EFFECT OF IMMOBILIZATION ON URINARY EXCRETION OF CREATINE AND CREATININE WITH CERTAIN POSSIBLE AMELIORATING MEASURES APPLIED

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INTRODUCTION

The undesirable metabolic and physiologic effects of simulated weightlessness on the musculo-skeletal system are well documented. Man's successful exploration of space depends upon research studies which simulate conditions of space travel. The ultimate objective of such research is the prevention of the deleterious effects of prolonged flights.

Since many of the problems encountered appear to be related to a lack of physical activity, the development of various exercise programs and their evaluation during prolonged bed rest periods become an important aspect of investigations designed to assure successful space missions. Although certain activities can be performed in the space vehicle, much less energy is expended than during similar activities under normal gravitational conditions. Thus, there is need for a program of physical stress and strain when no force of gravity exists and when confined by other factors of the life-support system of space existence— which is comparable to the activity normally needed to maintain body functions. Such programs, or devices, should be tried and evaluated during simulated hypodynamic conditions before they are adopted for space ventures.

The present study is an investigation of the effects of certain exercises and of a special exercise suit upon the urinary excretion of creatine and creatinine and is a part of the more extensive investigations undertaken by Texas Woman's University Research Institute to solve some of the problems encountered in space travel.

Creatinuria— that is, an increased concentration of creatine in the urine, has been observed in previous bed rest studies which involved human subjects (31) (33) as well as primates (76) at this Institute. The isotopic study of Schoenheyder and Christensen (100) on human subjects revealed that there was diminished uptake of creatine by the muscle during bed rest. Deitrick, Whedon, and Shorr (40) noted a decrease in muscle mass, a diminution of muscular strength, and a progressive reduction in creatine tolerance in four healthy men immobilized in bivalve casts. Such symptoms appear to be characteristic of various muscular diseases and dystrophies.

Our knowledge of the effects of exercise in the prevention of various metabolic disturbances during simulated weightlessness is extremely limited. Comparative exercise studies need to be made before astronauts can be expected to accomplish successful space missions and return without any muscular or other disorders. The possible beneficial effects of planned exercise programs as revealed by urinary

creatine and creatinine levels in the male human and in primates are thus investigated.

OBJECTIVES

The specific objectives of the present study are:

1. To include isometric and isotonic exercises during a 14-day bed rest period and to determine their effect on daily urinary creatine and creatinine levels of two human adult male subjects.

2. To compare statistically the above values with those obtained during a 14-day bed rest immobilization period of the same subjects.

3. To observe the 24-hour urinary creatine and creatinine levels of two additional recumbent male subjects.

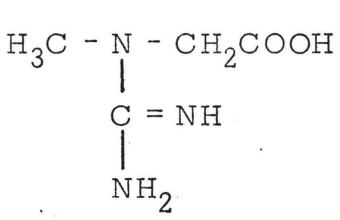
4. To evaluate the effect on creatine and creatinine excretion in five healthy adult males of wearing the gravitational acceleration simulation suits— designed by James Gatts, M.D., of the Republic Aviation Company during a 21-day bed rest period.

5. To make statistical comparisons of the excretion levels between all the periods of the two bed rest units, with and without exercise garment.

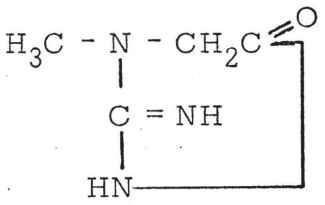
6. To analyze and to compare creatine and creatinine concentrations in the samples obtained from all the six control, exercised, and restrained primates, during 42 days of pre-, post-, and immobilization or exercise study.

REVIEW OF LITERATURE

Creatine is methyl guanidoacetic acid. Creatinine is the anhydride form of creatine. Their structural formulas are as follow.



Creatine



Creatinine

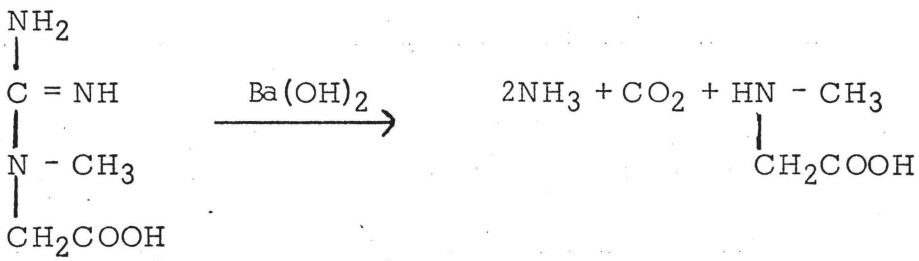
The role of creatine phosphate as a reservoir of energy for adenosine triphosphate in muscular contraction is well known. In addition, Harper (69) has reported that creatine phosphate is involved in the regeneration of ATP which is used in the resynthesis of acetylcholine in nerve tissue. Creatinine is a waste product derived from creatine phosphate and creatine.

ENDOGENOUS SOURCE OF CREATINE

Creatine is synthesized in the body and is not a necessary constituent of diet. The amino acids, glycine, arginine, and methionine, are the precursors of creatine. Definite proof that glycine is the precursor of creatine first came from Brand, Harris, Sandberg, and Ringer

in 1929 (18). They observed the highest increase in creatine excretion in five patients with progressive muscular dystrophy when the creatine-free diet was supplemented with glycine. Withdrawal of glycine resulted in a marked decrease in creatine excretion.

The first step in the synthesis of creatine is the formation of guanidoacetic acid (glycocyamine) from glycine and arginine. This reaction is catalyzed by the enzyme, glycine transamidinase, and is called "Transamidination" according to Borsook and Dubnoff (16). Guanidoacetic acid was proved to be the most potent creatine precursor of all the labeled nitrogenous compounds tested by Bloch and Schoenheimer (12). Their results definitely showed that amidine nitrogen is derived from arginine and sarcosine nitrogen from glycine which was based on the following reaction:



A more recent report by Gerber, Koszalka, Gerber, and Altman (59) of a study on rats carried out by using C^{14}-labeled glycine and arginine substantiated the above earlier experiment.

Guanidoacetate is methylated by S-adenosylmethionine to form creatine (15) (42). The enzyme that catalyzes this reaction is known as "guanidoacetate methylferase" (26). In 1951 Cohen (35), in studies using guinea pig and rabbit liver homogenates, demonstrated that the presence of ATP and Mg++ are essential for the methylation reaction.

SITE OF CREATINE SYNTHESIS

According to early vitro studies (12) (13), the transamidination reaction occurred in kidney and the methylation reaction in liver tissues. Since considerable species variation was observed regarding the site of creatine synthesis, Sandberg, Hecht, and Tyler (98) studied the major site of creatine synthesis in man. They compared the hepatic and renal levels with the arterial levels of arginine, glycocyamine, creatine, and creatinine in six men volunteers. The blood was drawn by catheterization. These investigators concluded that the liver was the major site of glycocyamine methylation and the principle site of creatine synthesis in man. The pancreas has been shown to be capable of synthesizing creatine in the dog (118) since appreciable quantities of both transamidinase (117) and guanidoacetate methyltransferase (118) were found in the organ. The experiments by Goldman and Moss (60) on nephrectomized rats using C^{14}-labeled glycine indicated that 40 to 60 per cent of creatine synthesis may be by extrarenal routes. Van Pilsum, Orsen, Taylor, Rozycki, and Pierce (110) showed that the spleen, skeletal muscle, heart muscle, lung, brain, and testes from various

mammals have measurable amounts of transamidinase activity in vitro. The total of all these tissue activities should approximate the total activity of the kidney and the pancreas.

The phenomenon of chemical feedback was evident in the effect of dietary creatine on creatine synthesis in the body. Walker (115) (116), in his experiments, found reduced levels of glycine transamidinase activity when creatine was added to the diet. Creatinine administration did not have this effect. The possible mechanism for this behavior was studied by Fitch, Hay, and Dinning (50). According to their study on rats, glycine and creatine compete for the enzyme site. Dietary creatine becomes a part of the total creatine pool of the body.

FORMATION OF CREATININE

The studies done by Borsook and Dubnoff (17) indicated that creatinine is formed directly by a nonenzymatic cleavage of phosphocreatine. They did not exclude the possibility of enzymic conversion. Van Pilsum and Hiller (111) did not observe the above reaction in the muscle extracts from normal rabbits; instead, they observed the conversion of creatine phosphate to creatine. Creatine itself can lose a molecule of water to form creatinine at a slow rate (24). The results of the experiments with isotopic creatinine in rats showed that the conversion of creatine to creatinine is irreversible (13). This conversion is under the

influence of temperature, pH, and other physicochemical characteristics of the medium (68).

Foods, such as meat and fish, contain significant amounts of both creatine and creatinine. The amount of preformed creatinine is increased after cooking (70).

METABOLISM OF CREATINE AND CREATININE

About 95 to 98 per cent of the body creatine is found in skeletal muscle. The rest is in the myocardium, neurons, spermatozoa, and uterine muscle (68). Fitch and Shields (49) described the mechanism of creatine entrance into muscle membranes. Their studies are of great value in understanding the creatinuria observed during immobilization and in conditions of muscular disorders. In the opinion of these workers, a mediated entry process exists which is capable of moving creatine into muscle against a concentration gradient and is not just a simple diffusion.

The capacity of the muscle to contract is related to its phosphocreatine content. The fact that phosphocreatine is the important storage form of energy in muscle and ATP is the more immediate source of the energy needed for muscle contraction is demonstrated by Lohmann (23). It is generally agreed that the contractility phenomenon involves a reaction between ATP and a contractile protein known as

actomyosin, resulting in the formation of ADP and inorganic phosphate. The energy liberated in the hydrolysis of ATP is used up for the mechanical work of contraction. The regeneration of ATP is accomplished by the Lohmann reaction:

$$\text{ATP} + \text{actomyosin} \xrightarrow{\text{actomyosin ATPase}} \text{ADP} + \text{inorganic phosphate} + \text{mechanical work}$$

$$\text{ADP} + \text{phosphocreatine} \xleftrightarrow[\text{or phosphokinase}]{\text{creatine phosphoryl transferase}} \text{ATP} + \text{creatine}$$

Cain, Infante, and Davies (23) found a linear relationship between the amount of work and the amount of breakdown of phosphocreatine in frog muscle. At a somewhat lessened rate, glycolysis in the muscle resulted in the further regeneration of ATP. When the muscle was at rest, the surplus amount of energy was stored as phosphocreatine which served as a ready reservoir of ATP for future contraction. Experiments in vitro demonstrated that the phosphorylation of creatine can occur in the presence of diphosphopyridine nucleotide-DPN- and phosphoenolpyruvate as a phosphate donor. DPN is indirectly involved in the reaction (37).

The existence of phosphocreatine bound to protein in the resting muscle of rat, rabbit, and frog was reported by Sacks and Fulford (97). The significance of this bound phosphocreatine is not known.

CREATINE AND CREATININE EXCRETION UNDER NORMAL CONDITIONS

Most of the creatine is excreted as creatinine through the kidneys (13) (73). When large doses of creatine were given to individuals in nitrogen balance, there was a perceptible increase in the output of creatinine but not in the output of creatine (94). Before accurate methods were developed, it was thought that creatinuria was a feminine or prepubertal characteristic. Albanese and Wangerin (3), using a modified Folin method, found a range of 184 to 352 milligrams of daily creatine excretions in a study of adult males on normal diets. Ages of the subjects varied from 19 to 35 years. Taylor and Chew (106) reported lower levels, 10 to 100 milligrams of daily creatine excretion for normal male subjects ranging in age from 20 to 24 years. In both of the above studies the amount of creatine excreted varied widely from day to day. For example, one individual whose urine was analyzed over a period of five years showed a range in excretion of 50 to 175 milligrams of creatine (106). According to the studies of Light and Warren (83) on adolescent boys whose ages ranged from 14 to 19 years, the average creatine excretion declined as age increased.

Infants, children, and some women especially during menstruation and pregnancy excrete considerable amounts of creatine in the urine. More than 100 normal children between the ages of three and 18 years

were studied repeatedly over a period of four years by Clark, Thompson, Beck, and Jacobson (34). These authors stated "Creatine excretion exceeds that of creatinine at the age of three years and then, on the average, gradually decreases to about a tenth of creatinine excretion at the age of 18 years. There are great differences in the creatine excretion of individual children".

Studies of animals and humans indicated a decrease in the ratio of creatinine to creatine during the geriatric period. This is believed to be the cause of muscular dystrophy in old age (64). In rats, after the twentieth month, the excretion of creatine increased while that of creatinine decreased (79). Research studies done on 23 men with the age range of 52 to 88 years at the University of Iowa, reported mean values of 0.06 to 0.121 grams (39) and 105.0 to 166.5 milligrams of creatine per day (96). In a group of 27 subjects all aged 90 years or more, daily urinary excretions of creatine ranged from 0.025 to 0.23 grams. The administration of sex hormones abolished the observed creatinuria in all subjects (77).

Grossman (63) showed that serum creatine was immediate source of urinary creatine. Serum creatine levels for men and women reached 0.7 per cent and 0.5 per cent, respectively, before creatine appeared in the urine.

CREATININE EXCRETION

Creatinine has been considered a normal constituent of urine since the development of the colorimetric method for creatinine determination by Folin in 1905 (52). It was a well established fact that creatinine elimination is remarkably constant and is a good indicator of the accuracy of 24 hour urine collection (52) (53) (101) (102). However, this concept is rapidly changing with the development of micromethods in analytical chemistry. The present opinion is that the constancy of urinary creatinine excretion in a 24 hour period is highly dependent on the subject under investigation (14) (113). Vestergard and Leverett (113) said "Great constancy in some, fair constancy in most, and poor constancy in some." In their opinion, the variability in glomerular filtration rate may be responsible for such individual differences. The subjects in their study were 10 women and eight men, aged 19 to 45 years.

The results of a more recent study at Cornell University (14) agreed with the above conclusions and showed that most of the time, the daily creatinine excretion does not vary more than 20 per cent of the mean excretion of adolescent boys, aged 14 to 17 years. Albanese and Wangerin (3) found variations of 10 to 25 per cent of the total creatinine excretion in 30 normal subjects studied for 38 to 60 days. In the light of these findings, it becomes necessary to determine the variation in the creatinine excretion of the individual under investigation, before the creatinine excretion is used for an index of muscle

mass or for any other measurement based on the constancy of creatinine excretion.

A diurnal variation in creatinine excretion was noted by Addis, Barnett, Ureen, and Lippman (1) and Eimer (45). The excretion of creatinine fell to a low level during the night and increased during the day. Seasonal variations were observed also by Fukasawa (55). Creatinine output and creatinine coefficient were found to be high in summer and low in winter.

Normal creatinine excretion was reported to be 1.0 to 2.0 grams for adult males and 0.8 to 1.5 grams for women (25). Clark and others (34) noticed that boys started excreting significantly higher amounts of creatinine than girls at the age of 12 years.

The term "creatinine coefficient" was introduced by Shaffer in 1908 (101), and is used to indicate the number of milligrams of creatinine nitrogen excreted per kilogram of body weight in 24 hours. Normal daily values for creatinine excretion in milligrams per kilogram of body weight ranged from 5.6 to 11.9 and 3.3 to 9.8 for men and women, respectively (75). These differences are indicative of differences in musculature (109). The values obtained for 14 college girls who had been engaged in vigorous physical activity were closer to those of men (75). Novak (87) observed significantly higher creatinine coefficient values for boys as compared to girls after the age of 14.5 years. Since the

creatinine coefficient reflects the lean body mass, lower values are to be expected in obese individuals as compared to normal people with no differences in daily creatinine excretion (44). Several investigators feel that the creatinine excretion (grams or milligrams per 24 hours) is a better index of muscle mass than the creatinine coefficient (87) (55). Thus, creatinine excretion has been used as a measure of muscle mass in human and animal studies (81) (44) (88) (93). Miller and Blyth (85) developed the regression equation based on this relationship:

LBM = 20.97 + 0.5161 (creatinine)

LBM = Lean body mass in kilograms

Creatinine = Creatinine excretion in milligrams per hour

In 90 per cent of their cases, lean body mass calculated from creatinine excretion agreed within ±13.1 per cent of the values determined densiometrically. Seventy-eight males and one female, with ages ranging from 20 to 26 years, served as subjects in the study. Shirling and Passmore (103) disagreed with the earlier workers and, in their opinion, creatinine excretion is related to surface area rather than to lean body mass.

EFFECT OF DIET ON CREATINE AND CREATININE EXCRETION

Creatinuria in 96 out of 97 male students was attributed to high carbohydrate intake by Hobson (74). Creatinuria was reduced to a

considerable extent by a low carbohydrate diet. The findings agreed with the study of Haldi and Bachmann (66) who induced creatinuria by the use of glucose and with greater efficiency by the use of fructose or a combination of the two. Creatinine excretion remained uninfluenced in both the studies.

Most of the research workers in this field, who have studied creatine and creatinine, seem convinced that dietary protein has little or no influence on the excretion of creatine and creatinine provided the protein intake is adequate (3) (6) (11) (47) (106). Extremely low or high protein intakes resulted in changes in creatinine excretion (6) (11). Fisher (47) on the basis of his experiments on rats indicated that dietary protein and amino acid content influenced the creatinine excretion in a manner which was hard to predict. Adult rats excreted increased amounts of creatinine in the urine when the diet provided the least amount of dietary nitrogen but contained free amino acids.

Creatinuria was produced in three adult men by the administration of large doses of caffeine (5). However, the amount of creatine excreted showed no relationship to the amount of caffeine ingested. Reports in the literature indicated that diet was not a major factor in the metabolic studies of creatine and creatinine as long as the same type of diet was provided throughout the study.

INFLUENCE OF HORMONES ON CREATINE AND CREATININE METABOLISM

Testosterone. Williamson and Gulick (124) studied the influence of testosterone on the distribution and excretion of creatine in rabbits. The administration of this hormone increased the retention of creatine in the muscles and lowered urinary excretion without appreciable changes in the blood creatine. Treatment of sexually underdeveloped dwarfs with testosterone propionate decreased the excretion of creatine (121). The mode of testosterone action was later demonstrated to be in creatine biosynthesis (80). The enzyme (amidinotranspherase) activity in male kidney homogenates was almost double that of females of the same age, but such a difference did not exist before puberty.

Thyroxine. In 1929, Carson (28) produced creatinuria in three individuals by administering thyroid extract. Creatinuria is common in conditions like thyrotoxicosis and hyperthyroidism (119) (108). In rabbits, the injection of thyroxine produced low levels of creatine and phosphocreatine in the muscles as compared to control animals (119). According to Fitch (48), there is a block in the entry of creatine into skeletal muscles in hyperthyroidism. The decreased incorporation of C^{14}-labeled creatine into the muscle of rats suffering from hyperthyroidism led to the above conclusion.

Vasopressin. Williams and Maury (123) produced creatinuria in rats by administering the antidiuretic hormone— vasopressin— dissolved in oil. Aqueous solutions of the hormone did not bring about any changes— creatinine was unaffected in both instances.

CREATINE AND CREATININE METABOLISM IN MUSCULAR DISORDERS

Progressive muscular dystrophy and many other conditions associated with muscular wasting are characterized by creatinuria and low muscle concentrations of creatine (51) (114) (68). Creatinine excretion is markedly diminished in progressive muscular dystrophy and probably is a better indication of the severity of the disease than creatinuria according to Hoagland, Gilder, and Shank (72). Ryan and others (95) compared the daily creatinine excretion of five normal healthy medical students with the excretion of two individuals suffering from muscle wasting. The average daily creatinine excretions of the normal men and the patients, respectively, were 27.9 and 12.4 milligrams per kilograms of body weight.

Nutritional muscular dystrophy which can be produced in experimental animals by feeding a diet deficient in vitamin E or choline is accompanied by creatinuria and diminished creatinine excretion (41) (58) (105).

Creatinuria and diminished creatine tolerance are observed in patients with Graves' disease and are indicative of a defect in creatine metabolism (92) (104). The creatine tolerance test is administered to determine quantitatively the functional state of muscle. In this test the excretion of creatine is measured during the 24 hours following the ingestion of a standard dose of 1.32 or 2.64 grams of creatine hydrate. Normal adult men and women excrete 20 per cent and 30 per cent of a test dose, respectively (25).

Many investigators have tried to study the mechanism of creatinuria and other disturbances in creatine metabolism associated with muscular disorders. Benedict and co-workers (7) fed N^{15}-labeled glycine to two patients with severe muscular dystrophy. The urinary creatine and creatinine levels of N^{15} were determined and analyzed. The results showed a higher isotopic concentration in creatine than creatinine. The authors concluded that excessive creatine in the urine of the muscular dystrophic patients represented freshly synthesized creatine which has been denied access to muscle. Similar observations were made by Gerber and co-workers (58) using vitamin E deficient rats and C^{14}-labeled creatine.

Among other pathological conditions in which creatine and creatinine metabolism are disturbed are: febrile diseases, diabetes mellitus (119), certain myopathies, eunuchoidism, postencephalitis,

Parkinsonism (25), leukemia (4), poliomyelitis (95), myoglobulinuria, and polymyositis (68).

THE EFFECT OF PHYSICAL EXERCISE ON CREATINE AND CREATININE LEVELS IN MUSCLE, BLOOD AND URINE

The effect of physical exercise on creatine and creatinine content of muscles has been studied mostly in animals. Usually, the animals are decerebrated and the muscles under investigation are deprived of blood supply during the study. Physical exertion is brought about by nerve stimulation using electric shock. Earlier experiments on cats (107), frogs (21), rabbits (22), and dogs (99) showed a significant decrease of creatine in the stimulated muscles. A highly significant increase in creatine phosphate in hypertrophied rat heart was demonstrated by Gangloff, Hemmings, and Krause (56). The activities of the enzymes— creatine phosphokinase and ATPase— in rat muscle were uninfluenced by exercise (90). These two enzymes are involved in muscular contraction. The rats were divided into four groups. Two of them were exercised daily by swimming for 30 minutes and the control groups were placed in the exercise tank for 20 seconds each day.

Ahlborg and Brohault (2) have recently studied the effect of exercise on the serum creatinine as well as serum creatine-phosphokinase levels in 12 healthy men who were 20 years old. The subjects performed standardized continuous physical exercises as energetically as possible

on a bicycle ergometer for two hours each day for 14 days. The results showed a slight rise in creatinine level at the end of exercise and a return to normal a day after exercise. Serum creatine phosphokinase also rose significantly during exercise but returned more slowly to normal level. Similar results were observed in a study by Vejjajiva and Teasdale (112) who measured the serum phosphokinase level in 12 healthy young medical students or doctors who underwent strenuous physical exercise by playing rugby. The effect of exercise on serum creatine level was studied in rats by Gsell, Von Hohn, and Schank (64). These research workers were motivated to do this study because of their previous observations (65) of muscular dystrophy among aged laboratory rats— which are usually kept without physical activity. Thus, 48 such rats were exercised for 12 consecutive months. The animals were run on a machine built as a conveyor belt at a speed of 14 miles per minute for 30 minutes, with a short rest after 15 minutes, six times a week. The results indicated that during exercise serum creatine values increased in good runners and either decreased or remained unchanged in poor runners. Another interesting observation was that serum creatine levels at rest were higher in poor runners than in good runners. These results give some insight into the problem of creatinuria due to immobilization.

A great deal of research data is now available which indicate that urinary creatinine excretion is uninfluenced by physical activity.

As early as 1908, Cathcart, Kennaway, and Leathes (29) stated that creatinine excretion was not altered before and after severe exertion in man. In a later report (30), which was performed on his co-author, no appreciable changes were observed during pre-work, work, and post-work periods regarding daily creatinine excretion. During the six-day work period, the subject performed a given amount of work on a hand lever ergometer. These results agree with the observations made by Hobson (79) on students engaged in various kinds of active sports.

Mitchell and Kruger (86) described an extensive study on rats. Eight rats were subjected to a five-day work period after a four-day rest period. Their physical activity was rigorously restricted by a small piece of wire mesh bent around the body. In the work period, they were put in a cylindrical cage revolving on a horizontal axis. The urine was collected by placing a suitably equipped pan under the cage. The results were consistent with the other studies in that, no variations in creatinine output could be attributed to increased physical activity. Carpentier and Brigaudet (27) found that athletic and non-athletic subjects did not differ significantly, in their response to physical activity with respect to the daily creatinine elimination. Garry (57) showed that no appreciable changes in creatine and creatinine excretion in man followed voluntary or involuntary muscular activity. Furthermore, tremor in the involuntary muscles of arms and legs was induced by pulling against powerful springs.

MUSCULAR ATROPHY AND CREATINURIA DUE TO DISUSE AND IMMOBILIZATION

The effect of immobilization on various metabolic and physiologic functions of the body has been a subject of interest since World War II, and of even greater interest since the beginning of man's exploration of space. In the past bed rest was used as a part of the prescribed treatment to speed convalescence and rehabilitation. In 1945, William (122) summarized some of the undesirable and detrimental effects of this practice, such as: phlebothrombosis, pulmonary embolism, hypostatic pneumonia, decubitus ulcers, constipation, myosthenia, osteoporosis, and nephrolithiasis.

In order to differentiate the effects of bed rest, as such, from effects which might arise from disease or trauma, Deitrick and others (40) undertook an extensive study in which four healthy adult men volunteers were immobilized for a period of six to seven weeks in bivalve casts which extended from the toes to the umbilicus. Under these circumstances, numerous metabolic and physiologic disturbances were observed. These included: a decrease in muscle mass, as much as an average of five pounds for each subject (calculated on the basis of nitrogen loss); a diminution of muscular strength; and the progressive reduction in creatine tolerance— with no significant changes in creatine and creatinine excretions.

When three of the subjects in a later study (120) were immobilized in an oscillation bed, which probably caused slight muscle contraction, definite improvements were observed. The decline in creatine tolerance, muscle mass, and muscular strength was less in this study.

In an earlier study by Chor and Dolkart (32), six young Macacas Rhesus monkeys were rested in a body and leg cast for one to 10 weeks. The leg that was not rested served as the control. Atrophy in the gastrocnemius-soleus muscle was measured by dissection and subsequent weighing. The results showed an increase in the percentage of muscular atrophy with an increase in the length of the immobilization period. In addition, the atrophied muscle appeared paler than that from the control side. Microscopic examination revealed that the muscle fibers from the former were more narrow in a cross section and more prominently straiated in a longitudinal section. The Q bands appeared denser than normal.

Muscular atrophy due to disuse has been extensively studied in animals by denervation (43) (84) (82). Reid (91) compared the effect of disuse and denervation upon the gastrocnemius muscle of the cat. He found that muscular atrophy is more pronounced in denervated muscle than in disused muscle. In a careful study by Fudema, Fizzell, and Nelson (54), the hind limbs of 10 cats were bilaterally immobilized by

an external fixation apparatus for a period of 101 days. The course of disuse atrophy was followed with the help of electromyograms. As expected, gradual, but highly significant decreases in electrical output were recorded. In addition, a decrease in the motor unit potential indicated a reduction in the mass of individual muscle fibers.

From the above studies, it is apparent that muscular atrophy could be expected during long space flights. At least partial solutions to many of the problems can be accomplished on earth by continued research using simulated hypodynamic states. Thus, bed rest and water immersion studies have become a vital part of the huge man-in-space program.

Graveline and Blake (61) and Graybiel and Clark (62) have reported the results of water immersion studies. The subjects were floated in a specially constructed water tank. After seven days of continuous water immersion, the subject of the study by Graveline and Blake exhibited decreased muscle tone, whereas muscle strength was maintained fully in all three men who participated in the study by Graybiel and Clark. However, the endurance was reduced in two of the three subjects as measured by treadmill. In this study the subjects were immersed in water for 10 hours every day for two weeks and the rest of the hours were spent in bed.

CREATINURIA DURING BED REST

In 1955, Heilskov and Schonheyder (71) reported that all three of their adult male subjects excreted significantly higher amounts of creatine in the urine during bed rest than during periods before and after bed rest. Recent research at Texas Woman's University also has revealed this fact (31) (33) (76). Schonheyder and Christensen (100) studied the possible mechanism of creatinuria due to bed rest. One gram of N^{15}-labeled glycine was administered to a 21 year old adult male on the eleventh day of a two weeks bed rest period. The subject was receiving a creatine-free diet. Creatine and creatinine were isolated as creatinine zinc chloride and analyzed for labeled nitrogen. The results showed a greater concentration of the isotope in the creatine than in the creatinine. These observations were similar to those of Benedict and co-workers (7) who studied the mechanism of creatinuria in patients with muscular dystrophy. Thus, during bed rest as in muscular dystrophy, there is diminished uptake of creatine by the muscle tissues resulting in the elimination of synthesized creatine in the urine.

CREATININE EXCRETION DURING IMMOBILIZATION

As early as 1908, Shaffer (102) studied the effect of diminished muscular activity on protein metabolism. During the six-day rest period, one subject had two days of complete bed rest and four days of partial bed rest during which he was allowed to spend a few hours each

day on a Morris chair. Another subject mostly spent all six days in bed. Nitrogen excretion increased during the bed rest period and decreased with increased activity. However, creatinine excretion did not fluctuate with muscular activity. This behavior of creatinine is being shown repeatedly in immobilization studies.

Birkhead and others (9) confined four healthy men to bed for forty-two days. During the 18 days before and after the bed rest period, the subjects took exercise on a bicycle ergometer twice daily for one-half hour. Of the compounds tested, urinary excretion of catecholamines, 17-ketosteroids, and creatinine did not show any consistent changes.

In the study by Cuthbertson (38), eight volunteer subjects, either in good health or with loose cartilage in the knee joint, were immobilized to various degrees. The urinary creatinine excretion was nearly constant in all the subjects throughout the study.

In an interesting study by Hale, Ellis, and Williams (67) the urinary creatinine values before and after 12 hours of simulated flight did not show significant changes for the 48 men who participated.

From this review of studies on immobilization, it is evident that gravitational force and activity play a vital role in maintaining the health and well-being of the skeletal muscle and the body as a whole.

Brannon, Rockwood, and Potts (19) have recently shown that a small amount of exercise may be enough to prevent the deleterious effects of relative inactivity on bone and muscle. The authors selected 30 men between the ages of 18 and 22 years and divided them into five groups. The subjects were confined to a 60 day period of varying activity and bed rest. The following are some details of their study:

CATEGORIES AND EXERCISES	
Category I:	Normal activity on isolated ward. Performed 5 BX twice daily (11 minute periods).
Category II:	Bed rest and isotonic exercises with weights.
Category III:	Bed rest and isotonic exercises without weights.
Category IV:	Bed rest and isometric (or dynamic tension) exercises.
Category V:	Bed rest and no exercise routine. Movements limited to turning, sitting, eating, washing, handwork, and bed pan.

NOTE: Isotonic and isometric exercises done for ten minute periods either 2, 4, or 6 times daily.

Based on their results, these investigators think that isometric exercises would be more suitable during space ventures.

An experimental chair which makes exercise possible in space flights has been described by Potts and Bowring (89).

PLAN OF PROCEDURE

The three studies which are reported in this disseration were 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 author has chosen one aspect of the studies, namely, creatine and creatinine excretion analyses during the bed rest periods. Descriptions are given of the three units of study: 1) bed rest with and without exercise, 2) bed rest with and without the special suits, and 3) immobilization-exercise study on primates. The details of each of these investigations, as well as the analytical procedures for creatine and creatinine are presented.

BED REST STUDY WITH AND WITHOUT PLANNED EXERCISE

(January 3 - March 21, 1966)

This study consisted of two 14-day Bed Rest Periods, each of which was preceded by a Pre-Bed Rest equilibration Period followed by a final reconditioning Period. Four healthy male subjects were chosen after extensive physical and psychological examinations. Of these subjects, two remained for the second Bed Rest unit. The age, weight,

height, as well as the former occupation of each of the subjects are given below.

Subject	Age (years)	Height (cm)	Weight (kg)	Former Occupation
G	42	73	200	Retired, U.S. Air Force member
L	35	71	201	Construction worker
P*	22	68	140	Sporting goods salesman
V*	21	68	170	University student

The subjects stayed in the metabolic ward of the Research Institute throughout the entire period of study. Specially trained dietitians planned the nutritionally adequate diets. These consisted of natural foods low in residue content. Weighed portions were served each subject, and plate wastes or spilled particles were weighed and recorded. Aliquots of the diets were analyzed for calcium and phosphorus and daily nutrient intakes determined for energy and other nutrients, using the computer technique for dietary calculation employed by the Texas Woman's University Research Institute. The basic dietary statistical cards are kept current as new foods and data on food composition become available. The daily intake of various nutrients are kept as nearly

*The two subjects who remained for the second bed rest

constant as possible throughout the study. The daily dietary specifications for all subjects were:

Calories	2,800
Protein	90 - 100 grams
Fat	120 grams
Carbohydrates	280 grams
Iron	18 - 20 milligrams
Calcium	800 milligrams
Magnesium	250 milligrams
Vitamin A	10,000 International Units
Vitamin D	400 International Units
Ascorbic acid	100 - 150 milligrams
Thiamine	1.6 milligrams
Riboflavin	1.8 milligrams
Niacin	21 milligrams

The subjects led a normal life during the 17-day Pre-Bed Rest Periods. They worked eight hours a day in one of the laboratories of the Research Institute. Throughout each of the Bed Rest Periods, the subjects remained recumbent in a horizontal position on a single bed with only one pillow. They were not allowed to raise their heads from the pillow, and the movements of arms and legs were very limited. They could read and watch television by using glasses equipped with

prismatic lenses. All of their hygienic needs were taken care of by trained orderlies. They were spoon-fed by the dietitians.

The subjects who participated throughout the entire study had four additional days of Post-Bed Rest, making a total of 18 days. The first Post-Bed Rest Period served as a reconditioning phase for the first Bed Rest. It also served as an equilibration period for the second Bed Rest. A planned exercise program was carried out during the Second Bed Rest, but otherwise there were no changes.

ISOMETRIC EXERCISE

Isometric exercise was described by Brannon and his associates (19) as follows.

"The length of the muscle group remains essentially the same during contraction against a fixed resistance and a portion of the liberated energy is converted to tension."

The resistance of the antagonist muscle contracting against a prime mover or the resistance of the bed was the theoretical basis of the isometric exercises employed.

Two types of isometric exercises were practiced. In the first type, the subject grasped his hands behind his head and tried to pull them apart with maximum strength applied for 60 seconds. In the

second exercise, the subject held his entire body rigid for 60 seconds. Each subject performed both exercises four times daily during the second 14-day Bed Rest.

ISOTONIC EXERCISE

Brannon and associates (19) also defined isotonic exercise as follows:

"The shortening of muscle group during contraction against a movable load in which a portion of the liberated energy is converted to work."

This exercise was executed by utilizing a special device which was developed by Dietlein and Rapp at the Manned Spacecraft Center, Houston, Texas. The device with its major parts is shown in Figure 1. Both upper and lower extremities can be exercised by stretching the rubber bungee cords, either by foot or by hand. In the present study, the upper extremities were exercised by having to stretch the exercising device by placing the feet in the nylon straps and holding the handle with the hands in a static position. The nylon-steel stop cable maintained the fixed isotonic work load of each pull by limiting the stretch of the rubber bungee cords. Each exercise period lasted for 30 seconds during which the subjects had to stretch and release 20 times as described. This was repeated four times each day.

The isometric and isotonic exercises described above and employed in this study were the same as those practiced by the Gemini VII astronauts throughout their 14-day orbital mission. This was the purpose for which this exerciser was designed at the National Aeronautics and Space Administration, Manned Spacecraft Center, Houston, by Dr. Lawrence Dietlein and Miss Rita Rapp.

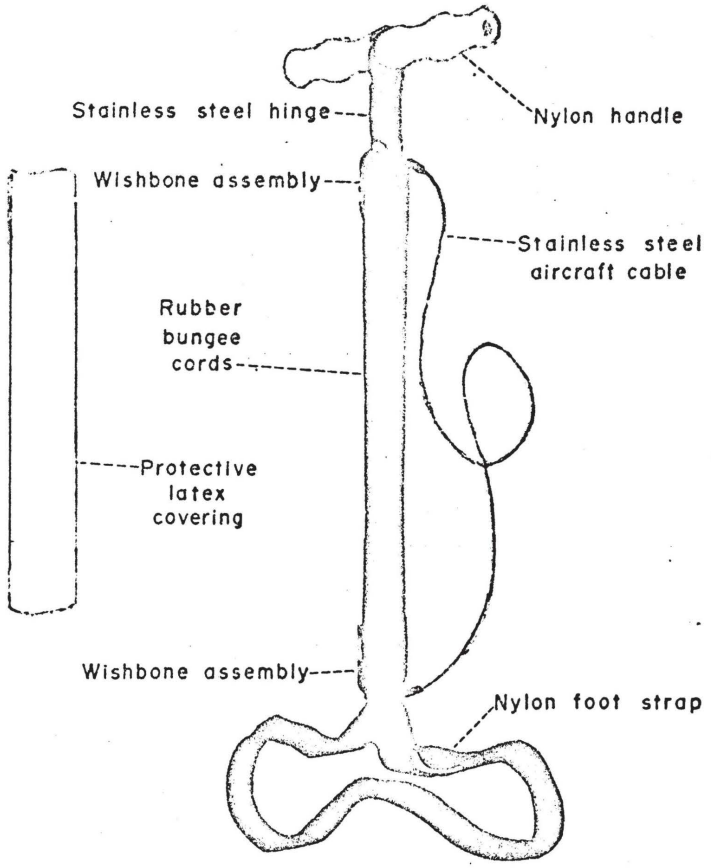


FIGURE 1. ISOTONIC EXERCISER DEVICE WITH MAJOR COMPONENTS CITED

RESULTS AND DISCUSSION

CREATINE EXCRETION

The data obtained on daily urinary excretion of creatine and creatinine for all of the subjects in this study were analyzed statistically using the "t" test. The average daily excretion values for each subject during the various periods and the results of the statistical analysis are given in Tables I - XVI.

Tables I through IV give the basic data on the analytical findings with respect to urinary creatine.

SUBJECT G

The average daily creatinine excretion for this subject during Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods was slightly above the normal range that was reported by Cantarow and Trumper (25). Creatine excretion showed marked changes from one period to another. As can be seen in Table I, this subject excreted significantly higher amounts of creatine during the Bed Rest immobilization Period than during the periods before and after Bed Rest. The average daily excretion during the Bed Rest Period was 156 milligrams as compared to 14 milligrams per day during the period before Bed Rest. This difference was statistically significant ($P < 0.02$). Following the Bed Rest Period,

there was a decline in the amount of creatine excreted, although the change in value showed very little significance when analyzed statistically.

These findings on creatine excretion are typical of Bed Rest immobilization. Subject G participated only in the first Bed Rest unit when no exercise program was included. See Table V.

SUBJECT L

The average creatine values for the Pre-Bed Rest, the Bed Rest, and the Recovery Periods were 72, 133, and 77 milligrams per day, respectively. The increase in the creatine excretion during the Bed Rest Period, however, was not statistically significant. The decrease in the output during the Post-Bed Rest Period also was not significant. See Table VI.

SUBJECT P

From the tables on this subject, it may be seen that there were no statistically significant differences between the two Bed Rest Periods with respect to the daily creatine excretion of Subject P. Although there was a decrease in creatine excretion following each Bed Rest Period, the difference observed was not statistically significant. The increased creatine output during the Bed Rest Periods 1 and 2 were statistically significant as compared with the equilibration period ($P < 0.05$, and $P < 0.01$, respectively). See Table VII.

SUBJECT V

Statistical comparisons of the average daily urinary creatine excretion show that the creatine excretion during the Bed Rest with exercise did not differ significantly from that of the first Bed Rest for this subject. Apparently, the addition of isometric and isotonic exercises to the Bed Rest immobilization did not reduce substantially the creatinuria observed during the Bed Rest without exercise.

The average daily creatine excretion was lowest during the initial equilibration period and highest during the final ambulatory period. The increased creatine excretion during the first and the second Bed Rest Periods were highly significant when compared separately with the values obtained during the Pre-Bed Rest Period ($P < 0.02$ in each case). See Table VIII.

TABLE I

URINARY CREATINE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: G

Equilibration Period	Bed Rest Period	Ambulatory Period
1) 000	18) 000	32) 487
2) 000	19) 000	33) 000
3) 000	20) 000	34) 12
4) 000	21) 000	35) 000
5) 000	22) 000	36) 000
6) 000	23) 47	37) 000
7) 000	24) 396	38) 100
8) 000	25) 58	39) 000
9) 000	26) 000	40) 000
10) 000	27) 386	41) 261
11) 000	28) 445	42) 387
12) 000	29) 610	43) 139
13) 541	30) 447	44) 236
14) 000	31) 000	45) 000
15) 000		
16) 000		
17) 000		

TABLE II

URINARY CREATINE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: L

Equilibration Period	Bed Rest Period	Ambulatory Period
1) 000	18) 000	32) 000
2) 000	19) 000	33) 000
3) 000	20) 000	34) 000
4) 000	21) 000	35) 000
5) 000	22) 000	36) 56
6) 420	23) 42	37) 000
7) 000	24) 670	38) 22
8) 384	25) 000	39) 000
9) 000	26) 000	40) 000
10) 000	27) 390	41) 192
11) 000	28) 253	42) 134
12) 000	29) 000	43) 387
13) 000	30) 197	44) 138
14) 63	31) 000	45) 144
15) 299		
16) 000		
17) 000		

TABLE III

URINARY CREATINE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: P

Equilibration Period	Bed Rest Period	Interim-Ambulatory Period	Bed Rest with Exercise Period	Final Ambulatory Period
1) 000	18) 000	32) 94	50) 000	64) 60
2) 000	19) 212	33) 000	51) 141	65) 158
3) 000	20) 000	34) 000	52) 000	66) 000
4) 000	21) 000	35) 000	53) 280	67) 70
5) 000	22) 000	36) 000	54) 685	68) 183
6) 000	23) 217	37) 000	55) 000	69) 331
7) 000	24) 99	38) 18	56) 000	70) 159
8) 000	25) 000	39) 000	57) 96	71) 748
9) 000	26) 000	40) 000	58) 000	72) 000
10) 000	27) 33	41) 184	59) 000	73) 000
11) 72	28) 699	42) 95	60) 94	74) 000
12) 000	29) 233	43) 000	61) 595	75) 000
13) 77	30) 000	44) 166	62) 246	76) 75
14) 58	31) 000	45) 189	63) 203	77) 000
15) 000		46) 295		
16) 000		47) 118		
17) 000		48) 114		
		49) 237		

TABLE IV

URINARY CREATINE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: V

Equilibration Period	Bed Rest Period	Interim-Ambulatory Period	Bed Rest with Exercise Period	Final Ambulatory Period
1) 000	18) 000	32) 35	50) 000	64) 133
2) 000	19) 000	33) 000	51) 264	65) 000
3) 000	20) 000	34) 000	52) 000	66) 295
4) 000	21) 000	35) 32	53) 154	67) 000
5) 000	22) 000	36) 000	54) 000	68) 227
6) 000	23) 000	37) 000	55) 000	69) 177
7) 000	24) 186	38) 000	56) 000	70) 39
8) 000	25) 000	39) 000	57) 000	71) 870
9) 000	26) 000	40) 000	58) 000	72) 000
10) 000	27) 227	41) 182	59) 000	73) 45
11) 000	28) 204	42) 117	60) 155	74) 000
12) 000	29) 179	43) 67	61) 53	75) 000
13) 000	30) 356	44) 29	62) 123	76) 81
14) 000	31) 000	45) 000	63) 58	77) 000
15) 000		46) 50		
16) 000		47) 167		
17) 52		48) 109		
		49) 102		

TABLE V

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: G

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest	14 156	Bed Rest	$P<0.02$
1 - 3	Pre-Bed Rest vs Post-Bed Rest	14 116	Post-Bed Rest	$P<0.05$
2 - 3	Bed Rest vs Post-Bed Rest	156 116	Bed Rest	N.S.

TABLE VI

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: L

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest	72 133	Bed Rest	N.S.
1 - 3	Pre-Bed Rest vs Post-Bed Rest	72 77	Post-Bed Rest	N.S.
2 - 3	Bed Rest vs Post-Bed Rest	133 77	Bed Rest	N.S.

TABLE VII

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: P

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	12 120	Bed Rest 1	$P<0.05$
1 - 3	Pre-Bed Rest vs Interim Period	12 73	Interim Period	$P<0.01$
1 - 4	Pre-Bed Rest vs Bed Rest 2	12 187	Bed Rest 2	$P<0.01$
1 - 5	Pre-Bed Rest vs Post Bed Rest	12 127	Post Bed Rest	$P<0.05$
2 - 3	Bed Rest 1 vs Interim Period	120 73	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	120 187	Bed Rest 2	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	120 127	Post-Bed Rest	N.S.
3 - 4	Interim Period vs Bed Rest 2	73 187	Bed Rest 2	$P<0.1$
3 - 5	Interim Period vs Post-Bed Rest	73 127	Post-Bed Rest	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	187 127	Bed Rest 2	N.S.

TABLE VIII

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: V

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	3 82	Bed Rest 1	$P<0.02$
1 - 3	Pre-Bed Rest vs Interim Period	3	Interim Period	$P<0.01$
1 - 4	Pre-Bed Rest vs Bed Rest 2	3 79	Bed Rest 2	$P<0.02$
1 - 5	Pre-Bed Rest vs Post-Bed Rest	3 133	Post-Bed Rest	$P<0.05$
2 - 3	Bed Rest 1 vs Interim Period	82 47	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	82 79	Bed Rest 1	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	82 133	Post-Bed Rest	N.S.
3 - 4	Interim Period vs Bed Rest 2	47 79	Bed Rest 2	N.S.
3 - 5	Interim Period vs Post-Bed Rest	47 133	Post-Bed Rest	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	79	Post-Bed Rest	N.S.

CREATININE EXCRETION

SUBJECT G

Tables IX through XII give the basic data on the findings of creatinine in the urine for the respective subjects.

Statistical analysis of the data on creatinine indicated that there were no significant differences between the periods of this study with respect to creatinine insofar as Subject G was concerned. See Table XIII.

SUBJECT L

The average daily creatinine excretion during all three periods of the first unit of the study for Subject L fell slightly above the normal range, which is 1,500 to 2,000 milligrams as reported by Cantarow and Trumper (25). Also, there was no statistically significant difference between the excretion of creatinine during the three units of the part of the study in which this subject took part. See Table XIV.

SUBJECT P

The highest mean value of daily creatinine excretion was observed during the second Bed Rest, as was true also for creatine. This was not significantly different from the other periods of the study, however. There was a statistically significant difference in the creatinine output in the first Bed Rest when compared to the interim period

and to the Post-Bed Rest ($P < 0.05$ and $P < 0.01$, respectively). See Table XV.

SUBJECT V

The average daily creatinine excretion during Bed Rest with exercise was 2,160 milligrams and without exercise was 2,406 milligrams. The probability that this was a real difference was less than 10 per cent and this can be considered as insignificant. The creatinine excretion during the second Bed Rest did not vary to any extent from all the periods of Bed Rest of both studies. A highly significant decrease in creatinine excretion was observed during the Post-Bed Rest Period when compared with the creatinine excretion of the first Bed Rest ($P < 0.001$). The decrease during the interim period following this Bed Rest was also significant ($P < 0.01$). Except for these two comparisons, it can be said that creatinine excretion did not vary greatly between the various periods of the study. See Table XVI.

The two subjects who continued on the study for the second Bed Rest of this series showed marked creatinuria during the second Bed Rest, in spite of the fact that they performed planned isometric and isotonic exercises four times a day. No statistically significant differences were observed between Bed Rest 1 and Bed Rest 2 with regard to creatine excretion for the two subjects. However, non-significant differences in creatine excretion were noticed during the interim period—

which served as the equilibration period for this Bed Rest unit as compared with Bed Rest 2. It would appear that the amount of exercise performed was insufficient to combat the creatinuria presumably due to overall inactivity.

The daily creatinine excretion did not vary greatly between the Bed Rest 2 Periods for Subjects P and V when the data were statistically analyzed. Slight, but non-significant differences, however, were noticed between the two Bed Rest Periods in both subjects.

Although the exercises performed were insufficient to decrease the creatine excretion, the results for the total calcium excretion by the two subjects during Bed Rest 2 surpassed that which was found in Bed Rest 1 with no performed exercise by a statistically significant difference. In addition, better retention of bone mass was observed through extensive densitometric measurements of radiographs.

In a similar study by Brannon, Rockwood, and Potts (19), an evaluation was made of planned isometric and isotonic exercises in preventing the musculo-skeletal disorders during a 60-day Bed Rest Period on 30 healthy male volunteers. Although creatine, creatinine, calcium and other substances were measured in the excreta, the results were not reported because of the inability to carry out precise metabolic studies on 30 subjects. Nevertheless, they discussed the effect of the exercise in sustaining muscular strength and muscle mass during

the bed rest period. No definite conclusions could be drawn by the authors from the results, although they noticed that the subjects who carried out the exercise routines had slight losses in strength and mass of the thigh while the subjects with no exercise schedule experienced substantial decreases during the bed rest. All of the bed rest subjects, with or without the exercise program, showed a decrease in the girth and the strength of the calf muscles. The severity of these physiological changes decreased with the increase in the amount of exercises of the types mentioned. The authors felt that isometric exercise is preferable in space flights since they do not require a special device or equipment. They called for more comparative exercise studies as a prerequisite to intelligent planning of long space ventures.

CREATINE AND CREATININE EXCRETION BY ALL THE SUBJECTS IN THE FIRST BED REST

The presence of an increased amount of creatine in the urine during the Bed Rest immobilization Period was noticed in all the four subjects as compared to the Pre- and Post-Bed Rest Periods. These differences were not significant (except for one subject) when compared with the Pre-Bed Rest Period and were significant in all subjects in comparison with the Post-Bed Rest Period.

With regard to the creatinine excretion, no statistically significant differences were obtained between Bed Rest and Pre-Bed Rest

Periods for three subjects and only a slightly significant difference in one subject ($P < 0.10$). Varying degrees of significance were noticed when the Bed Rest Period was compared with the Post-Bed Rest Period in all subjects except one. However, between the Periods of equilibration and recovery, the differences observed for all the subjects were found to be of no significance when the data were pooled.

These findings are in agreement with the previous Bed Rest studies conducted at the TWU Research Institute (31) (33), and those reported by other laboratories (71) (9) (38).

TABLE IX

URINARY CREATININE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: G

Equilibration Period	Bed Rest Period	Ambulatory Period
1) 3000	18) 2564	32) 2196
2) 2400	19) 2822	33) 2317
3) 2297	20) 2420	34) 2286
4) 2394	21) 2943	35) 1568
5) 2701	22) 2055	36) 4733
6) 3501	23) 2964	37) 2372
7) 2500	24) 2629	38) 1898
8) 2208	25) 2625	39) 2252
9) 2251	26) 2153	40) 2392
10) 2697	27) 2304	41) 2054
11) 2194	28) 2560	42) 2260
12) 2322	29) 2518	43) 2136
13) 2369	30) 2111	44) 1975
14) 2392	31) 2700	45) 1618
15) 2275		
16) 2830		
17) 2509		

TABLE X

URINARY CREATININE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: L

Equilibration Period	Bed Rest Period	Ambulatory Period
1) 1490	18) 2349	32) 2376
2) 1130	19) 2700	33) 2350
3) 2744	20) 2383	34) 2407
4) 2574	21) 2332	35) 1921
5) 2664	22) 2769	36) 2076
6) 1484	23) 2311	37) 1776
7) 2570	24) 2910	38) 2734
8) 2147	25) 2761	39) 1930
9) 1947	26) 2156	40) 2496
10) 2608	27) 2256	41) 1818
11) 2289	28) 2632	42) 2270
12) 2442	29) 3950	43) 1908
13) 2143	30) 2291	44) 1607
14) 2249	31) 2933	45) 2250
15) 2250		
16) 815		
17) 2620		

TABLE XI

URINARY CREATININE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: P

Equilibration Period	Bed Rest Period	Interim-Ambulatory Period	Bed Rest with Exercise Period	Final Ambulatory Period
1) 1070	18) 3441	32) 1673	50) 1928	64) 2128
2) 1960	19) 2253	33) 2023	51) 4211	65) 1955
3) 1872	20) 1946	34) 2042	52) 3296	66) 1601
4) 1989	21) 2016	35) 2052	53) 1786	67) 1499
5) 2029	22) 1961	36) 2343	54) 2698	68) 1499
6) 2040	23) 1942	37) 1820	55) 1618	69) 1921
7) 2100	24) 2189	38) 1234	56) 1737	70) 1617
8) 1795	25) 2430	39) 2450	57) 2224	71) 1290
9) 2394	26) 2022	40) 2020	58) 1538	72) 2067
10) 2258	27) 1876	41) 1735	59) 3263	73) 1797
11) 2050	26) 2567	42) 2037	60) 2091	74) 1616
12) 1424	29) 2059	43) 1943	61) 1435	75) 1968
13) 1989	30) 1945	44) 1647	62) 1670	76) 1166
14) 2161	31) 2533	45) 1855	63) 1853	77) 2411
15) 1870		46) 1896		
16) 2337		47) 1921		
17) 2140		48) 1804		
		49) 1859		

TABLE XII

URINARY CREATININE EXCRETION

(Milligrams Per 24 Hours)

SUBJECT: V

Equilibration Period	Bed Rest Period	Interim Ambulatory Period	Bed Rest with Exercise Period	Final Ambulatory Period
1) 1000	18) 1989	32) 2175	50) 2366	64) 2185
2) 1410	19) 2367	33) 1026	51) 2282	65) 1702
3) 2358	20) 2141	34) 2286	52) 1931	66) 2242
4) 2440	21) 2119	35) 1446	53) 1627	67) 1974
5) 2037	22) 3073	36) 2578	54) 2882	68) 1833
6) 2044	23) 2340	37) 4758	55) 1904	69) 2303
7) 2641	24) 2250	38) 1615	56) 2003	70) 1514
8) 2170	25) 2514	39) 2327	57) 2318	71) 1400
9) 2457	26) 2570	40) 1492	58) 1664	72) 1642
10) 2386	27) 2165	41) 1834	59) 2516	73) 2008
11) 2168	28) 2598	42) 1306	60) 2362	74) 2146
12) 1968	29) 2299	43) 1420	61) 1854	75) 2241
13) 2923	30) 2255	44) 1290	62) 2141	76) 2101
14) 1707	31) 2958	45) 2268	63) 2388	77) 1071
15) 2205		46) 2190		
16) 2999		47) 1896		
17) 2315		48) 2162		
		49) 1472		

TABLE XIII

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: G

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest	2529 2526	Pre-Bed Rest	N.S.
1 - 3	Pre-Bed Rest vs Post-Bed Rest	2529 2290	Post-Bed Rest	N.S.
2 - 3	Bed Rest vs Post-Bed Rest	2526 2290	Post-Bed Rest	N.S.

TABLE XIV

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: L

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest	2252 2556	Bed Rest	N.S.
1 - 3	Pre-Bed Rest vs Post-Bed Rest	2252 2136	Pre-Bed Rest	N.S.
2 - 3	Bed Rest vs Post-Bed Rest	2556 2136	Bed Rest	$P<0.02$

TABLE XV

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: P

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1970 2223	Bed Rest 1	$P<0.1$
1 - 3	Pre-Bed Rest vs Interim Period	1970 1927	Pre-Bed Rest	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	1970 2239	Bed Rest 2	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1970 1752	Pre-Bed Rest	$P<0.1$
2 - 3	Bed Rest 1 vs Interim Period	2223 1927	Bed Rest 1	$P<0.05$
2 - 4	Bed Rest 1 vs Bed Rest 2	2223 2239	Bed Rest 2	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	2223 1752	Bed Rest 1	$P<0.01$
3 - 4	Interim Period vs Bed Rest 2	1927 2239	Bed Rest 2	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1927 1752	Interim Period	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	2239 1752	Bed Rest 2	$P<0.1$

TABLE XVI

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

(Milligrams Per 24 Hours)

SUBJECT: V

Populations	Periods Compared	Means	Which Period is Greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	2326 2406	Bed Rest 1	N.S.
1 - 3	Pre-Bed Rest vs Interim Period	2326 1977	Pre-Bed Rest	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	2326 2160	Pre-Bed Rest	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	2326 1883	Pre-Bed Rest	$P<0.01$
2 - 3	Bed Rest 1 vs Interim Period	2406 1977	Bed Rest 1	$P<0.1$
2 - 4	Bed Rest 1 vs Bed Rest 2	2406 2160	Bed Rest 1	$P<0.1$
2 - 5	Bed Rest 1 vs Post-Bed Rest	2406 1883	Bed Rest 1	$P<0.001$
3 - 4	Interim Period vs Bed Rest 2	1977 2160	Bed Rest 2	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1977 1883	Interim Period	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	2160 1883	Bed Rest 2	$P<0.1$

BED REST STUDY WITH AND WITHOUT GRAVITATIONAL ACCELERATION SIMULATION SUIT

(June 13 - September 18, 1966)

This 97-day study also consisted of two Bed Rest units. The possible beneficial effect of wearing a gravitational acceleration simulation suit during a 21-day Bed Rest Period was evaluated. This suit was designed by James Gatts, M.D., of the Republic Aviation Company, Farmingdale, Long Island, New York. The suit provided a work load both to the upper and lower body. It had a fixed point at the iliac crest and elastic components around the upper and lower body to provide a graded force on the musculoskeletal system. The force exerted by this suit was believed by the inventor to provide a gravity vector along the axis of the body when weights were added approximately equal to the weight of the subject's own body.

Four healthy adult male subjects wore the above described suit during the second Bed Rest Period of the investigation. The study was divided into the following periods:

1. A 17 to 25 day Equilibration Period.
2. A 21-day Bed Rest Immobilization Period, during which no suit was worn.
3. A 16-day Interim Ambulatory Period.
4. A 21-day Second Bed Rest Period, during which the

gravitational acceleration simulation suit was worn for about eight hours each day.

5. A 14-day final Reconditioning Ambulatory Period.

The dietary management and all other aspects during the above periods were the same as described for the first study except for the fact that the exercise garment replaced the exercises, with the Bed Rest Periods 21 days in length rather than 14 days.

The subjects of this study were the following:

Subject	Age (years)	Height (cm)	Weight (kg)	Former Occupation
W	22	155	77.0	Student
V	21	150	69.0	Student
P*	22	149	63.0	Salesman
S	46	150	95.5	Retired (USAF)

*This subject participated in the previous study.

RESULTS AND DISCUSSION

CREATINE EXCRETION

Tables XVII through XX give the analytical data on the subjects of this study concerning urinary creatine excretion.

SUBJECT P

The average urinary creatine excretion by this subject increased from 22 milligrams per day during the Pre-Bed Rest Period to 138 milligrams during Bed Rest Period 1. It was found that this increase in urinary creatine excretion was highly significant as compared with the Pre-Bed Rest Period ($P < 0.01$). This increase in creatine excretion was greatly reduced during the final Post-Bed Rest Period, as well as during Bed Rest 2, with significant differences in both cases ($P < 0.01$), when the gravitational acceleration simulation suit was worn.

The creatine excretion during Bed Rest 2 did not differ significantly from other periods of the study. See Table XXI.

SUBJECT S

This subject needed only 18 days of initial equilibration since he had been in earlier studies, with his calcium metabolism stabilized. Marked creatinuria was observed during Bed Rest 1 as compared with

other periods. This large creatine excretion was highly significant when compared with the Pre-Bed Rest, with Bed Rest 2, during which the subject wore the exercise garment, and the final ambulatory periods ($P < 0.001$ in all three cases). The average creatine excretion during Bed Rest 1 was 307 milligrams/day, whereas, it was 107 milligrams/day during Bed Rest 2. The mean value for the interim period was practically the same as that of the second Bed Rest. See Table XXII. Thus, it would seem that the gravitational acceleration simulation suit had a beneficial effect on creatine metabolism during prolonged inactivity, except for other factors discussed later.

SUBJECT V

Table XXIII gives the daily creatine excretion in milligrams for Subject V. It can be seen that, during the second Bed Rest Period this subject excreted no creatine in the urine except a trace amount on the last day. During the first Bed Rest, on the other hand, amounts differing significantly ($P < 0.01$) were excreted. The average daily creatine excretion during this period was 117 milligrams which was the highest for any of the periods. The mean value for the daily creatine excretion during Bed Rest 2 was lower than during the Interim and the Pre-Bed Rest Periods and almost equal to that of the Post-Bed Rest Period.

SUBJECT W

During the entire period of Bed Rest 2, not even a trace amount

of creatine was excreted by this subject. It was essentially zero for each day of this period, as may be seen in the basic data tables. The average daily creatine excretion during the first Bed Rest, on the other hand, was 110 milligrams with a range of 0 to 685 milligrams. The difference between the two Bed Rest Periods was highly significant ($P < 0.02$). Following the Bed Rest 1, there was a definite decrease in creatine excretion as shown in Table XXIV.

With all of the data for creatine excretion for the four subjects of this study pooled together (Table XXV), daily creatine excretion during the Pre-Bed Rest was surpassed by that for Bed Rest 1 by a difference which was highly significant ($P < 0.001$). The Pre-Bed Rest and the Interim ambulatory period differed slightly ($P < 0.05$), whereas there was a highly significant difference between Bed Rest 1 and the Interim Period, with a higher excretion level in Bed Rest 1.

The amount of creatine excreted during Bed Rest 1 exceeded that during Bed Rest 2 by a highly significant difference ($P < 0.001$). It should be stated in this connection that the treatment of the subjects was not standardized during Bed Rest 2 when the Gatts Suits were worn as they were during the regular bed rest units conducted in these laboratories or during Bed Rest 1 of this series. Bed Rest 1 was supervised by the Director of the Texas Woman's University Research Institute and associates, while Bed Rest 2 was supervised by the inventor of the

Gatts Suit and by an associate of his. Not only did the subjects during Bed Rest 2 not maintain the degree of horizontal recumbency as was maintained by them during Bed Rest 1 and during other TWU bed rest periods, but the subjects underwent considerable activity, including extensive exercise in two subjects through a part of the period. The exercise did not involve the parts of the body which were x-rayed, and did not result in preservation of the skeletal system insofar as it was radiographed. Isometric exercise of the arms and exercise of the legs in some subjects, however, undoubtedly affected the muscular system.

The body weights of the subjects showed but small changes, as shown in the following summary:

BODY WEIGHT RECORDS OF THE SUBJECTS

(pounds)

Subject	Initial Weight	Beginning of 1st Bed Rest	End of 1st Bed Rest	Beginning of 2nd Bed Rest	End of 2nd Bed Rest
P	142 1/4	140 3/4	140	138 1/2	138
S	167	165	166	166	165
V	161	156 1/2	155	152	151
W	177 1/4	174 1/2	170	169 1/4	166

TABLE XVII

URINARY CREATINE EXCRETION

(Milligrams per 24 Hours)

SUBJECT P

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 000	26) 000	47) 251	63) 000	84) 76
2) 000	27) 133	48) 93	64) 000	85) 000
3) 000	28) 54	49) 000	65) 000	86) 000
4) 000	29) 63	50) 000	66) 000	87) 000
5) 58	30) 306	51) 000	67) 000	88) 000
6) 000	31) 000	52) 176	68) 000	89) 000
7) 000	32) 000	53) 000	69) 000	90) 000
8) 17	33) 89	54) 000	70) 000	91) 000
9) 18	34) 613	55) 000	71) 000	92) 000
10) 173	35) 512	56) 000	72) 000	93) 000
11) 000	36) 146	57) 34	73) 000	94) 000
12) 000	37) 30	58) 347	74) 000	95) 000
13) 000	38) 000	59) 000	75) 000	96) 000
14) 91	39) 134	60) 000	76) 000	97) 000
15) 000	40) 71	61) 000	77) 000	
16) 000	41) 150	62) 000	78) 000	
17) 81	42) 000		79) 51	
18) 000	43) 372		80) 34	
19) 000	44) 109		81) 92	
20) 000	45) 000		82) 125	
21) 000	46) 113		83) 000	
22) 39				
23) 71				
24) 000				
25) 000				

TABLE XVIII

URINARY CREATINE EXCRETION

(Milligrams per 24 Hours)

SUBJECT S

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 000	18) 142	39) 280	55) 000	76) 81
2) 000	19) 286	40) 147	56) 59	77) 245
3) 000	20) 226	41) 152	57) 27	78) 000
4) 000	21) 67	42) 164	58) 000	79) 000
5) 000	22) 173	43) 350	59) 81	80) 56
6) 315	23) 626	44) 206	60) 47	81) 000
7) 000	24) 154	45) 000	61) 93	82) 000
8) 38	25) 186	46) 000	62) 115	83) 56
9) 110	26) 901	47) 31	63) 155	84) 93
10) 154	27) 847	48) 46	64) 000	85) 000
11) 33	28) 111	49) 000	65) 197	86) 113
12) 44	29) 93	50) 114	66) 000	87) 000
13) 000	30) 257	51) 94	67) 183	88) 000
14) 68	31) 223	52) 000	68) 219	89) 000
15) 96	32) 208	53) 96	69) 116	
16) 143	33) 421	54) 28	70) 000	
17) 187	34) 401		71) 182	
	35) 269		72) 259	
	36) 404		73) 133	
	37) 206		74) 138	
	38) 239		75) 235	

TABLE XIX

URINARY CREATINE EXCRETION

(Milligrams per 24 Hours)

SUBJECT V

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 000	26) 53	47) 21	63) 000	84) 000
2) 000	27) 27	48) 000	64) 000	85) 000
3) 000	28) 126	49) 123	65) 000	86) 000
4) 000	29) 90	50) 53	66) 000	87) 000
5) 000	30) 000	51) 000	67) 000	88) 000
6) 000	31) 186	52) 23	68) 000	89) 000
7) 000	32) 116	53) 000	69) 000	90) 000
8) 000	33) 35	54) 189	70) 000	91) 000
9) 000	34) 583	55) 000	71) 000	92) 000
10) 000	35) 536	56) 000	72) 000	93) 000
11) 10	36) 68	57) 000	73) 000	94) 000
12) 382	37) 23	58) 000	74) 000	95) 000
13) 000	38) 000	59) 000	75) 000	96) 000
14) 208	39) 000	60) 000	76) 000	97) 000
15) 197	40) 000	61) 16	77) 000	
16) 000	41) 450	62) 000	78) 000	
17) 000	42) 100		79) 000	
18) 000	43) 000		80) 000	
19) 000	44) 45		81) 000	
20) 000	45) 25		82) 000	
21) 000	46) 000		83) 35	
22) 000				
23) 27				
24) 000				
25) 000				

TABLE XX

URINARY CREATINE EXCRETION

(Milligrams per 24 Hours)

SUBJECT W

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 000	26) 19	47) 666	63) 000	84) 000
2) 000	27) 200	48) 000	64) 000	85) 000
3) 000	28) 000	49) 73	65) 000	86) 000
4) 000	29) 000	50) 000	66) 000	87) 49
5) 000	30) 50	51) 104	67) 000	88) 000
6) 000	31) 384	52) 163	68) 000	89) 30
7) 000	32) 45	53) 000	69) 000	90) 000
8) 000	33) 58	54) 000	70) 000	91) 90
9) 000,	34) 685	55) 000	71) 000	92) 000
10) 000	35) 590	56) 000	72) 000	93) 000
11) 000	36) 23	57) 232	73) 000	94) 14
12) 000	37) 55	58) 000	74) 000	95) 78
13) 000	38) 20	59) 51	75) 000	96) 000
14) 000	39) 000	60) 104	76) 000	97) 000
15) 000	40) 000	61) 000	77) 000	
16) 79	41) 50	62) 000	78) 000	
17) 000	42) 000		79) 000	
18) 000	43) 102		80) 000	
19) 000	44) 000		81) 000	
20) 000	45) 000		82) 000	
21) 000	46) 20		83) 000	
22) 000				
23) 149				
24) 138				
25) 000				

TABLE XXI

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY (Milligrams per 24 Hours)

SUBJECT P

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	22 138	Bed Rest 1	$P < 0.01$
1 - 3	Pre-Bed Rest vs Interim Period	22 56	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	22 14	Pre-Bed Rest	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	22 5	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	138 56	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	138 14	Bed Rest 1	$P < 0.01$
2 - 5	Bed Rest 1 vs Post-Bed Rest	138 5	Bed Rest 1	$P < 0.01$
3 - 4	Interim Period vs Bed Rest 2	56 14	Interim Period	N.S.
3 - 5	Interim Period vs Post-Bed Rest	56 5	Interim Period	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	14 5	Bed Rest 2	N.S.

TABLE XXII

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT S

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	66 307	Bed Rest 1	$P < 0.001$
1 - 3	Pre-Bed Rest vs Interim Period	66 107	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	66 107	Bed Rest 2	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	66 46	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	307 107	Bed Rest 1	$P < 0.01$
2 - 4	Bed Rest 1 vs Bed Rest 2	307 107	Bed Rest 1	$P < 0.001$
2 - 5	Bed Rest 1 vs Post-Bed Rest	307 46	Bed Rest 1	$P < 0.001$
3 - 4	Interim Period vs Bed Rest 2	107 107	Same	N.S.
3 - 5	Interim Period vs Post-Bed Rest	107 46	Interim Period	$P < 0.1$
4 - 5	Bed Rest 2 vs Post-Bed Rest	107 46	Bed Rest 2	$P < 0.05$

TABLE XXIII

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT V

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	33 117	Bed Rest 1	$P < 0.05$
1 - 3	Pre-Bed Rest vs Interim Period	33 27	Pre-Bed Rest	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	33 2	Pre-Bed Rest	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	33 0	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	117 27	Bed Rest 1	$P < 0.1$
2 - 4	Bed Rest 1 vs Bed Rest 2	117 2	Bed Rest 1	$P < 0.01$
2 - 5	Bed Rest 1 vs Post-Bed Rest	117 0	Bed Rest 1	$P < 0.02$
3 - 4	Interim Period vs Bed Rest 2	27 2	Interim Period	$P < 0.05$
3 - 5	Interim Period vs Post-Bed Rest	27 0	Interim Period	$P < 0.1$
4 - 5	Bed Rest 2 vs Post-Bed Rest	2 0	Bed Rest 2	N.S.

TABLE XXIV

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT W

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	15 110	Bed Rest 1	$P < 0.05$
1 - 3	Pre-Bed Rest vs Interim Period	15 87	Interim Period	$P < 0.05$
1 - 4	Pre-Bed Rest vs Bed Rest 2	15 0	Pre-Bed Rest	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	15 19	Post-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	110 87	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	110 0	Bed Rest 1	$P < 0.02$
2 - 5	Bed Rest 1 vs Post-Bed Rest	110 19	Bed Rest 1	$P < 0.1$
3 - 4	Interim Period vs Bed Rest 2	87 0	Interim Period	$P < 0.05$
3 - 5	Interim Period vs Post-Bed Rest	87 19	Interim Period	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	0 19	Post-Bed Rest	$P < 0.01$

TABLE XXV

STATISTICAL COMPARISONS OF URINARY CREATINE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

FOUR SUBJECTS, P, S, V, AND W

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	31 166	Bed Rest 1	$P < 0.001$
1 - 3	Pre-Bed Rest vs Interim Period	31 60	Interim Period	$P < 0.050$
1 - 4	Pre-Bed Rest vs Bed Rest 2	31 31	Same	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	31 26	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	166 60	Bed Rest 1	$P < 0.001$
2 - 4	Bed Rest 1 vs Bed Rest 2	166 31	Bed Rest 1	$P < 0.001$
2 - 5	Bed Rest 1 vs Post-Bed Rest	166 26	Bed Rest 1	$P < 0.001$
3 - 4	Interim Period vs Bed Rest 2	60 31	Interim Period	$P < 0.050$
3 - 5	Interim Period vs Post-Bed Rest	60 26	Interim Period	$P < 0.100$
4 - 5	Bed Rest 2 vs Post-Bed Rest	31 26	Bed Rest 2	N.S.

CREATININE EXCRETION

The basic data emanating from the analysis of urinary creatinine for this study are given in Tables XXVI through XXIX.

SUBJECT P

The average daily creatinine excretion during all the periods was within the normal range for Subject P. Significant differences were observed when the excretion values of the final ambulatory period were compared either with Bed Rest 1 or Bed Rest 2, with the final Post-Bed Rest lower in each case. On the whole, there was not much fluctuation in creatinine excretion from period to period. See Table XXX.

SUBJECT S

Table XXXI shows that the wearing of the Suits which were undergoing study did not alter the creatinine excretion in the urine as shown by a comparison of the two Bed Rest Periods. For this subject there was very little change in the excretion of this compound from one period to another.

SUBJECT V

Creatinine excretion again did not vary to any great extent in Subject V, as shown in Table XXXII. The excretion of this compound during Bed Rest 1 surpassed that of the Pre-Bed Rest Period by a significant

difference which was significant ($P < 0.01$), although the difference between Bed Rest Period 1 and Bed Rest Period 2 was minor ($P < 0.10$).

SUBJECT W

When the two bed rest periods were compared for this subject, (Bed Rest 1 with no suit worn and Bed Rest 2 with the experimental suit worn), there was a slight difference shown in the two cases with Bed Rest 1 higher ($P < 0.10$). There also were some differences shown between certain of the ambulatory periods for Subject W. See Table XXXIII.

COMPARISON OF PERIODS IN THE STUDY

FOR ALL SUBJECTS COMBINED

Table XXXIV gives a resume' of the statistical findings when the urinary creatinine data were pooled for all four subjects. The excretion during Bed Rest 1 surpassed that during the Pre-Bed Rest by a difference which was significant ($P < 0.01$). The creatinine excreted in the urine during the Interim ambulatory period between the two bed rest periods exceeded that during the Pre-Bed Rest Period ($P < 0.05$). There also was a significant difference between Bed Rest 1 and the final Post-Bed Rest ($P < 0.02$).

With respect to the comparison of the creatinine excretion between Bed Rest 1 and Bed Rest 2, with data from all four subjects combined, there was no significant difference found.

TABLE XXVI

URINARY CREATININE EXCRETION

(Milligrams per 24 Hours)

SUBJECT P

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 1860	26) 2208	47) 1767	63) 1839	84) 1897
2) 1980	27) 1837	48) 2092	64) 1939	85) 1843
3) 3696	28) 1906	49) 2038	65) 1462	86) 1617
4) 1876	29) 1956	50) 2538	66) 1961	87) 1474
5) 1695	30) 1872	51) 1814	67) 1336	88) 1231
6) 1676	31) 1912	52) 1791	68) 2245	89) 1196
7) 1834	32) 1751	53) 1198	69) 1931	90) 1937
8) 1477	33) 1773	54) 2250	70) 2182	91) 1559
9) 1674	34) 2029	55) 1700	71) 1846	92) 1900
10) 1544	35) 1829	56) 2041	72) 1783	93) 1437
11) 1842	36) 2054	57) 1661	73) 1818	94) 2049
12) 1685	37) 1289	58) 2015	74) 2038	95) 859
13) 1826	38) 1942	59) 1869	75) 1921	96) 881
14) 940	39) 1856	60) 1152	76) 1950	97) 1408
15) 1426	40) 2091	61) 1936	77) 1836	
16) 2131	41) 1857	62) 2136	78) 2036	
17) 1179	42) 2086		79) 1866	
18) 1235	43) 1898		80) 1928	
19) 1144	44) 1331		81) 1889	
20) 1352	45) 1813		82) 2196	
21) 1904	46) 2054		83) 1636	
22) 2103				
23) 1947				
24) 2081				
25) 1936				

TABLE XXVII

URINARY CREATININE EXCRETION

(Milligrams per 24 Hours)

SUBJECT S

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 1790	19) 1894	40) 1519	56) 1676	77) 1485
2) 1980	20) 1908	41) 1833	57) 1803	78) 1456
3) 1560	21) 1860	42) 1480	58) 1725	79) 2052
4) 1798	22) 1833	43) 1386	59) 2002	80) 1690
5) 2129	23) 1917	44) 1706	60) 1654	81) 1643
6) 1619	24) 1710	45) 1645	61) 1813	82) 3417
7) 788+	25) 1577	46) 1829	62) 1635	83) 2266
8) 1043	26) 695	47) 2033	63) 2099	84) 1525
9) 1089	27) 2160	48) 1742	64) 2074	85) 1794
10) 1292	28) 1702	49) 1876	65) 2042	86) 1739
11) 1197	29) 2054	50) 1802	66) 1701	87) 1735
12) 1550	30) 1296	51) 2073	67) 1638	88) 1865
13) 1458	31) 1831	52) 1809	68) 1777	89) 2381
14) 1830	32) 1680	53) 999+	69) 1871	90) 1699
15) 1990	33) 1920	54) 2049	70) 1407	
16) 1954	34) 1719	55) 2035	71) 2216	
17) 1968	35) 1851		72) 1734	
18) 1833	36) 1772		73) 1792	
	37) 2663		74) 1594	
	38) 1684		75) 1967	
	39) 2348		76) 1492	

TABLE XXVIII

URINARY CREATININE EXCRETION

(Milligrams per 24 Hours)

SUBJECT V

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 2051	26) 2334	47) 2040	63) 2013	84) 2135
2) 1782	27) 2031	48) 1564	64) 2143	85) 2000
3) 1821	28) 1965	49) 2016	65) 1930	86) 1737
4) 1910	29) 1640	50) 2998	66) 1512	87) 1943
5) 1470	30) 2473	51) 1850	67) 1650	88) 1252
6) 1767	31) 1667	52) 1814	68) 2205	89) 1428
7) 2339	32) 2003	53) 1836	69) 2089	90) 2099
8) 1302	33) 1889	54) 1948	70) 2160	91) 1793
9) 2073	34) 2417	55) 1455	71) 1124	92) 2046
10) 2349	35) 1831	56) 2070	72) 2218	93) 1743
11) 1251	36) 1920	57) 1797	73) 1929	94) 1859
12) 2222	37) 1852	58) 1814	74) 1861	95) 2030
13) 1817	38) 2049	59) 1817	75) 1768	96) 1942
14) 1512	39) 1904	60) 1344	76) 1730	97) 2141
15) 1504	40) 2165	61) 1728	77) 2178	
16) 1694	41) 1806	62) 1831	78) 940	
17) 840	42) 2270		79) 1978	
18) 1187	43) 1947		80) 1969	
19) 1767	44) 2169		81) 1890	
20) 2061	45) 2153		82) 1972	
21) 1573	46) 1940		83) 1731	
22) 2244				
23) 1887				
24) 1819				
25) 1444				

TABLE XXIX

URINARY CREATININE EXCRETION

(Milligrams per 24 Hours)

SUBJECT W

Equilibration Period	First Bed Rest	Interim Ambulatory Period	Second Bed Rest	Final Ambulatory Period
1) 1770	26) 1892	47) 1656	63) 2997	84) 1935
2) 2135	27) 2148	48) 1939	64) 2159	85) 1858
3) 2061	28) 2074	49) 2385	65) 1935	86) 2086
4) 2008	29) 1712	50) 2641	66) 2238	87) 1785
5) 1940	30) 2332	51) 2197	67) 1627	88) 1509
6) 2169	31) 1836	52) 2035	68) 1312	89) 1766
7) 2098	32) 1908	53) 1785	69) 2149	90) 1589
8) 1132	33) 1699	54) 2049	70) 1796	91) 2106
9) 1555	34) 2129	55) 3116	71) 1103	92) 1631
10) 2692	35) 1869	56) 2115	72) 2006	93) 1848
11) 2078	36) 2541	57) 2140	73) 1800	94) 2331
12) 1662	37) 1820	58) 1867	74) 1997	95) 1242
13) 2353	38) 2050	59) 1911	75) 1473	96) 2223
14) 1789	39) 1857	60) 1597	76) 1827	97) 1711
15) 838+	40) 2029	61) 1972	77) 2419	
16) 1844	41) 2256	62) 2599	78) 2030	
17) 1532	42) 1791		79) 1894	
18) 2085	43) 1661		80) 1736	
19) 1612	44) 2370		81) 1956	
20) 2017	45) 2001		82) 2141	
21) 1890	46) 2045		83) 1696	
22) 2097				
23) 1335				
24) 2518				
25) 2391				

TABLE XXX

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT P

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1762 1874	Bed Rest 1	N.S.
1 - 3	Pre-Bed Rest vs Interim Period	1762 1875	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	1762 1888	Bed Rest 2	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1762 1521	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	1874 1875	Interim Period	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	1874 1888	Bed Rest 2	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	1874 1521	Bed Rest 1	$P < 0.001$
3 - 4	Interim Period vs Bed Rest 2	1875 1888	Bed Rest 2	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1875 1521	Interim Period	$P < 0.02$
4 - 5	Bed Rest 2 vs Post-Bed Rest	1888 1521	Bed Rest 2	$P < 0.001$

TABLE XXXI

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT S

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1604 1813	Bed Rest 1	$P < 0.1$
1 - 3	Pre-Bed Rest vs Interim Period	1604 1738	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	1604 1796	Bed Rest 2	$P < 0.1$
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1604 1910	Post-Bed Rest	$P < 0.1$
2 - 3	Bed Rest 1 vs Interim Period	1813 1738	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	1813 1796	Bed Rest 1	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	1813 1910	Post-Bed Rest	N.S.
3 - 4	Interim Period vs Bed Rest 2	1738 1796	Bed Rest 2	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1738 1910	Post-Bed Rest	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	1796 1910	Post-Bed Rest	N.S.

TABLE XXXII

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT V

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1747 2020	Bed Rest 1	$P < 0.01$
1 - 3	Pre-Bed Rest vs Interim Period	1747 1870	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	1747 1857	Bed Rest 2	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1747 1868	Post-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	2020 1870	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	2020 1857	Bed Rest 1	$P < 0.1$
2 - 5	Bed Rest 1 vs Post-Bed Rest	2020 1868	Bed Rest 1	$P < 0.1$
3 - 4	Interim Period vs Bed Rest 2	1870 1857	Interim Period	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1870 1868	Interim Period	N.S.
4 - 5	Bed Rest 2 vs Post-Bed Rest	1857 1868	Post-Bed Rest	N.S.

TABLE XXXIII

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

SUBJECT W

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1904 2001	Bed Rest 1	N.S.
1 - 3	Pre-Bed Rest vs Interim Period	1904 2125	Interim Period	N.S.
1 - 4	Pre-Bed Rest vs Bed Rest 2	1904 1919	Bed Rest 2	N.S.
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1904 1830	Pre-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	2001 2125	Interim Period	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	2001 1919	Bed Rest 1	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	2001 1830	Bed Rest 1	$P < 0.1$
3 - 4	Interim Period vs Bed Rest 2	2125 1919	Interim Period	N.S.
3 - 5	Interim Period vs Post-Bed Rest	2125 1830	Interim Period	$P < 0.05$
4 - 5	Bed Rest 2 vs Post-Bed Rest	1919 1830	Bed Rest 2	$P < 0.01$

TABLE XXXIV

STATISTICAL COMPARISONS OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY
(Milligrams per 24 Hours)

FOUR SUBJECTS, P, S, V, AND W

Populations	Periods Compared	Means	Which period is greater?	Probability
1 - 2	Pre-Bed Rest vs Bed Rest 1	1766 1927	Bed Rest 1	$P<0.01$
1 - 3	Pre-Bed Rest vs Interim Period	1766 1902	Interim Period	$P<0.05$
1 - 4	Pre-Bed Rest vs Bed Rest 2	1766 1865	Bed Rest 2	$P<0.10$
1 - 5	Pre-Bed Rest vs Post-Bed Rest	1766 1782	Post-Bed Rest	N.S.
2 - 3	Bed Rest 1 vs Interim Period	1927 1902	Bed Rest 1	N.S.
2 - 4	Bed Rest 1 vs Bed Rest 2	1927 1865	Bed Rest 1	N.S.
2 - 5	Bed Rest 1 vs Post-Bed Rest	1927 1782	Bed Rest 1	$P<0.02$
3 - 4	Interim Period vs Bed Rest 2	1902 1865	Interim Period	N.S.
3 - 5	Interim Period vs Post-Bed Rest	1902 1782	Interim Period	$P<0.10$
4 - 5	Bed Rest 2 vs Post-Bed Rest	1865 1782	Post-Bed Rest	N.S.

IMMOBILIZATION AND EXERCISE STUDY ON PRIMATES

(February 23 - April 6, 1967)

The primates which were chosen for this study were of the species Macacas Nemestrina, popularly known as the pigtail monkey. Six monkeys were divided into three groups, one group of which served as a control; whereas, the remaining two groups were studied under two different experimental conditions, namely— restraint and exercise. The entire study consisted of three 14-day Periods: Equilibration, Immobilization or Exercise, and Ambulatory Periods.

All of the monkeys had freedom of cage activity during the Pre- and Post-Experimental Periods, differing only during the experimental period. During this period, two primates were immobilized in specially designed restraint couches. Movements of the arms and legs were prevented by keeping these in metal tubes. The remaining group was forced to exercise in their cages for one hour every day during the same length of time.

All of the animals were fed weighed amounts of Purina monkey chow once a day in the morning. Any left over food was weighed the following day. They were given water ad libitum. The immobilized monkeys were given the chow by forceps.

The collection of urine and feces was done in the usual way while the monkeys were in their metabolism cages. The restrained monkeys were catheterized during 14 days of the immobilization period for urine collection. Feces were collected in a container under the couches.

URINARY CREATINE EXCRETION

Tables XXXV and XXXVI give the basic data concerning the excretion of creatine by the two immobilized primates in the study described in this dissertation. These were Primate - 21 and Primate - 25. Tables XXXVII and XXXVIII give the analytical data concerning creatine excretion by Primates - 65 and 69, the exercised animals. Tables XXXIX and XL give the same type of data concerning Primate - 5 and Primate - 52, the two control animals in this investigation.

Tables XLI, XLII, and XLIII present the results of the statistical comparison of urinary creatine excretion between pairs of the different periods of the study by the three groups of experimental primates. Table XLIV shows the weights of the animals before and after the experimental period of the study.

RESTRAINED PRIMATES

The two immobilized animals, Primates - 21 and 25, were restrained in couches during a 14-day period, preceded by a 14-day ambulatory period, followed by 15 days of recovery. When the primates

which were restrained were considered together, they showed a marked increase in creatine excretion, by a difference from the Pre-Restraint Period which was statistically significant ($P < 0.02$). These animals were only slightly different in creatine excretion during the Pre-Restraint and Post-Restraint Periods ($P < 0.10$). On the other hand, the Restraint Period surpassed the Post-Restraint Period in urinary creatine excretion by a highly significant difference ($P < 0.01$).

The individual animals differed in some of their period comparisons, although the trends in both primates were similar. See Table XLI.

EXERCISED PRIMATES

Table XLII shows the results of the statistical comparisons which were made between the various periods of the study for the exercised animals. The lowest mean value of daily creatine excretion was obtained during the exercise period in both primates.

When the creatine values were pooled for both exercise animals, the creatine excretion levels during the exercise period was lower than during each ambulatory period. The Pre-Exercise Period surpassed the Exercise Period by a difference which was highly significant ($P < 0.01$).

CONTROL PRIMATES

With respect to the control animals (Primates - 5 and 52), creatine excretion was not high during any period of the study. During

the period when the restraint and exercise periods were in progress, there was no statistically significant difference between the creatine excretion by the control animals and the same animals during ambulation except by Primate - 5 when the first period was compared with the second ($P < 0.05$). See Table XLIII.

WEIGHT CHANGES OF THE ANIMALS

Table XLIV shows the weight changes of the six primates in the study at the beginning and close of the experimental period.

Neither of the control animals changed in weight during the experimental period. Of the animals which followed a daily exercise program, Primate - 65 did not change in body weight, and Primate - 69 gained one-half pound. Of the restrained animals, Primate - 21 lost 2.75 pounds (12 per cent) of its weight during the 14-day restraint period, while Primate - 25 lost 4.75 pounds (17 per cent).

TABLE XXXV

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-21 (RESTRAINED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 000	15) 000	29) 000
2) 000	16) 000	30) 000
3) 000	17) 000	31) 6.26
4) 000	18) 000	32) 000
5) 000	19) 000	33) 000
6) 7.09	20) 000	34) 43.21
7) 31.34	21) 000	35) 10.22
8) 000	22) 000	36) 000
9) 85.60	23) 000	37) 7.25
10) 53.53	24) 000	38) 000
11) 000	25) 000	39) 3.20
12) 000	26) 000	40) 000
13) 000	27) 000	41) 000
14) 000	28) 000	42) 000
		43) 000

TABLE XXXVI

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-25 (RESTRAINED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 000	15) 000	29) 000
2) 000	16) 16.65	30) 30.76
3) 000	17) 81.01	31) 000
4) 000	18) 38.00	32) 000
5) 000	19) 90.78	33) 28.32
6) 67.44	20) 221.93	34) 000
7) 34.34	21) 170.06	35) 4.24
8) 73.08	22) 186.76	36) 2.03
9) 81.85	23) 293.91	37) 000
10) 47.98	24) 369.75	38) 000
11) 000	25) 399.33	39) 000
12) 000	26) 285.53	40) 2.92
13) 000	27) 42.34	41) 000
14) 000	28) 31.21	42) 000
		43) 52.78

TABLE XXXVII

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-65 (EXERCISED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 000	15) 000	29) 000
2) 51.27	16) 000	30) 000
3) 000	17) 000	31) 000
4) 000	18) 000	32) 000
5) 000	19) 9.38	33) 000
6) 78.44	20) 000	34) 24.07
7) 10.95	21) 000	35) 000
8) 000	22) 000	36) 000
9) 39.38	23) 000	37) 000
10) 24.94	24) 000	38) 42.13
11) 000	25) 000	39) 000
12) 000	26) 000	40) 3.90
13) 000	27) 000	41) 000
14) 000	28) 000	42) 000
		43) 000

TABLE XXXVIII

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-69 (EXERCISED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 000	15) 000	29) 000
2) 000	16) 000	30) 000
3) 000	17) 000	31) 000
4) 000	18) 000	32) 000
5) 000	19) 13.92	33) 000
6) 15.40	20) 000	34) 000
7) 28.59	21) 000	35) 67.64
8) 000	22) 000	36) 000
9) 59.63	23) 000	37) 000
10) 20.06	24) 000	38) 000
11) 000	25) 000	39) 000
12) 000	26) 000	40) 4.63
13) 000	27) 000	41) 000
14) 000	28) 000	42) 3.77
		43) 2.13

TABLE XXXIX

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-5 (CONTROL)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 5.22	15) 000	29) 000
2) 000	16) 000	30) 000
3) 000	17) 10.33	31) 000
4) 000	18) 000	32) 000
5) 000	19) 000	33) 17.98
6) 19.77	20) 000	34) 000
7) 21.46	21) 000	35) 000
8) 36.89	22) 000	36) 000
9) 41.98	23) 000	37) 000
10) 5.46	24) 000	38) 000
11) 000	25) 000	39) 000
12) 000	26) 000	40) 000
13) 000	27) 000	41) 000
14) 000	28) 000	42) 000
		43) 2.12

TABLE XL

URINARY CREATINE EXCRETION BY EXPERIMENTAL PRIMATE-52 (CONTROL)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 000	15) 000	29) 000
2) 000	16) 000	30) 000
3) 000	17) 000	31) 000
4) 000	18) 000	32) 000
5) 000	19) 000	33) 56.84
6) 41.44	20) 14.55	34) 000
7) 000	21) 000	35) 14.55
8) 000	22) 000	36) 17.42
9) 28.83	23) 000	37) 5.32
10) 40.60	24) 000	38) 000
11) 000	25) 000	39) 2.57
12) 000	26) 000	40) 5.22
13) 000	27) 000	41) 15.08
14) 000	28) 000	42) 000
		43) 4.64

TABLE XLI

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

Populations Compared	Means	"t" Value	Probability
Primate - 21 Pre-Restraint Period Restraint Period	 12.7 0.0	1.7405	$P < 0.10$
Primate - 21 Pre-Restraint Period Post-Restraint Period	 12.7 4.7	1.0425	N.S.
Primate - 21 Restraint Period Post-Restraint Period	 0.0 4.7	1.5119	N.S.
Primate - 25 Pre-Restraint Period Restraint Period	 21.8 159.1	3.5316	$P < 0.01$
Primate - 25 Pre-Restraint Period Post-Restraint Period	 21.8 8.1	1.4148	N.S.
Primate - 25 Restraint Period Post-Restraint Period	 159.1 8.1	4.1054	$P < 0.001$
Primates - 21 and 25 Pre-Restraint Period Restraint Period	 17.2 79.5	2.5303	$P < 0.02$
Primates - 21 and 25 Pre-Restraint Period Post-Restraint Period	 17.2 6.4	1.8007	$P < 0.10$
Primates - 21 and 25 Restraint Period Post-Restraint Period	 79.5 6.4	3.1401	$P < 0.01$

TABLE XLII

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

Populations Compared	Means	"t" Value	Probability
Primate - 65 Pre-Exercise Period Exercise Period	 14.6 0.7	2.0113	$P < 0.10$
Primate - 65 Pre-Exercise Period Post-Exercise Period	 14.6 4.7	1.3362	N.S.
Primate - 65 Exercise Period Post-Exercise Period	 0.7 4.7	1.1734	N.S.
Primate - 69 Pre-Exercise Period Exercise Period	 8.8 1.0	1.5917	N.S.
Primate - 69 Pre-Exercise Period Post-Exercise Period	 8.8 5.2	0.5422	N.S.
Primate - 69 Exercise Period Post-Exercise Period	 1.0 5.2	0.8599	N.S.
Primates - 65 and 69 Pre-Exercise Period Exercise Period	 11.7 0.8	2.6346	$P < 0.01$
Primates - 65 and 69 Pre-Exercise Period Post-Exercise Period	 11.7 4.9	1.3985	N.S.
Primates - 65 and 69 Exercise Period Post-Exercise Period	 0.8 4.9	1.4258	N.S.

TABLE XLIII

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

Populations Compared	Means	"t" Value	Probability
Primate - 5 Pre-Experimental Period Experimental Period	 9.3 0.7	2.0737	$P < 0.05$
Primate - 5 Pre-Experimental Period Post-Experimental Period	 9.3 1.3	1.9335	$P < 0.10$
Primate - 5 Experimental Period Post-Experimental Period	 0.7 1.3	0.4065	N.S.
Primate - 52 Pre-Experimental Period Experimental Period	 7.9 1.0	1.5085	N.S.
Primate - 52 Pre-Experimental Period Post-Experimental Period	 7.9 8.1	0.0321	N.S.
Primate - 52 Experimental Period Post-Experimental Period	 1.0 8.1	1.6706	N.S.
Primates - 5 and 52 Pre-Experimental Period Experimental Period	 8.6 0.9	2.6049	$P < 0.01$
Primates - 5 and 52 Pre-Experimental Period Post-Experimental Period	 8.6 4.7	1.1006	N.S.
Primates - 5 and 52 Experimental Period Post-Experimental Period	 0.9 4.7	1.6957	$P < 0.10$

TABLE XLIV

WEIGHT CHANGES BEFORE AND AFTER

EXPERIMENTAL PERIOD

Group	Primate	Weight Before Experimental Period (pounds)	Weight After Experimental Period (pounds)
Immobilized	P - 21	23	20 1/4
	P - 25	28	23 1/4
Exercised	P - 65	22	22
	P - 69	20 1/2	21
Control	P - 5	18 1/2	18 1/2
	P - 52	20	20

URINARY CREATININE EXCRETION

Table XLV through Table L gives the basic analytical data on urinary creatinine. Tables LI, LII, and LIII summarize the statistical analysis of the data on creatinine excretion in the urine.

RESTRAINED PRIMATES

Table LI shows that no statistically significant differences were found by means of the "t" test when the data for both restrained animals were pooled and the Pre-Restraint Period was compared with the Restraint Period with respect to urinary creatinine excretion. For each primate considered separately and for both primates considered together, a larger quantity of creatinine was excreted during Restraint than during the Pre-Restraint Period, although the difference was significant only with Primate - 21 alone, and then only to a minor degree of probability ($P < 0.10$). With respect to the comparisons between the Restraint and the Post-Restraint Periods, however, differences in the case of each primate considered alone and both animals considered together, the quantity of creatinine excreted in the urine during the Restraint Period surpassed that in the Post-Restraint phase of the study by differences which were highly significant ($P < 0.001$ in all three cases).

EXERCISED PRIMATES

Table LII shows that there was a highly significant decrease in excretion of urinary creatinine when the animals changed from the

Exercise to the Post-Exercise Period ($P < 0.001$ for each animal considered separately, or for both primates considered together). The same relationship was not found when the Pre-Bed Rest excretion was compared with the Pre-Exercise Period. A scrutiny of the data in the table shows that more creatinine was excreted during the Pre-Bed Rest than during the Post-Bed Rest Period for both animals, indicating the possibility that the preliminary period may not have had the same level of activity in the first as in the last period of the study. As a matter of observation, the two animals in this group were seen to continue exercising on their own account during a part of the Post-Exercise Period.

CONTROL PRIMATES

Table LIII shows that creatinine excretion did not change in Primate - 5 throughout any of the periods of the study. Primate - 52, on the other hand, showed statistically significant changes between some of the periods. No explanation is available for this difference.

TABLE XLV

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-21 (RESTRAINED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 252.28	15) 75.84	29) 205.80
2) 276.42	16) 411.32	30) 275.50
3) 247.00	17) 415.81	31) 25.38
4) 446.33	18) 474.81	32) 211.05
5) 344.50	19) 436.80	33) 306.24
6) 306.34	20) 414.57	34) 344.93
7) 259.94	21) 498.94	35) 27.30
8) 414.08	22) 434.50	36) 13.44
9) 397.21	23) 418.00	37) 14.00
10) 254.15	24) 435.43	38) 232.56
11) 360.40	25) 253.47	39) 20.24
12) 183.75	26) 205.02	40) 202.50
13) 322.87	27) 444.04	41) 23.50
14) 285.12	28) 389.85	42) 34.45
		43) 77.00

TABLE XLVI

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-25 (RESTRAINED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 376.20	15) 566.96	29) 354.60
2) 478.80	16) 209.55	30) 146.64
3) 419.64	17) 477.00	31) 50.71
4) 442.00	18) 630.00	32) 266.05
5) 273.00	19) 507.83	33) 76.59
6) 493.68	20) 469.94	34) 144.42
7) 102.12	21) 463.25	35) 152.50
8) 299.88	22) 362.48	36) 180.32
9) 441.00	23) 440.89	37) 208.15
10) 175.83	24) 412.50	38) 62.15
11) 402.00	25) 294.03	39) 138.02
12) 428.51	26) 326.56	40) 65.16
13) 472.12	27) 166.44	41) 173.24
14) 368.14	28) 49.92	42) 320.07
		43) 295.75

TABLE XLVII

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-65 (EXERCISED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 375.70	15) 446.00	29) 150.00
2) 381.45	16) 394.02	30) 402.93
3) 169.00	17) 455.81	31) 263.90
4) 418.00	18) 360.25	32) 159.38
5) 368.00	19) 420.19	33) 152.10
6) 343.00	20) 347.20	34) 332.00
7) 374.06	21) 228.33	35) 76.32
8) 415.26	22) 393.60	36) 244.94
9) 463.66	23) 296.52	37) 131.04
10) 231.34	24) 380.00	38) 363.30
11) 417.36	25) 366.52	39) 294.15
12) 269.50	26) 466.39	40) 28.14
13) 289.39	27) 354.70	41) 130.37
14) 427.04	28) 226.92	42) 85.54
		43) 45.22

TABLE XLVIII

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-69 (EXERCISED)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 397.00	15) 405.14	29) 223.59
2) 431.11	16) 379.50	30) 302.82
3) 203.84	17) 482.59	31) 72.58
4) 404.94	18) 309.66	32) 252.84
5) 407.39	19) 468.75	33) 260.52
6) 417.49	20) 365.89	34) 200.00
7) 108.75	21) 301.20	35) 39.69
8) 354.64	22) 269.44	36) 263.25
9) 353.87	23) 247.72	37) 253.50
10) 217.49	24) 317.42	38) 78.28
11) 328.32	25) 295.20	39) 238.12
12) 402.60	26) 488.03	40) 258.02
13) 319.55	27) 325.29	41) 192.39
14) 225.94	28) 223.30	42) 15.79
		43) 17.02

TABLE XLIX

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-5 (CONTROL)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 301.50	15) 100.00	29) 326.80
2) 283.31	16) 219.78	30) 27.60
3) 161.04	17) 256.87	31) 21.00
4) 329.67	18) 312.34	32) 191.40
5) 264.86	19) 335.38	33) 22.00
6) 319.50	20) 168.75	34) 84.76
7) 212.75	21) 63.25	35) 218.75
8) 301.80	22) 57.75	36) 180.00
9) 190.19	23) 30.42	37) 81.25
10) 26.23	24) 76.22	38) 264.48
11) 201.26	25) 234.50	39) 294.97
12) 69.84	26) 217.61	40) 173.25
13) 67.32	27) 253.89	41) 54.80
14) 64.80	28) 368.50	42) 27.75
		43) 79.91

TABLE L

URINARY CREATININE EXCRETION BY EXPERIMENTAL PRIMATE-52 (CONTROL)

(Milligrams Per 24 Hours)

Equilibration Period	Immobilization Period	Ambulatory Period
1) 376.20	15) 454.50	29) 210.00
2) 374.40	16) 330.86	30) 153.12
3) 214.18	17) 151.32	31) 12.92
4) 134.85	18) 214.50	32) 231.24
5) 276.67	19) 255.00	33) 15.43
6) 336.70	20) 371.25	34) 38.50
7) 336.72	21) 345.62	35) 330.00
8) 396.74	22) 173.16	36) 324.55
9) 286.13	23) 158.85	37) 244.03
10) 252.00	24) 256.89	38) 94.62
11) 313.87	25) 300.81	39) 64.75
12) 299.16	26) 382.03	40) 22.00
13) 284.37	27) 356.90	41) 106.00
14) 119.52	28) 346.71	42) 116.81
		43) 9.50

TABLE LI

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

Populations Compared	Means	"t" Value	Probability
Primate - 21 Pre-Restraint Period Restraint Period	310.7 379.2	1.7715	$P < 0.10$
Primate - 21 Pre-Restraint Period Post-Restraint Period	310.7 134.3	4.4893	$P < 0.001$
Primate - 21 Restraint Period Post-Restraint Period	379.2 134.3	5.2864	$P < 0.001$
Primate - 25 Pre-Restraint Period Restraint Period	369.5 384.1	0.2644	N.S.
Primate - 25 Pre-Restraint Period Post-Restraint Period	369.5 175.6	4.7016	$P < 0.001$
Primate - 25 Restraint Period Post-Restraint Period	384.1 175.6	4.1100	$P < 0.001$
Primates - 21 and 25 Pre-Restraint Period Restraint Period	340.1 381.6	1.2596	N.S.
Primates - 21 and 25 Pre-Restraint Period Post-Restraint Period	340.1 154.9	6.5392	$P < 0.001$
Primates - 21 and 25 Restraint Period Post-Restraint Period	381.6 154.9	6.7942	$P < 0.001$

TABLE LII

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

Populations Compared	Means	"t" Value	Probability
Primate - 65 Pre-Exercise Period Exercise Period	 353.1 366.9	0.4433	N.S.
Primate - 65 Pre-Exercise Period Post-Exercise Period	 353.1 190.6	4.0812	$P < 0.001$
Primate - 65 Exercise Period Post-Exercise Period	 366.9 190.6	4.5818	$P < 0.001$
Primate - 69 Pre-Exercise Period Exercise Period	 326.6 348.5	0.5990	N.S.
Primate - 69 Pre-Exercise Period Post-Exercise Period	 326.6 177.9	3.8278	$P < 0.001$
Primate - 69 Exercise Period Post-Exercise Period	 348.5 177.9	4.6735	$P < 0.001$
Primates - 65 and 69 Pre-Exercise Period Exercise Period	 339.8 357.7	0.7649	N.S.
Primates - 65 and 69 Pre-Exercise Period Post-Exercise Period	 339.8 184.3	5.7696	$P < 0.001$
Primates - 65 and 69 Exercise Period Post-Exercise Period	 357.7 184.3	6.7578	$P < 0.001$

TABLE LIII

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

Populations Compared	Means	"t" Value	Probability
Primate - 5 Pre-Experimental Period Experimental Period	199.6 192.5	0.1653	N.S.
Primate - 5 Pre-Experimental Period Post-Experimental Period	199.6 136.6	1.5410	N.S.
Primate - 5 Experimental Period Post-Experimental Period	192.5 136.6	1.3403	N.S.
Primate - 52 Pre-Experimental Period Experimental Period	285.8 292.7	0.1992	N.S.
Primate - 52 Pre-Experimental Period Post-Experimental Period	285.8 131.6	4.0298	$P < 0.02$
Primate - 52 Experimental Period Post-Experimental Period	292.7 131.6	4.0506	$P < 0.02$
Primates - 5 and 52 Pre-Experimental Period Experimental Period	242.7 242.6	0.0023	N.S.
Primates - 5 and 52 Pre-Experimental Period Post-Experimental Period	242.7 134.1	3.8501	$P < 0.02$
Primates - 5 and 52 Experimental Period Post-Experimental Period	242.6 134.1	3.6949	$P < 0.01$

SUMMARY AND CONCLUSIONS

Daily urinary creatine and creatinine excretion values were determined in two studies with human subjects, and in one study with primates. These studies were conducted at the Texas Woman's University Research Institute under the sponsorship of the National Aeronautics and Space Administration. The analytical procedure used for determination of creatine and creatinine was the modified Folin method as described by Biggs and Cooper (8), and outlined in the Appendix of this dissertation.

The first study in this series 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. No statistically significant differences were obtained between Bed Rests 1 and 2 with regard either to creatine or creatinine excretion for the two subjects studied in the second Bed Rest.

In the second study of the series, an evaluation of a gravitation acceleration simulation suit designed by James Gatts, M.D., of the Republic Aviation Company, was carried out during a 21-day horizontal Bed Rest Period in comparison with the same subjects during a similar Bed Rest Period without the suit. This garment provided a work load both to the upper and lower body. All four adult men who took part in the study showed a highly significant decrease in creatine excretion during the period during which the suit was worn as compared to a similar period without the suit ($P < 0.001$). Also, the amount of creatine excreted while the suit was worn was not significantly different from that excreted during the Pre-Bed Rest Periods. The highest mean values were obtained for each of the subjects during the first Bed Rest. In regard to creatinine excretion, no statistically significant differences were obtained for the two Bed Rest Periods, with and without the Gatts' suit.

In connection with this second study, however, it should be noted that the designer of the suit and a colleague supervised the men during the Bed Rest Period when the suits were worn, rather than members of the TWU staff, with the level of recumbency maintained during

Bed Rest Periods of the TWU series including the Bed Rest Period when the suits were not worn in this study definitely not followed.

The third study in the series was conducted on six primates of the species Macacas Nemestrina. The whole study 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 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 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 Exercise 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.

Although creatinuria was reduced when the gravitation acceleration simulation suit was worn, the effect probably was due to a lesser degree of recumbency during the Bed Rest Period when the suits were worn, as noted.

With respect to the second study of the period, it is probable that isometric and isotonic exercise might be more beneficial in reducing muscular atrophy during Bed Rest at earth gravity or during weightlessness in orbital flight if it were performed for longer periods of time, or more frequently during the day.

A P P E N D I X

PROCEDURES FOR THE MEASUREMENT OF CREATINE AND CREATININE IN URINE

Sample Collection and Storage

The urine collections were pooled daily every day in all the studies and for all the subjects who participated. It was 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. These two procedures would prevent the occasional contamination of urine with creatinine-splitting organisms. Jones (78) reported that these organisms produce an enzyme called creatinase which is responsible for this destruction and is most active at an alkaline pH.

Analytical Procedure:

The analytical procedure used for the determination of creatine and creatinine in urine was based on that of the modified Folin method as described by Biggs and Cooper (8). These workers evaluated four methods of measuring urinary creatinine (36) and concluded that the simplicity and excellent reliability of the modified Folin procedure make it the method of choice.

Reagents required:

1. Creatinine standard solution: containing 0.5 milligrams per milliliter of distilled water.

2. Creatine standard solution: the same concentration as creatinine.

3. 0.057 N picric acid solution: prepared by dissolving 30 grams of the compound in two liters of warm water, then refrigerating for 12 hours. The crystals which form are filtered out and the normality established by titrating with a standardized NaOH solution, using phenolphthaline as an indicator.

4. 2.4 N NaOH solution.

Method: To determine creatinine and creatine:

Pipette one milliliter of urine into a 100 milliliter volumetric flask, to which 10 milliliters of 0.057 N picric acid and 1.5 milliliters of 2.5 N sodium hydroxide are added. The contents are mixed thoroughly and allowed to stand for 10 minutes for color development. Distilled water is added up to the mark and after shaking well, is read at 540 millimicrons using the Coleman Spectrophotometer.

Since the colored compound resulting from Jaffe's reaction does not strictly follow beer's law, it was necessary to construct a calibration curve using varying creatinine concentrations over the working range and to calculate results from the graph.

Creatine was determined by converting it to creatinine. The same amount of urine sample was boiled with one milliliter of 0.057 N picric acid and approximately 60 milliliters of distilled water over an electric heater for one hour. Care was taken to maintain the volume at 20 milliliters or above by the addition of distilled water. After the contents are cooled at room temperature, add nine milliliters of picric acid and 1.5 milliliters of NaOH and proceed as described for creatinine.

Creatine concentration was obtained by subtracting the creatinine value from that of converted creatine plus creatinine and then multiplying by a correction factor of 1.16.

$$\text{Thus, creatine} = \left[(\text{creatinine} + \text{creatine}) - \text{creatinine}\right] \quad 1.16$$

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