A STUDY OF CREATININE METABOLISM DURING AMBULATION AND RECUMBENCY

A THESIS

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INTRODUCTION

The advent of space flight served as an impetus for the instigation of numerous biological investigations. Under the auspices of the National Aeronautic and Space Administration, the Nelda Childers Stark Laboratory for Human Nutrition Research of the Texas Woman's University has participated in research in the realm of aerospace problems by conducting a number of bed rest studies designed to determine metabolic changes experienced because of the condition of recumbency, which involves some relation to weightlessness.

Since numerous physiological changes have been observed to occur as a result of environmental changes, this same laboratory became interested in the phenomenon of circadian rhythm. The present study is the second investigation conducted at the TWU laboratory designed to delve into the possible existence of circadian periodicities for various metabolites by observing the reactions of experimental subjects who were maintained under controlled recumbency conditions. The establishment of circadian patterns for these metabolites could serve as useful criteria for space physiological science.

As the study of physical activity also is known to influence body metabolism, it has been of interest to scientists in this laboratory to ascertain the effect of programmed exercise on the physical status of subjects who are participating in immobilization studies.

For many years creatinine values have been used clinically as an index for assessing nutritional status. Controversy over the accuracy of employing the creatinine ratio for this purpose has stimulated many investigators to probe further into this phenomenon. The research presented in this report deals in particular with the aspects of circadian rhythm and programmed exercise relative to urinary creatinine excretion.

Specific Objectives

1. To analyze the urinary creatinine excretion values of eight men in a recumbency study;

2. To compare statistically the daily creatinine excretion values of the three different periods of the study which included an Equilibration Period, a Bed Rest Period, and a Recovery Period;

3. To determine whether or not a circadian rhythm in urinary creatinine excretions during a 56-day bed rest period could be established;

4. To investigate the effect of exercise on the excretory levels of urinary creatinine.

REVIEW OF LITERATURE

FORMATION OF CREATININE

Creatinine, which is one of the most important end products of amino acid catabolism, ranks second only to urea as the most abundant nitrogen containing compound in the urine. According to Borsook and Dubnoff (1) creatinine in the body is formed in the muscle from the spontaneous and nonenzymatic transformation of phosphocreatine.

As a result of an investigation using rabbit muscle extract as a catalyst, Caputto (2) demonstrated the existence of an enzymatic reaction in the formation of creatinine and glucose 1,6 diphosphate from creatine phosphate and glucose-1-phosphate. Similar investigations by Van Pilsum and Hiller (3) and Cappenter (4) verified these findings.

Mathews, Stacy, and Hoover (5) suggested that, in muscle, there occurs an irreversible reaction in which one molecule of water may be lost from free creatine to form creatinine which ultimately is excreted in the urine.

Bloch and Schoenheimer (6) fed isotopic creatinine to adult rats and found that most of the isotopic materials were excreted in the urinary creatinine, with no isotopic creatine being found in the body. These results indicated that biologically the conversion of creatine to creatinine is irreversible.

EXCRETION OF CREATININE

In the past it has been considered that the daily excretion of creatinine was independent of urine volume and was little influenced by diet or by exercise. Edwards et al (7) measured the daily creatinine excretion of five patients for 28 to 29 days. The results of this investigation showed that the standard deviation of all subjects ranged from 0.055 to 0.276 and that the coefficient of variation ranged from 5.6 to 22.3 per cent. On the basis of these data, these investigators suggested that the daily creatinine excretion was not constant in any given individual. This report, however, was criticized by other authorities.

Gabriel (8) feels that, before collecting any patient's urinary samples for analytical purposes, it is pertinent to know the normalcy of the individual's plasma-creatinine levels, sodium excretion, urinary sediment, and renal function. Epstein and Schriever (9) observed that, if the daily excretion of creatinine falls below one gram, one can suspect that a true daily sample collection has not been made.

Bailey and Wardener (10) measured the daily creatinine excretion of 18 patients whose renal functions were known to be normal. The results of this study supported the concept that the daily excretion of urinary creatinine is not constant in any given individual.

Picon-Reatigni et al (11) explained that the amount of creatinine in the urinary and intracellular fluid volume was not affected by body weight, fat or extracellular fluid, but depended to a high degree on fat-free body mass. According to these investigators, creatinine excretion is a most useful tool for determining nutritional status in relation to muscle mass. The normal adult excretes an average of 1.5 grams of creatinine in the urine daily.

McClugage, Booth, and Evans (12) observed that the amounts of creatinine excreted by obese patients were similar to that excreted by normal individuals. Obesity does not appear to increase muscular mass, however individuals suffering from malnutrition experience a reduction in muscle mass resulting in a decrease in urinary creatinine excretion. Nichols et al (13) demonstrated that a significant decrease in muscle respiration occurs in children suffering from malnutrition.

FACTORS AFFECTING THE EXCRETION OF CREATININE

Effect of Diet

Most of the literature reports that the amount of creatinine excretion appears to be fairly independent of the diet consumed. Chanutin (14) divided white rats into several groups, and fed a different type of protein diet to each group, with one group receiving a protein free diet. It was observed that the protein intake did not effect an alteration in the creatinine excretion of the rats. Kiriyama et al (15) and Albanese (16) conducted similar investigations using adult men as subjects, while De Jorge et al (17) observed the same phenomenon in snails. All of these investigators concurred in reporting diet to have little or no effect on urinary creatinine excretion.

Nakagawa et al (18) adjusted the diet of normal children with the addition of an amino acid supplement. The urinary excretion of creatinine by these children showed a slight variation in comparison with those having a normal diet.

Fisher (19) fed rats with diets varying both in protein and free amino acid content. He found that creatinine excretion was not affected by the level of protein intake. With the diet which contained lysine, methionine and threonine, however, increased creatinine excretion was observed within one week after feeding. He hypothesized that the addition of free amino acids to the diet could stimulate synthesis of creatine and subsequently creatinine excretion would be stimulated.

Although the level of protein in the diet does not affect the creatinine excretion directly, in growing children a high protein diet can increase muscular mass and indirectly increase the excretion of creatinine. Fry, Fox, and Fry (20) reported a higher creatinine excretion in children fed a wheat diet than in those maintained on a rice diet. The wheat diet provides larger amounts of the essential amino acids than does the rice diet. Srikantia et al (21) found serum creatinine and creatinine concentration to be low in children suffering from kwashiorkor. After receiving supplements of arginine and glycine, however, the serum levels of creatine and creatinine were increased in these same children.

Effects of Sex and Age

The low level of creatinine excretion which is noted during the early weeks of life gradually increases until adulthood, after which a fairly constant level is maintained. Young et al (22) studied the body composition of preadolescent and adolescent girls, and found that the excretion of creatinine increases with an increase in body size, physiological age, and absolute muscle mass. The ratio of creatinine excretion to body weight, however, remained constant.

Clark et al (23) determined creatinine for 800 healthy children ranging in age from three to 18 years.

Creatinine excretion increased from 0.36 grams per 24 hours at the age of five years to 1.58 grams at the age of 17 years. Howell (24) reports that in aged men a decrease occurred in the urinary excretion of creatinine, probably due to the decrease in muscle mass experienced in aging.

The higher creatinine excretion found in males as compared to that of females is thought to be related to the higher degree of muscle mass found in men. Clark (25) reported that boys begin to excrete significantly higher amounts of creatinine than girls at the age of 12 years. The ratio of creatinine excretion to body weight remains at about 21 to one for girls, but increases to 25 to one for boys.

Effect of Hormones

Hoagland, Gilder and Shank (26) studied the effect of hormones on male children with progressive muscular dystrophy including five normal male children. The results of this study indicated that the male hormone testosterone propionate accelerates the deposition of creatine in the muscle, thereby serving to strengthen the muscle system, without affecting a significant change in creatinine excretion.

Schrire and Zwarenstein (27) demonstrated that the urinary creatinine output is increased in animals following the administration of growth hormone.

Effects of Stress and Immobilization

Many bed rest studies have been conducted to determine the physiological changes occurring as a result of the conditions of prolonged weightlessness and partial immobilization which are associated with physical confinement in a space craft. Hoffman and Mack et al (28) conducted a study using primates in which one group was restrained in bed, while another group was caged and free of restraint. The restrained animals showed an increase in creatine excretion with levels of creatinine excretion in the urine remaining irregular. These investigators concluded that, due to restraint, there was a decrease in muscle mass, loss of body weight, and a weakening of skeletal muscle.

Scrimshaw et al (29) determined the urinary composition of university students during, before, and after examinations. They observed that, during examination periods, increased excretions of 17,21-dihydroxycorticosteroids, creatinine, nitrogen and sulfates were detected in the urine of freshmen while the average excretion of these substances by upperclassmen remained unchanged.

Whedon, Deitrick and Shorr (30) studied the effects of immobilization on the metabolic and physiologic functions of normal men. Creatinine excretion was observed to remain quite constant for all subjects participating in this study.

Hale, Ellis, and Williams (31) have reported that the urinary creatinine values of 48 men determined both before and after 12 hours of simulated flight showed no significant changes.

Effect of Exercise

The effect of muscular exercise on creatinine excretion is currently an object of extensive investigation. Cathcart, Kennaway, and Leathes (32) have noted that the excretion of creatinine is not changed by severe exercise. Mitchell and Kruger (33) made an extensive study in which eight rats were subjected to a four-day rest period after a five-day work period, and reported that no change was noted in the excretion either of total endogenous nitrogen or creatinine in the urine as a result of activity or inactivity. These investigators concluded that increased catabolism of muscle tissue could not be attributed to any increase in muscle activity.

Garry (34) determined the creatinine excretion in experimental animals over a six-day period. On the third day some of the animals were allowed to have voluntary activity while other animals were made to participate in various planned activities. The results of this study showed that voluntary activity did not significantly change creatinine excretion, but that involuntary activity induced a slight increase in creatinine excretion both during and following the exercise.

Hobson (35) studied the relationship between creatinine excretion and exercise on 97 physical education students. These subjects participated in a strenuous exercise regime every day except Saturday and Sunday. On these two days they participated in limited physical exercise. The severity of the exercise regime appeared to effect no significant change in the amount of creatinine excreted in the urine.

Haldi and Bachmann (36) reported that the addition of glucose or fructose or a mixture of both hexoses to the diets of subjects engaged in exercise prompted an increase in the creatinine excretion in the urine. These investigators suggest that exercise stimulates carbohydrate oxidation which is essential for creatine to creatinine metabolism, with a rise in urinary creatinine levels consequently being noted.

Norris and Weiser (37) studied the correlation between strenuous muscular exercise and creatinine excretion in two young men, and found a significant decrease in creatinine excretion to occur both during and after muscular exercise. In this same study, the other urinary constituents observed, such as total nitrogen, uric acid, chlorides and inorganic phosphates also exhibited a decrease in excretion. It was hypothesized by these investigators that following exercise there is a decrease in urinary volume and consequently there occurs a decrease in urinary metabolites.

Ahlborg and Brohult (38) studied the effect of exercise on the levels of serum creatinine and creatine-kinase in 12 healthy men and concluded that the levels both of creatinine and creatine-kinase are slightly elevated after exercise with serum creatinine showing a faster return to normal values.

Srivastava et al (39) conducted a series of four experiments to study the effect of muscular exercise on the excretion of creatinine and creatine in the urine. The army personnel employed as subjects in these studies were required to march three hours daily. The results of all four investigations showed that the 24-hour urinary creatinine excretion was elevated during the days of exercise as compared to normal values, and that the hourly excretion rate during the exercise period was higher than during the pre-exercise and recovery periods.

Umapathy (40) reported on a study using six primates as subjects during the experimental days. Two primates were forced to exercise for one hour each day, two served as controls, and two were placed in restraint. The results showed that the creatinine excretion of the experimental animals remained virtually unchanged during the pre-restraint and restraint periods with a decrease noted in the recovery stage. No markedly different levels were recorded for the control animals.

Montgomery (41) measured the urinary creatinine and creatine excretion of six male university students during a study containing two bed rest periods. During the first period, no exercise was taken by the subjects. During the second bed rest, one group of subjects engaged in regular programmed exercise, while the other group exercised at will. The results of this study showed that the creatinine excretion of all subjects during both bed rest periods was significantly higher than during the pre-bed rest period. The creatinine excretion during the exercise period was higher for the group exercising ad libitum than for the group exercising regularly.

CIRCADIAN VARIABILITY

It has been established that plants and animals vary their activities on an approximately 24-hour basis. This physiological behavior is referred to as a biological clock or circadian rhythm. Pittendrigh and Bruce (42) have suggested that biological clocks are divided into three categories: (a) Continuously consulted clocks, which are systems concerned with time-compensation; (b) Interval timers, or systems controlling discrete events; and (c) Clocks controlling "pure rhythms", or rhythms in which the amplitude of a continuous process varies with time.

The mechanisms controlling this phenomenon are not fully understood as yet, although one popular theory is that the control is purely endogenous in origin as physiological rhythms appear to have a self-sustained oscillation which remain free-running under constant conditions. Schweiger et al (43) found that the nucleus of unicellular green algae has the ability to determine the phase of the circadium rhythm of oxygen balance in the cytoplasm of the cell.

Aschoff (44) supports the opposing hypothesis that the constant condition of rhythms are affected by many exogenous factors. He believes that environmental factors such as light, temperature, noise and social contacts, all are involved in the control of biological rhythm. Body temperature and urinary excretion are the primary systems in which the daily rhythms are reflected.

Harker (45) states that most animals living in natural environments are nocturnal and diurnal. Hence light can be considered the most important factor affecting the phases of rhythm. Chandranshekaran and Loher (46) studied the effect of light on the phase of the circadian system of Drosophila. They reported that a light intensity of 10 lux had the maximum effect on phase shifts. Higher light intensities such as 3000 and 10,000 lux effected the same result as 10 lux light, whereas lower light intensities of 0.1, 0.3 and 1.0 lux were observed to delay phase shifts.

Besch (47) exposed rats to different light-dark fluctuations in an effort to determine activity responses to altered photoperiods. The results indicated that both peak and low activity periods displayed phase shifts which were related to the change in the light:dark cycle. These phase shifts were apparent immediately with exposure to longer days and were delayed when day length was decreased. Aschoff (44) observed that, with increasing intensities of illumination, the circadian period is shortened in diurnal animals and lengthened in nocturnal animals.

Underwood and Menaker (48) have treated both blind house and normal sparrows with cycles of (a) 12 hours light: 12 hours dark and (b) 16 hours light:8 hours dark, at intensities ranging from 20 to 500 lux. Under these conditions the blind house birds were found to have the same testicular response as the normal birds. The data from this study indicated the existence in this species of birds of a functional extraretinal photoreceptor which is capable of mediating a gonadal response to photoperiodic stimuli.

The effect of temperature on circadian rhythm has been noted by some investigators. Bateman (49) reported that the fruit fly Dacus tryoni is sensitive to temperature cycles when it is in the pupal stage. Richard (50) found that the rhythm of stripe formation on the shell of cuttle-fish is dependent on temperature.

Hayden and Lindberg (51) have found that a 24-hour cycle of pressure can act as a zeitgeber to entrain the endogenous circadian rhythm of body temperature in pocket mice maintained under constant conditions of environmental temperature and light.

Chook (52) noted that many people lose their correlation with clock time during illness, disorientation, or under drug influence. Ionizing radiation is known to accelerate the biological clock which controls the aging process resulting in premature death.

Frazier et al (53) made vigilance performance tests on three human subjects maintained in a highly constant environmental condition for a 14-day period. The results revealed the fact that confinement stress can alter circadian rhythmicity in the body, even when the physical environment and activity schedule are held highly constant.

Kosmolinsky and Dushkov (54) found that subjects who had a prolonged stay in a sealed chamber experienced an alteration in their biorhythmology. These investigators feel that the element of stress induces an alteration in psycho-physiological functions which leads to a change in circadian patterns.

Hymer, Mastro, and Griswold (55) found that exposure of rats to constant conditions of light or dark induced changes in DNA synthesis in the cells of the anterior pituitary.

According to Haberg, Peterson, and Silber (56) the blood level of corticosterone in mice shows a circadian periodity in which the peak blood level occurs about 10 hours after the beginning of the light period in a 12-hour light: 12-hour darkness cycle. The peak level of mitotic rhythm in the adrenal cortex was observed to occur when corticosterone concentration was minimal.

Feigin, Dangerfield and Beisel (57) studied the circadian periodocities of amino acids in normal and adrenalectomized mice. Adrenalectomized mice appeared to have a less definite circadian pattern for amino acid levels than did normal mice. These investigators hypothesized that corticosteroids are the major internal factor controlling the amino acid levels in blood, as the corticosteroids are known to be capable of inducing enzyme synthesis. It also was observed that a direct relationship exists between mitotic activity and amino acid concentration. When mitotic activity is highest, the circulating amino acid concentration is lowest; and conversely, when systhesis is low, the amino acid concentration is at its highest level.

Takebe et al (58) reported that the diurnal rhythm of adrenocortical function was dependent upon the rhythm of ACTH secretion in man, since both plasma ACTH and cortisol are observed to rise in the morning and fall at night.

Suomalainen (59) feels that, since the hypothalamus controls the states of sleep and wakefulness, this gland might be regarded as the major internal factor controlling circadian rhythms.

Mills (60) stated that the phenomenon of creatinine excretion being higher in the morning and lower over night reflects a variation of glomerular filtration rate. The renal rhythms of various substances were observed by Mills (61) by placing one man in solitude for 100 days. He found that, although creatinine excretion varied somewhat irregularly, the lowest level of this metabolite in the urine always was noted in the overnight sample, while sodium excretion was observed to be at its highest level during this same period.

Best, Kuhl and Friedeman (62) conducted a study using aged, normal, white male rats, and found that the highest level of creatinine excretion during a 24-hour period occurred between the hours of 6 A.M. and 10 A.M.

Hale, Ellis and Williams (63) studied a group of 12 healthy men for a period of one year for the purpose of establishing seasonal baselines for a number of urinary variables which are currently being used for assessing flight stress. Once a week urine samples were collected at the time of awakening and analyzed for hormones, electrolytes and creatinine. As seasonal mean values of creatinine-based ratios showed long-term cyclic shifting, these investigators reasoned that creatinine-based ratios could be used successfully for flight-stress studies.

The creatinine base ratio was used by Mautalen (64) to study the circadian rhythm both of total and free hydroxyproline excretion in the urine. Urinary total hydroxyproline excretion followed a circadian pattern with the peak excretion being observed from midnight to 0800 hours and the lowest excretion reported between 1200 to 2000 hours. Conversely, the creatinine excretion was higher during the daytime than overnight.

Although it has been established that living organisms have an inherent natural frequency, the basic mechanisms of circadian rhythmicity still are not clearly defined. Mills (60) has proposed a graphic description of the system controlling circadian rhythms as shown in Figure 1.

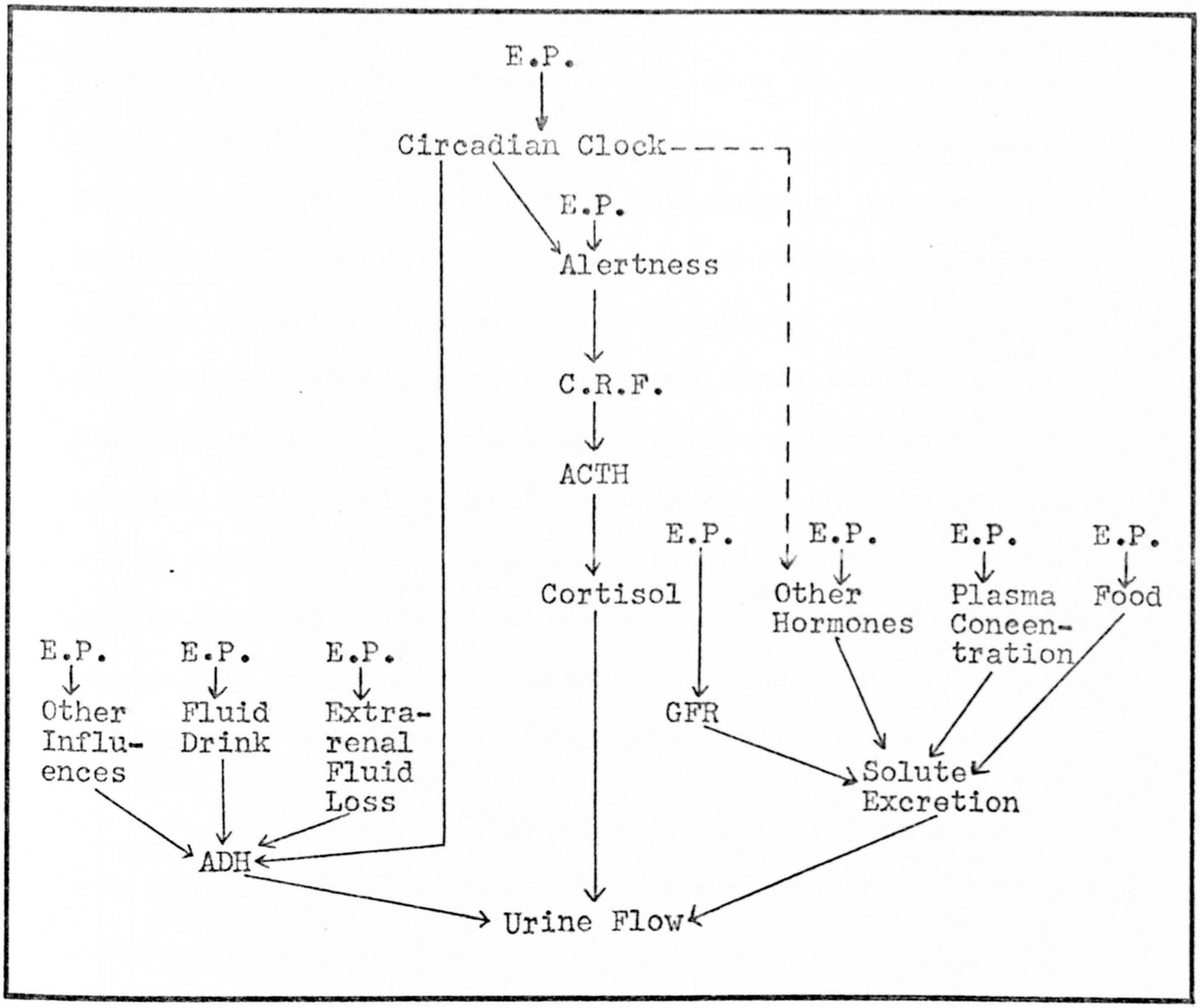

Figure 1. Diagrammatic representation of some factors possibly conducive to circadian periodicity in urine flow. E.P. indicates external periodic influences, including environment and habit.

(as shown by Mills (60))

P L A N   O F   P R O C E D U R E

The data presented in this thesis were obtained during a bed rest study conducted in the Nelda Childers Stark Laboratory for Human Nutrition Research on the campus of Texas Woman's University, by the Texas Woman's University Research Institute, as part of an extensive investigation sponsored by the National Aeronautics and Space Administration to study various metabolic reactions occurring in experimental subjects exposed to conditions similar to those encountered during space flight. This study was designed to examine such physiological factors as changes in bone density and various metabolic reactions as well as the circadian response of immobilized healthy adult males under carefully controlled environmental conditions, and to note the effectiveness of exercise as a possible ameliorating measure.

A detailed account of the creatinine excretions of the subjects participating throughout this investigation is presented in this report.

Periods of the Study

This study consisted of one bed rest period accompanied by pre-bed rest and post-bed rest ambulatory periods as follows:

Equilibration Period, 17 days, July 7 - July 24, 1969

Bed Rest Period, 56 days, July 24 - September 17, 1969

Post-Bed Rest Period, 15 days, September 17 - October 1, 1969

Subjects of the Study

Participating in this study were seven university students between 20 and 25 years of age and one older man 40 years old. The heights and weights of the subjects upon entering the study were as follows:

Subject	Age (years)	Height (inches)	Weight (pounds) Initial
1A	20	69	157
2A	23	69	150
3A	40	71	142
4A	20	66	155
6A	20	73	180
7A	22	71	175
8A	23	73	195
9A	22	70	135

GENERAL PROCEDURE USED IN THE STUDY

Throughout the entire study, the subjects were housed and fed in the metabolic ward of the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute. Specially trained dietitians supervised the preparation of the meals which were optimum in all major nutrients.

The food fed during the Pre-Bed Rest Equilibration Period was regular food chiefly purchased through the University Purchasing Department; that fed during the Bed Rest and the Post-Bed Rest Periods was flight food furnished by National Aeronautics and Space Administration Manned Spacecraft Center, Houston. The daily food intake of each subject was recorded throughout the study by individual foods.

This study was conducted under close medical supervision. A record was made of height and weight changes throughout the study. Male orderlies attended to the hygienic needs of the subjects during immobilization, and safeguarded the movements of the subjects.

Equilibration Period

During this period, which lasted for 17 days, the eight subjects led a normal life while engaged at various tasks in the laboratory. They were encouraged to participate in moderate exercise each day and were required to be in bed at 10:30 P.M. with the lights out and the bed cubicles darkened from 11:00 P.M. until 7:00 A.M. The meals which were prepared and consumed in the metabolic ward were planned to contain 2600 calories daily, including 1.0 gram of calcium and 100 grams of protein.

Daily urinary samples covering a 24-hour period were collected until six days before entering the period of immobilization, when the urinary voids were collected in three aliquots during a 24-hour period.

Bed Rest Period

This portion of the study covered a span of 56 days during which time the eight male subjects were immobilized. They assumed a horizontal position on a single bed equipped with one thin pillow. They were encouraged not to lift their heads, although very limited movement of the arms and legs was allowed. Reading was done with the aid of glasses equipped with prismatic lenses, and individual TV sets equipped with ear phones also were provided for the purpose of viewing TV on the hospital type television sets. During this period of immobilization trained male orderlies were present around-the-clock to attend to the hygienic needs of the subjects and to safeguard their movement.

This phase of the study was designed to investigate the phenomenon of circadian rhythm. A strict day-night regimen was maintained which provided 14 hours of daytime and 10 hours of night. All outdoor and hall windows in the metabolic ward were screened with opaque black paper and heavy drapes, thereby allowing no light whatever to penetrate the parts of the ward in which the experimental subjects were kept in bed. The light intensity in the 14 daytime hours in the various parts of the metabolic ward in which the men were in bed exhibited a mean of 30 foot candles when measured with a General Electric Type DW-68 Light Meter.

At 9:00 A.M. the day began by turning on the lights. This lasted until 11:00 P.M. when all lights including TV lamps were turned off. A thermometer was placed on the wall above the head of each subject and the temperature was maintained carefully within a range of 72° $\pm$ 2°F.

During this period of the study exercise was introduced as a variable through the use of the Exer-Genie and Exer-Grip Exercisers, modified at TWU for better use while the subject was in horizontal bed rest. Measurements of foot and hand action, squeeze, and isometrics were made with the Lufkin Anthropometric (woven) Tape with a Gurlick Spring Attachment (3176ME).

Four subjects were selected to take part in the Exercise Program with the other four subjects not exercising.

The subjects selected to exercise were 2A, 3A, 6A, and 7A. One subject, however, (3A), pleaded fatigue when the exercise program was one-half completed and was replaced by Subject 1A who followed the program the remainder of the 56-day period.

The entire exercise routine, which is given in Summary A, was performed three times daily under close supervision. During the 48-hour periods when blood was taken every four hours, the exercise was omitted. The exercise was carried out with the help of a metronome so that a regular routine rhythm was followed in all phases of the program. Stop watch timing was used and records were kept on each subject as to the time expended on each step of the schedule. The results are shown in Summary B.

Throughout this 56-day period, flight food was consumed by the subjects with a careful record of intake being kept by the dietitians. X-rays and blood tests were made on a routine basis throughout immobilization and urine samples were collected four times daily at 8 A.M., 12 Noon, 8 P.M., and 12 Midnight for the purpose of investigating the circadian rhythm phenomenon.

Post-Bed Rest Period

During this phase of the study which lasted 15 days, all subjects were ambulatory with some of the young men resuming studies at North Texas State University.

The subjects continued to consume astronaut food during this period of the study.

SUMMARY A

EXERCISE SCHEDULE

(Cylinder on Exerciser set at 8 pounds and Metronome speed of one beat per second.)

Step	Activity	Time
1	Isometric (EXER-GENIE)	10 seconds
2	Leg Exercise (EXER-GENIE).	6 minutes
3	Rest	2 minutes
4	Hand - Fingers (Gripper)	1 minute
5	Rest	2 minutes
6	Isometric (EXER-GENIE)	10 seconds
7	Arm Exercise (EXER-GENIE).	6 minutes
8	Rest	2 minutes
9	Hand - Fingers (Gripper)	1 minute
10	Rest	2 minutes
11	Isometric (EXER-GENIE)	10 seconds
12	Leg Exercise (EXER-GENIE)	6 minutes

Total	Isometric	30 seconds
	Isotonic	20 minutes

SUMMARY B

SUMMARY OF EXERCISE ACCOMPLISHMENT

Exercise Accomplishment	(2A) Smith (through entire bed rest study)	(6A) Kerr (through entire bed rest study)	(7A) Jordan (through entire bed rest study)	(3A) Bishop (from beginning of bed rest through August 22)	(1A) Agger (from August 23 through remainder of bed rest)
Per cent of days when full Exercise Schedule was followed	90.5%	88.1%	80.6%	0.0%	82.6%
Per cent of days when no exercise was followed	0.0%	0.0%	0.0%	2.4%	0.0%
Per cent of periods when no exercise was followed	3.2%	3.2%	6.4%	16.7%	2.9%
Average daily time expended on isometric exercise (seconds). Total daily time called for on this exercise, 90 seconds.	86.4	85.6	58.2	20.0	85.8
Average daily time expended on arm isotonic exercise (minutes). Total daily time called for on this exercise, 18 minutes	17.2	17.2	16.5	5.4	16.0
Average daily time expended on leg isotonic exercise (minutes). Total daily time called for on this exercise, 36 minutes.	34.3	34.3	33.1	7.0	32.6
Average daily time expended on use of hand grippers (minutes). Total daily time called for on this exercise (6 minutes)	5.8	5.8	5.8	0.7	5.6

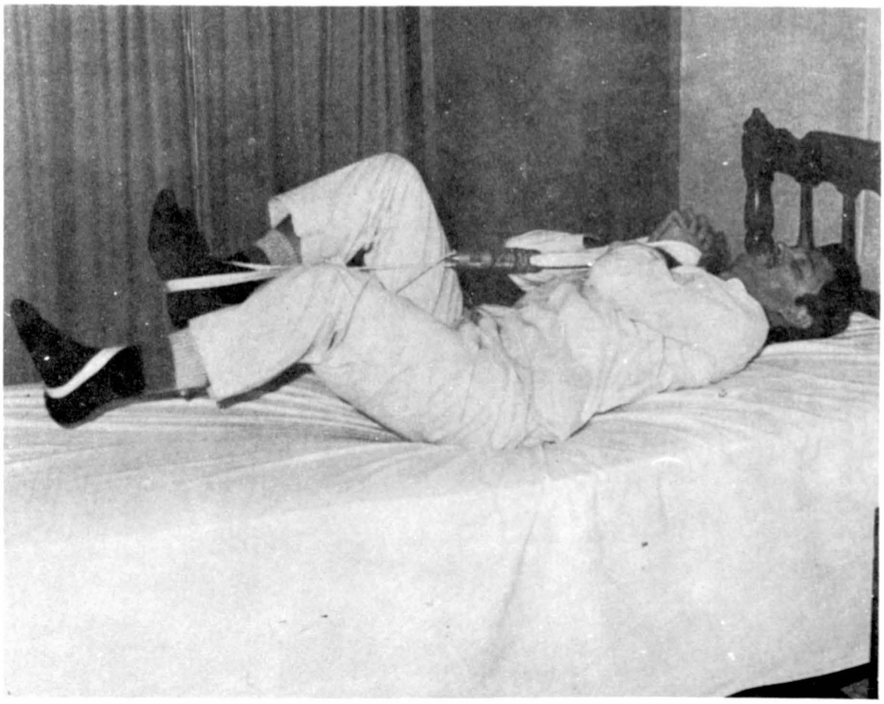

Figure 2. METHOD OF USING THE EXER-GENIE
IN ISOTONIC EXERCISE INVOLVING THE FEET

Procedure for Determination of Creatine and Creatinine in Urine

The methods used for the collection, the storage, and the analytical procedure for creatinine were followed as have been used in previous studies conducted in the TWU laboratories.

Sample Collection and Storage

Urine specimens were collected on an 8-hour basis. The urine was measured and stored under refrigeration in clean polyethylene bottles. These bottles had been washed with a 10 per cent hydrochloric acid solution in an effort to prevent contamination of the urine by the enzyme creatinase which is most active at an alkaline pH.

Preparation of Reagents

1. Creatinine Standard Solution (0.5 mg./ml.) - 0.5 grams of creatinine anhydride were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored under refrigeration.

2. Picric Acid Solution (0.057 N) - 30 grams of crystalline picric acid were dissolved in 2000 milliliters of warm water. After cooling to room temperature, this solution was refrigerated for 12 hours. As this is a supersaturated solution, crystals form which must be removed by filtration. In

order to establish the normality, the filtrate was titrated with standardized NaOH using phenolphthaline as an indicator. The solution then was stored at room temperature.

3. 2.5 N NaOH Solution - 100 grams of NaOH crystals were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored at room temperature.

Analytical Procedure

A color reaction for creatinine which first was described by Jaffe constituted the basis of a method developed by Folin in 1904 for the quantitative determination of creatinine in urine.

The method adopted for use in the TWU laboratories for the determinations of creatinine in the urine is the modified Folin procedure as developed by Biggs and Cooper (65).

Determination of Creatinine

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

Coleman Spectrophotometer at 540 millimicrons.

Calibration Curve for Urinary Creatinine

Since the colored compound formed does not follow Beer's Law, it is necessary to plot a graph and calculate results by reading from the graph. In order to construct a calibration curve, known amounts of the standard solutions are used to replace the urine samples and the determination procedures are applied to these standards.

The curves used were those made for concentrations of 0.5, 1.0, 2.0, and 3.0 milligrams of creatinine. This was done by pipetting 1.0, 2.0, 4.0 and 6.0 milliliters of the respective solutions into volumetric flasks and proceeding with the determinations.

Creatinine concentration may be read directly from the calibration curve. A typical standard curve is shown in Figure 3.

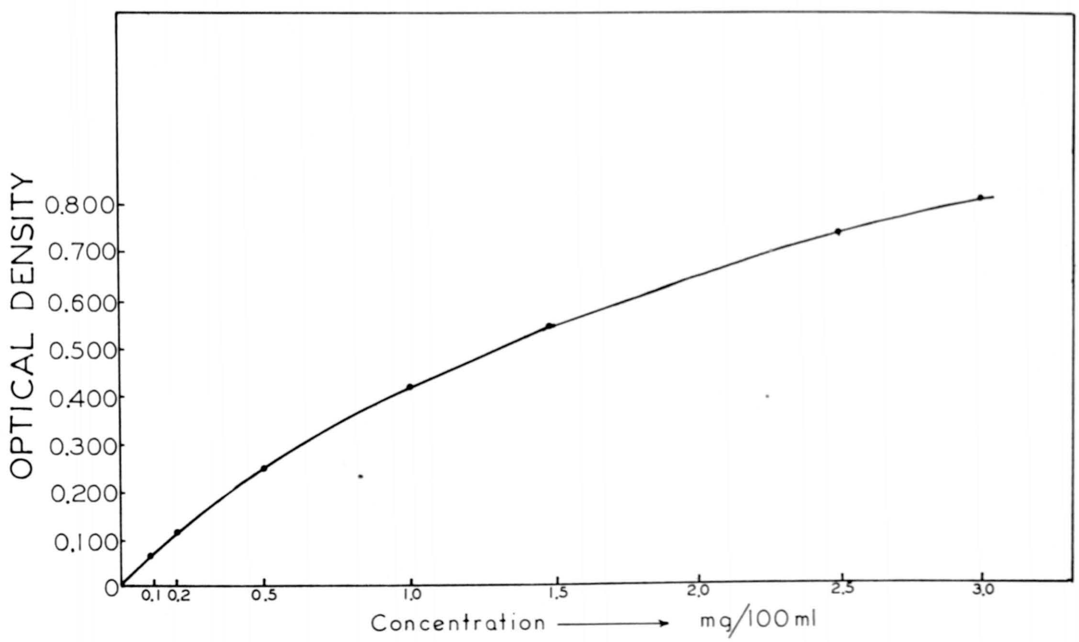

Figure 3. TYPICAL CALIBRATION CURVE FOR URINARY CREATININE

PRESENTATION OF FINDINGS WITH DISCUSSION

Eight healthy adult male subjects participated in an immobilization study which covered a span of 85 days and consisted of three different periods: Pre-Bed Rest; Bed Rest; and Post-Bed Rest. This study was designed to investigate changes in bone density, and to observe various metabolic reactions throughout all periods of the investigation as well as to determine circadian periodicities existing in the physiological functions of these subjects while they were engaged in a 56-day period of bed rest immobilization.

The daily urinary excretion data on creatinine, which constituted the author's contribution to the overall study, are recorded in the Appendix in Table I. Urinary creatinine excretion during four daily periods throughout Bed Rest are reported in Table II. The statistical comparisons of excretion between pairs of different periods are presented in Table III. The statistical comparisons of circadian rhythm are recorded in Table IV. The statistical data relating to exercise are shown in Table V, VI, VII, and VIII.

COMPARISON OF URINARY CREATININE EXCRETION DURING THE BED REST, PRE- AND POST-BED REST PERIODS

The statistical analyses of the urinary creatinine excretion data are shown in Table III (Appendix).

Creatinine Urinary Excretion by Subjects Which Exercised during Bed Rest

SUBJECT 2A

The average daily urinary excretion of creatinine by this subject with Pre-Bed Rest and Bed Rest compared statistically are presented in Table III, Part A. The amount of urinary creatinine excreted by this subject during Bed Rest was greater in quantity than during Pre-Bed Rest and Post-Bed Rest by a difference which was highly significant ($P < 0.001$) in both comparisons.

SUBJECT 6A

When the amount of urinary creatinine excreted by this subject during the Pre-Bed Rest Period was compared with that excreted during Bed Rest, a higher amount of urinary creatinine was recorded during Bed Rest than during Pre-Bed Rest, with a difference which was distinctly significant ($P < 0.02$).

The urinary excretion by Subject 6A differed little between Pre-Bed Rest and Post-Bed Rest, with no statistically significant difference between that excreted between Bed Rest and Post-Bed Rest.

SUBJECT 7A

During Bed Rest, the urinary creatinine excretion by this subject was greater than that during the Pre-Bed Rest

Period and Post-Bed Rest. This difference was highly significant in both instances ($P < 0.001$), as shown in Table III, Part A.

SUBJECTS 2A, 6A, 7A

As shown in Table III, Part B, when the data for all subjects who exercised throughout the Bed Rest Period were pooled together for purposes of statistical comparison, the amount of creatinine excreted in the urine during the Bed Rest Period was higher than that during the Pre-Bed Rest Period by a difference which was highly significant ($P < 0.001$).

Creatinine Urinary Excretion by Subjects Who Did Not Exercise Routinely during Bed Rest

SUBJECT 4A

A higher level of daily urinary creatinine was excreted by Subject 4A during Bed Rest than during Pre-Bed Rest. The difference was highly significant ($P < 0.001$), as shown in Table III, Part C. The same was true when the Bed Rest and the Post-Bed Rest Periods were compared. The excretion during the Pre-Bed Rest and the Post-Bed Rest Periods did not differ significantly.

SUBJECT 8A

The excretion of urinary creatinine by this subject was significantly higher ($P < 0.001$) during the Bed Rest

Period than during the Pre-Bed Rest or the Post-Bed Rest Periods, with the Pre- and Post-Bed Rest Periods not differing significantly from each other. Table III, Part C.

SUBJECT 9A

As shown in Table III, Part C, Subject 9A excreted an appreciably greater quantity of creatinine in the urine during the Bed Rest Period than during the Pre- or Post-Bed Rest. The difference was highly significant in both cases ($P < 0.001$). The Pre- and Post-Bed Rest Periods were not statistically different in this respect.

SUBJECTS 4A, 8A, 9A (No exercise)

Table III, Part D gives a resumé of the statistical findings when the urinary creatinine data were pooled together for the three subjects who did not exercise. The total excretion during the Bed Rest Period surpassed that of the Pre-Bed Rest and of the Post-Bed Rest Periods by differences which were highly significant in both cases ($P < 0.001$). There was no significant difference in creatinine excretion between the two ambulatory periods of this group.

Creatinine Urinary Excretion by Subjects Who Exercised Only One-Half of the Bed Rest Period

Table III, Part E gives the urinary creatinine statistical data for Subject 3A, who exercised during the first

28 days of the 56-day Bed Rest Period, and for Subject 1A, who exercised during the last 28 days.

It is shown in the table which was cited that Subject 3A, who exercised during the first half of the Bed Rest Period surpassed both the Pre- and Post-Bed Rest Periods during the part of the Bed Rest Period when he exercised by a highly significant amount of urinary creatinine excreatinine ($P < 0.001$), with no significant difference between the amounts excreted during the Pre- and Post Periods when the subjects were ambulatory.

The same was found for Subject 1A during the part of the Bed Rest Period when he exercised.

ALL SUBJECTS

When the data for all eight subjects were combined for the purpose of comparing the urinary creatinine excretion during Bed Rest with that of Pre-Bed Rest and Post-Bed Rest, Table III, Part F, shows that the amount excreted during the Bed Rest Period was appreciably greater than that of either of the two ambulatory periods in both instances. The results of this study support previous reports by Umapathy (40) and Montgomery (41) which indicated that the element of stress induced by long periods of immobilization might serve to enhance the excretion of creatinine in the urine.

CIRCADIAN PATTERN OF URINARY CREATININE EXCRETION DURING BED REST PERIOD

In order to study the variation of creatinine, the urine excreted at different times during the 24-hour period was collected into four aliquots which ended respectively at 8 A.M., 12 Noon, 8 P.M., and 12 Midnight. The statistical data regarding the excretion patterns are given in Table IV and in Figures 4 and 5.

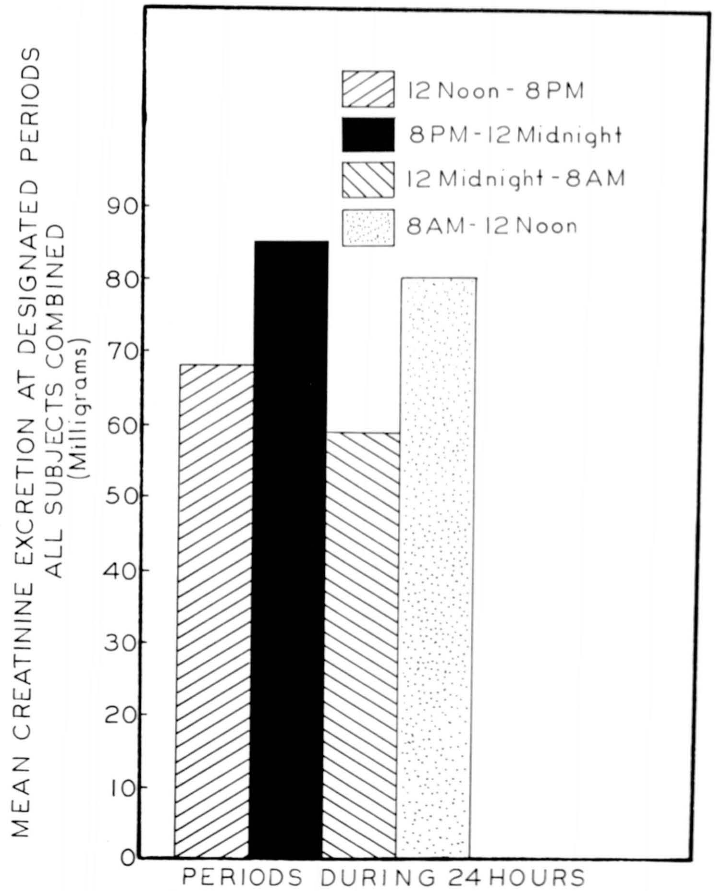

Figure 4. MEAN CREATININE EXCRETION AT DESIGNATED PERIODS, ALL SUBJECTS COMBINED

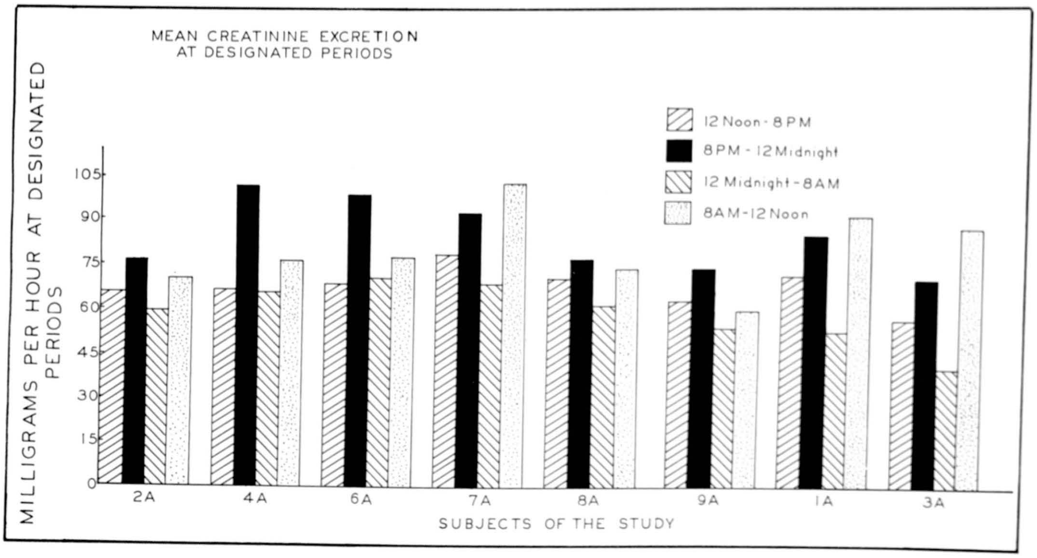

Figure 5. MEAN CREATININE EXCRETION, AT DESIGNATED PERIODS, OF INDIVIDUAL SUBJECTS

SUBJECT 2A

Table IV, Part A, shows that the quantity of creatinine excreted by this subject between 12 Noon and 8 P.M. was somewhat lower than that excreted between 8 P.M. and 12 Midnight ($P < 0.02$). No statistical significance was found between the 12 Noon to 8 P.M. period and the other periods of the day. The mean hourly value of the period from 8 P.M. to 12 Midnight was highest for this subject. A comparison of this period with the period from 8 A.M. to 12 Noon showed no significance, although a very high degree of significance ($P < 0.001$) was found when comparing the 8 P.M. to 12 Midnight period with the overnight period. Similarly, it was found that, in comparing the overnight period with the period from 8 A.M. to 12 Noon, the amount of creatinine excreted by this subject was far greater between 8 A.M. to 12 Noon ($P < 0.001$).

SUBJECT 6A

As shown in Table IV, Part B, the analysis of the data pertaining to Subject 6A showed that the amount of creatinine excreted in the urine from 8 P.M. to 12 Midnight surpassed the amount excreted from 12 Noon to 8 P.M. and from 12 Midnight to 8 A.M. by a difference which was highly significant ($P < 0.001$). The amount of urinary creatinine excreted during the period from 8 A.M. to 12 Noon was slightly higher than that excreted between 12 Noon and

8 P.M. ($P < 0.10$), and the creatinine excretion from 8 P.M. to 12 Midnight was higher than the excretion from 8 A.M. to 12 Noon ($P < 0.01$). In comparing the creatinine excretion from 12 Midnight to 8 A.M. both with that from 12 Noon to 8 P.M. and with that between 8 A.M. and 12 Noon, no statistically significant difference was found.

SUBJECT 7A

Table IV, Part C, contains the daily periodic data for creatinine excretion by Subject 7A. When pairs of the four collection periods for urine were compared for quantities of excreted creatinine by means of the "t" test, the amount excreted by Subject 7A from 12 Noon to 8 P.M. was lower than that excreted between 8 P.M. to 12 Midnight ($P < 0.02$). The mean hourly value from 12 Noon to 8 P.M. was much higher, however, than that from 12 Midnight to 8 A.M. ($P < 0.05$). The amount of creatinine excretion per hour between 12 Noon and 8 P.M. was distinctly lower than that from 8 A.M. to 12 Noon ($P < 0.01$). The creatinine excretions both from the 8 P.M. to 12 Midnight Period and the 8 A.M. to 12 Noon Period were significantly higher than the excretion from 12 Midnight to 8 A.M. ($P < 0.001$). There was no statistically significant difference between the 8 P.M. to 12 Midnight and the 8 A.M. to 12 Noon periods.

SUBJECTS 2A, 6A, 7A (Exercise)

When the data for the three subjects who exercised throughout the study were pooled together, it was found that the sub-period from 8 P.M. to 12 Midnight showed the highest level of creatinine excretion. When this period was compared with the periods from 12 Noon to 8 P.M. and from 12 Midnight to 8 A.M., a highly significant statistical difference was noted ($P < 0.001$). The sub-period from 8 A.M. to 12 Noon also showed a highly different level of creatinine excretion when compared with that from 12 Noon to 8 P.M. and from 12 Midnight to 8 A.M. ($P < 0.001$). A distinctly higher amount of creatinine excretion was noted to occur during the period from 12 Noon to 8 P.M. as compared to that from 12 Midnight to 8 A.M. ($P < 0.10$), with no significant difference being determined between the creatinine excretion from 8 P.M. to 12 Midnight and that from 8 A.M. to 12 Noon. See Table IV, Part D.

SUBJECT 4A

As shown in Table IV, Part E, when pairs of the four collection periods were compared for quantities of urinary creatinine excreted by means of the "t" test, the amount excreted by Subject 4A during the period from 8 P.M. to 12 Midnight was higher than that excreted between 12 Noon to 8 P.M. ($P < 0.01$). No significant difference was found between the 12 Noon to 8 P.M. period and the 12 Midnight to 8 A.M. period. The creatinine excretion from 8 A.M. to 12 Noon was slightly higher than that from 12 Noon to 8 P.M. ($P < 0.10$). During the period from 8 P.M. to 12 Midnight the creatinine excretion was significantly higher ($P < 0.001$) than during the periods from 12 Midnight to 8 A.M. and from 8 A.M. to 12 Noon. The creatinine excreted by this subject between 8 A.M. and 12 Noon was higher than that excreted between 12 Midnight and 8 A.M. ($P < 0.05$).

SUBJECT 8A

Table IV, Part F, contains the statistical data pertaining to the periodic comparison of the sub-periods of creatinine excretion for Subject 8A. The quantity of urinary creatinine excreted by this subject from 12 Noon to 8 P.M. surpassed that from 12 Midnight to 8 A.M. ($P < 0.02$). The creatinine excretion from 8 P.M. to 12 Midnight and from 8 A.M. to 12 Noon, exceeded both the excretion from 12 Midnight to 8 A.M. ($P < 0.01$). The periods from 12 Noon to 8 P.M. and from 8 P.M. to 12 Midnight were not significantly different than that of 8 A.M. to 12 Noon.

SUBJECT 9A

The amount of urinary creatinine excreted by Subject 9A, during the period from 8 P.M. to 12 Midnight was greater than from 12 Noon to 8 P.M. ($P < 0.05$). The quantity of creatinine excreted from 12 Noon to 8 P.M. surpassed the amount excreted from 12 Midnight to 8 A.M. ($P < 0.01$). The collection from 12 Noon to 8 P.M., however, was not significantly different from that between 8 A.M. and 12 Noon. The 8 P.M. to 12 Midnight samples contained a markedly higher level of creatinine excretion than the 12 Midnight to 8 A.M. samples ($P < 0.001$). The creatinine excretion from 8 P.M. to 12 Midnight surpassed that from 8 A.M. to 12 Noon by a significant difference ($P < 0.01$). A slightly higher excretion of creatinine occurred between 8 A.M. and 12 Noon than between 12 Midnight and 8 A.M. ($P < 0.10$). See Table IV, Part G.

SUBJECTS 4A, 8A, 9A (NO exercise)

The statistical information compiled from the combined data of the three subjects who did not exercise throughout the study is recorded in Table IV, Part H. The sub-period from 8 P.M. to 12 Midnight showed the highest level of creatinine excretion. A high level of significance ($P < 0.001$) was found when comparing the excretion levels of creatinine during this time, with those of the other three sub-periods, 12 Noon to 8 P.M., 12 Midnight to 8 A.M., and 8 A.M. to 12 Noon. No significant difference was evident between the 12 Noon to 8 P.M. and the 8 A.M. to 12 Noon collection periods. The urinary creatinine excretion during the period from 8 A.M. to 12 Noon was significantly greater than that during the period from 12 Midnight to 8 A.M. ($P < 0.001$). The higher creatinine excretion observed during the period from 12 Noon to 8 P.M. was significant when compared with the excretion from 12 Midnight to 8 A.M. ($P < 0.01$).

Subject 3A excreted the largest amount of urinary creatinine during the period from 8 A.M. until 12 Noon. The quantity of creatinine excreted during this period markedly surpassed the amounts excreted from 12 Noon to 8 P.M. and from 12 Midnight to 8 A.M. periods ($P < 0.001$), but was only moderately higher than that from 8 P.M. to 12 Midnight ($P < 0.05$). During the period from 8 P.M. to 12 Midnight, creatinine excretion was higher than between 12 Noon and 8 P.M. ($P < 0.01$). The samples collected from this subject between 12 Midnight and 8 A.M. contained the lowest level of urinary creatinine of any of the four periods of day. The quantity of creatinine excreted during this period was significantly lower ($P < 0.001$) than the amount excreted during the other three periods. See Table IV, Part I.

Subject 1A excreted the least amount of urinary creatinine during the period from 12 Midnight to 8 A.M. When comparing the amount of creatinine excreted during this period with the amounts excreted from 8 A.M. to 12 Noon, from 12 Noon to 8 P.M., and from 8 P.M. to 12 Midnight, a high level of statistical significance was observed ($P < 0.001$). This subject showed a slight increase in the excretion of creatinine between 8 P.M. and 12 Midnight as compared to the period from 12 Noon to 8 P.M. ($P < 0.05$). The quantity of creatinine excreted during the 8 A.M. to 12 Noon period significantly surpassed

($P < 0.01$) the amount excreted from 12 Noon to 8 P.M. No significant difference was found between the period from 8 P.M. to 12 Midnight and the period from 8 A.M. to 12 Noon. See Table IV, Part J.

ALL SUBJECTS

An analysis of the pooled data for all eight subjects showed that the highest level of urinary creatinine excretion among the four periods of the day occurred during the period from 8 P.M. to 12 Midnight. Creatinine excretion during this period was distinctly significant ($P < 0.001$) when compared with the periods from 12 Noon to 8 P.M. and 12 Midnight to 8 A.M. The amount of creatinine excreted by all subjects between 8 A.M. and 12 Noon was significantly higher than the amounts excreted during the periods from 12 Noon to 8 P.M. and 12 Midnight to 8 A.M. ($P < 0.001$). The creatinine excretion from 12 Noon to 8 P.M. surpassed that from 12 Midnight to 8 A.M. by a highly significant difference ($P < 0.001$) while the quantity of creatinine excreted from 8 P.M. to 12 Midnight was only slightly greater than the amount excreted from 8 A.M. to 12 Noon ($P < 0.10$).

Although some individual variations were observed in the periodicities of the subjects of the study, the pooled data for all subjects indicated the existence of a marked characteristic circadian cycle in urinary creatinine excretion, with the peak level of excretion being attained between 8 P.M. and 12 Midnight. Subjects 7A, 3A, 1A, however, excreted the highest level of creatinine in the urine between 8 A.M. and 12 Noon.

All subjects except 6A, experienced the lowest level of urinary creatinine excretion between the hours of 12 Midnight to 8 A.M. This finding is similar to reports by Mills (60) and Montgomery (41) who found creatinine excretion to be lowest during the overnight collection.

In comparing the combined data for the three individuals who exercised throughout the study shown in Table IV, Part D, with the combined data for the three subjects who did not exercise (Table IV, Part H), it is of note that a higher level of creatinine excretion for all periods was apparent in the group who exercised. The comparison between the periods for the two groups was the same, except in two instances. The group who did not exercise showed no level of statistical significance between the 12 Noon to 8 P.M. period and the 8 A.M. to 12 Noon period whereas the exercise group excreted a far greater amount ($P < 0.001$) of urinary creatinine during the period from 8 A.M. to 12 Noon.

The other contrast between the two groups was found in a comparison of the periods between 8 P.M. to 12 Midnight and 8 A.M. to 12 Noon. The groups which did not exercise excreted a distinctly higher ($P < 0.001$) amount of urinary creatinine between 8 P.M. and 12 Midnight while the values between the two periods for the exercise group showed no level of significance.

Comparison of Urinary Creatinine Excretion During Bed Rest As Influenced by Exercise

Table V, VI, VII, and VIII (Appendix) contain the data pertinent to the effect of exercise on urinary creatinine excretion.

SUBJECT 3A (Exercised First 28 Days of the Study)

The urinary creatinine excretion of this subject was significantly lower ($P < 0.01$) when he was participating in exercise as shown in Table V. Table V shows a comparison between the creatinine excretion during the time when each of the men exercised part time in comparison with the time when they did not exercise. Subject 3A complained continuously of fatigue during the time when he was supposed to exercise, and rarely completed an exercise program. Table VI, Part A, shows a comparison of the creatinine excretion at different periods of the day by this subject. During the period from 12 Noon to 8 P.M. when this subject exercised, the decreased excretion of creatinine was distinctly significant ($P < 0.001$) when compared to the period when this subject did not exercise. Also, exercise during the period from 8 P.M. to 12 Midnight appeared to effect a significant decrease ($P < 0.01$) in urinary creatinine excretion as compared to the time when no exercise was taken. No significant changes in creatinine excretion were noted from 12 Midnight to 8 A.M. and from 8 A.M. to 12 Noon due to activity or inactivity.

<u>SUBJECT</u> <u>1A</u> (Exercised Second Half of Study)

As shown in Table V, Subject 1A experienced an appreciable increase in the excretion of creatinine during the second half of the Bed Rest when he was engaged in programmed exercise. Upon comparing the creatinine excretion during the exercise period with that of the first 28 days when this subject did not exercise, a highly significant difference ($P < 0.001$) was found. No significant difference was reflected in the amounts of urinary creatinine excreted by this subject under different activity schedules, during the collection periods from 12 Noon to 8 P.M., from 8 P.M. to 12 Midnight, and from 12 Midnight to 8 A.M. During the period from 8 A.M. to 12 Noon, however, this subject excreted a significantly higher amount of creatinine when participating in exercise than when not exercising ($P < 0.001$). See Table VI, Part B.

<u>GROUP OF SUBJECTS WHO EXERCISED</u> (2A, 6A, 7A) <u>VERSUS GROUP WHO DID NOT EXERCISE</u> (4A, 8A, 9A)

A statistical comparison was made between the group of subjects which exercised (2A, 6A, 7A) and the group of subjects which did not exercise (4A, 8A, 9A) during the Bed Rest Period. The mean quantity of creatinine excretion per day was significantly higher for the group exercising than for the group not exercising ($P < 0.001$). See Table VII.

When the creatinine values for the same times of the day during Bed Rest were compared between the group exercising and the group not exercising, it was observed that in every period, a higher quantity of urinary creatinine was excreted by the group which exercised. No statistical significance was found between the creatinine excreted by the groups between 8 P.M. and 12 Midnight. A highly statistical difference ($P < 0.001$) was found between the excretory levels of the two groups between 8 A.M. and 12 Noon and lesser degrees of significance were noted for the Periods from 12 Noon to 8 P.M. ($P < 0.10$) and from 12 Midnight to 8 A.M. ($P < 0.01$). See Table VIII.

According to Tables V and VI, both Subject 3A and Subject 1A excreted more creatinine during the last half of the Bed Rest than during the first half of this period. Apparently, the stress of prolonged immobilization serves to stimulate an increase in the excretion of urinary creatinine. Also, prolonged immobilization seems to more significantly affect the excretory levels of urinary creatinine than does exercise. According to Table VII and VIII, however, exercise did influence the rate of creatinine excretion during the Bed Rest Period as the group of subjects participating in the exercise program throughout the period had a higher mean excretion of the metabolite.

SUMMARY AND CONCLUSIONS

Eight male university students participated in an immobilization study which covered a span of 85 days and consisted of a 16-day Pre-Bed Rest Period, a 56-day Bed Rest Period, and a 14-day Post-Bed Rest Period. This study was conducted during the summer of 1969 at the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute and was sponsored by the National Aeronautics and Space Administration. This investigation was designed to observe any changes occurring in bone density and various metabolic functions, including circadian response, in human subjects experiencing prolonged immobilization under carefully controlled environmental conditions.

This report deals with the metabolite, creatinine. The analytical method of Biggs and Cooper (65) was employed to determine the creatinine content of all urinary samples collected throughout this study.

Total 24-hour urinary creatinine excretion was found to be significantly higher ($P < 0.001$) for all subjects during the Bed Rest Period of the study as compared to the Pre- and Post-Bed Rest Periods. Immobilization appears to increase the excretion levels of creatinine, possibly because of stress. Creatinine excretions rapidly returned to normal levels in the Post-Bed Rest Ambulatory Period.

The circadian variation of creatinine also was studied during the 56-day Bed Rest Period. Throughout this period of the study, a strict regimen of 14 hours daytime and 10 hours nighttime was maintained, and urinary samples were collected four times daily. Most subjects excreted the lowest amount of urinary creatinine during the overnight period. Five of the subjects attained a peak in creatinine excretion between 8 P.M. and 12 Midnight while three of the subjects exhibited the highest excretion of this metabolite between 8 A.M. and 12 Noon.

The subjects were divided into groups which did and did not participate in programmed exercise which was introduced as a variable during the immobilization period. It was observed that the group which exercised excreted a significantly greater amount of creatinine in the urine than did the group which did not exercise ($P < 0.001$). Two individuals exercised for 28 days each, one the first half of the bed rest and the other the latter part. Both of these individauls excreted a greater amount of creatinine during the second half of the bed rest. This finding could have been related to the influence of the element of stress which is involved in prolonged immobilization.

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A P P E N D I X

TABLE I

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART A. SUBJECT 1A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1202	(1)	830	(29)	1980	(1)	758
(2)	X	(2)	1267	(30)	1823	(2)	752
(3)	656	(3)	1621	(31)	1601	(3)	652
(4)	919	(4)	1221	(32)	2022	(4)	843
(5)	1969	(5)	1445	(33)	1708	(5)	649
(6)	1004	(6)	1389	(34)	1922	(6)	X
(7)	1441	(7)	1424	(35)	1908	(7)	614
(8)	704	(8)	1117	(36)	1681	(8)	520
(9)	1210	(9)	1529	(37)	2159	(9)	2825
(10)	466	(10)	1404	(38)	2101	(10)	849
(11)	2127	(11)	1431	(39)	1906	(11)	X
(12)	1086	(12)	1577	(40)	1946	(12)	863
(13)	1216	(13)	1644	(41)	1923	(13)	X
(14)	956	(14)	1583	(42)	1705	Mean	942
(15)	1490	(15)	1805	(43)	1736		
(16)	1542	(16)	1598	(44)	1823		
Mean	1199	(17)	1718	(45)	1606		
		(18)	1798	(46)	1959		
		(19)	1958	(47)	1878		
		(20)	1370	(48)	1968		
		(21)	1844	(49)	1670		
		(22)	1983	(50)	1651		
		(23)	1819	(51)	1982		
		(24)	1180	(52)	1691		
		(25)	1683	(53)	1763		
		(26)	1855	(54)	1724		
		(27)	1999	(55)	1879		
		(28)	2014	(56)	1493		
				Mean	1702		

TABLE I, CONTINUED

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART B. SUBJECT 2A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1291	(1)	834	(29)	1700	(1)	1269
(2)	1017	(2)	814	(30)	1675	(2)	1012
(3)	1188	(3)	1506	(31)	1708	(3)	1357
(4)	607	(4)	1362	(32)	1754	(4)	984
(5)	1062	(5)	1892	(33)	1629	(5)	510
(6)	996	(6)	1669	(34)	1719	(6)	500
(7)	760	(7)	1188	(35)	1543	(7)	1192
(8)	896	(8)	1716	(36)	1593	(8)	574
(9)	740	(9)	1382	(37)	2014	(9)	1266
(10)	1156	(10)	1599	(38)	1412	(10)	1229
(11)	1081	(11)	1506	(39)	1716	(11)	656
(12)	1303	(12)	1432	(40)	1719	(12)	273
(13)	1371	(13)	1514	(41)	1739	(13)	638
(14)	1028	(14)	1307	(42)	1665	Mean	882
(15)	1200	(15)	1530	(43)	1598		
(16)	1064	(16)	1441	(44)	1620		
Mean	1048	(17)	1747	(45)	1514		
		(18)	1592	(46)	1517		
		(19)	1504	(47)	1821		
		(20)	1475	(48)	1596		
		(21)	1656	(49)	1786		
		(22)	1754	(50)	1270		
		(23)	1951	(51)	1759		
		(24)	1044	(52)	1723		
		(25)	1768	(53)	2038		
		(26)	1774	(54)	1599		
		(27)	1658	(55)	1990		
		(28)	1736	(56)	1088		
				Mean	1587		

TABLE I, CONTINUED

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART C. SUBJECT 3A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	680	(1)	689	(29)	1495	(1)	942
(2)	523	(2)	919	(30)	1415	(2)	1355
(3)	1140	(3)	1190	(31)	1194	(3)	630
(4)	935	(4)	1168	(32)	1403	(4)	1696
(5)	1148	(5)	1256	(33)	1523	(5)	1236
(6)	628	(6)	1454	(34)	1601	(6)	1323
(7)	958	(7)	1120	(35)	1227	(7)	695
(8)	761	(8)	1286	(36)	1738	(8)	1570
(9)	938	(9)	1851	(37)	1838	(9)	912
(10)	550	(10)	1238	(38)	1383	(10)	1421
(11)	668	(11)	843	(39)	1741	(11)	816
(12)	2033	(12)	1234	(40)	1507	(12)	620
(13)	1056	(13)	1724	(41)	1651	(13)	1134
(14)	468	(14)	1238	(42)	1687	Mean	1104
(15)	1734	(15)	1072	(43)	1297		
(16)	870	(16)	1313	(44)	1486		
Mean	943	(17)	1555	(45)	2042		
		(18)	1450	(56)	1777		
		(19)	1402	(47)	1537		
		(20)	736	(48)	1381		
		(21)	1515	(49)	1332		
		(22)	1513	(50)	1415		
		(23)	1504	(51)	1878		
		(24)	1065	(52)	1360		
		(25)	2059	(53)	1639		
		(26)	1061	(54)	1456		
		(27)	1609	(55)	1573		
		(28)	1604	(56)	1464		
				Mean	1423		

TABLE I, CONTINUED

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART D. SUBJECT 4A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1349	(1)	921	(29)	2230	(1)	1426
(2)	1125	(2)	1413	(30)	1808	(2)	951
(3)	1360	(3)	1142	(31)	1789	(3)	684
(4)	1348	(4)	1650	(32)	1944	(4)	1727
(5)	1188	(5)	1012	(33)	1838	(5)	1305
(6)	1800	(6)	1555	(34)	1988	(6)	1400
(7)	1662	(7)	1786	(35)	1743	(7)	1473
(8)	1166	(8)	1598	(36)	2002	(8)	1120
(9)	1121	(9)	1712	(37)	2063	(9)	2288
(10)	891	(10)	1300	(38)	1591	(10)	1185
(11)	984	(11)	1597	(39)	1802	(11)	1114
(12)	627	(12)	1293	(40)	2192	(12)	2154
(13)	1085	(13)	1751	(41)	2516	(13)	901
(14)	887	(14)	2308	(42)	1701	Mean	1364
(15)	1223	(15)	1833	(43)	2192		
(16)	1073	(16)	1465	(44)	1787		
Mean	1181	(17)	1560	(45)	1795		
		(18)	1782	(46)	1795		
		(19)	1834	(47)	1952		
		(20)	1257	(48)	1705		
		(21)	1899	(49)	1675		
		(22)	1925	(50)	1891		
		(23)	1995	(51)	1926		
		(24)	1305	(52)	1824		
		(25)	2214	(53)	2868		
		(26)	1704	(54)	1826		
		(27)	1987	(55)	1940		
		(28)	2123	(56)	1655		
				Mean	1785		

TABLE I, CONTINUED

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART E. SUBJECT 6A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1223	(1)	1283	(29)	2072	(1)	1221
(2)	1667	(2)	1507	(30)	2392	(2)	1465
(3)	1243	(3)	1303	(31)	1888	(3)	1844
(4)	1678	(4)	1462	(32)	1678	(4)	2196
(5)	1502	(5)	1360	(33)	2047	(5)	1675
(6)	1642	(6)	1668	(34)	2156	(6)	1802
(7)	2016	(7)	1539	(35)	1961	(7)	2013
(8)	1551	(8)	1568	(36)	2010	(8)	2233
(9)	1276	(9)	1764	(37)	2276	(9)	1919
(10)	1682	(10)	1535	(38)	1838	(10)	1837
(11)	1846	(11)	1512	(39)	2144	(11)	1524
(12)	1155	(12)	1505	(40)	2159	(12)	2025
(13)	1544	(13)	1640	(41)	2186	(13)	2190
(14)	1627	(14)	1801	(42)	2140	Mean	1842
(15)	2111	(15)	1514	(43)	1728		
(16)	2103	(16)	2034	(44)	2392		
Mean	1617	(17)	2187	(45)	2040		
		(18)	2006	(46)	2316		
		(19)	1481	(47)	1962		
		(20)	930	(48)	1770		
		(21)	1652	(49)	2149		
		(22)	2160	(50)	1843		
		(23)	1960	(51)	1631		
		(24)	1256	(52)	1783		
		(25)	1811	(53)	2090		
		(26)	1866	(54)	1825		
		(27)	2114	(55)	2111		
		(28)	2202	(56)	1794		
				Mean	1839		

TABLE I, CONTINUED

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART F. SUBJECT 7A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	X	(1)	1773	(29)	2094	(1)	1590
(2)	1297	(2)	1989	(30)	2330	(2)	2322
(3)	775	(3)	1484	(31)	1942	(3)	1838
(4)	674	(4)	1479	(32)	2180	(4)	1492
(5)	1275	(5)	1301	(33)	2014	(5)	1507
(6)	2708	(6)	1887	(34)	2551	(6)	992
(7)	1008	(7)	1963	(35)	2288	(7)	1639
(8)	1485	(8)	2164	(36)	2089	(8)	784
(9)	399	(9)	1928	(37)	2554	(9)	1344
(10)	1238	(10)	1937	(38)	1555	(10)	558
(11)	1601	(11)	1817	(39)	2195	(11)	1354
(12)	1688	(12)	2061	(40)	1876	(12)	1092
(13)	1622	(13)	1698	(41)	2080	(13)	1896
(14)	1242	(14)	1683	(42)	1792	Mean	1416
(15)	1407	(15)	2082	(43)	1649		
(16)	1451	(16)	1929	(44)	2005		
Mean	1325	(17)	2551	(45)	2062		
		(18)	1845	(46)	2057		
		(19)	2192	(47)	2290		
		(20)	1107	(48)	1987		
		(21)	1596	(49)	2222		
		(22)	2068	(50)	1768		
		(23)	1707	(51)	1957		
		(24)	1479	(52)	2067		
		(25)	1873	(53)	2114		
		(26)	2016	(54)	2002		
		(27)	2303	(55)	2273		
		(28)	2107	(56)	1687		
				Mean	1959		

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART G. SUBJECT 8A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1046	(1)	1420	(29)	1900	(1)	2023
(2)	1077	(2)	1577	(30)	1695	(2)	1090
(3)	1520	(3)	1356	(31)	1795	(3)	1038
(4)	1473	(4)	1240	(32)	1707	(4)	1523
(5)	1724	(5)	892	(32)	1929	(5)	1418
(6)	1892	(6)	2656	(34)	1687	(6)	1305
(7)	1101	(7)	1945	(35)	1485	(7)	1397
(8)	1646	(8)	1546	(36)	1810	(8)	1332
(9)	930	(9)	1764	(37)	2044	(9)	1734
(10)	1394	(10)	1606	(38)	1838	(10)	1247
(11)	1837	(11)	1374	(39)	1938	(11)	825
(12)	1340	(12)	1432	(40)	1798	(12)	864
(13)	1409	(13)	1584	(41)	1890	(13)	1413
(14)	1390	(14)	1304	(42)	1735	Mean	1324
(15)	1357	(15)	1542	(43)	1024		
(16)	2026	(16)	1967	(44)	1730		
Mean	1448	(17)	1849	(45)	1508		
		(18)	1771	(46)	1736		
		(19)	1664	(47)	1744		
		(20)	1414	(48)	1465		
		(21)	1720	(49)	2142		
		(22)	1914	(50)	1844		
		(23)	2343	(51)	1694		
		(24)	1075	(52)	1566		
		(25)	1864	(53)	1594		
		(26)	1556	(54)	1434		
		(27)	2080	(55)	1506		
		(28)	2108	(56)	1546		
				Mean	1685		

URINARY CREATININE EXCRETION

(milligrams per 24 hours)

PART H. SUBJECT 9A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
(1)	1079	(1)	1078	(29)	1571	(1)	951
(2)	1290	(2)	1803	(30)	1623	(2)	1219
(3)	587	(3)	1471	(31)	1380	(3)	X
(4)	621	(4)	1092	(32)	1602	(4)	1300
(5)	1032	(5)	1069	(33)	1610	(5)	1245
(6)	1051	(6)	1158	(34)	1685	(6)	650
(7)	1219	(7)	2560	(35)	1634	(7)	1333
(8)	1176	(8)	1234	(36)	1534	(8)	1087
(9)	808	(9)	1495	(37)	1742	(9)	1391
(10)	873	(10)	1089	(38)	1562	(10)	1104
(11)	1126	(11)	1415	(39)	1261	(11)	1357
(12)	996	(12)	1482	(40)	1627	(12)	719
(13)	1180	(13)	1333	(41)	1787	(13)	977
(14)	748	(14)	1097	(42)	1451	Mean	1111
(15)	1302	(15)	1290	(43)	1625		
(16)	955	(16)	1592	(44)	1583		
Mean	1003	(17)	1676	(45)	1518		
		(18)	1408	(46)	1512		
		(19)	2017	(47)	1466		
		(20)	938	(48)	1333		
		(21)	1465	(49)	1610		
		(22)	1543	(50)	1479		
		(23)	1614	(51)	1772		
		(24)	924	(52)	1563		
		(25)	1261	(53)	1353		
		(26)	1538	(54)	1445		
		(27)	1800	(55)	1517		
		(28)	1604	(56)	960		
				Mean	1479		

TABLE II

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART A. SUBJECT 1A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	28	28	28	68	(30)	87	86	66	66
(2)	67	68	19	77	(31)	65	33	26	186
(3)	84	33	79	70	(32)	57	118	86	100
(4)	53	57	44	54	(33)	86	78	59	59
(5)	61	75	42	81	(34)	106	75	65	65
(6)	48	39	62	90	(35)	100	91	62	62
(7)	75	56	59	33	(36)	62	110	71	45
(8)	42	59	24	88	(37)	90	84	92	92
(9)	78	48	61	58	(38)	75	112	88	88
(10)	42	42	49	128	(39)	101	48	91	44
(11)	49	97	62	40	(40)	94	46	28	196
(12)	66	55	80	47	(41)	85	63	20	209
(13)	73	66	61	79	(42)	62	91	19	174
(14)	88	50	39	93	(43)	99	64	18	156
(15)	57	142	75	45	(44)	63	136	77	42
(16)	29	179	59	44	(45)	42	108	80	49
(17)	63	76	67	96	(46)	77	111	13	199
(18)	87	38	63	114	(47)	81	78	26	178
(19)	77	139	67	67	(48)	59	175	14	171
(20)	42	67	53	85	(49)	77	55	19	170
(21)	94	100	63	48	(50)	93	75	13	128
(22)	108	63	77	64	(51)	125	31	25	165
(23)	86	86	33	131	(52)	23	167	75	59
(24)	59	63	6	102	(53)	108	72	51	51
(25)	59	112	73	46	(54)	63	109	70	56
(26)	80	89	78	60	(55)	33	168	25	187
(27)	101	105	78	36	(56)	84	X	X	X
(28)	74	140	72	72	Mean	72	85	53	92
(29)	86	130	73	47					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART B. SUBJECT 2A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	21	33	33	68	(30)	68	73	67	75
(2)	24	55	36	29	(31)	72	82	34	132
(3)	82	13	71	59	(32)	56	87	78	85
(4)	72	49	38	72	(33)	60	75	64	88
(5)	105	94	55	60	(34)	90	71	54	72
(6)	65	108	60	50	(35)	61	53	77	58
(7)	63	65	25	57	(36)	58	100	72	40
(8)	70	108	52	77	(37)	98	96	62	87
(9)	71	81	31	62	(38)	60	43	59	73
(10)	65	98	46	81	(39)	73	67	79	60
(11)	76	79	81	65	(40)	63	104	62	76
(12)	28	131	57	57	(41)	70	62	77	79
(13)	67	82	51	62	(42)	76	72	65	63
(14)	46	46	56	79	(43)	63	108	53	60
(15)	38	73	76	82	(44)	79	78	49	73
(16)	43	39	71	94	(45)	45	92	63	70
(17)	79	89	85	22	(46)	85	73	30	76
(18)	52	91	45	113	(47)	91	59	80	55
(19)	56	97	53	62	(48)	63	99	52	71
(20)	57	77	64	49	(49)	76	91	71	61
(21)	63	64	72	73	(50)	31	58	65	68
(22)	97	29	71	73	(51)	84	61	71	70
(23)	81	86	69	102	(52)	49	129	62	79
(24)	27	36	46	79	(53)	122	87	54	71
(25)	66	107	59	86	(54)	66	82	57	72
(26)	45	184	55	61	(55)	117	26	87	64
(27)	77	31	85	60	(56)	33	X	X	X
(28)	56	83	53	130	Mean	66	77	60	71
(29)	70	104	59	62					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART C. SUBJECT 3A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	28	28	28	40	(30)	25	78	27	171
(2)	33	44	51	19	(31)	47	53	53	47
(3)	61	63	40	28	(32)	30	84	76	56
(4)	28	37	42	107	(33)	56	110	51	56
(5)	53	52	55	46	(34)	84	37	20	155
(6)	84	78	34	49	(35)	35	83	32	90
(7)	57	32	45	45	(36)	90	70	63	59
(8)	51	103	33	53	(37)	59	80	34	138
(9)	68	88	58	124	(38)	66	42	24	124
(10)	56	45	25	101	(39)	79	94	26	131
(11)	47	35	31	22	(40)	33	117	63	69
(12)	65	67	37	37	(41)	69	87	31	127
(13)	83	68	73	51	(42)	43	106	71	89
(14)	55	36	36	91	(43)	57	93	39	39
(15)	43	32	44	61	(44)	37	139	32	94
(16)	41	61	46	92	(45)	94	61	86	90
(17)	65	77	31	120	(46)	90	78	21	145
(18)	57	98	39	74	(47)	60	67	28	142
(19)	74	90	30	53	(48)	32	113	20	128
(20)	23	44	10	75	(49)	70	65	22	95
(21)	59	78	43	96	(50)	59	83	26	101
(22)	51	94	35	112	(51)	109	41	43	125
(23)	78	65	36	83	(52)	53	68	50	67
(24)	29	86	49	49	(53)	67	93	61	61
(25)	48	52	8	351	(54)	48	86	58	66
(26)	22	44	48	77	(55)	66	88	25	124
(27)	76	55	50	96	(56)	77	X	X	X
(28)	71	70	63	63	Mean	57	71	41	88
(29)	60	69	68	46					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART D. SUBJECT 4A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	8	46	46	56	(30)	54	124	65	90
(2)	69	62	42	71	(31)	73	98	74	57
(3)	30	54	41	80	(32)	56	117	65	126
(4)	76	68	56	81	(33)	91	70	63	82
(5)	43	25	37	69	(34)	117	201	82	97
(6)	73	88	49	57	(35)	100	82	63	27
(7)	79	134	72	12	(36)	27	149	74	96
(8)	12	121	83	90	(37)	99	69	70	110
(9)	89	131	40	40	(38)	65	71	57	83
(10)	68	67	41	40	(39)	97	82	69	38
(11)	33	33	33	151	(40)	99	52	107	84
(12)	47	120	36	36	(41)	72	118	80	208
(13)	84	72	34	128	(42)	50	128	65	69
(14)	128	72	76	98	(43)	69	130	114	53
(15)	44	156	72	70	(44)	72	136	62	43
(16)	25	116	59	83	(45)	78	74	75	64
(17)	47	163	20	95	(46)	74	85	63	89
(18)	73	77	77	69	(47)	60	60	117	75
(19)	69	126	64	68	(48)	74	90	56	76
(20)	49	67	35	79	(49)	80	128	38	67
(21)	79	119	63	72	(50)	71	102	76	76
(22)	33	214	61	80	(51)	75	77	90	70
(23)	89	78	78	87	(52)	33	184	64	78
(24)	32	112	56	37	(53)	151	151	89	86
(25)	101	126	66	96	(54)	56	56	109	70
(26)	68	81	66	79	(55)	100	71	72	72
(27)	88	112	73	63	(56)	87	X	X	X
(28)	80	158	65	83	Mean	67	102	66	77
(29)	41	131	118	99					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART E. SUBJECT 6A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	66	51	51	38	(30)	39	256	71	122
(2)	82	70	40	63	(31)	75	118	74	56
(3)	81	41	51	23	(32)	77	31	70	95
(4)	38	80	72	49	(33)	91	91	75	89
(5)	46	79	52	68	(34)	95	90	78	104
(6)	86	102	57	29	(35)	97	92	69	66
(7)	96	31	29	104	(36)	66	113	78	104
(8)	57	43	41	153	(37)	79	146	83	100
(9)	78	54	87	56	(38)	82	86	55	100
(10)	80	69	40	75	(39)	98	109	72	88
(11)	80	83	48	39	(40)	60	91	115	100
(12)	48	47	80	74	(41)	58	140	98	98
(13)	92	86	46	46	(42)	15	179	107	113
(14)	77	39	82	94	(43)	113	14	7	28
(15)	42	47	33	82	(44)	82	97	89	160
(16)	50	151	76	106	(45)	56	164	76	83
(17)	73	129	83	105	(46)	112	100	84	86
(18)	28	148	95	108	(47)	76	109	74	82
(19)	31	71	84	68	(48)	74	74	78	64
(20)	35	79	28	29	(49)	87	105	92	74
(21)	64	94	78	36	(50)	60	132	83	45
(22)	102	110	70	85	(51)	52	79	72	82
(23)	173	134	89	33	(52)	39	127	77	88
(24)	28	44	73	68	(53)	97	73	72	112
(25)	62	107	77	69	(54)	51	177	72	44
(26)	72	107	69	78	(55)	85	126	75	82
(27)	90	84	105	54	(56)	86	X	X	X
(28)	51	191	79	98	Mean	69	99	71	78
(29)	49	127	73	71					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART F. SUBJECT 7A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	52	83	83	90	(30)	103	120	95	68
(2)	71	58	74	64	(31)	100	102	65	53
(3)	43	98	54	78	(32)	83	117	79	104
(4)	62	63	72	41	(33)	93	94	74	76
(5)	27	93	61	56	(34)	105	71	121	114
(6)	72	94	71	91	(35)	95	134	82	68
(7)	75	76	91	83	(36)	68	129	89	80
(8)	95	92	95	70	(37)	93	88	13	339
(9)	75	133	53	93	(38)	29	82	34	181
(10)	67	108	70	103	(39)	91	109	53	152
(11)	118	50	70	28	(40)	74	69	32	188
(12)	93	39	104	82	(41)	62	92	103	97
(13)	86	81	44	83	(42)	72	46	84	45
(14)	74	46	79	71	(43)	61	79	16	179
(15)	119	89	83	27	(44)	95	74	63	113
(16)	90	79	76	72	(45)	108	63	27	183
(17)	95	141	107	94	(46)	114	60	29	169
(18)	32	164	21	191	(47)	115	140	33	138
(19)	69	143	83	101	(48)	60	158	78	61
(20)	30	59	60	38	(49)	98	62	112	74
(21)	103	101	94	60	(50)	73	72	41	143
(22)	75	69	65	169	(51)	91	77	53	123
(23)	95	86	63	26	(52)	113	82	75	59
(24)	35	87	84	46	(53)	43	73	104	161
(25)	70	93	18	199	(54)	66	166	85	34
(26)	60	149	82	72	(55)	95	99	26	228
(27)	118	60	101	79	(56)	85	X	X	X
(28)	68	84	107	94	Mean	79	93	69	103
(29)	89	113	58	116					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART G. SUBJECT 8A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	38	55	55	61	(30)	57	107	67	70
(2)	76	54	60	69	(31)	96	65	28	137
(3)	107	37	15	58	(32)	63	92	65	80
(4)	65	47	53	27	(33)	95	81	71	69
(5)	38	86	18	25	(34)	83	28	63	103
(6)	75	47	68	109	(35)	99	70	20	64
(7)	102	63	66	88	(36)	64	128	59	79
(8)	69	50	54	91	(37)	86	126	62	91
(9)	63	79	79	78	(38)	79	78	65	94
(10)	74	63	51	90	(39)	113	76	54	75
(11)	85	49	50	25	(40)	82	23	99	66
(12)	55	66	68	48	(41)	85	58	79	89
(13)	74	71	54	70	(42)	81	66	55	97
(14)	43	51	58	51	(43)	38	52	31	68
(15)	64	62	58	80	(44)	79	57	66	86
(16)	63	65	95	111	(45)	47	87	62	73
(17)	65	143	65	64	(46)	85	73	56	78
(18)	73	96	69	60	(47)	77	105	59	61
(19)	60	64	84	64	(48)	57	74	59	64
(20)	44	114	62	28	(49)	85	125	85	70
(21)	57	87	69	92	(50)	74	122	58	75
(22)	93	71	67	86	(51)	71	41	88	66
(23)	107	69	111	83	(52)	67	76	45	88
(24)	32	46	59	42	(53)	54	117	52	70
(25)	81	104	57	85	(54)	44	81	55	81
(26)	66	96	46	69	(55)	26	97	79	63
(27)	107	56	91	68	(56)	77	X	X	X
(28)	85	121	74	88	Mean	71	77	62	74
(29)	73	94	75	88					

TABLE II, CONTINUED

URINARY CREATININE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(milligrams per hour during each period)

PART H. SUBJECT 9A

Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M. 12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)	Days	12 Noon-8 P.M. (mgs/hr.)	8 P.M.-12 Mid-night (mgs/hr.)	12 Mid-night-8 A.M. (mgs/hr.)	8 A.M.-12 Noon (mgs/hr.)
(1)	32	45	45	67	(30)	65	94	52	77
(2)	133	46	48	44	(31)	81	63	25	70
(3)	79	80	48	35	(32)	58	120	51	61
(4)	35	113	24	41	(33)	74	48	72	63
(5)	42	27	57	44	(34)	72	72	72	59
(6)	48	60	61	14	(35)	88	51	61	60
(7)	61	112	38	41	(36)	60	102	50	58
(8)	43	68	45	66	(37)	73	53	76	84
(9)	67	90	7	[illegible]	(38)	[illegible]	[illegible]	[illegible]	[illegible]
(10)	45	51	46	44	(39)	43	59	58	55
(11)	64	72	51	52	(40)	70	59	76	58
(12)	68	83	56	41	(41)	74	55	77	86
(13)	83	51	34	48	(42)	57	34	73	58
(14)	42	71	26	68	(43)	73	85	66	44
(15)	33	40	81	55	(44)	66	82	59	64
(16)	55	55	75	84	(45)	76	68	48	64
(17)	80	81	76	25	(46)	64	89	25	112
(18)	44	85	49	81	(47)	72	78	47	66
(19)	56	240	47	59	(48)	57	58	50	62
(20)	34	59	25	60	(49)	71	79	62	60
(21)	60	110	53	29	(50)	69	77	68	46
(22)	76	57	55	67	(51)	76	79	80	52
(23)	79	98	57	33	(52)	58	86	58	73
(24)	41	29	40	42	(53)	27	109	41	95
(25)	38	59	54	72	(54)	65	63	58	53
(26)	67	116	38	59	(55)	67	46	68	64
(27)	102	37	75	60	(56)	46	x	x	x
(28)	58	64	71	79	Mean	63	74	54	60
(29)	82	64	59	45					

TABLE III

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

PART A. SUBJECTS 2A, 6A, 7A (Exercised Throughout the Study, Individual Data)

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Subject 2A				
Pre-Bed Rest	1048	207	7.6736	P< 0.001
Bed Rest	1587	250		
Pre-Bed Rest	1048	207	1.4629	N.S.
Post-Bed Rest	882	354		
Bed Rest	1587	250	8.1174	P<0.001
Post-Bed Rest	882	354		
Subject 6A				
Pre-Bed Rest	1617	291	2.4525	P<0.02
Bed Rest	1839	317		
Pre-Bed Rest	1617	291	1.9213	P<0.10
Post-Bed Rest	1842	294		
Bed Rest	1839	317	0.0256	N.S.
Post-Bed Rest	1842	294		
Subject 7A				
Pre-Bed Rest	1325	514	6.0156	P<0.001
Bed Rest	1959	291		
Pre-Bed Rest	1325	514	0.4573	N.S.
Post-Bed Rest	1416	460		
Bed Rest	1959	291	5.1635	P<0.001
Post-Bed Rest	1416	460		

TABLE III, CONTINUED

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

PART B. SUBJECTS 2A, 6A, 7A (Exercised Throughout the Study, Combined Data)

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Bed Rest	1330 1795	427 327	7.9467	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	1330 1380	427 544	0.4670	N.S.
Bed Rest Post-Bed Rest	1795 1380	327 544	6.1189	$P < 0.001$

TABLE III, CONTINUED

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

PART C. SUBJECTS 4A, 8A, 9A (Did not exercise, Individual Data)

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Subject 4A				
Pre-Bed Rest Bed Rest	1181 1785	279 340	6.3337	$P<0.001$
Pre-Bed Rest Post-Bed Rest	1181 1364	279 450	1.2461	N.S.
Bed Rest Post-Bed Rest	1785 1364	340 450	3.6433	$P<0.001$
Subject 8A				
Pre-Bed Rest Bed Rest	1448 1685	308 304	2.6676	$P<0.01$
Pre-Bed Rest Post-Bed Rest	1448 1324	308 319	0.9851	N.S.
Bed Rest Post Bed Rest	1685 1324	304 319	3.7078	$P<0.001$
Subject 9A				
Pre-Bed Rest Bed Rest	1003 1479	216 275	6.2182	$P<0.001$
Pre-Bed Rest Post-Bed Rest	1003 1111	216 235	1.1744	N.S.
Bed Rest Post-Bed Rest	1479 1111	275 235	4.1862	$P<0.001$

TABLE III, CONTINUED

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

PART D. SUBJECTS 4A, 8A, 9A (Did NOT Exercise, Combined Data)

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	1210	327	8.0252	$P < 0.001$
Bed Rest	1650	333		
Pre-Bed Rest	1210	327	0.7847	N.S.
Post-Bed Rest	1270	365		
Bed Rest	1650	333	6.1660	$P < 0.001$
Post-Bed Rest	1270	365		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY FOR SUBJECTS WHO EXERCISED 28 DAYS EACH

(Subject 3A - First 28 Days,
Subject 1A - Second Half
of Study)

PART E. SUBJECTS 1A, 3A

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Subject 1A				
Pre-Bed Rest	1199	446	5.3426	$P<0.001$
Bed Rest	1702	266		
Pre-Bed Rest	1199	446	1.0259	N.S.
Post-Bed Rest	942	674		
Bed Rest	1702	266	5.7179	$P<0.001$
Post-Bed Rest	942	674		
Subject 3A				
Pre-Bed Rest	943	414	5.1296	$P<0.001$
Bed Rest	1423	287		
Pre-Bed Rest	943	414	1.0394	N.S.
Post-Bed Rest	1104	347		
Bed Rest	1423	287	3.3564	$P<0.001$
Post-Bed Rest	1104	347		

TABLE III, CONTINUED

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

PART F. ALL SUBJECTS

POPULATIONS COMPARED	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	1220	411	12.8712	$P < 0.001$
Bed Rest	1682	338		
Pre-Bed Rest	1220	411	0.6932	N.S.
Post-Bed Rest	1262	493		
Bed Rest	1682	338	10.1697	$P < 0.001$
Post-Bed Rest	1262	493		

TABLE IV

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART A. SUBJECT 2A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	66 77	21 29	2.3988	$P < 0.020$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	66 60	21 15	1.5693	N.S.
12 Noon - 8 P.M. 8 A.M. - 12 Noon	66 71	21 19	1.4384	N.S.
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	77 60	29 15	3.8533	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	77 71	29 19	1.3057	N.S.
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	60 71	15 19	3.3735	$P < 0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART B. SUBJECT 6A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	69 99	23 46	4.3581	$P<0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	69 71	23 21	0.4097	N.S.
12 Noon - 8 P.M. 8 A.M. - 12 Noon	69 78	23 30	1.7025	$P<0.101$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	99 71	46 21	4.1507	$P<0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	99 78	46 30	2.8911	$P<0.01$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	71 78	21 30	1.4022	N.S.

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART C. SUBJECT 7A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	79 93	24 31	2.4663	$P < 0.02$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	79 69	24 27	2.1226	$P < 0.05$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	79 103	24 59	2.6849	$P < 0.01$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	93 69	31 27	4.1990	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	93 103	31 59	1.1075	N.S.
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	69 103	27 59	3.7892	$P < 0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART D. SUBJECTS 2A, 6A, 7A (Exercised Throughout the Study, Combined Data)

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	71 90	23 37	5.3928	$P<0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	71 66	23 22	1.9130	$P<0.10$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	71 84	23 42	3.3534	$P<0.001$
8 P.M. 12 Midnight 12 Midnight - 8 A.M.	90 66	37 22	6.8660	$P<0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	90 84	37 42	1.3492	N.S.
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	66 84	22 42	4.6687	$P<0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART E. SUBJECT 4A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	67 102	28 41		
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	67 66	28 21	0.1982	N.S.
12 Noon - 8 P.M. 8 A.M. - 12 Noon	67 77	28 30	1.8979	$P < 0.10$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	102 66	41 21	5.8056	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	102 77	41 30	3.6035	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	66 77	21 30	2.2768	$P < 0.05$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART F. SUBJECT 8A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	71 77	20 27	1.1044	N.S.
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	71 62	20 18	2.5212	$P < 0.02$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	71 74	20 21	0.6368	N.S.
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	77 62	27 18	3.1880	$P < 0.01$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	77 74	27 21	0.5495	N.S.
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	62 74	18 21	3.1055	$P < 0.01$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART G. SUBJECT 9A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Proba- bility
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	63 74	19 32	2.1097	$P < 0.05$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	63 54	19 16	2.6937	$P < 0.01$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	63 60	19 20	0.8036	N.S.
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	74 54	32 16	4.0279	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	74 60	32 20	2.6402	$P < 0.01$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	54 60	16 20	1.7244	$P < 0.10$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART H. SUBJECTS 4A, 8A, 9A (Did NOT Exercise, Combined Data)

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	67 84	23 36	5.1668	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	67 61	23 19	2.7778	$P < 0.01$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	67 70	23 25	1.2698	N.S.
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	84 61	36 19	7.3580	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	84 70	36 25	3.9959	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	61 70	19 25	3.9426	$P < 0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART I. SUBJECT 3A (Exercised First 28 Days of Study)

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	57 71	20 25	3.2392	$P<0.01$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	57 41	20 17	4.4808	$P<0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	57 88	20 51	4.1680	$P<0.001$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	71 41	25 17	7.2932	$P<0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	71 88	25 51	2.1978	$P<0.05$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	41 88	17 51	6.3680	$P<0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART J. SUBJECT 1A (Exercised Second Half of Study)

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	72 85	22 38	2.1176	$P<0.05$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	72 53	22 24	4.2526	$P<0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	72 92	22 50	2.6146	$P<0.01$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	85 53	38 24	5.1296	$P<0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	85 92	38 50	0.7837	N.S.
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	53 92	24 50	5.0525	$P<0.001$

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY

PART K. ALL 8 SUBJECTS

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	68 85	23 36	8.2179	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	68 59	23 22	5.6467	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	68 80	23 40	5.5998	$P < 0.001$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	85 59	36 22	12.4914	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	85 80	36 40	1.7175	$P < 0.10$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	59 80	22 40	9.5390	$P < 0.001$

TABLE V

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION DURING BED REST BY SUBJECTS WHO EXERCISED 28 DAYS EACH (Subject 3A - First 28 Days, Subject 1A - Second Half of Study)

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Subject 1A				
No Exercise	1589	294	3.5324	$P < 0.001$
Exercise	1823	160		
Subject 3A				
No Exercise	1539	202	3.0334	$P < 0.010$
Exercise	1316	312		

TABLE VI

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY DURING BED REST BY SUBJECTS WHO EXERCISED 28 DAYS EACH

(Subject 3A - First 28 Days, Subject 1A - Second Half of Study)

PART A. SUBJECT 3A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	62	21	1.6635	$P<0.100$
Exercise	53	18		
8 P.M. - 12 Midnight				
No Exercise	82	24	2.9936	$P<0.010$
Exercise	62	21		
12 Midnight - 8 A.M.				
No Exercise	42	19	0.3602	N.S.
Exercise	40	14		
8 A.M. - 12 Noon				
No Exercise	97	34	1.1075	N.S.
Exercise	81	61		

TABLE VI, CONTINUED

STATISTICAL COMPARISON OF URINARY CREATININE EXCRETION AT DIFFERENT TIMES OF THE DAY DURING BED REST BY SUBJECTS WHO EXERCISED 28 DAYS EACH

(Subject 3A - First 28 Days, Subject 1A - Second Half of Study)

PART B. SUBJECT 1A

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	68	20	1.5164	N.S.
Exercise	77	24		
8 P.M. - 12 Midnight				
No Exercise	80	37	1.1570	N.S.
Exercise	92	39		
12 Midnight - 8 A.M.				
No Exercise	57	20	1.1223	N.S.
Exercise	49	28		
8 A.M. - 12 Noon				
No Exercise	71	25	3.6655	$P < 0.001$
Exercise	117	60		

TABLE VII

STATISTICAL COMPARISON BETWEEN URINARY CREATININE EXCRETION OF GROUPS OF SUBJECTS WHO EXERCISED (2A, 6A, 7A) AND WHO DID NOT EXERCISE (4A, 8A, 9A) THROUGHOUT BED REST

Populations Compared	Means (mg/24 hrs.)	Standard Deviation	"t" Value	Probability
Exercisers	1795	327	4.0115	$P < 0.001$
Non-Exercisers	1650	333		

TABLE VIII

STATISTICAL COMPARISON BETWEEN URINARY CREATININE EXCRETION OF GROUPS OF SUBJECTS WHO EXERCISED (2A, 6A, 7A) AND WHO DID NOT EXERCISE (4A, 8A, 9A) THROUGHOUT BED REST

Populations Compared	Means (mg/hr.)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
Exercisers	71	23	1.6548	$P < 0.1$
Non-Exercisers	67	23		
8 P.M. - 12 Midnight				
Exercisers	90	37	1.3325	N.S.
Non-Exercisers	84	36		
12 Midnight - 8 A.M.				
Exercisers	66	22	2.5809	$P < 0.01$
Non-Exercisers	60	19		
8 A.M. - 12 Noon				
Exercisers	84	42	3.4765	$P < 0.001$
Non-Exercisers	70	25		