A STUDY ON URINARY CREATINE AND CREATININE EXCRETION ON FOUR MACACA NEMESTRINA PRIMATES DURING RESTRAINT AND NON-RESTRAINT CONDITIONS

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We hereby recommend that the thesis prepared under our supervision by Ila Varia entitled A STUDY ON URINARY CREATINE AND CREATININE EXCRETION ON FOUR MACACA NEMESTRINA PRIMATES DURING RESTRAINT AND NON-RESTRAINT CONDITIONS

be accepted as fulfilling this part of the requirements for the Degree of Master of Science.

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INTRODUCTION

Man's desire to enter space in a specially designed capsule has presented countless problems. One area of primary concern to man has been the effect of weightlessness on the space crew exposed to this new environment. The physiological effects which have been attributed to such exposure include anorexia, nausea, disorientation, fatigue, urinary retention, diuresis, muscular incoordination, muscular atrophy, and demineralization of bone (1). A great deal of research has been done in order to prevent damage to the astronauts in long flights through space. Deviations from the normal physiological responses of the body may reflect the alteration of functions which occur. Therefore, the measurements of the urinary levels of certain metabolic excretions under condition simulating space travel are warranted.

This report describes an investigation conducted at Texas Woman's University Research Institute, in which four healthy male primates of the Macaca nemestrina type were used to determine the urinary excretion of creatine and creatinine during periods of equilibration, immobilization, and ambulation. The primate was selected by the National Aeronautics and Space Administration for bone density and metabolic studies to support the more limited measurements which can be made on man. This investigation was sponsored by the National Aeronautics and Space Administration (NASA).

Some of the physiological alterations resulting from space travel have been attributed to the confinement in the space capsule, resulting in the inability to move about in a normal manner. Increased urinary excretion of creatine has been observed during periods of immobilization (2). Immobility is associated with a decrease in muscle mass with a resulting increase of creatine in the urine. Whereas, under normal physiological conditions, creatine is converted to creatinine prior to excretion, the same is not true under conditions of inactivity.

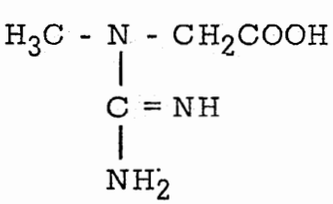
The specific objectives of the present study are as follows:

1. To make chemical analyses of the urine of four primates throughout the study to determine the daily excretion of creatine and creatinine during Pre-Bed Rest, Bed Rest, and Post-Bed Rest Periods.

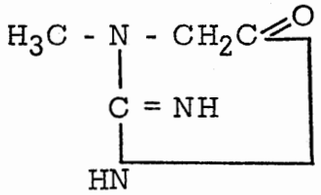
2. To analyze the data obtained for creatine and creatinine statistically in order to evaluate the effects of the various treatments on the urinary excretions.

REVIEW OF LITERATURE

Creatine is an essential physiological constituent of the body, whereas, creatinine is a waste product. Chemically, creatine is a methyl guanidoacetic acid and creatinine is its anhydride form. Structural formulas (2) are as follows:



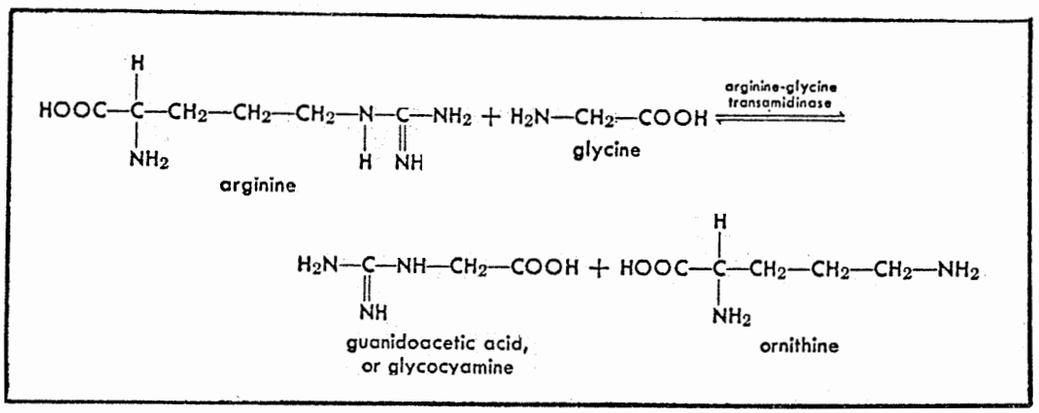
Creatine



Creatinine

Formation of Creatine

Transamination. The synthesis of creatine involves the amino acids- glycine, arginine, and methionine- which are referred to, also, as precursors of creatine (3). The initial reaction in the formation of creatine is the transfer of the amidine group of arginine to glycine, forming guanidoacetic acid (4). This reaction proceeds in the following manner:

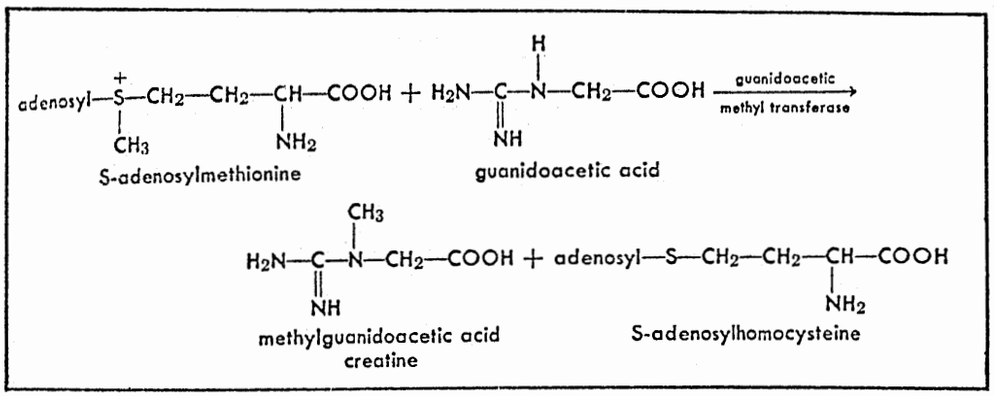


This process is reversible and proceeds without the addition of co-factors or adenosinetriphosphate. Transamination has been found in mammalian kidney and pancreas (5) (6), and in avian kidney and liver (3). The arginine-glycine transaminase activity of chick and duck liver is repressed by dietary creatine or guanidoacetate. Such repression may be of physiological significance, in that it leads to conservation of arginine, glycine, and methionine for other metabolic functions, including protein synthesis (7). Rabbits, deficient in vitamin E, exhibit very low kidney transaminase activity; and it appears that the reduction in enzyme activity is due to the feedback repression of transaminase produced by the excess creatine presented to the kidneys (8). Feedback repression of transaminase has been observed also in the liver of the developing chick embryo (9) (6).

Koszalka (10) injected guanidoacetic acid 2-C^{14}, or C^{14} labeled guanido-L-arginine monohydrochloride, in hepatectomized and hepatectomized-nephrectomized rats. The result with hepatectomized rats

indicated that both the pancreas and kidney synthesized significant amount of guanidoacetic acid in vivo, when labeled arginine was injected. Appreciable creatine synthesis, however, could be detected only in the pancreas. In contrast, creatine synthesis could be demonstrated in the kidneys, but not in the pancreas– when guanidoacetic acid 2-C^{14} was injected into hepatectomized rats. This suggested that guanidoacetate did not penetrate the cells of the pancreas. The importance of the pancreas in creatine synthesis was demonstrated in vitro using arginine C^{14}. It was found that the pancreas has about the same guanidoacetate methyltransferase activity as liver, which was five times that found in kidney.

Transmethylation. In the second stage of creatine synthesis, the net reaction is the transfer of a methyl group from methionine to guanidoacetic acid, forming creatine (11).

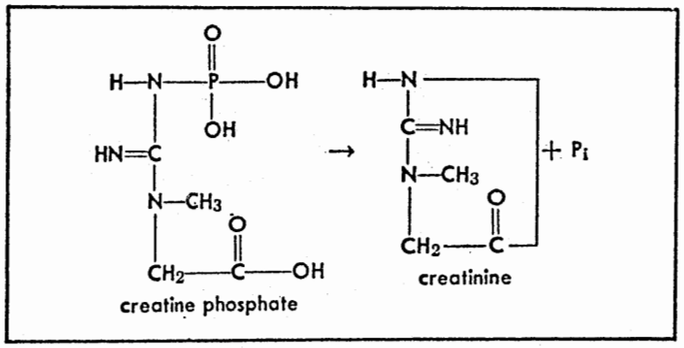


This complex process involves the conversion of methionine to "active methionine" or S-adenosylmethionine, following reaction with ATP in the

presence of the "methionine activation" enzyme. The guanidoacetic acid is methylated to creatine by S-adenosylmethionine (11). The enzyme that catalyzes this reaction is known as "guanidoacetic methylpherase" (12).

Formation of Creatinine

Phosphocreatine, formed in the muscle from creatine by reaction with adenosinetriphosphate, probably is the major source of urinary creatinine.



The formation of creatinine may occur non-enzymatically (13) or it may be enzyme catalyzed or both. Recent studies showed that incubation of rabbit muscle extract with creatine phosphate gave creatine, but not creatinine (14).

The administration of isotopic creatinine to rats resulted in the immediate excretion of most of the labeled material in the urine, with no isotope found in the body creatine. This study indicated that the conversion of creatinine to creatine does not occur biologically (15).

Urinary Excretion of Creatine and Creatinine

Creatine. Under normal conditions, creatine is excreted in the urine only to a slight extent. Prior to excretion, creatine phosphate is converted to creatinine, which is excreted via the kidneys.

The oral and parenteral administration of creatine to animals is followed by the excretion in the urine of only a fraction of the amount administered. A single dose of creatine caused no increase in urinary creatinine, although, after prolonged feeding of a large amount of creatine, the creatinine content of the urine was increased. Elevated urinary creatinine levels continued for several weeks after creatine ingestion was discontinued. Chanutin et al. (16) fed large amounts of creatine to rats and mice. They found that muscle creatine increased sharply the following day; after which, it remained practically constant. Liver and kidney creatine also increased when the creatine feeding stopped; the excess creatine rapidly disappeared from all tissue except the muscles, which retained excessive amounts for some time.

Creatinine. Creatinine always is present in the urine. It is an end product of creatine metabolism. Since most of the creatine of the body is located within skeletal muscle and since there is no evidence for enzymatic formation of creatinine (13), it is commonly assumed that urinary creatinine has its origin largely in skeletal muscle. The daily

output on a creatine-creatinine free diet is almost constant for a given individual (17).

The level of creatinine excretion is measured in terms of a 24-hour sample. In 1908, Shaffer (18) introduced the term "creatinine coefficient", which is used commonly to express the number of milligrams of creatinine nitrogen excreted per kilogram of body weight in 24 hours.

Effect of Diet

The effect of diet upon urinary creatinine has been controversial for more than 55 years. Folin (19) stimulated considerable interest in this subject when he pointed out that the amount of creatinine excreted in the urine by a given individual, receiving a meat-free diet, was a constant quantity; but, that it may be different for other individuals, and wholly independent of the quantitative changes in the total amount of nitrogen eliminated.

A series of experiments were carried out with adult rats to study the effect on creatinine excretion of variable daily intakes of protein and amino acid. The results indicated that the excretion of creatinine was not constant, but varied with the intake of protein and the content of amino acids. When the diet was composed of free amino acids, the lowest level of dietary nitrogen resulted in the highest daily creatinine excretion. The excretion was approximately three times as high as when the diet provided 15 per cent protein. On the other hand, the creatine

excretion decreased significantly when the amino acid deficient diets were fed. It was suggested that feeding free amino acids stimulated creatine synthesis more rapidly than did dietary protein (20).

Block and Schoenheimer (15), using N^{15} labeled creatine, were able to demonstrate conclusively that dietary creatine can be converted into creatinine. Despite this, however, other investigators have demonstrated that the rate of urinary creatinine excretion is relatively independent of the intake of dietary creatine. Massive doses of creatine were required to increase significantly the urinary creatinine excretion in dogs (21) and rabbits (22). Studies done in other laboratories help to explain these observations. Borsook and Dubnoff (13) demonstrated that phosphocreatine was the immediate precursor of creatinine. The studies of Walker (23) suggested that the regulation of creatine synthesis, in vivo, was governed by the level of creatine in the muscle.

Maw (24) demonstrated, in his experiments on rats, that at least 30 per cent of a dietary dose of creatinine was recovered in the urine. The work of Dinning et al. (25) with cattle indicated that dietary creatinine is independent of the nitrogen content of the diet. When studying five sheep on starvation, Ven Nieke et al. (26) found that creatinine output was 73 per cent lower on the average than during the pre-treatment period. These data suggested that the changes in the amount of creatinine excreted were, in part, the reflection of changes in the composition of lean body mass.

Creatine and Creatinine Metabolism in Muscular Disorders

Small amounts of creatine occur in the urine of the normal human adult. The presence of increased amounts of creatine in the urine is called "creatinuria". Creatinuria has been found associated with starvation, carbohydrate deprivation, diabetes, muscular dystrophy, hyperthyroidism, fevers, and malnutrition. In all these conditions, there is an increased catabolism of either muscular tissue or other tissue protein (17).

In muscular dystrophy, creatinuria results from a reduced rate of conversion of creatine to creatinine, rather than from an increased rate of creatine formation (27). The research of McGeer et al. (28) indicated that a significant degree of creatinuria occurred in dystrophic mice, when compared to the unaffected control group, if the dystrophic mice were less than two months old. The creatine to creatinine ratio of dystrophic mice seemed to decrease with age; whereas, unaffected mice showed no change.

Morgulis and Spencer (29) reported that, on the basis of their studies on rabbits during periods immediately prior to dystrophy, creatinine excretion remained practically constant while creatine excretion increased by leaps and bounds, and continued to rise during the period of progressive dystrophy.

Nutritional muscular dystrophy can be produced in experimental animals by feeding a diet deficient in vitamin E. Gerber et al. (30) injected creatine 2-C^{14} into the peritoneum of normal vitamin E deficient rats as well as those previously deficient who had been treated with α-tocopheryl acetate. They observed an increased ratio of specific activity of free creatine to muscle creatine in the vitamin E deficient animals. The increase in muscle creatine resulted in the excretion of excess creatine in urine, or creatinuria.

It has been demonstrated, also, that nutritional muscular dystrophy can be produced in experimental animals by feeding a diet deficient in choline (31) and vitamin C (32). This experimental muscular dystrophy was accompanied by creatinuria and by diminished excretion of creatinine.

Dinning and Day (33) reported that, on the basis of their studies on the Rhesus monkey, a large dose of folic acid would result in creatinuria. Before giving a large dose of folic acid to the monkey, the excretion of creatine was 34.7 milligrams per kilogram of body weight in 24 hours. After the administration of the folic acid, a very high excretion of creatine resulted. In one case, the creatine excretion reached a daily maximum of 140 milligrams per kilogram of body weight.

Creatinuria is common in hyperthyroidism. Grillo and Fossa (34), in studies on rats placed in a hyperthyroid status by a diet

containing 0.15 per cent propylthiouracil, or by a diet containing 2 per cent powdered thyroid, found normal levels of renal aminotransferase activity, which is necessary in the conversion of glycine to ornithine glucocyamine, or to creatine. It was further noted that, when the renal creatine level was above normal and the aminotransferase activity was below normal in these rats, the metabolic precursors for creatine synthesis were not altered by hyperthyroidism. The quantity of creatine formed by the hyperthyroid rat was less than was synthesized by normal rats. This suggested that the rate of creatine synthesis in hyperthyroidism is not increased, but rather that the retention of creatine by muscle is decreased, resulting in creatinuria. Wang (35) demonstrated the production of creatinuria in the rabbit by the injection of thyroxine.

Muscular Atrophy and Creatinuria Due to Disuse and Immobilization

Since immobilization alters various metabolic and physiologic functions of the body, it has been of interest to scientists to study these biological changes. Special concern regarding the effects of immobility has been aroused by man's efforts in space exploration.

Chor and Dolkart (36) studied the effects of immobilization in an experiment using six young Macaca Rhesus monkeys. A body and leg cast restrained these monkeys for periods from one to 10 weeks in duration. The leg that was not in the cast served as the control in each

case. These workers reported that an increased percentage of muscular atrophy was associated with an increase in the length of the period of immobility. Also, the atrophied muscle appeared paler than the muscle from the control limb.

It is well known that denervation of skeletal muscle is followed by marked changes in the weight, the chemical composition, and the physiological properties of the affected muscle. Hines and Knowlten (37) measured the creatine content of the muscles of 40 rats at periods of one day up to 28 days after denervation. One limb of the rat was denervated, while the opposite limb served as the control. Their results showed that the concentration of creatine in the denervated muscle was lower than in the control muscle within one week after denervation.

Reid (38) compared the effect of disuse and denervation on the gastrocnemius muscle of the cat. According to his report, muscular atrophy was more pronounced in denervated than in disused muscle in the cat.

Increased concentrations of creatine in the urine were observed during Bed Rest Periods, when compared with periods before or after Bed Rest, in studies conducted by Texas Woman's University Research Institute, which involved both human subjects (39) (40) (41) (2) and primates (2) (42).

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-Bed Rest (March 6 through April 28, 1968). The animals remained in their metabolism cages during this period.

2. Period of Bed Rest (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 another graduate student to dissect their bones for the analysis of active ^{45}Ca.

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 substracted from the daily intake. The animals were given water ad libitum. On the first day of bed rest 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.

PROCEDURE FOR DETERMINATION OF CREATINE AND CREATININE IN URINE

The method used for the collection, the storage, and the analytical procedures for creatine and creatinine was that which had been used in previous studies conducted in the Texas Woman's University Research Institute laboratory (39) (40) (41) (2).

ANALYTICAL PROCEDURE

Creatinine is determined easily by a colorimetric method based on a reaction which was first described by Jaffe in 1886. This reaction is based on the production of a red colored substance when creatinine solution is treated with picric acid and alkali. In 1904, Folin used this reaction for the quantitative determination of creatinine in urine (43). Creatine was determined by converting it to creatinine by the same reaction.

Numerous procedures have been devised for the measurement of creatinine, but it was found that not all of the methods are suitable for every application. The method of Folin has long been used as a routine procedure for the measurement of creatinine and creatine because of its speed and simplicity, even though it is well established that the color reaction is not specific for creatinine.

The method adopted for use in the TWU laboratory for the determination of creatinine and creatine in the urine is the modified Folin procedure as developed by Biggs and Cooper (44).

PREPARATION OF REAGENTS

1. Creatine standard solution (0.5797 mg./ml.) - 0.5797 grams of creatine anhydried were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored under refrigeration.

2. Creatinine standard solution (0.5 mg./ml.) - 0.5 grams of creatinine anhydried were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored under refrigeration.

3. Picric acid solution (0.057N) - 30 grams of crystalline picric acid were dissolved in 2000 milliliters of warm distilled water. After cooling to room temperature, this solution was refrigerated for 12 hours. The crystals which form are filtered out. In order to establish the normality, the filtrate was filtrated with standardized NaOH using phenolphthaline as an indicator. The solution then was stored at room temperature.

4. 2.5N NaOH solution - 100 grams of NaOH crystals were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored at room temperature.

DETERMINATION OF CREATININE

Into a clean 100 milliliter flask, pipette one milliliter of urine sample. To this add 10 milliliters of 0.057N picric acid and 1.5 milliliters of 2.5 NaOH. Allow the mixture to stand 10 minutes for color development. Add distilled water to volume and shake the flask well. The absorbancy of the solution is determined at 540 millimicrons.

DETERMINATION OF CREATINE

Into a clean 100 milliliter flask, pipette one milliliter of urine sample. To this add one milliliter of 0.057N picric acid, 65 milliliters distilled water, and two glass beads. The solution is boiled vigorously on an electric heater for a minimum of 40 minutes. The volume of the solution should never be reduced to less than 20 milliliters. Additional distilled water is added as needed. After the solutions are cooled to room temperature, nine milliliters of 0.057N picric acid and 1.5 milliliters of 2.5N NaOH are added. Allow the solution to stand 10 minutes for color development. Add distilled water to volume and shake the flask well, the absorbancy of the solution is determined at 540 millimicrons on the Coleman spectrophotometer.

Calibration Curve for Urinary Creatinine and Creatine

Since the colored compound resulting from Jaffe's reaction does not follow Beer's law strictly, it is necessary to plot a graph and

calculate results by reading from the graph. In order to construct a calibration curve, known amounts of the standard solutions are used to replace the urine samples; and determination procedures are applied to these standards.

A useful calibration curve either for creatinine or creatine can be plotted from the absorbance value obtained by using sample containing 0.5, 1.0, 2.0, 3.0 milligrams of creatine or creatinine, respectively. These amounts can be measured accurately by pipetting 1.0, 2.0, 4.0, and 6.0 milliliters of the respective solutions into volumetric flasks and proceeding with the determinations.

The concentration of urinary creatinine is obtained directly from the creatinine calibration curve. The creatine concentration is found by subtraction of the value for creatinine from the value for creatine and creatinine which is obtained from the converted creatine standard curve. This gives the creatine concentration in terms of creatinine and is converted to creatine concentration by multiplication with the correction factor 1.16.

$$\text{Creatine} = [(\text{Creatinine} + \text{Creatine}) - \text{Creatinine}] \times 1.16$$

RESULTS AND DISCUSSION

The data obtained on daily urinary excretion of creatine and creatinine for the primates in this study were analyzed statistically using the "t" test. The average daily excretion values for each primate during the various periods are given in Tables I and II (Appendix). Statistical comparisons for each primate between various periods are given in Tables III and IV (Appendix).

URINARY CREATINE EXCRETION

Primate 239

Table I, Part A gives the day by day excretion of creatine in urine during Pre-Bed Rest, Bed Rest, and Post-Bed Rest for Primate 239. As can be seen in Table I, Part A this primate excreted significantly higher amounts of creatine during Bed Rest than during the period of Pre-Bed Rest. The average daily excretion during the Bed Rest Period was 111 milligrams as compared to 31 milligrams per day during the period before Bed Rest. This difference was statistically significant ($P < 0.001$). See Table III.

Following the Bed Rest Period there was an increase in the amount of creatine excreted from 111 milligrams per day to 131 milligrams per day. This change in value showed no significance when analyzed statistically.

The average urinary creatine excretion by this primate increased from 31 milligrams per day during the Pre-Bed Rest Period to 131 milligrams during Post-Bed Rest. This change in value is highly significant ($P < 0.001$). In short, this primate had not returned to the normal status with respect to creatine excretion before the end of the study.

Primate 249

Table I, Part B gives day by day excretion of creatine in urine during the Pre-Bed Rest and Bed Rest Periods for Primate 249. This primate was sacrificed after the Bed Rest Period in order to have his bones for radioactive Ca^{45} by another graduate student.

The increase in creatine excretion by this primate from 51 milligrams per day during Pre-Bed Rest to 297 milligrams per day during Bed Rest was highly significant statistically ($P < 0.001$). See Table III.

Primate 419

Table I, Part C gives the daily creatine excretion in milligrams during different periods of the study for Primate 419.

The average creatine values for the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods were 37, 199, and 66 milligrams per day, respectively. The increase in creatine output during the Bed Rest Period was higher than that during the Pre-Bed Rest by a difference which was highly significant. The level of excretion during Bed Rest also was

superior to that during Post-Bed Rest ($P < 0.001$). The level of excretion during Post-Bed Rest also was higher than that during Pre-Bed Rest, also by a difference which again was highly significant. The ambulatory period after Bed Rest had not returned to its Pre-Bed Rest status with respect to urinary creatine although it was lower than bed rest excretion. There still was a significant difference between the Pre- and Post-Bed Rest levels ($P < 0.02$).

Primate 423

Table I, Part D gives daily creatine excretion values for Pre-Bed Rest and Bed Rest Periods for Primate 423. This primate was sacrificed after the Bed Rest Period in order to analyze his bones for radioactive Ca^{45} by another investigator, as noted for Primate 249.

Statistical comparison of the average daily urinary creatine excretion shows that the excretion during the Bed Rest with the Pre-Bed Rest Period was highly significant ($P < 0.001$).

With all of the data for creatine excretion for the four primates of this study pooled together, daily creatine excretion during the Pre-Bed Rest was surpassed by that for the Bed Rest by a difference which was highly significant ($P < 0.001$).

URINARY CREATININE EXCRETION

Table II gives the basic analytical data on urinary creatinine excretion in the urine. Table IV summarizes the statistical analysis of data on creatinine excretion.

Primate 239

Table II, Part A gives daily creatinine excretion during different periods of the study for Primate 239.

The average urinary creatinine excretion by this primate increased from 241 milligrams per day during the Pre-Bed Rest Period to 326 milligrams per day during Bed Rest. This change in value is statistically significant ($P < 0.01$). The decrease in the output during Post-Bed Rest as compared to the Bed Rest Period also was significant. The statistical comparison of the average daily creatinine excretion during Pre-Bed Rest with the Post-Bed Rest Period was significant only with a minor degree of probability ($P < 0.10$). See Table IV.

Primate 249

Table II, Part B gives the daily creatinine excretion during Pre-Bed Rest, and the Bed Rest Period for Primate 249. This primate was sacrificed after the Bed Rest Period in order to analyze its bones for radioactive Ca^{45}, as noted.

The decrease in average daily excretion of creatinine from the Pre-Bed Rest Period to the Bed Rest Period was statistically significant ($P < 0.01$).

Primate 419

Table II, Part C gives the daily creatinine excretion during different periods of the study for Primate 419.

In contrast to Primate 239, Primate 419 showed the decrease in creatinine excretion from the Pre-Bed Rest Period to the Bed Rest Period, however, the difference not to be statistically significant. The decrease in output from the Bed Rest Period to the Post-Bed Rest Period also was not significant. Like 239, a statistically significant comparison of the daily creatinine excretion during Pre-Bed Rest compared with the Post-Bed Rest Period was significant, with only a minor degree of probability ($P < 0.10$). See Table IV.

Primate 423

Table II, Part D gives the daily creatinine excretion values during the Pre-Bed Rest and the Bed Rest Periods for Primate 423. This primate was sacrificed after the Bed Rest Period in order to dissect his bones to be analyzed for radioactive Ca^{45}, as noted.

The increase in creatinine output during the Bed Rest Period as compared with the Pre-Bed Rest Period was statistically significant with only a minor degree of probability shown ($P < 0.10$).

SUMMARY AND CONCLUSIONS

Daily urinary creatine and creatinine excretion values were determined in this study on four male primates of species Macaca nemestrina. This study was conducted at the Texas Woman's University Research Institute. It was sponsored by the National Aeronautics and Space Administration.

The study lasted 97 days which included 54-day Pre-Bed Rest, 11-day Bed Rest, and 32-day Post-Bed Rest Periods. At the end of the Bed Rest Period, two of the four primates were sacrificed in order to dissect out bones in order to be analyzed for radioactive ^{45}Ca. During the Post-Bed Rest Period, the remaining two animals were kept in their metabolism cages.

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

Primate 239 excreted higher amounts of creatine during his Post-Bed Rest Period as compared either to the Pre-Bed Rest or Bed Rest Periods.

Primate 419 excreted higher amounts of creatine during the Post-Bed Rest Period as compared to the Pre-Bed Rest Period.

Both of these primates had very little activity when they were returned from their couches to their metabolism cages. This fact would explain the reason why both primates excreted higher amounts of creatine during the Post-Bed Rest Period as compared to the Pre-Bed Rest Period. It also was noticed that Primate 239 had almost no activity in his metabolism cage during the entire period of Post-Bed Rest, and he was not able to stand up in his cage until the last few days of the study. This fact should explain the reason for higher creatine excretion during his Post-Bed Rest Period than was found in the other animals.

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.

REFERENCES CITED

1. White, S. C., and Berry, C. A., Resume of Present Knowledge of Man's Ability to Meet the Space Environment, Aerospace Medicine, 35:43-48 (1964)

2. Umapathy, P. K., Effect of Immobilization on Urinary Excretion of Creatine and Creatinine with Possible Ameliorating Measures Applied, Dissertation, Texas Woman's University (1967)

3. Block, K., and Schoenheimer, R., The Biological Precursors of Creatine, Journal of Biological Chemistry, 138:167-194 (1941)

4. Borsook, H., and Dubnoff, J. W., The Formation of Glycocyamine in Animal Tissues, Journal of Biological Chemistry, 138:389-403 (1941)

5. Walker, J. B., Arginine Ornithine Transamidination in Kidney, Journal of Biological Chemistry, 221:771-776 (1956)

6. Walker, J. B., Role for Pancreas in Biosynthesis of Creatine, Proceedings of the Society for Experimental Biology and Medicine, 98:7-9 (1958)

7. Walker, J. B., Metabolic Control of Creatine Biosynthesis. II. Restoration of Transamidinase Activity Following Creatine Repression, Journal of Biological Chemistry, 236:493-498 (1961)

8. Fitch, C. D., Hsu, L., and Dinning, J. S., The Mechanism of Kidney Transamidinase Reduction in Vitamin E Deficient Rabbits, Journal of Biological Chemistry, 236:490-492 (1961)

9. Walker, M. S., and Waler, J. B., Repression of Transamidinase Activity During Embryonic Development, Journal of Biological Chemistry, 237:473-476 (1962)

10. Kuszalka, T. R., Extrahepatic Creatine Synthesis in the Rat. Role of the Pancreas and Kidney, Archives of Biochemistry and Biophysics, 122:400-405 (1967)

11. Cantoni, G. L., and Durell, J., Activation of Methionine for Transamethylation II. The Methionine-activating Enzyme Studies on the Mechanism of the Reaction, Journal of Biological Chemistry, 225:1033-1048 (1957)

12. Cantoni, G. L., and Vignos, P. J., Enzymatic Mechanism of Creatin Synthesis, Journal of Biological Chemistry, 209:647-659 (1954)

13. Borsook, H., and Dubnoff, J. W., The Hydrolysis of Phosphocreatine and the Origin of Urinary Creatine, Journal of Biological Chemistry, 168:493-510 (1947)

14. Van Pilsum, J. F., and Hiller, B., On the Proposed Origin of Creatinine from Creatine Phosphate, Archives of Biochemistry and Biophysics, 85:483-486 (1959)

15. Block, K., and Schoenheimer, R., Studies in Protein Metabolism XI. The Metabolic Relation of Creatine and Creatinine Studies with Isotopic Nitrogen, Journal of Biological Chemistry, 131: 111-119 (1939)

16. Chanutin, A., Studies of Creatine and Nitrogen Content of the Whole Rat after the Feeding of a Variety of Diets and after Nephrectomy, Journal of Biological Chemistry, 89:765-774 (1930)

17. Kleiner, S. I., and Orton, J. B., Biochemistry, Sixth Edition, The C. V. Mosby Company, St. Louis (1962)

18. Shaffer, A. P., The Excretion of Kreatinine and Kreatin in Health and Disease, American Journal of Physiology, 23:1-22 (1908)

19. Folin, O., Laws Governing the Chemical Composition of Urine, American Journal of Physiology, 13:66-102 (1905)

20. Fisher, H., Variations in the Urinary Creatinine Excretion of Rats Fed Diets with Protein and Amino Acid Content, Journal of Nutrition, 85:181-186 (1965)

21. Benedict, S. R., and Osterberg, E., Studies in Creatine and Creatinine Metabolism, Journal of Biological Chemistry, 56:229-252 (1923)

22. Meyers, V. C., and Fine, M. S., The Influence of the Administration of Creatinine and Creatine on the Creatinine Content of Muscle, Journal of Biological Chemistry, 16:169 (1913)

23. Walker, J. B., Metabolic Control of Creatine Biosynthesis. I. Effect of Dietary Creatine, Journal of Biological Chemistry, 235: 2357-2361 (1960)

24. Maw, G. A., The Fate of Ingested Creatinine in the Rat, Biochemical Journal, 235:2357 (1960)

25. Dinning, J. S., Gallup, W. D., and Briggs, H. M., Excretion of Creatinine and Creatine by Beef Steers, Journal of Biological Chemistry, 177:157-161 (1949)

26. Van Niekerk, B. H. D., Bensadoun, A., Paladines, O. L., and Reid, J. T., A Study of Some of the Conditions Affecting the Rate of Excretion and Stability of Creatinine in Sheep Urine, Journal of Nutrition, 79:373-380 (1963)

27. Gerard, R. W., Recent Advances in Knowledge of Metabolic Alterations in Muscular Dystrophy, American Journal of Physiological Medicine, 34:279-280 (1955)

28. McGeer, E. G., McGreer, P. L., Miller, Jr., Perry, D., and Nichol, C., Excretion of 4-Amino-5 Imidiazolecarboxyamide and Creatine: Creatine Rations in Human and Mouse Muscular Dystrophy, Canadian Journal of Biochemistry, 40:13-23 (1962)

29. Morguli, S., and Spencer, H. C., Metabolism Studies in Nutritional Muscular Dystrophy, Journal of Nutrition, 12:191-204 (1936)

30. Gerber, G. B., Gerber, G., Koszalka, T. R., and Emmel, J. M., Creatine Metabolism in Vitamin E Deficiency in the Rat, American Journal of Physiology, 202:453-460 (1962)

31. Shrivastava, V., Devi, A., and Sarkar, N. K., Biochemical Changes in Progressive Muscular Dystrophy. III. Nucleic Acid, Phosphorus and Creatine Metabolism in the Muscle, Liver, and Brain of Rabbits Maintained with a Choline-deficient Diet, Journal of Nutrition, 86:298-302 (1963)

32. Greben'Kov, S. G., Creatinuria as an Index of Normal Vitamin C Supply of the Animal Organisms, Vopr Pitaniya, 22:43-48 (1963)

33. Dinning, J. S., and Day, P. L., Creatinuria During Recovery from Aminopterin Induced Folic Acid Deficiency in the Monkey, Journal of Biological Chemistry, 181:897-903 (1949)

34. Grillo, M. A., and Fossa, T., Creatine Synthesis in Hypo- and Hyperthyroid Rats, Boll. Soc. Ital. Biol. Sper., 43:1594-1596 (1967)

35. Wang, E., Clinical and Experimental Investigations in Creatine Metabolism, Acta Medica Scandinavica, Supplementum, CV:Z-297 (1939)

36. Chor, H., and Dolkart, R. E., A Study of "Simple Disuse Dystrophy" in the Monkey, American Journal of Physiology, 117:626-630 (1936)

37. Hines, H. M., and Knowlton, G. C., Changes in the Skeletal Muscle of the Rat Following Denervation, American Journal of Physiology, 104:379-391 (1933)

38. Reid, G., A Comparison of the Effects of Disuse and Denervation Upon Skeletal Muscle, The Medical Journal of Australia, 2:165-167 (1941)

39. Montgomery, K. B., A Study of Nitrogen Balance and Creatine and Creatinine Excretion During Recumbency and Ambulation of Five Young Adult Human Males, Master's Thesis, Texas Woman's University (1968)

40. Chan, Shiu-Shiang, Excretion of Creatine and Creatinine in Adult Male Subjects during Immobilization and Ambulation, Master's Thesis, Texas Woman's University (1965)

41. Chung, Tze-Chuan Agnes, Creatine, Creatinine and Nitrogen Excretion by Bed Rest Recumbent Male Subjects, Master's Thesis, Texas Woman's University (1966)

42. Hoffman, R. A., Dozier, E. A., Mack, P. B., Hood, W. N., and Parrott, M. W., 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, 39:693-698 (1968)

43. Taussky, M. N., Creatinine and Creatine in Urine and Serum, Standard Method of Clinical Chemistry, 3:99-101 (1961)

44. Biggs, H. G., and Cooper, J. M., Modified Folin Methods for the Measurement of Urinary Creatine and Creatinine, Clinical Chemistry, 7:655-664 (1961)

APPENDIX

TABLE I

URINARY CREATINE EXCRETION

PART A. PRIMATE - 239

PRE-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	40	April 2	0
7	89	3	0
8	0	4	43
9	0	5	64
10	21	6	5
11	0	7	80
12	0	8	28
13	56	9	14
14	52	10	0
15	64	11	0
16	19	12	22
17	114	13	34
18	50	14	5
19	60	15	27
20	10	16	17
21	57	17	39
22	0	18	3
23	0	19	0
24	29	20	12
25	44	21	0
26	87	22	31
27	0	23	41
28	97	24	62
29	75	25	21
30	89	26	27
31	2	27	52
April 1	0	28	0
MEAN: 31			

BED REST	
Date	mg. per 24 hours
April 29	145
30	283
May 1	6
2	35
3	124
4	0
5	198
6	113
7	40
8	148
9	124
MEAN: 111	

POST-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
May 10	66	May 26	55
11	330	27	70
12	525	28	39
13	157	29	135
14	80	30	---
15	67	31	291
16	17	June 1	402
17	55	2	---
18	170	3	24
19	32	4	29
20	25	5	110
21	8	6	137
22	---	7	52
23	218	8	42
24	197	9	36
25	108	10	31
MEAN: 131			

TABLE I, CONTINUED

URINARY CREATINE EXCRETION

PART B. PRIMATE - 249

PRE-BED REST				BED REST	
Date	mg. per 24 hours	Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	86	April 2	0	April 29	97
7	202	3	0		
8	70	4	64	30	550
9	2	5	76		
10	0	6	192	May 1	535
11	0	7	0		
12	18	8	107	2	62
13	54	9	62		
14	40	10	0	3	571
15	56	11	0		
16	103	12	17	4	383
17	122	13	0		
18	27	14	5	5	166
19	37	15	53		
20	32	16	0	6	122
21	41	17	0		
22	0	18	0	7	223
23	0	19	0		
24	84	20	26	8	244
25	66	21	6		
26	122	22	26	9	312
27	115	23	76		
28	124	24	32		
29	147	25	0		
30	145	26	0	MEAN: 297	
31	0	27	29		
April 1	215	28	0		
MEAN: 51					

TABLE I, CONTINUED

URINARY CREATINE EXCRETION

PART C. PRIMATE - 419

PRE-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	44	April 2	0
7	129	3	3
8	121	4	50
9	31	5	256
10	22	6	178
11	43	7	82
12	9	8	18
13	0	9	25
14	27	10	0
15	14	11	0
16	78	12	0
17	60	13	15
18	49	14	0
19	10	15	21
20	12	16	14
21	27	17	0
22	29	18	11
23	8	19	0
24	27	20	0
25	37	21	0
26	45	22	44
27	79	23	54
28	86	24	6
29	49	25	14
30	76	26	11
31	0	27	46
April 1	8	28	14
MEAN: 37			

BED REST	
Date	mg. per 24 hours
April 29	151
30	312
May 1	188
2	263
3	183
4	94
5	312
6	109
7	113
8	151
9	312
MEAN: 199	

POST-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
May 10	52	May 26	70
11	262	27	95
12	198	28	195
13	12	29	0
14	65	30	41
15	78	31	0
16	96	June 1	24
17	74	2	27
18	71	3	---
19	44	4	82
20	60	5	135
21	24	6	36
22	42	7	16
23	0	8	25
24	34	9	---
25	46	10	84
MEAN: 66			

TABLE I, CONTINUED

URINARY CREATINE EXCRETION

PART D. PRIMATE - 423

PRE-BED REST				BED REST	
Date	mg. per 24 hours	Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	160	April 2	0	April 29	49
7	151	3	0	30	505
8	0	4	0	May 1	102
9	43	5	0	2	245
10	0	6	1	3	223
11	12	7	329	4	50
12	75	8	5	5	43
13	0	9	0	6	0
14	5	10	0	7	15
15	15	11	0	8	129
16	72	12	0	9	27
17	73	13	0		
18	0	14	22		
19	43	15	35		
20	0	16	5		
21	31	17	0		
22	0	18	0		
23	7	19	0		
24	37	20	11		
25	27	21	3		
26	36	22	30		
27	72	23	37		
28	52	24	108		
29	25	25	27		
30	83	26	8		
31	0	27	35		
April 1	9	28	7		
MEAN: 31				MEAN: 126	

TABLE II

URINARY CREATININE EXCRETION

PART A. PRIMATE - 239

PRE-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	311	April 2	156
7	294	3	336
8	325	4	253
9	173	5	246
10	463	6	278
11	212	7	237
12	231	8	169
13	318	9	204
14	260	10	195
15	252	11	241
16	196	12	231
17	208	13	205
18	231	14	198
19	124	15	271
20	309	16	168
21	236	17	181
22	149	18	200
23	178	19	213
24	169	20	201
25	123	21	395
26	95	22	140
27	155	23	169
28	309	24	229
29	239	25	313
30	311	26	542
31	170	27	428
April 1	261	28	289
MEAN: 241			

BED REST	
Date	mg. per 24 hours
April 29	343
30	424
May 1	429
2	142
3	349
4	481
5	395
6	439
7	317
8	200
9	71
MEAN: 326	

POST-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
May 10	104	May 26	153
11	298	27	140
12	537	28	135
13	288	29	106
14	215	30	---
15	237	31	272
16	30	June 1	182
17	201	2	---
18	305	3	65
19	78	4	59
20	165	5	191
21	15	6	121
22	---	7	110
23	465	8	162
24	184	9	148
25	526	10	100
MEAN: 193			

TABLE II, CONTINUED

URINARY CREATININE EXCRETION

PART B. PRIMATE - 249

PRE-BED REST				BED REST	
Date	mg. per 24 hours	Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	406	April 2	433	April 29	107
7	417	3	457		
8	442	4	313	30	370
9	248	5	238		
10	198	6	389	May 1	376
11	360	7	443		
12	480	8	395	2	15
13	430	9	522		
14	461	10	307	3	421
15	436	11	385		
16	433	12	321	4	433
17	411	13	593		
18	460	14	343	5	307
19	436	15	372		
20	343	16	458	6	300
21	313	17	475		
22	406	18	443	7	309
23	419	19	452		
24	377	20	445	8	266
25	354	21	180		
26	304	22	326	9	239
27	283	23	370		
28	454	24	332	MEAN: 286	
29	449	25	255		
30	448	26	346		
31	439	27	238		
April 1	433	28	297		
MEAN: 385					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION

PART C. PRIMATE - 419

PRE-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	346	April 2	166
7	326	3	368
8	308	4	196
9	368	5	253
10	353	6	341
11	395	7	366
12	326	8	240
13	333	9	197
14	341	10	286
15	421	11	266
16	339	12	336
17	271	13	157
18	474	14	319
19	325	15	225
20	360	16	315
21	357	17	267
22	331	18	152
23	203	19	317
24	306	20	181
25	335	21	278
26	185	22	244
27	354	23	252
28	396	24	268
29	338	25	172
30	248	26	292
31	146	27	220
April 1	171	28	217
MEAN: 288			

BED REST	
Date	mg. per 24 hours
April 29	209
30	316
May 1	385
2	309
3	315
4	287
5	212
6	253
7	223
8	238
9	236
MEAN: 271	

POST-BED REST			
Date	mg. per 24 hours	Date	mg. per 24 hours
May 10	92	May 26	218
11	488	27	261
12	363	28	287
13	390	29	263
14	211	30	276
15	205	31	387
16	244	June 1	144
17	265	2	209
18	207	3	---
19	151	4	288
20	294	5	300
21	141	6	376
22	219	7	254
23	277	8	143
24	262	9	---
25	96	10	295
MEAN: 254			

TABLE II, CONTINUED

URINARY CREATININE EXCRETION

PART D. PRIMATE - 423

PRE-BED REST				BED REST	
Date	mg. per 24 hours	Date	mg. per 24 hours	Date	mg. per 24 hours
March 6	250	April 2	83	April 29	492
7	332	3	104		
8	475	4	624	30	421
9	436	5	260		
10	380	6	339	May 1	419
11	398	7	374		
12	312	8	192	2	211
13	416	9	374		
14	359	10	361	3	347
15	381	11	361		
16	322	12	372	4	388
17	256	13	420		
18	379	14	338	5	381
19	374	15	517		
20	372	16	238	6	438
21	397	17	366		
22	394	18	383	7	331
23	128	19	373		
24	150	20	326	8	360
25	166	21	207		
26	107	22	151	9	408
27	207	23	190		
28	215	24	207		
29	183	25	234		
30	549	26	124		
31	172	27	162		
April 1	137	28	219		
MEAN: 314				MEAN: 389	

TABLE III

STATISTICAL COMPARISON OF URINARY CREATINE EXCRETION BETWEEN PAIRS FOR DIFFERENT PERIODS OF THE STUDY BY FOUR PRIMATES

Populations Compared	Means	Standard Deviation	"t" Value	Probability
PRIMATE 239				
Pre-Bed Rest	31	31	5.1993	$P < 0.001$
Bed Rest	111	82		
Pre-Bed Rest	31	31	5.2931	$P < 0.001$
Post-Bed Rest	131	127		
Bed Rest	111	82	0.4628	N.S.
Post-Bed Rest	131	127		
PRIMATE 419				
Pre-Bed Rest	37	47	8.6125	$P < 0.001$
Bed Rest	199	82		
Pre-Bed Rest	37	47	2.4203	$P < 0.02$
Post-Bed Rest	66	60		
Bed Rest	199	82	5.3310	$P < 0.001$
Post-Bed Rest	66	60		
PRIMATE 249				
Pre-Bed Rest	51	57	7.8221	$P < 0.001$
Bed Rest	297	180		
PRIMATE 423				
Pre-Bed Rest	31	55	3.5481	$P < 0.001$
Bed Rest	126	143		

TABLE IV

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION BETWEEN PAIRS FOR DIFFERENT PERIODS OF THE STUDY BY FOUR PRIMATES

Populations Compared	Means	Standard Deviation	"t" Value	Probability
PRIMATE 239				
Pre-Bed Rest	241	85	2.6763	$P < 0.01$
Bed Rest	326	127		
Pre-Bed Rest	241	85	1.9581	$P < 0.10$
Post-Bed Rest	193	131		
Bed Rest	326	127	2.7564	$P < 0.01$
Post-Bed Rest	193	131		
PRIMATE 419				
Pre-Bed Rest	288	76	0.6781	N.S.
Bed Rest	271	53		
Pre-Bed Rest	288	76	1.8247	$P < 0.10$
Post-Bed Rest	254	89		
Bed Rest	271	53	0.5920	N.S.
Post-Bed Rest	254	89		
PRIMATE 249				
Pre-Bed Rest	385	83	3.1865	$P < 0.01$
Bed Rest	286	122		
PRIMATE 423				
Pre-Bed Rest	314	144	1.6630	$P < 0.10$
Bed Rest	389	53		