A STUDY OF HYDROXYPROLINE AND NITROGEN METABOLISM DURING AMBULATION AND RECUMBENCY

A DISSERTATION
SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN NUTRITION IN THE GRADUATE DIVISION OF THE TEXAS WOMAN'S UNIVERSITY

COLLEGE OF HOUSEHOLD ARTS AND SCIENCES

BY
MARINA CUETO EILAND, B. S., M. S.

DENTON, TEXAS
AUGUST, 1970

# A C K N O W L E D G M E N T S

The author wishes to express her sincere gratitude and appreciation to the following persons who have helped her greatly in the conduct of the research itself and in the preparation of this dissertation:

To Dr. Pauline Beery Mack, Director of the Texas Woman's University Research Institute for her guidance, understanding, and invaluable direction during all of the author's graduate studies, and for allowing her to take part in the larger research project of which this dissertation is but a small part;

To Dr. Kathryn Montgomery, for giving generously of her time and guidance in carrying out the investigations and in discussing and clarifying the results as well as in aiding in the preparation of the manuscript. Without her encouragement and advice, the accomplishment of this study would not have been possible;

To Dr. Elsa Arciniegas Dozier for her assistance and direction of the study in its earlier phases;

To the National Aeronautics and Space Administration for its support of the overall research project of which this study was a part which made the graduate research assistantship the author has held possible;

To Dr. Jessie Bateman, Dean of the College of Household Arts and Sciences, for her kindness and interest and for participating as a member of the examining committee;

To Dr. Betty Alford for her kindness, encouragement, understanding, and invaluable teaching in the field of nutrition;

To Drs. Alice Milner and Wilma Brown for giving valuable instruction in the field of nutrition and their interest in this study;

To Dr. Ralph E. Pyke and Mr. Walter Gilchrist for giving generously of their time and assistance in the laboratory work connected with this study;

To Bill Stover for the preparation of the graphs;

To Jessie M. Ashby, Reba Fry, Lenoir O'Rear, and Martin Cudd for their aid in coding and preparing the data in the TWU Institute Statistical Laboratory and in the TWU Data Processing Center;

To Eugene Van Hooser for his unselfish aid in the laboratory;

Special recognition goes to Michael Eiland, the author's husband, and to her son Mike for their thoughtfulness, interest, understanding, and valuable encouragement during her graduate study;

To her mother, Mrs. Petronila Cuets, and her parents-in-law for their everlasting love, understanding, and advice and for encouraging her to continue her graduate study;

To the memory of her father who placed a high value on education and would have encouraged her to pursue this study; and

To all of her brothers and sisters in her country Peru who helped and encouraged the author to achieve her desire for advanced study in this country.

Finally, thanks go to the experimental subjects Brogoitti, Creamer, Gantt, Bishop, Smith, Agger, Jordan, and Kerr who played a valuable role in this research project.

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INTRODUCTION

The dawn of the space age with its attendant orbital space flights and moon explorations has brought about numerous new fields of study in nutrition. Texas Woman's University was invited early in the space program to take part in many of the space missions, both by studying the Astronauts themselves and by performing supporting bed rest studies.

Of primary importance has been the study of calcium loss and the accompanying loss of bone density during space flights. In order to study this phenomenon better, TWU has focussed much attention on the urinary excretion of the amino acid hydroxyproline as an index to measure bone density loss. Since hydroxyproline is found almost exclusively in collagen, a major constituent of bone and of other body tissues, its use as a research tool in the measurement of several endocrine and skeletal disorders is very valuable.

In order to gain an understanding of hydroxyproline and its research applications, this author has selected for her doctoral dissertation the study of the urinary hydroxyproline excretion of eight human subjects. The study included an Equilibration Period, a Bed Rest Period, and a Post-Bed Rest Period. A study of a definite circadian rhythm pattern for urinary hydroxyproline excretion has been included in the study because this would be a useful criterion in the study of different diseases likely to alter collagen metabolism and the rates of bone resorption and formation.

The study in this dissertation was conducted at the Nelda Childers Stark Laboratory for Human Nutrition Research in the Texas Woman's University Research Institute, under the sponsorship of the National Aeronautics and Space Administration.

For this study, the author used the Prockop-Udenfriend method (1) for the measurements of urinary hydroxyproline. This measurement is carried out by the oxidation of imino acid hydroxyproline to Δ^1-pyrroline-4-hydroxy-2-carboxylic acid, and pyrrole-2-carboxylic acid, followed by the formation of a chromophore with p-dimethylaminobenzaldehyde. This investigation involves a number of reagents and biochemical techniques according to the method of Radakhrishnan and Meister (2).

The specific objectives of this study were the following:

1. To analyze all urinary samples collected throughout the entire study in order to determine the quantity of hydroxyproline excreted by eight male subjects during the following periods:

A. Period of Equilibration, July 7 through July 24, 1969

B. Period of Bed Rest, July 24 through September 17, 1969

C. Period of Post Bed Rest, September 17 through October 1, 1969

2. To observe the 24-hour urinary hydroxyproline levels of the eight healthy men participating in the investigation.

3. To ascertain the existence of a circadian rhythm pattern for urinary hydroxyproline through the interpretation of the excretory data recorded for each man during various periods of the day.

4. To evaluate the effect of programmed exercise on the urinary excretion of hydroxyproline of the eight adult males during the bed rest period of the study.

5. To compare statistically the different periods of the study.

Because of the fact that nitrogen is a component of hydroxyproline, analyses also were run on this element for the objective of finding the quantity of this element excreted per hour and also the four periods during which aliquots were collected.

REVIEW OF LITERATURE

What is the meaning of "Circadian Rhythm"? The term first was introduced by Halberg (3) as a replacement for "diurnal" which was rather ambiguous and which more properly refers to daytime as opposed to "nocturnal" for night. Halberg coined the term from two Latin words, "circe" (about) and "diem" (day), and defined it as that period which is synchronized with or trained to the period of the earth's rotation. Both Bruce (4) and Halberg (3) reported that rhythms could be trained by periodic environmental factors which are called "Zeitgebers". Light and temperature seem to be the most predominant Zeitgebers; they have been observed to alter or to damp out some circadian rhythms. The terms used to describe cycles with lengths differing slightly from 24 hours ("free-running") are "infradian" and "supradian" which signify shorter and longer periods, respectively.

ENVIRONMENTAL FACTORS

Most human beings live their lives following an alternating pattern of light and dark with an almost constant cycle length of 24 hours. This cycle determines a pattern of behavior including periods of activity, rest, meals, etc. It is possible to alter these patterns either by exogenous or endogenous influences, such as travel, change of work shift, change of sleeping hours, etc. Aschoff (5) (6) states that, in this regard, it is of interest to note that the organism always manages to survive the modification.

An example of a circadian rhythm known to everyone is the phenomenon of body temperature: each day it reaches its maximum in the evening and falls to its low point in the early morning. This modern age of high-speed jet travel has uncovered another circadian rhythm: that of time zone adjustment. Klein, et al (7), after studying several U.S.A.F. pilots, reported that the organism requires a period of from one to several days to readjust its various rhythms after traveling through time zones.

BIOLOGICAL CLOCKS

Aschoff (8), Bunning (9), and Pittendrigh (10) have reported on clinical and physiological studies which have shown that apparently all organs and body functions exhibit some sort of rhythmicity. These rhythms are all expressions of a so-called "biological clock" which is considered to be a basic feature in most living systems. The phenomenon of a biological clock has reached such importance in the study of human physiology that the famous Cold Spring Harbor Symposia on Quantitative Biology devoted its 1960 reunion to the presentation and review of research material relative to this subject.

Development Of Rhythmicity In Infancy

According to Hellbrugge (11), periodicity in different functions begins at different times during the first year of a human infant's life. A study conducted by Kleitman and Ramsaroop (12) indicated that the major rhythms to be developed are a slow nocturnal pulse at the age of six weeks, day-night periodicity in body temperature also at six weeks, long nocturnal sleeps by 26 weeks, and periodic urine flow by four weeks.

Effects Of Light And Darkness

Brown (13) observed the effects of total darkness and total light on two groups of subjects and concluded that the most common example of a circadian rhythm is that of nocturnal sleep. Lobban (14) observed that Eskimos when living in the perpetual daylight of arctic midsummer exhibited sleep patterns very different from those exhibited during the period of midwinter darkness. It is of interest that the sleep patterns of midsummer tended to be very irregular. According to Sweeny and Hastings (15), however, the actual hours of sleep in any one 24-hour period are not substantially different during these two seasons.

The results of studies by Aschoff (6) indicated that after a short period of adjustment, people who had been isolated from sunlight and other outside distractions soon exhibited a free-running rhythm covering a period slightly longer than 24 hours in duration.

Kleitman (16) has concluded that the infradian sleep rhythm of the infant is of sub-cortical origin while the circadian rhythm of the adult is a cortical function.

Temperature And Cardiovascular Systems

The temperature of the human body depends on the balance between heat production and loss. Kleitman and Ramsaroop (12) have accumulated a great amount of data on temperature and heart rate. Their observations show that there is an increase in minute pulse rate of between 10 and 15 beats per degree Fahrenheit of rectal temperature, depending on the subject. Hellbrugge (11) noted that neither temperature nor pulse rate show a circadian periodicity at birth with both developing their rhythms at an age of 4 to 6 weeks.

Sharp (17) suggests that the probable cause of body temperature rhythm may be variations in the heat-loss mechanisms. Since the amplitude of the internal temperature rhythm is very similar in both temperate and humid climates, it appears that the rhythm is in the temperature-regulating mechanism, rather than in any single contribution to it, such as cutaneous blood flow or metabolic rate.

Renal System

In the past, diminished urine flow at night has been ascribed to the diminished intake of fluids. In 1889, Lahr (18) demonstrated that, if he remained recumbent on a constant fluid intake for 24 hours, his urine flow was least during the eight hour sleep period. It is now felt that urine flow is the result of a number of potentially independent influences. The rate of liberation of posterior antidiuretic hormone (ADH) is considered to be the most important internal factor influencing the flow of urine.

Gunn, et al (19) have reported that the kidney's disposition of various organic constituents is presumably not nervous in origin but chemical, since a transplanted human kidney shows its native circadian function. Mills (20) has suggested that circadian oscillations might be indeed a property of renal tissues themselves. Figure 1 depicts factors influencing the circadian periodicity of urine flow.

The endogenous nature of the excretory rhythms was demonstrated by Mills and Stanbury (21) using a group of subjects who spent 48 hours in a basement room from which daylight was excluded and followed an exact 12-hour cycle of sleeping, meals, and activity. They found that there was an individual variation in renal tubular activity, which occurred rhythmically over a 24-hour period, thereby influencing

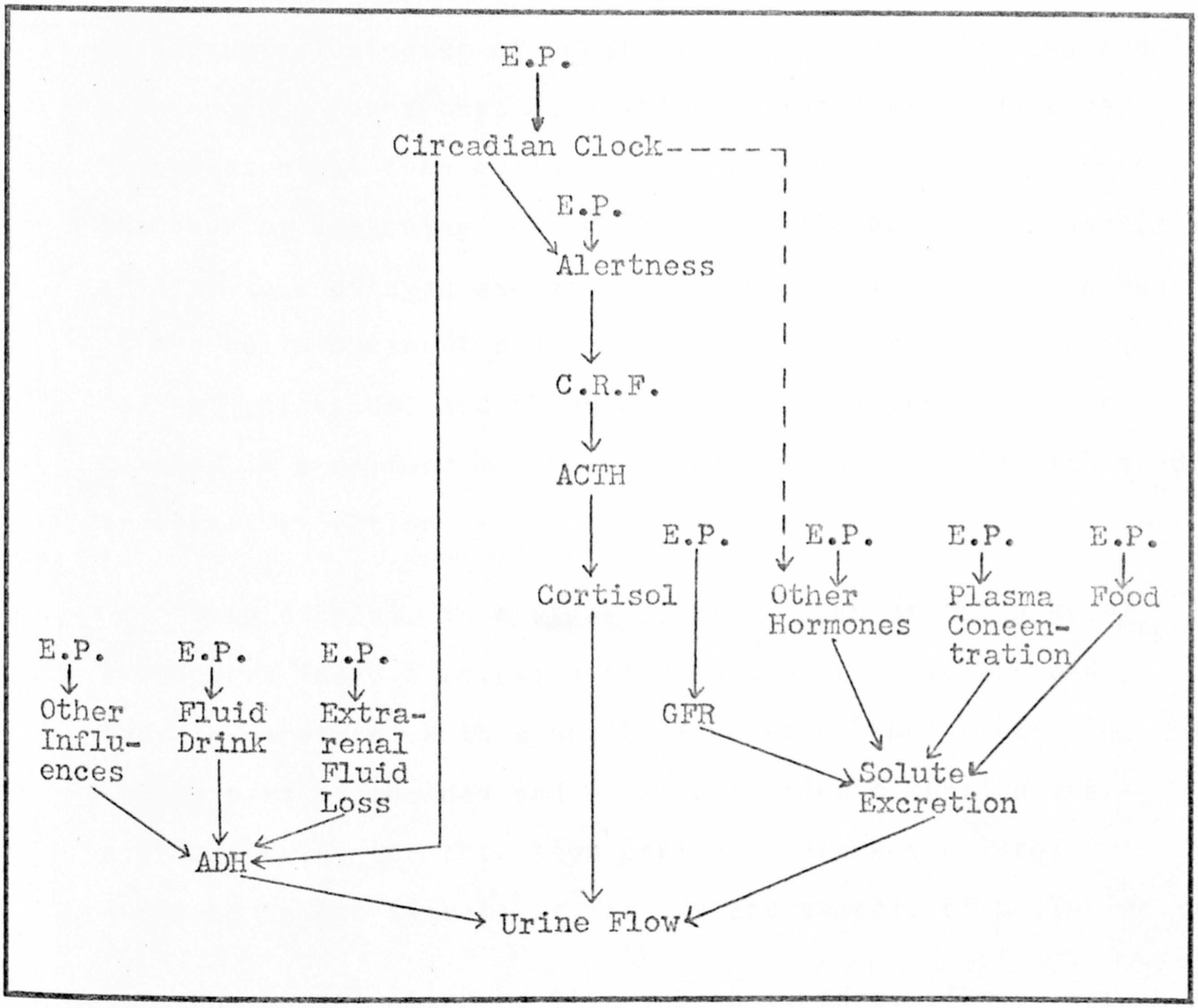

Figure 1. Diagrammatic representation of some factors possibly conducive to circadian periodicity in urine flow as shown by Mills (20).

urinary pH, output of all measured electrolytes except phosphate, and total urine volume.

While overnight urine usually is acid, pH alone is not an adequate indicator of renal function. Campbell (22) and Simpson (23) found that acid and ammonium excretions were higher at night than during the day, reaching a peak around the hour of awakening. Campbell (22) attributed high nocturnal acidity to a delayed excretion of certain fixed acids formed in the cells during the day. Stanbury (24) reported that sodium, potassium, and chloride excretion increased or decreased in a uniform manner, occurring concurrently with acid or alkali excretion.

Simpson (25), in a study of urine samples taken every two hours, found a relationship between urine volume and body temperature, with a negative water balance occurring during part of the day and water retention occurring overnight. During the retention period, there was a large increase in the titratable acidity and ammonia of the urine.

Cohen (26) observed five subjects, three of whom were taking food and two of whom were fasting, and found a rise in urinary alkalinity to occur following food consumption. Acidosis was present in the fasting subjects after 24 hours.

Sodium and potassium excretion was not studied extensively until the invention of the flame photometer. Using this instrument, Mills and Stanbury (27) determined the excretion of sodium, potassium, and chloride to be low at night and high during the day, rising to a peak between the hours of 0900 and 1200 and gradually declining during the night.

Mills (20) feels that variations in the glomular filtration rate (GFR) are related directly to variations occurring in the excretion of sodium and chloride.

Hematological System

Sharp (28) has reported that eosinophil counts fall during the first hours after awakening and that there is a similar decline in lymphocytes, with a concurrent rise in neutrophil counts.

Halberg (29) calls attention to the fact that social habit seems to be of some importance in determining the timing of the eosinophil rhythm since the fall of its count apparently starts earlier in the morning for people who are early risers. Light also is cited as being an important factor affecting this rhythm since the count change occurs earlier in the day during the summer months.

According to Renborn (30), circadian periodicities have been noted to exist in hematocrit, hemoglobin concentration, red cell count and size, and plasma protein concentration, with high values being observed during the day and low values occurring at night. This same investigator also noted the existence of periodicities occurring in the sedimentation rate and in the clotting of blood. He demonstrated further the presence of a circadian rhythm pattern in erythrocyte production by studying the reticulyte counts of recuperating patients experiencing active blood regeneration.

Hamilton et al (31) have stated that plasma iron concentration displays maximal values around the time of awakening, with lower values being observed in the late evening and early night.

Pathological Factors

Circadian rhythms may be of value in the diagnosis of pathological conditions. Also, they may play a part in pathogenesis. According to Halberg (32), any disturbance of the regularity or amplitude of the rhythm of temperature may be an early indication of a pathological process. He further states that circadian rhythm structure is involved in the temporal organization of physiologic change. He places great emphasis on "hours of changing resistance", whereby an injection of a substance into one person at a certain hour may cure an illness while the same substance injected into another body at the same hour may cause death. This he attributes to a function of the changing phase relations among an organism's circadian rhythms.

Adrenal Cortex Activity

The existence of a circadian periodicity in adrenal cortex activity was reported first by Pincus (33). The results of a study conducted by Bartter et al (34) showed that plasma corticosteroids attain peak values around or before the hour of awakening. Studies conducted by Doe et al (35) and Migeon et al (36), showed that changes in the levels of urinary corticosteroids occurred two to three hours after these same changes were noted in the plasma concentration, regardless of whether the plasma concentration was changed spontaneously or as a result of intravenous administration of cortisol.

Skeletal Processes

Peterkovsky and Udenfriend (37), as well as Simmons and Nichols (38) have demonstrated that, in many tissues the processes involved in cell proliferation and cell function exhibit maximum and minimum hours of activity within a single 24-hour period. Simmons (39) (40) conducted studies which indicated that the mitotic activity of chondrocytes in the growth cartileges from the long bones of mice and rats varies diurnally.

Preliminary studies by Simmons (39) indicated that a diurnal periodicity existed in the mitosis of cells of the epiphyseal cartileges of young, growing mice, thus indicating the rate of growth to be greater during the day than during night. He was, however, unable to establish the actual hour of minimum and maximum growth activity definitely at this time.

In a subsequent study, using distal femurs of albino rats, Simmons (40) was able to establish a mitotic index, which showed a maximum occurring at 1800 hours and with a minimum occurring around 0200 hours.

In vivo studies conducted by Simmons and Nichols (38) showed a diurnal variation of 40 per cent to exist in the uptake of radioglycine by osteoclasts in the metaphysis of the distal femurs of rats. The hours of peak glycine incorporation were between 1400 and 1800, with values at other hours of the day remaining fairly constant (Figure 2). In the femurs, maximum uptake of the radioglycine occurred between the hours of 1300 and 1700 with a minimum activity recorded during the period from 0100 to 0500 hours (Figure 3).

Studies conducted by Mack and associates (41) (42) have revealed small but significant losses of bone mass by both Gemini Astronauts and experimental subjects participating in immobilization studies. The per cent loss ranged from 2.46 for Gemini VII Astronauts to 10.23 for Gemini V Astronauts. The duration of the orbital flights (four to 14 days) did not appear to be the sole factor influencing the losses of bone mass, since the astronauts of the longest flight

(Gemini VII) experienced lower changes in bone density. It was felt also that the inclusion of an exercise program in the Gemini VII flight and the greater consumption of food probably contributed to the lower losses in bone density. The results of the immobilization studies which were conducted both with and without accompanying exercise, closely parallelled the data compiled from the Gemini flights. The astronauts regained their pre-flight levels of bone density within 10 days after splashdown, while the bed rest subjects reached their equilibrium from five to seven days after bed rest.

Hydroxyproline Excretion

According to Neuman and Logan (43), collagen synthesis and degradation are reflected in the urinary excretion of hydroxyproline, since this amino acid is found essentially only in this tissue. Prockop and Kivirikko (44) have cited the desirability of determining hydroxyproline excretion for use as an index in the clinical assessment of several endocrine and skeletal disorders. Sjordsma (45) has stated that levels of urinary hydroxyproline excretion in urine are of value as a research tool; and they appear to indicate turnover rates and to provide a valuable index of collagen metabolism in animals.

Prockop (46) was the first investigator to make an extensive study of human biological material for hydroxyproline content. Total hydroxyproline in the urine consists of one to three per cent free hydroxyproline and more than 95 per cent in a peptide bound form.

On a meat and gelatin diet, total urinary hydroxyproline may reflect connective tissue turnover. Dull (47) has suggested that the determination of a 24-hour total hydroxyproline excretion determined while a patient is maintained on a meat and gelatin free diet might prove to be as sensitive an index of bone protein turnover as calcium excretion is for bone mineral metabolism.

Because of the difficulty of evaluating non-hospitalized patients, there has been very little research conducted to determine whether or not a periodicity exists relative to the excretion of hydroxyproline. In a recent study using 17 healthy subjects, with the exception of one 23 year old man with idiopathic hypercalciuria, Mautalen (48) determined both free and total urinary hydroxyproline excretion as related to creatinine excretion. He used the laboratory method of Prockop and Udenfriend (1) as modified by Kivirikko and associates (49) to determine total urinary hydroxyproline. Free hydroxyproline was determined by the use of the same method except for the omission of the acid hydrolysis. Calcium and creatinine determinations were made using the methods of Clark and Collip (50) and Tausky (51).

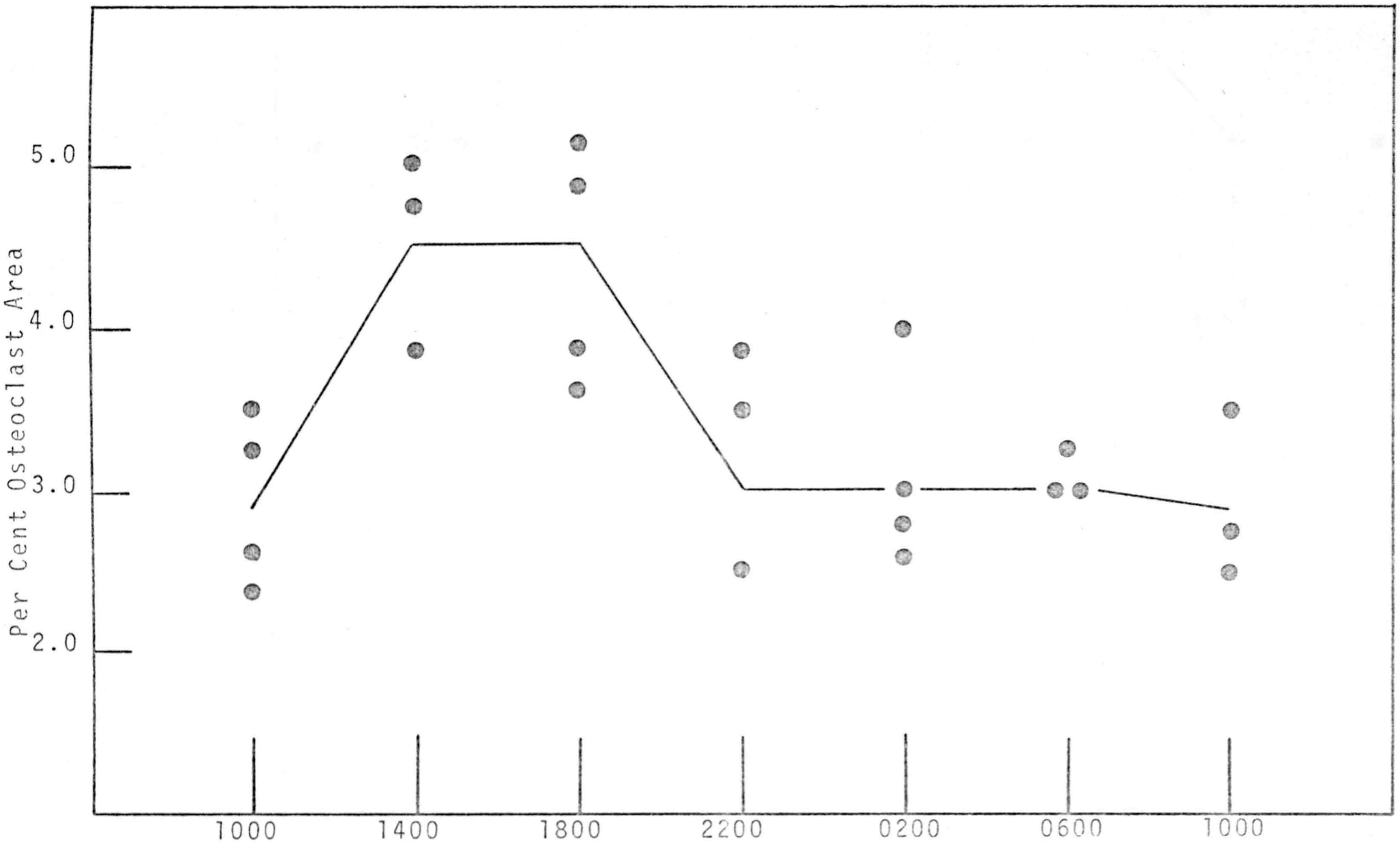

Figure 2. Diurnal changes in the per cent of distal femoral metaphyseal area occupied by osteoclasts as shown by Simmons and Nichols (38).

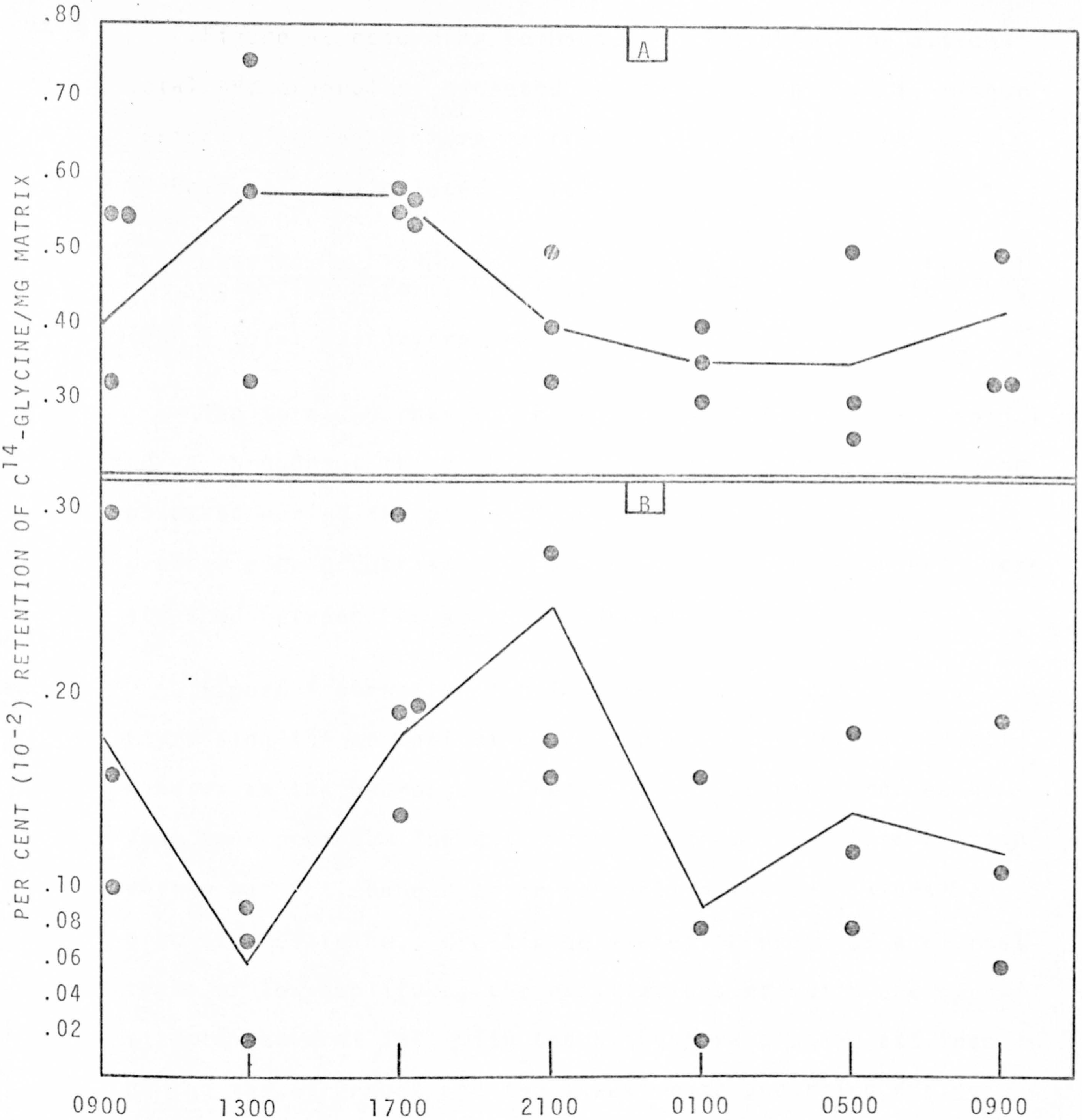

Figure 3. The 24-hour changes in glycine -2-C^{14} incorporation into rat femurs as shown by Simmons and Nichols (38).

A = Distal epiphyseal-metaphyseal segments
B = Shaft segments

Figure 4, according to Mautalen (48), shows the average total hydroxyproline excreted by the group in each four-hour period. The results are expressed as per cent of the mean 24-hour value calculated as follows:

$$\text{\% of mean for period 8 to 12 hrs} = \frac{\text{mg of THP 8 to 12 hrs} \times 10}{\text{mg of THP 8 to 12 hrs} + \text{12 to 16 hrs} + \ldots + \text{4 to 8 hrs}}$$

(THP = total hydroxyproline)

The total hydroxyproline excretion followed a sinusoidal curve throughout the 24 hours, with the lowest amounts being observed during the period from 1200 to 1600 hours with a gradual rise occurring thereafter until the peak amounts were attained between the hours of 0400 and 0800.

Figure 4 shows the total hydroxyproline values after expressing the content of each sample as per gram of creatinine or as the hydroxyproline: creatinine ratio for each four hour period. The data show clearly that the circadian rhythm has not changed after correcting the THP values per gram of creatinine. Creatinine excretion presents a diurnal cycle of low amplitude, the oscillations of which are opposite to those of THP, with the highest rate being attained during the afternoon and the lowest rate occurring during the period from 0000 to 0800 hours.

The 24-hour urinary total hydroxyproline was within a normal range (Figure 5), except for the patient with the

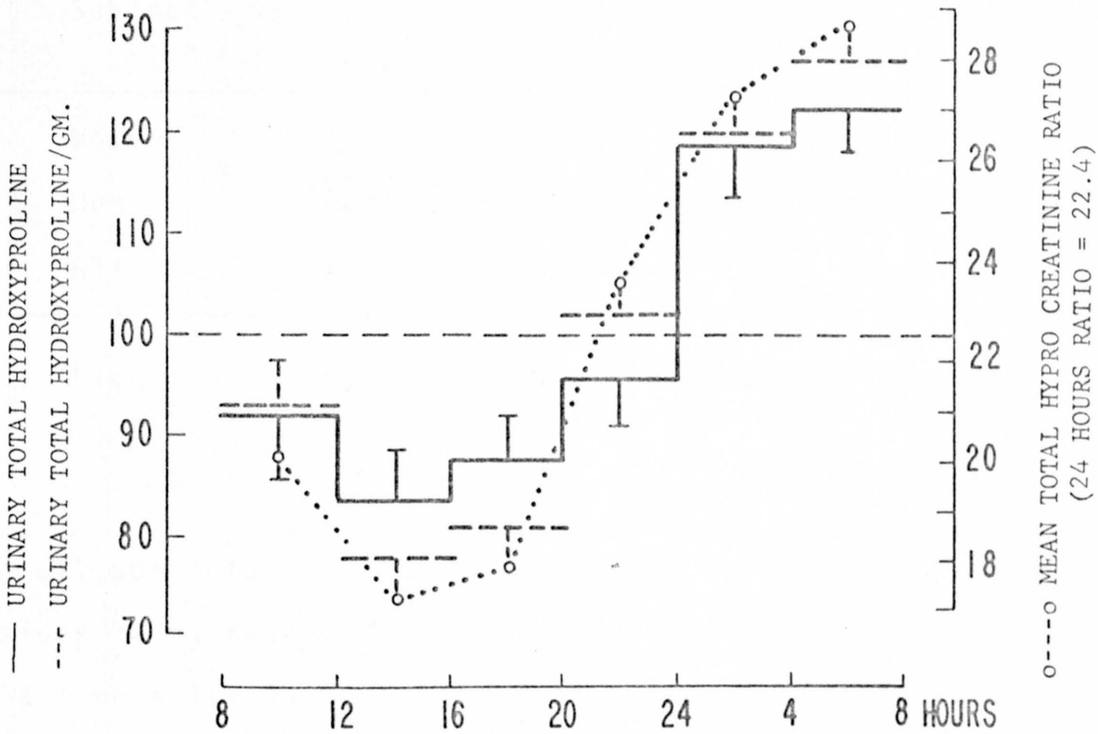

Figure 4. Mean urinary total hydroxyproline excretion every 4 hours (solid line) and after correction per creatinine excretion of each period (dotted line) in 17 control subjects. Results are expressed as percentage of the mean 24 hour value. Vertical bars represent ± S.E.M. Open circles depict hydroxyproline (milligrams): Creatinine (grams) ratio in each period according to Mautalen (48).

renal disorder whose excretion was above normal.

Subject	Total Hydroxyproline		
	mg/24 hrs	mg/M^2/24 hrs	Hypro (mg): Creatinine (gm)
Women	20.8 $\pm$ 1.8	12.9 $\pm$ 1.3	23.4 $\pm$ 2.9
Men	27.0 $\pm$ 3.4	14.2 $\pm$ 1.5	18.2 $\pm$ 1.5
All	23.3 $\pm$ 1.8	13.4 $\pm$ 0.9	21.3 $\pm$ 1.9

Figure 5. Total hydroxyproline excretion in normal subjects, (mean $\pm$ 1 S.E.M.), according to Mautalen (48).

In all subjects, a definite cycle was observed with the peak values being reached during the hours from 0000 to 0800 and the lowest levels occurring during the morning or early afternoon. Two subjects, however, showed their highest excretion in the period from 0800 to 1200 hours with the lowest level occurring during the period from 2000 to 0400 hours.

In order to study the reproducibility of the circadian rhythm of THP excretion further, urine was collected every four hours for three consecutive days from the patient with renal disease (initials V.N.). The results in Figure 6 show that the pattern was practically the same for each of the three days. The lowest excretion was recorded during the period from 1200 to 1600 hours and the peak was reached between midnight and 0800 hours.

The urinary excretion of free hydroxyproline in five normal subjects was 636 ± 79 (average ± 1 S.E.M.) per day. Figure 7 shows the mean free hydroxyproline excretion for four-hour periods, the results being expressed as per cent of the mean 24-hour value. There is an apparent circadian rhythm noted, with the peak excretion being attained during the period from 1200 to 2000 hours.

The comparison of total and free hydroxyproline for all five subjects (Figure 7) shows that these components are excreted in a dissimilar circadian pattern. Peak excretion of free hydroxyproline occurs at the same time (1200 to 2000 hours) as the low of total hydroxyproline and, conversely, peak total hydroxyproline is attained when the low level of free hydroxyproline values are noted.

Since the length of the rhythmic cycle of urinary hydroxyproline excretion appears to be of 24 hours duration, environmental factors probably play an important role in this phenomenon. Mautalen (48) states that this does not preclude the possibility of an internal clock that has been gradually trained by external influences. His study confirms earlier observations by Aschoff (6) and Carruthers et al (52), in which they found that the calcium excretion of subjects following a normal diet reached their lowest level during the overnight period. It has been observed by Mintz (53), however, that persons either in a basal, fasting, resting state or receiving a liquid metabolic diet every four hours reversed this pattern with the peak excretions occurring overnight.

Based on the observations of Weiss and Klein (54) that urinary excretion of peptide and free hydroxyproline represent only a small fraction of endogenous production, Mautalen (48) further surmised that the circadian pattern of hydroxyproline could be determined by discrete changes in the rates of peripheral metabolic degradation and renal tubular reabsorption. According to Katz and Kappas (55), these values also could reflect true changes in the rate of collagen degradation conditioned by circadian changes in physical conditions or hormonal secretion.

Figure 7 shows that, in the study conducted by Mautalen (48), the free hydroxyproline: peptide hydroxyproline ratio in urine changed throughout the day. Morrow et al (56), determined this same ratio in urine samples of premature and full term infants up to one year of age and reported that a similar ratio was found in samples collected at various times of the day. This finding suggests that circadian variabilities both in peptide and free hydroxyproline excretions probably are established later in life According to Litval (57) and Whitehead (58) the best method of expressing urinary hydroxyproline excretion is per gram of creatinine, while Howells (59) favors the use of a hydroxyproline: creatinine ratio. Mautalen (48) feels that this would be valid only if the urinary excretion of hydroxyproline showed the same diurnal cycle. It appears preferable to express the urinary hydroxyproline excretions as milligrams per 24 hours per square meter of body surface area as suggested by Jasin and colleagues (60).

Other Amino Acids

Physiological rhythms of amino acids have been the topic of recent studies. Squibb (61) has reported a circadian periodicity of serum amino acids in growing chickens, and Rapoport and his co-workers (62) have established that, in mice tryptophan exhibits a circadian variability.

Feigin et al (63) studied eight subjects aged 19 to 26 years and obtained determinations both of total and individual amino acid levels at 0800 and 2000 hours daily for five days. The results of this study showed that the values of the total blood amino acids in each of the men were consistently higher at 2000 hours than at 0800 hours. The total concentration of amino acids in the plasma of these same subjects, as measured by column chromatography, averaged 34.5 per cent higher in the evening (Figure 8) than in the morning, indicating a highly significant increase, statistically, over the morning concentrations ($P < 0.001$). Additionally, as shown in Figure 8, it was found that all free plasma amino acids, with the exceptions of citrulline, display a similar rhythmicity. The per cent of variation between the morning and evening values ranged from 26.1 to 100.0 with a mean difference of 44.2 per cent (excluding citrulline). The absolute differences varied for each amino acid with the greatest differences being recorded for alanine, glutamine, and lysine. The highest percentages of increase were noted in methionine, isoleucine, and leucine.

Wurtman et al (64) arrived at similar results in another study. They collected blood samples at regular intervals during a 24-hour period from 23 subjects on diets containing various amounts of protein. The samples then were assayed for 16 amino acids. The results showed the peak values for tyrosine, phenylalanine, and tryptophan to occur at or after 1030 hours, and minimum values to occur at or slightly after 0200 hours. Since it was impossible to submit more than two of the plasma samples collected daily from any one subject to autoanalysis, these investigators hypothesized from the data accumulated that the concentration of total amino acids in the plasma varies with a periodic phasing similar to that exhibited by tyrosine.

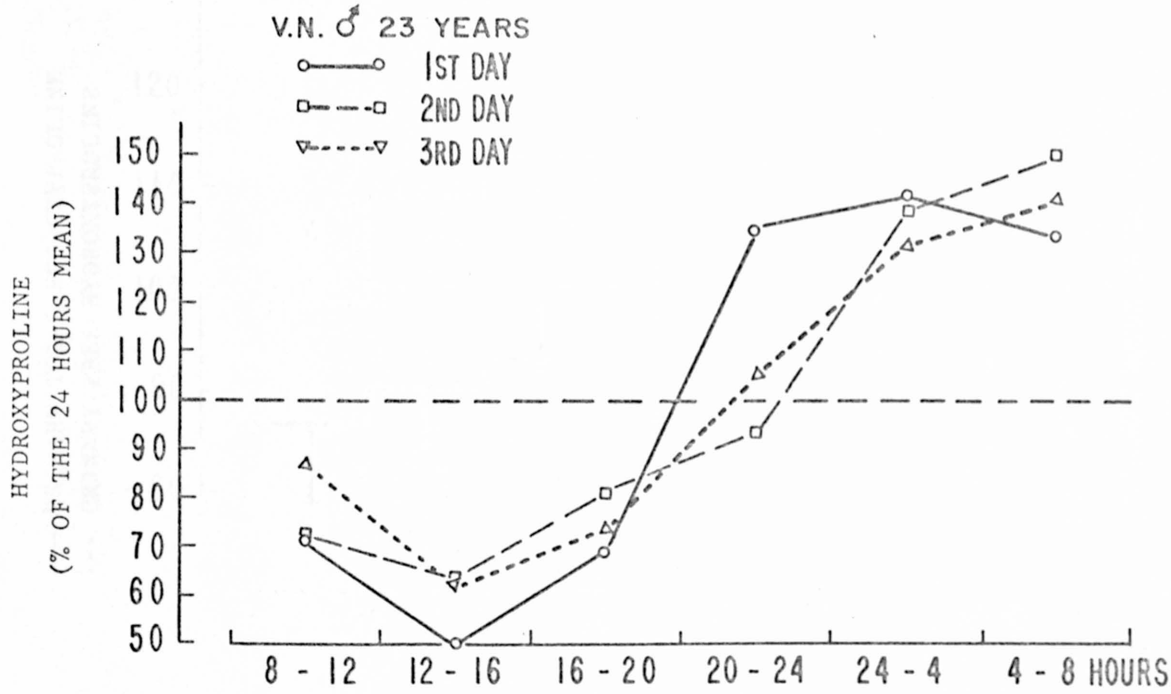

Figure 6. Urinary total hydroxyproline excretion every 4 hours during 3 consecutive days in V. N. results are expressed as percentage of the mean 24 hour value according to Mautalen (48).

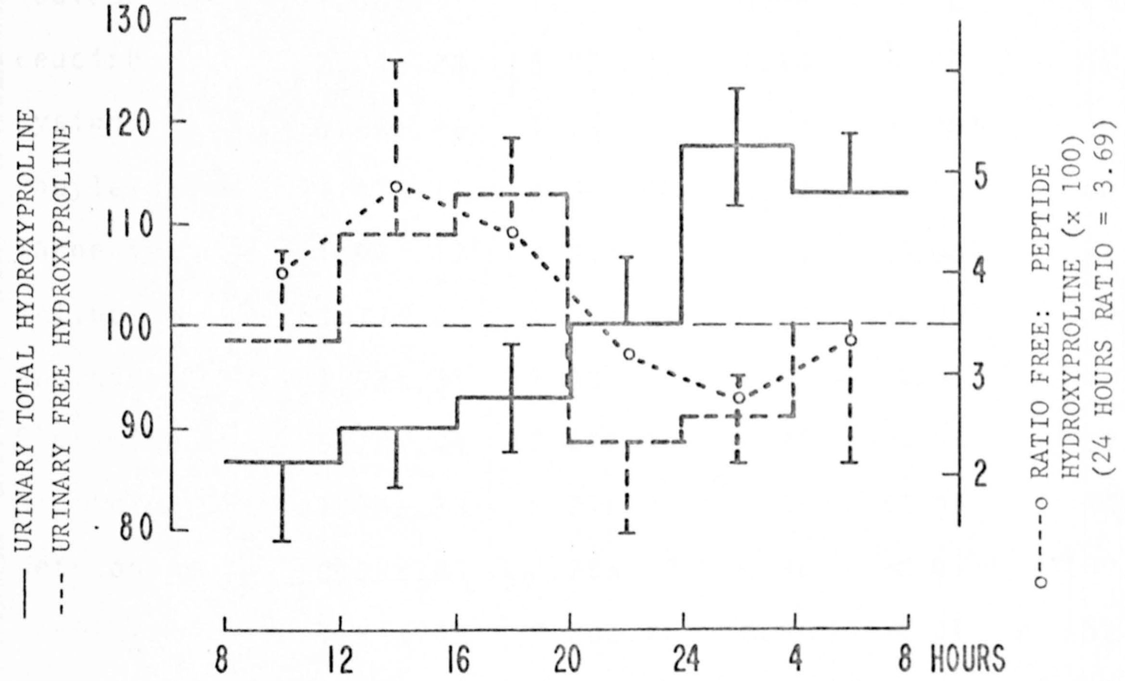

Figure 7. Mean urinary excretion of total and free hydroxyproline every 4 hours in 5 control subjects. Results are expressed as percentage of the mean 24 hour value. Vertical bars represent $\pm$ 1 S.E.M. Open circles depict free: Peptide hydroxyproline ratio (X 100) according to Mautalen (48).

	Mean ± 1 S.E. (mg %) of 8 Subjects				
Amino Acid	0800 hrs	2000 hrs	Diff.	Paired t Test p Value	% Increase
Glycine	1.98±.71	2.64±.10	0.66	<.0001	33.3
Alanine	4.29±.33	6.24±.18	1.95	<.001	49.6
Isoleucine	1.08±.11	1.76±.06	0.68	<.001	63.0
Leucine	2.04±.28	3.20±.19	1.16	<.001	56.9
Lysine	3.66±.26	5.13±.19	1.47	<.001	40.2
Pheylalanine	1.47±.11	2.06±.08	0.59	<.001	40.1
Throenine	1.97±.17	3.04±.16	1.07	<.001	54.3
Valine	3.13±.22	4.37±.11	1.24	<.001	39.6
Arginine	1.93±.31	2.80±.27	0.87	<.01	45.1
Glutamine	5.78±.44	7.48±.25	1.70	<.01	29.4
Histidine	1.55±.30	2.21±.20	0.66	<.01	42.6
Methionine	0.38±.04	0.76±.07	0.38	<.01	100.0
Ornithine	1.84±.18	2.80±.26	0.96	<.01	52.2
Glutamine	2.76±.36	3.52±.46	0.76	<.05	27.5
Proline	3.49±.41	4.43±.55	0.94	<.05	26.9
Serine	2.11±.28	2.65±.26	0.54	<.05	26.1
Tyrosein	1.17±.14	1.77±.13	0.60	<.05	51.3
Citrulline	0.73±.03	0.86±.07	0.13	n/s	17.8
Total Amino Acids	41.64±2.01	56.02±2.17	14.38	<.001	34.5

Figure 8. Plasma Amino Acid Periodicity in Normal Men According to Feigin, et al (63).

* Per cent increase = $\frac{\text{(mean 2000 hrs value - mean 0800 hrs value)}}{\text{mean 0800 value}} \times 100$

Effect of Stress, Exercise, and Immobilization

Hiroshige and Sato (65) recently published the results of a study conducted for the purpose of ascertaining the postnatal development of circadian rhythm and stress-induced changes in hypothalmic corticotropin-releasing activity CRF in rats.

Their findings indicated that a circadian periodicity of CRF activity appeared between the 14th and 21st day of post-natal life. Response to stress was evident, however, as early as the seventh day. These investigators concluded that there is a dissociation between the onset time of the circadian rhythm and the stress induced changes in hypothalamic CRF.

Besch (66) has reported on a study conducted to measure the locomotor responses of rats exposed to different types of artificial days. Using various, but equal, combinations of light and darkness, he found the rats to be most active during periods of darkness and cold, and least active during light and warm periods.

Lovinbond (67) conducted a study in which 120 ulcer-susceptible rats were placed under restraint in order to determine the frequency of gastric lesions. Using a light stimulus and a buzzer which had been associated in previous training with feeding and electric shock, respectively, he found that gastric lesions were exacerbated by the simultaneous presentation of the two stimuli, but were ameliorated by the stimulus of feeding and the absence of shock. The investigator interpreted these results as supporting the hypothesis that conflicting phenomena are generated when a stimulus pattern tends to elicit incompatible responses, with no one response being able to achieve dominance.

Recently another study of gastric ulceration and changes in motility of the stomach was made by Boles and Russell (68). After 36 hours of exposure to a physical stress (immobilization) and a chemical stress (reserpine), both individually and combined, it was found that all rats exposed to restraint and reserpine were ulcerated. On the other hand, 60 per cent of the restraint-only had lesions, while only 30 per cent of the no-restraint rats showed any gastric damage. Increased gastric motility was observed in the restraint-only groups while the reserpine and restraint groups showed a progressive decrease in motility. There were no significant differences in total stomach acidity among the groups. The investigators therefore concluded that stomach acidity does not produce lesions but that the lesions are related to changes in gastric motility.

Stini (69) conducted a study in a rural mountain community in Columbia, to investigate long term effects due to the stress induced by diet deficiencies. The residents of this town live in a continual state of malnutrition characterized by a lack of protein and an excess of carbohydrates. As a result of this imbalance, Kwashiorkor and Marasmus are seen frequently. This investigator found the skeletal maturation of most of the children examined to be below U.S. standards. The female children seemed to experience a form of "catch-up" growth beginning in the pre-adolescent years. The males however appeared to be more severely retarded throughout adolescence with a pronounced reduction in stature being noted as well as an accompanying reduction in sexual dimorphism for overall body size. The Kwashiorkor and Marasmus affected children showed a more severe retardation in skeletal growth, but no significant difference was noted in their sexual response.

When considering the classification of exercise, it must be recognized that exercise itself is a vague term. Different kinds, intensities, and durations of exercise accomplish very different results. Mayer (70) lists three main types of exercise: a) moderate, b) limbering, coordinating and strengthening, and c) constant, vigorous physical labor. Moderate exercise, such as walking, is beneficial for the mesomorphic individual. Limbering, co-ordinating and strengthening types are of less significance as far as health is concerned. This research worker, however, reports that exercises to strengthen the back and stomach are useful in preventing "low back pain". Constant, vigorous, physical labor, while not adaptable to the modern sedentary life, appears to permit an individual to handle a diet high in saturated fat without an accompanying increase in serum cholesterol.

Davis and associates (71) have listed several biophysical values which can be derived from muscular activity. These benefits include: a) stimulation of growth and development; b) aid in body internal adaptation; c) improvement of the efficiency of the living organism; d) desirable emotional releases; e) restoration of skeletal atrophy; f) increase in adaptability to external forces; and prevention of bodily injury.

Dagenais et al (72) conducted a study to determine the effects of carbohydrate-rich and protein-rich meals on cardiac out-put, heart rate, blood pressure, and oxygen consuption of normal subjects in the resting state. The study was designed further to observe the changes occurring in these criteria when the subjects engaged in exercise. It was concluded that the protein and carbohydrates induced changes which were equal in intensity under conditions either of rest or exercise.

Wisham and associates (73) studied 10 subjects to determine whether exercise produces an increased blood flow to the extremities of persons with and without peripheral arterial disease, and to find quantitatively the changes in blood flow produced by a series of exercises requiring progressively greater muscular effort. These investigators reported that exercise is an effective stimulus for increasing blood flow both during participation and for a short time afterwards with the rate of increase being noted paralleling the amount of muscular effort exerted. The results of this study indicated that exercise as therapy did not produce long term effectiveness.

Buckler (74) reported on a study conducted to determine the ability to secrete growth hormone in response to "Bovril". Using one group of 35 men and 18 women and a separate group of 13 men who had been treated with estrogens, he found that all women, except three, and most of the men below the age of 30 years showed a marked rise in hormone levels. Of the estrogen treated men, nine showed a definite response. With the addition of exercise to the study, it was found that the degree of exercise required to stimulate hormone release in women appeared to be less than that required to effect the same response in men. Continuous moderate exercise did not produce any increase in hormone output. Strenuous exercise for as little as four minutes, however, produced very high levels of hormone within six minutes.

Stanek and associates (75) observed that, although post-pneumonectomy patients had a lower cardio-pulmonary blood volume than normal subjects, the pressure in the pulmonary blood volume than normal subjects, the pressure in the pulmonary artery both at rest and during exercise was 20 to 27 per cent higher than would be expected in completely healthy subjects of similar age. With the increase significantly being related to the age of the patients the comparison of younger and older patients showed no differences at rest. During exercise, however, the older patients exhibited a higher pulmonary arterial pressure as well as a higher pulmonary resistance, a higher alveolar-arterial oxygen tension difference, and a lower arterial oxygen tension.

Bevegard and associates (76) reported on an exercise study in which six young, healthy male subjects were observed as to their circulatory adaptation to the following: a) arm exercises, b) leg exercises, and c) combined arm and leg exercises by cardiac catheterization both in supine and sitting positions. It was noted that hemodynamic and ventilatory responses were equal during leg exercise, and that ventilatory responses were equal during leg exercise and combined arm-leg exercises. During arm exercise, however, total ventilation, heart rate, and lactate formation all were significantly higher for any given oxygen uptake. These investigators reported that the observed differences in circulatory adaptation during arm vs. leg exercises indicated the presence of a higher sympathetic tone during arm exercise.

Lamb and associates (77) measured the cardiac output and the coronary blood flow in all subjects engaged in 20 minutes of steady state exercise and in four control subjects. A 0.737 correlation coefficient was yielded when the per cent increase in cardiac output times systolic pressure was compared with the per cent increase of coronary blood flow during exercise. A comparison of the per cent increase in cardiac output with the per cent increase in coronary blood flow at rest yielded a correlation coefficient of 0.719. These findings demonstrated a relationship to exist between an increase in pressure volume and an increase in coronary blood flow.

A study was conducted by Buskirk and associates (78) to investigate basal and resting oxygen consumption as affected by specific dynamic action, exercise, and the impact of climate.

Moderate exercise alone did not alter resting oxygen consumption, although a small diurnal elevation was observed to occur in fasting subjects, either with or without exercise. Climate, per se, did not appear to affect either basal oxygen consumption or resting metabolism except at 2000 hours in a hot - dry climate, when the basal oxygen consumption recorded was significantly higher than the values reported for 2000 hours in other climates.

PLAN OF PROCEDURE

The data presented in this dissertation were obtained during a bed rest study conducted in the Nelda Childers Stark Laboratory for Human Nutrition Research on the campus of the Texas Woman's University as a part of an extensive investigation sponsored by the National Aeronautics and Space Adminstration 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 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 hydroxyproline excretions of the subjects participating in this investigation is presented in this report.

PERIODS OF THE STUDY

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

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

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

Post Bed Rest Period, 15 days, September 17 to 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	Height (inches)	Weight (pounds)
1A	20	69	157
2A	23	69	150
3A	40	71	142
4A	20	66	155
5A	20	73	180
6A	22	71	175
7A	23	73	195
8A	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, and spoon fed the participants.

The food fed during the Pre-Bed Rest Equilibration Period was regular food purchased chiefly through the University Purchasing Department. That which was fed during the Bed Rest and Post-Bed Rest Periods was flight food furnished by the Manned Spacecraft Center, Houston. The daily intake of each subject was recorded each day throughout the study by the chief dietitian according to 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 hygenic 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 laboratories of the Institute. 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 24-hour urinary samples were collected until six days before entering the period of immobilization, when the urinary voids were accumulated in four aliquots during a 24-hour period. The periods ran from 8 A.M. until 12 (noon), from noon until 8 P.M., from 8 P.M. until midnight, and from midnight until 8 A.M.

Bed Rest Period

This portion of the study covered a span of 56 days during which time the eight male subjects were thoroughly immobilized. They assumed a horizontal position on a single bed equipped with one 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 an individual TV equipped with ear phones also was provided each subject for the purpose of viewing the TV program of his choice. 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 movements.

This phase of the study was designed to investigate many physiologic changes including the phenomenon of circadian rhythms. 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 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., day began by turning on the lights with the light lasting until 11:00 P.M., when all lights, including TV, were turned off. A calibrated thermometer was placed on the wall above the head of each subject and the temperature was carefully maintained within a range of 72 $\pm$ 2 F., by examination of the temperature each hour of the day and night.

During this period of the study, exercise was introduced as a variable through the use of a completely modified Exer-Genie, together with a Exer-Grip exerciser. 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, (3A) however, pleaded fatigue after 28 days of the exercise program, and was replaced by Subject 1A who followed the program the remaining 28 days of bed rest.

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. This is shown in Summary B. Figure 9 shows a subject engaged in exercise.

Throughout this 56-day period, astronaut 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 on a routine basis throughout immobilization; and urine samples of all excretions 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.

SUMMARY A

E X E R C I S E   S C H E D U L E

(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-GENIO)	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

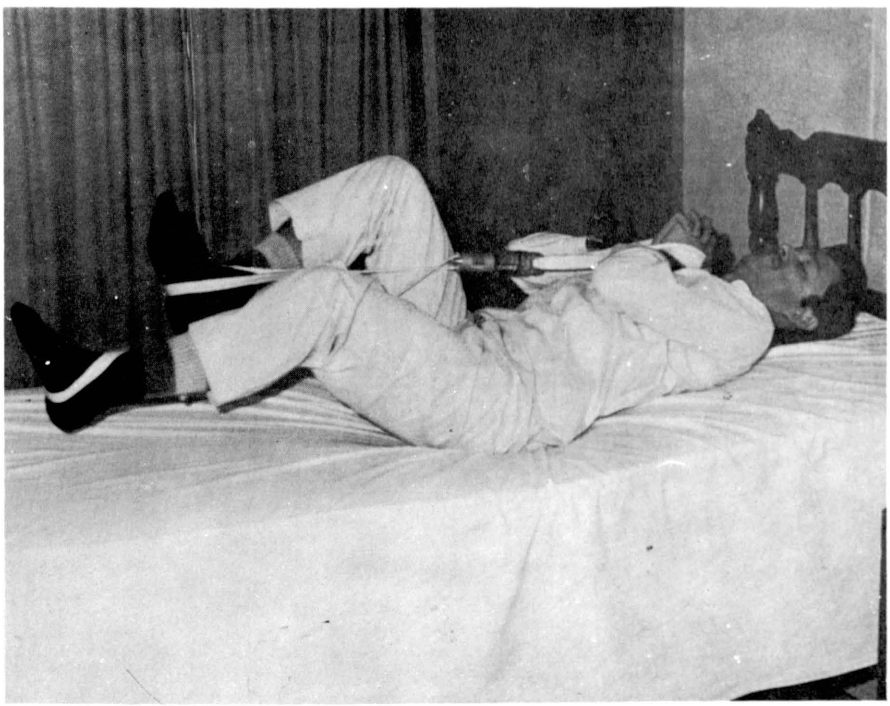

Figure 9. METHOD OF USING THE EXER-GENIE IN ISOTONIC EXERCISE INVOLVING THE FEET

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. Urine collections were made on a 24-hour basis during the greater part of this period.

PROCEDURE FOR THE DETERMINATION OF HYDROXYPROLINE IN URINE

There has been a great need for a satisfactory method for the determination of hydroxyproline to facilitate the study of the composition of proteins. The uniquely high hydroxyproline content of collagen suggests the desirability of an accurate method for the determination of this amino acid in small quantitites as a means of estimating the amount of collagen in a mixture of proteins. Troll (79) has reported one method for the specific assay of this imino acid. Other methods for the analysis of hydroxyproline have been described by Grunbaum (80), Huttere (81), Rosano (82), Stegemann (83), and Stein and Muting (84). According to Prockop and Udenfriend (1) these methods can be used only with relatively pure solutions.

Since nearly all mammalian hydroxyproline is found in collagen, the incorporation in vivo of radiosisotopes into hydroxyproline has been used frequently in studies on the degradation of collagen. Mitoma (85) and Nueman (86) have developed methods for the quantitative analysis of Hydroxyproline which involve oxidation of the imino acid to a pyrrole and then the condensation of the pyrrole with p-dimethylamino-benzaldehyde to give a chromophore suitable for spectrophotometry.

Prockop and Udenfriend (1) have described a method for hydroxyproline assay which extracts pyrrole into toluene to separate it from interfering materials and to insure specificity of the color reaction. Figure 10 depicts the oxidation and decarboxylation of hydroxyproline to pyrrole.

This technique was later modified by Prockop (87) so that it could be applied to samples containing C^{14} and tritium-labled hydroxyproline. The final solutions of pyrrole can be counted directly in a liquid scintillation counter. The specific activity of the pyrrole then is readily determined by taking an aliquot of the toluene solution for quantitative assay. Interfering radioactive materials are removed by solvent extrations before the pyrrole is formed, since the initial oxidation products of hydroxyproline are not extracted from alkaline solutions by chloroform or toluene. This procedure can be applied to urine and crude tissue preparations.

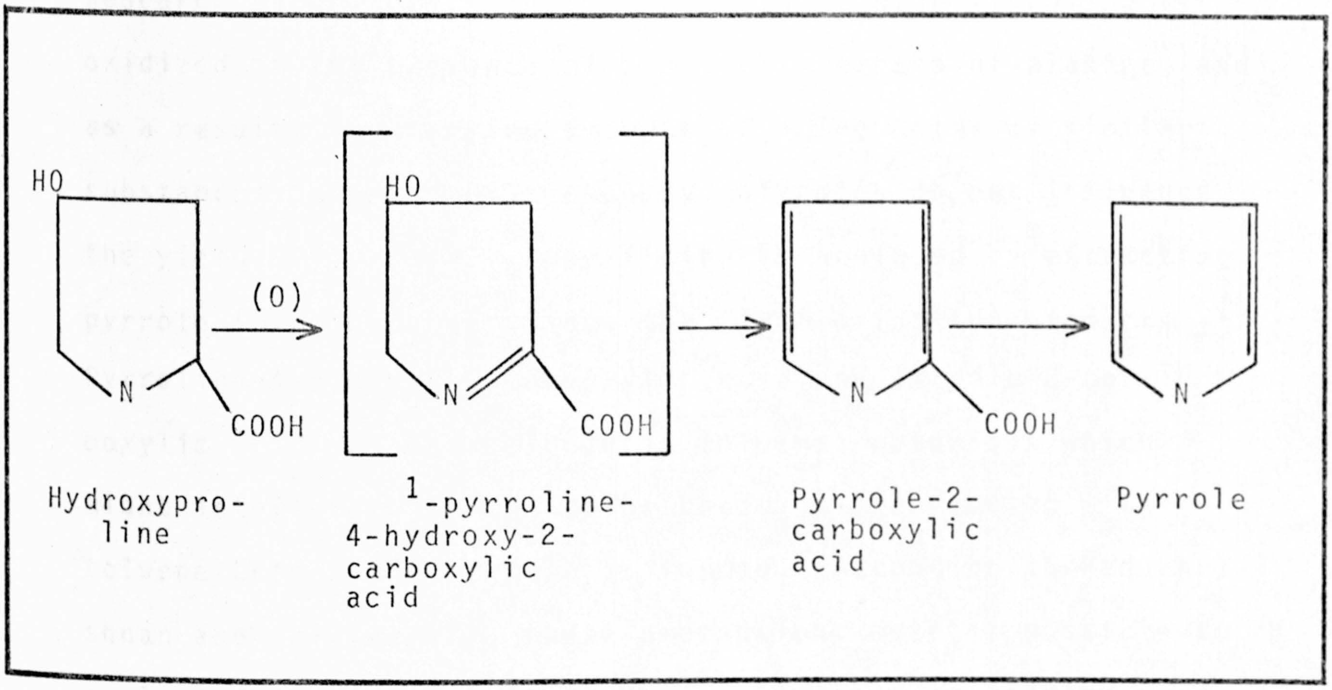

Figure 10. Oxidation and decarboxylation of hydroxyproline to pyrrole as shown by Prockop and Udenfriend (1).

Thus a number of samples can be applied to urine and crude tissue preparations, with a number of samples analyzed simultaneously.

For the purposes of this experiment, the method developed by Prockop and Udenfriend (1) has been adopted by the TWU Research Laboratory. In this procedure, hydroxyproline is oxidized in the presence of a measured excess of alanine, and as a result, the varying amounts of amino acids or similar substances in urine or tissue hydrolyzates do not influence the yield of pyrrole. Specificity is achieved by extracting pyrrole into toluene. Since the first oxidation products _[1] Pyrroline-4-hydroxy-2-carboxylic acid and pyrrole-2-carboxylic acid are not soluble in toluene, materials which might interfere with the color reaction are removed with toluene before the pyrrole is formed. According to Radakhrishnan and Meister (2), these precautions make it possible to analyze 5.0 µg. hydroxyproline in solutions containing over 50 mg. of other amino acids.

PROCEDURE ADOPTED IN THE TWU LABORATORIES

1. Urine Collection and Storage

A 24-Hour urine specimen is collected in a plastic bottle. The number of the subject as well as the volume of urine and the data are recorded on the plastic bottle with the urine kept in a frozen state until ready for extration. No chemical preservative is added to the samples during this period.

When the specimen is removed from the deep freeze, it is permitted to thaw. Then the specimen is mixed thoroughly before an aliquot is placed in a pyrex tube for analysis.

2. Total Urinary Hydroxyproline

A. From 0.1 to 1.0 milliliters of urine is placed in a pyrex test tube and each sample is analyzed in duplicate.

B. Add 2.0 milliliters of concentrated hydrochloric acid (hydrolysis is maximum at 6 N), and 1.0 milliliters of distilled water.

C. Seal tubes with a blow torch. These can be stored for several days in the deep freeze.

D. Autoclave for three hours (124 C). Store in the deep freeze (-20 C) until analyzed.

3. Removal of Pigments

A. Break seal on autoclaved samples with pliers and towel.

B. Pour contents of tubes into round-bottom glass centrifuge tubes.

(Note: If a small break has occurred in a tube during autoclaving, the volume may be increased due to condensation of water during cooling. Make a note of such a tube, but run sample through entire procedure. Rather than adding 4.0 ml. distilled water, however, make up to 8.0 ml. according to graduations of centrifuge. If the samples duplicate well. Results may be interpreted as valid.)

C. Add 4.0 ml. of distilled water to the total volume of 8.0 ml. (Final normality should not be less than 2 N).

D. Add an amount not exceeding the equivalent of 1.0 ml. Dowex-Charcoal resin (0.5 ml. is usually sufficient) and mix well by repeated inversion using parafilm.

E. Centrifuge 15 minutes at 2,000 rpm. (during centrifugation, standards and blanks may be made.)

F. Take 4.0 ml. of supernatant liquid immediately with 4.0 ml. volumetric pipette, being careful not to disturb the resin precipitate, and put into large culture tubes. These, with standards and blanks, may be stored for one or two days at -20 C.

4. Preparation of Standard and Reagent Blanks

A. Stock standard is 100 µmole hydroxyproline per ml. Working standard is made at the time of pigment removal by adding 0.1 ml. of 100 µmole/ml. stock standard to a 10 ml. volumetric flask and making to volume with distilled water. Working standard thus contains 1.0 µm. per ml. Range of the calorimetric determination is from 0.1 to 1.0 µm. Usually two 0.5 um. standards are used. 0.5 ml. working standard is placed in a centrifuge tube and 5.5 ml. of distilled water is added (to make 6.0 ml.) followed by 2.0 ml. concentrated HCl. This is mixed and 4.0 ml. are added to a large culture tube at the same time as decolorized samples are being put into culture tubes. Degeneration during storage therefore will reflect in the standard.

B. Reagent blank: 6.0 ml. distilled water plus 2.0 ml. HCl, mixed with 4.0 ml. are added to a culture tube. Two blanks are sufficient.

5. Colorimetric Analysis

A. Allow samples in culture tubes to come to room temperature.

B. Add one drop 1.0 per cent phenolphthalein.

C. Add 2.5 ml. 5 N KOH.

D. Add 1 N KOH dropwise until a faint pink color develops. Colors may vary, but if only one drop has produced color, the pH will not be sufficiently high to alter reactions; buffer will be added later.

E. Add KCl in minimal excess to sat ur ation (2.5 to 3.0 cc. of granular salt).

F. Add 2 ml. borate buffer.

G. Add 1 ml. 10% alanine. Mix with the Vortex.

H. Add 2.0 ml. 0.2M chloramine-T, made fresh daily.

I. Let oxidation proceed at room temperature for 20 min.

J. After 20 minutes, the reaction is stopped by adding 6.0 ml. sodium thiosulfate solution. Mix well with Vortex.

K. Add approximately 10 ml. toluene and shake at high speed with shaker for 2 minutes. (At this point, turn on boiling water bath.)

L. Centrifuge 10 minutes at 750 rpm.

M. Remove toluene phase via vacuum. being careful not to remove any of aqueous phase, even if this requires leaving a small residue of toluene.

N. Cap tubes and place in boiling water bath for 30 minutes.

O. Cool under running tap water. When the next step is instituted, the procedure must be carried straight through to completion.

P. Add exactly 10 ml. toluene.

Q. Shake for two minutes on shaker at high speed. (Check to make sure Coleman is on at this point.)

R. Centrifuge 10 minutes at 750 rpm.

S. Take exactly 5.0 ml. of clear toluene phase with a 5.0 ml. volumetric pipette and put in Coleman test tube.

T. Add precisely 2.0 ml. Ehrlich's reagent using a 10 ml. serological pipette. Mix with Vortex.

U. Allow to stand for 15 minutes.

V. Read at 560 mμ. on Coleman. (Valid range of OD reading is from 0.150 to 1.50, although best is below 1.0.)

6. Calculations

A. $K = \frac{\text{Gms. of hydroxyproline in 25 } \mu\text{m/ml solution of standard}}{\text{OD Standard}}$

B. $K \times OD = \frac{\text{Mg of hydroxyproline}}{\text{100 ml.}}$

C. $\frac{\text{Mg of hydroxyproline}}{\text{100 ml.}} \times \frac{\text{total volume of urine}}{100} =$ mg. of hydroxyproline/24 hours

Comments

1. Accuracy is required when preparing samples for autoclaving and at each step through addition of 4.0 ml. decolorized sample to culture tubes. Thereafter, precision is not so critical, since the final product is extracted into a constant volume of 10 ml. of toluene. Then toluene and final color reaction reagents again must be added carefully.

2. Storage at -20 C at various stages as described is not known to be harmful to stability of hydroxyproline, but the length of time these remain stable is not worked out.

The less storage time, in general, the better. Where there are known limitations, these have been noted in the procedure above.

3. If too much KCl is added, there is often difficulty in separating aqueous and toluene phases; also, any KCl present when color reagent is added will cause failure. Care also must be taken not to use too little.

4. Reagents used in massive amounts so that close watch should be kept on available stock are: sodium thiosulfate, d-alanine, p-dimethylaminobenzaldehyde, toluene, and KOH.

5. It is recommended that the paper of Prockop and Udenfriend from Analytical Biochemistry 1:228-239 (1960) entitled "Analysis of Hydroxyproline" be studied before the procedure is attempted so that the theory presented here and further application of the procedure can be appreciated.

6. Amounts of Ehrilich's reagent and toluene phase have been reduced in this procedure from those described in the reported method for the following purposes: (a) to conserve Ehrlich's reagent, (b) to produce a handy working volume, and (c) to allow enough toluene phase to remain to repeat this step if necessary. Do not discard contents of culture tubes until reading has occurred for reason (c) above.

Reagents

1. Borate buffer, pH 8.7: 61.84 gm. boric acid and 225 gm. potassium chloride are mixed into about 800 ml. of distilled water. The pH is adjusted to 8.7 with a 5 N potassium hydroxide solution and the final volume is made up to one liter.

2. Alanine solution: 10 gm. of alanine are dissolved in about 90 ml. of distilled water, the pH adjusted to 8.7 with a 5N potassium hydroxide solution, and the final volume is made up to 100 ml.

3. Chloramine-T solution (Eastman Organic Chemicals): 0.2 M solution in Methyl Cellosolve is prepared daily. Then 2.8 gm. chloramine-T is made to 50 ml. with methyl cellosolve; then mix thoroughly.

4. Sodium Thiosulfate: solution is 3.6 M in distilled water and is stored under toluene at room temperature for several weeks.

5. p-Dimethylaminobenzaldehyde, or Ehrlich's reagent: (Analytical grade; Matheson, Coleman, and Bell.) Concentrated sulfuric acid, 27.4 ml., is added slowly to 200 ml. of absolute alcohol in a beaker and the mixture cooled. p-Dimethylaminobenzaldehyde, 120 gm., is added to 200 ml. of absolute alcohol in another beaker, and

then the acid-ethanol mixture is slowly stirred into the first beaker. The solution can be stored in the refrigerator for several weeks; the crystals which precipitate on cooling are redissolved by warming the solution briefly.

6. Dowex-Charcoal Resin: 50 gm. analytical grade Dowex 1 and 20 gm. Norit A are added to about 100 ml. 6 N HCl. Wash several times with 6 N HCl in Buchner funnel until effluent is no longer yellowish. Dry with several rinses of 1:1 ethanol:ether.

7. Hydroxy-L-Proline: 1.31 gm. of hydroxyproline is mixed into about 90 ml. of distilled water. The final volume is made up to 100 ml. and stored in the refrigerator.

8. Phenolphthalein: 1 gm. of phenolphthalein is mixed into about 50 ml. of ethanol. The final volume is made up to 100 ml.

9. Potassium Chloride: Analytical grade, granular.

10. Toluene: Reagent grade.

11. Potassium Hydroxide (5 N): 280.55 gm. of potassium hydroxide is dissolved in about 900 ml. of distilled water. The final volume is made up to 1 liter. For a 1 N solution, 56.11 gm. of potassium hydroxide is dissolved in about 900 ml. of distilled water. The final volume is made up to 1 liter.

PRESENTATION OF FINDINGS WITH DISCUSSION

During the summer of 1969, eight healthy human males participated in an immobilization study conducted at the Texas Woman's University Research Institute. This study, which covered a span of 85 days, including a 16 day Pre-Bed Rest Period, a 56 day Bed Rest Period, and a 13 day Post-Bed Rest Period, was designed to investigate metabolic changes and circadian rhythms of excreted urinary metabolites from individuals subjected to immobilization under carefully controlled environmental conditions.

The daily urinary excretion data on hydroxyproline are recorded in the Appendix in Table I. Urinary hydroxyproline excretion during four daily periods throughout Bed Rest are reported in Table II. The statistical comparisons of urinary hydroxyproline excretion between pairs of the different periods are presented in Table III. The statistical comparisons on circadian rhythms are shown in Table IV and Figures 11 and 12. The statistical data pertaining to exercise are recorded in Tables V, VI, VII, and VIII.

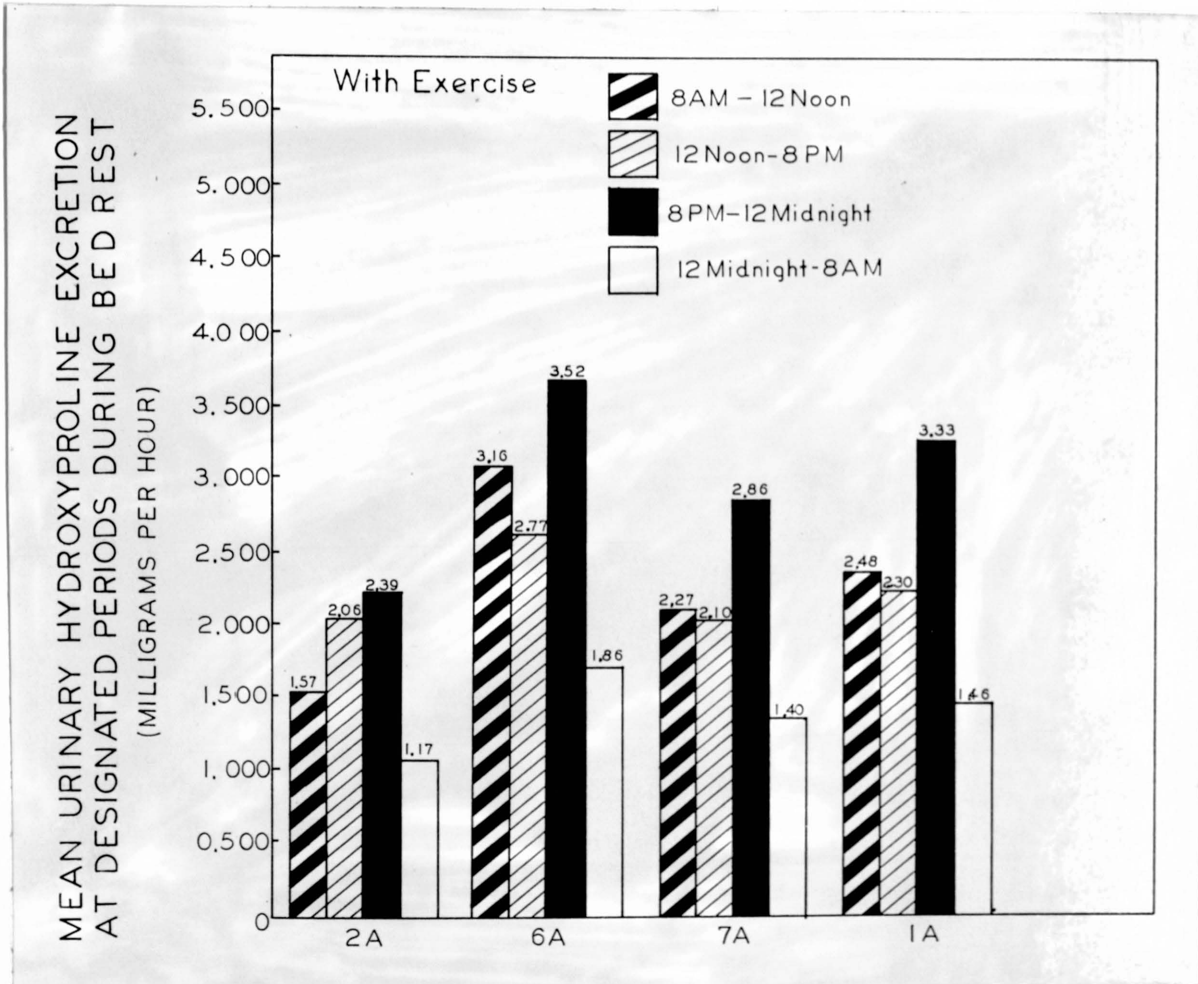

Figure 11. Circadian Rhythms shown for Mean Urinary Hydroxyproline for the Subjects Who Exercised. (Note: Subject 1A Exercised during the Last 28 days of the Study.)

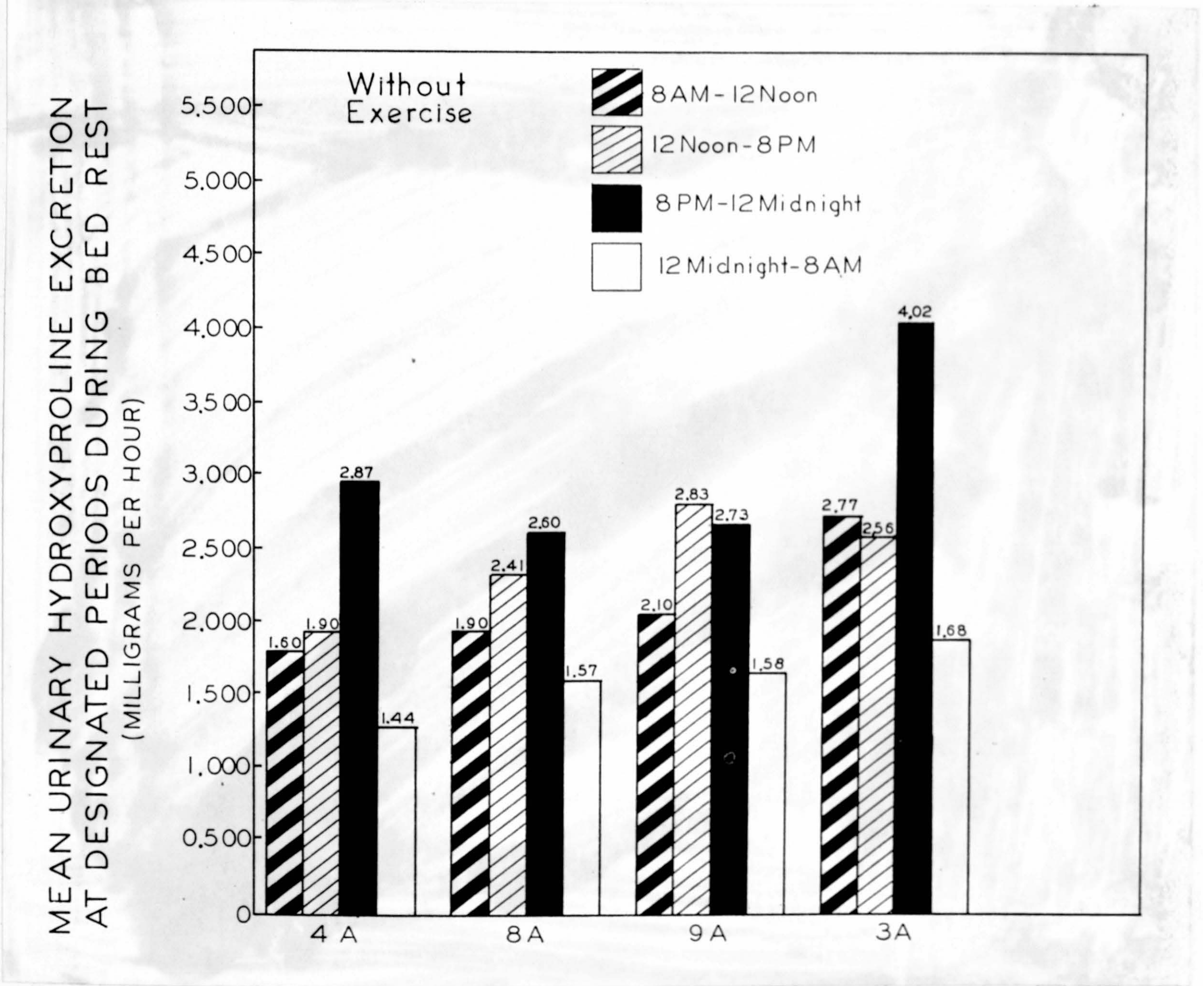

Figure 12. Circadium Rhythms shown for Mean Urinary Hydroxyproline for the Subjects Who did not Exercise. (Note: Subject 3A Exercised throughout the first 28 Days, the Period Represented in this Graph.)

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

Table III in the Appendix contains the statistical data pertaining to urinary hydroxyproline excretion during the different periods of the study.

SUBJECT 2A

The amount of hydroxyproline excreted in the urine by this subject during Bed Rest was appreciably greater in quantity than during the ambulatory periods of the study. When comparing the excretion during Bed Rest with both the Pre-Bed Rest and Post-Bed Rest Periods, statistically a highly significant increase ($P < 0.001$) was noted during the Bed Rest Period. The hydroxyproline excretion during Pre-Bed Rest was determined to be significantly higher than during Post-Bed Rest ($P < 0.01$). See Table III, Part A.

SUBJECT 6A

As shown in Table III, Part A, Subject 6A excreted more hydroxyproline in the urine during Bed Rest than during the other two periods of the study. Although no significant difference was noted between the amounts recorded for the Bed Rest Period and the Pre-Bed Rest Period, a comparison of the Bed Rest Period with the Post-Bed Rest Period proved to be highly significant ($P < 0.001$). A slightly higher amount of hydroxyproline was excreted in the urine during the Pre-Bed Rest Period than was found in the Post-Bed Rest Period ($P < 0.02$).

SUBJECT 7A

Table III, Part A., shows that, when the amount of hydroxyproline excreted by this subject during the Bed Rest Period was compared with the amounts excreted during both the Pre-Bed Rest and Post-Bed Rest Periods, the excretion during the Bed Rest Period greatly surpassed that of the other two periods in both cases ($P < 0.001$). The urinary hydroxyproline level was higher during the Pre-Bed Rest Period than during the Post-Bed Rest Period. The difference, however, was not significant.

SUBJECTS 2A, 6A, 7A
(Subjects who exercised throughout the Bed Rest)

As shown in Table III, Part B, when the data for all subjects who exercised during Bed Rest were pooled together for statistical comparison, the amount of hydroxyproline excreted in the urine during Bed Rest greatly surpassed the quantities reported both for the Pre-Bed Rest and Post-Bed Rest Periods by a difference which was highly significant ($P < 0.001$). The urinary excretion of hydroxyproline by this group of subjects during the Pre-Bed Rest Period was found to be significantly higher ($P < 0.01$) than during the Post-Bed Rest Periods.

SUBJECT 4A

The excretion of urinary hydroxyproline by Subject 4A was significantly greater during the Bed Rest Period than both with the Pre-Bed Rest and Post-Bed Rest Period ($P < 0.001$), as shown in Table III, Part C. During the period of Pre-Bed Rest, the urinary hydroxyproline excretion by this subject was very slightly higher than during the period of Post-Bed Rest ($P < 0.10$).

SUBJECT 8A

According to Table III, part C, a higher level of hydroxyproline was excreted by Subject 8A during the period of Bed Rest than during the period of Pre-Bed Rest. The difference was somewhat significant ($P < 0.02$). In comparing the Bed Rest Period with the Post-Bed Rest Period, it was found that the urinary hydroxyproline level during Bed Rest greatly surpassed that of the latter period ($P < 0.001$). It also was found that the urinary excretion of hydroxyproline by Subject 8A during the Pre-Bed Rest Period was higher than during the Post-Bed Rest Period by a significant difference ($P < 0.05$).

SUBJECT 9A

During Bed Rest the urinary hydroxyproline excretion by Subject 9A was determined to be higher than during Pre-Bed Rest by a difference which was somewhat significant ($P < 0.02$).

A highly significant difference ($P < 0.001$) was found between the Bed Rest and Post-Bed Rest Periods with the higher excretion of hydroxyproline in the urine being reported during Bed Rest. This subject excreted significantly more hydroxyproline in the urine during the Pre-Bed Rest Period as compared to the Post-Bed Rest Period ($P < 0.02$). See Table III, Part C.

SUBJECTS 4A, 8A, 9A

(Subjects who did not exercise during the Bed Rest)

Tabe III, Part D, gives a resumé of the statistical findings when the urinary hydroxyproline data were pooled for the three subjects who did not exercise. The total hydroxyproline excretion during the Bed Rest Period surpassed that of both the Pre-Bed Rest and Post-Bed Rest Periods by a highly significant difference ($P < 0.001$). It was found that a highly significant difference ($P < 0.001$) also existed between the urinary hydroxyproline levels recorded for the Pre-Bed Rest and the Post-Bed Rest Periods with the higher level being observed during the period of Pre-Bed Rest.

SUBJECT 3A

(Subject Exercising First Half of Bed Rest)

Table III, Part E., shows that the average daily hydroxyproline excretion by this subject was greater in quantity during the Bed Rest Period than during either the Pre-Bed

Rest or Post-Bed Rest Periods by a difference which was highly significant in both cases ($P < 0.001$). During the Pre-Bed Rest Period the amount of urinary hydroxyproline excreted by this subject was slightly higher than during the Post-Bed Rest Period. The difference, however, was not statisticaly significant.

SUBJECT 1A

(Subject Exercising Last Half of Bed Rest)

Although this subject excreted somewhat more hydroxyproline in the urine during the Bed Rest Period in comparison with the Pre-Bed Rest Period, the difference was not found to be significant. In comparing the Bed Rest and Post-Bed Rest·Periods, however, the hydroxyproline level reported for the Bed Rest Period greatly surpassed that of the latter period ($P < 0.001$). The amount of urinary hydroxyproline excreted by this subject during the Pre-Bed Rest Period was appreciably higher than the amount excreted during the Post-Bed Rest Period with the difference being highly significant ($P < 0.001$). See Table III, Part E.

ALL SUBJECTS

When the data for all eight subjects were combined for the purpose of statistical comparison, it was found that the amount of urinary hydroxyproline excreted during the Bed Rest Period greatly exceeded the amounts reported for the two ambulatory periods. A highly significant difference ($P < 0.001$) was determined in comparing the Bed Rest Period with both the Pre-Bed Rest Period and the Post-Bed Rest Period. It also was found that the amount of hydroxyproline excreted in the urine during the Pre-Bed Rest Period was greater than the quantity reported for the Post-Bed Rest Period with the difference being highly significant ($P < 0.001$).

CIRCADIAN PATTERN OF URINARY HYDROXYPROLINE EXCRETION DURING BED REST PERIOD

In order to study the diurnal variation of hydroxyproline, 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 of the excretion patterns are given in Table IV (Appendix).

SUBJECT 2A

Table IV, Part A, shows that the quantity of hydroxyproline excreted by this subject between 12 Noon and 8 P.M. was lower than between 8 P.M. and 12 Midnight, the difference being slightly significant ($P < 0.10$). The mean hourly value of urinary excretion was highest for this subject during the period from 8 P.M. to 12 Midnight. A statistical comparison of the period from 12 Noon to 8 P.M. with the period from 12 Midnight to 8 A.M. showed that this subject had an increase in the excretion of urinary hydroxyproline during the period from 12 Noon to 8 P.M., the difference being highly significant ($P < 0.001$).

In comparing the period from 12 Noon to 8 P.M. with that from 8 A.M. to 12 Noon, the larger excretion was noted to occur between 12 Noon and 8 P.M. The difference was distinctly significant ($P < 0.01$). When the period from 8 P.M. to 12 Midnight was compared with the period from 12 Midnight to 8 A.M., the higher excretion was found during the period from 8 P.M. to Midnight. The difference was highly significant ($P < 0.001$). It also was found that the urinary hydroxyproline excretion between 8 P.M. and 12 Midnight greatly surpassed that of the period from 8 A.M. to 12 Noon. Statistically, the difference was highly significant ($P < 0.001$). In comparing the period from 12 Midnight to 8 A.M. with that of 8 A.M. to 12 Noon, it also was found that there was a significant increase ($P < 0.01$) in the excretion of hydroxyproline during the 8 A.M. to 12 Noon period.

SUBJECT 6A

Table IV, Part B, shows in an analysis of the data pertaining to Subject 6A that the amount of urinary hydroxyproline excreted by this subject from 8 P.M. to 12 Midnight surpassed the amount excreted from 12 Noon to 8 P.M. ($P < 0.02$).

In comparing the amount excreted from 12 Midnight to 8 A.M. with that from 12 Noon to 8.P.M., it was found that the latter surpassed the former by a difference which was highly significant ($P < 0.001$). Although a slightly higher excretion was found to occur during the period from 8 A.M. to 12 Noon as compared with that of 12 Noon to 8 P.M., the difference was not significant.

In comparing the amount of urinary hydroxyproline excreted during the period from 8 P.M. to 12 Midnight with the period from 12 Midnight to 8 A.M., a higher excretion was observed during the 8 P.M. to 12 Midnight period, with the difference highly significant ($P < 0.001$). In comparing the period 8 P.M. to 12 Midnight with the period 8 A.M. to 12 Noon, the difference in urinary hydroxyproline excretion was not determined to be statistically significant.

The amount of urinary hydroxyproline excreted by this subject from 8 A.M. to 12 Noon greatly surpassed the amount excreted between 12 Midnight and 8 A.M. Statistically, the difference was highly significant ($P < 0.001$).

SUBJECT 7A

Table IV, Part C, contains the daily periodic data for Subject 7A.

When the amount of urinary hydroxyproline excreted during the period 8 P.M. to 12 Midnight was compared with the amount excreted from 12 Noon to 8 P.M., the higher amount was found for the period between 8 P.M. and 12 Midnight. The difference was distinctly significant ($P < 0.01$).

The mean hourly value of urinary hydroxyproline excreted from 12 Noon to 8 P.M. was higher than the value reported for the 12 Midnight to 8 A.M. period. The difference between these two periods was highly significant ($P < 0.001$). In comparing the period from 12 Noon to 8 P.M. with the period from 8 A.M. to 12 Noon, it was found that, although the excretion was somewhat higher between 8 A.M. and 12 Noon, the difference was not found to be statistically significant.

In comparing the amount of urinary hydroxyproline excreted during the 8 P.M. to 12 Midnight period with that excreted during the 12 Midnight to 8 A.M. period, a higher excretion was noted during the former period. The difference between the two periods was highly significant ($P < 0.001$).

The urinary hydroxyproline excreted by this subject during the period from 8 P.M. to 12 Midnight surpassed the amount excreted between 8 A.M. and 12 Noon by a difference which was somewhat significant ($P < 0.002$). In comparing the amount of urinary hydroxyproline excretion between the hours of 12 Midnight and 8 A.M. with the excretion between 8 A.M. and 12 Noon, a greater quantity of hydroxyproline was found in the urine during the period from 8 A.M. to 12 Noon. The difference found was highly significant ($P < 0.001$).

SUBJECTS 2A, 6A, 7A

(Subjects Who Exercised Throughout Bed Rest)

When the data for the three subjects who exercised throughout the study were pooled, it was found that a higher excretion of urinary hydroxyproline appeared during the period from 8 P.M. to 12 Midnight, as compared to that from 12 Noon to 8 P.M., with the difference being highly significant ($P < 0.001$). Further it was found that the hydroxyproline excretion between 12 Noon and 8 P.M. surpassed the amount excreted during the 12 Midnight to 8 A.M. period by a highly significant difference ($P < 0.001$).

In comparing the urinary hydroxyproline excretion during the period from 12 Noon to 8 P.M., on the other hand, with that from 8 A.M. to 12 Noon, the mean values were the same, with no significant statistical difference observed. There was a distinctly higher level of urinary hydroxyproline excreted by these subjects during the period from 8 P.M. to 12 Midnight as compared to the periods from 12 Midnight to 8 A.M. and 8 A.M. to 12 Noon. Both comparisons indicated a highly significant difference between the rates of hydroxyproline excretion for these periods undergoing comparison ($P < 0.001$).

A higher urinary hydroxyproline level was noted to occur during the period 8 A.M. to 12 Noon as compared with the 12 Midnight to 8 A.M. period. The difference was found to be highly significant in this case ($P < 0.001$).

SUBJECT 4A

As shown in Table IV, Part F, when pairs of the collection periods were compared as to the quantities of urinary hydroxyproline excreted by Subject 4A, it was found that, during the period from 8 P.M. to 12 Midnight this subject excreted a far greater amount of hydroxyproline in the urine than during the other three daily periods ($P < 0.001$).

When a statistical comparison was made between the hydroxyproline excretion levels between 12 Noon and 8 P.M. and between 12 Midnight and 8 A.M., the former surpassed the latter by a level which was significant at the 1.0 per cent level of confidence. In comparing this same period, 12 Noon to 8 P.M., with the period from 8 A.M. to 12 Noon, the higher level of hydroxyproline excretion again was noted to occur between 12 Noon and 8 P.M. with a difference which was barely significant ($P < 0.10$).

A slightly higher amount of hydroxyproline excretion was noted to appear in the urine during the period from 8 A.M. to 12 Noon in comparison with that occurring from 12 Midnight to 8 A.M. The difference, however, was not significant.

<u>SUBJECT 8A</u>

Table IV, Part F, contains the statistical data pertaining to Subject 8A which is related to circadian rhythms. The quantity of urinary hydroxyproline excreted by this subject from 8 P.M. to 12 Midnight surpassed that excreted from 12 Noon to 8 P.M., although the difference was not significant.

In comparing the quantity of urinary hydroxyproline excreted between 12 Midnight and 8 A.M. with that excreted between 12 Noon and 8 P.M., the greater level was observed to occur during the 12 Noon to 8 P.M. period. The difference was determined to be highly significant ($P < 0.001$).

When the excretion of urinary hydroxyproline during the period from 12 Noon to 8 P.M. was compared with that from 8 A.M. to 12 Noon, a higher excretion was apparent during the 12 Noon to 8 P.M. period. The difference was significant ($P < 0.02$).

During the period 8 P.M. to 12 Midnight, the urinary excretions of hydroxyproline by this subject was higher than during the period from 12 Midnight to 8 A.M., with the difference being highly significant ($P < 0.001$). In comparing the same period, (8 P.M. to 12 Midnight) with the 8 A.M. to 12 Noon period, the higher excretion again was noted during the 8 P.M. to 12 Midnight period. The difference was found to be distinctly significant ($P < 0.01$).

The period 8 A.M. to 12 Noon, as compared with that of 12 Midnight to 8 A.M., shows a slightly higher rate of urinary hydroxyproline excretion which was determined to have a statistically significant difference ($P < 0.05$).

SUBJECT 9A

The amount of urinary hydroxyproline excreted by Subject 9A during the period from 12 Noon to 8 P.M. was slightly greater than that excreted from 8 P.M. to 12 Midnight, although the difference was not statistically significant.

In comparing the periods 12 Noon to 8 P.M. and 12 Midnight to 8 A.M., a higher level of urinary hydroxyproline excretion was recorded during the period from 12 Noon to 8 P.M. The difference was highly significant ($P < 0.001$). When the same period, 12 Noon to 8 P.M., was compared with that from 8 A.M. to 12 Noon, a significantly higher ($P < 0.01$) rate of hydroxyproline excretion was noted during the period 12 Noon to 8 P.M. ($P < 0.01$).

A higher quantity of hydroxyproline was excreted in the urine of this subject during the period from 8 P.M. to 12 Midnight as compared with that of 12 Midnight to 8 A.M., with the difference being highly significant ($P < 0.001$).

In comparing the amount of urinary hydroxyproline excreted by Subject 9A during the period 8 P.M. to 12 Midnight with the amount from 8 A.M. to 12 Noon, a higher level of excretion appeared between 8 P.M. and 12 Midnight. The difference was determined to be significant ($P < 0.01$).

The urinary excretion of hydroxyproline during the period from 8 A.M. to 12 Noon was higher than the excretion from 12 Midnight to 8 A.M. The difference was found to be significant at the 1.0 per cent level of significance ($P < 0.01$).

SUBJECTS 4A, 8A, 9A

(Subjects Who Did Not Exercise During Bed Rest)

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.

A higher excretion of hydroxyproline was found during the period from 12 Noon to 8 P.M. as compared with the periods from 12 Midnight to 8 A.M. and from 8 A.M. to 12 Noon. In both comparisons, the difference was found to be highly significant ($P < 0.001$).

When the urinary hydroxyproline excretion during the period 8 P.M. to 12 Midnight was compared with the excretion between 12 Noon and 8 P.M., a slightly higher excretion level was found to exist between 8 P.M. and 12 Midnight ($P < 0.05$). The amount of urinary hydroxyproline excreted by this group of subjects during the period from 8 P.M. to 12 Midnight greatly surpassed the levels observed from the periods 12 Midnight to 8 A.M. and 8 A.M. to 12 Noon. In both instances, the difference was highly significant ($P < 0.001$).

A greater quantity of urinary hydroxyproline was excreted during the period 8 A.M. to 12 Noon, as compared with the 12 Midnight to 8 A.M. periods, with the difference being highly significant ($P < 0.001$).

SUBJECT 3A

(Subjects Who Exercised First 28 Days of the Study)

This subject excreted the largest amount of urinary hydroxyproline over the period between 8 P.M. and 12 Midnight. The quantity excreted during this period markedly surpassed the amounts excreted during the periods from 12 Noon to 8 P.M., 12 Midnight to 8 A.M. and 8 A.M. to 12 Noon ($P < 0.001$).

During the period from 12 Noon to 8 P.M., the hydroxyproline excretion of this subject was appreciably greater than during the period from 12 Midnight to 8 A.M., with the difference being highly significant ($P < 0.001$).

The urinary hydroxyproline excretion of Subject 3A between 8 A.M. and 12 Noon, was slightly higher than between 12 Noon and 8 P.M. The difference, however, was not statistically significant.

A higher excretion of urinary hydroxyproline by this subject also was observed to occur during the period from

8 A.M. to 12 Noon as compared with the 12 Midnight to 8 A.M. period. The difference was highly significant ($P < 0.001$).

SUBJECT 1A

(Subject Who Exercised Second Half of the Study)

As shown in Table IV, Part J, Subject 1A excreted the greatest amount of urinary hydroxyproline during the period from 8 P.M. to 12 Midnight. When this amount was compared with the quantities excreted during the periods from 12 Noon to 8 P.M. and from 12 Midnight to 8 A.M. the differences were found to be highly significant ($P < 0.001$).

When the 12 Noon to 8 P.M. period was compared with the 12 Midnight to 8 A.M. period, a significantly higher excretion of hydroxyproline was found to occur from 12 Noon to 8 P.M. ($P < 0.001$).

Although the difference was not significant, a slightly higher excretion of hydroxyproline occurred during the period from 8 A.M. When a comparison was made with the period from 12 Noon to 8 P.M. a lower level of urinary hydroxyproline was observed during the period from 8 A.M. to 12 Noon than during the period from 8 P.M. to 12 Midnight with the difference distinctly significant ($P < 0.001$).

There was a higher excretion of urinary hydroxyproline by this subject during the period from 8 A.M. to 12 Noon as compared with the period from 12 Midnight to 8 A.M.

Statistically, this difference was highly significant ($P < 0.001$).

ALL SUBJECTS

An analysis of the pooled data for all eight subjects showed that, among the four daily collection periods, the highest level of urinary hydroxyproline excretion occurred during the period from 8 P.M. to 12 Midnight. During this period the hydroxyproline excretion was distinctly higher ($P < 0.001$) in comparison with the periods of 12 Noon to 8 P.M., 12 Midnight to 8 A.M. and 8 A.M. to 12 Noon.

The urinary hydroxyproline excretion during the period 12 Noon to 8 P.M. greatly surpassed that of the period 12 Midnight to 8 A.M. with the difference being highly significant ($P < 0.001$).

In comparing the hydroxyproline urinary excretion between 12 Noon and 8 P.M. with that from 8 A.M. to 12 Noon, it was found that the excretion between 12 Noon and 8 P.M. was slightly higher ($P < 0.10$).

When the higher amount of urinary hydroxyproline which was excreted between 8 A.M. and 12 Noon was compared with the amount excreted between 12 Midnight and 8 A.M, the difference was found to be highly significant ($P < 0.001$).

Although some variations were observed in the periodicities of the individual subjects of this study, the pooled data for all subjects indicated that a characteristic circadian cycle does prevail in the excretion of urinary hydroxyproline. The peak level of excretion was attained between 8 P.M. and 12 Midnight, and the lowest hydroxyproline values were recorded during the period from 12 Midnight to 8 A.M. This study comfirms earlier observations by Mautalen (48) which showed maximum total hydroxyproline levels occurring overnight with minimum levels being reached during the morning and early afternoon.

COMPARISON OF URINARY HYDROXYPROLINE EXCRETION DURING BED REST AS INFLUENCED BY EXERCISE

Tables V, VI, VII, and VIII, (Appendix) contain the data pertaining to the effect of exercise on urinary hydroxyproline excretion.

SUBJECT 3A

(Subject Who Exercised During the First 28 Days of the Study)

The total urinary hydroxyproline excretion by this subject was significantly lower ($P < 0.001$) when he was participating in exercise, during the first half of the study, as shown in Table V.

Subject 1A excreted somewhat more hydroxyproline when he was not exercising during the first half of the study, although there was no statistically significant difference between the periods of exercise and that of no exercise. See Table V.

Table VI, Part A, gives a comparison of the hydroxyproline excretion at different periods of the day by Subject 3A. Between 12 Noon and 8 P.M. the decreased excretion of hydroxyproline which was noted when this subject exercised was distinctly significant ($P < 0.01$) as compared to the period when this subject did not exercise. Also, exercise during the period from 8 P.M. to 12 Midnight stimulated a highly significant decrease ($P < 0.001$) in urinary hydroxyproline excretion as compared with the time during these hours when exercise was not taken.

No significant changes in hydroxyproline excretion were noted during the period from 12 Midnight to 8 A.M. because of exercising or not exercising during the day.

During the period from 8 A.M. to 12 Noon, there was a higher excretion of urinary hydroxyproline reported when this subject had not exercised. The difference, however, was not significant.

SUBJECT 1A

(Subject Who Exercised During the Second Half of the Study)

As shown in Table V, during the second half of the Bed Rest Period when this subject was engaged in programmed exercise, the urinary excretion of hydroxyproline was lower thanduring the period of the Bed Rest when no exercise was taken.

Table VI contains the statistical comparisons of the urinary hydroxyproline excreted during the day of exercise with that excreted during the day of no exercise. No significant difference was detected in the amount of hydroxyproline excreted by this subject when participating in different activity schedules during the collection periods from 12 Noon to 8 P.M., 8 P.M. to 12 Midnight, and 8 A.M. to 12 Noon.

For the period of 12 Midnight to 8 A.M, however, there was an elevation in hydroxyproline excretion during the time when this subject had not exercised during the day, with the difference being significant ($P < 0.02$).

GROUP OF SUBJECTS WHO EXERCISED (2A, 6A, 7A) vs.
GROUP WHO DID NOT EXERCISE (4A, 8A, 9A)

A statistical comparison was made between the group of subjects which exercised (2A, 6A, 7A) and the group of subjects who did not exercise (4A, 8A, 9A) during the Bed Rest Period of the study. As shown in Table VII, the mean quantity of hydroxyproline excreted from 12 Noon to 8 P.M. and from 8 P.M. until Midnight was not significantly different for the group exercising than for the group not exercising. The same was true for the period from 8 A.M. until Noon. See Figure 13.

When the hydroxyproline values for the same times of the day during Bed Rest were compared between the two exercise groups, it was found that the only statistical difference occurred in the period from 8 A.M. to 12 Noon. During this period the group which exercised excreted an appreciably greater amount of hydroxyproline in the urine ($P < 0.001$) than did the group which did not exercise. See Table VIII.

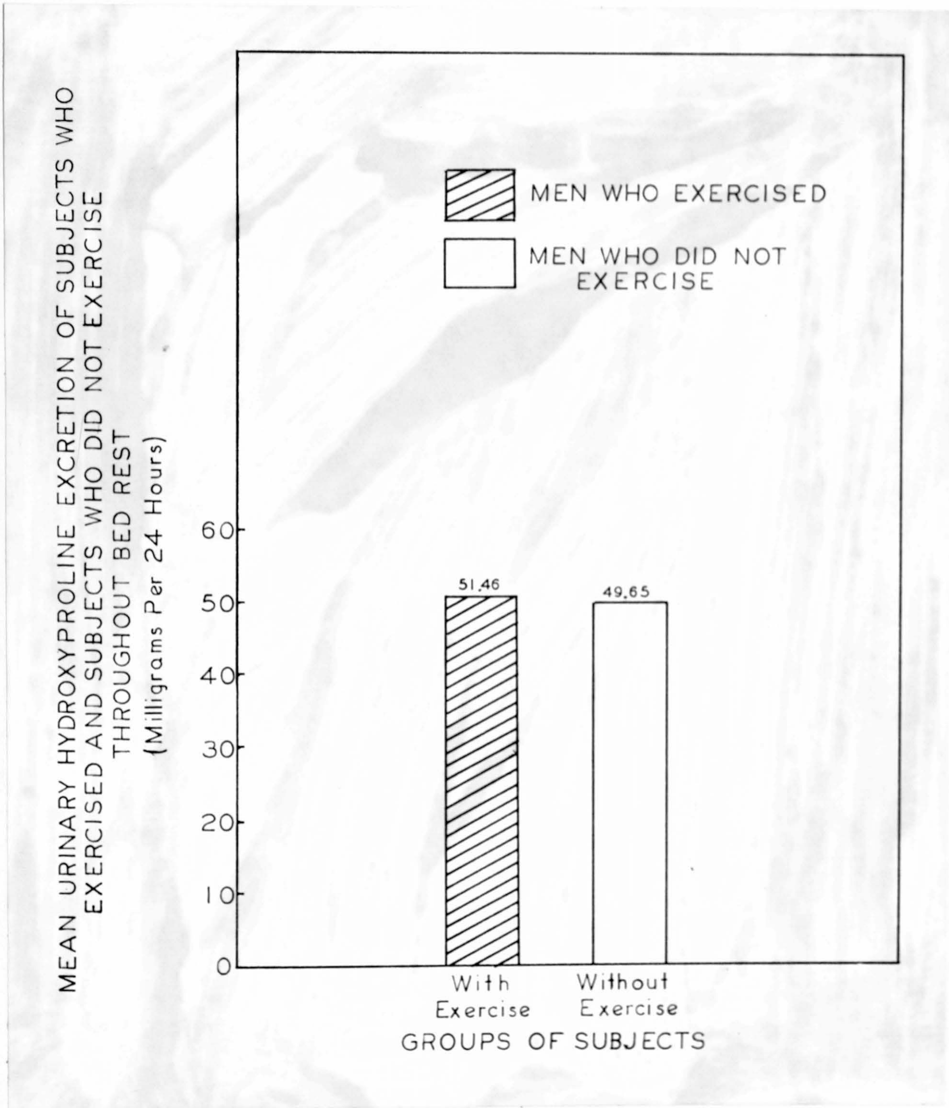

Figure 13. Mean Urinary Hydroxyproline Excretion by Subjects Who Exercised and Those Who did not Exercise.

SUMMARY AND CONCLUSIONS

The present investigation is one of a series of immobilization studies to be conducted under the auspices of the National Aeronautics and Space Administration at the Texas Woman's Research Institute on the campus of Texas Woman's University. During the summer of 1969, the eight healthy adult males participating in this Bed Rest study were housed and fed at the Nelda Childers Stark Laboratory for Human Research in the metabolic ward of the Research Institute. The study covered a span of 85 days which included a 16-day Pre-Bed Rest Period, a 56-day Bed Rest Period, and a 13-day Post-Bed Rest Period. Throughout the entire study, the subjects were radiographed in order to find any losses which may have occurred in bone density and for any metabolic changes occurring in physiologic functions. This report is concerned with the metabolism of hydroxyproline in the eight subjects under surveillance. All urine samples were analyzed for hydroxyproline content using the method of Prockop and Udenfriend (1).

Total 24-hour urinary hydroxyproline values increased in all subjects during the Bed Rest Period as compared with the Pre-Bed Rest Period. The overall average excretion of hydroxyproline during the Pre-Bed Rest Period was 38.01 milligrams per 24 hours, while that per 24 hours during the Bed Rest

Period was 52.17 milligrams. This represented an increase having a statistical significance of ($P < 0.001$).

During the Post-Bed Rest Period, all subjects showed a significant decrease ($P < 0.001$) in urinary hydroxyproline as compared with the Pre-Bed Rest Period. The overall average of hydroxyproline excretion during the Post-Bed Rest Period was 22.70 milligrams per 24 hours. Similarly, a statistical difference of ($P < 0.001$) was determined when the Post-Bed Rest Period was compared with the Bed Rest Period as to the quantity of hydroxyproline excreted in the urine, with a far greater amount being reported during the Bed Rest Period. It appears that immobilization markedly increases the excretion of hydroxyproline in the urine. Possibly the element of stress which is connected with a long period of immobilization may have served to enhance hydroxyproline excretion.

During the 56-day Bed Rest portion of this study, the environmental conditions in the metabolic ward were carefully controlled as to light and temperature for the purpose of studying whatever circadian rhythms may have occurred in various metabolites. A 14-hour day and 10-hour night schedule and a temperature of 72° F. were maintained in the metabolic ward at all times.

Urinary samples were collected four times daily during this phase of the study for analytical purposes. The urinary hydroxyproline excretion of all subjects reached a minimum level during the overnight period. In seven of the eight subjects, the maximum level of urinary hydroxyproline was observed between 8 P.M. and 12 Midnight. One subject showed a peak excretion between 12 Noon and 8 P.M. In comparing the pooled data for all subjects statistically, both the low (12 Midnight to 8 A.M.) and the high (8 P.M. to 12 Midnight) levels of excretion were found to differ by highly significant amounts ($P < 0.001$) when related to the other periods of the day. Apparently, a marked circadian periodicity does exist in the excretion of hydroxyproline in the urine.

Exercise was introduced as a variable during the Bed Rest Period with three subjects participating in programmed exercise throughout this phase of the study and three subjects not exercising. Two subjects exercised 28 days each, one the first half of the Bed Rest and the other the latter half of the study.

No significant difference was found in the 24-hour total of urinary hydroxyproline excreted by the two groups. It is of note, however, that a highly significant difference ($P < 0.001$) was reported in the excretion of hydroxyproline by these groups between 8 A.M. and 12 Noon, with the exercise group excreting the larger amount.

The subject who exercised at intervals during the first half of the study showed a significant rise in the urinary excretion of hydroxyproline during the second part of the Bed Rest Period ($P < 0.001$). No significant difference was observed between the exercise periods for the other subject, although the excretion of hydroxyproline was lower when exercise was part of the schedule.

The results of this study pertaining to the effect of exercise on urinary hydroxyproline excretion suggest an apparent individual variation. Since it is impossible to line these variances definitely thus far with intensity of participation in exercise, it is felt that further investigation in this area is indicated.

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RESULTS ON URINARY NITROGEN AND NITROGEN BALANCE

PROCEDURE FOR THE DETERMINATION OF NITROGEN

The Micro-Kjeldahl method as described by Archibald (1) has been adopted for use in the T.W.U. Research Institute Laboratory for the determination of nitrogen in urine and feces. This method is based on the principle that organic matter of a sample to be analyzed is oxidized by heating it with sulfuric acid in the presence of a catalyst. All combined nitrogen in the sample thereby is converted to ammonium sulfate nitrogen. An excess of alkali then is added and the ammonia which thus is liberated is distilled into acid solution. The ammonia is distilled into an accurately measured small excess of standard H_2SO_4. The aid not neutralized by the ammonia then is titrated with standard NaOH.

Reagents

The reagents are the following:

1. Powered potassium sulfate, K_2SO_4 (ammonia free).
2. Concentrated sulfuric acid, c.p. H_2SO_4, sp. gr. 1.84.
3. Mercuric sulfate solution. Add 12 ml. of concentrated H_2SO_4 to distilled water and make up to 100 ml. with distilled water. Dissolve 10 g. of red mercuric oxide in this solution.
4. Zinc dust (not granulated zinc), ammonia-free.
5. Sodium hydroxide, approximately 10 N. Dissolve 400 g. of NaOH in distilled water and dilute to 1 liter.

6. Standard 71.4 millimolar ammonium chloride solution. Used for checking the micro-Kjeldahl distillation procedure. Dissolve 0.3820 g. of NH_4Cl, analytical reagent grade, in distilled water and dilute to 100 ml. One ml. contains 1 mg. of nitrogen.
7. Acetate buffer, o.2 M, pH 5. Dissolve 27.22 g. of sodium acetate in water and make up to 1 liter. Add 427 ml. of 0.2 N acetic acid (standardized by titration against 0.1 N NaOH with phenolphthalein as an indicator).
8. Alizarin red solution, 0.1% in distilled water.
9. 0.01428 N (N/70) H_2SO_4. Prepare by diluting 14.28 ml. of 1 N acid to 1000 ml. with distilled water.
10. 0.01428 N (N/70) NaOH solution. Store in a plastic container of a paraffin-lined bottle. Protect against CO_2 by a soda lime tube. Standardize the solution daily by titration against 10 ml. portions of the 0.01428 N H_2SO_4, with the same pH and volume at the end point as is described below for titration of distilled ammonia.
11. Boiling chips, Nortons Alundum chips, No. 14, black.

PROCEDURE:

Into a Pyrex glass tube 22 - 25 m.m. x 200 m.m., measure 0.5 g. of K_2SO_4 and the sample of the unknown which contains 0.2 - 2.0 mg. of nitrogen (e.g., 0.1 ml. of urine or.2 - .3 gm. feces which has been homogenized previously with an appropriate amount of distilled water in a Waring Blendor. Then add 0.5 ml.

of the mercuric sulfate solution, 1.0 ml. of concentrated sulfuric acid, and 3 boiling chips. Boil mixture gently until the water is boiled off. Then adjust the heat so that the concentrated digest boils with very slight motion. This digestion continues for 30 minutes after the mixture has become entirely clear. About 2 minutes after completion of the digestion, but before the contents solidify, wash down the sides of the tube with 3 ml. of distilled water. Grease the lip of the digestion tube lightly with silicone grease to avoid loss during the quantitative transfer to the distillation apparatus. Steam out the still for 30 minutes before each series of distillations.

Transfer the contents of the digestion tube into the distillation flask with four portions of distilled water, approximately 2 ml. each. Add 0.2 g. of zinc dust to the third washing in the funnel of the distillation apparatus. Admit the mixture to the flask and follow with the fourth washing. Deliver 4 ml. of 10 N NaOH into the distillation flash and distill into 10.00 ml. of 0.01428 N H_2SO_4, with the tip of the condensor below the surface of the acid. Distill enough liquid to insure quantitative transfer of the ammonia to the standard acid. The volume of liquid which must be so transferred by distillation will vary from one apparatus to another, and should be predetermined for each distillation apparatus by trial runs with the standard 71.0 millimolar ammonium chloride solution.

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

CALCULATIONS:

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

PRESENTATION OF FINDINGS WITH DISCUSSION - NITROGEN

Included in this dissertation is a report on the analytical results compiled on urinary nitrogen pertaining to the 1969 investigation as well as nitrogen balance data on each of the eight 1969 bed rest subjects. The combination of hydroxyproline and nitrogen were chosen because of the relationship between these two metabolites.

The daily urinary nitrogen excretion data are recorded in Table IX in the Appendix. The urinary nitrogen excretion during four daily periods throughout Bed Rest are reported in Table X. Table XI contains the statistical comparisons of urinary nitrogen excretion between pairs of the different periods. The statistical comparisons on circadian rhythm patterns are shown in Table XII and Figures 14 and 15. The statistical data pertaining to exercise are recorded in Tables XIII, XIV, XV, and XVI, and in Figure 16. Table XVII contains the data on coefficient of correlation values between urinary hydroxyproline and urinary nitrogen excretion, while the nitrogen balance data are reported in Table XVIII.

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

The statistical data pertaining to urinary nitrogen excretions during the different periods of the study are recorded in Table XI in the Appendix.

SUBJECT 2A

As shown in Part A of Table XI, the amount of urinary nitrogen excreted in the urine by Subject 2A during the Bed Rest Period surpassed that excreted during both the Pre-Bed Rest and Post-Bed Rest Periods by a difference which was found to be highly significant in both cases ($P < 0.001$). A statistical comparison of the two ambulatory periods showed that the urinary nitrogen excretion reported during the Pre-Bed Rest Period was distinctly higher than the amount noted during the Post-Bed Rest Period ($P < 0.001$).

SUBJECT 6A

When the amount of urinary nitrogen excreted by this subject during the Bed Rest Period was compared with the amount excreted during the Pre-Bed Rest Period, no significant difference was noted. There was, however, a highly significant difference in the urinary nitrogen excretions reported for the periods of Bed Rest and Post-Bed Rest with the higher level being observed during the former period ($P < 0.001$). The urinary nitrogen level of the Pre-Bed Rest Period was determined to be significantly higher ($P < 0.05$) than that of the Post-Bed Rest Period. See Table XI, Part A.

SUBJECT 7A

When making a statistical comparison of the urinary nitrogen excretion by Subject 7A during Bed Rest with both the Pre-Bed Rest and Post-Bed Rest Periods, a highly significant increase was noted during the Bed Rest Period ($P < 0.001$). No significant difference was found in comparing the Pre-Bed Rest Period with the Post-Bed Rest Period. See Table IX, Part A.

SUBJECTS 2A, 6A, 7A

(Subjects Who Exercised Throughout the Bed Rest)

As shown in Table X, Part B, when the data for all subjects who exercised during Bed Rest were pooled together for statistical comparison, the amount of nitrogen excreted in the urine during the Bed Rest greatly surpassed the quantities reported both for the Pre-Bed Rest and Post-Bed Rest Periods by a difference which was highly significant ($P < 0.001$). The urinary excretion of nitrogen by this group of subjects during the Pre-Bed Rest Period was found to be significantly higher ($P < 0.01$) than during the Post-Bed Rest Period.

SUBJECT 4A

The excretion of urinary nitrogen by Subject 4A during the Bed Rest Period was considerably greater than during the Post-Bed Rest Period ($P < 0.001$). On the other hand, no statistical difference was found between the amounts of nitrogen excreted during the Bed Rest and Pre-Bed Rest Periods by this subject. It also was observed that the quantity of nitrogen excreted in the urine during the Pre-Bed Rest Period greatly surpassed the amount excreted during the Post-Bed Rest Period ($P < 0.001$). See Table XI, Part C.

SUBJECT 8A

During Bed Rest the urinary nitrogen excretion of Subject 8A increased significantly over the amount reported for the Pre-Bed Rest Period ($P < 0.05$). A highly significant difference was found between the Bed Rest and Post-Bed Rest Periods, with the higher excretion of nitrogen in the urine occurring during the Bed Rest Period ($P < 0.001$). It also was found that there was a highly significant difference between the urinary nitrogen excreted during the Pre-Bed Rest than the Post-Bed Rest Periods ($P < 0.001$). See Table XI, Part C.

SUBJECT 9A

Table XI, Part C, shows that, when the amount of urinary nitrogen excreted by this subject during the Bed Rest Period was compared with the amounts excreted during both the Pre-Bed Rest and Post-Bed Rest Periods, the excretion during the Bed Rest Period greatly surpassed that of the other two periods in both cases ($P < 0.001$). When the Pre-Bed Rest Period was compared with the Post-Bed Rest Period, it was found that the amount of urinary nitrogen excreted during the Pre-Bed Rest Period was distinctly higher ($P < 0.01$).

<u>SUBJECTS</u> <u>4A</u>, <u>8A</u>, <u>9A</u>

(Subjects Who Did Not Exercise During the Bed Rest)

An analysis of the pooled data for the three subjects who did not exercise showed that, during the Bed Rest Period, the amount of nitrogen excreted in the urine greatly surpassed the amounts excreted during both the Pre-Bed Rest and Post-Bed Rest Periods with the difference being highly significant in both cases ($P < 0.001$). It also was found that the quantity of nitrogen excreted in the urine by this group of subjects was appreciably greater during the Pre-Bed Rest Period than during the Post-Bed Rest Period ($P < 0.001$). See Table XI, Part D.

<u>SUBJECT</u> <u>3A</u>

(Subject Exercising First Half of the Bed Rest)

As shown in Table XI, Part E, the urinary nitrogen excretion by this subject during Bed Rest greatly surpassed the excretions both of the Pre-Bed Rest and Post-Bed Rest Periods with the difference being highly significant in both cases ($P < 0.001$). When the Pre-Bed Rest and Post-Bed Rest Periods were compared statistically, no significant difference was determined.

SUBJECT 1A

(Subject Exercising Last Half of the Study)

According to Table XI, Part E, this subject excreted a significantly higher amount of nitrogen in the urine during the Bed Rest Period than during the Pre-Bed Rest Period ($P < 0.05$). When the Bed Rest Period and the Post-Bed Rest Period were compared statistically, it was found that the level of urinary nitrogen during the Bed Rest Period greatly exceeded the amount reported for the Post-Bed Rest Period ($P < 0.001$). In comparing the levels reported for the Pre-Bed Rest and Post-Bed Rest Periods, a highly significant difference again was noted with a far greater amount of urinary nitrogen being excreted during the period of Pre-Bed Rest ($P < 0.001$).

ALL SUBJECTS

When statistical comparisons were made of the pooled data for all eight subjects, it was found that the highest level of urinary nitrogen excretion occurred during the Bed Rest Period of the study. When this period was compared both with the Pre-Bed Rest and Post-Bed Rest Periods, a highly significant difference was evident ($P < 0.001$). It further was observed that, during the Pre-Bed Rest Period the quantity of nitrogen excreted in the urine greatly surpasses the amount excreted during the Post-Bed Rest Period ($P < 0.001$).

CIRCADIAN PATTERN OF URINARY NITROGEN DURING BED REST PERIOD

In order to study the diurnal variation of nitrogen, the urine excreted at different times of the day 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, as has been noted. The statistical data concerning the excretion patterns are given in Table XII and in Figures 14 and 15.

SUBJECT 2A

Table XII, Part A, shows that the quantity of nitrogen excreted by this subject between 12 Noon and 8 P.M. was lower than that between 8 P.M. and 12 Midnight. The difference was highly significant ($P < 0.001$).

A statistical comparison of the period from 12 Noon to 8 P.M. with the period from 12 Midnight to 8 A.M. showed that this subject had an increase in the level of urinary nitrogen during the period from 12 Noon to 8 P.M., with the difference being highly significant ($P < 0.001$).

In comparing the period from 12 Noon to 8 P.M. with that of 8 A.M. to 12 Noon, a slight increase in the excretion of urinary nitrogen was noted during the period from 12 Noon to

8 P.M., although the difference between these periods was not significant.

When the period from 8 P.M. to 12 Midnight was compared with the period from 12 Midnight to 8 A.M., the higher excretion was found to occur between 8 P.M. and 12 Midnight with the difference highly significant ($P < 0.001$). It also was found that the urinary nitrogen excretion between 8 P.M. and 12 Midnight greatly surpassed that of the period from 8 A.M. to 12 Noon. Statistically, the difference was highly significant ($P < 0.001$). In comparing the period from 12 Midnight to 8 A.M. with the 8 A.M. to 12 Noon Period, it was noted that the excretion of nitrogen was distinctly higher ($P < 0.001$) during the period from 8 A.M. to 12 Noon.

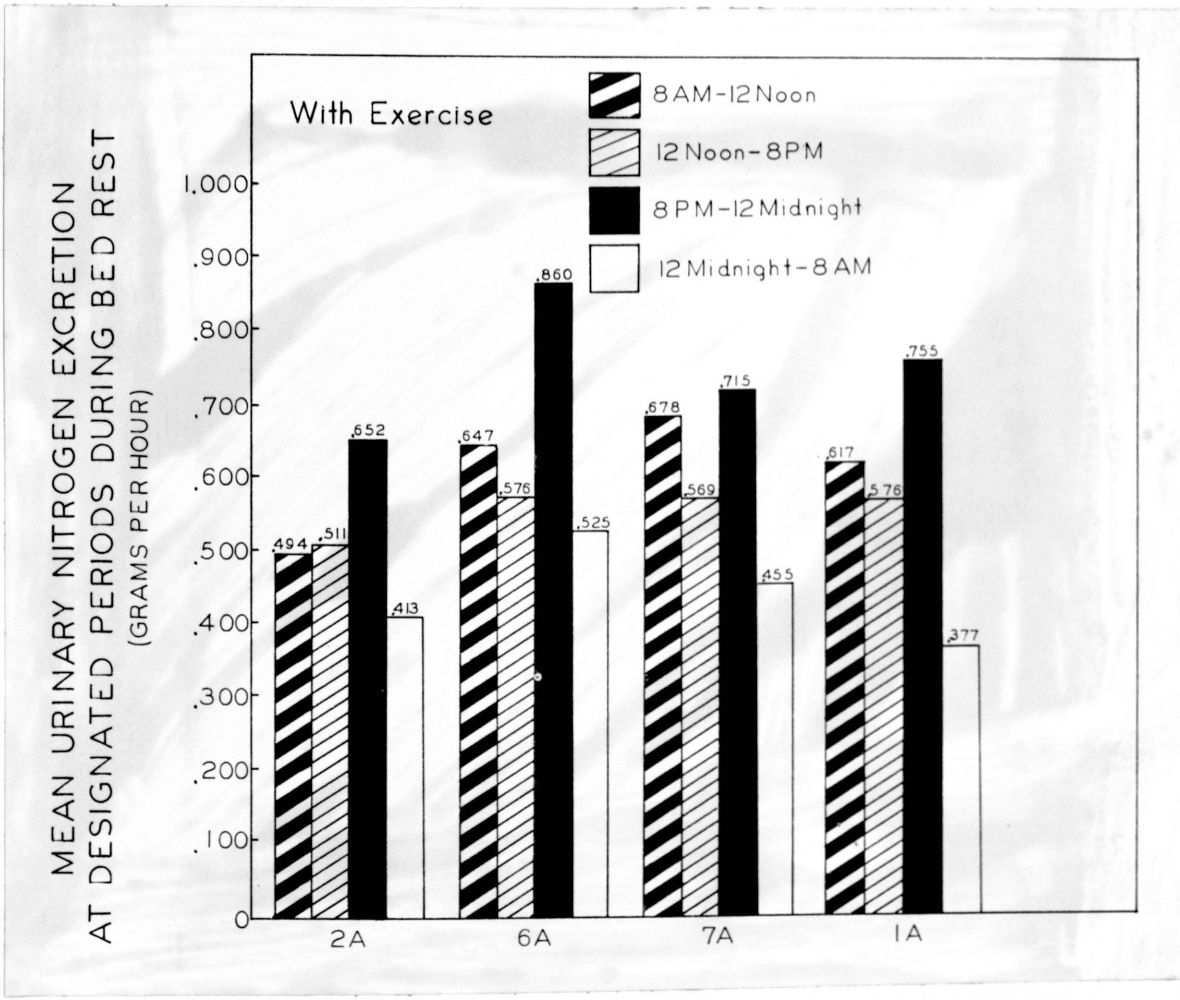

Figure 14. Circadian Rhythms shown for Mean Urinary Nitrogen for the Subjects Who Exercised. (Note: Subject 1A Exercised during the Last 28 days of the Study.)

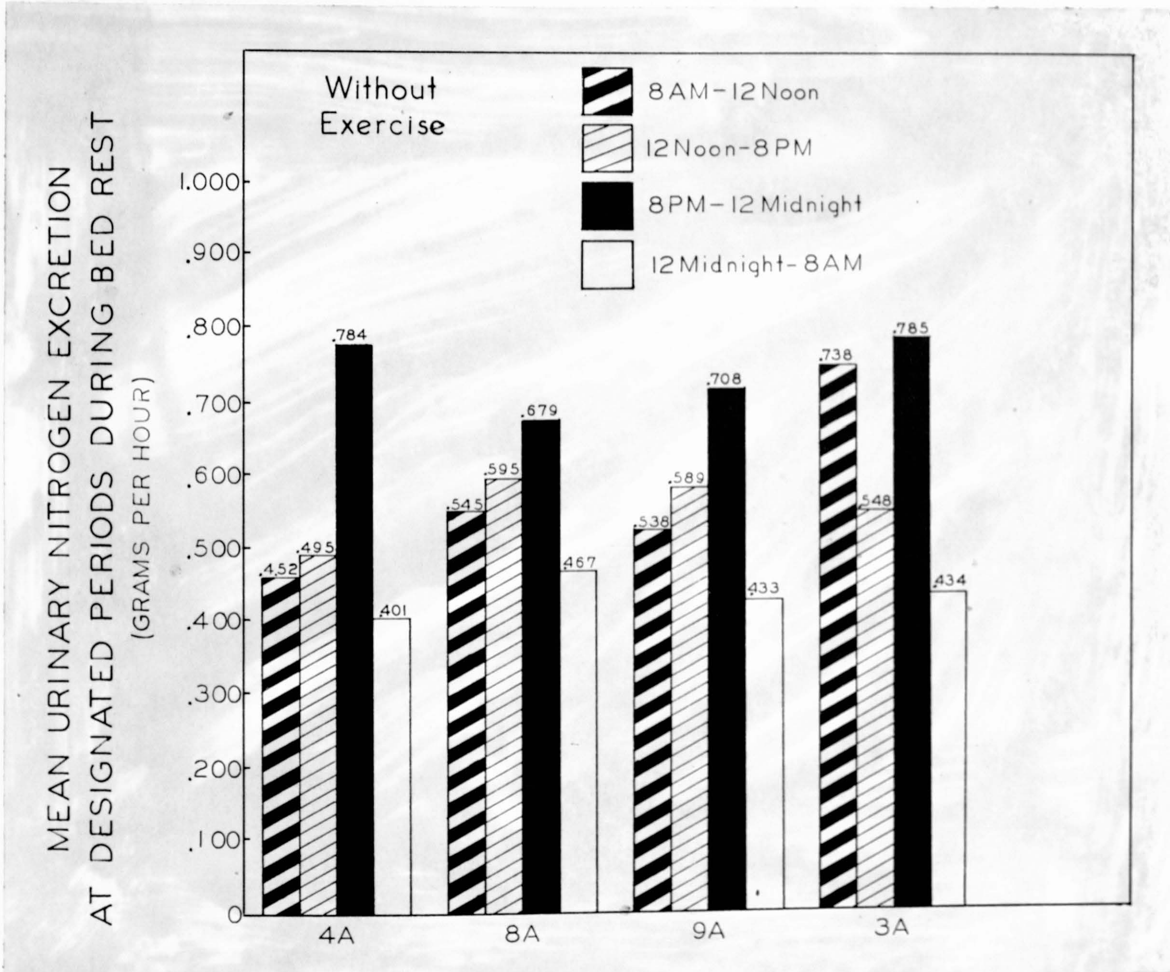

Figure 15. Circadium Rhythms shown for Mean Urinary Nitrogen for the Subjects Who did not Exercise. (Note: Subject 3A Exercised throughout the first 28 Days of The Bed Rest Period, but did not Exercise During the Last 28 Days, the Period Represented in This Graph.)

SUBJECT 6A

Table XII, Part B, shows in an analysis of the data pertaining to Subject 6A, that the amount of urinary nitrogen excreted between the Period 8 P.M. to 12 Midnight greatly surpassed the amount excreted from 12 Noon to 8 P.M. ($P < 0.001$).

In comparing the excretion of urinary nitrogen from 12 Noon to 8 P.M. with that from 12 Midnight to 8 A.M., it was found that the difference was not statistically significant. When comparing this same period, 12 Noon to 8 P.M., with the 8 A.M. to 12 Noon Period, it was found that, during the latter, a significantly higher amount of urinary nitrogen was excreted by this subject ($P < 0.05$).

A statistical comparison of the amount of urinary nitrogen excreted between 8 P.M. and 12 Midnight with that excreted between 12 Midnight and 8 A.M. showed that an appreciably greater quantity ($P < 0.001$) was reported for the period 8 P.M. to 12 Midnight. Similarly, in comparing the period 8 P.M. to 12 Midnight with the period 8 A.M. to 12 Noon, a higher excretion level was noted between 8 P.M. and 12 Midnight. The difference was found to be highly significant ($P < 0.001$).

The amount of urinary nitrogen excreted by this subject from 12 Midnight to 8 A.M. surpassed the amount excreted between 8 A.M. and 12 Noon by a highly significant difference ($P < 0.001$).

<u>SUBJECT</u> <u>7A</u>

Table XII, Part C, contains the daily periodical data for Subject 7A. When the amount of nitrogen excreted during the period from 8 P.M. to 12 Midnight was compared with the amount excreted from 12 Noon to 8 P.M., a higher amount was reported for the period 8 P.M. to 12 Midnight, which was found to be highly significant ($P < 0.001$).

The mean hourly value of urinary nitrogen excreted from 12 Noon to 8 P.M. was higher than the value reported for the 12 Midnight to 8 A.M. period. Statistically, the difference was highly significant ($P < 0.001$).

In comparing the period from 12 Noon to 8 P.M. with the period from 8 A.M. to 12 Noon, it was found that the slight increase in urinary nitrogen excretion which was observed during the period from 8 A.M. to 12 Noon was statistically significant ($P < 0.02$).

A highly significant difference was found between the amount of urinary nitrogen excreted by this subject from 8 P.M. to 12 Midnight with 12 Midnight to 8 A.M. with the greater excretion reported during the period from 8 P.M. to 12 Midnight ($P < 0.001$).

When a statistical comparison was made between the excretion levels of urinary nitrogen from 8 P.M. to 12 Midnight and from 8 A.M. to 12 Noon, no significant difference was determined.

The urinary nitrogen excreted by this subject during the period from 8 A.M. to 12 Noon surpassed the amount excreted between 12 Midnight and 8 A.M. by a difference which proved to be highly significant ($P < 0.001$).

<u>SUBJECTS</u> <u>2A</u>, <u>6A</u>, <u>7A</u>

(Subjects Who Exercised Throughout the Bed Rest)

When the data for the three subjects who exercised throughout the study were pooled together for comparative purposes, a highly statistical difference was found between the greater quantity of urinary nitrogen which appeared during the period from 8 P.M. to 12 Midnight as compared with that of the 12 Noon to 8 P.M. Period. See Table XII, Part D. Similarly, it was found that excretion between 12 Noon and 8 P.M. was higher than the excretion between 12 Midnight and 8 A.M. Again, the difference was found to be highly significant ($P < 0.001$).

In comparing the amount of nitrogen excreted during the 12 Noon to 8 P.M. Period with that of the 8 P.M. to 12 Noon Period, it was found that the level reported for the latter period surpassed the former by a difference which was somewhat superior ($P < 0.02$).

In comparing the amount of urinary nitrogen excreted by this subject during the 12 Midnight to 8 A.M. Period with the amount excreted from 8 P.M. to 12 Midnight, a highly significant difference was found, with the 8 P.M. to 12 Midnight urinary collection reflecting the highest level of nitrogen. The difference was highly significant ($P < 0.001$).

A higher urinary nitrogen level also was found to occur during the period from 8 P.M. to 12 Midnight as compared with the 8 A.M. to 12 Noon Period. The difference was found to be highly significant ($P < 0.001$). Similarly, a higher excretion of urinary nitrogen eas found during the 8 A.M. to 12 Noon Period as compared with the 12 Midnight to 8 A.M. Period. The difference was highly significant ($P < 0.001$).

SUBJECT 4A

As shown in Table XII, Part E, when pairs of the collection periods were compared as to the quantities of urinary nitrogen excreted by Subject 4A, it was found that a significantly higher amount was excreted during the period from 8 P.M. to 12 Midnight as compared with the period from 12 Noon to 8 P.M. ($P < 0.001$). In comparing the period 12 Noon to 8 P.M. with the period from 12 Midnight to 8 A.M., the highest level appeared during the period from 12 Noon to 8 P.M. Statistically, the difference was highly significant ($P < 0.001$). No statistical difference was noted in comparing the urinary nitrogen levels between 12 Noon and 8 P.M. and 8 A.M. and 12 Noon.

The amount of nitrogen excreted in the urine by Subject 4A between 8 P.M. and 12 Midnight greatly surpassed the excretions reported for the periods from 12 Midnight to 8 A.M. and 8 A.M. to 12 Noon. Both comparisons revealed a highly significant difference between the rates of urinary nitrogen excretion for the periods undergoing comparison ($P < 0.001$ in both cases).

The difference observed in the levels of urinary nitrogen excretion from 12 Midnight to 8 A.M. and from 8 A.M. to 12 Noon was determined to be barely significant ($P < 0.10$) with the higher level being observed in the period from 8 A.M. to 12 Noon.

SUBJECT 8A

Table XII, Part F, contains the statistical data pertaining to Subject 8A which is related to cirdadian rhythmicity. The slight increase noted in urinary nitrogen excretion during the period 8 P.M. to 12 Midnight as compared with the period from 12 Noon to 8 P.M. was determined to be slightly significant ($P < 0.10$). In comparing the period from 12 Noon to 8 P.M. with that from 12 Midnight to 8 A.M., the excretion of urinary nitrogen during the former period exceeded the excretion between 12 Noon and 8 P.M. by a difference which was highly significant ($P < 0.001$). No significant difference was found when a comparison was made between the 12 Noon to 8 P.M. Period and the 8 A.M. to 12 Noon Period.

The amount of urinary nitrogen excreted between 8 P.M. and 12 Midnight surpassed the amount excreted between 12 Midnight and 8 A.M. The difference was highly significant ($P < 0.001$). The increase in urinary nitrogen found during the period 8 P.M. to 12 Midnight also was statistically significant at the one per cent level of confidence, when compared with the excretion between 8 A.M. and 12 Noon ($P < 0.01$).

When the period of 12 Midnight to 8 A.M. was compared with the 8 A.M. to 12 Noon Period, it was found that the higher excretion of urinary nitrogen which was apparent in the period from 8 A.M. to 12 Noon showed a difference which was statistically significant ($P < 0.001$).

SUBJECT 9A

The amount of urinary nitrogen excreted by Subject 9A during the period from 8 P.M. to 12 Midnight was higher as compared with the 12 Noon to 8 P.M. Period, with a difference which was higher at the two per cent level ($P < 0.02$).

In comparing the periods 12 Noon to 8 P.M. and 12 Midnight to 8 A.M., the higher excretion of urinary nitrogen which was recorded during the 12 Noon to the 8 P.M. Period was found to be highly significant ($P < 0.001$). Although the amount of nitrogen excreted in the urine by this subject between 12 Noon and 8 P.M. slightly exceeded the amount between 8 A.M. and 12 Noon, the difference was not found to be statistically significant.

A higher excretion of nitrogen was detected during the 8 P.M. to 12 Midnight Period as compared both with the 12 Midnight to 8 A.M. and the 8 A.M. to 12 Noon Periods. In both cases, the difference was highly significant ($P < 0.001$).

In comparing the amount of urinary nitrogen excreted by Subject 9A during the period 8 A.M. to 12 Noon with the amount excreted during the 12 Midnight to 8 A.M. Period, a higher level of excretion appeared between 8 A.M. and 12 Noon. The difference was determined to be highly significant ($P < 0.001$).

SUBJECTS 4A, 8A, 9A

(Subjects Who Did Not Exercise During Bed Rest)

The statistical information compiled from the combined data of the three subjects who did not exercise throughout the study is recorded in Table XII, Part H.

A higher excretion of urinary nitrogen by this group of subjects was found during the period from 8 P.M. to 12 Midnight as compared with the periods 12 Noon to 8 P.M., 12 Midnight to 8 A.M., and 8 A.M. to 12 Noon. In the statistical comparisons for all these periods, the difference was found to be highly significant ($P < 0.001$).

A highly significant difference also was determined when comparing the mean hourly value of urinary nitrogen excretion between 12 Noon and 8 P.M. with the value for the 12 Midnight to 8 A.M. Period ($P < 0.001$). The higher value was observed between 12 Noon and 8 P.M. Further, it was observed that during the 8 A.M. to 12 Noon Period the excretion of urinary nitrogen surpassed that of the 12 Midnight to 8 A.M. period by a difference which was highly significant ($P < 0.001$).

SUBJECT 3A

(Subject Who Exercised First 28 Days of the Study)

This subject excreted the largest amount of urinary nitrogen over the period from 8 P.M. to 12 Midnight. The quantity excreted during this period markedly surpassed the amounts excreted during the periods from 12 Midnight to 8 A.M. and from 12 Noon to 8 P.M. The difference was highly significant in both cases ($P < 0.001$). When this same period, 8 P.M. to 12 Midnight, was compared with the period from 8 A.M. to 12 Noon, no significant difference was noted in the amount of urinary nitrogen excreted by this subject during these two collection periods.

It also was observed that the amount of nitrogen excreted in the urine by Subject 3A between 8 A.M. and 12 Noon greatly surpassed the amounts excreted from 12 Noon to 8 P.M. and 12 Midnight to 8 A.M. ($P < 0.001$). A highly significant difference ($P < 0.001$) also was found in comparing the 12 Noon to 8 P.M. Period with the 12 Midnight to 8 A.M. Period with the higher mean hourly value of urinary nitrogen excretion being reported for the 12 Noon to 8 P.M. Period.

SUBJECT 1A

(Subject Who Exercised Second Half of the Study)

The analytical data for Subject 1A are shown in Table XII, Part J. This subject excreted the greatest amount of urinary nitrogen during the period from 8 P.M. to 12 Midnight. When the level of nitrogen excreted in the urine over the period 8 P.M. to 12 Midnight was compared with the amounts reported for the periods 12 Noon to 8 P.M. and 12 Midnight to 8 A.M., the differences were found to be highly significant ($P < 0.001$).

The higher excretion of urinary nitrogen which was apparent during the period from 12 Noon to 8 P.M. as compared with the Period from 12 Midnight to 8 A.M. was determined to surpass the latter by a highly significant difference ($P < 0.001$). In comparing the same period, 12 Noon to 8 P.M., with the period from 8 A.M. to 12 Noon, however, the difference did not prove to be significant.

The higher amount of urinary nitrogen excreted by this subject during the period 8 P.M. to 12 Midnight as compared with the period 8 A.M. to 12 Noon was statistically significant ($P < 0.02$). A higher excretion of urinary nitrogen by this subject also was observed to occur during the period from 8 A.M. to 12 Noon as compared with the 12 Midnight to 8 A.M. Period. The difference was highly significant ($P < 0.001$).

ALL SUBJECTS

An analysis of the pooled data for all eight subjects showed that, among the four daily collection periods the highest level of urinary nitrogen excretion occurred during the period from 8 P.M. to 12 Midnight. The higher level of nitrogen excreted in the urine during this period was distinctly significant, when compared with the levels observed in the periods 12 Noon to 8 P.M., 12 Midnight to 8 A.M., and 8 A.M. to 12 Noon ($P < 0.001$).

During the period from 12 Noon to 8 P.M., the amount of nitrogen excreted in the urine by all subjects greatly surpassed the amount recorded for the period from 12 Midnight to 8 A.M. by a difference which was highly significant ($P < 0.001$). In comparing the urinary nitrogen excretion between 12 Noon and 8 P.M. with that from 8 A.M. to 12 Noon, it was noted that the excretion between 8 A.M. and 12 Noon was somewhat higher ($P < 0.05$).

When the higher amount of urinary nitrogen which was excreted between 8 A.M. and 12 Noon was compared with that excreted between 12 Midnight and 8 A.M., the former surpassed the latter period by a difference which was found to be highly significant ($P < 0.001$).

For all subjects combined, the lowest urinary nitrogen values were recorded during the period from 12 Midnight to

8 A.M. while the peak level of excretion was attained between 8 P.M. and 12 Midnight.

COMPARISON OF URINARY NITROGEN EXCRETION DURING BED REST AS INFLUENCED BY EXERCISE

The statistical information related to the effects of exercise on the urinary excretion of nitrogen is recorded in Tables XIII, XIV, XV, and XVI (Appendix), and in Figure 16.

SUBJECT 3A

(Subject Who Exercised During the First 28 Days of the Study)

According to Table XIII, this subject excreted slightly more nitrogen in the urine while he was participating in exercise. The difference, however was not found to be statistically significant. The statistical comparison between exercise and no exercise for Subject 1A followed this same pattern.

The statistical comparisons of the urinary nitrogen excretion at different periods of the day by Subject 3A are found in Table XIV, Part A. For the periods 12 Noon to 8 P.M., 8 P.M. to 12 Midnight, and 8 A.M. to 12 Noon, no significant changes were noted to occur as a result of exercising or not exercising. A slight increase ($P < 0.10$) was reflected in the urinary nitrogen level from 12 Midnight to 8 A.M. when this subject engaged in exercise.

SUBJECT 1A

(Subject Who Exercised During the Second Half of the Study)

During the second half of the Bed Rest Period when this subject participated in the exercise program, there was a slight elevation in urinary nitrogen excretion. Table XIII, however, shows that this increase was not found to be significantly higher than the amount of nitrogen excreted by this subject during the period when no exercise was taken.

According to Table XIV, Part B, no significant difference was detected in the amount of nitrogen excreted by this subject during the collection periods 12 Noon to 8 P.M. and 8 P.M. to 12 Midnight which could be related to participation in exercise. For the period 12 Midnight to 8 A.M. there was a slightly significant decrease ($P < 0.10$) in the amount of urinary nitrogen excreted by this subject during the time when this subject did not exercise. For the period 8 A.M. to 12 Noon, however, an elevation in urinary nitrogen excretion was observed to occur when this subject participated in exercise during the day with the difference being slightly significant ($P < 0.10$).

GROUP OF SUBJECTS WHO EXERCISED DAILY (2A, 6A, 7A) VS. GROUP WHO DID NOT EXERCISE (4A, 8A, 9A)

As shown in Table XV, when 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 of the study, it was observed that the group of subjects who exercised excreted a higher quantity of nitrogen in the urine than did the group of subjects who did not exercise. The difference was determined to be statistically significant ($P < 0.02$). The mean levels of 24-hour urinary nitrogen excretion by the two groups during Bed Rest are depicted graphically in Figure 16.

When the urinary nitrogen levels for the same times of the day were compared between the two exercise groups, no statistical changes were found to exist in the periods from 12 Noon to 8 P.M. and 8 P.M. to 12 Midnight. A statistical difference of ($P < 0.05$) was recorded for the period of 12 Midnight to 8 A.M. with a greater quantity of nitrogen being excreted in the urine by the group of subjects who exercised.

There was a highly significant difference ($P < 0.001$) between the amounts of urinary nitrogen reported for the two groups between 8 A.M. and 12 Noon with the level for the exercise group again exceeding the level for the group not exercising. See Table XVI.

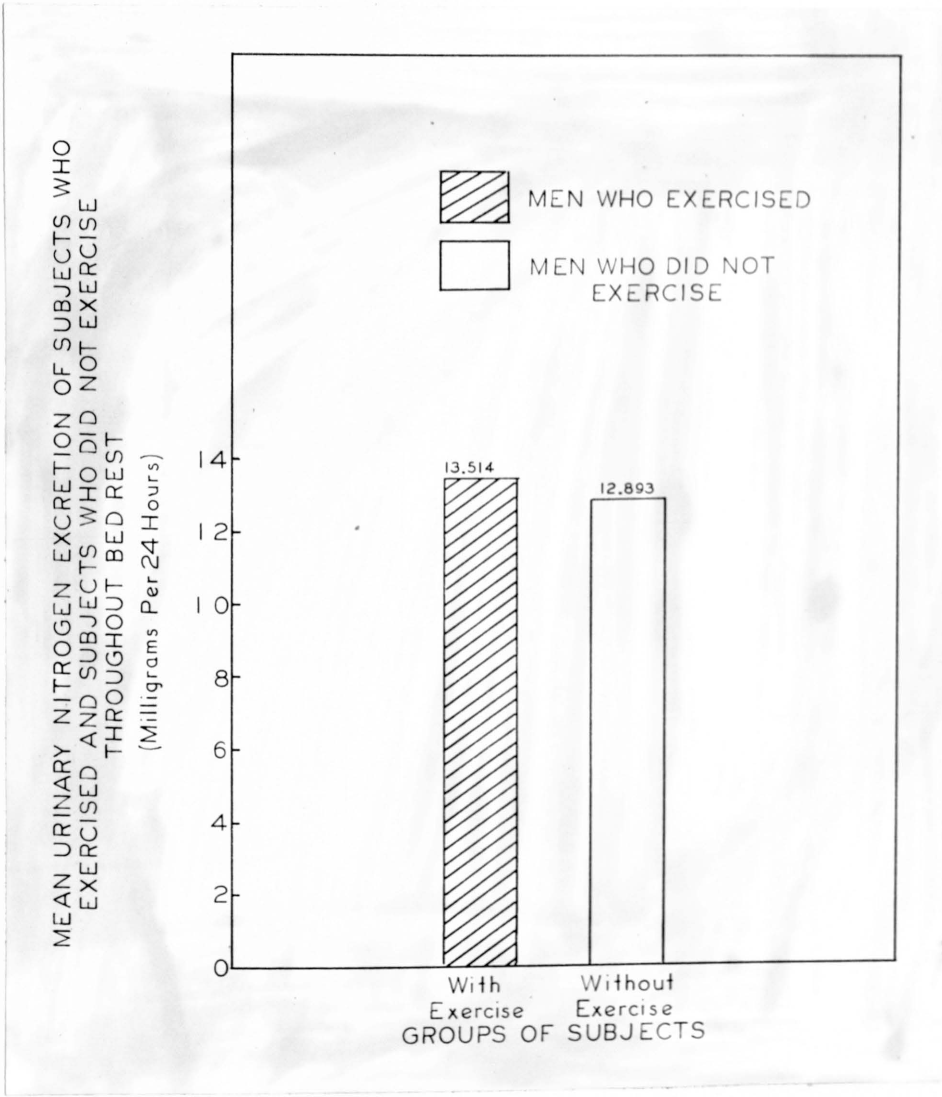

Figure 16. Mean Urinary Nitrogen Excretion by Subjects Who Exercised and Those Who did not Exercise.

CORRELATION BETWEEN URINARY HYDROXYPROLINE AND URINARY NITROGEN EXCRETIONS AND NITROGEN BALANCE DATA DURING THE BED REST PERIOD OF THE STUDY

It was of interest to this author to determine whether or not there was any correlation between the amounts of urinary hydroxyproline and urinary nitrogen excreted by the subjects participating in this study. Urinary nitrogen was used for the comparisons, as it generally is felt to be a valid index, since the urine contains the major portion of the total nitrogen excretion.

According to Chow et al (2) fecal nitrogen is insignificant as compared to total intake of nitrogen. Montgomery (3) stated that in a former study conducted at the T.W.U. Research Institute it was determined that 96.6% of the total nitrogen excreted by human subjects was found in the urine with the feces containing only 3.4% of the total nitrogen.

Further, it has been reported that the stress of immobilization causes an increase in urinary nitrogen with a corresponding decrease in fecal nitrogen. Scrimshaw (4) (5) has conducted studies on university men undergoing examinations in which he has observed the phenomenon of increased urinary nitrogen excretion with a decrease in nitrogen balance. In summarizing former studies conducted at the T.W.U. Research

Institute, Chung (6), Mayer (7), and Montgomery (3), have observed that, when individuals are immobilized there is an increase in the nitrogen content of the urine and a decrease in nitrogen excreted in the feces.

The correlation coefficients between hydroxyproline and nitrogen are given in Table XVII in the Appendix and the nitrogen balance data are presented in Table XVIII.

SUBJECT 1A

Table XVII indicates that, during the Bed Rest Period of the study there was no significant correlation between the amounts of hydroxyproline and nitrogen which were excreted into the urine by Subject 1A. This subject was maintained in positive nitrogen balance (+ 2.2) for the overall 56-day period. See Table XVIII, Part A.

SUBJECT 2A

As shown in Table XVII, a significant relationship between the urinary excretions of hydroxyproline and nitrogen by this subject. The "r" value was 0.3922 and the level of significance was ($P < 0.01$). Positive nitrogen balance (+ 2.9) was reported for Subject 2A for the Bed Rest Period of the study. See Table XVIII, Part B.

SUBJECT 3A

When the statistical comparison was made between the amounts of hydroxyproline and nitrogen excreted in the urine by Subject 3A, no significant correlation was determined. See Table XVII. Table XVIII, Part C, shows that this subject had a mean positive nitrogen balance (+ 1.1) for the Bed Rest Period of the study.

SUBJECT 4A

A significant relationship was found between the urinary hydroxyproline excretion and the urinary nitrogen excretion of Subject 4A during Bed Rest. Table XVII shows that a correlation coefficient of 0.4024 for these two factors had a high level of significance ($P < 0.01$). Table XVIII, Part D, shows that this subject had a mean nitrogen balance of + 2.2.

SUBJECT 6A

Table XVII indicates that there was a positive correlation between the excretion of hydroxyproline and nitrogen in the urine by Subject 6A. The correlation factor (0.4099) was highly significant ($P < 0.01$). Table XVIII, Part E, reports that this subject was maintained in positive nitrogen balance (+ 1.3) throughout the 56-day Bed Rest Period.

SUBJECT 7A

A comparison of the urinary hydroxyproline and nitrogen excretions by Subject 7A revealed that there was a positive correlation between these two metabolites. The "r" value of 0.3518 was determined to have a level of significance of ($P < 0.02$), as shown in Table XVII. The nitrogen balance data for this subject which is reported in Table XVIII, Part F, gives + 2.3 as the mean positive balance for Subject 7A during Bed Rest.

SUBJECT 8A

According to Table XVII, a positive correlation existed between the amounts of urinary hydroxyproline and urinary nitrogen reported for this subject during the 56-day Bed Rest. The correlation factor of 0.4778 was determined to be highly significant ($P < 0.001$). For the same period of the study, this subject had a mean overall nitrogen balance of + 2.4 as shown in Table XVIII, Part G.

SUBJECT 9A

During the Bed Rest Period, as shown in Table XVII, there was a positive correlation between the amount of hydroxyproline and nitrogen excreted in the urine by Subject 9A. The correlation factor was 0.4413 and the level of significance was ($P < 0.01$). Table XVIII, Part H, contains the nitrogen balance data for this subject which indicates that a mean positive balance of + 3.0 was maintained throughout Bed Rest.

ALL SUBJECTS

When the data for all eight subjects were pooled for comparison, a correlation coefficient of 0.4266 was found between the urinary excretion of hydroxyproline and the urinary excretion of nitrogen. This was found to be highly significant ($P < 0.001$) as shown in Table XVII. It is of note that all eight subjects were maintained in overall positive nitrogen balance during the 56-day period of Bed Rest. Montgomery(3) and Sampley (8) have reported that, in previous studies conducted at the T.W.U. Research Institute all subjects were maintained in positive nitrogen balance during Bed Rest Periods. Undoubtedly this can be attributed partially to the high protein diet which is fed these subjects during immobilization.

SUMMARY AND CONCLUSIONS - NITROGEN

The second portion of this dissertation is concerned with the nitrogen metabolism of the eight young men participating in this study. All urinary and fecal samples were analyzed for total nitrogen using the method described by Archibald (1).

Total 24-hour urinary nitrogen values for all eight subjects were highest during the Bed Rest Period of the study. In comparing the mean daily excretion for this period, both with the means for the Pre-Bed Rest and Post-Bed Rest Periods, a highly significant difference was found ($P < 0.001$). The mean daily excretion of nitrogen for all subjects during the Pre-Bed Rest Period was observed to surpass that of the Post-Bed Rest Period with a higher significant difference ($P < 0.001$). Apparently the stress of immobilization induces an increase in the excretion of urinary nitrogen.

During the Bed Rest Period of the study an investigation was conducted into the existence of a circadian pattern in the excretion of urinary nitrogen. Samples were collected four times daily for analytical purposes. In all eight subjects the maximum level of urinary nitrogen excretion occurred during the period from 8 P.M. to 12 Midnight, while the minimum level of nitrogen in the urine was reported between 12 Midnight and 8 A.M. In comparing the pooled data statistically for all subjects, both the low (12 Midnight to 8 A.M.) and the high (8 P.M. to 12 Midnight) levels of excretion were determined to be significantly higher than the amounts excreted during the other periods of the day ($P < 0.001$). It appears evident that the excretion of nitrogen in the urine follows a circadian pattern.

The effect of exercise on the excretion of nitrogen was followed by dividing the subjects into groups of those which exercised and those who did not exercise during the period of Bed Rest. Two subjects each exercised only one-half of the Bed Rest Period. A slightly significant increase was noted in the amount of nitrogen excreted in the urine by the group of subjects which exercised as compared to the group of subjects which did not exercise ($P < 0.02$). No significance was determined between the amounts of nitrogen excreted in the urine by the two subjects who participated in exercise during 28 days of the study, although both of these subjects excreted slightly more urinary nitrogen while engaged in the exercise program. The results of this study indicate that more nitrogen is excreted in the urine when individuals accelerate their activity.

The urinary hydroxyproline and urinary nitrogen excretions of the individual subjects during the period of bed rest were compared in order to determine whether or not any correlation existed between the two factors. In two of the subjects no significant relationship was found between the amounts of these metabolites excreted in the urine. In six of the subjects, however, a positive correlation coefficient was established between the excretion of hydroxyproline and nitrogen in the urine. A comparison of the pooled data for all eight subjects proved to be highly significant ($P < 0.001$). The results of this study suggest that a definite positive correlation exists between the urinary excretion of hydroxyproline and the urinary excretion of nitrogen.

All eight subjects were observed to remain in overall positive nitrogen balance throughout the 56-day Bed Rest Period of the study. It should be mentioned that, throughout this period of the investigation, these subjects were given a high protein diet which averaged at least 100 grams protein daily.

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APPENDIX

TABLE I

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART A. SUBJECT 1A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	27.80	1	27.82	29	60.92	1	15.67
2	24.90	2	69.83	30	59.07	2	11.68
3	22.42	3	59.23	31	63.42	3	14.51
4	33.52	4	54.34	32	50.51	4	22.16
5	94.47	5	66.19	33	50.18	5	14.59
6	55.08	6	90.73	34	59.38	6	-----
7	20.02	7	63.36	35	50.42	7	14.84
8	42.51	8	41.95	36	42.76	8	10.06
9	32.94	9	55.05	37	36.64	9	21.46
10	76.34	10	42.28	38	76.70	10	21.46
11	51.26	11	41.38	39	46.18	11	20.09
12	37.02	12	60.65	40	45.85	12	6.01
13	34.49	13	63.03	41	45.39	13	-----
14	83.97	14	43.97	42	53.32		
15	59.73	15	65.22	43	59.84	Mean	14.89
16	-----	16	75.37	44	53.73		
		17	57.12	45	39.71		
Mean	47.76	18	45.64	46	54.45		
		19	52.54	47	36.42		
		20	50.76	48	80.19		
		21	56.56	49	52.00		
		22	50.03	50	42.55		
		23	50.17	51	49.71		
		24	67.10	52	45.75		
		25	42.82	53	59.59		
		26	46.54	54	43.03		
		27	45.60	55	60.13		
		28	51.38	56	39.63		
				Mean	53.03		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART B SUBJECT 2A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	18.20	1	35.87	29	33.79	1	7.81
2	22.27	2	45.03	30	51.40	2	10.09
3	14.82	3	80.83	31	42.35	3	19.20
4	14.50	4	62.57	32	30.18	4	22.26
5	21.51	5	45.08	33	35.78	5	7.55
6	56.11	6	56.21	34	50.92	6	10.21
7	30.40	7	30.45	35	38.53	7	9.18
8	15.25	8	38.84	36	24.15	8	19.18
9	22.32	9	47.80	37	51.39	9	23.39
10	29.95	10	33.04	38	31.36	10	26.11
11	31.87	11	51.07	39	29.66	11	14.80
12	24.11	12	37.60	40	22.65	12	8.93
13	24.38	13	45.30	41	28.45	13	13.86
14	23.39	14	31.32	42	55.02		
15	39.45	15	43.33	43	51.40	Mean	14.52
16	32.52	16	36.27	44	50.47		
		17	27.25	45	44.52		
Mean	26.31	18	36.15	46	33.11		
		19	35.86	47	44.35		
		20	37.66	48	40.50		
		21	43.63	49	41.50		
		22	31.99	50	41.11		
		23	35.45	51	41.79		
		24	38.87	52	36.78		
		25	38.48	53	39.71		
		26	30.52	54	56.21		
		27	52.00	55	44.58		
		28	47.03	56	13.19		
				Mean	40.70		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART C SUBJECT 3A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	21.54	1	39.62	29	68.70	1	26.50
2	24.81	2	58.57	30	55.39	2	21.98
3	26.28	3	56.43	31	66.73	3	19.95
4	33.26	4	64.92	32	51.94	4	26.86
5	30.14	5	59.28	33	53.94	5	47.39
6	55.90	6	67.33	34	55.86	6	30.82
7	38.10	7	51.83	35	77.13	7	32.01
8	35.99	8	63.75	36	53.25	8	36.70
9	97.16	9	48.16	37	66.94	9	52.95
10	27.57	10	50.05	38	54.73	10	43.36
11	16.61	11	43.34	39	94.76	11	28.32
12	61.97	12	51.04	40	80.72	12	25.96
13	69.42	13	76.99	41	60.20	13	52.67
14	40.82	14	61.94	42	67.28		
15	36.29	15	50.73	43	89.73	Mean	34.27
16	40.14	16	60.98	44	81.62		
		17	42.49	45	95.60		
Mean	41.00	18	31.20	46	73.14		
		19	39.63	47	64.49		
		20	28.28	48	72.58		
		21	41.10	49	44.37		
		22	60.27	50	105.03		
		23	54.98	51	88.55		
		24	39.15	52	57.39		
		25	36.39	53	79.71		
		26	42.10	54	69.23		
		27	88.16	55	89.06		
		28	39.67	56	45.58		
				Mean	60.93		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART D. SUBJECT 4A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	19.87	1	21.23	29	47.54	1	21.59
2	25.03	2	26.23	30	36.73	2	16.82
3	29.58	3	34.47	31	35.79	3	13.68
4	22.31	4	31.10	32	31.46	4	35.63
5	20.68	5	22.53	33	50.62	5	18.68
6	24.32	6	25.00	34	38.43	6	15.44
7	23.53	7	49.31	35	45.34	7	21.98
8	28.85	8	42.01	36	50.37	8	11.98
9	20.36	9	42.90	37	50.00	9	33.99
10	22.90	10	42.81	38	35.71	10	24.64
11	12.82	11	53.47	39	73.83	11	16.39
12	34.41	12	47.97	40	43.62	12	23.66
13	47.53	13	54.74	41	67.40	13	9.45
14	29.67	14	24.50	42	59.76		
15	26.74	15	51.36	43	69.41	Mean	19.37
16	15.99	16	32.37	44	57.69		
		17	41.21	45	57.69		
Mean	25.29	18	48.01	46	54.64		
		19	42.80	47	53.54		
		20	40.30	48	45.65		
		21	33.17	49	63.24		
		22	51.28	50	45.13		
		23	41.41	51	44.31		
		24	32.03	52	28.72		
		25	67.21	53	40.71		
		26	47.45	54	37.32		
		27	60.46	55	37.70		
		28	47.48	56	26.67		
				Mean	44.36		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION
(MILLIGRAMS 24 HOURS)

PART E. SUBJECT 6A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	42.37	1	83.27	29	88.29	1	7.81
2	36.00	2	85.50	30	87.35	2	23.04
3	22.13	3	80.86	31	62.76	3	45.14
4	23.61	4	69.79	32	50.85	4	42.81
5	78.81	5	99.94	33	66.97	5	33.93
6	125.52	6	42.17	34	80.17	6	72.86
7	111.21	7	33.22	35	42.23	7	40.83
8	70.72	8	67.37	36	46.48	8	21.43
9	73.10	9	87.46	37	98.38	9	35.63
10	136.00	10	61.22	38	52.85	10	26.28
11	41.74	11	88.31	39	59.00	11	23.75
12	36.36	12	51.03	40	72.20	12	20.07
13	37.02	13	65.80	41	59.57	13	36.86
14	32.15	14	77.12	42	58.52		
15	45.90	15	49.70	43	61.78	Mean	32.04
16	33.91	16	61.70	44	59.13		
		17	70.96	45	72.61		
Mean	59.16	18	64.72	46	65.93		
		19	31.96	47	102.69		
		20	51.20	48	41.08		
		21	47.41	49	86.42		
		22	57.87	50	64.03		
		23	60.55	51	68.61		
		24	37.17	52	51.42		
		25	44.63	53	72.05		
		26	56.27	54	59.11		
		27	57.90	55	56.25		
		28	54.46	56	35.38		
				Mean	63.62		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART F. SUBJECT 7A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1		1	27.81	29	53.62	1	18.07
2	19.00	2	62.50	30	62.18	2	24.93
3	26.29	3	52.69	31	46.50	3	49.54
4	20.04	4	40.08	32	39.35	4	33.94
5	22.23	5	44.05	33	74.13	5	24.98
6	37.25	6	61.69	34	45.03	6	34.20
7	17.86	7	43.52	35	73.19	7	14.24
8	22.70	8	46.40	36	54.68	8	12.60
9	30.33	9	50.16	37	62.43	9	23.26
10	39.70	10	59.92	38	45.48	10	17.49
11	44.69	11	57.19	39	41.00	11	16.55
12	32.38	12	46.48	40	57.20	12	14.67
13	36.49	13	64.58	41	45.49	13	18.31
14	24.96	14	30.24	42	79.94		
15	22.76	15	59.66	43	32.23	Mean	22.34
16	30.30	16	39.90	44	36.55		
		17	58.53	45	36.22		
Mean	28.45	18	53.37	46	33.26		
		19	39.55	47	44.62		
		20	40.39	48	46.88		
		21	56.04	49	57.16		
		22	54.70	50	56.37		
		23	53.42	51	31.45		
		24	44.52	52	53.27		
		25	49.98	53	52.71		
		26	50.63	54	51.14		
		27	60.64	55	40.42		
		28	54.86	56	48.13		
				Mean	50.07		

TABLE I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART G. SUBJECT 8A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	22.46	1	59.87	29	66.68	1	28.83
2	24.58	2	57.71	30	56.93	2	17.50
3	27.01	3	48.19	31	39.80	3	16.27
4	23.13	4	40.43	32	52.69	4	23.21
5	62.29	5	66.27	33	58.27	5	25.71
6	48.40	6	59.42	34	70.43	6	22.38
7	40.13	7	64.96	35	58.66	7	67.44
8	30.99	8	26.15	36	22.62	8	13.36
9	29.45	9	46.79	37	57.66	9	29.75
10	26.66	10	44.00	38	69.74	10	24.15
11	69.84	11	42.93	39	39.51	11	15.88
12	86.25	12	41.63	40	60.18	12	18.29
13	39.44	13	89.17	41	49.68	13	10.86
14	31.62	14	48.43	42	47.81		
15	24.31	15	80.01	43	27.87	Mean	22.99
16	20.44	16	37.54	44	35.21		
		17	52.53	45	27.63		
Mean	37.94	18	98.57	46	54.77		
		19	46.58	47	48.78		
		20	67.04	48	50.57		
		21	48.00	49	32.72		
		22	46.15	50	33.66		
		23	52.44	51	31.76		
		24	57.29	52	37.82		
		25	57.81	53	44.12		
		26	40.47	54	32.52		
		27	57.70	55	23.40		
		28	42.14	56	32.59		
				Mean	49.72		

T A B L E I, (Continued)

URINARY HYDROXYPROLINE EXCRETION

(MILLIGRAMS 24 HOURS)

PART H. SUBJECT 9A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr	Day	Mg/24 hr
1	24.38	1	18.32	29	101.32	1	26.49
2	19.21	2	37.17	30	73.05	2	34.91
3	16.65	3	46.88	31	56.25	3	-- --
4	12.05	4	52.71	32	54.42	4	29.87
5	14.42	5	55.85	33	48.89	5	8.05
6	20.82	6	36.51	34	50.74	6	25.97
7	54.49	7	35.91	35	54.95	7	26.19
8	48.70	8	47.79	36	42.06	8	9.57
9	71.09	9	65.17	37	64.40	9	28.63
10	74.00	10	39.75	38	50.95	10	29.99
11	39.64	11	54.92	39	49.14	11	8.60
12	32.83	12	54.53	40	67.89	12	10.89
13	24.03	13	53.47	41	46.47	13	22.67
14	47.26	14	49.96	42	60.78		
15	61.87	15	46.37	43	63.60	Mean	20.73
16	59.36	16	49.10	44	75.36		
		17	60.13	45	43.44		
Mean	38.80	18	39.92	46	100.14		
		19	54.40	47	66.22		
		20	43.45	48	47.45		
		21	45.96	49	78.39		
		22	64.51	50	54.53		
		23	45.64	51	55.91		
		24	36.91	52	48.33		
		25	35.54	53	45.66		
		26	49.36	54	46.50		
		27	78.84	55	46.15		
		28	44.67	56	36.05		
				Mean	54.87		

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(MILLIGRAMS PER HOUR)

PART A. SUBJECT 1A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr	Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	1.06	1.06	1.06	1.62	29	2.55	4.54	2.04	1.52
2	4.36	3.90	0.88	3.08	30	2.59	4.04	1.85	1.85
3	3.75	2.09	1.90	1.44	31	2.85	2.18	0.75	6.48
4	2.46	2.19	1.36	3.77	32	1.32	4.86	1.56	2.01
5	3.89	3.71	1.89	1.27	33	2.37	3.58	1.41	1.41
6	7.36	3.32	1.65	1.35	34	2.65	2.88	2.22	2.22
7	4.14	1.92	1.76	2.12	35	2.75	3.30	1.27	1.27
8	1.35	2.26	1.69	2.15	36	1.27	4.24	1.51	0.90
9	2.15	4.16	1.75	1.82	37	2.10	2.49	0.91	0.91
10	1.40	1.96	1.94	1.92	38	1.63	9.12	2.26	2.26
11	1.93	2.24	1.47	1.32	39	2.73	1.52	1.60	1.38
12	2.88	2.01	1.75	3.89	40	2.56	1.08	0.65	3.97
13	2.36	4.21	2.26	2.31	41	2.34	2.60	0.69	2.70
14	1.26	3.50	1.37	2.25	42	1.87	4.16	0.91	3.61
15	1.18	7.28	1.38	3.91	43	2.04	2.20	1.75	5.19
16	2.54	6.66	2.02	3.10	44	1.37	5.54	1.76	1.38
17	2.54	3.07	1.79	2.55	45	1.38	4.00	0.69	2.03
18	2.25	1.87	1.59	1.86	46	1.87	4.70	0.96	3.25
19	2.68	4.25	1.18	1.18	47	2.00	1.85	0.75	1.73
20	1.39	2.18	3.13	1.48	48	1.59	5.67	1.52	8.16
21	2.37	4.35	1.63	1.80	49	2.98	3.90	0.66	1.83
22	2.89	2.40	1.45	1.43	50	1.57	2.81	1.03	2.62
23	2.41	1.86	1.11	3.63	51	2.04	1.07	0.72	5.39
24	1.33	3.52	1.23	5.63	52	1.14	2.50	2.06	2.55
25	1.95	2.28	1.72	1.35	53	3.97	1.92	1.68	1.68
26	1.99	3.13	1.44	1.64	54	1.70	2.72	1.52	1.61
27	2.13	3.89	1.05	1.15	55	1.33	4.76	1.01	5.59
28	1.55	5.65	1.36	1.36	56	2.41	1.27	1.27	1.27
					Mean	2.30	3.33	1.46	2.48

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST (MILLIGRAMS PER HOUR)

PART B. SUBJECT 2A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night - 8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night 8 A.M.	8 A.M.-12 Noon
Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr	Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	0.67	2.22	2.22	0.97	29	2.40	1.34	0.50	1.29
2	3.38	1.72	0.76	1.26	30	3.37	2.55	1.25	1.06
3	6.05	2.75	0.90	3.57	31	2.11	2.13	0.73	2.77
4	4.26	2.66	0.77	2.94	32	1.08	1.87	1.45	0.61
5	3.12	1.77	0.92	1.42	33	1.77	2.16	1.03	1.20
6	2.75	5.02	1.12	1.31	34	2.72	3.29	0.93	2.14
7	0.82	1.91	0.77	2.54	35	1.30	1.69	2.27	3.24
8	1.09	3.95	0.89	1.82	36	0.82	1.51	1.04	0.82
9	3.00	3.10	1.00	0.86	37	3.14	2.77	1.12	1.55
10	1.10	2.10	0.98	2.02	38	1.90	1.97	0.43	1.21
11	3.26	2.87	1.12	1.13	39	1.80	1.79	0.51	1.02
12	1.75	2.78	0.89	1.26	40	1.18	1.68	5.09	0.60
13	1.29	3.47	1.37	2.55	41	1.44	1.32	0.48	1.97
14	2.74	1.10	1.09	5.31	42	1.76	3.93	1.94	2.43
15	1.38	4.03	1.17	1.70	43	2.44	4.58	0.79	1.81
16	1.44	1.36	1.74	1.36	44	4.33	2.50	1.04	1.64
17	0.75	1.70	1.50	0.61	45	1.94	3.75	0.78	1.93
18	1.77	1.65	0.77	2.33	46	1.32	1.82	0.92	2.00
19	1.37	2.20	1.43	1.17	47	2.67	1.32	1.24	1.98
20	1.94	1.28	1.29	1.69	48	1.64	3.25	1.08	1.44
21	2.12	2.09	1.69	1.20	49	1.68	1.84	1.15	2.88
22	1.64	0.91	1.33	1.15	50	1.63	1.06	1.48	3.01
23	1.45	2.19	1.34	1.08	51	2.63	1.80	1.10	1.40
24	1.95	2.12	1.29	1.12	52	1.13	4.27	0.87	0.93
25	1.53	3.08	0.98	1.53	53	2.50	2.32	0.40	1.83
26	0.40	4.78	0.51	1.02	54	3.80	2.63	1.07	1.68
27	2.88	2.64	1.67	1.28	55	2.50	1.69	1.67	1.12
28	1.94	3.38	1.17	2.17	56	0.59	0.54	0.54	0.54
					Mean	2.06	2.39	1.17	1.57

T A B L E I I

URINARY HYDROXYPROLINE EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

PART C   SUBJECT 3A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night - 8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night 8 A.M.	8 A.M.-12 Noon
Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr	Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	1.30	1.30	1.30	3.40	29	1.90	3.47	2.29	5.32
2	3.10	2.14	1.39	3.53	30	1.63	5.72	0.76	3.36
3	2.60	3.00	1.30	3.50	31	2.99	6.36	1.27	1.81
4	3.50	3.05	1.31	3.56	32	1.41	2.60	2.02	3.52
5	0.88	4.35	3.23	2.26	33	3.52	3.27	1.07	1.02
6	1.85	6.48	2.56	1.54	34	2.52	5.02	0.66	2.60
7	1.18	4.23	2.20	1.97	35	3.08	7.66	2.11	1.24
8	2.60	3.88	2.25	2.35	36	1.24	3.68	1.20	4.75
9	1.40	2.48	1.17	4.42	37	4.75	3.64	0.64	2.33
10	1.97	2.80	1.58	2.62	38	2.63	3.08	1.90	1.55
11	1.72	1.58	1.89	2.04	39	6.35	4.40	0.87	4.86
12	1.88	2.44	2.19	2.19	40	2.42	8.94	1.39	3.63
13	1.70	2.80	5.36	2.45	41	3.63	4.76	1.07	2.39
14	3.54	4.39	0.74	2.53	42	4.30	3.07	1.55	2.06
15	3.00	1.52	1.37	2.43	43	7.50	4.95	0.83	0.83
16	2.20	6.04	1.50	1.80	44	3.40	3.18	3.16	4.09
17	1.65	1.47	0.87	4.11	45	4.09	6.73	2.54	3.92
18	1.32	2.94	0.74	0.75	46	3.92	4.77	1.35	2.98
19	0.75	1.86	1.94	2.68	47	3.24	4.47	0.93	3.32
20	1.34	1.62	0.58	1.62	48	2.30	7.72	0.91	4.00
21	1.78	2.33	1.19	2.00	49	1.94	2.72	0.93	2.64
22	1.82	4.78	1.32	4.00	50	2.66	10.87	3.87	2.52
23	1.80	3.81	2.09	2.15	51	2.33	4.25	3.18	6.87
24	1.35	4.38	0.82	0.82	52	2.30	2.67	2.09	2.91
25	1.51	2.50	0.55	2.48	53	2.91	5.80	2.77	2.77
26	2.76	1.38	1.38	0.87	54	1.29	6.93	2.73	2.34
27	6.09	4.46	1.61	2.14	55	2.34	4.85	2.91	6.89
28	2.39	3.25	0.63	0.63	56	1.59	2.19	2.19	1.67
					Mean	2.56	4.02	1.68	2.77

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST (MILLIGRAMS PER HOUR)

PART D. SUBJECT 4A

Day	12 Noon-8 P.M. Mg/hr	8 P.M.-12 Mid-night Mg/hr	12 Mid-night - 8 A.M. Mg/hr	8 A.M.-12 Noon Mg/hr	Day	12 Noon-8 P.M. Mg/hr	8 P.M.-12 Mid-night Mg/hr	12 Mid-Night 8 A.M. Mg/hr	8 A.M.-12 Noon Mg/hr
1	0.40	1.34	1.34	0.50	29	1.25	4.49	1.06	2.79
2	1.11	1.49	0.98	0.94	30	1.34	3.15	1.29	0.78
3	1.99	1.81	0.88	1.06	31	2.09	1.64	1.18	0.77
4	1.53	1.05	0.97	1.72	32	1.04	3.29	0.77	0.95
5	1.25	1.22	0.51	0.90	33	2.03	3.62	1.44	2.10
6	1.22	1.15	0.70	1.25	34	0.93	4.97	0.68	1.35
7	2.17	3.04	1.83	1.31	35	3.12	2.22	1.11	0.65
8	1.31	3.72	1.07	2.03	36	0.65	6.85	1.39	1.67
9	2.58	3.07	0.83	0.83	37	2.07	3.92	0.65	3.16
10	2.62	1.87	0.46	2.69	38	1.16	2.56	1.06	1.92
11	2.60	2.60	1.83	1.91	39	4.44	3.19	2.34	1.71
12	1.28	5.30	1.37	1.37	40	1.50	1.59	2.88	0.56
13	3.16	2.89	1.60	1.28	41	2.47	5.98	2.50	0.96
14	1.28	0.94	1.08	0.47	42	1.58	4.06	2.10	3.51
15	1.06	4.61	2.72	6.73	43	3.51	3.90	2.73	0.97
16	1.34	2.53	0.96	0.96	44	1.30	6.95	1.57	1.74
17	1.38	3.40	0.33	0.96	45	2.96	4.04	1.61	1.25
18	2.23	1.80	1.80	2.13	46	1.69	4.01	1.77	2.73
19	2.13	3.49	0.85	1.26	47	1.03	1.03	4.08	2.14
20	1.94	1.39	1.41	2.02	48	1.85	2.24	2.13	1.23
21	2.02	1.62	0.97	0.68	49	2.82	3.37	2.03	2.74
22	1.20	5.31	1.53	2.06	50	2.14	3.00	1.24	1.24
23	3.00	1.74	0.53	0.54	51	1.85	1.85	1.29	2.94
24	1.97	1.31	0.94	0.90	52	0.81	1.68	1.26	1.30
25	4.38	3.06	1.80	1.39	53	1.01	1.01	2.49	2.19
26	2.11	3.34	1.50	1.31	54	0.50	0.50	2.83	2.17
27	4.05	2.17	1.83	1.20	55	2.17	3.27	0.61	0.60
28	1.69	4.38	1.49	1.12	56	2.05	0.64	0.64	0.64
					Mean	1.90	2.87	1.44	1.60

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(MILLIGRAMS PER HOUR)

PART E. SUBJECT 6A

Day	12 Noon-8 P.M. Mg/hr	8 P.M.-12 Mid-night Mg/hr	12 Mid-night - 8 A.M. Mg/hr	8 A.M.-12 Noon Mg/hr	Day	12 Noon-8 P.M. Mg/hr	8 P.M.-12 Mid-night Mg/hr	12 Mid-Night 8 A.M. Mg/hr	8 A.M.-12 Noon Mg/hr
1	0.56	5.72	5.72	2.54	29	2.74	6.19	1.59	7.23
2	5.38	5.57	1.32	2.33	30	2.98	5.39	1.87	6.76
3	3.26	4.96	1.17	6.40	31	2.62	3.87	1.50	3.57
4	5.35	1.82	1.53	1.87	32	2.05	3.47	1.05	3.06
5	8.35	3.86	1.52	1.40	33	3.16	2.26	1.32	5.51
6	1.74	2.91	1.01	2.15	34	4.45	2.44	2.05	4.61
7	1.42	0.89	1.14	2.30	35	1.40	2.20	1.99	1.82
8	3.00	4.52	1.38	3.57	36	1.82	1.45	1.92	2.70
9	8.48	0.94	0.90	2.18	37	3.30	7.99	1.26	7.48
10	3.72	2.06	1.64	2.53	38	2.73	2.61	0.98	3.19
11	5.91	2.50	2.07	3.62	39	3.73	2.08	1.17	2.89
12	1.25	3.23	2.31	2.41	40	2.31	2.77	3.59	3.49
13	2.22	3.65	2.79	2.79	41	1.38	4.47	2.56	2.56
14	5.29	1.86	2.21	2.42	42	0.78	3.69	3.18	3.03
15	1.25	5.27	1.14	2.38	43	3.03	1.97	1.52	4.37
16	1.30	8.47	0.45	3.44	44	2.36	1.83	1.14	5.94
17	4.12	3.06	1.62	3.20	45	3.82	4.60	2.03	1.85
18	2.49	5.52	1.71	2.25	46	2.92	3.40	2.27	2.70
19	0.70	1.51	1.51	2.06	47	5.93	3.80	1.57	6.88
20	1.87	2.95	1.45	3.23	48	2.22	2.22	0.97	1.69
21	2.27	2.05	1.84	1.58	49	2.24	6.22	3.17	4.64
22	3.02	3.23	1.27	2.67	50	1.58	5.10	3.25	1.26
23	2.47	3.12	2.41	2.26	51	2.27	2.24	3.88	2.62
24	1.67	1.51	1.16	2.12	52	1.28	3.46	2.00	2.83
25	1.06	2.39	2.09	2.46	53	3.09	3.05	2.50	3.78
26	2.17	3.46	1.91	2.45	54	1.66	5.02	2.03	2.38
27	2.65	3.29	2.01	1.88	55	0.98	3.73	1.87	4.62
28	1.75	5.64	1.42	1.64	56	1.50	1.46	1.46	1.46
					Mean	2.77	3.52	1.86	3.16

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST (MILLIGRAMS PER HOUR)

PART F. SUBJECT 7A

Day	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight - 8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight 8 A.M.	8 A.M.-12 Noon
	Mg/hr	Mg/hr	Mg/hr	Mg/hr		Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	1.22	1.09	1.09	1.23	29	1.81	3.15	2.25	2.14
2	5.28	1.07	1.49	1.08	30	3.20	2.11	2.12	2.80
3	3.57	2.04	1.22	1.56	31	2.61	2.85	1.28	1.00
4	2.24	1.79 3.70	0.82	2.11	32	1.93	2.72	1.05	1.16
5	2.01	2.97	1.45	1.12	33	3.08	6.82	1.98	1.60
6	1.92	4.80	2.45	1.90	34	2.04	2.00	1.05	3.08
7	2.14	1.51	1.46	2.16	35	2.46	7.40	2.40	1.19
8	2.45	1.27	1.75	1.94	36	1.19	3.93	0.99	5.36
9	2.30	3.67	1.59	1.10	37	1.27	7.45	0.45	4.71
10	2.08	2.23	2.36	3.88	38	0.56	2.23	1.88	4.27
11	2.64	2.30	2.66	1.43	39	0.91	2.74	0.73	4.23
12	2.60	2.54	1.03	1.82	40	4.82	1.67	0.52	1.96
13	2.40	2.78	3.44	1.69	41	1.53	2.08	2.08	2.10
14	0.97	2.42	0.93	1.35	42	1.45	2.32	0.48	10.1 1.01
15	3.89	2.80	1.12	2.09	43	1.18	2.13	0.92	1.74
16	1.96	2.34	1.30	1.11	44	1.20	2.29	1.00	2.45
17	1.20	4.07	1.48	5.20	45	1.88	1.24	0.98	2.12
18	1.87	4.21	0.83	3.75	46	1.38	1.45	1.14	1.85
19	0.88	3.72	0.75	2.90	47	2.13	2.88	0.68	3.42
20	1.22	2.03	1.83	1.97	48	1.69	5.38	0.48	2.00
21	2.75	3.33	1.75	1.70	49	2.12	3.22	2.24	2.37
22	2.13	2.54	1.55	3.79	50	3.33	2.10	1.22	2.90
23	2.34	2.42	0.94	4.37	51	1.76	1.51	0.70	1.44
24	2.87	0.89	1.50	1.50	52	3.88	2.46	0.85	1.40
25	2.46	2.68	0.58	3.75	53	0.98	2.71	3.17	2.16
26	1.66	4.27	1.84	1.40	54	1.13	4.41	2.23	1.66
27	4.83	2.14	0.98	1.39	55	0.83	3.84	1.02	2.57
28	1.42	4.55	2.13	2.07	56	4.75	0.45	0.45	1.18
					Mean	2.19	2.86	1.40	2.27

TABLE III

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST (MILLIGRAMS PER HOUR)

PART G. SUBJECT 8A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night - 8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night 8 A.M.	8 A.M.-12 Noon
Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr	Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	0.54	3.48	3.48	3.44	29	1.65	3.98	2.08	5.25
2	3.77	1.54	1.70	1.96	30	2.22	3.42	2.29	1.79
3	3.30	1.21	1.12	1.99	31	2.30	2.31	0.68	1.68
4	2.17	1.99	1.06	1.65	32	2.24	4.35	1.32	1.71
5	5.21	2.16	1.06	1.88	33	3.62	2.73	2.01	0.57
6	3.72	2.64	0.54	3.68	34	5.31	1.45	1.64	2.27
7	3.71	2.02	2.23	2.34	35	4.15	1.88	1.69	1.11
8	0.74	0.87	0.94	2.32	36	1.11	1.37	0.38	1.33
9	1.84	3.11	1.42	2.06	37	1.98	5.13	1.61	2.11
10	1.90	2.34	1.82	1.24	38	3.87	4.65	1.97	1.11
11	1.25	2.88	2.21	0.93	39	2.33	2.55	0.69	1.29
12	2.20	1.53	1.40	1.68	40	2.20	0.57	4.37	1.32
13	4.26	7.90	2.04	1.79	41	2.15	1.40	2.73	1.28
14	1.28	7.56	1.17	1.99	42	1.73	2.80	1.88	1.94
15	4.65	5.56	1.74	1.68	43	1.07	0.95	1.17	1.54
16	1.13	1.68	1.42	2.63	44	1.23	1.00	1.79	1.78
17	2.29	4.15	1.29	1.86	45	0.83	2.28	0.96	1.04
18	6.09	5.68	1.95	2.88	46	1.77	1.68	3.52	1.44
19	2.88	1.30	1.88	0.83	47	2.18	2.30	1.19	3.15
20	5.30	1.18	1.94	1.10	48	3.15	1.42	1.06	2.82
21	2.34	3.17	1.41	1.34	49	1.97	1.34	0.78	1.34
22	1.97	2.06	1.44	2.67	50	1.45	3.41	0.47	1.18
23	3.17	2.13	1.48	1.67	51	1.47	0.81	1.58	1.04
24	2.86	2.23	2.43	1.51	52	1.66	0.80	1.70	1.92
25	2.11	4.34	1.24	3.41	53	1.36	2.10	1.26	3.70
26	1.27	3.35	1.44	1.35	54	0.96	1.78	1.21	2.01
27	3.55	3.54	1.17	1.44	55	0.65	1.18	1.05	1.27
28	2.06	3.18	0.75	1.75	56	1.06	1.25	1.25	2.26
					Mean	2.41	2.60	1.57	1.90

TABLE II

URINARY HYDROXYPROLINE EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(MILLIGRAMS PER HOUR)

PART H. SUBJECT 9A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night - 8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-Night 8 A.M.	8 A.M.-12 Noon
Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr	Day	Mg/hr	Mg/hr	Mg/hr	Mg/hr
1	0.56	0.67	0.67	1.45	29	8.20	2.86	2.10	1.88
2	2.03	0.76	1.40	1.67	30	3.90	4.36	1.47	3.17
3	2.87	1.69	1.36	1.56	31	3.24	3.95	1.28	1.08
4	3.92	1.72	1.10	1.44	32	2.06	4.58	1.57	1.77
5	4.08	1.55	1.33	1.64	33	2.17	2.22	0.97	3.73
6	0.95	2.48	1.61	1.54	34	2.70	2.51	1.43	1.94
7	2.19	4.02	1.10	2.76	35	1.51	3.82	2.21	2.49
8	2.34	2.43	1.50	1.84	36	2.49	1.37	0.89	2.38
9	3.39	4.29	0.32	4.61	37	1.73	2.11	2.91	4.71
10	1.66	2.27	0.87	2.60	38	1.64	5.40	8.46	2.38
11	2.81	3.48	1.70	1.23	39	0.51	2.02	2.09	5.06
12	2.86	2.40	2.20	1.10	40	5.64	1.87	1.63	0.56
13	2.45	1.82	1.72	3.23	41	1.70	1.58	2.27	2.10
14	3.07	2.05	1.37	1.57	42	2.64	1.20	3.30	2.13
15	1.47	3.35	1.76	1.79	43	3.50	2.59	1.91	2.49
16	1.73	2.41	1.94	2.53	44	5.88	2.41	1.61	1.46
17	2.43	2.62	2.58	2.40	45	2.49	2.03	1.05	1.76
18	1.36	2.87	0.93	2.54	46	8.03	4.04	0.64	3.66
19	1.28	7.41	0.85	1.93	47	5.28	2.10	0.91	2.09
20	2.59	2.68	0.84	1.34	48	2.73	1.34	1.47	2.14
21	1.83	4.49	1.10	1.15	49	6.35	2.86	1.24	1.56
22	5.19	1.55	1.10	1.98	50	3.44	1.68	1.43	3.63
23	2.58	3.01	1.08	1.08	51	3.16	3.93	1.12	1.48
24	1.23	2.55	1.28	1.67	52	2.73	1.93	1.43	1.85
25	1.29	2.40	1.22	1.49	53	1.70	3.66	1.25	1.85
26	1.89	5.51	0.48	2.10	54	1.90	2.69	1.54	2.07
27	4.72	3.56	2.89	0.93	55	2.42	2.20	1.55	1.40
28	1.18	2.75	1.61	2.85	56	2.69	0.91	0.91	0.91
					Mean	2.83	2.73	1.58	2.10

TABLE III

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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 Bed Rest	26.31 40.70	10.28 10.78	4.6251	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	26.31 14.52	10.28 6.12	3.5056	$P < 0.01$
Bed Rest Post-Bed Rest	40.70 14.52	10.78 6.12	8.5173	$P < 0.001$
SUBJECT 6A				
Pre-Bed Rest Bed Rest	59.16 63.62	35.39 16.89	0.6784	NS
Pre-Bed Rest Post-Bed Rest	59.16 32.04	35.39 15.33	2.4842	$P < 0.02$
Bed Rest Post-Bed Rest	63.62 32.04	16.89 15.33	6.1937	$P < 0.001$
SUBJECT 7A				
Pre-Bed Rest Bed Rest	28.45 50.07	7.97 10.94	6.9764	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	28.45 22.34	7.97 10.33	1.6668	NS
Bed Rest Post-Bed Rest	50.07 22.34	10.94 10.33	8.3363	$P < 0.001$

TABLE III (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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	38.18 51.46	26.66 16.19	4.1967	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	38.18 22.96	26.66 13.33	3.2693	$P < 0.01$
Bed Rest Post-Bed Rest	51.46 22.96	16.19 13.33	10.4524	$P < 0.001$

TABLE III (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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	25.29 44.36	7.77 12.21	5.7659	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	25.29 19.37	7.77 8.01	1.9129	$P < 0.10$
Bed Rest Post-Bed Rest	44.36 19.37	12.21 8.01	7.0861	$P < 0.001$
SUBJECT 8A				
Pre-Bed Rest Bed Rest	37.94 49.72	18.70 15.36	2.4949	$P < 0.02$
Pre-Bed Rest Post-Bed Rest	37.94 22.99	18.70 14.43	2.2646	$P < 0.05$
Bed Rest Post-Bed Rest	49.72 22.99	15.36 14.43	5.7264	$P < 0.001$
SUBJECT 9A				
Pre-Bed Rest Bed Rest	38.80 54.87	20.34 21.89	2.5576	$P < 0.02$
Pre-Bed Rest Post-Bed Rest	38.80 20.73	20.34 9.72	2.7450	$P < 0.02$
Bed Rest Post-Bed Rest	54.87 20.73	21.89 9.72	5.3621	$P < 0.001$

TABLE III (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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 Bed Rest	34.01 49.65	17.69 17.51	5.3954	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	34.01 21.04	17.69 11.19	3.9638	$P < 0.001$
Bed Rest Post-Bed Rest	49.65 21.04	17.51 11.19	9.8922	$P < 0.001$

TABLE III (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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 Bed Rest	47.76 53.03	22.67 11.99	1.1574	NS
Pre-Bed Rest Post-Bed Rest	47.76 14.89	22.67 5.48	4.5369	P < 0.001
Bed Rest Post-Bed Rest	53.03 14.89	11.99 5.48	10.5171	P ≤ 0.001
SUBJECT 3A				
Pre-Bed Rest Bed Rest	41.00 60.93	20.14 17.39	3.7839	P < 0.001
Pre-Bed Rest Post-Bed Rest	41.00 34.27	20.14 10.89	1.0108	NS
Bed Rest Post-Bed Rest	60.93 34.27	17.39 10.89	5.1550	P < 0.001

TABLE III (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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 Bed Rest	38.01 52.17	22.69 16.76	7.6534	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	38.01 22.70	22.69 12.58	6.1829	$P < 0.001$
Bed Rest Post-Bed Rest	52.17 22.70	16.76 12.58	17.0883	$P < 0.001$

TABLE IV

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

PART A. SUBJECT 2A

Populations Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.06 2.39	1.05 1.01	1.6715	$P < 0.10$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.06 1.17	1.05 0.67	5.2514	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.06 1.57	1.05 0.67	2.8762	$P < 0.01$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M	2.39 1.17	1.01 0.67	7.3984	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.39 1.57	1.01 0.67	4.9565	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.17 1.57	0.67 0.67	3.1098	$P < 0.01$

TABLE IV (Continued)

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

PART B. SUBJECT 6A

Populations Compared	Means (mg / hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.77 3.52	1.68 1.67	2.3440	$P < 0.02$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.77 1.86	1.68 0.87	3.5184	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.77 3.16	1.68 1.53	1.2704	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	3.52 1.86	1.67 0.87	6.4900	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	3.52 3.16	1.67 1.53	1.1693	NS
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.86 3.16	0.87 1.53	5.4221	$P < 0.001$

TABLE IV (Continued)

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

PART C. SUBJECT 7A

Populations Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.19 2.86	1.07 1.46	2.7257	P < 0.01
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.19 1.40	1.07 0.69	4.4939	P < 0.001
12 Noon - 8 P. M. 8 A.M. - 12 Noon	2.19 2.27	1.07 1.12	0.4080	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	2.86 1.40	1.46 0.69	6.6151	P < 0.001
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.86 2.27	1.46 1.12	2.3377	P < 0.02
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.40 2.27	0.69 1.12	4.8336	P < 0.001

TABLE IV (Continued)

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

PART D. SUBJECTS 2A, 6A, 7A

(Exercised throughout the study, combined data)

Populations Compared	Means (mg / hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M - 12 Midnight	2.34 2.93	1.34 1.48	3.8033	P < 0.001
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.34 1.48	1.34 0.80	7.0968	P < 0.001
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.34 2.34	1.34 1.33	0.0211	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M	2.93 1.48	1.48 0.80	11.0725	P < 0.001
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.93 2.34	1.48 1.33	3.8293	P < 0.001
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.48 2.34	0.80 1.33	7.0898	P < 0.001

TABLE IV (Continued)

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

PART E. SUBJECT 4A

Populations Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	1.90 2.87	0.90 1.51	4.0530	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	1.90 1.44	0.90 0.73	2.8936	$P < 0.01$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	1.90 1.60	0.90 1.00	1.6609	$P < 0.10$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	2.87 1.44	1.51 0.73	6.2386	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.87 1.60	1.51 1.00	5.1618	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.44 1.60	0.73 1.00	0.9008	NS

TABLE IV (Continued)

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

PART F. SUBJECT 8A

Populations Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.41 2.60	1.30 1.58	0.6697	NS
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.41 1.57	1.30 0.73	4.1375	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.41 1.90	1.30 0.84	2.4482	$P < 0.02$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	2.60 1.57	1.58 0.73	4.3420	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.60 1.90	1.58 0.84	2.8893	$P < 0.01$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.57 1.90	0.73 0.84	2.1440	$P < 0.05$

TABLE IV (Continued)

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

PART G. SUBJECT 9A

Populations Compared	Means (mg/ hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.83 2.73	1.63 1.26	0.3406	NS
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.83 1.58	1.63 1.10	4.6533	P < 0.001
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.83 2.10	1.63 0.94	2.8296	P < 0.01
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	2.73 1.58	1.26 1.10	5.0552	P < 0.001
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.73 2.10	1.26 0.94	2.9429	P < 0.01
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.58 2.10	1.10 0.94	2.6524	P < 0.01

TABLE IV (Continued)

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

PART H. SUBJECTS 4A, 8A, 9A

(Did not exercise, combined data)

Population Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.38 2.73	1.37 1.46	2.2758	$P < 0.05$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.38 1.53	1.37 0.87	6.7341	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.38 1.87	1.37 0.95	3.9849	$P < 0.001$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	2.73 1.53	1.46 0.87	9.0923	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	2.73 1.87	1.37 0.95	6.4180	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.53 1.87	0.87 0.95	3.3188	$P < 0.001$

TABLE IV (Continued)

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

PART I. SUBJECT 3A

(Exercised First 28 days of study)

Population Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.56 4.02	1.33 1.97	4.5222	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.56 1.68	1.33 0.93	3.9575	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.56 2.77	1.33 1.34	0.8247	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	4.02 1.68	1.97 0.93	7.8833	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	4.02 2.77	1.97 1.34	3.8556	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.68 2.77	0.93 1.34	4.8881	$P < 0.001$

TABLE IV (Continued)

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

PART J. SUBJECT 1A

(Exercised second half of study)

Population Compared	Means (mg/ hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P. M.- 12 Midnight	2.30 3.33	1.03 1.59	4.0053	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.30 1.46	1.03 0.49	5.3487	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.30 2.48	1.03 1.51	0.7616	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	3.33 1.46	1.59 0.49	8.2474	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	3.33 2.48	1.59 1.51	2.8279	$P < 0.01$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.46 2.48	0.49 1.51	4.7339	$P < 0.001$

TABLE IV (Continued)

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

PART K. ALL SUBJECTS

Population Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	2.38 3.04	1.32 1.61	6.7547	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	2.38 1.52	1.32 0.82	11.6305	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	2.38 2.23	1.32 1.27	1.6642	$P < 0.10$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	3.04 1.52	1.61 0.82	17.7523	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	3.04 2.23	1.61 1.27	8.3324	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	1.52 2.23	0.82 1.27	9.9111	$P < 0.001$

TABLE V

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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 3A				
Exercise	52.31	13.63	4.3181	$P < 0.001$
No Exercise	70.18	16.19		
SUBJECT 1A				
Exercise	51.72	10.61	0.7593	NS
No Exercise	54.21	13.00		

TABLE VI

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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/ hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise Exercise	3.05 2.10	1.43 1.04	2.7509	$P < 0.01$
8 P.M. - 12 Midnight				
No Exercise Exercise	4.97 3.13	2.08 1.34	3.8234	$P < 0.001$
12 Midnight - 8 A.M.				
No Exercise Exercise	1.74 1.63	0.90 0.95	0.4054	NS
8 A.M. - 12 Noon				
No Exercise Exercise	3.07 2.49	1.49 1.49	1.5989	NS

TABLE VI (Continued)

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	2.49	1.26	1.4083	NS
Exercise	2.09	0.65		
8 P.M. - 12 Midnight				
No Exercise	3.29	1.45	0.1750	NS
Exercise	3.37	1.72		
12 Midnight - 8 A.M.				
No Exercise	1.62	0.44	2.4823	$P < 0.02$
Exercise	1.30	0.49		
8 A.M. - 12 Noon				
No Exercise	2.20	1.07	1.4185	NS
Exercise	2.79	1.83		

TABLE VII

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE 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	51.46	16.19	0.9806	NS
Non-Exercisers	49.65	17.51		

TABLE VIII

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE EXCRETION AT DIFFERENT TIMES OF THE DAY BY GROUPS OF SUBJECTS WHO EXERCISED (2A, 6A, 7A) AND WHO DID NOT EXERCISE (4A, 8A, 9A) THROUGHOUT BED REST

Populations Compared	Means (mg/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
Exercisers Non-Exercisers	2.34 2.38	1.34 1.37	0.2851	NS
8 P.M. - 12 Midnight				
Exercisers Non-Exercisers	2.93 2.73	1.48 1.46	1.1952	NS
12 Midnight - 8 A.M.				
Exercisers Non-Exercisers	1.48 1.53	0.80 0.87	0.5725	NS
8 A.M. - 12 Noon				
Exercisers Non-Exercisers	2.34 1.87	1.33 0.95	3.6965	$P < 0.001$

TABLE IX

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART A. SUBJECT 1A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	12.374	1	9.744	29	15.135	1	4.735
2	10.146	2	13.416	30	12.075	2	4.032
3	10.283	3	13.395	31	7.645	3	3.672
4	11.200	4	18.098	32	14.340	4	4.275
5	10.309	5	14.103	33	14.340	5	4.042
6	11.628	6	13.755	34	14.627	6	- -
7	5.456	7	12.683	35	13.040	7	4.095
8	12.513	8	13.323	36	12.064	8	3.692
9	7.920	9	14.265	37	14.968	9	15.473
10	13.727	10	14.689	38	11.948	10	4.928
11	16.156	11	10.837	39	12.971	11	4.934
12	12.948	12	13.663	40	14.546	12	1.320
13	16.590	13	12.295	41	13.201	13	- -
14	11.677	14	7.644	42	14.571		
15	14.414	15	12.717	43	13.107	Mean	5.018
16	- -	16	13.874	44	14.719		
		17	12.775	45	13.141		
Mean	11.822	18	12.394	46	13.787		
		19	13.530	47	11.975		
		20	11.725	48	13.834		
		21	12.651	49	13.799		
		22	14.022	50	15.229		
		23	11.304	51	14.413		
		24	9.465	52	14.065		
		25	12.727	53	14.250		
		26	14.697	54	13.748		
		27	12.425	55	13.188		
		28	13.849	56	9.144		
				Mean	13.044		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART B. SUBJECT 2A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	8.004	1	6.770	29	14.254	1	3.520
2	9.006	2	12.132	30	13.411	2	6.281
3	9.500	3	14.987	31	12.992	3	8.470
4	5.664	4	10.535	32	13.260	4	11.628
5	7.502	5	12.609	33	13.636	5	2.450
6	7.885	6	12.842	34	13.163	6	2.583
7	8.064	7	7.892	35	11.961	7	4.392
8	7.552	8	11.648	36	12.222	8	1.760
9	10.701	9	12.625	37	16.846	9	3.905
10	7.956	10	8.770	38	9.335	10	5.352
11	8.772	11	12.035	39	9.014	11	4.214
12	11.529	12	9.956	40	14.009	12	1.372
13	11.922	13	11.323	41	12.501	13	2.880
14	7.093	14	10.345	42	14.386	Mean	4.524
15	13.266	15	12.918	43	13.432		
16	9.622	16	12.380	44	12.913		
		17	13.998	45	11.406		
Mean	9.002	18	12.438	46	11.156		
		19	12.312	47	11.934		
		20	10.608	48	10.850		
		21	12.658	49	13.396		
		22	12.802	50	11.444		
		23	14.242	51	10.782		
		24	8.340	52	11.579		
		25	14.341	53	12.935		
		26	11.869	54	10.010		
		27	13.266	55	10.180		
		28	13.352	56	3.912		
				Mean	12.140		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART C. SUBJECT 3A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	5.340	1	12.130	29	15.606	1	11.668
2	7.770	2	13.501	30	13.180	2	5.673
3	9.860	3	10.482	31	11.550	3	10.675
4	11.592	4	13.594	32	12.375	4	7.956
5	8.415	5	12.960	33	14.102	5	9.016
6	7.536	6	13.793	34	14.746	6	5.335
7	9.680	7	14.371	35	11.356	7	10.368
8	8.514	8	14.628	36	12.614	8	9.443
9	12.485	9	10.995	37	12.889	9	10.183
10	7.272	10	13.787	38	11.278	10	6.600
11	7.786	11	12.704	39	15.832	11	6.048
12	14.354	12	15.319	40	13.461	12	9.576
13	13.318	13	16.061	41	16.443	13	2.778
14	5.416	14	14.106	42	15.868		
15	15.660	15	15.743	43	12.888	Mean	8.101
16	7.146	16	14.435	44	13.676		
		17	16.640	45	15.365		
Mean	9.509	18	14.153	46	14.524		
		19	14.087	47	14.736		
		20	14.277	48	13.517		
		21	16.300	49	12.974		
		22	15.212	50	14.890		
		23	14.780	51	17.736		
		24	13.611	52	12.119		
		25	13.682	53	14.975		
		26	10.745	54	14.272		
		27	16.318	55	14.808		
		28	13.818	56	12.678		
				Mean	14.137		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART D. SUBJECT 4A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	12.516	1	11.317	29	14.869	1	6.695
2	15.762	2	12.688	30	12.723	2	5.595
3	16.241	3	11.578	31	10.512	3	3.534
4	13.200	4	9.746	32	10.949	4	9.713
5	11.124	5	11.328	33	13.569	5	3.313
6	15.851	6	11.495	34	13.963	6	6.120
7	17.169	7	15.887	35	11.520	7	6.364
8	17.464	8	12.729	36	12.107	8	3.640
9	15.576	9	12.027	37	14.331	9	7.457
10	12.750	10	10.705	38	11.974	10	4.680
11	5.208	11	10.480	39	12.081	11	4.345
12	8.096	12	8.555	40	11.018	12	11.223
13	8.676	13	11.313	41	14.532	13	3.240
14	9.677	14	7.052	42	8.652		
15	12.102	15	13.634	43	16.355	Mean	5.847
16	9.284	16	12.164	44	12.759		
		17	12.799	45	14.284		
Mean	12.544	18	14.171	46	11.754		
		19	11.706	47	12.282		
		20	9.769	48	10.370		
		21	12.744	49	13.411		
		22	13.238	50	13.091		
		23	12.453	51	11.583		
		24	8.880	52	11.014		
		25	14.796	53	14.597		
		26	13.310	54	12.276		
		27	13.064	55	10.453		
		28	12.333	56	8.944		
				Mean	11.629		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART E. SUBJECT 6A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	9.434	1	14.152	29	16.178	1	9.196
2	13.509	2	12.614	30	18.740	2	10.540
3	12.614	3	13.629	31	13.326	3	11.096
4	13.298	4	16.297	32	13.915	4	10.943
5	11.378	5	12.594	33	16.869	5	9.440
6	16.830	6	15.816	34	18.012	6	15.640
7	35.616	7	12.856	35	14.221	7	11.371
8	13.536	8	17.773	36	15.771	8	10.450
9	13.056	9	14.572	37	17.444	9	11.272
10	12.064	10	15.948	38	12.413	10	12.727
11	14.861	11	13.203	39	14.808	11	10.465
12	11.865	12	13.855	40	13.807	12	11.610
13	15.419	13	15.943	41	15.668	13	10.168
14	15.300	14	14.419	42	17.490		
15	17.625	15	14.107	43	13.016	Mean	11.147
16	12.591	16	14.596	44	14.480		
		17	18.877	45	16.614		
Mean	14.937	18	13.911	46	17.966		
		19	9.817	47	15.608		
		20	12.831	48	11.729		
		21	15.602	49	17.177		
		22	19.112	50	14.927		
		23	14.342	51	16.765		
		24	10.024	52	13.276		
		25	14.063	53	15.642		
		26	14.462	54	14.903		
		27	16.168	55	14.551		
		28	15.197	56	10.463		
				Mean	14.537		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART F. SUBJECT 7A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	- -	1	12.005	29	14.566	1	10.706
2	12.056	2	11.093	30	15.340	2	12.771
3	12.152	3	14.841	31	15.692	3	10.350
4	4.949	4	12.895	32	15.657	4	8.591
5	7.300	5	12.239	33	15.848	5	6.611
6	14.161	6	14.777	34	16.051	6	9.858
7	5.152	7	13.962	35	14.987	7	7.383
8	9.010	8	14.201	36	13.927	8	3.616
9	3.770	9	13.809	37	14.585	9	7.732
10	8.360	10	12.629	38	12.590	10	4.272
11	6.335	11	12.858	39	13.695	11	6.820
12	12.689	12	14.085	40	14.417	12	5.233
13	10.770	13	12.238	41	14.766	13	9.010
14	8.073	14	11.877	42	19.708		
15	10.298	15	15.242	43	9.657	Mean	7.919
16	8.692	16	12.932	44	14.528		
		17	18.541	45	14.603		
Mean	8.917	18	12.779	46	13.758		
		19	13.725	47	14.563		
		20	11.852	48	16.060		
		21	16.261	49	13.486		
		22	14.967	50	13.134		
		23	10.154	51	11.309		
		24	9.110	52	12.156		
		25	12.166	53	12.487		
		26	13.411	54	13.852		
		27	13.302	55	15.682		
		28	13.988	56	10.544		
				Mean	13.143		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART G. SUBJECT 8A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	9.579	1	9.185	29	17.704	1	13.192
2	10.464	2	11.914	30	14.803	2	7.191
3	12.036	3	12.500	31	12.814	3	5.738
4	8.694	4	14.063	32	13.687	4	7.140
5	19.228	5	14.562	33	16.510	5	6.615
6	16.544	6	13.028	34	15.969	6	6.513
7	9.612	7	15.378	35	11.627	7	7.533
8	12.155	8	11.468	36	13.461	8	3.924
9	10.260	9	14.343	37	17.106	9	7.156
10	1.768	10	14.954	38	14.584	10	7.503
11	14.246	11	13.623	39	12.033	11	3.570
12	13.331	12	11.794	40	13.948	12	5.644
13	12.003	13	15.504	41	11.512	13	5.255
14	14.580	14	10.025	42	13.222		
15	14.438	15	14.036	43	10.532	Mean	6.690
16	10.111	16	13.124	44	12.938		
		17	17.779	45	11.629		
Mean	11.819	18	15.699	46	15.974		
		19	13.585	47	10.950		
		20	13.558	48	9.605		
		21	17.155	49	12.862		
		22	16.490	50	11.687		
		23	15.186	51	11.338		
		24	10.111	52	9.763		
		25	16.568	53	11.798		
		26	14.445	54	9.547		
		27	16.664	55	9.443		
		28	14.546	56	10.999		
				Mean	12.939		

TABLE IX, (Continued)

URINARY NITROGEN EXCRETION

(GRAMS PER 24 HOURS)

PART H. SUBJECT 9A

Pre-Bed Rest		Bed Rest				Post-Bed Rest	
Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr	Day	Gm/24 hr
1	7.119	1	6.595	29	15.152	1	6.322
2	5.985	2	16.996	30	15.316	2	9.162
3	5.508	3	9.427	31	12.379	3	- -
4	2.501	4	14.155	32	11.754	4	7.176
5	7.300	5	14.365	33	14.882	5	3.360
6	11.600	6	12.694	34	13.714	6	4.199
7	10.600	7	14.151	35	13.366	7	6.518
8	9.604	8	14.472	36	13.148	8	4.462
9	5.940	9	15.474	37	15.478	9	7.168
10	11.446	10	13.097	38	12.290	10	5.553
11	6.101	11	11.936	39	9.397	11	4.209
12	10.640	12	12.594	40	13.338	12	3.383
13	6.612	13	14.945	41	13.314	13	4.699
14	11.473	14	8.293	42	12.521		
15	12.131	15	11.120	43	13.406	Mean	5.518
16	9.642	16	14.965	44	12.645		
		17	15.412	45	14.506		
Mean	8.388	18	11.924	46	12.334		
		19	17.188	47	11.386		
		20	17.301	48	10.501		
		21	21.630	49	12.843		
		22	12.660	50	12.185		
		23	12.284	51	12.637		
		24	11.947	52	13.552		
		25	11.670	53	17.614		
		26	15.921	54	13.296		
		27	9.685	55	10.346		
		28	11.820	56	10.333		
				Mean	12.740		

TABLE X

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART A. SUBJECT 1A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr	Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.312	0.312	0.312	0.876	29	0.536	1.294	0.534	0.348
2	0.465	0.817	0.569	0.468	30	0.672	0.761	0.304	0.304
3	0.491	0.487	0.533	0.814	31	0.406	0.244	0.096	0.662
4	1.152	0.750	0.420	0.630	32	0.466	0.953	0.573	0.552
5	0.550	1.035	0.398	0.594	33	0.744	0.818	0.426	0.426
6	0.890	0.418	0.390	0.462	34	0.777	0.672	0.477	0.477
7	0.838	0.420	0.240	0.594	35	0.750	0.671	0.363	0.363
8	0.551	0.866	0.399	0.564	36	0.363	0.957	0.514	0.305
9	0.656	0.748	0.480	0.546	37	0.573	1.072	0.508	0.508
10	0.801	0.531	0.444	0.651	38	0.358	0.958	0.438	0.438
11	0.473	0.720	0.496	0.052	39	0.748	0.384	0.557	0.249
12	0.840	0.305	0.468	0.497	40	0.645	0.370	0.234	1.508
13	0.532	0.488	0.452	0.620	41	0.520	0.514	0.164	1.418
14	0.207	0.461	0.268	0.499	42	0.582	0.960	0.136	1.246
15	0.333	1.154	0.409	0.540	43	0.619	0.578	0.199	1.062
16	0.406	1.258	0.390	0.618	44	0.476	1.189	0.619	0.300
17	0.460	0.690	0.425	0.732	45	0.300	1.069	0.601	0.414
18	0.662	0.351	0.379	0.662	46	0.557	1.080	0.175	0.902
19	0.566	1.082	0.390	0.390	47	0.551	0.550	0.193	0.954
20	0.511	0.630	0.373	0.533	48	0.412	1.302	0.108	1.116
21	0.590	0.830	0.402	0.348	49	0.765	0.522	0.128	1.142
22	0.762	0.508	0.523	0.427	50	0.835	0.818	0.268	0.784
23	0.480	0.592	0.225	0.824	51	0.885	0.261	0.194	1.184
24	0.391	0.575	0.057	0.895	52	0.331	1.438	0.531	0.356
25	0.447	0.959	0.462	0.403	53	0.866	0.741	0.363	0.363
26	0.627	0.840	0.574	0.433	54	0.489	1.032	0.497	0.434
27	0.638	0.600	0.491	0.250	55	0.261	1.271	0.219	1.065
28	0.541	1.069	0.437	0.437	56	0.569	0.287	0.287	0.287
					Mean	0.76	0.755	0.377	0.617

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART B. SUBJECT 2A

Day	12 Noon-8 P.M. Gm/hr	8 P.M.-12 Midnight Gm/hr	12 Midnight-8 A.M. Gm/hr	8 A.M.-12 Noon Gm/hr	Day	12 Noon-8 P.M. Gm/hr	8 P.M.-12 Midnight Gm/hr	12 Midnight-8 A.M. Gm/hr	8 A.M.-12 Noon Gm/hr
1	0.161	0.280	0.280	0.532	29	0.593	0.861	0.481	0.557
2	0.378	1.284	0.304	0.385	30	0.689	0.657	0.423	0.473
3	0.780	0.282	0.682	0.540	31	0.532	0.527	0.293	1.072
4	0.497	0.398	0.338	0.466	32	0.453	0.770	0.536	0.569
5	0.541	0.979	0.357	0.378	33	0.575	0.707	0.518	0.517
6	0.457	1.201	0.375	0.347	34	0.695	0.692	0.477	0.505
7	0.505	0.619	0.319	0.457	35	0.516	0.374	0.547	0.491
8	0.438	0.855	0.403	0.377	36	0.491	0.758	0.488	0.290
9	0.616	0.758	0.354	0.459	37	0.774	1.064	0.468	0.663
10	0.304	0.570	0.228	0.560	38	0.454	0.180	0.388	0.471
11	0.491	0.825	0.421	0.360	39	0.404	0.488	0.435	0.339
12	0.287	0.880	0.346	0.342	40	0.494	0.677	0.538	0.511
13	0.531	0.563	0.243	0.719	41	0.519	0.455	0.516	0.599
14	0.662	0.475	0.160	0.469	42	0.723	0.612	0.483	0.574
15	0.647	0.512	0.454	0.515	43	0.574	1.023	0.355	0.428
16	0.384	0.430	0.687	0.521	44	0.624	0.653	0.435	0.458
17	0.572	0.856	0.656	0.187	45	0.374	0.839	0.414	0.438
18	0.433	0.809	0.361	0.713	46	0.647	0.657	0.196	0.446
19	0.459	0.915	0.412	0.421	47	0.520	0.450	0.525	0.444
20	0.501	0.398	0.354	0.544	48	0.443	0.753	0.322	0.431
21	0.534	0.593	0.521	0.462	49	0.640	0.349	0.488	0.745
22	0.708	0.319	0.502	0.462	50	0.395	0.554	0.525	0.468
23	0.559	0.782	0.487	0.687	51	0.394	0.496	0.467	0.478
24	0.236	0.395	0.349	0.520	52	0.369	0.909	0.382	0.484
25	0.556	1.018	0.425	0.604	53	0.786	0.721	0.242	0.457
26	0.264	1.243	0.402	0.393	54	0.462	0.631	0.279	0.389
27	0.749	0.250	0.557	0.455	55	0.720	0.210	0.289	0.317
28	0.469	0.699	0.422	0.858	56	0.026	0.231	0.231	0.231
					Mean	0.511	0.652	0.413	0.494

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART C. SUBJECT 3A

Day	12 Noon-8 P.M. Gm/hr	8 P.M.-12 Mid-night Gm/hr	12 Mid-night-8 A.M. Gm/hr	8 A.M.-12 Noon Gm/hr	Day	12 Noon-8 P.M. Gm/hr	8 P.M.-12 Mid-night Gm/hr	12 Mid-night-8 A.M. Gm/hr	8 A.M.-12 Noon Gm/hr
1	0.485	0.485	0.485	0.608	29	0.605	0.700	0.630	0.731
2	0.585	0.853	0.508	0.336	30	0.369	0.791	0.290	1.188
3	0.449	0.435	0.429	0.430	31	0.417	0.576	0.465	0.549
4	0.430	0.716	0.481	0.861	32	0.288	0.767	0.621	0.508
5	0.663	0.907	0.429	0.150	33	0.508	1.104	0.493	0.420
6	0.593	0.639	0.602	0.419	34	0.783	0.657	0.204	1.057
7	0.607	0.810	0.462	0.645	35	0.388	0.875	0.331	0.525
8	0.600	0.956	0.531	0.439	36	0.525	0.784	0.477	0.365
9	0.356	0.570	0.188	1.092	37	0.365	0.996	0.318	0.861
10	0.580	0.605	0.333	1.017	38	0.431	0.446	0.338	0.835
11	0.429	0.345	0.811	0.352	39	0.660	0.957	0.270	1.142
12	0.789	0.643	0.536	0.536	40	0.344	1.181	0.457	0.583
13	0.644	0.798	0.804	0.322	41	0.583	0.866	0.267	0.961
14	0.721	0.575	0.328	0.854	42	0.375	0.966	0.662	0.928
15	0.680	0.521	0.686	0.683	43	0.509	0.941	0.426	0.426
16	0.461	0.719	0.559	0.851	44	0.416	0.955	0.414	0.805
17	0.798	0.741	0.349	1.148	45	0.805	0.791	0.392	0.657
18	0.481	1.084	0.440	0.613	46	0.657	0.704	0.201	1.211
19	0.613	1.680	0.361	0.505	47	0.703	0.702	0.266	1.043
20	0.650	0.792	0.196	1.086	48	0.330	1.044	0.275	1.125
21	0.569	0.922	0.522	0.972	49	0.558	0.888	0.233	0.776
22	0.458	0.975	0.444	1.024	50	0.545	0.495	0.403	0.844
23	0.644	0.835	0.423	0.726	51	0.819	0.494	0.659	0.983
24	0.288	0.803	0.675	0.675	52	0.394	0.788	0.430	0.594
25	0.484	0.787	0.124	1.418	53	0.594	1.016	0.518	0.518
26	0.286	0.493	0.493	0.636	54	0.458	0.998	0.519	0.616
27	0.896	0.581	0.413	0.882	55	0.616	1.002	0.275	0.918
28	0.753	0.717	0.411	0.411	56	0.675	0.475	0.475	0.443
					Mean	0.548	0.785	0.434	0.738

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART D. SUBJECT 4A

	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M. 12 Noon
Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr	Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.341	0.597	0.597	0.355	29	0.344	1.325	0.429	0.846
2	0.603	0.843	0.389	0.344	30	0.473	1.141	0.284	0.528
3	0.726	0.486	0.319	0.319	31	0.484	0.661	0.337	0.326
4	0.569	0.387	0.233	0.446	32	0.417	0.910	0.336	0.322
5	0.495	0.729	0.375	0.364	33	0.582	0.705	0.435	0.652
6	0.413	0.851	0.349	0.500	34	0.193	1.484	0.563	0.497
7	0.758	0.947	0.564	0.382	35	0.694	0.588	0.313	0.280
8	0.382	1.262	0.308	0.515	36	0.280	1.206	0.420	0.421
9	0.712	0.890	0.231	0.231	37	0.697	0.504	0.460	0.766
10	0.626	0.450	0.272	0.431	38	0.536	0.599	0.459	0.406
11	0.372	0.372	0.326	0.853	39	0.661	0.664	0.353	0.329
12	0.348	0.618	0.275	0.275	40	0.390	0.320	0.590	0.475
13	0.665	0.927	0.294	0.056	41	0.591	1.068	0.419	0.546
14	0.056	0.546	0.388	0.330	42	0.319	0.140	0.445	0.495
15	0.148	1.268	0.783	0.278	43	0.495	1.056	0.804	0.432
16	0.461	0.863	0.408	0.440	44	0.524	1.013	0.341	0.448
17	0.364	1.294	0.072	1.059	45	0.633	0.930	0.470	0.438
18	0.799	0.410	0.410	0.716	46	0.617	0.556	0.320	0.508
19	0.716	0.795	0.047	0.406	47	0.363	0.363	0.752	0.478
20	0.228	0.850	0.314	0.509	48	0.505	0.570	0.326	0.361
21	0.509	1.062	0.349	0.408	49	0.500	1.059	0.405	0.484
22	0.221	1.651	0.384	0.449	50	0.567	0.856	0.428	0.428
23	0.633	0.518	0.353	0.625	51	0.508	0.508	0.488	0.395
24	0.248	0.764	0.330	0.299	52	0.190	1.266	0.347	0.415
25	0.756	0.940	0.326	0.596	53	0.483	0.483	0.828	0.544
26	0.739	0.682	0.385	0.397	54	0.418	0.418	0.615	0.585
27	0.716	0.809	0.375	0.277	55	0.573	0.427	0.347	0.347
28	0.489	1.017	0.322	0.445	56	0.591	0.263	0.263	0.263
					Mean	0.495	0.784	0.401	0.452

TABLE X
(Continued)

URINARY NITROGEN EXCRETION DURING
FOUR DAILY PERIODS THROUGHOUT THE BED REST
(GRAMS PER HOUR)

PART E. SUBJECT 6A

Day	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M.-12 Noon
	Gm/hr	Gm/hr	Gm/hr	Gm/hr		Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.360	0.726	0.726	0.640	29	0.486	1.240	0.540	0.753
2	0.698	0.598	0.360	0.441	30	0.365	1.991	0.525	0.914
3	0.465	1.012	0.546	0.374	31	0.496	0.923	0.491	0.436
4	0.830	0.441	0.611	0.752	32	0.597	0.896	0.435	0.520
5	0.543	0.020	0.203	0.638	33	0.761	0.797	0.627	0.644
6	0.759	0.780	0.552	0.553	34	0.972	0.686	0.538	0.796
7	0.790	0.236	0.300	0.798	35	0.712	0.662	0.445	0.580
8	0.585	1.146	0.511	1.106	36	0.580	0.927	0.550	0.754
9	0.809	0.169	0.560	0.780	37	0.531	1.229	0.536	0.998
10	0.844	0.575	0.474	0.776	38	0.521	0.791	0.349	0.573
11	0.708	0.735	0.411	0.327	39	0.801	0.572	0.417	0.695
12	0.564	0.609	0.576	0.575	40	0.360	0.677	0.698	0.659
13	0.672	0.931	0.570	0.570	41	0.455	1.215	0.598	0.598
14	0.810	0.510	0.489	0.497	42	0.097	1.468	1.080	0.550
15	0.294	1.547	0.388	0.617	43	0.550	0.625	0.397	0.735
16	0.515	0.901	0.462	0.793	44	0.529	0.484	0.504	1.070
17	0.735	1.098	0.629	0.893	45	0.447	1.463	0.579	0.639
18	0.262	1.030	0.613	0.698	46	0.869	0.882	0.504	0.864
19	0.196	0.795	0.370	0.527	47	0.802	0.720	0.477	0.625
20	0.479	0.657	0.553	0.488	48	0.514	0.514	0.464	0.462
21	0.704	0.782	0.652	0.406	49	0.588	1.037	0.650	0.781
22	0.798	1.162	0.423	0.675	50	0.481	0.994	0.608	0.561
23	0.529	0.984	0.606	0.333	51	0.876	0.640	0.563	0.675
24	0.219	0.553	0.505	0.504	52	0.366	0.735	0.537	0.780
25	0.390	0.924	0.589	0.634	53	0.686	0.641	0.510	0.878
26	0.543	0.851	0.516	0.646	54	0.386	1.200	0.641	0.471
27	0.760	0.696	0.648	0.532	55	0.551	1.012	0.456	0.613
28	0.430	1.331	0.459	0.690	56	0.572	0.368	0.368	0.368
					Mean	0.576	0.860	0.525	0.647

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART F. SUBJECT 7A

	12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Midnight	12 Midnight-8 A.M.	8 A.M.-12 Noon
Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr	Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.234	0.585	0.585	0.779	29	0.622	0.964	0.388	0.702
2	0.538	0.448	0.463	0.322	30	0.707	0.868	0.554	0.446
3	0.476	0.774	0.582	0.821	31	0.940	0.741	0.481	0.339
4	0.639	0.509	0.463	0.513	32	0.595	0.994	0.491	0.749
5	0.440	0.733	0.561	0.325	33	0.612	0.871	0.545	0.777
6	0.420	0.694	0.712	0.736	34	0.791	0.618	0.553	0.707
7	0.607	0.556	0.527	0.666	35	0.569	0.979	0.591	0.447
8	0.704	0.557	0.506	0.572	36	0.447	0.879	0.585	0.539
9	0.605	1.051	0.285	0.602	37	0.612	1.013	0.110	1.188
10	0.351	0.834	0.420	0.782	38	0.185	0.667	0.419	1.274
11	0.694	0.527	0.426	0.450	39	0.504	0.873	0.260	1.023
12	0.654	0.488	0.568	0.611	40	0.798	0.459	0.205	1.141
13	0.634	0.608	0.371	0.444	41	0.570	0.613	0.615	0.709
14	0.518	0.540	0.491	0.415	42	0.949	0.878	0.592	0.968
15	0.755	0.673	0.574	0.656	43	0.366	0.375	0.121	1.067
16	0.638	0.542	0.535	0.347	44	0.713	0.602	0.470	0.664
17	0.559	1.280	0.751	0.762	45	0.880	0.468	0.221	0.982
18	0.299	1.259	0.151	1.035	46	0.728	0.479	0.246	1.014
19	0.379	0.990	0.525	0.636	47	0.685	1.160	0.188	0.736
20	0.473	0.513	0.550	0.404	48	0.749	1.224	0.429	0.435
21	0.664	0.772	0.516	0.960	49	0.540	0.495	0.658	0.482
22	0.599	0.506	0.485	1.066	50	0.634	0.653	0.276	0.812
23	0.499	0.541	0.363	0.274	51	0.470	0.462	0.354	0.718
24	0.265	0.285	0.585	0.292	52	0.655	0.465	0.456	0.354
25	0.528	0.754	0.130	0.997	53	0.290	0.610	0.700	0.531
26	0.428	1.153	0.474	0.395	54	0.480	1.250	0.509	0.236
27	0.530	0.557	0.610	0.491	55	0.552	0.677	0.184	1.772
28	0.492	0.581	0.698	0.535	56	0.584	0.392	0.392	0.293
					Mean	0.569	0.715	0.455	0.678

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART G. SUBJECT 8A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night 8 A.M.	8 A.M. 12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr	Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.219	0.457	0.457	0.490	29	0.620	0.984	0.674	0.853
2	0.709	0.399	0.392	0.380	30	0.627	1.021	0.469	0.489
3	0.752	0.574	0.344	0.361	31	0.554	0.527	0.238	1.092
4	0.599	0.546	0.605	0.564	32	0.600	0.964	0.374	0.510
5	0.665	1.121	0.235	0.720	33	0.839	0.712	0.574	0.591
6	0.703	0.489	0.482	0.399	34	0.963	0.474	0.472	0.649
7	0.638	0.554	0.720	0.575	35	0.441	0.581	0.467	0.509
8	0.580	0.394	0.340	0.633	36	0.509	1.050	0.386	0.527
9	0.549	0.752	0.600	0.537	37	0.644	1.271	0.471	0.776
10	0.766	0.490	0.561	0.595	38	0.660	1.018	0.425	0.458
11	0.648	0.446	0.609	0.446	39	0.829	0.721	0.287	0.056
12	0.604	0.338	0.488	0.428	40	0.760	0.022	0.756	0.432
13	0.770	0.489	0.586	0.677	41	0.453	0.444	0.501	0.527
14	0.437	0.713	0.204	0.513	42	0.527	0.750	0.481	0.541
15	0.660	0.552	0.463	0.712	43	0.380	0.495	0.431	0.518
16	0.399	0.425	0.625	0.808	44	0.642	0.489	0.485	0.493
17	0.649	1.378	0.569	0.632	45	0.358	0.827	0.394	0.576
18	0.677	0.992	0.518	0.543	46	0.718	0.895	0.529	0.605
19	0.543	0.532	0.641	0.497	47	0.454	0.738	0.341	0.410
20	0.778	0.589	0.405	0.435	48	0.506	0.586	0.251	0.300
21	0.759	1.168	0.468	0.666	49	0.495	0.563	0.606	0.450
22	0.911	0.643	0.518	0.623	50	0.582	0.901	0.290	0.276
23	0.835	0.473	0.508	0.612	51	0.494	0.265	0.568	0.442
24	0.389	0.380	0.486	0.398	52	0.444	0.554	0.303	0.395
25	0.656	1.139	0.467	0.756	53	0.363	1.012	0.354	0.503
26	0.532	0.956	0.514	0.564	54	0.310	0.768	0.260	0.479
27	0.800	0.610	0.680	0.596	55	0.158	0.618	0.466	0.496
28	0.574	0.840	0.484	0.683	56	0.552	0.334	0.334	0.727
					Mean	0.594	0.679	0.467	0.545

TABLE X

(Continued)

URINARY NITROGEN EXCRETION DURING

FOUR DAILY PERIODS THROUGHOUT THE BED REST

(GRAMS PER HOUR)

PART H. SUBJECT 9A

	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon		12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr	Day	Gm/hr	Gm/hr	Gm/hr	Gm/hr
1	0.119	0.254	0.254	0.650	29	0.759	0.693	0.578	0.423
2	1.328	0.390	0.425	0.353	30	0.641	0.930	0.472	0.674
3	0.486	0.360	0.399	0.228	31	0.658	0.601	0.193	0.791
4	0.571	1.017	0.420	0.540	32	0.469	0.838	0.341	0.48
5	0.741	0.360	0.633	0.483	33	0.614	0.492	0.736	0.529
6	0.601	0.375	0.513	0.570	34	0.596	0.612	0.562	0.499
7	0.612	0.945	0.391	0.587	35	0.675	0.472	0.464	0.592
8	0.648	0.672	0.506	0.638	36	0.592	0.861	0.370	0.500
9	0.725	1.008	0.070	1.272	37	0.621	0.540	0.727	0.634
10	0.666	0.634	0.358	0.594	38	0.535	0.799	0.402	0.400
11	0.496	0.816	0.368	0.441	39	0.327	0.540	0.341	0.472
12	0.581	0.751	0.430	0.375	40	0.644	0.564	0.536	0.409
13	0.723	0.732	0.425	0.710	41	0.512	0.384	0.554	0.812
14	0.560	0.803	0.360	0.078	42	0.464	0.401	0.673	0.455
15	0.428	0.605	0.415	0.488	43	0.511	0.740	0.598	0.394
16	0.638	0.475	0.604	0.783	44	0.594	0.782	0.394	0.404
17	0.703	0.841	0.663	0.280	45	0.634	0.733	0.530	0.564
18	0.384	0.846	0.398	0.571	46	0.645	0.766	0.105	0.817
19	0.517	1.964	0.403	0.495	47	0.551	0.634	0.299	0.512
20	1.009	0.663	0.430	0.535	48	0.532	0.488	0.278	0.517
21	1.351	1.262	0.485	0.473	49	0.580	0.741	0.459	0.390
22	0.538	0.581	0.455	0.573	50	0.520	0.916	0.364	0.362
23	0.476	0.960	0.411	0.337	51	0.608	0.636	0.442	0.424
24	0.653	0.309	0.495	0.383	52	0.690	0.826	0.349	0.484
25	0.330	0.738	0.440	0.640	53	0.543	1.806	0.378	0.755
26	0.569	1.172	0.386	0.900	54	0.615	0.603	0.370	0.750
27	0.225	0.378	0.592	0.409	55	0.486	0.337	0.201	0.875
28	0.277	0.563	0.615	0.609	56	0.397	0.447	0.447	0.447
					Mean	0.589	0.708	0.433	0.538

TABLE XI

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (gm/24 hrs)	Standard Deviation	"t" Value	Probability
SUBJECT 2A				
Pre-Bed Rest Bed Rest	9.002 12.140	1.943 2.142	4.7624	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	9.002 4.524	1.943 2.770	4.7525	$P < 0.001$
Bed Rest Post-Bed Rest	12.140 4.524	2.142 2.770	10.2296	$P < 0.001$
SUBJECT 6A				
Pre-Bed Rest Bed Rest	14.937 14.537	5.700 2.074	0.0731	NS
Pre-Bed Rest Post-Bed Rest	14.937 11.147	5.700 1.571	2.1713	$P < 0.05$
Bed Rest Post-Bed Rest	14.537 11.147	2.074 1.571	5.9100	$P < 0.001$
SUBJECT 7A				
Pre-Bed Rest Bed Rest	8.917 13.143	2.992 1.930	7.3046	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	8.917 7.919	2.992 2.561	0.8750	NS
Bed Rest Post-Bed Rest	13.143 7.919	1.930 2.561	8.8742	$P < 0.001$

TABLE XI (Continued)

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (Gm/24 hrs)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Bed Rest	10.996 13.516	4.819 2.384	4.8731	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	10.996 7.995	4.819 3.540	3.1287	$P < 0.01$
Bed Rest Post-Bed Rest	13.506 7.995	2.384 3.540	11.5086	$P < 0.001$

TABLE XI (Continued)

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

PART C. SUBJECTS 4A, 8A, 9A

(Did not exercise, individual data)

Populations Compared	Means (gm/24 hrs)	Standard Deviation	"t" Value	Probability
SUBJECT 4A				
Pre-Bed Rest Bed Rest	12.544 11.629	3.534 1.851	0.6390	NS
Pre-Bed Rest Post-Bed Rest	12.544 5.847	3.534 2.414	5.3880	$P < 0.001$
Bed Rest Post-Bed Rest	11.629 5.847	1.851 2.414	9.9235	$P < 0.001$
SUBJECT 8A				
Pre-Bed Rest Bed Rest	11.819 12.939	3.773 2.301	1.9777	$P < 0.05$
Pre-Bed Rest Post-Bed Rest	11.819 6.690	3.773 2.254	4.0224	$P < 0.001$
Bed Rest Post-Bed Rest	12.939 6.690	2.301 2.254	9.1995	$P < 0.001$
SUBJECT 9A				
Pre-Bed Rest Bed Rest	8.388 12.740	2.762 2.474	6.4729	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	8.388 5.518	2.762 1.703	2.9542	$P < 0.01$
Bed Rest Post-Bed Rest	12.740 5.518	2.474 1.703	9.9634	$P < 0.001$

TABLE XI (Continued)

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

PART D. SUBJECTS 4A, 8A, 9A

(Did not exercise, combined data)

Populations Compared	Means (gm/24 hrs)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Bed Rest	10.916 12.891	3.839 2.294	4.3975	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	10.916 6.047	3.839 2.208	6.8012	$P < 0.001$
Bed Rest Post-Bed Rest	12.891 6.047	2.294 2.208	16.5626	$P < 0.001$

TABLE XI (Continued)

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (gm/24 hrs)	Standard Deviation	"t" Value	Probability
SUBJECT 1A				
Pre-Bed Rest Bed Rest	11.822 13.044	2.80 1.81	2.0749	$P < 0.05$
Pre-Bed Rest Post-Bed Rest	11.822 5.018	2.80 3.44	5.1208	$P < 0.001$
Bed Rest Post-Bed Rest	13.044 5.018	1.81 3.44	10.8965	$P < 0.001$
SUBJECT 3A				
Pre-Bed Rest Bed Rest	9.501 14.137	3.02 1.57	7.6577	$P < 0.001$
Pre-Bed Rest Post-Bed Rest	9.509 8.101	3.02 2.51	1.2514	NS
Bed Rest Post-Bed Rest	14.137 8.101	1.57 2.51	10.2979	$P < 0.001$

TABLE XI (Continued)

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

PART F. ALL SUBJECTS

Populations Compared	Means (gm/24 hrs)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	10.875	4.089	8.6629	$P < 0.001$
Bed Rest	13.284	2.228		
Pre-Bed Rest	10.875	4.089	7.9463	$P < 0.001$
Post-Bed Rest	6.901	3.179		
Bed Rest	13.284	2.228	23.7409	$P < 0.001$
Post-Bed Rest	6.901	3.179		

TABLE XII

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

PART A. SUBJECT 2A

Populations Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.520 0.659	0.140 0.259	3.4515	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.520 0.417	0.140 0.114	4.1517	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.520 0.499	0.140 0.141	0.7523	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.659 0.417	0.259 0.114	6.2506	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.659 0.499	0.259 0.141	3.9556	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.417 0.499	0.114 0.141	3.3120	$P < 0.001$

TABLE XII (Continued)

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

PART B. SUBJECT 6A

Populations Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.576 0.860	0.191 0.331	5.4822	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.576 0.525	0.191 0.126	1.6385	NS
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.576 0.647	0.191 0.172	2.0489	$P < 0.05$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.860 0.525	0.331 0.126	6.9771	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.860 0.647	0.331 0.172	4.2022	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.525 0.647	0.126 0.172	4.2294	$P < 0.001$

TABLE XII (Continued)

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

PART C. SUBJECT 7A

Populations compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.569 0.715	0.162 0.250	3.6073	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.569 0.455	0.162 0.161	3.6510	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.569 0.678	0.162 0.301	2.3614	$P < 0.02$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.715 0.455	0.250 0.161	6.4220	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.715 0.678	0.250 0.301	0.6869	NS
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.455 0.678	0.161 0.301	4.8119	$P < 0.001$

TABLE XII (Continued)

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

PART D. SUBJECT 2A, 6A, 7A

(Exercised throughout the study, combined data)

Populations Compared	Means (gm/ hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.555 0.745	0.168 0.295	7.2153	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.555 0.466	0.168 0.142	5.2014	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.555 0.609	0.168 0.230	2.4385	$P < 0.02$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.745 0.466	0.295 0.142	10.9747	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.745 0.609	0.295 0.230	4.6889	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.466 0.609	0.142 0.230	6.7990	$P < 0.001$

TABLE XII (Continued)

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

PART E. SUBJECT 4A

Populations Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.495 0.784	0.174 0.328	5.7256	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.495 0.401	0.174 0.147	3.0323	$P < 0.01$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.495 0.452	0.174 0.166	1.3109	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.784 0.401	0.328 0.147	7.8373	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.784 0.452	0.328 0.166	6.6424	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.401 0.452	0.147 0.166	1.6959	$P < 0.10$

TABLE XII (Continued)

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

PART F. SUBJECT 8A

Population Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.594 0.679	0.164 0.275	1.9424	$P < 0.10$
12 Noon - 8 P.M. 12 Midnight - 8 A.M	0.594 0.467	0.164 0.127	4.5015	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.594 0.545	0.164 0.156	1.5966	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.679 0.467	0.275 0.127	5.1448	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.679 0.545	0.275 0.156	3.1133	$P < 0.01$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.467 0.545	0.127 0.156	2.8436	$P < 0.01$

TABLE XII (Continued)

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

PART G. SUBJECT 9A

Populations Compared	Means (gm/hour)	Standard Deviation	Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.589 0.713	0.201 0.316	2.4323	$P < 0.02$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.589 0.438	0.201 0.135	4.5851	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.589 0.542	0.201 0.188	1.2444	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.713 0.438	0.316 0.135	5.8788	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.713 0.542	0.316 0.188	3.4061	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.438 0.542	0.135 0.188	3.3149	$P < 0.001$

TABLE XII (Continued)

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

PART H. SUBJECT 4A, 8A, 9A

(Did not exercise, combined data)

Populations Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.559 0.725	0.186 0.310	5.9123	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.559 0.435	0.186 0.139	6.8783	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.559 0.513	0.186 0.176	2.3269	$P < 0.02$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.725 0.435	0.310 0.139	10.9926	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M.-12 Noon	0.725 0.513	0.310 0.176	7.6674	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.435 0.513	0.139 0.176	4.4692	$P < 0.001$

TABLE XII (Continued)

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

PART I. SUBJECT 3A

(Exercised First 28 days of study)

Population Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.548 0.785	0.150 0.227	6.3745	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.548 0.434	0.150 0.151	3.9370	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.548 0.738	0.150 0.280	4.3894	$P < 0.001$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.785 0.434	0.227 0.151	9.4331	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.785 0.738	0.227 0.280	0.9637	NS
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.434 0.738	0.151 0.280	7.0253	$P < 0.001$

TABLE XII (Continued)

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

PART J. SUBJECT 1A

(Exercised second half of study)

Populations Compared	Means (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.576 0.755	0.185 0.307	3.6727	$P < 0.001$
12 Noon - 8 P.M 12 Midnight - 8 A.M.	0.576 0.377	0.185 0.145	6.1958	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.576 0.617	0.185 0.309	0.8364	NS
8 P.M. - 12 Midnight 12 Midnight - 8 A.M.	0.755 0.377	0.307 0.145	8.1786	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.755 0.617	0.307 0.309	2.3307	$P < 0.02$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.377 0.617	0.145 0.309	5.1547	$P < 0.001$

T A B L E X I I (Continued)

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

PART K. ALL SUBJECTS

Populations Compared	Means (gm/hour)	Deviation	Value	Probability
12 Noon - 8 P.M. 8 P.M. - 12 Midnight	0.558 0.744	0.175 0.295	11.4040	$P < 0.001$
12 Noon - 8 P.M. 12 Midnight - 8 A.M.	0.558 0.439	0.175 0.145	11.0340	$P < 0.001$
12 Noon - 8 P.M. 8 A.M. - 12 Noon	0.558 0.590	0.175 0.241	2.2375	$P < 0.05$
8 P.M. - 12 Midnight 12 Midnight - 8 A.M	0.744 0.439	0.295 0.145	19.5317	$P < 0.001$
8 P.M. - 12 Midnight 8 A.M. - 12 Noon	0.744 0.590	0.295 0.241	8.5153	$P < 0.001$
12 Midnight - 8 A.M. 8 A.M. - 12 Noon	0.439 0.590	0.145 0.241	11.2832	$P < 0.001$

TABLE XIII

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (Gm/24 hrs)	Standard Deviation	"t" Value	Probability
SUBJECT 3A				
Exercise	14.060	1.571	0.4130	NS
No Exercise	13.882	1.583		
SUBJECT 1A				
Exercise	13.333	1.691	0.8500	NS
No Exercise	12.911	1.880		

TABLE XIV

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise Exercise	0.523 0.572	0.151 0.145	1.2081	NS
8 P.M. - 12 Midnight				
No Exercise Exercise	0.824 0.748	0.199 0.245	1.2315	NS
12 Midnight - 8 A.M				
No Exercise Exercise	0.396 0.471	0.130 0.159	1.8598	$P < 0.10$
8 A.M. - 12 Noon				
No Exercise Exercise	0.773 0.704	0.258 0.293	0.9011	NS

T A B L E   X I V   (Continued)

STATISTICAL COMPARISON OF URINARY NITROGEN 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 (gm/hour)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	0.576	0.193	0.0257	NS
Exercise	0.575	0.177		
8 P.M. - 12 Midnight				
No Exercise	0.717	0.278	0.9283	NS
Exercise	0.795	0.330		
12 Midnight - 8 A.M.				
No Exercise	0.412	0.109	1.8477	$P < 0.10$
Exercise	0.340	0.168		
8 A.M. - 12 Noon				
No Exercise	0.542	0.183	1.8711	$P < 0.10$
Exercise	0.697	0.387		

TABLE XV

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

Populations Compared	Means (gm/24 hrs)	Standard Deviation	"t" Value	Probability
Exercisers	13.514	2.381	2.3979	$P < 0.02$
Non-Exercisers	12.893	2.292		

TABLE XVI

STATISTICAL COMPARISON OF URINARY NITROGEN EXCRETION AT DIFFERENT TIMES OF THE DAY BY GROUPS OF SUBJECTS WHO EXERCISED (2A, 6A, 7A) AND WHO DID NOT EXERCISE (4A, 8A, 9A) THROUGHOUT BED REST

Populations Compared	Means (Gm/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
Exercisers	0.555	0.168	0.2286	NS
Non-Exercisers	0.559	0.186		
8 P.M. - 12 Midnight				
Exercisers	0.745	0.295	0.6005	NS
Non-Exercisers	0.725	0.310		
12 Midnight - 8 A.M.				
Exercisers	0.466	0.142	1.9787	$P < 0.05$
Non-Exercisers	0.435	0.139		
8 A.M. - 12 Noon				
Exercisers	0.609	0.230	4.2596	$P < 0.001$
Non-Exercisers	0.513	0.176		

TABLE XVII

CORRELATION COEFFICIENTS BETWEEN URINARY NITROGEN AND URINARY HYDROXYPROLINE EXCRETION OF SUBJECTS PARTICIPATING IN A 56 DAY BED REST

(MG/24 HOURS)

Subjects	Correlation Coefficients	Level of Significance
Subject 1A	0.0957	NS
Subject 2A	0.3922	$P < 0.01$
Subject 3A	0.1573	NS
Subject 4A	0.4024	$P < 0.01$
Subject 6A	0.4099	$P < 0.01$
Subject 7A	0.3518	$P < 0.02$
Subject 8A	0.4778	$P < 0.001$
Subject 9A	0.4413	$P < 0.01$
ALL EIGHT SUBJECTS	0.4266	$P < 0.001$

TABLE XVIII

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART A. SUBJECT 1A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	16.1	14.4	0.6	15.0	+1.1
7-25-69	16.4	9.7	0.6	10.3	+6.1
7-26-69	16.5	13.4	0.6	14.0	+2.5
7-27-69	15.2	13.4	0.6	14.0	+1.2
7-28-69	17.3	18.1	0.6	18.7	-1.4
7-29-69	16.5	14.1	0.6	14.7	+1.8
7-30-69	15.6	13.8	0.6	14.4	+1.2
7-31-69	18.8	12.7	0.6	13.3	+5.5
8-1-69	17.6	13.3	0.6	13.9	+3.7
8-2-69	15.3	14.3	0.6	14.9	+0.4
8-3-69	14.1	14.7	0.6	15.3	-1.2
8-4-69	18.8	10.8	0.6	11.4	+7.4
8-5-69	18.1	13.7	0.6	14.3	+3.8
8-6-69	15.3	12.3	0.6	12.9	+2.4
8-7-69	13.5	7.6	0.6	8.2	+5.3
8-8-69	18.8	12.7	0.6	13.3	+5.5
8-9-69	17.3	13.9	0.6	14.5	+2.8
8-10-69	14.8	12.8	0.6	13.4	+1.4
8-11-69	13.3	12.4	0.6	13.0	+0.3
8-12-69	18.8	13.5	0.6	14.1	+4.7
8-13-69	17.3	11.7	0.6	12.3	+5.0
8-14-69	15.3	12.7	0.6	13.3	+2.0
8-15-69	13.3	14.0	0.6	14.6	-1.3
8-16-69	18.8	11.3	0.6	11.9	+6.9
8-17-69	17.4	9.5	0.6	10.1	+7.3
8-18-69	15.3	12.7	0.6	13.3	+2.0
8-19-69	14.1	14.7	0.6	15.3	-1.2
8-20-69	18.3	12.4	0.6	13.0	+5.3
8-21-69	17.3	13.8	0.6	14.4	+2.9
8-22-69	15.2	15.1	0.6	15.7	-0.5
8-23-69	13.3	12.1	0.6	12.7	+0.6
8-24-69	18.5	7.6	0.6	8.2	+10.3
8-25-69	17.4	14.3	0.6	14.9	+2.5
8-26-69	15.2	14.3	0.6	14.9	+0.3
8-27-69	12.7	14.6	0.6	15.2	-2.5
8-28-69	18.8	13.0	0.6	13.6	+5.2
8-29-69	17.1	12.1	0.6	12.7	+4.4
8-30-69	15.1	15.0	0.6	15.6	-0.5
8-31-69	12.0	11.9	0.6	12.5	-0.5

T A B L E X V I I I (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART A., CONTINUED. SUBJECT 1A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-1-69	18.4	13.0	0.6	13.6	+4.8
9-2-69	16.2	14.5	0.6	15.1	+1.1
9-3-69	15.2	13.2	0.6	13.8	+1.4
9-4-69	12.8	14.6	0.6	15.2	-2.4
9-5-69	18.5	13.1	0.6	13.7	+4.8
9-6-69	16.5	14.7	0.6	15.3	+1.2
9-7-69	14.3	13.1	0.6	13.7	+0.6
9-8-69	11.8	13.8	0.6	14.4	-2.6
9-9-69	18.5	12.0	0.6	12.6	+5.9
9-10-69	13.7	13.8	0.6	14.4	-0.7
9-11-69	14.0	13.8	0.6	14.4	-0.4
9-12-69	11.9	15.2	0.6	15.8	-3.9
9-13-69	16.5	14.4	0.6	15.0	+1.5
9-14-69	16.3	14.1	0.6	14.7	+1.6
9-15-69	14.5	14.2	0.6	14.8	-0.3
9-16-69	9.7	13.7	0.6	14.3	-4.6
9-17-69	17.7	13.2	0.6	13.8	+3.9
Mean	15.8	13.0	0.6	13.6	2.2

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART B. SUBJECT 2A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	16.1	9.6	0.6	10.2	+5.9
7-25-69	16.4	6.8	0.6	7.4	+9.0
7-26-69	16.5	12.2	0.6	12.8	+3.7
7-27-69	15.2	15.0	0.6	15.6	-0.4
7-28-69	15.7	10.5	0.6	11.1	+4.6
7-29-69	16.5	12.6	0.6	13.2	+3.3
7-30-69	15.6	12.8	0.6	13.4	+2.2
7-31-69	18.8	7.9	0.7	8.6	+10.2
8-1-69	18.2	11.6	0.7	12.3	+5.9
8-2-69	15.3	12.6	0.7	13.3	+2.0
8-3-69	13.7	8.8	0.7	9.5	+4.2
8-4-69	18.8	12.0	0.7	12.7	+6.1
8-5-69	18.2	10.0	0.7	10.7	+7.5
8-6-69	15.0	11.3	0.7	12.0	+3.0
8-7-69	14.1	10.3	0.7	11.0	+3.1
8-8-69	18.8	12.9	0.7	13.6	+5.2
8-9-69	17.8	12.4	0.7	13.1	+4.7
8-10-69	15.3	14.0	0.7	14.7	+0.6
8-11-69	14.1	12.4	0.7	13.1	+1.0
8-12-69	18.8	12.3	0.7	13.0	+5.8
8-13-69	16.6	10.6	0.7	11.3	+5.3
8-14-69	14.8	12.7	0.7	13.4	+1.4
8-15-69	13.7	12.8	0.7	13.5	+0.2
8-16-69	18.8	14.2	0.7	14.9	+3.9
8-17-69	18.2	8.3	0.7	9.0	+9.2
8-18-69	15.3	14.3	0.7	15.0	+0.3
8-19-69	14.1	11.7	0.7	12.4	+1.7
8-20-69	18.2	13.3	0.7	14.0	+4.2
8-21-69	17.6	13.3	0.7	14.0	+3.6
8-22-69	15.3	14.2	0.7	14.9	+0.4
8-23-69	13.7	13.4	0.7	14.1	-0.4
8-24-69	18.5	13.0	0.7	13.7	+4.8
8-25-69	18.2	13.3	0.7	14.0	+4.2
8-26-69	14.8	13.6	0.7	14.3	+0.5
8-27-69	13.3	13.2	0.7	13.9	-0.6
8-28-69	18.8	12.0	0.7	12.7	+6.1
8-29-69	18.2	12.2	0.7	12.9	+5.3
8-30-69	14.4	16.8	0.7	17.5	-3.1
8-31-69	13.3	9.3	0.7	10.0	+3.3

T A B L E X V I I I (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART B., CONTINUED. SUBJECT 2A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-1-69	18.5	9.0	0.7	9.7	+8.8
9-2-69	18.2	14.0	0.7	14.7	+3.5
9-3-69	14.9	12.5	0.7	13.2	+1.7
9-4-69	13.3	14.4	0.7	15.1	-1.8
9-5-69	18.2	13.4	0.7	14.1	+4.1
9-6-69	13.6	12.9	0.7	13.6	±0.0
9-7-69	14.9	11.4	0.7	12.1	+2.8
9-8-69	12.7	11.2	0.7	11.9	+0.8
9-9-69	17.4	11.9	0.7	12.6	+4.8
9-10-69	16.9	10.8	0.7	11.5	+5.4
9-11-69	14.7	13.4	0.7	14.1	+0.6
9-12-69	13.6	11.4	0.7	12.1	+1.5
9-13-69	16.6	10.8	0.7	11.5	+5.1
9-14-69	13.7	11.6	0.7	12.3	+1.4
9-15-69	8.7	12.9	0.7	13.6	-4.9
9-16-69	4.4	10.0	0.7	10.7	-6.3
9-17-69	18.5	10.2	0.7	10.9	+7.6
Mean	15.7	12.1	0.7	12.8	+2.9

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART C. SUBJECT 3A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	13.2	7.1	0.3	7.4	+5.8
7-25-69	16.6	12.1	0.3	12.4	+4.2
7-26-69	16.5	13.5	0.3	13.8	+2.7
7-27-69	15.2	10.5	0.3	10.8	+4.4
7-28-69	17.3	13.6	0.3	13.9	+3.4
7-29-69	16.5	13.0	0.3	13.3	+3.2
7-30-69	15.6	13.8	0.3	14.1	+1.5
7-31-69	18.8	14.4	0.5	14.9	+3.9
8-1-69	17.4	14.6	0.5	15.1	+2.3
8-2-69	15.3	11.0	0.5	11.5	+3.8
8-3-69	14.1	13.8	0.5	14.3	-0.2
8-4-69	18.8	12.7	0.5	13.2	+5.6
8-5-69	18.2	15.3	0.5	15.8	+2.4
8-6-69	15.3	16.0	0.5	16.5	-1.2
8-7-69	13.1	14.1	0.5	14.6	-1.5
8-8-69	18.8	15.7	0.5	16.2	+2.6
8-9-69	17.0	14.4	0.5	14.9	+2.1
8-10-69	15.3	16.6	0.5	17.1	-1.8
8-11-69	13.0	14.2	0.5	14.7	-1.7
8-12-69	18.8	14.1	0.5	14.6	+4.2
8-13-69	17.7	14.3	0.5	14.8	+2.9
8-14-69	15.3	16.3	0.5	16.8	-1.5
8-15-69	14.1	15.2	0.5	15.7	-1.6
8-16-69	18.8	14.8	0.5	15.3	+3.5
8-17-69	15.8	13.6	0.5	14.1	+1.7
8-18-69	12.5	13.7	0.5	14.2	-1.7
8-19-69	14.1	10.7	0.5	11.2	+2.9
8-20-69	17.7	16.3	0.5	16.8	+0.9
8-21-69	17.9	13.8	0.5	14.3	+3.6
8-22-69	11.7	15.6	0.5	16.1	-4.4
8-23-69	14.1	13.2	0.5	13.7	+0.4
8-24-69	18.7	11.5	0.5	12.0	+6.7
8-25-69	17.3	12.4	0.5	12.9	+4.4
8-26-69	15.3	14.1	0.5	14.6	+0.7
8-27-69	13.8	14.7	0.5	15.2	-1.4
8-28-69	15.8	11.4	0.5	11.9	+3.9
8-29-69	15.6	14.7	0.5	15.2	+0.4
8-30-69	15.3	12.9	0.5	13.4	+1.9
8-31-69	13.8	11.3	0.5	11.8	+2.0

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART C., CONTINUED. SUBJECT 3A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-1-69	17.7	15.8	0.5	16.3	+1.4
9-2-69	15.6	13.5	0.5	14.0	+1.6
9-3-69	15.3	16.4	0.5	16.9	-1.6
9-4-69	11.0	15.9	0.5	16.4	-5.4
9-5-69	18.6	12.9	0.5	13.4	+5.2
9-6-69	18.2	13.7	0.5	14.2	+4.0
9-7-69	15.3	15.4	0.5	15.9	-0.6
9-8-69	13.7	14.5	0.5	15.0	-1.3
9-9-69	18.8	14.7	0.5	15.2	+3.6
9-10-69	15.8	13.5	0.5	14.0	+1.8
9-11-69	15.3	13.0	0.5	13.5	+1.8
9-12-69	11.4	14.9	0.5	15.4	-4.0
9-13-69	18.8	17.7	0.5	18.2	+0.6
9-14-69	14.3	12.1	0.5	12.6	+1.7
9-15-69	14.6	15.0	0.5	15.5	-0.9
9-16-69	11.4	14.3	0.5	14.8	-3.4
9-17-69	16.4	14.8	0.5	15.3	+1.1
Mean	15.7	14.1	0.5	14.6	+1.1

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART D. SUBJECT 4A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	15.9	9.3	0.3	9.6	+6.3
7-25-69	16.3	11.3	0.3	11.6	+4.7
7-26-69	16.5	12.7	0.3	13.0	+3.5
7-27-69	15.2	11.6	0.3	11.9	+3.3
7-28-69	17.1	9.7	0.3	10.0	+7.1
7-29-69	16.5	11.3	0.3	11.6	+4.9
7-30-69	15.6	11.5	0.3	11.8	+3.8
7-31-69	18.8	15.9	0.4	16.3	+2.5
8-1-69	18.0	12.7	0.4	13.1	+4.9
8-2-69	15.3	12.0	0.4	12.4	+2.9
8-3-69	14.1	10.7	0.4	11.1	+3.0
8-4-69	18.8	10.5	0.4	10.9	+7.9
8-5-69	18.2	8.6	0.4	9.0	+9.2
8-6-69	14.6	11.3	0.4	11.7	+2.9
8-7-69	13.3	7.1	0.4	7.5	+5.8
8-8-69	18.8	13.6	0.4	14.0	+4.8
8-9-69	16.9	12.2	0.4	12.6	+4.3
8-10-69	14.8	12.8	0.4	13.2	+1.6
8-11-69	11.9	14.1	0.4	14.5	-2.6
8-12-69	17.9	11.7	0.4	12.1	+5.8
8-13-69	16.5	9.8	0.4	10.2	+6.3
8-14-69	14.8	12.7	0.4	13.1	+1.7
8-15-69	12.1	13.2	0.4	13.6	-1.5
8-16-69	17.9	12.4	0.4	12.8	+5.1
8-17-69	17.3	8.9	0.4	9.3	+8.0
8-18-69	14.8	14.8	0.4	15.2	-0.4
8-19-69	13.3	13.3	0.4	13.7	-0.4
8-20-69	17.9	13.1	0.4	13.5	+4.4
8-21-69	17.3	12.3	0.4	12.7	+4.6
8-22-69	14.8	14.9	0.4	15.3	-0.5
8-23-69	12.1	12.7	0.4	13.1	-1.0
8-24-69	16.9	10.5	0.4	10.9	+6.0
8-25-69	16.7	10.9	0.4	11.3	+5.4
8-26-69	14.8	13.6	0.4	14.0	+0.8
8-27-69	12.1	14.0	0.4	14.4	-2.3
8-28-69	17.0	11.5	0.4	11.9	+5.1
8-29-69	16.5	12.1	0.4	12.5	+4.0
8-30-69	14.8	14.3	0.4	14.7	+0.1
8-31-69	12.1	12.0	0.4	12.4	-0.3
9-1-69	16.9	12.1	0.4	12.5	+4.4

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART D., CONTINUED. SUBJECT 4A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-2-69	16.7	11.0	0.4	11.4	+5.3
9-3-69	14.8	14.5	0.4	14.9	-0.1
9-4-69	11.6	8.7	0.4	9.1	+2.5
9-5-69	16.8	16.4	0.4	16.8	0.0
9-6-69	15.8	12.8	0.4	13.2	+2.6
9-7-69	13.8	14.3	0.4	14.7	-0.9
9-8-69	12.1	11.8	0.4	12.2	-0.1
9-9-69	14.5	12.3	0.4	12.7	+1.8
9-10-69	16.3	10.4	0.4	10.8	+5.5
9-11-69	12.1	13.4	0.4	13.8	-1.7
9-12-69	11.4	13.0	0.4	13.4	-2.0
9-13-69	15.8	11.6	0.4	12.0	+3.8
9-14-69	16.5	11.0	0.4	11.4	+5.1
9-15-69	10.9	14.6	0.4	15.0	-4.1
9-16-69	11.4	12.3	0.4	12.7	-1.3
9-17-69	16.7	10.5	0.4	10.9	+5.8
Mean	14.2	11.6	0.4	12.0	+2.2

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART E. SUBJECT 6A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	16.1	12.6	0.3	12.9	+3.2
7-25-69	16.4	14.2	0.3	14.5	+1.9
7-26-69	16.5	12.6	0.3	12.9	+3.6
7-27-69	15.2	13.6	0.3	13.9	+1.3
7-28-69	16.5	16.3	0.3	16.6	-0.1
7-29-69	16.5	12.6	0.3	12.9	+3.6
7-30-69	15.6	15.8	0.3	16.1	-0.5
7-31-69	18.8	12.9	0.5	13.4	+5.4
8-1-69	18.2	17.8	0.5	18.3	-0.1
8-2-69	15.3	14.8	0.5	15.3	±0.0
8-3-69	14.1	15.9	0.5	16.4	-2.3
8-4-69	17.7	13.2	0.5	13.7	+4.0
8-5-69	17.8	13.9	0.5	14.4	+3.4
8-6-69	15.3	15.9	0.5	16.4	-1.1
8-7-69	14.1	14.4	0.5	14.9	-0.8
8-8-69	18.8	14.1	0.5	14.6	+4.2
8-9-69	17.6	14.6	0.5	15.1	+2.5
8-10-69	15.3	18.9	0.5	19.4	-4.1
8-11-69	13.3	13.9	0.5	14.4	-1.1
8-12-69	18.8	9.8	0.5	10.3	+8.5
8-13-69	18.2	12.8	0.5	13.3	+4.9
8-14-69	15.3	15.6	0.5	16.1	-0.8
8-15-69	13.5	19.1	0.5	19.6	-6.1
8-16-69	18.8	14.3	0.5	14.8	+4.0
8-17-69	18.2	10.0	0.5	10.5	+7.7
8-18-69	14.9	14.1	0.5	14.6	+0.3
8-19-69	13.6	14.5	0.5	15.0	-1.4
8-20-69	18.6	16.2	0.5	16.7	+1.9
8-21-69	17.5	15.2	0.5	15.7	+1.8
8-22-69	15.3	16.2	0.5	16.7	-1.4
8-23-69	12.0	18.7	0.5	19.2	-7.2
8-24-69	18.8	13.3	0.5	13.8	+5.0
8-25-69	18.2	13.9	0.5	14.4	+3.8
8-26-69	15.3	16.9	0.5	17.4	-2.1
8-27-69	12.9	18.0	0.5	18.5	-5.6
8-28-69	18.8	14.2	0.5	14.7	+4.1
8-29-69	17.8	15.8	0.5	16.3	+1.5
8-30-69	15.3	17.4	0.5	17.9	-2.6
8-31-69	14.0	12.4	0.5	12.9	+1.1
9-1-69	18.8	14.8	0.5	15.3	+3.5

T A B L E X V I I I (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART E., CONTINUED. SUBJECT 6A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-2-69	16.8	13.8	0.5	14.3	+2.5
9-3-69	15.3	15.7	0.5	16.2	-0.9
9-4-69	13.6	17.5	0.5	18.0	-4.4
9-5-69	18.8	13.0	0.5	13.5	+5.3
9-6-69	18.2	14.5	0.5	15.0	+3.2
9-7-69	15.3	16.6	0.5	17.1	-1.8
9-8-69	12.9	18.0	0.5	18.5	-5.6
9-9-69	18.8	15.6	0.5	16.1	+2.7
9-10-69	17.1	11.7	0.5	12.2	+4.9
9-11-69	15.3	17.2	0.5	17.7	-2.4
9-12-69	12.3	14.9	0.5	15.4	-3.1
9-13-69	18.8	16.8	0.5	17.3	+1.5
9-14-69	16.0	13.3	0.5	13.8	+2.2
9-15-69	15.3	15.6	0.5	16.1	-0.8
9-16-69	10.5	14.9	0.5	15.4	-4.9
9-17-69	18.8	14.6	0.5	15.1	+3.7
Mean	16.2	14.5	0.4	14.9	+1.3

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART F. SUBJECT 7A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	15.9	8.7	0.4	9.1	+6.8
7-25-69	16.6	12.0	0.4	12.4	+4.2
7-26-69	16.5	11.1	0.4	11.5	+5.0
7-27-69	15.2	14.8	0.4	15.2	±0.0
7-28-69	16.9	12.9	0.4	13.3	+3.6
7-29-69	16.5	12.2	0.4	12.6	+3.9
7-30-69	15.6	14.8	0.4	15.2	+0.4
7-31-69	18.8	14.0	0.6	14.6	+4.2
8-1-69	17.9	14.2	0.6	14.8	+3.1
8-2-69	15.3	13.8	0.6	14.4	+0.9
8-3-69	13.5	12.6	0.6	13.2	+0.3
8-4-69	18.8	12.9	0.6	13.5	+5.3
8-5-69	18.2	14.1	0.6	14.7	+3.5
8-6-69	15.3	12.2	0.6	12.8	+2.5
8-7-69	13.1	11.9	0.6	12.5	+0.6
8-8-69	18.8	15.2	0.6	15.8	+3.0
8-9-69	18.2	12.9	0.6	13.5	+4.7
8-10-69	15.3	18.5	0.6	19.1	-3.8
8-11-69	13.8	12.8	0.6	13.4	+0.4
8-12-69	18.8	13.7	0.6	14.3	+4.5
8-13-69	18.2	11.8	0.6	12.4	+5.8
8-14-69	15.3	16.3	0.6	16.9	-1.6
8-15-69	13.3	15.0	0.6	15.6	-2.3
8-16-69	17.9	10.2	0.6	10.8	+7.1
8-17-69	18.2	9.1	0.6	9.7	+8.5
8-18-69	15.2	12.2	0.6	12.8	+2.4
8-19-69	13.3	13.4	0.6	14.0	-0.7
8-20-69	18.5	13.3	0.6	13.9	+4.6
8-21-69	16.8	14.0	0.6	14.6	+2.2
8-22-69	14.8	14.6	0.6	15.2	-0.4
8-23-69	14.1	15.3	0.6	15.9	-1.8
8-24-69	18.6	15.7	0.6	16.3	+2.3
8-25-69	17.9	15.7	0.6	16.3	+1.6
8-26-69	15.0	15.8	0.6	16.4	-1.4
8-27-69	13.3	16.1	0.6	16.7	-3.4
8-28-69	18.5	15.0	0.6	15.6	+2.9
8-29-69	14.8	13.9	0.6	14.5	+0.3
8-30-69	14.7	14.6	0.6	15.2	-0.5
8-31-69	13.3	12.6	0.6	13.2	+0.1

T A B L E X V I I I (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART F., CONTINUED. SUBJECT 7A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-1-69	15.8	13.7	0.6	14.3	+1.5
9-2-69	14.9	14.4	0.6	15.0	-0.1
9-3-69	13.9	14.8	0.6	15.4	-1.5
9-4-69	13.8	19.7	0.6	20.3	-6.5
9-5-69	16.4	9.7	0.6	10.3	+6.1
9-6-69	14.6	14.5	0.6	15.1	-0.5
9-7-69	13.7	14.6	0.6	15.2	-1.5
9-8-69	11.8	13.8	0.6	14.4	-2.6
9-9-69	16.3	14.6	0.6	15.2	+1.1
9-10-69	14.3	16.1	0.6	16.7	-2.4
9-11-69	14.2	13.5	0.6	14.1	+0.1
9-12-69	13.0	13.1	0.6	13.7	-0.7
9-13-69	18.4	11.3	0.6	11.9	+6.5
9-14-69	12.5	12.2	0.6	12.8	-0.3
9-15-69	14.2	12.5	0.6	13.1	+1.1
9-16-69	12.3	13.9	0.6	14.5	-2.2
9-17-69	17.9	15.7	0.6	16.3	+1.6
Mean	15.9	13.1	0.5	13.6	+2.3

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART G. SUBJECT 8A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	15.9	10.1	0.8	10.9	+5.0
7-25-69	16.3	9.2	0.8	10.0	+6.3
7-26-69	16.5	11.9	0.8	12.7	+3.8
7-27-69	14.7	12.5	0.8	13.3	+1.4
7-28-69	16.8	14.1	0.8	14.9	+1.9
7-29-69	16.5	14.6	0.8	15.4	+1.1
7-30-69	15.6	13.0	0.8	13.8	+1.8
7-31-69	18.8	15.4	0.3	15.7	+3.1
8-1-69	18.2	11.5	0.3	11.8	+6.4
8-2-69	15.3	14.3	0.3	14.6	+0.7
8-3-69	14.1	15.0	0.3	15.3	-1.2
8-4-69	18.8	13.6	0.3	13.9	+4.9
8-5-69	18.2	11.8	0.3	12.1	+6.1
8-6-69	15.3	15.5	0.3	15.8	-0.5
8-7-69	14.1	10.0	0.3	10.3	+3.8
8-8-69	18.8	14.0	0.3	14.3	+4.5
8-9-69	18.2	13.1	0.3	13.4	+4.8
8-10-69	14.8	17.8	0.3	18.1	-3.3
8-11-69	14.1	15.7	0.3	16.0	-1.9
8-12-69	18.8	13.6	0.3	13.9	+4.9
8-13-69	18.2	13.6	0.3	13.9	+4.3
8-14-69	15.3	17.2	0.3	17.5	-2.2
8-15-69	14.1	16.5	0.3	16.8	-2.7
8-16-69	18.8	15.2	0.3	15.5	+3.3
8-17-69	18.2	10.1	0.3	10.4	+7.8
8-18-69	15.3	16.6	0.3	16.9	-1.6
8-19-69	14.1	14.4	0.3	14.7	-0.6
8-20-69	18.8	16.7	0.3	17.0	+1.8
8-21-69	18.2	14.5	0.3	14.8	+3.4
8-22-69	15.3	17.7	0.3	18.0	-2.7
8-23-69	14.1	14.8	0.3	15.1	-1.0
8-24-69	18.8	12.8	0.3	13.1	+5.7
8-25-69	18.2	13.7	0.3	14.0	+4.2
8-26-69	15.3	16.5	0.3	16.8	-1.5
8-27-69	14.1	16.0	0.3	16.3	-2.2
8-28-69	18.7	11.6	0.3	11.9	+6.8
8-29-69	18.2	13.5	0.3	13.8	+4.4
8-30-69	14.8	17.1	0.3	17.4	-2.6
8-31-69	14.1	14.6	0.3	14.9	-0.8
9-1-69	15.8	12.0	0.3	12.3	+3.5

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART G., CONTINUED. SUBJECT 8A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-2-69	16.5	13.9	0.3	14.2	+2.3
9-3-69	14.2	11.5	0.3	11.8	+2.4
9-4-69	13.3	13.2	0.3	13.5	-0.3
9-5-69	18.2	10.5	0.3	10.8	+7.4
9-6-69	17.3	12.9	0.3	13.2	+4.1
9-7-69	14.8	11.6	0.3	11.9	+2.9
9-8-69	11.5	16.0	0.3	16.3	-4.8
9-9-69	11.6	10.9	0.3	11.2	+0.4
9-10-69	14.2	9.6	0.3	9.9	+4.3
9-11-69	11.2	12.9	0.3	13.2	-2.0
9-12-69	10.2	11.7	0.3	12.0	-1.8
9-13-69	16.6	11.3	0.3	11.6	+5.0
9-14-69	14.9	9.8	0.3	10.1	+4.8
9-15-69	9.0	11.8	0.3	12.1	-3.1
9-16-69	13.3	9.5	0.3	9.8	+3.5
9-17-69	18.0	9.4	0.3	9.7	+8.3
Mean	15.8	12.9	0.5	13.4	+2.4

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART H. SUBJECT 9A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
7-24-69	15.9	9.6	0.3	9.9	+6.0
7-25-69	16.6	6.6	0.3	6.9	+9.7
7-26-69	16.5	17.0	0.3	17.3	-0.8
7-27-69	14.7	9.4	0.3	9.7	+5.0
7-28-69	17.3	14.2	0.3	14.5	+2.8
7-29-69	16.5	14.4	0.3	14.7	+1.8
7-30-69	15.6	12.7	0.3	13.0	+2.6
7-31-69	18.8	14.2	0.4	14.6	+4.2
8-1-69	18.2	14.5	0.4	14.9	+3.3
8-2-69	15.3	15.5	0.4	15.9	-0.6
8-3-69	14.1	13.1	0.4	13.5	+0.6
8-4-69	18.8	11.9	0.4	12.3	+6.5
8-5-69	18.2	12.6	0.4	13.0	+5.2
8-6-69	15.3	14.9	0.4	15.3	±0.0
8-7-69	14.1	8.3	0.4	8.7	+5.4
8-8-69	18.8	11.1	0.4	11.5	+7.3
8-9-69	18.2	15.0	0.4	15.4	+2.8
8-10-69	15.0	15.4	0.4	15.8	-0.8
8-11-69	14.1	11.9	0.4	12.3	+1.8
8-12-69	18.8	17.2	0.4	17.6	+1.2
8-13-69	17.4	17.3	0.4	17.7	-0.3
8-14-69	15.3	21.6	0.4	22.0	-6.7
8-15-69	14.1	12.7	0.4	13.1	+1.0
8-16-69	18.8	12.3	0.4	12.7	+6.1
8-17-69	18.2	11.9	0.4	12.3	+5.9
8-18-69	15.3	11.7	0.4	12.1	+3.2
8-19-69	14.1	15.9	0.4	16.3	-2.2
8-20-69	18.8	9.7	0.4	10.1	+8.7
8-21-69	18.2	11.8	0.4	12.2	+6.0
8-22-69	15.3	15.2	0.4	15.6	-0.3
8-23-69	14.1	15.3	0.4	15.7	-1.6
8-24-69	18.8	12.4	0.4	12.8	+6.0
8-25-69	18.2	11.7	0.4	12.1	+6.1
8-26-69	15.3	14.9	0.4	15.3	±0.0
8-27-69	14.1	13.7	0.4	14.1	±0.0
8-28-69	18.8	13.4	0.4	13.8	+5.0
8-29-69	18.2	13.1	0.4	13.5	+4.7
8-30-69	15.3	15.5	0.4	15.9	-0.6
8-31-69	14.1	12.3	0.4	12.7	+1.4

TABLE XVIII (Continued)

BALANCE DATA FOR NITROGEN DURING A 56-DAY BED REST ON EVALUATION OF FLIGHT FOODS UNDER HYPOKINETIC CONDITIONS

PART H., CONTINUED SUBJECT 9A

Date	Intake	Excretion			Balance
		Urinary	Fecal	Total	
9-1-69	18.8	9.4	0.4	9.8	+9.0
9-2-69	18.2	13.3	0.4	13.7	+4.5
9-3-69	15.3	13.3	0.4	13.7	+1.6
9-4-69	14.1	12.5	0.4	12.9	+1.2
9-5-69	18.8	13.4	0.4	13.8	+5.0
9-6-69	17.3	12.6	0.4	13.0	+4.3
9-7-69	15.3	14.5	0.4	14.9	+0.4
9-8-69	13.5	12.3	0.4	12.7	+0.8
9-9-69	9.3	11.4	0.4	11.8	-2.5
9-10-69	16.9	10.5	0.4	10.9	+6.0
9-11-69	13.9	12.8	0.4	13.2	+0.7
9-12-69	13.1	12.2	0.4	12.6	+0.5
9-13-69	16.2	12.6	0.4	13.0	+3.2
9-14-69	17.3	13.6	0.4	14.0	+3.3
9-15-69	11.1	17.6	0.4	18.0	-6.9
9-16-69	10.3	13.3	0.4	13.7	-3.4
9-17-69	16.1	10.3	0.4	10.7	+5.4
Mean	16.1	12.7	0.4	13.1	+3.0