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UTILIZATION OF NITROGEN BY THE ANIMAL ORGANISM.
VII. FACTORS INFLUENCING THE AMINO ACID REQUIREMENT OF THE ADULT MALE ALBINO RAT

by

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INTRODUCTION

A continuous process of protein or nitrogen turnover consisting of catabolism and anabolism takes place in the animal organism throughout its life span. The function of dietary protein, therefore, is to supply the amino acids in proper amounts and kinds to meet the demands for the vital metabolic activities and to provide the basic building materials for the growth and repair of body tissues.

A deficit of food proteins afflicts a large portion of the world population today, particularly in those underdeveloped areas where people receive significantly too little or too poor a quality of protein for the support of adequate nutrition. As evidence is the wide prevalence of kwashiorkor and other syndromes of human protein malnutrition - a situation complicated today by rapid increases in the world population.

It was pointed out at a conference sponsored by the Food and Agriculture Organization of the United Nations, the World Health Organization, and the Josiah Macy Jr. Foundation in June of 1955 that in attempting to establish protein requirements a consideration of world problems was necessary (Elvehjem, 1956). Even though the basic relation of nutrition to amino acid intake was emphasized at the conference, it was apparent that workers in practical nutrition were not yet ready to shift from a consideration of protein requirements to a consideration of amino acid requirements.

Perhaps this is well at the present stage of nutritional history because it is only of late that the complexity of the many factors underlying the utilization of amino acids has begun to be appreciated. Recent studies (Elvehjem, 1956) describing the results of imbalance between the components of an amino acid mixture illustrate this point. The data also raise the question as to what the composition of the most physiologically efficient mixture of amino acids may be.

That natural proteins even though they have a high biological value may not contain amino acids in the best proportions for the maintenance of nitrogen equilibrium in the adult organism has been suggested by the work of Clark (1950). She hypothesized that the amino acids present in one of the most nutritionally efficient proteins known, i.e., lactalbumin, could be made more efficient by alterations of certain of its amino acids.

The present study was planned to explore this hypothesis. Three basic mixtures of amino acids were prepared, one of which represented the combination of amino acids present in the lactalbumin preparation tested by Clark. These mixtures were so formulated that they contained different proportions of the amino acids, methionine, phenylalanine, cystine, and tyrosine as well as of the non-essential acids. Factors that influence the utilization of amino acid mixtures used as the sole source of dietary nitrogen were studied also, i.e., the total nitrogen content of the ration, omission of non-essential nitrogen, single vs. mixtures of non-essential amino acids, variation in the optical form of certain amino acids, variation in the non-nitrogenous portion of the ration, and variation in caloric value of the diet.

It was hoped that the study would contribute basic knowledge leading to a better understanding of the interrelations of amino acids in establishing and interpreting protein requirements.

REVIEW OF LITERATURE

The Reference Protein

In the experiment herein reported, lactalbumin was chosen as "the reference protein", the original amino acid mixture fed being compounded to resemble the amino acid composition of this protein as closely as possible.

That lactalbumin has nutritional properties that make it an excellent reference protein has been demonstrated by various workers in the field over the years.

Ability of a protein to promote growth is an excellent tool for measuring the nutritional properties of a protein. In 1919, Osborne, Mendel, and Ferry studied the nutritive value of lactalbumin in terms of its ability to support growth in young rats. The superiority of lactalbumin over casein for the maintenance of growth was demonstrated during an 11-week feeding period. Rats gained approximately 2 gm. of body weight per gram of lactalbumin consumed as compared with 1.7 gm. for each gram of casein eaten.

Researchers studying the nutritional value of proteins also may use either "biological value" (Mitchell, 1924) or "nitrogen balance index" (Nasset and Ely, 1953), or "biological efficiency" (Marshall, 1943, and Brush, Willman and Swanson, 1947) as indices of the ability of a specific protein to maintain body tissue. The last index takes into account, which biological value does not, the marked depressions in the excretion of urinary nitrogen that may occur when proteins of exceptionally high quality are fed following an interval on a protein-free diet.

In a feeding test in 1924, Mitchell fed a diet containing 3 per cent of lactalbumin. He noted that rats fed the lactalbumin-containing diet excreted 5 mg. less nitrogen per day in the urine than was excreted in the preceding period of low-nitrogen feeding. He did not attempt to explain the decreased excretion of urinary nitrogen induced by the incorporation of lactalbumin into the diet or to calculate the biological value. However, his data permit the calculation of biological efficiency, and when they were so treated by Metz (1947), lactalbumin received a value of 110. Metz also made an intensive study of the biological efficiency of lactalbumin when fed to adult rats, and the value she recorded was 106.

Metz also showed that lactalbumin excelled casein when evaluated in terms of biological efficiency. It also was equivalent in nutritive value to the mixed proteins present in dried whole egg and superior to those in certain other foods (rice, lyophilized milk, and yeast).

Tests based on the estimation of biological value also show that lactalbumin rates higher nutritionally than certain other proteins. When protein was incorporated into test rations at the 8 per cent level, Kik (1938) found that lactalbumin had a biological value of 81; casein, 69. With only 5 per cent of lactalbumin in the ration, Chick et al. (1935) showed that lactalbumin had a biological value of 92; casein, 68.

Melnick and Cowgill in 1937 reported the results of a study in which they evaluated lactalbumin, serum protein, casein, and gliadin with respect to the minimal amount of each that was essential for the dog in attaining nitrogen equilibrium. Respective biological values were 100, 80, 73, and 33.

It is interesting that although the superiority of lactalbumin over casein has been demonstrated consistently in studies in which the rat has been used as the experimental animal, a report by Mueller and Cox (1947) comparing the relative effectiveness of these proteins for the maintenance of nitrogen equilibrium in man, failed to show this distinction. This paper is pertinent in that it suggests that there is still much to be learned about the utilization proteins by different species.

Thus, on the basis of most evidence, it seemed suitable to consider the use of lactalbumin as a reference protein when the investigations current in the Nutrition Laboratory of the Home Economics Research Department on protein needs and protein utilization were extended to quantitative studies of the amino acid requirements of the adult male rat for body maintenance.

Amino Acid Replacement of Dietary Protein

After threonine was isolated and identified as an essential amino acid by the group working in Rose's laboratory (McCoy *et al.*, 1935-1936), it became feasible for the first time to induce a favorable state of nutrition in animals by the feeding of diets containing mixtures of highly purified amino acids instead of intact proteins. Rose and his co-workers were able to induce satisfactory growth in young animals by feeding diets containing about 12 per cent of physiologically active amino acids as the source of nitrogen (Rose, Oesterling, and Womack, 1948). Clark (1950) obtained nitrogen equilibrium in adult rats when the ration furnished daily 74 mg. of nitrogen derived solely from amino acids. This amount was essentially the same as that provided by a 4 per cent lactalbumin diet which supported nitrogen equilibrium successfully. Womack and co-workers in 1953 demonstrated that the adult male rats fed, *ad libitum*, a diet containing a mixture of 19 amino acids in a quantity equivalent to 3.8 per cent of the diet maintained nitrogen equilibrium.

Certain studies employing the rat as the test animal will be reviewed to illustrate the nature of some of the problems involved when amino acids represent the sole source of dietary nitrogen.

Role of non-essential acids

The nutritive roles of the various essential amino acids were established by the removal, one by one, from the diet of each amino acid (Rose, 1938). It now is accepted, generally, that only nine amino acids, namely, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine must exist preformed in the diet if growth is to be sustained. The tenth acid, arginine, can be synthesized by the rat but not at the rate commensurate with the needs of the organism for the attainment of maximum growth (Scull and Rose, 1930; and Borman, *et al.*, 1946). Therefore, Rose in 1938 classified arginine as an essential amino acid for the rat. He stated in an early paper without the submission of evidence that rats grew equally well when fed a diet containing nitrogen provided either by a mixture of the 10 essential amino acids only or by one containing all the components of an intact protein (1938).

Later (1943), Albanese and Irby reported that young rats lost weight steadily when they were fed a diet containing the essential amino acids as the sole source of dietary nitrogen in the approximate proportions that they are found in casein and in a quantity equivalent to a 14 per cent casein diet.

Subsequent studies at Rose's laboratory (Rose, Oesterling, and Womack, 1948) did not support his earlier premise that the 10 essential amino acids are adequate for the support of growth in the young rat. The divergence in results apparently was due to defects in the basal diets employed in the initial studies. Upon improvement of the basal ration, young rats gained only 79 gm. when they were fed a diet containing 10 essential amino acids as the sole source of dietary nitrogen during a 28-day period. The feeding of a diet containing 19 amino acids supported a mean gain in body weight of 108 gm. The quantities of nitrogen furnished by physiologically active amino acids in each diet were identical. Hence, the authors concluded that a mixture containing 10 essential amino acids was considerably less effective than a mixture containing 10 essential and 9 non-essential acids as the sole source of dietary nitrogen. The authors further suggested that the task of synthesizing 9 non-essential amino acids simultaneously "appears to present too great a burden upon the chemical resources of the cells to permit the latter to keep pace with the needs of the organism for optimum growth" (p. 758).

The utilization of amino acids by adult albino rats has been studied in Cannon's laboratory at the University of Chicago. Their investigations are interesting inasmuch as they take into account the influence of previous nutritional state on the utilization of the dietary supply of nitrogen. Frazier _et_ _al_. (1947) showed that protein-depleted rats gained weight rapidly when they were fed a diet containing a mixture containing both essential and non-essential amino acids simulating the acids present in a reference diet providing 10 per cent of casein. Rate of gain was equivalent to that produced by the feeding of the intact protein diet. They observed also that food consumption was reduced immediately when any one of the 9 amino acids essential for rat growth (arginine not included) was omitted even though its nitrogen equivalent was replaced by glutamic acid. The omission of arginine from the ration did not affect weight gain or appetite. They obtained the same results when the essential acids only were provided.

Workers from the same laboratory later demonstrated that the well nourished adult rat when fed amino acids equivalent to a 10 per cent casein diet required only 9 of the 10 essential acids for the maintenance of appetite, weight, and nitrogen equilibrium (Wissler, _et_ _al_., 1948). Removal of arginine from the essential acid mixture produced variable depressions in appetite and state of nitrogen balance. These workers came to the conclusion that the "non-essential" amino acids may be considered dispensable. Whether they are justified in the view may be questioned. Their rations furnished about 1500 mg. of nitrogen per day, an amount probably three times the requirement for the maintenance of nitrogen equilibrium in the adult rat. Sufficient essential nitrogen was furnished to permit *in vivo* the synthesis of the non-essential acids. Thus, these data do not prove that the essential amino acids alone, combined as they were in this experiment, are capable of maintaining nitrogen equilibrium when the quantity of total nitrogen provided is approximately the minimum amount needed for nitrogen equilibrium.

In Swanson's laboratory, Kuehl (1949) and Clark (1950) showed that following the feeding of a diet containing a mixture of 18 amino acids patterned qualitatively and quantitatively after those present in one day's feed of the reference diet, i.e., a 4 per cent lactalbumin diet, the nutriture of the animals approximated that of rats administered the reference diet. Animals fed a diet containing a mixture of essential amino acids equivalent to those provided by the protein in one day's quota of a 4 per cent lactalbumin passed into severe negative balance. It should be noted, however, that the nitrogen administered was quantitatively equivalent to only a 2 per cent protein diet. Therefore, the data of these authors are not conclusive either.

In 1953, Litwack, Williams, and Elvehjem observed that the formation of xanthine oxidase in the liver of rats was increased markedly when non-essential acids were added to a ration containing only essential amino acids. These observations are in accord with an earlier statement (Williams and Elvehjem, 1950) that liver xanthine oxidase activity is very sensitive to amino acid changes in the diet.

In general, evidence to date seems to be in favor of the premise that some source of non-essential nitrogen needs to be present if maximum nutritional benefit is to be derived from diets containing amino acids as the sole source of nitrogen.

Source of non-essential nitrogen in diets

Single amino acids. Ramasarma *et al.* in 1949 studied the adequacy of a pure amino acid mixture for the support of growth in rats that was patterned after a mixture formulated by Womack and Kade (1944). Rate of growth was very poor when essential amino acids only were used as the sole source of dietary nitrogen. Addition of glutamic acid produced an immediate improvement. A linear relationship was observed between rate of growth and the quantity of glutamic acid provided. Rose, Oesterling, and Womack (1948) also showed that glutamic acid was growth-stimulatory when it was added to a ration containing only the 10 essential amino acids. However, its omission from a ration containing 19 amino acids did not reduce the growth rate significantly.

Whether or not glutamic acid is as effective nutritionally in supplementing the essential amino acid as is a mixture of non-essential amino acids has been debated. Ramasarma *et al.* (1949) observed that the single amino acid enhanced growth to lesser extent than did a mixture of non-essential acids. Frost (1949), however, concluded that glutamic acid could replace all of the other non-essential acids in the diet of the repleting rat.

Glycine, according to studies made at Illinois, indicates that this amino acid can serve as the sole source of non-essential nitrogen also (Rose, 1949).

Inorganic nitrogen. Inorganic sources of nitrogen may also serve in supplementary capacity to the essential nitrogen acids. This fact was demonstrated in 1944 by Hegsted. He found that a mixture of amino acids considered essential for the growth of a specific strain of microorganisms was not sufficient for its culture unless supplemented with some source of inorganic nitrogen.

It has been shown that the nitrogen required for the synthesis of non-essential amino acids in growth processes of the rat also may be derived from several sources (Rose, Smith, Womack, and Shane, 1949). Ammonium salts and urea are effective. Urea seems less efficacious than diammonium citrate, (Rose *et al.*, 1949; Lardy and Feldott, 1950). It is significant, in this connection, that nitrogen fed as ammonium citrate or urea exerted the same effect as a mixture of non-essential acids upon the formation of xanthine oxidase in the liver (Williams and Elvehjem, 1950).

Quantity of non-essential nitrogen in diets

Rose concluded his studies of the amino acid requirements of man by determining the approximate amount of nitrogen which is required for the synthesis of the non-essential amino acids when the diet furnishes the 8 essentials each at its safe level of intake (Rose and Wixom, 1955). It appeared that equilibrium could be achieved with rations in which the essential amino acids provided 1.9 gm. of nitrogen daily and the non-essential 2.0 gm. of nitrogen, i.e., approximately a 1:1 proportion.

Factors Related to Utilization of Amino Acids

Utilization of dietary amino acids is influenced by a number of factors. Some of these have been recognized, the influence of others is not understood clearly at the present time. The requirement for any individual amino acid may be determined by the level of intake of certain other acids. It seems, also, that dietary components other than nitrogen play an important role in determining degree of amino acid utilization. Before the amino acids required by the adult male albino rat for body maintenance can be established, some of the factors that may influence the utilization of nitrogen need to be clarified. Certain of these factors will be discussed below.

Imbalance of amino acids

It more or less has been conceded that mixtures of good quality proteins contain balanced supplies of amino acids. Elvehjem has written (1956) that rats experience no kidney damage even when the ration contains as much as 90 per cent of a high quality protein. However, when the dietary protein is incomplete, serious results may follow the feeding of diets in such proteins. He believes that as the quantity of an inadequate protein in the diet is increased, the requirement for certain amino acids increases and the effect may be more deleterious than if this protein of poor biologic value were used at lower levels.

It was from observations like these that appreciation of the significance of amino acid imbalance developed. The idea grew out of early studies on the relationship of tryptophan and niacin in maintaining normal growth and preventing pellagra (Krehl, et al., 1946, and 1946a). Then came the suggestion of fat in the liver may serve as index of amino acid imbalance (Harper, et al., 1953). The roles played by various amino acids in controlling deposition of fat in the liver is being studied in many laboratories presently. This review will confine itself to a discussion of the effects of alterations in the methionine, phenylalanine, and threonine contents of a diet on utilization of nitrogen.

Methionine. In 1947, Brush, Willman, and Swanson showed that methionine is the most powerful of the essential amino acids in sparing body nitrogen in adult rats partially depleted of body stores of the element. Analyses of the whole carcass of the experimental animals indicated that tissues were degraded to secure this amino acid for the synthesis of functional proteins and metabolites needed for the support of life.

Anderson and Combs (1952) studied the effect of methionine and glucose ratios on growth of chicks. It was observed that the consumption of feed was greatly reduced when additional DL-methionine (3%) was added to the adequate basal diet. When chicks were force-fed a glucose-methionine mixture, they lost significantly less weight than did the chicks force-fed an equal amount of glucose solution. It was suggested, therefore, that the slow rate of growth of the chicks fed an excess of DL-methionine might be due to, in part, the self-restriction of the feed intake.

The addition to the diet of excess DL-methionine, however, appears to affect adversely the growth rate of several species. Russell et al. (1952) reported that a normal rate of growth occurred in white rats when they were fed a diet containing the mixture of essential amino acids formulated by Rose and Womack (1946). The feeding of extra DL-methionine, however, induced a retardation of growth.

Excessive quantities of methionine in the diet also has an unfavorable effect upon nitrogen balance in dogs (Roth and Allison, 1949). This disturbance was counteracted by glycine or arginine.

Wyzan, Kade, and Shepherd (1950) found that the retention of nitrogen in dogs was enhanced by supplementing casein hydrolysates with methionine up to 40 mg. of nitrogen per kilogram as methionine. The addition of more than 50 per cent of the total amino acids as methionine resulted in serious physical deterioration apparently creating an imbalance and seriously impairing utilization of nitrogen. The authors did not attribute the effects of the imbalance directly to the labile methyl group or sulfur atom since excess choline chloride, cystine, and cysteine were fed without adverse effect.

Tucker and Eckstein (1937) and Channon _et al._ (1938) showed that the deposition of lipids in the livers of rats fed protein rations deficient in choline was influenced adversely by the addition of cystine to the diet. On the other hand, methionine exerted a favorable action.

Considerable controversy had centered around the question of whether the lipotropic activity of a protein can be ascribed to its content of cystine and methionine. It is clear that methionine is the only amino acid that can reduce the concentration of fat in livers of animals fed diets deficient in choline (Eckstein, 1952).

More recent studies dealing with the role of methionine when fed in conjunction with low protein diets have disclosed some interesting information. Studies from Elvehjem's laboratory (Harper, *et al.*, 1954) demonstrated that as little as 0.1 per cent DL-methionine exerted a lipogenic effect on rats fed a threonine-low 9 per cent casein diet that was supplemented with choline but not with cystine. The accumulation of fat in the liver, however, was appreciably reduced by supplementation of the ration with threonine. The authors indicated that their reference diet containing 9 per cent of casein was primarily deficient in sulfur amino acids. When methionine was added, the secondary deficiency of threonine became evident. It was suggested that the proportions as well as the actual quantities of amino acids are of considerable importance in respect to the accumulation of fat in the liver. This effect is especially interesting since methionine long has been recognized as a lipotropic factor. The effect must be separate and distinct from the effect of choline and the sparing effect of methionine on choline. These results are not limited to weanling rats, since a similar accumulation of liver fat is obtained with adult animals when the level of casein is reduced to 5 per cent (Harper *et al.*, 1954a).

Phenylalanine. In 1940, Moss and Schoenheimer, by feeding labelled amino acids showed that the conversion of phenylalanine to tyrosine proceeded even when high levels of tyrosine were fed along with phenylalanine. They also found that mice fed a phenylalanine-low but otherwise adequate diet (tyrosine and other amino acids being supplied in adequate amounts) converted a smaller proportion of the absorbed phenylalanine to hepatic tyrosine than did animals fed the diet containing adequate phenylalanine.

Hier _et al._ (1944) reported that the supplementation of DL-phenylalanine at the 5 per cent level exerted an inhibitory effect on growth in young rats. On the other hand, large amounts of phenylalanine added to a casein diet were metabolized by dogs without any ill effects (Wyzan, _et al._, 1950).

Investigators in Elvehjem's laboratory (Benton, _et al._, 1956a) demonstrated that a ration containing 3 per cent DL-phenylalanine caused a depression of growth in young rats when it was fed in conjunction with a basal diet containing 9 per cent casein supplemented with tryptophan and methionine. The growth depressing effect was completely overcome by the addition of threonine. At a higher level of phenylalanine intake, threonine, however, was not completely effective in restoring the growth rate. It should be noted also that under their experimental conditions the feeding of phenylalanine caused a reduction in the concentration of fat in the livers whether or not threonine was added to the diet.

Threonine. It has been found that threonine is another amino acid that is particularly active in determining balance or imbalance between amino acids as judged by rate of growth of rats and deposition of fat in the liver.

Threonine when added to a basal diet containing 9 per cent of casein, 0.2 per cent of cystine, and 0.2 per cent choline exerted a depressing effect on growth (Singal, et al., 1953 and Ebisuzaki, et al., 1952). Hepatic fat was normal. When tryptophan was supplied, growth improved but livers contained 14 per cent of fat. When both amino acids were added simultaneously the animals grew and hepatic fat was maintained at a normal concentration. A comparable situation prevailed when a mixture of amino acids provided the dietary nitrogen (Singal, et al., 1953a)

These workers believe that hydroxyl groups per se do not account for the lipotropic action of threonine. It is interesting that activity occurs only when choline is present in the diet. Ebisuzaki et al. (1952) have advanced the theory that excess dietary threonine causes an imbalance by decreasing the availability of amino acids in whole casein, probably by the inhibition of digestive processes.

The concentration of fat in the livers of rats fed low protein diets containing pork, beef or egg albumin is dependent on the level of dietary protein when the rations are supplemented appropriately with choline, tryptophan, and methionine (Winje, Harper, Benton, Boldt, and Elvehjem, 1954).

The addition of threonine, however, always reduced hepatic fat. On the other hand, excessive deposition of liver fat was not observed when fibrin was the dietary protein (6%, 7.5% or 9%). It was suggested that maintenance of hepatic fat at normal concentrations in rats fed low protein diets containing choline "depend upon the presence of a specific ratio of amino acids".

The phenomenon of fat deposition in livers also was observed in adult rats by Harper, et al. (1954a). When they fed a basal diet containing 5 per cent of casein, well-nourished adult rats maintained body weight during a 3-week period even though the concentration of fat in the dry liver increased to 26 per cent. When the basal diet was supplemented with 0.4 per cent DL-threonine, these mature rats were able to maintain a normal concentration of fat in the liver, i.e., 14 per cent on the dry basis, and gained 22 gm. in body weight during the experimental period (3 weeks).

Threonine also plays a role in repletion diets. Young adult male albino rats were rendered hypoproteinemic and moderately anemic by the feeding of a low protein but otherwise adequate diet for a period from two to three months (Steffee, et al., 1950). A repletion diet deficient in threonine induced a rather high concentration of fat in the livers.

Optical forms of amino acids

The work of several groups of investigators has shown that the D-isomers of certain amino acids are not utilized by the animal organism to the same degree as the L-forms. The early studies of du Vigneaud, et al. (1932), and Berg (1934) showed that D-tryptophan could be utilized as effectively as the naturally occurring L-tryptophan for the support of growth in rats. However, Nasset and Ely in 1953 showed that under rigidly controlled conditions, adult rats required twice as much D- as L-tryptophan to achieve nitrogen equilibrium.

Rose (1949) reported that D-tryptophan could not be utilized by the adult human male for the maintenance of nitrogen equilibrium.

The reviews by Berg (1942, 1953, and 1955), by Albanese (1947), and by Neuberger (1948) indicate that the rat is unable to utilize the D-forms of isoleucine, leucine, lysine, and threonine. Valine is partially available and histidine more readily so. D-methionine and D-phenylalanine can be fully inverted apparently. Data from Nasset and Anderson (1951) suggest that the D-component of DL-methionine is fully utilized at or near nitrogen equilibrium but may not be utilized at all when nitrogen balance is decidely negative.

The comparative nutritional values of a mixture of essential amino acids containing a mixture composed entirely of the L-forms of the acids and of one composed of DL-forms was determined by Pilsum and Berg in 1950. Both mixtures provided the same quantities of biological active amino acids and supplied twice the minimal amount of essential amino acids recommended by Rose (1937) for inducing growth in the rat. Rats fed either the mixture of L- or DL- essential amino acids grew approximately the same. Growth was retarded, however, when the rats were fed a diet containing a mixture of DL-essential acids which provided twice the quantity of each acid present in the L-acid mixture. Later studies from the same laboratory (Phillips and Berg, 1954) suggested that animal organism might be limited in its capacity to retain the D-amino acids or to effect their inversion.

The author has had occasion to study the relative efficiency of D- and L-threonine during the course of the present investigation. The studies of Singal, Hazan, Sydensticker, and Littlejohn (1953) are, therefore, of special interest. These workers found that in supplementing a low protein diet with threonine, both growth and lipotropic properties were characteristic only of the L-form of threonine. The unnatural isomer was entirely without effect. The D-form, however, did not interfere with the utilization for growth of any of the L-form present.

Therefore, the physiological availability of the optical isomers of the natural amino acids appeared to be an important factor in the evaluation of the adequacy of amino acid mixtures for the support of protein nutrition.

Berg (1955, p. 581) summarizes as follows:

> It is probably fair to say that the D-amino acids are somewhat less well utilized metabolically because they must first be inverted. ...they are much more readily excreted than are the L-amino acids. (therefore) No clear-cut nutritional inhibition has been attributed to the D-amino acids in the mammal, as is the case with many microorganisms.

Variation in non-nitrogenous components of the diet

The protein-sparing effect of carbohydrate is well known and was reviewed by Munro in 1951. The addition of extra energy in the form of carbohydrate to sub-maintenance diets that contained adequate amounts of protein caused proportional improvement in nitrogen balance (Munro and Naismith, 1953). The maximum sparing effect is manifested when the protein and carbohydrate are fed at the same time (Geiger *et al.*, 1950).

It has been observed by several investigators that the chick can not utilize various carbohydrates to the same extent. Johansson *et al.* in 1947 showed that the number of coliforms in the intestinal flora of hens fed a dextrin diet was 10 times that of a hen given a sucrose diet. The authors suggested that when dextrin was incorporated in the diets a more favorable medium for the bacterial synthesis of vitamins was established than was the case when sucrose provided the carbohydrate. Anderson *et al.* (1950) found that the D-tryptophan was utilized by the chick to a greater extent when corn was incorporated into the diet than when glucose was used. The difference seemed to be the result of a change in the bacterial flora in the digestive tract because the nutritive value of D-tryptophan was decreased upon the addition of sulfasuxidine to the diet. Monson, et al. in 1950 demonstrated that the dextrin ration passed through the gastro-intestinal tract of the chick more slowly than did one containing sucrose, and allowed time for the intestinal synthesis of some unknown factor or factors for the growth of chicks.

Becker, et al. in 1954 tested the ability of baby pigs to utilize various sources of carbohydrates when fed in connection with an adequate diet. All pigs failed to gain any weight when they were on a sucrose or fructose regimen. When glucose was fed as the dietary carbohydrate, performance was very satisfactory in terms of survival and rate of growth. Later studies from the same laboratory (Becker and Terrill, 1954) showed that the feeding of diets containing carbohydrates from different sources, namely, glucose, sucrose, dextrin, and cornstarch to the 9-week old pigs produced equally satisfactory growth. The response, however, was very poor upon the feeding of lactose as the dietary carbohydrate. A moderate diarrhea was occasionally observed. The severity of the adverse effect of the lactose containing diet was retarded markedly by the presence of an antibiotic in the diet.

It has been shown also that rats respond differently when fed diets containing carbohydrate from different sources. Early studies at Elvehjem's laboratory (Sarma, et al., 1946 and Elvehjem, 1948) have presented definite evidence that increased synthesis of niacin, pyridoxine, biotin, and folic acid in young rats occurred when dextrin was used as the dietary carbohydrate instead of sucrose. Later studies from the same laboratory (Lyman and Elvehjem, 1950) indicated that the slower passage of dextrin ration through the digestive tract permitted a slower digestion and more economical utilization of the other dietary components. The relative nutritional efficiency of sucrose and glucose in growing rats was investigated by Lamb in 1950. It was found that differences were slight but quite constantly in favor of sucrose.

Harper and Katayama in 1953 showed the influence of various carbohydrates on the utilization of low protein rations by the young rat. The rate of growth of rats was much slower when fed a diet containing 9 per cent of casein and sucrose as the dietary carbohydrate than when given the same casein diet containing dextrin. It is very interesting to note that the rats fed the sucrose diet containing 9 per cent casein and supplemented with lysine, valine, threonine, tryptophan and histidine grew almost at the same rate as those receiving the 9 per cent casein diet containing cornstarch. Dextrin supported growth equivalent to that obtained with cornstarch.

Evidence has been obtained concerning the protein-sparing effect of various carbohydrates in adult rats. Womack, Marshall, and Parks in 1953 showed that adult rats fed low levels of amino acids were in negative nitrogen balance when the dietary carbohydrate was sucrose, whereas nitrogen equilibrium was attained when the carbohydrate was corn dextrin or when the essential amino acid intake was increased. These authors also showed that the fat content of the livers of the animals indicated that rats receiving sucrose and amino acids had more fat in the liver than those fed the corresponding diet with corn dextrin. Later studies from the same laboratory (Marshall and Womack, 1953) found that when the quantities of essential amino acids were doubled differences in nitrogen balances were eliminated for both well-nourished and protein-depleted animals fed diets containing either dextrin or sucrose. The supplementation of glutamic acid increased the nitrogen balance of the animals fed sucrose but had no effect on the group receiving dextrin. Both well-nourished animals and protein-depleted animals responded the same. It is interesting to note that upon the feeding of an amino acid diet containing sucrose, the well nourished animals contained significantly more protein in the livers than those rats on the corresponding diet containing corn dextrin. These authors further suggested that dextrin present in the diet may aid the transformation of protein or amino acids into liver protein. This then might reduce nitrogen excretion and perhaps prevent the accumulation of fat in the livers, also.

Variation in the energy value of the diet

The important bearing of the energy content of a ration upon the utilization of protein has been demonstrated by several laboratories. If available calories fail to meet the demand for energy, proteins are diverted from their normal roles in the maintenance and synthesis of tissue and functional proteins to provide fuel (Beattie, Herbert, and Bell, 1948).

The experiments of Willman, Brush, Clark, and Swanson (1947) and Swanson (1951) indicated that fats have a greater nitrogen-sparing effect than carbohydrates in protein-deficient rats fed a low caloric diet. These authors found that when the diets of partially protein-depleted rats were restricted to or below 50 per cent of the normal caloric value, catabolism of tissues was accelerated to a greater degree in rats receiving low-fat diets than in those fed fat. In the growing rat and mouse, the critical level of caloric value is 80 and 95 per cent, respectively (Bosshardt, et al., 1946).

The effect of the energy value of the ration on nitrogen balance in the dog has been studied in Allison's laboratory.

Rosenthal and Allison in 1951 reported that when dogs were fed diets of low-energy value, their first response was an increase in the excretion of urinary nitrogen without any change in the nitrogen balance index of the dietary protein. The second response was a decrease in the nitrogen balance index reflecting a shift in the mechanisms of nitrogen metabolism. Resistance to caloric restriction was correlated, in part, with the magnitude of the protein stores of the body. Later work from the same laboratory (Rosenthal, 1952) showed that nitrogen utilization might be reduced, when large amounts of fat were fed in conjunction with diets restricted in calories and protein, thereby confirming the work described above.

Leverton and co-workers in 1951 studied the effect of the energy value of the diet on nitrogen utilization in young women. They found there was a highly significant reduction in nitrogen excretion when the daily caloric value of the ration was increased from 1800 to 2400. The nitrogen sparing action of the extra calories was greatest at the lowest protein intake.

Calloway and Spector in 1954 reported that young men, normal in weight and nutritional status, lost 12 grams of nitrogen daily during fasting and that this protein deficit was reduced by supplying 700 protein-free calories. No further advantage in the sparing of body protein was achieved when the energy value of the diet was increased to 2800 calories daily. The nitrogen balance remained negative throughout (approximately 7 grams). Data also showed that the nitrogen balance was not improved substantially when protein was fed with food of low energy value. Apparently, the protein fed under these conditions was burned largely as a source of energy. On a fixed and adequate protein intake, energy level was the deciding factor in nitrogen balance. With a fixed and adequate energy value of the diet, protein level was the determinant.

Garcia (1955) raised the question as to when the need for calories takes precedence over that for protein at minimal intakes of both food energy and protein. It was found in the adult female albino rats that nitrogen retention was increased with increments in dietary protein intake when food consumption was unrestricted. After caloric restriction was imposed, nitrogen losses occurred regardless of the intake of dietary protein. However, nitrogen equilibrium was re-attained within 30 to 35 days of restricted feeding.

Rose _et_ _al_. in 1954 reported that three human subjects were able to maintain positive nitrogen when fed diets yielding 34 calories per kilogram of body weight daily if casein was the sole source of the dietary nitrogen. The isonitrogenous replacement of whole casein by a mixture of amino acids recognized as components of casein induced negative nitrogen balance. Increasing the energy value of the diet from 35 to 45 calories per kilogram of body weight led promptly to positive nitrogen balance. It seems, therefore, that diets fed to human subjects must have a relatively high energy value if amino acids provide the sole source of dietary nitrogen. This observation was unexpected and disconcerting to these investigators. They are unable to offer any satisfactory explanation to account for the phenomena. They suggest that the rapid absorption of the amino acid diet may lead to spillage in the urine or to an accelerated catabolism. These workers also advance the idea that perhaps "liberation of amino acids from proteins during alimentary digestion proceeds in the order and at the rate best adapted for their economical use in anabolic reactions" (p. 341). Is more energy consumed when amino acids enter the circulation more or less simultaneously?

EXPERIMENTAL PROCEDURE

Plan of the Experiment

The present investigation was directed toward an elucidation of some of the factors that may influence the nitrogen requirement of the adult male albino rat when purified amino acids provided the sole source of dietary nitrogen. It seemed that feeding an assortment of amino acids simulating those present in a "pure" protein whose biological efficiency was well established offered an interesting and sound approach to the study.

Lactalbumin was chosen as the reference protein. Clark (1950) already had shown that response of rats to the incorporation either of lactalbumin at the 4.27% level in an adequate ration or of a mixture of amino acids equal in kind and quantity to those in the lactalbumin diet was similar when measured in terms of nitrogen balance. The mixture she formulated and variations thereof were used in the present experiment.

Clark's basal mixture consisted of two parts; one containing the essential amino acids only, the other the non-essential amino acids. The acids present in the essential amino acid mixture were equivalent to those in one day's feed of the lactalbumin diet and assortment was designated as

EAA-I. Similarly, the acids in mixture of the non-essential amino acids known as NEAA-I, simulated those in the ration containing 4.27% of the reference protein. It was not possible to obtain all the non-essential amino acids found in lactalbumin. The mixture used contained 7 non-essential amino acids. The EAA-I mixture provided 34 mg. of nitrogen per day; the NEAA-I, 40 mg. of nitrogen. Thus, the total amount of nitrogen furnished per day was 74 mg., the equivalent of that provided by one day's feed of the 4.27% lactalbumin diet.

However, before Clark's work could be extended, it became necessary to re-examine the total over-all adequacy of the ration. At the time she conducted her research, rice bran polish served as the source of the unidentified B vitamins; folic acid and vitamin B_{12} were not yet available for general experimental purposes. However, very shortly after the present experiment was initiated, production of rice bran polish ceased, and it became necessary to determine whether rats responded in the same way to the feeding of diets containing folic acid and vitamin B_{12} as they had to rations in which rice bran polish was used. This study of the influence of vitamin B_{12} and folic acid on the utilization of nitrogen constituted Series I of the present investigation.

Series II was concerned with a study of the influence of variations in the nitrogenous components of the ration, i.e., amino acids, upon the nitrogen utilization. Attention was directed to methionine-cystine-phenylalanine-tyrosine relations. This investigation was designated as Study A. This exploration was based on 5 experiments. In the first experiment, the influence of feeding graded doses of the mixture of non-essential amino acids (NEAA-I) with the mixture of essential amino acids (EAA-I) was evaluated in terms of the increments of cystine and tyrosine thus provided.

In the second experiment, the effect of the omission of cystine and tyrosine from NEAA-I was determined. In the third experiment of this study, the cystine and tyrosine supplied by NEAA-I were replaced isonitrogenously by methionine and phenylalanine and nitrogen utilization determined. This modification of the essential amino acid mixture was designated as EAA-II. The total nitrogen of the daily diet was kept within the nitrogen content of the 4.27% lactalbumin ration (74 mg. N). In the fourth experiment, utilization of nitrogen was observed when graded doses of a mixture of non-essential amino acids lacking in cystine and tyrosine (NEAA-II) was fed in conjunction with EAA-II. In the last experiment of this study, the influence of further increases in the methionine and phenylalanine content of the essential amino acid mixture was evaluated. The new mixture thus produced was called EAA-III.

In Study B of Series II, the effect of substituting a single non-essential amino acid for the mixture of 7 non-essential amino acids was tested when different mixtures of essential amino acids were employed. Glutamic acid was used as the substitute amino acid.

In Study C of Series II, the ability of mixtures composed of the essential amino acids only to support protein nutrition was investigated.

In Study D, the effect of varying the optical form of one amino acid on the relative efficiencies of two essential amino acid mixtures was determined, threonine being chosen for this determination.

Series III was designed to study the influence on protein nutrition of variations in the non-nitrogenous components of rations containing amino acids as the sole source of dietary nitrogen. In this series, the role played by the carbohydrate moiety of the ration was studied, the effect of the substitution of cornstarch, dextrose, and sucrose for the dextrin in the control diet on nitrogen utilization being determined in Study A of this series. In Study B, the effect of varying the caloric value of the experimental diet on nitrogen utilization was evaluated, the respective rations studied providing approximately 65, 50, and 35 calories per rat per day.

Several criteria were chosen to evaluate the efficacy of nitrogen utilization under the experimental conditions described above. Ability of the rats fed the various experimental diets to establish nitrogen balance was determined first. Bases of reference were the rations containing the intact reference protein, lactalbumin, and the diet containing the mixture of amino acids simulating those present in lactalbumin.

However, as indicated in the "REVIEW OF LITERATURE", nitrogen equilibrium may not represent an adequate measure of the state of protein nutrition in the adult rat. Hepatic nitrogen, hepatic fat, and hepatic moisture also are sensitive indicators. The composition of the liver may digress from normal even though nitrogen equilibrium occurs. Therefore, it was considered necessary to evaluate the relative protein nutrition of the various experimental groups simultaneously in terms of body weight, nitrogen balance, and concentrations of nitrogen, fat, and moisture in the liver.

Experimental Groups

Thirteen experimental groups were used in the three series of experiments described above. Each, when necessary, contained an appropriate control group. The number of rats in each experimental group varied from four to ten. In the main, most of the groups contained six animals.

The groups of experimental animals employed and the diets fed to them are summarized in Table 1, the symbol (C) after any diet designating a control ration.

Table 1. Experimental plan

SERIES I. EVALUATION OF BASIC DIET

Diet 1: 4.27% lactalbumin diet (C)

Diet 2: Amino acid diet, EAA-I plus NEAA-I, supplemented with 100 mg. of rice bran polish daily (C)

Diet 3: Amino acid diet, EAA-I plus NEAA-I, supplemented with 8 mcg. of folic acid daily

Diet 4: Amino acid diet, EAA-I plus NEAA-I, supplemented with 8 mcg. of folic acid and 0.75 mcg. of vitamin B_{12} daily

Diet 5: Stock ration customarily used in the Nutrition Laboratory (C)

SERIES II. INFLUENCE OF VARIATIONS IN THE NITROGENOUS COMPONENTS OF THE DIET

STUDY A: Influence of methionine-cystine-phenylalanine-tyrosine relations

Experiment One: Effect of feeding graded doses of non-essential amino acid nitrogen (NEAA-I) in conjunction with 34 mg. of nitrogen from essential amino acids (EAA-I) per day

Diet 6: Plus 10 mg. of N from NEAA-I

Diet 6A: Plus 20 mg. of N from NEAA-I

Diet 6B: Plus 30 mg. of N from NEAA-I

Diet 4: Plus 40 mg. of N from NEAA-I (C)

Diet 6C: Plus 50 mg. of N from NEAA-I

Diet 6D: Plus 60 mg. of N from NEAA-I

Table 1. Continued

Experiment Two: Effect of omission of either cystine or tyrosine from the non-essential amino acid mixture when fed in conjunction with 34 mg. of nitrogen provided by essential amino acids (EAA-I) per day[a]

Diet 4:	Plus NEAA-I 74 mg. N daily (C)
Diet 7:	Plus NEAA-I minus cystine 74 mg. N daily
Diet 8:	Plus NEAA-I minus tyrosine 74 mg. N daily

Experiment Three: Influence of isonitrogenously replacing cystine and tyrosine with methionine and phenylalanine, respectively, and feeding in conjunction with essential amino acids, total nitrogen per day being held at 74 mg.[b]

Diet 4:	34 mg. essential N(EAA-I) and 40 mg. non-essential N(NEAA-I) (C)
Diet 9:	36 mg. essential N(EAA-I) plus 2 mg. methionine N) and 38 mg. non-essential N(NEAA-I) minus 2 mg. cystine N)

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[a] When either cystine or tyrosine or both were omitted from the non-essential amino acid mixture, the remaining non-essential amino acids were increased proportionately to keep the day's supply of non-essential amino acid nitrogen at 40 mg. In all cases, the EAA-I mixture provided 34 mg. of nitrogen per day.

[b] Cystine and tyrosine provided by NEAA-I were replaced isonitrogenously by methionine and phenylalanine, singly or as a pair. The latter substitution gave a mixture of essential acids, hereafter known as EAA-II and providing 38 mg. of nitrogen daily. The mixture of non-essential amino acids remaining after the removal of both cystine and tyrosine was called NEAA-II. It provided 36 mg. of nitrogen daily instead of 40 mg.

Table 1. Continued

Diet 10: 36 mg. essential N(EAA-I plus 2 mg. phenylalanine N) and 38 mg. non-essential N(NEAA-I minus 2 mg. tyrosine N)

Diet 11: 38 mg. essential N(EAA-II) and 36 mg. non-essential N(NEAA-I minus 2 mg. cystine N, minus 2 mg. tyrosine N)

Experiment Four: Effect of feeding graded doses of NEAA-I containing no cystine and tyrosine in conjunction with 38 mg. of nitrogen provided by a modified mixture of essential amino acids (EAA-II[a])

Diet 12: Plus 10 mg. of N from NEAA-II

Diet 12A: Plus 20 mg. of N from NEAA-II

Diet 12B: Plus 30 mg. of N from NEAA-II

Diet 12C: Plus 40 mg. of N from NEAA-II (C)

Diet 12D: Plus 50 mg. of N from NEAA-II

Diet 12E: Plus 60 mg. of N from NEAA-II

Experiment Five: Effect of further increasing the methionine and phenylalanine contents of the essential amino acid mixture (EAA-III[b]), and feeding 39.5 mg. of nitrogen per day in conjunction with non-essential amino acids nitrogen provided by graded doses of NEAA-II

Diet 13: Plus 10 mg. of N from NEAA-II

Diet 13A: Plus 34.5 mg. of N from NEAA-II (C)

Diet 13B: Plus 45 mg. of N from NEAA-II

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[a] The cystine and tyrosine supplied by NEAA-I were replaced isonitrogenously by methionine and phenylalanine, respectively. The modification of the essential amino acid mixture was designated as EAA-II

[b] Extra nitrogen, 0.5 and 1.0 mg. provided by methionine and phenylalanine, respectively, was added to EAA-II. The new mixture was called EAA-III. It provided 39.5 mg. of nitrogen per day.

Table 1. Continued

Diet 13C: Plus 60 mg. of N from NEAA-II

STUDY B: Effect of substituting a single non-essential amino acid for mixture of seven non-essential amino acids and effect of feeding with two different mixtures of essential amino acids, total nitrogen per day being held at 74 mg.

Diet 4: EAA-I (34 mg. N) plus NEAA-I (40 mg. N) (C)

Diet 14: EAA-I (34 mg. N) plus glutamic acid (40 mg. N)

Diet 15: EAA-II (38 mg. N) plus glutamic acid (36 mg. N)

Diet 18C: EAA-II (38 mg. N) plus NEAA-II (40 mg. N) (C)

STUDY C: Efficacy of mixtures containing essential amino acids only for the support of protein nutrition

<u>Experiment One</u>: Comparison of three mixtures containing variable amounts of methionine and phenylalanine at two levels of feeding

Diet 16: EAA-I providing 34 mg. of N per day

Diet 16A: EAA-I providing 74 mg. of N per day (C)

Diet 17: EAA-II providing 38 mg. of N per day

Diet 17A: EAA-II providing 74 mg. of N per day

Diet 18: EAA-III providing 39.5 mg. of N per day

Diet 18C: EAA-III providing 74 mg. of N per day

<u>Experiment Two</u>: Effect of feeding EAA-III in quantities providing graded doses of nitrogen

Diet 18: 39.5 mg. of N per day

Diet 18A: 50 mg. of N per day

Table 1. Continued

Diet 18B: 60 mg. of N per day

Diet 18C: 74 mg. of N per day (C)

Diet 18D: 80 mg. of N per day

STUDY D: Effect of variation in the optical form of one amino acid in the essential amino acid mixture

Experiment One: Effect when essential amino acid nitrogen only as present in EAA-I and EAA-II, is fed

Diet 17: EAA-II containing 0.2% L-threonine (38 mg. N per day)

Diet 19: EAA-II containing 0.4% DL-threonine (38 mg. N per day) (C)

Diet 20: EAA-III containing 0.2% L-threonine (39.5 mg. N per day)

Diet 18: EAA-III containing 0.4% DL-threonine (39.5 mg. N per day) (C)

Experiment Two: Effect when dietary nitrogen is provided by both essential and non-essential amino acid nitrogen, EAA-III (39.5 mg. N) and NEAA-II (34.5 mg. N)

Diet 21: EAA-III containing 0.2% L-threonine

Diet 22: EAA-III containing 0.4% L-threonine

Diet 13A: EAA-III containing 0.4% DL-threonine (C)

SERIES III: INFLUENCE OF VARIATION IN THE NON-NITROGENOUS COMPONENTS OF THE DIET

STUDY A: Effect of using different sources of carbohydrate in the diet containing EAA-III (39.5 mg. N per day) and NEAA-II (34.5 mg. N per day)

Diet 13A: With 67.5% dextrin (C)

Diet 23: With 67.5% cornstarch

Diet 24: With 67.5% dextrose

Table 1. Continued

	Diet 25:	With 67.5% sucrose
STUDY B:	Effect of feeding the amino acid diet containing EAA-III (39.5 mg. N per day) and NEAA-II (34.5 mg. N per day), but at different levels of caloric intake	
	Diet 13A:	When 65 calories per day are offered (C)
	Diet 26:	When 50 calories per day are offered
	Diet 27:	When 35 calories per day are offered

Experimental Animals

Adult male albino rats approximately six months old (24 ± 2 weeks) were used. They were Wistar stock, strain A, and had been inbred for more than 100 generations by brother and sister mating. From the time the rats were weaned at 28 days of age until the beginning of the experiment, two animals were housed together in pairs in a round wire-meshed cage. They were maintained on a stock diet which contained approximately 23% protein. They were weighed weekly. Only those animals free from respiratory infections and exhibiting normal physical characteristics were used. The animals chosen for any experiment were distributed in such a manner that the average weights of the animals in the groups making up the experiment conformed as closely as possible. In general, only animals weighing 340 - 370 gm. were chosen. Prior to the initiation of the experiment, the experimental animals were transferred into individual cages for several days in order that they might adjust to separate housing.

Experimental Diets

The basic diets

The composition of the protein-free diets used as the bases for the formulation of the various rations fed in the present experiment is shown in Table 2. The nitrogenous component of any ration always was introduced at the expense of the dietary carbohydrate in formulae given. Two diets were fed at reduced levels of intake in Study B of Series III, and the composition of these basic diets, therefore, was modified in order that one day's feed might provide the same quantities of Osborne and Mendel salts, ruffex and sodium chloride as the diets fed the other experimental groups.

The butterfat was prepared by melting several pounds of butter purchased from the local market. When the butter was thoroughly melted, the milk solids which floated on the top were carefully removed by skimming. The clear liquid was filtered through a clean cheese cloth placed in a hot water funnel and then stored in a deep freeze until ready for use.

The basic diet was supplemented with a mixture of vitamins believed to be adequate for the maintenance of the

Table 2. Composition of the nitrogen-free basal diets used in Series I, II, and III of the experiment, nitrogen-containing components being added at the expense of carbohydrate.

Dietary component	Series I and II: Diets 1-22 inclusive[a]	Series III				
		Study A			Study B	
		Diet 23	Diet 24	Diet 25	Diet 26	Diet 27
	gm.	gm.	gm.	gm.	gm.	gm.
Dextrin[b]	73	--	--	--	71.8	69.8
Cornstarch[c]	--	73	--	--	--	--
Dextrose[d]	--	--	73	--	--	--
Sucrose[d]	--	--	--	73	--	--
Butterfat[e]	10	10	10	10	9.6	8.9
Swiftn'ing[f]	10	10	10	10	9.6	8.9
O. & M. salts[1]	4	4	4	4	5.1	7.1
Ruffex[b]	2	2	2	2	2.6	3.6
NaCl[g]	1	1	1	1	1.3	1.8
Total	100	100	100	100	100.0	100.0
Total calories	472	472	472	472	460	439

[a] Except for Diet 5, which was stock ration customarily used in the Nutrition Laboratory

[b] Fisher Scientific Co.

[c] Penick & Ford, Inc.

[d] Pfanstiehl Chemical Co.

[e] From butter purchased on the local market

[f] Swift & Co.

[g] Baker Chemical Co.

[1] Osborne, T. B. and Mendel, L. B. (1919), J. Biol. Chem. 37, 557-601

adult rat, the composition of which is given in Table 3. The mixture was prepared as described by Clark (1950, p. 97). The daily dose was 500 mg. A preparation known as rice bran polish factor II was first used as a source of unidentified B vitamins. It was replaced, however, after the completion of Series I, by vitamin B_{12} and folic acid. The vitamin B_{12} was not a part of the vitamin mixture; it was administered intraperitoneally.

The reference diet

A preparation of lactalbumin of high quality secured from Harris Laboratories, Division of Bristol-Myers of New York was used as the reference protein in the present experiment. The specific concentrations of 15 of the amino acids present in the preparation was determined by means of microbiological assay (see Table 4). At the time these assays were made, methods were not available for the analyses of threonine, alanine, and serine. The respective concentrations of these acids in the reference lactalbumin were estimated from data provided by Schmidt (1944).

It was found that this preparation of lactalbumin contained 12.725 per cent nitrogen, 1.04 per cent ash, and 8.18 per cent moisture (Kuehl, 1949). Therefore, the nitrogen content on the ash-free moisture-free basis was 14.01 per cent. This gives 7.14 as the factor for the conversion of the nitrogen value to protein.

Table 3. Vitamin supplements added to basal diets.

Components	Allowance per rat per day		
	Diets 1 & 2	Diet 3	Diet 4[a]
Dry mixture			
Biotin, crystalline[b]	1 mcg.	1 mcg.	1 mcg.
Thiamine[b]	40 mcg.	40 mcg.	40 mcg.
Pyridoxine[c]	40 mcg.	40 mcg.	40 mcg.
Riboflavin[b]	60 mcg.	60 mcg.	60 mcg.
Ca pantothenate[b]	100 mcg.	100 mcg.	100 mcg.
Niacin[d]	500 mcg.	500 mcg.	500 mcg.
Ascorbic acid[e]	1 mg.	1 mg.	1 mg.
Choline chloride[c]	5 mg.	5 mg.	5 mg.
Inositol[c]	10 mg.	10 mg.	10 mg.
Para-amino benzoic acid[c]	10 mg.	10 mg.	10 mg.
Rice bran polish, Factor II[f]	100 mg.	---	---
Folic acid[c]	---	8 mcg.	8 mcg.
Dextrin[g] to total weight of	500 mg.	500 mg.	500 mg.
Cod liver oil[h]	50 mg.	50 mg.	50 mg.
Alpha-tocopherol solution[i]	50 mg.	50 mg.	50 mg.
Vitamin B_{12}, crystalline[j]	---	---	0.75 mcg.

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[a] After the completion of Series, this mixture was used throughout the courses of the present experiment

[b] General Biochemicals Inc.

[c] Nutritional Biochemical Corp.

[d] Wyeth Inc.

[e] Merck & Co.

[f] Borden Co.

[g] Fisher Scientific Co.

[h] E. R. Squibb & Sons

[i] Five gm. of alpha-tocopherol (Nutritional Biochemical Corp.) was added to 328 gm. of Wesson oil. The daily dose of 50 mg. of this solution contained 0.75 mg. of alpha-tocopherol

[j] Eli Lilly & Co.

Table 4. Amino acids present in lactalbumin as determined by microbiological assay[a]

Amino acid	Per cent amino acid in lactalbumin	Mg. amino acids in 4.27 gm. lactalbumin
Arginine	2.56	109.1
Histidine	1.65	70.3
Isoleucine	5.00	213.0
Leucine	9.71	413.6
Lysine	7.57	322.5
Methionine	1.86	79.2
Phenylalanine	3.29	140.2
Tryptophan	1.66	70.7
Valine	5.13	218.5
Aspartic acid	8.50	362.1
Cystine	1.82	77.5
Glutamic acid	14.60	622.0
Glycine	1.63	69.4
Proline	4.35	185.3
Tyrosine	3.08	131.2

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[a] Analyzed through the courtesy of Dr. M. S. Dunn, University of California, at Los Angeles

Early studies (Swanson, et al., 1948) had demonstrated that a synthetic diet containing 3.5 - 4.0 per cent lactalbumin protein supported nitrogen equilibrium in the adult rat. Clark (1950) had demonstrated that about 4.27 per cent of the preparation of lactalbumin used in the present experiment supported nitrogen equilibrium. This amount of lactalbumin preparation, therefore, was used again in the reference ration and supplied 3.88 per cent protein.

Weighed amounts of the dry ingredients of the reference diet were tabled carefully on a large sheet of cellophane. They were then transferred into a Hobart mixer, the melted fat added, and all mixed at a medium speed for 30 minutes. Any fat adhering to the paddle or bowl was scraped into the mixture at intervals.

Analysis showed that the reference diet contained 583 mg. of nitrogen per 100 gm., the basic portion furnishing 40 mg. of this amount. The reference diet thus contained 543 mg. of nitrogen per 100 gm., which was equivalent to 3.88 gm. of protein, the quantity initial calculations indicated would be present. One day's feed (13 gm.), therefore, contained approximately 74 mg. of nitrogen provided by lactalbumin protein. The selection of 13 gm. as the quota of ration to be fed daily, however, was based on the quantity which the rat consumed when the reference diet was offered ad libitum.

The amino acid-containing diets

At the time the present experiment was initiated, natural isomers of several amino acids were not readily obtainable. Therefore, DL-forms of several amino acids, i.e., isoleucine, methionine, phenylalanine, threonine, valine alanine, and aspartic acid were used. It has been shown, however, that the rat does not utilize both optical isomers of certain of these acids equally well. For this reason, twice the required amounts of isoleucine, threonine, and valine were included in the essential amino acid mixture. No correction was made for DL-forms of methionine, phenylalanine, alanine, and aspartic acid since the rat appears to use both optical isomers of these amino acids efficiently. The water present in L-histidine was taken into account in the preparation of the mixture. The hydrochloric acid present in L-histidine and L-lysine was neutralized by the incorporation of appropriate amounts of sodium carbonate (see Clark, p. 102).

A report in the literature indicated DL-serine, the only form available for general experimental purpose which exerted a nephrotoxic effect (Fishman and Artom, 1944). Therefore, it was omitted from the mixture and replaced isonitrogenously by glycine, since serine and glycine appear to be interconvertible (Goldsworthy, et al., 1949).

Thirty-seven different mixtures of amino acids were used in the present experiment. The amino acids fed daily, in general, consisted of two parts, one containing the essential amino acids (EAA) only, the other, the non-essential amino acids (NEAA). In the basic reference mixture, each part was formulated so that its amino acids would be equal in kind and quantity to those present in one day's feed (13 gm.) of the reference diet containing 4.27 per cent lactalbumin preparation. The composition of this reference mixture is shown in Table 5, (Diet 4, made up by EAA-I plus NEAA-I). The amino acid mixtures used in making two other basic diets, i.e., Diet 11 containing EAA-II plus NEAA-II and Diet 13A containing EAA-III plus NEAA-II are shown therein also. The total nitrogen provided daily by these three different mixtures, was kept within the nitrogen content of one day's feed (13 gm.) of the diet containing 4.27 per cent of the lactalbumin preparation (74 mg. N). The quantity of each individual amino acid to be incorporated in an amino acid mixture was calculated as described by Clark (p. 107, Table 8). The formulae in less detailed form for the remaining amino acid mixtures studies appear in Table 6.

Before the experiment was initiated, the amino acids employed were analyzed for their nitrogen concentration. The values all were within a satisfactory range.

The desired amounts of amino acids were weighed on an analytical balance when humidity was relatively low, then

Table 5. Composition of three basal mixtures of amino acids used in experimental diet

Amino acid fed	Optical form utilized by rat	Mg. amino acid/100 gm. diet as formulated			Mg. utilizable amin 100 gm. diet		
		EAA-I and NEAA-I (Diet 4)	EAA-II and NEAA-II (Diet 11)	EAA-III and NEAA-III (Diet 13A)	EAA-I and NEAA-I (Diet 4)	EAA-II and NEAA-II (Diet 11)	E N (
		mg.	mg.	mg.	mg.	mg.	
L-arginine.HCl[a]	L	132	132	132	109	109	
L-histidine.HCl.H_2O[b]	DL	95	95	95	70	70	
DL-isoleucine[a]	L	426	426	426	213	213	
L-leucine[a]	L	414	414	414	414	414	
L-lysine.HCl[a]	L	405	405	405	322	322	
DL-methionine[a]	DL	79	232	286	79	232	
DL-phenylalanine[a]	DL	140	330	407	140	330	
DL-threonine[a]	L	395	395	395	198	198	
L-tryptophan[a]	DL	71	71	71	71	71	
DL-valine[a]	L	437	437	437	218	218	
Total		2594	2937	3068	1834	2177	
DL-alanine[a]	DL	164	164	157	164	164	
DL-aspartic acid[a]	DL	580	580	555	580	580	
L-cystine[b]	L	124	---	---	124	---	
L-glutamic acid[b]	L	996	996	955	996	996	
Glycine[b]	-	320	320	307	320	320	
L-proline[a]	-	297	297	284	297	297	
L-tyrosine[c]	DL	210	---	---	210	---	
Total		2691	2357	2258	2691	2357	
Grand total		5285	5294	5326	4525	4534	

[a] Nutritional Biochemicals Corp.

[b] Merck & Co.

[c] Eastman Kodak Co.

rimental diets.

ilizable amino acid/ . diet			Mg. utilizable amino acid/ 1 day's feed (13 gm.)			Mg. utilizable amino acid nitrogen/ 1 day's feed (13 gm.)		
4)	EAA-II and NEAA-II (Diet 11)	EAA-III and NEAA-III (Diet 13A)	EAA-I and NEAA-I (Diet 4)	EAA-II and NEAA-II (Diet 11)	EAA-III and NEAA-III (Diet 13A)	EAA-I and NEAA-I (Diet 4)	EAA-II and NEAA-II (Diet 11)	EAA-III and NEAA-III (Diet 13A)
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
	109	109	14	14	14	4.6	4.6	4.6
	70	70	9	9	9	2.5	2.5	2.5
	213	213	28	28	28	3.0	3.0	3.0
	414	414	54	54	54	5.8	5.8	5.8
	322	322	42	42	42	8.0	8.0	8.0
	232	286	10	30	37	0.9	2.9	3.5
	330	407	18	43	53	1.5	3.6	4.5
	198	198	26	26	26	3.0	3.0	3.0
	71	71	9	9	9	1.3	1.3	1.3
	218	218	28	28	28	3.4	3.4	3.4
	2177	2308	238	283	300	34.0	38.0	39.5
	164	157	21	21	20	3.3	3.3	3.2
	580	555	75	75	72	7.9	7.9	7.6
	---	---	16	--	--	1.9	---	---
	996	955	130	130	124	12.4	12.4	11.8
	320	307	42	42	40	7.7	7.7	7.4
	297	284	39	39	37	4.7	4.7	4.5
	---	---	27	--	--	2.1	---	---
	2357	2258	350	307	294	40.0	36.0	34.5
	4534	4566	588	590	594	74.0	74.0	74.0

Table 6. Composition of 33 modifications of the three basal mixtures of amino acids.

Amino acid fed	Diet 6	Diet 6A	Diet 6B	Diet 6C	Diet 6D	Diet 7	Diet 8	Diet 9
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
L-arginine.HCl	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
L-histidine.HCl.H_2O	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
DL-isoleucine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-leucine	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
L-lysine.HCl	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
DL-methionine	0.9	0.9	0.9	0.9	0.9	0.9	0.9	2.9
DL-phenylalanine	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
DL-threonine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
L-tryptophan	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
DL-valine	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
Total	34.0	34.0	34.0	34.0	34.0	34.0	34.0	36.0
DL-alanine	0.8	1.7	2.5	4.2	5.0	3.5	3.5	3.3
DL-aspartic acid	2.0	4.0	6.0	9.9	11.9	8.3	8.3	7.9
L-cystine	0.5	0.9	1.4	2.3	2.8	---	2.0	---
L-glutamic acid	3.1	6.1	9.2	15.4	18.5	12.9	13.0	12.3
Glycine	1.9	3.9	5.8	9.7	11.6	8.1	8.2	7.7
L-proline	1.2	2.4	3.5	5.9	7.0	5.0	5.0	4.7
L-tyrosine	0.5	1.0	1.6	2.6	3.2	2.2	---	2.1
Total	10.0	20.0	30.0	50.0	60.0	40.0	40.0	38.0
Grand total	44.0	54.0	64.0	84.0	94.0	74.0	74.0	74.0

of amino acids.

Diet 8	Diet 9	Diet 10	Diet 12	Diet 12A	Diet 12B	Diet 12C	Diet 12D	Diet 12E	Diet 13	Diet 13B	Diet 13C
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
4.6	4.6	4.6-	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6	4.6
2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
0.9	2.9	0.9	2.9	2.9	2.9	2.9	2.9	2.9	3.5	3.5	3.5
1.5	1.5	3.6	3.6	3.6	3.6	3.6	3.6	3.6	4.5	4.5	4.5
3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4
34.0	36.0	36.0	38.0	38.0	38.0	38.0	38.0	38.0	39.5	39.5	39.5
3.5	3.3	3.3	0.9	1.8	2.8	3.7	4.6	5.5	0.9	4.1	5.5
8.3	7.9	7.9	2.2	4.4	6.6	8.8	11.0	13.2	2.2	9.9	13.2
2.0	---	2.0	---	---	---	---	---	---	---	---	---
13.0	12.3	12.3	3.4	6.8	10.3	13.7	17.1	20.5	3.4	15.3	20.5
8.2	7.7	7.7	2.2	4.3	6.4	8.6	10.8	12.9	2.2	9.9	12.9
5.0	4.7	4.7	1.3	2.6	3.9	5.2	6.6	7.9	1.3	5.8	7.9
---	2.1	---	---	---	---	---	---	---	---	---	---
40.0	38.0	36.0	10.0	20.0	30.0	40.0	50.0	60.0	10.0	45.0	60.0
74.0	74.0	74.0	48.0	58.0	68.0	78.0	88.0	98.0	49.5	84.5	99.5

Table 6. Continued

Amino acid fed	Diet 14	Diet 15	Diet 16	Diet 16A	Diet 17	Diet 17A	Diet 18	Diet 18A	Diet 18B
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
L-arginine.HCl	4.6	4.6	4.6	10.0	4.6	9.0	4.6	5.8	7.0
L-histidine.HCl.H_2O	2.5	2.5	2.5	5.4	2.5	4.9	2.5	3.2	3.8
DL-isoleucine	3.0	3.0	3.0	6.5	3.0	5.8	3.0	3.8	4.6
L-leucine	5.8	5.8	5.8	12.6	5.8	11.3	5.8	7.3	8.8
L-lysine.HCl	8.0	8.0	8.0	17.4	8.0	15.6	8.0	10.1	12.2
DL-methionine	0.9	2.9	0.9	2.0	2.9	5.6	3.5	4.4	5.3
DL-phenylalanine	1.5	3.6	1.5	3.3	3.6	7.0	4.5	5.7	6.8
DL-threonine	3.0	3.0	3.0	6.5	---	---	3.0	3.8	4.6
L-threonine	---	---	---	---	3.0	5.8	---	---	---
L-tryptophan	1.3	1.3	1.3	2.8	1.3	2.5	1.3	1.6	2.0
DL-valine	3.4	3.4	3.4	7.4	3.4	6.6	3.4	4.3	5.2
Total	34.0	38.0	34.0	74.0	38.0	74.0	39.5	50.0	60.0
DL-alanine	---	---	---	---	---	---	---	---	---
DL-aspartic acid	---	---	---	---	---	---	---	---	---
L-glutamic acid	40.0	36.0	---	---	---	---	---	---	---
Glycine	---	---	---	---	---	---	---	---	---
L-proline	---	---	---	---	---	---	---	---	---
Total	40.0	36.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0
Grand total	74.0	74.0	34.0	74.0	38.0	74.0	39.5	50.0	60.0

Diet 17A	Diet 18	Diet 18A	Diet 18B	Diet 18C	Diet 18D	Diet 19	Diet 20	Diet 21	Diet 22
mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
9.0	4.6	5.8	7.0	8.6	9.3	4.6	4.6	4.6	4.6
4.9	2.5	3.2	3.8	4.7	5.1	2.5	2.5	2.5	2.5
5.8	3.0	3.8	4.6	5.6	6.1	3.0	3.0	3.0	3.0
11.3	5.8	7.3	8.8	10.9	11.7	5.8	5.8	5.8	5.8
15.6	8.0	10.1	12.2	15.0	16.2	8.0	8.0	8.0	8.0
5.6	3.5	4.4	5.3	6.6	7.1	2.9	3.5	3.5	3.5
7.0	4.5	5.7	6.8	8.4	9.1	3.6	4.5	4.5	4.5
---	3.0	3.8	4.6	5.6	6.1	3.0	---	---	---
5.8	---	---	---	---	---	---	3.0	3.0	6.0
2.5	1.3	1.6	2.0	2.4	2.6	1.3	1.3	1.3	1.3
6.6	3.4	4.3	5.2	6.4	6.9	3.4	3.4	3.4	3.4
74.0	39.5	50.0	60.0	74.0	80.0	38.0	39.5	39.5	42.5
---	---	---	---	---	---	---	---	3.2	3.2
---	---	---	---	---	---	---	---	7.6	7.6
---	---	---	---	---	---	---	---	11.8	11.8
---	---	---	---	---	---	---	---	7.4	7.4
---	---	---	---	---	---	---	---	4.5	4.5
00.0	00.0	00.0	00.0	00.0	00.0	00.0	00.0	34.5	34.5
74.0	39.5	50.0	60.0	74.0	80.0	38.0	39.5	74.0	77.0

transferred to a mortar and ground to a fine powder. They were tabled carefully together and the mixture analyzed for the concentration of nitrogen immediately. The values all were within a satisfactory range.

As with the reference diet, the amino acids replaced an equal weight of carbohydrate in the basic nitrogen-low diet (see Table 7). Other diets were formulated similarly. Every precaution was taken to insure quantitative transfer and thorough blending of amino acid mixtures with other ingredients. For example, the amino acid mixture and the appropriate quantity of sodium carbonate were transferred to a large sheet of cellophane and tabled; the Osborne and Mendel salts were placed in the beaker that had contained the amino acids to remove the small fraction of ground amino acids that adhered to the beaker despite careful brushing; the salts were tabled with the amino acids; then, sodium chloride and Ruffex were carefully added. When these ingredients were thoroughly mixed carbohydrate was introduced portion by portion and the mixture tabled carefully before it was placed in the Hobart mixer and blended with the melted fat.

Administration of experimental diets

It has been observed that rats eating *ad libitum* rations that were deficient in amino acids immediately showed a loss of appetite (Frazier, *et al.*, 1948, Caedo, 1955). The re-

Table 7. Composition of the reference and basal diets as fed exclusive of vitamin supplements. (For amount of utilizable nitrogen provided see Table 5.)

Dietary component	Reference diet	Diet 4	Diet 11	Diet 13A
	gm.	gm.	gm.	gm.
Lactalbumin	4.27			
EAA-I and NEAA-I		5.46[a]		
EAA-II and NEAA-II			5.47[a]	
EAA-III and NEAA-II				5.50[a]
Dextrin	68.73	67.54	67.53	67.50
Butterfat	10.00	10.00	10.00	10.00
Swiftn'ing	10.00	10.00	10.00	10.00
O. & M. salts	4.00	4.00	4.00	4.00
Ruffex	2.00	2.00	2.00	2.00
NaCl	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00

[a] Including several racemic acids

duced caloric intake, then, further affects the nitrogen utilization (Calloway and Spector, 1954).

In the early studies, Clark (1950) showed that the average daily food intakes of 11 adult rats were 12.3, 12.6, 12.9, and 13.0 gm. in four successive periods of four days each when they were fed *ad libitum* a ration containing 14 per cent protein from lactalbumin. These rats had been transferred directly from the stock colony and weighed approximately 325 gm. In order to control the daily intake of nitrogen and to keep the energy value constant, the daily quota of 13 gm. of the solid diet providing approximately 60 calories was made into homogenate which could be introduced into the stomach of the experimental animal by means of a catheter.

The experimental diets were homogenized for force-feeding as described by Clark (1950, p. 119). Generally, 15 doses of feed were made up at one time. In this case, 250 gm. of experimental diet, 9.6 of dry vitamin mixture, 0.96 gm. of cod liver oil, 0.96 gm. of an alpha-tocopherol solution, and appropriate volume of distilled water to make the final homogenate to a volume of 465 ml. were used. The resulting homogenate was placed in two half-pint jars and refrigerated immediately and continually except at feeding intervals. Twenty-four ml. of this homogenate contained one day's feed (equivalent to 13 gm. of solid diet) and one day's allowance of the dry vitamin mixture, 50 mg. of cod liver oil, and 50 mg. of alpha-tocopherol solution. The quantity was force-fed in three equal portions daily. A 20 ml. veterinary syringe fitted with blunt needle carrying a No. 8 French catheter was used (Kuehl, 1949, Clark, 1950). Early studies (Clark, 1950) showed that the adult rats were force-fed three times a day and that they utilized nitrogen as efficiently as the animals that were fed ad libitum.

The force-feeding technique offers a simple solution to the problem of ensuring a definite intake of specific nutrient but it may not provide the most effective condition for evaluating nitrogen utilization. It is clear that the capacity of the stomach may have been exceeded, especially during the first few days after the practice of the force-feeding of the experiment rations. The technique developed in the present experiment, however, appeared to be satisfactory in the preliminary studies. No difficulty was experienced in administering the food homogenate throughout the experiment. All the animals were free from diarrhea and distension even on the first day when force-feeding was initiated. They rested comfortably in normal position after they were fed each time and were alert and active between the feeding intervals.

Two diets were fed at reduced levels of intake in Study B of Series III. Therefore, the quantities of the amino acid-containing diet required in the homogenate were different.

The respective amounts needed for Diets 26 and 27 were 197 and 142 gm. One day's feed of Diet 26 contained 10.2 gm. of dry diet, of Diet 27, 7.3 gm.

Evaluation of Nitrogen Utilization

Determination of nitrogen balance

Manner of conducting balance test. The nitrogen metabolism study was divided into two periods, i.e., (1) a preliminary period in which the animals became adjusted to the force-feeding technique and to the diets administered, and (2) the balance period. The respective intervals for these two periods were 12 and 7 days.

The rats were force-fed thrice daily at approximately 6-hour intervals. Precautions were taken to avoid any disturbance which might result from over-loading the gastro-intestinal tract. The rats were given only 1 ml. of homogenate per feeding the first day, 2 ml. the second day, 4 ml. the third day, 8 ml. the fourth day and thereafter. On the day preceding the initiation of the balance period, the quantity of homogenate was reduced from 24 ml. to 20 ml. and the last feeding given at 4 P.M. The experimental animals were transferred to the wide-meshed metabolism cages at 8 A.M. on the first day of the balance period. On the last day of the test, the feeding schedule was similar to that on the day preceding the balance period. At the conclusion of the balance period, animals were removed from the metabolism cages at the same hour that they had been placed on the test.

Collection of urine. Urine was collected on circles of high quality filter paper containing only a slight trace of nitrogen. Before use these papers were soaked over night in a 95 per cent ethyl alcohol solution containing 10 per cent glacial acetic acid and air-dried. Nine treated filter papers were placed on a Pyrex plate under the metabolism cage prior to the initiation of the balance period. One filter paper was removed every morning and thereafter and placed in a 1000 ml. wide-mouthed Erlenmeyer flask containing 200 ml. of 20 per cent hydrochloric acid. The flask was covered with a beaker to avoid evaporation. On the last day of the metabolism period, the three remaining papers were removed and transferred to the same flask. Then the cage and plate were washed quantitatively with hot distilled water applied under pressure. The rinsings were transferred to the flask containing the filter papers. The contents were stirred to break up the papers and then filtered quantitatively through a Buchner funnel fitted into a 2000 ml. suction flask. Residues in the funnel were rinsed several times with hot distilled water to wash out all the urine. The extracted urine then was transferred quantitatively to a 2000 ml. volumetric flask. When the solution had cooled to room temperature, it was made to volume. A sample of the well-mixed urine was stored in a 12-ounce pharmacy bottle until time of analysis.

The ability of the author to recover nitrogen quantitatively after the dispersion of a standard solution of $(NH_4)_2SO_4$ over the bottom of the metabolism cage fitted with treated filter papers has been demonstrated (Table I Appendix). Aliquots of the $(NH_4)_2SO_4$ solution were trickled from a pipette twice a day over the wire mesh for the 7 days involved in the balance test. The top filter paper was removed each day just as it was during the progress of the balance test.

Collection of feces. Feces were removed daily from the filter papers. They were brushed free of hair and placed in 125 ml. Erlenmeyer flasks containing 50 ml. of 20 per cent hydrochloric acid and covered with a beaker. Ferric oxide was used to mark the beginning and termination of the balance intervals. On the first day of the balance period food colored with ferric oxide was fed. It may be recalled that no food was offered to the rats for 16 hours prior to the initiation of the balance test. Therefore, any red feces that appeared later were ascribed to the test food, any brown to the previous feeding. The brown feces were discarded. The ferric oxide-colored food was administered again on the day following the termination of the balance test and feces were collected up to the time when red feces appeared.

At the termination of the metabolism period, the flask containing feces was autoclaved under 15 pounds of pressure per square inch for two hours. The digest then was rubbed through a fine sieve into a 250 ml. volumetric flask and diluted to volume with distilled water at room temperature. After the sample was well mixed, it was transferred into an 8-ounce pharmacy bottle and stored for analysis.

Analysis of nitrogen. The nitrogen content of the food consumed and the urine and feces excreted during the balance period were determined. Since the food homogenate was mixed, an aliquot of 8 ml. of each diet tested was transferred each day during the 7 days involved in the balance period into a 125 ml. Erlenmeyer flask containing 50 ml. of 20 per cent hydrochloric acid. At the termination of the metabolism period, the flask containing food was treated as the one containing the feces. From this, a 10 ml. aliquot was taken for analysis of nitrogen. The respective aliquots of urine and feces, however, were 50 and 25 ml.

The Kjeldahl-Gunning procedure for nitrogen analysis was followed. Urine aliquot was digested in Kjeldahl flask with 20 ml. of concentrated sulfuric acid, 10 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide for an hour and 15 minutes. The aliquot from food digest or feces, however, was digested with 20 ml. of sulfuric acid, 15 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide for about one and one-half hours.

Determination of hepatic constituents

Preparation of organ for analysis. The experimental animal was anesthetized by the intraperitoneal injection of 0.5 ml. of nembutal solution which contained 15 mg. of pentobarbital sodium at the termination of the nitrogen metabolism period. The liver was removed from the anesthetized animal as soon as an incision was made. The organ was blotted on filter papers to remove excess blood. A cross-section of the largest lobe and the tip of the longest lobe of liver were placed in a tared glass weighing bottle for the determination of moisture and fat. The remaining portion of the liver for the determination of hepatic nitrogen was placed in a 125 ml. previously weighed Erlenmeyer flask and weighed immediately. It was then covered with 50 ml. of 20% hydrochloric acid and autoclaved under for two hours. The digest was pressed through a fine sieve into a 250 ml. volumetric flask and diluted with distilled water to volume at room temperature. Finally, the aliquot was transferred into an 8-ounce pharmacy bottle and ready for analysis.

Analysis of hepatic nitrogen. Ten ml. aliquot of the liver acid digest was used for the analysis of nitrogen in a manner similar to fecal sample as described previously.

Analysis of hepatic moisture. Small tared glass weighing bottles were at hand; tissues were sampled as quickly as possible and placed directly into the weighing bottles. They were covered promptly and weighed immediately in order to minimize moisture losses. The bottles containing tissue samples were dried at a temperature of 85°C. in an electric oven for 72 hours and then dried to constant weight (±0.2 mg.) in a vacuum oven held at 80°C.

Analysis of hepatic fat. The determination of fat was patterned after the method of Bloor (1929) which gives an estimation of the quantity of fat present in terms of total alcohol-ether soluble substances.

The moisture-free sample was finely ground in a mortar with one-half teaspoon of acid-washed sand and transferred quantitatively into a 125 ml. Erlenmeyer flask. The weighing bottle, mortar, and pestle were rinsed with three portions of a 3 to 1 mixture of absolute alcohol anhydrous ethyl ether mixture. Approximately 25 ml. of this alcohol-ether mixture was added and the sample then heated on a steam bath with shaking for five minutes.

The sample was cooled to room temperature and filtered through a fat-free filter paper into a 200 ml. volumetric flask. The filter paper containing the ground sample was washed several times with small portions of the alcohol-ether mixture. When the ground sample appeared dry, it was extracted with anhydrous ethyl ether for eight hours in a

Goldfish extractor. This ether extract plus rinsings from the extraction cup was added into the 200 ml. volumetric flask containing the alcohol filtrate and the solution was made to volume with anhydrous ethyl ether. After mixing, 50 ml. aliquot was measured from a pipette into a large glass weighing bottle. It was evaporated to dryness on a steam bath and brought to constant weight (±0.2 mg.) in an air oven held at 80°C. These steps were followed exactly in all analyses.

The ability of the author to recover the hepatic fat from a standard preparation is shown in Table II (Appendix).

Determination of carcass constituents

Preparation of carcass for analysis. The carcass constituents of the rats used in Study B of Series III were analyzed. The whole carcass, which included the fur, skin, skeleton, viscera (liver removed) and blood was ground into a homogenous sample by putting through a Hobart grinder equipped with a 3 mm. mesh sieve. The carcass was chilled first and the resulting product was thoroughly mixed and sampled.

One hundred grams of carcass from each animal was placed in an Erlenmeyer flask and covered with 300 ml. of 20 per cent hydrochloric acid for analysis of nitrogen. The flask then was autoclaved under pressure for two hours. The contents of each flask were rubbed quantitatively through a fine sieve into a 1000 ml. volumetric flask and diluted to volume with distilled water at room temperature. These mixtures were thoroughly mixed and a sample was transferred into a pharmacy bottle and then stored until time for analysis.

Analysis of carcass nitrogen. Total carcass nitrogen again was determined by the Kjeldahl-Gunning procedure. Ten ml. aliquot of the carcass digest was used and the analysis was proceeded with in a manner similar to the fecal sample as described previously.

Analysis of carcass moisture. Triplicated samples of 2 gm. each from any rat were placed in tared glass weighing bottles for analysis of body moisture. They were analyzed in a manner similar to liver samples as described previously.

Analysis of carcass fat. The moisture-free samples were analyzed for body fat by the method of Bloor (1929) as described previously (see Analysis of hepatic fat).

RESULTS AND DISCUSSION

Series I. Evaluation of the Basic Diet

In 1950, Clark reported that equilibrium was characteristic of the nitrogen metabolism of the rats fed either a synthetic diet containing 4 per cent of a lactalbumin preparation or one providing a mixture of amino acids equal in kind and quantity to those present in one day's feed, i.e., 13 gm. of the reference diet containing lactalbumin. In Clark's experimental diets, rice bran polish served as the source of unidentified B vitamins. When her experiments were initiated, neither folic acid nor vitamin B_{12} was available for general experimental purposes. However, very shortly after the initiation of the present experiment, the production of rice bran polish ceased. Hence, re-examination of the over-all adequacy of the ration for maintenance of protein nutrition when folic acid and vitamin B_{12} replaced the rice bran polish seemed advisable before Clark's investigation was extended.

Nitrogen utilization in the present experiment was measured in terms of nitrogen balance, weight of liver, and concentration of moisture, nitrogen, and fat in the liver. Five diets were fed. Diets 1 and 2 represented the control rations. Diet 1 contained the lactalbumin preparation at the

4.27 per cent level; Diet 2, the mixture of amino acids equal in kind and quantity to those present in the lactalbumin diet. In these two diets, rice bran polish was used. Diets 3 and 4 contained the same amino acids present in Diet 2 and differed only by the virtue of the vitamin composition. Folic acid (8 mcg./day) replaced the rice bran polish in Diet 3; vitamin B_{12} (0.75 mcg./day) and folic acid (8 mcg./day) were substituted for it in Diet 4. The last diet (Number 5) containing approximately 23 per cent protein, was the stock ration customarily used in the Nutrition Laboratory of the Home Economics Research Department of the Iowa Agriculture Experiment Station. Rats maintained on it were used to secure data about composition of liver of normal rats.

The animals representing all experimental groups appeared physically normal in every respect. Their fur was clean and sleek, their muscles firm. They were neither nervous nor irritable. On the average, rats fed all test diets maintained body weight (±2 gm.) throughout the experimental period (Table III, Appendix).

Data pertaining to the nitrogen metabolism of the five groups are presented in Table 8. The metabolism test indicated that the lactalbumin control diet (Diet 1) administered in the present series was not entirely adequate for the support of nitrogen equilibrium. The average nitrogen balance in the 7-day period was -33 mg. Early studies by Küehl (1949),

Table 8. Average nitrogen retention and average composition of livers in rats fed die one day's feed of a 4.27% lactalbumin ration.

Series

Diet No.	Source of dietary nitrogen	Supplement to members of vitamin B-complex present in ration[a]	Nitrogen force-fed per day	No. of rats	Body weight	Nitrogen metabolism per 7 days		
						Nitrogen fed	Nitrogen balance	Pe H_2
			mg.		gm.	mg.	mg.	
1	Lactalbumin	Rice bran polish	74	6	357	536	-33	6
2	EAA-I plus NEAA-I	Rice bran polish	74	6	353	541	-34	6
3	EAA-I plus NEAA-I	Folic acid	74	6	347	576	-37	6
4	EAA-I plus NEAA-I	Folic acid plus vitamin B_{12}	74	4	336	576	-30	6
5	Mixed natural food[b]	---	602	55	350	4212[c]	427[c]	6

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[a] Rice bran polish, 100 gm./day; folic acid, 8 mcg./day; vitamin B_{12} 0.75 mcg./day

[b] Offered *ad libitum*

[c] Based on 9 rats: average weight 329 gm.

in rats fed diets containing nitrogen provided by amino acid mixtures simulating

Series I

gen meta- per 7 days		Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
n	Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Rat fat nit gen
	mg.		gm.	gm.	mg.			mg.			
	-33	68.2	8.84	2.14	266	3.0	12.5	675	7.5	24.0	2.5
	-34	67.7	8.67	2.03	261	3.0	12.8	769	8.9	27.4	2.9
	-37	64.3	8.97	1.97	256	2.9	13.0	1254	13.7	37.3	4.9
	-30	66.3	8.52	2.06	252	3.0	12.4	831	9.7	28.8	3.3
	427[c]	69.2	9.01	2.30	349	3.9	15.2	477	5.3	17.2	1.4

75 mcg./day

iets containing nitrogen provided by amino acid mixtures simulating

ies I

Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
	gm.	gm.	mg.			mg.			
68.2	8.84	2.14	266	3.0	12.5	675	7.5	24.0	2.5
67.7	8.67	2.03	261	3.0	12.8	769	8.9	27.4	2.9
64.3	8.97	1.97	256	2.9	13.0	1254	13.7	37.3	4.9
66.3	8.52	2.06	252	3.0	12.4	831	9.7	28.8	3.3
69.2	9.01	2.30	349	3.9	15.2	477	5.3	17.2	1.4

however, indicated that the rats fed a diet containing the lactalbumin preparation at the 4.4 per cent level may maintain positive nitrogen balance. On the average, her rats stored about 42 mg. of nitrogen in the test period (7 days).

It is interesting to find that the average nitrogen balances of the rats which received Diets 2, 3, and 4 were essentially the same and also very similar to those of the rats administered the lactalbumin control diet. Their nitrogen balances during the 7-day period were -34, -37, and -30 mg., respectively. The rats fed, ad libitum, the stock ration containing approximately 23 per cent protein were in positive nitrogen balance. They stored an average of 427 mg. of nitrogen during the 7-day test period, balances ranging from 15 to 733 mg. (Table III, Appendix).

The data pertaining to the characteristics of the livers of the rats in Series I are shown in Table 8. Values of individual rats are presented in Table IV (Appendix). The average weights of the livers of the rats in the groups fed the synthetic diets (Diets 1-4) did not appear to differ either on the fresh or on the dry, fat-free basis. Moisture contents of the organs ranged from 64.3 to 68.2 per cent in these groups. On the fresh and on the dry, fat-free basis, the percentages of nitrogen were approximately the same. Total hepatic "fat"[a] varied from 675 to 1254 mg. in Groups 1-4; the percentage of hepatic fat on the dry basis from 24.0 to

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[a] Fat throughout this thesis represents alcohol-ether soluble materials.

28.8 per cent. Differences in percentages of hepatic fat were significant at the 5 per cent level ($F_{3,18} = 4.39$). When tested by analysis of variance, data from the group fed Diet 3 contributed the significant variation.

An inverse relationship between per cent of water and per cent of fat was observed. The displacement of water by fat was most marked in the livers of rats of the group fed Diet 3.

As one might expect from the difference between the nitrogen content of the stock ration and the four experimental diets, the livers of the rats fed the stock ration were not only larger but also contained more nitrogen than did the organs of the rats administered the experimental diets. However, the livers of rats given Diet 5 were considerably less fatty than those of other groups.

However, when only the groups fed Diets 2 and 4 were compared, the data demonstrated clearly that the substitution of folic acid and vitamin B_{12} (Diet 4) for the rice bran polish (Diet 2) produced no change in the response of the rats when evaluated in terms of change in body weight, nitrogen retention, and concentration of nitrogen and fat in the livers. These vitamins were used, therefore, in all future experiments as a replacement for rice bran polish.

It should be noted, however, that all four experimental diets were lipogenic. Later experiments will offer explanation for the phenomenon. This study suggests the lipotropic activity of vitamin B_{12} when added to a ration supplemented with folic acid. Its presence in the diet caused a decrease in total hepatic fat from an average value of 1254 mg. to 831 mg. Thus, the protective action of the vitamin against deposition of liver fat is demonstrable in adult as well as in young rats fed a low protein diet (9% casein) supplemented with 0.02 per cent choline chloride (Hawk and Elvehjem, 1953). The lipogenic activity of the ration suggested that an experimental framework had been found for a study of amino acid imbalance. The nutritional adequacy of the nitrogenous moiety of the diet, therefore, was examined in Series II in terms of its ability to support nitrogen equilibrium and maintain a normal concentration of hepatic fat.

Series II. Influence of Variation in the Nitrogenous Components of the Diet

Study A. Methionine-cystine-phenylalanine-tyrosine relations

Results of the experiment reported in the previous series showed that rats fed either the reference diet containing lactalbumin or the diets providing a mixture of amino acids quantitatively equivalent to those in the intact protein were in slight negative nitrogen balance and that their livers were fatty. The mixture of non-essential amino acids contained cystine and tyrosine which are related, chemically and physiologically, to methionine and phenylalanine, respectively. In view of this fact, the observations immediately raised three questions. Did the mixture of amino acids contain adequate amounts of methionine and phenylalanine? What role did cystine and tyrosine play in meeting the respective requirements for methionine and phenylalanine? Were cystine and tyrosine instruments in the development of the fatty livers observed?

Experiment one. It was thought that the part played by cystine and tyrosine in meeting the requirement for methionine and phenylalanine for body maintenance could be demonstrated by the feeding of graded doses of non-essential amino acids in combination with the mixture containing essential amino acids only. The amount of fat accumulated in the livers of each experimental group also could be taken as indicative of the lipogenic property of cystine in this experimental situation.

Therefore, in Diets 6, 6A, 6B, 4, 6C, and 6D, the basal essential amino acid mixture (EAA-I) was supplemented, respectively, with 10, 20, 30, 40, 50, and 60 mg. of nitrogen per day from the non-essential amino acids (NEAA-I).

Results are presented in Table 9. The average nitrogen balance of the rats fed the essential amino acid diet supplemented with 10 mg. of non-essential nitrogen was -187 mg.

Table 9. Average nitrogen retention and average composition of livers of rats fed die of nitrogen from NEAA-I.

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I				Nitrogen fed	Nitrogen balance	Pe H_2
		Total	Cystine N					
	mg.	mg.	mg.		gm.	mg.	mg.	
6	34	10	0.47	6	344	408	-187	6
6A	34	20	0.94	6	350	476	-108	6
6B	34	30	1.40	6	353	541	- 34	6
4	34	40	1.87	10	358	618	8	6
6C	34	50	2.34	5	375	697	100	6
6D	34	60	2.81	5	377	762	88	6

f rats fed diets containing nitrogen provided by EAA-I supplemented with graded doses

Series II
Study A
Experiment One

n meta- r 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-187	69.0	8.34	1.93	246	3.0	12.8	654	7.9	25.4	2.7
-108	68.5	8.52	2.04	263	3.1	12.9	645	7.6	24.0	2.5
- 34	67.7	8.67	2.03	261	3.0	12.8	769	8.9	27.4	2.9
8	67.3	9.19	2.11	274	3.0	13.0	849	9.3	28.6	3.1
100	67.0	8.93	2.12	262	2.9	12.4	827	9.3	28.0	3.2
88	65.5	9.05	2.07	260	2.9	12.5	1019	11.2	33.0	3.9

for the 7-day period. The negative balances became progressively less as the amounts of non-essential amino acid nitrogen were increased. Approximate nitrogen equilibrium was obtained when the diet provided 40 mg. of non-essential nitrogen. It is interesting to note in this and in later experiments that this diet containing a mixture of amino acids quantitatively equivalent to those present in one day's feed of the reference diet supported equilibrium.

When the non-essential nitrogen of the ration was increased to 50 mg., the rats passed into positive nitrogen balance storing, on the average, 100 mg. of nitrogen in the 7-day period. An increase in the non-essential nitrogen moiety from 50 to 60 mg. per day, however, did not improve the nitrogen retention. The nitrogen balances and nitrogen intakes are highly correlated, i.e., $r = 0.973$. The regression of nitrogen balance on amount of non-essential nitrogen fed per 300 gm. of body weight per 7 days is represented by the equation $Y = 4.96\ x - 193$.

Average body weight of the animals in each group reflected the nitrogen balances. This was especially true of the two extremes. The rats whose diets were supplemented with 10 mg. of non-essential nitrogen lost an average of 8 gm. in the balance period (Table V, Appendix). Losses were averted by the feeding of 20, 30, and 40 mg. of nitrogen from the non-essential amino acid mixture (NEAA-I). Furthermore, the rats gained an average of 5 gm. when 50 or 60 mg. of non-essential nitrogen were provided.

Thus, it appears that under this set of dietary conditions, non-essential amino acids play an important part in establishing the protein nutrition of rats maintained on diets whose nitrogen is derived entirely from pure amino acids. Whether or not the best possible state of nutrition developed was studied by examining data pertaining to the livers (Table 9) is not decided.

The data show that the average weights of the dry, fat-free livers of rats representing the various groups were not different (see individual data also in Appendix, Table VI). However, the total amount of fat in the liver was associated with the amount of cystine provided by the diet. For example, when expressed on the dry basis, 25.4 per cent of the liver was fat when the diet contained 4.0 mg. of cystine (10 mg. non-essential nitrogen) and 33.0 per cent when 24.2 mg. were present (60 mg. of non-essential nitrogen).

It is also interesting that the proportion of fat to nitrogen in the livers increased step-wise as the quantity of dietary cystine increased. Thus dietary manipulation has induced an imbalance between liver components.

However, the concentration of tyrosine also has been systematically increased in the rations. Has it played a role? The problem was studied in the next experiment.

Experiment two. In this experiment, the relative importance of cystine and tyrosine in determining the nutritive value of a mixture of amino acids simulating in kind and quantity the acids present in one day's feed of a 4.27 per cent lactalbumin ration was studied further by the dietary elimination first of cystine and then of tyrosine. In response to the removal of cystine, nitrogen balance dropped from 8 mg. (control group fed Diet 4) to -184 mg./7 days (Table 10). In the original mixture, therefore, cystine appears to serve simultaneously as an important source of organic sulfur and as a sparer of methionine. These results indicate that lactalbumin does not contain enough methionine to meet the requirement for both of the sulfur-containing amino acids (cystine and methionine).

The omission of tyrosine also was reflected in a drop in nitrogen balance but the change was not nearly as severe as when cystine was eliminated.

The average weights of the livers of the rats fed the three experimental diets are shown in Table 10, together with the average nitrogen contents of the organs. It is very interesting to note that the rats administered the control diet (Diet 4) not only maintained nitrogen equilibrium but also had heavier livers of greater nitrogen content than the animals in the groups fed Diets 7 and 8. Thus, the importance of cystine and tyrosine in determining the nutritive value of

Table 10. Average nitrogen retention and average composition of livers of rats fed die
either cystine or tyrosine has been omitted.

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I				Nitrogen fed	Nitrogen balance	Per H_2O
		Total	Modification					
	mg.	mg.			gm.	mg.	mg.	
4	34	40	---	10	358	618	8	6
7	34	40	No cystine N	5	352	624	-184	6
8	34	40	No tyrosine N	4	357	622	- 8	6

f rats fed diets containing nitrogen provided by EAA-I plus NEAA-I from which

Series II
Study A
Experiment Two

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
8	67.7	9.19	2.11	274	3.0	13.0	849	9.3	28.6	3.1
-184	69.4	7.81	1.83	228	2.9	12.5	560	7.2	23.3	2.5
- 8	66.9	8.30	1.90	250	3.0	13.2	845	10.2	30.8	3.4

an intact protein has been demonstrated from another angle.

The respective concentrations of fat in the livers of the three groups indicate that the assortment of amino acids found in lactalbumin may not represent balanced mixtures of amino acids. Livers of the control group fed amino acids simulating lactalbumin contained 28.6 per cent of fat on the dry basis - about 12 per cent more than is found in the hepatic tissues of stock rats described in Series I. In response to the elimination of cystine, the concentration of hepatic fat dropped to 23.3 per cent. When tyrosine was removed from the non-essential amino acid mixture, the concentration of fat in the liver was about 2 per cent more than in the control group (30.8% vs. 28.6%). Possibly tyrosine has some depressing influence on the lipogenic effect of cystine.

Omission of cystine from the diet reduced the ratio of fat to nitrogen in the liver also. When tyrosine was omitted, the ratio remained high. The respective ratios for rats fed the amino acid mixture simulating lactalbumin, the mixture devoid of cystine, and the mixture devoid of tyrosine were 3.1, 2.5, and 3.4, respectively.

Experiment three. In this experiment, the problem of methionine-cystine-phenylalanine-tyrosine relations and dietary requirements thereof was approached from another angle. It was designed to determine the effect on the nutritive value of the amino acid mixture approximating the amino acids in lactalbumin, of the isonitrogenous replacement of the non-essential amino acids, cystine and tyrosine with the related essential amino acids, methionine and phenylalanine, respectively. Again, response was evaluated in terms of nitrogen balance and characteristics of livers.

Three experimental diets were formulated and the results of their administration to rats compared with those obtained when animals were fed the control diet (Diet 4) in which the composition of the amino acid mixture fed approximated the amino acid composition of lactalbumin. In Diet 9, the quantity of nitrogen provided by methionine was equivalent to the total contributed by the methionine and cystine in the control diet (Diet 4). Tyrosine was replaced isonitrogenously by phenylalanine in Diet 10. Finally, in Diet 11, both methionine and phenylalanine were increased at the expense of cystine and tyrosine, respectively.

Rats fed the control diet did not gain weight during the balance test (Table V, Appendix). Also, on the average, the body weights of the rats receiving the diet in which cystine or tyrosine was replaced isonitrogenously by either methionine or phenylalanine remained constant during the 7-day nitrogen metabolism period (Table IX, Appendix). It was interesting, however, to find that the rats administered Diet 11, in which both cystine and tyrosine were replaced isonitrogenously by the appropriate essential amino acids, gained an average of 5 gm. during the balance period.

Results pertaining to the nitrogen metabolism of the control rats and the three experimental groups fed Diets 9, 10, and 11 are presented in Table 11. Analysis of variance showed that the differences in nitrogen balance among the three experimental groups were highly significant ($F_{2,14}$= 8.32). When cystine was replaced with methionine, retention of nitrogen was maintained at the same plane as it was in the control rats, i.e., 5 mg. and 8 mg./7 days, respectively. Tyrosine could not be replaced by phenylalanine as effectively as cystine was by methionine, however. The average nitrogen retention dropped to -19 mg./7 days. When both cystine and tyrosine were replaced isonitrogenously by methionine and phenylalanine, respectively, the average nitrogen balance improved distinctly, the average balance being 70 mg. per rat during the 7-day period. This retention is associated, it may be recalled, with a gain in body weight.

These results offer an example of the importance of the proportionality of amino acids in establishing the nutritive value of a mixture of amino acids, in this case, phenylalanine vs. methionine, or tyrosine vs. cystine. Relations are shown in Table 12.

An increase in the methionine content of the ratio to

Table 11. Average nitrogen retention and average composition of livers of rats fed diet tyrosine being replaced isonitrogenously by methionine and phenylalanine, res

Series
Study
Experiment

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed				No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential nitrogen provided by		Non-essential nitrogen provided by NEAA-I				Nitrogen fed	Nitrogen balance	Per H_2O
	EAA-I	Other sources	Total	Modification					
	mg.	mg.	mg.			gm.	mg.	mg.	
4	34	-	40	---	10	358	618	8	67.
9	34	2 mg. methionine N	38	No cystine	6	369	605	5	68.
10	34	2 mg. phenylalanine N	38	No tyrosine	6	371	619	-19	67.
11	34	2 mg. methionine N 2 mg. phenylalanine N	36	No cystine No tyrosine	5	379	622	70	69.

f rats fed diets containing nitrogen provided by EAA-I plus NEAA-I, cystine and nylalanine, respectively.

Series II
Study A
Experiment Three

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
8	67.7	9.19	2.11	274	3.0	13.0	849	9.3	28.6	3.1
5	68.5	9.57	2.24	302	3.2	13.5	774	8.1	25.7	2.6
-19	67.8	10.08	2.28	304	3.0	13.4	971	9.6	29.8	3.2
70	69.1	9.29	2.24	282	3.0	12.6	618	6.7	21.6	2.2

Table 12. Changes in proportions of sulfur and benzene ring-containing amino acids, nitrogen balance, and concentrations of hepatic fat.

Diet No.	Description of diet[a]	Nitrogen from four amino acids/rat/day[a]					Pa/Me ratio	Nitrogen balance/rat/7 days	Hepatic fat	
		Me	Cys	PA	Ty	Total				
		mg.	mg.	mg.	mg.	mg.		mg.	mg.	%
4	AA-LA (control)	0.9	1.9	1.5	2.1	6.5	1.6	8	849	28.6
9	Cys N replaced by Me N	2.9	--	1.5	2.1	6.5	0.4	5	774	25.7
10	Ty N replaced by PA N	0.9	1.9	3.6	--	6.5	4.0	-19	971	29.8
11	Cys and Ty N replaced by Me and Pa N	2.9	--	3.6	--	6.5	1.2	70	618	21.6

[a] Abbreviations are as follows:

Me:	methionine
Cys:	cystine
PA:	phenylalanine
Ty:	tyrosene
AA:	amino acids
LA:	lactalbumin
N:	nitrogen

the point where the methionine nitrogen to phenylalanine nitrogen ratio shifted from 1.6 to 0.4 was not manifested in any adverse results when balance was the index of evaluation of nutritive value; neither were any beneficial results observed. However, increasing dietary phenylalanine so that the ratio changed from 1.6 to 4.0 was associated with negative balance. Workers at Elvehjem's laboratory have observed that increasing the quantity of phenylalanine in the ration results in an inhibition of growth in young rats (Benton, et al., 1956a).

The total sulfur content of Diets 4, 9, and 10 was the same. Extra phenylalanine introduced an imbalance. However, when the total sulfur of the ration was provided in the form of methionine only as in Diet 11 (phenylalanine nitrogen to methionine nitrogen ratio: 1.2), the same amount of phenylalanine was no longer antagonistic. Thus, it seems that in these low nitrogen rations equivalent to 4 per cent of protein that the imbalance induced by excess phenylalanine is related to the presence of cystine. Also, a comparison of the balances, 8 mg. vs. 70 mg. per 7 days is an index of the degree to which cystine and tyrosine may reduce the nutritive efficiency of the amino acid mixture. It seems, therefore, that the nutritive value of lactalbumin is limited by its methionine and phenylalanine contents. The related non-essential acids in the intact protein, however, have some protective action, seemingly sparing the essential amino acids for metabolic purposes other than for their own synthesis.

The imbalances as judged by nitrogen retention are mirrored in concentrations of hepatic fat also (Tables 11 and 12). The average values of the total hepatic fat in the three experimental groups varied from 618 mg. to 971 mg; the percentages from 21.6 per cent to 29.8 per cent on the dry basis. Differences in percentages of hepatic fat were highly significant ($F_{2,14}$= 82.50).

The substitution of cystine nitrogen by methionine nitrogen (Diet 9) reduced the total fat in the liver from 849 to 774 mg. On the percentage basis expressed in terms of dry weight, the concentration dropped from 28.6 per cent to 25.7 per cent. Dietary phenylalanine added at the expense of tyrosine in the presence of cystine (Diet 10) maintained liver fat at a somewhat higher concentration than that characteristic of the livers of the control rats, apparently increasing the lipogenic influence of cystine. But when both non-essentials were replaced by the related essential acids, (Diet 11) the mixture was lipogenic no longer, total fat dropping to 618 mg. and percentage-wise on the dry basis to 21.6 per cent. Thus in respect to hepatic fat also, excess phenylalanine in the presence of cystine creates an imbalance but is beneficial when cystine is replaced by methionine.

The roles of the two non-essential acids in controlling hepatic fat might be evaluated as follows: Cystine is lipogenic. Removal of tyrosine with or without its isonitrogenous replacement with phenylalanine results in increased fat. So, it appears that tyrosine exerts a lipotropic action of mild degree when the diet contains cystine combined with small amounts of methionine.

The nitrogen present in the livers of the animals in the three experimental groups was about the same. However, the hepatic fat to hepatic nitrogen rations, 2.6, 3.2, and 2.2, indicated a definite improvement in the integrity of the tissue was induced by increasing the methionine and phenylalanine values of the ration at the expense of cystine and tyrosine.

On the basis of the present observations, methionine appears to play the major role and phenylalanine the secondary in stimulating nitrogen retention and controlling liver fat. Both methionine and phenylalanine are necessary for maximum effects, however. This is a clear example of the importance of evaluating the need for one amino acid in relation to other amino acids present.

Experiment four. The experiment just recorded demonstrates that the nutritive efficiency of the amino acid mixture simulating the amino acids present in one day's feed of a diet containing the reference protein, lactalbumin, can be improved by alterations in its methionine, cystine, phenylalanine, and tyrosine contents within the total nitrogen framework of the original ration (74 mg. per day).

Womack, et al. (1953) has noted that the total intake of nitrogen is important in relation to the utilization of amino acid mixture. It seemed of interest, therefore, to determine the minimal amount of non-essential nitrogen required to establish nitrogen equilibrium when the apparently more efficient mixture of essential amino acids (EAA-II) developed in Experiment three was fed instead of the mixture EAA-I resembling lactalbumin. It may be recalled that 38 mg. of nitrogen from the new mixture of essential amino acids (EAA-II) when fed in conjunction with 36 mg. of non-essential nitrogen induced storage of nitrogen (70 mg.).

In this experiment, Diets 12, 12A, 12B, 12C, 12D, and 12E were fed, the essential amino acid mixture (EAA-II) being supplemented, respectively, with 10, 20, 30, 40, 50, and 60 mg. of nitrogen per day as provided by a mixture of non-essential amino acids containing no cystine and tyrosine (NEAA-II). Measurements, again, were made in terms of nitrogen balance and liver composition.

Data depicting the composition of the livers of rats fed these various rations will be considered first. They are presented in Table 13. The picture is very different

Table 13. Average nitrogen retention and average composition of livers of rats fed di
of nitrogen from NEAA-II.

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II			Nitrogen fed	Nitrogen balance	Pe H_2
	mg.	mg.		gm.	mg.	mg.	
12	38	10	6	350	425	-34	
12A	38	20	6	345	471	-30	
12B	38	30	6	355	529	0	
12C	38	40	6	351	584	6	
12D	38	50	6	355	617	-18	
12E	38	60	6	351	654	-20	

f rats fed diets containing nitrogen provided by EAA-II supplemented with graded doses

Series II
Study A
Experiment Four

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-34	69.9	8.34	2.12	264	3.2	12.6	395	4.8	15.8	1.5
-30	70.2	8.18	2.04	270	3.3	13.3	397	4.8	16.3	1.5
0	69.4	8.24	2.08	278	3.4	13.4	431	5.2	17.1	1.6
6	69.7	7.73	1.92	265	3.4	13.8	420	5.4	17.8	1.6
-18	69.5	7.82	1.96	265	3.4	13.5	427	5.5	17.9	1.6
-20	69.1	7.70	1.91	260	3.4	13.6	470	6.1	19.7	1.8

than the one obtained when EAA-I served as the source of the essential amino acids.

Feeding of the various rations in which the cystine-tyrosine free mixture of non-essential amino acids was used in conjunction with the methionine-phenylalanine enriched mixture of essential amino acids, in general, resulted in livers of normal water and fat content, no matter at what level the mixture of non-essential amino acids were fed. The data from any group in this test series compared very favorably with those obtained in a study of 50 stock rats whose livers, on the average, contained 69.3 per cent of moisture and 17.2 per cent of fat on the dry basis.

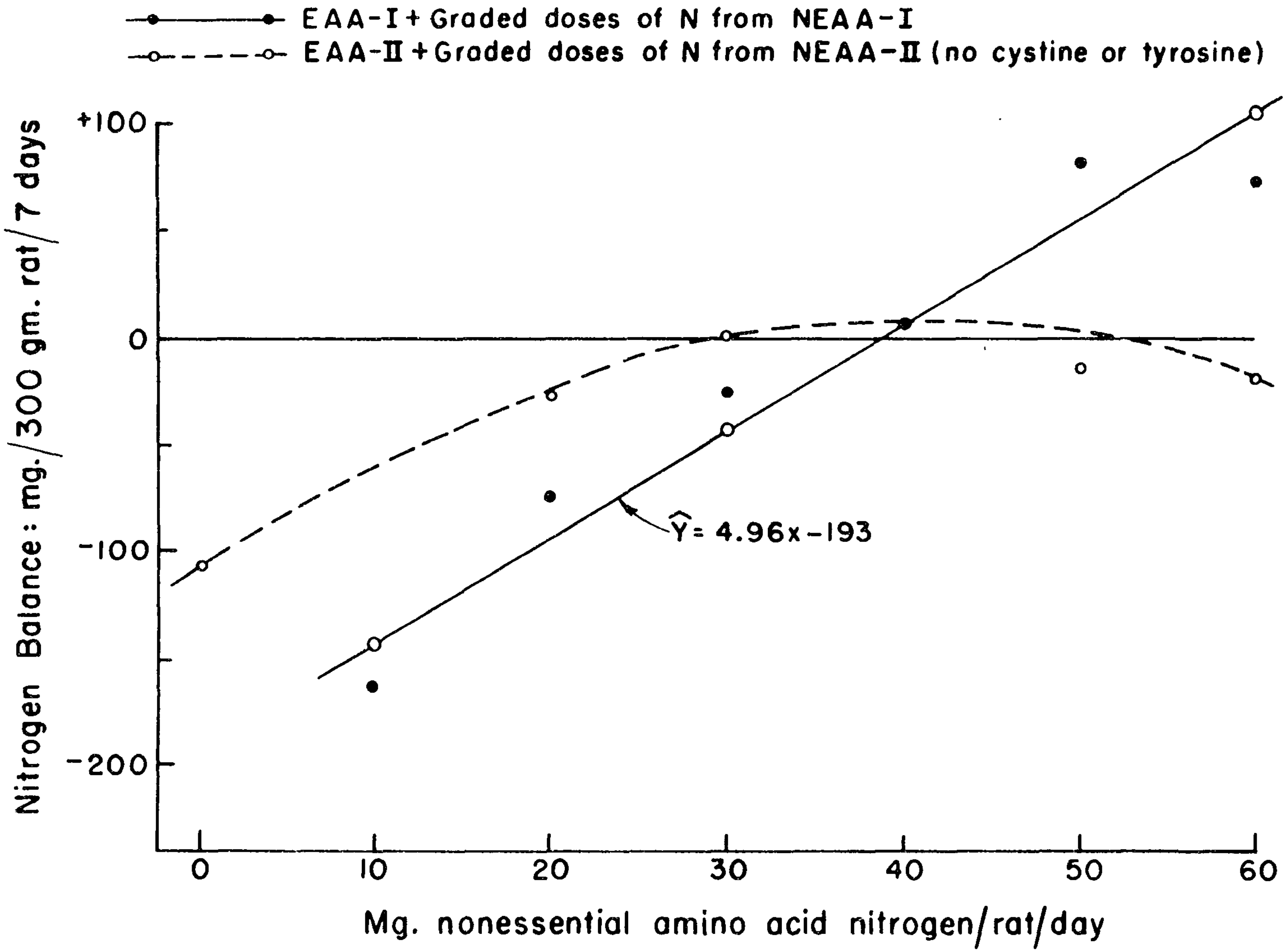
These data are in contrast to those obtained when the amino acid mixture contained graded doses of cystine and tyrosine. In this experiment (Table 9), there was a lower than normal water content and a higher than normal concentration of fat in the liver with each increment in the amount of non-essential amino acid nitrogen fed. Data describing the livers of rats in each of the two groups when the non-essential mixture of amino acids provided 40 mg. of nitrogen will illustrate the difference. At this level of feeding, rats receiving no cystine and tyrosine had livers with 69.7 per cent moisture, 420 mg. of total fat, and 17.8 per cent of fat on the dry basis; those receiving these acids had livers with the following values: 67.3 per cent, 849 mg., and 28.6 per cent, respectively. It is interesting that at this level of feeding Diets 12C and 4 contained the same amounts of the sulfur-containing acids and of the acids carrying the benzene ring. These data lend weight to the observations on the interactions between methionine, phenylalanine, cystine, and tyrosine on the production of fat in the liver.

There is another item of interest in Table 13. The feeding of the largest amount of nitrogen from non-essential amino acids, i.e., 60 mg. per day, tended to produce an increase in liver fat, increasing it from about 430 mg. to 470 mg. Does this mean that an excess of non-essential amino acid nitrogen may be lipogenic even when no cystine and tyrosine are present?

The average quantities and average percentage of nitrogen in the livers were essentially the same, no matter at what level the mixture of non-essential amino acids were fed. No differences among the average ratios of fat to nitrogen in the livers of groups 12-12D, i.e., 1.5-1.6 occurred. The respective ratio for the rats fed Diet 12E (60 mg. of nitrogen from non-essential amino acids) where hepatic fat was highest was relatively large (1.8).

The nitrogen balances secured in the present experiment with the feeding of graded doses of non-essential amino acid nitrogen free of cystine and tyrosine also were very different from those obtained in Experiment one. Average values for nitrogen retentions of both groups are plotted against the quantities of non-essential nitrogen provided in Figure 1. In the case of the rats fed EAA-I simulating the essential amino acids in one day's quota of the 4.27 per cent lactalbumin diet, a linear relationship existed between nitrogen balances per 300 gm. rat and the amounts of nitrogen provided by the non-essential amino acid mixture, the regression being $Y = 4.96 X - 193$. The correlation coefficient is 0.973. Maximum retention was reached when 50 to 60 mg. of non-essential amino acid nitrogen was administered. In contrast, note the curve when the modified essential amino acid mixture (EAA-II) was supplemented with graded doses of nitrogen provided by a mixture of non-essential amino acids lacking cystine and tyrosine (NEAA-II). It shows that retention improved with each increment of non-essential nitrogen until 40 mg. was provided daily. Up to this point, retention in every instance exceeded that occurring when the essential amino acids were furnished in the form of the mixture resembling lactalbumin. Beyond this point, however, no further increases in retention occurred as the non-essential nitrogen was increased. It should be noted that when 40 mg. of non-essential nitrogen were provided by either mixture of non-essential amino acids, i.e., NEAA-I or NEAA-II, the nitrogen arising from methionine and phenylalanine in

Figure 1. Nitrogen balances at graded dose of non-essential nitrogen fed in conjunction with two different mixtures of essential amino acids.


EAA-I + Graded doses of N from NEAA-I
EAA-II + Graded doses of N from NEAA-II (no cystine or tyrosine)
Nitrogen Balance: mg./300 gm. rat/7 days
+100
0
-100
-200
Ŷ = 4.96x - 193
0
10
20
30
40
50
60
Mg. nonessential amino acid nitrogen/rat/day

mixture EAA-II was exactly the same, 6.5 mg., as that originating from methionine, phenylalanine, cystine, and tyrosine in the original mixture of EAA-I plus NEAA-I. When mixture EAA-I plus NEAA-I provided more than 40 mg. of nitrogen daily, the amount of cystine and tyrosine increased correspondingly. This did not occur when mixture EAA-II plus NEAA-II was fed, its contribution of sulfur-containing amino acids and phenylalanine having been established at a constant value in the nitrogenous portion of the essential amino acid mixture. It seems, therefore, that the superiority of the first amino acid mixture at the higher levels of the non-essential amino acid feeding might be explained by the action of the increased quantities of cystine and tyrosine provided.

To study this possibility, the influence of a further increase in the methionine and phenylalanine contents of the essential amino mixture eas evaluated in the following experiment.

Experiment five. A new essential amino acid mixture was formulated containing additional methionine and phenylalanine and was designated as EAA-III. It provided a total of 8 mg. of nitrogen from methionine and phenylalanine, which was the amount of nitrogen provided by the four amino acids, methionine, cystine, phenylalanine, and tyrosine when maximum retention of nitrogen was observed, i.e., when

55 mg. of non-essential nitrogen provided by EAA-I was fed in conjunction with 34 mg. of nitrogen from EAA-I (Figure 1).

Four diets were fed in the present experiment. In Diets 13, 13A, 13B, and 13C, 39.5 mg. of essential amino acid nitrogen provided by mixture EAA-III was supplemented with 10, 34.5, 45, and 60 mg. of non-essential nitrogen from a mixture of non-essential amino acids containing no cystine or tyrosine (EAA-II).

Results of feeding these rations are shown in Table 14 and Figure 2. The curves in Figure 2 depict the respective responses of rats, measured in terms of nitrogen balance, when the three different mixtures of essential amino acids under study were fed with graded doses of the non-essential amino acids. The upper curve refers to results obtained by feeding the mixture EAA-III containing increased quantities of methionine and phenylalanine in conjunction with graded doses of non-essential nitrogen (NEAA-II). Again, as was the case following the feeding of the mixture EAA-I as indicated by the lower curve, nitrogen balance appeared to be related in a linear fashion to the amount of non-essential amino acid administered. The equation for the regression of nitrogen balance per 300 gm. of body weight per 7 days on mg. of non-essential nitrogen intake is $Y = 3.183 - 72$. The correlation coefficient is 0.985.

The new mixture of essential amino acids (III) was definitely superior to either of the other two mixtures

Table 14. Average nitrogen retention and average composition of livers of rats fed di
doses of nitrogen from NEAA-II.

Serie
Stud
Experim

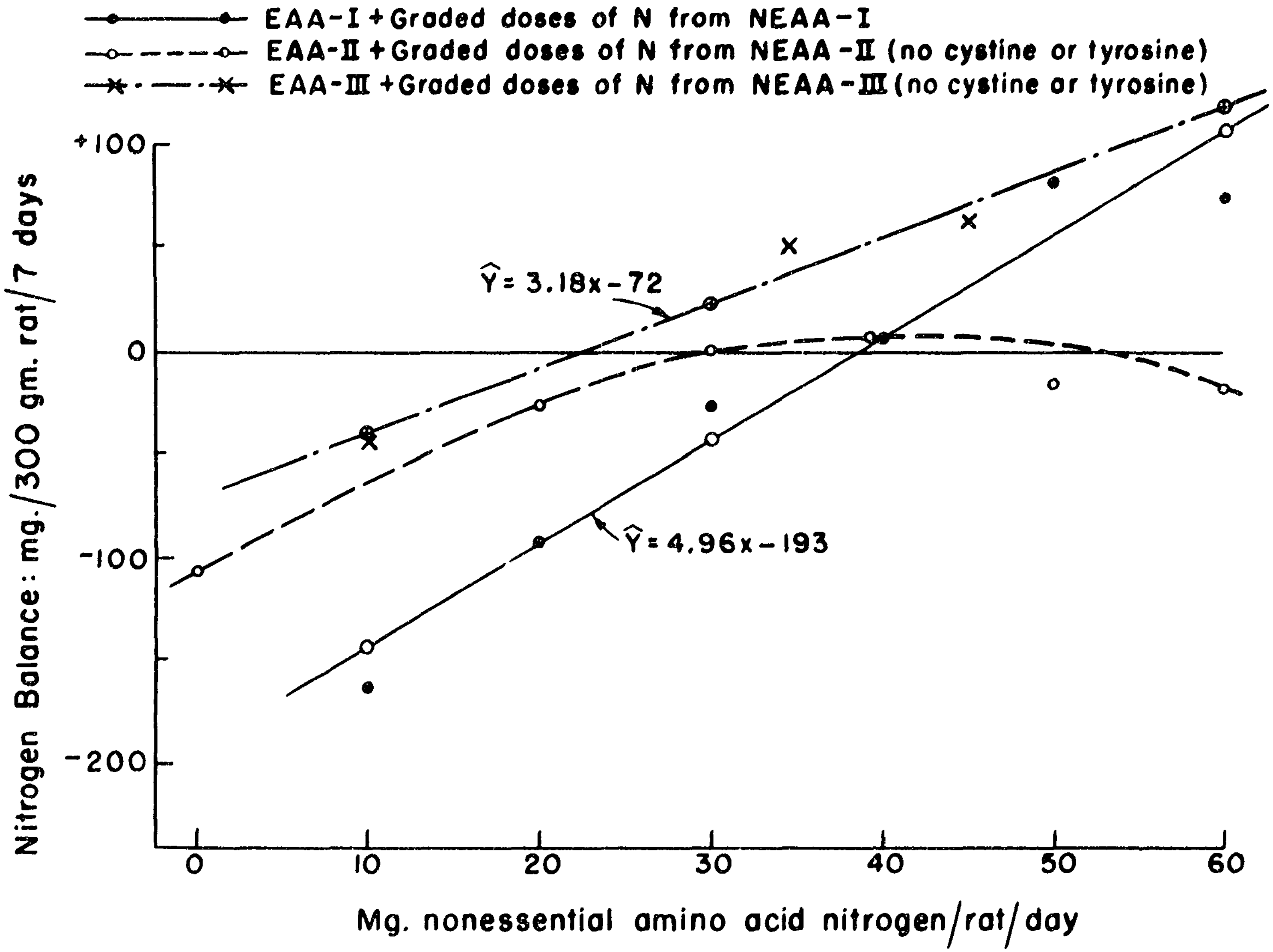
Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential amino acid nitrogen provided by EAA-III	Non-essential nitrogen provided by NEAA-II			Nitrogen fed	Nitrogen balance	Pe: H_2
	mg.	mg.		gm.	mg.	mg.	
13	39.5	10	6	336	415	-49	
13A	39.5	34.5	12	342	586	56	
13B	39.5	45	6	346	660	71	
13C	39.5	45	5	341	810	135	

f rats fed diets containing nitrogen provided by EAA-III supplemented with graded

Series II
Study A
Experiment Five

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-49	69.8	8.00	1.82	259	3.2	14.3	590	7.4	24.6	2.3
56	69.6	8.17	1.94	270	3.3	14.0	550	6.7	22.1	2.0
71	69.0	8.03	1.89	263	3.3	13.9	596	7.4	23.9	2.3
135	68.5	7.77	1.83	261	3.4	14.3	620	8.0	25.3	2.4

Figure 2. Nitrogen balances at graded doses of nonessential nitrogen fed in conjunction with three different mixtures of essential amino acids.

EAA-I + Graded doses of N from NEAA-I
EAA-II + Graded doses of N from NEAA-II (no cystine or tyrosine)
EAA-III + Graded doses of N from NEAA-III (no cystine or tyrosine)
+100
0
-100
-200
Nitrogen Balance: mg./300 gm. rat/7 days
$\hat{Y} = 3.18x - 72$
$\hat{Y} = 4.96x - 193$
0
10
20
30
40
50
60
Mg. nonessential amino acid nitrogen/rat/day

formulated. With EAA-III, equilibrium was achieved when supplemented with 22.5 mg. of nitrogen from non-essential acids; with EAA-II, equilibrium was attained with 30 mg. of non-essential nitrogen, with EAA-I, with 40 mg. In other words, the total amounts of nitrogen required per day for the establishment of equilibrium were 62 mg., 68 mg., and 74 mg., respectively. At this point, mixtures of essential and non-essential acids, I, II, and III provided 8 mg., 6.5 mg., and 6.5., respectively, of nitrogen from acids carrying sulfur or the benzene ring. That the nutritive efficiency of an amino acid mixture can be enriched by increasing the quota of these acids to 8 mg./day in the form of methionine and phenylalanine is illustrated in the upper and lower curves. These curves suggest that the greatest retention can be supported by increasing nitrogen from these sources to 8 mg. within a total framework of 95 to 100 mg. of nitrogen daily.

Some very interesting data were accumulated concerning the deposition of the hepatic fat when the three different amino acid mixtures were fed (see Table 15).

Mixture Number 3 had lipotropic properties when compared to mixture Number 1 but was not as effective in this respect as mixture Number 2. These differences are highly significant ($F_{2,19}$= 21.12 in terms of actual quantities and $F_{2,19}$= 25.01 on a relative dry basis).

The effect may be attributed to the 1.5 mg. of nitrogen derived from the addition of extra methionine and phenylalanine, since non-essential amino acid nitrogen fed daily at the level of 45 mg. with EAA-II (Table 14) did not stimulate the deposition of hepatic fat. Furthermore, hepatic fat was maintained at approximately the same concentration at any level of non-essential amino acid nitrogen feeding with EAA-III (Table 14). Why mixture EAA-III takes

Table 15. Variations in lipotropic properties of three mixtures of amino acids.

Mixture No.	Amino acid mixture fed		Total N	Hepatic fat	
	Essential acids	Non-essential acids		Total	Relative dry basis
			mg.	mg.	%
1	EAA-I (34 mg. N)	NEAA-I (40 mg. N)	74	831	28.8
2	EAA-II (38 mg. N)	NEAA-II (40 mg. N)	78	420	17.8
3	EAA-III (39.5 mg. N)	NEAA-II (34.5 mg. N)	74	550	22.1

on some lipogenic properties needs further investigation.

Study B. Single vs. a mixture of non-essential acids

In Study B, the respective capacities of cystine and tyrosine to spare methionine and phenylalanine was demonstrated from another angle. The experiment was planned initially to determine whether or not a single non-essential amino acid could be substituted for a mixture of acids without influencing the retention of nitrogen.

In the first phase of the test, 40 mg. of nitrogen from glutamic acid was fed in conjunction with 34 mg. of nitrogen from EAA-I (Diet 14) and results compared with those obtained by feeding the original mixture (Diet 4).

The inability of glutamic acid to replace the other non-essential amino acids occurring in lactalbumin as a source of dietary non-essential nitrogen in the supplementation of the essential amino acids simulating those present in the reference protein (EAA-I) was determined. All the animals receiving Diet 14 lost weight, losses ranging from 9 to 29 mg. during the experimental period (Table XV, Appendix). These losses were reflected in the nitrogen retentions.

In contrast to the average balance of 8 mg./7 days for the group receiving the control ration (Diet 4), the average nitrogen balance for the glutamic acid-fed rats was -253 mg. per 7 days with a range in values from -306 to -194 mg. (Table 16). The values for the individual rats are presented

Table 16. Average nitrogen retention and composition of livers of rats fed diets cont
from a mixture of non-essential amino acids or from glutamic acid alone.

Serie
Stud

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed				No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Essential nitrogen		Non-essential nitrogen				Nitrogen fed	Nitrogen balance	Pe H_2
	Quantity	Source	Quantity	Source					
	mg.		mg.			gm.	mg.	mg.	
4	34	EAA-I	40	NEAA-I	10	358	618	8	6
14	34	EAA-I	40	Glutamic acid	6	333	578	-253	6
15	38	EAA-II	36	Glutamic acid	6	345	576	- 5	6
12C	38	EAA-II	40	NEAA-II	6	351	584	5	6

fed diets containing nitrogen provided by EAA-I or EAA-II supplemented with nitrogen
acid alone.

Series II
Study B

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
8	67.7	9.19	2.11	274	3.0	13.0	849	9.3	28.6	3.1
-253	69.1	7.97	2.04	241	3.0	12.0	434	5.4	17.7	1.8
- 5	69.0	8.71	2.30	265	3.0	11.6	407	4.6	15.0	1.5
5	69.7	7.73	1.92	265	3.4	13.8	420	5.4	17.8	1.6

in Table XV, Appendix. The average nitrogen balance of the animals receiving the diet containing EAA-I and 40 mg. of nitrogen from glutamic acid was even more negative than the one induced by administering the diet providing EAA-I and only 10 mg. of non-essential nitrogen from a mixture of several non-essential amino acids; the average nitrogen balance of this group (Diet 6 in the previous study) was -187 mg./7 days.

The immediate reaction to these results was one of surprise, but then it was recalled that a prior experiment (as reported herein) had demonstrated that cystine and tyrosine played a very important role in establishing the nutritive value of a mixture of amino acids simulating the amino acids found in lactalbumin. Therefore, it was decided to determine whether or not glutamic acid would behave differently when it was fed as a sole source of non-essential nitrogen in combination with EAA-II which carried additional methionine and phenylalanine in amounts isonitrogenously equivalent to the cystine and tyrosine present in one day's feed of the 4.27 per cent lactalbumin diet.

The rats responded very well to the feeding of Diet 15. On the average, body weight was maintained (±2 gm.) in the experimental period (see Table XV, Appendix). The average nitrogen was -5 mg. per 7 days, values ranging from -47 to 21 mg. (Table 16 and Table XV, Appendix). The control group receiving the mixture of several non-essential amino acids (Diet 12C) responded nearly the same (5 vs. -5 mg.).

Thus, it seems that the unfavorable outcome of the feeding of Diet 14 might be explained on the relatively low amounts of methionine, cystine, phenylalanine, and tyrosine present in the test mixture. Furthermore, it is also evident that the glutamic acid can replace all other non-essential amino acids except cystine and tyrosine. These results also lend support to the idea that the original mixture of essential amino acids was inadequate in respect to methionine and phenylalanine and that cystine and tyrosine had been acting in sparing capacities.

The data pertaining to the characteristics of the livers of the rats in the present study are shown in Table 15. On the fresh basis, the average weights of the organs of rats in Groups 4, 12C, 14, and 15 varied from 7.73 to 9.13 gm.; on the dry, fat-free basis, from 1.92 to 2.30 gm. The total quantity of hepatic nitrogen of the rats ingesting Diet 14, however, was less than that in other groups, an observation supporting those pertaining to losses in nitrogen from body tissue.

The concentrations of fat in the livers again show the lipogenic property of cystine. In response to the removal of cystine, total and relative quantities of fat in the livers decreased significantly (Diets 12C, 14, and 15).

It may be noteworthy that in this experiment a severe negative nitrogen retention was associated with what could be considered a normal amount of hepatic fat.

Study C. Efficacy of essential amino acids only

Data obtained in the studies described in the previous sections demonstrated that the role played by non-essential nitrogen in the determination of the nutritive value of mixtures of amino acids depended upon the composition of the mixture of essential acids with which the non-essential nitrogen was fed. This was true regardless of whether a mixture or a single amino acid provided the non-essential nitrogen. In view of the commonly accepted idea that mixtures of essential amino acids must be fortified with some sources of non-essential nitrogen for the highest biological efficiency (Rose, et al., 1948), it was decided, in the words of Clark (1950), to test the "essentiality of the non-essential amino acids" with various mixtures of the essential members. In other words, in this experiment the efficacy of several mixtures containing only essential amino acids for the support of protein nutrition was determined. Mixtures already described that represented three degrees of nutritional efficiency were used.

Experiment one. Six experimental diets containing three different mixtures of essential amino acids were fed. These mixtures varied in their nutritional efficiency, this being determined as shown in previous sections by their methionine-cystine-phenylalanine-tyrosine contents. Diet 16 was so formulated that it contained a mixture of 10 essential amino acids (34 mg. of nitrogen) simulating those present in one day's feed of the reference diet (EAA-I). This was the least efficient mixture. In Diet 16A, the amino acids present in EAA-I were increased proportionally to provide 74 mg. of essential nitrogen in one day's feed. In Diets 17 and 17A, respectively, 38 and 74 mg. of essential nitrogen from EAA-II were provided. In Diets 18 and 18A, respectively, 39.5 and 74 mg. of essential nitrogen were furnished from EAA-III, the mixture believed to be the most efficient of the three for the support of nitrogen retention. Measurements were made in terms of nitrogen balance and liver composition.

Average nitrogen balances for the six experimental groups are shown in Table 17, and data pertaining to the individual rats are presented in Table XVii, in the Appendix.

The data show that the rats given only the 10 essential amino acids in a mixture modeled after the essential acids provided by lactalbumin in a 4 per cent protein ration (Diet 16) dropped into a severe negative nitrogen balance. On the average, they lost 252 mg. of tissue nitrogen in the 7-day period. This is in harmony with weight losses observed (see Table XVII, Appendix), i.e., an average of 5 gm. of body

Table 17. Average nitrogen retention and average composition of livers of rats fed of varying nutritive value being fed.

Ser
St
Exper

Diet	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed		No. of rats	Body weight	Nitrogen metabolism per 7 days		
	Source	Quantity			Nitrogen fed	Nitrogen balance	P H
		mg.		gm.	mg.	mg.	
16	EAA-I only	34	6	344	349	-252	
16A	EAA-I only	74	5	369	691	- 87	
17	EAA-II only	38	6	341	319	-151	
17A	EAA-II only	74	6	349	602	22	
18	EAA-III only	39.5	6	350	359	-158	
18A	EAA-III only	74	5	365	609	42	

of rats fed diets containing essential amino acid nitrogen only, three mixtures

Series II
Study C
Experiment One

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-252	68.6	8.29	1.93	245	3.0	12.7	678	8.2	26.0	2.8
- 87	69.0	8.80	2.12	281	3.2	13.2	603	6.9	22.2	2.1
-151	68.6	8.11	1.91	267	3.3	14.0	639	7.9	25.2	2.4
22	67.2	8.01	1.97	271	3.4	13.7	651	8.1	24.8	2.4
-158	69.6	8.17	1.97	267	3.3	13.6	517	6.3	20.8	1.9
42	69.3	8.35	1.97	283	3.4	14.5	598	7.2	23.4	2.1

weight during the nitrogen metabolism period. Thus, unsupplemented with non-essential acids, essential acids providing 34 mg. of total nitrogen are inadequate for the support of protein nutrition. There is not enough nitrogen present to meet the needs for these specific acids nor to allow for their transformation into the non-essential acids metabolically required for functioning of the body.

It is interesting to note that increasing the quantity of EAA-I to provide 74 mg. of essential nitrogen equivalent to the total nitrogen present in one day's feed of the reference diet decreased but did not avert the tissue catabolism. The average nitrogen balance of the group fed Diet 16A shifted to -87 mg. All of the animals in this group maintained their body weight (±2 gm.) during the nitrogen metabolism period. However, the animals did not utilize the dietary supply of nitrogen from the essential amino acids (74 mg./day) as effectively as they did when the ration contained 74 mg. of nitrogen arising from both essential and non-essential amino acids. It may be recalled that the feeding of 34 mg. of essential nitrogen from EAA-I is in conjunction with 40 mg. of non-essential nitrogen from NEAA-I supported nitrogen equilibrium successfully (Diet 4, balance being 8 mg./7 days).

When the rats were fed Diets 17 and 18 containing 38 mg. of essential nitrogen from EAA-II and 39.5 mg. from EAA-III, respectively, the average nitrogen balances were -151 and -158. These values were markedly less than the figure representing the catabolism when 34 mg. of nitrogen from EAA-I was fed. It should be noted that EAA-II and EAA-III are considerably richer in methionine and phenylalanine than EAA-I.

The metabolism test indicated that rats were in positive balance when fed diets containing 74 mg. of essential nitrogen either from EAA-II (Diet 17A) or from EAA-III (Diet 18A). When EAA-I was offered in this quantity (74 mg. daily), the balance remained negative. On the average, group 17A stored about 22 mg. of nitrogen and group 18A 42 mg. during the 7-day metabolism period.

Thus, essential amino acids fed without supplementation with non-essential nitrogen may support nitrogen retention when fed at a level providing the same amount of nitrogen per day as may be derived from a 4.27 per cent lactalbumin diet, only when the quantities and proportions of certain essential amino acids contained therein are adequate. Methionine and phenylalanine appear to be the controlling acids (Table 18). Also, this experiment emphasizes again the important role that cystine and tyrosine play in determining the nutritive value of the intact lactalbumin protein.

The data pertaining to the characteristics of livers of the rats are shown in Table 17. Values obtained from individual animals are presented in Table XVIII, Appendix. The average weights of the livers of the rats in the six experimental groups did not appear to differ either on the

Table 18. Role of methionine and phenylalanine in relation of nitrogen retention when different mixtures of essential amino acids providing 74 mg. of nitrogen are fed.

Sources of nitrogen	Diet 4: Control diet EAA-I plus NEAA-I 74 mg.N	Diet 16A: EAA-I 74 mg.N	Diet 17A: EAA-II 74 mg.N	Diet 18A: EAA-III 74 mg.N
	mg.	mg.	mg.	mg.
N from acids containing sulfur	2.8	2.0	5.6	6.6
N from acids containing benzene ring	3.7	3.3	7.0	8.4
Total N from both source	6.5	5.3	12.6	15.0
N balance/ 300 gm. rat/ 7 days	7	-70	19	35

of the rats are shown in Table 17. Values obtained from individual animals are presented in Table XVIII, Appendix.

The average weights of the livers of the rats in the six experimental groups did not appear to differ either on the fresh or on the dry, fat-free basis. The organ in only one group showed a moisture content of less than 69%. The average quantities of nitrogen in the livers were essentially the same except in the case of the livers obtained from rats fed Diet 16. The average value for this group was 245 mg. as against 267 to 283 mg. for the other groups. Less nitrogen was present also on the relative basis. This is the group that was in the most acute negative balance and it seems that only when catabolism is of severe order that any significant decrement occurs in concentration of hepatic nitrogen.

Increasing the quantity of EAA-I from 34 mg. of essential nitrogen (Diet 16) to 74 mg. (Diet 16a) daily caused an immediate drop in the concentration of fat in the livers. The respective values were 26.0 per cent and 22.2 per cent on the dry basis. This drop may reflect the increased amounts of methionine and phenylalanine provided by ration 16a.

The feeding of mixture EAA-III, again resulted in some increase in concentration of fat in livers. For example, increasing the quantity of essential nitrogen from 39.5 mg. (Diet 18) to 74 mg. daily (Diet 18a) enhanced the accumulation of fat; the respective percentages of fat were 20.8 and 23.4 on the dry basis. Total fat contents varied from 517 to 678 mg. Analysis of variance for groups 16, 16a, 18, and

18a indicated that the difference in values relating to Diet 16 and Diet 18 was significantly different at the 1 per cent level (t = 21.85).

Possibly the detrimental effect of too much methionine and phenylalanine may be illustrated in the group fed Diet 18a containing 74 mg. of nitrogen. The hepatic fat in this group is higher than that of group 18, but the difference is significant only at the 5 per cent level.

It should be noted that Diets 17 and 17a contained 0.2 per cent L-threonine as in all other diets. Therefore, discussion of the concentration of fat present in the livers of rats fed these two diets (Diet 17 and 17a) will be deferred to a later section.

Experiment two. Data described in the previous section demonstrated that the ability of the essential amino acids to support a favorable nitrogen balance when fed at a level providing the same amount of total nitrogen per day as may be derived from one day's food quota of the intact protein diet depended on the composition of the mixture in respect to methionine and phenylalanine. The present experiment was planned to determine the specific quantity of a mixture containing the essential acids only needed for the maintenance of nitrogen equilibrium. Essential amino acid mixture III (EAA-III) was chosen for this test because it had been demonstrated that retention of considerable degree occurred when it was fed at a level approximately the total nitrogen provided per day by a 4.27 per cent lactalbumin diet. It seemed to owe its superiority to its relatively high content of methionine and phenylalanine.

The mixture was fed in quantities providing graded doses of essential nitrogen. Diets 18, 18A, 18B, 18C, and 18D provided 39.5, 50, 60, 74, and 80 mg. of essential nitrogen, respectively. Nitrogen retention and liver composition were used as the criteria for the evaluation of the results.

It is interesting to note that the average body weight of the animals in each experimental group reflected the nitrogen intake. The average change in body weight of the rats in the five experimental groups, 18, 18A, 18B, 18C, and 18D were -4, -2, 0, 8, and 8 gm., respectively, during the experiment.

Data pertaining to the nitrogen metabolism of the five groups are presented in Table 19 and Figure 3. A linear relationship existed between nitrogen balance and quantity of essential nitrogen fed. The equation for the regression of nitrogen balance per 300 gm. of body weight per 7 days on intake of essential nitrogen is $Y = 5.08 - 340$. The correlation coefficient is 0.981. Nitrogen equilibrium was obtained when the diet provided approximately 67 mg. of essential nitrogen (also equal to the daily total dietary nitrogen).

Table 19. Average nitrogen retention and average composition of livers of rats fed die amino acids (EAA-III).

Series
Study
Experimen

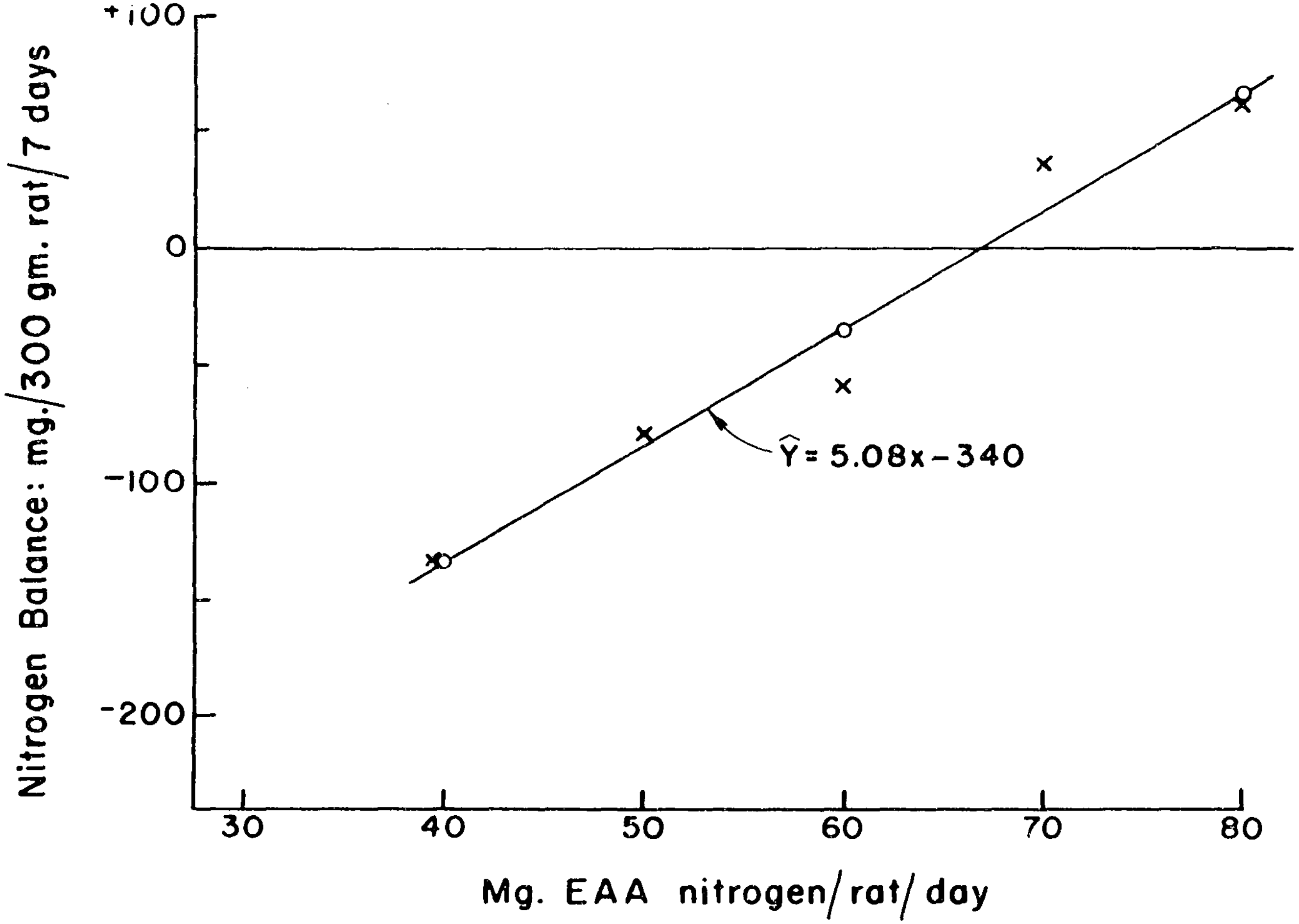
Diet No.	Quantity of nitrogen in daily dose of EAA-III	No. of rats	Body weight	Nitrogen metabolism per 7 days		
				Nitrogen fed	Nitrogen balance	Per H_2O
	mg.		gm.	mg.	mg.	
18	39.5	6	350	359	-158	6
18A	50	6	352	455	- 95	6
18B	60	6	354	530	- 71	6
18C	74	5	365	609	42	6
18D	80	5	361	680	75	6

of rats fed diets containing graded doses of nitrogen provided by essential

Series II
Study C
Experiment Two

n meta- r 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-158	69.6	8.17	1.97	267	3.3	13.6	517	6.3	20.8	1.9
- 95	69.6	7.95	1.82	266	3.4	14.7	582	7.4	24.3	2.2
- 71	69.2	8.20	1.94	270	3.3	14.1	595	7.2	23.6	2.2
42	69.3	8.35	1.97	283	3.4	14.5	598	7.2	23.4	2.1
75	69.0	8.92	2.17	290	3.2	13.4	597	6.9	22.3	2.1

Figure 3. Regression of nitrogen balance on milligrams of nitrogen per day administered in the form of essential amino acids.


+100
0
-100
-200
30
40
50
60
70
80
Nitrogen Balance: mg./300 gm. rat/7 days
Mg. EAA nitrogen/rat/day
$\hat{Y} = 5.08x - 340$

It is interesting to compare this value with the data secured in Experiment five of Study A (Figure 2) in which the total nitrogen content of the diet was 62 mg. per day. Only 39.5 mg. of essential nitrogen from EAA-III was needed for the support of nitrogen equilibrium in this case when the essential acids were supplemented with 22.5 mg. of non-essential nitrogen.

With essential amino acids only present, a total of 67 mg. of nitrogen was needed; with the mixture of essential and non-essential acids, 62 mg. It appears, therefore, that there is some physiological cost in the metabolic conversion of essential acids to the equally important "non-essential" amino acids required in metabolism.

Thus, the present experiment has demonstrated that nitrogen balance may be achieved when the only source of dietary nitrogen is from essential amino acids, providing the mixture contains adequate amounts of certain amino acids, i.e., methionine and phenylalanine in balanced proportions. This can occur when the amount of nitrogen present is equivalent to the minimal quantity of nitrogen from a high quality protein needed for nitrogen equilibrium. Insofar as the author knows, nitrogen equilibrium with essential amino acids only has been demonstrated heretofore at this level of nitrogen intake.

The average water contents of the livers of rats in the five experimental groups were essentially the same. Only in one group (18D) did the organ seem to be unduly large. This is the group that received the largest amount of essential amino acids in the ration and that also exhibited the highest retention of nitrogen. The total amount of nitrogen in the livers appears to be associated with the quantity of the essential nitrogen provided by the diet. Values were essentially the same until the dietary nitrogen approached the value for total nitrogen needed for equilibrium, thereafter they increased in a linear fashion with each increment of essential acid fed (Figure 4). It is interesting to speculate that these increments in hepatic nitrogen indicate that the essential amino acids are being stock-piled in the liver for conversion into non-essential amino acids.

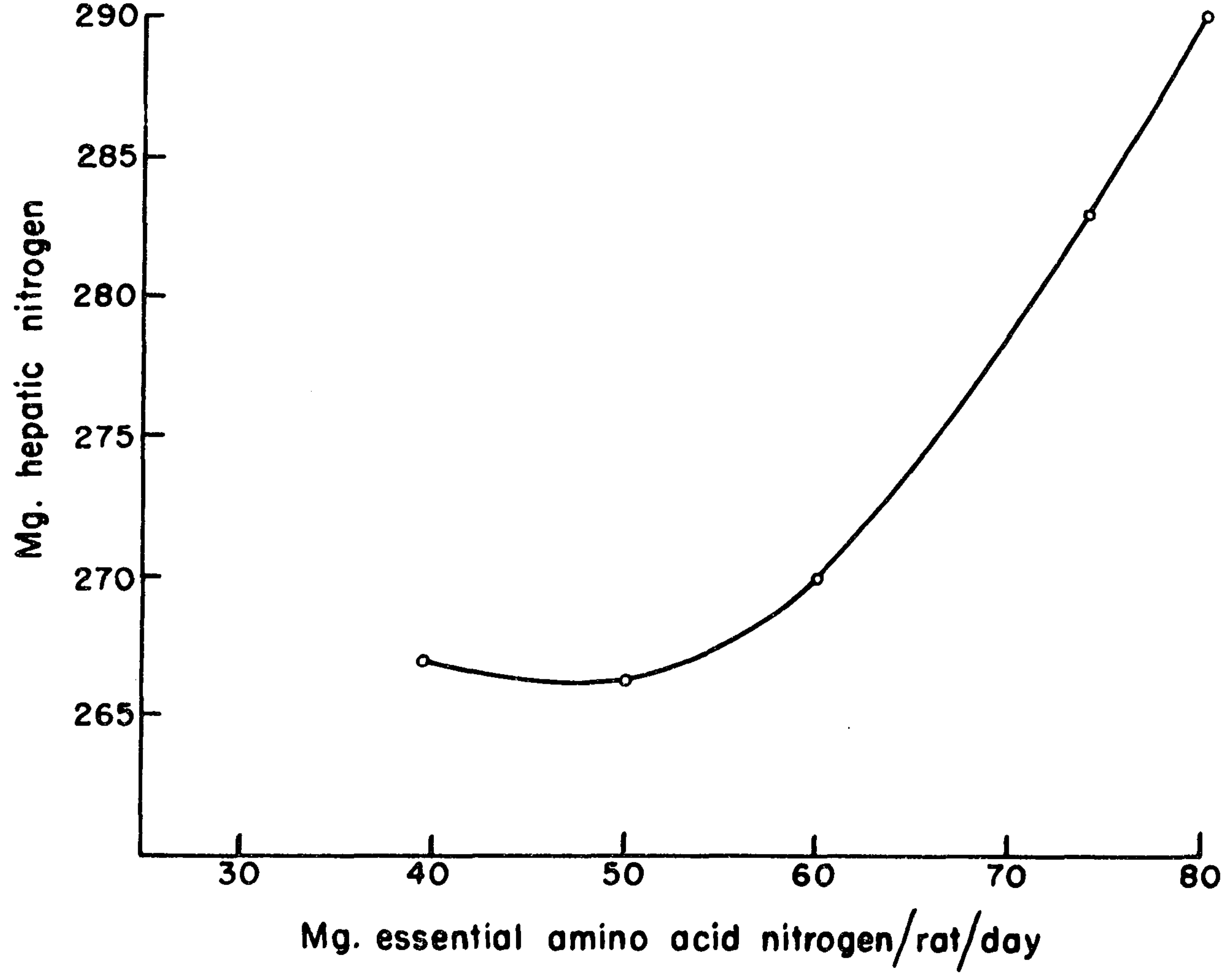
The data in Table 19 also show that the average weight of fat in the organs representing the various groups were not different except in the case of the group fed Diet 18. Its average value (517 mg.) was less than the figures obtained from other groups (582-598 mg.).

Again, the results raise the question in regard to what effect the increased quantities of methionine and phenylalanine used in this specific mixture may be exerting on hepatic fat.

Study D. Optical form of amino acid

In the course of the investigation reported herein, a

Figure 4. Nitrogen balance in relation to the quantity of nitrogen present in livers of rats fed graded doses of essential amino acids only.


290
285
280
275
270
265
Mg. hepatic nitrogen
30
40
50
60
70
80
Mg. essential amino acid nitrogen/rat/day

preparation of L-threonine became available. It seemed of interest to determine whether or not varying the optical form of threonine used in the experimental rations had any influence on the relative efficiencies of two different amino acid mixtures.

Experiment one. Four diets were formulated. Diets 17 and 19 contained mixtures of only the essential amino acids (EAA-II) in amounts providing 38 mg. of nitrogen per day. These diets contained 0.2 per cent L-threonine and 0.4 per cent DL-threonine, respectively. Diets 20 and 18 contained 39.5 mg. of essential nitrogen from EAA-III, providing 0.2 per cent L-threonine or 0.4 per cent DL-threonine, respectively.

The results are presented in Table 20. Individual values are shown in Tables XXI and XXII, Appendix.

The metabolism test indicated that the substitution of 0.2 per cent L-threonine for 0.4 per cent DL-threonine in the diets containing either mixture of essential acids only, i.e., EAA-II or EAA-III, elevated the tissue catabolism (Diets 17 and 20).

It is very interesting that the substitution of 0.2 per cent L-threonine for 0.4 per cent DL-threonine present in EAA-II (Diets 19 and 17) produced a significant increase in the concentration of fat in the liver (13.6% vs. 25.2%, on the dry basis). The increase is statistically significant

Table 20. Average nitrogen retention and average composition of livers of rats fed die
threonine is present in either the L- or DL- form.

Series
Study
Experime

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	No. of rats	Body weight	Nitrogen metabolism per 7 days		
					Nitrogen fed	Nitrogen balance	Per H_2O
				gm.	mg.	mg.	
17	EAA-II only	0.2%L-	6	341	319	-151	6
19	EAA-II only	0.4%DL-	8	350	351	-122	7
20	EAA-III only	0.2%L-	6	340	309	-141	6
18	EAA-III only	0.4%DL-	6	321	347	- 97	7

f rats fed diets containing nitrogen provided by EAA-II or EAA-III in which the

Series II
Study A
Experiment One

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-151	68.8	8.11	1.91	267	3.3	14.0	639	7.9	25.2	2.4
-122	70.3	8.21	2.10	262	3.2	12.4	332	4.0	13.6	1.3
-141	68.0	8.31	2.02	257	3.1	12.8	641	7.7	24.2	2.5
- 97	70.2	7.58	1.74	247	3.3	14.2	521	6.9	23.1	2.1

(t = 14.5). In 1953, Elvehjem's group (Harper, et al.) demonstrated that DL-threonine exerted a specific role in reducing liver fat deposition in the young rats. In the present experiment, the D-threonine must be utilized because it simultaneously reduced catabolism and liver fat.

Therefore, the lipogenic property of Diets 17 and 17a that was described in Experiment one of Study C might be explained on the basis of the present observations. It should be recalled that Diets 17 and 17a contained, respectively, 38 and 74 mg. of essential nitrogen from EAA-II as the sole source of the dietary nitrogen. In each diet, L-threonine constituted 0.2 per cent of the ration.

The response of the substitution of 0.2 per cent L-threonine for 0.4 per cent DL-threonine in EAA-III (Diets 18 and 20) was very different. No significant difference was observed between the concentrations of the hepatic fat of the animals in these two groups. Thus, it looks as though relations between threonine, methionine, and phenylalanine may be important in controlling fat deposition in the liver. It should be noted again, however, that on the whole, EAA-III has some lipogenic properties.

Experiment two: In this experiment, the effectiveness of D-threonine in promoting nitrogen retention and reducing fat deposition in the liver was evaluated when it was incorporated in a mixture containing both the essential and non-essential amino acids.

Three experimental diets were formulated. All diets contained 39.5 mg. of essential nitrogen from EAA-III and 34.5 mg. of non-essential nitrogen from NEAA-II, amounts equivalent to the acid present in one day's quota of the reference diet. Diets 21, 22, and 13A provided, respectively, 0.2 per cent L-threonine, 0.4 per cent L-threonine, and 0.4 per cent DL-threonine. Again, response was evaluated in terms of nitrogen balance and characteristics of the livers.

Data obtained from the three experimental groups are shown in Table 21 and Tables XXII and XXIII in the Appendix. The feeding of the diet containing either 0.4 per cent L-threonine (Diet 22) or 0.4 per cent DL-threonine (Diet 13A) produced a higher nitrogen retention than did the one containing 0.2 per cent L-threonine (Diet 21). When the diet contained 0.4 per cent threonine either as the L- or the DL- acid, rats utilized them equally well. Again, evidence is clear cut that the D-form of threonine is utilized by the adult rat in the nitrogen economy. Thus, lactalbumin appears to be low in threonine as well as in methionine and phenylalanine.

The average weights of the livers of the rats in the groups fed the three different diets (Diets 21, 22, and 13A) did not appear to differ either on the fresh or on the dry, fat-free basis. Average moisture contents of the organ also

Table 21. Average nitrogen retention and composition of livers of rats fed diets cont is present in either the L- or DL- form.

Seri
Stu
Experi

Diet No.	Nitrogen in daily dose provided by	Quantity and form of threonine fed	No. of rats	Body weight	Nitrogen metabolism per 7 days	
					Nitrogen fed	Nitrogen balance
				gm.	mg.	mg.
21	EAA-III(39.5 mg.N) NEAA-II(34.5 mg.N)	0.2%L-	6	344	541	-13
22	EAA-III(39.5 mg.N) NEAA-II(34.5 mg.N)	0.4%L-	6	340	568	32
13A	EAA-III(39.5 mg.N) NEAA-II(34.5 mg.N)	0.4%DL-	6	346	612	46

fed diets containing nitrogen provided by EAA-III and NEAA-II in which the threonine

Series II
Study D
Experiment Two

en meta- er 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
-13	69.0	8.11	1.92	263	3.2	13.8	590	7.3	23.6	2.2
32	69.0	8.31	1.97	275	3.3	14.0	611	7.4	23.7	2.2
46	69.3	8.42	2.02	268	3.2	13.3	558	6.6	21.6	2.1

were similar. Actual and relative quantities of hepatic nitrogen were approximately the same. Total hepatic fat varied from 558 to 611 mg.; the percentage of hepatic fat on the dry basis from 21.6 to 23.7 per cent. Differences in percentage of hepatic fat, however, were not statistically significant.

Series III. Influence of Variation in the Non-nitrogenous Components of the Diet

Study A. Variations in the source of dietary carbohydrate

As indicated in the "REVIEW OF LITERATURE", various investigators have demonstrated that under their experimental conditions certain dietary carbohydrates when incorporated into rations containing either intact proteins or mixtures of amino acids only, exerted a favorable influence on rate of growth, synthesis of B-vitamins in the intestine, nitrogen retention, and fatty composition of liver. The purpose of the present investigation was to study the influence of different carbohydrates on retention of nitrogen when they were incorporated into diets containing the amino acid mixture found most favorable to nitrogen retention.

Four diets were used in the study. Diet 13A, as a control diet, contained dextrin and EAA-III plus NEAA-II. In Diets 23, 24, and 25, dextrin was replaced, respectively by starch, dextrose, and sucrose. It should be noted, however, that all diets contained approximately 68% of carbohydrate and provided 74 mg. of total nitrogen in one day's quota of feed that originated from EAA-III (39.5 mg. N) and NEAA-II (34.5 mg. N).

Table 22 presents average data pertaining to the four groups of animals fed diets containing carbohydrates from various sources (Diets 13A, 23, 24, and 25). Individual values are shown in Tables XXV and XXVI, Appendix.

The average nitrogen balance of the rats indicated that the replacement of dextrin by starch did not introduce any appreciable change in the retention of nitrogen (62 mg. *vs.* 45 mg./days). The replacement of dextrin by either dextrose (Diet 24) or sucrose (Diet 25), however, resulted in negative nitrogen balances, i.e., -135 and -59 mg. per 7 days. The differences in the nitrogen balances of the rats in these groups and those fed the control ration (Diet 13A) were highly significant ($F_{2,11}$= 16.59). The catabolism of the rats upon the feeding of dextrose was higher than that characteristic of any other group.

The average weights of the livers of the rats in the groups fed the four different experimental diets (Diets 13A, 23, 24, and 25) did not appear to differ, either on the fresh or on the dry, fat-free basis. Average moisture contents of the organ also were similar.

The feeding of the four different experimental diets

Table 22. Average nitrogen retention and average composition of livers of rats when th
containing different sources of carbohydrate.

Series
Study

Diet No.	Source of carbohydrate in ration	Quantity and source of nitrogen in one day's quota of feed	No. of rats	Body weight	Nitrogen metabolism per 7 days		
					Nitrogen fed	Nitrogen balance	Per H_2O
				gm.	mg.	mg.	
13A	Dextrin	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	4	331	581	45	68
23	Starch	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	5	333	612	62	68
24	Dextrose	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	4	336	563	-135	69
25	Sucrose	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	6	333	559	-59	69

f rats when the amino acid mixture (EAA-III plus NEAA-II) was incorporated in diets

Series III
Study A

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract)			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
45	68.4	7.97	1.97	266	3.4	13.5	548	6.9	21.8	2.1
62	68.7	7.76	1.88	255	3.3	13.6	542	7.0	22.4	2.1
-135	69.3	7.56	1.84	257	3.4	14.0	482	6.4	20.8	1.9
-59	69.6	7.95	1.93	277	3.5	14.4	486	6.1	20.1	1.8

seemed to produce no change in the concentration of hepatic nitrogen that could be considered significant, as measured by either actual or relative quantities.

Neither did the kind of carbohydrate used in the ration affect the actual and relative quantities of fat in the liver to any significant extent.

Study B: Variations in the energy value of the diets

Since Rose, Coon, and Lambert (1954) have stated that they have no satisfactory explanation for the unfavorable retentions of nitrogen exhibited by human subjects receiving amino acids in diets adequate to meet their energy requirements, it seemed important to study the relation of variations in caloric value of the ration on nitrogen balance under the experimental situations described in the present investigation. Data are presented in Table 23 and in Tables XXVII and XXVIII of the Appendix. In addition to the measurements used up to this point for evaluation of response of the rats, studies were made of carcass composition.

Three experimental diets were formulated. Diet 13A was the control diet. All diets contained 10 per cent of hydrogenated lard (Swiftn'ing) and 10 per cent of butterfat. The absolute quantities of fat and carbohydrate were reduced proportionately in Diets 26 and 27 so as to provide approximately 50 and 35 calories in one day's quota of food in contrast to the 65 calories yielded by the control ration.

Table 23. Average nitrogen retention and average composition of livers of rats when th
of varying caloric value.

Series
Stud

Diet No.	Caloric value of one day's quota of diet	Quantity and source of nitrogen in one day's quota of diet	No. of rats	Body weight	Nitrogen metabolism per 7 days		
					Nitrogen fed	Nitrogen balance	Per H_2O
	Cal.			gm.	mg.	mg.	
13A	65	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	6	352	625	80	6
26	50	EAA-III (39.5 mg.N) NEAA-II (34.5 mg. N)	8	338	666	17	6
27	35	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	6	312	685	-159	6

of rats when the amino acid mixture (EAA-III plus NEAA-II) was incorporated in diets

Series III
Study B

meta- 7 days	Liver			Hepatic nitrogen			Hepatic fat (alcohol-ether extract			
Nitrogen balance	Per cent H_2O	Fresh weight	Dry fat-free weight	Total	Per cent fresh weight	Per cent dry fat-free weight	Total	Per cent fresh weight	Per cent dry weight	Ratio fat: nitrogen
mg.		gm.	gm.	mg.			mg.			
80	69.4	7.96	1.88	264	3.3	14.1	554	7.0	22.8	2.1
17	69.4	7.04	1.68	266	3.8	15.8	474	6.7	22.0	1.8
-159	69.4	6.40	1.58	250	3.9	15.8	379	5.9	19.3	1.5

It should be noted that the day's allotment of each ration contained the same quantities of other dietary components. All provided 74 mg. of nitrogen from the combination of EAA-III (39.5 mg. N) and NEAA-II (34.5 mg. N). One day's feed in the solid form of Diets 13A, 26, and 27 weighed 13, 10.2, and 7.3 gm., respectively.

The inability of Diets 26 and 27 to support protein nutrition was evident even in an early phase of the experiment. All rats in group 26 lost weight (Appendix, Table XXVII), the average loss in the experimental period of group 26 being 11 gm.; of group 27, 40 gm. The feeding of the control diet (Diet 13A) which provided approximately 65 calories daily permitted the rats to gain on the aver 10 gm. during the same period (range 7 to 16 gm.).

The nitrogen balances of the rats fed diets of varied energy value were striking also. The difference in nitrogen balances between the three groups were highly significant ($F_{2,17} = 606.71$).

All the rats in the control group (Diet 13A) were in positive nitrogen balance. On the average, the group stored about 80 mg. of nitrogen (range 47 to 102 mg. during the 7-dy metabolism period). Greater individual variation in nitrogen retention, however, was associated with the feeding of Diet 26 than was the case in the rats receiving the control diet (Diet 13A). The mean retention of nitrogen of rats fed Diet 26 was positive, i.e, 17 mg. with a range in values from -43 to 105 mg. Comparison of the nitrogen balances of groups 13A and 26 indicated that the difference between them was significant at the 5 per cent level (t_{12} = 2.35). The rats given the diet providing approximately 35 calories in one day's quota (Diet 27) dropped into a severely negative nitrogen balance. The average nitrogen balance was -159 mg. per 7 days.

Thus retention of nitrogen was related to the caloric value of the diet. It should be noted that the rats maintained nitrogen equilibrium when offered a ration providing 50 calories in one day's quota. This was the approximate energy value of the food consumed by the rats when fed, *ad libitum*, the reference diet containing a lactalbumin preparation at the 4 per cent level. It is very interesting that the rats in this experiment receiving amino acids as the sole source of dietary nitrogen do not seem to need calories over their regular requirement as suggested by Rose, *et al.* in 1954. It is suggested that the need for additional calories under his experimental situations may be explained on the basis of the efficiency of the amino mixture fed. Further evidence for this hypothesis is presented in Table 24 in which retentions obtained during the course of this investigation with various mixtures of amino acids may be compared when the diet furnishes 65 calories per day.

The average nitrogen balance of the rats fed Diet 4 furnishing 65 calories per day and containing a mixture of amino acids simulating those present in one day's quota of the reference diet (EAA-I plus NEAA-I), was 8 mg. during the 7-day metabolism period. The simultaneous substitution of cystine and tyrosine nitrogen with methionine and phenylalanine nitrogen, respectively, (Diet 12C) induced a similar

Table 24. Composition of amino acid mixtures and nitrogen retention at a constant level of caloric intake.

Diet	Amino acid mixture 74 mg. N/day	Daily caloric value of force-fed diet	Nitrogen balance in the 7-day test period
4	EAA-I plus NEAA-I	65	8
12C	EAA-II plus NEAA-II	65	6
13A	EAA-III plus NEAA-II	65	80
26	EAA-III plus NEAA-III	50	17

retention (6 mg.) at this level of caloric intake. Increases in the quantities of methionine and phenylalanine at the expense of the non-essential amino acid mixture as was done in Diet 13A resulted in positive nitrogen balance (80 mg./

7 days). Also, this diet (Diet 13A) fed in amounts providing only 50 calories per day was as effective in promoting nitrogen balance as were less efficient mixtures (Diets 4 and 12C) fortified with 65 calories per day.

Data pertaining to the characteristics of the livers as shown in Table 23 and Table XXVIII in the Appendix are interesting. The average relative quantities of moisture in the livers of the rats fed three different experimental diets were identical. The mean weights of the organ, however, either on the fresh or on the dry, fat-free basis, were statistically different at the 1 per cent level ($F_{2,17}=$ 48.27 and $F_{2,16}=$ 2.34). The rats fed the low calorie diet had the smallest liver.

The effect of caloric restriction is especially discernable when groups fed Diets 13A and 27 are compared (i.e., 65 calories per day vs. 35). As noted above, the mean weight of the liver of the group fed 35 calories per day was less than that of the rats fed 65 calories. This was due to a loss in hepatic fat since moisture contents are identical. With the less fatty liver, then, one would expect some increase in the relative concentration of hepatic nitrogen, which was the case. Results obtained with the group fed Diet 26 are not as clear, but this is the more variable group receiving calories close to the critical level.

The actual and relative quantities of fat in the organ of rats fed the three experimental diets were statistically different at the 1 per cent level ($F_{2,17}$= 31.29 and $F_{2,17}$= 16.6).

The present experiments suggest that the weights of livers and the concentration of fat in the organ reflect the caloric value of the experimental diets.

The whole carcasses of rats maintained on three different experimental diets were analyzed for moisture, total nitrogen, and fat. Data pertaining to these determinations are shown in Table 25 and Table XXIX, Appendix.

A definite loss in the total water present in the whole carcass resulted from the feeding of a diet providing approximately 35 calories per day instead of 50 or 65 calories. The difference between the three groups was statistically significant at the 1 per cent level ($F_{2,17}$= 24.42). On the relative basis, moisture content of the carcass increased as the caloric value of the diet decreased. The carcasses of the positive control group (Diet 13A) contained an average of 125 gm. of dry matter; those of group 26, 111 gm.; and of group 27, 96 gm. The respective relative values for quantity of dry matter present were 37.0, 34.6, and 33.1. Both actual and relative quantities of dry matter present in carcasses of rats fed the three experimental diets were different at the 1 per cent level ($F_{2,17}$= 71.78 and $F_{2,17}$= 59.0,

Table 25. Average carcass composition of rats when the amino acid mixture (EAA-III plu

Series
Stud

Diet	Caloric value of one day's quota of diet	Quantity and source of nitrogen in one day's quota of diet	No. of rats	Carcass weight (liver-free)	Moisture		Dry	
					Per cent	Total weight	Per cent	Tota weig
	Cal.			gm.		gm.		gm.
13A	65	EAA-III (39.5 mg.N) EAA-II (34.5 mg.N)	6	338	63.0	213	37.0	125
26	50	EAA-III (39.5 mg.N) EAA-II (34.5 mg.N)	8	321	65.4	210	34.6	111
27	35	EAA-III (39.5 mg.N) EAA-II (34.5 mg.N)	6	290	66.9	194	33.1	96

re (EAA-III plus NEAA-II) was incorporated in diets of varying caloric value.

Series III
Study B

	Dry matter				Carcass nitrogen				Carcass fat (alcohol-ether extract)			
l ght	Dry		Dry, fat-free		Total	Per cent expressed in terms of			Total	Per cent expressed in terms of		Ratio fat: nitrogen
	Per cent	Total weight	Per cent	Total weight		Fresh weight	Dry weight	Dry fat-free weight		Fresh weight	Dry weight	
		gm.		gm.	gm.				gm.			
3	37.0	125	23.2	78.5	11.7	3.5	9.3	14.8	46.3	13.7	37.0	4.0
	34.6	111	23.0	74.0	11.6	3.6	10.4	15.6	37.1	11.7	33.6	3.2
	33.1	96	24.3	70.5	10.9	3.8	11.3	15.4	25.5	8.8	26.6	2.3

respectively).

The per cent of dry, fat-free matter in the carcasses of group 27 was about the same as that of animals in groups fed Diets 13A and 26. However, the actual quantities of dry, fat-free matter in the carcasses of groups 13A, 26, and 27 were different, i.e., 78.5, 74.0, and 70.5 gm., respectively. These values depicting relative amounts of dry, fat-free tissue are in accord with the retentions of nitrogen characteristic of the three groups of rats.

Both sets of observations are confirmed by the significant loss in the total nitrogen from the whole carcass that followed the feeding of Diet 27 which provided approximately 35 calories in one day's quota of food. The total quantities of body nitrogen of the rats fed Diet 13A (65 calories per day) and Diet 26 (50 calories per day) were essentially the same.

It was found that the feeding of Diets 26 and 27 produced a significant change in the concentration of fat in the carcass. This observation bears out the idea that the caloric requirement is increased when amino acid diets are fed. The rats apparently have been forced to draw on body stores of fat for additional calories. That this occurs in the group fed 50 calories per day (Diet 26) despite maintenance of satisfactory nitrogen balance again suggests that this is a critical level of energy for maximum utilization of dietary nitrogen coming solely from amino acids.

Evaluation of Amino Mixtures Formulated

It was thought that it might be interesting to evaluate the composition of the amino acid mixtures formulated in the present investigation in relation to the minimal requirements of the various amino acids for maintenance of nitrogen balance in man as set forth by Rose, Wixom, Lockhart, and Lambert in 1955. In this evaluation, the relative amounts in the two formulae of each essential amino acid in respect to the quantity of tryptophan was used as indices for comparison. Theoretically, utilization is determined quantitatively by the amino acid present in the smallest amount, i.e., "the law of minimum". Tryptophan is the amino acid that occurs in smallest amounts in natural proteins.

The quantities of each amino acid in the three mixtures tested in the present experiment and the amounts recommended by Rose, *et al.* are shown in Table 26. The proportionality relationships with tryptophan expressed as unity appear in Table 27.

The amino acid mixture used by Rose in his animal studies in which the essentiality of certain amino acids was established on the basis of the amino acid content of casein (Rose, 1931). Several modifications were made in this mixture over the course of time (Rose, *et al.*, 1949). Then when the Illinois investigations shifted to a study of human re-

Table 26. Composition of amino acid mixtures supporting body maintenance.

Amino acid	Quantity of utilizable amino acids			
	Iowa formulae for rat maintenance			Rose's tentative recommendation for minimum intake
	Mixture 1	Mixture 2	Mixture 3	
	mg.	mg.	mg.	gm.
Tryptophan	9.2	9.2	9.2	0.25
Methionine	10.3	30.2	37.1	1.10
Cystine	16.1	---	---	---
Total S-containing acids	26.4	30.2	37.1	1.10
Phenylalanine	18.2	45.5	52.9	1.10
Tyrosine	27.3	---	---	---
Total -Containing acids	45.5	45.5	52.9	1.10
Arginine	14.2	14.2	14.2	---
Histidine	9.1	9.1	9.1	---
Isoleucine	27.7	27.7	27.7	0.70
Leucine	53.8	53.8	53.8	1.10
Lysine	41.9	41.9	41.9	0.80
Threonine	51.4	51.4	51.4	0.50
Valine	28.4	28.4	28.4	0.80

Table 27. Ratios of amino acid components to tryptophan in amino diets supporting body maintenance

Amino acids	Ratio of quantity of each amino acid to tryptophan			
	Iowa formulae for rat maintenance			Rose's tentative recommendation for human beings
	Mixture 1	Mixture 2	Mixture 3	
Tryptophan	1.0	1.0	1.0	1.0
Methionine	1.1	3.3	4.0	4.4
Cystine	1.8	---	---	---
Total S-containing acids	2.9	3.3	4.0	4.4
Phenylalanine	2.0	4.9	5.8	4.4
Tyrosine	3.0	---	---	---
Total -containing acids	4.9	4.9	5.8	4.4
Arginine	1.5	1.5	1.5	---
Histidine	1.0	1.0	1.0	---
Isoleucine	3.0	3.0	3.0	2.8
Leucine	5.8	5.8	5.8	4.4
Lysine	4.6	4.6	4.6	3.2
Threonine	5.6	5.6	5.6	2.0
Valine	3.1	3.1	3.1	3.2

quirements for the various amino acids, the initial amino acid mixture used was the one which supported the best growth in the rat of any preparation tested in their laboratory up to that time and which had had its origin in the composition of casein (Rose, et al., 1950). As the requirement for each essential amino acid was established, the composition of the test mixture was modified accordingly (Rose, 1949). From these data then, minimum requirements eventually were evoked (Rose, et al., 1955).

The ratios of the concentrations of the various amino acids to that of tryptophan in Mixture 1 (Table 27) actually are the ratios that describe the protein, lactalbumin, with the exception of the concentration of threonine. Threonine was incorporated into this mixture at the level in which the L-form occurs in lactalbumin. Only DL-threonine was originally available, so the amino acid was fed in doses providing twice the quantity indicated by the composition of lactalbumin. However, it was demonstrated that the D-form was utilizable under the experimental conditions imposed and that the feeding of either L- or DL-threonine at the 0.4 per cent level definitely improved nitrogen retention over that obtained by the feeding of 0.2 per cent L-threonine, the level dictated by the composition of lactalbumin.

The changes in the ratios of methionine and phenylalanine to tryptophan in Mixtures 2 and 3 are associated with progressively greater efficiencies of the amino acid combinations in respect to their ability to support retention of nitrogen.

Comparison of the amino acid-tryptophan ratios presented in Table 27 show that there is a close similarity between many of the ratios for the most efficient mixture formulated in the present investigation (Mixture 3) and Rose's values for minimal amino acid requirements in adult human beings.

One of the most interesting digressions is in respect to phenylalanine, the ratio being 5.8 in the formula of the amino acid mixture for maintenance of nitrogen equilibrium in the rat and 4.4 in the list of minimal requirements of amino acids in man. It may be recalled that phenylalanine was involved definitely in the delicate balance with methionine whereby the efficiency of Mixture No. 1 was increased in respect to its ability to support a normal fat concentration in the liver and to maintain nitrogen equilibrium at a decreased level of total nitrogen intake (Mixture No. 2). In the presence of cystine and limited amounts of methionine, excess phenylalanine was definitely lipogenic and conducive to the establishment of negative nitrogen balance (Table 12). With the total sulfur content of the diet made up of methionine only, phenylalanine was lipotropic and efficient in regard to the promotion of retention of nitrogen. It is believed that the present experiment indicates that the adult rat needs a higher level of phenylalanine than heretofore believed and that the proportion of phenylalanine to tryptophan may be of importance in determining balance between ingredients of an amino acid mixture. Under the conditions laid down by Rose in his studies with adult men it has, of course, been impossible to secure data pertaining to composition of liver.

The change in the methionine-tryptophan ratios in Mixtures 2 and 3 from 3.3 to 4.0 illustrates the key position of methionine and the part it plays in controlling nitrogen metabolism. In Mixture 2, it was definitely lipotropic and exerted a favorable effect on retention of nitrogen; in Mixture 3, although the larger quantity of this acid improved the utilization of the mixture, it simultaneously imparted some lipogenic properties. One wonders what transpired in the human beings when the methionine-tryptophan ratio was 4.4.

Rose and Wixom used the DL- form of the amino acid in the investigation describing the human requirement for threonine (1955), and therefore introduced twice the amount called for by their formula into the amino acid mixture employed. If the observations of the present experiments are confirmed in regard to the physiological utilization of D-threonine by the adult rat, and if the results of an animal experiment are applicable to man, an alteration in Rose's recommendation value may be indicated.

In the progress of Rose's experiments, the proportions of the amino acids listed in his tentative requirements were increased gradually so that they now diverge rather sharply from the composition of casein in respect to methionine and phenylalanine as set forth by Steffee, et al. in 1950. This experience has a counterpart in the experiment reported herein. In this case, digression from the composition of lactalbumin occurred. In both instances, it was demonstrated that natural proteins may not contain amino acids in the best proportions for the maintenance of nitrogen balance. But it is very interesting that two different approaches involving two different species and different reference proteins have led to results surprisingly comparable.

The proportionality figures vary in respect to leucine and lysine also. Whether the excess proportions of these two acids in Mixture No. 3 have any antagonistic action should be studied in the future.

SUMMARY

Eventually protein requirements will be considered in terms of the requirements for the specific amino acids. A program of research has been in progress for some years in the Nutrition Laboratory of the Home Economics Research Department that has as its objective the determination, qualitatively and quantitatively, of the amino acids needed for the maintenance of body substance by the adult male albino rat. Results obtained in these investigations indicated that certain questions needed clearance before the ultimate objective could be attained. These questions may be listed as follows: In what way do variations in the amounts and proportions of methionine, phenylalanine, cystine, and tyrosine influence the nutritive value of an amino acid mixture for body maintenance? Is the D-form of threonine physiologically available? What effect do variations in the source of carbohydrate or in the total energy value of the diet have on utilization of nitrogen when amino acids provide the sole source of dietary nitrogen? The present investigation dealt with these problems and is a continuation of the work initiated by Kuehl (1949) and Clark (1950).

The basic amino acid mixture used in the study herein reported was modeled after the amino acid composition of lactalbumin, a "single" protein of well-established nutritive

value. It had been shown that the feeding of a ration containing 4.27 per cent of the specific lactalbumin preparation tested supported nitrogen equilibrium in the adult animal. This ration, therefore, became the reference diet and the initial amino acid mixture was formulated to simulate the assortment of acids provided by the amount of this ration that would provide 60 calories per day. The concentration of the various amino acids in the lactalbumin preparation was determined microbiologically.

Three different mixtures of amino acids were formulated. The first mixture contained an assortment of essential and non-essential amino acids (i.e., EAA-I plus NEAA-I), approximating those occurring in the lactalbumin present in one day's quota of the reference diet. All of the amino acids occurring in lactalbumin except serine and hydroxyglutamic acid could be secured for incorporation into the mixture. It should be noted that the portion of this mixture representing the non-essential acids contained cystine and tyrosine in the amounts present in the lactalbumin preparation.

In the second mixture, the cystine and tyrosine present in Mixture 1 were replaced isonitrogenously by methionine and phenylalanine, respectively. The second mixture (EAA-II plus NEAA-II), therefore, was considerably enriched in respect to methionine and phenylalanine. The total quantities of nitrogen provided by the sulfur-containing amino acids (methionine and cystine) and by the acids carrying the benzene ring (phenylalanine and tyrosine) were the same in Mixtures 1 and 2.

In Mixture 3, the methionine and phenylalanine present represented the total sulfur and aromatic (⬡) acids occurring in the amount of non-essential nitrogen, i.e., 55 mg., that gave the best nitrogen retention when Clark (1950) fed the first mixture of essential acids (EAA-I) with graded doses of NEAA-I.

It should be noted, however, that the total amount of nitrogen furnished per day from rations containing any one of these three mixtures was 74 mg., the equivalent of that provided by the daily feeding of the reference diet containing lactalbumin, and which supported nitrogen equilibrium.

Adult male albino rats, approximately six months old, were used in the experiment. In order to control the total daily intake of food and its caloric value, all experimental diets were administered by means of a forced feeding technique. The energy value of each day's quota was 60 calories. The relative protein nutrition of the groups of rats given the various experimental diets was evaluated in terms of body weight, nitrogen balance, and composition of liver in respect to nitrogen, fat, and moisture.

The investigation was divided into three series. In

Series I, the adequacy of the basic diet was evaluated. Folic acid and vitamin B_{12} were available when the present study was initiated and it became necessary to determine the effect of substituting them for the rice bran polish used in the diets in the early phases of the program as a source of the then not clearly identified vitamins. When Mixture 1 provided the sole source of the dietary nitrogen, the substitution of folic acid and vitamin B_{12} for rice bran polish did not change the response of the rats as measured in terms of nitrogen retention and fat content of liver. These vitamins thereafter were incorporated routinely into the test rations in lieu of rice bran polish which had become unavailable.

In Series II, the influence of variations in the amounts and proportions of certain acids in the mixture of amino acids on the protein nutrition of the animals was studied. Previous work in the laboratory directed to a study of the essentiality of the non-essential amino acids (Clark, 1950) had shown that when graded doses of the mixture of non-essential acid (NEAA-I) were fed in conjunction with 34 mg. of essential nitrogen as provided by Mixture 1 (EAA-I), the regression of nitrogen balance on quantity of non-essential nitrogen provided was linear, and that nitrogen equilibrium was attained when the diet provided approximately 74 mg. of nitrogen from a combination of EAA-I and NEAA-I, the equivalent of that provided by one day's quota of the reference diet that supported nitrogen equilibrium. It was thought that the improvement in nitrogen retention associated with each increment in non-essential nitrogen was ascribable to the increases in the cystine and tyrosine values of the diet that ensued. Therefore, in the present study, methionine, phenylalanine, cystine, and tyrosine relations were explored.

It was found that all diets were lipogenic. Their activity in this respect was related directly to the amount of non-essential nitrogen fed. It was inferred that cystine was the lipogenic agent. It was found also that rats fed the 4 per cent lactalbumin diet had fatty livers.

To test this hypothesis, cystine was removed from the ration. Its omission from NEAA-I resulted in marked nutritive failure as measured in terms of nitrogen balance. The concentration of fat in the liver, however, was normal. The omission of tyrosine from the non-essential mixture induced a slightly negative balance. The livers of these animals were fatty. When cystine and tyrosine were replaced isonitrogenously by methionine and phenylalanine, respectively, rats retained increased amounts of nitrogen and maintained a normal concentration of fat in the liver. Rats fed the 4 per cent lactalbumin diet, however, had fatty livers resulting, as the evidence above indicates, from imbalance in amounts and proportions of methionine, phenylalanine, cystine, and tyrosine present.

When the essential nitrogen from EAA-II was fed in conjunction with graded doses of a mixture of non-essential amino acids lacking in cystine and tyrosine (NEAA-II), retention of nitrogen, as compared with that obtained when Mixture 1 was used in the initial experiment, improved with each increment up to the point where 40 mg. of non-essential nitrogen was provided daily. It was believed that the superiority of amino acid Mixture 1 when more than 40 mg. of non-essential amino acids were fed, could be attributed to the increased amounts of cystine and tyrosine present.

Studies with the feeding of essential Mixture 3 proved this point. When this mixture was fed with graded doses of non-essential nitrogen, nitrogen balance again appeared to be related in a linear fashion to the amount of non-essential amino acids administered. However, the response was superior to that obtained when either Mixture 1 or Mixture 2 was supplemented similarly with non-essential nitrogen. Considerably less non-essential nitrogen was needed for the establishment of nitrogen equilibrium with Mixture 3 than with either Mixtures 1 or 2. Thus the dependence of the biological efficiency of an amino acid mixture on its methionine and phenylalanine content was demonstrated.

In the next experiment, evidence was obtained from another angle of interrelations between methionine, phenylalanine, cystine, and tyrosine that affect the nutritive value of an amino acid mixture. In this test, a single non-essential amino acid replaced the combination of acids present in NEAA-I. Glutamic acid was chosen for the test and was fed in amounts to bring the total daily nitrogen intake up to 74 mg. When this acid was added alone to EAA-I, the animals passed into acute negative balance and had livers normal in respect to fat. However, when it was added to EAA-II, very satisfactory balance ensued. The fat content of these livers was normal. In the first instance, the absence of dietary cystine prevented the occurrence of fatty livers even in the presence of acute negative balance; in the second, methionine exerted its lipotropic properties. The experiment also showed that as in the case of growth, the nature of the non-essential nitrogen does not need to be defined providing the essential acids fed contain appropriate amounts of methionine and phenylalanine.

Efficacy of mixtures containing essential amino acids only for the support of protein nutrition was determined and provided further data relating to the importance of methionine and phenylalanine. Mixture 1 (EAA-I) fed in the amount that provided the essential amino acids present in one day's feed of a 4.27 per cent lactalbumin diet did not support nitrogen equilibrium. Neither did it do so when fed in twice this quantity. Mixture 2 (EAA-II) fed in doses providing 38 mg. of nitrogen daily and without supplementary non-essential amino acids definitely lowered the break-down of body tissue that occurred when EAA-I was given. When the amount of EAA-II was doubled the mixture supported nitrogen equilibrium. The response with Mixture 3 was essentially the same. Mixture 3 also was fed in graded doses. Nitrogen balance was related linearly to quantity fed. This is the first time that it has been shown that the essential amino acids can support nitrogen equilibrium in the absence of fortifying non-essential nitrogen when the total nitrogen is held at a minimal value, i.e., that equivalent to the nitrogen in a quantity of high quality intact protein which will prevent loss of body tissue.

Effect of varying the optical form of the amino acid in the essential amino acid mixture was determined, threonine being chosen for the determination. The feeding of the diet containing either 0.4 per cent L-threonine or 0.4 per cent DL-threonine produced a greater retention of nitrogen than did the one containing 0.2 per cent L-threonine. The nitrogen retention of the rat fed a diet containing 0.4 per cent DL-threonine was similar to the value obtained when rats were fed the same diet containing 0.4 per cent L-threonine. When supplementing the essential mixture, EAA-II, unfortified by non-essential nitrogen, DL-threonine was more lipotropic than L-threonine.

The last series was designed to study the influence of variation in the non-nitrogenous components of the amino acid diet on protein nutrition. The effect of substituting starch, dextrose, or sucrose for the dextrin in the control diet was determined. The rats ingesting the dextrose and sucrose rations passed into negative nitrogen balance. The average percentage of the hepatic fat remained essentially the same in all groups.

In the last experiment, the effect of feeding the amino acid diet at different levels of food energy value was determined. Mixture 3 (EAA-III plus NEAA-II) was used as the source of dietary nitrogen. The respective rations provided approximately 65, 50, and 35 calories per rat per day. Retention of nitrogen was associated with the energy value of the diet. It ranged from 80 mg./7 days when the energy value of the day's ration was 65 calories to an acute negative nitrogen balance, -159 mg./7 days, when the caloric value was reduced to 35 calories. The rats, however, maintained nitrogen equilibrium when offered food providing approximately 50 calories per day. This was the approximate energy value of the food consumed by the rats, when they were fed, *ad libitum*, the reference lactalbumin diet. These latter observations are not in line with those of Rose in which adult men were unable to maintain nitrogen equilibrium when fed diets containing amino acids unless the energy value of the ration was well above the estimated daily allowance.

Carcasses of rats maintained on these rations of varying caloric value were analyzed for moisture, total nitrogen, and fat. Definite losses in total water, fat, dry, fat-free matter, and nitrogen occurred as the result of feeding of a diet providing approximately 35 calories per day instead of 65 calories. While a diet yielding approximately 50 calories daily promoted retention of nitrogen, there were losses of carcass fat. This observation may mean that the energy value of the ration was approaching a critical level under this specific feeding regime.

The three mixtures of amino acids formulated in the present investigation were compared with values for the amounts of the various amino acids needed for the maintenance of human beings as set forth by Rose in his tentative minimal recommendations.

CONCLUSIONS

Lactalbumin, although it rates high in regard to biological efficiency, does not contain certain amino acids in proportions that are the most efficient for the retention of nitrogen or for the maintenance of a normal concentration of hepatic fat in the adult male albino rat. Its limiting amino acids are methionine, phenylalanine, and threonine. Although cystine and tyrosine augment the amounts of the respective companion essential acids present, they render the intact protein lipogenic. The concentration of methionine in lactalbumin is not high enough to offset the lipogenic influence of cystine.

Amino acids when fed as the sole source of dietary nitrogen and when combined in an assortment quantitatively resembling the amino acids present in lactalbumin behave like the intact protein, not only in respect to ability to support nitrogen retention but in favoring the deposition of hepatic fat. Additional methionine incorporated at the expense of cystine into a mixture of the same total nitrogen value renders the amino acid mixture lipotropic and increases its nutritive value in respect to nitrogen retention. Extra methionine beyond this point makes the amino diet lipogenic again.

Additional phenylalanine in an amino acid diet containing cystine and a low concentration of methionine results in an imbalanced mixture. The imbalance may be removed by the isonitrogenous replacement of cystine by methionine. A ratio of 1.2 between phenylalanine and methionine is favorable.

Glutamic acid can replace a mixture made up of several non-essential amino acids only if the essential amino acid mixture is balanced in respect to its methionine and phenylalinine content.

Nitrogen equilibrium is achieved at a reduced level of total nitrogen intake when modifications are made in the amounts of methionine, phenylalanine, and threonine furnished by an amino acid mixture modeled after the composition of lactalbumin.

The essential amino acids, only, when fed as the sole source of nitrogen in a diet of low total nitrogen value (i.e., equivalent to that of a 4 per cent protein diet) are capable of supporting nitrogen equilibrium and a normal concentration of hepatic fat providing the amino acids are present in balanced proportions.

In contrast to observations made by other investigations, the D-form of threonine may be utilized as effectively as its natural isomer for the support of nitrogen retention and normal hepatic tissue in the adult rat. The requirements of threonine for the attainment of nitrogen equilibrium in the adult male rat appear to be twice as great as heretofore suggested.

Retention of nitrogen in adult rats is related to the caloric value of the diet. It appears that a mixture of amino acids serving as the sole source of dietary nitrogen can support nitrogen equilibrium in the adult rat when the energy value of the daily diet approximates the animal's caloric requirement providing methionine and phenylalanine are present in balanced amounts. However, further study of influence of proportionality between amino acids is needed for losses in carcass fat indicated that food energy approximating the rat's daily requirement, i.e., 50 calories represents a critical level of caloric intake for the establishment of nitrogen equilibrium. With reduction in the caloric value of the amino acid ration below the daily energy requirement of the rat, nitrogen equilibrium does not occur.

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APPENDIX

Table I Recovery of nitrogen from a standard solution of ammonium sulfate distributed at intervals over metabolism cages.

Cage No.	Quantity of nitrogen theoretically present	Quantity of nitrogen recovered	Per cent of nitrogen recovered
	mg.	mg.	
1	500	495	99.1
		494	99.0
		497	99.4
		496	99.2
2	500	496	99.2
		495	99.1
		494	99.0
		493	98.9
3	500	494	99.3
		497	99.4
		497	99.4
		495	99.1

Table II Recovery of lipid from a preparation of hepatic lipid of pig[a].

Determination No.	Quantity of lipid theoretically present	Quantity lipid (alcohol-ether extract) recovered	Per cent of lipid (alcohol-ether extract) recovered
	mg.	mg.	
1	414	407	98.5
2	237	236	99.4
3	473	468	99.0
4	407	603	99.4
5	521	515	98.9
6	416	412	99.1
7	437	434	99.2
8	518	516	99.7
9	437	435	99.5
10	527	522	99.1

[a] An ethylene dichloride extracted lipid from pork liver, Armour and Co.

Table III Body weights and nitrogen metabolism of rats fed diets containing nitrogen

Serie

Diet No.	Source of dietary nitrogen	Supplement to vitamin B-complex	Nitrogen force-fed per day	Rat number	Body weight		
					Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period
			mg.		gm.	gm.	gm.
1	Lactalbumin	Rice bran polish (100 mg./day)	74	50131	353	8	1
				50139	361	4	0
				50146	350	1	-3
				50158	379	10	2
				50184	356	4	4
				50199	345	8	2
				Average	357	6	1
2	EAA-I plus NEAA-I	Rice bran polish (100 mg./day)	74	50292	368	7	2
				50327	327	-2	2
				50333	353	-14	-6
				50341	352	1	0
				50377	343	7	3
				50392	374	2	2
				Average	353	0	0
3	EAA-I plus NEAA-I	Folic acid (8 mcg./day)	74	55021	369	-1	3
				55077	333	2	3
				55258	333	8	3
				55084	373	-1	3
				55037	318	12	8
				55051	357	1	1
				Average	347	4	4

fed diets containing nitrogen provided by amino acid mixture simulating a 4.27% lactalbumin diet.

Series I

Body weight			Nitrogen metabolism per 7 days					
age ing - ism od	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
n.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
53	8	1	536	200	353	553	-17	-14
51	4	0	536	223	331	554	-18	-15
50	1	-3	536	233	343	576	-40	-34
79	10	2	536	236	300	536	0	0
56	4	4	536	242	358	600	-64	-54
45	8	2	536	259	335	594	-58	-50
57	6	1	536	232	337	569	-33	-28
58	7	2	541	207	364	571	-30	-24
27	-2	2	541	192	360	552	-11	- 9
53	-14	-6	541	205	389	594	-53	-45
52	1	0	541	205	366	571	-30	-25
43	7	3	541	219	369	564	-23	-20
74	2	2	541	195	381	600	-59	-47
53	0	0	541	204	371	575	-34	-28
69	-1	3	576	194	440	634	-58	-47
33	2	3	576	190	443	633	-57	-51
33	8	3	576	172	439	611	-35	-32
73	-1	3	576	181	459	641	-64	-52
18	12	8	576	199	381	580	- 4	- 4
57	1	1	576	177	402	579	- 3	- 3
47	4	4	576	186	427	613	-37	-32

Table III Continued

Diet No.	Source of dietary nitrogen	Supplement to vitamin B-complex	Nitrogen force-fed per day	Rat number	Body weight: Average during metabolism period	Body weight: Change from beginning to end of period on diet	Body weight: Change during metabolism period
			mg.		gm.	gm.	gm.
4	EAA-I	Folic acid	74	55076	317	2	-1
	plus	(8 mcg./day)		55023	329	0	0
	NEAA-I	plus		55256	321	-7	-2
		vitamin B_{12}		55031	378	-6	-3
		(0.75 mcg./day)		Average	336	-3	-2
5	Mixed	----	602	43159	338	0	0
	natural			43147	364	1	1
	food[a]			43165	315	3	3
				43158	308	10	10
				43159	297	1	1
				43175	340	1	1
				43151	309	3	3
				43152	338	2	2
				43167	348	0	0
				Average	329	2	2

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[a] Offered *ad libitum*

Body weight		Nitrogen metabolism per 7 days					
Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
2	-1	576	186	376	562	+14	+13
0	0	576	181	432	613	-37	-34
-7	-2	576	183	414	597	-21	-20
-6	-3	576	214	437	651	-75	-59
-3	-2	576	191	415	606	-30	-25
0	0	3875	574	3164	3738	+137	+122
1	1	4207	618	3346	3964	+243	+200
3	3	3771	504	3252	3756	+ 15	+ 14
10	10	4002	523	3094	3617	+385	+375
1	1	4233	590	2950	3540	+693	+699
1	1	4361	368	3264	3632	+729	+642
3	3	4438	686	3495	4181	+257	+250
2	2	4284	658	2970	3628	+656	+582
0	0	4744	721	3290	4011	+733	+633
2	2	4213	582	3203	3785	+428	+391

Table IV Liver weights and concentrations of moisture, fat and nitrogen in the liver
lactalbumin (74 mg.N/day) or from an amino acid mixture simulating a 4.27%

Serie

Diet No.	Source of dietary nitrogen	Supplement to vitamin B-complex	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weight of liver		
						Fresh	Dry	Dry fat-f
				gm.		gm.	gm.	gm.
1	Lact-albumin	Rice bran polish (100 mg./day)	50131	353	68.2	8.85	2.81	2.20
			50139	361	67.4	8.83	2.88	2.17
			50146	350	68.6	8.69	2.73	2.19
			50158	379	67.3	8.95	2.92	2.10
			50184	356	70.3	9.22	2.74	2.10
			50199	345	67.1	8.47	2.78	2.06
			Average	357	68.2	8.84	2.81	2.14
2	EAA-I plus NEAA-I	Rice bran polish (100 mg./day)	50292	368	67.4	9.24	3.02	2.14
			50327	327	66.7	8.36	2.79	1.86
			50333	353	68.1	8.28	2.64	1.97
			50341	352	67.6	8.54	2.77	2.04
			50377	343	68.0	8.24	2.64	1.95
			50392	374	68.2	9.33	2.96	2.23
			Average	353	67.7	8.67	2.80	2.03
3	EAA-I plus NEAA-I	Folic acid (8 mcg./day)	55021	369	66.5	9.38	3.14	2.17
			55077	333	66.2	8.49	2.87	2.07
			55258	333	60.5	8.90	3.51	1.80
			55084	373	63.5	9.62	3.51	2.00
			55037	318	69.1	7.61	2.35	1.81
			55051	357	59.5	9.85	3.95	1.97
			Average	347	64.3	8.97	3.22	1.97

trogen in the livers of rats fed diets containing nitrogen provided by 4.27%
simulating a 4.27% lactalbumin diet. (74 mg.N/day)

Series I

Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
				Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
gm.	gm.	gm.	mg.				mg.		
8.85	2.81	2.20	263	3.0	9.3	12.0	615	7.0	21.9
8.83	2.88	2.17	263	3.0	9.1	12.1	706	8.0	24.5
8.69	2.73	2.19	272	3.1	9.1	12.4	543	6.2	19.9
8.95	2.92	2.10	263	2.9	9.0	12.5	821	9.2	28.1
9.22	2.74	2.10	275	3.0	10.1	13.1	639	6.3	23.4
8.47	2.78	2.06	262	3.1	9.4	12.7	724	8.5	26.0
8.84	2.81	2.14	266	3.0	9.5	12.5	675	7.5	24.0
9.24	3.02	2.14	284	3.1	9.4	13.2	871	9.4	28.9
8.36	2.79	1.86	230	2.8	8.3	12.4	931	11.1	33.4
8.28	2.64	1.97	257	3.1	9.7	13.0	667	8.0	25.3
8.54	2.77	2.04	263	3.1	9.5	12.9	728	8.5	26.3
8.24	2.64	1.95	248	3.0	9.4	12.7	684	8.3	25.9
9.33	2.96	2.23	287	3.1	9.7	12.9	734	7.9	24.8
8.67	2.80	2.03	261	3.0	9.3	12.8	769	8.9	27.4
9.38	3.14	2.17	275	2.9	8.8	12.7	975	10.4	31.0
8.49	2.87	2.07	276	3.3	9.6	13.3	798	9.4	27.8
8.90	3.51	1.80	234	2.6	6.7	13.0	1708	19.2	48.6
9.62	3.51	2.00	272	2.8	7.7	13.6	1521	15.8	43.3
7.61	2.35	1.81	225	3.0	9.6	12.4	540	7.1	23.0
9.85	3.95	1.97	253	2.6	6.4	12.8	1980	20.1	50.1
8.97	3.22	1.97	256	2.9	8.1	13.0	1254	13.7	37.3

Table IV Continued

Diet No.	Source of dietary nitrogen	Supplement to vitamin B-complex	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weight of liver		
						Fresh	Dry	Dry fat-
				gm.		gm.	gm.	gm
4	EAA-I plus NEAA-I	Folic acid (8 mcg./day) plus vitamin B_{12} (0.75 mcg./day)	55076	317	66.1	8.38	2.84	1.9
			55023	329	68.0	7.24	2.32	1.7
			55256	321	66.9	8.34	2.76	1.9
			55031	378	64.1	10.13	3.64	2.6
			Average	336	66.3	8.52	2.89	2.0

Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
				Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
gm.	gm.	gm.	mg.				mg.		
8.38	2.84	1.95	233	2.8	8.2	11.9	884	10.6	31.2
7.24	2.32	1.74	255	3.5	11.0	14.6	570	7.9	24.8
8.34	2.76	1.90	230	2.8	8.3	12.1	866	10.4	31.4
10.13	3.64	2.64	288	2.8	7.9	10.9	1005	9.9	27.6
8.52	2.89	2.06	252	3.0	8.8	12.4	831	9.7	28.8

Table V Body weights and nitrogen metabolism of rats fed diets containing nitrogen

Seri
Stu
Experime

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Body weight		
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I			Average during metabolism period	Change from beginning to end of period on diet	Ch du me bo pe
		Total	Cystine N				
	mg.	mg.	mg.		gm.	gm.	
6	34	10	0.47	50162	355	-12	
				50191	373	- 6	
				50265	338	- 4	
				50325	333	-14	
				50342	335	-22	
				50358	331	-15	
				Average	344	-13	
6A	34	20	0.94	50190	358	4	
				50263	341	1	
				50289	323	- 1	
				50329	359	- 5	
				50344	351	- 1	
				50366	366	0	
				Average	350	0	
6B	34	30	1.40	50292	368	7	
				50327	327	- 2	
				50333	353	-14	
				50341	352	1	
				50377	343	7	
				50392	374	2	
				Average	353	0	

diets containing nitrogen provided by EAA-I supplemented with graded doses of nitrogen from NEAA-I

Series II
Study A
Experiment One

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitroge balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
355	-12	-6	408	194	421	615	-207	-175
373	- 6	-2	408	214	402	616	-208	-167
338	- 4	-7	408	218	232	550	-142	-126
333	-14	-12	408	211	408	619	-211	-190
335	-22	-10	408	206	348	554	-146	-131
331	-15	- 9	408	233	382	615	-207	-188
344	-13	- 8	408	213	382	595	-187	-163
358	4	4	476	202	361	563	- 87	- 73
341	1	10	476	218	369	587	-111	- 98
323	- 1	2	476	207	417	624	-148	-137
359	- 5	-8	476	207	347	554	- 78	- 65
351	- 1	-8	476	210	358	568	- 92	- 79
366	0	-6	476	226	381	607	-131	-107
350	0	-1	476	212	372	584	-108	-93
368	7	2	541	207	364	571	- 30	- 24
327	- 2	2	541	192	360	552	- 11	- 9
353	-14	-6	541	205	389	594	- 53	- 45
352	1	0	541	205	366	571	- 30	- 25
343	7	3	541	195	369	564	- 23	- 20
374	2	2	541	219	381	600	- 59	- 47
353	0	0	541	204	371	575	- 34	- 28

Table V Continued

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Body weight		
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I			Average during metabolism period	Change from beginning to end of period on diet	Chang durin meta- bolis perio
		Total	Cystine N				
	mg.	mg.	mg.		gm.	gm.	gm.
4	34	40	1.87	50176	363	6	2
				50295	343	8	1
				50318	352	- 4	2
				50334	354	10	1
				50357	348	4	0
				50374	340	6	- 4
				50422	358	- 4	2
				50436	352	14	4
				50444	385	- 4	- 4
				50463	376	- 3	- 2
				50464	356	- 1	- 2
				Average	357	3	0
6C	34	50	2.34	50462	394	-14	0
				50485	355	0	6
				50537	410	- 4	6
				50609	366	3	5
				50620	352	14	10
				Average	375	0	5
6D	34	60	2.81	50536	357	5	7
				50549	379	- 1	2
				50553	408	- 4	4
				50606	353	8	8
				50621	389	-10	6
				Average	377	- 1	5

Body weight			Nitrogen metabolism					
rage ing a- ism iod	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/
m.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
63	6	2	615	204	369	573	42	35
43	8	1	615	191	383	574	41	36
52	- 4	2	615	212	407	619	- 4	- 3
54	10	1	615	201	393	594	21	18
48	4	0	615	211	392	603	12	10
40	6	- 4	615	195	365	560	55	49
58	- 4	2	621	206	435	641	- 20	- 17
52	14	4	621	199	387	579	42	37
85	- 4	- 4	621	216	427	643	- 22	- 17
76	- 3	- 2	621	223	482	705	- 84	- 67
56	- 1	- 2	621	211	410	621	0	0
57	3	0	618	206	404	610	8	7
94	-14	0	697	211	410	621	76	59
55	0	6	697	168	373	541	156	132
10	- 4	6	697	198	454	652	45	33
66	3	5	697	215	392	607	90	73
52	14	10	697	180	382	502	135	115
75	0	5	697	194	402	597	100	82
57	5	7	762	194	396	590	172	144
79	- 1	2	762	197	480	677	85	66
08	- 4	4	762	224	521	745	17	12
53	8	8	762	200	437	637	125	107
89	-10	6	762	222	501	723	39	30
77	- 1	5	762	207	467	674	88	72

Table VI Liver weights and concentrations of moisture, fat and nitrogen in the livers
with graded doses of nitrogen.

Series
Study
Experime

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I					Fresh
		Total	Cystine N				
	mg.	mg.	mg.		gm.		gm.
6	34	10	0.47	50162	355	69.7	8.6
				50191	373	69.7	9.1
				50265	338	69.0	8.4
				50325	333	68.9	8.5
				50342	335	68.5	7.3
				50358	331	68.5	7.8
				Average	344	69.0	8.3
6A	34	20	0.94	50190	358	68.8	8.8
				50263	341	69.5	8.2
				50289	323	68.2	8.0
				50329	359	68.1	8.5
				50344	351	67.6	8.1
				50366	366	68.8	9.2
				Average	350	68.5	8.5
6B	34	30	1.40	50292	368	67.4	9.2
				50327	327	66.7	8.3
				50333	353	68.1	8.2
				50341	352	67.6	8.5
				50377	343	68.0	8.2
				50392	374	68.2	9.3
				Average	353	67.7	8.6

the livers of rats fed diets containing nitrogen provided by EAA-I supplemented

Series II
Study A
Experiment One

cent ture iver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
.7	8.63	2.61	1.91	244	2.8	9.3	12.8	702	8.1	26.8
.7	9.18	2.78	2.16	270	3.0	9.7	12.5	628	6.8	22.6
.0	8.42	2.62	1.97	246	2.9	9.4	12.5	643	7.6	24.6
.9	8.58	2.67	2.01	235	2.7	8.8	11.7	656	7.6	24.6
.5	7.35	2.32	1.69	235	3.2	10.1	13.9	632	8.6	27.2
.5	7.88	2.48	1.82	248	3.2	10.0	13.6	666	8.4	26.8
.0	8.34	2.58	1.93	246	3.0	9.6	12.8	654	7.9	25.4
.8	8.85	2.79	2.17	278	3.1	10.0	12.8	592	6.7	21.4
.5	8.27	2.51	1.96	252	3.0	10.0	12.9	552	6.7	22.0
.2	8.06	2.57	1.92	249	3.1	9.7	12.9	644	8.0	25.1
.1	8.53	2.73	1.97	247	2.9	9.1	12.5	754	8.8	27.7
.6	8.18	2.65	2.00	274	3.4	10.4	13.7	648	7.9	24.5
.8	9.21	2.88	2.20	279	3.0	9.7	12.7	678	7.4	23.6
.5	8.52	2.68	2.04	263	3.1	9.8	12.9	645	7.6	24.0
.4	9.24	3.02	2.14	284	3.1	9.4	13.2	871	9.4	28.9
.7	8.36	2.79	1.86	230	2.8	8.3	12.4	931	11.1	33.4
.1	8.28	2.64	1.97	257	3.1	9.7	13.0	667	8.0	25.3
.6	8.54	2.77	2.04	263	3.1	9.5	12.9	728	8.5	26.3
.0	8.24	2.64	1.95	248	3.0	9.4	12.7	684	8.3	25.9
.2	9.33	2.96	2.23	287	3.1	9.7	12.9	734	7.9	24.8
.7	8.67	2.80	2.03	261	3.0	9.3	12.8	769	8.9	27.4

Table VI Continued

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I					Fre
		Total	Cystine N				
	mg.	mg.	mg.		gm.		gm
4	34	40	1.87	50176	363	67.6	9.
				50295	343	66.0	8.
				50334	354	67.7	9.
				50357	348	67.8	9.
				50374	340	66.6	8.
				50422	358	67.5	8.
				50436	352	67.9	8.
				50444	385	71.0	10.
				50463	376	67.6	9.
				50464	356	67.5	8.
				Average	358	67.7	9.
6C	34	50	2.34	50462	394	67.9	9.
				50485	355	64.4	8.
				50537	410	66.8	9.
				50609	366	67.2	8.
				50620	352	68.8	8.
				Average	375	67.0	8.
6D	34	60	2.81	50536	357	65.3	8.
				50549	379	65.3	9.
				50553	408	66.0	9.
				50606	353	66.2	8.
				50621	389	66.6	9.
				Average	377	65.9	9.

r cent isture liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
7.6	9.29	3.01	2.05	263	2.8	8.7	12.8	957	10.3	31.8
6.0	8.92	3.03	2.01	257	2.9	8.5	12.8	1023	11.5	33.7
7.7	9.35	3.02	2.18	276	3.0	9.2	12.6	833	8.9	27.6
7.8	9.45	3.04	2.11	285	3.0	9.4	13.5	926	9.8	30.5
6.6	8.83	2.95	2.05	263	3.0	8.9	12.8	896	10.2	30.4
7.5	8.96	2.91	2.21	282	3.1	9.7	12.8	704	7.9	24.2
7.9	8.92	2.86	2.08	266	3.0	9.3	12.8	784	8.8	27.4
1.0	10.19	2.96	2.24	288	2.8	9.7	12.9	714	7.0	24.2
7.6	9.25	3.00	2.10	281	3.0	9.4	13.4	906	9.8	30.2
7.5	8.71	2.83	2.08	275	3.2	9.7	13.2	748	8.6	26.4
7.7	9.19	2.96	2.11	274	3.0	9.2	13.0	849	9.3	28.6
7.9	9.36	3.00	2.25	277	3.0	9.2	12.3	756	8.1	25.2
4.4	8.55	3.05	1.94	241	2.8	7.9	12.4	1110	13.0	36.4
6.8	9.63	3.20	2.34	293	3.0	9.2	12.5	861	8.9	26.9
7.2	8.50	2.79	2.07	257	3.0	9.2	12.4	716	8.4	25.7
8.8	8.61	2.69	2.00	241	2.8	9.0	12.1	693	8.0	25.7
7.0	8.93	2.95	2.12	262	2.9	8.9	12.4	827	9.3	28.0
5.3	8.99	3.11	2.02	248	2.8	8.0	11.9	1093	12.2	35.1
5.3	9.29	3.22	2.12	259	2.8	8.0	12.2	1105	11.9	34.3
6.0	9.72	3.30	2.24	277	2.8	8.4	12.4	1068	11.0	32.3
6.2	8.14	2.75	1.85	246	3.0	8.9	13.3	901	11.1	32.7
6.6	9.10	3.04	2.11	271	3.0	8.9	12.8	927	10.2	30.5
5.9	9.05	3.09	2.07	260	2.9	8.4	12.5	1019	11.2	33.0

Table VII Body weights and nitrogen metabolism of rats fed diets containing nitrogen
has been omitted.

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Body weight		
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I			Average during metabolism period	Change from beginning to end of period on diet	Ch du me bo pe
		Total	Modification				
	mg.	mg.			gm.	gm.	
7	34	40	No cystine nitrogen	50533	358	-2	
				50554	356	-18	
				50613	344	-1	
				50653	374	-2	
				50675	330	-2	
				Average	352	-5	
8	34	40	No tyrosine nitrogen	50544	385	2	
				50622	333	20	
				50674	357	8	
				50713	350	1	
				Average	357	8	

diets containing nitrogen provided by EAA-I plus NEAA-I from which either cystine or tyrosine

Series II
Study A
Experiment Two

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
358	-2	6	624	202	589	791	-167	-134
356	-18	0	624	202	640	842	-213	-182
344	-1	0	624	175	621	796	-172	-150
374	-2	-4	624	199	591	790	-166	-133
330	-2	-4	624	210	612	822	-198	-180
352	-5	0	624	197	511	808	-184	-156
385	2	5	622	188	448	636	- 14	- 11
333	20	10	622	188	380	568	54	48
357	8	2	622	191	458	649	- 27	- 23
350	1	1	622	212	457	669	- 47	- 40
357	8	4	622	195	436	630	- 8	- 6

Table VIII Liver weights and concentrations of moisture, fat and nitrogen in the live from which either cystine or tyrosine has been omitted.

Series
Study
Experime

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed			Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by EAA-I	Non-essential nitrogen provided by NEAA-I					Fres
		Total	Modification				
	mg.	mg.			gm.		gm.
7	34	40	No cystine nitrogen	50533	358	69.2	7.9
				50554	356	68.8	8.2
				50613	344	70.1	8.0
				50653	374	69.3	7.8
				50675	330	69.7	6.9
				Average	352	69.4	7.8
8	34	40	No tyrosine nitrogen	50544	385	67.4	8.6
				50622	333	66.2	8.2
				50674	357	66.9	8.4
				50713	350	67.2	7.8
				Average	357	66.9	8.3

gen in the livers of rats fed diets containing nitrogen provided by EAA-I plus NEAA-I

Series II
Study A
Experiment Two

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.2	7.95	2.44	1.82	204	2.6	8.3	11.2	624	7.8	25.5
68.8	8.26	2.58	1.91	239	2.9	9.3	12.5	670	8.1	26.0
70.1	8.06	2.41	1.92	243	3.0	10.1	12.7	497	6.2	20.6
69.3	7.81	2.40	1.83	238	3.0	9.9	13.0	569	7.3	23.7
69.7	6.96	2.11	1.67	216	3.1	10.2	12.9	440	6.3	20.9
69.4	7.81	2.39	1.83	228	2.9	9.6	12.5	560	7.2	23.3
67.4	8.68	2.83	2.05	267	3.1	9.4	13.0	772	8.9	27.3
66.2	8.27	2.79	1.81	227	2.7	8.1	12.5	981	11.8	35.1
66.9	8.40	2.78	1.94	257	3.1	9.2	13.2	843	10.0	30.3
67.2	7.83	2.57	1.78	251	3.2	9.8	14.1	784	10.0	30.6
66.9	8.30	2.74	1.90	250	3.0	9.1	13.2	845	10.2	30.8

Table IX Body weights and nitrogen metabolism of rats fed diets containing nitrogen
isonitrogenously by methionine and phenylalanine, respectively.

Series
Study
Experiment

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed				Rat number	Body weight	
	Essential nitrogen provided by		Non-essential nitrogen provided by NEAA-I			Average during metabolism period	Change from beginning to end of period on diet
	EAA-I	Other sources	Total	Modification			
	mg.	mg.	mg.			gm.	gm.
9	34	2 mg. methionine N	38	No cystine	50414	368	4
					50421	357	6
					50433	380	-5
					50451	380	10
					50464	380	-1
					50468	349	8
					Average	369	4
10	34	2 mg. phenylalanine N	38	No tyrosine	50416	367	-2
					50423	375	5
					50734	365	2
					50442	352	2
					50450	384	- 2
					50470	381	7
					Average	371	2
11	34	2 mg. methionine N 2 mg. phenylalanine N	36	No cystine No tyrosine	50448	413	-12
					50476	371	5
					50502	371	2
					50535	390	2
					50588	349	11
					Average	379	2

diets containing nitrogen provided by EAA-I plus NEAA-I, cystine and tyrosine being replaced respectively.

Series II
Study A
Experiment Three

Body weight			Nitrogen metabolism					
Average during meta-bolism period	Change from beginning to end of period on diet	Change during meta-bolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
368	4	2	605	206	377	583	22	18
357	6	3	605	209	380	589	16	14
380	-5	-2	605	210	439	649	-44	-34
380	10	2	605	194	405	599	6	5
380	-1	1	605	201	402	603	2	2
349	8	0	605	216	362	578	27	23
369	4	0	605	205	394	599	5	1
367	-2	0	619	216	429	645	-26	-21
375	5	2	619	219	429	648	-29	-22
365	2	-1	619	202	404	606	13	11
352	2	2	619	228	401	629	-10	- 9
384	- 2	-2	619	205	443	648	-29	-23
381	7	1	619	227	425	652	-39	-26
371	2	0	619	216	422	638	-19	-15
413	-12	0	622	198	437	635	-13	-10
371	5	4	622	203	355	558	64	52
371	2	6	622	166	319	485	137	111
390	2	5	622	195	381	576	46	35
349	11	7	622	175	328	505	117	101
379	2	5	622	188	364	552	70	58

Table X Liver weights and concentrations of moisture, fat and nitrogen in the livers
and tyrosine being replaced isonitrogenously by methionine and phenylalanine,

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed				Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by		Non-essential nitrogen provided by NEAA-I					
	EAA-I	Other sources	Total	Modification				Fres
	mg.	mg.	mg.			gm.		gm.
9	34	2 mg. methionine N	38	No cystine	50414	368	68.6	9.4
					50421	357	69.5	9.9
					50433	380	67.9	9.6
					50451	380	67.9	9.5
					50464	380	68.7	9.8
					50468	349	68.4	8.9
					Average	369	68.5	9.5
10	34	2 mg. phenylalanine N	38	No tyrosine	50416	367	67.9	9.6
					50423	375	66.9	10.1
					50734	365	67.6	8.8
					50442	352	67.9	9.7
					50450	384	66.8	10.5
					50470	381	69.4	11.6
					Average	371	67.8	10.0
11	34	2 mg. methionine N 2 mg. phenylalanine N	36	No cystine No tyrosine	50448	413	68.7	9.8
					50476	371	68.8	9.3
					50502	371	68.6	9.2
					50535	390	70.9	9.9
					50588	349	68.7	8.0
					Average	379	69.1	9.2

in the livers of rats fed diets containing nitrogen provided by EAA-I plus NEAA-I, cystine
phenylalanine, respectively.

Series II
Study A
Experiment Three

er cent oisture n liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
68.6	9.45	2.97	2.22	300	3.2	10.1	13.5	752	8.0	25.3
69.5	9.91	3.02	2.22	304	3.1	10.1	13.7	804	8.1	26.6
67.9	9.62	3.09	2.36	316	3.3	10.2	13.4	727	7.6	23.6
67.9	9.54	3.06	2.28	294	3.1	9.6	12.9	785	8.2	25.6
68.7	9.89	3.10	2.30	309	3.1	10.0	13.5	801	8.1	25.8
68.4	8.99	2.84	2.06	288	3.2	10.1	13.9	776	8.6	27.3
68.5	9.57	3.01	2.24	302	3.2	10.0	13.5	774	8.1	25.7
67.9	9.65	3.10	2.17	263	2.7	8.5	12.1	933	9.7	30.1
66.9	10.12	3.35	2.34	315	3.1	9.4	13.5	1009	10.0	30.1
67.6	8.80	2.85	2.05	288	3.3	10.1	14.0	798	9.1	28.0
67.9	9.77	3.13	2.23	302	3.1	9.6	13.6	903	9.2	28.8
66.8	10.57	3.51	2.43	321	3.0	9.1	13.2	1081	10.2	30.8
69.4	11.60	3.54	2.44	337	2.9	9.5	13.8	1103	9.5	31.1
67.8	10.08	3.25	2.28	304	3.0	9.4	13.4	971	9.6	29.8
68.7	9.89	3.10	2.42	320	3.2	10.3	13.2	673	6.8	21.7
68.8	9.35	2.92	2.28	272	2.9	9.3	11.9	636	6.8	21.8
68.6	9.20	2.89	2.25	281	3.0	9.7	12.5	636	6.9	22.0
70.9	9.94	2.90	2.28	282	2.8	9.7	12.3	611	6.2	21.1
68.7	8.06	2.52	1.98	257	3.2	10.2	12.9	534	6.6	21.2
69.1	9.29	2.86	2.24	282	3.0	9.8	12.6	618	6.7	21.6

Table XI Body weights and nitrogen metabolism of rats fed diets containing nitrogen p
from NEAA-II.

Series
Study
Experiment

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight		
	Essential nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II		Average during metabolism period	Change from beginning to end of period on diet	Cha dur met bol per
	mg.	mg.		gm.	gm.	gm
12	38	10	56975	358	-16	-
			56996	342	- 1	
			57044	361	- 8	
			57094	348	4	
			57120	356	- 8	-
			57132	332	11	
			Average	350	- 3	
12A	38	20	56995	330	-14	
			57008	339	- 8	
			57043	363	- 6	
			57065	326	3	
			57101	348	- 2	-
			57118	366	6	
			Average	345	- 4	
12B	38	30	56974	340	5	
			57001	366	10	
			57018	362	- 1	-
			57055	346	8	
			57093	363	- 1	-
			57117	354	8	
			Average	355	2	

ets containing nitrogen provided by EAA-II supplemented with graded doses of nitrogen

Series II
Study A
Experiment Four

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
358	-16	-4	425	191	326	517	-92	-77
342	- 1	7	425	132	283	415	10	9
361	- 8	0	425	177	283	460	-35	-29
348	4	3	425	194	261	455	-30	-26
356	- 8	-2	425	169	290	459	-34	-29
332	11	6	425	180	265	445	-20	-18
350	- 3	2	425	174	285	459	-34	-28
330	-14	4	471	190	331	521	-50	-46
339	- 8	0	471	205	318	523	-52	-46
363	- 6	0	471	172	293	465	6	- 5
326	3	3	471	198	306	504	-33	-30
348	- 2	-2	471	178	322	500	-29	-25
366	6	3	471	196	297	493	-22	-18
345	- 4	1	471	190	311	501	-30	-26
340	5	1	529	185	360	550	-21	-19
366	10	2	529	181	283	464	65	53
362	- 1	-1	529	181	369	550	-21	-17
346	8	5	529	181	366	547	-18	-16
363	- 1	-3	529	180	415	595	-66	-55
354	8	9	529	189	280	469	60	51
355	2	5	529	183	346	529	0	0

Table XI Continued

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight	
	Essential nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II		Average during metabolism period	Change from beginning to end of period on diet
	mg.	mg.		gm.	gm.
12C	38	40	57009	345	-11
			57054	328	- 4
			57081	346	0
			57119	361	- 1
			57135	365	1
			57171	360	14
			Average	351	0
12D	38	50	56985	366	2
			57007	346	9
			57042	347	1
			57078	342	6
			57103	365	2
			57131	362	6
			Average	355	4
12E	38	60	56991	332	4
			57041	368	2
			57079	347	2
			57125	353	5
			57134	362	7
			57172	343	6
			Average	351	4

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
345	-11	-5	584	184	409	593	- 9	- 8
328	- 4	3	584	189	401	590	- 6	- 5
346	0	1	584	182	399	581	3	3
361	- 1	-2	584	178	369	547	37	30
365	1	-1	584	170	403	573	11	9
360	14	5	584	188	393	581	3	2
351	0	0	584	182	396	578	6	5
366	2	3	617	186	450	636	-19	-16
346	9	15	617	185	378	563	54	47
347	1	-4	617	212	432	644	-27	-23
342	6	12	617	212	445	657	-40	-35
365	2	-7	617	215	502	717	-100	-82
362	6	12	617	176	419	595	22	18
355	4	5	617	198	438	635	-18	-15
332	4	2	654	167	432	599	55	50
368	2	2	654	216	530	748	-94	-76
347	2	1	654	188	493	681	-27	-23
353	5	-2	654	180	543	723	-69	-58
362	7	2	654	187	441	628	26	21
343	6	2	654	214	452	666	-12	10
351	4	1	654	192	482	674	-20	-17

Table XII Liver weights and concentrations of moisture, fat and nitrogen in the live
supplemented with graded doses of nitrogen from NEAA-II.

Series
Study
Experimen

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II				Fr
	mg.	mg.		gm.		g
12	38	10	56975	358	71.0	9
			56996	342	70.4	7
			57044	361	68.9	8
			57094	348	69.7	8
			57120	356	69.4	8
			57132	332	69.9	7
			Average	350	69.9	8
12A	38	20	56995	330	69.9	7
			57008	339	70.5	8
			57043	363	70.3	8
			57065	326	70.7	7
			57101	348	69.8	8
			57118	366	70.1	8
			Average	345	70.2	8
12B	38	30	56974	340	70.4	8
			57001	366	70.1	8
			57018	362	70.2	8
			57055	346	69.2	7
			57093	363	67.4	8
			57117	354	69.1	7
			Average	355	69.4	8

en in the livers of rats fed diets containing nitrogen provided by EAA-II

Series II
Study A
Experiment Four

er cent oisture n liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				gm.		
71.0	9.68	2.81	2.42	282	2.9	10.0	11.7	392	4.0	14.0
70.4	7.74	2.30	1.93	254	3.3	11.1	13.2	368	4.8	16.0
68.9	8.17	2.54	2.10	262	3.2	10.3	12.5	441	5.4	17.4
69.7	8.14	2.47	2.06	249	3.1	10.1	12.1	404	5.0	16.3
69.4	8.38	2.56	2.15	279	3.3	10.9	13.0	416	5.0	16.2
69.9	7.96	2.40	2.04	264	3.3	11.0	12.9	350	4.4	14.6
69.9	8.34	2.51	2.12	264	3.2	10.6	12.6	395	4.8	15.8
69.9	7.24	2.18	1.77	252	3.5	11.6	14.2	412	5.7	18.9
70.5	8.22	2.42	2.05	278	3.4	11.5	13.6	377	4.6	15.5
70.3	8.56	2.54	3.00	278	3.2	10.9	13.3	447	5.2	17.6
70.7	7.66	2.25	1.90	255	3.3	11.4	13.4	350	4.6	15.6
69.8	8.41	2.54	2.24	277	3.2	10.9	12.4	300	3.5	11.8
70.1	8.96	2.68	2.18	279	3.1	10.4	12.8	496	5.5	18.5
70.2	8.18	2.44	2.04	270	3.3	11.1	13.3	397	4.8	16.3
70.4	8.35	2.47	2.02	284	3.4	11.5	14.0	449	5.4	18.2
70.1	8.62	2.57	2.17	295	3.4	11.5	13.6	408	4.7	15.8
70.2	8.57	2.56	2.16	289	3.4	11.3	13.4	392	4.6	15.3
69.2	7.92	2.44	2.08	264	3.3	10.8	12.7	360	4.5	14.7
67.4	8.01	2.61	2.09	279	3.5	10.7	13.3	518	6.5	19.8
69.1	7.99	2.39	1.93	256	3.2	10.7	13.3	459	5.8	18.6
69.4	8.24	2.51	2.08	278	3.4	11.1	13.4	431	5.2	17.1

Table XII Continued

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II				Fres
	mg.	mg.		gm.		gm.
12C	38	40	57009	345	69.7	7.2
			57054	328	70.2	6.9
			57081	346	70.7	7.7
			57119	361	69.2	8.0
			57135	365	68.5	7.7
			57171	360	69.8	8.6
			Average	351	69.7	7.7
12D	38	50	56985	366	69.2	8.6
			57007	346	69.9	7.4
			57042	347	69.8	7.61
			57078	342	69.2	7.4
			57103	365	69.6	7.8
			57131	362	69.2	7.9
			Average	355	69.5	7.8
12E	38	60	56991	332	70.0	7.2
			57041	368	69.4	8.6
			57079	347	69.8	7.4
			57125	353	68.2	7.21
			57134	362	68.4	7.9
			57172	343	68.8	7.71
			Average	351	69.1	7.7

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				gm.		
69.7	7.25	3.00	1.87	267	3.7	12.2	14.3	329	4.5	15.0
70.2	6.90	2.06	1.73	251	3.6	12.2	14.5	331	4.8	16.1
70.7	7.75	2.27	1.90	259	3.3	11.4	13.6	368	4.8	16.2
69.2	8.06	2.48	3.00	268	3.3	10.8	12.8	385	4.8	15.5
68.5	7.79	2.46	1.87	264	3.4	10.8	14.1	586	7.5	23.9
69.8	8.62	2.60	2.08	279	3.2	10.7	13.4	520	6.0	20.0
69.7	7.73	2.34	1.92	265	3.4	11.3	13.8	420	5.4	17.8
69.2	8.60	2.64	2.18	288	3.4	10.9	13.2	468	5.4	17.7
69.9	7.46	2.24	1.86	254	3.4	11.3	13.6	384	5.2	17.1
69.8	7.64	2.31	1.86	269	3.5	10.7	14.5	448	5.9	19.4
69.2	7.42	2.29	1.90	249	3.4	10.9	13.1	389	5.2	17.0
69.6	7.80	2.37	1.92	261	3.3	11.0	13.6	453	5.8	19.1
69.2	7.98	2.46	2.04	269	3.4	10.9	13.2	421	5.3	17.1
69.5	7.82	2.39	1.96	265	3.4	11.0	13.5	427	5.5	17.9
70.0	7.25	2.17	1.76	241	3.3	11.1	13.7	410	5.7	18.9
69.4	8.63	2.64	2.14	294	3.4	11.1	13.8	506	5.9	19.1
69.8	7.47	2.26	1.82	261	3.5	11.6	14.3	438	5.9	19.4
68.2	7.21	2.29	1.87	250	3.5	10.9	13.4	425	6.0	18.5
68.4	7.90	2.50	1.94	259	3.3	10.4	13.4	560	7.1	22.4
68.8	7.74	2.41	1.93	256	3.3	10.6	13.2	481	6.2	19.9
69.1	7.70	2.38	1.91	260	3.4	10.9	13.6	470	6.1	19.7

Table XIII Body weights and nitrogen metabolism of rats fed diets containing nitrog

Serie
Stud
Experime

Diet	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight		
	Essential amino acid nitrogen provided by EAA-III	Non-essential nitrogen provided by NEAA-II		Average during metabolism period	Change from beginning to end of period on diet	Ch du me bo pe
	mg.	mg.		gm.	gm.	
13	39.5	10	58562	391	-16	
			58564	334	8	
			58570	328	4	
			58588	326	2	
			58736	300	- 2	
			58767	339	3	
			Average	336	0	
13A	39.5	34.5	58554	345	9	
			58563	374	10	
			58569	329	9	
			58587	358	5	
			58731	285	7	
			58760	343	10	
			58895	342	- 8	
			58832	345	5	
			58881	359	- 7	
			59056	349	4	
			59002	339	0	
			59062	345	7	
			Average	343	4	

ets containing nitrogen provided by EAA-III supplemented with graded doses of nitrogen from NEAA-II

Series II
Study A
Experiment Five

Body weight			Nitrogen metabolism					
rage ing a- ism iod	Change from beginning to end of period on diet	Change during meta-bolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
m.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
91	-16	0	415	182	327	509	-94	-72
34	8	7	415	184	303	487	-72	-65
28	4	5	415	166	302	468	-53	-49
26	2	4	415	190	279	469	-54	-50
00	- 2	5	415	170	274	444	-29	-29
39	3	6	415	167	246	413	2	2
36	0	4	415	176	288	464	-49	-44
45	9	8	559	191	287	478	81	70
74	10	7	559	198	298	496	63	50
29	9	9	559	190	335	525	34	31
58	5	7	559	180	341	521	38	32
85	7	8	559	169	294	463	96	101
43	10	6	559	175	307	482	77	67
42	- 8	4	612	194	405	599	13	11
45	5	3	612	167	356	523	89	77
59	- 7	1	612	203	419	622	-10	- 8
49	4	7	612	219	364	583	29	25
39	0	6	612	180	373	553	59	52
45	7	7	612	187	324	511	101	88
43	4	6	586	188	342	530	56	50

Table XIII Continued

Diet	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight		
	Essential amino acid nitrogen provided by EAA-III	Non-essential nitrogen provided by NEAA-II		Average during metabolism period	Change from beginning to end of period on diet	Cha dur met bol per
	mg.	mg.		gm.	gm.	g
13B	39.5	45	58857	334	7	:
			59034	348	2	
			58899	343	3	
			58878	342	6	
			59065	355	0	
			58999	352	9	
			Average	346	4	
13C	39.5	60	58990	356	3	
			59014	336	10	
			59033	327	5	
			59088	329	6	
			59804	355	3	
			Average	341	5	

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
334	7	10	660	195	392	587	73	66
348	2	3	660	193	374	567	93	80
343	3	5	660	170	387	557	103	90
342	6	5	660	208	407	615	45	40
355	0	4	660	220	392	612	48	40
352	9	6	660	195	400	595	65	56
346	4	6	660	197	392	589	71	62
356	3	2	810	220	503	723	87	73
336	10	7	810	195	436	631	179	160
327	5	5	810	174	434	608	202	185
329	6	3	810	223	502	725	85	77
355	3	7	810	208	481	689	121	102
341	5	5	810	204	471	675	135	119

Table XIV Liver weights and concentrations of moisture, fat and nitrogen of livers with graded doses of nitrogen from NEAA-II.

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Experim

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential amino acid nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II				Fr
	mg.	mg.		gm.		g
13	39.5	10	58562	391	70.5	9
			58564	334	71.2	8
			58570	328	70.1	7
			58588	326	69.4	7
			58736	300	69.0	6
			58767	339	69.0	7
			Average	336	69.8	8
13A	39.5	34.5	58554	345	69.5	7
			58563	374	69.7	8
			58569	329	70.2	8
			58587	358	69.2	8
			58731	285	72.0	6
			58760	343	69.3	7
			58895	342	69.1	7
			58832	345	69.8	8
			58881	359	69.5	8
			59056	349	68.7	8
			59002	339	69.8	8
			59062	345	69.0	8
			Average	342	69.6	8

gen of livers of rats fed diets containing nitrogen provided by EAA-III supplemented

Series II
Study A
Experiment Five

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
70.5	9.24	2.73	2.14	300	3.2	11.0	14.0	590	6.4	21.6
71.2	8.62	2.48	1.87	278	3.2	11.2	14.9	611	7.1	24.7
70.1	7.74	2.32	1.69	250	3.2	10.8	14.8	622	8.0	26.8
69.4	7.65	2.34	1.76	245	3.2	10.5	13.9	584	7.6	24.9
69.0	6.96	2.16	1.61	227	3.3	10.5	14.1	552	7.9	25.5
69.0	7.79	2.42	1.83	255	3.3	10.5	13.9	581	7.5	24.1
69.8	8.00	2.41	1.82	259	3.2	10.8	14.3	590	7.4	24.6
69.5	7.62	2.32	1.81	264	3.5	11.4	14.6	512	6.7	22.0
69.7	8.48	2.56	1.99	290	3.4	11.3	14.6	575	6.8	22.4
70.2	8.05	2.40	1.87	275	3.4	11.5	14.7	534	6.6	22.2
69.2	8.97	2.76	2.12	298	3.3	10.8	14.1	638	7.1	23.1
72.0	6.83	1.91	1.48	232	3.4	12.1	15.7	435	6.4	22.8
69.3	7.86	2.41	1.85	269	3.4	11.2	14.5	560	7.1	23.2
69.1	7.81	2.42	1.86	258	3.3	10.7	13.9	558	7.1	23.1
69.8	8.18	2.47	1.94	256	3.1	10.4	13.2	528	6.4	21.4
69.5	8.50	2.59	2.02	283	3.3	10.9	14.0	570	6.7	22.0
68.7	8.68	2.72	2.14	275	3.2	10.1	12.9	572	6.6	21.1
69.8	8.67	2.62	2.13	271	3.1	10.3	12.7	489	5.6	18.7
69.0	8.67	2.69	2.05	265	3.1	9.9	12.9	633	7.3	23.6
69.6	8.20	2.49	1.94	270	3.3	10.9	14.0	550	6.7	22.1

Table XIV Continued

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed		Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential amino acid nitrogen provided by EAA-II	Non-essential nitrogen provided by NEAA-II				F
	mg.	mg.		gm.		
13B	39.5	45	58857	334	69.4	7
			59034	348	68.8	8
			58899	343	69.0	7
			58878	342	68.4	7
			59065	355	68.8	8
			58999	352	69.6	8
			Average	346	69.0	8
13C	39.5	60	58990	356	68.2	7
			59014	336	68.9	7
			59033	327	68.8	7
			59088	329	68.8	7
			59804	355	67.8	8
			Average	341	68.5	7

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.4	7.42	2.27	1.72	242	3.3	10.7	14.1	553	7.5	24.4
68.8	8.12	2.53	1.91	266	3.3	10.5	13.9	616	7.6	24.4
69.0	7.90	2.45	1.87	261	3.3	10.7	14.0	581	7.4	23.7
68.4	7.97	2.52	1.89	259	3.2	10.3	13.7	625	7.8	24.8
68.8	8.52	2.66	1.98	284	3.3	10.7	14.3	680	8.0	25.6
69.6	8.24	2.50	1.98	266	3.2	10.6	13.4	521	6.3	20.8
69.0	8.03	2.49	1.89	263	3.3	10.6	13.9	596	7.4	23.9
68.2	7.82	2.49	1.79	271	3.5	10.9	15.1	702	9.0	28.2
68.9	7.99	2.48	1.87	265	3.3	10.7	14.2	618	7.7	24.9
68.8	7.17	2.24	1.68	248	3.5	11.1	14.8	557	7.8	24.9
68.8	7.36	2.29	1.72	247	3.4	10.8	14.4	571	7.8	24.9
67.8	8.49	2.73	2.08	275	3.2	10.1	13.2	650	7.6	23.8
68.5	7.77	2.45	1.83	261	3.4	10.7	14.3	620	8.0	25.3

Table XV Body weights and nitrogen metabolism of rats fed diets containing nitrogen of non-essential amino acids or from glutamic acid alone.

Serie
Stud

Diet	Distribution of nitrogen in daily dose of amino acid mixture fed				Rat number	Body weight		
	Essential nitrogen		Non-essential nitrogen			Average during metabolism period	Change from beginning to end of period on diet	Char duri meta boli peri
	Quantity	Source	Quantity	Source				
	mg.		mg.			gm.	gm.	gm
14	34	EAA-I	40	Glutamic acid	55022	318	-13	-1
					55036	336	-29	-8
					55259	323	-24	-9
					55068	328	-14	-1
					55097	361	-16	-4
					55073	331	- 9	-3
					Average	333	-18	-4
15	38	EAA-II	36	Glutamic acid	55122	334	2	-3
					55257	337	7	0
					55052	373	- 1	-1
					55100	335	- 4	0
					54993	321	- 6	0
					55067	370	- 6	0
					Average	345	- 1	-1

diets containing nitrogen provided by EAA-I or EAA-II supplemented with nitrogen from a mixture
d alone.

Series II
Study B

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
318	-13	-1	578	189	663	852	-274	-259
336	-29	-8	578	203	681	884	-306	-273
323	-24	-9	578	196	631	827	-249	-231
328	-14	-1	578	189	629	818	-240	-220
361	-16	-4	578	194	641	835	-257	-214
331	- 9	-3	578	185	587	772	-194	-176
333	-18	-4	578	192	639	831	-253	-229
334	2	-3	576	177	393	570	6	5
337	7	0	576	179	393	572	4	4
373	- 1	-1	576	186	369	555	21	17
335	- 4	0	576	184	381	565	11	10
321	- 6	0	576	184	417	601	-25	-23
370	- 6	0	576	197	426	623	-47	-38
345	- 1	-1	576	185	396	581	- 5	- 4

Table XVI Liver weights and concentration of moisture, fat and nitrogen of livers of
with nitrogen from a mixture of non-essential amino acids or from glutamic

Series
Study

Diet No.	Distribution of nitrogen in daily dose of amino acid mixture fed				Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	
	Essential nitrogen		Non-essential nitrogen					
	Quantity	Source	Quantity	Source				Fresh
	mg.		mg.			gm.		gm.
14	34	EAA-I	40	Glutamic acid	55022	318	68.2	8.02
					55036	336	69.7	7.89
					55259	323	69.6	7.33
					55068	328	69.9	7.39
					55097	361	67.4	9.31
					55073	331	69.6	7.89
					Average	333	69.1	7.97
15	38	EAA-II	36	Glutamic acid	55122	334	69.7	9.05
					55257	337	69.1	8.70
					55052	373	69.1	9.00
					55100	335	69.9	7.81
					54993	321	67.9	8.00
					55067	370	68.0	9.68
					Average	345	69.0	8.71

n of livers of rats fed diets containing nitrogen provided by EAA-I or EAA-II supplemented from glutamic acid alone.

Series II
Study B

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry-fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
68.2	8.02	2.55	2.18	256	3.2	10.0	11.8	374	4.7	14.9
69.7	7.89	2.39	1.99	209	2.6	8.8	10.5	402	5.1	16.8
69.6	7.33	2.23	1.73	244	3.3	11.0	14.1	502	6.8	22.4
69.9	7.39	2.22	1.89	248	3.4	11.2	13.2	336	4.5	15.1
67.4	9.31	3.04	2.48	249	2.6	8.2	10.0	558	6.0	18.4
69.6	7.89	2.40	1.96	242	3.1	10.1	12.3	433	5.5	18.1
69.1	7.97	2.47	2.04	241	3.0	9.9	12.0	434	5.4	17.6
69.7	9.05	2.74	2.29	251	2.8	9.2	11.0	452	5.0	16.5
69.1	8.70	2.69	2.16	273	3.1	10.1	12.7	535	6.2	19.9
69.1	9.00	2.78	2.33	291	3.2	10.5	12.5	450	5.0	16.2
69.9	7.81	2.35	2.10	248	3.2	10.6	11.8	252	3.2	10.7
67.9	8.00	2.57	2.22	241	3.0	9.4	10.9	350	4.4	13.6
68.0	9.68	3.09	2.69	288	3.0	9.3	10.7	401	4.1	12.9
69.0	8.71	2.70	2.30	265	3.0	9.8	11.6	407	4.6	15.0

Table XVII Body weights and nitrogen metabolism of rats fed diets containing essenti being fed.

Seri
Stu
Experim

Diet No.	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight		
	Source	Quantity		Average during metabolism period	Change from beginning to end of period on diet	Cha dur meta boli peri
		mg.		gm.	gm.	g
16	EAA-I only	34	50147	344	-12	-4
			50161	365	-15	-9
			50324	347	-16	-6
			50330	346	- 7	4
			50343	329	-17	-7
			50359	332	-16	-7
			Average	344	-14	-5
16a	EAA-I only	74	50429	351	0	-4
			50435	371	-19	1
			50443	384	4	
			50477	382	- 6	-2
			50483	359	4	
			Average	369	- 4	-1
17	EAA-II only	38	57689	367	-16	1
			57697	363	- 9	2
			57726	334	-13	3
			57768	349	-13	-2
			58338	322	-19	0
			58368	312	-13	1

d diets containing essential amino acid nitrogen only, three mixtures of varying nutritive value

Series II
Study C
Experiment One

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
344	-12	-4	349	206	410	616	-267	-233
365	-15	-9	349	196	411	607	-260	-212
347	-16	-6	349	214	375	589	-240	-207
346	- 7	4	349	198	446	644	-295	-257
329	-17	-7	349	193	373	566	-217	-198
332	-16	-7	349	199	385	584	-235	-212
344	-14	-5	349	201	400	601	-252	-220
351	0	-4	691	201	549	750	- 59	- 50
371	-19	1	691	216	611	827	-136	-110
384	4	0	691	220	523	743	- 52	- 40
382	- 6	-2	691	241	606	847	-156	-123
359	4	2	691	196	528	724	- 33	- 27
369	- 4	-1	691	215	564	778	- 87	- 70
367	-16	1	319	222	291	513	-194	-159
363	- 9	2	319	211	257	468	-149	-123
334	-13	3	319	197	274	471	-152	-136
349	-13	-2	319	182	252	434	-115	- 99
322	-19	0	319	179	247	426	-107	-100
312	-13	1	319	236	272	508	-189	-182

Table XVII Continued

Diet No.	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed		Rat number	Body weight	
	Source	Quantity		Average during metabolism period	Change from beginning to end of period on diet
		mg.		gm.	gm.
17a	EAA-II only	74	57695	338	- 4
			57739	354	-14
			57744	382	- 4
			57753	336	7
			58330	325	- 3
			58360	359	- 4
			Average	349	- 4
18	EAA-III only	39.5	59563	338	2
			59443	369	- 4
			59612	339	- 5
			59537	347	-10
			59512	363	- 2
			59578	343	- 4
			Average	350	- 4
18a	EAA-III only	74	59541	377	9
			59506	379	7
			59444	357	5
			59577	356	12
			59561	354	8
			Average	365	8

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
338	- 4	6	602	177	415	592	10	9
354	-14	2	602	226	405	631	-29	-25
382	- 4	2	602	195	397	592	10	8
336	7	7	602	214	397	611	- 9	- 8
325	- 3	5	602	194	322	516	86	80
359	- 4	3	602	195	342	537	65	54
349	- 4	4	602	200	380	580	22	19
338	2	0	359	199	297	496	-137	-122
369	- 4	5	359	199	304	503	-144	-117
339	- 5	2	359	214	315	529	-170	-150
347	-10	0	359	214	349	563	-204	-176
363	- 2	2	359	203	315	518	-159	-131
343	- 4	1	359	185	311	496	-137	-120
350	- 4	2	359	202	315	517	-158	36
377	9	7	609	186	384	570	39	31
379	7	5	609	186	403	589	20	16
357	5	5	609	184	366	550	59	50
356	12	8	609	191	372	563	46	39
354	8	2	609	185	377	562	47	40
365	8	5	609	186	380	567	42	35

Table XVIII Liver weights and concentrations of moisture, fat and nitrogen of livers three mixtures of varying nutritive value being fed.

Serie
Stud
Experim

Diet No.	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed: Source	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed: Quantity	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Fre
		mg.		gm.		gm.
16	EAA-I only	34	50147	345	69.0	8.
			50161	365	68.4	9.
			50324	347	68.1	8.
			50330	346	67.8	8.
			50343	329	68.7	7.
			50359	332	69.4	8.
			Average	344	68.6	8.
16A	EAA-I only	74	50429	357	68.7	8.
			50435	401	69.3	9.
			50443	392	69.1	8.
			50477	390	69.6	8.
			50483	372	68.2	8.
			Average	382	69.0	8.
17	EAA-II only	38	57689	367	68.4	8.6
			57697	363	68.8	8.6
			57726	334	68.8	8.3
			57768	349	68.2	8.6
			58338	322	68.4	7.9
			58368	312	68.9	6.4
			Average	341	68.6	8.1

ogen of livers of rats fed diets containing essential amino acid nitrogen only,

Series II
Study C
Experiment One

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.0	8.28	2.57	1.91	247	3.0	9.6	12.9	655	7.9	25.5
68.4	9.08	2.86	2.07	271	3.0	9.5	13.1	794	8.7	27.7
68.1	8.23	2.62	1.87	240	2.9	7.6	12.8	752	9.1	28.7
67.8	8.14	2.62	1.97	248	3.0	9.5	12.6	645	7.9	24.6
68.7	7.64	2.39	1.74	234	3.1	9.8	13.4	652	8.8	27.2
69.4	8.39	2.57	2.00	231	2.8	8.9	11.6	568	6.8	22.1
68.6	8.29	2.61	1.93	245	3.0	9.2	12.7	678	8.2	26.0
68.7	8.59	2.68	2.02	258	3.0	9.6	12.8	665	7.8	24.8
69.3	9.92	3.64	2.46	316	3.2	8.7	12.8	581	5.8	19.1
69.1	8.44	2.62	2.04	266	3.2	10.2	13.0	580	6.9	22.2
69.6	8.44	2.53	2.00	282	3.3	11.0	14.1	565	6.7	22.1
68.2	8.57	2.73	2.10	282	3.3	10.3	13.4	625	7.3	22.9
69.0	8.80	2.73	2.12	281	3.2	10.0	13.2	603	6.9	22.2
68.4	8.69	2.75	2.10	301	3.5	11.0	14.3	654	7.5	23.8
68.8	8.66	2.70	2.08	292	3.4	10.8	14.0	626	7.2	23.2
68.8	8.30	2.59	1.96	281	3.4	10.9	14.3	626	7.6	24.2
68.2	8.62	2.74	2.00	256	3.0	9.3	12.8	743	8.6	27.1
68.4	7.93	2.51	1.90	249	3.1	9.9	13.1	608	7.7	24.3
68.9	6.46	2.01	1.44	223	3.4	11.1	15.5	573	8.9	28.5
68.6	8.11	2.55	1.91	267	3.3	10.5	14.0	639	7.9	25.2

Table XVIII Continued

Diet No.	Source and quantity of essential nitrogen in daily dose of amino acid mixture fed		Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	W
	Source	Quantity				Fresh
		mg.		gm.		gm.
17A	EAA-II only	74	57695	338	67.6	7.85
			57739	354	67.1	8.22
			57744	382	67.3	8.42
			57753	336	66.5	7.61
			58330	325	68.6	7.74
			58360	359	66.3	8.22
			Average	349	67.2	8.01
18	EAA-III only	39.5	59563	338	70.3	7.84
			59443	369	69.9	8.38
			59612	339	70.0	7.44
			59537	347	68.6	8.25
			59512	363	68.9	8.84
			59578	343	69.8	8.24
			Average	350	69.6	8.17
18A	EAA-III only	74	59541	377	69.2	8.53
			59506	379	70.0	8.38
			59444	357	68.7	7.58
			59577	356	68.6	9.17
			59561	354	70.0	8.10
			Average	365	69.3	8.35

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
67.6	7.85	2.54	1.95	272	3.5	10.7	13.9	595	7.6	23.4
67.1	8.22	2.71	2.00	289	3.5	10.7	14.4	710	8.6	26.2
67.3	8.42	2.75	2.08	291	3.5	10.6	14.0	670	8.0	24.3
66.5	7.61	2.55	1.80	244	3.2	9.6	13.6	747	9.8	29.3
68.6	7.74	2.43	1.86	256	3.3	10.5	13.8	566	7.3	23.2
66.3	8.22	2.77	2.15	272	3.3	9.8	12.7	619	7.5	22.4
67.2	8.01	2.62	1.97	271	3.4	10.3	13.7	651	8.1	24.8
70.3	7.84	2.33	1.81	269	3.4	11.5	14.9	520	6.6	22.4
69.9	8.38	2.52	1.96	279	3.3	11.1	14.2	559	6.7	22.2
70.0	7.44	2.24	1.78	264	3.6	11.8	14.8	454	6.1	20.3
68.6	8.25	2.59	2.06	260	3.2	10.0	12.6	532	6.4	20.5
68.9	8.84	2.75	2.20	275	3.1	10.0	12.5	554	6.3	20.2
69.8	8.24	2.49	2.01	257	3.1	10.3	12.8	483	5.9	19.4
69.6	8.17	2.49	1.97	267	3.3	10.8	13.6	517	6.3	20.8
69.2	8.53	2.63	1.95	295	3.5	11.2	15.1	674	7.9	25.7
70.0	8.38	2.52	1.94	292	3.5	11.6	15.1	580	6.9	23.0
68.7	7.58	2.37	1.78	267	3.5	11.3	15.0	597	7.9	25.2
68.6	9.17	2.88	2.28	280	2.0	9.7	12.3	596	6.5	20.7
70.0	8.10	2.43	1.89	280	3.5	11.5	14.8	544	6.7	22.4
69.3	8.35	2.56	1.97	283	3.4	11.1	14.5	598	7.2	23.4

Table XIX Body weights and nitrogen metabolism of rats fed diets containing graded dos

Series
Study
Experimen

Diet No.	Quantity of nitrogen in daily dose of EAA-III	Rat number	Body weight		
			Average during meta-bolism period	Change from beginning to end of period on diet	Chang durin meta-bolis perio
	mg.		gm.	gm.	gm.
18A	50	59519	360	-4	2
		59487	334	0	5
		59570	344	-3	-1
		59490	369	-3	0
		59625	362	-5	3
		59579	346	3	0
		Average	352	-2	2
18B	60	59562	350	-6	2
		59505	345	-1	10
		59580	340	-3	0
		59528	359	9	7
		59594	376	0	1
		59536	355	2	2
		Average	354	0	3
18D	80	59564	341	7	3
		59626	379	14	3
		59593	372	2	4
		59491	349	10	11
		59534	364	5	5
		Average	361	8	5

iets containing graded doses of nitrogen provided by essential amino acids only (EAA-III).

Series II
Study C
Experiment Two

Body weight			Nitrogen metabolism					
verage uring eta- olism eriod	Change from beginning to end of period on diet	Change during meta- bolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
360	-4	2	455	211	337	544	-89	-74
334	0	5	455	193	329	522	-67	-60
344	-3	-1	455	211	342	553	-98	-86
369	-3	0	455	207	360	567	-112	-91
362	-5	3	455	241	359	600	-145	-120
346	3	0	455	190	323	513	-58	-50
352	-2	2	455	209	341	550	-95	-80
350	-6	2	530	208	402	610	-80	-69
345	-1	10	530	182	348	530	0	0
340	-3	0	530	210	387	597	-67	-59
359	9	7	530	201	356	557	-27	-23
376	0	1	530	213	478	691	-161	-128
355	2	2	530	213	411	624	-94	-80
354	0	3	530	204	397	601	-71	-60
341	7	3	680	176	401	577	103	91
379	14	3	680	182	404	586	94	74
372	2	4	680	181	448	629	51	41
349	10	11	680	187	410	597	83	71
364	5	5	680	196	438	634	46	38
361	8	5	680	185	420	605	75	63

Table XX Liver composition and concentrations of moisture, fat and nitrogen of livers by essential amino acids (EAA-III).

Series
Study
Experiment

Diet No.	Quantity of nitrogen in daily dose of EAA-III	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	We
					Fresh
	mg.		gm.		gm.
18A	50	59519	360	69.9	7.86
		59487	334	70.5	8.03
		59570	344	70.7	7.73
		59490	369	68.2	7.79
		59625	362	69.4	8.76
		59579	346	68.7	7.50
		Average	352	69.6	7.95
18B	60	59562	350	69.4	8.13
		59505	345	70.0	7.40
		59580	340	69.3	6.77
		59528	359	69.8	8.40
		59594	376	68.0	9.95
		59536	355	68.5	8.51
		Average	354	69.2	8.20
18D	80	59564	341	69.4	7.92
		59626	379	69.5	8.58
		59593	372	68.7	9.53
		59491	349	68.9	9.01
		59534	364	68.0	9.53
		Average	361	69.0	8.92

trogen of livers of rats fed diets containing graded doses of nitrogen provided

Series II
Study C
Experiment Two

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.9	7.86	2.47	1.80	275	3.5	11.1	15.3	669	8.2	27.1
70.5	8.03	2.10	1.52	241	3.0	11.5	15.9	574	8.1	27.3
70.7	7.73	2.10	1.64	250	3.2	11.9	15.2	458	6.4	21.8
68.2	7.79	2.48	1.93	277	3.6	11.2	14.4	546	7.0	22.0
69.4	8.76	2.68	2.04	288	3.3	10.8	14.1	642	7.4	24.0
68.7	7.50	2.59	1.99	263	3.5	10.2	13.2	601	7.3	23.2
69.6	7.95	2.40	1.82	266	3.4	11.1	14.7	582	7.4	24.3
69.4	8.13	2.49	1.88	278	3.4	11.2	14.8	603	7.4	24.2
70.0	7.40	2.22	1.66	255	3.4	11.5	15.4	558	7.5	25.1
69.3	6.77	2.08	1.60	242	3.6	11.6	15.1	475	7.0	22.9
69.8	8.40	2.54	1.94	277	3.3	10.9	14.3	599	7.1	23.6
68.0	9.95	3.18	2.45	294	3.0	9.2	12.0	733	7.4	23.0
68.5	8.54	2.69	2.08	272	3.2	10.1	13.1	603	7.0	22.4
69.2	8.20	2.53	1.94	270	3.3	10.7	14.1	595	7.2	23.6
69.4	7.92	2.42	1.85	282	3.6	11.6	15.2	571	7.2	23.6
69.5	8.58	2.62	1.97	293	3.4	11.2	14.9	646	7.5	24.7
68.7	9.53	2.99	2.42	308	3.2	10.3	12.7	569	6.0	19.0
68.9	9.04	2.81	2.23	258	2.8	9.2	11.6	579	6.4	20.6
68.0	9.53	3.04	2.36	300	3.2	9.9	12.7	682	7.2	22.4
69.0	8.92	2.78	2.17	290	3.2	10.4	13.4	597	6.9	22.3

Table XXI Body weights and nitrogen metabolism of rats fed diets containing nitrogen the L- or DL- form.

Serie
Stud
Experim

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	Rat number	Body weight		
				Average during metabolism period	Change from beginning to end of period on diet	Cha dur met bol per
				gm.	gm.	g
17	EAA-II only	0.2% L-	57689	367	-16	
			57697	363	- 9	
			57726	334	-13	
			57768	349	-13	
			58338	322	-19	
			58368	312	-13	
			Average	341	-14	
19	EAA-II only	0.4% DL-	57181	360	7	
			57188	363	5	
			57198	364	4	
			57203	348	7	
			57211	345	7	
			57218	350	5	
			57226	321	4	
			57237	345	6	
			Average	350	5	
20	EAA-III only	0.2% L-	57659	364	-11	
			57667	340	- 2	
			57738	338	-15	
			57779	341	- 8	
			58332	341	-17	
			58367	315	-29	
			Average	340	-14	

iets containing nitrogen provided by EAA-II or EAA-III in which the threonine is present in either

Series II
Study D
Experiment one

Body weight			Nitrogen metabolism					
verage uring ta- olism eriod	Change from beginning to end of period on diet	Change during meta-bolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
367	-16	1	319	222	291	513	-194	-159
363	- 9	2	319	211	257	468	-149	-123
334	-13	3	319	197	274	471	-152	-136
349	-13	-2	319	182	252	434	-115	- 99
322	-19	0	319	179	247	426	-107	-100
312	-13	1	319	236	272	508	-189	-182
341	-14	1	319	204	266	470	-151	-133
360	7	3	351	176	295	471	-120	-100
363	5	2	351	172	268	440	- 89	- 74
364	4	3	351	189	330	519	-168	-139
348	7	-5	351	192	288	480	-129	-111
345	7	10	351	170	285	455	-104	- 90
350	5	10	351	183	295	478	-127	-109
321	4	-1	351	174	255	429	- 78	- 73
345	6	-7	351	193	316	509	-158	-137
350	5	2	351	181	292	473	-122	-104
364	-11	1	309	193	253	446	-137	-113
340	- 2	4	309	179	264	443	-134	-118
338	-15	-3	309	177	270	447	-138	-122
341	- 8	3	309	199	283	482	-173	-152
341	-17	-1	309	192	248	440	-131	-115
315	-29	1	309	185	254	439	-130	-124
340	-14	1	309	188	262	450	-141	-124

Table XXI Continued

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	Rat number	Body weight		
				Average during metabolism period	Change from beginning to end of period on diet	Chang duri metabolis peric
				gm.	gm.	gm.
18	EAA-III only	0.4% DL-	58539	328	- 9	4
			58561	312	5	5
			58565	325	- 1	2
			58576	348	- 5	4
			58729	280	- 1	3
			58757	328	- 5	6
			Average	321	- 3	4

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen	Nitrogen balance	Nitrogen balance/ 300 gm.
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
328	- 9	4	347	153	261	414	-67	-61
312	5	5	347	174	266	440	-93	-89
325	- 1	2	347	160	258	418	-71	-65
348	- 5	4	347	185	298	483	-135	-116
280	- 1	3	347	164	269	433	-80	-90
328	- 5	6	347	190	287	477	-130	-119
321	- 3	4	347	171	273	444	-97	-90

Table XXII Liver weights and concentrations of moisture, fat and nitrogen of livers o which the threonine is present in either the L- or DL-form.

Serie
Stud
Experim

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weig	
						Fresh	
				gm.		gm.	g
17	EAA-II only	0.2%L-	57689	367	68.4	8.69	2
			57697	363	68.8	8.66	2
			57726	334	68.8	8.30	2
			57768	349	68.2	8.62	2
			58338	322	68.4	7.93	2
			58368	312	68.9	6.46	2
			Average	341	68.6	8.11	2
19	EAA-II only	0.4%DL-	57181	360	70.4	8.24	2
			57188	363	70.1	8.80	2
			57198	364	70.6	8.20	2
			57203	348	70.2	7.85	2
			57211	345	70.2	8.38	2
			57218	350	70.3	8.60	2
			57226	321	70.7	7.65	2
			57237	345	70.0	7.95	2
			Average	350	70.3	8.21	2

itrogen of livers of rats fed diets containing nitrogen provided by EAA-II or EAA-III in
orm.

Series II
Study D
Experiment One

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
68.4	8.69	2.75	2.10	301	3.5	11.0	14.3	654	7.5	23.8
68.8	8.66	2.70	2.08	292	3.4	10.8	14.0	626	7.2	23.2
68.8	8.30	2.59	1.96	281	3.4	10.8	14.3	627	7.6	24.2
68.2	8.62	2.74	2.00	256	3.0	9.3	12.8	743	8.6	27.1
68.4	7.93	2.51	1.90	249	3.1	9.9	13.1	608	7.7	24.3
68.9	6.46	2.01	1.44	223	3.4	11.1	15.5	573	8.9	28.5
68.6	8.11	2.55	1.91	267	3.3	10.5	14.0	639	7.9	25.2
70.4	8.24	2.44	2.08	258	3.1	10.6	12.4	358	4.4	14.7
70.1	8.80	2.63	2.28	275	3.1	10.5	12.0	345	3.9	13.1
70.6	8.20	2.41	2.07	270	3.3	11.2	13.0	335	4.1	13.9
70.2	7.85	2.34	2.03	258	3.3	11.0	12.7	311	4.0	13.3
70.2	8.38	2.50	2.15	262	3.1	10.5	12.2	349	4.2	14.0
70.3	8.60	2.55	2.20	261	3.0	10.2	11.8	349	4.1	13.7
70.7	7.65	2.24	2.00	241	3.2	10.8	12.0	240	3.1	10.7
70.0	7.95	2.38	2.01	269	3.4	11.3	13.4	371	4.7	15.6
70.3	8.21	2.44	2.10	262	3.2	10.7	12.4	332	4.0	13.6

Table XXII Continued

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weigh	
						Fresh	I
				gm.		gm.	gn
20	EAA-III only	0.2%L-	57659	364	68.2	8.74	2.
			57667	340	67.4	7.95	2.
			57738	338	68.4	8.27	2.
			57779	341	67.7	8.88	2.
			58332	341	68.3	8.29	2.
			58367	315	67.8	7.72	2.
			Average	340	68.0	8.31	2.
18	EAA-III only	0.4%DL-	58539	328	70.7	8.02	2.
			58561	312	70.4	7.52	2.
			58565	325	70.0	7.44	2.
			58576	348	70.0	8.27	2.
			58729	286	69.9	6.65	2.
			58757	328	70.3	7.59	2.
			Average	321	70.2	7.58	2.

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
68.2	8.74	2.78	2.15	281	3.2	10.1	13.1	629	7.2	22.8
67.4	7.95	2.60	1.90	258	3.2	9.9	13.6	691	8.7	26.6
68.4	8.27	2.61	2.02	250	3.0	9.6	12.4	592	7.2	22.7
67.7	8.88	2.87	2.16	263	3.0	9.2	12.2	704	7.9	24.5
68.3	8.29	2.62	2.02	256	3.1	9.8	12.7	602	7.3	23.0
67.8	7.72	2.49	1.86	235	3.0	9.4	12.6	627	8.1	25.2
68.0	8.31	2.66	2.02	257	3.1	9.7	12.8	641	7.7	24.2
70.7	8.02	2.35	1.83	258	3.2	11.0	14.1	524	6.5	22.3
70.4	7.52	2.22	1.67	231	3.1	10.4	13.8	552	7.3	24.8
70.0	7.44	2.23	1.72	244	3.3	10.9	14.2	507	6.8	22.7
70.0	8.27	2.48	1.93	266	3.2	10.7	13.8	556	6.7	22.4
69.9	6.65	2.00	1.53	228	3.4	11.4	14.9	475	7.1	23.7
70.3	7.59	2.25	1.74	255	3.4	11.3	14.7	513	6.8	22.8
70.2	7.58	2.25	1.74	247	3.3	11.0	14.2	521	6.9	23.1

Table XXIII Body weights and nitrogen metabolism of rats fed diets containing nitro
in either the L- or DL- form.

Seri
Stu
Experi

Diet No.	Nitrogen in daily dose provided by	Quantity and form of threonine fed	Rat number	Body weight		
				Average during metabolism period	Change from beginning to end of period on diet	Cha du met bol pe
				gm.	gm.	g
21	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.2% L-	58889	336	-4	
			59041	332	0	
			59001	326	-6	
			58858	362	-1	
			58993	361	-6	
			59063	347	1	
			Average	344	-3	
22	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.4% L-	59015	344	-5	
			58992	350	3	
			58867	345	3	
			59087	345	-17	
			59802	321	-14	
			59118	333	9	
			Average	340	-4	
13A	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.4% DL-	58895	342	-8	
			58832	345	5	
			58881	359	-7	
			59056	349	4	
			59002	339	0	
			59062	345	7	
			Average	346	0	

l diets containing nitrogen provided by EAA-III and NEAA-II in which the threonine is present

Series II
Study D
Experiment Two

Body weight			Nitrogen metabolism					
Average during meta- bolism period	Change from beginning to end of period on diet	Change during meta- bolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
336	-4	7	541	208	308	516	25	22
332	0	8	541	190	325	515	26	23
326	-6	6	541	188	435	623	-82	-76
362	-1	4	541	205	391	596	-55	-46
361	-6	1	541	191	359	550	- 9	- 7
347	1	7	541	190	338	528	13	11
344	-3	6	541	195	359	554	-13	-12
344	-5	3	568	203	377	580	-12	-10
350	3	7	568	199	347	546	22	19
345	3	5	568	177	300	477	91	79
345	-17	1	568	200	341	541	27	23
321	-14	1	568	169	338	507	61	57
333	9	1	568	199	368	567	1	1
340	-4	3	568	191	345	536	32	28
342	-8	4	612	194	405	599	13	11
345	5	3	612	167	356	523	89	77
359	-7	1	612	203	419	622	-10	- 8
349	4	7	612	219	364	583	29	25
339	0	6	612	180	373	553	59	52
345	7	7	612	187	324	511	101	88
346	0	5	612	192	374	566	46	41

Table XXIV Liver weights and concentrations of moisture, fat and nitrogen of liverso in which the threonine is present in either the L- or DL-form.

Series
Study
Experimen

Diet No.	Nitrogen in daily dose provided by	Quantity and optical form of threonine in ration	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Fr
				gm.		g
21	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.2% L-	58889	336	68.5	8
			59041	332	68.8	7
			59001	326	69.6	7
			58858	362	69.3	8
			58993	361	68.4	8
			59063	347	69.4	8
			Average	344	69.0	8
22	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.4% L-	59015	344	68.8	8
			58992	350	68.5	8
			58867	345	69.1	8
			59087	345	69.4	7
			59802	321	69.3	8
			59118	333	68.7	9
			Average	340	69.0	8
13A	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	0.4% DL-	58895	342	69.1	7.
			58832	345	69.8	8
			58881	359	69.5	8.
			59056	349	68.7	8.
			59002	339	69.8	8.
			59062	345	69.0	8.
			Average	346	69.3	8.

gen of liversof rats fed diets containing nitrogen provided by EAA-III and NEAA-II
rm.

Series II
Study D
Experiment Two

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
68.5	8.28	2.60	1.95	270	3.3	10.4	13.8	652	7.9	25.0
68.8	7.76	2.42	1.86	262	3.4	10.8	14.1	566	7.3	23.4
69.6	7.01	2.13	1.56	235	3.4	11.0	15.1	570	8.1	26.7
69.3	8.58	2.63	2.06	274	3.2	10.4	13.3	575	6.7	21.8
68.4	8.57	2.69	2.05	269	3.1	10.0	13.1	638	7.4	23.8
69.4	8.48	2.59	2.05	269	3.2	10.4	13.1	541	6.4	20.9
69.0	8.11	2.51	1.92	263	3.2	10.5	13.8	590	7.3	23.6
68.8	8.03	2.50	1.87	278	3.5	11.1	14.9	633	7.9	25.3
68.5	8.91	2.80	2.09	285	3.2	10.2	13.6	716	8.0	25.5
69.1	8.01	2.48	1.87	278	3.5	11.2	14.9	606	7.6	24.5
69.4	7.31	2.24	1.73	261	3.6	11.6	15.1	510	7.0	22.7
69.3	8.37	2.57	2.00	257	3.1	10.0	12.8	573	6.8	22.3
68.7	9.19	2.88	2.25	291	3.2	10.1	12.9	630	6.8	21.9
69.0	8.31	2.58	1.97	275	3.3	10.6	14.0	611	7.4	23.7
69.1	7.81	2.41	1.86	258	3.3	10.7	13.9	558	7.1	23.1
69.8	8.18	2.47	1.94	256	3.1	10.4	13.2	528	6.4	21.4
69.5	8.50	2.59	2.02	283	3.3	10.9	14.0	570	6.7	22.0
68.7	8.68	2.72	2.14	275	3.2	10.1	12.9	572	6.6	21.1
69.8	8.67	2.62	2.13	271	3.1	10.3	12.7	489	5.6	18.7
69.0	8.67	2.69	2.05	265	3.1	9.8	12.9	633	7.3	23.6
69.3	8.42	2.58	2.02	268	3.2	10.4	13.3	558	6.6	21.6

Table XXV Body weights and nitrogen metabolism of rats when the amino acid mixture (
sources of carbohydrate.

Serie
Stu

Diet No.	Source of carbohydrate in ration	Quantity and source of nitrogen in one day's quota of feed	Rat number	Body weight	
				Average during metabolism period	Change during metabolism period
				gm.	gm.
13A	Dextrin	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	59325	336	4
			59389	320	7
			59380	339	5
			59352	330	9
			Average	331	6
23	Starch	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	59371	338	7
			59382	324	4
			59340	346	5
			59361	327	8
			59395	331	5
			Average	333	6
24	Dextrose	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	59324	334	-1
			59398	335	-3
			59124	358	-5
			59381	315	4
			Average	336	-1
25	Sucrose	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	59355	311	7
			59383	319	3
			59360	332	5
			59399	325	9
			59327	373	4
			59326	339	5
			Average	333	6

en the amino acid mixture (EAA-III plus NEAA-II) was incorporated in diets containing different

Series III
Study A

	Body weight		Nitrogen metabolism					
	Average during metabolism period	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
	336	4	581	107	412	519	62	56
	320	7	581	181	364	545	36	34
	339	5	581	177	372	549	32	28
	330	9	581	166	364	530	51	46
e	331	6	581	158	378	536	45	41
	338	7	612	112	388	500	112	99
	324	4	612	119	402	521	91	84
	346	5	612	116	440	556	56	49
	327	8	612	108	460	568	44	40
	331	5	612	129	477	606	6	5
e	333	6	612	117	433	550	62	55
	334	-1	563	137	588	725	-162	-146
	335	-3	563	121	512	633	- 70	-63
	358	-5	563	151	648	799	-236	-198
	315	4	563	149	485	634	- 71	- 68
e	336	-1	563	140	559	699	-135	-119
	311	7	559	153	488	641	-82	-79
	319	3	559	114	403	517	42	40
	332	5	559	129	556	685	-126	-114
	325	9	559	164	457	621	-62	-57
	373	4	559	135	495	630	-71	-57
	339	5	559	143	467	610	-51	-45
e	333	6	559	140	478	618	-59	-52

Table XXVI Liver weights and concentration of moisture, fat and nitrogen of livers of
incorporated in diets containing different sources of carbohydrate.

Serie
Stud

Diet No.	Source of carbohydrate in ration	Quantity and source of nitrogen in one day's quota of feed	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weig
						Fresh
				gm.		gm.
13A	Dextrin	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	59325	336	67.7	7.76
			59389	320	68.5	7.58
			59380	339	68.8	7.94
			59352	330	68.7	8.59
			Average	331	68.4	7.97
23	Starch	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	59371	338	68.8	7.70
			59382	324	68.6	7.64
			59340	346	68.2	7.56
			59361	327	69.0	8.19
			59395	331	69.1	7.73
			Average	333	68.7	7.76
24	Dextrose	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	59324	334	69.6	7.52
			59398	335	69.3	7.89
			59124	358	68.4	7.68
			59381	315	69.8	7.15
			Average	336	69.3	7.56

itrogen of livers of rats when the amino acid mixture (EAA-III plus NEAA-II) was carbohydrate.

Series III
Study A

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
67.7	7.76	2.51	1.95	264	3.4	10.5	13.5	557	7.2	22.2
68.5	7.58	2.39	1.93	259	3.4	10.8	13.4	460	6.1	19.2
68.8	7.94	2.47	1.91	275	3.5	11.1	14.4	568	7.2	23.0
68.7	8.59	2.69	2.08	264	3.1	9.8	12.7	606	7.0	22.6
68.4	7.97	2.52	1.97	266	3.4	10.6	13.5	548	6.9	21.8
68.8	7.70	2.40	1.84	256	3.3	10.6	13.9	564	7.3	23.4
68.6	7.64	2.40	1.88	252	3.3	10.7	13.4	524	6.9	21.8
68.2	7.56	2.40	1.79	260	3.4	10.5	14.5	611	8.1	25.4
69.0	8.19	2.54	2.06	265	3.2	10.8	12.9	472	5.8	18.6
69.1	7.73	2.39	1.85	244	3.2	10.4	13.2	540	7.0	22.6
68.7	7.76	2.43	1.88	255	3.3	10.5	13.6	542	7.0	22.4
69.6	7.52	2.29	1.83	246	3.3	10.7	13.4	456	6.1	20.0
69.3	7.89	2.42	1.97	261	3.3	10.8	13.2	453	5.8	18.7
68.4	7.68	2.43	1.85	274	3.6	11.3	14.8	580	7.5	23.9
69.8	7.15	2.16	1.71	248	3.5	11.5	14.5	448	6.3	20.7
69.3	7.56	2.32	1.84	257	3.4	11.1	14.0	482	6.4	20.8

Table XXVI Continued

Diet No.	Source of carbohydrate in ration	Quantity and source of nitrogen in one day's quota of feed	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weig	
						Fresh	
				gm.		gm.	g
25	Sucrose	EAA-III (39.5 mg. N) NEAA-II (34.5 mg. N)	59355	311	69.9	7.86	2.
			59383	319	70.0	8.03	2.
			59360	332	69.5	7.73	2.
			59399	325	69.8	7.79	2.
			59327	373	68.8	8.76	2.
			59326	339	69.6	7.50	2.
			Average	333	69.6	7.95	2.

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.9	7.86	2.36	1.93	265	3.4	11.2	13.7	434	5.6	18.5
70.0	8.03	2.41	1.94	273	3.4	11.3	14.1	471	5.9	19.5
69.5	7.73	2.36	1.94	286	3.7	12.1	14.7	416	5.4	17.7
69.8	7.79	2.36	1.85	270	3.5	11.4	14.6	505	6.5	21.4
68.8	8.76	2.73	2.14	301	3.4	11.0	14.1	596	6.8	21.8
69.6	7.50	2.28	1.79	266	3.6	11.7	14.9	492	6.6	21.6
69.6	7.95	2.42	1.93	277	3.5	11.4	14.4	486	6.1	20.1

Table XXVII Body weights and nitrogen metabolism of rats when the amino acid mixture value.

Series
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Diet	Caloric value of one day's quota of diet	Quantity and source of nitrogen in one day's quota of diet	Rat number	Body weight		
				Average during metabolism period	Change from beginning to end of period on diet	Chan duri meta boli peri
	Cal.			gm.	gm.	gm
13A	65	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60619	358	11	1
			60571	336	7	1
			60555	350	16	1
			60692	352	8	
			60587	349	10	1
			60601	365	7	
			Average	352	10	1
26	50	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60631	340	-9	
			60581	336	-12	
			60558	327	-9	
			60664	350	-16	
			60614	328	-8	
			60695	333	-7	
			60685	339	-18	
			60556	353	-9	
			Average	338	-11	
27	35	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60665	310	-36	
			60333	329	-42	-
			60602	309	-41	
			60557	314	-42	-
			60618	301	-43	-
			60573	312	-38	-
			Average	312	-40	-

when the amino acid mixture (EAA-III plus NEAA-II) was incorporated in diets of varying caloric

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Study B

Body weight			Nitrogen metabolism					
Average during metabolism period	Change from beginning to end of period on diet	Change during metabolism period	Nitrogen fed	Fecal nitrogen	Urinary nitrogen	Total nitrogen excreted	Nitrogen balance	Nitrogen balance/ 300 gm. rat
gm.	gm.	gm.	mg.	mg.	mg.	mg.	mg.	mg.
358	11	11	625	206	340	546	79	66
336	7	12	625	214	318	532	93	83
350	16	12	625	201	329	530	95	81
352	8	8	625	190	333	523	102	87
349	10	15	625	226	333	559	66	57
365	7	7	625	222	356	578	47	39
352	10	11	625	210	335	545	80	69
340	-9	7	666	208	438	648	18	16
336	-12	7	666	216	457	673	-7	-6
327	-9	7	666	235	440	675	-9	-8
350	-16	4	666	217	459	676	-10	-9
328	-8	7	666	199	435	634	32	29
333	-7	9	666	186	363	549	117	105
339	-18	5	666	246	468	714	-48	-43
353	-9	5	666	202	422	624	42	36
338	-11	6	666	214	435	649	17	15
310	-36	1	685	167	663	830	-145	-140
329	-42	-3	685	173	669	842	-157	-143
309	-41	3	685	172	664	836	-151	-147
314	-42	-3	685	168	640	808	-123	-118
301	-43	-1	685	165	691	856	-171	-170
312	-38	-5	685	167	723	890	-205	-197
312	-40	-1	685	169	675	844	-159	-152

Table XXVIII Liver weights and concentrations of moisture, fat and nitrogen of livers incorporated in diets of varying caloric value.

Series
Study

Diet No.	Caloric value of one day's quota of diet	Quantity and source of nitrogen in one day's quota of diet	Rat number	Average body weight in nitrogen metabolism period	Per cent moisture in liver	Weig	
						Fresh	D
	Cal.			gm.		gm.	g
13A	65	EAA-III (39.5 mg. N)	60619	358	69.7	8.62	2
		NEAA-II (34.5 mg. N)	60571	336	69.2	7.94	2
			60555	350	68.8	7.92	2
			60692	352	69.9	7.91	2
			60587	349	69.7	7.59	2
			60601	365	69.4	7.80	2
			Average	352	69.4	7.96	2.
26	50	EAA-III (39.5 mg. N)	60631	340	69.0	7.10	2
		NEAA-II (34.5 mg. N)	60581	336	69.1	6.61	2
			60558	327	70.1	7.02	2.
			60664	350	68.6	7.11	2.
			60614	328	70.2	6.75	2.
			60695	333	69.6	7.27	2
			60685	339	69.1	7.02	2.
			60556	353	69.6	7.46	2.
			Average	338	69.4	7.04	2
27	35	EAA-III (39.5 mg. N)	60665	310	69.2	6.59	2.
		NEAA-II (34.5 mg. N)	60633	329	68.6	6.60	2.
			60602	309	70.2	6.44	1.
			60557	314	69.0	6.46	2.
			60618	301	70.0	6.16	1.
			60573	312	69.4	6.17	1.
			Average	312	69.4	6.40	1.

nd nitrogen of livers of rats when the amino acid mixture (EAA-III plus NEAA-II) was

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Study B

Per cent moisture in liver	Weight of liver			Hepatic nitrogen				Hepatic fat (alcohol-ether extract)		
	Fresh	Dry	Dry fat-free	Total	Per cent in tissue on basis of			Total	Per cent in tissue on basis of	
					Fresh weight	Dry weight	Dry fat-free		Fresh weight	Dry weight
	gm.	gm.	gm.	mg.				mg.		
69.7	8.62	2.62	2.05	275	3.2	10.5	13.4	563	6.5	21.5
69.2	7.94	2.45	1.92	251	3.2	10.3	13.1	527	6.6	21.5
68.8	7.92	2.47	1.83	260	3.3	10.5	14.2	647	8.2	26.2
69.9	7.91	2.38	1.86	264	3.3	11.1	14.2	528	6.7	22.1
69.7	7.59	2.30	1.77	263	3.5	11.4	14.9	532	7.0	23.2
69.4	7.80	2.38	1.86	271	3.5	11.4	14.6	527	6.8	22.1
69.4	7.96	2.43	1.88	264	3.3	10.9	14.1	554	7.0	22.8
69.0	7.10	2.20	1.74	256	3.6	11.6	14.7	458	6.4	20.8
69.1	6.61	2.04	1.63	256	3.9	12.5	15.7	413	6.2	20.2
70.1	7.02	2.10	1.63	253	3.6	12.1	15.5	466	6.6	22.2
68.6	7.11	2.23	1.76	277	3.9	12.4	15.8	471	6.6	21.1
70.2	6.75	2.01	1.56	257	3.8	12.8	16.1	454	6.7	22.6
69.6	7.27	2.21	1.72	274	3.8	12.4	15.9	489	6.7	22.1
69.1	7.02	2.17	1.65	265	3.8	12.2	16.0	518	7.4	23.8
69.6	7.46	2.27	1.74	286	3.8	12.6	16.4	527	7.1	23.2
69.4	7.04	2.15	1.68	266	3.8	12.3	15.8	474	6.7	22.0
69.2	6.59	2.03	1.64	253	3.8	12.4	15.5	397	6.0	19.5
68.6	6.60	2.07	1.65	263	4.0	12.7	15.9	420	6.4	20.3
70.2	6.44	1.92	1.58	250	3.9	13.0	15.8	342	5.3	17.8
69.0	6.46	2.00	1.61	260	4.0	13.0	16.1	393	6.1	19.6
70.0	6.16	1.86	1.49	230	3.7	12.4	15.4	366	5.9	19.7
69.4	6.17	1.89	1.53	244	4.0	12.9	15.9	357	5.8	18.8
69.4	6.40	1.96	1.58	250	3.9	12.7	15.8	379	5.9	19.3

Table XXIX Carcass composition of rats when the amino acid mixture (EAA-III plus NEA

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Diet No.	Caloric value of one day's quota of diet	Quantity and source of nitrogen in one day's quota of diet	Rat number	Carcass weight	Moisture		
					Per cent	Total weight	P c
	Cal.			gm.		gm.	
13A	65	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60619	345	62.5	216	3
			60571	323	64.0	207	3
			60555	337	62.5	211	3
			60692	336	63.5	213	3
			60587	334	62.6	209	3
			60601	352	63.2	222	3
			Average	338	63.0	213	3
26	50	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60631	326	64.7	211	3
			60581	320	65.5	210	3
			60558	316	65.3	206	3
			60664	333	65.6	219	3
			60614	312	66.9	209	3
			60695	314	65.4	205	3
			60685	320	64.4	206	3
			60556	331	65.0	215	3
			Average	321	65.4	210	3
27	35	EAA-III (39.5 mg.N) NEAA-II (34.5 mg.N)	60665	290	65.8	191	3
			60333	303	67.1	203	3
			60602	287	67.5	194	3
			60557	291	67.3	196	3
			60618	282	66.8	188	3
			60573	288	67.0	193	3
			Average	290	66.9	194	3

I plus NEAA-II) was incorporated in diets of varying caloric value.

Series III
Study B

sture	Dry matter				Carcass nitrogen				Carcass fat (alcohol-ether extract)		
Total weight	Dry		Dry, fat-free		Total	Per cent expressed in terms of			Total	Per cent expressed in terms of	
	Per cent	Total weight	Per cent	Total weight		Fresh weight	Dry weight	Dry fat free-weight		Fresh weight	Dry weight
gm.		gm.		gm.	gm.				gm.		
216	37.5	129	23.1	79.7	11.9	3.4	9.2	14.9	49.3	14.3	38.2
207	36.0	116	24.3	78.4	11.4	3.5	9.9	14.6	37.6	11.6	32.4
211	37.5	126	22.5	75.7	11.6	3.4	9.2	15.4	50.3	14.9	39.8
213	36.5	123	23.5	79.1	11.7	3.5	9.5	14.7	43.9	13.1	35.9
209	37.4	125	22.9	76.6	11.6	3.5	9.3	15.2	48.4	14.5	38.7
222	36.8	130	23.2	81.6	11.2	3.4	8.6	13.7	48.4	13.8	37.4
213	37.0	125	23.2	78.5	11.7	3.5	9.3	14.8	46.3	13.7	37.0
211	35.3	115	23.0	75.0	11.8	3.6	10.2	15.7	40.0	12.3	34.7
210	34.5	110	22.8	73.1	11.6	3.6	10.6	15.9	36.9	12.7	36.9
206	34.7	110	22.9	72.4	11.2	3.6	10.2	15.5	37.6	11.9	33.6
219	34.4	114	23.9	79.6	11.9	3.6	10.4	14.9	32.0	9.6	28.0
209	33.1	103	24.2	75.4	11.3	3.6	11.0	15.0	27.6	8.8	26.7
205	34.6	109	22.1	69.5	11.3	3.6	10.4	16.3	39.5	12.6	36.4
206	35.6	114	22.7	72.5	11.4	3.6	10.0	15.8	41.5	13.0	36.4
215	35.0	116	22.5	74.4	11.9	3.6	10.2	16.0	41.6	12.6	35.9
210	34.6	111	23.0	74.0	11.6	3.6	10.4	15.6	37.1	11.7	33.6
191	34.2	99	24.3	70.5	10.8	3.7	10.9	15.8	28.5	9.8	28.7
203	32.9	100	24.0	72.7	11.3	3.7	11.3	15.5	27.3	9.0	27.4
194	32.5	93	25.0	71.6	10.8	3.8	11.7	15.1	21.4	7.4	22.9
196	32.7	95	23.8	69.3	10.8	3.7	11.4	15.6	25.7	8.8	27.0
188	33.2	94	24.6	69.4	10.6	3.8	11.3	15.3	24.6	8.7	26.3
193	33.0	95	24.1	69.3	10.9	3.8	11.4	15.7	25.7	8.9	27.1
194	33.1	96	24.3	70.5	10.9	3.8	11.3	15.4	25.5	8.8	26.6