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MEAT IN NUTRITION. XIII. THE BIOLOGICAL VALUE OF CANNED AUTOCLAVED PORK MUSCLE

by

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INTRODUCTION

It was not until the last decade of the nineteenth century that physiologists became aware of the significance of differences discovered in the composition and chemical behavior of the proteins and suspected that the uniform nutritional value heretofore attributed to them was an erroneous assumption. Rubner (1897) was probably the first to postulate that proteins may vary in physiological usefulness. It is now a well known fact that proteins from various sources differ in the extent to which they meet the demands of the organism for this nutrient.

Until 1900 very little was known about the quantitative distribution of the amino acids in the proteins. Thus, it is not surprising that there was little appreciation of their relation to the nutritive value of the proteins and that in many nutrition experiments, quantity rather than quality was emphasized. At this time, Fischer as well as Kossel and his co-workers developed chemical techniques for the isolation of the di-amino and the mono-amino acids. Results obtained with the use of these new methods soon showed that the amino acid content of the proteins varied widely. For example, gliadin was found deficient in lysine (Osborne and Mendel, '11; and Van Slyke, '11) and gelatine was proved entirely

devoid of cystine, valine, isoleucine, tyrosine, tryptophane and hydroxyglutamic acid (Dakin, '20).

With these findings in mind, Osborne and Mendel ('12) initiated the classical studies which led to the development of the concept, "indispensable amino acids." The biological feeding experiment as developed by them, in spite of certain limitations, has yielded results of great significance in studies pertaining to the nutritive value of the proteins. Early investigations involved the use of diets in which the nitrogen was supplied in the form of an isolated protein. In 1912, Osborne and Mendel demonstrated that rats maintained their weight over a long period of time but did not grow when gliadin was the sole source of protein in the test ration. Inability to grow, they believed, was due to the lack of lysine since gliadin had been found deficient in this amino acid by chemical analysis. These early studies also presented evidence of the dietary importance of tryptophane and lysine (Osborne and Mendel, '14). The rôle played by these amino acids in maintaining good nutrition was found to be different, tryptophane being essential for maintenance of body weight and lysine for growth.

In 1915, Osborne and Mendel found that cystine was also essential and at the same time pointed out that the quantity of amino acid present in a protein was important. Casein fed at a high level (18 per cent) was adequate, whereas growth

was retarded when it was incorporated in the diet at a level of 9 per cent. With the work of Osborne and Mendel as a background, Sherman ('25) demonstrated that growth was a linear function of the quantity of cystine in the diet.

These experiments led to differentiation of proteins into three classes defined by Sherman ('37) as (1) "Complete"; maintaining life and providing for normal growth of the young when used as the sole protein food; (2) "Partially incomplete": maintaining life but not supporting normal growth; and (3) "Incomplete"; incapable either of maintaining life or of supporting growth when fed as the sole protein.

Rose and his co-workers have amplified the early experiments in a most note-worthy manner. The results have been summarized recently by Dr. Rose ('38). Mixtures of purified amino acids were used as the sole source of nitrogen in the ration in all of their experiments. Their work has clarified the nutritional status of the various amino acids in the diet.

Investigators were not content to allow studies pertaining to the nutritive value of the proteins to stop with qualitative studies of the type heretofore described. They soon stepped to the quantitative estimations. As a result there have appeared in the literature methods for evaluating numerically the "biological value" of a protein.

REVIEW OF LITERATURE

Development of the Method for the Determination of the Biological Value of Proteins

The early studies of Osborne and Mendel with isolated proteins, the improvement of methods used for the estimation of the quantitative distribution of the amino acids, and the development of the "nitrogen-balance" technique have formed a foundation upon which has been built a long series of experiments, designed to measure the so-called "biological value of protein."

However, investigations up to the present time have yielded information inadequate from several standpoints. For instance, in the experiments which were discussed in the previous section, growth and maintenance were used as the criteria for judging the findings and have left the questions of the ability of proteins to meet the physiological demands of reproduction, lactation, middle age, and old age all unanswered. As early as 1920, Osborne and Mendel pointed out the importance of these factors but they believed even though the tests were not made that "... there are too many other nutritive factors involved in successful nutrition to enable

us, upon the basis of our present knowledge, to charge any failures of nutrition in the second generation to chemical inadequacy of protein. ... It seems to us, however, that if an animal is able to attain adult size upon a diet which furnishes protein from a single source, the nutritive value of this protein is clearly established."* The adequacy of specific proteins for reproduction, lactation and the maintenance of a normal life span is still an unexplored field and all of our present concepts of protein values need revaluation in the light of these factors. Experimental findings obtained in experiments of this wider sort today are not as likely to be obscured as they were in the time of Osborne and Mendel since we appreciate more clearly than formerly the food factors necessary for the support of good nutrition.

Not only were the physiological indices used for judging the value of the protein inadequate in the early experiments but they were qualitative rather than quantitative in nature. Great progress has been made in developing experiments for the evaluation of the biological value of proteins from this standpoint. McCollum and Davis ('15) were among the first to introduce the quantitative viewpoint in an attempt to more accurately compare the nutritional efficiency of one protein with another. They demonstrated that with rations of the same

*Osborne, T. B., and Mendel, L. B., J. Biol. Chem., 41; p. 279. (1920).

energy value, 6 per cent of milk protein was adequate for growth whereas the proteins of the whole wheat kernel fed at the same level supported growth at a subnormal rate, equal to that produced by the incorporation of as little as 4 per cent of milk protein.

In 1916, Osborne and Mendel, too, felt the need of a more refined method for the estimation of the biological value of a protein. They attempted to compare the value of proteins for growth by feeding the same quantity of food for a certain interval, the protein factor being the only variable. However, they found it difficult to force the animals to eat the same amount of food and in 1919, Osborne, Mendel,and Ferry developed a method whereby the relative value of proteins for growth could be expressed numerically. They reasoned that "Since food intake is quite closely regulated by the calorific requirements of the animal approximately as much food is eaten under otherwise similar conditions whether this contains a high or low percentage of protein."* They, therefore, fed diets containing varying percentages of the same proteins and then evaluated results in terms of the gain in weight made per gram of protein ingested. Thus, they were able to estimate the relative growth-promoting power of different proteins, and they found that proteins varied widely. However, Sherman ('20) in his work

*Osborne, T. B., Mendel, L. B., and Ferry, E. L., J. Biol. Chem. 37; p. 223, (1919).

on protein requirement for maintenance pointed out that proteins which vary in growth-promoting power may be more nearly equally efficient for maintenance of normal weight in adults.

Hoagland and Snider ('26) have used the method of Osborne, Mendel, and Ferry ('19) extensively in the estimation of the nutritional efficiency of many proteins. Their work illustrated the relative ease with which this may be done when the protein is of good quality as well as the difficulties met in obtaining comparable values for inferior proteins. For example, test animals which received beef extract as the source of protein made negative total gains (range - 2 gm. to -63 gm.). Thus, they were unable to assign a numerical value to this protein. On the other hand, when veal was the source of protein, the animal added weight in a consistent manner so that when growth was expressed in terms of gains made per gram of protein intake, the range in results was very small (2.24 gm. to 2.42 gm.).

From these findings it is evident that a method whereby the "low" proteins could be evaluated was needed. The nitrogen balance method has proved to be such a method. However, the nitrogen balance method does not allow the estimation of the comparative efficiency of the proteins for the support of vital life functions such as growth, reproduction, and lactation inasmuch as it permits evaluations in terms of maintenance only. Seegers ('37) is the only investigator

who has attempted a solution of the many problems involved in the utilization of protein during gestation.

Thomas ('09) introduced the term "biological value" and was the first to conceive the idea of determining the value of a protein by the nitrogen balance method. He developed the technique, using himself as the experimental subject. He early recognized the necessity of accounting for the "wear and tear" quota of body protein. In order to determine this latter value he fed himself a nitrogen-free diet during which time he determined the daily output of nitrogen in the feces and the urine. These values he believed represented his minimum protein requirement and that theoretically, it was necessary that the diet provide at least this much protein, so as to prevent body loss of nitrogen. During the next period he supplemented the nitrogen-free diet with a given amount of the protein food under examination and again determined the nitrogen output in both urine and feces. The difference in the quantity of nitrogen excreted in the urine under the two conditions represented the amount of food nitrogen excreted by way of the urine. From the results, Thomas calculated the biological value of the protein, which he defined as the number of parts of body nitrogen replaceable by 100 parts of the nitrogen of the protein food. He used three methods of calculation, each differing from the other according to the way he dealt with

the nitrogen of the feces. He recognized that part of this nitrogen was the result of endogenous metabolism and that part of it came from undigested food. However, he did not have a satisfactory method of differentiating these two fractions. He investigated 16 foods and assigned the biological value of 100 per cent to milk, meat and fish and used these as the "measuring stick" for his other proteins. His technique was in many ways faulty. For example, the experimental periods varied in length with no preliminary periods and no attempt was made to insure a constant intake of either nitrogen or calories from day to day, nor were the different forms of nitrogen fed at comparable levels. Thomas believed that protein is better utilized when ingested in small portions several times during the day than when all of it is eaten at one meal. However, he practiced this distributed intake in only a few of his experiments. Also, in calculating the biological value of his proteins he selected data arbitrarily discarding certain data without any apparent reason. However, in spite of all this, his ideas form the basis for subsequent methods developed for establishing the biological value of proteins by the so-called "balance sheet method."

Method of Determining Biological Value of Protein

Development of technique

General considerations. In spite of the fact that Thomas developed the idea of the biological value in 1909, it was not until 1924 that Mitchell applied the technique to the biological feeding experiment, and presented a method which has been followed since that time with but few alterations. He used the rat as his test animal. The formula which Mitchell used when stated in its simplest form expressed biological value as 100 x $\frac{\text{Body N spared}}{\text{Food N absorbed}}$ in which "Body N spared" was the difference between the absorbed nitrogen and the nitrogen of endogenous origin. Certain specific data must be collected for the calculation of the biological value of a protein by this formula. They are: (1) the nitrogen in the feces of endogenous origin; (2) the nitrogen of the feces of exogenous origin; (3) the nitrogen in the urine of endogenous origin; (4) the nitrogen in the urine of exogenous origin; and (5) the nitrogen of the food absorbed.

In order to obtain these data the assay period is divided into two parts. In the first interval, a low nitrogen diet is fed for a period sufficiently long to establish

equilibrium. Data secured during this period for the quantity of nitrogen in the food, feces, and urine, are used as a measure of the nitrogen resulting from tissue metabolism. In the second part of the assay, the food protein under study is offered as the sole source of nitrogen in the test ration. Nitrogen analysis of the food consumed and of the urine and feces provided the necessary data for the calculation of the biological values.

In order to obtain trustworthy results, the nitrogenous metabolism study must be conducted under certain definite conditions. These conditions as given by Mitchell are: (1) the diet must contain only the protein (or mixture of proteins) that is being investigated; (2) the diets must contain no non-protein nitrogenous substances except those present in the food under investigation; and (3) the food intake must be so adjusted as to composition and amount that dietary protein will not be used as source of energy.

In addition to these considerations, investigators interested in the development of this type of experiment have found that certain details of experimental procedure may influence the validity of results. These are: methods used for the estimations of metabolic fecal nitrogen and urinary nitrogen of body origin, the kind of experimental diets used in the assay periods, and the relative lengths of the nitrogen-free and protein-feeding periods.

Determination of metabolic fecal nitrogen. Mitchell ('24) states, "The 'biological value' of a protein, as the term was applied originally by Karl Thomas, referred to the utilization by the body of the products of protein digestion. The biological value was expressed as the percentage of the absorbed nitrogen which was retained by the body for repair or the construction of nitrogenous tissue. Hence, differences of digestibility of different proteins did not affect their biological values."* Indirectly, however, the influence of the digestibility is felt in the calculation of the absorbed nitrogen. This involves the estimation of nitrogen eliminated in the feces. However, this excretion contains nitrogen from two sources, i.e., (1) undigested food, and (2) nitrogenous material derived from bacteria, epithelial cells, the digestive secretions, bile and mucus, and under abnormal conditions pus and blood. The nitrogen from these latter sources is known as the metabolic fecal nitrogen and must be differentiated. Investigators disagree on the constancy of the factor. Martin and Robinson ('22) measured the fraction by the determination of the daily excretion of nitrogen when a diet practically devoid of the element was fed. Although they assumed in their calculations that the metabolic fecal nitrogen was constant regardless of the quantity of food consumed, these workers make the

*Mitchell, H. H., J. Biol. Chem., 58; p. 878 (1924).

statement that they believe the failure to take the metabolic nitrogen of the feces into account was a factor limiting the accuracy in biological values determined by Thomas.

Mitchell ('24) is of the opinion that the metabolic fecal nitrogen is closely related to the quantity of food consumed. Schneider ('34) has presented 1160 determinations, many of which were taken from the literature, to support the view that the fecal metabolic nitrogen in the rat consists of two fractions, one related to the body weight and the other to the intake of dry food. Adolph and Wu ('34), however, considered the intake of dry food as the only contributing factor and even went so far as to use a constant figure, 1.4 gm., to express the metabolic fecal nitrogen per gram of food intake for all intake levels throughout their experiments.

On the other hand, Chick, Hutchinson, and Jackson ('35) have found only a rough general proportionality between fecal nitrogen and the intake of food upon a low nitrogen diet. They write "Seeing that in any case the amount of the fecal endogenous nitrogen has relatively little influence on the value of the expression used for calculating the biological value..., we have taken the mean value of the actual faecal nitrogen excreted in all the nitrogen-free experiments performed on any rat, as a reasonably good estimate of the endogenous faecal nitrogen for that rat,..."*

*Chick, H., Hutchinson, J.C.D., and Jackson, H.M., Biochem. J., 29; p. 1708 (1935).

This idea of a lack of correlation between level of food intake and fecal endogenous nitrogen has been supported by French and Mattill ('36).

Seegers and Mattill ('35) have avoided the controversy by attempting to feed the nitrogenous ration at the same level as the non-nitrogenous diet.

Another factor which influences the quantity of metabolic nitrogen appearing in the feces is the amount of roughage in the diet (Mitchell, '24; and Adolph and Wu, '34). Its importance will be discussed in a later section.

Mitchell ('24) has summarized the relative importance of the metabolic fecal nitrogen figure in calculating the biological value in the following paragraph, in which he agrees in his general conclusions with Chick, Hutchinson, and Jackson ('35):

> "An unavoidable error enters into the method here, but is not thought to be serious, since an underestimation of the metabolic nitrogen in a period of protein feeding would lead to an overestimation both of the food nitrogen retained in the body and of the absorbed nitrogen, the numerator and the denominator, respectively, of the fraction determining the biological value sought after." *

Estimation of urinary nitrogen of body origin. The quantity of nitrogen in the urine resulting from the catabolism of dietary protein is complicated by the fact that

*Mitchell, H. H., J. Biol. Chem., 58; p. 880 (1924).

the urine also contains nitrogen resulting from the catabolism of the body tissues as well as that coming from food sources. Since the two types, i.e., the endogenous and exogenous nitrogen, cannot be separated by chemical means, they must be distinguished by an indirect method. The method suggested by Thomas ('09) and later by Martin and Robinson ('22) was to estimate the nitrogen arising from endogenous sources by the feeding of a nitrogen-free diet. Theoretically when such a diet contains sufficient calories, it prevents the oxidation of body protein for energy and it is assumed that the nitrogen which appears in the urine represents the quantity catabolized in the daily wear and tear of body tissue. Mitchell ('24), in developing his method, used data thus obtained to represent the urinary nitrogen of body origin for the determination of biological values. When this method of calculation is used it must be assumed that the basal metabolic rate of an animal is constant at all times because as Smuts ('35) has shown there is a close relationship between the total endogenous excretion of nitrogen in the urine of the warm-blodded animal and its basal metabolism. He believed that the nitrogen catabolism of any species of animal may be calculated from its basal heat production. Forbes, Kriss, and Miller ('34) have found that within certain limits there is a progressive increase in the rate of rise of heat production as food consumption is increased. Therefore, the food intake during the nitrogen-free period should be relatively the same as during the

test period if the endogenous urine nitrogen of the first period is to be used as a measure of the endogenous nitrogen in the latter period. Also, it must be assumed that the catabolism continues at the same rate when protein is fed as it does when no protein is included in the diet. However, it is known that the quantity of protein in the diet may affect the basal metabolism of rats. Horst, Mendel, and Benedict ('33) for instance, found no significant difference in the basal metabolism of rats fed diets of high protein and medium protein content, but when the rats were given a diet of low protein value, the basal rate was definitely lowered.

Since early times the influence of muscular exercise on protein metabolism has been discussed and many conflicting opinions have been advanced. Mitchell, Beadles, and Kruger in 1927 studied the question extensively and their results showed that considerable muscular effort, either static or motive, can be performed with no appreciable increase in the total endogenous urinary nitrogen. Therefore, the activity or nervous tension of the animal should not affect the nitrogen excretion. The above workers did find, however, that if the food consumed did not furnish sufficient energy for the specific activity, the muscle tissue itself was sacrificed to the point where there was an apparent increase in the quantity of metabolic nitrogen in the urine.

This again indicates the need for adequate calories in formulating the experimental diets used in this type of experiment.

Another factor (Ashworth and Brody, '33; Chick, Hutchinson, and Jackson, '35; and French and Mattill, '36) which has been found to affect the quantity of endogenous nitrogen in the urine is the length of time the animal is maintained on the nitrogen-free diet. These workers all observed that the excretion of endogenous nitrogen tended to diminish with time. Chick and her co-workers believed that a nitrogen-free period, two to three days long, was adequate since it included the initial rapid fall in nitrogen excretion. French and Mattill ('36) found that for adult animals and for mature animals, four days were necessary before collections could be started in the nitrogen-low feeding period but that an interval this long was not sufficient preparation for the adolescent. In the latter group of rats, constancy was not established even in ten days.

When these factors are considered, it would seem, as Chick and her co-workers ('35) so cogently state, "The estimated endogenous urinary nitrogen (as required for the calculation of biological value of proteins by the balance sheet method) must be regarded as a somewhat arbitrary quantity."*

*Chick, H., Hutchinson, J.C.D., and Jackson, H. M., Biochem. J., 29; p. 1707, (1935).

Experimental diets used in the assay period. The success or failure of the nitrogen balance determination for the estimation of the biological value of protein depends to a large degree upon the proper selection of the experimental ration. Two types of diets are needed, (1) the low-nitrogen diet for the determination of nitrogen of endogenous origin and, (2) the diet containing the protein to be assayed.

The formulation of the low-nitrogen diet presents the more serious difficulty. Since the animals receiving this diet rapidly lose their appetites, it must be chosen with great care. Many workers use diets containing no protein but adequate in all of the other necessary food constituents. In this case, Boas-Fixsen and her co-workers ('30) have emphasized the necessity of preventing loss of appetite by supplying sufficient vitamin B.

Mitchell ('24), however, has advocated the use of 4.0 per cent whole egg in the low-nitrogen diet as this protein has a very high biological value, i.e., 96 and, thus, does not affect the endogenous nitrogen. This type of diet was also used by Morgan ('31) in some of her experiments for the determination of the quantity of nitrogen excreted of endogenous origin. The quantity of protein that should be included in the diet in the second phase of the assay period has been discussed by certain investigators. Thomas, in his early work ('09), assumed that the protein was used

to the same degree regardless of the level of intake but it has since been pointed out by Boas-Fixsen ('35) that unless all of the absorbed nitrogen is built up into a compound such as "Vorratseiweiss," this assumption cannot be justified. Mitchell ('24), Morgan ('31), and Boas-Fixsen and Jackson ('32) have all found that the biological value of a protein decreases as the percentage of protein in the test ration is increased. Therefore, when the biological value of two proteins are to be compared, determinations must be made at the same level of intake. It is advisable to use a low level (5 per cent) since the proteins do not seem to be metabolized economically at the higher levels (Chick, Hutchinson, and Jackson, '35).

The inclusion of the vitamin B-complex in both the low-nitrogen diet and the diet containing the test protein has presented a serious difficulty in the past because all known rich sources of the nutrient are high in nitrogen. Yeast is such a food and one that workers have been forced to rely upon for supplying vitamin B. When it is added to the diet a considerable quantity of a protein of uncertain biological value is introduced. Various values have been ascribed to yeast proteins; for example, Still and Kock ('28) have assigned a biological value of 24 to yeast proteins in which case its use would lower the value of the protein being assayed. On the other hand, Mitchell ('24) reported a bio-

logical value of 85. If the latter value is correct, the inclusion of a small amount of yeast protein in the diet should not exert a measurable influence on the biological value of the protein being tested.

The importance of the other vitamins in relation to nitrogen metabolism has not been thoroughly investigated. Boas-Fixsen ('30) has shown that vitamin B_2 does not exert an influence on the economical use of ingested nitrogen. The work of Morgan and Osburn ('25) has indicated that vitamin A is necessary for normal nitrogen metabolism. Since very few studies have been made on this topic, it seems wise to include sufficient quantities of vitamins A, D, and B-complex in the test ration.

It is agreed by all workers that the intake of calories in the form of carbohydrate and fat must be great enough to prevent the use of food protein or body protein for fuel. The question has been raised as to the proportion of each of these that should be included in the two test diets needed for conducting the experiment because in certain abnormal nutritional states, the amount of carbohydrate ingested affects the intensity of the protein catabolism. During periods of nitrogen hunger or in fasting (Murlin, '07; and Cathcart, '09), an excessive carbohydrate intake prevents the breakdown of body protein. Likewise, in hyper-thyroidism and febrile conditions, nitrogen equilibrium can be obtained by

feeding diets containing large amounts of carbohydrates (Shaffer and Coleman, '09; and Kendall, '29). Under normal conditions, extra carbohydrate spares protein if the two are ingested at the same time (Larson and Chaikoff '37). To prevent too great a sparing action, it seems advisable to furnish considerable of the calories in the form of fat. Mitchell ('34) has shown that while substitution of fat for starch in the diet decreased its digestibility, the excretion of metabolic nitrogen in the feces was not altered.

It has been stated that the quantity and quality of the roughage in the diet affects the digestibility of the protein. Adolph and Wu ('34) believed that this was due to the laxative influence since crude fiber from different sources did not have the same effect. For this reason the only variation in the contents of the low-nitrogen diet and the test diet should be the source of the protein studied.

Age and size of the test animal employed. The biological values recorded in the literature have been determined with the use of rats ranging in age from the very young to the adult animal. Mitchell used animals varying from 60 to 250 gms. in weight for establishing the details of his method. Morgan ('31) likewise apparently believed that age made no appreciable difference in the final result, for she used rats of all ages in her experiments. Boas-Fixsen and her co-

workers have employed the adult animal in all of their determinations.

Although the workers are not consistent in the type of the test animals used, age is a factor that we feel should be considered. Mitchell ('24) using young rats obtained a decrease in the biological value when the percentage of the protein was increased in the diet. He thought that this observation might be explained by assuming that protein can be used more economically for the maintenance of body tissue than for growth. Thus, when the protein is supplied in the diet at a low level, almost all is used for maintenance and as the percentage is increased, more and more of it is used for growth. When the younger animal is used the physiological effect of both maintenance and growth on the biological value must be considered, whereas, with the adult animal the influence of maintenance, only, enters into the picture. French and Mattill ('36) found that the adolescent animal apparently never reached a constant endogenous nitrogen excretion. They ascribed this non-attainment of an approximate constancy to a heightened activity of certain of the endocrine glands in the growth period, that speeds up and complicates protein metabolism. It is doubtful if any biological value obtained with the adolescent animal possesses any significance.

Test periods. In conducting nitrogen balance experi-

ments, two types of test periods are necessary, (1) the "nitrogen-free" period, and (2) the period in which the animal receives the protein to be assayed. The relative sequence of these periods in the experimental set-up and the length of each apparently have a decided influence upon the final results. Both periods are preceded by a "preliminary period" when the animals receive the specific test ration for a short time before the actual collection of metabolic products is initiated. This plan eliminates the influence of previous feeding on the nitrogen excretion.

When the animal receives a diet very low in nitrogen, problems arise because the food intake (Mason and Palmer, '35) progressively decreases. Therefore, from this point of view it is highly desirable to make the preliminary period as short as possible. Mason and Palmer ('35) found that the endogenous nitrogen excretion of adult rats decreased only very slightly after the third day of feeding the low nitrogen diet. This confirmed the work of Mitchell, Nevens, and Kendall ('22) and of Chick and her co-workers ('35). However, Ashworth and Brody ('33), and French and Mattill ('36) found that in growing animals an equilibrium might not be reached until 10 to 28 days had elapsed. In light of these findings, French and Mattill ('36) have suggested that the protein-free feeding test be made after the animal has been fed the low protein diet for at least one week. The important point is to continue the experiment until endo-

genous catabolism has reached a more or less constant level (Mindhede, '26). Many investigators have used the average results obtained from two nitrogen-free periods for the estimation of endogenous nitrogen metabolism, one preceding and one following the feeding of the test protein. However, experience has shown that the value obtained in the second period is often much lower than the one secured in the first period. The question thus arose as to which figure represented the true value. In order to meet this difficulty Seeger and Mattill ('35) arranged the feeding régime so that the order of the protein-feeding and the nitrogen-free periods was not the same for all animals of a test series. This precaution permitted an average distribution of the variations introduced by the relative relation of the nitrogen-free period to the rest of the experiment.

The length of the collection periods used by the different laboratories vary from two to seven days. However, the majority of workers use seven days.

Number of assays possible with one animal. The number of tests made with each animal varies in the different laboratories. Morgan and Kern ('34) and Seegers and Mattill ('35) tested three proteins with one animal. However, Chick, Boas-Fixsen, Hutchinson, and Jackson ('35) kept the animals on the experiment for nearly three months and tested as many as eight proteins with each animal in this interval.

In the report of Mitchell and Carman ('26) one animal was used for six assay periods. Later, Mitchell and Beadles ('37) described experiments extending over five or six balance periods. However, in his early work Mitchell ('34) stated, "It is also advisable not to use one group of rats for a very extended series of metabolism studies, since the conditions imposed seem to undermine the conditions of the rats as reflected in progressively diminishing appetite."*

In our experience, many tests made on one animal so impaired its nutritional state, involving as they do several nitrogen-free periods, that the last tests were probably invalid. The ideal experimental situation is to use a rat for one assay only.

Factors that affect the biological value of proteins

The discussion in the preceding paragraphs indicates that assays relating to the determination of the biological value of even one specific protein may vary greatly according to the experimental conditions imposed. These might be called "experimental variations" and in most instances represent error. However, the biological value of a protein may be altered by factors that do not fall in this category. For instance, many recent studies have shown that the biological value of a protein depends upon the treatment to which it

*Mitchell, H. H., J. Biol. Chem., 58; p. 901 (1924).

was subjected prior to the test. The effect of toasting, cooking, autoclaving, and extraction with alcohol have all been investigated. All have been reported as causal agents in the lowering of the biological value. The exact change in the molecule which causes the denaturation is not definitely understood. Contributors on the subject have assumed that it is a mild hydrolysis (Chick and Martin, '10; Wu and Wu, '25; and Lepeschkin, '22).

In the present study we are especially interested in the effect of heat upon the biological value and the digestibility of protein. Morgan in 1931 reported that the differences observed between the biological values of raw and toasted cereal-proteins and of raw and toasted casein were more than six times as great as their probable error, but that the digestibility of the toasted protein and that of the raw was but little different. The results of experiments by Chick and her co-workers ('35) have confirmed the findings of Morgan concerning the effect of heat on biological values but not on digestibility. They found that heating casein at approximately 150°C. reduced its digestibility from 93 to 73 per cent. Similarly the digestibility of lactalbumin fell from 95 to 69 per cent. These latter findings are in accord with the work of Maynard and Tunison ('32) and of Schneider ('32). They demonstrated that high temperatures affected both the biological value and the digesti-

bility of fish meals. Morgan and Kern in a later publication ('34) found the supplementary feeding of lysine and histidine with the heat-treated protein produced a favorable effect, thus, indicating that some damage had occurred to the molecule.

In direct contrast to the reports of the above investigators are the findings of Seegers, Schultz, and Mattill ('36) who showed that heating casein at 120°C. for two hours or at 150°C. for 30 minutes had no effect on its biological value.

Morgan and Kern ('34) have also found that the heating of beef muscle under various conditions, i.e., (1) boiling in water until the internal temperature reached 84°C., (2) autoclaving at 15 pounds of pressure for 7 minutes, and (3) autoclaving at 15 pounds of pressure for 1 hour, lowers the biological value. There was no appreciable change in the digestibility of the meat. Maynard and Tunison ('32), too, believed that the method of heating had a decided effect, since the nutritive value of the protein of vacuum-dried fish meal was superior to flame-dried meal. On the other hand, Seegers, Schultz, and Mattill ('36) in three series of experiments on 24 animals demonstrated that the biological value of finely ground beef muscle dried at a low temperature and fed at a level of 5 per cent protein was not lowered by previous autoclaving for one hour at 15 pounds of pressure.

In their opinion, heat may affect various proteins differently. In contrast to the influence of heat on beef proteins, the biological value and the digestibility of liver proteins were slightly lowered by heating for two weeks at 100°C. However, when liver proteins were extracted with alcohol, a striking change in biological value occurred. It is their belief that the change in the nutritive value thus induced is the result of digestive processes that have produced a product for absorption containing amino acids in proportions that are not representative of the original protein. They do not believe the decreased digestibility is due to simple denaturation because proteins denatured in vitro seem to be as well digested as the original. They think that since the alcohol-extracted liver proteins are not as efficiently digested as untreated liver the change may be of a stereochemical nature.

Thus, the concept of the nutritive value of a protein must include not only its biological value but its coefficient of digestibility as well. The nitrogen balance method has been used in making most of the more recent studies and by this method the two factors may be taken into consideration.

PURPOSE OF THE STUDY

In recent studies, conducted in the Nutrition laboratory of the Foods and Nutrition Subsection of the Iowa Agricultural Experiment Station, dealing with the effect on the well-being of the albino rat of feeding canned, autoclaved pork muscle as the sole source of protein in an otherwise synthetic diet, many observations have been made that cannot be explained adequately in light of known nutritional facts. For example, the first generation of animals that was fed the test ration grew at approximately a normal rate and maintained their adult weight for 150 to 200 days (Dyar '35). After this, their weight gradually declined, and they became gaunt, very thin, and the muscles of the back legs became so atrophied that the animal exhibited a condition similar to a paralysis of that region. Dyar also observed that the animals born of parents receiving the diet containing the canned autoclaved pork muscle did not grow to as great an adult size when given the pork diet as did those animals fed the meat diet for one generation only.

The reproduction and lactation performance (Dyar, '35; and Gray '36) of the animals receiving the canned autoclaved pork muscle was also much poorer than that of a group of control animals receiving a mixed grain diet which had been

proved capable of supporting all the functions of life from generation to generation. However, when the rats were maintained on the diet supplemented with 2 gm. of raw liver daily, they were able to produce and rear young in a manner that compared favorably with that of the animals fed the grain ration (Rogosheski, '36).

Although the pork diet was deemed adequate, many nutritional factors may have served as contributing causes of these observations. For example, the diet as formulated possibly may have been unwittingly deficient in vitamin E, in certain members of the vitamin B-complex, or in some as yet unrecognized dietary factor. Or perhaps the process of autoclaving induced a rancidity that rendered important dietary constituents impotent (Mattill, '27). Or again, autoclaving may have altered the proteins of the meat muscle so that they were no longer adequate as was supposed (Morgan and Kern, '34) for the maintenance of life functions in the experimental animals.

Before any dietary deficiency could be ascribed as a causal agent in the physiological disturbances observed, it seemed necessary first to establish the nutritional status of the source of protein used in the test diet. The investigation herein reported deals with this problem.

EXPERIMENTAL

Design of Experiment

The nutritional deficiency ascribed by Dyar ('35) to the feeding of a diet containing dried canned autoclaved pork muscle may be due to the failure of the test animal to metabolize the nitrogen of the diet efficiently. The nitrogen in the test diet known as Pork I, was derived from the proteins of the pork muscle and the yeast used as a source of the vitamin B-complex.

Dyar's study indicated that normal utilization probably occurred early in experimental history but that a definite break in the mechanism took place when the animal was approximately one year old that was reflected in loss of body weight. Inability to maintain adult weight has not been noted in rats fed the stock colony ration (Steenbock V). For this reason, male rats reared on the Steenbock V diet in the pre-assay period were used as the control series in the present experiment for determining whether or not, continued maintenance on the Pork I diet for a period of one year resulted in physiological disturbances that affected the protein metabolism. Nitrogen utilization of the pro-

tein-mixture represented in the Pork I diet was determined in normal rats fed the Steenbock V ration at successive intervals in the first year of life. Nitrogen balance studies were made when the animals were three, six and 10 months old. The proteins appearing in pork muscle and yeast were used in making the test. The data thus collected allowed the calculation of the efficiency of the protein mixture in terms of the biological value and the coefficient of digestibility at the three age-levels.

In the selection of the specific life periods for study, many factors were considered. French and Mattill ('36) have shown that the results of nitrogen balance experiments obtained from adolescent rats were not reliable. The male animals of our stock colony are sexually mature when they are three months old (Earhart, '35) since sister mates have produced, on the average, one litter by that time. At this age then, adolescence does not introduce a factor of unreliability. The period of most rapid growth has also been reached at the end of three months (See figure 1.). Thus, in studying nitrogen utilization at intervals in the life of the mature rat, it seemed logical to start with a group, three months old. The biological value obtained with the group of animals studied when six months old probably represents the true biological value of the specific mixture of proteins tested inasmuch as animals of our colony have then

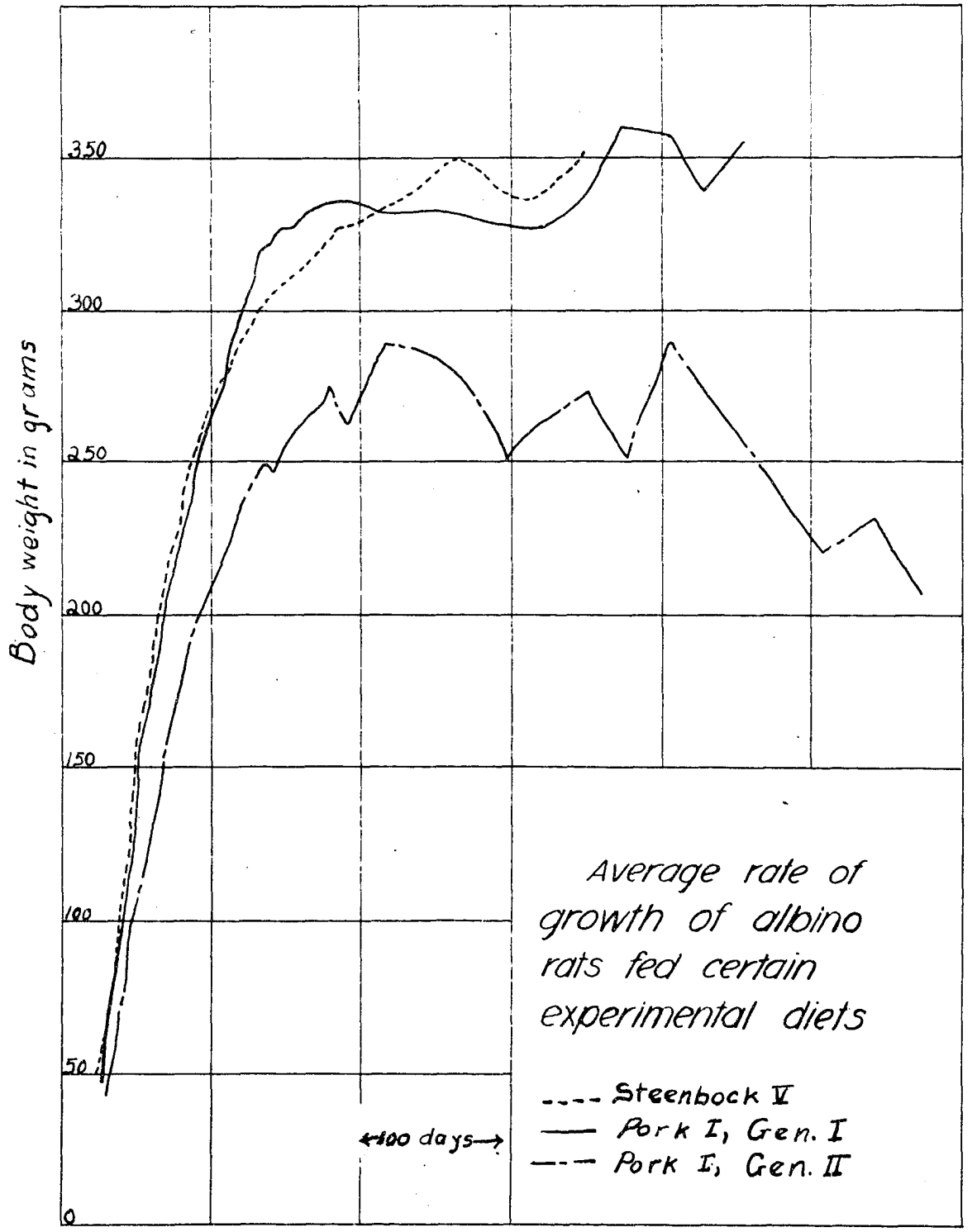
Body weight in grams
350
300
250
200
150
100
50
0
100 days
Average rate of growth of albino rats fed certain experimental diets
Steenbock V
Pork I, Gen. I
Pork I, Gen. II

Figure I.

attained their maximum adult weight (See Figure 1.) and reached the height of reproductive activity (Earhart, '35). Theoretically, this period should represent the portion of the life cycle of the rat when the nitrogen metabolism related to maintenance is most constant. For these reasons, the biological value and the coefficient of digestibility obtained at this age have been taken as the "yard stick" for measuring the influence of experimental factors on efficiency of nitrogen utilization. The ten-month interval was chosen because a study of the growth of a group of animals reared on the pork diet, designated as Pork I, showed that the animals maintained their adult weight until they were approximately this old after which they gradually declined in body weight. The interval apparently represents a critical period in the life cycle of the animal fed the Pork I diet. Since the stock rats do not exhibit loss in body weight, animals of this age were chosen for the final group in the control series for estimating whether or not continued maintenance on the Pork I diet resulted in physiological disturbances that affected protein utilization.

In the first experimental series, two groups of animals were fed, instead of the Steenbock ration, the Pork I diet until they were three months old and 10 months old, respectively. The complete series (A, Table I), therefore, gives information not only of the influence of age on the

biological utilization of the nitrogen but of the effect of previous dietary history as well.

As has been pointed out before, the animals reared on the pork-containing diet were less successful in the producing and rearing of young than were animals fed the stock colony ration (Dyar, '35; and Gray, '36). Definite improvement was obtained in the reproduction performance by the addition of two gm. of fresh liver daily to the Pork I ration (Rogosheski, '36). Growth records of nurslings throughout the entire lactation period exceeded those of nurslings in the stock colony. These facts pointed to the possibility that liver contained amino acids that were capable of making good the suspected deficiencies of the Pork I ration.

The results recorded by Rogosheski ('36) suggested a second experimental series (Series B, Table I) whereby the biological utilization of the collection of proteins present in pork muscle, liver, and yeast might be established. In this instance, the mixture of test proteins represented all sources of nitrogen in the Pork 7 diet, consisting of Pork I ad libitum and 2 gm. of fresh liver daily. Time permitted the study of nitrogen utilization at the 10-month interval only. It was believed that if the addition of the raw liver to the ration exerted a beneficial influence on nitrogen metabolism, it should be evident at the time just

prior to the nutritional failure observed in the animals maintained on the Pork I diet.

It was realized that in the experiments heretofore described the biological values and coefficients of digestibility obtained did not represent the true measure of biological utilization of pork muscle, but of the mixture of the nitrogen-containing substances present in rations Pork I and Pork 7. Since workers have disagreed as to the effect of heat on the biological value of meat proteins, it seemed desirable to check this point. For the final experiment (Series C, Table I), dried canned autoclaved pork muscle was fed as the test protein. Rats grown for series A, that already had been used for one assay were employed as the experimental animals.

The plan of the experiment and the test groups used are summarized in table I.

Experimental Animals

The animals used in the experiments herein reported were albino rats (Mus norvegicus albinus) of Wistar stock, strain A, inbred by brother and sister matings for 70 generations. The stock from which they were taken had been reared for 20 generations on a modification of Steenbock's grain diet (Steenbock, '25). The constituents of this diet were kept as constant as possible from season to season

Table I. Groups of test animals used in the experiment

Experiment number	Proteins tested in the assay period	Pre-experimental diet	Age of animal at time of assay	Number of assays per experimental group
A	Pork muscle and yeast	Steenbock V (Group 1)	3 mo. 6 mo. 10 mo.	9 9 9
		Pork I (Group 2)	3 mo. 10 mo.	9 8
B	Pork muscle, liver and yeast	Pork 7	10 mo.	10
C	Pork muscle only	Steenbock V (Group 1)	3 mo. 6 mo. 10 mo.	8 9 8
		Pork I (Group 2)	3 mo. 10 mo.	6 -

(Swanson, Stevenson, and Nelson, '38). Increments in weight at successive age intervals in different generations of these animals were the same (Timson, '32). With a uniform average rate of growth fixed by inbreeding, any significant deviation observed in our experiments may be attributed to the influence of the diet. Fifty-five mature male rats were used. Mating was not allowed. The rats were taken from litters reduced in number to eight during the first week of life, and were weaned at 28 days of age. At the time of weaning they were housed individually in round wire mesh cages with raised bottoms.

After weaning the animals were fed the stock ration for 14 days, at which time each rat was given the pre-experimental diet to which it had been assigned.

Each age-group was taken from the colony at a different time so that all animals would be ready for the balance studies in a consecutive series. For this reason litter mates could not be distributed over the various phases of the experiment but, in so far as was possible, a representative of each litter was placed in each lot within an age-group.

Assay Period

General considerations

When the animals reached the proper age for the experiment for which they were planned, nitrogen balances were determined. During this period the rats were housed in round, large meshed (1/4 inch) cages, the bottoms of which were raised one and one-half inches from the surface of the pyrex plate on which the cage was placed. During the assay period the cages were kept on large tables in a well ventilated room where no other rats were housed. The food was offered in a one-half pint jar which was wired to the side of the cage. Distilled water was available from a fountain attached to the outside of the cage (See FigureII). On the bottom of the pyrex plate two layers of acid-washed filter paper, cut to fit the dish closely, were placed to absorb the urine. In preparing these papers, they were first allowed to soak 24 hours in a two per cent acetic acid solution. Excess moisture was removed with suction. They were then soaked five minutes in a fresh acid solution, excess liquid removed as before. This process was continued until the rinsing solution was colorless. Then the papers were soaked 10 hours in a solution containing 88 per cent alcohol (95 per-cent), 10 per cent glacial acetic acid, and 2 per cent of

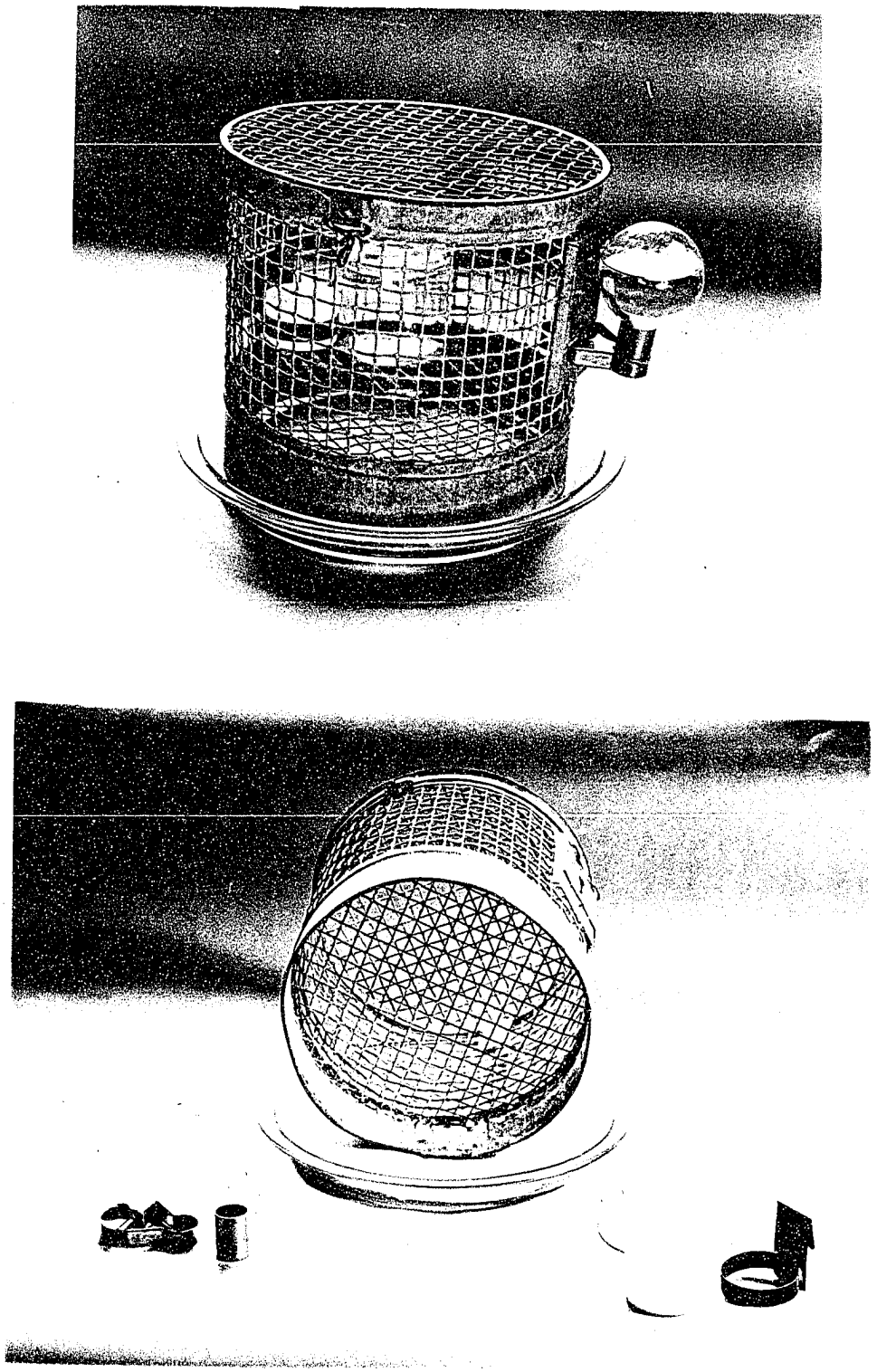

Figure II. Cage used for collection of samples.

thymol. They were then air-dried, wrapped in heavy paper and stored in a tight metal container. The washing made the filter paper nearly nitrogen-free.

Divisions of the assay period

The assay period was divided into four main intervals, i.e., two nitrogen-low feeding periods each composed of an adjustment period of four days duration and a collection period of seven days duration, and two periods of feeding the protein being assayed each of which was composed of an adjustment period (four days) and the collection period (seven days). The order of these intervals are shown in Table II. The two low nitrogen-feeding periods were used in order to secure as accurate an estimation as possible of the nitrogen of endogenous origin. In the original plan each animal was to be used for assaying proteins from two sources so as to reduce the number of rats and balance periods needed. In actual experience several of the animals maintained on the meat-containing diets for 10 months were not physically able to withstand two periods of the low protein feeding. Therefore, only those animals that were considered physically fit were used throughout the four phases of the experiment. Adjustments necessary will be discussed later in the text.

Table II. Feeding schedule of rats during the assay periods

Diets fed in the assay period	Divisions of feeding intervals	Duration of interval
		Days
Low-nitrogen food	Preliminary	4
	Collection	7
Protein food	Preliminary	4
	Collection	7
Low-nitrogen food	Preliminary	4
	Collection	7
Protein food	Preliminary	4
	Collection	7

The same type of food was provided in the adjustment period as was fed the rats during the collection period. They were allowed to eat ad libitum during the adjustment period and the average daily weight of food consumed during this period was offered to the animal each day during the collection period. In nearly every case all of the food offered was consumed. The calorie intake of the animals during these periods was entirely adequate and did not differ significantly from the calorie intake of stock animals of the same age (See Table III). Seegers and Mattill ('35b) fed the same weight of food during the protein-feeding interval as was given during the period of low nitrogen-feeding. The animals used in our experiments lost weight during the period of protein-feeding when this plan was used; therefore, they were allowed to establish a plane of food intake for each of the four intervals.

All collection periods were of seven days duration. The necessary amount of food for the first day of the collection period was weighed on a torsion balance, to this quantity of diet, approximately 30 mg. of carmine was added. The rats were offered this food at 10 P.M., eight hours before the initiation of the first collection period which was made at 6 A.M. the following morning. The rats with their food containers, were transferred at this time to the collection cage. This procedure eliminated the influence

Table III. Body weights and weekly caloric intakes of animals, 10 months old, fed certain diets

Steenbock V diet			Low-nitrogen diet			Test ration I	
Rat number	Weight	Weekly caloric intake	Rat number	Weight	Weekly caloric intake	Weight	Weekly caloric intake
	gm.			gm.		gm.	
14856	370	279	16900	382	368	394	399
14603	312	177	16918	262	274	273	294
14602	313	222	17170	358	341	379	356
15534	333	177	17317	321	322	337	392
14973	300	300	17379	281	205	302	268
15086	330	242	17670	294	273	304	336
15088	364	242	17853	316	338	324	350
14912	360	279	17949	361	321	379	430
14872	341	281	17990	249	234	270	155
Av.	336	218		314	297	329	329

of the nitrogen lag in the urine from the previous diet. On the sixth day carmine was again added to the food provided in order to mark the feces resulting from the food consumed on the last day of the collection period. At 4 P.M. of this day, the food was removed from the cage. The animal, however, remained in the collection cage until six o'clock the following morning. This again took care of the nitrogen lag.

Collection of Samples for Analysis

The feces and urine-soaked papers were placed in flasks containing a 20 per cent hydrochloric acid solution to which had been added 1 per cent of thymol.

The feces were collected at 8 A.M. and 4 P.M. The two collections were made to prevent contamination by urine since Schneider ('35) has pointed out that this may be an important uncontrolled factor, especially where one is dealing with small amounts of fecal nitrogen. At the end of the test period the bottoms of the cages and the pyrex plates were thoroughly washed with hot 20 per cent hydrochloric acid from a wash bottle. These washings were added to the flask containing the filter paper saturated with urine.

Blank collections were made also. Empty cages con-

taining the filter paper were treated in the same manner as the cages containing the rats. Analyses of these collections showed the quantity of nitrogen in the papers, and in the solutions, as well as that which might result from the absorption of ammonia from the air of the laboratory.

One-gram aliquots of food were weighed on the first, third and sixth days of the collection period, mixed, and analyses made on composite samples thus obtained.

Diets

Of the pre-experimental period

During the growth period the animals of the control group (Experiment A, group 1) received the regular stock colony ration, Steenbock V, which is a modification of the stock ration described by Steenbock ('23). It had the following percentage composition:

Ground yellow cornmeal	64
Linseed oil meal	16
Crude casein	5
Alfalfa	2
NaCl	0.5
$CaCO_3$	0.5
Yeast	1.5
Irradiated yeast	0.5

Wheat germ 10.0

It was supplemented daily with liquified Klim* to which was added cod liver oil (one teaspoon per quart) and trace minerals (2 ml. per quart of a solution containing KI, $MgSO_4$, $K_2Al_2(SO_4)$ and $CuSO_4$). In addition the rats received 5 gm. of raw lean beef and 10 gm. of lettuce on alternate days.

The first series of experimental rats received the Pork I diet during the period of rearing (Experiment A, group 2). It contained the following ingredients:

Autoclaved canned pork muscle (dried to one-half its original weight)	25 gm.
Cornstarch	53 gm.
Yeast	5 gm.
Agar agar	2 gm.
NaCl	1 gm.
Osborne and Mendel salt mixture**.....	4 gm.
Butter fat	8 gm.
Cod liver oil	2 gm.

The pork used in formulating this diet came from a lot of paired green skinned hams weighing 20 - 22 pounds. All of the external fat was removed by trimming and the muscle was ground once through a medium plate of a meat grinder. One pound of the ground muscle was placed in a No. 2 enamel-lined tin can, sealed, and autoclaved for 65

*Klim, The Borden Co., New York.

**Osborne, T.B., and Mendel, L.B., J. Biol. Chem., 37, 223-229. 1919.

minutes at 15 pounds of pressure. The cans were cooled quickly by dropping into a vat of cold water. When thoroughly cooled, they were stored at room temperature. When a can of meat was opened the interstitial fat, which had collected on the top, was removed and the remaining muscle tissue was spread one-half inch thick on trays, 1000 gm. to a tray, and dried to one-half of its original weight in a current of warm air. The temperature of the drying oven was maintained at 85°-95°C.

The animals in experiment B used for testing the efficiency of liver in supplementing the proteins of the Pork I diet were fed the Pork I diet already described supplemented with 2 gm. of fresh liver daily. This diet was known as Pork 7. The calves' liver used for supplementary feeding to the rats of the pork fed groups was purchased fresh twice weekly and cut into pieces weighing 2 gm. ± 25 mg. These pieces were placed in the freezing compartment of a mechanical refrigerator.

Group 1 of experiment C employed for determining the biological adequacy of pork muscle alone also received the stock ration in the growing period while group 2 of this experiment was maintained on Pork I diet during this interval.

The diets were mixed twice weekly and stored in two quart containers in an ice refrigerator. A fresh supply was offered the rats daily and the weight of food consumed

was recorded.

Of the assay period

As already noted, in the assay period the rats were subjected to four collection periods each of seven days duration. In the first and third intervals, the animals were given a diet low in nitrogen (See Table IV) and in the second and fourth periods they received the diets containing 5 per cent of the respective proteins being tested.

The five groups of animals in Series A of the experiment were given the diet containing the proteins of pork and yeast during the second nitrogen balance period. The composition of the diet is shown in Table IV. The animals in this series which were fed the Pork I ration for 10 months also received the test ration I in the fourth balance period.

The animals of Series B, which had received the Pork 7 diet in the pre-experimental period were given test ration III in the second and fourth intervals. This ration consisted of test ration I supplemented by 2 gm. of frozen raw liver daily. The animals of the last series (Series C) used for determining the biological value of pork muscle alone received test ration II in the fourth balance period. This ration was formulated by replacing the yeast of test ration I with cornstarch and feeding 0.2 mg. of pure vitamin B_1

Table IV. The composition of the diets used during the assay periods

Ingredient	Diets			
	Low nitrogen*	Test ration no. I.	Test ration no. II.*	Test ration no. III.
	per cent	per cent	per cent	per cent
Cod-liver oil	2	2	2	2
Butter fat	8	8	8	8
Agar-agar	2	2	2	2
NaCl	1	1	1	1
Osborne and Mendel salt mixture	4	4	4	4
Cornstarch	83	70	75	70
Autoclaved pork muscle (dried to one-half original weight)	-	8	8	8
Yeast	-	5	-	5
Liver (fresh frozen)	-	-	-	2 gm.

*Daily supplements of 200 mg. of Lilly liver extract number 343, as a source of the vitamin G-complex (Salmon and Guerrant, '31), and 0.2 mg. of vitamin B_1 crystals were also given (Betabion, Merck and Co. Inc., Rahway, N.J.).

crystals and 200 mg. of Lilly liver extract 343* daily to provide the vitamin B-complex. This was necessary to diminish the quantity of nitrogen coming from sources other than the pork muscle in the diet.

Method of Analysis of Urine, Feces, and Food

Hydrochloric acid digests of the urine and feces were prepared (Stearns, '29) by heating the six-day collections for eight hours over a hot water bath. The following day the solutions were made up to volume (urine, 1000 ml.; feces, 250 ml.). The solutions were transferred to eight-ounce glass bottles capped with bakelite stoppers and stored at room temperature until time for analysis.

For the analysis of the nitrogen content, the Kjeldahl-Gunning method was employed. Fifteen ml. of the urine digest was introduced into an 800 ml. Kjeldahl flask. To this was added 25 ml. of concentrated sulphuric acid, 0.9 gm. of mercuric oxide, and 15 gm. of potassium sulphate. The mixture was digested in the usual manner until it was colorless, after which it was heated for two hours. After cooling, 300 ml. of tap water was added. The mercury was reduced by the addition of 1 gm. of sodium hypo-phosphate and an excess (60 ml.) of a concentrated solution of sodium

*The liver extract was furnished by the Eli Lilly Co., Indianapolis, Ind.

hydroxide was carefully added. The sodium hydroxide was added from a burette. A "pinch" of granulated zinc was used to prevent "bumping." The flask was attached to the distillation rack and thoroughly mixed. The contents were brought to a boil and distilled as rapidly as possible for one-half hour. About 150 ml. of distillate was collected during this time. The nitrogen was collected as ammonium hydroxide in a standard hydrochloric acid solution. The amount of nitrogen was determined by titrating the remainder of the hydrochloric acid with a standard sodium hydroxide solution with methyl red as the indicator. The HCl and NaOH solutions were approximately 0.1 N. The concentration of the standard alkali solution was checked against potassium acid thalate (U.S.B.S.) once each month and the alkali was standardized against the acid solution at least once each week.

Since most of the ammonia passes over during the first 10 minutes of the distillation it is advisable to have at least 75 ml. of liquid in the receiving flask. Therefore, 50 ml. of carbon dioxide-free water was added to the 25 ml. of standard hydrochloric acid solution used. The tip of the distilling tube reached within one-fourth inch of the bottom of the flask. There is always a tendency toward a local concentration of ammonia. This was prevented by shaking the distillate from time to time during the first 10

minutes. Brooke ('32) has observed that the type of trap used between the Kjeldahl flask and the condenser greatly affects the accuracy of the determination. The type shown in the illustration (Figure III) was used in our determinations and proved satisfactory.

The nitrogen in the feces was determined in the same way. Twenty-five ml. aliquots of the acid digest were used.

Samples taken from the food composites were weighed onto glazed paper and transferred quantitatively to the Kjeldahl flasks. Samples weighing approximately three grams were used. They were digested and distilled by the same method as was used for the urine and feces.

Accuracy of the Analytical Method and of the Urine Collection

The reliability of the method used for analyzing for nitrogen was checked by determining the quantity of nitrogen in 25 mg. of chemically pure creatinine and in a standard creatinine solution containing 0.372 mg. per ml. (See Table V). The theoretical quantity of nitrogen in all of the samples tested was 9.292 mg. When the dry creatinine was used the sample analyzed 9.404 mg. giving an error of 1.21 per cent. The average per cent error obtained with the solid material and with the solution was 1.00. This was thought to be within the limits of the accuracy of the ex-

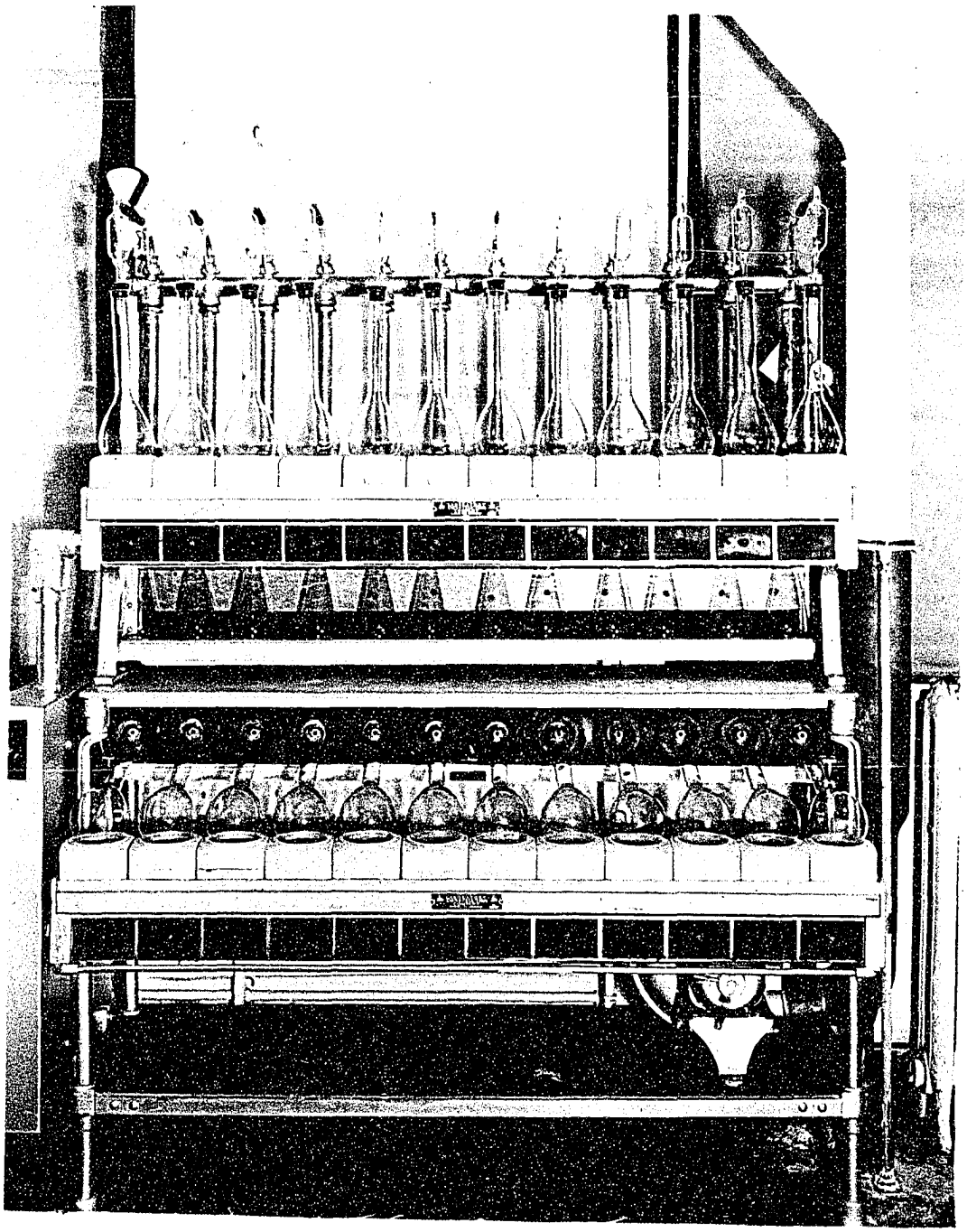

Figure III. Apparatus used for making nitrogen analyses.

perimental procedure.

The acid digests contained colloidal-like particles which settled out on standing and which adhered to the pipette when the sample was measured. It was, therefore, thought advisable to check the accuracy of the measurement and at the same time the homogeneity of the aliquots used for the nitrogen analysis. Two series of nitrogen determinations were made on a urine digest. Twelve determinations were made in each series. The procedure was as follows:

Series I:

The samples were measured from a 25 ml. pipette. The pipette was allowed to drain for 30 seconds and the last drop of the solution was removed by touching the tip of the pipette to the side of the flask. The digest was heated for two hours after it was colorless.

Series II:

The samples were measured from a 25 ml. pipette as before. The pipette was then rinsed with approximately 15 ml. of tap water to remove the last trace of urine. The washings were added to the contents of the digestion flask. The digest was heated for two hours after it was colorless.

The results of these tests are shown in Table VI. The differences observed in the mean results are not statistically significant, and since the method of measuring de-

Table V. Accuracy of the method used for nitrogen analyses

Creatinine	Size of sample	Determined nitrogen	Theoretical nitrogen	Error	
	mg.	mg.	mg.	mg.	per cent
Dry	25	9.384	9.292	0.092	1.00
	25	9.554	9.292	0.262	2.82
	25	9.274	9.292	-0.018	-0.20
	Average	9.404		0.112	1.21
	ml.				
Standard solution containing 0.372 mg. per ml.	25	9.384	9.292	0.092	1.00
	25	9.330	9.292	0.038	0.40
	Average	9.357		0.065	0.70

Table VI. Accuracy of method used for measuring aliquots of an acid digest from one solution of urine

Series I Measured without rinsing pipette			Series II Pipette rinsed after measuring		
Size of sample	0.1347N HCl	Nitrogen	Size of sample	0.1347N HCl	Nitrogen
ml.	ml.	mg.	ml.	ml.	mg.
25	8.93	16.84	25	9.46	17.84
25	9.40	17.73	25	9.23	17.41
25	9.07	17.11	25	9.04	17.05
25	8.90	16.78	25	8.96	16.90
25	8.99	16.96	25	9.30	17.54
25	8.99	16.96	25	9.15	17.26
25	8.92	16.82	25	8.94	16.86
25	8.79	16.58	25	9.30	17.54
25	8.68	16.37	25	9.11	17.18
25	8.85	16.69	25	9.26	17.46
25	8.93	16.84	25	8.68	16.37
25	8.93	16.78	25	9.54	17.99
Average		16.87 ± 0.37*			17.28 ± 0.53*

*Standard deviation.

scribed in Series I was simpler than that in Series II it was chosen as the procedure for the measuring of the samples.

The method used for the collection of urine and its analysis introduced many possibilities of error. Therefore, its accuracy was checkedby a recovery experiment in which a standard ammonium chloride solution was dripped onto the filter paper through the floor of the metabolism cage. Thirty-one ml. were sprinkled over the floor of the cage in 1 ml. portions over a period of two days. The papers were collected and the cages washed as described previously. The actual quantity of nitrogen in the 31 ml. portion of the solution used was 232.04 mg.; 233.53 mg. were recovered, an error of 0.60 per cent (See Table VII). The method used is therefore more accurate than the old method developed by Mevens ('21) wherein he attempted to remove all traces of nitrogen from the papers by washing with suction. His error was 2 to 3 per cent.

Table VII. Reliability of the method used for urine collection

Cage number	NH_4Cl solution	Nitrogen in NH_4Cl solution	Nitrogen in NH_4Cl soaked papers	Nitrogen recovered
	ml.	mg.	mg.	mg.
1	31.0	232.04	232.07	228.68
2	31.0	232.04	237.50	234.11
3	31.0	232.04	241.20	237.81
4	Blank	Blank	3.39	
Average				233.53

PLANS ADOPTED FOR THE CALCULATION OF BIOLOGICAL VALUES AND COEFFICIENTS OF DIGESTIBILITY

The Metabolic Fecal Nitrogen

For the calculation of biological values, two factors are essential. They are: (1) the quantity of nitrogen absorbed, and (2) the quantity of absorbed nitrogen retained by the body. The amount of nitrogen absorbed is represented by the difference between ingested nitrogen and nitrogen of undigested food. The nitrogen in the feces from undigested food is theoretically the quantity of nitrogen in the feces over and above the amount which has its origin in digestive juices, epithelial cells, mucus, bacteria, blood, and pus. That is, it is the amount of nitrogen in the feces of exogenous origin. As has been pointed out before, there is considerable controversy as to the method of determining the endogenous fecal nitrogen. Some of the workers (Mitchell, '24; and Adolph and Wu, '34) believe that endogenous fecal nitrogen is directly related to the dry weight of food consumed, others (Schneider, '35), that it is related to both food intake and body weight, while still others (Boas-Fixsen and Jackson, '32) think that it is related to nei-

ther of these but that it is a constant value for each individual animal. Therefore, before the biological value of the proteins of canned autoclaved pork muscle could be calculated from the data collected in the present experiment it was necessary to determine whether or not any correlation existed between body weight of the experimental animal, the weight of dry food ingested, and the output of fecal nitrogen during the feeding period of the low-nitrogen diet. The individual data from which these correlations were calculated are shown in Tables VIII, IX, and X. A close examination of the individual values and of the averages (Table XI) indicated that probably no significant correlation existed between the factors. In many instances, the average fecal nitrogen excreted in the period of low-nitrogen feeding by two unlike groups of animals was almost identical in spite of differences observed in body weight and quantity of food consumed. For example, inspect the results obtained in the experiments with the third and fourth groups listed in Table XI. The significance of these observations was borne out by simple correlation (See Table XII). The coefficients varied widely and while a certain few bordered on significance, one case only could be judged highly significant, i.e., body weight and fecal nitrogen excretion of the animals that had received the mixed grain diet for a period of six months. Neither were the differences between the fecal nitrogen

Table VIII. Food consumption, fecal nitrogen excretion, and body weight of male albino rats, three months old, receiving a low-nitrogen diet

Pre-experimental diet	Rat number	Dry weight of food consumed	Fecal nitrogen	Body weight
		gm.	mg.	gm.
Steenbock V	19695	50.63	78.64	164
	19702	30.05	77.65	207
	19703	48.99	63.15	202
	19768	63.75	69.31	228
	19807	53.55	94.76	229
	19808	46.81	92.93	220
	20338	41.32	82.48	198
	20399	37.89	89.57	161
	20431	42.58	80.91	166
Av.		46.17	81.04	197.2
Pork I	19693	54.64	110.72	193
	19694	54.37	109.03	190
	19701	56.19	79.24	196
	19766	48.72	89.37	200
	19767	63.57	137.23	213
	19806	59.56	122.28	205
	20305	46.73	86.81	180
	20307	59.09	101.96	184
	20398	51.96	116.53	200
Av.		54.98	105.89	195.7

Table IX. Food consumption, fecal nitrogen excretion, and body weight of male albino rats, six months old, receiving a low-nitrogen diet

Pre-experimental diet	Rat number	Dry weight of food consumed	Fecal nitrogen	Body weight
		gm.	mg.	gm.
Steenbock V	19235	48.85	123.40	264
	19345	54.14	98.68	267
	19332	75.28	140.20	303
	19344	78.29	125.33	269
	19328	55.50	98.68	256
	19334	59.33	112.00	257
	19296	81.34	120.31	311
	19233	70.90	137.11	311
	19245	67.08	138.65	334
Av.		65.69	121.59	283.6

Table X. Food consumption, fecal nitrogen excretion, and body weight of male albino rats, 10 months old, receiving a low-nitrogen diet

Pre-experimental diet	Rat number	Dry weight of food consumed	Fecal nitrogen	Body weight
		gm.	mg.	gm.
Steenbock V	16900	88.34	118.65	394
	16918	65.57	112.13	268
	17170	81.80	101.84	365
	17317	77.14	122.73	326
	17324	--	--	--
	17379	49.18	88.57	286
	17670	65.39	106.65	294
	17853	81.05	111.08	320
	17949	77.04	132.28	368
	17990	56.01	80.72	250
Av.		71.28	108.29	319.0
Pork I	16726	43.71	168.55	219
	16789	34.60	91.40	255
	*16790	33.69	68.65	194
	16893	54.64	83.34	306
	*17186	60.11	101.65	342
	17469	49.18	93.94	295
	17894	27.14	76.13	272
Av.		43.29	97.67	269.0
Pork 7	16727	60.11	93.70	273
	16728	61.01	107.67	260
	16791	38.25	68.18	210
	16895	45.54	71.01	273
	16896	54.64	91.66	342
	17470	65.12	116.97	340
	17895	63.75	85.20	274
Av.		55.49	90.63	281.7

*First low-nitrogen feeding period.

Table XI. Summary: Average food consumption, fecal nitrogen excretion, and body weight of male albino rats receiving a low-nitrogen diet

Pre-experimental diet	Age-group	Number animals	Average weight of food consumed	Average body weight	Average fecal nitrogen
			gm.	gm.	mg.
Steenbock V	3 mo.	9	46.17	197.2	81.04 ± 8.9*
	6 mo.	9	65.69	283.6	121.59 ± 15.1*
	10 mo.	9	71.28	319.0	108.29 ± 16.2*
Pork I	3 mo.	9	54.98	195.7	105.89 ± 18.2*
	10 mo.	7	43.29	269.0	97.67 ± 33.2*
Pork 7	10 mo.	7	55.49	281.7	90.63 ± 18.3*

*Standard deviation.

Table XII. Data showing the relation of food consumption and body weight to fecal nitrogen excreted in the low-nitrogen feeding period

Pre-experimental diet	Age-group	Number of animals	Simple correlation coefficients		
			Food consumption and fecal nitrogen	Body weight and fecal nitrogen	Value necessary for significance*
Steenbock V	3 mo.	9	-0.219	+0.002	0.798
Pork I		9	+0.652	+0.630	0.798
	Both groups	18	+0.454	+0.122	0.590
Steenbock V	6 mo.	9	+0.561	+0.790	0.798
Steenbock V	10 mo.	9	+0.712	+0.652	0.798
Pork I and Pork 7**		14	+0.341	+0.006	0.661
	Both groups	23	+0.452	+0.324	0.526

*Highly significant.
**Each group too small to treat individually.

values of the six groups significant (value of T, 1.4; value necessary for significance, 3.51). Thus, we can only conclude in light of these observations that fecal nitrogen of endogenous origin is a specific value for each rat, represented by the nitrogen excreted in the feces during the low-nitrogen feeding period. All calculations in the present experiment have been made on this basis. The conclusion supports the view advanced by Boas-Fixsen and her co-workers ('35). It is interesting to recall that they, as we, have employed adult rats consistently in protein utilization experiments.

In some cases the quantity of body nitrogen excreted in the feces during the low-nitrogen feeding period exceeded the total fecal nitrogen excreted in the assay period. In these cases, it was assumed that all of the food nitrogen was absorbed. This plan has also been used by Mitchell and Carman ('26).

The Urinary Nitrogen of Endogenous Origin

The quantity of absorbed nitrogen retained by the animal body may be estimated by determining the difference between the urinary nitrogen output during the assay period and the urinary nitrogen of endogenous origin. The excretion of nitrogen during the low-nitrogen feeding period may

be taken as the quantity of endogenous urinary nitrogen. This value is influenced by the previous food ingested by the animal, making it necessary to feed the low-nitrogen diet for a few days before the collections are started. The length of time necessary for the adjustment to take place has been discussed in a previous section. Since workers disagree as to how long a period is required for the stabilization of the nitrogen excretion, the time needed by the animals used in the present experiment to adjust to the low-nitrogen intake was determined with a group of rats grown especially for this purpose. These animals were reared on the various pre-experimental diets and were representative of the three age-groups used in the investigation. They were given the nitrogen-low diet for three consecutive collection periods of 11 days each. The results of the test are shown in Table XIII. In every case, the urinary output of nitrogen was markedly lower in the second test period than in the first, while the quantity excreted in the third period was only slightly lower than that in the second period. Mitchell and Carman ('26) have observed that the change in the values of endogenous urinary nitrogen is a linear function of time. Our data do not confirm their finding (Table XIII). We believe that the animals reached an approximate nitrogen equilibrium in the second period of low-nitrogen feeding in this experiment.

Table XIII. The body weight and urinary nitrogen excretion of a group of male albino rats for three consecutive low-nitrogen feeding periods

Age-group	Pre-experimental diet	Rat number	First period		Second period		Third period	
			Body weight	Urine nitrogen	Body weight	Urine nitrogen	Body weight	Urine nitrogen
			gm.	mg.	gm.	mg.	gm.	mg.
3 mo.	Pork I	20060	220	425.97	204	314.55	190	276.52
		20059	200	317.15	177	275.26	166	264.74
	Steenbock V	20061	228	543.92	206	329.00	190	302.76
		19954	255	487.58	238	340.79	218	325.07
6 mo.	Steenbock V	19469	250	516.42	227	331.60	217	327.67
		19464	242	539.99	320	398.47	301	378.75
10 mo.	Pork I	17186	338	449.55	312	327.67	298	294.90
		16893	323	365.03	304	308.02	293	309.29

To test this assumption further, comparisons were made of the quantity of urinary nitrogen and fecal nitrogen excreted during the two low-nitrogen feeding periods that were used in all of the experiments of the present study (Tables XIV, XV, and XVI). The data are summarized in Table XVII. In every individual case, the excretion of nitrogen in the urine was lower in the second period of low-nitrogen feeding than in the first. The fecal nitrogen remained roughly constant. In the original plan, we had hoped to use the average excretion of nitrogen in the urine in the two periods of low-nitrogen feeding as the index of urinary endogenous nitrogen. The findings precluded the use of the plan. It was decided, instead, that the nitrogen excretion of each animal during the second low-nitrogen feeding period would be taken as representative of the nitrogen of endogenous origin. The adoption of this plan eliminated mathematical adjustments in the calculations. The fact that the excretion of the element by the special group of rats used to test this problem was relatively constant after a period corresponding to the second nitrogen-free feeding period of the actual experiment validates this decision.

It may be interesting at this point to note the quantity of endogenous urinary nitrogen excreted per gram of body weight by the rat. The rate of tissue metabolism in animals varying in regard to dietary history and age was found to be

Table XIV. The body weight, fecal nitrogen excretion, and urinary nitrogen excretion of male albino rats, three months old, receiving a low-nitrogen diet

Pre-experimental diet	Rat number	First period			Second period		
		Body weight	Fecal nitrogen	Urine nitrogen	Body weight	Fecal nitrogen	Urine nitrogen
		gm.	mg.	mg.	gm.	mg.	mg.
Steenbock V	19695	172	78.64	256.94	164	78.64	191.41
	19702	216	88.97	394.54	207	77.65	264.80
	19703	203	94.53	518.55	202	63.15	226.77
	19768	226	106.05	515.08	228	69.31	259.54
	19807	226	125.91	297.14	229	94.76	263.74
	19808	222	114.19	403.66	220	92.93	266.33
	20338	203	115.15	393.34	198	82.48	264.93
	20399	169	116.33	293.70	161	89.57	242.56
	20431	182	94.09	259.61	166	80.91	235.96
Av.		202.1	103.76	359.17	197.2	81.04	246.23
Pork I	19693	192	113.00	300.17	193	110.72	222.84
	19694	185	85.20	315.88	190	109.03	211.72
	19701	195	90.16	323.74	196	70.24	267.40
	19766	200	102.48	315.88	200	89.37	233.30
	19767	213	126.51	370.96	213	137.23	259.83
	19806	202	135.25	344.72	205	122.28	354.11
	20305	183	124.97	288.31	180	86.81	238.49
	20307	188	136.60	368.30	184	101.96	253.30
	20398	205	89.37	357.91	200	116.33	250.42
Av.		195.9	111.50	331.76	195.7	105.89	250.16

Table XV. The body weight, fecal nitrogen excretion, and urinary nitrogen excretion of male albino rats, six months old, receiving a low-nitrogen diet

Pre-experimental diet	Rat number	First period			Second period		
		Body weight	Fecal nitrogen	Urine nitrogen	Body weight	Fecal nitrogen	Urine nitrogen
		gm.	mg.	mg.	gm.	mg.	mg.
Steenbock V	19235	282	100.46	479.72	264	123.40	343.39
	19345	274	104.71	487.44	267	98.68	369.63
	19332	320	113.01	480.98	303	140.20	394.54
	19344	289	107.22	478.39	269	125.33	244.49
	19328	262	104.13	522.14	236	98.68	309.36
	19334	263	110.50	441.09	257	112.00	390.09
	19296	318	118.22	460.40	311	120.31	444.36
	19233	327	125.18	586.41	311	137.11	394.54
	19245	348	136.95	554.64	334	138.65	439.09
Av.		298.1	113.38	499.02	283.6	121.59	368.83

Table XVI. The body weight, fecal nitrogen excretion, and urinary excretion of male albino rats, 10 months old, receiving nitrogen diet

Pre-experimental diet	Rat number	First period			S
		Body weight	Fecal nitrogen	Urine nitrogen	Body weight
		gm.	mg.	mg.	gm.
Steenbock V	16900	388	114.38	484.20	382
	16918	272	87.13	355.96	262
	17170	369	113.54	502.56	358
	17317	321	113.60	425.97	321
	17324	242	91.95	485.65	-
	17379	311	92.75	329.00	281
	17670	308	104.07	462.54	291
	17853	329	68.52	656.68	316
	17949	366	114.94	540.13	361
	17990	271	85.01	372.49	249
Av.		317.7	98.59	461.52	313.4
Pork I	16726	308	112.12	440.96	219
	16789	334	49.55	983.91	255
	16790	194	68.65	420.20	-
	16893	316	96.85	418.31	306
	17186	342	101.65	407.40	325
	17469	331		351.32	295
	17894	294	108.43	378.82	272
Av.		302.7		377.88	278.7
Pork 7	16727	289	109.73	371.52	273
	16728	277	106.78	387.25	260
	16791	219	72.23	366.64	210
	16895	314	68.08	477.36	273
	16896	355	88.45	465.36	342
	17191	195	57.90	1093.92	-
	17470	368	81.68	391.94	340
	17895	279	86.97	390.54	274
Av.		287.0	83.98	468.44	281.7

ody weight, fecal nitrogen excretion, and urinary nitrogen
tion of male albino rats, 10 months old, receiving a low-
gen diet

diet	Rat number	First period			Second period		
		Body weight	Fecal nitrogen	Urine nitrogen	Body weight	Fecal nitrogen	Urine nitrogen
		gm.	mg.	mg.	gm.	mg.	mg.
	16900	388	114.38	484.20	382	118.63	309.16
	16918	272	87.13	355.96	262	112.13	238.49
	17170	369	113.54	502.56	358	101.84	142.12
	17317	321	113.60	425.97	321	122.73	322.48
	17324	242	91.95	485.65	-	--	--
	17379	311	92.75	329.00	281	88.57	361.70
	17670	308	104.07	462.54	291	106.65	308.02
	17853	329	68.52	656.68	316	111.08	352.84
	17949	366	114.94	540.13	361	132.28	390.61
	17990	271	85.01	372.49	249	80.72	290.97
		317.7	98.59	461.52	313.4	108.29	301.82
	16726	308	112.12	440.96	219	168.55	379.33
	16789	334	49.55	983.91	255	91.40	265.17
	16790	194	68.65	420.20	-	--	--
	16893	316	96.85	418.31	306	83.34	267.64
	17186	342	101.65	407.40	325	98.54	136.64
	17469	331		351.32	295	93.94	356.51
	17894	294	108.43	378.82	272	76.13	365.23
		302.7		377.88	278.7	101.98	295.09
	16727	289	109.73	371.52	273	93.70	308.21
	16728	277	106.78	387.25	260	107.67	302.25
	16791	219	72.23	366.64	210	68.18	251.36
	16895	314	68.08	477.36	273	71.01	298.37
	16896	355	88.45	465.36	342	91.66	317.44
	17191	195	57.90	1093.92	-	--	--
	17470	368	81.68	391.94	340	116.97	349.98
	17895	279	86.97	390.54	274	85.20	298.37
		287.0	83.98	468.44	281.7	90.63	303.71

Table XVII. The average body weight, fecal nitrogen excretion, and urinary nitrogen excretion of male albino rats receiving a low-nitrogen diet

Age-group	Pre-experimental diet	First period			Third period		
		Body weight	Fecal nitrogen	Urine nitrogen	Body weight	Fecal nitrogen	Urine nitrogen
		gm.	mg.	mg.	gm.	mg.	mg.
3 mo.	Steenbock V	202.1	103.76	359.17	197.2	81.04	246.23
	Pork I	195.9	111.50	331.76	195.7	105.89	256.16
6 mo.	Steenbock V	298.1	113.38	499.02	283.6	121.59	368.83
10.mo.	Steenbock V	317.7	98.59	461.52	313.4	106.29	301.82
	Pork I	302.7		377.88	278.7	101.98	295.09
	Pork 7	287.0	83.98	468.44	281.7	90.63	303.71

roughly constant in the present experiment. Endogenous urinary nitrogen excretions ranged from 1.0 to 1.4 mg. per gram of body weight. The values obtained with the use of the different experimental groups were not significantly different. Therefore, the average value, 1.2 mg. of nitrogen per gram of body weight may be taken to represent the tissue catabolism of the rat. This closely approximates the value (1.3 mg.) reported by Morgan and Kern ('34) when they used adult rats for experimental purposes.

The Calculation of the Biological Value and the Coefficient of Digestibility

The biological value of a protein may be represented by $100 \times \frac{\text{food nitrogen retained}}{\text{food nitrogen absorbed}}$. The type of data necessary for the calculation of the biological value of any protein is shown in Table XVIII.

Table XVIII. Data necessary for the calculation of the biological value and the coefficient of digestibility

Excretions in low-nitrogen feeding period		Excretions in assay period		Nitrogen consumed
Metabolic fecal nitrogen	Metabolic urinary nitrogen	Fecal nitrogen	Urinary nitrogen	
mg.	mg.	mg.	mg.	mg.
110.72	222.84	155.70	376.82	897.74

The procedure for the calculations are as follows:

155.70 mg. N - 110.72 mg. N = 44.98 mg. N, which is the quantity of nitrogen present in the undigested food;

397.74 mg. N - 44.98 mg. N = 852.76 mg. N, which is the quantity of nitrogen absorbed from the food;

376.82 mg. N - 222.84 mg. N = 153.98 mg. N, which is the quantity of urinary nitrogen derived from food nitrogen;

852.76 mg. N - 153.98 mg. N = 698.76 mg. N, which is the quantity of absorbed nitrogen retained by the body;

$\frac{698.70}{852.76} \times 100 = 81.9$, the biological value of the protein.

It will be noted that the nitrogen derived from the low-nitrogen diet was omitted in the calculation. This value is relatively constant for the entire group of animals since all received the major part of the nitrogen in their vitamin supplement (99.02 mg.) which was given in the same quantity each day. For this reason we have not used it in our calculations. It has also been pointed out by Boas-Fixsen and her co-workers ('32) that "The nitrogen ingested during the nitrogen-free experiment is so small in amount, compared with the other values involved that it can be disregarded."*

*Boas-Fixsen, M.A., and Jackson, H.M., Biochem. J. 26; p. 1920 (1932)

The coefficient of digestibility is the percentage of the food nitrogen ingested that is absorbed. The coefficient calculated from the above data is,

$$\frac{852.76}{897.74} \times 100 \text{ or } 95.0.$$

RESULTS AND DISCUSSION

Biological Value

The biological value of the proteins of canned autoclaved pork muscle and yeast

The biological value. The biological value of the proteins of canned autoclaved pork muscle and yeast as determined with animals that had been reared for six months on a mixed grain diet (Series A, group 1) was 76.3 (See Table XIX). As has been pointed out before, this group is the one that we are using as the "measuring stick" for comparing the results obtained in other experiments. We regard the value, 76.3, as the true biological value of the proteins of the Pork I diet.

The individual determinations obtained with this group of rats were very consistent having a relatively small range, i.e., 72.0 to 83.8 and a standard deviation of only 4.4. As Mitchell, Burroughs, and Beadles ('36) have stated, "The variability of individual determinations of the biological value of a protein is the resultant not only of technical error in method, but also of individual differences in protein utilization in metabolism."* They found the variability,

*Mitchell, H.H., Burroughs, Wise, and Bealdes, J.R., J. Nutrition, 11; p. 259 (1936)

Table XIX. The biological value of the proteins of canned autoclaved pork muscle and yeast determined with male albino rats, six months old reared on a mixed grain diet (Series A, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19235	264	-24	129.67	123.40	343.39	
	19345	267	-18	133.00	98.68	369.63	
	19332	303	-22	146.27	140.20	394.54	
	19344	269	-18	148.15	125.33	361.94*	
	19328	236	- 4	133.85	98.68	415.75*	
	19334	257	-18	136.26	112.00	380.09	
	19296	311	-14	150.38	120.31	444.36	
	19233	311	-10	143.52	137.11	394.54	
	19245	334	-16	141.12	138.65	439.09	
Av.		283.6	-16.0	140.25	121.59	393.70	
Protein feeding period	19235	296	+ 8	1132.06	170.89	519.08	83.8
	19345	291	+22	1220.18	213.57	652.75	74.4
	19332	328	+ 8	1103.98	207.78	684.25	72.0
	19344	286	- 8	813.46	149.47	599.00	70.0
	19328	250	- 4	647.86	128.70	576.76	73.9
	19334	279	+14	1107.85	187.89	619.98	76.8
	19296	331	+18	1162.08	194.07	635.70	82.4
	19233	333	+22	1196.94	204.11	697.30	73.2
	19245	357	+22	1269.57	208.36	677.66	80.1
Av.		305.7	+11.3	1072.66	184.98	629.16	76.3

*Average of two periods.

as measured by the standard deviation, to be 3.7 in 30 of their more recent determinations. The standard deviations of the groups varied from 1.7 to 7.2. The variation in results obtained in similar determinations of biological value by Morgan and Kern ('34) in four test groups when re-calculated in terms of the standard deviation was 3.5 to 7.6. Seegers and Mattill ('35) reported the variation of their experiments as probable error. The range in the value of their probable errors was 1.8 to 1.1. These results compared very favorably to the probable errors reported by Morgan and Kern ('34). Thus, the variability of the results obtained in our laboratory does not differ greatly from that observed by other workers.

The effect of age of the test animal. Since one of the objectives of the experiment was to determine the efficiency of protein metabolism at different periods in the life cycle of the rat, the biological value of the proteins of the Pork I diet was determined with test animals reared on the stock ration for three months, six months, and 10 months (Series A, group 1). The results obtained are recorded in Tables XIX, XX, and XXI. It may be observed that a progressive lowering of the biological value of the proteins occurred as the animal increased in age. The values obtained and their standard deviations were: (1)when the rats were three months old, 83.4 ± 6.3; (2) when six months old, 76.3 ± 4.4, and

Table XX. The biological value of the proteins of canned autoclaved pork muscle and yeast determined with male albino rats, three months old, reared on a mixed grain diet (Series A, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19695	164	- 8	138.16	78.64	191.41	
	19702	207	-22	122.25	77.65	264.80	
	19703	202	-15	136.90	63.15	226.77	
	19768	228	- 8	143.40	69.31	259.54	
	19807	229	-14	132.65	94.76	263.74	
	19808	220	-12	128.42	92.93	266.33	
	20338	198	-16	128.42	80.42	264.93	
	20399	161	- 6	125.98	89.57	242.56	
	20431	166	- 8	129.32	80.91	235.96	
Av.		197.2	-12.1	131.72	81.04	246.23	
Protein feeding period	19695	175	+ 1	595.23	109.73	321.14	77.0
	19702	229	+10	790.05	139.61	385.35	83.4
	19703	221	+14	814.53	147.76	330.34	85.8
	19768	235	+ 2	978.95	186.68	445.62	78.4
	19807	244	+17	965.26	184.10	467.93	76.7
	19808	236	+12	877.77	177.55	440.36	78.1
	20338	213	+ 6	894.83	135.82	357.78	88.3
	20399	171	- 3	561.86	106.30	281.92	92.8
	20431	182	0	675.36	105.70	298.97	90.3
Av.		211.8	+ 6.6	783.76	143.69	369.93	83.4

Table XXI. The biological value of the proteins of canned autoclaved pork muscle and yeast determined with male albino rats, 10 months old, reared on a mixed grain diet (Series A, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	16900	382	-24	136.95	118.63	309.16	
	16918	262	-12	127.17	112.13	238.49	
	17170	358	-14	134.14	101.84	142.12	
	17317	321	-10	158.65	122.73	322.48	
	17379	281	-10	133.26	88.57	361.70	
	17670	291	- 6	144.54	106.65	308.02	
	17853	316	- 8	149.93	111.08	352.84	
	17949	361	-14	148.76	132.28	390.61	
	17990	249	- 2	135.18	80.72	290.97	
Av.		313.4	-11.1	140.95	108.29	301.82	
Protein feeding period	16900	394	± 8	987.24	151.54	714.56	57.5
	16918	273	± 6	727.44	136.83	469.36	67.2
	17170	379	± 2	806.88	142.67	424.64	63.1
	17317	337	±10	1102.35	187.67	568.90	76.2
	17379	302	± 4	715.27	132.47	474.46	85.2
	17670	304	± 8	897.60	166.73	563.63	69.5
	17853	324	± 8	951.00	177.15	634.36	68.2
	17949	379	±18	859.07	187.02	728.80	58.0
	17990	270	-12	308.95	84.77	494.17	33.4*
Av.		329.1	± 5.8	817.31	151.87	563.65	67.8

* Omitted, because the animal had diarrhea during the assay period

(3) when 10 months old, 67.8 ± 9.0. The standard deviations show that the animals in the younger and older groups were more variable in their reactions to the experiment than were the animals composing the six-month group. The significance of the differences observed in the mean values was tested by an analysis of variance. The differences were found highly significant as judged by values of F (F = 9.86; value necessary for significance, 3.37). Therefore, it must be concluded the animal utilizes a specific set of proteins less and less efficiently at progressive intervals in its life history.

These findings are in accord with the meagre data presented by Basu and Basak ('37) with pairs of animals of the same body weight which indicated that as rats increased in size (which we have interpreted to mean an increase in age), the biological value decreased (Table XXII).

Table XXII. Data reported by Basu and Basak ('37)*

Sundried polished rice		Sundried polished rice	
Body weight	Biological value	Body weight	Biological value
104	93	102	94
146	80	143	82
163	77	160	77
200	76	202	83
224	75	222	69
		290	75

*Basu, K.P., and Basak, M.N., Ind. J. Med. Res., 24; p. 1046 and 1047 (1937)

The significance of the results obtained in this experiment will be discussed further in a later section.

The effect of the pre-experimental diet. The effect of long maintenance on a meat diet upon the utilization of protein by the rat was studied in this experiment. The data pertaining to the biological value of the proteins of canned autoclaved pork muscle and yeast as determined with animals reared on the pork-containing diet (Series A, group 2) are presented in Tables XXIII and XXIV. The average value obtained when three-month old animals, whose pre-experimental diet was Steenbock V, were used (See Table XXX). Again as was found with the use of the animals of Series A, group 1, the biological value of the test proteins was lower when the rats were 10 months old than when they were three. The value obtained with the use of rats, 10 months old, was higher, although not statistically different, than the corresponding value secured with rats of this age in the control group (Series A, group 1). Therefore, continued feeding of the Pork I diet has not impaired protein utilization. It should be noted that the value approximated the "true" biological value of the proteins of the Pork I diet (Six-month group, series A, group 1). Thus, a general upward trend is indicated which may mean that in the long period of pork administration, the animals have become so accustomed to metabo-

Table XXIII. The biological value of the proteins of canned autoclaved pork muscle and yeast determined with male albino rats, three months old, reared on the Pork I diet (Series A, group 2)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19693	193	-10	138.50	110.72	222.84	
	19694	190	- 9	138.30	109.03	211.72	
	19701	196	- 9	139.62	79.24	267.40	
	19766	200	- 8	132.94	89.37	233.30	
	19767	213	- 6	143.27	137.23	239.83	
	19806	205	-10	136.43	122.28	354.11	
	20305	180	-16	132.28	86.81	238.49	
	20307	184	- 4	141.07	101.96	233.30	
	20398	200	- 8	136.00	116.33	250.40	
Av.		195.7	- 8.7	137.60	105.89	250.16	
Protein feeding period	19693	210	+12	897.74	155.70	376.82	81.9
	19694	206	+11	807.67	140.21	364.37	80.3
	19701	210	+ 8	793.97	125.84	370.96	86.1
	19766	210	+12	841.50	149.74	382.62	80.9
	19767	223	+ 7	890.12	181.12	436.43	76.8
	19806	215	+15	903.45	180.71	411.52	93.2
	20305	182	+ 4	562.28	105.11	344.85	80.4
	20307	188	+12	712.88	117.91	483.78	73.2
	20398	215	+ 6	665.04	120.26	338.13	86.7
Av.		206.6	+9.7	786.07	141.94	389.94	82.2

Table XXIV. The biological value of the proteins of canned autoclaved pork muscle and yeast determined with male albino rats, 10 months old, reared on the Pork I diet (Series A, group 2)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	16726	219	-14	112.65	168.55	379.33	
	16789	255	-18	118.25	91.40	265.17	
	16790	194	- 8	100.91	68.65	420.20*	
	16793	306	- 8	127.22	83.34	267.64	
	17186	342	-16	156.44	101.65	407.40*	
	17469	295	-14	137.04	93.94	365.51	
	17894	272	-21	116.06	76.13	365.23	
Av.		269.0	-14.1	124.08	97.66	352.92	
Protein feeding period	16726	263	-34	249.45	88.27	786.79	--
	16789	285	-14	432.67	90.24	390.04	71.1
	16790	174	- 8	362.03	68.52	365.54	-- died
	16893	321	+ 2	727.44	135.96	467.49	70.4
	17186	342	+ 4	706.02	143.81	492.08	87.2
	17469	329	- 9	659.85	132.84	488.91	80.1
	17894	302	+ 1	862.56	181.32	577.04	72.1
	16789	254	-12	710.61	146.41	531.64	59.4
	16893	311	-10	655.59	120.04	385.40	81.0
	17469	305	+ 6	824.74	127.50	511.16	81.6
Av.		288.6	- 7.4	619.10	123.49	499.61	75.4

*First low-nitrogen feeding period

lizing the specific set of proteins tested, that utilization actually has been enhanced.

The biological value of the proteins of canned autoclaved pork muscle, yeast and raw liver

Whether or not the addition of raw liver to the Pork I diet supplemented the proteins of this diet was studied by determining the biological value of the mixture of proteins represented in pork, liver, and yeast. Rats, 10 months old, fed the Pork 7 diet in the pre-assay period were used for the experiment. The results of these determinations are shown in Table XXV. The liver apparently does not contain any amino acids that supplement the pork proteins in any effective way since the biological value of the mixture (71.3) was not significantly different from that of the proteins of pork and yeast (Series A, group 1, biological value 67.8; Series A, group 2, biological value 75.3). Therefore, we may conclude that the improvement in reproduction, lactation, and growth of young observed when the Pork I diet was supplemented with fresh liver was not due to a better protein mixture.

The biological value of the proteins of canned autoclaved pork muscle

In this experiment, the biological value of the pro-

Table XXV. The biological value of the proteins of canned autoclaved pork muscle, yeast, and liver determined with male albino rats, 10 months old, reared on the Pork 7 diet (Series B)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		Gm.	Gm.	Mg.	Mg.	Mg.	
Low-nitrogen feeding period	16727	273	-10	122.62	93.70	308.21	
	16728	260	- 8	138.92	107.64	302.25	
	16791	310	-12	120.27	68.10	251.36	
	16895	273	-19	134.52	71.01	298.37	
	16896	342	-12	129.38	91.66	317.44	
	17470	340	- 4	155.20	116.97	349.98	
	17895	278	- 4	134.44	95.20	298.37	
Av.		282.3	- 9.9	129.88	90.62	303.71	
Protein feeding period	16727	285	- 3	946.56	127.55	722.04	54.8
	16728	275	-10	746.15	136.22	635.55	45.3**
	16791	224	- 4	767.90	97.44	474.55	69.8
	16895	305	-10	825.27	129.29	650.44	54.1*
	16896	357	- 6	1033.11	90.15	567.44	75.8
	17470	371	- 2	1028.62	97.25	669.44	67.0
	17895	286	- 8	869.68	110.02	461.33	80.7
	16727	271	-10	808.35	143.36	289.32	--
	16728	266	0	796.61	128.75	447.20	81.3
	16791	216	0	836.01	189.34	491.92	67.7
	16895	250	- 8	466.37	48.47	623.12	--
	17470	355	- 7	1134.21	160.07	651.41	75.6
Av.		288.4	- 0.5	881.24	121.41	580.31	71.3

*Refused liver supplement, therefore, omitted in average.
**Animal had diarrhea during test period.

teins of canned autoclaved pork muscle has been determined. The study was conducted for two reasons. In the first place it was thought that the proteins of the yeast which served as the source of the vitamin B-complex in the Pork I ration might affect the biological value of the protein of canned autoclaved pork muscle. In the second place, the question has continually risen in the laboratory in connection with the project, "Meat in Nutrition," as to whether or not the autoclaving process altered the proteins so that they were no longer nutritionally adequate.

In this series (Series C) it may be recalled that the vitamin B-complex was supplied by the pure vitamin B_1 crystals and Lilly liver extract 343.

The results of the experiment are shown in Table XXVI. The average biological value of the pork muscle determined with rats six months old, reared on the Steenbock V ration, was found to be 79.3, standard deviation of 5.9. This value did not differ significantly from the biological value (76.3) obtained when yeast was used as the source of the vitamin B-complex. Mitchell ('24) found the biological value of the proteins of yeast to be relatively high (85). Thus, as was shown to be actually the case, the inclusion of yeast in the test ration should not exert any appreciable effect upon the biological value of the test protein. This finding will validate any experiments conducted in the past,

Table XXVI. The biological value of the proteins of canned autoclaved pork muscle determined with male albino rats, six months old, reared on a mixed grain diet (Series C, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19235	264	-24	129.67	123.40	343.39	
	19345	267	-18	133.00	98.68	369.63	
	19332	303	-22	146.27	140.20	394.54	
	19344	269	-18	148.15	125.33	381.94*	
	19328	236	- 4	133.85	98.68	415.75*	
	19334	257	-18	136.26	112.00	380.09	
	19296	311	-14	150.38	120.31	440.36	
	19233	311	-10	143.52	137.11	394.54	
	19245	334	-16	141.12	138.65	439.09	
Av.		285.6	-16.0	140.25	121.59	393.70	
Protein feeding period	19235	257	-14	473.83	115.74	458.74	75.6
	19345	270	0	667.48	146.24	511.09	77.2
	19332	314	0	740.54	168.00	608.19	70.0
	19344	271	+ 2	652.63	154.90	456.14	84.9
	19328	252	+ 4	587.29	149.20	467.93	70.4**
	19334	266	+ 4	649.66	151.56	492.84	81.5
	19296	320	+12	772.02	156.68	557.04	84.7
	19233	315	- 2	618.77	150.86	481.05	85.7
	19245	341	+ 7	748.26	175.96	557.04	83.4
Av.		289.6	+ 1.4	656.72	152.12	510.01	79.3

*Average of two periods.
**Calculated on second endogenous urinary nitrogen only.

when investigators were forced to rely upon yeast as a source of the vitamin B-complex in the test ration.

The biological value obtained for the pork muscle (76.3) is very similar to the value assigned this protein by Mitchell, Beadles, and Kruger ('27). These workers found pork tenderloin to have a biological value of 79 and in general, they found that different cuts possessed rather constant biological values, averaging about 74. Fresh pork, ground and dried, was used by Mitchell and his co-workers. Since the value 76.3 obtained in the laboratory so closely approximates the average value observed by Mitchell (74) we can conclude that the process of autoclaving does not lower the biological value of the pork muscle. In this conclusion, we agree with Mattill who observed that the proteins of beef were not affected by autoclaving. On the other hand, Morgan and Kern ('34) are of the opinion that prolonged and drastic heating decreases the biological value of beef proteins. In view of the results obtained, we cannot ascribe the nutritional failure observed in animals reared on the experimental diet, Pork I, to the deleterious effect of heat.

When this protein was tested with the use of two groups of rats three months old and 10 months old, respectively, less efficient utilization occurred when the rats were 10 months old than when they were only three months old (Tables

Table XXVII. The biological value of the protein of canned autoclaved pork muscle determined with male albino rats, three months old, reared on a mixed grain diet (Series C, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19695	164	- 8	138.16	78.64	191.41	
	19702	207	-22	122.25	77.65	264.80	
	19703	202	-15	136.90	63.15	226.77	
	19768	228	- 8	143.40	69.31	259.54	
	19807	229	-14	132.65	94.76	263.74	
	19808	220	-12	128.42	92.93	266.33	
	20338	198	-16	128.42	82.48	264.93	
	20399	161	- 6	125.98	89.57	242.56	
	20431	166	- 8	129.32	80.91	255.96	
Av.		197.2	-12.1	131.72	81.04	246.23	
Protein feeding period	19695	168	+ 4	446.51	106.47	332.93	66.4
	19702	207	+ 6	523.14	77.06	269.99	99.0
	19703	210	+ 8	568.28	116.78	315.88	82.7
	19768	231	- 2	586.10	134.44	358.77	80.9
	19807	235	+ 6	672.20	124.94	363.10	84.5
	19808	223	+ 6	627.46	117.99	346.05	86.8
	20338	-	-	--	--	--	
	20399	162	- 4	424.92	89.57	258.21	96.3
	20431	171	+ 2	505.53	89.76	251.68	96.8
Av.		200.9	+3.2	544.27	107.12	312.08	86.9

Table XXVIII. The biological value of the proteins of canned autoclaved pork muscle determined with male albino rats, 10 months old, reared on a mixed grain diet (Series C, group 1)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	16900	382	-24	136.95	118.63	309.16	
	16918	262	-12	127.17	112.13	238.49	
	17170	358	-14	134.14	101.84	142.12	
	17317	321	-10	159.65	122.73	322.48	
	17379	281	-10	135.26	88.57	361.70	
	17670	291	- 6	144.54	106.65	308.02	
	17853	316	- 8	149.93	111.08	352.84	
	17949	361	-14	148.76	132.28	390.61	
	17990	249	- 2	135.18	80.72	290.97	
Av.		313.4	-11.1	140.95	108.29	301.82	
Protein feeding period	16900	406	+ 8	685.32	130.60	522.04	68.4
	16918	274	-12	418.82	106.37	402.88	60.2
	17170	-	-	--	--	--	--
	17317	327	+18	778.56	146.37	488.91	78.0
	17379	278	- 4	554.62	116.29	443.29	84.5
	17670	281	+ 6	595.60	141.01	446.55	75.3
	17853	322	0	704.34	81.07	467.93	83.7
	17949	361	- 3	712.92	141.13	526.94	80.6
	17990	240	0	543.94	103.34	376.09	83.7
Av.		311.1	+ 1.6	624.26	120.77	459.33	76.9

XXVII and XXVIII). The differences while not as great as those observed in the previous control determinations (Series A, group 1) were significant. Thus, further evidence of the effect of age upon results secured in the determination of biological values was obtained. The animals reared on the Pork I ration for three months gave a slightly lower average biological value for the proteins (See Table XXIX) than did the corresponding animals reared on the grain diet. However, the differences were not significant. We were unable to check this value using animals that had been reared on Pork I ration until 10 months old since a large number of the animals in this test did not withstand the rigid dietary regime imposed during the assay period.

A summary of the results obtained in all experimental series of this investigation is given in Table XXX.

The physiological significance of the partition of nitrogen in the urine

A study of the partition of nitrogen in the urine revealed the physiological significance of the decrease in the biological value of pork proteins as the animals increased in age. The data are recorded in Table XXXI.

The degree to which the rat utilizes the nitrogen of its food changes as the animal becomes older. In the three-month-old rats of Series A, group 1, 33 per cent of the nitro-

Table XXIX. The biological value of the protein of canned autoclaved pork muscle determined with male albino rats, three months old, reared on the Pork I diet (Series C, group 2)

Period of assay	Rat number	Average body weight	Gain during test period	Nitrogen consumed	Fecal nitrogen	Urine nitrogen	Biological value
		gm.	gm.	mg.	mg.	mg.	
Low-nitrogen feeding period	19693	193	-10	138.50	110.72	222.84	
	19694	190	- 8	138.30	109.03	211.72	
	19701	196	- 8	139.62	79.24	267.40	
	19766	200	- 8	132.94	89.37	233.30	
	19767	213	- 6	143.27	137.23	259.83	
	19806	205	-10	136.43	122.28	354.11	
	20305	180	-16	132.28	86.81	238.49	
	20307	184	- 4	141.07	101.96	233.30	
	20398	200	- 8	136.00	116.33	250.40	
Av.		195.7	- 8.7	137.60	105.89	250.16	
Protein feeding period	19693	212	+12	628.27	138.82	399.80	70.5
	19694	210	+16	662.73	139.22	353.91	77.5
	19701	214	+12	633.62	132.06	374.89	81.5
	19766	222	+16	704.90	194.69	408.99	70.7
	19767	230	+ 8	704.90	178.28	452.68	67.9
	19806	218	+ 8	564.65	112.81	297.57	--
	20305	-	-	--	--	--	--
	20307	-	-	--	--	--	--
	20398	209	+10	552.55	122.24	271.33	96.2
Av.		216.4	+11.7	635.95	145.45	365.60	77.4

Table XXX. Summary; biological values

I. Arranged according to the protein assayed.

Experimental series	Assay protein	Pre-experimental diet	Age of test animal	Number of assays
			mo.	
A	Pork and yeast	Steenbock V group 1	3	9
			6	9
			10	9
		Pork I group 2	3	9
			10	8
B	Pork, yeast, and liver	Pork 7	10	8
C	Pork	Steenbock V group 1	3	8
			6	9
			10	8
		Pork I group 2	3	6
			10	-

II. Arranged according to the age of the animal

Age of test animal	Experimental series	Pre-experimental diet	Assay protein	Number of assays
3 mo.	A	Steenbock V	Pork and yeast	9
	A	Pork I		9
	C	Steenbock V	Pork	8
	C	Pork I	Pork	6
6 mo.	A	Steenbock V	Pork and yeast	9
	C	Steenbock V	Pork	9
10 mo.	A	Steenbock V	Pork and yeast	9
	A	Pork I		8
	B	Pork 7	Pork, yeast, and liver	8
	C	Steenbock V	Pork	8

)logical values

:o the protein assayed.

:ein	Pre-experimental diet	Age of test animal	Number of assays	Biological value		
				Mean	Standard deviation	Range
		mo.				
	Steenbock V group 1	3	9	83.4	6.3	16
		6	9	76.3	4.4	13
		10	9	67.8	9.0	25
	Pork I group 2	3	9	82.2	5.6	20
		10	8	75.4	8.6	25
ıst, ɔr	Pork 7	10	8	71.3	7.7	26
	Steenbock V group 1	3	8	86.9	9.7	33
		6	9	79.3	5.9	20
		10	8	76.9	8.8	24
	Pork I group 2	3	6	77.4	10.4	26
		10	-	--	--	--

to the age of the animal

ıl	Pre-experimental diet	Assay protein	Number of assays	Biological value		
				Mean	Standard deviation	Range
	Steenbock V	Pork and yeast	9	83.4	6.3	16
	Pork I		9	82.2	5.6	20
	Steenbock V	Pork	8	86.9	9.7	33
	Pork I		6	77.4	10.4	26
	Steenbock V	Pork and yeast	9	76.3	4.4	13
	Steenbock V	Pork	9	79.3	5.9	20
	Steenbock V	Pork and yeast	9	67.8	9.0	25
	Pork I		8	76.9	8.8	23
	Pork 7	Pork, yeast, and liver	8	71.3	7.7	26
	Steenbock V	Pork	8	76.9	8.8	24

Table XXXI. Data to show the relationship between the age of the test animal and percentage of absorbed nitrogen excreted in the urine, and the percentage of urine nitrogen derived from food

Pre-experimental diet	Source of proteins	Age of test animal Mo.	Per cent of absorbed nitrogen excreted in the urine	Per cent of exogenous nitrogen in the urine	Biological value
Steenbock V	Pork and yeast	3	16	33	83.4
		6	22	37	76.3
		10	32	46	67.8
Pork I	Pork and yeast	3	18	36	86.9
		10	23	29	75.4
Steenbock V	Pork alone	3	12	21	82.2
		6	18	23	79.3
		10	25	34	76.9
Pork 7	Pork, yeast, and liver	10	32	32	71.3

gen in the urine was derived from food sources. Later, at six months, 37 per cent of the urine nitrogen was of exogenous origin, and at 10 months, 46 per cent. The same change in the proportion of exogenous nitrogen in the urine occurred in 10 rats comprising Series C, but was not as marked in the urine of animals of Series A, group 2.

The percentage of the absorbed food nitrogen that appeared in the urine excreted by the various experimental groups was also calculated. A progressive increase in the percentage of absorbed food nitrogen excreted as the rats increased in age was consistently demonstrated in all groups. If the average is taken of the values obtained with the use of each age group of animals, results may be summarized as follows: at three months of age, 16 per cent of the absorbed nitrogen of the food appeared in the urine; at six months, 20 percent; and 10 months, 28 per cent. As the rat became older, less and less of the dietary protein was utilized. Explanations for the phenomenon may be found in the principles of the general theory of protein metabolism.

Although the period of most rapid growth is over when the rat is three months old, the body continues to increase in size thereafter. New tissue is being laid down constantly until the animal is at least five or six months old. The small quantity of nitrogen derived from food in the urine of rats in the present experiment when they were three

months may be accounted for by the increased demands of the body for "building stones" to synthesize new tissue over and above the quantity of nitrogenous material required for maintenance. However, when the rat is six months old, body demands for nitrogen are largely those of maintenance and little is required for storage as new tissue. We, therefore, should expect to find, as we did, an increased quantity of nitrogen excreted in the urine that had its origin in food protein. At 10 months, metabolic processes begin to slacken. The animal is less active. Cell activity has decreased. Since wear and tear of body tissues are reduced even the bodily demands for nitrogen, for purposes of maintenance, decrease. In the experimental groups, 10 months old, the increased percentage of nitrogen excreted in the urine coming from food is a reflection of normally lowered metabolism.

It is to be noted that while the partition of the nitrogen in the urine of stock animals progressively increasing in age and fed the proteins present in the pork diets followed this trend, the excretion of food nitrogen was slightly higher in all age-groups in this series than it was in corresponding groups where the rats were given the proteins of pork alone. This was probably due to the fact that some of the nitrogen-carrying constitutents of the yeast cannot be utilized by the test animal for either building or repair purposes.

Although the findings are in accord with metabolic theory, another reason for the increased quantity of food nitrogen appearing in the urine of the older animal must not be overlooked, especially since theoretically rats are still in the prime of life when they are 10 months old. The observations may also mean that the oldest animal was losing unduly large quantities of nitrogen through the kidney tissues. Moise and Smith ('27) have pointed out that glomerular and tubular changes are present as normal occurrence in the kidneys of rats fed an adequate diet containing casein as its protein for 150 days after unilateral nephrectomy. In the rat, glomeruli continue to be formed for the first 100 days of life. Injuries produced during this time are probably repaired through processes of growth. In older animals this does not take place.

But if kidney damage is responsible for the increased excretion of food nitrogen in the older rats the lesion must be attributed only to the effect of age, and not to the influence of the pre-experimental diet, because the animals reared for 10 months on the meat-containing diets (Pork I and Pork 7) excreted no greater a proportion of the absorbed nitrogen than did the animals reared for 10 months on the mixed grain diet. This is very interesting, since the animals fed the meat diets often excreted urine which contained blood that could be detected by gross ob-

servation. The kidneys of some of the old animals also had a mottled appearance upon autopsy. In spite of this, the biological value of the proteins tested shows that these animals were utilizing nitrogen in a manner that was normal for rats of their age.

Since factors such as continued growth at three months and a decreased rate of metabolism with possible kidney damage at 10 months, complicate the picture, our hypothesis has been confirmed that an experimental animal approximately six months old is the best type of animal for use in the establishing the biological value of a protein.

Digestibility

The digestibility of the various protein mixtures that have been assayed for biological values was determined by calculating the coefficients of digestibility. This determination is as important as that of the biological value since it is impossible to secure a complete picture of the ability of the animal to utilize the protein ingested without this information. Two situations may occur. First, Seegers and Mattill ('35) have shown that the low biological value of alcohol-extracted liver is due to its poor digestibility because the amino acids absorbed are not representative of the original protein. Second, even though the biological value may be high as is the case with autoclaved

beef muscle (Seegers, Schultz, and Mattill, '36), in reality the animals derive benefit from only one-half of the proteins eaten because only this proportion is digested (Seegers, and Mattill '35).

The coefficients of digestibility obtained with the use of all groups in the present experiment were very constant. Individual data pertaining to each experimental group are shown in Tables XXXII - XXXVII. The digestibility of the various proteins tested ranged from 92 to 96 per cent in spite of differences in age of the test rats and the kind of pre-experimental diet to which the animals were accustomed. Thus, the proteins in pork muscle alone, in the Pork I diet, and in the Pork 7 diet do not appear to differ in degree to which they are acted upon by the enzymes of the intestinal tract.

Mitchell ('27) has determined the biological value of pork but he does not give the coefficient of digestibility of its proteins. However, a comparison of the values that he presented for the total nitrogen in the feces and the fecal nitrogen of endogenous origin indicate that the proteins of pork were well digested. Morgan and Kern ('34) testing the autoclaved proteins of beef muscle, found the coefficient of digestibility (98) to be as high as that characteristic of raw meat. Seegers and Mattill ('35), however, describe a progressive lowering of the digestibility

Table XXXII. The coefficient of digestibility of certain protein mixtures determined with male albino rats, three months old, reared on a mixed grain diet (Series A, group 1, and series C, group 1)

Rat number	Fecal nitrogen of endogenous origin	Canned autoclaved pork muscle and yeast				Canned autoclaved pork muscle			
		Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.		mg.	mg.	mg.	
19695	78.64	109.73	31.09	595.23	94.8	106.47	27.83	446.51	93.8
19702	77.65	139.61	61.96	790.05	92.2	77.06	0.59	523.14	99.8
19703	63.15	147.76	84.61	814.53	89.6	116.78	53.63	568.28	90.6
19768	69.31	186.68	117.37	978.95	88.0	134.44	65.13	586.10	88.9
19807	94.76	184.10	89.34	965.26	90.8	124.94	30.18	672.20	95.5
19808	92.93	177.55	84.62	877.77	90.4	117.99	25.06	627.46	96.0
20338	82.48	135.82	53.34	849.83	93.7	--	-	--	--
20399	89.57	106.30	16.73	561.86	97.0	89.57	0.00	424.92	100.0
20431	80.91	105.70	24.79	675.36	96.4	89.76	8.85	505.53	98.2
Av.					92.5				95.3

Table XXXIII. The coefficient of digestibility of certain protein mixtures determined with male albino rats, six months old, reared on a mixed grain diet (Series A, group 1, and series C, group 1)

Rat number	Fecal nitrogen of endogenous origin	Canned autoclaved pork muscle and yeast				Canned autoclaved pork muscle			
		Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.		mg.	mg.	mg.	
19235	123.40	170.89	47.49	1132.06	95.8	115.74	--	473.83	--
19345	98.68	213.57	114.89	1220.18	90.6	146.24	47.56	667.48	92.9
19332	140.20	207.78	67.58	1103.98	93.9	168.00	27.80	740.54	96.3
19344	125.33	149.47	24.14	813.46	97.0	154.90	29.57	652.63	95.5
19328	98.68	128.70	30.02	647.86	95.4	149.20	50.52	587.29	91.4
19334	112.00	187.89	75.89	1107.85	93.2	151.56	39.56	649.66	93.9
19296	120.31	194.07	73.76	1162.08	93.7	156.68	36.37	772.02	95.3
19233	137.11	204.11	67.00	1196.94	94.4	150.86	13.75	618.77	97.8
19245	138.65	208.36	69.71	1269.57	94.5	175.96	37.31	748.26	95.0
Av.					94.3				94.8

Table XXXIV. The coefficient of digestibility of certain protein mixtures determined with male albino rats, 10 months old, reared on a mixed grain diet (Series A, group 1 and series C, group 1)

Rat number	Fecal nitrogen of endogenous origin	Canned autoclaved pork muscle and yeast				Canned autoclaved pork muscle			
		Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.		mg.	mg.	mg.	
16900	118.63	151.54	32.91	987.24	96.7	130.60	11.97	685.32	98.2
16918	112.13	136.83	24.70	727.44	96.6	106.37	--	418.82	
17170	101.84	142.67	40.83	806.88	94.9	--	--	--	
17317	122.73	187.67	64.94	1102.35	94.1	146.37	23.64	778.56	97.0
17379	88.57	132.47	43.90	715.27	93.9	116.29	27.72	554.62	95.0
17670	106.65	166.73	60.08	897.60	93.3	141.01	34.36	595.60	94.2
17853	111.08	177.15	65.97	951.00	93.1	81.07	--	704.34	
17949	132.28	187.02	54.74	859.07	93.6	141.13	8.85	712.92	98.8
17990	80.72	84.77	4.05	308.95	98.7	103.34	22.62	543.94	95.2
Av.					95.0				96.4

Table XXXV. The coefficient of digestibility of certain protein mixtures determined with male albino rats, three months old, reared on the Pork I diet (Series A, group 2, and series C, group 2)

Rat number	Fecal nitrogen of endogenous origin	Canned autoclaved pork muscle and yeast				Canned autoclaved pork muscle			
		Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.		mg.	mg.	mg.	
19693	110.72	155.70	44.98	897.74	95.0	138.82	28.10	628.27	95.5
19694	109.03	140.21	31.18	807.67	96.1	139.22	30.19	662.73	95.5
19701	79.24	125.84	46.60	793.97	94.1	132.06	52.82	633.62	91.3
19766	89.37	149.74	60.37	841.50	92.8	194.69	105.32	704.90	85.1
19767	137.23	181.12	43.89	890.12	95.1	178.28	41.05	704.90	94.2
19806	122.28	180.71	58.43	903.45	93.5	112.81	--	564.65	--
20305	86.81	105.11	18.30	562.28	96.8	--	--	--	--
20307	101.96	117.91	15.95	712.88	97.8	--	--	--	--
20398	116.33	120.26	3.93	665.04	99.4	122.24	5.91	552.55	98.9
Av.					95.6				93.4

Table XXXVI. The coefficient of digestibility of certain protein mixtures determined with male albino rats, 10 months old, reared on the Pork I diet (Series A, group 1)

Rat number	Fecal nitrogen of endogenous origin	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.	
16726	168.55	88.27	80.28	249.45	67.8
16789	91.40	90.24	1.16	432.67	99.7
16790	68.65	68.52	0.13	362.03	99.9
16893	83.34	135.96	52.62	727.44	92.8
17186	101.65	143.81	42.16	706.02	94.0
17469	93.94	132.84	38.90	658.85	94.1
17894	76.13	181.32	105.19	862.56	87.8
16789	91.40	146.41	55.01	710.61	92.2
16893	83.34	120.04	36.70	655.59	94.4
17469	93.94	127.50	33.56	824.74	95.9
Av.					91.9

Table XXXVII. The coefficient of digestibility of the proteins of the Pork 7 diet determined with male albino rats, 10 months old, reared on the Pork 7 diet (Series B)

Rat number	Fecal nitrogen of endogenous origin	Fecal nitrogen	Nitrogen from undigested food	Nitrogen consumed	Coefficient of digestibility
	mg.	mg.	mg.	mg.	
16727	93.70	127.55	33.85	948.56	96.4
16728	107.67	135.22	27.58	746.15	96.3
16791	68.18	97.44	29.26	767.90	96.2
16895	71.01	129.29	58.28	825.27	92.9
16896	91.66	90.15	1.51	1033.11	98.5
17470	116.97	97.25	--	1028.62	
17895	85.20	110.02	24.82	869.68	97.2
16727	93.70	143.36	49.66	808.35	93.9
16728	107.64	128.73	21.09	796.61	97.4
16791	68.18	189.34	121.16	866.01	86.0
16895	71.01	48.47	--	460.37	--
17470	116.97	160.07	43.10	1184.21	96.4
Av.					95.1

of beef protein as the intensity of heat is increased. The results obtained in this investigation are the first recorded that ascribe a high biological value and a high coefficient of digestibility to autoclaved meat muscle. The pork proteins used in our test diet, the feeding of which leads invariably to nutritional disaster are, therefore, of high biological efficiency.

The relative digestibility of pork alone, and of a combination of pork, yeast, and liver was 95 and 96 per cent respectively (Table XXXVIII). We had more or less expected to find that the proteins of the canned autoclaved pork muscle alone were more completely digested than those of the Pork I diet, since Mitchell ('24) gave the coefficient of digestibility of yeast proteins as 77. Proteins having such a low coefficient of digestibility when incorporated in a diet might appreciably affect the apparent digestibility of the proteins of the original diet.

The question of the relation of the quantity of endogenous nitrogen excreted in the feces to the weight of the food consumed has been discussed in the Review of Literature. The coefficients were calculated with the use of uncorrected fecal endogenous nitrogen figures. We believe that the constancy of the individual values obtained for the coefficient of digestibility supports the view that endogenous fecal nitrogen is independent of the weight of

Table XXXVIII. Summary; coefficients of digestibility

Experimental series	Assay protein	Pre-experimental diet	Age of test animal	Number of assays	Coefficients of digestibility	
					Mean	Standard deviation
			mo.			
A	Pork and yeast	Steenbock V, group 1	3	9	92.5	3.8
			6	9	94.3	1.7
			10	9	95.0	1.9
		Pork I, group 2	3	9	95.6	2.6
			10	8	91.9	9.2*
B	Pork, yeast, and liver	Pork 7	10	8	95.1	3.6
C	Pork	Steenbock V, group 1	3	8	95.3	4.7
			6	9	94.8	2.1
			10	8	96.4	2.0
		Pork I, group 2	3	6	93.4	4.9
			10	-	--	-

*When rat having low coefficient (67.8) is omitted, mean = 94.6, standard deviation 3.8.

the food consumed. We believe these data lend support to the method we have adopted. Error in the estimating of endogenous nitrogen in the feces affects the coefficient of digestibility whereas it does not affect the biological value.

Factors Affecting the Variability in Individual Determinations

The variations observed in the individual determinations of the standard assay obtained with the use of the animals six months old and reared on the Steenbock V diet (Series A, group 1) have been discussed. The mean biological value was 76.3, standard deviation, 4.4. It has been shown that the variation, measured in terms of the standard deviation approximated that of Morgan and Kern ('34), Seegers and Mattill ('35), and Mitchell, Burroughs, and Beadles ('36), Mitchell, probably being the authority in the field of protein utilization.

However, the variations obtained within every determination made with other experimental groups employed in this study were greater than the variation obtained with the control group, as shown by ranges in biological values and the standard deviations. These data are tabulated in Table XXX. Ranges in individual results secured with the different experimental groups varied from 13 to 33; the average range was 20. The variation is approximately that recorded by other

workers in the field (See Table XXXIX), and probably should not be considered as unreasonable, since the whole determination is fraught with many possible sources of error from the chemical, biological, and manipulative standpoints. As Mitchell ('36) has pointed out, the error represented in estimation of biological values is an accumulation of not only the unavoidable error in technique but also of the individual variability of the test animal. Since the standard determination was apparently so much more reliable than any other obtained, it seemed important to search our data for reasons for the increased variability observed in the other experimental lots.

While it is possible to secure a uniformity in experiments conducted with rats three months old equal to that obtained with animals six months old, it is just as likely that the use of animals of this age may introduce increased variation (Table XXX). A rat in this age-group may chance to be that animal of an average group that continues active growth longer than usual. A biological value obtained with the use of such an animal will be high because the protein of the diet is being deposited as new tissues. For example, with three of the rats three months old, biological values of 96, 96, and 99 were obtained, meaning if the possibility of experimental error is excluded, that almost perfect utilization of the protein occurred. It was these animals that were wholly

Table XXXIX. The variability in biological values within assay groups obtained by different investigators

Worker	Protein studied	Range	Length of range	Average value
1. Mitchell	Milk	81-100	19	93
2. Morgan and Kern	Raw meat	59-73	14	67
3. Boas-Fixsen and Jackson	Whole maize	63-92	29	84
4. Braman	Cottonseed meal	75-80	15	78
5. Basu and Basak	Sun-dried polished rice	69-94	25	80
6. Seegers and Mattill	Dried whole liver	38-63	25	88
7. Schneider	Fish meal	56-82	26	71
8. Bethke and co-workers	Linseed meal	58-79	21	70

1. Mitchell, H. H., J. Biol. Chem., 58; p. 907 (1924).
2. Morgan, A. F., and Kern, G. E., J. Nutrition, 7; p. 371 (1934).
3. Boas-Fixsen, M. A., and Jackson, H. M., Biochem. J., 26; p. 1928 (1932).
4. Braman, W. W., J. Nutrition, 4; p. 255 (1931).
5. Basu, K. P., and Basak, M. N., Ind. J. Med. Res., 24; p. 1046 (1937).
6. Seegers, W. W., and Mattill, H. A., J. Nutrition, 10; p. 208 (1935).
7. Schneider, B. H., J. Agr. Res., 44; p. 725 (1932).
8. Bethke, R. M., Bohstedt, G., Sassaman, H. L., Kennard, D. C., and Edington, B. H., J. Agr. Res., 36; p. 860 (1928).

responsible for the increased variation in the results obtained with two lots of this age-group.

The data (Table XXX) also show that employment of animals 10 months old may be expected to introduce extreme variation consistently. The range in the results of the four sets of determinations made with this age-group was very close to 25, in contrast to one of 13 obtained in the test made with the standard group.

In light of these observations, we found it interesting to examine in a preliminary fashion supporting data obtained in the assays as well as many gross observations, to find if possible, the underlying cause of the variability. This study resolved itself into two divisions, (1) the relation of the characteristics of the growth of the animal in the pre-experimental period to the variability of the individual biological values obtained in the various assays, and (2) the relation of the physical condition of the animals in the specific groups at the time of the assay to the uniformity of the final results of the experiment.

Relation of rate of growth of animal in pre-experimental period to variability

The individual curves depicting the rate of growth for the 55 animals used in the assays were plotted. The curves for each group studied are shown in Figures IV, V, VI, VII VIII and IX. At the end of three months the rats in all

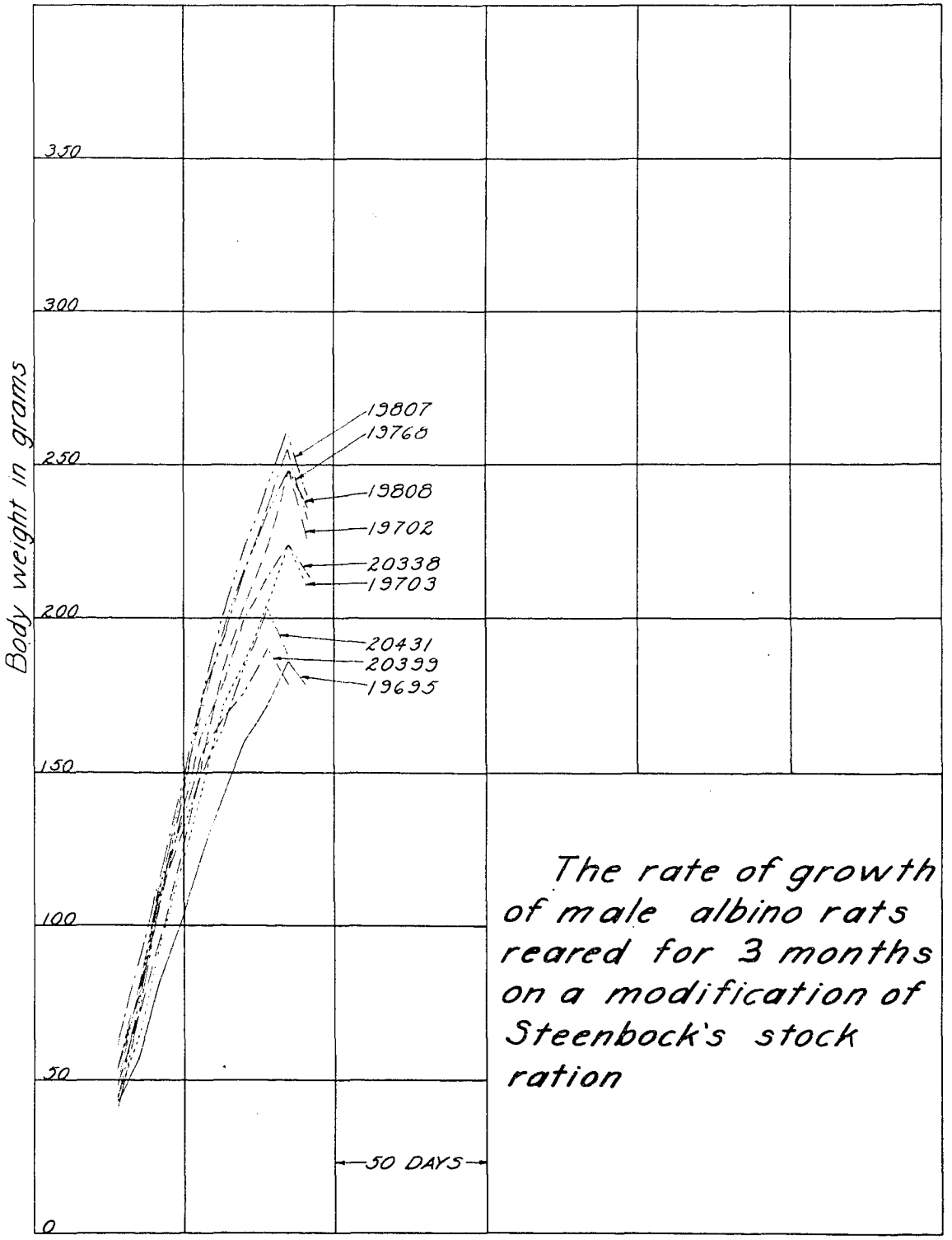

Figure IV. Growth of rats 3 months old belonging to Series A, group 1 and Series C, group 1.

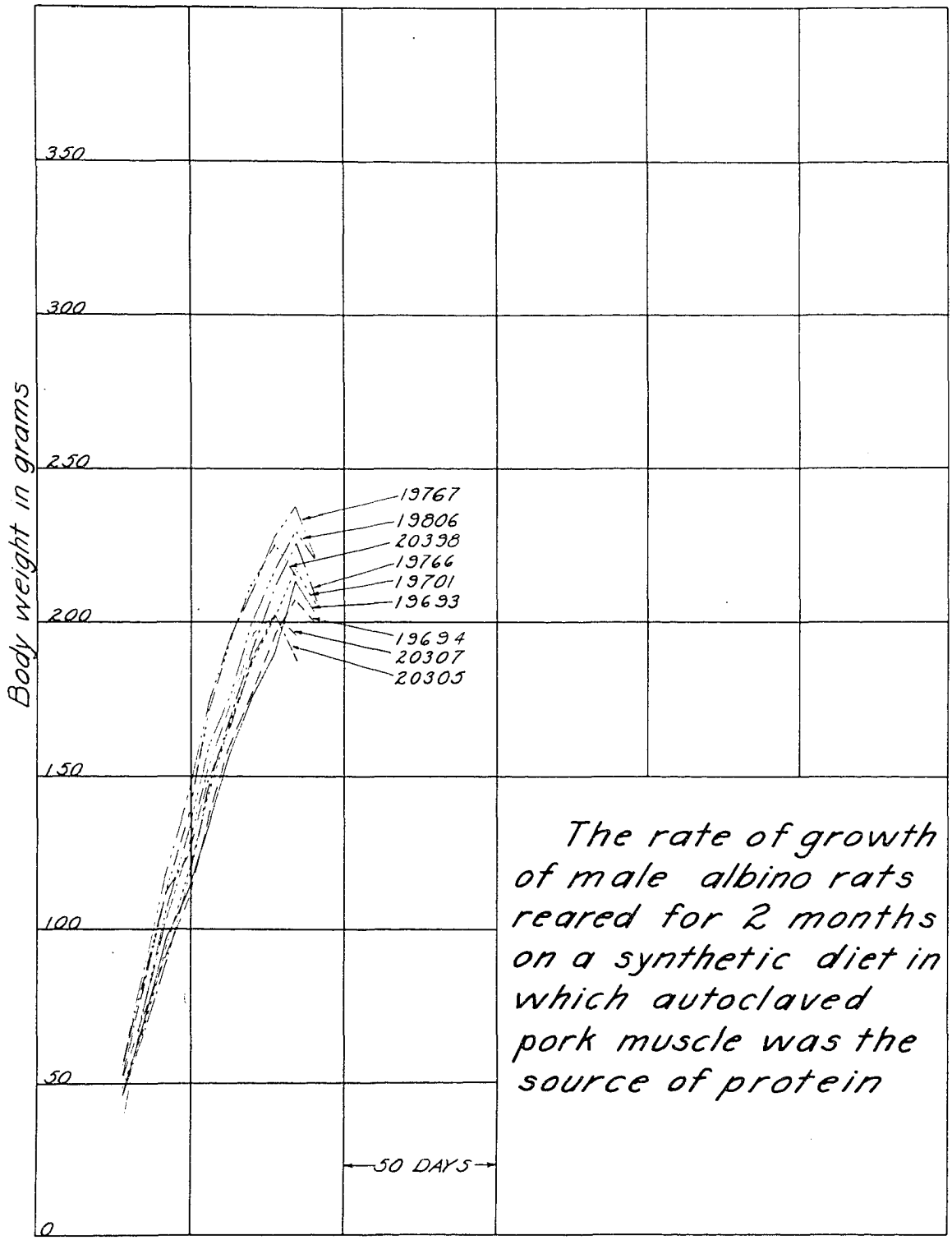

Figure V. Growth of rats 3 months old belonging to Series A, group 2, and Series C, group 2.

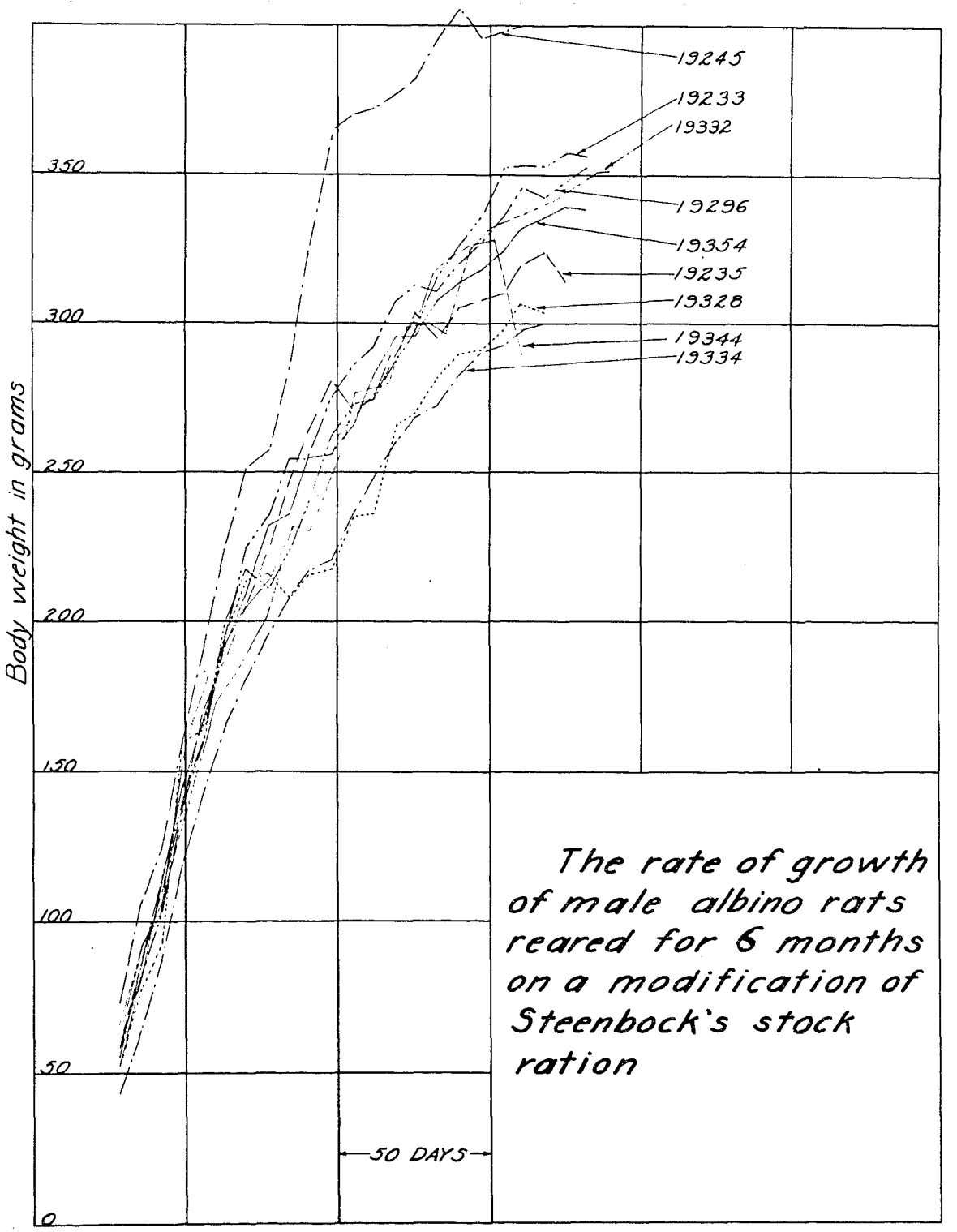

Figure VI. Growth of rats 6 months old belonging to Series A, group 1 and Series C, group 1.

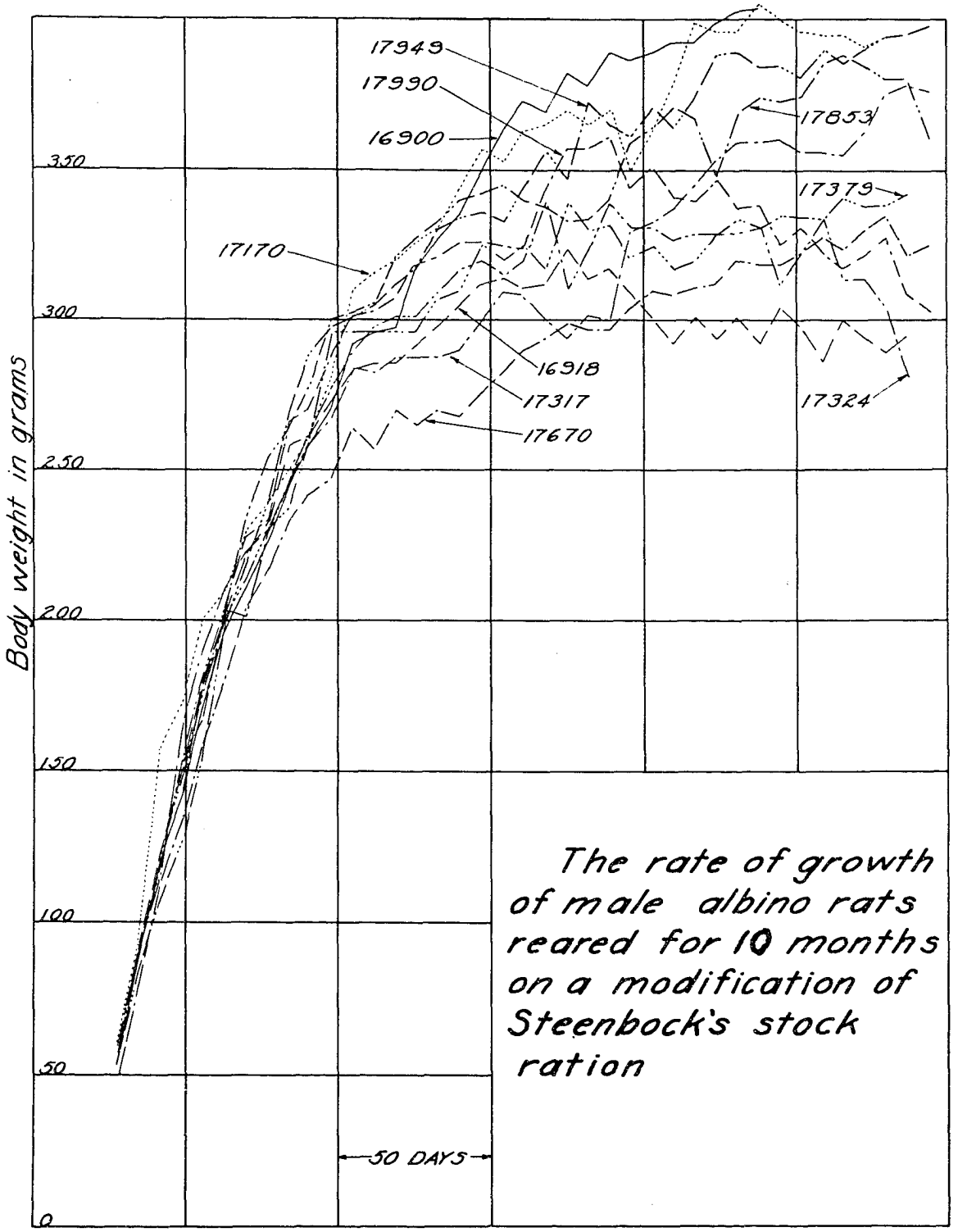

Figure VII. Growth of rats 10 months old belonging to Series A, group 1 and Series C, group 1.

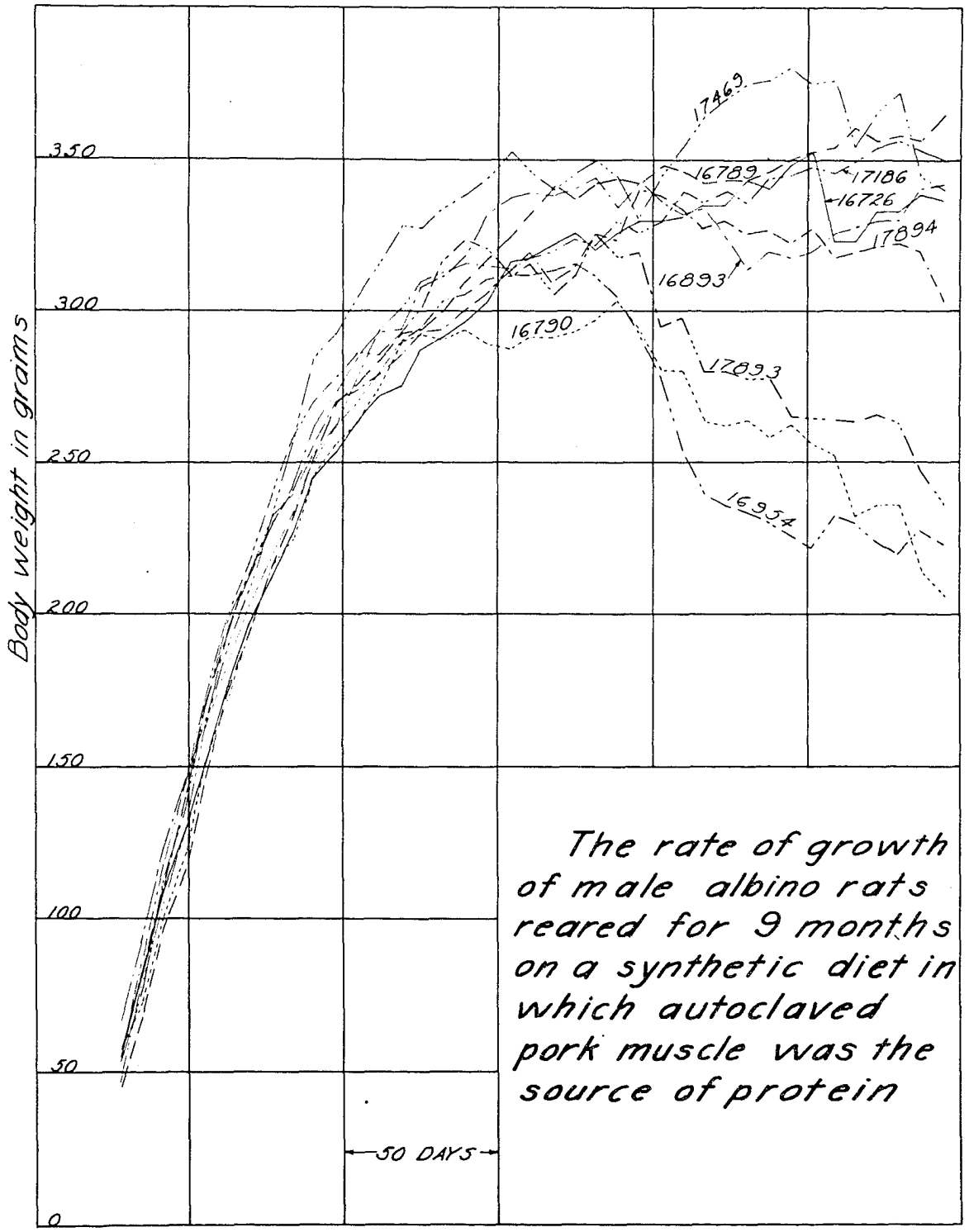

Figure VIII. Growth of rats 10 months old belonging to Series A, group 2.

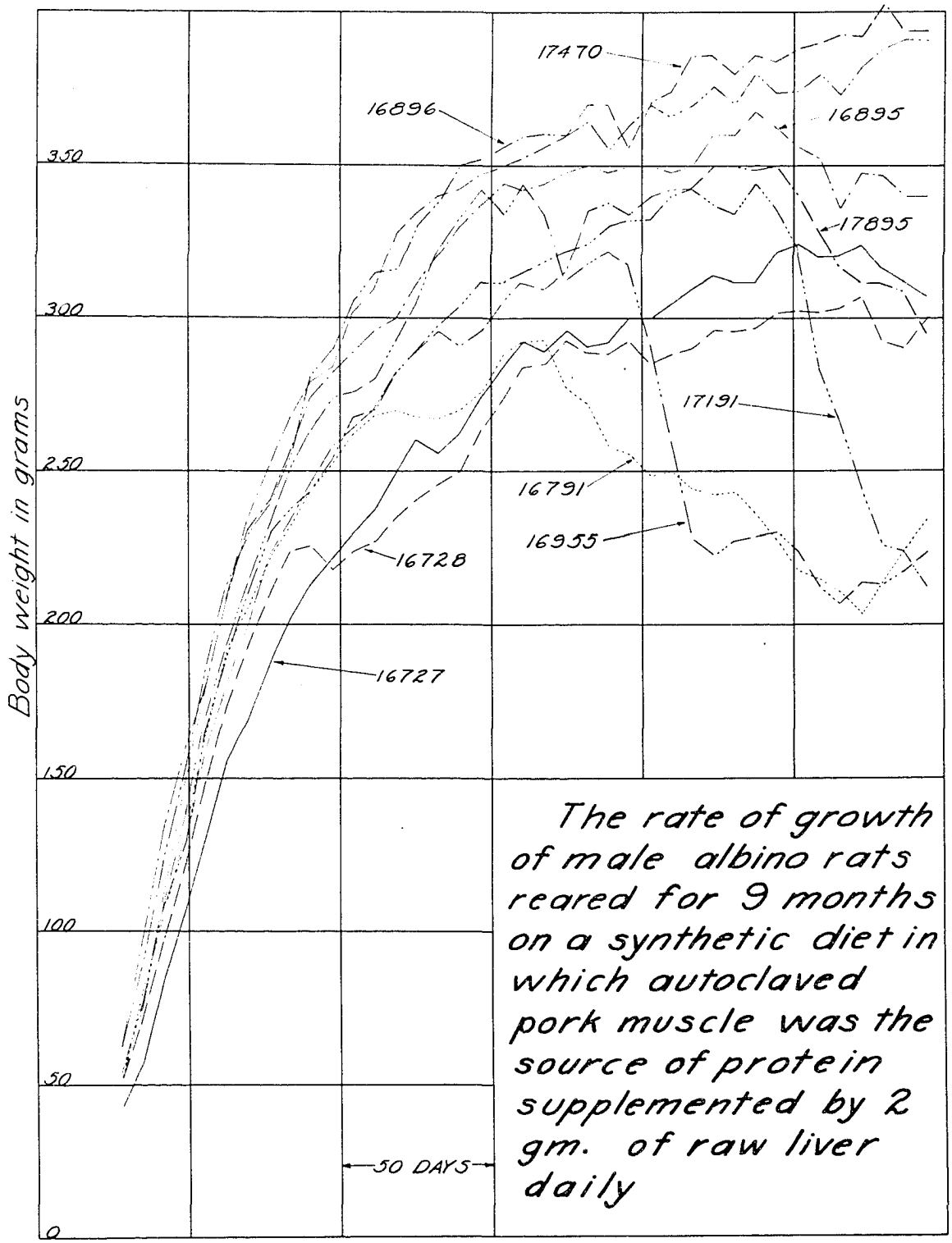

Figure IX. Growth of rats 10 months old belonging to Series B.

experimental groups were of normal body weight. The range in body weight was approximately 75 grams. The groups grown for six months were also normal in size, the extreme difference in body weights being 80 grams. Such a range in body weights can be expected in groups of normal animals of these ages (Timson '32). However, the variations in the size of the individual animals at the end of 10 months of growth was much greater than that observed at the earlier periods of life history, being 211 grams. The ranges in biological values obtained for the three age-groups do not appear to be associated with the degree of variation noted in body weights of the three age-groups studied as shown in the tabulations listed below:

Table XL. Range in body weight of test animals and the variations in biological values

Age of animals	Range in body weight	Range in biological values obtained			
		Test 1	Test 2	Test 3	Test 4
	gm.				
3 mo.	75	16	20	33	26
6 mo.	80	13	20		
10 mo.	211	25	24	28	26

The variations in individual biological values obtained with the groups three months old were as great as those observed in the groups of animals 10 months old in 50 per cent of the assays made. Therefore, variations in body weight of test rats apparently do not affect the variation in biological

values obtained. This idea was further supported when the data pertaining to the four sets of determinations made with rats 10 months old were analysed. The individual body weights of rats reared on the Steenbock V ration varied by 120 grams, those of the rats fed the Pork I ration by 142 grams, and those on the Pork 7 diet by 170 grams. An increase in variability in body weight of the experimental animal did not increase the variability of the biological value obtained with each experimental group (See Table below).

Table XLI. Range in body weight of rats, 10 months old, and the variations in biological values

Pre-experimental diet	Range in body weight at the time of the assay	Range in biological values obtained in each assay	Average biological value
	gm.		
Steenbock V	120	25	67.8
		24	76.9
Pork I	142	28	75.4
Pork 7	170	26	76.9

Thus, the studies indicate that variation in results obtained in the biological analysis of proteins does not arise from differences in weights of rats composing any test group. Further studies are to be made relative to this subject.

The great variability in body weight in the groups of rats receiving the pork diets was due to the fact that certain of the animals were notaable to maintain their adult weight

after they had reached 170 days of age. Drastic losses occurred in some of the animals. We wondered whether or not the rats that exhibited great losses in body weight were the animals that caused the variation in results obtained in groups they represented. We feared that possibly the use of these rats in our final analyses introduced error.

In order to study this problem, the animals were divided into two classes, those that maintained their adult weight and those that did not maintain their adult weight. Of the 28 animals in the three groups, 10 rats lost weight. Of these 10, five died during the assay, and one of the others lost so much weight during the collection period that the biological value could not be determined. The remaining four yielded results, which were well within the range of the biological values obtained with the animals that were able to maintain their weight (See Table XLII).

Therefore, the inclusion of data pertaining to these animals in final analysis did not lead to wrong conclusions.

The physical condition of the animals

An examination of the average rate of growth on the animals, 10 months old (Figure X), showed that in early periods of the life history the rate of growth of the animals receiving the pork rations was as good as that of the animals receiving the Steenbock V diet. However, when the animals fed

Table XLII. The biological values obtained with animals that had failed to maintain their adult weight, and average biological value of the assay represented and its range

Rat number	Biological value obtained with use of this rat	Average biological value obtained in the assay	Range of individual biological values of the assay
17790	84.7	76.9	24
17894	72.1	75.4	28
16791	69.8	76.9	26
17895	80.7	76.9	26

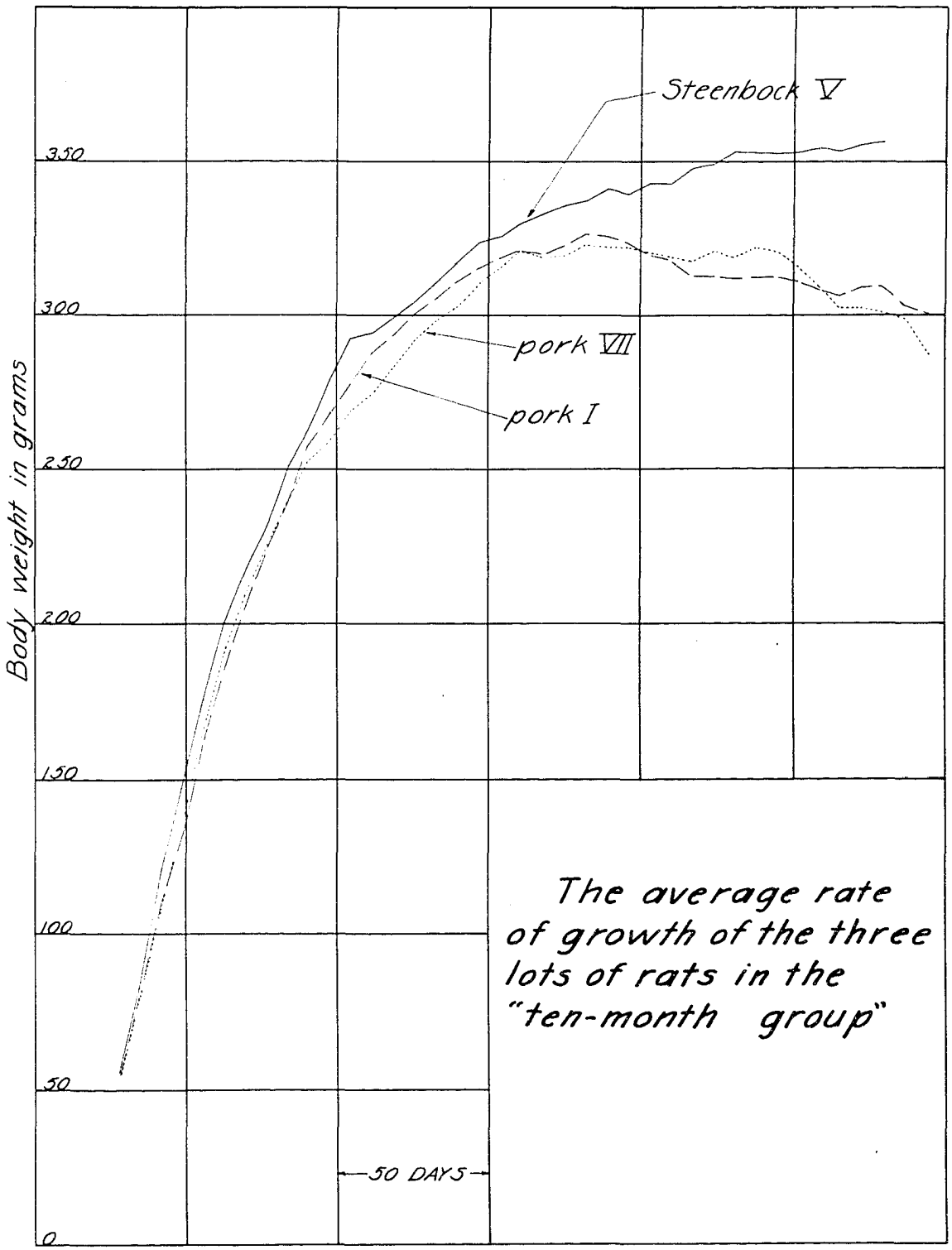
Steenbock V
pork VII
pork I
Body weight in grams
350
300
250
200
150
100
50
0
50 DAYS
The average rate of growth of the three lots of rats in the "ten-month group"

Figure X.

the Pork I diet were approximately 160 days old they started to decline slowly in weight. At this time they began to exhibit many signs of malnutrition. The picture described by Dyar was confirmed in all essentials. Although the data presented in a previous section show that the condition cannot be attributed either to poor quality or to poor utilization of the proteins in that diet fed. The use of animals showing all symptoms of malnutrition for the determination of biological values concerned us. It seemed very possible their use might introduce variations of sufficient size to invalidate the experiment. The loss in the adult weight of the animals reared on the Pork 7 diet was even greater than that of the animals fed the Pork I diet. Apparently the proteins of the liver supplement had no supplementary value in maintaining the adult rat in a state of good nutrition.

The contours of the growth curves were reflected in the physical condition of the animal. The animals in all groups at three months were in excellent condition, having clean, smooth, and glossy fur, bright orange-colored teeth, deep pink eyes and paws, normal nervous tension, and a youthful appearance. They were alert and active. The animals at six months were also in excellent condition. They looked mature, having developed an accumulation of fat pads which gave them the heavy appearance typical of well-nourished rats of this age. The animals at 10 months receiving the mixed grain diet

were, on the whole, active and clean, with smooth, well-groomed fur and good posture. Of the 9 rats in the group, all but two were classed as being in good or excellent condition (Table XLIII). At autopsy no obvious abnormalities were observed in the internal organs. However, five of the group had mild lung infections, indicated by pus and mucus in the lung tissues. One had an infection in the inner ear.

Animals, 10 months old, that had been grown on the Pork I diet were dirty, their fur was dull, they were thin, extremely gaunt, lethargic and inactive and many showed signs of dyspnea. Of this group of nine animals, three were judged as being in good nutritional condition, and all others in a fair or poor state. Six of the animals had ulcers of the stomach, one had pits on the surface of the kidney, and all had severe infections of the lungs. Malnutrition was especially marked in the animals receiving the liver supplement in addition to the Pork I diet. Of this group of rats fed Pork 7, only one was rated as being in good shape, the others were physical wrecks. At autopsy, seven of the nine animals had stomach ulcers, three had mottled kidneys, and all had lung infection.

In spite of the inferior physical condition of the 10 month animals reared on the pork diets the variations in the individual determinations of the biological values determined with them was not any greater than the variations in values obtained when the physically superior group was used. (See

Table XLIII. The physical condition of the animals reared for 10 mont
various pre-experimental diets as determined by gross ob

Pre-experimental diet	Rat number	Condition before the assay period	
		General condition	Normality of excretions
Steenbock V	16900	Good	Normal
	16918	Fair	"
	17170	Good	"
	17317	Excellent	"
	17379	"	"
	17670	Good	"
	17853	Fair	"
	17949	Good	"
	17990	"	Slight diarrhea
	*17324	Poor	" "
Pork I	16726	Good	Normal
	16789	Fair	Blood in urine
	16893	Good	Normal
	17186	"	"
	17469	Fair	"
	17893	"	"
	17894	"	"
	*16790	Poor	Diarrhea
	*16954	"	"
Pork 7	16727	Poor	Normal
	16728	"	"
	16791	"	"
	16895	Fair	"
	16896	Good	Blood in urine
	17470	Fair	Normal
	17895	"	"
	*17191	Poor	Blood in urine
	*16955	"	Diarrhea

*Animals died
**Data for autopsy unfortunately lost

reared for 10 months on the
ermined by gross observations

ne assay period ality of excretions	Condition at autopsy Quantity of body fat	Stomach	Kidneys	Degree of lung infection	Occurrence of other infections
Normal	++	No ulcers	Normal	2	None
"	+	" "	"	5	"
"	++	" "	"	1	"
"	-+	" "	"	0	"
"	+	" "	"	0	"
"	**	**	**	**	**
"	++	No ulcers	Normal	0	None
"	+	" "	"	0	Ear
light diarrhea	**	**	**	**	**
" "	-	No ulcers	Normal	21	None
Normal	-	Ulcers	Mottled	2	None
lood in urine	-	"	"	12	"
Normal	+	No ulcers	Normal	4	"
"	+	Lining rough	"	5	"
"	+	" "	Mottled	19	"
"	-	Ulcers	Normal	24	"
"	**	**	**	**	**
Diarrhea	-	Ulcers	*	20	None
"	-	"	*	20	"
Normal	-	Ulcers	Normal	7	None
"	+	No ulcers	"	8	"
"	-	Ulcers	"	5	"
"	+	"	Pitted	29	"
lood in urine	+	"	Normal	13	"
Normal	++	No ulcers	"	8	"
"	**	**	**	**	**
lood in urine	-	Ulcers	*	15	None
Diarrhea	-	"	*	20	Penis

range of individual biological values obtained with each group, Table XLI). Therefore, it seems that the poor physical condition induced by the feeding of the pork-containing diet did not affect the variability of the biological value determinations.

SUMMARY

Dyar ('35) reported that a diet containing dried canned autoclaved pork muscle as its source of protein is nutritionally inadequate for the support of normal life processes such as growth, adult maintenance, and the propagation of the species in the albino rat. This early study indicated that although growth progressed at a normal rate during the early life history of the male animal, after 10 months the rat no longer was able to maintain its adult weight and sign after sign of nutritional failure appeared. The proteins in the diet employed by Dyar were derived from autoclaved canned pork muscle and the yeast which served as the source of the vitamin B-complex. The present investigation was planned to determine whether or not the nutritional failure was due to inefficient utilization of the proteins.

Two factors may have induced inefficient utilization. First, autoclaving the protein-mixture represented in the pork may render it less valuable from a nutritional standpoint than the untreated protein. Second, continued maintenance on the diet containing pork may cause pathological changes in the animal so that it no longer utilizes protein

of the good quality in a normal way.

In order to study these points two experiments were planned. In the first experiment, the utilization of the protein represented in the Pork I diet (canned autoclaved pork muscle and yeast) was tested with the use of normal rats taken from the stock colony at successive intervals in their adult life. Results obtained with these groups were compared with those obtained in a second experiment wherein the availibility of the proteins of the Pork I diet to the animal was determined with animals of the same age as the control groups of the first experiment but reared during their entire life history on the Pork I diet.

The biological utilization of the protein-mixture of the Pork I ration has been determined in terms of its biological value and its coefficient of digestibility. These values were estimated by the balance-sheet method described by Mitchell ('24). The method used in calculating results was slightly different than that used by Mitchell, since we were unable to demonstrate that the quantity of endogenous fecal nitrogen was related to food intake. Therefore, we designated the quantity of nitrogen excreted in the feces during the period of low nitrogen feeding as the nitrogen of endogenous origin.

Nitrogen balance studies were made when the animals were three, six, and 10 months old. The biological values

determined with the use of animals of these various ages were 83, 76, and 68 respectively. The gradual decline in the values as the animal became progressively older may be explained on the basis of the principles of protein metabolism. At three months, the animal is still growing, and no doubt large quantities of the nitrogen are laid down in the form of new tissue, therefore, the apparently high biological value results. The rat at six months requires protein only for maintenance. At 10 months the rate of metabolism of the animal has decreased so that even less is needed for maintenance at this age than formerly. The tenability of this view is confirmed by the small quantity of nitrogen derived from food sources that was found in the urine of the younger animal, in contrast to the larger proportion present in the urine excreted by the two groups of older animals. The increased percentage of food nitrogen in the urine might also be explained on the basis of loss of nitrogen through the kidney tissues, since Moise and Smith ('27) have found that under normal conditions, kidney damage appears in rats of this age.

The biological value, 76, obtained with rats six months old probably represents the true biological value of the specific proteins of the Pork I ration, since, the animals at this age have just reached their maximum adult weight and also, their protein needs for maintenance are probably

characteristic of the normal adult rat. The biological value obtained with animals of this age was used in all comparisons as the "measuring stick" of efficiency. This experiment shows that the autoclaved proteins of the test diet are well utilized by the rat.

The utilization of the proteins of the Pork I ration by two groups of animals that had been maintained upon the pork-containing ration (Pork I) for three months and 10 months, respectively, was compared to utilization by the control animals. It was found that each group was able to utilize the proteins of the canned autoclaved pork muscle and yeast as efficiently as animals of the same age reared on the stock colony diet (biological values, 82 and 75). This shows that continued maintenance on the diet containing autoclaved pork muscle did not produce physiological or pathological disturbances that affected the ability of the animal to utilize the proteins of its diet.

Improvement in reproduction, lactation, and growth of young had been noted in previous experiments when test rats were fed the Pork I ration supplemented by raw liver. This finding raised the question whether or not the good results were due to an improvement in the quality of the dietary proteins. An experiment was planned to investigate this possibility. Rats were grown on the supplemented diet. When they were 10 months old they were used to determine the

biological values of the proteins of the diet. The results of this experiment were compared to those obtained in the preceeding series. The proteins of the Pork I diet when supplemented with raw liver were no better utilized than were the proteins of the unsupplemented ration.

The studies reported above do not give an index of the biological value of pork muscle but of the proteins of pork muscle and yeast. The comparatively high biological value obtained for the mixture may be due to the amino acids present in the yeast. If so, as supplementary material they may obscure the effect of heating. Investigators have disagreed as to the influence of increased temperature upon the biological value of proteins. Therefore, balance studies were made using the canned autoclaved pork muscle alone as the source of the protein. The biological value thus obtained (78) was similar to that obtained when pork and yeast were the sources of the protein (76). It agrees with the biological value reported by Mitchell for the proteins of raw pork muscle. Therefore the process of autoclaving probably has not reduced the biological value of the pork muscle incorporated in the test ration.

Determinations of biological value do not give the entire picture of protein utilization. For example, even though a biological value is high, a low coefficient of digestibility means that in the final analysis only the

portion of the ingested protein represented by the coefficient of digestibility is available for use by the animal. In the present investigation, the digestibility of the protein may have been reduced by the canning process. Therefore, the coefficients of digestibility of the proteins were determined for the various groups represented in the three series of the experiment. The results were striking in their similarity. Almost complete digestion of the protein mixtures was observed in all cases.

A preliminary study has been made of some of the factors that may influence the variability of results obtained in determinations of biological values. Variations in body weight of the test animal, the failure of the experimental rat to maintain adult weight, and the physical condition of the test animal apparently do not affect the uniformity of any specific assay.

CONCLUSIONS

1. The biological value of the proteins represented in the pork-containing diet fed as the experimental ration in the project of the Foods and Nutrition Subsection, Iowa Agricultural Experiment Station entitled, Meat in Nutrition is high. Therefore, the inability of the test animal reared on the Pork I ration to maintain adult weight and the resulting nutritional failure is not due to the poor quality of the proteins of the diet.

2. Continued maintenance on the pork diet for as long as 10 months does not influence the ability of the animal to utilize the proteins of the meat diet.

3. Pork proteins are utilized less efficiently as the test animal increases in age.

4. Liver does not contain a collection of amino acids that are capable of improving the quality of the proteins of the pork-containing ration. Any improvement in the nutritional state of the rat heretofore attributed to the addition of liver to the test ration must be ascribed to some other liver component.

5. The nutritional failure induced by feeding the ration

containing canned autoclaved pork muscle is not due to a low digestibility of the proteins. The ulcers present in the lining of the stomach of the rats reared on the pork diets do not affect the ability of the animal to digest the proteins of the rations.

6. Since the biological value of the proteins of pork muscle which have been autoclaved for one hour and five minutes at 15 pounds of pressure is as high as that reported by Mitchell for raw pork muscle, the biological value of the proteins of the meat diet apparently is not lowered by the autoclaving process.

7. The following items should receive consideration when the technique for determining the biological value of a protein is being established:

A. Yeast apparently can be included in the test ration as a source of the vitamin B-complex without affecting the results obtained in a determination of a biological value of a specific protein;

B. Endogenous fecal nitrogen is not related to the dry weight of food consumed or to the body weight of the animal. Instead it seems to be a constant value for each individual rat;

C. Nitrogenous tissue catabolism in the period of low-nitrogen feeding does not become constant

until 11 days have elapsed;

D. A rat approximately five or six months old is the best type of experimental animal to employ in conducting a determination for the biological value of a protein;

E. The pre-assay diet of the test animal does not affect the utilization of protein in the assay period.

F. Poor physical condition of the test animal does not seem to increase the variability of the determination of biological value.

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