Iowa State University
Digital Repository

Retrospective Theses and Dissertations

Iowa State University Capstones, Theses and Dissertations

1946

Utilization of nitrogen by the animal organism: I. When methionine serves as the main source of nitrogen in the diet of the rat

Miriam Kelly Brush
Iowa State College

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Part of the Dietetics and Clinical Nutrition Commons, and the Human and Clinical Nutrition Commons

Recommended Citation

Brush, Miriam Kelly, "Utilization of nitrogen by the animal organism: I. When methionine serves as the main source of nitrogen in the diet of the rat" (1946). *Retrospective Theses and Dissertations*. 13596.
https://lib.dr.iastate.edu/rtd/13596

This Dissertation is brought to you for free and open access by the Iowa State University Capstones, Theses and Dissertations at Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.

www.manaraa.com

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

NOTE TO USERS

This reproduction is the best copy available.

UMI®

UTILIZATION OF NITROGEN BY THE ANIMAL ORGANISM
I. WHEN METHIONINE SERVES AS THE MAIN SOURCE OF NITROGEN IN THE DIET OF THE RAT

by

Miriam Brush

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

Major Subject: Nutrition

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Department

Signature was redacted for privacy.

Dean of Graduate College

STATE AMES IA LIBRARY

Iowa State College
1946

UMI Number: DP12284

UMI®

UMI Microform DP12284

Copyright 2005 by ProQuest Information and Learning Company.
All rights reserved. This microform edition is protected against
unauthorized copying under Title 17, United States Code.

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

QP141
B838u

TABLE OF CONTENTS

Page

LIST OF TABLES IN BODY OF THESIS.......................... v

LIST OF FIGURES.. vii

INTRODUCTION... 1

IDENTIFICATION OF THE SULFUR-CONTAINING AMINO ACIDS.................................... 2

NUTRITIONAL IMPORTANCE OF THE SULFUR-CONTAINING AMINO ACIDS.................................... 3

PHYSIOLOGICAL ROLE OF METHIONINE................... 5

PURPOSE OF PRESENT INVESTIGATION.................... 6

REVIEW OF LITERATURE..................................... 8

CONCEPTS OF PROTEIN METABOLISM...................... 8

DEPRESSION OF "ENDOGENOUS" URINARY NITROGEN EXCRETION.. 19

FUNCTIONS OF METHIONINE............................. 28

Formation of Structural Protein............ 29

Provision of Cystine........................ 30

Methionine as a Precursor of Cystine.. 30

Mode of Action of the Sulfhydryl Group.............................. 32

Detoxification........................ 33

Formation of Taurine.................. 35

Provision of Labile Methyl Groups.......... 36

Creatine.............................. 37

T 8035

Page

Choline.......................... 38

Other Methylated Compounds.......... 39

SUMMARY AND PROPOSED THEORY OF PROTEIN METABOLISM.......................... 39

PLAN OF EXPERIMENT.......................... 42

EXPERIMENTAL PROCEDURE.......................... 46

NITROGEN BALANCE TECHNIQUE.......................... 46

Details of Procedure Used.......................... 46

Selection and Care of Animals.......................... 48

Diets Fed.......................... 51

Collection of Samples for Analysis.......................... 53

Urine.......................... 53

Feces.......................... 54

Method of Analysis of Urine, Feces, and Food.......................... 55

TISSUE ANALYSIS.......................... 57

Experimental Material.......................... 57

Preparation of the Whole Carcass...... 57

Extirpation and Hydrolysis of Liver... 58

Extirpation and Hydrolysis of Muscle.. 59

Preparation of Tissue Extracts........ 60

Analytical Methods.......................... 60

Moisture Analysis.......................... 60

Determination of Fat.......................... 60

Determination of Methionine........... 61

Page

Procedure........................ 61

Reagents........................... 63

Specificity of reaction.......... 63

Reproducibility, accuracy, and agreement with other methods..... 64

RESULTS AND DISCUSSION.. 66

EXPERIMENT 1.. 56

EXPERIMENT 2.. 78

EXPERIMENT 3.. 85

EXPERIMENT 4.. 97

EXPERIMENT 5.. 110

EXPERIMENT 6.. 115a

Whole Carcass Analysis........................ 115a

Liver Analysis............................. 119

Muscle Analysis............................ 124

Nature of Storage Protein.................. 128

SUMMARY.. 133

CONCLUSIONS.. 140

LITERATURE CITED.. 142

ACKNOWLEDGMENTS.. 152

APPENDIX.. 153

LIST OF TABLES IN APPENDIX........................ 176

LIST OF TABLES IN BODY OF THESIS

Page

1. Excretion of nitrogen by rats fed lactalbumin...... 20
2. Methionine in various proteins, calculated to 16.0 per cent N.............................. 29
3. Data relating to nitrogen metabolism expressed per 100 gm. of body weight, of rats fed the nitrogen-low diet in two consecutive metabolism periods.. 49
4. Composition of complete amino acid mixture......... 67
5. Average data descriptive of body size, caloric requirement, and caloric intake of rats fed various nitrogenous supplements to a nitrogen-low diet (Exp. 1).................................. 68
6. Nitrogen metabolism data (Exp. 1)................... 70
7. Body tissue equivalent of nitrogen balance in period I.. 75
8. Body tissue equivalent of body nitrogen spared..... 77
9. Average data descriptive of body size, caloric requirement, and caloric intake of rats fed various nitrogenous supplements to a nitrogen-low diet (Exp. 2).................................. 80
10. Nitrogen metabolism data (Exp. 2).................. 81
11. Body tissue spared by methionine................... 84
12. Urinary excretion of creatinine in the two periods of the nitrogen balance test................ 85
13. Average data descriptive of body size, caloric requirement, and caloric intake of rats fed various nitrogenous supplements to a nitrogen-low diet (Exp. 3).................................. 87
14. Nitrogen metabolism data (Exp. 3).................. 89a,b

page

15. Average data descriptive of body size, caloric requirement, and caloric intake of rats fed various nitrogenous supplements to a nitrogen-low diet (Exp. 4)............................... 99

16. Nitrogen metabolism data (Exp. 4)............... 101a,b

17. Analysis of variance of body nitrogen spared by 33 rats fed six different quantities of methionine....................................... 106

18. Average data descriptive of body size, caloric requirement, and caloric intake of rats fed various nitrogenous supplements to a nitrogen-low diet (Exp. 5)............................... 112

19. Nitrogen metabolism data (Exp. 5)............... 113

20. Analysis of variance of body nitrogen spared by three groups of rats fed methionine, cystine, and choline, respectively.........................115a

21. Average weight, methionine content and total nitrogen content of the whole carcasses of rats fed the stock colony diet, and of ones fed the low-nitrogen diet alone or with nitrogenous supplements............................ 115c

22. Change in total nitrogen of whole carcasses of rats after nitrogen depletion and supplementation.................................. 116

23. Nitrogen content of the whole carcass of rats, as determined by various investigators.......... 118

24. Moisture and fat content of hepatic and muscle tissue of animals fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements................ 120

25. Weight, methionine content, and total nitrogen content of livers of rats fed a nitrogen-low diet alone and with supplementary methionine.. 124

26. Methionine content of muscles of rats fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements..127

LIST OF FIGURES

Page

1. Depressions and elevation of urinary excretion of nitrogen per 100 sq. cm. of body surface caused by addition of egg proteins, a mixture of the ten essential amino acids, methionine, and a mixture of the ten essential amino acids minus methionine to the diet of rats fed a nitrogen-low ration.................................... 73

2. Body nitrogen spared per 100 sq. cm. of body surface by addition of egg proteins, a mixture of the ten essential amino acids, and methionine to the diet of rats fed a nitrogen-low ration..... 74

3. Depressions and elevations of urinary excretion of nitrogen per 100 sq. cm. of body surface caused by addition of each of the ten essential amino acids to the diet of rats fed a nitrogen-low ration.. 88

4. Body nitrogen spared per 100 sq. cm. of body surface by addition of each of the ten essential amino acids to the diet of rats fed a nitrogen-low ration.. 91

5. Depressions of urinary excretion of nitrogen per 100 sq. cm. of body surface caused by the addition of increasing amounts of methionine and of egg proteins to the diet of rats fed a nitrogen-low ration.. 102

6. Body nitrogen spared per 100 sq. cm. of body surface by addition of increasing amounts of methionine and of egg proteins to the diet of rats fed a nitrogen-low ration...................... 103

5A. Depression of urinary excretion of nitrogen per 100 sq. cm. of body surface caused by addition of crystalline methionine and of methionine supplied by egg proteins to the diet of rats fed a nitrogen-low ration............................ 107

Page

6A. Body nitrogen spared per 100 sq. cm. of body surface by addition of crystalline methionine and of methionine supplied by egg proteins to the diet of rats fed a nitrogen-low ration....... 108

7. Modifications in nitrogen metabolism caused by addition of methionine, cystine, and choline to the diet of rats fed a nitrogen-low ration, calculated per 100 sq. cm. of body surface....... 114

INTRODUCTION

The greatest advance in the study of the utilization of the nitrogenous constituents of the diet by the animal organism occurred less than half a century ago when it was realized that proteins were important not for themselves but for the specific amino acids they supplied. In the classical work of Osborne and Mendel (1914), there can be found proof of the validity of this concept as well as the idea, in embryo, of the modern theory of the dynamic nature of protein metabolism. Since the amino acids, "die Bausteine" of the old German writers, rather than the complete molecule represent the functional groups in nitrogen metabolism, the amino acids themselves must be examined to understand the importance of each and the means by which it contributes to normal life processes. Within recent years, evidence has accumulated which indicates that methionine, one of the sulfur-containing amino acids, may play a leading role in the control of certain physiological reactions in the body. The present work represents an attempt to evaluate the extent and nature of this control. A brief review of the knowledge concerning the part sulfur-containing amino acids play in metabolism will afford a basis for discussion of the particular problems involved.

IDENTIFICATION OF THE SULFUR-CONTAINING AMINO ACIDS

Cystine was isolated from a urinary calculus by Wollaston in 1805, 15 years before Braconnot obtained glycine, the most common amino acid, from the hydrolysis of gelatin. Not until 1837, however, was cystine recognized as a sulfur-containing compound by Baudrimont and Malaguti, and several years passed before the exact combination of investigative conditions was achieved which demonstrated its presence in natural proteins. In 1899, Morner and Embden independently prepared cystine from horn, succeeding where previous workers, who chose proteins less rich in sulfur, had failed. The structure of cystine was proved in 1903 when Erlenmeyer synthesized a substance from benzoylserine ethyl ester and phosphorous pentasulfide which was identical with the naturally occurring compound (Schmidt, 1944, pp. 3, 4).

For several years, cystine was thought to be the only sulfur-containing amino acid. However, in 1920-1921, a bacteriologist, Mueller (1923), noted that certain protein hydrolysates stimulated the growth of a hemolytic streptococcus while others did not. He prepared active fractions containing sulfur in forms other than cystine from casein hydrolysates which supplied the necessary factor for growth stimulus. With the use of mercuric and cupric salts, he

isolated a new sulfur-containing amino acid which, contrary to expectations, had no specific stimulating effect on the growth of streptococci. Barger and Coyne in 1928 synthesized a compound identical with Mueller's naturally occurring amino acid which they named methionine, because of its characteristic methylthiol group.

NUTRITIONAL IMPORTANCE OF THE SULFUR-CONTAINING AMINO ACIDS

Osborne and Mendel (1915) showed that young rats fed a diet containing limited amounts of casein suffered curtailment of growth, but when the diet was supplemented with cystine growth was immediately resumed. They concluded that cystine was an amino acid indispensable for growth, and that casein was deficient in this essential nutrient. H. B. Lewis two years later, in 1917, observed a marked decrease in the quantity of nitrogen lost in the urine when supplementary cystine was given to rats maintained on a nitrogen-poor diet. The experiment demonstrated, in his eyes, the need of the body for cystine in the fulfillment of some essential function and its attempt to supply the metabolite by breakdown of its own tissue when dietary cystine was not available. As soon as methionine was isolated and identified it was discovered that it, as well as cystine, caused a stimulation of growth in animals maintained on certain

protein-restricted diets. When pure crystalline amino acids became available, Rose and his co-workers (Womack, Kemmerer, and Rose, 1937) demonstrated, by means of growth measurements, the essential nature of certain of the amino acids. Contrary to previous beliefs, cystine proved not to be an essential amino acid. Methionine, however, was needed by the animal. This discovery necessitated reinterpretation of earlier data. Evidently experimenters had, in their work, supplied diets which were not only cystine-deficient but very low in methionine as well. Later, Womack and Rose (1941) showed that one-sixth of the methionine requirements of the rat could be met by cystine. Within certain limits, therefore, the administration of either methionine or cystine might be expected to bring about an improvement of nutritional status. The possibility that cystine may play a specific role should not be overlooked. Recently Madden and his co-workers (1943) indicated that cystine was more effective in stimulating the production of plasma protein than methionine. The general consensus, however, seems to be that methionine is capable of conversion to cystine so that it indirectly fulfills all other functions of the latter substance.

PHYSIOLOGICAL ROLE OF METHIONINE

With the knowledge established that methionine was essential for normal health, it was next in interest to discover why this compound was so important and how it functioned in the animal body. Early workers were satisfied with the answer that it was necessary for the establishment of a normal rate of growth and for the maintenance of tissues. More specific requirements, however, were soon revealed. Lewis' laboratory (Witter, 1944), for example, has repeatedly demonstrated the importance of the sulfur-containing amino acids in the detoxification of aromatic halogens administered to experimental animals. More recently, this knowledge has been applied in the treatment of human disorders. Methionine and cystine may be given with benefit to individuals suffering from certain types of liver poisoning (Eddy, 1945). English workers(Croft and Peters, 1945) investigating the nature of the therapeutic value of protein-feeding after severe burns, found that a dietary supplement of methionine sharply reduced the urinary output of nitrogen and improved the physiological condition of experimental animals subjected to hot-water burns. White and Lewis (1932), Stekol (1935), and Miller (1944) independently recorded a surprising depression in the excretion of urinary nitrogen by feeding methionine (and in some cases, cystine)

to animals reared on a nitrogen-low diet. The two sulfur-containing amino acids appear to exert opposite effects on the deposition of fat in the liver (McHenry and Patterson, 1944); one, methionine, being lipotropic and the other, cystine, lipogenic. Addition of methionine to the diet has proved beneficial in cases of renal hemorrhage; in some way it acts to maintain normal renal tissue (McHenry and Patterson, 1944; Griffith, 1941). Such multifarious functions of methionine and cystine suggest that these compounds are of fundamental importance in the physiology of the animal organism.

PURPOSE OF PRESENT INVESTIGATION

Thus, organic sulfur in the form of methionine has been shown to be indispensable for life, and to effect certain important changes in body processes. The immediate questions are: How does methionine work? What are its physiologically important structural groupings? How does the body handle the methionine in its food supply? Studies employing isotopic carbon and sulfur have revealed that methionine serves as a donor of methyl groups in the synthesis of creatine and of choline, and that its sulfur in conjunction with the carbon chain of serine may be used to build cystine. Workers in the Nutrition laboratory of the Iowa State

College (Willman *et al.*, 1945) have been especially interested in the depression of urinary nitrogen reported by various workers when methionine is fed to nitrogen-depleted animals, since they had previously observed that egg proteins similarly fed caused a very dramatic fall in the quantity of nitrogen excreted in the urine. This depression is unexplainable by conventional theories but fits in well with modern concepts of the dynamic nature of protein metabolism.

The objective of the present study was to investigate why egg protein exerted this depressant effect and to determine whether or not methionine was related to the phenomenon.

REVIEW OF LITERATURE

CONCEPTS OF PROTEIN METABOLISM

Before the time of Otto Folin in the early twentieth century, few attempts had been made to develop a comprehensive theory of protein metabolism. Certain facts had been established. It was recognized that physical exercise did not increase protein destruction, and that synthesis of protein from simpler nitrogenous compounds was possible. The capacity of proteins to maintain nitrogen equilibrium was recognized as a function of their amino acid content. But lack of adequate experimental evidence prevented testing of old ideas and elaboration of new ones.

Folin, however, developed the analytical methods necessary for testing theories of protein metabolism and also saw experimental possibilities that proved fruitful in developing new concepts. His most important experiment in this connection (Folin, 1905) was one in which he correlated variations in certain of the components of human urine with variations in the nitrogen content of the diet. His subject was maintained for several days on a diet consisting largely of milk and eggs, then for a longer period on potatoes, starch and cream, and returned for a brief time to the

milk-egg diet. Urine was collected daily and analyzed for total nitrogen, urea, ammonia, creatinine, uric acid, and inorganic, ethereal and neutral sulfur. The analyses showed that after the subject became adjusted to each diet the concentration of certain substances in the urine, such as total nitrogen, urea, ammonia, and inorganic and ethereal sulfur varied directly with the protein content of the diet. Others, like creatinine and neutral sulfur, were independent of the nature of the diet.

These observations led Folin to certain deductions as to the nature of protein metabolism. He concluded that there were two kinds of catabolism, one variable or exogenous, which was dependent upon the protein ingested, and the other constant or endogenous, the result of the daily "wear and tear" of the protein structures of the organism. The metabolic processes resulting in these constant end-products he assumed to be indispensable for the continuation of life, and, therefore, "to represent the lowest level of nitrogen metabolism achievable" (Folin, 1905). Food protein was drawn upon to replace these losses, and any excess was either discarded or stored. Beyond this, no synthesis of protein in the body was assumed, the structural units of the body being considered static. Although at first Folin did not recognize the possibility of storage,

later he explained the lag in establishment of a constant level of nitrogen excretion after a marked change in diet by either a filling or depletion of the storehouse for amino acids which existed in muscle and other tissues (Folin and Denis, 1912). He attributed the complete retention of a single amino acid by an animal on a nitrogen-poor diet to the filling of an empty storehouse, and the elimination of this amino acid by an animal on a nitrogen-rich diet, to the lack of space in an already full storehouse. Folin's concept of a constant "endogenous" nitrogen metabolism has been criticized and modified, but it is interesting to trace its persistence through all the alterations of more recent theory and application.

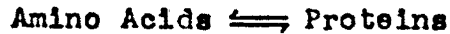
Osborne and Mendel were pioneers in the investigation of the quality of proteins from various sources in regard especially to the requirements of growth and maintenance in the animal organism, and were among the first to question Folin. They found that some proteins were adequate for both growth and maintenance, others for maintenance alone, while still others were unsatisfactory for either purpose. Knowledge of the deficiencies of certain proteins in specific amino acids led Osborne and Mendel to supplement the proteins with the lacking amino acids. Their experiments (1912) revealed, for example, that gliadin which, untreated, was adequate only for bodily maintenance, provided material

for rapid growth as well when supplemented with lysine.

These results caused Osborne and Mendel (1914) to examine Folin's theory of protein metabolism. They refined and modified it, but did not discard it completely.. They suggested that the "endogenous" metabolism postulated by Folin might represent a requirement for specific amino acids for the synthesis of enzymes or hormones or for other protein or non-protein reactions. In their opinion, the entire protein molecule might undergo degradation only to liberate specific amino acids. In light of later discoveries, it may be noted that they proposed (Osborne and Mendel,1915) that the minimum requirement for protein might best be met not by a protein most like body protein, but by one rich in the specific amino acids needed for the synthesis of new tissue or of a specific hormone, enzyme, etc. Mitchell (1924), in commenting on this theory, said that if it were correct, animals maintained on a diet containing complete proteins should excrete less nitrogen than when on a nitrogen-free diet, since in the former case, the "endogenous" requirement would be met by food protein. This last is an interesting statement in view of the special balance technique developed in Mitchell's laboratory for the measurement of the nutritive value of proteins.

Henry C. Sherman, one of the leading nutritionists of the early twentieth century, also questioned Folin's theory.

Sherman believed (1924) that the experimental evidence collected in his laboratory as to the adequacy of cereal proteins for the maintenance of body tissues in both man and rats necessitated a new concept of protein metabolism. He thought in terms of an equilibrium in the cells between proteins and amino acids:



Amino Acids ⇌ Proteins

Any amino acid supplied in the food would drive the reaction to the right and thus function in maintenance. Thus, by the law of mass action, the hydrolysis of tissue protein would be checked by amino acids derived from the food, thereby retarding the "endogenous" catabolism of Folin. The more complete the food protein, the greater would be the retardation of tissue protein hydrolysis. Sherman's concept eliminated the possibility of a constant tissue metabolism, and made it susceptible to the quantity, as well as to the quality of the protein in the diet.

As time went on, more and more evidence accumulated that a dynamic rather than a static state characterized protein metabolism. This idea was early expressed and developed in detail by Borsook and Keighley (1935). To them, the frequently observed lag in attaining nitrogen balance when passing from one level of nitrogen intake to another and the constancy of the free amino nitrogen content

of the tissues despite extreme variations in diet and nutritional state were evidence of a "continuing" metabolism. Its extent was dependent on the level of nitrogen balance, *i.e.*, on the amount of available nitrogen stores, and was on any one day independent of that day's intake. Borsook and Keighley believed that this continuing metabolism, defined as "the nitrogen metabolized on any one day which is already present in the tissues", was a measure of the synthetic processes normally in operation, and was of a magnitude far greater than the "endogenous" metabolism of Folin. In men, Borsook and Keighley suggested, it might amount to 50 per cent or more of the nitrogen intake.

Direct proof of the dynamic nature of protein metabolism rests largely on studies reported from Schoenheimer's laboratory (Schoenheimer, 1942) in which the course of nitrogen metabolism was followed with the use of labelled elements. Schoenheimer and his co-workers fed amino acids containing heavy hydrogen and heavy nitrogen (N_{15}) to experimental animals, and after appropriate intervals analyzed tissues and excreta for the heavy atom content of the various amino acids found therein. Were the body proteins static except for endogenous wear and tear, the nitrogen excreted in the urine would be derived largely from the diet. However, the incorporation of the marked molecules into body tissue up to at least 50 per cent of the amount fed agrees precisely

with the estimate of Borsook and Keighley for the continuing metabolism, and indicates the ready interchange between food protein and body protein. The isolation in amino acids in body tissues of tagged nitrogen derived from different dietary amino acids proved that continuous transamination of amino acids took place in the body. Only lysine, once deaminated, was incapable of accepting an amino group from another amino acid. Thus, body protein is not stable and unchangeable until worn out, but continuously in the state of flux, and all the reactions of which the organism is capable are continually in operation. There is, however, a balance between synthesis and degradation, loss and replacement, indicated by the maintenance of constant weight and of nitrogen equilibrium in the experimental animals, and by the constancy in amount and composition of the body components.

Whipple and his group working at the University of Rochester early made a definite contribution to the understanding of protein metabolism. Work beginning as early as 1919 has been summarized in a report by Madden and Whipple in 1940. By means of plasmapheresis in dogs maintained in good health on a low-protein diet, this laboratory demonstrated the existence of the reserve stores of proteins which Folin had earlier postulated. Continuous weekly

bleedings were necessary to reduce the plasma proteins to a constant minimum level, and the quantity of protein thus removed was indicative of the basal output of plasma protein. Larger amounts of blood had to be removed in the earlier weeks than later, and the protein in this, over and above the basal output, represented the reserve store of the animal. It was found that dogs had in reserve enough protein to form one to two times the normal quantities of circulating plasma protein. That this flow of organ protein to plasma was reversible was demonstrated by the accumulation of stores when plasmapheresis was discontinued and by the ability of injected plasma to replenish body reserves.

Whipple evaluated the plasma-protein rebuilding capacity of various foods by determining the amount of plasma protein over and above the basal output produced by the feeding of 100 gm. of the food. His work (Robscheit-Robbins, Miller, and Whipple, 1943) showed also that proteins valuable for plasma protein regeneration were not necessarily effective in the building of hemoglobin. Reserve protein stores, however, were drawn upon for the production of the latter as well as for the former. This observation supports the earlier idea of Osborne and Mendel that body tissues are broken down not for the whole protein molecule but for specific amino acids, in this case, different amino acids being needed for the formation of plasma protein than for

hemoglobin. Whipple's work shows clearly the dynamic relationship between food proteins, plasma proteins, reserve proteins in the liver and tissue proteins, and demonstrates the fluidity of body processes.

Thus, a variety of evidence seems to suggest complete lack of distinction between varying forms of nitrogen metabolism in the body. There is a constantly shifting structure, with building and destruction, interchange of amino acids in the nutrient media and those in the tissue protein, with some regulating force keeping everything in balance.

Mitchell (1944) would separate out from this metabolic pool some few processes which are uninfluenced by any external factors, but which represent, according to him, Folin's "endogenous" metabolism. The constant rate of conversion of creatine to creatinine and its subsequent excretion would be one of these processes. This conversion-degradation process is apart from all the others which Folin included in his original concept, and thus makes the expected level of "endogenous" metabolism lower. It still, however, would allow for the employment of the concept in certain experimental procedures the results of which by their very constancy seem to justify it. Lysine, which Schoenheimer showed could not be reversibly deaminated and reaminated, might be another process to be included in Mitchell's "endogenous" metabolism.

Various workers at Iowa State College* have re-evaluated the older theories of protein metabolism, and modified them in the light of their demonstration of a fall in the excretion of total nitrogen and creatinine obtained by feeding egg proteins to adult animals previously maintained on a nitrogen-low diet. These results, although surprising in the light of the experiences of others using the balance test, are nonetheless logical, and may be explained by the theory postulated by Osborne and Mendel as early as 1914, i.e., protein requirements for bodily maintenance are, in actuality, requirements for specific amino acids for specific purposes. There is, as demonstrated by Schoenheimer, continual activity among protein and amino acid moieties within the body. The removal of an amino acid molecule for use will depend on the need for it in a specific process like, for example, the synthesis of thyroxine or pepsin. If the rest of the protein molecule from which the amino acid came cannot be used elsewhere or stored it will be deaminated and its nitrogen excreted. In a nitrogen-impoverished state, then, body protein is broken down as specific amino acids are needed, and the excess discarded. While this non-utilizable nitrogen may be the equivalent to

---

*Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799

Folin's "endogenous" metabolism, it is not the constant characteristic postulated by him.

The feeding of a "perfect" protein which has such composition that all its amino acids meet the requirements of the body for its vital functions would cause a marked depression in total urinary nitrogen. Theoretically, if the animal were in nitrogen equilibrium, the excretion would drop to a point represented by the elimination of catabolites resulting from certain irreversible body processes, plus metabolites which escape reconversion. Eggs seem to contain an assortment of proteins that approach this criterion. Mitchell found evidence years ago (1924) that lactalbumin caused a similar depression in the excretion of urinary nitrogen; however, the magnitude of the depression seemed insignificant to him. Perhaps there are other proteins such as those of yeast or rice whose pattern of amino acids may be ideal for the varied needs of the body.

At the present time, there is general acceptance of the dynamic nature of protein metabolism, as a result of the discoveries of Schoenheimer, Whipple, Swanson, and others. However, no comprehensive scheme clarifying the intermediate and ultimate fate of the components of the protein molecule in a dynamic system has been elaborated, and one sizeable school, that of Mitchell, still upholds a modified Folin theory, with its elements of constant and of variable metabolism.

DEPRESSION OF "ENDOGENOUS" URINARY NITROGEN EXCRETION

In Folin's opinion, the continual "wear and tear" of body tissues results in a constant "endogenous" urinary excretion of nitrogen which persists throughout all alterations in the composition of the diet. However, a fall in the urinary nitrogen beyond the excretion characteristic of an animal living on a nitrogen-low diet has been reported by various investigators in a number of experimental animals fed a variety of supplements. These observations represent an important addition to the work of Schoenheimer and of Whipple and support the suggestion of a dynamic protein metabolism.

Lewis was first to report a definite fall in the excretion of nitrogen in the urine when, in 1917, he fed single doses of cystine to dogs maintained on a low-nitrogen diet. The substitution of 0.1 gm. of nitrogen from cystine for an equivalent amount of the nitrogen in the basal low-nitrogen diet caused a depression in urinary nitrogen equivalent to 10 times the quantity in the dietary supplement. Lewis believed at the time that the decrease in the loss of nitrogen was evidence of a specific demand for cystine by the dog, and that it established the essential nature of this amino acid.

That proteins as well as amino acids can effect such a depression is shown in a report of H. H. Mitchell in 1924. Mitchell measured the excretion of nitrogen in the urine of rats fed in successive periods a low-nitrogen diet, the same diet fortified with 3 per cent of lactalbumin, and finally the original low-nitrogen ration. His data are reproduced in the table below. Even the average results obtained with five rats did not seem marked enough to convince Mitchell of the reality of the depression.

TABLE 1

Excretion of nitrogen by rats fed lactalbumin

Experimental day	Ration	Daily intake of N	Daily urinary N	Av. weight of rats	Urinary N* per 100 gm. body weight
		mg.	mg.	gm.	
10-12	Low N	4	19.9	124	16.0
13-15	Lactalbumin	34	14.8	122	12.1
16-18	Low N	3	16.0	120	13.3

*Not in original table. Calculated from data in preceding columns.

Even in the face of such evidence, he stated that "there seems to be a true basal catabolism of nitrogenous substances in the tissues" (p. 902).

Belief in the constancy of this basal catabolism as

postulated by Folin was shaken by the discovery of Ackerson and Blish (1926) that the so-called "endogenous" loss of nitrogen of non-molting hens markedly increased in molting birds. With the realization that this change occurred simultaneously with the extraordinary demand for cystine for the building of feathers, the workers suggested that it might be due to a breaking down of large quantities of body protein in an effort to secure cystine for the synthesis of keratin. Experimental proof as to the validity of this speculation was obtained by feeding cystine equivalent to 16 mg. of nitrogen to molting hens maintained on a low-nitrogen diet. Analysis of the total excreta in the nitrogen-low and cystine-feeding periods showed that the addition of the amino acid produced a fall in nitrogen excretion of 231 mg. Thus, according to the calculations of Ackerson and Blish, the addition to a nitrogen-low diet of the specific amino acid needed at molting in an amount supplying only 16 mg. of nitrogen spared 65 gm. of muscle protein. This is a concrete example of the belief of Swanson* that body tissues are raided for specific amino acids, and that all but the needed substance will be discarded as waste in the urine.

---

*Swanson, P.P., Conference on developing concepts of protein metabolism, sponsored by Subsistence Research and Development Laboratory, Military Planning Division, Office of The Quartermaster General, Ames, Iowa. Report. March 5 and 6, 1946.

After the discovery of the second sulfur-containing amino acid, methionine, and one capable of replacing cystine for purposes of growth, White and Lewis (1932) tried the effect of feeding the new compound on the nitrogen excretion of dogs maintained on a nitrogen-low diet. They observed a depression of urinary nitrogen similar to the one that Lewis (1917) had obtained years before with cystine. These workers, however, were primarily interested in the mechanism of detoxification of aromatic halogens by the sulfur-containing amino acids, and they made little attempt to explain the fall in excretion of nitrogen at the time. Later*, Lewis expressed the belief that one of the factors limiting normal metabolic processes in experimental animals maintained on a low nitrogen diet is the deficiency of sulfur-containing amino acids, and that the addition of either cystine or methionine will spare body protein, a phenomenon that will be reflected in a depressed excretion of nitrogen.

Stekol and Schmidt (1933) were unable to confirm the results of White and Lewis by feeding methionine to dogs on a diet containing 44 per cent of protein. However, when Stekol (1935) added methionine to a diet containing less protein (22 per cent), both adult dogs and pups fed the

*Lewis, H. B., Ann Arbor, Mich. Private communication. 1946.

ration showed the now expected depression of urinary nitrogen.

Up to this time, the lowering of the "endogenous" excretion of nitrogen had been noted only when the sulfur-containing amino acids, cystine and methionine, and one protein, lactalbumin, known to be high in cystine, had been added to a nitrogen-low diet. Nielsen, Gerber, and Corley (1939), examining the possibility of independent action of each of the amino acids, fed rations to dogs containing only 0.01 - 0.02 per cent of nitrogen until the urinary excretion of nitrogen was constant. Several of the amino acids were then added, one by one, to the diet. Of the amino acids tested, cystine and, to a somewhat lesser extent, lysine and histidine reduced the excretion of nitrogen both during and after the experimental period. The investigators suggested that the amounts of these substances retained in the body exerted a sparing action on body protein continuing for several days.

Burroughs, Burroughs, and Mitchell (1940) recognized that this interpretation of the body-sparing action of certain amino acids involved a depression of the "endogenous" catabolism and was, therefore, incompatible with Folin's theory. Using rats, they failed to demonstrate clearly a depression by any of the amino acids which they fed, including methionine, lysine, and histidine. Neither was the incorporation of egg proteins in the ration effective in this

respect. However, the data presented reveal that at the time the supplements were fed the animals were still losing "labile" protein stores, and could not be considered in a constant state of nitrogen metabolism, a condition necessary if conclusions were to be valid. The conclusion that these experiments prove the independence of the endogenous and exogenous types of nitrogen metabolism seems based on inconclusive evidence.

Work of Miller (1944) re-emphasized the existence of substances capable of depressing the urinary nitrogen of dogs maintained on a low protein diet. Methionine and cystine, when fed separately in doses of 1.25 gm. per day over periods of 10 or more days, caused a fall in urinary nitrogen which was maintained for the duration of the feeding. It is possible, according to Miller, that the marked protein-sparing action of these two sulfur-containing amino acids is due to the requirement of the body for them in the elaboration of essential oxidative systems. In this instance, glutathione, particularly, might be involved.

Another illustration of the sparing action of protein in general and of methionine especially appears in the work of Croft and Peters (1945) with experimentally produced burns. They interpret the large losses of nitrogen in the urine following injury of tissues by burns as evidence of the raiding of tissue proteins for the amino acids particularly

needed for the formation of new skin. Then the nitrogen of the unwanted amino acids, arising from the degradation of tissue proteins, is eliminated as urea. In experiments designed to test this theory, Croft and Peters fed diets containing various proteins and amino acids to rats which had been burned according to a standard technique. Rats given a basal 10 per cent casein diet excreted 300 mg. more nitrogen in the 13 days following the burn than they had in a similar period previous to burning. Inclusion of 8 per cent additional casein in the basal diet cut the excess loss to 55 mg. The addition of methionine (1 per cent) was even more effective, less than 30 mg. of excess nitrogen being excreted. That methionine is specific in sparing body protein under these circumstances seems indicated by the fact that the inclusion of 1 per cent of cystine in the ration had no ameliorative effect on the nitrogen loss.

Willman _et al_. (1945) have reported a sparing action of fresh whole egg, dried whole egg, egg white, and egg yolk on the body protein of rats partially depleted of their tissue reserves of nitrogen. When any one of these substances was added to the nitrogen-low diet of the rats, the quantity of nitrogen excreted in the urine was decreased by one-third. As a result of a demonstration that the feeding of liver and muscle tissue extirpated from nitrogen-depleted animals caused a rise rather than a fall in urinary

nitrogen, they suggested that egg proteins were more effective than the rat's own body protein in meeting the inescapable physiological requirements for life. These workers* showed, in addition, that increasing concentrations of dried whole egg in the ration ranging from 1 to 4 per cent caused identical depressions of urinary nitrogen, whereas pork, administered in like fashion, reflected its lower nutritional value for the animal by an increase in urinary nitrogen with each increase in the amount of protein fed. Mitchell, the originator of the balance test as a tool for evaluating the nutritional value of proteins, has not been able to demonstrate this body-sparing action of egg proteins (Mitchell and Carman, 1926). Instead, he has concluded that they are exactly the equivalent of body protein, and, therefore, can be incorporated in the basal diet in the standard nitrogen balance test. However, Mitchell fed the egg proteins at a level at which the animals were in positive nitrogen balance, and it is not surprising that he did not detect any depression of "endogenous" urinary nitrogen. A decrease in creatinine excretion concomitant with the fall in urinary nitrogen in Willman's animals fed the egg diets points to a real, though as yet unexplained, change in the state of nitrogen metabolism of nitrogen-depleted rats when

*Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

fed egg proteins.

Allison, Anderson, and Seeley (1945) confirmed the work of Willman _et al._ (1945) in experiments employing another animal. They demonstrated that the dog, when properly depleted by the combination of the administration of a low-protein diet and plasmapheresis, reacted similarly to the rat, the same depression in the excretion of urinary nitrogen being seen when egg proteins were fed. Nor did the reduction in the excretion occur when casein served as the protein supplement. These investigators, also, interpreted their data to mean a sparing of body nitrogen by the egg proteins.

Schwimmer (1945), experimenting with young adult human males as subjects, was unable to demonstrate the decrease in urinary nitrogen that the addition of egg albumin to a nitrogen-low diet produces in the rat and the dog. He, however, utilized an experimental plan in which he compared the urinary excretion of nitrogen in a group of subjects maintained on a nitrogen-low diet with that of another group fed the nitrogen-low diet plus egg albumin. It is noteworthy, however, that results thus obtained indicated no increase in urinary nitrogen excretion despite the increase in nitrogen intake with the egg feeding. Swanson* also fed dried egg albumin to human subjects living on a nitrogen-

*Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

low diet. When four individuals, subjected to a dietary regime similar to that used in the animal test, were fed egg albumin, there was no significant increase in the amount of nitrogen excreted in the urine. Further control of experimental conditions, especially in regard to depletion time, caloric intake, and digestibility of the supplementary protein would undoubtedly make it easier to demonstrate the "egg effect", if such occurs, in human beings.

Without doubt the concept of body-sparing action of specific proteins and amino acids is verified by experimental findings. It has been demonstrated in several species of animals and by many groups of workers. The next step is to learn how and why this phenomenon takes place.

FUNCTIONS OF METHIONINE

The experiments so far reported reveal in almost every instance that methionine possesses definite tissue-sparing powers. It seems that this compound must be so essential for the physiological life processes of the body that, unless it is provided in the diet, large quantities of tissue protein are broken down to supply it. This section is concerned with the fundamental reactions in which methionine takes part, the formation of structural protein, the provision of cystine, and the provision of labile methyl groups.

Formation of Structural Protein

Methionine exists as a distinct entity in the animal organism only as it participates in the structural composition of body proteins. The following table (Block and Bolling, 1945, pp. 174-198) indicates the concentration of methionine in various commonly occurring animal and vegetable proteins.

TABLE 2

Methionine in Various Proteins
Calculated to 16.0 Per Cent N

Protein	Methionine	Protein	Methionine
	per cent		per cent
Arachin	0.6	Gelatin	0.8
Brain	3	Hair	0.5-0.8
Casein	3.5	Hemoglobin	0.5-1.8
Corn gluten	5.5	Insulin	0
Egg	5	Liver	3.2
Elastin	0.4	Muscle	3.2
Entire animals	3	Rice	3.4
Fibrin	2.2	Serum proteins	2.1

Insulin, of all proteins so far analyzed, has no detectable methionine. All other proteins have concentrations varying from 0.4 per cent in elastin to approximately 5 per cent in egg proteins and corn gluten.

Provision of Cystine

Methionine, as has been shown, is an indispensable component of the diet. One of its functions is the provision of cystine. Cystine, either preformed or fabricated in the body is essential for normal life processes and, in many cases, exerts a sparing action on methionine.

Methionine as a Precursor of Cystine

The role of methionine as a precursor of the physiologically indispensable cystine will be examined from various experimental angles. Work of Weichselbaum (1935) showed that cystine when eliminated from the diet was associated with a specific set of deficiency symptoms. Weichselbaum, raising rats on a standard cystine-deficient diet, produced marked curvature of the spine, partial paralysis of the throat, and ultimate death. Even when the animals had progressed to the moribund stage because of the deficient diet, complete return to normal could be insured by the addition of cystine to the ration. In general, it has been believed that the feeding of methionine is as adequate for any purpose requiring cystine as is cystine itself. Tarver and Schmidt (1939) fed rats methionine containing isotopic sulfur and were able to isolate the "tagged" element from the cystine of body proteins. Thus, they

demonstrated that the ready substitutability of methionine for cystine results from its conversion <u>in vivo</u> into the latter compound.

There are, however, two cases in which methionine is not as satisfactory in meeting body needs as is preformed cystine. Robscheit-Robbins, Miller, and Whipple (1943) found that when any protein or when a mixture containing 11 amino acids was fed to their hypoproteinemic, anemic dogs, production of hemoglobin had high priority over formation of plasma protein. The ratio of production could be modified in favor of plasma protein by only one substance, cystine. This, when substituted for methionine in the amino acid mixture, stimulated plasma protein production far beyond previous levels over a period of seven days. After that time, however, other body processes failed, reflecting the lack of methionine, and the animals lost weight and declined in health. Seligman and Fine (1943) confirmed the finding of Robscheit-Robbins, <u>et al</u>. by the dietary administration of isotopic substances. They fed cystine, homocystine, or methionine containing isotopic sulfur to dogs made hypoproteinemic by Whipple's method of plasmapheresis, and found the highest concentration of isotopic cystine in plasma protein after the feeding of cystine itself. The inferiority of methionine to cystine in plasma protein production may

lie in the fact that the body utilizes the former compound as a source of both labile methyl groups and cystine (Mulford and Griffith, 1942), and, thus, less cystine is available at any one time than is needed under optimal conditions for the production of plasma protein.

Evidence of the superiority of preformed cystine over methionine may also be found in the report of Weichselbaum (1935). The specific deficiency symptoms produced in rats on a cystine-deficient diet could be prevented in their early stages by the administration of either cystine or methionine, but once the animal had advanced to the moribund stage, only cystine had an ameliorative effect. Since post-mortem examination showed hemorrhagic lesions in the liver, it is possible that in the damaged organ the conversion of methionine to cystine could not take place.

Mode of Action of the Sulfhydryl Group

The sulfhydryl group of cystine has long been recognized as an important, indeed, an essential cog in intermediary metabolism. Hammett (1933) believes that the natural regulation of growth as represented by increase in number of cells is due to equilibrium between the sulfhydryl group and its partially oxidized derivatives. His suggestion that glutathione, a tripeptide made up of glutamic acid, cysteine, and glycine, is a key compound is strengthened by a report

of Quastel (1933) showing that glutathione remarkably speeds up the carbon dioxide output of a glucose-yeast mixture. It apparently has an important role in the dynamics of glucose metabolism.

Detoxification

One of the chief components of the chemical defense mechanism of the body against foreign poisons is cystine. Since the time of Howland and Richards (1909), numerous investigators have observed an increase in the neutral sulfur fraction of the urinary sulfur excreted by dogs following the administration of organic halogen compounds. The rise has been interpreted as meaning a conjugation of the foreign substances with tissue cystine to form mercapturic acids, which are then excreted in the neutral sulfur fraction of the urine. Feeding of cystine or methionine with the halogen compound further increased the output of neutral sulfur, an increase due almost entirely to a greater excretion of mercapturic acids (Stekol, 1936).

Cystine plays an important role in maintaining the liver in a normal condition despite exposure to certain halogen compounds. Miller, Ross, and Whipple (1940) found that dogs subjected to chloroform anaesthesia suffered liver damage in direct relation to the extent to which their body stores of protein had been depleted. The administration of

cystine within 24 hours preceding or three hours following the use of the anaesthetic exerted remarkable protective effect (Miller and Whipple, 1942). It is likely that the cystine and chloroform combined, and after acetylation were excreted as the mercapturic acid. Methionine was equally effective in this detoxification mechanism, indicating its conversion to cystine *in vivo*. Its overall beneficial effect, however, was greater than that of cystine, probably because of its lipotropic methyl groups. Cystine, on the other hand, tended to impair *general* liver function by its lipogenic action.

It is not difficult to correlate the protective role of cystine in certain cases of liver poisoning with the concept of the essential nature of glutathione. Just as iodoacetic acid is known to inhibit certain stages of intermediary carbohydrate metabolism by blocking the sulfhydryl group of glutathione (Quastel, 1933; Michaelis and Schubert, 1934) so may chloroform or aromatic halogens such as brombenzene interfere with the action of the tripeptide, either by loose combination with it or by actual withdrawal of the cysteine for detoxification purposes. If the hepatic cells are dependent upon these enzyme-catalyzed carbohydrate oxidations for life, in the absence of the enzyme they will die; necrosis of hepatic tissue will occur. Administration of cystine before anaesthesia or within a short enough time

following it that the enzyme changes are reversable may result in the formation of extra glutathione. Thereupon, the hepatic cells can again function, despite the presence of the poison. Weight is given to this speculation by the finding of Binet, Weller, and Gondard in 1937 that chloroform poisoning in rats caused a decrease in the concentration of glutathione in the liver.

Formation of Taurine

The sulfur-containing amine, taurine, is found solely in bile, where it is important in the control of intestinal digestion, especially as regards the emulsification of fats. Virtue and Doster-Virtue (1937) provided presumptive evidence for the origin of taurine in cystine by experiments with bile-fistula dogs. The animals were given cholic acid in excess in order to deplete the taurine and taurine precursors in the tissues, and to provide an ample supply for later conjugation. After the feeding of cystine, they excreted increased alcohol-soluble material in the bile which was, in all probability, taurine. The likely means of synthesis of taurine in the body involves the oxidation of cystine to cysteic acid, and the decarboxylation of the latter compound to form the amine. That enzymes for such purposes exist in animal tissues has been demonstrated by Medes (1939), who isolated an enzyme from rat liver capable

of catalyzing the oxidation of cystine to cysteic acid, and by Blaschko (1942), who found in dog liver a decarboxylase specific for the conversion of cysteic acid to taurine. Earlier (1936), White and Fishman performed the *in vitro* synthesis of taurine from cysteic acid.

Isotopic studies by Tarver and Schmidt (1942) showed conclusively that the physiological origin of taurine was in cystine. These investigators administered methionine containing radioactive sulfur to bile-fistula dogs previously depleted of taurine by cholic acid feeding, and isolated taurine which contained radioactive sulfur from the bile of these animals. Similar isotopic work by Schoenheimer (1942), indicating the complete interchange of the amino acid components of both food and tissue proteins in a "metabolic pool", was criticized by Mitchell (1944) as being applicable only to the "labile" protein reserves, since the animals were well-fed, not starved, at the time of study. Tarver and Schmidt, pointing out that their dogs had been starved for periods of six to eight days prior to the critical experiment, stated that their results showed beyond doubt that the entire cellular protein is involved in the dynamic interchange.

The Provision of Labile Methyl Groups

Methionine is also needed to supply labile methyl

groups which, under all ordinary circumstances, the body cannot synthesize (du Vigneaud, et al., 1945). Other substances such as choline, lecithin, phosphorylcholine, and betaine contain methyl groups which are labile. Isotopic studies (Simmonds, et al., 1943) have indicated that the body utilizes the methyl radicals of these compounds chiefly for the formation of methionine, which is the key substance in the transmethylation process.

Creatine

The formation of creatine, long shrouded in uncertainty, was demonstrated clearly, once isotopes were available, by du Vigneaud and his associates (1940), Borsook and Dubnoff (1940), and Bloch and Schoenheimer (1941). The amidine group from arginine combines with glycine to form guanidinoacetic acid, which is methylated by methionine. The last step in the synthesis is irreversible; that is, creatine, once methylated, cannot transfer its methyl group to any other compound. Since creatine catalyzes carbohydrate metabolism by providing energy-rich phosphate radicals for the oxidation of glucose, hence is essential for muscle construction, and since it is continually excreted in the urine as creatinine, its formation represents one of the chief demands of the body for methyl groups.

Choline

The methyl group of methionine may also be used in the <u>in vivo</u> synthesis of choline, a substance of marked lipotropic value. Du Vigneaud and his associates (1941) "tagged" the methyl groups of methionine by substituting deuterium for hydrogen, and fed this isotopic substance to rats. Analysis of the animals' tissues revealed choline which contained methyl groups with a high deuterium content, giving proof of the transfer of the methyl radical from methionine to ethanolamine (Stetten, 1942) to form choline.

Without choline or its precursors, methionine and betaine, hemorrhagic degeneration of the kidneys and fatty infiltration of the liver occur (Griffith and Wade, 1940; Griffith, 1941). The effectiveness of the labile methyl-containing compounds seems to lie in their role in the synthesis of lecithin and, hence, of phospholipids. The phospholipids are recognized as essential for the transport of fatty acids in the liver. A sufficient supply of these compounds failing, as would occur in a choline- and methionine- deficient diet, fatty acids would pile up in the liver and the typical "fatty liver" would result. Phospholipids are, in some more obscure way, also responsible for maintaining the normal structure of the kidney.

Other Methylated Compounds

Without doubt, the animal organism requires methyl groups for other reactions than those of which there is at present definite evidence. Epinephrine, one of the hormones of the adrenal gland, is formed from tyrosine by decarboxylation and subsequent oxidation and methylation (Schuler, Bernhardt, and Reindel, 1936). The source of the methyl groups is probably ultimately methionine. Niacin, a member of the vitamin B complex, is excreted in part as trigonelline, and it has been suggested (Quick, 1944) that the methyl groups for this reaction are obtained from methionine. It seems obvious that both these methylation processes are irreversible, and that they would make a continual, inescapable drain upon the body's supply of methyl radicals. In addition, other reactions, as yet unidentified, but essential to normal body processes, may also involve methylation.

SUMMARY AND PROPOSED THEORY OF PROTEIN METABOLISM

The important and quantitatively impressive position of methionine as a structural unit in the molecules of protein which constitute the musculature and soft tissues of the body has been elaborated in the classical definitions of the function of protein. The equally important capacity

of methionine to form glutathione and creatine, essential elements in the carbohydrate oxidation system, and phospholipids, compounds needed for the normal metabolism of fat, has been firmly established.

That amino acids might play a role in nutrition very similar to that of the vitamins, in which they function in minute quantities to regulate body processes, was evident with the realization that the activity of the thyroid gland in regulating metabolism depended upon the amino acid, thyroxine. With the exception of the large group of hormones from the sex glands and from the adrenal cortex, which are related to the steroids, in general the products of the endocrine glands are composed of amino acids, and the digestive enzymes so far isolated seem protein in nature. It has been pointed out that the amino acid, methionine, as a precursor of glutathione, creatine, and the phospholipids, is essential for the metabolism of carbohydrates and of fats. Can the generalization be made that proteins, outside of their structural function, exist in order to make possible the utilization of carbohydrates and fats for energy purposes? In the adult, then, amino acids would be required in very small amounts in order to act as regulators of body functions, and a minute amount of one amino acid administered to a deficient animal could have dramatic results.

The end result of the feeding of extremely small quantities of methionine to protein-deficient animals is a decrease in the quantity of nitrogen excreted in the urine. Whether or not the role of methionine in building glutathione, creatine, and phospholipids is sufficient to account for this sparing action is incapable of precise answer at the present time. However, the demonstrated importance of these compounds for life itself suggests that at least a large portion of the effect may be explained on this basis.

PLAN OF EXPERIMENT

The nature of the body-sparing action of dried whole eggs, which follows their incorporation in the basal nitrogen-low diet of partially depleted rats, has been examined in the present investigation. The study represents a progressive series of experimental units in which the problem was eventually attacked from six angles. These lines of approach are described below:

1. Whether or not the body-sparing action of the dehydrated eggs was due to their amino acid content was unknown. The effect of the dried eggs was referred specifically to their nitrogenous constituents by replacing them in the test ration with a mixture of the ten essential amino acids furnishing an equivalent quantity of nitrogen. Urinary excretion of nitrogen and nitrogen balance were used as indices of measurement.

2. A survey of the literature, together with communications from Dr. Samuel Lepkovsky, coordinator of the Committee on Food Research, Office of the Quartermaster General, Military Planning Division, United States Army, suggested that methionine played an outstandingly important role in nitrogen metabolism; therefore, this amino acid, alone, was added to the basal nitrogen-low diet, and its

effect assayed. As a control to this experiment, the effect of the omission of methionine from the mixture of essential amino acids was tested. The discovery, by this means, that the sulfur-containing amino acid, methionine, was as effective as were egg proteins in reducing the urinary excretion of nitrogen formed the basis of the remaining experimental units.

3. In order to determine if methionine was unique among the essential amino acids in its nitrogen-sparing properties, the other nine essential amino acids were added, one by one, to the basal diet, and the urinary excretion of nitrogen and the nitrogen balance measured.

4. Previous experiments in the Nutrition laboratory of the Foods and Nutrition Department at the Iowa State College had revealed, in contrast to results obtained in similar tests with other proteins, that the capacity of egg proteins to depress the urinary excretion of nitrogen was consistent quantitatively whether they were added to the basal diet in amounts equivalent to 1, 2, 3, 4, or 5 per cent. In the present study, supplementary methionine was fed in daily doses varying from 11 to 175 mg., its action at the six levels of graded intake being compared with that of egg proteins.

5. The physiological mechanism whereby methionine might exert its effect was investigated by determining the

the action of the related compounds, cystine and choline, on nitrogen metabolism

6. In the last unit of the study, methionine and total nitrogen in the whole carcass, liver, and muscle of rats reared under various experimental conditions were determined, in an attempt to gain some indication of the mode of action of the amino acid. Whole carcasses of animals maintained on the basal low-nitrogen diet were so analyzed, as well as those representing animals reared on the basal diet supplemented by methionine. These data were compared with data obtained from similar analyses of a positive control group of animals, fed the regular stock colony diet used in the laboratory, and of a group fed the low-nitrogen diet supplemented with egg proteins. Livers extirpated from animals maintained on the nitrogen-low diet and from others on the same basal diet supplemented with methionine were analyzed for methionine and total nitrogen, making possible an estimate of the effect of the supplement on liver composition. The methionine content of muscle removed from animals maintained on the low-nitrogen diet, alone, or supplemented with egg proteins or methionine, was compared with that of animals fed the standard stock colony diet.

In the first five units, the classical nitrogen balance test as developed by Mitchell (1924) and standardized

in this laboratory (Marshall, 1943; Willman*) was used to measure the response of rats to the various dietary regimes. In the sixth unit, the tissues examined were those of rats treated exactly like the animals in the balance test in regard to dietary manipulation, the animals being sacrificed at appropriate intervals in the regular balance period.

*Willman, W. Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

EXPERIMENTAL PROCEDURE

NITROGEN BALANCE TECHNIQUE

Details of Procedure Used

The standard method for the determination of the biological value of a protein as developed by Mitchell (1924) is based on the estimation of nitrogen balances in two consecutive periods, *i.e.*, a nitrogen-low feeding period followed by one in which the basal low-nitrogen diet is supplemented by a source of protein. The quantity of nitrogen excreted in urine and feces of rats during a period of nitrogen starvation is assumed to be indicative of the "endogenous" nitrogen metabolism. The changes in nitrogen content of urine and feces effected by the addition of a definite amount of the test protein of known nitrogen content reflect the value of that protein in meeting the protein requirements of the animal. Mitchell has defined the biological value of proteins as "the percentage of the absorbed nitrogen (nitrogen intake minus fecal nitrogen of dietary origin) that is not eliminated in the urine (p.901).

Work of Marshall (1943) in the Nutrition laboratory of the Iowa State College revealed that the Mitchell formula for determining biological value could not be applied to

egg proteins, since the feeding of these, at a 4 per cent level, caused a decrease rather than an increase in the excretion of urinary nitrogen. A new method was, therefore, evolved (Marshall, pp. 79-81) utilizing the fact that the feeding of egg proteins brought about a reduction in the large negative nitrogen balances characteristic of the nitrogen-low period. Relation of this reduction, expressed as "body nitrogen spared", to the food nitrogen absorbed was used as an index of the "biological efficiency" of the protein fed.

In the present investigation, the procedure of Marshall was followed, with modifications and refinements introduced by Willman*. The balance test, 29 days in length, was divided into two main periods, as follows:

Nitrogen-low feeding period

Preliminary depletion period	11 days
Collection period	7 days

Nitrogen-feeding period

Adjustment period	4 days
Collection period	7 days

Many of Marshall's animals were in positive balance in the second collection period, when 4 per cent of egg proteins were incorporated in the nitrogen-low ration. Since such a condition makes possible utilization of the excess protein

*Willman, W. Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

for energy purposes or for conversion to fat and carbohydrate, a false biological value is obtained. In all later studies, therefore, Willman reduced the quantity of protein fed to an amount equivalent to 3.5 per cent of the ration. Recent evidence has confirmed the conclusion of Marshall that an 11-day period of protein deprivation is adequate for the depletion of the body stores of protein to such an extent as to bring the animal to a constant plane of nitrogen metabolism. Table 3, showing data collected by the author and by other workers in the Nutrition laboratory of the Iowa State College, demonstrates the constancy of three indices of nitrogen metabolism from the first to the second 7-day collection period, in animals fed the basal low-nitrogen diet, without supplement, for the entire 29 days of the balance experiment. Improvements, to be described in detail in later sections, were made in the method of collection of feces and urine. Daily intakes of food in the protein-feeding period were made equivalent to those in the nitrogen-low-feeding period by restriction of food consumption to the average daily intake during the 7-day collection period when the rats received the nitrogen-low diet.

Selection and Care of Animals

Approximately six-month old male albino rats of the

TABLE 3

Data relating to nitrogen metabolism, expressed per 100 gm. of body weight, of rats fed the nitrogen-low diet in two consecutive metabolism periods

Index	Test	Period I	Period II
		mg.	mg.
Urinary nitrogen	1	87	90
	2	141	147
	3	97	97
	4	110	96
	5	111	102
Urinary creatinine	1	32	30
	2	33	30
Liver nitrogen	1	64	65

Wistar stock, inbred for 94 generations, and maintained from the time of weaning on the stock colony diet (described below) were used for all units of the experiment. Only those animals were chosen which were in good physical condition, especially with regard to freedom from any respiratory infection.

During the 11-day depletion period, the experimental animals were kept in pairs in regular cages. They were allowed free access to the low-nitrogen diet and to water. They were fed 0.5 gm. of the standard synthetic vitamin mixture daily. Food was removed from each cage 10 hours before

the time the first collection period was started. By this means, metabolic end-products reflecting the previous diet did not contaminate the urine of the collection period. On the first morning of the collection period, the animals were placed in individual wide-meshed metabolism cages, which rested on Pyrex glass plates, lined with nine nitrogen-free filter papers. On these papers, acid-treated to prevent loss of ammonia, the major portion of the urine fell and dried. The vitamin mixture was fed daily as before, and the intake of the low-nitrogen diet, to which the animals were allowed unrestricted access, was recorded.

On the morning of the 19th day (first day of the nitrogen-feeding period), the animals, after another 10-hour starvation period, were changed to regular cages, and for the next four days were fed the low-nitrogen diet and the vitamin mixture, supplemented either by protein or by crystalline amino acids. No collections were made during this adjustment period, but intake of the low-nitrogen diet was limited daily to one-seventh of the total amount consumed during the preceding 7-day period.

Collections were again initiated after the four days of adjustment to the nitrogen-containing ration, following the usual 10-hour period of starvation. On the morning of the 23rd day, the animals were transferred to metabolism cages resting on Pyrex plates lined with filter papers.

For the next seven days, they were fed the low-nitrogen diet in daily amounts equivalent to one-seventh of that consumed during the first collection period. The nitrogenous supplement was fed apart from the basal diet. Urinary collections were completed on the morning of the 30th day. It was necessary, however, to collect feces until all representing the nitrogen-feeding period had been excreted, as shown by the appearance of colored feces marking the first post-experimental day.

Diets Fed

All animals were maintained on the standard stock colony diet of the laboratory, known as Steenbock V, from the time of weaning until the time that the experiment was initiated, when the rats were about six months old. A number of animals of this age were used as the source of positive control material in the analysis of whole carcass, liver and muscle for methionine and total nitrogen. Ingredients of the basal portion of the stock ration were:

Ingredient	Amount
Yellow cornmeal	64.0 gm.
Crude casein	5.0
Linseed meal	16.0
Ground alfalfa	2.0
Sodium chloride	0.5
Calcium chloride	0.5
Yeast (Pabst)	1.5
Irradiated yeast (Pabst)	0.5
Wheat germ	10.0
	100.0 gm.

This ration, fed ad libitum, was supplemented daily with 12 ml. of milk containing a mixture of the trace elements and cod liver oil (Clark, 1945). In addition, 5 gm. of fresh ground beef and 10 gm. of fresh cabbage or carrots were fed three times a week.

The basal low-nitrogen diet fed throughout the balance experiment was of the following composition:

Dextrin	73 gm.
Butterfat	10
Lard	10
Osborne and Mendel salt mixture	4
Ruffex	2
Sodium chloride	1
	100 gm.

This ration contained approximately 0.06 per cent of nitrogen. Its nitrogen content was determined exactly before the initiation of each experiment. The nitrogen-low ration was supplemented with a mixture of vitamins made up from materials which were synthetic except for rice bran polish and cod liver oil.. The composition of the mixture and the daily doses of each vitamin are shown below:

Thiamin	40 micrograms
Riboflavin	60 micrograms
Pyridoxine	40 micrograms
Inositol	10 mg.
p-amino benzoic acid	10 mg.
Nicotinic acid	0.5 mg.
Calcium pantothenate	0.1 mg.
Ascorbic acid	1.0 mg.
Choline	5.0 mg.
Biotin	1.0 microgram
Rice bran polish	100 mg.
α-tocopherol	0.75 mg.
Cod liver oil	50.0 mg.

Nitrogenous supplements fed during both the adjustment and collection periods of the nitrogen-feeding period were weighed on cellophane on an analytical balance with an accuracy of $\pm$ 1 mg. They were offered to the animals in the cups containing the standard vitamin mixture, and were, in general, immediately eaten. If this was not the case, no food was given until the supplement was completely consumed.

Collection of Samples for Analysis

Urine

High-quality filter papers containing only traces of nitrogen were soaked overnight in a 10 per cent solution of glacial acetic acid in 95 per cent ethyl alcohol, and air-dried. Nine were placed on the Pyrex plates under each metabolism cage, and one removed each day during a collection period until the final day, when the last three were removed. The papers from each cage were placed in wide-mouthed Erlenmeyer flasks containing 400 ml. of 20 per cent HCl, and covered with two layers of cellophane impervious to moisture. At the end of a collection period, each cage with its Pyrex plate was quantitatively washed with hot, distilled water applied under pressure and the washings transferred to the Erlenmeyer flask containing the filter papers corresponding to the particular cage. The acid

extract from the filter papers was poured quantitatively through a Büchner funnel fitted into a two-liter suction flask. The somewhat disintegrated papers were then transferred to the funnel and washed with hot water until all of the urine was extracted, and rinsed into the suction flask below, after which the Erlenmeyer flask was thoroughly washed. The contents of the suction flask were transferred quantitatively to a two-liter volumetric flask, and made up to volume, after cooling to room temperature. Pharmacy bottles (12-oz.) were filled with the adequately mixed urine sample, and the excess discarded. Recovery experiments (Table 1, APPENDIX) in which a known amount of standard ammonium sulfate was sprinkled on cages at regular intervals over a 7-day period and carried through the entire cage-washing procedure, upheld the validity of this method of collection.

Feces

In order to demarcate sharply the feces that represented the metabolic and digestive processes of any 7-day collection period, the nitrogen-low diet was colored red with ferric oxide (0.1 gm. per 100 gm. of diet) on the first day of each period. The feces representing the food ingested on this day were, therefore, red in color. The first red feces excreted and all following feces were collected

and brushed free of food and hair. On the morning of the 19th and 30th days, food colored red was again fed. The excretion of feces colored red marked the food eaten the day after the collection period terminated. Collections, therefore, continued until red feces appeared. The fecal material was placed in 125 ml. Erlenmeyer flasks containing 50 ml. of 20 per cent HCl, and when the collection from each period was complete, the total suspension was digested on a water bath at approximately 80° C. for four hours (Stearns, 1929). The digest was rubbed through a fine sieve, transferred quantitatively to a 250 ml. volumetric flask, and made to volume. After thorough mixing, the material was transferred to an 8-oz. pharmacy bottle and stored until time of analysis.

Method of Analysis of Urine, Feces, and Food

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

Aliquots of urine extract of appropriate size were digested in a Kjeldahl flask with 20 ml. of concentrated sulfuric acid, 10 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide, for one and one-fourth hours, allowed to cool, and diluted with 200 ml. of tap water. Experimentation by the present investigator had shown this period to be adequate for complete digestion of urine samples. After

reduction of excess mercury with 1 ml. of 4.5 per cent sodium sulfide solution, a slight excess of concentrated sodium hydroxide above the amount needed to neutralize the sulfuric acid was added, and the ammonia thus released was distilled into a known amount of 0.1 N hydrochloric acid. The hydrochloric acid not neutralized by the ammonia was titrated with standard sodium hydroxide solution, approximately 0.1N, with the use of a methylene blue-methyl red indicator. Table 2 in the APPENDIX illustrates the accuracy of the present investigator in applying this technique to the analysis of a standard creatinine solution.

Twenty-five ml. aliquots of the fecal digests were measured by means of a large-bore pipette and, after digestion for one and one-half hours with 20 ml. of concentrated sulfuric acid, 15 gm. of potassium sulfate, and 0.7 gm. of mercuric oxide, were treated similarly to the urine samples.

Weighed portions of the nitrogen-low diet and of the protein or amino acid supplements were transferred quantitatively to Kjeldahl flasks and treated just as were the fecal digests. The diets were also analyzed for moisture and fat, according to methods to be described below, in order to make calculations on a moisture-free, fat-free basis possible.

TISSUE ANALYSIS

Experimental Material

For this phase of the experiment, animals were maintained on the experimental diets for the intervals of time called for by the nitrogen balance test as used in the Nutrition laboratory of the Iowa State College, and were sacrificed at the end of the customary 29 days.

Preparation of the Whole Carcass

The animal had access to food until the time the experiment was terminated. Then it was anaesthetized under ether, the intestinal contents removed, and the entire carcass sealed in a tin can and quick-frozen at -40° C. The carcass was stored at -10° C. until needed for analysis. At this time, the entire carcass was ground in a hand meat-grinder and transferred quantitatively to a two-liter Erlenmeyer flask containing 400 ml. of 20 per cent HCl. All grinder parts and any instruments used in the procedure were washed thoroughly and the washings added to the contents of the flask. Approximately 600 ml. of water was needed for the process. The ground carcass was autoclaved at 125° C. and 15 lb. pressure for 7 hours. Table 3 in the APPENDIX indicates that these conditions were adequate for the complete

hydrolysis of proteins, yet gentle enough that none of the methionine was destroyed. After cooling to room temperature, the digests were made to volume in a two-liter volumetric flask and sufficient portions of the well-mixed material for replicate analyses were stored in 12-oz. bottles.

The total nitrogen present in the digests was determined according to the Kjeldahl-Gunning technique previously described. Before investigation of the methionine content, 100 ml. portions of the digests were shaken gently with one-half teaspoon of activated charcoal* and heated to approximately 60° C., in order to remove highly colored degradation products. Lavine (1943) has shown that no loss of methionine is entailed in the decolorization of digests in acid solution. After cooling, the mixtures were filtered through fine filter paper and 15 ml. aliquots of the water-clear filtrates taken for methionine determinations.

Extirpation and Hydrolysis of Liver

The rats were starved for 10 hours preceding the time of killing, for the purpose of eliminating the variable effect of food consumption on the composition of liver tissue. After ether anaesthesia, 5 ml. of blood was removed from the heart of each animal, in order that liver tissue itself

*Nuchar, from West Virginia Pulp and Paper Co., 230 Park Ave., N.Y.C.

and not liver tissue plus a large quantity of blood might be analyzed. The entire liver was removed, trimmed free of connective tissue and blood vessels, and blotted to remove surface blood. Approximately 1 gm. was placed in a weighing bottle for moisture and fat analyses, the remainder in a weighed, stoppered 125 ml. Erlenmeyer flask for methionine analysis. To the latter portion, after weighing, 4 ml. of 20 per cent HCl and 10 ml. of water were added. The flask was capped with tinfoil and autoclaved for 7 hours at 125° C. and 15 lb. pressure. In the instances where both methionine and total nitrogen analyses were performed on the same liver, approximately equal portions of the organ were reserved for each analysis, moisture and fat determinations being omitted.

Extirpation and Hydrolysis of Muscle

Animals whose livers had been removed for analysis as indicated above were the source of muscle tissue for methionine and moisture and fat determinations. The left gastrocnemius muscle was extirpated as completely as possible and placed in a weighing bottle for moisture and fat analysis. The right gastrocnemius, similarly removed, was placed in a 125 ml. Erlenmeyer flask, weighed, and prepared for methionine analysis in the same manner as were the liver samples.

Preparation of Tissue Extracts

The samples of liver and muscle, after autoclaving, were treated with one-fourth teaspoon of activated charcoal, heated to 60° C., and filtered through fine filter paper into a 50 ml. volumetric flask. After careful washing of the Erlenmeyer flask, filter paper, and funnel with warm water, the contents of the volumetric flask were cooled and diluted to 50 ml. with distilled water.

Analytical Methods

Moisture Analysis

Weighed samples of liver and muscle were dried in an air oven at 105° C. for 7 days, or until their weight was constant within 0.5 mg.

Determination of Fat

The determination of fat was based on the method of Bloor (1929), which gives values for fat in terms of total alcohol-ether soluble substances. Moisture-free samples of liver and muscle were ground in a mortar with one-half teaspoon of acid-washed sand and transferred quantitatively to a 125 ml. Erlenmeyer flask. Weighing bottle and mortar were rinsed with three 1-ml. portions of absolute alcohol. Approximately 35 ml. of a 3 to 1 mixture of alcohol and

anhydrous ethyl ether were added to the sample, and the mixture boiled on a steam bath, with shaking, for five minutes.

After cooling to room temperature the solution was filtered quantitatively through Whatman no. 45 fat-free filter paper into a 200 ml. volumetric flask. The filter paper holding the ground tissue was washed with several small portions of the alcohol-ether mixture and it, with its contents, was extracted for five hours with anhydrous ethyl ether in a Goldfisch extraction apparatus.

The ether extract, plus rinsings of the extraction cup, was filtered into the 200 ml. volumetric flask containing the alcohol-ether filtrate, and the solution made to volume with ether. Fifty ml. aliquots of this solution were measured into large weighing bottles, evaporated to dryness on a steam bath at 80-90° C., and brought to constant weight in an air oven at 80° C.

Determination of Methionine

Procedure. The method of Albanese, Frankston, and Irby (1944) for the estimation of methionine was used in this experiment, after unsuccessful attempts to reproduce the procedure of McCarthy and Sullivan (1941). The former workers, utilizing the discovery of Toennies and Callan in 1939 that methionine and cystine were oxidized by hydrogen peroxide at different rates, found that by proper control

of experimental conditions a method could be developed which was specific for methionine.

After decolorization of the digests with charcoal and dilution to 50 ml., 15 ml. aliquots of tissue or food extracts were measured into 125 ml. Erlenmeyer flasks, and diluted to 17 ml. with distilled water. Blanks composed of 17 ml. of distilled water, and aliquots of a standard solution made up of 2 ml. of a methionine solution (10.0688 mg. per 2 ml.) and 15 ml. of distilled water were also measured. To each reaction flask, 3 ml. of a hydrogen peroxide-perchloric acid oxidizing mixture was added with mixing, and the flask stoppered. In exactly one hour the reaction was terminated by dilution with 30 ml. of distilled water. After addition of 200ml. of a 1 per cent potassium iodide solution made up in 0.1 per cent ammonium molybdate solution, the free iodine released by the action of the excess hydrogen peroxide on the potassium iodide was titrated with approximately 0.1N sodium thiosulfate solution, with a 1 per cent solution of soluble starch in saturated sodium chloride as the final indicator. From the blank and standard titrations, a factor was obtained which indicated the number of milligrams of methionine equivalent to 1 ml. of standard sodium thiosulfate solution. From this, and the titrations of each of the unknown samples, the number of milligrams of

methionine in each food or tissue digest was determined. A sample calculation is recorded in the APPENDIX (Table 4).

Reagents. The original directions prescribed an oxidizing mixture made up of 96 ml. of an 80 per cent perchloric acid solution and 4 ml. of 30 per cent hydrogen peroxide, diluted to 300 ml. with distilled water. It was found, however, that even hydrogen peroxide taken from freshly opened bottles was not as active as the solution Albanese, et al. evidently had available. The use of double the prescribed volume of hydrogen peroxide was necessary, therefore, to provide a mixture strong enough to oxidize the methionine present in the usual aliquot. Once prepared, this mixture was stable at least 8 weeks if refrigerated, giving the same factor with standard sodium thiosulfate solution over the entire period (Table 5, APPENDIX).

Twenty liters of 0.1N sodium thiosulfate solution, made up in carbon dioxide-free water, with 1 per cent amyl alcohol, was likewise stable for several weeks, as indicated by Table 6 in the APPENDIX.

Specificity of reaction. Albanese, et al. (1944) warn in their original article that unless the concentrations of both hydrogen peroxide and perchloric acid in the oxidizing mixture are carefully controlled, cystine and tryptophane will be oxidized by hydrogen peroxide at a rate fast enough to interfere with the estimation of methionine. To determine

the critical concentration of these two constituents of the oxidizing mixture at which point all the methionine in a digest would be measured and none of the cystine or tryptophane, several oxidizing mixtures were prepared (Table 7, APPENDIX). These contained varying concentrations of hydrogen peroxide, and were added to standard solutions of methionine, cystine, and tryptophane. As Table 8 in the APPENDIX shows, at no point was there interference by tryptophane, but at too high and too low concentrations of hydrogen peroxide, cystine was oxidized speedily enough to interfere. The oxidizing mixture best suited for the determination of methionine was one containing 56 ml. of perchloric acid and 4 ml. of 30 per cent hydrogen peroxide, diluted to 175 ml. with distilled water (Table 9, APPENDIX).

Reproducibility, accuracy, and agreement with other methods. Repeated analyses of a sample of dried eggs by the method outlined above gave results agreeing closely from time to time (Table 10, APPENDIX). The constancy of the values for methionine content obtained with varying periods of autoclaving indicated that the particular time chosen was adequate for the complete release of methionine from the protein without destruction (Table 3, APPENDIX).

Crystalline methionine, added in known amounts to the egg proteins before autoclaving, was recovered to an extent which indicated that no destruction or loss of methionine

took place during the entire process (Table 11, APPENDIX).

The results obtained with eggs are in close agreement with others recorded in the literature, as is indicated by Table 12 in the APPENDIX.

RESULTS AND DISCUSSION

EXPERIMENT 1

The relation of the body-sparing action of dried whole eggs to their nitrogenous constituents was determined in the first unit of the present study. Three groups of animals were used. One group, as the negative control, was fed the basal low-nitrogen diet throughout the entire balance period of 29 days. To the second group, egg proteins were offered in a quantity equivalent to 3.5 per cent of the ration consumed in the previous collection period when the low-nitrogen diet was fed. The egg proteins were fed separately and supplied 421 mg. of nitrogen daily. The third group of animals received as supplement to the basal diet 374 mg. of nitrogen per day derived from a mixture of the ten essential amino acids. Crystalline amino acids were used, each supplying one-tenth of the total nitrogen of the mixture. The composition of the amino acid mixture and the form in which each amino acid was fed are shown in Table 4.

Average data pertaining to the three test groups are presented in Table 5 which describe the three groups of experimental animals in respect to certain characteristics, i.e., body weight, surface area, caloric requirement, and

TABLE 4

Composition of complete amino acid mixture

Amino acid, as fed	Amount of *l*-amino acid needed to supply 40 mg. N per day	Amount of amino acid, as fed, needed to supply 40 mg. N per day
	mg.	mg.
l-lysine	203	203
l-tryptophane	291	291
l-histidine hydrochloride	148	148
dl-phenylalanine	471	471
l-leucine	374	374
dl-isoleucine*	374	748
dl-threonine*	340	680
dl-methionine	436	436
dl-valine	668	668
l-arginine	124	124

*Twice the quantity of isoleucine and threonine needed to supply 40 mg. N per day was incorporated in the mixture, because the *d*-forms of these amino acids are not utilizable.

TABLE 5

Average data descriptive of body size, caloric requirement, and caloric
various nitrogenous supplements to a nitrogen-low diet (Exp.

Supplement fed	Number of animals in group	Nitrogen fed in supplement per day in Period II	Average body weight		Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement**	
			Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
		mg.	gm.	gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.
None N-low)	5	0	272	254	- 8	- 8	362	347	27.4	26.3
Dried whole egg	4	421	286	284	-11	+ 3	372	371	28.2	28.1
10 essential amino acids	5	374	240	229	- 9	+ 6	335	328	25.4	24.9

*In this and all subsequent tables in the body of the thesis, Period I refers to the fir
maintained on a nitrogen-low diet, and Period II to the second collection period, when
supplement in addition to the basal ration.

**Calculated on basis of 75.8 cal. per sq. meter, average of results of Smuts (1935), Mi
and Benedict, Horst, and Mendel (1934).

***Estimated as 1 1/2 times the basal caloric requirement.

TABLE 5

ıf body size, caloric requirement, and caloric intake of rats fed
;enous supplements to a nitrogen-low diet (Exp. 1)*

ody	Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement**		Estimated total daily caloric requirement***		Av. daily food intake in grams		Av. daily food intake in calories	
.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
m.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal.	cal.	gm.	gm.	cal.	cal.
54	- 8	- 8	362	347	27.4	26.3	41.1	39.5	12.0	11.2	57.5	53.7
84	-11	+ 3	372	371	28.2	28.1	42.3	42.1	11.5	11.4	55.3	54.7
29	- 9	+ 6	335	328	25.4	24.9	38.1	37.4	9.5	8.7	45.6	41.8

body of the thesis, Period I refers to the first collection period, when the animals were
riod II to the second collection period, when the animals were offered a nitrogenous
n.

meter, average of results of Smuts (1935), Mitchell and Carman (1924), Benedict (1932),

ric requirement.

caloric adequacy of the food consumed. It should be noted that the group of animals fed the egg proteins and the one fed the mixture of essential amino acids both gained weight during the last six days of the second collection period, an indication in itself of the marked body-sparing properties of the two supplements. Comparison of the estimated total daily caloric requirement of the animals with the daily food intake in calories shows in this and in the four experiments to follow that, with one exception (see Table 15), the energy-producing value of the food consumed by the animals more than met the calculated caloric requirement. Caloric deprivation, thus, was eliminated as a factor that might influence nitrogen metabolism. The relative importance of this relationship was discussed by Allison and Anderson (1945).

Metabolic data showing the response of the three groups of rats to the experimental diets fed are shown in Table 6. The quantity of nitrogen excreted in the urine in the two periods of the test, and the respective nitrogen balances are expressed in terms of absolute values and relatively, i.e., per 100 gm. body weight and per 100 sq. cm. body surface.

The characteristic depression in excretion of urinary nitrogen first noted by Marshall (1943) following the incorporation of egg proteins in a nitrogen-low ration again

TABLE 6

Nitrogen metabolism data (Exp. 1)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per. I	Per. II	Dif.	Per. I	Per. II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
EXPRESSED AS ABSOLUTE VALUES						
N-low	260	244	-16	-345	-333	12
Dried whole egg	275	212	-63	-384	+ 77	461
10 essential amino acids	319	288	-31	-401	+ 35	436
EXPRESSED AS N PER 100 GM. BODY WEIGHT						
N-low	97	97	0	-136	-133	3
Dried whole egg	97	77	-20	-136	+ 26	162
10 essential amino acids	136	119	-17	-170	+ 16	186
EXPRESSED AS N PER 100 SQ. CM. BODY SURFACE						
N-low	72	70	- 2	-101	- 96	5
Dried whole egg	74	58	-16	-103	+ 20	123
10 essential amino acids	99	87	-12	-120	+ 11	131

occurred. A comparison based on absolute values indicates that a similar, although not as marked, depression resulted from the addition of a mixture of the ten essential amino acids to the basal ration of the third group of experimental animals. However, when differences in body size are eliminated by expression of the data in terms of body weight or surface area, the two dietary supplements in the quantities provided (421 mg. of nitrogen from egg proteins daily and 374 mg. from amino acids) were equally effective in depressing the urinary excretion of nitrogen.

The degree of improvement in nitrogen balance brought about by the addition of a nitrogenous supplement to the diet of animals fed a nitrogen-low ration may be considered as an index of the nutritive value of the supplement (Willman, et al., 1945; Allison, Anderson, and Seeley, 1945). In the Nutrition laboratory of the Iowa State College, this decrease in negative nitrogen balance has been termed "body nitrogen spared", since it represents the nitrogen from the body of the animal which would have been lost, had no supplement been fed. Data in Table 6, expressed in terms of absolute values or of body size, show that the mixture of the ten essential amino acids shared with egg the capacity of sparing body nitrogen. It seems certain, as a result of this experiment, that the body-sparing properties of dried whole eggs reside in the essential amino acids which compose

the egg proteins. Graphic presentation of the metabolic data appears in Figures 1 and 2.

An attempt was made to test the validity of the concept, "body nitrogen spared". Data representing the first metabolism period in all groups of rats studied in the present investigation formed the basis for this evaluation. Actual loss in body weight incurred during the last six days of the nitrogen-low collection period was related to the quantity of body tissue represented by the value of the negative nitrogen balance in the same period.

Analyses of carcasses of 36 animals by the author, to be presented in more detail in Experiment 6, revealed that the average nitrogen content of the carcasses of rats fed a nitrogen-low diet for 29 days, during the last 11 of which a small amount of supplementary nitrogen was supplied, was 2.6 per cent nitrogen, or 16.2 per cent protein (N x 6.25). This average figure was used to convert the value for the nitrogen balance, reduced by one-seventh to correspond with the 6-day period over which the actual weight loss was observed, to its equivalent in body tissue. In Table 7, the observed loss in body weight of each group of rats during the last six days of the nitrogen-low collection period may be compared with the loss in body weight calculated from the nitrogen balances for the same group. The average value for the observed weight loss was 10 gm.;

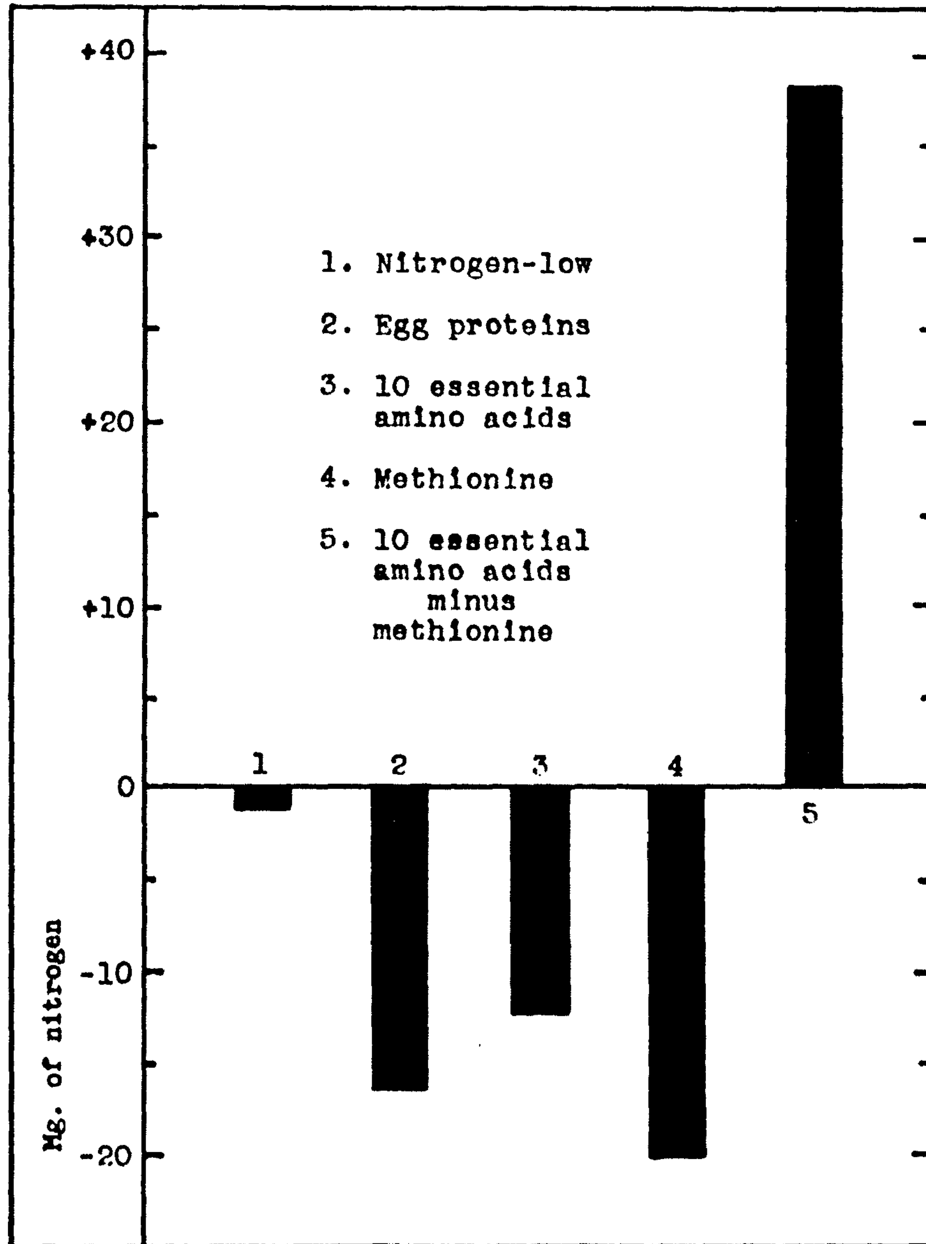


FIGURE 1
DEPRESSIONS AND ELEVATION OF URINARY EXCRETION OF NITROGEN PER 100 SQ. CM. OF BODY SURFACE CAUSED BY ADDITION OF EGG PROTEINS, A MIXTURE OF THE TEN ESSENTIAL AMINO ACIDS, METHIONINE, AND A MIXTURE OF THE TEN ESSENTIAL AMINO ACIDS MINUS METHIONINE TO THE DIET OF RATS FED A NITROGEN-LOW RATION

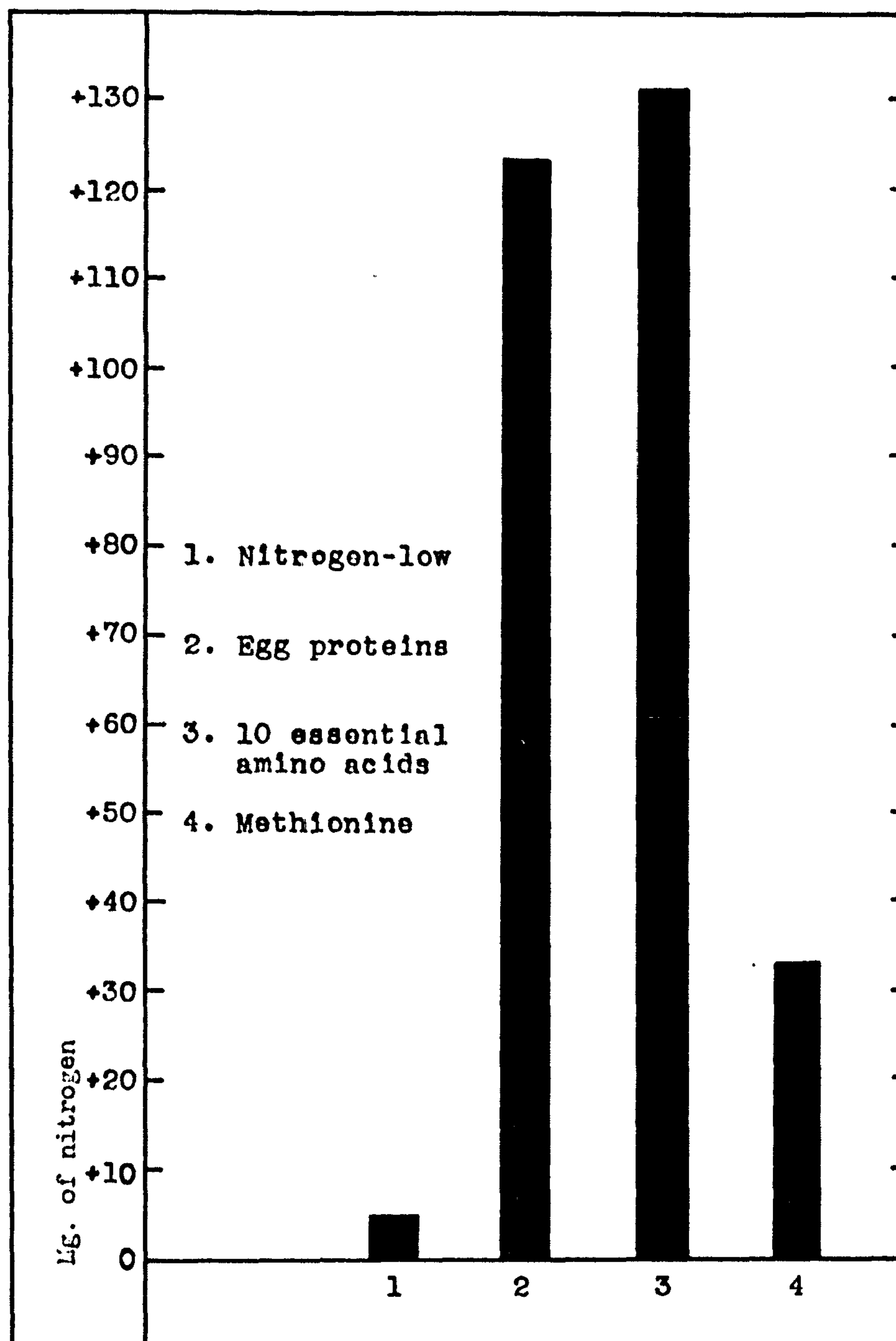


FIGURE 2
BODY NITROGEN SPARED PER 100 SQ. CM. OF BODY SURFACE BY ADDITION OF EGG PROTEINS, A MIXTURE OF THE TEN ESSENTIAL AMINO ACIDS, AND METHIONINE TO THE DIET OF RATS FED A NITROGEN-LOW DIET

TABLE 7

Body tissue equivalent of nitrogen balance in period I

Group	Loss in body wt. last 6 days of Per. I	Nitrogen balance in Per. I	Nitrogen balance x 6/7	Body tissue equivalent
	gm.	mg.	mg.	gm.
N-low	8	345	296	11
Egg	11	384	329	12
10 essential amino acids	9	401	344	13
Methionine	7	374	321	12
Arginine	5	353	302	12
Histidine	6	373	320	12
Leucine	11	392	336	13
Valine	6	429	368	14
Lysine	10	365	313	12
Isoleucine	11	363	311	12
Phenylalanine	8	371	318	12
Threonine	9	351	300	12
Tryptophane	10	351	306	12
Methionine				
1 mg.	9	334	286	11
2 mg.	7	332	284	11
3 mg.	13	236	202	8
4 mg.	12	332	284	11
8 mg.	11	317	272	11
16 mg.	8	332	284	11
Egg 1%	10	298	255	10
2%	15	309	265	10
3%	14	294	252	10
4%	11	301	258	10
5%	11	292	250	10
Cystine	9	256	219	8
Choline	3	257	220	9
Average	10			11

that of the calculated, 11 gm. The nitrogen balance reflects changes in body weight in the period when the nitrogen-low diet is fed, and is indicative of the destruction of body tissue brought about by continuing demands of the body for amino acids in the face of the dietary deprivation of nitrogen.

This method of interpretation was next applied to the data characteristic of the subsequent period of the balance test when a nitrogenous supplement definitely body-sparing in action was fed. Data in Table 8 shows that the addition of egg proteins or of a mixture of the ten essential amino acids not only prevented the substantial loss in body weight that occurred in the same animals during the nitrogen-low feeding period, but caused a definite gain in weight. Thus, the loss prevented by the supplement plus the actual increment in weight must be equivalent to the body tissue represented by the body nitrogen spared, i.e., the difference in the nitrogen balances in the two periods. This hypothesis is supported by the fact that the group of animals maintained on a nitrogen-low diet throughout the entire balance test suffered exactly the same loss in weight in Period II as in Period I. In this case, only an insignificant amount (12 mg.) of body nitrogen was spared. With continuance on the nitrogen-deficient diet, this particular group of animals made slightly more economical use of body supplies than

TABLE 8

Body tissue equivalent of body nitrogen spared

Supplement	Change in wt. last 6 days		Body wt. loss prevented	Body N spared	Body N spared x 6/7	Body tissue equivalent
	Per.I	Per.II				
	gm.	gm.	gm.	mg.	mg.	gm.
None	- 8	-8	0	12	10	0
Egg	-11	+3	14	461	395	15
10 essential amino acids	- 9	+6	15	436	374	14

they did in the early days of the experiment. However, the quantity of body nitrogen spared, when converted to its equivalent in body tissue, was so small that it may be taken to agree with the observation of a constant rate of weight loss during both periods.

The relation of the body tissue equivalent of the "body nitrogen spared" to _weight loss prevented_ by the addition of egg proteins or of the complete amino acid mixture is indicated in Table 8. The similarities of the two values in each experimental group achieved by such different means, argue for the validity of the concept of "body nitrogen spared". The results of this analytical method of treatment of the data obtained in Experiment 1 suggest that,

under carefully controlled conditions, determination of the changes in the body weight which result from the addition of a nitrogenous supplement to the diets of animals fed a nitrogen-low ration may be used as an index of the nutritive value of the supplement.

EXPERIMENT 2

The results obtained in Experiment 1 indicate that the marked body-sparing property of dried whole eggs is due primarily to their amino acid content. Frequent reports in the literature of the effectiveness of methionine in improving the state of nitrogen metabolism of various experimental animals, as well as correspondence with Dr. Samuel Lepkovsky, suggested that this amino acid might be specifically responsible, in part at least, for the effect of both the proteins of eggs and of the complete amino acid mixture on urinary excretion of nitrogen and on nitrogen balance. Therefore, in Experiment 2, the efficacy of methionine alone and of a mixture containing all of the essential amino acids except methionine was tested. Three groups of animals received methionine; a fourth, a mixture of the ten essential amino acids minus methionine. In the three replications of the methionine experiment, one group of animals received a quantity of methionine supplying 3 mg. of

nitrogen per day, and the other two, studied three months apart, a quantity of methionine furnishing 4 mg. of nitrogen per day.

Table 9 presents pertinent data concerning the group of animals fed the incomplete amino acid mixture and the groups fed methionine. All animals, it may be seen, lost less weight in the second metabolism period than in the first, indicating the favorable action of the supplement in decreasing the degree of breakdown of body tissue.

Metabolic data based on the response of the four experimental groups to the administration of the various supplements are assembled in Table 10. It is evident that a greater depression in the excretion of urinary nitrogen resulted from the addition of methionine to the basal ration than from the addition of dehydrated eggs (Figure 1). However, the quantities of nitrogen furnished by the two supplements were of very different order, the egg supplying 421 mg. of nitrogen per day, and the methionine only 3 or 4 mg. Allison and his co-workers (1946) later found that the dog, if adequately depleted of its labile stores of nitrogen, responds similarly to the administration of methionine.

Omission of methionine from the mixture of the essential amino acids caused an increase in the urinary excretion of nitrogen of 110 mg., as compared with the decrease in

TABLE 9

Average data descriptive of body size, caloric requirement, and calori
various nitrogenous supplements to a nitrogen-low diet (Ex

Supplement fed	Number of animals in group	Average body weight		Change in weight last 6 days of collection		Average body surface area		Basal daily caloric requirement		Est: tot: cal quii
		Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.
		gm.	gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal
Methionine	6	213	195	-13	-5	312	296	23.6	22.4	35.
	6	257	239	- 7	-7	350	335	26.5	25.4	39.
	5	273	248	-12	-6	363	342	27.5	25.9	41.
10 essential amino acids minus methionine	6	260	238	-15	-10	353	334	26.8	25.3	40.

* See footnotes to Table 5.

TABLE 9

' body size, caloric requirement, and caloric intake of rats fed
nous supplements to a nitrogen-low diet (Exp. 2)*

ange in ight last da$s of llection		Average body surface area		Basal daily caloric requirement		Estimated total daily caloric requirement		Av. daily food intake in grams		Av. daily food intake in calories		Nitrogen fed in supplement per day in Period II
r.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	PerII	
m.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal.	cal.	gm.	gm.	cal.	cal.	mg.
3	-5	312	296	23.6	22.4	35.4	33.6	10.5	9.4	50.4	45.2	3
7	-7	350	335	26.5	25.4	39.8	38.1	1014	9.8	49.8	47.0	4
2	-6	363	342	27.5	25.9	41.3	38.9	9.3	8.8	44.6	42.3	4
5	-10	353	334	26.8	25.3	40.2	38.0	9.5	8.6	45.6	41.2	37

TABLE 10

Nitrogen metabolism data (Exp. 2)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
EXPRESSED AS ABSOLUTE VALUES						
Methionine						
3 mg. N	236	149	-87	-347	-210	137
4 mg. N	278	200	-78	-374	-248	126
4 mg. N	332	245	-87	-454	-308	146
10 essential amino acids minus methionine	308	418	+110	---	---	---
EXPRESSED AS N PER 100 GM. BODY WEIGHT						
Methionine						
3 mg. N	112	77	- 35	-166	-109	57
4 mg. N	108	84	- 24	-146	-104	42
4 mg. N	122	100	- 22	-166	-124	42
10 essential amino acids minus methionine	118	176	+ 58	---	---	--
EXPRESSED AS N PER 100 SQ. CM. BODY SURFACE						
Methionine						
3 mg. N	76	50	-26	-112	-71	41
4 mg. N	80	60	-20	-107	-74	33
4 mg. N	91	72	-19	-125	-90	35
10 essential amino acids minus methionine	87	125	+38	---	--	--

excretion of 31 mg. which followed the feeding of the complete amino acid mixture. Some of the incomplete mixture was, nonetheless, utilized, since of the 259 mg. of nitrogen fed, 149 mg. did not appear as "extra" nitrogen in the urine. In a similar experiment, Robscheit-Robbins and Miller (1946), using dogs as experimental subjects, found that administration of an amino acid mixture complete except for methionine caused an increase in the amount of nitrogen excreted in the urine.

Calculation of the amount of body nitrogen spared showed that methionine had some ability to conserve body tissue, but, as is shown graphically in Figure 2, it was much less effective than egg proteins or than the mixture of the ten essential amino acids. This is not surprising, since the metabolic requirements of the body are far too varied to permit their satisfaction by any one amino acid; a mixture of complete proteins, such as those of eggs, or a mixture of the essential amino acids should be more capable of meeting a variety of demands.

The three tests in which methionine was fed were essentially replicates of one another. It is interesting to observe in Table 10 that in the two groups fed identical quantities of methionine the urinary nitrogen and nitrogen balance data, expressed in terms of body weight or of body surface area, are identical in almost every instance. Such

reproducibility of results argues for their validity, and upholds the belief that methionine is an effective agent in the sparing of body nitrogen.

The data illustrating the influence of methionine represent an interesting phenomenon. Here is an example of a specific capacity on the part of a single substance to spare urinary nitrogen coincident with a direct loss in body weight. Casual comparison of the relative ability of egg proteins and of methionine in decreasing losses of nitrogen in the urine (Figure 1) might lead to the conclusion that methionine, because of its somewhat greater effect in this respect, makes a more important contribution than egg proteins to nitrogen metabolism. However, the data in Table 11 show that rats fed supplementary methionine continued to lose weight during the second metabolism period. Catabolism proceeded at a slower rate than during the nitrogen-low collection period, so that actually a loss of 5 gm. of weight was prevented. Concomitant with this loss in body weight, the metabolism data indicate a sparing of body substance. Conversion of body nitrogen spared by the addition of methionine to its equivalent in body tissue shows an average sparing of body tissue that amounts to 5 gm., a value identical with the average loss in weight prevented during the same period. Thus, methionine when fed as a supplement to a nitrogen-low ration does not cause a gain in body

TABLE 11

Body tissue spared by methionine

Supplement	Change in wt. last 6 days		Body wt. loss prevented	Body N spared	Body N spared x 6/7	Body tissue equivalent
	Per. I	Per. II				
	gm.	gm.	gm.	mg.	mg.	gm.
Methionine						
3 mg. N	-13	-5	8	137	117	5
4 mg. N	- 7	-7	0	126	108	4
4 mg. N	-12	-6	6	146	125	5
Average	-11	-6	5	136	117	5

weight nor exert the maximum body-sparing capacity. However, it is important that rats fed methionine as a dietary supplement lose 5 gm. less than they would have had no supplement been given. This is particularly notable because it is caused by the addition of a very small quantity of a single amino acid.

It was noted in the REVIEW OF LITERATURE that the depression in the urinary excretion of nitrogen which follows the incorporation of egg proteins in a nitrogen-low diet was paralleled by a simultaneous depression in the urinary excretion of creatinine. In light of the observed depression in the excretion of nitrogen brought about by methionine administration, creatinine determinations were made on the

same material that was analyzed for nitrogen. The data in Table 12, showing a depression in the excretion of creatinine resulting from the feeding of either egg proteins or of methionine, casts further doubt on the constancy of the "endogenous" metabolism, as postulated by Folin.

TABLE 12

Urinary excretion of creatinine in the two periods of the nitrogen balance test

Supplement	Urinary creatinine		
	Period I	Period II	Difference
	mg.	mg.	mg.
Egg	106	82	24
Methionine	97	70	27

EXPERIMENT 3

Evidence from Experiment 2, that one amino acid, methionine, depressed the urinary excretion of nitrogen as much as egg proteins or a complete mixture of the essential amino acids, led to the next step, *i.e.*, the testing in the same fashion of all of the amino acids considered essential for the nutrition of the rat. Each of the ten amino acids was fed as the sole supplement to the nitrogen-low ration of

ten groups of rats composed of six animals each. The amino acids were fed in a quantity supplying 4 mg. of nitrogen per day. This standard amount was suggested by the work of Wolf and Corley (1939). Data descriptive of various characteristics of these experimental groups are presented in Table 13.

Graphic illustration of the effect on the urinary excretion of nitrogen of the feeding of the individual amino acids, expressed in Figure 3 in terms of surface area, shows that methionine was outstanding in its ability to decrease urinary losses of nitrogen. Arginine and histidine caused significant, although less marked, depressions (see Table 14). The action of these basic amino acids may be explained in viewof the requirements of the body for these substances for the synthesis of nucleoproteins. Arginine also participates in the urea cycle, which is one suggested mechanism by which excess nitrogen is excreted by the body. The feeding of isoleucine, lysine, phenylalanine, tryptophane, and thoronine seems to induce increments in the quantity of nitrogen excreted in the urine. The significance of the data, however, may be questioned, for the increases observed are within two standard deviations of the mean excretion of urinary nitrogen in rats fed the nitrogen-low diet.

Changes in urinary excretion of nitrogen, however, do

TABLE 13

Average data descriptive of body size, caloric requirement, and
fed various nitrogenous supplements to a nitrogen-low d

Supplement fed	Number of animals in group	Nitrogen fed in supplement per day in period II	Average body weight		Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement	
			Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I.	Per.[illegible]
		mg.	gm.	gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.
Methionine	6	4	257	239	- 7	- 7	350	335	26.5	25.[illegible]
Arginine	4	4	245	227	- 5	- 6	340	324	25.8	24.[illegible]
Histidine	6	4	271	248	- 6	- 6	361	342	27.4	25.[illegible]
Leucine	6	4	237	224	-11	- 9	333	322	25.2	24.[illegible]
Valine	2	4	280	263	- 6	- 6	366	353	27.7	26.[illegible]
Isoleucine	6	4	262	243	-11	- 6	354	338	26.8	25.[illegible]
Lysine	6	4	248	230	-10	- 4	342	327	25.9	24.[illegible]
Phenylalanine	6	4	268	248	- 8	- 9	358	342	27.1	25.[illegible]
Tryptophane	6	4	264	244	-10	- 6	355	340	26.9	25.[illegible]
Threonine	5	4	276	260	- 9	- 7	365	352	27.7	26.[illegible]

*See footnotes to Table 5.

TABLE 13

ive of body size, caloric requirement, and caloric intake of rats
itrogenous supplements to a nitrogen-low diet (Exp. 3)*

ly	Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement		Estimated total daily caloric requirement		Average daily food intake in grams		Average daily food intake in calories	
I	Per.I	Per.II	Per.I	Per.II	Per.I.	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal.	cal.	gm.	gm.	cal.	cal.
	- 7	- 7	350	335	26.5	25.4	39.8	38.1	10.4	9.8	49.8	47.0
	- 5	- 6	340	324	25.8	24.6	38.7	36.9	11.2	8.6	53.7	41.2
	- 6	- 6	361	342	27.4	25.9	41.1	38.9	11.7	10.3	56.1	49.4
	-11	- 9	333	322	25.2	24.4	37.8	36.6	10.3	8.9	49.4	42.7
	- 6	- 6	366	353	27.7	26.8	41.6	40.2	12.3	11.5	59.1	55.3
	-11	- 6	354	338	26.8	25.6	40.2	38.4	11.0	10.5	52.8	50.4
	-10	- 4	342	327	25.9	24.8	38.9	37.2	10.9	9.9	52.3	47.5
	- 8	- 9	358	342	27.1	25.9	40.7	38.9	11.5	10.1	55.3	48.5
	-10	- 6	355	340	26.9	25.8	40.4	38.7	11.1	9.8	53.3	47.0
	- 9	- 7	365	352	27.7	26.7	41.6	40.1	11.1	10.3	53.3	49.4

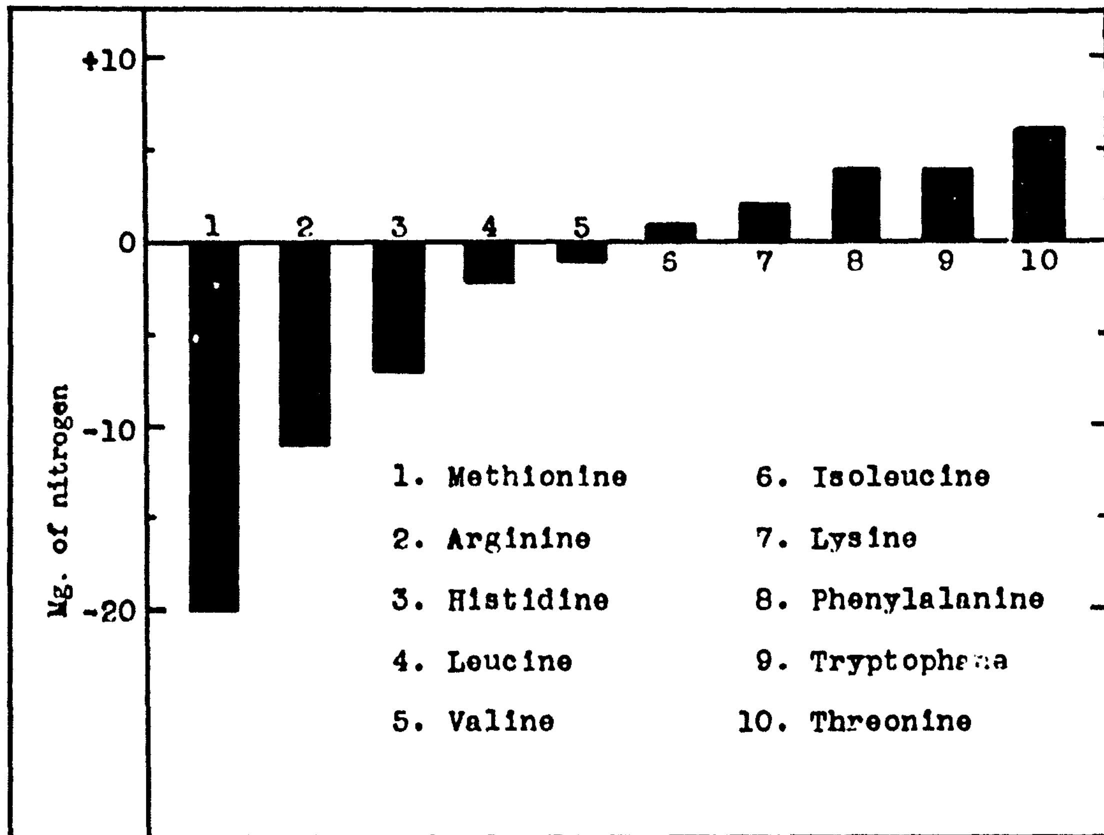


FIGURE 3
DEPRESSIONS AND ELEVATIONS OF URINARY EXCRETION OF NITROGEN PER 100 SQ. CM. OF BODY SURFACE CAUSED BY ADDITION OF EACH OF THE TEN ESSENTIAL AMINO ACIDS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

TABLE 14

Nitrogen metabolism data (Exp. 3)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
EXPRESSED AS ABSOLUTE VALUES						
Methionine	278	200	-78	-374	-248	126
Arginine	259	211	-48	-353	-262	91
Histidine	274	236	-38	-373	-298	75
Leucine	280	264	-16	-392	-309	83
Valine	307	294	-13	-429	-390	39
Isoleucine	276	272	- 4	-363	-342	21
Lysine	259	251	- 8	-365	-301	64
Phenylalanine	266	267	+ 1	-371	-420	-49
Tryptophane	269	288	+19	-357	-328	29
Threonine	249	262	+13	-351	-335	16
EXPRESSED AS N PER 100 GM. BODY WEIGHT						
Methionine	108	84	-24	-146	-104	42
Arginine	106	94	-12	-145	-116	29
Histidine	102	95	- 7	-138	-120	18
Leucine	119	119	0	-167	-140	27
Valine	110	112	+ 2	-150	-150	0
Isoleucine	107	112	+ 5	-140	-140	0
Lysine	105	111	+ 6	-148	-131	17
Phenlalanine	100	109	+ 9	-138	-167	-29
Tryptophane	102	111	+ 9	-136	-134	2
Threonine	90	101	+11	-127	-131	- 4
EXPRESSED AS N PER 100 SQ. CM. BODY SURFACE						
Methionine	80	60	-20	-107	- 74	33
Arginine	76	65	-11	-104	- 81	23
Histidine	76	69	- 7	-103	- 87	16
Leucine	84	82	- 2	-118	- 96	22

Continued on next page

TABLE 14 (continued)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
Valine	83	82	- 1	-113	-110	3
Isoleucine	80	81	+ 1	-103	-101	2
Lysine	75	77	+ 2	-107	- 92	15
Phenyl-alanine	74	78	+ 4	-103	-123	-20
Tryptophane	76	80	+ 4	-101	- 97	4
Threonine	68	74	+ 6	- 96	- 96	0

not present the final and complete picture. The effect of nitrogenous supplements in the maintenance of body tissue is more important. Figure 4 and Table 14 show that methionine, arginine and histidine, as well as leucine and lysine which caused little noticeable change in the urinary excretion of nitrogen, spared sizable amounts of body tissue. Since all groups of animals used in Experiment 3 ingested the same quantity of nitrogen, the sparing properties of leucine and lysine could exist only by virtue of a decrease in the fecal excretion of nitrogen. Perhaps this change occurs through modification of intestinal bacteria or by decrease in losses of digestive enzymes. In the case of lysine, the body-sparing capacity may be a reflection of a specific need for the third of the basic amino acids. In this connection it is interesting that lysine is the one amino acid which cannot be reaminated once it is deaminated in the body (Schoenheimer, 1942). An apparent stimulus to the breakdown of body tissue by phenylalanine was indicated by the negative value for body nitrogen spared. It is possible that this may be connected with the synthesis of the related amino acid, di-iodotyrosine.

The body-nitrogen-sparing capacity characteristic of each amino acid when fed singly evidently persists when it is administered as part of a complete amino acid mixture. The sum of the various quantities of body nitrogen spared

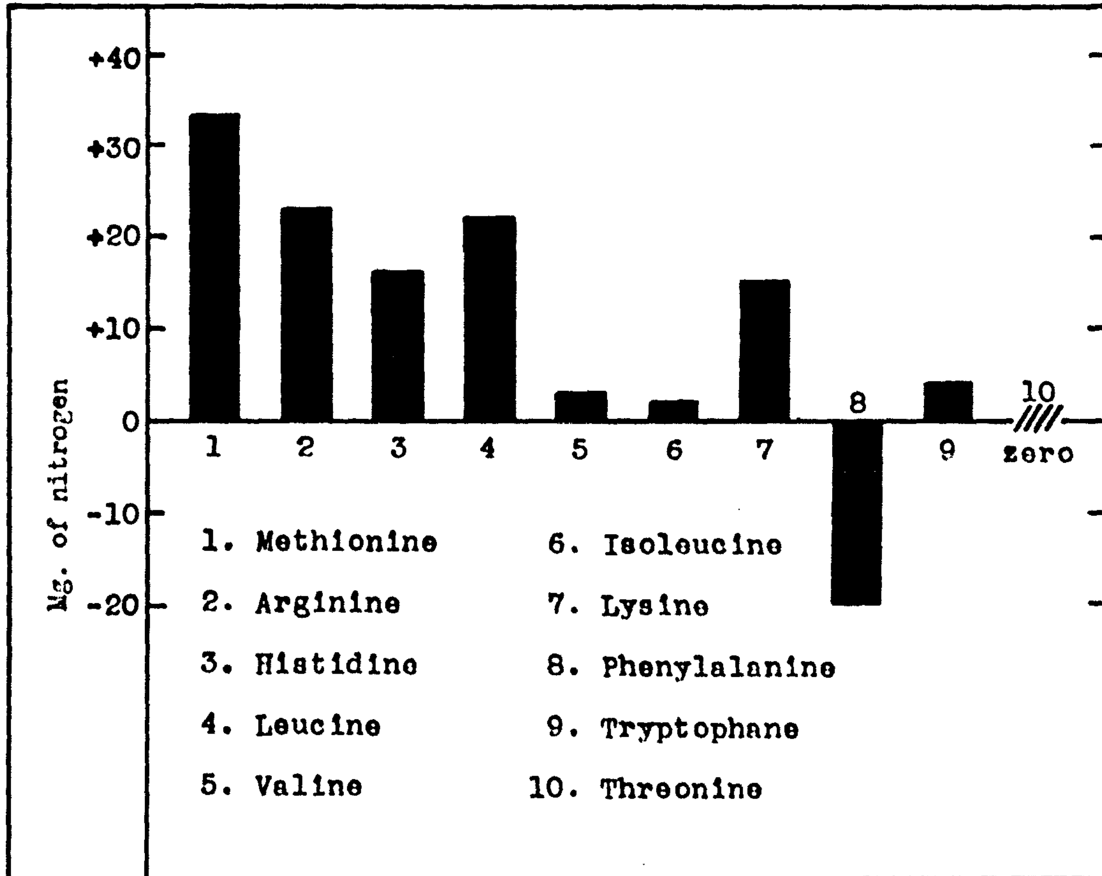


FIGURE 4
BODY NITROGEN SPARED PER 100 SQ. CM. OF BODY SURFACE BY ADDITION OF EACH OF THE TEN ESSENTIAL AMINO ACIDS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

by each amino acid (Table 14) is 395 mg. of nitrogen, while the mixture of the ten essential amino acids (Table 6) spares 436 mg. of body nitrogen, probably an insignificant difference in view of the errors inherent in the methods involved.

An unexpected and interesting observation was made with reference to the group of animals fed tryptophane as a supplement to their basal diet. During the first few days of the period of nitrogen supplementation, a bright yellow pigment was excreted in the urine of the partially depleted rats. Lepkovsky and his co-workers (1943) reported the presence of a yellow compound identified as xanthurenic acid in the urine of pyridoxine-deficient animals fed tryptophane. The product obtained in this instance was evidently not xanthurenic acid, since a green color did not develop on the addition of ferric ammonium sulfate. No other attempts at identification were made. The yellow pigment gradually disappeared from the urine as the tryptophane feeding was continued, suggesting that the animals were adapting to an unusual dietary situation in which the main source of nitrogen was a single amino acid, tryptophane. Equally puzzling results may have occurred with the feeding of the other amino acids, but because the end-products did not happen to be readily visible, they were not detected.

Undoubtedly a thoroughly reliable picture of the relative importance of each of the essential amino acids cannot be gained by the means employed in the present experiment. However, the general conclusion can be drawn that some of the amino acids, especially methionine, and to a lesser extent, arginine, histidine, leucine and lysine, spare significant amounts of body tissue, while the other five essential amino acids do not. The results indicate that, although all ten compounds are essential, in that normal body functions cannot long proceed without them, there are varying degrees of "essentiality". The five amino acids which, in this experiment, spared body nitrogen, probably play very important roles in the "master" reactions which control body function.

It seems clear that the so-called "endogenous" metabolism _can_ be depressed. It is interesting to compare this study with a similar experiment reported by Burroughs, Burroughs, and Mitchell in 1940 which was specifically designed to investigate the possibility of depressing the "endogenous" metabolism by the ingestion of small amounts of individual amino acids. These workers interpreted their data to mean that no significant depression occurred in the urinary excretion of nitrogen, when any one of the ten essential amino acids was added to the diet of rats fed a nitrogen-low ration, and, hence, no sparing of body tissue.

Since their conclusions differ from those of the author drawn from the results of Experiment 3, the experimental procedure of Burroughs, Burroughs, and Mitchell and their results will be examined in an attempt to understand the cause of the divergency.

Burroughs, Burroughs, and Mitchell declare (pp. 272, 273) that any indications of a depression in endogenous metabolism induced by the addition of a nitrogenous supplement to the basal diet, to be valid, should be obtained from animals whose protein stores have been reduced to zero by the continued feeding of diets low in nitrogen. Any changes occurring in the excretion of urinary nitrogen in the test period should also be definite and reproducible, and, therefore, distinguishable from the normal variations in the amount of nitrogen excreted by animals on an unsupplemented nitrogen-low diet. Their own animals were fed a nitrogen-low diet for five to six days before initiation of the experiment. It is evident from data reported in the first table of their article that the body stores of nitrogen of these animals had not been depleted to zero in this period, since the average urinary excretion of nitrogen in three consecutive 3-day periods following a 5-day depletion period was 192, 171, and 150 mg., respectively. Workers in this laboratory agree that the depletion of body stores of nitrogen is of paramount importance in an experiment of

this nature. They believe that a large part of the explanation of the different results obtained by the two groups of workers may be found in the fact that the animals of Burroughs and co-workers had not been reduced to a constant state of nitrogen metabolism. Evidence that this had been effected in the laboratory of the author has already been presented in Table 3.

Ignoring this inconsistency, Burroughs, Burroughs, and Mitchell felt that the fall in the excretion of nitrogen in the three periods by animals on a nitrogen-low diet was rectilinear, and that the excretion in the second of the three 3-day periods was the average of the excretion of the first and third periods. They used this relationship in the determination of the effect of single amino acids on urinary nitrogen excretion, by feeding the nitrogenous supplement in Period 2, and comparing the nitrogen excretion of that period with the average of the excretions in Periods 1 and 3. Work in the author's laboratory and in that of Allison (Allison, Anderson, and Seeley, 1945) showed that there is a lag or "hangover" in the effect of proteins or amino acids on the excretion of nitrogen in rats and in dogs in a subsequent period of nitrogen deprivation. The accuracy of any value for urinary nitrogen obtained in the three days following a change in diet may, therefore, be questioned. Undoubtedly, in the experiments of Burroughs, Burroughs, and

Mitchell, the urinary nitrogen excreted by the rats in the third 3-day period reflected to a marked degree the nitrogen fed in the preceding 3-day period of supplementation.

Burroughs, Burroughs, and Mitchell also used the same animals, without realimentation, for successive experiments, repeating the schedule of nitrogen-low feeding period and period of nitrogen supplementation at least three times, in the testing of three different amino acids. Thus the animals lost more of their body stores of protein as the periods of nitrogen-low feeding went on, and they could not be expected to react in the same manner to nitrogen supplementation. This may explain the great variation reported in replicate tests of the same amino acids, a condition which Burroughs, Burroughs, and Mitchell themselves suggest may invalidate results.

It may be of interest to examine the data pertaining to one specific amino acid, methionine. Of the six animals whose response to methionine feeding was measured, three showed statistically significant depressions in the urinary excretion of nitrogen. These were regarded, however, as irregular, and explainable, perhaps, on the basis of an unduly high excretion of nitrogen in the first nitrogen-low period. The amount of nitrogen in the urine rose only slightly, or fell further in the three days after methionine feeding ceased, a period regarded by Burroughs, Burroughs

and Mitchell as a control, nitrogen-low feeding period. As previously noted, the effect of methionine was probably still being demonstrated in this period, and a true measurement of the nitrogen excretion on a nitrogen-low diet was not obtained. These three animals whose behavior was more in accord with that observed by the present investigator, and by others whose work has been reported in the REVIEW OF LITERATURE, were ones which had not been used previously, to test any other amino acids. The three animals in which methionine caused no depression of urinary nitrogen had received doses of several other amino acids previous to the administration of methionine.

The factors of undepleted animals, very short experimental periods (each three days in length), lack of any opportunity for adjustment to changes in diet, and repeated use of the same animals distinguish the experiment of Burroughs, Burroughs, and Mitchell from Experiment 3 of the present study. The presence of such conditions is contrary to the stipulations for a valid experiment set up by the former workers themselves, and seems sufficient to cast doubt upon the correctness of their conclusions.

EXPERIMENT 4

In general, it has been the experience of investigators that the quantitative feeding of graded amounts of proteins

to animals previously fed a low-nitrogen diet caused a progressive increase in the quantity of nitrogen excreted in the urine. In view of the fact that egg proteins fed at a level equivalent to 3.5 per cent of the basal ration caused a decrease in the excretion of urinary nitrogen, workers in the Nutrition laboratory of the Iowa State College fed graded amounts of egg proteins, ranging from 1 to 5 per cent of the basal diet, and measured the response of the animals in terms of the excretion of urinary nitrogen and nitrogen balance. The unexpected finding* that the depression in the excretion of urinary nitrogen was maintained at the same point whether 1, 2, 3, or 4 per dent of egg proteins were furnished in the diet led to the present experiment, in which methionine was fed in graded doses. The response of the animals to the amino acid supplement was compared with that of similar groups of animals to egg proteins.

Six groups of animals were fed graded amounts of methionine, which supplied from 1 to 16 mg. of nitrogen per day. Again in Table 15, information with regard to body size, caloric intake, and caloric requirement is presented. It is evident that the animals fed the various quantities of

*Unpublished data, Files, Foods and Nutrition Section, Iowa Agricultural Experiment Station, Project 799.

TABLE 15

Average data descriptive of body size, caloric requirement, and
fed various nitrogenous supplements to a nitrogen-low di

Supplement fed	Number of animals in group	Nitrogen fed in supplement per day in period II	Average body weight		Change in weight last 6 days of collection period		Average body surface area		Basal da caloric quiremen	
			Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Pe
		mg.	gm.	gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	c
Methionine	5	1	262	246	-9	-8	354	341	26.8	2
	6	2	255	239	-7	-5	348	335	26.4	2
	6	3	213	195	-13	-5	312	296	23.6	2
	5	4	273	248	-12	-6	363	342	27.5	2
	6	8	261	241	-11	-5	353	337	26.8	2
	5	16	270	250	-8	-5	360	344	27.3	2
Dried whole egg	4	104	255	241	-10	-4	348	337	26.4	2
	4	210	256	242	-15	0	349	337	26.5	2
	4	298	241	233	-14	+4	337	330	25.5	2
	3	411	249	242	-11	+3	342	336	25.9	2
	4	506	261	256	-11	+2	352	349	26.7	2

* See footnotes to Table 5.

TABLE 15

tive of body size, caloric requirement, and caloric intake of rats
nitrogenous supplements to a nitrogen-low diet (Exp. 4)*

e body ght	Change in weight last 6 days of collection period		Average body surface area		Basal daily calorid requirement		Estimated total daily caloric requirement		Average daily food intake in grams		Average daily food intake in calories	
Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal.	cal.	gm.	gm.	cal.	cal.
246	-9	-8	354	341	26.8	25.8	40.2	38.7	10.8	10.1	51.8	48.5
239	-7	-5	348	335	26.4	25.4	39.6	38.1	9.8	9.4	47.0	45.2
195	-13	-5	312	296	23.6	22.4	35.4	33.6	10.5	9.4	50.4	45.2
248	-12	-6	363	342	27.5	25.9	41.3	38.9	9.3	8.8	44.6	42.3
241	-11	-5	353	337	26.8	25.5	40.2	38.3	10.1	915	48.5	45.6
250	-8	-5	360	344	27.3	26.1	41.0	39.1	11.8	10.3	56.6	49.4
241	-10	-4	348	337	26.4	25.5	39.6	38.3	9.7	9.4	46.6	45.2
242	-15	0	349	337	26.5	25.5	39.8	38.3	9.9	8.1	47.5	38.9
233	-14	+4	337	330	25.5	25.0	38.3	37.5	9.3	9.0	44.6	43.2
242	-11	+3	342	336	25.9	25.5	38.9	38.3	8.9	8.1	42.7	38.9
256	-11	+2	352	349	26.7	26.5	40.1	39.8	9.4	8.1	45.2	38.9

methionine lost somewhat less weight in Period II than in Period I, indicating some sparing of body tissue. On the other hand, the addition of egg proteins to the ration at every level prevented the loss of as much weight as would have occurred had no supplement been given; in addition, in the groups fed egg proteins in amounts equivalent to from 2 to 5 per cent of the basal ration, there was an actual gain in weight in the period of nitrogen supplementation. Egg proteins, it seems, have high nutritive value for the nitrogen-depleted animal, even when fed in small doses.

Data relating to the nitrogen metabolism of the several groups of rats employed in this experiment are presented in Table 16, on both the absolute and relative basis. The same data, in terms of surface area, are shown graphically in Figures 5 and 6. Care should be exercised, in interpreting the graphs, to compare only the trend in urinary nitrogen excretion and nitrogen balance resulting from the addition of varying amounts of egg proteins to the basal diet, with the trend in these same values resulting from the feeding of increasing quantities of crystalline methionine. The absolute degree of the depressions or the specific amounts of body nitrogen spared are not comparable, since milligrams of methionine nitrogen, in one case, and per cent of egg proteins, in the other case, were used as the bases for pictorial arrangement. The trends, however,

TABLE 16

Nitrogen metabolism data (Exp. 4)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
			EXPRESSED AS ABSOLUTE VALUES			
Methionine						
1 mg.N	334	202	-132	-462	-293	169
2 mg.N	332	214	-118	-438	-270	168
3 mg.N	236	149	- 87	-347	-210	137
4 mg.N	332	245	- 87	-454	-308	146
8 mg.N	317	244	- 73	-441	-285	156
16 mg.N	332	281	- 51	-480	-266	214
Egg, 1%	298	216	- 82	-415	-221	194
2%	309	236	- 75	-426	-135	291
3%	294	216	- 78	-427	- 44	383
4%	301	228	- 73	-421	- 13	408
5%	292	289	- 3	-410	+ 70	480
			EXPRESSED AS N PER 100 GM. BODY WEIGHT			
Methionine						
1 mg.N	128	82	- 46	-176	-119	57
2 mg.N	131	90	- 41	-172	-113	59
3 mg.N	112	77	- 35	-166	-109	57
4 mg.N	122	100	- 22	-166	-124	42
8 mg.N	122	102	- 20	-169	-118	51
16 mg.N	124	112	- 12	-179	-101	78
Egg, 1%	117	89	- 28	-162	- 92	70
2%	120	97	- 23	-166	- 58	108
3%	122	92	- 30	-178	- 19	159
4%	118	115	- 3	-164	- 7	157
5%	112	114	+ 2	-159	+ 26	185

Continued on next page

TABLE 16 (continued)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
EXPRESSED AS N PER 100 SQ. CM. BODY SURFACE						
Methionine						
1 mg.N	94	59	-35	-130	-86	44
2 mg.N	96	64	-32	-126	-80	46
3 mg.N	76	50	-26	-112	-71	41
4 mg.N	91	72	-19	-125	-90	35
8 mg.N	90	70	-18	-125	-84	41
16 mg.N	92	81	-11	-134	-77	57
Egg, 1%	85	64	-21	-119	-66	53
2%	88	70	-18	-122	-40	82
3%	87	65	-22	-127	-13	114
4%	86	84	- 2	-120	- 4	116
5%	83	83	0	-117	+20	137

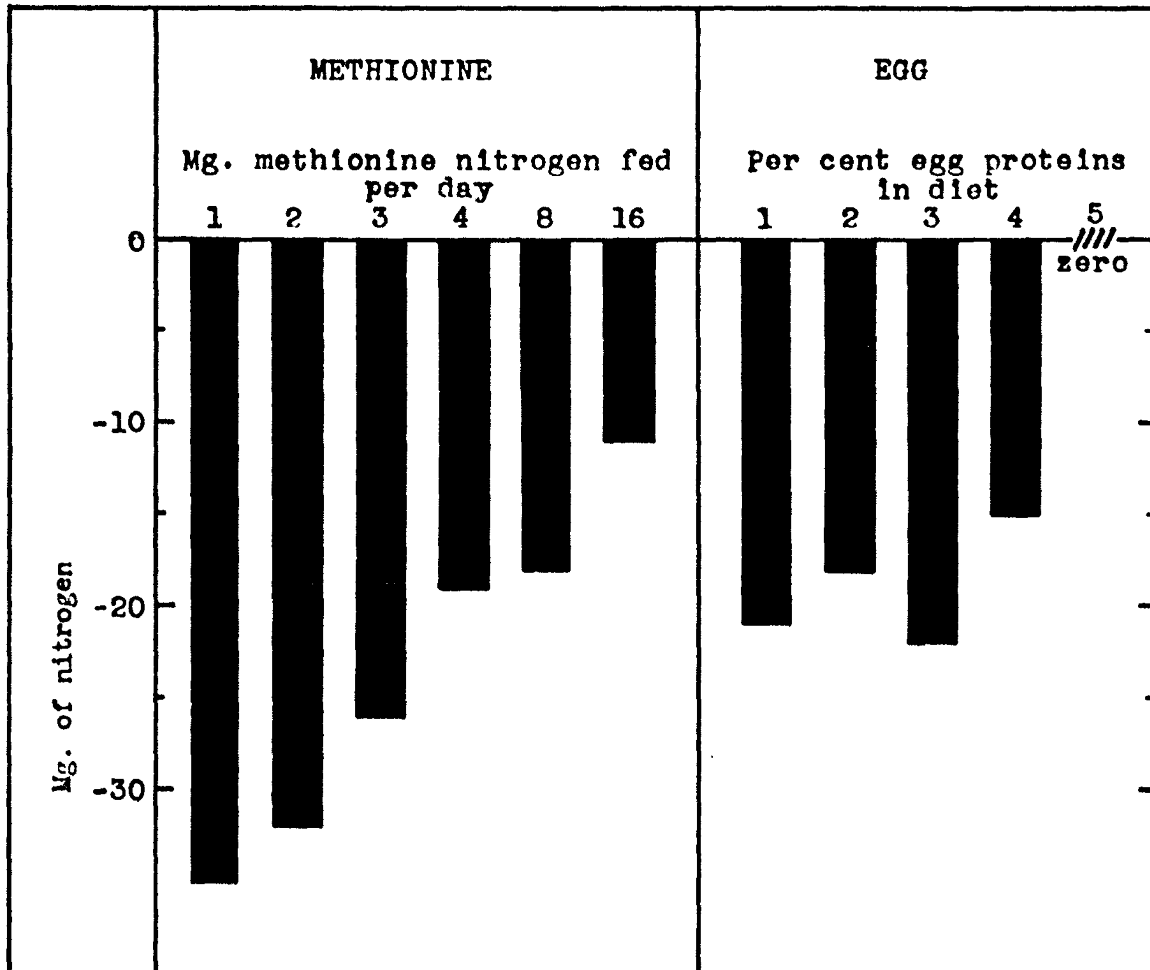


FIGURE 5
DEPRESSIONS OF URINARY EXCRETION OF NITROGEN PER 100 SQ. CM. OF BODY SURFACE CAUSED BY THE ADDITION OF INCREASING AMOUNTS OF METHIONINE AND OF EGG PROTEINS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

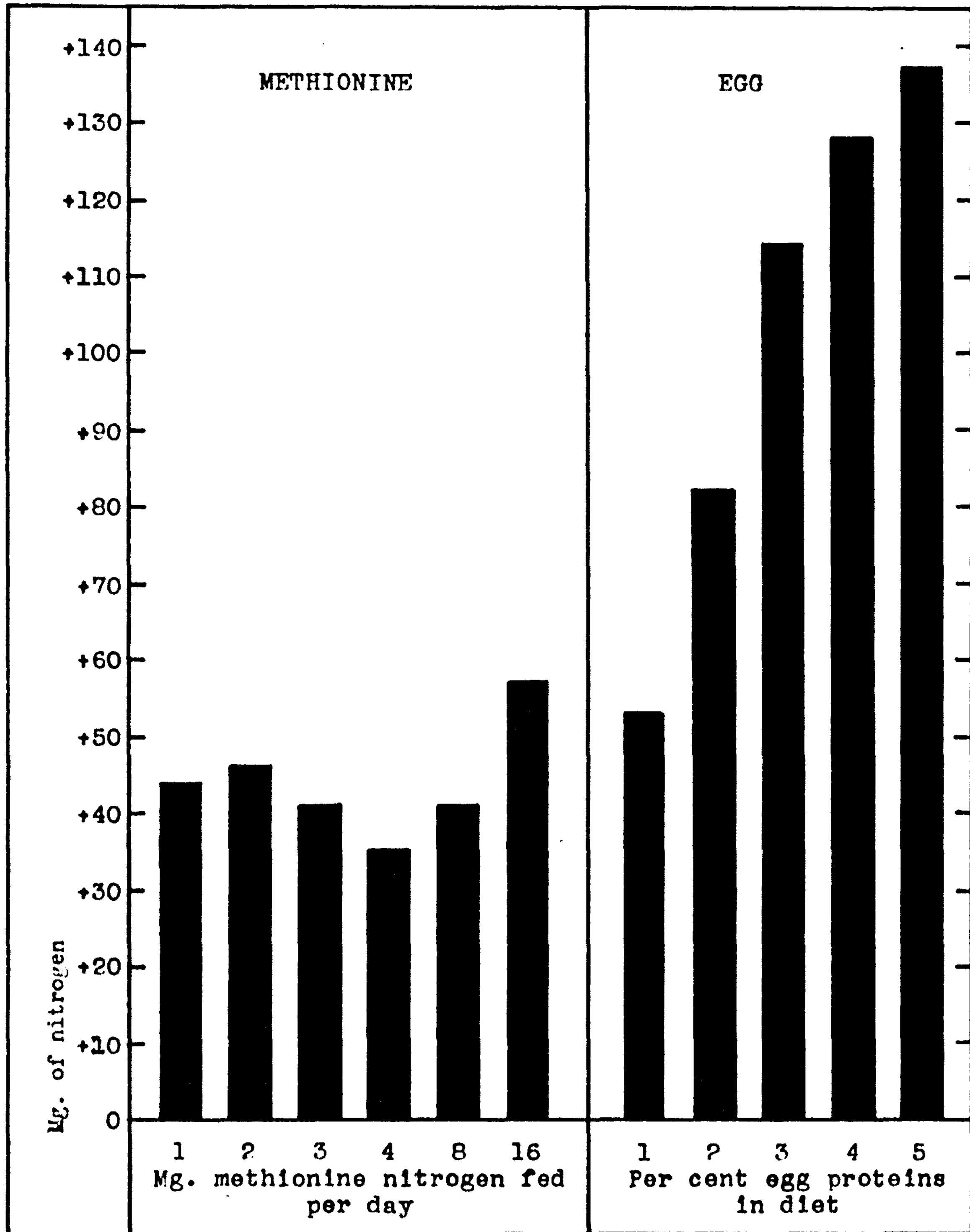


FIGURE 6
BODY NITROGEN SPARED PER 100 SQ. CM. OF BODY SURFACE BY ADDITION OF INCREASING AMOUNTS OF METHIONINE AND OF EGG PROTEINS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

are clear. All levels of egg proteins from 1 to 4 per cent caused a depression in the excretion of urinary nitrogen of the same magnitude. The feeding of 5 per cent egg proteins, a quantity which, as may be seen from Table 16, was sufficient to enable the animals to achieve positive nitrogen balance, was associated with an excretion of nitrogen identical with that of the previous low-nitrogen feeding period.

This condition did not hold when methionine, offered to six groups of rats at ascending levels of intake (11 to 175 mg. per day) served as the nitrogenous supplement to the diet. As the quantity of dietary methionine was increased there was a progressive decrease in the extent of the depression in the excretion of urinary nitrogen. Data presented in Figure 5 suggest that the methionine requirement of the rat, as measured by the addition of small amounts of methionine to the diet of partially depleted rats maintained on a low-nitrogen ration, is very small, probably less than 11 mg. With the ingestion of increasing amounts, no more is utilized, hence, the excess is excreted and the depression in the excretion of urinary nitrogen is less at the higher intakes than when the smallest amount is fed. Theoretically, the decrease in the depression of the urinary excretion of nitrogen observed in increasing the quantity of methionine fed above the amount required should be equal

to the nitrogen contained in the excess methionine. Physiological variations in the behavior of animals, particularly evident with small groups, undoubtedly obscure this relationship; however, examination of the figures for the depression in urinary nitrogen, expressed in absolute values, in Table 16, shows that this general relationship is evident when the effects at the 1 and the 8 mg. level are compared. The 49 additional milligrams of nitrogen ingested at the latter level were associated with a depression in the excretion of nitrogen 59 milligrams less than that occurring at the 1 mg. level.

From Figure 6 it is evident that increasing quantities of egg proteins in the diet caused increased sparing of body nitrogen; in contrast, 16 mg. of nitrogen daily from methionine was no more efficient than 1 mg. of methionine nitrogen. Apparent differences in the quantity of body nitrogen spared at the six levels of intake are insignificant according to statistical analysis (Table 17). If, as postulated in the paragraph above, the body's requirement for methionine is less than 11 mg., it is logical that, however large the quantities of methionine ingested, no additional body nitrogen would be spared, once the requirement is satisfied. Egg proteins, on the other hand, contain substances other than methionine, and as the amount of egg

TABLE 17

Analysis of variance of body nitrogen spared by 33 rats fed six different quantities of methionine

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	32	4672.2	146.0
Quantities of methionine fed	5	1316.4	263.3
Rats fed same quantity	27	3355.8	124.3
		F = 2.1 not significant	

ingested is increased, there are more of the other constituents supplied to meet other specific functional needs of the organism, thereby preventing further breakdown of body tissue.

Proof that eggs contain substances in addition to methionine which are capable of depressing the urinary excretion of nitrogen and of sparing body protein is offered in Figures 5A and 6A. Here the depression of urinary nitrogen and the body nitrogen spared are plotted against mg. of methionine nitrogen fed. The methionine present in the various quantities of egg proteins fed was calculated from determination of the methionine content of another portion of the same sample of dried eggs. When egg proteins equivalent to 1, 2, or 3 per cent of the basal diet and containing

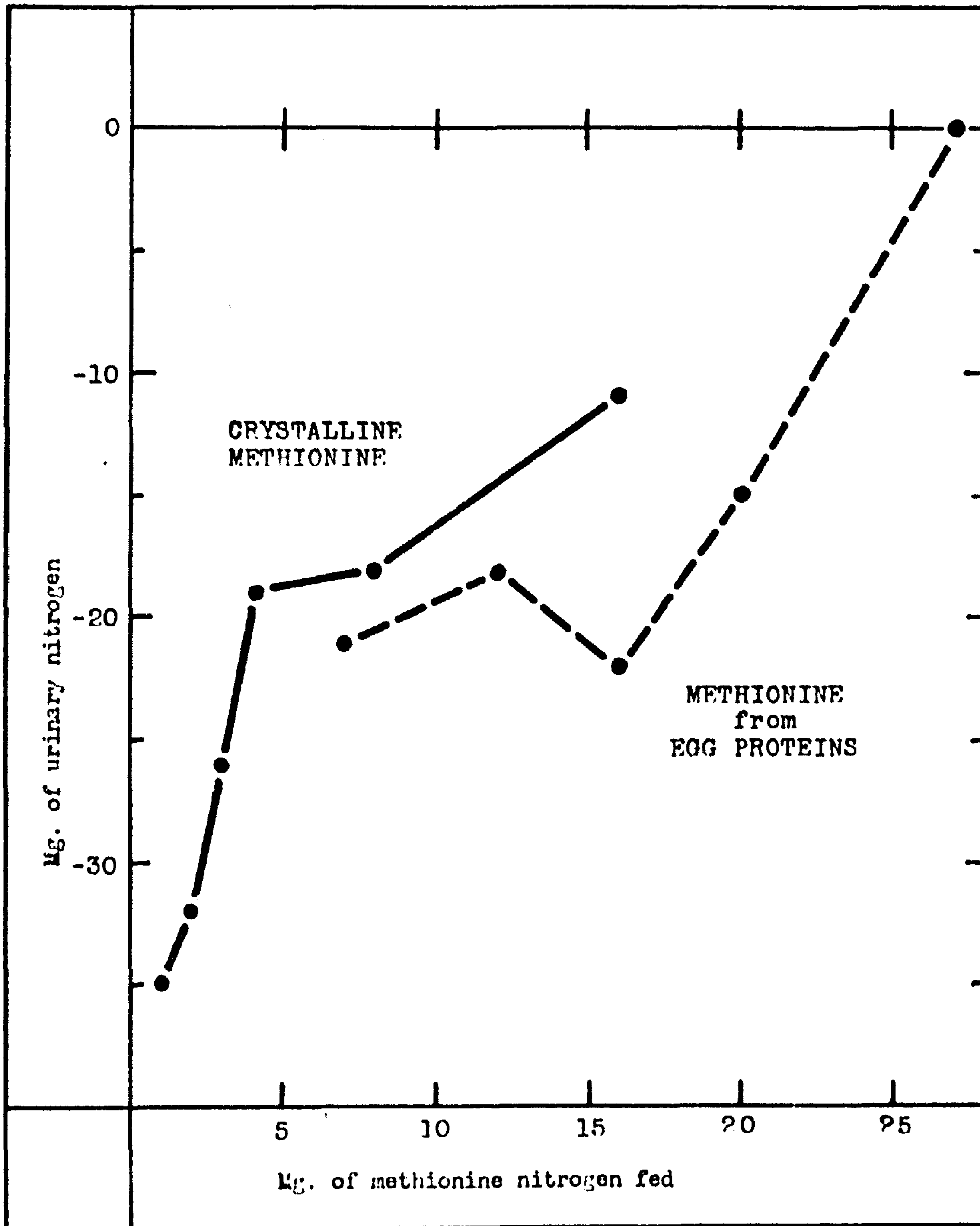


FIGURE 5A
DEPRESSION OF URINARY EXCRETION OF NITROGEN PER 100 SQ. CM. OF BODY SURFACE CAUSED BY ADDITION OF CRYSTALLINE METHIONINE AND OF METHIONINE SUPPLIED BY EGG PROTEINS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

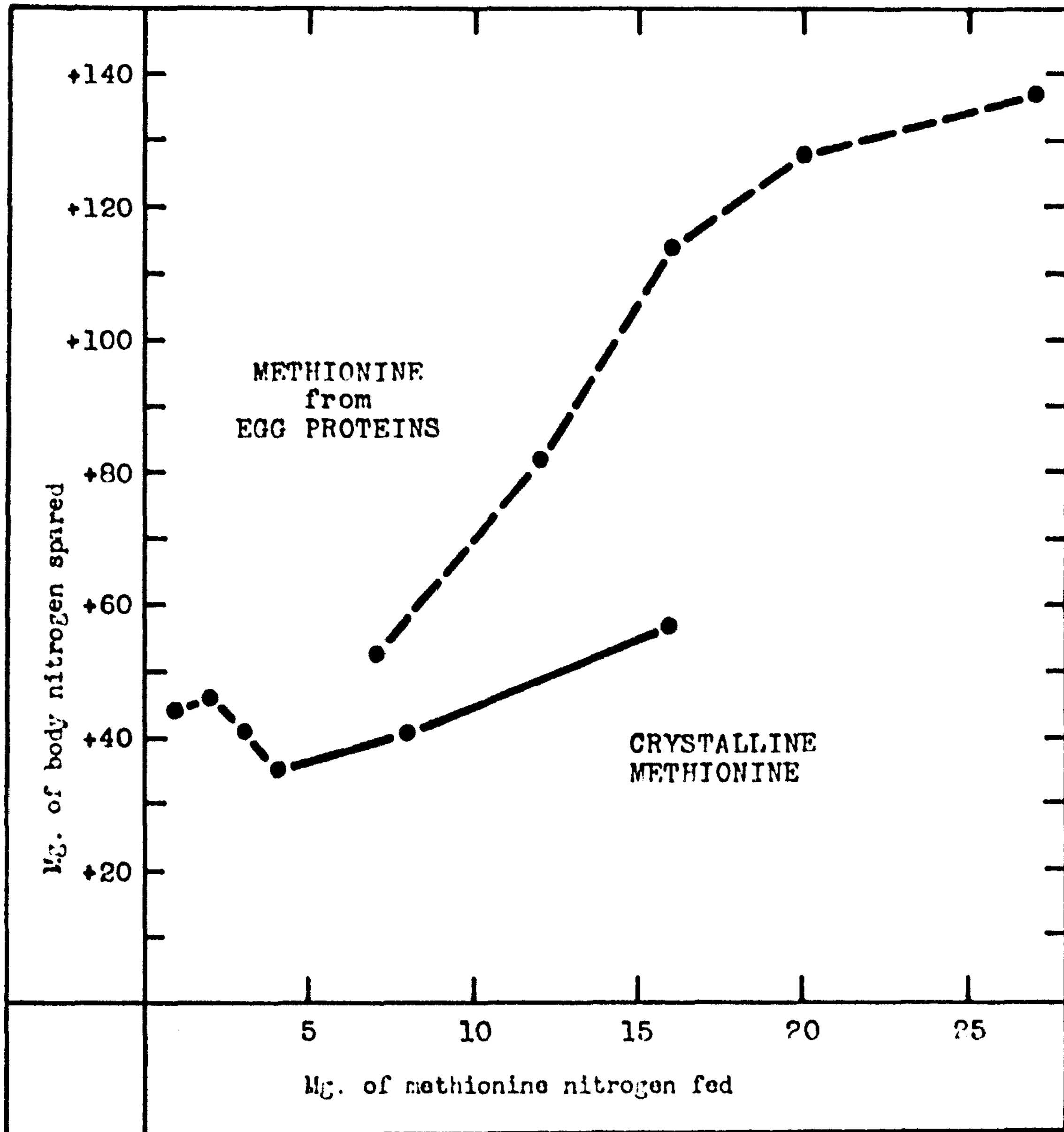


FIGURE 6A

BODY NITROGEN SPARED PER 100 SQ. CM. OF BODY SURFACE BY ADDITION OF CRYSTALLINE METHIONINE AND OF METHIONINE SUPPLIED BY EGG PROTEINS TO THE DIET OF RATS FED A NITROGEN-LOW RATION

7, 12, and 16 mg. of methionine nitrogen, respectively, were fed, approximately the same depression of the urinary excretion of nitrogen occurred in all three cases. Figure 5A shows that an amount of egg protein sufficient to furnish 16 mg. of methionine nitrogen caused a depression of the urinary excretion of nitrogen twice that which results when 16 mg. of methionine nitrogen were fed in the form of crystalline methionine, _i.e._, 22 mg. as compared with 11 mg. At this point, then, egg proteins caused a depression of urinary nitrogen excretion far greater than can be accounted for on the basis of their methionine content alone. The other essential amino acids in egg proteins were undoubtedly exerting an additional nitrogen-sparing effect. With the feeding of egg proteins in amounts which supplied more than 16 mg. of methionine nitrogen per day, the depression in the excretion of nitrogen decreased, disappearing altogether at the 5 per cent level. This was probably due to the excretion in the urine of the excess non-utilizable amino acids.

Only when egg proteins are fed at the 1 per cent level of intake does it seem likely that their body-sparing effect is due largely to their methionine content. Perhaps, at this low level no other amino acids are present in amounts sufficient for any protein-sparing action, while methionine stands out because of its quantitatively greater sparing

powers (illustrated in Experiment 3) and because of the large quantity of this amino acid found in egg proteins. When egg proteins were fed in amounts ranging from 2 to 5 per cent of the basal ration (Figure 6A), the body nitrogen spared was far greater than could be attributed to the methionine content.

This experiment shows that at a low level of feeding of egg proteins, the nitrogen-sparing action of the eggs is largely due to methionine. At higher levels, the presence of the other essential amino acids increases the body-nitrogen-sparing capacity of egg proteins far beyond that achievable by methionine alone.

EXPERIMENT 5

A comparison in Experiment 3, of the effect on nitrogen metabolism of the individual essential amino acids showed that, although four or five amino acids possessed definite body-sparing powers when added to a nitrogen-low diet, methionine was the most powerful, both in depressing the urinary excretion of nitrogen and in sparing body nitrogen. The fifth experiment was undertaken in an attempt to elucidate the means by which methionine acts. It is possible that its chief function may be the supply of methyl groups or of organic sulfur. Therefore, three groups of rats were

subjects for the regular nitrogen balance test; to one group, 4 mg. of nitrogen in the form of methionine was offered daily, the second received 4 mg. of nitrogen from cystine, and the third, 4 mg. of nitrogen from choline per day in addition to the choline routinely supplied in the synthetic vitamin mixture. The latter two compounds are ones which have been shown to be derivable from methionine by normal physiological processes and ones which are important for synthesis of body tissue and regulation of body functions. Table 18 presents information with regard to certain characteristics of these three groups of animals.

According to the metabolic data presented both in Table 19 and in Figure 7, cystine and choline share the ability of methionine to decrease urinary losses of nitrogen and to spare body nitrogen. All three compounds spared approximately the same amount of body nitrogen, when the data are expressed in terms of surface area. Any apparent differences are statistically insignificant (Table 20). This finding agrees with the work of Mulford and Griffith (1942), which indicates that the same molecule of methionine cannot be used for two purposes, _i.e._, to supply labile methyl groups and sulfur. Were the reverse true, one molecule of methionine should be able to spare body nitrogen equivalent to the sum of that spared by cystine and choline individually. The similarity of the effects of cystine and

TABLE 18

Average data descriptive of body size, caloric requirement, and c
fed various nitrogenous supplements to a nitrogen-low die

Supplement fed	Number of animals in group	Nitrogen fed in supplement per day in period II	Average body weight		Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement	
			Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
	.	mg.	gm.	gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.
Methionine	6	4	257	239	-7	-7	350	335	26.5	25.4
Cystine	6	4	241	224	-9	-4	337	332	25.5	24.4
Choline	5	4	233	217	-3	-3	330	316	25.0	24.0

*See footnotes to Table 5.

TABLE 18

riptive of body size, caloric requirement, and caloric intake of rats
us nitrogenous supplements to a nitrogen-low diet (Exp. 5)*

ge body ight	Change in weight last 6 days of collection period		Average body surface area		Basal daily caloric requirement		Estimated total daily caloric requirement		Average daily food intake in grams		Average daily food intake in calories	
Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II	Per.I	Per.II
gm.	gm.	gm.	sq.cm.	sq.cm.	cal.	cal.	cal.	cal.	gm.	gm.	cal.	cal.
239	-7	-7	350	335	26.5	25.4	39.8	38.1	10.4	9.8	49.8	47.0
224	-9	-4	337	332	25.5	24.4	38.3	36.6	10.6	9.8	50.8	47.0
217	-3	-3	330	316	25.0	24.0	37.5	36.0	9.9	9.4	47.5	45.2

TABLE 19

Nitrogen metabolism data (Exp. 5)

Supplement	Urinary nitrogen			Nitrogen balance		
	Per.I	Per.II	Dif.	Per.I	Per.II	Body nitrogen spared
	mg.	mg.	mg.	mg.	mg.	mg.
EXPRESSED AS ABSOLUTE VALUES						
Methionine	278	200	-78	-374	-248	126
Cystine	256	197	-59	-338	-259	79
Choline	257	221	-36	-401	-276	125
EXPRESSED AS N PER 100 GM. BODY WEIGHT						
Methionine	108	84	-24	-146	-104	42
Cystine	108	88	-20	-140	-116	24
Choline	111	102	- 9	-174	-128	46
EXPRESSED AS N PER 100 SQ. CM. BODY SURFACE						
Methionine	80	60	-20	-107	- 74	33
Cystine	76	61	-15	-100	- 80	20
Choline	78	70	- 8	-122	- 87	35

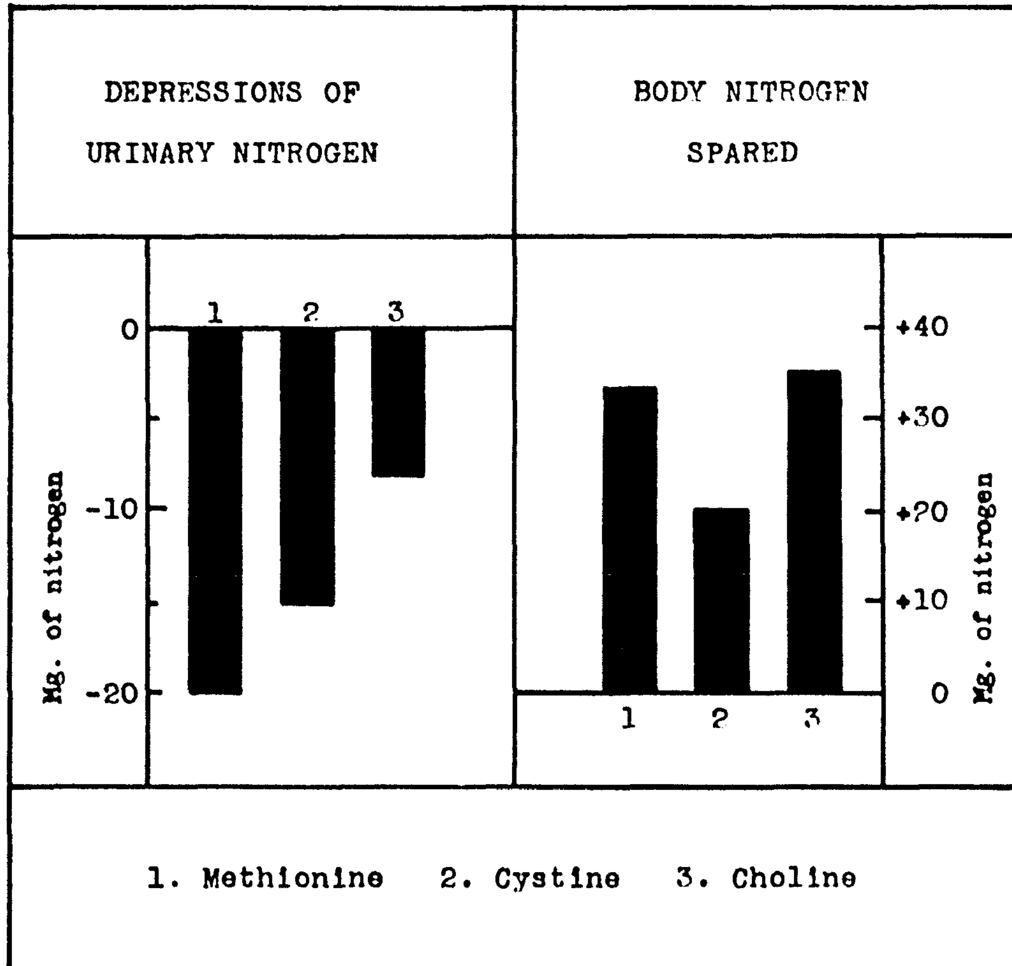


FIGURE 7
MODIFICATIONS IN NITROGEN METABOLISM CAUSED BY ADDITION OF METHIONINE, CYSTINE, AND CHOLINE TO THE DIET OF RATS FED A NITROGEN-LOW RATION
CALCULATED PER 100 SQ. CM. OF BODY SURFACE

TABLE 20

Analysis of variance of body nitrogen spared by three groups of rats fed methionine, cystine, and choline, respectively

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	16	2860.5	
Between groups of rats	2	652.5	326.3
Between rats of same group	14	2208.0	157.7
	F = 2.07 not insignificant		

choline with that of methionine suggests that the latter compound in sparing body nitrogen acts largely through its capacity to form cystine and choline. Any other function, in which methionine may participate, however important, is evidently not outstanding quantitatively.

EXPERIMENT 6

Whole Carcass Analysis

In the first section of Experiment 6, the whole carcasses of rats maintained on various dietary regimes were analyzed for total nitrogen and for methionine. The values obtained with animals fed the nitrogen-low diet for 18 days and for 29 days were compared with data representing rats

fed the basal ration supplemented with methionine or with egg proteins, according to the usual balance test technique. A positive control group fed the stock colony diet (Steenbock V) was also analyzed. Table 21 shows data relating to body weight, total nitrogen, and methionine content of these groups of animals.

A definite loss in the total nitrogen present in the whole carcass resulted from the feeding of the nitrogen-low diet. The carcasses of the positive control group contained an average of 8.38 gm. of nitrogen; those of the nitrogen-low groups, 6.41 and 6.69 gm. By inspection of the data, the total nitrogen in the whole carcass of rats fed various quantities of methionine does not seem to differ significantly from that of rats maintained on an unsupplemented nitrogen-low diet for 18 or 29 days, and the conclusion might be drawn that methionine is not associated with the formation of new tissue. But before this statement can be made, it is necessary to determine the influence that the differences in body weight existing among the groups has on the picture. The nitrogen content of each group of animals after 18 days on the nitrogen-low diet was calculated on the basis of its body weight at that time and the per cent of nitrogen in the carcasses of rats as analyzed after maintenance on the basal diet for 18 days.

TABLE 21

Average weight, methionine content and total nitrogen content of the whole carcasses of rats fed the stock colony diet, and of ones fed the low-nitrogen diet alone or with nitrogen supplements

Supplement	Body wt. when analyzed	Body wt. after 18 days depletion	Total nitrogen	Nitrogen per 100 gm. body weight	Total methionine	Methionine per 100 gm. body weight	Methionine N / Total N
	gm.	gm.	mg.	gm.	mg.	mg.	%
Stock colony	326	—	8.38	2.58	182	56	2.2
N-low, 18 days	253	287*	6.41	2.52	132	52	2.1
N-low, 29 days	249	279	6.69	2.68	132	53	2.0
Egg	289	296	7.75	2.69	159	55	2.1
Methionine							
1 mg. N	233	254	6.67	2.86	118	51	2.2
2 mg. N	226	247	6.25	2.77	123	54	2.0
4 mg. N	235	238	5.52	2.36	123	51	2.2
16 mg. N	225	259	6.37	2.82	145	63	2.2

*Body wt. before depletion

Comparison of the average nitrogen content of the carcasses of each group at 18 days, calculated in this manner, with the nitrogen content observed at 29 days indicated the change in nitrogen content brought about by the feeding of the nitrogen-low diet, alone or supplemented, for an additional 11 days. The formula used in this calculation is given below:

A = per cent of nitrogen in carcasses of animals fed N-low diet for 18 days

B = body weight after 18 days of N-low feeding of animals analyzed after 29 days of N-low feeding

C = A x B, estimated total nitrogen of carcasses of each group, after 18 days of N-low feeding

D = Observed total nitrogen content at 29 days— C, represents change in total nitrogen content with continuance on diet for 11 more days

Table 22 shows the changes in total nitrogen, calculated as indicated above and thus corrected for body weight differences among the groups. The nitrogen content before depletion of the animals fed a nitrogen-low diet for 18 days is calculated in a similar fashion, and compared with the positive control animals (Table 22).

It is evident from Table 22 that when body weight differences are eliminated, there is no change in the total nitrogen content of the whole carcass of the partially-depleted animals, whether they are continued on a low-nitrogen diet or on one supplemented by various quantities

TABLE 22

Change in total nitrogen of whole carcasses of rats after nitrogen depletion on supplementation

Supplement added to basal low-nitrogen diet in second metabolism period	Change in nitrogen content of carcass
	gm.
No supplement	-0.34
Methionine	
Equivalent to 1 mg. N	+0.26
Equivalent to 2 mg. N	+0.03
Equivalent to 4 mg. N	-0.48
Equivalent to 16 mg. N	-0.16
Egg proteins equivalent to 374 mg. N	+1.04
Nitrogen-low diet for 18 days, compared with animal at beginning of test	-1.00

of methionine for 11 days after the first metabolism period of 18 days. This is consistent with the relatively small body-sparing capacity of methionine revealed in Experiment 4. The feeding of egg proteins for the last 11 days of the balance test caused an increment in body nitrogen of 1.04 gm., the equivalent of 40 gm. of body tissue. The observed difference in weight between the animals maintained on the nitrogen-low diet for 18 days, and those fed the basal diet plus egg proteins for 11 days after this, is 36 gm. (Table 21). Animals maintained on the nitrogen-low diet for 18 days lose 1.0 gm. of body nitrogen, as compared with the nitrogen content of the positive control animals. This is the equivalent of 39 gm. of body tissue, exactly equal to the observed difference in weight between the two groups of animals (Table 21).

From the data in Table 23, comparison may be made of the relative amount of nitrogen in the whole carcass of rats as determined by the author and by two other investigators.

The total methionine content of the whole carcass paralleled the total nitrogen content of the same material. Therefore, the composition of the body carcass with regard to methionine nitrogen, is constant; as Table 21 shows, the range is from 2.0 to 2.2 per cent. The body-sparing action of methionine is evidently not related to a gain in either

TABLE 23

Nitrogen content of the whole carcass of rats, as determined by various investigators

Investigator	Number of animals	Age	Per cent nitrogen range	average
Brush	24	7	2.4-2.9	2.6
Medes, Floyd, and Cammaroti (1944)	3	4-6	2.7-2.9	2.8
Mitchell, Burroughs, and Beadles (1936)	8	4	2.5-3.1	2.8

the total nitrogen or the methionine content of the carcass. Rather, it may serve as a catalytic agent, governing essential body processes. The total methionine content of the whole carcass of rats fed egg proteins is higher than that of those fed the nitrogen-low diet alone or supplemented by methionine, to the same extent that the nitrogen content of the carcasses of the first group is higher than that of the carcasses of the second group. The ratio of the methionine nitrogen to total nitrogen, however, is 2.1 per cent, indicating that the composition of the whole carcass with regard to methionine is unchanged. The positive control animals, likewise, have body tissue of the same composition, since the ratio of methionine nitrogen to total nitrogen is the same as in all other groups, 2.2. Medes, Floyd, and

Cammaroti(1944) reported the methionine content of the whole carcass of the three rats whose nitrogen content is given in Table 23 as 59, 63, and 96 mg. The ratio of methionine nitrogen to total nitrogen in these three cases is 1.3 per cent, a value much lower than the 2.2 per cent obtained in the present study. It is possible that the reason for the divergency lies in the fact that different means were used for the determination of methionine. The method of Lavine (1943), employed by Medes and co-workers, is said to give lower values for methionine than the method of Albanese, Frankston, and Irby, used by the present investigator.

Liver Analysis

The moisture and fat contents of hepatic and muscle tissue obtained from animals fed a variety of nitrogenous supplements to the basal low-nitrogen diet were determined in order to express the nitrogen and methionine analyses on a dry, fat-free basis. These data, which are of interest in themselves, are presented in Table 24. The constancy of composition of muscle tissue with regard to these two constituents is striking when compared with hepatic tissue. Clark (1945) in a similar study demonstrated, also, that muscle tissue was remarkably constant in its nitrogen content as well as in moisture and fat, even though severe dietary limitations had been placed on the experimental animals.

TABLE 24

Moisture and fat content of hepatic and muscle tissue of animals fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements

Supplement	Liver		Muscle	
	Moisture	Fat	Moisture	Fat
	%	% of dry weight	%	% of dry weight
Stock colony (Steenbock V)	71.2	15.7	75.9	7.5
N-low (18 days)	70.9	24.3	75.6	6.9
N-102 (29 days)	70.6	20.2	75.8	9.3
Egg (3.5%)	71.3	23.4	75.7	7.2
Methionine				
1 mg. N/day	71.1	25.5	76.0	8.8
2 mg. N/day	70.6	28.2	75.8	7.4
4 mg. N/day	69.5	31.9	75.7	8.7
8 mg. N/day	70.4	29.1	75.4	8.6
16 mg. N/day	70.7	27.5	76.0	7.8
Cystine	68.1	30.3	75.9	8.6
Choline	72.1	21.0	75.8	9.3
Mixture of 10 essential amino acids	72.5	22.7	75.7	8.7

That hepatic tissue is much more sensitive to changes in diet than is muscle is illustrated in Table 24. The values shown there differ from those of Clark numerically, but the groups analyzed in the two studies show moisture and fat contents which are in the same relation to each other.

The lowest concentration of fat in the liver, 15.7 per cent of the dry weight, was found in the positive control animals. The feeding of a nitrogen-low diet for 18 or 29 days caused a rise from this level to an average of 22 per cent. It may be that, on a protein-deficient diet, an interference occurs in the transport of body fat. Therefore, part of it accumulates in the liver. This condition was not corrected by the addition to the basal low-nitrogen diet of egg proteins, choline, or a mixture of the ten essential amino acids. The feeding of methionine, in various quantities, as a supplement to the nitrogen-low diet caused a decided increase in the concentration of fat in the liver, with values as high as 32 per cent. Cystine, likewise, increased the concentration of fat in the liver to 30 per cent.

In general, a high concentration of fat in the liver has not been associated with physiological well-being. The incidence of abnormally high values in the case of the rats fed methionine, occurring simultaneously with the body-sparing action demonstrated previously, lends uncertainty

to the meaning of a high concentration of fat in the liver. The higher concentration of liver fat may be harmful, as it is usually considered. Egg proteins, choline, and the essential amino acids (all of which fail to lower the concentration to that present in the livers of animals in the positive control group), and methionine and cystine (which bring about an increase in liver fat), then, may have certain undesirable attributes, in opposition to their beneficial body-sparing properties. On the other hand, the increase in liver fat may be an indication of the mobilization of fat for metabolic purposes, and, therefore, desirable. It is well known that the body lays down stores of fat in depots for use in event of nutritional adversity; the administration of certain dietary supplements may make possible the utilization and transport of this storage fat in a way not possible when the animal is fed only a nitrogen-low ration. It would be interesting to examine the livers of the various groups of animals histologically, in order to determine any physical changes coincident with modifications in the content of fat.

Studies from Whipple's laboratory have shown that the liver is probably the storehouse of most of the body's reserves of protein. Changes in nitrogen metabolism caused by the feeding of amino acids or proteins to partially

depleted animals should, therefore, be reflected in this place. Demonstration (in Experiment 2) that methionine had dramatic effects on the urinary excretion of nitrogen in depleted animals fed a nitrogen-low diet led to the present experiment, in which hepatic tissue of animals fed a low-nitrogen diet for 29 days, and of ones fed a similar diet supplemented by methionine for the last 11 days of the 29-day period, were analyzed for methionine and total nitrogen. Table 25 presents data obtained in these analyses.

When the weights of the livers of the animals in the first group were adjusted for the difference in total body weight between the two groups and were calculated on a moisture-free, fat-free basis, the total nitrogen content was 138 and 154 mg. in the nitrogen-low and methionine-supplemented groups, respectively. The relative concentration of nitrogen was 8.46 and 10.4 per cent. The total methionine content of the group given the deficient ration was correspondingly less, 45 mg., as compared with 50 mg. As a result, the ratio of methionine nitrogen to total nitrogen was the same in the hepatic tissue of each group, 3.0 per cent. No precise values for the concentration of methionine in the liver of the rat have been found in the literature, although Block and Bolling (1945) have suggested, on the basis of the amount in the livers of other

TABLE 25

Weight, methionine content, and total nitrogen content of livers of rats fed a nitrogen-low diet alone and with supplementary methionine

Diet	Body weight	Total weight of fresh liver	Weight of dry, fat-free liver*	Total nitrogen	Per cent N in dry, fat-free liver	Total methionine	Methionine N / Total N
	gm.	gm.	gm.	mg.	%	mg.	%
N-low	267	6.41	1.50	127	8.46	41	3.00
Adjusted to:	290	6.96	1.63	138	8.46	45	3.00
N-low plus methionine	290	6.96	1.47	154	10.4	50	2.94

animals and in other animal tissues in general, that the methionine concentration in rat livers is probably about 3 per cent.

In light of the constancy of composition of the protein fraction of hepatic tissue thus demonstrated, the increase in the per cent of nitrogen which occurred with methionine feeding suggests that additional protein is deposited in the liver cells, identical in chemical composition with the protein already present. Since the hepatic tissue is the same size, with or without supplementary methionine, the increase in protein must occur at the expense of glycogen. These hypotheses are supported by the report of Harrison and Long (1945) of a study of the effect of various proteins and amino acids on the regeneration of liver protein in the rat following a 48-hour fast. Their results, obtained under different experimental conditions than the ones of the present study, showed that the sulfur-containing amino acids, methionine, cystine, and probably homocystine, when added to a diet inadequate in its content of casein, were outstanding in their ability to increase the amount of liver protein regenerated after fasting.

The amount of methionine fed the supplemented group was the same as that which, in Experiment 2, caused a marked depression in the urinary excretion of nitrogen, and some sparing of body nitrogen. The analysis of hepatic tissue

when adjusted showed an increase in total nitrogen in the liver after methionine feeding of 16 mg., an amount equivalent to 0.6 gm. of body tissue. According to the results of the nitrogen balance test, methionine supplementation spared body tissue equivalent to 5.0 gm. The difference, 4.4 gm., unaccounted for by changes in the composition of the liver, apparently represents the results of the action of methionine in fulfilling other specific functions throughout the body.

Muscle Analysis

The gastrocnemius muscles of animals fed a variety of diets were analyzed for methionine. Since the muscles could not be removed completely, results are presented in Table 26 in terms of mg. of methionine per gm. of dry, fat-free tissue. The constancy of composition of muscle tissue, already demonstrated with regard to moisture, fat, and nitrogen, is maintained in the case of a specific amino acid. It is likely that any other condition, indicative of a physiological attempt to change the nature of one of the structural elements of the body, would mean a moribund state.

The loss through the urine of large quantities of nitrogen during a period of nitrogen deprivation is accompanied by an equivalent loss in total body weight. The

TABLE 26

Methionine content of muscles of rats fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements

Supplement	Mg. methionine per gm. dry, fat-free muscle
	mg.
Stock colony	37.8
N-low, 18 days	36.2
N-low, 29 days	36.8
Egg	35.3
Methionine, 1 mg. N	38.4
2 mg. N	35.9
4 mg. N	38.2
8 mg. N	37.7
16 mg. N	38.3

addition of methionine to the ration decreases the magnitude of these losses. When no methionine is available in the diet, the animal evidently is not capable of removing methionine, alone, from his body tissues, but must break down the entire structural unit for this one specific substance. Such raiding of the tissues is reflected in a decrease in the total amount of muscle in the body, although what remains has the same composition, with regard to methionine, as was characteristic of the muscle protein of the well-fed animal. Methionine, when added to the diet, caused no modification in the composition of body tissue. The body-sparing ability of arginine, histidine, leucine, and lysine (demonstrated in Experiment 3) suggests that these amino acids, too, affect the destruction and the regeneration of body tissue in the same manner as does methionine.

Nature of Storage Protein

There has long been discussion of the possibility of the existence of "storage protein". The reality of such substance has been demonstrated by Whipple in his work on the regeneration of plasma proteins in hypoproteinemic dogs. The chief question has been as to the nature of the storage protein--whether it is a unique form of protein chemically and structurally, analogous to the carbohydrate

stores of glycogen in the liver, or whether it differs only physiologically from other, more stable forms of protein in the body.

In 1922, Martin and Robison pointed out that the urinary nitrogen excreted during the first few days by animals fed a protein-free diet decreased in a regular fashion. They proposed a simple logarithmic equation which conforms to the idea that the amount of storage nitrogen removed from the body on any given day is proportional to the amount still present. Various methods have been employed to determine whether or not the labile protein thus lost differs chemically from the protein remaining in the body.

The chief source of the labile nitrogen excreted early in a nitrogen-low feeding period was identified by Addis, Poo, and Lew (1936 a, b, and c) as the liver. They found a 20 per cent loss in hepatic nitrogen in the early stages of nitrogen starvation, in a period when other tissues and organs lost 4 per cent. There was no indication, however, of the nature of the nitrogen thus lost. These workers stated that the only satisfactory evidence of a specific storage protein would be the isolation of a definitely characterized protein from the livers of well-fed animals, and the demonstration of its disappearance on fasting.

Since both nitrogen and sulfur are constituents of the protein molecule, studies of the relation between these

two elements in urine or in tissue should give some indication of the nature of the protein from which they are derived. Wilson (1933) found that the urine excreted by experimental subjects early in a period of nitrogen-starvation had a higher sulfur-to-nitrogen ratio than that excreted later, a fact which he thought due to an early splitting off of the sulfur from the protein molecule. This would leave a modified protein molecule, of lower sulfur content than before. However, Lee and Lewis reported (1934) that the feeding of a protein deficient in sulfur did not change the composition of the proteins of the liver despite large changes in the urinary nitrogen-sulfur ratio. Analysis of livers from well-fed, fasted, and fasted and re-fed animals showed a ratio of cystine nitrogen to total nitrogen of 1.2 per cent in all cases. From this evidence, it would seem that the differences which Wilson found when correlated with the work of Addis, Poo, and Lew, mean that the nitrogen and sulfur excreted in the urine early in a period of nitrogen deprivation arise from the most labile source of protein, the liver, and that later other organs and tissues lose protein. Differences in composition, then, between liver proteins and the proteins characteristic of the other organs or tissues would be reflected in different nitrogen-sulfur ratios in the urine.

Luck (1936) carried out analyses suggested by Addis,

Poo, and Lew, in an effort to isolate "storage" protein if it did exist as a chemical entity. He analyzed liver proteins on the basis of four arbitrary chemical fractionations, and demonstrated that on going from conditions of low to high protein feeding, there was a uniform increase of 1.55 per cent in each of the fractions. This, he believed, showed that all of the liver proteins were "storage" proteins; there is no reserve material which is chemically distinct from the basic structural proteins of the organ.

The problem was attacked by Kosterlitz (1944) in a somewhat different way. He analyzed the protein of the livers of rats fasted for 24 or 48 hours, and of ones fed a variety of diets high in protein. Determination of total protein, phospholipin protein and of the number of cells (obtained by counting the number of nuclei in uniform thin sections of the tissue) showed a constancy in the ratio of total protein to phospholipin protein, and in the number of cells. Thus, the loss of liver weight and liver nitrogen which occurs with fasting is not due to a decrease in the number of liver cells but to a decrease in the volume of each individual cell. The total protein and phospholipin protein which are lost evidently form a structural part of the cytoplasm of the liver. Kosterlitz confirmed this hypothesis by histological examination of the livers of rats fed low-nitrogen diets. Such an examination revealed

a diminution in stainable cytoplasm and mitochondria, and an apparent vacuolization, due to increased glycogen. This worker considers the fact that changes in diet are reflected in liver cytoplasm as more important than simple physical storage or the depletion of such stores would be, since many of the enzymatic activities of the liver cell are associated with the cytoplasm.

The demonstration in the present study that the concentration of methionine in rat tissue, whether whole carcasses, liver or muscle, does not change despite dietary changes, is further corroborative evidence of the lack of any chemically distinct storage protein. It indicates that the function of methionine is not the deposition of methionine, as such, in the body, and that its value for the animal, reflected in its effect in depressing the urinary excretion of nitrogen and in sparing body nitrogen, lies in its ability to regulate certain body processes, which are essential to the life of the animal.

SUMMARY

In 1943, Marshall, in the Nutrition laboratory of the Iowa State College, observed a depression in the urinary excretion of nitrogen of rats maintained on a nitrogen-low diet, following the incorporation of dried whole eggs in the ration. This depression, contraindicated by all concepts of a constant "endogenous" nitrogen metabolism, was accompanied by the sparing of body nitrogen as calculated from nitrogen balance data, and by an actual gain in body weight during the period of supplementary egg feeding. Clark (1945) investigated the mechanisms by which the dehydrated eggs accomplished these beneficial results, in an attempt to determine the role which egg proteins played in the normal nitrogen metabolism of the rat. She found that the most important function of egg proteins, of those measured, was the building-up of the reserves of plasma protein which had been lowered through the ingestion of a nitrogen-low diet for 18 days. Regeneration of liver protein evidently took place next, after demands for plasma protein had been met. There was no indication as to which of the many constituents of dried whole eggs were responsible for the observed effects, nor as to a more precise explanation of the changes occurring within the body during

protein-deprivation and protein-feeding.

The present study reports the identification of the specific components of the dried eggs which were effective in sparing body nitrogen, an evaluation of the relative capacity of each of these compounds with regard to body-sparing ability, and results of further investigation into the mechanism of body-sparing action.

The standard nitrogen balance test was employed, groups of albino rats being used as the test animals. The effect on urinary nitrogen and on nitrogen balance of the addition of certain nitrogenous supplements to the basal low-nitrogen diet was studied. Two mixtures of the essential amino acids, one complete and the other lacking only methionine, were tested, as well as cystine, choline, and each of the ten essential amino acids individually. The response of the rats to the feeding of graded levels of methionine and of egg proteins on urinary excretion and on nitrogen balance were also measured.

The distribution of nitrogen and methionine in the bodies of the rats was determined. Animals maintained under the conditions of the nitrogen balance test were the source of material for these analyses of tissues. The whole carcass and hepatic and muscle tissues of animals fed a variety of nitrogenous supplements during the last 11 days of the nitrogen balance test were analyzed. The quantities of

moisture and fat present in muscle and hepatic tissues were also determined.

The feeding of a supplementary mixture of the ten essential amino acids caused a depression in the urinary excretion of nitrogen and a body-sparing effect essentially the same as that resulting from a similar administration, to partially depleted adult rats, of egg proteins (fed in an amount supplying approximately the same quantity of nitrogen). The body nitrogen spared in each case was equal, when converted to its equivalent in body tissue, to the observed weight gained by the two groups of animals in the second metabolism period.

Methionine fed as the sole nitrogenous supplement to a nitrogen-low diet induced a fall in the urinary excretion of nitrogen in rats previously maintained on a nitrogen-low ration, and spared body nitrogen equivalent to approximately 5 gm. of tissue. Animals fed methionine failed to gain weight, although they lost 5 gm. less than they would have, had no methionine been offered.

In contrast to the effect of the complete amino acid mixture, only two-thirds of the nitrogen of a mixture composed of all the essential amino acids except methionine was retained, when fed to protein-depleted rats on a nitrogen-low ration.

When each of the ten essential amino acids was fed

separately to ten groups of animals subjected to the customary nitrogen balance test, methionine, arginine, and histidine caused a noticeable depression in the urinary excretion of nitrogen. In addition to these three amino acids, leucine and lysine, also, spared some body tissue, although they effected no change in the quantity of nitrogen excreted in the urine. Phenylalanine appeared to stimulate the breakdown of body tissue, as indicated by an increase in the negative nitrogen balance in the period of nitrogen feeding. Some disturbance in metabolism was evident following the feeding of tryptophane, when a yellow pigment was excreted in the urine.

Egg proteins fed to groups of rats in amounts equivalent to 1 to 4 per cent of the nitrogen-low ration consumed during the first balance period caused identical depressions in the urinary excretion of nitrogen at each level of intake. When egg proteins made up 5 per cent of the ration, no depression occurred, because the animals passed into positive nitrogen balance. In contrast, the body nitrogen spared increased with each increase in the quantity of egg proteins fed.

The magnitude of the depression in the excretion of urinary nitrogen decreased with increasing amounts of methionine in the diet. However, the same amount of body nitrogen was spared at each level of feeding. The

requirement of partially depleted rats for methionine appeared to be less than 11 mg. per day. Glynn, Himsworth, and Neuberger (1945) have reported that hypoproteinemia in rats, correlated under their specific experimental conditions _only_ with methionine deficiency, may be prevented by 8 mg. of methionine per day.

The effect of cystine and choline was similar to that of methionine both in regard to the extent to which the urinary excretion of nitrogen was depressed, and the quantity of body nitrogen spared.

The nitrogen content of the whole carcasses of rats decreased from 8.38 gm. immediately prior to depletion to 6.41 gm., as the animals were maintained on a nitrogen-low diet for 18 days. The body weight declined correspondingly. Neither the administration of methionine as a supplement to the nitrogen-low diet, nor the feeding of the nitrogen-low diet alone, for an additional 11 days, had any further effect on the nitrogen content of the whole carcass. On the other hand, the addition of egg proteins to the diet for the last 11 days of the balance test brought the nitrogen content of the whole carcass to 7.75 gm., a quantity exactly equal, when converted to its equivalent in body tissue, to the observed gain in body weight during this period. The per cent of nitrogen in all carcasses was the same, 2.6 per cent.

The total methionine content of the whole carcass fell

during the first 18 days of nitrogen-low feeding, but remained constant thereafter, no matter whether the nitrogen-low diet was fed, or that diet fortified with methionine. Egg proteins fed during the last 11 days of the balance period caused an increase in the total methionine corresponding to the increase in total nitrogen and in body weight. Nonetheless, the per cent of methionine nitrogen to total nitrogen in the carcasses of all groups analyzed remained constant.

The fat content of muscle tissue was constant despite dietary changes. Hepatic tissue reflected the dietary lack of nitrogen by a rise in the concentration of fat, a condition not corrected by the feeding of supplementary egg proteins, choline, or a mixture of the ten essential amino acids. It responded to supplementary methionine and cystine by a marked increase in fat content.

The livers of animals fed a nitrogen-low diet for 29 days contained less nitrogen than did the same organ removed from animals supplemented in the last 11 days of the nitrogen balance test with methionine. The concentration of methionine in the livers of the unsupplemented group was correspondingly lower, so that the ratio of methionine nitrogen to total nitrogen was the same for both groups, 3.0 per cent.

The concentration of methionine per gram of dry, fat-free muscle was constant, whatever dietary modification was imposed.

CONCLUSIONS

Thus, it seems that the effects of egg proteins may be ascribed to their content of essential amino acids. Of these, methionine is the most powerful in sparing body protein; arginine, histidine, leucine, and lysine are only slightly less so. No single amino acid, however, can be very important quantitatively in sparing body tissue in the partially depleted animal, in view of the complex demands of the body for the synthesis of its structural and functional components. Egg proteins and a mixture of the essential amino acids are much more effective in this respect, as indicated by tha quantities of body nitrogen spared and by the observed gains in weight.

Determination of the effect of administration of graded doses of both egg proteins and of crystalline methionine indicates that only at about the 1 per cent level might the body-sparing action of egg proteins be said to be due to their methionine content. Above that, other amino acids are present in sufficiently large amounts to make their effect felt, and increasing quantities of body tissue are spared.

Analyses of whole carcass, liver, and muscle of adult rats indicate that the effect of methionine on nitrogen

metabolism does not represent the over-all picture of nitrogen metabolism, wherein amino acids in the metabolic pool, arising from food and tissue sources, are used in the maintenance of body tissue, and in the synthesis of functional protein and of nitrogenous and non-nitrogenous metabolites. The lack of gain in body weight and the relatively small increment in labile reserves in the liver when methionine is fed to the depleted animal, suggest that methionine does not act in the maintenance of body tissues. The powerful effect of methionine, and of arginine, histidine, leucine, and lysine, in decreasing the losses of nitrogen in the urine of animals maintained on a low-nitrogen diet is evidence that the body does raid its tissues for specific metabolites for the synthesis of specific functional substances.

LITERATURE CITED

Ackerson, C. W., and Blish, M. J.
1926. The effect of cystine on the endogenous metabolism of molting hens
Poultry Sci. 5:162-165.

Addis, T., Poo, L. J., and Lew, W.
1936a. The quantities of protein lost by the various organs and tissues of the body during a fast
J. Biol. Chem. 115:111-116.

Addis, T., Poo, L. J., and Lew, W.
1936b. Protein loss from liver during a two day fast
J. Biol. Chem. 115:117-118.

Addis, T., Poo, L. J., and Lew, W.
1936c. The rate of protein formation in the organs and tissues of the body
J. Biol. Chem. 116:343-352.

Albanese, A. A., Frankston, J., and Irby, V.
1944. The estimation of methionine in protein hydrolysates and human urine
J. Biol. Chem. 156:293-302.

Allison, J. B., and Anderson, J. A.
1945. The relation between absorbed nitrogen, nitrogen balance and biological value of proteins in adult dogs
J. Nutr. 29:413-420.

Allison, J. B., Anderson, J. A., and Seeley, R. D.
1945. The determination of the nitrogen balance index in normal and hypoproteinemic dogs
Preprinted manuscripts, Conference on Proteins and Protein Hydrolysates in Nutrition. The New York Academy of Sciences, Dec. 7 and 8.

Baernstein, H. D.
1936. A new method for the determination of methionine in proteins
J. Biol. Chem. 115:25-32.

Barger, G., and Coyne, F. P.
1928. The amino acid methionine; constitution and synthesis
Biochem. J. 22:1417-1425.

Benedict, F. G., Horst, K., and Mendel, L. B.
1932. The heat production of unusually large rats during prolonged fasting
J. Nutr. 5:581-597.

Binet, L., Weller, G., and Gondard, H.
1937. Le faux du glutathion dans le foie altéré
Compt. rend. Soc. de biol. 124:1141-1143.

Binkley, F., and du Vigneaud, V.
1942. The formation of cysteine from homocysteine and serine by liver tissue of rats
J. Biol. Chem. 144:507-511.

Blaschko, H.
1942. l (-)-cysteic acid decarboxylase of dog's liver
J. Physiol. 101:6P-7P.

Bloch, K., and Schoenheimer, R. J.
1941. The biological precursors of creatine
J. Biol. Chem. 138:167-194.

Bloch, R. J., and Bolling, D.
1945. The amino acid composition of proteins and foods.
pp. 174-198. Springfield, Ill., Charles C. Thomas.

Bloor, W. R.
1929. The oxidative determination of phospholipid (lecithin and cephalin) in blood and tissues
J. Biol. Chem. 83:273-286.

Borsook, H., and Dubnoff, J. W.
1940. The formation of creatine from glycocyamine in the liver
J. Biol. Chem. 132:559-574.

Borsook, H., and Keighley, G. L.
1935. The "continuing" metabolism of nitrogen in animals
Proc. Roy. Soc. [London], Series B, 118:488-521.

Burroughs, E. W., Burroughs, H. S., and Mitchell, H. H.
1940. The independence of the endogenous and the exogenous metabolism of nitrogen
J. Nutr. 20:271-283.

Clark, H. E.
1945. The biological value of egg proteins. II. Their role in the maintenance of a normal nitrogen metabolism in the adult rat
Unpublished M. S. Thesis. Ames, Ia., Iowa State College Library.

Croft, P. B., and Peters, R. A.
1945. Nitrogen loss after thermal burns
Lancet [London] 248:266-271.

Eddy, J. H., Jr.
1945. Methionine in the treatment of toxic hepatitis
Am. J. Med. Sci. 210:374-377.

Folin, O.
1905. A theory of protein metabolism
Am. J. Physiol. 13:117-138.

Folin, O., and Denis, W.
1912. Protein metabolism from the standpoint of blood and tissue analysis
J. Biol. Chem. 11:87-95.

Glynn, L. E., Himsworth, H. P., and Neuberger, A.
1945. Pathological states due to deficiency of the sulfur-containing amino acids
Brit. J. Exp. Path. [London] 126:326-337.

Griffith, W. H.
1941. The nutritional importance of choline
J. Nutr. 22:239.

Griffith, W. H., and Wade, J. J.
1940. Choline metabolism II. The interrelationship of choline, cystine, and methionine in the occurrence and prevention of hemorrhagic degeneration in young rats
J. Biol. Chem. 132:627-637.

Hammett, F. S.
1933. Growth retardation by the partially oxidized sulfhydryl of cysteine
Science 77:190-191.

Harrison, H. C., and Long, C.N.H.
1945. The regeneration of liver protein in the rat
J. Biol. Chem. 161:545-557.

Hess, W. C., and Sullivan, M. X.
1943. The cysteine, cystine, and methionine content of proteins
J. Biol. Chem. 151:635-642.

Horst, K., Mendel, L. B., and Benedict, F. G.
1934. The influence of previous diet, growth, and age upon the basal metabolism of the rat
J. Nutr. 8:139-162.

Howland, J., and Richards, A. N.
1909. An experimental study of the metabolism and pathology of delayed chloroform poisoning
J. Exp. Med. 11:344-371.

Lavine, T. F.
1943. An iodometric determination of methionine
J. Biol. Chem. 151:281-297.

Lee, W. C., and Lewis, H. B.
1934. The effect of fasting, refeeding, and of variations in the cystine content of the diet on the composition of the tissue proteins of the white rat
J. Biol. Chem. 107:650-659.

Lepkovsky, S., Roboz, E., and Haagen-Smit, A. J.
1943. Xanthurenic acid and its role in the tryptophane metabolism of pyridoxine-deficient rats
J. Biol. Chem. 149:195-201.

Lewis, H. B.
1917. The metabolism of sulfur. II. The influence of small amounts of cystine on the balance of nitrogen in dogs maintained on a low protein diet
J. Biol. Chem. 31:363-377.

Luck, J. M.
1936. Liver proteins I. The question of protein storage
J. Biol. Chem. 115:491-510.

McCarthy, T. E., and Sullivan, M. X.
1941. A new and highly specific colorimetric test for methionine
J. Biol. Chem. 141:871-876.

McHenry, E. W., and Patterson, J. M.
1944. Lipotropic factors
Physiol. Rev. 24:128-167.

Madden, S. C., and Whipple, G. H.
1940. Plasma proteins: their source, production and utilization
Physiol. Rev. 20:194-217.

Madden, S. C., _et al_.
1939. Blood plasma protein production as influenced by amino acids
J. Exp. Med., 69:721-738.

Madden, S. C., _et al_.
1940. Blood plasma protein production and utilization
J. Exp. Med. 71:283-297.

Madden, S. C., _et al_.
1943. Ten amino acids essential for plasma protein production effective orally or intravenously
J. Exp. Med. 77:277-295.

Marshall, M. W.
1943. The biological value of egg proteins. I. Effect of dehydration
Unpublished M. S. Thesis. Ames, Ia., Iowa State College Library.

Martin, C. J., and Robison, R.
1922. The minimum nitrogen expenditure of man and the biological value of various proteins for human nutrition
Biochem. J. 16:407-447.

Medes, G.
1939. Metabolism of sulfur. VIII. Oxidation of the sulfur-containing amino acids by enzymes from the liver of the albino rat
Biochem. J. 33:1559-1569.

Medes, G., Floyd, N. F., and Cammaroti, M. S.
1944. Synthesis of methionine and cystine by rats on a choline-free diet with homocystine as the only sulfur-containing amino acid
Growth 8:89-94.

Michaelis, L., and Schubert, M. P.
1934. The reaction of iodoacetic acid on mercaptans and amines
J. Biol. Chem. 106:331-341.

Miller, L. L.
1944. The metabolism of dl-methionine and l-cystine in dogs on a very low protein diet
J. Biol. Chem. 152:603-611.

Miller, L. L., Ross, J. F., and Whipple, G. H.
1940. Methionine and cystine, specific protein factors preventing chloroform liver injury in protein-depleted dogs
Am. J. Med. Sci. 200:739-756.

Miller, L. L., and Whipple, G. H.
1942. Liver injury, liver protection, and sulfur metabolism
J. Exp. Med. 76:421-435.

Mitchell, H. H.
1924. A method of determining the biological value of protein
J. Biol. Chem. 58:873-903.

Mitchell, H. H.
1944. Determination of the nutritive value of the proteins of food products
Ind. and Eng. Chem., Anal. Ed. 16:696-700.

Mitchell, H. H., Burroughs, W., and Beadles, J. R.
1936. The significance and accuracy of biological values of proteins computed from nitrogen metabolism data
J. Nutr. 11:257-274.

Mitchell, H. H., and Carman, G. G.
1926. The biological value of the nitrogen of mixtures of patent white flour and animal foods
J. Biol. Chem. 68:183-215.

Mueller, J. H.
1923. A new sulfur-containing amino acid isolated from the hydrolytic products of protein
J. Biol. Chem. 56:157-169.

Mulford, D. J., and Griffith, W. H.
1942. Choline metabolism
J. Nutr. 23:91-100.

Nielsen, E. K., Gerber, L. P., and Corley, R. C.
1939. Retention of the nitrogen of amino acids administered singly or in mixtures to dogs fed diets low in protein
Am. J. Physiol. 126:215-222.

Osborne, T. B., and Mendel, L. B.
1912. The role of gliadin in nutrition
J. Biol. Chem. 12:473-510.

Osborne, T. B., and Mendel, L. B.
1914. Amino acids in nutrition and growth
J. Biol. Chem. 17:325-349.

Osborne, T. B., and Mendel, L. B.
1915. The comparative nutritive value of certain proteins in growth and the problem of the protein minimum
J. Biol. Chem. 20:351-378.

Quastel, J. H.
1933. Glutathione, iodoacetic acid and glucose metabolism
Nature 131:206.

Quick, A. J.
1944. Relation of amino acids to biologically important products and the role of certain amino acids in detoxication in Sayhun, M. Outline of the amino acids and proteins.
pp. 158-178, New York, N.Y., Reinhold Publishing Corp.

Robscheit-Robbins, F. S., and Miller, L. L.
1946. Amino acid utilization in simultaneous hypo-proteinemia and anemia. Elimination of one essential from growth mixture
Proc. Fed. Am. Soc. Exp. Biol. 5:226.

Robscheit-Robbins, R. S., Miller, L. L., and Whipple, G. H.
1943. Hemoglobin and plasma protein
J. Exp. Med. 77:375-396.

Schmidt, C.L.A., editor
1944. The chemistry of the amino acids and proteins with addendum.
2d ed. pp. 3, 4. Springfield, Ill., Charles C. Thomas.

Schoenheimer, R.
1942. The dynamic state of body constituents.
Cambridge, Mass., Harvard Univ. Press.

Schuler, W., Bernhardt, H., and Reindel, W.
1936. Die Tryaminbildung aus Tryosin mit überlebenden Gewebschnitten und deren Beziehung zur Adrenalinsynthese
Z. physiol. Chem. 243:90-102.

Schwimmer, D.
1945. Life raft ration studies
Mimeographed manuscript. Welfare Island, New York City. N. Y. Medical College, Metropolitan Hospital Research Unit.

Seligman, A. M., and Fine, J.
1943. Traumatic shock. I. The production of radioactive plasma protein from amino acids containing radioactive sulfur
J. Clin. Investigation 22:265-273.

Sherman, H. C.
1920. Protein requirement of maintenance in man and the nutritive efficiency of bread protein
J. Biol. Chem. 41:97-109.

Simmonds, S., *et al.*
1943. The utilization of the methyl groups of choline in the biological synthesis of methionine
J. Biol. Chem. 149:519-525.

Smuts, B. D.
1935. The relation between the basal metabolism and the endogenous nitrogen metabolism with particular reference to the estimation of the maintenance requirement of protein
J. Nutr. 9:403-433.

Stearns, G.
1929. Method of preparation of fecal digests for nitrogen and mineral analysis
J. Lab. and Clin. Med. 14:954-957.

Stekol, J. A.
1935. Metabolism of *l*- and *dl*-methionine in adult and growing dogs maintained on diets of various protein contents
J. Biol. Chem. 109:147-157.

Stekol, J. A.
1936. The determination of p-bromophenylmercapturic acid in the urine of the dog
J. Biol. Chem. 113:279-288.

Stekol, J. A., and Schmidt, C.L.A.
1933. The fate of *dl*-methionine, glycine, and *d*-glutamic acid and *l*-pyrrolidone carboxylic acid in the dog
Univ. of Calif. Publ. in Physiol. 8:31-42.

Stetten, D., Jr.
1942. The fate of dietary serine in the body of the rat
J. Biol. Chem. 144:501-506.

Tarver, H., and Schmidt, C.L.A.
1939. The conversion of methionine to cystine: experiments with radioactive sulfur
J. Biol. Chem. 130:67-80.

Tarver, H., and Schmidt, C.L.A.
1942. Radioactive sulfur studies
J. Biol. Chem. 146:69-84.

Toennies, G., and Callan, T. P.
1939. Methionine studies. III. A comparison of oxidative reactions of methionine, cysteine and cystine
J. Biol. Chem. 129:481-490.

du Vigneaud, V., et al.
1940. The transfer of the methyl group from methionine to choline and creatine
J. Biol. Chem. 134:787-788.

du Vigneaud, V., et al.
1941. The utilization of the methyl group of methionine in the biological synthesis of choline and creatine
J. Biol. Chem. 140:625-641.

du Vigneaud, V., et al.
1944. On the mechanism of the conversion in vivo of methionine to cystine
J. Biol. Chem. 155:645-651.

du Vigneaud, V., et al.
1945. Synthesis of labile methyl groups in the white rat
J. Biol. Chem. 159:755-756.

Virtue, R. W., and Doster-Virtue, M. E.
1937. Studies on the production of taurocholic acid in the dog
J. Biol. Chem. 119:697-705.

Weichselbaum, T. E.
1935. Cystine deficiency in the albino rat
Quart. J. Exp. Physiol. [London] 25:363-367.

White, A., and Fishman, J. B.
1936. The formation of taurine by the decarboxylation of cysteic acid
J. Biol. Chem. 116:457-461.

White, A., and Lewis, H. B.
1932. The metabolism of sulfur. XIX. The distribution of urinary sulfur in the dog after the oral administration of monobromobenzene as influenced by the character of the dietary protein and by the feeding of l-cystine and dl-methionine
J. Biol. Chem. 98:607-624.

Willman, W., et al.
1945. Biological efficiency of egg proteins
Proc. Fed. Am. Soc. Exp. Biol. 4:164.

Wilson, H.E.C.
1933. Experiment on the nature of the labile role of sulphur in metabolism
J. Physiol. 77:240-246.

Witter, R. F.
1944. The metabolism of monobromobenzene, benzene, benzyl chloride, and related compounds in the rabbit
Unpublished Ph.D. Thesis. Ann Arbor, Mich., Univ. of Michigan Library.

Wolf, P. A., and Corley, R. C.
1939. Significance of amino acids for the maintenance of nitrogen balance in the adult white rat
Am. J. Physiol. 127:589-596.

Womack, M., Kemmerer, K. S., and Rose, W. C.
1937. The relation of cystine and methionine to growth
J. Biol. Chem. 121:403-410.

Womack, M., and Rose, W. C.
1941. The partial replacement of dietary methionine by cystine for purposes of growth
J. Biol. Chem. 141:375-379.

ACKNOWLEDGMENTS

Dr. Pearl Swanson has guided the preparation of this thesis throughout its development. The author is very appreciative of the stimulus to thorough analysis and interpretation of data and formulation of new ideas, which have resulted from this association.

The author, likewise, values highly the opportunity she has had for enjoyable and challenging working co-operation with Miss Wanda Willman, and the help which other fellow-workers in the laboratory have given.

APPENDIX

TABLE 1

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

Cage number	Total nitrogen	Theoretical quantity of nitrogen present	Recovery of nitrogen
	mg.	mg.	per cent
1	369.3	370.3	99.7
	370.3		100.0
	369.3		99.7
2	371.0	370.3	100.2
	370.6		100.1
	370.6		100.1
3	368.6	370.3	99.6
	368.3		99.4
	368.3		99.4
		Average	99.8

TABLE 2

Concentration of nitrogen in 25 ml. of a standard creatinine solution (13.49 mg. per cent N)

0.1N HCl neutralized	Nitrogen in 25 ml. aliquot	Theoretical quantity of nitrogen present	Recovery of nitrogen
ml.	mg.	mg.	per cent
16.61	23.25	23.25	100.00
16.60	23.24		99.96
16.61	23.25		100.00
16.62	23.27		100.09
16.61	23.25		100.00
16.60	23.24		99.96
16.60	23.24		99.96
16.60	23.24		99.96
16.60	23.24		99.96
		Average	99.98

TABLE 3

Methionine content of eggs determined after varying intervals of autoclaving

Temperature	Pressure	Time	Methionine
°C.	lb.	hr.	per cent
125	17	4	4.76
		5	4.90
		6	4.86
		7	4.94
		8	4.90

TABLE 4

Sample calculations, methionine content of proteins

A = ml. 0.1 N sodium thiosulfate required for water blank

B = ml. 0.1 N sodium thiosulfate required for methionine standard

C = ml. 0.1 N sodium thiosulfate required for unknown

B' = A - B, corrected titer of methionine standard

C' = A - C, corrected titer of unknown

F = mg. methionine in standard/B'

Mg. methionine in protein sample = C' x F

TABLE 5

Factor showing the methionine equivalent of 1.00 ml. of standard sodium thiosulfate solution, as determined at different times

Date	Factor
February 11, 1946	8.055
February 13	8.055
March 26	8.055
March 30	8.055
May 10	8.055

TABLE 6

Standardization of sodium thiosulfate

Potassium iodate	Aliquot of potassium iodate	Quantity of thiosulfate used in titration	Normality of sodium thiosulfate
	ml.	ml.	
Solution I Jan. 18	50.00	51.45	
		51.44	
		51.44	
		51.44	0.09720
Solution I April 10	50.00	51.46	
		51.47	
		51.56	0.09716
Solution II April 10	50.00	51.42	
		51.42	
		51.41	0.09724

TABLE 7

Composition of oxidizing mixtures tested in the estimation of methionine

Number	Hydrogen peroxide	Perchloric acid	Final volume
	ml.	ml.	ml.
1	4	40	125
2	4	48	150
3	4	56	175
4	4	64	200
5	4	72	225
6	4	80	250
7	4	88	275
8	4	96	300

TABLE 8

Oxidation of cystine and tryptophane by hydrogen peroxide

Oxidizing mixture	Sodium thiosulfate used		
	Blank	Cystine*	Tryptophane**
	ml.	ml.	ml.
1	11.27	10.17	11.27
2	10.20	10.20	10.20
3	9.18	9.18	9.18
4	8.07	8.05	8.07
5	7.15	7.10	7.15
6	6.43	6.36	6.43
7	5.80	5.71	5.79
8	5.39	5.29	5.38

*Solution contained 10.03 mg. cystine.

**Solution contained 10.02 mg. tryptophane.

TABLE 9

Oxidation of cystine, methionine, and tryptophane by oxidizing mixture 3

Sample*	Sodium thio-sulfate	Blank titer—unknown titer	Total concentration of standard solutions
	ml.	ml.	mg.
Blank	9.18		
2 ml. methionine standard	7.93	1.25	10.07
4 ml. methionine standard	6.68	2.50	20.14
2 ml. cystine standard	9.18	0	10.03
2 ml. tryptophane standard	9.18	0	10.03
2 ml. methionine st. + 2 ml. cystine st.	7.93	1.25	
2 ml. methionine st. + 2 ml. tryptophane st.	7.93	1.25	
2 ml. methionine st., 2 ml. cystine st., + 2 ml. tryptophane st.	7.93	1.25	

*Total volume = 17 ml.

$$F = \frac{10.0688}{1.25} = 8.055$$

Recovery based on internal standard
= 2.50 x F = 2.50 x 8.055 = 20.1375

Theoretical amount of methionine present = 20.1376

Per cent recovery = 100.00

TABLE 10

Methionine content of spray-dried whole eggs calculated to 16.0 per cent N

Date	Methionine
	per cent
December 7, 1945	4.98
December 11, 1945	4.86
January 1, 1946	5.04
May 10, 1946	4.86
	Average 4.94

TABLE 11

Recovery of crystalline methionine after its addition to a fixed quantity of egg proteins

Methionine in egg proteins	Quantity of methionine added	Total methionine determined	Crystalline methionine recovered	Recovery of methionine
mg.	mg.	mg.	mg.	per cent
33.74	50.34	83.44	49.70	98.93
33.47	50.34	82.98	49.51	98.36
33.79	50.34	84.16	50.37	100.06
33.91	50.34	83.99	50.08	99.48
				Av. 99.21

TABLE 12

Methionine content of eggs, as reported by various investigators

Investigator	Methionine
	per cent
Baernstein (1936)	4.49 - 5.07
Block and Bolling (1945)	4.9
Lavine (1943)	4.58
Brush (present study)	4.94

TABLE 13…

Data relating to the nitrogen metabolism of i…
of nitrogen during the period of n…

Supplement	Rat number	Nitrogen-low period							
		Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitr… bala… per 1… sq. c…
		gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
Methionine	39380	244	339	288	118	85	-391	-160	-11[illegible]
	38946	285	373	283	99	76	-399	-140	-10[illegible]
	39200	258	351	296	115	84	-383	-148	-10[illegible]
	39381	249	344	312	125	91	-398	-160	-11[illegible]
	39227	251	345	260	104	75	-350	-139	-10[illegible]
	39292	257	350	230	90	66	-323	-126	- 9[illegible]
Arginine	39428	220	319	239	109	75	-340	-154	-10[illegible]
	39222	258	351	258	100	74	-341	-132	- 9[illegible]
	39405	245	316	249	115	79	-340	-157	-10[illegible]
	39047	286	373	291	102	78	-390	-136	-10[illegible]
Threonine	39055	271	361	232	86	64	-334	-123	- 9[illegible]
	39228	234	331	233	100	70	-318	-136	- 9[illegible]
	39440	219	318	266	121	84	-340	-155	-10[illegible]
	39074	293	379	241	82	64	-370	-126	- 9[illegible]
	39071	308	390	291	94	74	-383	-124	- 9[illegible]
Valine	39068	347	419	382	110	91	-518	-149	-12[illegible]
	39339	213	313	307	109	83	-341	-160	-10[illegible]
Histidine	39438	274	364	322	118	88	-409	-149	-11[illegible]
	39204	294	380	284	97	75	-389	-132	-10[illegible]
	39048	276	365	238	86	65	-342	-124	- 9[illegible]
	39075	278	367	223	80	61	-328	-118	- 8[illegible]
	39378	239	335	297	124	89	-371	-155	-11[illegible]
	39364	266	357	279	105	78	-399	-150	-11[illegible]
Tryptophane	39011	301	385	297	99	77	-389	-129	-10[illegible]
	38945	287	374	263	92	70	-317	-110	- 8[illegible]
	39483	259	352	281	108	80	-364	-140	-10[illegible]
	39176	264	328	239	103	73	-314	-136	- 9[illegible]
	39332	244	339	277	114	82	-397	-163	-11[illegible]
	39417	259	352	257	99	73	-360	-139	-10[illegible]

Continued o[n] next p…

TABLE 13

gen metabolism of individual rats fed various sources
ing the period of nitrogen supplementation

gen e	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.	Period of nitrogen supplementation: Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.
	mg.	mg.	gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
	-160	-115	228	326	188	82	58	-221	-97	-68
	-140	-107	261	353	203	78	58	-254	-97	-72
	-148	-109	235	332	187	80	56	-246	-105	-74
	-160	-116	230	328	241	105	74	-289	-126	-88
	-139	-101	237	334	190	80	57	-242	-102	-72
	-126	- 92	244	339	194	80	57	-232	- 95	-68
	-154	-106	204	305	219	108	72	-267	-131	-88
	-132	- 97	240	336	203	85	60	-240	-100	-71
	-157	-108	195	297	204	105	69	-238	-122	-80
	-136	-104	268	359	216	81	60	-304	-113	-85
	-123	- 92	256	349	246	96	70	-310	-121	-89
	-136	- 96	217	316	243	112	77	-312	-144	
	-155	-107	192	294	432	225	147	-455	-237	-155
	-126	- 98	274	364	272	99	75	-371	-135	-102
	-124	- 98	293	379	285	97	75	-348	-122	- 92
	-149	-124	331	408	361	109	88	-473	-143	-116
	-160	-109	196	298	226	116	76	-308	-157	-103
	-149	-112	256	349	253	99	73	-331	-129	- 95
	-132	-102	236	333	217	92	65	-266	-113	- 80
	-124	- 94	257	350	252	98	72	-305	-119	- 87
	-118	- 89	263	355	223	85	63	-313	-119	- 88
	-155	-111	224	322	226	101	71	-276	-123	- 86
	-150	-112	251	345	242	96	70	-300	-120	- 87
	-129	-101	282	370	305	108	82	-383	-136	-104
	-110	- 85	265	357	282	106	79	-333	-126	- 93
	-140	-104	237	334	307	130	92	-360	-152	-108
	-136	- 96	212	312	223	105	72	-271	-128	- 87
	-163	-117	229	327	231	101	71	-290	-127	- 89
	-139	-102	241	337	282	117	84	-333	-138	- 99

Continued on next page

TABLE 13 (cont

Supplement	Rat number	Nitrogen-low period							
		Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nit bal per sq.
		gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	m
Phenylalanine	39294	284	372	283	100	76	-374	-132	-1
	39168	229	327	196	86	60	-279	-122	-
	39062	321	400	326	102	82	-448	-140	-1
	39396	284	372	285	100	77	-391	-138	-1
	39344	250	344	277	111	80	-391	-156	-1
	39280	243	339	230	95	68	-342	-141	-1
Lysine	39177	278	367	286	103	78	-414	-149	-1
	39432	227	325	244	105	75	-336	-148	-1
	39439	278	367	293	105	80	-406	-146	-1
	39190	232	329	243	105	74	-342	-147	-1
	39487	273	363	266	97	73	-383	-140	-1
	39346	198	299	223	113	74	-308	-156	-1
Leucine	39373	256	349	255	99	73	-338	-132	-
	39441	260	353	302	116	86	-443	-170	-1
	39367	230	328	309	134	94	-420	-183	-1
	39431	214	314	243	114	74	-320	-154	-1
	39484	246	341	288	117	84	-378	-154	-1
	39351	215	314	280	119	91	-392	-208	-1
Isoleucine	39462	290	376	322	111	86	-432	-149	-1
	39347	211	311	257	122	83	-348	-165	-1
	39205	238	334	255	107	76	-332	-139	-
	39054	288	375	255	89	78	-327	-114	-
	39482	273	363	288	105	79	-362	-133	-1
	39433	273	363	281	110	77	-378	-138	-1
Cystine	39473	226	324	245	108	76	-347	-154	-1
	39485	223	322	241	108	75	-284	-127	-
	39449	265	357	272	103	76	-344	-130	-
	39463	245	340	246	100	72	-331	-135	-
	39206	252	346	271	108	78	-392	-156	-1
	39430	235	332	262	112	79	-329	-140	-

Continued on next

TABLE 13 (continued)

en e	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.	Period of nitrogen supplementation							
			Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.
	mg.	mg.	gm.	sq. cm.	mg.	mg.	mg.	mg.	mg.	mg.
	-132	-101	264	356	280	106	79	-412	-156	-116
	-122	- 85	205	306	240	117	78	-376	-183	-123
	-140	-112	299	384	306	102	80	-508	-170	-132
	-138	-105	263	355	293	111	82	-460	-157	-130
	-156	-113	230	328	265	115	81	-414	-180	-126
	-141	-101	224	322	227	101	70	-352	-157	-109
	-149	-113	261	354	272	104	77	-374	-143	-106
	-148	-103	206	307	231	112	75	-284	-138	- 93
	-146	-111	262	354	263	100	74	-335	-128	- 95
	-147	-104	217	316	215	99	68	-272	-125	- 86
	-140	-106	252	346	263	105	76	-311	-123	- 90
	-156	-103	179	282	262	146	93	-229	-128	- 81
	-132	- 97	273	363	263	96	72	-310	-114	- 85
	-170	-125	241	337	282	117	84	-322	-134	- 96
	-183	-128	208	308	274	132	89	-344	-165	-112
	-154	-105	197	299	229	116	77	-258	-131	- 86
	-154	-111	222	321	264	119	82	-308	-139	- 96
	-208	-142	200	301	268	134	89	-310	-155	-103
	-149	-115	268	359	300	112	84	-380	-142	-106
	-165	-112	193	295	251	130	85	-318	-165	-108
	-139	- 99	224	322	231	103	72	-286	-128	- 89
	-114	- 87	266	357	285	107	80	-365	-128	-102
	-133	-100	255	348	286	112	82	-346	-136	- 99
	-138	-104	253	347	279	110	80	-357	-141	-103
	-154	-107	210	310	227	108	73	-281	-134	- 91
	-127	- 88	210	310	184	88	59	-247	-118	- 80
	-130	- 96	244	339	193	79	57	-336	-138	- 99
	-135	- 97	231	328	194	84	59	-245	-106	- 75
	-156	-113	230	328	193	84	59	-232	-101	- 71
	-140	- 99	219	318	192	88	60	-213	- 97	- 67

ontinued on next page

TABLE 13 (cont

Supplement	Rat number	Nitrogen-low period							
		Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nit bal per sq.
		gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	m
Choline	39583	234	331	248	106	75	-386	-155	-1
	39679	241	337	281	117	83	-422	-175	-1
	40042	205	306	247	120	81	-456	-222	-1
	39863	254	248	253	100	73	-371	-146	-1
	39747	229	327	258	113	79	-371	-162	-1
Dried whole egg (3.5 per cent)	39050	348	420	297	85	71	-411	-118	-
	39063	287	374	307	107	82	-416	-145	-1
	39486	220	319	232	106	73	-326	-148	-1
	39049	290	376	263	91	70	-381	-121	-1
N-low	39046	325	403	250	77	62	-377	-116	-
	39461	246	341	243	99	71	-352	-143	-1
	39376	233	330	242	104	73	-316	-136	-
	39472	263	355	284	108	80	-426	-162	-1
	39305	293	379	279	95	74	-356	-122	-
10 essential amino acids	38317	245	340	324	132	95	-416	-170	-1
	38057	206	307	319	155	104	-409	-198	-1
	38058	317	397	362	114	91	-450	-142	-1
	38238	196	298	325	166	109	-385	-196	-1
	38387	237	334	268	113	98	-344	-145	-1
10 essential amino acids minus methionine	40198	236	333	249	106	75			
	40095	243	339	274	113	81			
	40088	251	345	313	125	91			
	40231	291	377	363	125	96			
	40180	254	348	278	109	80			
	40171	287	374	367	128	98			
Dried whole eggs 1 per cent	38256	248	343	285	115	83	-410	-165	-12
	38261	243	339	304	125	90	-409	-168	-12
	38339	252	346	279	111	81	-385	-153	-11
	38340	277	366	321	116	88	-455	-164	-12

Continued on next

TABLE 13 (continued)

Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.	Period of nitrogen supplementation: Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.
mg.	mg.	gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
-155	-117	220	319	204	93	64	-279	-127	-88
-175	-125	222	321	243	109	76	-291	-131	-91
-222 >1154	-149	189	291	221	117	76	-261	-138	-90
-146	-107	238	334	227	95	68	-285	-120	-85
-162	-113	214	314	209	98	67	-263	-123	-84
-118	-98	343	416	184	54	44	+132	+38	+32
-145	-111	282	370	256	91	69	+ 38	+13	+10
-148	-102	220	319	202	92	63	+ 28	+13	+ 9
-121	-101	289	376	204	71	54	+112	+39	+30
-116	-94	302	386	253	84	66	-350	-116	-91
-143	-109	226	324	240	106	74	-334	-148	-103
-136	-96	211	311	218	103	70	-287	-136	-92
-162	-120	250	344	231	92	67	-341	-136	-99
-122	-94	280	369	276	98	75	-357	-128	-97
-170	-122	232	329	309	133	94	+ 38	+17	+12
-198	-133	197	299	250	127	84	+ 17	+ 9	+ 6
-142	-113	303	386	386	101	100	+ 54	+18	+14
-196	-129	184	287	205	111	71	+ 45	+24	+16
-145	-103	229	329	287	125	87	+ 24	+11	+ 7
		218	317	+334	153	105			
		227	325	379	167	117			
		230	328	452	196	138			
		264	356	472	179	133			
		230	328	406	176	124			
		256	349	466	182	133			
-165	-120	234	331	212	90	64	-219	-94	-66
-168	-121	228	326	201	88	62	-229	-100	-70
-153	-111	241	337	208	86	62	-200	-83	-59
-164	-124	262	354	241	92	68	-237	-90	-67

Continued on next page

TABLE 13 (continue

Supplement	Rat number	Nitrogen-low period							
		Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitro bala per 1 sq. c
		gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg
Dried whole eggs									
2 per cent	38336	227	325	242	107	74	-333	-147	-10
	38337	241	337	300	124	89	-407	-169	-12
	38348	285	373	335	118	90	-474	-166	-12
	38349	271	362	358	132	99	-491	-181	-13
3 per cent	38234	249	344	302	121	88	-418	-168	-12
	38287	236	333	294	124	88	-442	-187	-13
	38285	241	337	273	113	81	-388	-161	-11
	38256	236	333	307	130	92	-457	-194	-13
4 per cent	38160	282	370	279	99	75	-383	-136	-10
	38216	181	284	253	140	89	-353	-195	-12
	38215	283	371	319	113	86	-470	-166	-12
5 per cent	38188	237	334	287	121	89	-401	-169	-12
	38295	238	334	246	103	74	-371	-159	-11
	38296	304	387	339	112	88	-452	-149	-11
	38304	263	355	298	113	84	-417	-158	-11
Methionine									
1 mg. N	40155	250	344	368	147	107	-499	-200	-14
	40160	290	376	365	126	97	-484	-167	-12
	40111	265	357	322	122	90	-477	-180	-13
	40241	249	344	291	117	85	-414	-166	-12
	40232	257	350	324	126	92	-435	-169	-12
2 mg. N	40154	236	333	281	119	84	-378	-160	-11
	40135	254	348	336	132	97	-451	-178	-13
	40110	272	362	302	111	83	-428	-157	-11
	40218	251	345	337	134	98	-420	-167	-12
	40206	271	362	341	126	94	-458	-169	-12
	40181	244	339	397	163	117	-490	-201	-14

Continued on next p

TABLE 13 (continued)

en e	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.	Period of nitrogen supplementation							
			Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.
	mg.	mg.	gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
	-147	-102	209	309	191	91	62	-98	-47	-32
	-169	-121	230	328	197	86	60	-116	-50	-35
	-166	-127	272	362	239	88	66	-118	-43	-33
	-181	-136	257	350	319	124	91	-209	-81	-60
	-168	-122	241	337	232	96	69	-48	-20	-14
	-187	-133	229	327	217	95	66	-54	-24	-16
	-161	-115	234	331	202	86	61	-44	-19	-13
	-194	-137	228	326	212	93	65	-32	-14	-10
	-136	-103	274	364	218	80	60	+58	+21	+16
	-195	-124	172	275	212	123	77	-51	-30	-18
	-166	-127	280	369	254	91	69	+46	+16	+12
	-169	-124	228	326	288	126	88	-21	- 9	- 6
	-159	-111	238	334	243	102	73	+127	+53	+38
	-149	-117	296	381	290	98	76	+93	+31	+24
	-158	-118	262	354	337	129	95	+82	+31	+23
	-200	-145	236	333	219	93	66	-302	-128	-91
	-167	-129	270	361	225	83	62	-335	-124	-93
	-180	-134	250	344	207	83	60	-301	-120	-87
	-166	-120	229	367	181	79	55	-259	-113	-79
	-169	-124	243	339	181	74	53	-270	-111	-80
	-160	-114	220	319	172	78	54	-223	-101	-70
	-178	-130	238	334	206	86	62	-206	- 86	-62
	-157	-118	252	346	214	85	62	-292	-116	-84
	-167	-122	239	335	241	101	72	-303	-127	-90
	-169	-126	254	348	221	87	64	-299	-118	-86
	-201	-144	232	329	231	100	70	-295	-127	-90

Continued on next page

TABLE 13 (continued)

Supplement	Rat number	Nitrogen-low period							
		Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrog balanc per 10 sq. cm
		gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
Methionine									
3 mg. N	38236	259	352	263	102	75	-372	-144	-106
	38168	221	320	230	104	72	-369	-167	-115
	38155	173	276	264	153	96	-379	-219	-137
	38159	200	301	228	114	76	-333	-166	-111
	37957	185	287	199	108	69	-301	-163	-105
	37958	242	338	228	94	68	-329	-136	- 97
4 mg. N	40134	301	385	367	122	95	-519	-172	-135
	40009	248	343	276	111	80	-402	-162	-117
	40162	295	380	340	115	89	-460	-156	-121
	40233	284	372	368	130	99	-487	-171	-131
	40177	237	333	309	130	93	-401	-169	-120
8 mg. N	40142	275	365	318	116	87	-478	-174	-131
	40159	247	342	322	130	94	-429	-174	-126
	40143	266	357	364	137	102	-483	-182	-135
	40108	243	339	280	115	83	-397	-163	-117
	40116	260	352	302	116	86	-420	-162	-119
	40176	272	362	315	116	87	-437	-161	-121
16 mg. N	40150	276	365	315	114	86	-466	-169	-128
	40133	278	367	298	107	81	-436	-157	-119
	40090	257	350	364	142	104	-508	-198	-145
	40188	255	348	366	144	105	-505	-198	-145
	40219	282	370	319	113	86	-486	-172	-131

LE 13 (continued)

		Period of nitrogen supplementation							
Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.	Body weight	Body surface area	Urinary nitrogen	Urinary nitrogen per 100 gm.	Urinary nitrogen per 100 sq. cm.	Nitrogen balance	Nitrogen balance per 100 gm.	Nitrogen balance per 100 sq. cm.
mg.	mg.	gm.	sq.cm.	mg.	mg.	mg.	mg.	mg.	mg.
-144	-106	241	337	184	76	55	-277	-115	-82
-167	-115	209	309	144	69	46	-213	-102	-69
-219	-137	156	260	159	102	61	-217	-139	-84
-166	-111	175	278	134	76	48	-187	-107	-67
-163	-105	168	271	123	73	45	-176	-105	-65
-136	- 97	222	321	147	66	46	-188	- 85	-59
-172	-135	279	368	255	91	69	-335	-120	-91
-162	-117	227	325	196	86	60	-224	- 99	-69
-156	-121	268	359	266	99	74	-341	-127	-95
-171	-131	257	350	246	96	70	-330	-128	-94
-169	-120	209	309	264	126	85	-310	-148	-100
-174	-131	257	350	241	94	69	-342	-133	-98
-174	-126	229	327	233	102	71	-257	-112	-79
-182	-135	237	333	267	113	80	-281	-118	-84
-163	-117	230	328	236	103	72	-272	-118	-83
-162	-119	240	336	229	95	68	-241	-100	-72
-161	-121	253	347	259	102	75	-314	-124	-91
-169	-128	259	352	291	112	83	-288	-111	-82
-157	-119	259	352	254	98	72	-235	-91	-67
-198	-145	233	330	297	127	90	-259	-87	-78
-198	-145	238	334	250	105	75	-234	-98	-70
-172	-131	262	354	311	119	88	-315	-120	-89

TABLE 14

Weights, methionine content, and total nitrogen content of the whole carcasses of individual rats fed the stock colony diet, and of ones fed the low-nitrogen diet alone or with nitrogenous supplements

Supplement	Rat number	Body weight	Total nitrogen	Total methionine
		gm.	mg.	mg.
Stock colony	40182	317	8856	186
	40155	350	8260	196
	40160	310	8036	164
N-low, 18 days	40111	288	7557	141
	40241	240	5897	129
	40232	232	5783	126
N-low, 29 days	40150	246	6292	137
	40135	298	8319	154
	40110	204	5457	104
Egg	40218	236	7445	135
	40206	284	6815	152
	40181	346	6980	190
Methionine 1 mg. N	40153	224	6793	141
	40134	238	6992	153
	40099	238	6222	139
2 mg. N	40162	219	5337	107
	40233	210	6269	127
	40177	248	7157	136
4 mg. N	40142	234	5477	126
	40159	226	5774	114
	40143	244	5317	116
16 mg. N	40108	212	5670	138
	40116	236	6499	143
	40176	226	6952	143

TABLE 15

Moisture and fat content of hepatic and muscle tissue of animals fed the stock colony diet, and of ones fed the nitrogen-low diet alone, and with nitrogenous supplements

Supplements	Rat number	Liver		Muscle	
		Moisture	Fat	Moisture	Fat
		%	% of dry wt.	%	% of dry wt
Stock colony	39380	71.1	16.1	76.4	8.7
	39346	72.1	15.3	75.6	7.1
	39200	70.6	15.8	75.7	6.7
N-low 18 days	39480	70.9	22.4	76.1	6.1
	39495	71.2	24.2	75.7	8.3
	39152	70.8	26.3	75.2	6.2
N-low 29 days	39406	70.4	18.7	76.0	9.8
	39461	70.2	21.5	75.6	10.1
	39376	71.2	20.4	75.7	7.9
Egg	40164	71.5	23.6	76.0	5.4
	40141	70.7	24.0	75.7	6.7
	40207	71.5	23.8	75.5	5.2
	40087	71.3	23.8	76.0	7.9
	40230	71.3	21.6	75.2	10.8
	40185	71.1	23.7	75.9	7.0
Methionine					
1 mg. N	40170	69.7	29.3	76.2	9.4
	40198	71.0	28.6	76.1	9.5
	40095	72.7	18.5	75.6	7.4
2 mg. N	40088	70.9	24.3	76.2	6.5
	40231	70.7	30.1	75.5	8.0
	40180	70.1	30.1	75.8	7.7
4 mg. N	40171	68.7	31.6	75.7	8.3
	40133	70.9	29.2	75.7	8.8
	40090	68.8	34.9	75.7	8.9
8 mg. N	40188	70.3	27.5	75.6	7.9
	40165	70.4	30.4	75.3	9.2
	40219	70.6	29.6	——	——

Continued on next page

TABLE 15 (continued)

Supplements	Rat number	Liver		Muscle	
		Moisture	Fat	Moisture	Fat
		%	% of dry wt.	%	% of dry wt
Methionine 16 mg. N	40149	70.7	24.1	75.5	9.4
	40132	73.1	22.5	76.3	7.2
	40089	68.3	36.0	76.2	6.8
Cystine	39473	68.5	30.9	75.8	7.9
	39485	68.7	29.7	75.9	9.4
Choline	39677	71.6	22.6	75.4	8.5
	39583	71.7	23.0	75.7	8.9
	39679	72.9	17.4	76.1	10.5
Mixture of 10 essential amino acids	38317	72.6	23.6	75.8	9.6
	38057	73.3	22.5	75.5	8.5
	38058	71.7	21.9	75.5	8.0

TOTAL 16

Weight, methionine content, and total nitrogen content of livers of rats fed a nitrogen-low diet, alone and with supplementary methionine

Diet	Rat number	Total wt. of fresh liver	Methionine in fresh liver	Total nitrogen in fresh liver
		gm.		
N-low	40751	6.2079	36.9	115.6
	40757	6.4797	42.0	123.4
	40759	6.5047	37.9	132.6
	40760	6.4417	45.2	135.9
N-low plus methionine	40765	7.8446	54.4	182.2
	40767	6.6457	44.6	146.8
	40784	6.4927	39.3	126.9
	40786	7.3293	58.8	181.2
	40788	6.5071	43.3	132.3

TABLE 17

Methionine content of muscle of individual rats fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements

Supplement	Rat number	Methionine per gm. dry, fat-free muscle
		mg.
Stock colony	39380	37.8
	39346	34.8
	39200	40.7
N-low, 18 days	39480	35.8
	39495	36.1
	39152	36.6
N-low, 29 days	39406	36.9
	39461	36.3
	39376	37.2
Egg	40164	34.7
	40141	34.2
Methionine		
1 mg. N	40170	36.9
	40198	36.8
	40095	41.4
2 mg. N	40088	37.3
	40231	36.3
	40180	34.0
4 mg. N	40170	41.2
	40133	33.1
	40090	40.6
8 mg. N	40188	39.2
	40165	36.3
16 mg. N	40149	45.9
	40132	36.1
	40089	33.0

LIST OF TABLES IN APPENDIX

Page

1. Nitrogen recovered from cages sprinkled with aliquots of standard ammonium sulfate solution.... 154

2. Concentration of nitrogen in 25 ml. of a standard creatinine solution (13.49 mg. per cent N).. 155

3. Methionine content of eggs determined after varying intervals of autoclaving.................. 156

4. Sample calculations, methionine content of proteins.. 157

5. Factor showing the methionine equivalent of 1.00 ml. of standard sodium thiosulfate solution, as determined at different times........ 158

6. Standardization of sodium thiosulfate............. 159

7. Composition of oxidizing mixtures tested in the estimation of methionine...................... 160

8. Oxidation of cystine and tryptophane by hydrogen peroxide............................... 161

9. Oxidation of cystine, methionine, and tryptophane by oxidizing mixture 3...................... 162

10. Methionine content of spray-dried whole eggs calculated to 16.0 per cent N................... 163

11. Recovery of crystalline methionine after its addition to a fixed quantity of egg proteins...... 164

12. Methionine content of eggs, as reported by various investigators............................ 165

13. Data relating to the nitrogen metabolism of individual rats fed various sources of nitrogen during the period of nitrogen supplementation.................................. 166

Page

14. Weight, methionine content, and total nitrogen content of the whole carcasses of individual rats fed the stock colony diet, and of ones fed the low-nitrogen diet alone or with nitrogenous supplements.. 172

15. Moisture and fat content of hepatic and muscle tissue of animals fed the stock colony diet, and of ones fed the nitrogen-low diet alone, and with nitrogenous supplements........................ 172

16. Weight, methionine content, and total nitrogen content of livers of rats fed a nitrogen-low diet, alone and with supplementary methionine....... 174

17. Methionine content of muscle of individual rats fed the stock colony diet, and of ones fed the nitrogen-low diet alone and with nitrogenous supplements.. 175