22.0	22.5	15.1	3.3	3.6	8.2	18.0
3.6	A-4	242	6.6	-	-	16.1	-	-	-	-
4.6	A-5	251	7.1	-	-	16.3	-	-	-	-
6.7	A-6	279	7.8	17.5	19.5	15.6	2.9	3.0	-	-
8.6	A-7	283	7.5	19.5	19.7	15.7	2.7	2.8	-	-
10.3	A-8	301	8.2	21.6	20.7	15.4	3.1	3.2	8.4	17.2
12.3	A-9	293	8.3	17.8	18.6	15.9	2.6	2.8	9.7	17.3
14.8	A-10	305	7.3	19.0	20.1	15.6	3.0	3.0	9.0	17.1
18.0	A-11	285	7.2	18.3	18.4	16.0	2.8	2.9	9.7	17.8
25.7	A-12	286	7.2	15.7	16.8	15.9	2.6	2.7	8.4	13.2

Table 17. Characteristic of blood in rats of Series B and C, the well nourished and control animals

Diet	Group	Av. body weight	Serum protein concentration	Blood volume		Hemoglobin			Erythrocytes	
				Total	Total/ 300 gm.	Relative concentration	Total amt.	Total amt./ 300 gm. body wt.	Millions/ cmm.	Millions x 10^4
		gm.	gm. %	ml.	ml.	gm. %	gm.	gm.		
1.6	B-1	268	7.5	17.1	19.2	16.2	2.7	3.0	9.4	16.1
3.2	B-2	308	7.5	15.5	15.6	16.3	2.5	2.4	9.0	14.0
3.5	B-3	309	8.0	16.1	16.2	16.8	2.7	2.6	9.5	15.3
6.9	B-4	325	7.6	18.4	17.4	15.9	3.0	2.7	9.2	16.0
8.3	B-5	324	8.6	16.5	15.6	16.7	2.5	2.6	9.3	14.5
10.0	B-6	334	7.6	25.4	22.8	16.1	4.2	3.8	9.1	23.1
12.1	B-7	318	7.7	16.3	15.6	16.2	2.4	2.5	8.9	14.5
18.0	B-8	324	8.0	19.6	18.0	16.7	3.2	3.0	9.4	18.4
25.0	B-9	306	7.5	21.7	23.4	17.1	3.7	3.6	8.8	19.1
Steenbock XVII	C-1	326	7.2	17.2	16.2	15.9	2.8	2.5	8.2	14.1
Nitrogen low	C-2	282	6.7	16.4	17.4	16.1	2.6	2.8	8.9	14.6

CHANGES IN BLOOD

Data relating to the characteristics of the blood in the various groups of experimental animals are tabulated in Tables 16 and 17.

Serum Proteins

The average concentration of proteins in the serum of the blood in the negative control rat was 6.7 gm. per cent; in the positive control, 7.2 gm. per cent. All well nourished rats maintained concentrations equivalent to this or slightly better. The well nourished rats were forced to make considerable physiological adjustment when their diets were changed from Steenbock XVII at the beginning of the experiment to the diets containing minimal amounts of protein. The data indicate that in these groups of animals, body reserves were sufficient to support a normal concentration of proteins in the serum.

The picture in the undernourished group of animals was interesting. The effects on the concentration of serum proteins of realimenting the undernourished rat were quite striking. When the rations furnished protein equivalent to 1.5 to 4.6 per cent of the diet, the relative concentrations of protein in the sera did not shift from the value characteristic of the animal receiving no protein in its diet (6.7 gm. per cent). Thereafter, with each increment in dietary protein, the

concentration of serum proteins in the blood increased until the diet provided 12.3 per cent of protein. Then the quantity of serum proteins in the blood dropped.

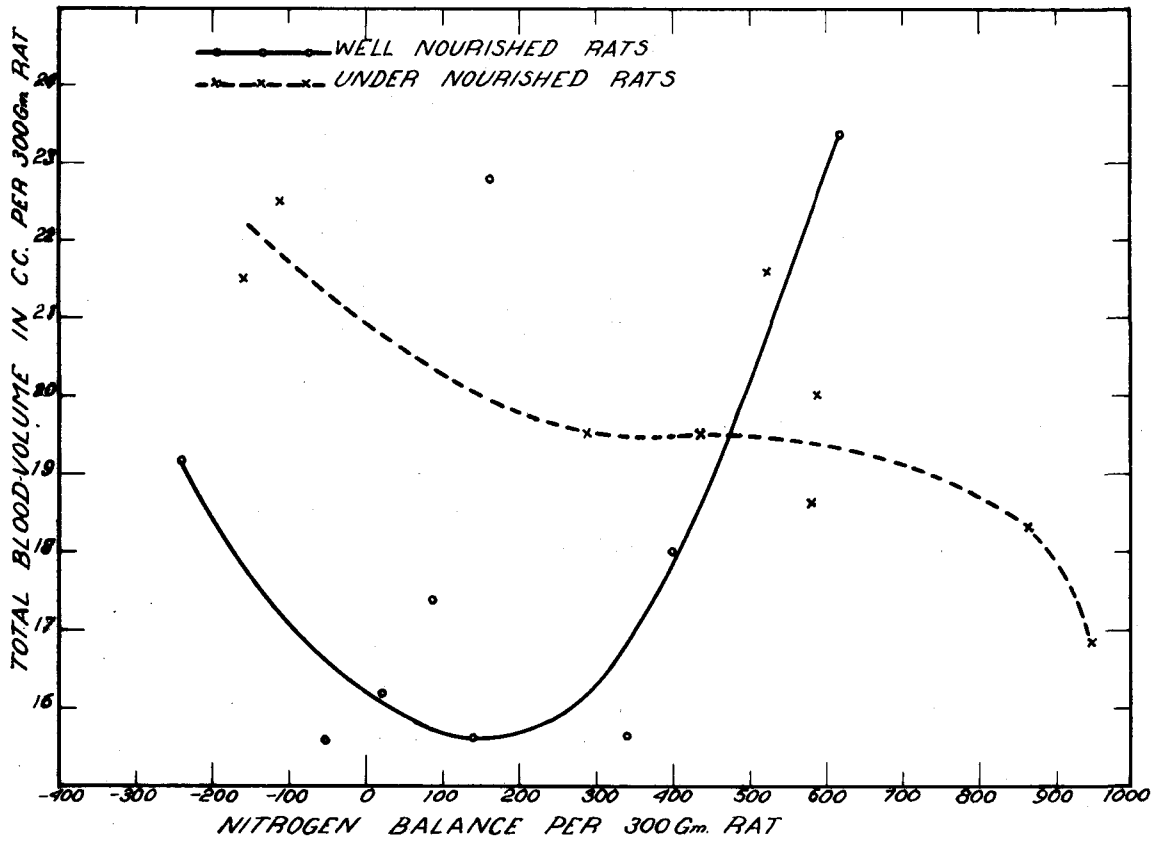
This situation seems to bear a relation to the changes diet produced in the concentration of liver nitrogen (Figure 6). In the first four groups of animals described above in which the concentrations of serum proteins in the blood remained relatively constant, a continuous repletion of hepatic nitrogen occurred. This response seemed to have, therefore, a priority value over the manufacture of serum proteins.

Blood Volume

The feeding of egg diets to well nourished rats seemed to induce the formation of an increased volume of blood. Data from group to group were not too consistent, however. The curve that the author believes represents the relation between volume of blood and nitrogen balance is shown in Figure 8.

The positive control rats showing a positive balance of 390 mg. of nitrogen had a blood volume of 16.2 ml. per 300 gm. rat; the negative controls, 17.4 ml. per 300 mg. rat. The author has found from the present and other studies she has conducted that the average blood volume of 43 normal rats is 16.3 ml. per 300 gm. rat. Depleted rats fed the 1.5 per cent protein diet had a negative balance of 224 mg. per 300 gm. rat and a blood volume 21.5 ml. Thus it appears

-A-



-B-

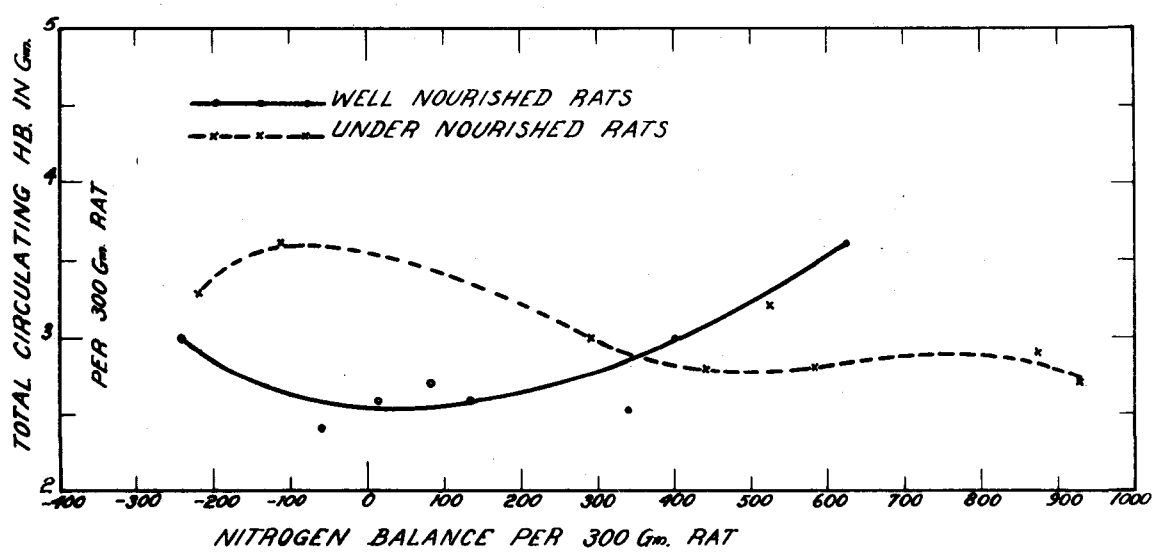


Figure 8. Relationship of blood volume and of quantity of total circulating hemoglobin to quantity of food nitrogen retained.

that the blood volume does not decrease when protein is missing or very low in the diet. However, this statement needs fortification by carcass analysis for fat, water and nitrogen. With data available at present, the preliminary deduction might be made from this picture, that placing well nourished rats on diets containing minimal amounts of protein resulted first in a diminished blood stream. It would seem that it was built up speedily when more protein was added to the diet so that balances of 600 mg. or more of nitrogen were produced. Rats consuming rations containing 18 to 25 per cent of egg protein had a definitely greater volume of blood than the control rats.

It looks as though repletion of an undernourished animal resulted in an immediate increase in the volume of blood. The group given the 18 per cent protein diet had a positive balance of 876 mg. of nitrogen per 300 gm. rat and a blood volume of 18.4 ml. This may be due to hydration of the blood, or it may represent an artefact due to the differences in the composition of the animal carcass. That the first statement does not explain the situation is indicated by studying relative and total concentrations of hemoglobin in the blood (See a following section and Figure 8). The curve depicted in Figure 8 shows that the blood decreased and tended to return to figures more nearly characteristic of the control rat as the concentration of protein in the diet increased.

Erythrocytes

The feeding of egg-containing diets to well nourished rats seemed to stimulate the production of red cells. The blood of the Steenbock control rats contained 8.2 millions of red cells per cmm. of blood whereas all well nourished rats fed the various protein rations had values of 8.8 to 9.5 millions. When the data are expressed as the total number of circulating cells, differences were not as marked between the groups fed the 1.5 to 6.9 per cent protein diets. However, when the ration contained more protein than this, total values for all groups but one were exceedingly high.

The same phenomena of increased production of red cells occurred when undernourished rats received egg diets. With 1.5 per cent of protein in the diet, the total number of circulating cells was normal; thereafter there were 17 to 18 millions of cells x 10^4 present. That this increased concentration was definitely related to increased nitrogen balances in this series is shown in Figure 9. That such graded response may not occur in the well nourished at the lower levels of protein intake and of nitrogen balance is indicated in the figure also. Only when the nitrogen balance became very high did the egg diets cause an increase in the total number of red cells in the well nourished rats. It may be recalled that the concentration of nitrogen in the liver likewise increased when these nitrogen balances were attained. Apparently the state of nutrition is stimulatory to the production of red cells.

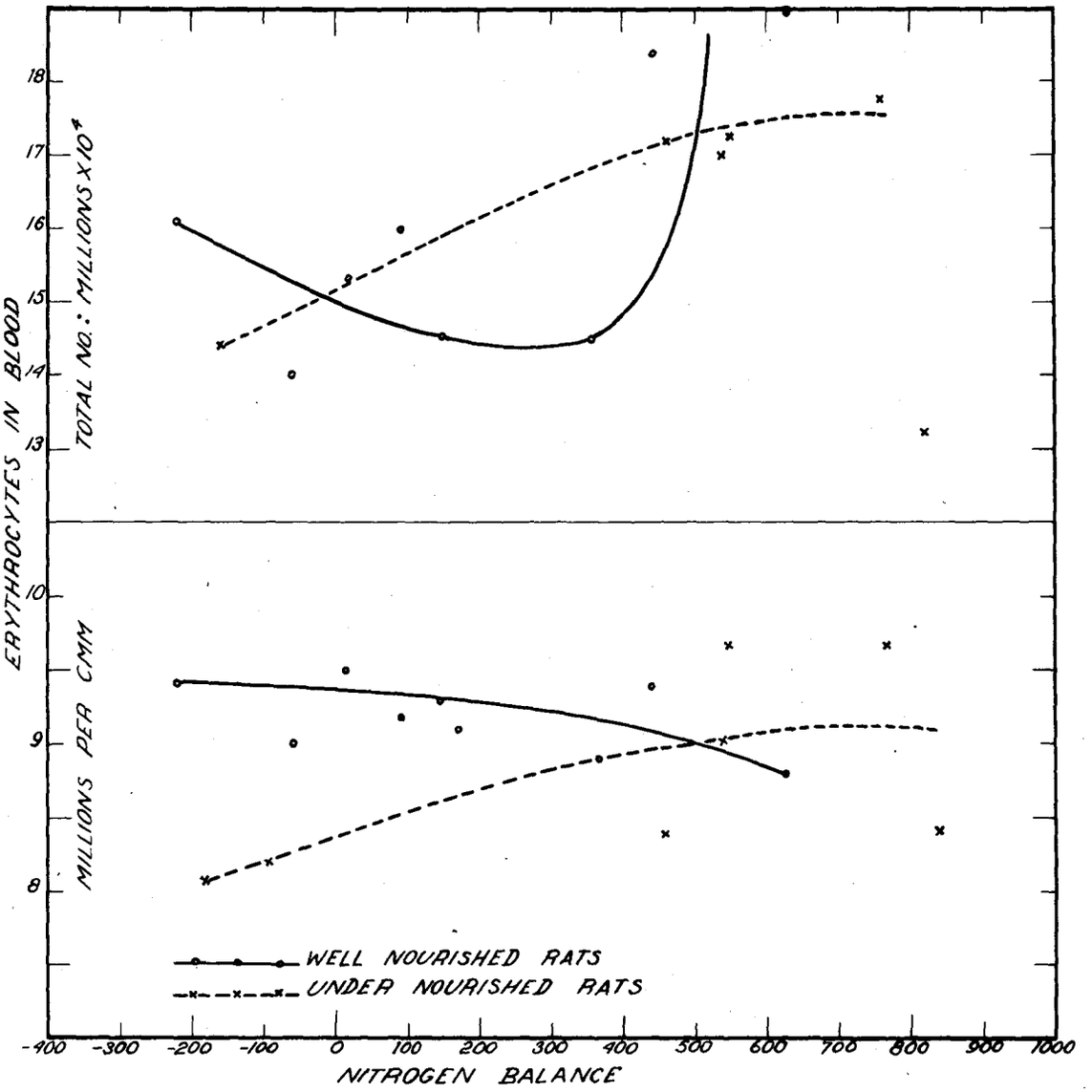


Figure 9. Relationship of number of erythrocytes in the blood to quantity of food nitrogen retained.

Hemoglobin

Increasing the protein in the diet induced approximately the same type of effect on the total quantity of hemoglobin circulating in the blood as it did on the number of red cells (Figure 8) in the case of the well nourished rats.

However, hemoglobin production did not seem to follow red cell production in the case of the undernourished rats. Although values for total circulating hemoglobin were somewhat above normal, protein enrichment of the diet did not induce hemoglobin production as it had stimulated formation of red cells. In fact, the amount present seemed to decrease as the protein of the diet increased. This means that cells containing relatively less hemoglobin were formed in the rats representing the groups that received the most protein.

DISCUSSION

The analyses herein presented indicate clearly that animals, either well nourished or undernourished in respect to protein, utilize their dietary protein in different ways. Nitrogen equilibrium is achieved at a significantly smaller intake of nitrogen by the undernourished than by the well nourished rats. The greater retentions of nitrogen exhibited by the undernourished rats at all levels of protein intake confirm the findings of Allison, Seeley, Brown and Ferguson (1946). These workers suggest that there is a different type of internal supplementation in the depleted and normal animals. They observed regular shifts in body fluids and the retention of nitrogen as the protein stores of the animal were altered. Some evidence of such shifts may be discernible in the data presented herein in respect to blood volume.

Results from the present investigation also show the effect of different quantities of protein intake on the distribution of total protein within the body. In the undernourished animals, the quantity of nitrogen in the liver is directly referable to the nitrogen balance until positive balances of 200-300 mg. are attained. Then the nitrogen concentration is maintained at a fairly constant level even though the balance may reach 1000 mg. Again the undernourished rats deposit nitrogen in the liver in larger quantities than the well nourished.

The plateau value is reached at a very early interval. However, larger amounts of dietary protein (18 and 25 per cent) did force a rise in the concentration of nitrogen in the liver. It is interesting in this connection to note that Addis, Lee, Lew and Poo (1939) observed that maximum storage was induced by feeding a diet containing 26 per cent of casein. Forty-three per cent of dietary casein was less effective than 18 per cent. Addis and his group suggested that these changes in liver protein may be associated with changes in other essential cell constituents. Harrison and Long (1945) believe that deposition of protein in liver is such a fundamental characteristic of protein metabolism that the deposition of the element there may be used as an index for measuring the nutritional value of a protein.

Thus, it has been demonstrated that nitrogen equilibrium, often used as a criterion of adequate protein nutrition, does not cover maximal storage in the liver that well may serve in increasing the resistance of the body in times of emergency.

The use that the body makes of food protein in fabricating blood and its elements has interested investigators for a long time. Whipple (1938) and his associates suggest that good dietary proteins are utilized to replete protein stores in organs and tissues, their repletion being reflected in a moderate way in the blood protein. The data in this experiment show how good stores of body protein protect the animal when dietary protein is minimal and how a normal concentration of serum protein is maintained. The data show also how in

the depleted rat, liver protein is maintained at the expense of serum when the food contains less than 6.7 per cent of protein. At concentrations greater than this, the amount of protein increased in the blood until the diet contained 12.3 per cent. Addis et al.(1939) observed this break when the dietary protein was greater than 16 per cent.

The feeding of egg diets seemed stimulatory to the production of red cells even in the well nourished rats. The response of the undernourished rats was interesting, increases in number of cells being directly related to nitrogen balance over a varying range in the protein content of the ration. Protein enrichment of the diet did not induce hemoglobin production to the extent that it had stimulated formation of red cells. In fact, the amount present seemed to decrease as the protein of the diet increased. This means that cells containing relatively less hemoglobin were formed in the rats representing the groups that received the most protein.

It is regretted that the omission of the determination of the volume of the red cells precluded the estimation of total circulating serum proteins. The data regarding the synthesis of hemoglobin herein reported indicates that the egg diets do not favor the production of hemoglobin. Robscheit-Robbins and Whipple (1949) write that "much more total protein (hemoglobin and plasma protein) is produced on a meat diet than on an egg diet of comparable amount" p. 354.

Swanson et al. (1948) have also reported the stimulatory effect of a 14 per cent lactalbumin diet on the production of serum protein.

Effects on red cell and hemoglobin formation were less marked. This is in accord with findings of Robscheit-Robbins and Whipple (1949). Apparently there is ample evidence that different proteins serve different functions in the body.

The data all suggest the high nutritive value of egg protein. That nutritive value is higher when the protein is utilized by the undernourished rat than by the well nourished is indicated below. It can be proved that the biological efficiency (Brush, William and Swanson, 1947) and nitrogen balance index (Allison and Anderson, Seeley 1946) are one and the same. The nutritional value of egg proteins have therefore been calculated with the use of the two indexes.

Table 18. Nutritive value of egg proteins

Index	Well nourished rats	Undernourished rats
Nitrogen balance index	102	134
Biological efficiency	108	120

The author finds the implication of these findings very interesting. They are directly applicable to the problems of India. Economy in nutrition during growth and maintenance depends upon the proper adjustment between the quantity of protein in the diet and the total energy supplied. The efficient use that an undernourished group of people might make of the addition of a small dose of supplementary protein of high biological value is challenging in thinking of the

nutritional problems of India. Also the way in which generous amounts of high quality protein will fill tissue stores with protein is of significance. Such stores are of great advantage to the individual in being able to develop a resistance to infection and emergencies that may arise through accident, famine, etc. Their importance in the maintenance of health is recognized.

SUMMARY AND CONCLUSIONS

The investigation reported herein is a study of the utilization well nourished and undernourished rats make of their supplies of dietary protein. Diets whose protein content ranged from 1.5 to 25.7 per cent of the diet by weight were used in the experiment. These rations were fed for a period of 34 days to one series of rats that had been removed directly from the stock colony. They were offered also for 11 days to an undernourished group of animals that had been prepared for the experiment by maintenance on nitrogen-low diet for 21 days.

Nitrogen utilization was evaluated in terms of changes in body weight, nitrogen balance, changes in the composition of organs, and changes in the blood stream.

The principal findings have been summarized in the Discussion.

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APPENDIX

Table A. Body weights at various experimental intervals of rats in Series A, the under-nourished animals

Rat number	Egg protein	At beginning of experiment	At end of depletion period	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
444722	1.5	304	252	246	- 6
44849	1.5	305	251	245	- 6
44856	1.5	312	254	247	- 7
44963	1.5	274	239	233	- 6
45036	1.5	298	246	237	- 9
44788	1.5	348	294	287	- 7
45890	1.5	356	298	290	- 8
45913	1.5	312	246	236	-10
45949	1.5	302	258	250	- 8
46052	1.5	318	254	244	-10
46067	1.5	264	266	258	- 8
46126	1.5	278	226	216	-10
46127	1.5	338	286	280	- 6
46238	1.5	312	276	268	- 8
46268	1.5	310	268	260	- 8
46340	1.5	340	303	298	- 6
46346	1.5	328	289	284	- 5
46396	1.5	295	248	240	- 8
Average		307	257	250	- 7
44723	2.6	290	261	260	- 1
44848	2.6	284	258	258	0
44857	2.6	244	213	212	- 1
44798	2.6	334	301	307	+ 6
44964	2.6	254	225	224	- 1
45034	2.6	254	228	225	- 3
Average		299	248	248	0
45891	3.0	342	285	280	- 5
45941	3.0	288	244	246	+ 2
45962	3.0	292	248	249	+ 1
46039	3.0	278	230	230	0
46068	3.0	304	249	248	- 1
46125	3.0	310	270	274	+ 4
Average		302	251	253	+ 2

Table A. (continued)

Rat number	Egg protein	At beginning of experiment	At end of depletion period	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
44724	3.6	286	264	261	- 3
44845	3.6	256	229	229	0
44858	3.6	286	240	249	+ 9
44951	3.6	266	232	230	- 2
44980	3.6	249	208	220	+12
44991	3.6	320	279	283	+ 4
Average		291	242	245	+ 3
44729	4.6	294	270	277	+ 7
44844	4.6	284	248	249	+ 1
44863	4.6	252	223	221	- 2
44950	4.6	280	245	251	+ 6
44981	4.6	244	218	225	+ 7
45026	4.6	252	231	232	+ 1
Average		275	239	242	+ 3
44733	6.7	334	285	279	- 6
44816	6.7	330	281	284	+ 3
44865	6.7	310	266	278	+12
44948	6.7	284	234	249	+15
44985	6.7	292	244	248	+ 4
45014	6.7	292	243	253	+10
45900	6.7	294	252	274	+22
45940	6.7	244	206	224	+18
45963	6.7	316	278	296	+18
46032	6.7	286	238	250	+12
46069	6.7	324	284	300	+16
46210	6.7	324	272	292	+20
Average		302	257	270	+13

Table A. (continued)

Rat number	Egg protein	At beginning of experiment	At end of depletion period	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
45014	8.6	269	226	246	+20
44765	8.6	317	268	283	+15
44812	8.6	256	209	218	+ 9
44891	8.6	308	266	268	+ 4
44951	8.6	326	280	293	+13
46190	8.6	316	274	294	+20
46262	8.6	312	280	324	+44
46269	8.6	264	-	-	-
46333	8.6	316	275	310	+35
46342	8.6	332	286	326	+40
46438	8.6	310	276	302	+26
Average		307	261	286	+22
44992	10.3	324	271	300	+29
45012	10.3	314	260	274	+14
44778	10.3	365	302	321	+19
44811	10.3	290	261	286	+20
44893	10.3	276	238	255	+17
46215	10.3	290	246	260	+14
46261	10.3	322	270	288	+18
46282	10.3	354	304	342	+38
46332	10.3	344	293	334	+41
46339	10.3	342	289	308	+19
46382	10.3	366	303	326	+23
46410	10.3	328	281	294	+13
46437	10.3	298	253	278	+25
45902	10.3	266	218	250	+32
45924	10.3	308	260	284	+24
45965	10.3	393	252	288	+36
46023	10.3	342	284	320	+36
46083	10.3	298	258	281	+26
46102	10.3	310	262	292	+30
Average		307	262	287	25

Table A. (continued)

Rat number	Egg protein	At beginning of experiment	At end of depletion period	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
44930	12.3	314	270	286	+16
44994	12.3	315	265	273	+ 8
45010	12.3	280	230	231	+ 1
44782	12.3	346	265	285	+20
44810	12.3	308	264	283	+19
45903	12.3	316	278	297	+19
45923	12.3	330	278	297	+19
45977	12.3	300	268	281	+13
46028	12.3	304	253	261	+ 8
46088	12.3	282	235	265	+30
46110	12.3	324	263	281	+18
46216	12.3	300	261	286	+25
46260	12.3	314	272	290	+18
46283	12.3	322	276	304	+28
46327	12.3	320	274	304	+30
46369	12.3	306	259	278	+19
46381	12.3	356	308	336	+28
46412	12.3	294	254	278	+24
46430	12.3	308	254	292	+38
Average		311	270	290	20
45906	14.8	331	289	313	+24
45920	14.8	356	303	321	+18
45978	14.8	280	234	247	+13
46024	14.8	336	283	305	+22
46104	14.8	254	260	283	+23
46096	14.8	316	213	231	+18
46217	14.8	310	257	232	-25
46259	14.8	320	274	288	+14
46288	14.8	330	279	326	+47
46326	14.8	336	293	330	+37
46348	14.8	362	311	348	+37
46370	14.8	300	253	284	+31
46415	14.8	382	315	348	+33
46429	14.8	332	287	306	+19
46441	14.8	330	283	316	+41
Average		312	266	291	+25

Table A. (concluded)

Rat number	Egg protein	At beginning of experiment	At end of depletion period	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
44897	18.0	324	279	299	+20
44925	18.0	266	230	237	+ 7
44995	18.0	266	222	245	+23
45009	18.0	276	226	249	+23
44787	18.0	320	274	298	+24
45907	18.0	352	290	336	+46
45914	18.0	363	308	336	+28
46018	18.0	282	240	272	+32
46030	18.0	300	248	282	+34
46091	18.0	322	276	310	+34
46112	18.0	293	252	280	+28
46232	18.0	336	293	292	- 1
46251	18.0	324	293	312	+19
46289	18.0	344	299	316	+17
46318	18.0	298	266	286	+20
46325	18.0	350	290	316	+26
46367	18.0	300	263	286	+23
46416	18.0	310	275	282	+ 7
46425	18.0	294	265	272	+12
Average		318	269	303	+34
45911	25.7	318	263	271	+ 8
46019	25.7	298	248	268	+20
46022	25.7	286	256	272	+16
46097	25.7	304	247	265	+18
45961	25.7	290	267	251	-16
46090	25.7	316	255	260	+14
46241	25.7	310	267	282	+15
46267	25.7	316	276	300	+24
46317	25.7	294	264	284	+20
46367	25.7	372	334	356	+22
46368	25.7	328	282	302	+20
46380	25.7	352	305	312	+ 7
46417	25.7	378	327	354	+27
46424	25.7	302	259	278	+19
46442	25.7	362	313	312	- 1
Average		322	274	294	+14

Table B. Body weights at various experimental intervals of rats in Series B, the well-nourished animal

Rat number	Egg protein per cent	At beginning of experiment gm.	After 24 hrs. starvation gm.	At end of balance period gm.	Change in weight in balance period
47186	1.6	345	330	297	-33
47192	1.6	316	298	268	-30
47302	1.6	318	302	259	-43
47318	1.6	312	298	268	-30
47330	1.6	340	322	287	-35
47513	1.6	330	308	279	-29
47583	1.6	330	310	271	-39
47591	1.6	330	312	273	-39
47633	1.6	302	284	266	-18
47656	1.6	306	282	272	-10
Average		318	305	274	-31
47518	3.2	346	332	320	-12
47525	3.2	354	342	328	-14
47540	3.2	362	346	314	-32
47580	3.2	354	340	331	- 9
47636	3.2	350	328	313	-15
47251	3.2	336	312	309	- 3
47256	3.2	352	328	317	-11
47369	3.2	358	328	307	-21
47191	3.2	340	316	306	-10
47376	3.2	320	304	290	-14
Average		347	328	314	-14
47483	3.5	326	316	296	-20
47564	3.5	340	324	310	-14
47581	3.5	336	326	308	-16
47589	3.5	326	310	315	+ 5
47596	3.5	330	314	301	-13
47170	3.5	354	346	347	+ 1
47206	3.5	342	322	291	-31
47229	3.5	356	328	280	-48
47249	3.5	350	330	316	-14
47316	3.5	344	316	307	- 9
Average		329	329	308	-21

Table B. (continued)

Rat number	Egg protein per cent	At beginning of experiment gm.	After 24 hrs. starvation gm.	At end of balance period gm.	Change in weight in balance period
47519	6.9	352	332	351	+19
47553	6.9	332	304	318	+14
47587	6.9	350	324	341	+17
47605	6.9	334	310	331	+21
47634	6.9	346	328	334	+ 6
47272	6.9	350	322	325	+ 3
47311	6.9	336	314	326	+12
47336	6.9	314	292	316	+24
47344	6.9	334	312	329	+17
47379	6.9	314	292	303	+11
Average		336	313	328	+15
47194	8.3	324	312	319	+ 7
47259	8.3	322	308	331	+23
47295	8.3	360	336	358	+22
47346	8.3	346	326	345	+19
47339	8.3	330	312	315	+ 3
47535	8.3	318	300	311	+11
47566	8.3	338	318	334	+16
47590	8.3	342	322	342	+20
47598	8.3	334	312	312	0
47635	8.3	322	300	311	+11
Average		325	315	328	+15
47433	10.0	356	342	340	- 2
47541	10.0	372	350	333	-17
47573	10.0	362	342	349	+ 7
47582	10.0	360	342	340	- 2
47604	10.0	356	328	340	+12
47237	10.0	358	336	351	+15
47248	10.0	344	324	351	+27
47273	10.0	350	328	345	+17
47353	10.0	356	332	341	+ 9
47419	10.0	340	320	343	+23
Average		355	334	343	+15

Table B. (concluded)

Rat number	Egg protein	At beginning of experiment	After 24 hrs. starvation	At end of balance period	Change in weight in balance period
	per cent	gm.	gm.	gm.	
47558	12.1	322	302	325	+23
47579	12.1	332	310	319	+ 9
47588	12.1	336	316	339	+23
47610	12.1	328	310	306	- 4
47626	12.1	300	280	301	+21
47418	12.1	316	296	327	+31
47308	12.1	332	308	335	+28
47368	12.1	318	308	335	+27
47338	12.1	324	302	329	+22
47377	12.1	314	296	320	+24
Average		322	303	325	+22
47169	18.2	360	336	353	+17
47252	18.2	334	316	329	+13
47258	18.2	348	332	347	+15
47282	18.2	358	334	337	+ 3
47287	18.2	332	312	326	+14
47534	18.2	316	300	315	+15
47559	18.2	344	324	338	+14
47572	18.2	314	302	313	+11
47603	18.2	334	312	323	+11
47627	18.2	308	292	308	+16
Average		334	315	328	+13
47520	25.0	300	280	279	- 1
47565	25.0	318	296	306	+10
47606	25.0	324	302	317	+15
47625	25.0	294	280	287	+ 7
47631	25.0	304	286	279	- 7
47667	25.0	322	302	310	+ 8
47362	25.0	300	295	299	+ 4
47354	25.0	320	298	309	+11
47228	25.0	330	310	330	+20
47198	25.0	332	310	319	+ 9
47417	25.0	335	318	313	- 5
Average		316	298	305	+ 8

Table C. Body weights at various experimental intervals of rats in Series C, the positive and negative animals

Rat number	At beginning of experiment	After 24 hrs. starvation	At end of balance period	Change in weight in balance period
	gm.	gm.	gm.	
		Steenbock XVII diet		
47124	351	351	356	+ 5
47162	362	354	343	-11
47168	322	322	334	+12
47185	338	338	333	- 5
47193	336	336	329	- 7
47204	366	362	347	-15
47230	368	368	360	- 8
47662	338	320	328	+ 8
47664	342	320	331	+11
47677	326	310	319	+ 9
47729	314	280	333	+53
47754	334	320	323	+ 3
47685	318	300	289	-11
47655	280	266	279	+13
47661	324	306	318	+12
47676	316	302	307	+ 5
47686	308	296	307	+11
47692	362	342	354	+12
47726	332	316	326	+10
47753	308	290	308	+18
Average	339	330	328	- 2
		Nitrogen low diet		
47526	358	342	293	-49
47543	376	360	311	-49
47548	382	366	311	-55
47595	376	362	304	-58
47156	362	348	288	-60
47171	360	350	286	-64
47184	352	346	292	-54
47221	346	330	285	-45
47236	350	340	270	-70
47250	376	368	295	-73
47257	378	370	303	-67
47265	342	334	273	-61
47317	346	338	273	-65
Average	364	356	291	-65

Table D. Nitrogen balances of rats fed varying amounts of egg protein in Series A, the under-nourished animals

Rat number	Egg protein	Body weight		Amount of food consumed		Amount of nitrogen consumed		Fecal nitrogen		Urinary nitrogen		Total nitrogen excretion		Nitrogen balances	
		Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II
	per cent	gm.		gm.		mg.		mg.		mg.		mg.		mg.	
44722	1.70	259	253	71.0	68.0	55.4	202.3	151.5	126.6	286.2	208.0	437.6	334.6	-382.2	-132.3
44849	1.70	251	245	74.2	71.5	57.1	222.3	163.7	140.9	279.7	216.4	443.3	357.4	-386.2	-135.1
44856	1.70	254	247	74.3	71.5	57.2	222.3	150.6	137.1	291.2	210.6	441.8	347.7	-384.7	-125.4
44963	1.70	239	233	67.4	66.4	53.5	207.7	172.9	145.6	244.7	183.4	417.6	329.0	-364.1	-121.3
45036	1.70	246	237	70.5	66.7	55.2	208.6	156.5	120.4	269.4	216.7	425.9	337.1	-370.7	-128.5
44788	1.70	294	287	79.0	79.0	59.7	243.75	169.3	180.9	266.6	214.5	435.9	395.4	-376.2	-151.7
Average		257	250	72.7	70.5	56.4	217.8	160.8	141.9	272.9	208.3	433.7	350.2	-377.3	-133.7
44723	2.6	261	260	81.0	81.0	60.7	375.8	166.6	181.0	311.9	211.7	478.5	392.6	-417.8	- 16.8
44848	2.6	258	258	74.6	74.6	57.4	347.5	148.8	162.7	265.7	180.3	414.5	343.0	-357.2	+ 4.5
44857	2.6	213	212	67.0	67.0	53.3	314.0	134.3	158.8	257.3	194.9	391.7	353.7	-338.3	- 39.8
44798	2.6	301	308	98.7	98.7	70.1	454.1	215.3	205.9	380.0	264.6	595.3	470.5	-525.2	- 16.5
44964	2.6	225	224	68.7	68.7	54.2	321.5	166.0	180.2	260.2	179.5	426.2	359.7	-372.0	- 38.2
45034	2.6	228	225	68.5	68.5	54.1	320.6	133.5	142.1	294.9	196.0	428.1	338.1	-364.0	- 17.5
Average		248	248	76.4	76.4	58.3	355.5	160.7	171.8	295.0	204.5	455.7	376.3	-397.4	- 20.7
45891	3.1	285	281	77.6	77.6	98.5	412.4	197.1	206.5	378.6	287.3	575.6	493.8	-476.1	- 81.4
45941	3.1	244	246	78.5	78.5	99.3	417.9	238.7	226.8	289.0	208.9	527.7	435.7	-428.3	- 17.8
45962	3.1	248	249	78.5	75.5	99.3	402.9	192.5	211.8	293.4	266.0	485.9	477.8	-386.6	- 74.8
46039	3.1	230	230	72.4	72.4	93.6	387.5	216.3	191.5	345.0	238.6	561.3	430.0	-467.7	- 42.6
46068	3.1	249	248	76.4	76.4	97.4	407.4	195.3	197.4	337.7	273.3	532.9	470.7	-435.6	- 63.3
46125	3.1	270	274	93.2	86.2	113.1	456.4	203.0	219.5	296.2	268.2	499.2	487.7	-386.1	- 31.3
Average		254	255	79.4	77.7	100.2	414.4	207.1	208.9	323.3	301.8	530.5	465.9	-430.0	- 51.9

Table D. (continued)

Rat number	Egg protein	Body weight		Amount of food consumed		Amount of nitrogen consumed		Fecal nitrogen		Urinary nitrogen		Total nitrogen excretion		Nitrogen balances	
		Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II
	per cent	gm.		gm.		mg.		mg.		mg.		mg.		mg.	
44724	3.6	264	261	65.6	65.6	52.6	440.2	146.2	163.9	302.4	268.9	448.6	428.8	-396	- 19
44845	3.6	229	229	65.0	65.0	52.3	406.5	136.4	149.6	278.9	205.2	415.3	354.8	-363	+ 52
44858	3.6	240	249	79.7	79.7	60.0	494.4	153.7	170.2	251.7	185.9	405.4	356.0	-345	+138
44951	3.6	232	230	65.8	65.8	52.7	411.3	162.3	160.7	256.2	213.9	418.5	374.6	-365	+ 36
44980	3.6	208	220	66.3	66.3	53.0	414.3	152.7	151.2	246.7	162.9	399.4	314.2	-346	+100
44991	3.6	279	283	81.4	81.4	61.0	504.6	162.3	189.4	296.5	223.7	458.9	413.1	-398	+ 92
Average		242	245	70.6	70.6	55.3	440.2	153.3	164.2	272.1	209.4	434.3	373.6	-379.1	+166.6
44729	4.6	270	277	82.7	82.7	61.6	648.8	161.4	187.7	315.3	191.8	476.6	379.5	-415	+269
44844	4.6	248	249	64.3	59.3	51.9	470.3	136.6	142.7	278.9	236.9	415.5	379.6	-366	+ 90
44863	4.6	223	221	70.0	67.3	54.9	530.6	117.7	150.6	272.4	211.6	390.2	362.2	-335	+169
44950	4.6	245	251	87.5	87.5	64.2	685.4	202.1	192.2	277.8	219.0	479.9	411.2	-416	+272
44981	4.6	218	225	95.6	72.9	68.5	574.0	159.1	143.9	230.7	177.8	389.8	321.7	-321	+252
45026	4.6	231	232	73.0	73.0	56.5	574.8	172.4	186.2	276.6	209.7	449.1	395.9	-392	+179
Average		239	242	78.8	73.8	59.6	581.3	158.2	167.2	275.3	207.8	433.5	375.0	-313.9	+206.3
44733	6.9	285	279	83.6	83.6	62.1	942.4	208.8	231.1	367.1	308.6	575.9	539.6	-513.8	+402.8
44816	6.9	281	284	73.1	73.1	56.6	826.2	166.8	195.5	375.5	320.3	542.3	517.9	-485.7	+308.3
44865	6.9	266	278	81.2	79.1	60.9	892.7	189.2	231.8	348.2	342.4	537.4	574.3	-476.8	+318.4
44948	6.9	234	249	74.1	74.1	57.1	837.4	197.8	206.8	266.3	258.2	464.0	464.9	-407.9	+372.5
44985	6.9	244	248	69.4	55.8	54.6	635.0	183.4	170.8	289.0	281.7	472.4	452.5	-417.8	+182.6
45014	6.9	243	253	75.2	75.2	57.7	849.5	157.9	212.8	326.7	279.2	484.6	492.0	-426.9	+357.5
Average		259	268	76.1	73.5	58.1	830.5	184.0	208.5	328.8	298.4	512.8	506.9	-454.4	+323.7

Table D. (continued)

Rat number	Egg protein	Body weight		Amount of food consumed		Amount of nitrogen consumed		Fecal nitrogen		Urinary nitrogen		Total nitrogen excretion		Nitrogen balances	
		Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II
	per cent	gm.		gm.		mg.		mg.		mg.		mg.		mg.	
45014	9.1	226	246	76.4	75.2	58.3	1098.2	177.0	167.4	269.4	309.9	446.4	477.3	-388.1	+620.9
44765	9.1	268	283	75.4	75.4	57.8	1111.1	139.0	140.7	279.2	403.5	418.2	544.2	-361.6	+566.9
44812	9.1	209	218	69.1	69.1	54.4	1019.8	175.9	145.2	227.6	357.5	403.5	503.0	-349.1	+670.7
44891	9.1	266	268	57.6	57.6	48.3	853.0	123.3	113.6	298.6	431.2	421.9	544.8	-373.6	+309.2
44931	9.1	280	293	78.0	78.0	59.2	1148.8	161.4	156.5	291.2	412.7	452.6	569.2	-393.4	+579.6
Average		250	263	71.3	71.1	55.6	1046.2	155.3	144.7	273.2	383.0	428.4	527.7	-372.8	+518.5
44992	10.6	271	300	82.5	79.5	61.6	1358.2	189.9	268.9	303.5	410.8	493.4	679.7	-431.8	+678.5
45012	10.6	260	274	78.1	78.1	59.2	1334.6	166.6	249.1	338.8	546.0	505.4	795.1	-446.2	+539.5
44778	10.6	302	321	93.1	93.1	67.2	1587.5	167.7	237.2	317.5	442.4	485.2	679.6	-418.0	+907.9
44811	10.6	268	286	74.1	94.1	57.1	1267.1	179.6	214.8	289.2	477.9	468.9	692.8	-411.8	+574.3
44893	10.6	238	255	72.8	72.8	56.4	1245.2	149.2	259.0	248.1	482.4	397.3	741.4	-340.9	+503.8
Average		268	287	80.1	79.5	60.3	1358.5	170.6	245.8	299.4	471.9	470.0	717.8	-409.8	+640.8
44930	12.3	270	286	71.3	71.3	55.6	1465.2	148.1	260.5	270.5	773.1	418.6	1033.6	-363.0	+431.6
44994	12.3	265	273	77.2	77.2	58.7	1585.0	141.2	232.8	344.2	701.4	485.3	934.2	-426.6	+650.8
45010	12.3	230	231	54.0	54.0	46.4	1114.0	140.1	208.0	383.9	720.2	524.0	928.2	-477.6	+185.8
44782	12.3	265	285	78.9	78.9	59.6	1619.5	178.5	266.0	361.2	674.2	539.7	930.2	-480.1	+689.3
44810	12.3	264	283	74.8	74.8	57.5	1536.3	175.6	265.7	276.9	692.4	452.5	958.2	-395.0	+578.1
45903	12.3	278	297	76.3	76.3	97.3	1521.1	212.5	297.5	346.6	733.6	559.1	1031.1	-461.8	+490.0
45923	12.3	278	297	85.0	85.0	105.5	1691.2	235.6	329.7	370.2	910.0	605.7	239.0	-500.3	+451.5
45977	12.3	268	281	74.5	74.5	95.6	1485.4	190.1	264.6	289.0	724.1	479.0	988.9	-383.4	+496.7
46028	12.3	253	261	80.2	77.5	98.4	1543.6	203.4	310.8	293.4	738.1	496.8	1048.9	-398.4	+494.7
46088	12.3	235	265	73.2	65.7	94.3	1312.3	191.1	366.8	299.6	738.1	490.7	104.9	-396.8	+207.4
46110	12.3	263	281	79.6	79.6	100.4	1585.5	242.2	310.8	372.4	881.4	614.6	1192.2	-514.2	+393.3
Average		269.7	284	74.6	78.8	77.1	1493.1	184.6	229.0	327.9	749.9	512.5	1028.4	-435.4	464.2

Table D. (concluded)

Rat number	Egg protein	Body weight		Amount of food consumed		Amount of nitrogen consumed		Fecal nitrogen		Urinary nitrogen		Total nitrogen excretion		Nitrogen balances	
		Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II	Pd.I	Pd.II
	per cent	gm.		gm.		mg.		mg.		mg.		mg.		mg.	
45906	14.4	289	313	88.2	87.5	108.5	2038.1	177.5	331.1	313.6	944.7	491.1	1275.8	-383.6	+763.3
45920	14.4	303	321	85.5	85.5	105.9	1991.4	235.5	354.2	354.5	1085.3	590.0	1439.5	-484.1	+551.9
45978	14.4	234	247	71.5	71.5	102.8	1669.4	181.3	279.7	303.3	846.7	484.3	1126.4	-381.5	+543.0
46024	14.4	283	305	83.3	83.3	103.9	1941.5	215.6	324.5	338.8	1031.5	554.4	1356.0	-450.5	+585.5
46104	14.4	267	283	94.7	94.6	114.5	2202.3	219.8	384.0	312.5	875.3	532.3	1259.2	-418.4	+949.1
46096	14.4	213	231	74.5	73.0	95.6	1704.1	181.3	306.3	241.9	1045.0	423.2	1351.2	-327.6	+352.9
Average		265	283	82.9	82.6	105.2	1924.5	201.8	326.1	310.7	971.4	512.5	1301.4	-407.6	+624.3
44897	18.2	279	299	80.5	78.1	60.5	2287.4	195.5	285.5	281.7	1128.1	477.2	1413.6	-416.7	+873.8
44925	18.2	230	237	66.0	63.4	52.8	1860.2	149.1	203.3	287.6	1029.3	436.7	1237.5	-383.9	+622.7
44995	18.2	222	245	75.9	75.9	58.0	2223.5	163.9	297.6	252.0	1143.5	415.9	1441.0	-357.9	+782.5
45009	18.2	226	249	81.2	81.2	60.9	2377.5	181.3	186.5	316.4	1027.3	497.7	1213.8	-436.8	+1163.7
44787	18.2	274	298	81.2	77.9	60.9	2281.6	175.1	296.5	271.0	1251.3	446.1	1547.8	-385.2	+733.8
Average		246	266	77.0	75.3	58.6	2206.0	173.0	254.9	281.7	1115.9	454.7	1370.8	-396.1	+835.5
45911	25.7	263	271	80.1	76.7	100.8	3184.8	201.6	351.1	290.1	1909.0	491.7	2260.1	-390.9	+924.0
46019	25.7	248	268	84.4	82.4	104.9	3418.4	206.2	411.6	279.4	2004.2	485.6	2415.8	-380.7	+1002.6
46022	25.7	256	272	70.3	70.3	91.6	2921.1	198.5	439.9	370.7	1891.7	569.2	2331.6	-477.6	+589.2
46097	25.7	247	265	81.3	81.3	102.0	3373.9	196.7	408.5	275.5	2116.8	472.2	2525.3	-370.2	+848.7
45961	25.7	267	251	87.2	-	107.5	2812.8	161.4	-	300.7	1738.8	462.1	-	-354.6	+877.2
46090	25.7	255	269	84.7	81.3	105.2	3375.1	237.7	371.0	312.5	2126.9	550.1	2497.9	-444.9	
Average		256	266	81.3	78.4	102.0	3181.0	200.3	396.4	304.8	1964.6	505.1	2406.1	-403.1	+848.5

Table E. Nitrogen balances of rats fed varying amounts of egg protein in Series B, the well-nourished rats

Rat number	Egg protein	Body weight	Weight of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	per cent	gm.	gm.	mg.	mg.	mg.	mg.	mg.
47186	1.6	297	86.1	238.0	196.4	271.6	468.0	-230.0
47192	1.6	268	65.7	186.2	132.0	280.0	412.0	-225.8
47302	1.6	259	78.8	319.5	196.7	245.3	441.9	-222.5
47318	1.6	268	85.7	237.0	190.5	258.7	449.3	-212.3
47330	1.6	287	85.8	237.2	-			-
47513	1.6	279	84.9	235.0	209.3	245.3	454.6	-219.6
47583	1.6	271	86.8	237.2	215.3	272.7	488.0	-250.7
47591	1.6	273	83.9	234.4	182.0	258.7	440.7	-208.3
47633	1.6	266	80.6	224.0	206.2	227.9	434.1	-210.0
47656	1.6	272	86.7	239.5	184.5	249.2	433.7	-194.1
Average		274	82.2	226.9	189.7	257.3	446.6	-219.2
47518	3.2	320	89.9	473.8	227.9	286.2	514.0	- 40.2
47525	3.2	328	90.7	477.9	188.3	352.6	510.9	- 33.0
47540	3.2	314	90.3	475.9	253.1	313.6	566.7	- 90.8
47580	3.2	331	95.8	503.7	220.5	312.5	533.0	- 29.3
47636	3.2	313	89.7	472.8	238.7	332.1	570.8	- 98.0
47251	3.2	309	74.6	396.5	184.2	282.8	467.0	- 70.6
47256	3.2	317	79.1	419.2	203.4	281.1	484.5	- 65.3
47369	3.2	307	81.7	432.4	186.5	262.6	449.1	- 16.7
47191	3.2	306	79.1	419.4	211.4	285.0	496.4	- 77.2
47376	3.2	290	81.9	433.4	236.3	290.1	526.3	- 92.9
Average		314	85.3	450.1	215.0	300.9	511.9	- 61.3

Table E. (continued)

Rat number	Egg protein	Body weight	Weight of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	per cent	gm.	gm.	mg.	mg.	mg.	mg.	mg.
47483	3.5	296	88.8	513.8	217.0	295.1	512.1	+ 1.6
47564	3.5	310	88.2	510.4	217.0	292.9	509.9	+ 0.5
47581	3.5	308	89.6	518.2	212.5	295.1	507.6	+10.6
47589	3.5	315	90.6	523.8	216.0	287.8	503.8	+20.0
47596	3.5	301	90.0	520.4	212.5	292.9	505.3	+15.1
47170	3.5	347	81.0	470.3	228.6	295.1	523.7	-53.4
47206	3.5	291	86.1	498.7	223.7	240.8	464.5	+34.2
47229	3.5	280	87.8	508.2	224.6	240.8	465.4	+42.8
47249	3.5	322	83.9	486.5	215.0	252.6	467.6	+18.8
47316	3.5	307	90.0	520.4	216.9	295.1	512.0	+ 8.5
Average		308	88.2	510.4	217.5	275.1	492.6	+17.8
47519	6.9	351	72.9	821.9	274.4	591.9	766.3	+55.6
47553	6.9	318	79.3	892.4	258.3	499.0	757.3	+135.1
47587	6.9	341	85.9	965.1	263.6	543.2	806.8	+158.3
47605	6.9	331	80.6	906.7	262.2	550.5	812.6	+ 94.1
47634	6.9	354	88.3	991.5	289.5	637.3	926.7	+ 64.8
47272	6.9	325	84.5	949.6	245.3	637.3	882.6	+ 67.1
47311	6.9	326	76.0	856.1	238.4	532.6	770.9	+ 85.2
47336	6.9	316	82.4	926.5	258.3	499.0	757.3	+169.3
47344	6.9	329	81.0	911.1	258.3	564.5	822.8	+ 88.3
47379	6.9	303	85.6	961.8	246.3	658.6	904.8	+ 56.8
Average		328	81.7	918.3	259.5	571.9	820.8	+ 97.5

Table E. (continued)

Rat number	Egg protein	Body weight	Weight of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	per cent	gm.	gm.	mg.	mg.	mg.	mg.	mg.
47194	8.3	319	76.9	1040.5	246.4	736.4	982.8	+57.7
47259	8.3	331	84.2	1137.5	257.0	576.8	833.8	+303.7
47295	8.3	358	85.3	1152.1	308.4	780.1	1088.5	+63.6
47346	8.3	345	88.9	1199.9	264.5	775.0	1039.5	+160.4
47339	8.3	315	60.4	821.4	193.8	661.4	855.1	
47535	8.3	311	86.0	1161.3	211.1	701.1	912.2	+249.1
47566	8.3	334	86.3	1165.4	294.4	800.8	1095.2	+ 70.2
47590	8.3	342	86.3	1172.0	307.0	750.4	1057.4	+114.7
47598	8.3	312	85.6	1156.1	312.6	627.8	940.3	+216.8
47635	8.3	309	83.7	1130.9	266.7	790.2	1056.9	+ 74.0
Average		330	84.8	1146.7	273.7	725.6	999.3	+148.6
47433	10.0	340	90.3	1442.4	270.9	1094.2	1365.1	+ 77.3
47541	10.0	333	86.0	1374.7	314.3	1020.9	1335.2	+ 39.5
47573	10.0	349	85.6	1368.4	282.8	1074.1	1356.9	+ 11.5
47582	10.0	333	82.9	1325.8	267.1	1064.6	1331.6	-5.8
47604	10.0	340	89.3	1426.7	298.9	944.2	1243.1	+183.6
47237	10.0	351	82.5	1319.5	259.8	738.6	998.5	+321.0
47248	10.0	351	76.4	1223.4	257.0	679.3	936.3	+287.0
47273	10.0	345	82.4	1317.9	285.6	815.9	1101.5	+216.4
47353	10.0	341	84.6	1352.6	267.0	824.9	1091.9	+260.8
47419	10.0	343	81.2	1299.0	257.0	749.3	1006.3	+292.7
Average		343	84.6	1352.8	278.5	897.5	1176.0	+177.4

Table 5. (continued)

Rat number	Egg protein per cent	Body weight gm.	Weight of food consumed gm.	Amount of nitrogen consumed mg.	Fecal nitrogen mg.	Urinary nitrogen mg.	Total nitrogen excreted mg.	Nitrogen balance mg.
47558	12.1	325	90.7	1770.7	302.8	1138.5	1441.2	+331.5
47579	12.1	319	85.9	1678.0	270.2	1000.7	1270.9	+407.1
47588	12.1	339	89.5	1747.6	305.9	960.4	1266.3	+521.3
47610	12.1	306	90.5	1766.9	290.5	1132.3	1422.8	+344.0
47626	12.1	301	90.5	1766.9	299.6	996.2	1295.8	+471.0
47418	12.1	327	76.7	1500.4	261.1	811.4	1072.5	+427.9
47308	12.1	336	74.6	1459.9	226.4	910.6	1136.9	+322.9
47368	12.1	335	82.7	1616.3	250.3	982.8	1233.1	+383.2
47338	12.1	329	80.0	1564.1	256.8	1155.8	1412.6	+151.5
47377	12.1	320	82.1	1604.7	-	924.0	-	-
Average		325	84.0	1640.6	271.2	1005.4	1276.6	+368.2
47169	18.2	353	86.7	2545.8	317.1	1645.3	1962.4	+583.4
47252	18.2	329	69.8	2053.3	284.6	1257.8	1542.4	+510.9
47258	18.2	347	83.9	2464.2	326.8	1820.6	2147.3	+316.8
47282	18.2	337	67.2	1977.5	266.7	1573.6	1840.3	+137.2
47287	18.2	326	86.6	2542.8	350.0	1906.8	2256.8	+286.0
27534	18.2	315	78.8	2315.5	312.2	1121.1	1433.3	+882.2
47559	18.2	338	86.1	2528.3	387.8	1885.5	2273.3	+254.9
47572	18.2	313	83.2	2443.8	353.2	1689.0	2042.1	+401.7
47603	18.2	323	82.6	2426.3	339.2	1515.4	1854.6	+471.8
47627	18.2	308	84.6	2484.6	341.6	1687.3	2028.9	+455.7
Average		328	80.9	2378.2	327.9	1610.3	1938.1	+440.1

Table E. (concluded)

Rat number	Egg protein	Body weight	Weight of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	per cent	gm.	gm.	mg.	mg.	mg.	mg.	mg.
47520	25.0	279	73.3	2994.6	330.1	2128.6	2458.6	+536.0
47565	25.0	306	80.8	3299.0	379.4	2194.1	2573.5	+725.5
47606	25.0	317	84.3	3441.0	412.0	2488.1	2900.0	+541.0
47625	25.0	287	83.5	3408.6	371.0	2438.8	2809.8	+598.8
47631	25.0	279	80.4	3282.8	371.0	2223.2	2594.2	+688.6
47667	25.0	310	82.0	3347.7	330.1	2391.2	2721.3	+626.4
47362	25.0	299	79.9	3262.5	338.2	2395.1	2733.4	+529.1
47354	25.0	309	77.2	3152.9	341.6	2102.8	2444.4	+708.5
47228	25.0	330	76.4	3120.4	347.5	2409.7	2757.2	+363.2
47198	25.0	319	79.2	3234.0	345.6	2061.9	2407.4	+826.7
47417	25.0	313	77.1	3148.8	339.2	2021.6	2360.8	+788.0
Average		305	79.4	3239.7	354.0	2254.4	2608.4	+631.3

Table F. Nitrogen balances of rats fed varying amounts of egg protein in Series C, the positive control animals

Rat number	Body weight	Amount of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	gm.	gm.	mg.	mg.	mg.	mg.	mg.
			Steenbock XVII diet				
47124	356	85.5	3807.5	514.3	3202.1	3716.4	+ 91.1
47162	343	92.4	4068.2	635.3	3101.3	3736.6	+331.6
47168	334	89.4	3954.9	594.7	3140.5	3735.2	+219.7
47185	333	66.7	3087.3	544.7	3010.6	3555.3	-468.0
47193	329	89.3	5951.1	618.2	3106.9	3725.1	+226.0
47204	347	85.3	3800.0	544.7	3159.5	3704.3	+ 95.7
47230	360	86.0	3826.4	573.2	3037.4	3610.6	+215.8
47662	328	99.0	4281.1	667.5	3223.4	3890.8	+390.3
47664	331	106.3	4592.9	707.7	3211.0	3918.7	+674.1
47677	319	99.3	4328.4	633.2	3335.4	3968.5	+359.9
47729	333	102.2	4436.0	737.5	3068.8	3806.3	+631.7
47754	323	110.1	4736.5	712.3	3139.4	3851.6	+884.8
47685	289	81.4	3652.2	593.6	2680.2	3273.8	+378.4
47655	279	94.9	4160.2	685.3	2841.4	3526.7	+633.5
47661	318	98.6	4302.0	846.3	3332.0	4178.3	+123.7
47676	307	87.3	3875.1	569.8	3147.2	3717.0	+158.1
47686	307	85.1	3792.0	551.3	2897.4	3448.7	+343.3
47692	354	100.1	4358.7	693.7	2985.9	3679.6	+679.0
47726	326	99.7	4343.5	672.0	3281.6	3953.6	+389.9
47753	308	94.6	4150.9	649.6	3112.5	3762.1	+388.8
Average	328	94.4	4152.0	643.1	3118.3	3761.4	+390.2

Table F. (concluded)

Rat number	Body weight	Amount of food consumed	Amount of nitrogen consumed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance
	gm.	gm.	mg.	mg.	mg.	mg.	mg.
			Nitrogen low diet				
47526	293	83.3	80.1	222.3	295.1	517.4	-437.3
47543	311	87.2	83.0	244.7	285.6	530.3	-447.3
47548	311	85.2	81.6	251.7	311.9	563.6	-482.0
47595	304	88.5	83.9	249.9	283.9	533.8	-449.9
47156	288	81.1	78.5	169.7	297.9	467.6	-388.1
47171	286	73.8	73.2	144.2	257.6	401.8	-328.6
47184	292	90.9	85.7	191.5	283.4	474.9	-389.2
47221	285	67.0	68.2	174.9	285.0	459.9	-391.7
47236	270	61.1	63.9	155.7	264.9	420.6	-356.7
47250	295	81.3	78.7	153.9	306.3	460.2	-381.5
47257	303	88.4	83.8	150.9	307.2	458.1	-374.3
47265	273	84.7	81.1	259.9	303.0	562.9	-481.7
47317	273	85.1	81.4	245.0	310.8	555.8	-474.4

Table G. Average food intake and food nitrogen absorbed in Series A, the under-nourished animals

Protein in diet	Food nitrogen consumed	Fecal nitrogen		Egg nitrogen in feces	Food nitrogen absorbed
		At end of depletion period	At end of repletion period		
per cent	mg.	mg.	mg.	mg.	mg.
1.5	163	161	142	-	163
2.6	298	161	172	11	287
3.0	414	207	209	2	412
3.6	385	152	164	12	373
4.6	521	158	167	9	512
6.7	773	184	208	24	749
8.6	922	167	189	22	900
10.3	990	155	145	-	990
12.3	1410	191	280	89	1321
14.8	1660	180	285	105	1555
18.0	2148	173	255	82	2066
25.7	3181	200	396	196	2985

Table H. Average food intake and food nitrogen absorbed in Series B, the well-nourished animals

Protein in diet	Food nitrogen consumed	Faecal nitrogen		Egg nitrogen in faeces	Food nitrogen absorbed
		Metabolic nitrogen	Nitrogen after end of balance period		
per cent	mg.	mg.	mg.		
1.6	227	180	190	10	217
3.2	450	180	215	35	415
3.5	510	180	218	38	472
6.9	918	180	260	80	838
8.3	1147	180	274	94	1053
10.0	1353	180	279	99	1256
12.1	1641	180	271	91	1550
18.2	2378	180	328	148	2230
25.4	3239	180	354	174	3065

Table I. Weights of fresh liver, liver nitrogen, liver fat and moisture in Series A, the under-nourished animals

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
45913	1.5	227	5.8	150	302	71.8
45949	1.5	238	6.2	155	475	69.5
46268	1.5	256	9.0	160	418	72.5
46340	1.5	290	7.4	189	525	70.9
44722	1.5	250	6.4	167	424	72.3
44849	1.5	242	5.4	159	392	70.7
44856	1.5	245	6.9	173	413	73.0
44963	1.5	217	5.5	148	342	71.2
Average		248	6.3	167	430	71.0
447233	2.6	263	6.6	189	425	71.8
44848	2.6	258	6.0	170	420	71.4
44857	2.6	212	6.0	169	380	70.0
44798	2.6	307	7.5	227	475	71.8
Average		260	6.5	189	425	71.2
45891	3.0	275	7.7	225	409	70.6
45941	3.0	245	6.0	211	631	67.8
45962	3.0	245	6.5	210	441	70.3
46039	3.0	212	5.1	180	298	71.7
46125	3.0	270	6.8	225	501	71.2
46155	3.0	286	7.5	235	399	71.3
46274	3.0	226	6.7	201	283	72.1
Average		273	6.7	211	399	71.0
44724	3.6	268	7.1	230	489	71.6
44845	3.6	236	7.5	210	400	71.5
44858	3.6	258	5.9	220	434	71.3
44951	3.6	230	7.9	225	-	72.7
44980	3.6	217	6.6	215	401	69.1
Average		242	7.0	220	431	71.3

Table I. (continued)

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
44729	4.6	286	8.4	220	598	70.0
44844	4.6	259	7.7	192	334	70.6
44863	4.6	225	7.2	209	241	70.8
44950	4.6	257	6.4	208	360	70.3
44991	4.6	230	6.1	202	-	70.7
Average		251	7.1	206	383	70.6
46032	6.7	252	6.2	253	458	69.6
46210	6.7	294	7.2	320	685	68.1
44733	6.7	309	8.5	227	513	71.2
44816	6.7	295	7.2	244	324	72.4
44865	6.7	302	7.2	208	464	70.6
44948	6.7	248	6.1	203	313	72.8
44985	6.7	261	7.0	208	389	71.2
Average		273	7.0	286	421	70.1
45014	8.6	261	7.4	205	-	70.2
44765	8.6	296	7.2	227	-	72.2
44812	8.6	220	5.9	197	-	70.7
44891	8.6	267	7.5	238	-	73.0
45925	8.6	313	7.4	299	317	69.2
45964	8.6	290	7.4	282	317	68.6
Average		301	7.4	291	317	70.3
44992	10.3	313	8.1	290	-	71.2
45012	10.3	283	7.9	290	-	71.0
44778	10.3	335	9.8	295	-	70.8
44811	10.3	303	7.0	268	-	70.2
45965	10.3	288	6.7	283	400	71.8
45023	10.3	284	7.0	297	382	70.0
46215	10.3	266	8.0	335	438	72.0
Average		285	7.6	290	410	71.0

Table I. (concluded)

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
44930	12.3	297	9.3	229	-	71.7
44994	12.3	294	10.4	306	-	73.7
45010	12.3	240	7.5	261	-	72.4
44782	12.3	287	8.6	300	-	70.9
46216	12.3	294	10.9	280	389	72.4
46260	12.3	296	10.9	274	402	72.8
Average		295	10.1	277	391	72.6
45906	14.8	325	8.3	342	351	68.8
45920	14.8	330	8.9	359	361	70.2
45978	14.8	245	6.4	287	309	69.4
46024	14.8	310	7.9	330	301	69.6
46104	14.8	320	9.4	340	349	69.7
46096	14.8	244	7.2	284	309	69.9
46259	14.8	300	9.6	249	301	73.0
46288	14.8	330	10.6	275	331	71.0
Average		305	9.0	285	334	70.8
44897	18.0	313	8.7	364	-	72.5
44925	18.0	230	6.7	304	-	71.0
44995	18.0	266	7.2	329	-	70.0
45009	18.0	266	6.9	321	-	70.6
46030	18.0	280	7.1	318	418	68.9
46091	18.0	288	7.2	304	395	71.2
46232	18.0	286	7.2	279	203	72.0
46289	18.0	308	10.3	316	360	70.4
Average		296	7.9	304	343	71.0
45911	25.7	290	7.7	311	404	69.6
46019	25.7	275	6.9	268	-	70.2
46022	25.7	282	7.3	299	457	69.6
46097	25.7	282	8.7	340	574	70.0
45961	25.7	229	6.4	232	398	73.1
46090	25.7	280	8.2	332	554	69.8
46317	25.7	288	7.1	237	269	71.9
46368	25.7	293	7.7	276	307	71.3
Average		283	7.5	277	382	71.0

Table J. Weights of fresh liver, liver nitrogen, liver fat and moisture in Series B, the well-nourished animals

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
47186	1.6	286	7.4	212	485	74.2
47192	1.6	252	6.0	182	430	70.5
47302	1.6	242	6.8	197	373	73.3
47318	1.6	250	5.9	196	374	70.7
47330	1.6	276	5.9	223	351	71.3
47513	1.6	274	8.0	202	468	70.2
47583	1.6	270	7.4	208	497	71.2
47591	1.6	266	7.4	211	478	68.9
47633	1.6	262	7.0	190	451	70.4
47656	1.6	269	7.8	186	507	70.8
Average		265	7.0	201	442	71.1
47518	3.2	288	6.9	214.9	513	68.6
47525	3.2	304	7.4	225.6	459	69.8
47540	3.2	290	7.2	227.2	433	70.9
47580	3.2	294	7.1	226.3	443	70.9
47636	3.2	272	7.0	246.0	423	70.7
47251	3.2	310	8.4	264.2	653	69.8
47256	3.2	320	8.5	264.2	641	68.4
47369	3.2	314	8.4	279.3	599	69.3
47191	3.2	320	8.2	267.4	568	68.9
47376	3.2	309	7.8	-	530	69.2
Average		300	7.7	248.4	526	69.7
47483	3.5	326	6.9	234	431	69.7
47564	3.5	286	6.6	209	417	70.6
47581	3.5	316	8.4	219	515	70.9
47589	3.5	312	7.6	237	574	68.7
47596	3.5	302	6.8	240	440	69.5
47170	3.5	292	9.1	241	452	71.9
47206	3.5	298	7.7	279	513	69.9
47229	3.5	300	6.9	258	-	-
47249	3.5	296	7.6	259	486	76.5
47316	3.5	298	7.9	257	562	68.4
Average		303	7.6	243	486	70.8

Table J. (continued)

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
47519	6.9	318	8.0	266	450	69.9
47553	6.9	306	-	206	-	-
47587	6.9	300	7.2	238	481	70.6
47605	6.9	318	7.6	264	561	68.8
47634	6.9	296	6.9	252	485	68.6
47272	6.9	344	8.3	283	608	68.6
47311	6.9	310	8.0	276	505	69.4
47336	6.9	340	9.0	299	526	69.6
47344	6.9	320	7.9	272	503	69.3
47379	6.9	326	8.0	290	419	69.3
Average		318	7.8	265	503	69.6
47194	8.3	304	7.6	270	629	66.1
47259	8.3	314	8.0	243	587	68.5
47295	8.3	348	8.3	290	586	68.4
47346	8.3	328	7.3	252	415	68.7
47339	8.3	302	5.4	201	475	-
47535	8.3	308	8.2	250	532	69.0
47566	8.3	325	7.8	290	437	70.0
47590	8.3	334	8.7	293	537	69.3
47598	8.3	308	7.9	254	483	69.9
47635	8.3	300	6.8	234	417	69.4
Average		317	7.6	258	501	68.7
47433	10.0	334	8.2	266	563	68.1
47541	10.0	334	7.9	271	679	67.2
47573	10.0	333	8.3	274	593	69.2
47582	10.0	330	-	-	-	-
47604	10.0	324	7.5	280	537	68.5
47237	10.0	336	8.5	278	500	69.7
47248	10.0	314	8.0	285	482	69.4
47273	10.0	346	8.6	295	580	67.9
47353	10.0	326	8.6	285	453	69.4
47419	10.0	352	8.8	283	551	68.9
Average		331	8.2	279	564	68.7

Table J. (concluded)

Rat number	Egg-protein per cent	Body weight	Liver			
			Fresh weight	Nitrogen	Fat	Per cent moisture
		gm.	gm.	mg.	mg.	gm.
47558	12.1	318	8.4	263	542	69.7
47579	12.1	322	8.2	267	605	68.4
47588	12.1	326	8.3	291	533	68.5
47610	12.1	316	7.5	237	536	67.8
47626	12.1	306	7.4	262	482	68.8
47418	12.1	330	9.0	291	594	68.7
47308	12.1	300	7.8	252	338	70.3
47368	12.1	334	7.9	258	467	68.7
47338	12.1	304	8.5	273	513	69.2
47377	12.1	302	7.9	271	626	66.9
Average		316	7.9	267	524	68.7
47169	18.2	344	7.6	285	504	68.5
47252	18.2	312	7.2	275	380	69.5
47258	18.2	332	7.1	228	383	69.4
47282	18.2	324	6.9	253	440	-
47287	18.2	324	7.8	313	477	69.2
47534	18.2	316	8.6	279	499	70.0
47559	18.2	338	8.8	293	557	68.6
47572	18.2	310	7.8	285	439	68.6
47603	18.2	316	8.3	287	455	69.3
47627	18.2	298	7.3	261	488	68.2
Average		321	7.8	276	463	68.9
47520	25.0	288	7.2	270	356	69.4
47565	25.0	298	6.9	251	432	69.0
47606	25.0	306	7.0	271	339	69.0
47625	25.0	308	7.3	267	373	69.4
47631	25.0	304	6.9	272	353	69.4
47667	25.0	274	7.0	256	362	69.4
47362	25.0	300	7.6	285	395	69.3
47354	25.0	308	8.4	297	454	68.9
47228	25.0	278	7.1	272	412	68.9
47198	25.0	274	7.6	272	365	67.9
47417	25.0	302	7.9	272	402	68.4
Average		295	7.4	271	385	68.8

Table K. Weights of fresh liver, liver nitrogen, liver fat and moisture in Series C, the positive and negative control animals

Rat number	Body weight	Liver			
		Fresh weight	Nitrogen	Fat	Per cent moisture
	gm.	gm.	mg.	mg.	gm.
		Steenbock XVII			
47124	352	9.0	381	525	68.5
47162	362	8.2	373	496	63.0
47168	340	-	-	-	-
47185	338	8.5	354	505	68.3
47193	320	8.0	313	588	69.3
47204	332	9.3	354	512	69.6
47230	350	9.4	386	-	69.2
47662	356	8.7	354	422	69.2
47664	320	9.0	-	434	68.0
47677	320	7.9	365	372	69.3
47729	308	7.6	321	405	68.8
47754	325	8.8	304	418	69.7
47685	316	9.3	357	524	69.0
47655	281	7.8	301	353	69.3
47661	276	7.5	295	433	68.3
47676	310	8.7	345	441	68.8
47686	300	6.2	322	393	70.0
47692	344	8.1	323	383	69.0
47726	320	9.1	347	436	70.0
47753	306	9.4	356	455	69.6
Average	322	8.6	342	453	68.8
		Nitrogen low diet			
47526	276	6.5	-	408	71.2
47543	272	6.0	221	333	70.9
47548	280	6.7	137	496	59.0
47595	270	6.0	208	481	71.2
47156	260	4.8	206	276	71.2
47171	284	6.7	212	453	70.0
47184	292	6.8	316	611	70.6
47221	264	6.0	206	372	71.9
47236	264	6.0	181	513	70.7
47250	278	6.9	201	616	69.2
47257	291	7.2	206	564	69.8
47265	294	6.9	224	527	70.1
47317	290	7.3	237	617	69.5
Average	281	6.8	206	510	69.7

Table L. Characteristics of blood in rats of Series B, the under-nourished animals

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
44722	1.5	250	6.9	-	13.7	-	-
44849	1.5	242	5.8	-	14.9	-	-
44856	1.5	245	5.8	-	17.2	-	--
44963	1.5	217	6.6	-	17.0	-	-
45890	1.5	284	-	16.4	-	-	-
45913	1.5	227	10.4	17.0	-	-	-
45949	1.5	240	7.8	13.9	-	-	-
46052	1.5	238	7.0	-	14.7	2.4	-
46067	1.5	249	7.6	16.8	-	-	-
46126	1.5	204	7.0	16.6	-	-	-
46127	1.5	274	-	-	14.5	-	8.2
46238	1.5	258	-	23.3	17.3	4.0	8.7
46268	1.5	250	-	17.8	15.7	2.8	8.1
46340	1.5	288	6.2	21.3	14.8	3.2	7.9
46346	1.5	272	-	18.9	15.4	2.9	7.8
46396	1.5	232	-	16.1	15.0	2.4	7.6
Average		247	6.8	17.8	15.3	2.8	8.1
45891	3.0	275	7.5	27.3	-	-	-
45941	3.0	243	5.0	-	-	-	-
45962	3.0	245	7.3	-	14.1	-	7.3
46039	3.0	212	7.6	15.9	-	-	-
46068	3.0	-	-	-	-	-	-
46125	3.0	270	7.2	-	15.6	-	9.4
46132	3.0	280	-	18.9	15.6	2.9	8.1
46155	3.0	282	6.1	25.8	16.2	4.2	8.5
46266	3.0	298	-	-	15.4	-	9.7
46274	3.0	230	7.3	22.8	16.5	3.8	8.3
46305	3.0	276	-	28.0	15.4	4.3	8.2
46341	3.0	290	7.1	25.8	14.5	3.8	7.4
46373	3.0	256	6.8	-	15.4	-	8.1
46387	3.0	266	6.1	20.0	15.1	3.1	7.5
46398	3.0	232	-	17.8	14.9	2.7	8.1
46439	3.0	300	-	20.4	15.2	3.1	8.2
Average		260	6.8	22.0	15.1	3.3	8.2

Table L. (continued)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
44724	3.6	268	7.1	-	14.9	-	-
44845	3.6	236	6.5	-	16.4	-	-
44858	3.6	258	5.9	-	15.5	-	-
44951	3.6	230	6.9	-	18.4	-	-
44980	3.6	217	6.6	-	15.4	-	-
Average		242	6.6	-	16.1	-	-
44729	4.6	286	7.7	-	16.1	-	-
44844	4.6	259	6.7	-	17.3	-	-
44863	4.6	225	7.7	-	17.4	-	-
44950	4.6	257	7.0	-	15.9	-	-
44981	4.6	230	6.4	-	14.8	-	-
Average		251	7.1	-	16.3	-	-
44733	6.7	309	7.0	-	15.2	-	-
44816	6.7	295	7.2	-	16.7	-	-
44865	6.7	302	6.4	-	14.3	-	-
44948	6.7	248	8.7	-	18.8	-	-
44985	6.7	261	6.8	-	15.5	-	-
45900	6.7	278	7.6	21.1	-	-	-
45940	6.7	223	6.5	14.4	-	-	-
45963	6.7	296	5.8	17.3	-	-	-
46032	6.7	252	6.9	17.3	-	-	-
46069	6.7	302	5.8	17.5	-	-	-
46210	6.7	294	-	-	15.1	2.9	-
Average		279	7.8	17.5	15.6	2.9	-
45014	8.6	261	6.9	-	16.0	-	-
44765	8.6	296	5.8	-	15.5	-	-
44812	8.6	220	8.6	-	17.6	-	-
44891	8.6	267	7.7	-	18.5	-	-
45901	8.6	343	4.3	14.6	-	-	-
45925	8.6	313	8.3	26.1	-	-	-
45964	8.6	290	-	18.7	14.4	2.7	-
46031	8.6	325	6.3	20.5	-	-	-
46070	8.6	283	6.0	17.0	-	-	-
46117	8.6	278	7.4	20.5	-	-	-
Average		283	7.5	19.5	15.7	2.7	-

Table L. (continued)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
44992	10.3	313	7.5	-	15.2	-	-
45012	10.3	283	6.7	-	16.7	-	-
44778	10.3	335	6.9	-	14.7	-	-
44811	10.3	303	7.0	-	16.4	-	-
45902	10.3	257	9.4	-	-	-	-
45924	10.3	295	8.4	19.8	-	-	-
45965	10.3	288	-	21.3	15.3	3.2	-
46023	10.3	328	8.7	-	-	-	-
46083	10.3	284	8.7	20.6	-	-	-
46102	10.3	260	6.3	21.3	-	-	-
46215	10.3	266	8.8	21.8	15.4	3.4	11.2
46261	10.3	302	9.5	15.6	14.9	2.3	10.2
46282	10.3	342	-	25.8	15.1	3.9	10.2
46332	10.3	332	-	22.3	15.3	3.4	10.4
46339	10.3	322	-	20.0	13.9	2.8	9.0
46382	10.3	336	-	24.5	16.1	4.0	10.2
46410	10.3	300	-	21.8	15.1	3.3	9.3
46437	10.3	288	-	14.4	14.3	2.1	9.9
Average		301	8.2	21.6	15.4	3.1	10.2
44980	12.3	297	7.1	-	16.1	-	-
44994	12.3	294	7.0	-	15.4	-	-
45010	12.3	240	7.3	-	18.4	-	-
44782	12.3	287	7.7	-	19.0	-	-
45903	12.3	314	6.3	19.8	-	-	-
45923	12.3	308	7.2	22.3	-	-	-
45977	12.3	285	6.1	17.3	-	-	-
46028	12.3	272	-	16.2	15.3	2.5	-
46088	12.3	278	5.2	14.4	-	-	-
46110	12.3	295	5.1	15.0	-	-	-
46216	12.3	294	8.9	17.2	14.9	2.6	10.1
46260	12.3	296	8.7	17.8	15.3	2.7	10.2
46283	12.3	322	-	-	15.8	-	10.4
46327	12.3	312	-	18.2	14.4	2.6	9.1
46369	12.3	296	-	16.3	14.2	2.3	9.3
46381	12.3	340	-	18.5	15.6	2.9	10.1
46412	12.3	288	-	17.8	14.5	2.6	9.4
46430	12.3	302	-	18.2	13.9	2.5	8.8
Average		293	8.3	17.8	15.9	2.6	9.7

Table L. (continued)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
45906	14.8	325	5.4	17.5	-	-	-
45920	14.8	330	-	-	-	-	-
45978	14.8	245	-	15.1	15.3	2.3	7.9
46024	14.8	310	6.8	21.2	-	-	-
46104	14.8	320	-	19.7	15.9	3.1	7.8
46096	14.8	244	6.3	15.3	-	-	-
46217	14.8	238	8.5	18.2	18.2	3.3	11.2
46259	14.8	300	7.3	20.4	15.1	3.1	9.9
46288	14.8	330	5.8	18.2	16.1	2.9	11.2
46326	14.8	332	6.8	21.8	15.8	3.5	10.5
46348	14.8	350	6.6	21.8	14.8	3.2	9.4
46370	14.8	290	7.1	20.4	15.2	3.1	9.5
46415	14.8	350	-	-	15.2	-	9.9
46429	14.8	-	6.4	19.2	15.8	3.0	10.1
46441	14.8	332	-	-	14.5	-	8.3
Average		305	7.3	19.0	15.5	3.0	9.0
44897	18.0	313	7.1	-	16.5	-	-
44925	18.0	230	7.0	-	17.3	-	-
44995	18.0	266	7.0	-	15.7	-	-
45009	18.0	266	6.6	-	17.1	-	-
45907	18.0	333	6.6	22.0	-	-	-
45914	18.0	344	-	-	-	-	-
46018	18.0	270	-	-	-	-	-
46030	18.0	280	-	18.2	15.7	2.9	-
46091	18.0	313	5.3	16.6	-	-	-
46112	18.0	288	7.6	21.9	-	-	-
46232	18.0	286	-	15.8	16.5	2.3	10.2
46251	18.0	302	-	15.1	15.3	2.3	9.9
46289	18.0	308	-	17.5	15.2	2.7	9.4
46318	18.0	276	-	17.5	15.2	2.7	9.7
46325	18.0	308	-	17.5	16.0	2.8	9.8
46367	18.0	282	-	18.9	14.7	2.8	9.1
46416	18.0	274	-	17.8	15.8	2.8	9.5
Average		285	7.2	18.3	16.0	2.8	9.7

Table L. (concluded)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
45911	25.7	290	7.9	14.4	-	-	-
46019	25.7	275	8.6	17.3	-	-	-
46022	25.7	282	7.8	-	16.2	-	-
46097	25.7	282	7.7	16.6	15.9	2.65	8.0
45961	25.7	229	8.0	15.0	-	-	8.5
46090	25.7	280	7.9	15.9	-	-	-
46241	25.7	282	5.7	14.4	16.2	2.3	8.9
46267	25.7	298	-	-	14.6	-	7.9
46317	25.7	288	-	16.9	16.6	2.6	8.3
46367	25.7	342	-	14.4	15.7	2.3	9.1
46368	25.7	298	7.2	13.1	15.1	2.0	8.1
46380	25.7	296	-	20.0	16.8	3.4	9.1
46417	25.7	330	-	16.9	14.9	2.5	8.3
46424	25.7	264	-	15.8	15.6	2.5	8.7
46442	25.7	294	-	14.4	16.1	2.3	8.8
Average		286	7.2	15.7	15.9	2.6	8.4

Table M. Characteristics of blood in rats of Series B, the well-nourished animals

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood.
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
47186	1.6	290	8.0	16.7	16.6	2.6	11.1
47192	1.6	258	-	15.0	16.6	2.3	10.6
47302	1.6	250	8.0	-	15.8	-	9.3
47318	1.6	260	-	14.4	16.4	2.4	9.1
47330	1.6	280	7.9	15.4	16.4	2.5	9.3
47513	1.6	276	6.7	-	17.2	-	9.8
47583	1.6	266	7.6	117.9	18.1	3.3	9.8
47591	1.6	270	7.4	21.0	14.9	3.1	8.4
47633	1.6	262	7.4	18.6	15.3	2.9	8.6
47656	1.6	270	6.7	18.1	15.0	2.7	8.3
Average		268	7.5	15.5	16.2	2.7	9.4
47251	3.2	300	7.2	16.5	17.2	2.8	8.6
47256	3.2	308	8.4	16.7	15.9	2.7	8.6
47369	3.2	302	7.0	16.5	13.3	2.2	9.0
47191	3.2	300	7.9	-	16.1	-	8.4
47376	3.2	280	7.7	15.6	17.8	2.8	8.4
47518	3.2	316	7.4	-	16.8	-	9.5
47525	3.2	322	7.1	14.5	16.5	2.4	10.6
47540	3.2	316	7.5	16.1	15.5	2.5	8.3
47580	3.2	324	7.3	14.3	16.8	2.4	9.6
47636	3.2	310	7.8	13.2	16.8	2.2	-
Average		308	7.5	15.5	16.3	2.5	9.0
47170	3.5	340	9.1	17.2	14.8	2.6	9.9
47206	3.5	296	9.2	16.5	17.9	3.0	9.4
47229	3.5	318	9.0	-	15.5	-	9.4
47249	3.5	316	9.1	16.9	16.8	2.8	9.8
47316	3.5	302	8.1	16.9	16.5	2.8	9.8
47483	3.5	298	-	-	18.6	-	10.5
47564	3.5	302	8.4	15.4	16.4	2.5	8.5
47581	3.5	302	8.4	15.3	17.0	2.6	9.1
47589	3.5	300	8.1	15.4	16.8	2.6	9.3
47596	3.5	300	10.4	-	17.1	-	-
Average		309	8.0	16.1	16.8	2.7	9.5

Table M. (continued)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
47272	6.9	320	7.4	17.7	16.3	2.9	9.6
47311	6.9	320	8.3	17.2	17.3	3.0	9.4
47336	6.9	306	7.7	-	15.6	-	8.6
47344	6.9	320	7.3	17.7	15.3	2.7	8.5
47379	6.9	300	-	16.5	-	-	9.2
47519	6.9	346	7.9	22.3	15.7	3.2	9.8
47553	6.9	316	7.6	16.6	16.1	2.7	9.0
47587	6.9	342	6.9	21.3	15.6	3.3	9.4
47605	6.9	328	7.2	17.7	15.6	2.8	-
47634	6.9	336	7.4	-	14.8	-	-
Average		325	7.6	18.4	15.9	2.9	9.2
47194	8.3	310	8.9	16.2	17.6	2.9	9.1
47259	8.3	326	9.4	16.9	15.7	2.7	9.3
47295	8.3	350	9.4	-	16.5	-	9.4
47346	8.3	346	8.4	17.5	17.8	3.1	9.4
47339	8.3	306	9.4	16.0	17.5	2.8	9.5
47535	8.3	308	9.1	16.0	16.0	2.6	9.3
47566	8.3	334	7.1	16.6	16.4	2.7	9.4
47590	8.3	340	7.6	16.4	16.4	2.7	9.0
47598	8.3	314	8.7	16.0	16.4	2.6	9.0
47635	8.3	308	8.2	-	16.2	-	-
Average		324	8.6	16.5	16.7	2.5	9.3
47237	10.0	342	7.7	26.0	16.5	4.3	9.5
47248	10.0	342	7.9	30.8	17.2	5.3	9.2
47273	10.0	336	7.4	-	16.3	-	9.0
47353	10.0	336	7.5	22.5	16.5	3.7	10.0
47419	10.0	334	7.8	21.6	14.7	3.2	9.0
47433	10.0	332	7.7	26.2	16.7	4.4	9.5
47541	10.0	316	7.6	23.0	15.7	3.6	9.0
47573	10.0	342	7.4	28.3	15.7	4.4	9.2
47582	10.0	324	7.7	-	16.5	-	9.3
47604	10.0	336	7.1	27.5	15.5	4.3	7.3
Average		334	7.6	25.4	16.1	4.2	9.1

Table M. (concluded)

Rat number	Egg-protein per cent	Body weight	Serum protein concentration	Blood volume	Hemoglobin		Erythrocytes per cmm. of blood
					Concentration	Total weight	
		gm.	gm.%	ml.	gm.%	gm.	millions
47418	12.1	322	7.9	16.7	14.7	2.5	8.6
47308	12.1	322	8.1	16.9	17.1	2.9	9.0
47368	12.1	330	7.7	-	17.3	-	8.3
47338	12.1	322	7.4	16.5	16.5	2.7	9.1
47377	12.1	312	-	15.6	15.3	2.4	8.3
47558	12.1	322	7.8	16.0	16.4	2.6	9.8
47579	12.1	308	7.9	16.2	17.0	2.8	9.8
47588	12.1	332	7.9	15.7	14.8	2.3	8.6
47610	12.1	302	7.6	16.4	16.5	2.7	9.8
47626	12.1	300	7.0	-	-	-	-
Average		318	7.7	16.3	16.2	2.4	8.9
47169	18.0	360	-	19.9	17.2	3.2	10.0
47252	18.0	322	-	17.7	16.6	2.9	10.3
47258	18.0	346	8.8	-	16.8	-	9.4
47282	18.0	326	8.9	18.2	16.4	3.0	9.4
47287	18.0	326	8.4	18.2	15.3	2.8	9.3
47534	18.0	310	6.9	20.4	18.1	3.7	11.4
47559	18.0	328	6.9	22.5	16.2	3.7	8.4
47572	18.0	308	7.3	18.9	16.4	3.1	8.7
47603	18.0	312	7.4	20.4	16.5	3.4	8.9
47627	18.0	300	7.3	-	16.8	-	8.1
Average		324	8.0	19.6	16.7	3.2	9.4
47362	25.0	292	-	20.9	16.9	3.5	9.2
47354	25.0	304	7.7	20.9	17.1	3.6	9.3
47223	25.0	320	8.5	21.3	17.3	3.7	8.5
47198	25.0	312	-	-	16.5	-	8.5
47417	25.0	306	7.7	20.9	15.3	3.2	8.9
47520	25.0	276	6.5	21.2	16.8	3.6	9.8
47565	25.0	302	6.7	20.6	18.6	3.8	8.4
47606	25.0	314	7.4	23.3	17.0	4.0	8.1
47625	25.0	282	6.7	22.2	16.5	3.7	8.9
47631	25.0	280	7.5	-	19.0	-	8.7
47667	25.0	306	6.9	23.3	17.3	4.0	8.8
Average		306	7.5	21.7	17.1	3.7	8.8

Table N. Characteristic of blood in rats of Series C, the positive and negative control animals

Rat number	Body weight	Serum protein concentration	Blood volume	Hemoglobin Concentration	Hemoglobin Total weight	Erythrocytes per cmm. of blood
	gm.	gm.%	ml.	gm.%	gm.	millions
			Steenbock XVII diet			
47124	354	7.3	19.8	17.4	3.4	9.2
47135	362	7.6	20.5	16.8	3.4	8.8
47162	338	7.0	-	16.3	-	8.4
47168	336	7.0	15.8	15.9	2.5	8.3
47185	316	6.9	16.0	15.9	2.5	7.9
47193	328	7.1	15.8	16.0	2.5	7.9
47204	346	7.1	21.0	16.3	3.4	9.0
47230	350	7.3	19.8	17.5	3.5	8.9
47662	328	6.9	16.4	15.8	2.6	9.1
47664	326	6.9	16.4	15.8	2.6	9.1
47677	326	7.4	-	15.6	-	9.2
47729	330	6.9	-	15.8	-	9.3
44754	326	7.2	16.4	15.7	2.6	9.0
47685	284	6.8	16.1	15.8	2.5	8.5
47655	280	7.1	16.1	15.0	2.4	8.8
47661	316	7.1	16.0	15.2	2.4	9.3
47676	302	7.0	17.0	15.7	2.7	9.2
47686	308	7.8	-	15.4	-	8.4
47692	352	6.7	18.8	14.9	2.8	-
47726	324	7.0	16.4	16.5	2.8	-
47753	312	-	15.9	16.0	2.5	-
Average	326	7.2	17.2	15.9	2.8	8.2
			Nitrogen low diet			
47156	268	6.6	14.9	15.5	2.3	9.4
47171	268	6.6	13.6	16.8	2.3	8.6
47184	276	6.5	16.1	16.0	2.6	9.2
47221	266	6.5	-	16.2	-	9.1
47236	258	6.7	13.0	15.2	2.0	8.9
47250	280	6.7	18.8	15.6	2.9	8.9
47257	284	-	13.6	16.6	2.3	9.5
47265	256	-	17.6	16.8	3.0	8.1
47317	256	6.5	15.5	15.9	2.5	8.3
47526	286	6.6	-	16.3	-	8.5
47543	296	6.6	-	15.9	-	8.6
47548	300	6.7	18.4	16.2	3.0	9.0
47595	296	6.7	16.3	16.0	2.6	-
Average	282	6.7	16.4	16.1	2.6	8.9