NITROGEN METABOLISM OF MACACA NEMESTRINA PRIMATES DURING IMMOBILIZATION AND AMBULATION

A DISSERTATION

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF DOCTOR OF PHILOSOPHY IN NUTRITION

IN THE GRADUATE DIVISION OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF HOUSEHOLD ARTS AND SCIENCES

BY

SIADA ABD ELMAGID TOLBA, B.S., M.S.

DENTON, TEXAS

AUGUST, 1968

Texas Woman's University

Denton, Texas

August 19 68

We hereby recommend that the dissertation prepared under our supervision by Siada Abd Elmagid Tolba

entitled NITROGEN METABOLISM OF MACACA NEMESTRINA PRIMATES DURING IMMOBILIZATION AND AMBULATION

be accepted as fulfilling this part of the requirements for the Degree of Doctor of Philosophy.

Committee:

Pauline Beery Mack
Chairman
Jessie W Bateman
Elsa A Dorsey
Dora R. Lynn
Esther R. Broome

Accepted:

J. L. Morgan
Dean of Graduate Studies

ACKNOWLEDGMENTS

The author of this dissertation wishes to express her sincere appreciation and gratitude to those who gave so willingly of their time to aid her throughout this investigation.

To Dr. Pauline Beery Mack, Director of the Research Institute, Texas Woman's University, for her encouragement and guidance during the author's graduate program, and for assistance in the writing of this dissertation.

To Dr. Jessie W. Bateman, Dean of the College of Household Arts and Sciences, for her personal interest in the author's graduate program in all of its aspects.

To Dr. Betty B. Alford for her interest and encouragement throughout the graduate program and for her assistance in the completion of this work. The author appreciates so much her help in courses and in all ways.

To Dr. Dora R. Tyer, for her assistance and encouragement throughout the graduate program of the author.

To Dr. Wilma A. Brown, for her interest in the completion of the author's graduate program.

To Dr. Ralph E. Pyke, and Dr. Alice Milner for their inspirational teaching and help.

To the Embassy of the United Arab Republic of Egypt whose staff provided the scholarship and enough time which has made the study possible.

To her father, Mr. Abd Elmagid Tolba and her mother who helped her to accomplish advanced study.

To her brother, Mr. Mohamed Kamel Tolba, who encouraged and helped her so much to study. The author appreciates his assistance and kindness.

TABLE OF CONTENTS

page

page

LIST OF TABLES

INTRODUCTION

The study covered in this report describes an investigation conducted in the Nelda Childers Stark Laboratory for Human Nutrition Research, a component part of the Texas Woman's University Research Institute. In this investigation, four healthy male primates of the Macaca nemestrina strain, generally referred to as pigtail monkeys, were used in a study of nitrogen excretion changes during bed rest and during ambulation.

Primates were chosen by the National Aeronautics and Space Administration as test animals because of their physiological and anatomic resemblance to man. The major objective of this primate investigation has been the preparation of primates of the type chosen for space flight, thus adding more information to the investigation of space science.

The existence of the weightless state in a space vehicle, and the lack of physical activity are expected to cause disturbances in the metabolic pathways of various metabolites, among them, nitrogen.

The major objectives of this study have been the following:

1. To determine the effects of prolonged couch restraint on Macaca nemestrina as measured by urinary and fecal excretion of nitrogen.

2. To increase knowledge about the normal physiology of primates of the Macaca nemestrina strain insofar as nitrogen is concerned, thus gaining information of help during space flight in the Biosatellite program.

Nitrogen also is an important component of the human diet because of its presence as a major element in protein.

The author's interest in the subject of nitrogen is evidenced by her selection of nitrogen as a topic for the research upon which this dissertation is based. In her master's thesis, the author, as a former Agricultural Engineer in the United Arab Republic of Egypt has worked on possible methods of improving the quantity and quality of the protein in the Egyptian diet. Her Summary in this Master's thesis follows:

"She has reviewed briefly the literature of this subject in American and British scientific journals, and particularly has studied the publications of the United States Department of Agriculture with reference to family low-cost and moderate-cost dietary plans. On the basis of the latter she has devised a low-cost and a moderate-cost dietary plan for Egyptian families, and has calculated the costs of the foods included in these plans in terms of Egyptian food prices as obtained from compiled Egyptian tables of food costs, as well as recent food cost information obtained from Egyptian families who have shopped for food in representative stores in order to secure this information.

"It was discovered in these studies that as families progressed from the extremely low to higher income groups that people with very little food increased cereal consumption as the first change; as the income increases, additional meat and fat replace some of the cereals; further additions to the income lead to the consumption of a larger supply of milk, fruit, and vegetables. Thus, the protein needs are more apt to be met if a family prospers.

"From charts of the amino acid content of selected foods, as well as the family low cost and moderate cost food plans, representative recommended menus have been designed for Egyptian families which are complete in their nutritional provision, including the adequacy of their amino acid content.

"Methods of supplementing Egyptian diets with animal and vegetable foods which will amplify the diets with respect to amino acid content have been suggested.

"The author found that through the supplementation of the various diets with certain foods, they would be more adequate as to their amino acid content. Vegetable foods themselves may be used as supplements if the choice of vegetables is sound. For instance, cottonseed flour is nearly twice as high in arginine as soya flour, and contains many of the essential amino acids. Also, a combination of cottonseed meal, peanut meal, Brazil nut meal, and corn meal would

supplement each other insofar as amino acids are concerned. This mixture would greatly enrich white wheat flour, which is low in lysine, methionine, and tryptophan. This combination of meals could supplement corn meal which is low in lysine and tryptophan, or rice flour which is also low in the same amino acids.

"The author expects to carry on nutrition work of this type in Egypt so that better balanced diets can be worked out which will come within the budgetary means of Egyptian families with low and moderately low income.

"She realizes that the population as a whole will need to be educated about the importance of good nutrition before the acceptance of new protein mixtures as human food is widespread. The response has been poor up to the present time which is probably due to a lack of understanding of the nutritional value of these new foods, a lack of experience in recipe development, and a popular resistance to change in traditional foods."

REVIEW OF LITERATURE

There has been a growing interest in nitrogen metabolism during conditions of immobilization, not only because of its relation to space flight but also because continuous immobilization is associated with illness.

Cantarow and Schepartz (1) stated that much of the information on the metabolism of proteins is measured frequently in terms of nitrogen excretion. Urine is the major route of nitrogen excretion. Fecal nitrogen is not a major outcome of ingested protein. Its quantity varies, however, with the bulk of the diet and does not normally represent unabsorbed dietary protein.

From a study conducted by Murlin, Hayes, and Johnson (2), on the correlation between the biological value of protein and the percentage of nitrogen in the urine, it was found that, with a very poor protein intake, the amount of nitrogen appearing in the urine is greater proportionately.

Primates have been selected by the National Aeronautics and Space Administration for certain biological experiments which will be of aid to astronauts who are to perform space flights. Harris, Moor, and Wanner (3) have stated that, in selecting an animal species for research

in human nutrition, the animal should resemble the human being as closely as possible in metabolism, degradation, and excretion of nutrients.

Guinea pigs, rabbits, dogs, pigs, and rats have been used extensively in metabolic studies by many investigators. Wanner, Moor, Bronner, Pearson, and Harris (4), however, have presented evidence which indicates that none of these animals can compare with the monkey as substitutes for human beings in general studies of metabolism.

Some Animal Experiments on Nitrogen Metabolism

A biostatistical analysis of three experiments carried out by Bock et al (5) to determine the nitrogen exchange in 114 growing Wistar-ra rats fed a protein free diet showed that endogenous losses of nitrogen into the urine and the intestines were largely influenced by the liver weight of the animals, and the amount of dry substance ingested with the food.

When dogs and rats were placed on a protein-free diet by Allison and Wannemacher (6), the urinary excretion of nitrogen per day decreased rapidly during the first few days. After a loss of 8.0 per cent of body nitrogen, the decrease in the rate of nitrogen excretion was retarded until about 25 per cent of body nitrogen had been lost, after

which the urinary excretion of this element became rather constant. The nitrogen loss was attributed to the labile protein reserves of the body. Thus endogenous excretion of nitrogen could not be considered a constant until the animals were severely depleted. The rats lost 50 per cent of their body proteins before they succumbed to the rigors of the protein-free diet.

Van Zandt (7) reported on the effects of a new type of restraint garment on nitrogen metabolism of Macaca nemestrina primates. The data compiled in this study indicated that the loss of nitrogen during a restraint study was significantly higher than that during ambulation.

Brooks (8) also reported on nitrogen losses in a study with Macaca nemestrina primates. During the restraint period urinary nitrogen excretion was significantly higher than during the pre-restraint and post-restraint periods. This was consistent with the distinct losses of weight in the restraint animals during that period.

Hoffman, Dozier, Mack, Hood, and Parrott (9) conducted a study on 11 primates, fed two diets which were similar in provision of calories, but which differed in content of major nutrients. Two groups of animals were put on the respective diets and were placed in restraint on couches for 35 days followed by 35 days of reconditioning. One group of animals on each of the diets served as non-restrained controls. There was a massive increase in urinary nitrogen excretion in both the restrained

dietary groups immediately after restraint was imposed, consistent with an increase in body weight loss during this period. The level of excretion began a descent after the second week, simultaneously with the increase in body weight change in the two groups.

The Medico-Biological Station of the Academy of Medical Science USSR at Sukhumi has been in existence for a quarter of a century (10). It has extensive facilities for experimentation with monkeys. In a Sukhumi experiment, three healthy monkeys were used. This study established the fact that the average quantity of urine excreted during 24 hours by these primates amounted to 220-500 milliliters. The specific gravity fluctuated between 1,002 and 1,010, while the pH as a rule was above pH 7. The quantity of nitrogen excreted during 24 hours in the urine ranged from 1.57 to 3.0 grams; that of ammonia was 45 to 166 milligrams. The residual nitrogen varied from 18 to 42 milligrams. Because of the close morpho-physiological resemblance of monkey to man, the primate can, in a number of cases, not only be valuable but irreplaceable subjects for medical experiments.

A comprehensive review of literature on primates has been given by Varner (11) and by Al-Shawi (12), former graduate students at the Texas Woman's University.

PLAN OF PROCEDURE

GENERAL DESIGN OF THE STUDY

The study which is reported in this thesis was conducted at the Nelda Childers Stark Laboratory for Human Nutrition Research of the Texas Woman's University Research Institute under the sponsorship of the National Aeronautics and Space Administration. The data presented in this report were obtained from four healthy male primates of the Macaca nemestrina strain, generally referred to as pigtail monkeys.

The entire study consisted of the three following periods:

1. Period of Pre-Restraint (March 6 through April 28, 1968). The animals remained in their metabolism cages during this period.

2. Period of Restraint (April 29 through May 9, 1968). The animals were placed on couches and were fed by means of forceps. At the end of this period two of the four primates were sacrificed in order for two other graduate students to dissect their bones for the analysis of active ^{45}Ca, the animals having been injected with radioactive calcium during the

study in order that students of calcium metabolism might find the metabolic pathways of calcium. Also it was desired by these investigators to find whether calcium was deposited in the bones.

3. Period of Post-Bed Rest (May 10 through June 10, 1968). During this period, the remaining two animals were kept in their metabolism cages.

FEEDING METHOD

The diet of the primates consisted of purina monkey chow and apples. The food was weighed in individual portions each morning. If any food was not consumed before the next feeding, it was weighed and subtracted from the daily intake. The animals were given water ad libitum. On the first day of restraint, the four primates received an intravenous injection of 50 μc of radioactive ^{45}Ca as part of a study being conducted by two other graduate students.

COLLECTION OF EXCRETA

Excreta were collected from primates in metabolism cages in the usual manner. The urine was collected in bottles. During the immobilization period urine was collected from primates by means of urinary catheters.

The urine collections were kept on a daily basis, and were measured and stored in polyethylene bottles which were washed with a 10 per cent hydrochloric acid solution. The samples were refrigerated until they were used.

Nitrogen analyses were run on the urine and feces of the experimental animals by the Micro-Kjeldahl method (13), the details which follow.

MICRO-KJELDAHL METHOD FOR NITROGEN

REAGENTS:

1. Powdered potassium sulfate, K_2SO_4 (ammonia free).

2. Concentrated sulfuric acid, c. p. H_2SO_4, sp. gr. 1.84.

3. Mercuric sulfate solution. Add 12 ml of concentrated H_2SO_4 to water, and make up to 100 ml with water. Dissolve 10 g of red mercuric oxide in this solution.

4. Zinc dust (not granulated zinc), ammonia-free.

5. Sodium hydroxide, approximately 10 N. Dissolve 400 g of NaOH in water and dilute to 1 liter.

6. Standard 71.4 millimolar ammonium chloride solution. Used for checking the micro-Kjeldahl distillation procedure. Dissolve 0.3820 g of NH_4Cl, analytical reagent grade, in water and dilute to 100 ml. One ml contains 1 mg of nitrogen.

7. Acetate buffer, 0.2 M, pH 5. Dissolve 27.22 g of sodium acetate in water and make up to 1 liter. Add 427 ml of 0.2 N acetic acid, (standardized by titration against 0.1 N NaOH with phenolplthalein as an indicator).

8. Alizarin red solution, 0.1% in water.

9. 0.01428 N (N/70) H_2SO_4. Prepare by diluting 14.28 ml of 1 N acid to 1000 ml with water.

10. 0.01428 N (N/70) NaOH solution. Store in a plastic container or a paraffin-lined bottle. Protect against CO_2 by a soda lime tube. Standardize the solution daily by titration against 10 ml portions of the 0.01428 N H_2SO_4, with the same pH and volume at the end point as is described below for titration of distilled ammonia.

11. Boiling chips. Nortons Alundum chips, No. 14, black.

PROCEDURE:

Into a Pyrex glass tube 22 - 25 mm x 200 mm, measure 0.5 g of K_2SO_4 and the sample of unknown which contains 0.2 - 2.0 mg. of nitrogen (e. g. 0.1 ml of serum or urine). Then add 0.5 ml of the mercuric sulfate solution, 1.0 ml of concentrated sulfuric acid, and 3 boiling chips. Boil mixture gently until the water is boiled off. Then adjust the heat so that the concentrated digest boils with very slight motion. This digestion continues for 30 minutes after the mixture has become entirely clear. About 2 minutes after completion of the digestion, but before the contents solidify, wash down the sides of the tube with 3 ml of water. Grease the lip of the digestion tube lightly with silicone grease to avoid loss during the quantitative transfer to the distillation apparatus. Steam out the still for 30 minutes before each series of distillations. Transfer the contents of the digestion tube into the distillation flask with four portions of water, approximately 2 ml each. Add 0.2 g of zinc dust to the third washing in the funnel of the distillation apparatus. Admit the mixture to the flask and follow with the fourth washing. Deliver 4 ml of 10 N NaOH into the distillation flask and distill into 10.00 ml of 0.01428 N H_2SO_4, with the tip of the condensor below the surface of the acid. Distill enough liquid to insure quantitative transfer of the ammonia to the standard acid. The volume of liquid which must be so transferred by distillation will vary from one apparatus to another and should be predetermined for each

distillation apparatus by trial runs with the standard 71.0 millimolar ammonium chloride solution. (Use 1 ml of standard and run it like urine samples, should recover 1.0 mg of nitrogen.) Then continue the distillation for 1 minute after the receiving flask has been lowered so that the tip of the condensor tube is above the surface of the standard acid. To the distillate add 0.8 ml of 1% alizarine red indicator solution and titrate with 0.01428 N NaOH from a 10-ml buret until the color matches that of acetate buffer solution in a control flask. The volume of liquid at the end of the titration should be nearly equal to that in the control flask. To prepare the control, measure into a 125-ml Erlenmeyer flask 7 ml of 0.2 N acetate buffer, 63 ml of distilled water, and 0.8 ml of 0.1% alizarin red solution. A new control should be made up every 3 days or more often if mold growth becomes visible therein. Blank analyses are run through the entire procedure.

CALCULATIONS:

Milligrams nitrogen in sample analyzed equals (ml of 0.01428 N NaOH required to back-titrate the blank minus ml required to titrate distillate of unknown) multiplied by 0.2.

PRINCIPLE:

Organic matter of the sample to be analyzed is oxidized by heating it with sulfuric acid in the presence of a catalyst. All combined nitrogen in the sample is thereby converted to ammonium sulfate

nitrogen. An excess of alkali is then added and the ammonia which is thus liberated is distilled into acid solution. The ammonia is distilled into an accurately measured small excess of standard H_2SO_4. The acid not neutralized by the ammonia is then titrated with standard NaOH.

REFERENCE:

Standard Methods of Clinical Chemistry. Volume II, 1958, page 91 (13).

RESULTS OF THE STUDY

EXCRETION OF NITROGEN

The data obtained on urinary and fecal nitrogen excretion are given in Tables I, II, III, and IV for the respective primates in this study. The data for the major periods of the study also appear in the four tables.

Table V, Table VI, and Table VII show the statistical comparisons of urinary nitrogen excretion, fecal nitrogen excretion, and total nitrogen excretion, respectively, between different periods of the study for the four primates. The tables are presented in the Appendix.

The nitrogen excretion for primates in this study changed from period to period as will be noted from the Tables just cited.

URINARY NITROGEN EXCRETION

Primate 239

The mean urinary nitrogen excretion for Primate 239 was higher both for the Restraint and Post-Restraint Periods than during the Pre-Restraint Period. The mean increase in urinary nitrogen during Restraint was significantly higher ($P < 0.05$) than during the Pre-Restraint Period.

The other comparisons were non-significant, although the mean for the Bed Rest, or Restraint Period was somewhat higher. See Table V.

All-in-all, the highest level of excretion by this animal occurred during the Bed Rest, or Restraint Period. See Table I.

Primate 249

Table II gives the quantity of total daily urinary nitrogen excreted by Primate 249 during the Pre-Bed Rest, and the Bed Rest Periods. The daily average of urinary nitrogen excretion during Pre-Bed Rest was 2,727 milligrams. During the Bed Rest Period, the daily average was 2,832 milligrams. This denotes an increase in urinary nitrogen excretion during Bed Rest, although this difference in values was not statistically significant.

This animal was sacrificed at the end of the Restraint Period so that another graduate student could use certain of the bones in a radioactive calcium experiment.

Primate 419

In contrast to Primate 239, Primate 419 showed a greater difference in urinary nitrogen excretion between the Pre-Restraint and the Restraint Periods. The average quantity of urine excreted by Primate 239 was 2,491 milligrams during Pre-Bed Rest and 3,302 milligrams during Bed Rest, while for Primate 419, urinary nitrogen excretion was 1,965

milligrams during Pre-Bed Rest, and 3,756 milligrams during the Bed Rest Periods. See Table III.

Statistically the mean for urinary nitrogen excreted during Bed Rest, or Restraint, for this primate was higher than the mean for other periods of Pre-Restraint or Post-Restraint ($P < 0.001$ in both comparisons). The average nitrogen excretion also was significantly higher during Post-Restraint than during the period preceding Restraint ($P < 0.02$).

Primate 423

As in the case of Primate 249, Primate 423 was sacrificed immediately after the close of the Restraint Period. In contrast with the former animal, Primate 423 excreted a quantity of urinary nitrogen during the Restraint Period which was significantly higher statistically than during Pre-Restraint ($P < 0.001$). The two mean 24-hour values were 2,529 milligrams during the Pre-Restraint and 4,182 milligrams during the Restraint Period. During Restraint, there was an increase in the amount of urinary nitrogen excretion from 1,886 milligrams on the first day to 4,650 milligrams per day on the final day. See Table IV.

FECAL NITROGEN EXCRETION

Primate 239

In contrast to the results for urinary nitrogen, fecal nitrogen decreased from one study period to the next.

The fecal nitrogen excreted day-by-day for this primate was 444 milligrams during Pre-Restraint, 255 milligrams during the Restraint or Bed Rest Period, and 237 milligrams during the Post-Restraint Period.

Statistically, fecal nitrogen excretion both for the Restraint and Post-Restraint Periods was significantly lower than for the initial study period. See Tables I and VI.

Primate 249

The mean fecal nitrogen excreted by this primate was 283 milligrams during the Pre-Restraint, and 180 milligrams during the Restraint Periods. See Table II.

The difference in the fecal nitrogen excretion during the Restraint Period was lower than that during the Pre-Restraint Period, by a statistically significant difference ($P < 0.02$).

Primate 419

During the Pre-Restraint Period, Primate 419 excreted 147 milligrams of fecal nitrogen per 24-hours. During the Restraint phase of the

study, 118 milligrams of fecal nitrogen were excreted per 24 hours. See Table III. The difference in this factor was significant ($P < 0.01$) in behalf of the Restraint Period.

In a comparison of the fecal nitrogen excreted during the Restraint and Post-Restraint Periods, the values were 118 milligrams daily during Restraint, and 87 milligrams during Post-Restraint. Statistically there was no significant difference between the fecal nitrogen excretion values during these two periods, although the quantity during Restraint was somewhat higher.

Primate 423

The daily average of fecal nitrogen excretion was 245 milligrams during Pre-Restraint for this animal and 122 during Restraint. See Table IV.

There was a highly significant decrease in fecal nitrogen excretion during the Restraint Period ($P < 0.001$).

TOTAL NITROGEN EXCRETION

Primate 239

The total nitrogen excreted by Primate 239 was 2,935 milligrams, 3,578 milligrams, and 2,885 milligrams during the Pre-Restraint, the Restraint, and the Post-Restraint Periods, respectively. This shows an

increase in total nitrogen excretion with Restraint followed by a decrease in total output during the Post-Restraint Period.

When statistical comparisons were made (Table VII), there was only a slightly significant difference between the Pre-Restraint and the Restraint Periods ($P < 0.10$), respectively, with the same level of difference between the Restraint and the Post-Restraint Periods of the study. When the Pre-Restraint and Post-Restraint Periods of the study were compared by the "t" test, however, the levels of excretion by this primate were not statistically significant.

Primate 249

Table II gives the quantities of total nitrogen excreted during the Pre-Restraint, the Restraint, and the Post-Restraint Periods by Primate 249. The average total quantities of nitrogen excreted were identical for the Pre-Restraint and the Restraint Periods. The quantities of total nitrogen excreted during these two periods were 2,727 and 2,832 milligrams, respectively. The difference between these two periods was not significant.

Primate 419

During the Restraint Period Primate 419 excreted the highest mean daily quantity of total nitrogen of any of the phases of the study (3,958

milligrams), with 2,321 milligrams during Pre-Restraint and 1,833 milligrams daily in the Post-Restraint phase (Table II).

A statistical analysis of the data showed that the total excretion of nitrogen by this animal during Restraint surpassed that of the Pre-Restraint Period by a highly significant difference ($P < 0.001$). See Table VII.

In the same table, the excretion of total nitrogen during Restraint exceeded that excreted during the Post-Restraint phase, again by a highly significant difference ($P < 0.001$). A "t" test comparison of the Pre- and Post-Bed Rest Periods showed that the former surpassed the latter by a difference which also was highly significant ($P < 0.001$).

Primate 423

The total nitrogen excreted day-by-day by this primate was 2,529 milligrams during Pre-Restraint. The daily average of total nitrogen excretion increased 1,653 milligrams during the Restraint Period. Statistically, the Restraint Period surpassed the Pre-Restraint Period by a highly significant difference ($P < 0.001$).

RELATION OF BODY WEIGHT CHANGE TO TOTAL NITROGEN EXCRETION

The losses in body weight during Restraint were estimated as follows:

Animal	Per Cent Loss
Primate 239	10.0
Primate 249	14.0
Primate 419	12.0
Primate 423	14.5

The losses in weight were related to the mean total loss in nitrogen during Restraint. Failure to consume the food offered them was largely responsible for weight loss, which came largely from the muscle mass.

GENERAL DISCUSSION

Many authors have suggested that the metabolism of protein can be expressed in terms of nitrogen. Nitrogen which enters the body of mammals as food is ultimately stored in the form of body protein, or it is eliminated through urine and feces as nitrogenous substances. As noted, the urine constitutes the major carrier for the elimination of nitrogen.

These factors are important for nitrogen balance, synthesis, and metabolism.

For the purposes of this study, factors were controlled: calorie intake was relatively adequate and constant; protein intake was kept as constant as possible from one period to another; and water intake was controlled. No infections, even of seemingly mild degree were present. Intestinal parasites were absent by virtue of extensive treatment of the primates at NASA Ames Research Center, Moffett Field, California before they were shipped to the Texas Woman's University.

DIETARY NITROGEN INTAKE AND EXCRETION

Nitrogen intake and output from mammals depends on the quality and quantity of protein in the diet; the nitrogen content of food; the calorie content of the diet; and the environmental temperature.

In determination of nitrogen balance in children has proved a particularly sensitive method for evaluating the effects of amino acid supplementation of cereal protein, and also has given satisfactory results for this purpose in adults.

De Costa and his associates (14) found that animals and men sometimes failed to gain weight despite evidence of positive nitrogen balance. This suggested an additional unaccounted for route of

excretion of nitrogen other than urine and feces. When mice were fed nitrogen-glycine in airtight metabolic chambers, it was noted that the atmosphere was enriched by isotopic nitrogen. This means that an alternative route of excretion of nitrogen is possible through the skin, the lungs, or through flatus.

Arrayane (15) made a report of body protein deficit in protein in a malnourished population by creatinine measurements. He found that urinary creatinine excretion measurements are useful in field studies as an indirect estimate of muscle mass changes. These measurements may be of great value in assessing the relative degree of protein depletion of population groups suffering from shortage of dietary protein.

Blondell (16) suggested that patients with acute renal failure show negative nitrogen balance and enhanced urea production.

Before treatment, nitrogen balance was negative in four studies, and positive in five studies with values ranging from -0.4 to 1.9 grams per day.

EFFECT OF NITROGEN IN GROWTH OF CHILDREN

Protein is one of the essential factors in the growth of children including body weight and body height. By radiographic measurements, weight may gain rapidly, but body length may not be greatly altered, because of many factors.

It is recognized that the development of muscle mass is a parameter which is dependent on protein nutrition, if the needs for other essential nutrients are satisfied. Nitrogen balance of children usually is positive since increase in size is sacrificed in order to conserve weight. This has been found in the Pennsylvania-Texas population studies.

Deshpande (17), in his study of rats, found that the rate of growth of young rats was increased from three to 21 grams per week when their diet, containing 78 per cent of white flour was supplemented with 0.5 per cent of L-lysine and 0.4 per cent of threonine. The improvement in growth was obtained when seven more essential amino acids were added.

Although lysine was limiting for growth, liver fat did not accumulate when the diet contained 78 per cent of white flour. Fatty infiltration, which occurred when the flour was fed at a 5.4 per cent protein level, was prevented by a lysine supplement.

The maximum growth, however, was obtained when intact protein formed part of the supplement; but growth was not as rapid when the protein was replaced by equivalent quantities of crystaline essential and non-essential amino acids.

Mack et al (18) fed lysine to 42 sub-adolescent orphanage children with the following conclusions:

"The effects on growth and bone density of adding lysine to the food of forty-two sub-adolescent orphanage children consuming a diet which provided 62 grams of protein daily (ratio of protein from animal to that from vegetable sources 1.56:1, lysine:tryptophane ratio 5:1) have been studied. A group of forty-two other children on the same basic diet as the experimental group, with a similar distribution in respect to sex, age and response to height-weight, bone density and biochemical tests, served as controls. Bone density was measured by the method of radiographic densitometry.

"L-lysine monohydrochloride to the extent approximately of 70 milligrams per kilograms of body weight was added to the food of the experimental group daily for the duration of the study, which was five and a half months. The lysine:tryptophane ratio in the basic diet plus the lysine supplement was 7.9:1.

"The experimental children receiving the lysine supplement gained in growth to a significantly greater extent than did the control children. They also had statistically significant gains in bone density in two anatomic sites, whereas the control children did not have significant bone density increases in the regions measured. Bone density gains in the experimental group in a section of the distal end of the radius, which passes through the epiphysis and diaphysis, were greater than those in a section of the os calcis which does not include the more rapidly growing portion of the bone."

Sanberlich (19) found in a study with rats that amino acid imbalance was produced by feeding a casein gelatine diet or a casein oxidized diet. He suggested the following:

The reduction in growth of the animals due to the imbalance could be corrected by dietary supplements of tryptophan, and not by addition of casein alone.

Protein digestion and absorption was similar for all groups.

The percentage of free and total tryptophan (and certain other amino acids) excreted in the urine by the rats on the imbalanced diets was two- to three-fold over that excreted by control animals or by animals receiving supplements of tryptophan alone.

PROTEIN AND MALNUTRITION

Numerous observations have indicated the importance of protein in hemoglobin formation. Anemia develops in rats fed a diet abnormally low in protein and be corrected by feeding protein, according to Orten (20). Numerous investigators have shown that, in dogs fed abundant iron and protein-free or low protein diets, globulin can be readily formed from plasma, casein, or hemoglobin. There appears to be a "dynamic equilibrium" between plasma protein cell protein with the result that protein deficiency affects hemoglobin synthesis as well as the production of other body proteins, and increased demands for hemoglobin will call upon the general body protein pool.

A study was made by Walton (21) on 300 nonselected samples of blood, which were analyzed by means of a modification of the urease method for determining the amount of urea nitrogen. However, aliquots of the samples also were processed in automatic testing and recording apparatus, which was designed in such a manner that a method could be used as a means of determining the amount of urea nitrogen in dialyzates of blood. A critical study of the data indicated that there was no clinical significance in the difference between the results of the two methods, but that non-urea substances in a series of 50 specimens of blood of

uremic patients, the effect on the color developed in the carbamido-diacety monoxime reaction was not clinically significant.

Koryakina (22) did a study on nitrogen balance in burnt dogs treated with the serum of convalescent burnt dogs which had been treated with the serum of healthy animals. No toxicity of the serum was observed in the first group, while the blood of the second group retained its toxicity during the whole experiment period. The treatment with immune blood serum had an inhibitory effect on the catabolic process, although the negative nitrogen balance was half that observed after the first burn.

PROTEIN IN RELATION TO KWASHIORKOR IN CHILDREN

The author's interest in protein and nitrogen malnutrition has led her to study the literature of this field in relation to kwashiorkor, which is less prominent in Egypt than in many other far East countries, but is found there to a certain extent.

Davidson, Passmore, and Orr (23) have described this protein-calorie deficiency disease as follows:

"Kwashiorkor is a nutritional deficiency disease due predominantly to an inadequate intake of utilisable protein, occurring principally in

children shortly after weaning and characterised by muscular wasting, oedema, irritability or mental apathy, failure to grow and a liability to a damaged liver.

'The fundamental biochemical lesion is caused by a deficiency of amino acids necessary for protein synthesis.....

"The immediate cause of the disease is often an acute infection (e.g. measles or gastroenteritis); this increases the rate of breakdown of tissue proteins and also may decrease the dietary intake of protein .....

History

"Cicely Williams (1933) was the first to record that 'some amino acid or protein deficiency' might be an aetiological factor in kwashiorkor, the name given to this disease by the Ga tribe living in and around Accra, the capital of the Gold Coast (now Ghana). Later, Dr. Williams (1958) wrote that the definition of kwashiorkor which was originally given to her is 'the sickness the older child gets when the next baby is born'; this translation indicates the circumstances in which the disease most commonly develops, namely an ignorance of the best foods to give children during the weaning period, or an inability to provide them for one reason or another. She noted that the same condition has been described in 1906 in Germany, in 1924 in Indo-China, in 1926 in Mexico

and in 1928 in East Africa. In fact the disease occurs in any part of the world if the dietary conditions described below are present. Elsewhere in Africa doctors have frequently misinterpreted the descriptive term 'kwashiorkor' to mean 'red boy' or 'pale-coloured child.' Children suffering from kwashiorkor frequently have pale-coloured skins and mucous membranes and depigmented.....

"Descriptive names for this syndrome which have been used in the past 25 years by clinicians include 'infantile pellagra,' 'malignant malnutrition,' 'syndrome dépigmentation-oedème,' 'distrofia pluricarencial.' The Joint FAO/WHO Expert Committee on Nutrition decided that 'protein-calorie deficiency disease' was better suited to describe its basic aetiology than 'kwashiorkor.' However, the Ghana word seems well established in the international medical literature, and like the Malayan 'beriberi' will probably hold its own against more scientific newcomers.

Aetiology and Incidence

"As with other deficiency diseases all the clinical features cannot be attributed to a single dietary defect; but there is little doubt that the predominant features are due to a deficiency of protein, either absolute or relative to energy requirements..... At periods of life when there is great need for both protein and energy to meet the demands of growth, as in infancy, childhood and at puberty, or during pregnancy and

lactation, dietary restriction of either protein or calories, or both as is most often the case, will precipitate the development of kwashiorkor. If the customary diet of a population is limited in protein and calories to around the levels of minimal requirements, the disease may be precipitated in epidemic proportions by outbreaks of febrile illnesses such as malaria, measles or whooping-cough. A helminthic or other infection may bring out the disease in a child belonging to a family whose diet is barely adequate. Here are good examples of the importance of consuming a recommended allowance of any nutrient which provides a safety margin above the minimum requirement for growth and maintenance. In addition, failure to utilise properly such dietary protein as is available may result from a lack of digestive enzymes, itself caused by a quantitative or qualitative deficiency of dietary protein.

"The syndrome has been described at all ages and in both sexes. Undoubtedly it is most common in those infants or toddlers whose mothers wean their children from the breast on to diets which are mainly composed of starchy gruels containing too little protein and providing too little energy. This may be on account of ignorance of good nutritional practices or lack of money to buy better foods. In the less-developed countries, where the majority of the world's population live, such mothers too often do not have ready access to suitable protein-rich foods with which to supplement these gruels.

"Kwashiorkor is a rare disease in countries in which literacy, education and developed food-processing industries have made it possible for all classes of the population to obtain and consume diets which provide sufficient energy and nutrients. Occasional cases are encountered in New York, Paris, London and other cities which include among their populations immigrants from less-developed countries. Such people are unaccustomed to urban living conditions and are inexpert in purchasing diets suitable for the younger members of their families.

"In the rural areas of many underdeveloped countries families grow their own food and employ methods of crop culture and food processing adapted over the centuries by circumstances to be compatible with the continuing existence of the community. Many traditional methods of crop production and food preparation must be commended and should be retained. Others should be modified and made more effective by the introduction of new techniques suitable to local conditions. Although food supplies may be sufficient in these rural areas, cases of kwashiorkor occur, often as the result of failure to comply with traditional dietary habits for reasons outwith the control of the family group. For instance a mother may die, or develop breast abscesses and her milk fail, when her child is a few weeks or months old; no aunt, grandmother or grand-aunt may be in lactation at this critical time to act as a foster-nurse. In many rural areas the food supplies may become scarce each year in the season before the harvesting of the staple food,

and the incidence of kwashiorkor may increase at this time. The incidence of kwashiorkor in children from birth to nine years of age, living in the rural areas of Nigeria, was found by Nicol (1959) to vary between 2 and 5 per cent, being highest in areas where the staple foods are yams, cassava or other starchy tubers, and lowest where cereals such as sorghum and millets (Sorghum vulgare, Pennisetum typhoideum and Eleusine coracana) provide most of the dietary calories and protein. The incidence of kwashiorkor in other African countries is probably of the same order, most cases occurring between the ages of 1 and 4 years.

"A much higher incidence of kwashiorkor may be found in the rural areas of countries which are much more densely populated than those of the African continent, such as the Indonesian islands and Bengal in India, and also in rapidly growing towns in the tropics. Here, lack of land to grow sufficient foodstuffs to provide the customary diets, and the high cost in the city markets of protein-rich foods, whether fresh or processed, are factors additional to lack of nutritional knowledge, seasonal food shortages and misfortune which operate to produce the syndrome. Paediatric wards in city hospitals usually contain severe cases of kwashiorkor, and the out-patient departments treat many more patients who are less seriously ill.....

Clinical Features

"Failure to grow is a constant and essential feature of the disease. The child's weight is usually much below standard for his age, but real weight may be masked by oedema. The muscles are always wasted and this is particularly noticeable around the chest; the wasting of the legs and around the hips is frequently concealed by oedema. If the disease has resulted from a dietary restriction of both calories and protein, muscle wasting and an almost complete lack of subcutaneous fat is the most striking feature, but the feet are oedematous ('marasmic kwashiorkor'). Subcutaneous fat is often plentiful in children whose diets have provided ample calories but little protein. The child is often apathetic and this is a most serious prognostic feature. A return from apathy to irritability is a sign of successful initial treatment. The hair is nearly always affected in African children; in Asiatics the changes are less frequent and less marked. There is an alteration in texture. The hair becomes fine, straight and soft, it loses its curl and lustre and is often sparse. The hair of African children may show a variety of pigmentary changes. Shades range from brownish-black and brown to a pale greyish-brown or even a straw colour. The extent of these changes in the hair is not necessarily an indication of the severity of the disease. Children who have been unwell for a long time but never seriously ill, may show them most markedly. Alterations in the skin are often present, especially in severe cases. These include

pigmentation, depigmentation, desquamation and ulceration. The legs, buttocks and perineal areas are most frequently involved, but any region may be affected. This is in contrast to pellagra in which the dermatosis occurs mainly on the exposed surfaces.....

The liver may be greatly enlarged and extend down to the umbilicus. Anorexia and vomiting occur and there is often a distressing diarrhoea, with the passage of stools containing much undigested food. This feature may be secondary to the failure of the pancreas to secrete digestive juices, especially lipase. Important losses of potassium may result from the diarrhoea. There is nearly always some anaemia, but it is not usually very severe. Both the degree of anaemia and its nature are largely determined by associated infections and other dietary deficiencies.

Prognosis

"Kwashiorkor is often a severe malady with a fatal termination, but it exists in a great variety of forms and may be so mild as to escape attention; in such cases complete recovery is the rule. The name 'pre-kwashiorkor' has been suggested for the state of subclinical protein deficiency in children in which a failure in growth, a reduction in the serum albumin and an increased liability to diarrhoea and respiratory infections are the salient clinical features. There is good reason to believe that pre-kwashiorkor is extremely common in regions where the

fully developed syndrome is found. But even mild degrees of fatty degeneration of the liver which heal with slow absorption of the fat, may leave a liver peculiarly susceptible to infections and other poisons. This combination of factors may lead to cirrhosis of the liver in late adolescence or early adult life- a condition which is common in many parts of the tropics. Many children who suffer from the disease pass through their formative years with their physical and mental faculties partially impaired by their inadequate diet. The importance of this in the educational and physical development of Africans is not yet fully appreciated.....

Prevention

"This obviously depends upon eliminating the aetiological factors. Education in nutrition, the introduction of improved farming methods, including irrigation, and the development of food industries whereby protective foods may be made available in large towns at a reasonable cost to the consumer, are all important. In each country careful thought must be given to the provision of protein-rich foods made from local crops which are suitable both for infant feeding and for supplementing diets low in protein.

'Such local research has been supported on a world-wide basis since 1957 by a generous grant made by the Rockefeller Foundation. This grant is administered by the Committee on Protein Malnutrition of the

Food and Nutrition Board of the United States. Its research programme is concerned with the identification, production and utilisation of locally grown protein-rich foods of both vegetable and animal origin.....

"Concentrates of vegetable protein made from oil-seed cakes, when combined with easily digestible sugars or cereal flours, have been found to be satisfactory substitutes for milk in the diet of infants. Flours made from pulses, suitably prepared to eliminate antimetabolites or toxic factors, can also improve the biological value of such mixtures. By mixing one vegetable product with another, deficiencies of amino acids in single proteins can be made good. For instance, sesame flour, which has a low content of lysine but ample amounts of methionine and tryptophan, can be blended with bean flour, which is rich in lysine, to make a mixture of high biological value. Most of the edible pulses have a high lysine content and so make good supplements to cereals such as wheat, sorghum and the millets.

"Even small amounts of foods of animal origin such as dried milk or concentrates of fish protein, which are not readily available and are expensive in countries where kwashiorkor is prevalent, are of great value when mixed with high-protein vegetable foods. A mixture of one part of dried skimmed milk with three parts of groundnut flour, or one part of casein mixed with ten parts of groundnut flour, is as effective in the prevention and treatment of kwashiorkor as dried skimmed milk alone.

The first is prepared by Cow & Gate in Nigeria and marketed as Arlac. The basic research which made the production of this product possible and established its nutritional and therapeutic value was carried out by the Nutrition Unit of the Nigerian Federal Ministry of Health and the Northern Nigerian Ministries of Health and of Trade and Industry. The second product, called Amama, is prepared and marketed in several African countries by Glaxo. The United Nations agencies, WHO, FAO and UNICEF, advised by a panel of nutritionists drawn from different parts of the world (the Protein Advisory Group), have assisted many countries to develop the local production of protein-rich foods suitable for the prevention and treatment of kwashiorkor. Examples are Saridele prepared from soya beans and sesame in Indonesia, fish-flour and sorghum in Senegal, and a mixture of cotton-seed flour with maize and sorghum in Central America (Incaparina: the name is derived from the initials of the Institute of Nutrition for Central America and Panama). Another very satisfactory but more expensive product has been prepared on a commercial scale in England from soya beans and chickpeas (Cicer arietinum), to which are added small amounts of methionine and vitamin B_{12}.

"Concerted action by research units and foundations, government departments, international agencies and commercial interests, for promoting the production of such protein-rich foods, coupled with the

nutritional education of all classes of the populations of underdeveloped countries, is essential if this world-wide cause of morbidity and mortality is to be eliminated."

Many special diets have been devised to cope with protein deficiencies in children. Srikantia and Copalan (24), members of the staff of the Nutrition Research Laboratories, Indian Council of Medical Research, have described a fish protein concentrate in the treatment of kwashiorkor as follows:

"Skim milk has been used extensively in the treatment of kwashiorkor and found satisfactory. Diets based upon vegetable sources of protein have also initiated satisfactory clinical cure although they have been found inferior in their ability to regenerate serum albumin. On the other hand, blends of vegetable protein and milk have been reported to compare favorably with milk alone, from both a clinical and biochemical standpoint. Fish offers an inexpensive source of large amounts of protein. The long coast line and the numerous inland rivers in India offer considerable possibilities for the production and utilization of fish as a major source of protein in the prevention and control of protein malnutrition. Recently several processes have been developed to produce deodorized, defatted fish protein concentrates. Although some studies on experimental animals have shown that the nutritive value of fish flours may vary widely, depending upon the species of fish used and the

processing procedure employed, it is possible to obtain samples high in protein.....

The authors summarize their study as follows:

"Fifty-seven children suffering from kwashiorkor were hospitalized and given diets in which the major source of protein was from either fish protein concentrate (FPC) or skim milk. Diets containing fish protein concentrates were not well accepted by a large proportion of the children. In those who did consume it, both the clinical and biochemical responses compared favorably with those obtained in children receiving the diets containing skim milk, except for the increase in body weight after the disappearance of edema. Analysis of the cooked diets showed that the amounts of available lysine were lower in the FPC diets than in the skim milk diets. However, supplementation studies, using pure L-lysine, suggested that the lower gain in body weight could not be ascribed to this factor.

"Fish flour does not appear to be a satisfactory substitute for skim milk in the treatment of kwashiorkor, not only because of its non-acceptance by a large number of children but also because of its inability to promote body weight gains."

EXERCISE IN RELATION TO NITROGEN EXCRETION

Although Brooks (8), cited earlier, reported that, during a restraint period imposed on primates, urinary excretion of nitrogen was significantly higher than during pre-restraint. She found, on the other hand, that animals carrying on regular exercise exhibited no significant change in excretion of urinary nitrogen.

Salter (25) reported with human subjects that, within fairly wide limits, variations in muscular activity did not affect the nitrogen balance or protein requirement. When exercise was increased suddenly to the point where the individual remained in a chronic state of severe fatigue, however, nitrogen losses occurred that could not be compensated for by alterations in the caloric intake. An increase in activity, on the other hand, increased nitrogen excretion only when the carbohydrate and fat consumption were insufficient to cover the increased caloric expenditure. Permanent increase in muscular activity was found to be accompanied by a hypertrophy of the body musculature. This change usually was found to increase the amount of nitrogen retained if there was an increase in protein consumption.

Yoshimura and Yamaji (26) made a study of protein metabolism in students who were subjected to long continuing heavy exercise. They found that a positive protein balance developed, although the amount of

protein consumed was held constant and at the level optimum for an individual in a resting state. However, the calories were raised to meet the increased energy expenditure. After 10 days, while nitrogen balance of the experimental subject became positive, anemia and hypoproteinemia appeared. This was due to an increased state of erythrocyte destruction, and was accompanied by acceleration of red cell formation in the bone marrow. However, the anemia disappeared spontaneously after two to three weeks, and did not reappear in the subjects who had been trained for heavy exercise. Excess protein in the diet also prevented the subjects from having the anemia and hypoproteinemia due to muscular exercise.

Lack of any type or degree of exercise was studied in five healthy adult males by Dozier, Chen, and Mack (27). The men were kept immobilized for 30 days in bed and were not allowed to raise their heads from their one pillow throughout the period, with all of their physical needs cared for by orderlies and dietitians.

It was observed in this study that immobilization produced a definite increase in urinary nitrogen excretion when compared with the excretion during an ambulatory control period during which the men were eating the same diet and were engaging in moderate exercise.

All five subjects had significantly higher urinary nitrogen excretion during the immobilization period than in the control period of

ambulation and moderate exercise immediately preceding it. The average daily urinary nitrogen excretion for these two periods were 18.77 and 15.93 grams, respectively.

It also was observed by these investigators that, during the recovery period following immobilization, all five subjects exhibited a sharp decrease in nitrogen excretion. During this 30-day recovery period, the nitrogen excretion fell to an average of 13.40 grams per day.

NITROGEN NUTRITION IN RELATION TO INJURY, SURGERY, AND CONVALESCENCE

The immediate need for protein in cases of severe disease can be met by giving repeated transfusions of plasma and of whole blood. If the disease responds satisfactorily, the nutritional state of the patient will respond to special feeding methods (28).

Cuthbertson (29) in 1930 was the first to show that injury is followed by an increased excretion of urinary nitrogen due to a breakdown of proteins throughout the body. The resultant negative nitrogen balance is usually of the order of 12 to 15 grams/day, corresponding to a loss of 350 to 450 grams of muscle and other lean tissues.

A subject also may go into negative nitrogen balance following surgery. This rate of loss persists for 2 to 3 days and then decreases, provided convalescence is proceeding satisfactorily. A negative

nitrogen balance may, however, persist for as long as a week, even in cases in which the operation has been entirely satisfactory. Potassium loss from the cells also occurs and is often higher than the corresponding nitrogen losses.

The cause of this tissue breakdown in response to injury or surgery is almost certainly due to an increased secretion of cortical hormones by the adrenal glands. This is part of the normal physiological response to stress and is mediated through the pituitary gland and increased secretion of ACTH. It is not possible to prevent this loss of tissue by dietary measures and, in so far as it seems to be a physiological response, there is little justification for attempting to do so. However, the loss must be made good when convalescence is established.

In assessing the nutritional state of persons who have had severe injuries or have undergone major surgical operations, the measurement of body weight is the most useful single test, according to these authors. Balances are now available which enable patients who are seriously ill to be weighed with a minimum of disturbance. These are being increasingly used in surgical clinics. The interpretation of changes in weight is, however, not always easy. Loss of tissues may be masked by the accumulation of oedema.

Protein deficiency is undoubtedly the most important nutritional deficiency in surgical practice. The level of serum albumin is a useful index of the state of protein repletion. The level in the serum is in part determined by the state of hydration. Thus dehydration leads to a reduction in plasma volume, and in consequence the level of albumin may be within normal limits, despite the fact that the total quantity of serum albumin may be greatly reduced. The use of the haematocrit and the measurement of the total blood volume may help in assessing these changes.

RELATIONSHIP OF HORMONES TO NITROGEN EXCRETION

Numerous authors have stated that there is a relationship between steroids and nitrogen excretion. One author, Chen (30) carried out a study on the excretion of urinary 17-hydroxycorticosteroids by the same four primates as were used in the author's study described in this dissertation. It is not known whether or not there was a cause and effect relationship between nitrogen excretion and excretion of 17-hydroxycorticoids. Nevertheless, both substances were similarly affected by restraint, with the following results emanating from the latter study, as described by the author of this study:

"The urinary 17-hydroxycorticosteroids which were isolated from the primate urine come from adrenal glands. This hormone is

concerned principally with the intermediary metabolism of protein, carbohydrate, and fat. It also has been reported that this hormone may have some effect on certain vitamins and minerals, although the exact effects are not settled.

"Four healthy male primates of the Macaca nemestrina strain participated in a restraint study which included a 54-day pre-restraint period, an 11-day restraint period, and a 32-day post-restraint period. At the end of the bed rest period, two of the four primates were sacrificed in order to dissect out their bones for the analysis of radioactive ^{45}Ca. During the post-bed rest period, the remaining two animals were kept in their metabolism cages.

"One of the interesting findings of this study was the fact that the amounts of 17-hydroxycorticosteroids excreted in the urine varied from primate to primate, although certain trends were found with all of the animals.

"During the pre-restraint period, the overall average excretion of total urinary 17-hydroxycorticosteroids among all four primates was 1.72 mg./24 hours.

"The overall average of total urinary 17-hydroxycorticosteroids excretion during restraint among the four primates was 5.23 mg./24 hours. This increase was highly significant for all four primates.

"During the post-restraint period only two animals remained in the study, and their average 17-hydroxycorticosteroids excretion was 1.72 mg./24 hours. This value was not significantly different from that obtained in the pre-restraint periods of the same animals.

"On the other hand, a statistically significant decrease was found during the post-restraint period as compared with the value obtained during the restraint period for the same animals."

RELATIONSHIP BETWEEN EXCRETION OF CREATINE, CREATININE, AND NITROGEN IN THE URINE

Another investigator, Varia (31) made daily tests on the urine for creatine and creatinine excreted by the same primates as those which featured in the study covered in this dissertation. The following findings are reported by this investigator:

"The results indicated that, in all four primates, daily creatine excretion during Pre-Restraint was surpassed by the Restraint Period by a difference which was highly significant ($P < 0.001$). During the Pre-Restraint Period, the mean creatine excretion among all four primates was 30 milligrams per day, and during the Restraint Period, it was 318 milligrams per day. During the Post-Restraint Period only two primates

remained in the study, which excreted increased amounts of creatine as compared with the Pre-Restraint phase of the study.

"Both of these primates had very little activity when they were returned from their couches to their metabolism cages. This fact would provide the reason why both primates excreted higher amounts of creatine during the Post-Restraint Period as compared to the Pre-Restraint Period.

"With regard to creatinine excretion, there was not any statistically significant change among all four primates in any of the different periods of the study."

Dozier, Chan, and Mack (32) carried out an experiment on the excretion of creatine and creatinine by five healthy adult male subjects during immobilization and ambulation. The subject who had the greatest excretion of urinary nitrogen during immobilization also experienced a greater excretion of urinary creatinine than did any of the other three subjects, with no increased excretion of creatinine by the other three subjects. Nitrogen excretion was increased in the urine to some extent in all subjects.

A statistically significant rise in the excretion of creatine in the subjects during the Bed Rest Period was found. It also was apparent that, during the Recovery Period, the excretion of urinary creatine went

back to normal excretion levels. This agrees with the general concept that creatine is not excreted in significant amounts in the urine of normal adult males.

A study was conducted by Umapathy (33) on daily urinary creatine and creatinine excretion values in one study with human subjects, and in one study with primates.

The human study consisted of two 14-day Bed Rest Periods with and without planned isometric and isotonic exercises. Four adult male subjects participated in the first Bed Rest unit and two of these subjects remained for the second Bed Rest Period. The exercises were repeated four times a day during Bed Rest 2.

The results showed that three of four subjects excreted significantly higher amounts of creatine during bed rest with no exercise than during the equilibration period. Although there was a considerable decline in the excretion of creatine following bed rest, the decrease was not significant for any of the subjects. On the other hand, creatinine excretion values exhibited less fluctuation from one period to another for each of the subjects.

The study of this series which was conducted on six primates of the species Macaca Nemestrina, time was divided equally into three 14-day periods: equilibration, experimental, and recovery. Two

monkeys served as controls, two were restrained without activity, and two were forced to exercise for one hour each day. Following the equilibration period of normal cage activity, two primates were immobilized in specially designed restraint couches and the second group was forced to exercise for one hour a day during this period. In the recovery period all of the primates were allowed to have normal cage activity.

The results indicated that, during the equilibration period as well as the recovery period, there generally were no statistically significant differences between the three groups of monkeys with regard to the quantity of creatine excreted. As expected, the restrained monkeys excreted more creatine during the experimental (restraint) period than did the other two groups of monkeys ($P < 0.001$ in each comparison).

During the Exercise Period, the exercised animals excreted less creatine than during the Pre-Exercise or the Post-Exercise Periods. The difference in the first instance was statistically significant when the data for both primates in this group were pooled.

With regard to urinary creatinine excretion, the exercised animals excreted less creatinine during the Exercise Period than during the Pre-Exercise phase of the study, although the differences were not statistically significant. The same primates during the Exercise Period excreted far more creatinine than during the Post-Restraint Period, probably

because they were observed to exercise voluntarily in their cages for protracted periods of time after the supervised Excercise Period had ended.

The control animals did not excrete markedly different levels of creatine or creatinine during the various periods of the primate study.

The results of the above studies indicate that a suitable exercise program could prevent some of the undesirable physiologic and biologic changes that might occur during long space ventures.

SUMMARY

The purpose of this study was to determine the physiological effects of immobilization on the amount of urinary and fecal nitrogen excreted by four healthy male primates of the Macaca nemestrina strain.

This experiment took 97 days, divided between the Pre-Restraint Period 54 days, the Restraint Period 11 days, and the Post-Restraint Period 32 days.

During the Post-Restraint Period, two of the four primates were sacrificed so that the bones could be analyzed for radioactive Ca^{45} by another investigator. During the Post-Restraint Period, the remaining two animals were kept in their metabolism cages.

The study as a whole includes the quality and quantity of protein provided in the diet of the primates, biochemical tests for the nitrogen excretion in urine and feces of the primates, and a statistical comparison of urinary, fecal, and total nitrogen excretion between pairs of the different exploratory periods for the four primates.

The results showed that these primates excreted the highest amount of urinary nitrogen and total nitrogen during the Restraint Period, with the lowest amount during the Pre-Restraint Period.

Because of the author's great interest in nitrogen and protein metabolism in her own country, Egypt, she has reviewed the subject of nitrogen in relation to the health of populations, particularly of children, with especial emphasis on kwashiorkor, a severe protein deficiency disease common to some extent in Egypt and to a greater extent in other African countries. She also has reviewed work which has been done on inexpensive dietary sources of protein, on exercise in relation to nitrogen retention, on nitrogen nutrition in relation to injury, surgery, and convalescence, on the relation of nitrogen excretion to hormone excretion, and on the relation of urinary nitrogen excretion to the excretion of creatine and creatinine.

REFERENCES CITED

1. Cantarow, A., and B. Schepartz, Biochemistry, Third Edition, W. B. Saunders Company, Philadelphia (1962)

2. Murlin, J. R., A. D. Hayes, and K. Johnson, Correlation between the Biological Value of Protein and the Percentage of Creatinine Nitrogen in the Urine, Journal of Nutrition, 51:149 (1953)

3. Harris, Robert S., Joan R. Moor, and Robert L. Wanner, Calcium Metabolism of the Normal Rhesus Monkey, American Journal of Clinical Investigation, 40:1766-1774 (1961)

4. Wanner, R. L., J. R. Moor, F. Bronner, N. S. Pearson, and R. S. Harris, Species Differences in the Excretion of Intravenously Injected Calcium^{45}, Federation Proceedings, 15:575 (1956)

5. Bock, J. D., F. Horszczaruk, and L. Angelowa, Nitrogen Metabolism of Growing Albino Rats during a Protein-Free Diet, Archiv Tierernaehrung, 14(13):157-165 (1964)

6. Allison, James B., and Robert W. Wannemacher, Jr., The Concept and Significance of Labile and Over-all Protein Reserves of the Body, American Journal of Clinical Nutrition, 16:445-462 (1965)

7. Van Zandt, Dorothy Perkins, The Effect of a New Type Restraint Garment on the Bone Mass and the Calcium, Phosphorus, and Nitrogen Metabolism of Macaca Nemestrina Primates, Master's Thesis, The Texas Woman's University (August, 1967)

8. Brooks, Paula Denise, A Study of Restraint and of Programmed Exercise on Bone Density and Metabolism of Macaca Nemestrina Primates, Master's Thesis, The Texas Woman's University (August, 1967)

9. Hoffman, Rudolf A., Elsa A. Dozier, Pauline Beery Mack, William N. Hood, and Marshall W. Parrott, Physiologic and Metabolic Changes in Macaca Nemestrina Primates on Two Types of Diets During Thirty-five Days of Restraint Followed by Thirty-five Days of Non-Restraint. I. Body Weight Changes, Food Consumption, and Urinary Excretion of Nitrogen, Creatine, and Creatinine, Aerospace Medicine, 39:693 (1968)

10. Sokaloverova, I. M., Spontaneous Diabetes Mellitus in a Monkey. A Chapter in Theoretical and Practical Problems of Medicine and Biology in Experiments on Monkeys, Edited by I. A. Utkin, Pergamon Press, New York (1960)

11. Varner, Charlene L. M., A Comparison of the Mineral Content of Bones in Macaca Nemestrina Primates as Determined by Radiographic Bone Density and Chemical Analytic Methods, Unpublished Doctor's Dissertation, College of Household Arts and Sciences, Texas Woman's University (1966)

12. Al-Shawi, Aliya N., A Study of the Effects of Diet and Restraint on the Bone Mass in Seventeen Anatomical Sites of Primates (Macaca Nemestrina), Unpublished Doctor's Dissertation, College of Household Arts and Sciences, Texas Woman's University (1966)

13. Standard Method of Clinical Chemistry, Volume II, page 91, Academic Press (1958)

14. De Costa, G. et al, Dietary Nitrogen Excretion, British Medical Journal, 2:1493 (1965)

15. Arrayane, Guillermo, Estimation of Body Protein Deficit in Protein-malnourished Populations by Creatinine Excretion Measurements, Proceedings of the Symposium on Protein Nutrition Metabolism, 1962, 47-55 (Published 1963)

16. Blondell, N., S. Goodman, F. Del Greco, and N. M. Simon, Urea Production and Nitrogen Balance in Chronic Arotemia, Journal of Laboratory and Clinical Medicine, 64:842 (1964)

17. Deshpande, P. D., A. E. Harper, and C. A. Elvehjem, Amino Acid Imbalance on Low Fibrin Diets, Journal of Biological Chemistry, 230:327 (1958)

18. Mack, Pauline Beery, George P. Vose, Conrad L. Kinard, and Helen Buck Campbell, Effects of Lysine-Supplemented Diets on Growth and Skeletal Status of Preadolescent Children, American Journal of Clinical Nutrition, 11:255 (1962)

19. Sanderlich, H. E., Amino Acid Imbalance as Related to Methionine, Isoleucine, and Tryptophan Requirements of the Rat or Mouse, Journal of Nutrition, 59:353 (1956)

20. Orten, A. U., and J. M. Orten, The Role of Dietary Protein in Hemoglobin Formation and Anemia Correction in Experiments with Animals, Journal of Nutrition, 26:21 (1946); and ibid, 30:137 (1945)

21. Walton, and Benjamin Fingerhut, Determination of Nitrogen with the Diacetyl Method and an Automatic Dialyzing Apparatus, American Journal of Clinical Pathology, Volume 28, page 681 (1957)

22. L'vova, V. V., and I. K. Koryakina, Autointoxication in the Nitrogen Balance Alteration Following Thermal Burns in Dogs, Byulletin Eksperimental 'noi Biologii i Meditsiny (Bulletin of Experimental Biology and Medicine), 58(7), 36-41 (1964)

23. Davidson, Sir Stanley, R. Passmore, and Lord Boyd Orr, Human Nutrition and Dietetics, Chapter 28, page 399, The Williams and Wilkins Company, Baltimore (1963)

24. Srikantia, S. G., and C. Copalan, Fish Protein Concentrates in the Treatment of Kwashiorkor, American Journal of Clinical Nutrition, Volume 18, page 34 (1968)

25. Salter, James M., Protein Metabolism, Chapter 1 in Diseases of Metabolism, Edited by Garfield G. Duncan, W. B. Saunders Company, Philadelphia (1964)

26. Yoshimura, H., Federation Proceedings, 20:103 (1961)

27. Dozier, Elsa Arciniegas, Lance Ya-Nan Chen, and Pauline Beery Mack, Urinary Nitrogen Excretion during a 30-Day Bed Rest Period by Five Healthy Adult Males in Comparison with that during Ambulation (in publication)

28. Davidson, Sir Stanley, R. Passmore, and Lord Boyd Orr, Human Nutrition and Dietetics, Chapter 47, page 673, The Williams and Wilkins Company, Baltimore (1963)

29. Cuthbertson, David Paton, The Influence of Prolonged Muscular Rest on Metabolism, Biochemical Journal, 23:1328 (1929)

30. Chen, Dorothy Daw-Bing, Urinary 17-Hydroxycorticosteroid Excretion by Four Macaca Nemestrina Primates as Influenced by Conditions of Stress and Immobilization, A Thesis in Nutrition of the Texas Woman's University (1968)

31. Varia, Ila, A Study on Urinary Creatine and Creatinine Excretion in Four Macaca Nemestrina Primates during Restraint and Non-Restraint Conditions, A Thesis in Nutrition of the Texas Woman's University (1968)

32. Dozier, Elsa Arciniegas, Shiu-Shiang Chan, and Pauline Beery Mack, Excretion of Creatine and Creatinine by Healthy Adult Human Males during Immobilization and Ambulation (in publication)

33. Umapathy, Padma Kaparthi, Effect of Immobilization on Urinary Excretion of Creatine and Creatinine, A Dissertation submitted in Partial Fulfillment of the Requirements for the Ph.D. in Nutrition at the Texas Woman's University (1967)

A P P E N D I X

TABLE I

URINARY, FECAL, AND TOTAL NITROGEN EXCRETED BY PRIMATE 239

DURING THE INVESTIGATION

(Milligrams per 24 Hours)

8	2080	421	2501	23	2773	34	2807
9	2660	451	3111	24	3400	83	3483
10	2682	512	3194	25	4070	83	4153
11	2100	257	2357	26	3087	230	3317
12	5425	476	5901	27	4030	204	4234
13	2320	220	2540	28	2448	294	2742
14	2728	234	2962	29	1863	978	2841
15	1961	235	2196	30	X	259	259
16	2356	518	2874	31	3936	393	4329
17	2315	380	2695	June 1	5640	342	5982
18	2320	318	2638	2	3336	251	3587
19	2430	264	2694	3	2120	212	2332
20	2495	362	2857	4	1650	357	2007
21	2160	303	2463	5	2058	542	2600
22	1100	396	1496	6	3400	360	3760
23	3162	335	3497	7	3600	238	3838
24	2964	272	3236	8	4590	250	4840
25	2610	432	3042	9	2475	234	2709
26	5655	139	5794	10	1712	456	2168
27	1771	160	1931				
28	2409	155	2564				
Mean	2491	444	2935	Mean	2648	237	2885

TABLE II

URINARY, FECAL, AND TOTAL NITROGEN EXCRETED BY PRIMATE 249 DURING THE INVESTIGATION

(Milligrams per 24 Hours)

19	4453	318	4771
20	1952	434	2386
21	1250	359	1609
22	2345	426	2771
23	2257	380	2637
24	2465	279	2744
25	2546	358	2904
26	2614	334	2948
27	2295	347	2642
28	2356	248	2604
29	2854	343	3197
30	2833	317	3150
31	2718	231	2949
April 1	2614	397	3011
2	2650	330	2980
3	2754	299	3053
4	2440	428	2868
5	2430	249	2679
6	2496	269	2765
7	2964	288	3252
8	2220	287	2507
9	3432	182	3614
10	1725	347	2072
11	2376	234	2610

25	2645	339	2984
26	3375	168	3543
27	2673	167	2840
28	2777	185	2962
Mean	2728	283	3011

RESTRAINT PERIOD

Date (1968)	Urinary	Fecal	Total
April 29	825	141	966
30	3012	118	3130
May 1	4317	871	5188
2	1137	178	1315
3	3465	212	3677
4	478	26	504
5	3937	164	4101
6	3120	32	3152
7	4122	32	4154
8	3783	X	3783
9	2957	203	3160
Mean	2887	179	3066

TABLE III

30	2250	564	2814	14	2035	301	2336
31	2268	368	2636	15	2295	215	2510
April 1	2204	498	2702	16	1860	112	1972
2	1590	299	1889	17	1870	136	2006
3	2201	484	2685	18	1456	268	1724
4	2296	523	2819	19	963	233	1196
5	2632	386	3018	20	2343	209	2552
6	2686	382	3068	21	891	200	1091
7	2438	415	2853	22	1045	114	1159
8	1762	444	2206	23	1650	253	1903
9	1168	232	1400	24	1127	106	1233
10	2065	536	2601	25	1705	427	2132
11	2656	288	2944	26	1567	210	1777
12	2480	332	2812	27	1624	163	1787
13	1095	297	1392	28	1788	216	2004
14	1870	237	2107	29	1512	204	1716
15	2160	137	2297	30	1612	332	1944
16	2304	158	2462	31	1475	412	1887
17	1746	319	2065	June 1	1620	219	1839
18	1526	249	1775	2	1192	244	1436
19	2397	214	2611	3	1112	278	1390
20	2100	350	2450	4	1040	246	1286
21	2187	149	2336	5	1520	199	1719
22	1759	216	1975	6	1995	194	2189
23	2252	236	2488	7	1155	237	1392
24	2349	284	2633	8	1330	44	1374
25	2282	244	2526	9	1100	85	1185
26	2612	125	2737	10	870	111	981
27	2362	133	2495				
28	2442	195	2637				
Mean	1963	354	2317	Mean	1623	210	1833

TABLE IV

URINARY, FECAL, AND TOTAL NITROGEN EXCRETED BY PRIMATE 423 DURING THE INVESTIGATION

(Milligrams per 24 Hr.)

17	1595	266	1861
18	2107	296	2403
19	3036	198	3234
20	2072	424	2496
21	2322	250	2572
22	2037	551	2588
23	2130	226	2356
24	2035	403	2438
25	1100	130	1230
26	2382	306	2688
27	3060	323	3383
28	1533	193	1726
29	1147	139	1286
30	3485	348	3833
31	2082	266	2348
April 1	2090	233	2323
2	1035	278	1313
3	3488	199	3687
4	4026	344	4370
5	2560	231	2791
6	2352	381	2733
7	2552	320	2872
8	2092	250	2342
9	2115	223	2338
10	2009	223	2232
11	2307	208	2515
23	2350	263	2613
24	2229	120	2349
25	2772	153	2925
26	2139	230	2369
27	2450	119	2569
28	2160	57	2217
Mean	2283	246	2529

RESTRAINT PERIOD

Date (1968)	Urinary	Fecal	Total
April 29	1886	337	2223
30	6732	X	6732
May 1	4422	X	4422
2	3062	38	3100
3	3595	103	3698
4	4097	38	4135
5	4000	104	4104
6	4845	188	5033
7	4054	165	4219
8	3314	151	3465
9	4650	217	4867
Mean	4060	122	4182

TABLE V

STATISTICAL COMPARISONS OF URINARY NITROGEN EXCRETION BETWEEN PAIRS FOR DIFFERENT PERIODS OF THE STUDY FOR THE FOUR PRIMATES IN THE INVESTIGATION

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Primate 239				
Pre-Restraint	2491	1146	2.0143	$P < 0.05$
Restraint	3302	1319		
Pre-Restraint	2491	1146	0.5037	N.S.
Post-Restraint	2648	1664		
Restraint	3302	1319	1.1298	N.S.
Post-Restraint	2648	1664		
Primate 249				
Pre-Restraint	2727	946	0.3007	N.S.
Restraint	2832	1313		
Primate 419				
Pre-Restraint	1965	615	6.9402	$P < 0.001$
Restraint	3756	1204		
Pre-Restraint	1965	615	2.4845	$P < 0.02$
Post-Restraint	1623	582		
Restraint	3756	1204	7.2909	$P < 0.001$
Post-Restraint	1623	582		
Primate 423				
Pre-Restraint	2283	532	7.5651	$P < 0.001$
Restraint	4060	1157		

TABLE VI

STATISTICAL COMPARISONS OF FECAL NITROGEN EXCRETION BETWEEN PAIRS FOR DIFFERENT PERIODS OF THE STUDY FOR THE FOUR PRIMATES IN THE INVESTIGATION

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Primate 239				
Pre-Restraint	444	177	2.7026	P< 0.01
Restraint	285	152		
Pre-Restraint	444	177	4.8528	P< 0.001
Post-Restraint	237	202		
Restraint	285	152	0.6837	N.S.
Post-Restraint	237	202		
Primate 249				
Pre-Restraint	283	72	2.5704	P< 0.02
Restraint	180	231		
Primate 419				
Pre-Restraint	354	147	3.1407	P< 0.01
Restraint	202	118		
Pre-Restraint	354	147	4.9447	P< 0.001
Post-Restraint	210	87		
Restraint	202	118	0.2339	N.S.
Post-Restraint	210	87		
Primate 423				
Pre-Restraint	246	97	3.7369	P< 0.001
Restraint	122	99		

TABLE VII

STATISTICAL COMPARISONS OF TOTAL NITROGEN EXCRETION BETWEEN PAIRS FOR DIFFERENT PERIODS OF THE STUDY FOR THE FOUR PRIMATES IN THE INVESTIGATION

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Primate 239				
Pre-Restraint	2935	1183	1.6648	P< 0.10
Restraint	3578	1291		
Pre-Restraint	2935	1183	0.1552	N.S.
Post-Restraint	2885	1728		
Restraint	3578	1291	1.7142	P< 0.10
Post-Restraint	2885	1728		
Primate 249				
Pre-Restraint	3010	938	0.0012	N.S.
Restraint	3012	1404		
Primate 419				
Pre-Restraint	2321	596	6.4590	P< 0.001
Restraint	3958	1200		
Pre-Restraint	2321	596	3.5711	P< 0.001
Post-Restraint	1833	602		
Restraint	3958	1200	7.1774	P< 0.001
Post-Restraint	1833	602		
Primate 423				
Pre-Restraint	2529	543	7.1077	P< 0.001
Restraint	4182	1108		