CIRCADIAN RHYTHMS AND THE EFFECT OF EXERCISE ON URINARY HYDROXYPROLINE EXCRETION IN HEALTHY ADULT MEN DURING RECUMBENCY AND AMBULATION

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We hereby recommend that the thesis prepared under our supervision by Marla McBee entitled CIRCADIAN RHYTHMS AND THE EFFECT OF EXERCISE ON URINARY HYDROXYPROLINE EXCRETION IN HEALTHY ADULT MEN DURING RECUMBENCY AND AMBULATION

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

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INTRODUCTION

Forty to fifty-five per cent of body collagen is in the matrix of the bone and hydroxyproline constitutes approximately 13 per cent of collagen. It is thought that the level of excretion of urinary hydroxyproline peptides reflects the metabolism of collagen and thus provides a valuable index of bone and collagen metabolism.

One of the complications of prolonged weightlessness is that of a loss of skeletal calcium and bone substance. Studies of astronauts have revealed abnormal losses of calcium and reduced bone density as a result of weightlessness incurred during space flights.

The nearest condition to weightlessness, corresponding to the conditions present in space capsules, that man has been able to simulate is recumbency. This thesis is based on an immobilization study conducted by a team of research workers at the Texas Woman's University Research Institute. This study included many biochemical analyses, of which the author was responsible for the urinary hydroxyproline determinations, with the purpose of evaluating the effects of bed rest immobilization on bone density as well as physiologic and metabolic changes in the healthy young adult male.

REVIEW OF THE LITERATURE

HYDROXYPROLINE

Hydroxyproline is a nonessential amino acid which constitutes approximately 13 per cent of collagen and one to two per cent of elastin. It is present in no other tissues in the body in significant amounts as reported by Ziff et al. (79).

Hydroxy-L-proline was discovered by Fisher(20) in gelatin hydrolysate in 1902. Gustavson(28)(29) reported its presence in gelatins and collagens, Steward et al. (72) found it bound in alfalfa protein, Sisakyan(66) in sugar-beet protein, Belozerskii(3) in Sarcina lutea, Losee et al. (45) in dentine protein, Machly and Paleus(46) in horse-radish peroxidase, Hackman(30) in lutea proteins of insect cuticle, and Brockmann and Pampus(6) in the antibiotic actinomycin $X_{o\beta}$.

Auclair et al. (2) reported that hydroxy-L-proline occurs in the free state in pollen, Joslyn and Stepka(3)(5) found it in prunes, Auclair and Dubrevil(1) in the haemolymph of Drososophila melanogaster, Davis and Williams(12) in the sporulation medium of Bacillus alobigii, and Drilhon et al. (13) in blood and Malpighian tubes of the larvae of Bombyx mori infected with polyhedral disease.

Collagen is one of the scleroproteins and accounts for about 30 per cent of the body proteins. It exerts an architectural function

throughout the body, most notably in skin, tendons, cartilage, blood vessels, connective tissue, organ capsules, and bone matrix. Sjoerdsma et al. (65) reported its fundamental importance in the pathogenesis of various diseases, in the processes of growth and aging, and in the healing of wounds. Underfriend(75) reported that collagen is not only the most abundant, but one of the most unusual animal proteins in that it is devoid of cysteine and tryptophan and contains more than 30 percent glycine and such unusual amino acids as hydroxyproline and hydroxylysine.

Tropocollagen is believed to be the basic building block of collagen. It is composed of three helical polypeptide chains wound around each other to give a superhelix. This monomer has a molecular weight of about 300, 000 and is about 15 Å in diameter and 2, 800 Å in length. According to Gross(27) these polypeptide subunits are initially linked only by weak forces such as hydrogen bonds, but with maturation the subunits become bound to each other in covalent linkage. In a study reported by Sjoerdsma et al. (65) the molecules overlap each other by about one quarter of their length, which accounts for the characteristic 640 to 700 Å bands observed under the electron microscope. It has been shown by Rubin and co-workers(61) that there are three forms of tropocollagen, depending on the degree of intramolecular cross-linking of the subunits present. These have been referred to as α, β, and γ forms. The exact nature of the

intramolecular cross-linkages present in α, β, and γ-tropocollagen is unknown, but they have been shown to be split by pepsin, and this has led to the suggestion that they are primarily peptide bonds. One of the chief characteristics of collagen is the property of being converted by boiling water or acid to gelatin. This process as observed by Oser(50) does not seem to be one of simple hydrolysis but rather a physical change, the separation of the three strands of the collagen helix.

FORMATION OF HYDROXYPROLINE IN COLLAGEN

The biosynthesis of collagen differs from the conventional scheme for the synthesis of protein in respect that hydroxyproline and hydrosylysine are not incorporated in collagen. Stetten and Schoenheimer(71) and Stetter(70) showed that free proline could serve as a precursor of collagen hydroxyproline, but free hydroxyproline did not incorporate in collagen *in vivo*. These observations have been verified *in vitro* and *in vivo* by several investigators(26)(78)(51). Other investigators(5)(37) have established the same relationship between lysine and hydroxylysine.

A great deal of information concerning the overall mechanism of hydroxylation has been obtained with intact chick embryos. Fujimoto and Tamiya(22) have shown that the hydroxyl moiety of collagen hydroxyproline is derived from molecular oxygen and not from water.

Other investigators(17)(54) provided evidence that proline is hydroxylated by an oxygenase mechanism. Proline is converted to hydroxyproline by a direct displacement type of hydroxylation, where one hydrogen is released from carbon atom in position four of the pyrrolidine ring.

Direct oxygenase mechanism in hydroxylation of proline was confirmed by Fujita et al. (23) by the preparation of cis- and trans-4-^{3}H-L-proline with the observation that only the tritium at the trans-position to the carboxyl group was lost during the synthesis of collagen hydroxyproline in chick embryos. Other investigators(57)(76) think that the mechanism of the hydroxylation of lysine is most likely to be similar to that of proline.

Investigations for the biosynthesis of collagen are generally based on the appearance of protein-bound hydroxyproline or hydroxylysine. Juva(36) found that both peptide synthesis and hydroxylation are involved in the conversion of proline and lysine to protein-bound hydroxyproline and hydroxylysine respectively. Hydroxylation of proline and lysine can occur at the stage of free proline or lysine, prolyl- or lysyl- adenylate, prolyl- or lysyl-sRNA, or ribosomal peptide-bound proline or lysine, or after the release of peptide chains from ribosomes. The parent amino acid destined for the hydroxylation must occur in a sequestered or bound form, that distinguishes it from the proline or lysine, which is incorporated into collagen and

other proteins but remains unhydroxylated.

There has been considerable controversy as to the site at which proline is hydroxylated. Several investigators(73)(11)(47)(33) believe that hydroxylation occurs prior to the formation of the peptide linkage, in which case it is suggested that activated proline or prolyl-sRNA are the immediate precursors. At this time the most widely accepted theory is that proline is hydroxylated after it is incorporated into peptide or protein linkages. Other investigators(70)(51) hold this opinion. Recently Juva(36) demonstrated that after the synthesis of the polypeptide precursor of collagen, a system containing a soluble fraction of chick embryo homogenate could be hydroxylated.

Undenfriend(74) has suggested a generalized scheme for the hydroxylating mechanism: The ribosome accepts amino acyl sRNA molecules destined for collagen. As polymerization proceeds, two conditions begin to be met which allow proline and lysine residues in peptide linkages to become substrates for the hydroxylase: (1) the peptide grows to a minimum size, and (2) the two amino acids become incorporated into definite sequences which can be recognized by the specific hydroxylases. In the presence of sufficient hydroxylating enzymes and cofactors, hydroxylation keeps pace with peptide synthesis so that when the protein chain is completed it is fully hydroxylated. Under such conditions hydroxylation occurs during

the process of translation. When hydroxylation is limited, ribosomal peptide-synthesizing mechanisms are, for a time at least, unaffected and continue to elaborate the usual chain, which is, however, deficient in or devoid of hydroxylated residues. No large quantities of unhydroxylated "collagen" have been accumulated experimentally. It should be noted that collagen is composed of two different peptide chains and that each contains hydroxylated amino acids. The same enzyme may be responsible for hydroxylation of both chains.

METABOLISM

In metabolic balance studies Sjoerdsma et al. (65) found that 85 per cent of 50 mg. of bound hydroxyproline ingested undergoes intestinal absorption and that about seven and one half mg./day is recovered in the feces. Of the hydroxyproline that is absorbed, more than 90 per cent is the free amino acid. The blood plasma contains free, peptide-bound, and protein-bound hydroxyproline. Most of the free hydroxyproline is rapidly metabolized to carbon dioxide and water. The major source of peptide-bound hydroxyproline in blood is thought to be soluble collagen in bone, skin, tendons, and other collagen depots. Both free and peptide hydroxyproline are filtered by the glomerulus. The free amino acid undergoes 99 per cent tubular resorption and less than one mg./day appears in the urine. Peptide-bound hydroxyproline is only 80 per

cent resorbed and about 25 mg./day is excreted.

When Lindstedt and Prockop(43) followed the specific activity of urinary hydroxyproline after the injection of radioactive proline into young rats, they found evidence suggestive of at least three different pools of hydroxyproline, with half lives of one, five, and 50 to 100 days. The pool with the long half-life was thought to be insoluble collagen. The other pools were considered to represent the metabolically active soluble collagens which contribute most to urinary hydroxyproline.

Prockop has since expanded these studies(53). Proline-C^{14} was injected into young rats which were killed after 15 hours, one week, or four weeks. The skin of each rat was removed, homogenized, and a "soluble collagen" fraction was then extracted from the residue with hot water. The remaining carcass of the rat was treated in a similar manner to obtain additional soluble and insoluble collagen fractions. After 15 hours the specific activity of the hydroxyproline in the urine was one and a half times greater than that in soluble collagen and three times greater than that in insoluble collagen. After four weeks, the specific activity of urinary hydroxyproline was three to five times greater than that in soluble collagen but less than that in insoluble collagen. These results suggest that some forms of body collagen are metabolically active. Part of the soluble collagen is degraded rapidly, and some catabolism of insoluble collagen also

occurs. These data indicate that catabolism of insoluble collagen furnishes a significant contribution to urinary hydroxyproline, in addition to that derived from the soluble collagen pool.

The first biological material from man studied extensively for hydroxyproline content was urine. Sjoerdsma and associates(65) found that total urinary hydroxyproline consists of one to three per cent free hydroxyproline and more than 95 per cent in a peptide-bound form. Fourteen different hydroxyproline peptides have been isolated and identified in human urine. The dipeptide, prolyl-hydroxy-proline peptides and the tripeptide, glycyl-prolyl-hydroxy-proline about 15 per cent of the hydroxyproline peptides excreted. The amino acid composition and sequences of 75 per cent of isolated urinary peptides are identical with known sequences in collagen. The composition of 10 of the remaining 12 peptides is also consistent with their origin from collagen.

Prockop and Sjoerdsma(55) studied several factors that might affect the urinary excretion of hydroxyproline. They found that urinary excretion of hydroxyproline did not decrease when subjects were changed from a normal to a low hydroxyproline diet, or to an isocaloric low protein diet. No diurnal variation in urinary hydroxyproline was found, and hydration or dehydration did not alter excretion.

Ingestion of hydroxyproline as the free amino acid resulted in

an increased excretion of free hydroxyproline, but no change in the excretion of bound hydroxyproline, the peptide form which normally accounts for nearly all of the hydroxyproline in urine. Ingestion of large amounts of hydroxyproline in the form of gelatin, however, increased the excretion of hydroxyproline peptides.

These findings suggest that the bound hydroxyproline normally found in urine arises from collagen breakdown and that the amount excreted is an index of the rate of degradation of this protein.

HYDROXYPROLINE AND GROWTH

Forty per cent of body collagen is in the matrix of the bone and this bone collagen has been found to turn over more rapidly than collagen in other tissues by Gould et al. (25). It has been suggested by Dull and associates(15) that bone provides a reservoir of mature collagen, the catabolism of which gives rise to most urinary hydroxyproline. Smiley and Ziff(67) think that the reorganization of bone structure which occurs during growth could provide an area of continuous breakdown and resynthesis of collagen.

In 1956 Ziff, Kibrick, Dresner, and Gribetz(79) conducted a study in which total urinary excretion of hydroxyproline was determined in patients with rheumatoid arthritis, collagen diseases,

miscellaneous diseases, in normal individuals, and in a group of children, both normal and with a variety of diseases.

Because hydroxyproline is present almost entirely in collagen, it was felt that if there were a significant deviation from normal in the metabolism of collagen or an increase in degradation of this protein in patients with collagen diseases, these changes might be reflected by increased levels of excretion of this amino acid.

While there was no significant difference in the excretion of total hydroxyproline in the groups of normal individuals, patients with miscellaneous diseases, rheumatoid arthritis, and collagen diseases, the excretion of total hydroxyproline in a miscellaneous group of children was two to three times greater than that of the adult group. This increased excretion did not appear to be related to the presence or absence of disease. It was suggested that the level of urinary hydroxyproline might be related to the size of the metabolically active, soluble collagen pool available for breakdown.

Other investigations by Jason et al. (34) were carried out to determine the relationship between growth and hydroxyproline excretion in man. Total hydroxyproline excretion was determined in normal and hospitalized adults and children, in children with dwarfism, and in patients with acromegaly. Total excretion was significantly greater

in children in the 10- to 14-year old group than in adults.

When calculated per unit body surface area, it was greater in all childhood age groups. Peak levels of excretion per square meter coincided with periods of active growth. Children with dwarfism showed decreased excretion of hydroxyproline. Administration of growth hormone to two children with pituitary dwarfism and of thyroid hormone to three children with cretinism resulted in a prompt rise in excretion, which attained levels characteristic of rapidly growing children. After cessation of growth hormone administration, excretion fell to pretreatment levels. One patient with active acromegly excreted markedly increased amounts of hydroxyproline. After irradiation therapy, these fell toward normal.

Growth hormone is known to be responsible for collagen deposition in growing individuals. Daughday and Marez(10) have demonstrated this in vitro by the incorporation of proline-C^{14} into the collagen of cartilage under the influence of growth hormone. It has been demonstrated by Snipes and Kostyo(69) that, like insulin, growth hormone promotes cellular uptake of amino acids and increases protein synthesis. Korner(41) has demonstrated accerated incorporation of amino acids into protein using cell-free rat liver preparations from hypophysectomized animals treated with growth hormone. He attributed this effect to the microsome.

It is of interest that only the hormones concerned with bone metabolism are effective in altering hydroxyproline excretion. Scrow (63) observed that growth hormone and thyroid hormone promote bone matrix formation and maturation, while Reiss et al. (60) found gonadal hormones to cause cessation of bone growth. Since gonadal hormones also lower gonadotropin levels, they appear to bear a reciprocal relationship to hydroxyproline excretion. Rasmussen(59) observed that parathyroid hormone causes bone mineral resorption with consequent disruption of bone collagen but apparently has no effect on formation of new bone matrix.

Growth hormone does not have the same effect on premature infants and on newborn infants as it does on older children and adults. It has been found by Ducharme and Grumbach(14) that the premature infant is relatively unresponsive to the action of exogenous growth hormone. Chiumello et al. (7) conducted an investigation to determine the relationship between bone growth and metabolism of premature infants who were treated with human growth hormone.

They found that it was not possible to register any increase of linear bone growth after hormone administration. At the same time urinary hydroxyproline excretion did not change significantly. They concluded that the metabolic response of premature infants to human growth hormone differs consistently from that normally observed in

more mature subjects. Similiarly, Vest et al. (77) reported that growth hormone acts on metabolism in newborn infants in essentially the same way in older children or adults, but to a lesser extent.

HYDROXYPROLINE AND DISEASE

Beginning with the report by Ziff et al. (79), who found that urinary hydroxyproline levels are increased with growing children, other investigators began studying the levels of excretion of urinary hydroxyproline in relation to such "disease states" as malabsorption, bone mineral loss, distruction of collagen, "collagen" disease, endocrine diseases, and states characterized by increased osteoblastic activity.

The urinary excretion of hydroxyproline was found to be increased in patients with adult celiac disease and other malabsorption states, such as regional enteritis, pancreatic carcinoma, gastroileostomy, radiation of the small intestine, and primary biliary cirrhosis. Improvement of the malabsorption was found to be associated with a return toward normal of the hydroxyproline excretion level. Other investigators(8)(62) believe that the increased hydroxyproline excretion reflects an increased turnover of collagen and may be related to the osteomalacia which accompanies malabsorption states.

Subjects undergoing a gradual loss of bone mineral such as

that which occurs, for example, in senile osteoporosis as reported by Lenzi et al. (42), or in hyperparathyroidism without bone lesions, as described by Klein et al. (39, had urinary hydroxyproline values within the normal ranges. Apparently, such a gradual loss of even considerable amounts of collagen from the bone matrix over an extended period in the absence of new bone synthesis, produces only a negligible increment in daily urinary hydroxyproline. However, this does not explain the increase in the proportion of free to bound hydroxyproline in the urine as reported by Lenzi et al. (42) in patients with senile osteoporosis.

A number of other conditions in which destruction of body collagen occurs also are associated with marked elevation of hydroxyproline excretion. The elevated excretion in patients with large burns has been reported by Klein et al. (40). Hyperparathyroidism with bone lesions has been reported by Klein et al. (39) and by Dull et al. (15), and to some extent Paget's disease, also reported by Dull and associates(15), would fall in this catagory. Wound repair in burn patients would be associated with a spurt in new collagen synthesis as well as a breakdown.

Hydroxyproline excretion has been found to be normal in such "collagen diseases" as scleroderma and dermatomyositis by Smith and associates(68). Patients with rheumatoid arthritis have been shown to have excretion values in the normal and high-normal range by Ziff et al. (79). It has also been found by Smith et al. (68) that urinary

hydroxyproline excretion was increased in individuals in the active phase of rheumatoid arthritis, but not in patients in the chronic phase of this disease.

Undenfriend(75) observed increased hydroxyproline excretion in such endocrine diseases as acromegaly, hyperthyroidism, hypergonadotropin conditions, and in one patient with the Fanconi syndrome. However, normal excretion was found in patients with hypopituitarism, hypothyroidism, Cushing's syndrome, pheochromocytoma, and aldosteroma. On giving hormonal agents, increased excretion was present only after thyroid hormone administration, and no change was observed on giving ACTH, TSH, cortisol, or an anabolic agent

Increased hydroxyproline excretion has been observed in patients having increased osteoblastic activity such as growth by Vest et al. (77), acromegaly by Benoit et al. (4), Paget's disease by Dull and Henneman(15) and Goidanich et al. (24), after the administration of growth hormone by Benoit et al. (4), recovering from breaks and fractures by Crabbe et al. (8), metastatic cancer of the bone by Platt et al. (52) and Prockop(53), and in Marfan's syndrome by Sjoerdsma and associates(64).

HYDROXYPROLINE AND MENTAL RETARDATION

Efron reports(16) that large amounts of free hydroxyproline were found to accumulate in the blood and urine of a thirteen-year-old mentally retarded girl.

The urinary hydroxyproline was identified as L-4-hydroxyproline. The patient has no apparent collagen disease, and the peptide-bound hydroxyproline, which is known to reflect collagen turnover, was normal.

Evidence that the accumulation of free hydroxyproline in the patient is the result of deficient activity of the enzyme hydroxyproline oxidase, which normally catalyzes the first step in hydroxyproline degradation, was presented.

The relation between the mental retardation and the elevated hydroxyproline concentration is not established. This is the only known patient with the disorder, and the biochemical defect was discovered in the course of a survey of a retarded population.

The blood hydroxyproline concentration was not lowered by a hydroxyproline-free diet. No therapy is at present available for this disorder.

If it should be established that hydroxyprolinemia causes mental retardation, there is no immediate prospect of therapy. Unlike phenylketonuria, in which essential amino acids are accumulated, the plasma level of hydroxyproline in hydroxyprolinemia cannot be lowered by removal of the amino acid from the diet. This is likely to be true of all inborn errors of metabolism with accumulation of amino acids that are nonessential and freely synthesized. No therapy would be possible until other means besides simple dietary management become available

to lower the plasma concentration of the accumulated amino acid.

CIRCADIAN RHYTHMS

Physiological rhythms are now well-recognized throughout the plant and animal kingdom. Many body functions undergo variations recurring at roughly 24-hour intervals, hence the term "circadian" was coined by Halberg(31). (Circa, "about", and dies, "days").

Curiosity about circadian rhythms is relatively recent, largely prompted by observations of desynchronization after time-zone changes in the course of flight. Struehold(74) reports that participants of such flights experience discomfort, fatigue, and loss of efficiency in adjustment to a new time table. Similiar rhythm dislocations occur in submarine crews, astronauts, and in industrial workers on night work. Other investigators(21)(38) suggest that confinement stress can lead to alterations of circadian rhythmicity.

The most commonly recognized example of a circadian rhythm in man is the recurrent phenomenon of nocturnal sleep. Other circadian rhythms which have been clearly demonstrated by several investigators (44)(19)(49)(9)(48)(58)(18) are those of fluctuations in the blood eosinophil count, the serum iron content, of body temperature, heart rate and blood pressure, of urine production, of urine electrolytes, amino-acids, and hormones. No circadian rhythm of urinary hydroxyproline excretion was found in the literature.

Hellbrugge(32) observed that diurnal rhythms of some physiologic functions in men, like body temperature, sleep-wakefulness and motility cannot be found until after birth. It is Halberg's(31) opinion that the periodic character of circadian rhythms - their periodicity as such - seems to be an acquisition of the species rather than the individual; it is more endogenous than exogenous. It is primarily the external timing of rhythms rather than their periodicity which is dictated largely by the environment.

PLAN OF PROCEDURE

The data presented in this thesis were obtained on six healthy adult human male subjects who participated in a study designed to provide a 1.0 gram dietary level of calcium, 100 grams of protein, and 2400 calories, together with optimum levels of other major nutrients, per day during two 28-day bed rest phases with preliminary, interim, and subsequent ambulatory periods during which corresponding diets were fed.

The study was conducted at the Nelda Childers Stark Laboratory for Human Nutrition Research of the Texas Woman's University Research Institute, as part of a more extensive investigation sponsored by the National Aeronautics and Space Administration. The study was designed to examine the response of healthy subjects to conditions which closely resemble those encountered by astronauts during space flight.

PERIODS OF STUDY

This study consisted of two bed rest periods accompanied by ambulatory periods as follows:

Equilibration Period, 29 days, June 2 - July 2, 1968

Bed Rest Number One, 28 days, July 2 - July 30, 1968

Interim Ambulatory Period, 14 days, July 30 - August 13, 1968

Bed Rest Number Two, 28 days, August 13 - September 10, 1968

Post-Bed Rest Period, 14 days, September 10 - September 24, 1968

A record was made of height and weight changes throughout the study. Male orderlies attended to the hygienic needs of the subjects during immobilization.

Name	Age (years)	Height (inches)	Weight (pounds) Beginning	End	Occupation
AA	25	70 1/4	152.5	147	University Student
BB	22	72	152	149.5	University Student
EE	22	74 1/2	181	176.25	University Student
FF	20	69 1/2	155	149	University Student
GG	24	70 1/4	170	164.75	University Student
HH	21	69	170	165.5	University Student

Equilibration Period

During this period, which lasted 29 days, the subjects led a normal life. They worked at various tasks in the laboratory for eight hours per day. The diet, high in all nutrients, was fed to each of the subjects during this time in order to equilibrate all of them before the first bed rest began, with particular attention given to the calcium level.

Bed Rest Number One

The first bed rest covered a span of 28 days, during which time the men were completely immobilized. They assumed a horizontal position on a single bed equipped with one pillow. They did not 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 television was watched on hospital type television sets. During this period of immobilization, male orderlies were present round-the-clock to attend to the hygienic needs of the subjects and to prevent undue movement. All the subjects were spoon fed and a record was kept of all items of their individual food consumption per meal.

During this phase of the study 50 microcuries of ^{47}calcium in the form of $^{47}CaCl_3$ was incorporated in 40 milliliters of milk on the first morning of immobilization. Blood and urine samples then were collected simultaneously at 15 minutes after ingestion of the isotope, and then at 45, 135, 255, 465, and 765 minutes after collection of the initial sample. Thereafter, blood and urine were collected simultaneously at 8 A. M. every other day for two weeks. The dietary intake during immobilization was planned to contain 1.0 gram of calcium and 100 grams of protein per day, as noted.

During immobilization, x-rays were taken each day. Each man was lifted by three or four men from the bed to a mobile stretcher cart for transportation across the hall to the x-ray laboratory, where they

were carefully lifted onto the x-ray table. The x-ray table, the stretcher and the bed were made the same height.

Urine and serum samples were collected six times on the first day of immobilization. Urine samples were collected four times at three-hour intervals on the second day of immobilization. Thereafter, urine samples were collected at 8 A.M., 12 Noon, and 8 P.M. daily. Serum samples were collected at 8 A.M. every other day until no radioactivity was detected, which in this bed rest was about three weeks.

INTERIM AMBULATORY PERIOD

During this 14-day period of mobilization, the six subjects engaged daily in compulsory physical activity, golf and bowling. They worked in the laboratory for short periods during the times when they were not engaged in the physical exercise program. Meals were consumed in the metabolic ward under the supervision of the dietitian. These meals were planned to contain 1000 milligrams of calcium each day and 100 grams of protein.

Bed Rest Number Two

The variable in this 28-day bed rest was the introduction of an exercise program, using the Exer-Genie and Exer-Grip Exercisers. Measurements of hand and foot action, squeeze, and isometrics were made with the Lufkin Anthropometric (woven) Tape with a Gurlick Spring Attachment (3176 ME). The measurements of hand and foot action

were started on the second day of immobilization, while the squeeze exercise was introduced on the third day and isometrics were begun on the fourth day of immobilization. Three subjects were allowed to exercise "at will" and three men volunteered to exercise according to a set schedule under strict supervision.

As occurred in Bed Rest One, ^{47}calcium was ingested in 40 milliliters of milk on the first morning of immobilization. Serum and urine samples were collected four times at three-hour intervals. These four collections occurred one hour later than those of Bed Rest One. Serum again was collected at 8 A.M. every other morning until no radioactivity could be detected.

In this bed rest, exercise was studied, three subjects at will and three per schedule, as noted. Daily urinary output was kept separate for each man for each void, and this method of collection increased the total number of urine samples obtained in Bed Rest Number One. Each subject was encouraged to have an 8 A.M. and a 12 Noon sample, although they sometimes were not able to produce a sample at these times. Each of the individual urine samples was analyzed for urinary nitrogen, total calcium, ^{47}calcium, phosphorus, 17-ketosteroids, 17-hydroxycorticosteroids, hydroxyproline, creatine, and creatinine.

With the above listed exceptions of exercise, frequency of urine

collection, and collection of urine and serum four times of the second day of immobilization, the conditions and procedures followed during this bed rest were the same as those described in Bed Rest Number one.

Post-Bed Rest Period

This final period of the study lasted for 14 days. During this time the subjects engaged in supervised physical activity as much as their physical condition permitted. During this final period the six subjects continued to consume 1000 milligrams of calcium per day, and 100 grams of protein.

ANALYSIS OF HYDROXYPROLINE FROM URINE

The method of analysis of urinary hydroxyproline used is a modification of the method presented by Prockop and Udenfriend(56).

Total Urinary Hydroxyproline:

1. Urine is collected under 20 ml toluene for 24 hours and kept refrigerated, and then frozen until used.
2. One ml urine is placed in a tube that can be sealed and the volume made up to 2.0 cc with H_2O in duplicate.
3. Add 2.0 ml concentrated HCl(Hydrolysis is maximum at 6N).
4. Seal tubes with blowtorch. These can be stored several days in deep freeze.
5. Auto clave three hours (124°), store in deep freeze (-20°C) until use.

Removal of Pigments:

6. Break seal on autoclaved samples with pliers and towel.
7. Add 4.0 cc H_2O - to total volume of 8.0 cc (Final normality should not be less than 2N).
8. Add an amount not exceeding the equivalent of one cc Dowex-Charcoal resin (0.5 cc usually sufficient) and mix well with Vortex.
9. Centrifuge 15 minutes at 2,000 RPM. (During centrifugation standards and blanks may be made.)
10. Take 4.0 cc of supernatant immediately with 4 cc volumetric pipette, being careful not to disturb resin precipitate, and put in large

culture tubes. These with standards and blank may be stored at -20° C for one or two days.

Preparation of Standard and Reagent Blanks:

11. Stock standard is 100 umole hydroxyproline per ml. Working standard is made at time of pigment removal by adding 0.1 ml of the 100 umole/ml stock standard to a 10cc volumetric flask and making volume with H_2O). Working standard is thus 1.0 umole per ml. Range of the colorimetric determination is from 0.1 to 1.0 umole. Usually two 0.5 umole standards are used:

    1.0 ml working standard is placed in a centrifuge tube and 11ml H_2O added, followed by 4cc concentrated HCl. This is mixed and 4.0cc added to a large culture tube at the same time as decolorized samples are being put in culture tubes. Degeneration during storage will therefore reflect in the standard.

12. Reagent blank: 12 cc H_2O and 4 cc HCl mixed with 4 cc added to a culture tube.

Colorimetric Analysis:

13. Add one drop 1% phenolphthalein.
14. Add 2.5 ml 5N KOH.
15. Add 1 N KOH dropwise to faint pink color. Colors may vary, but if only one drop produces color, pH will not be high enough to alter reactions, since buffer will be added later.
16. Add KCl in minimal excess to saturation.

17. Add 2 ml borate buffer.
18. Add 1 ml 10% alanine. Mix with Vortex.
19. Add 2 cc 0.2 Mlchloramine T, made fresh daily.
20. Let oxidation proceed at room temperature for 20 minutes.
21. After 20 minutes reaction is stopped by adding 6.0 ml 3.6 M sodium thiosulfate. Mix with Vortex.
22. Add approximately 10 cc toluene and shake at high speed with shaker for 10 minutes.
23. Centrifuge 1/4 to 1/2 speed for 10 minutes.
24. Remove toluene phase via vacuum, being careful not to remove any of aqueous phase, even if this requires leaving a small residual of toluene.
25. Cap tubes and place in boiling water bath for 30 minutes.
26. Cool under running tap. When next step is instituted procedure must be carried straight through to completion.
27. Add exactly 10 ml toluene.
28. Shake 2 minutes on shaker.
29. Centrifuge 1/4 to 1/2 speed for 10 minutes.
30. Take exactly 5 cc toluene phase with 5 ml volumetric pipette and put in cuvette.
31. Add precisely 2 cc Ehrlich's reagent and stir rapidly.
32. Allow to stand 15 minutes.
33. Read at 560 on Zeiss, DU, or DB. (Valid range of OD readings is from .150 to 1.50 although best if below 1.0).

PRESENTATION OF RESULTS

The daily excretions of urinary hydroxyproline for each subject are shown in Table I in the Appendix. The periodic excretions of urinary hydroxyproline during Bed Rest I and the Interim Period are shown in Tables II, and III. The statistical comparisons are shown in Tables IV, V, and VI.

URINARY HYDROXYPROLINE EXCRETION AND RECUMBENCY

The six subjects excreted an average of 32,943 mg. of hydroxyproline per 24 hours during the Pre-Bed Rest Period, 63.760 mg./24 hours during Bed Rest I, 38.087 mg./24 hours during the Interim Period, 56.274 mg./24 hours during Bed Rest II, and 47.674 mg./24 hours during the Post-Bed Rest Period.

The excretion of urinary hydroxyproline was significantly higher during Bed Rest I as compared with the Pre-Bed Rest Period ($P<.001$), and with the Interim Period ($P<.001$).

URINARY HYDROXYPROLINE EXCRETION AND EXERCISE

At the initiation of Bed Rest II, three subjects (Subjects BB, FF, and HH) volunteered to exercise according to a daily routine. The others (Subjects AA, EE, and GG) exercised "at will". The subjects who exercised regularly had a mean 24 hour hydroxyproline excretion of 56.082

mg. which statistically was significant ($P<0.05$) when compared with Bed Rest I in which there was no exercise. The subjects who exercised "at will" had a mean daily excretion of 56.466 mg. which also was statistically significant ($P<0.05$). The urinary hydroxyproline excretion for all six subjects during Bed Rest II with exercise was significantly lower ($P<0.01$) than the hydroxyproline excretion from Bed Rest I with no exercise. This is exemplified in Figure I.

CIRCADIAN RHYTHMS AND HYDROXYPROLINE EXCRETION

Urine was collected during Bed Rest I and the Interim Period at periods ending at 8 A.M., 12 Noon, and 8 P.M. to observe the possibility of a circadian rhythm of hydroxyproline excretion in man. In Bed Rest I the average excretion of all six subjects was the highest period ending at 12 Noon (3.328 mg./hour), the second highest the period ending at 8 P.M. (2.963 mg./hour) and the lowest the period ending at 8 A.M. (2.165 mg./hour). By employing the "t" test it was shown that the hydroxyproline excretion was significantly higher in the period ending 12 Noon ($P<0.001$). This is illustrated in Figure II.

In the Interim Period the average excretion of all six subjects was again the highest in the period ending at 12 Noon (3.626 mg./hour), the second highest the period ending at 8 P.M. (2.080 mg./hour), and the lowest the period ending at 8 A.M (2.000 mg./hour). There was no significant difference in the excretions between the periods ending at

8 P. M. and 8 A. M. but the urinary hydroxyproline excretion during the period ending at 12 Noon was highly significant ($P<0.001$) when compared with the other two periods. This is illustrated in Figure III.

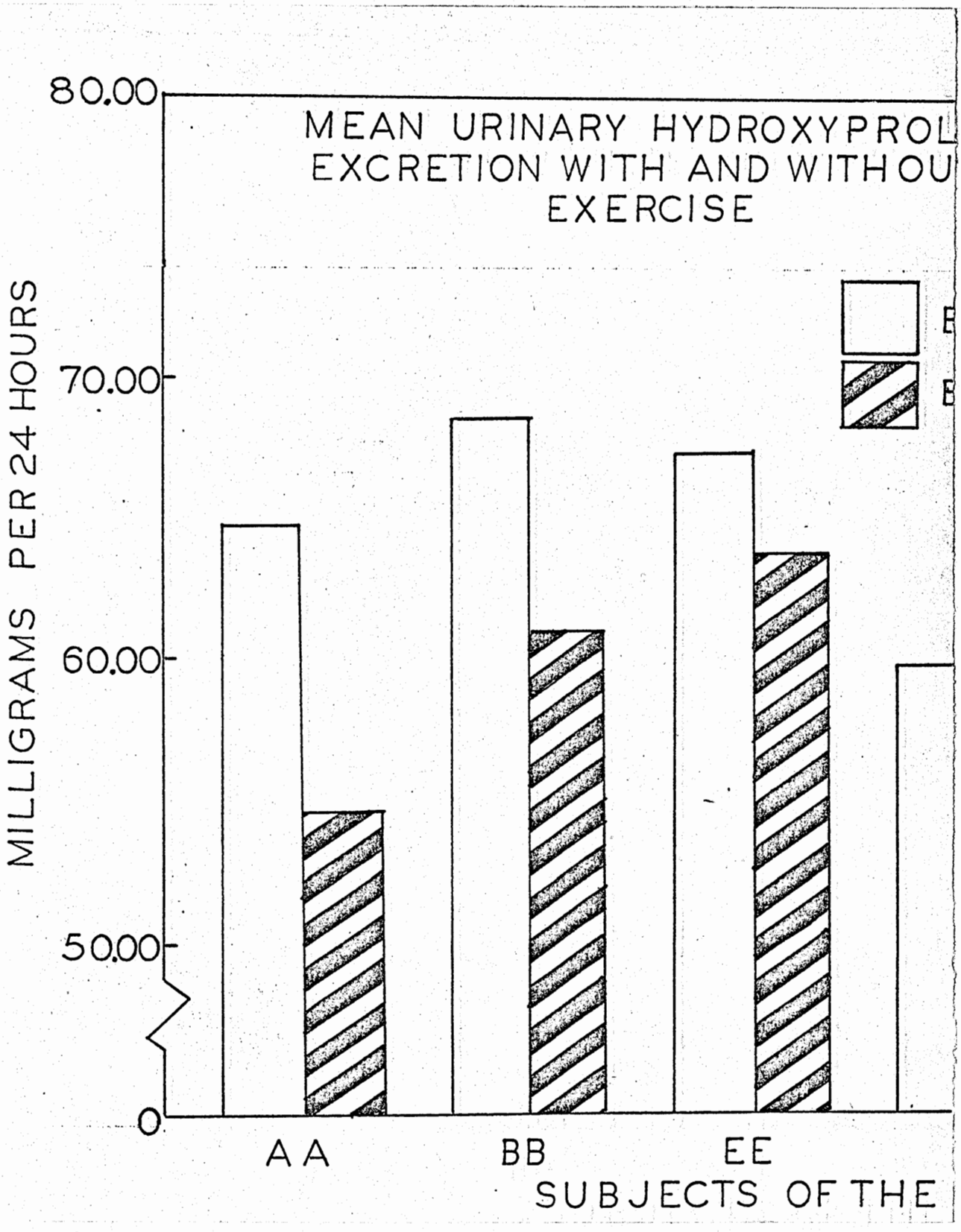

Figure I. COMPARISON OF MEAN URINARY HYDROXYPROLINE EXCRETION DURING BED REST I (NO EXERCISE) AND BED REST II (EXERCISE)

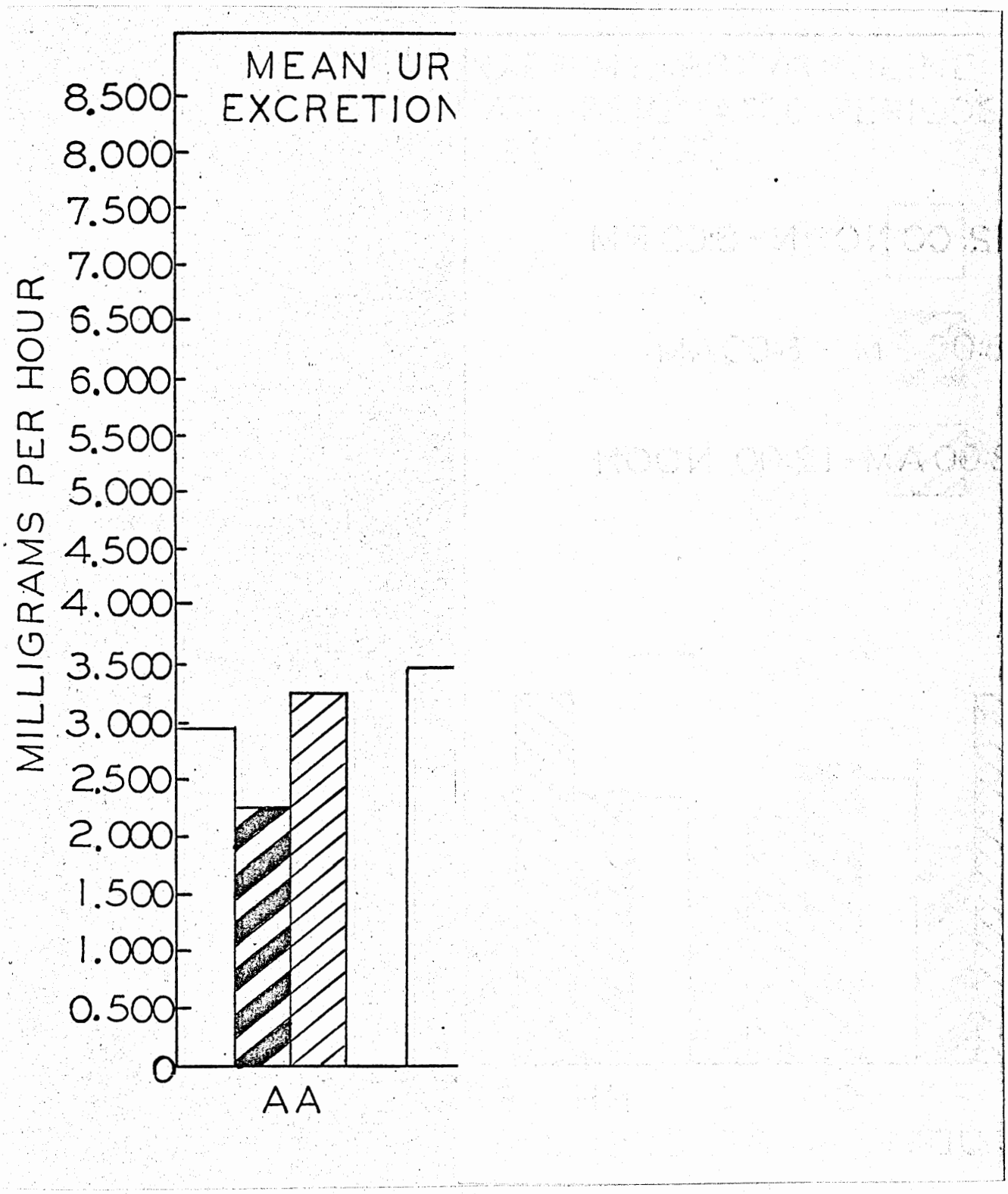

Figure II. COMPARISON OF MEAN HYDROXYPROLINE EXCRETION DURING BED REST I (NO EXERCISE) AT THE THREE PERIODS OF COLLECTION

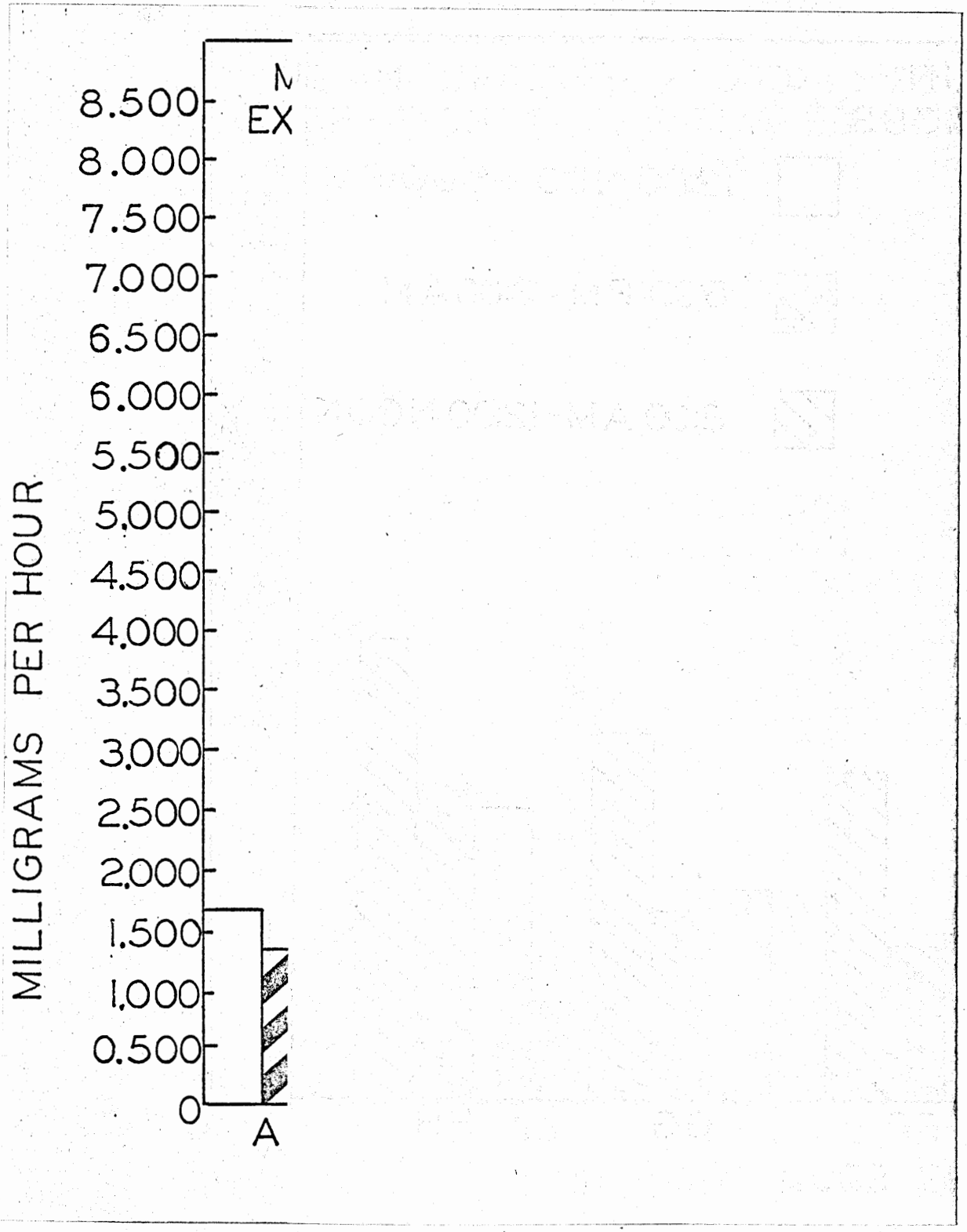

Figure III. COMPARISON OF MEAN URINARY HYDROXYPROLINE EXCRETION DURING THE INTERIM PERIOD AT THE THREE PERIODS OF COLLECTION

SUMMARY AND CONCLUSIONS

A study of 113 days duration was conducted at the Texas Woman's University Research Institute on six male university students, ranging in age from 20 to 25 years, as part of a more extensive Bed Rest Study. The purpose of this study was threefold: (a) to study the effect of immobilization; (b) to observe the possibility of a circadian rhythm in the excretion of hydroxyproline; and (c) to study the effect of exercise on hydroxyproline excretion.

Urinary hydroxyproline excretion was found to rise significantly during periods of recumbency as compared to periods of ambulation. Exercise, whether strictly enforced or voluntary, was found to decrease significantly the urinary hydroxyproline during recumbency in Bed Rest II as compared to Bed Rest I. A circadian rhythm of urinary hydroxyproline excretion was observed with the highest level occurring during the period ending at 12 Noon.

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A P P E N D I X

TABLE I

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per 24 hours)

PART A. SUBJECT BB (Exercised Regularly During Bed Rest II)

Equilibration Period	Bed Rest Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 36.63	(1) 43.40	(1) 29.12	(1) 39.55	(1) 52.27
(2) 73.20	(2) 29.48	(2) 29.63	(2) 78.95	(2) 44.27
(3) 22.74	(3) 34.10	(3) 31.44	(3) 53.40	(3) 37.18
(4) 15.96	(4) 22.67	(4) 47.76	(4) 70.68	(4) 23.97
(5) 49.83	(5) 34.66	(5) 15.05	(5) 107.90	(5) 94.78
(6) 31.28	(6) 19.78	(6) 32.20	(6) 71.22	(6) 50.96
(7) 15.30	(7) 72.97	(7) 37.04	(7) 36.98	(7) 43.70
(8) 53.29	(8) 115.83	(8) 75.31	(8) 76.26	(8) 40.17
(9) 40.18	(9) 88.30	(9) 60.56	(9) 71.40	(9) 33.54
(10) 21.63	(10) 76.09	(10) 30.60	(10) 53.53	(10) 40.48
(11) 74.40	(11) 50.25	(11) 52.55	(11) 45.48	(11) 47.75
(12) 17.60	(12) 54.60	(12) 61.71	(12) 64.16	(12) 31.50
(13) 56.40	(13) 36.11	(13) 37.88	(13) 54.62	(13) 36.63
(14) 17.30	(14) 53.87	(14) 28.05	(14) 58.35	(14) 44.99
(15) 74.96	(15) 42.18	X	(15) 52.13	X
(16) 44.02	(16) 95.96	X	(16) 56.18	X
(17) 31.34	(17) 63.70	X	(17) 61.10	X
(18) 77.00	(18) 85.90	X	(18) 44.84	X
(19) 38.34	(19) 22.49	X	(19) 47.23	X
(20) 8.19	(20) 62.04	X	(20) 51.07	X
(21) 37.80	(21) 91.26	X	(21) 72.09	X
(22) 39.97	(22) 97.30	X	(22) 63.25	X
(23) 30.59	(23) 41.90	X	(23) 72.23	X
(24) 45.44	(24) 48.00	X	(24) 75.96	X
(25) 14.90	(25) 51.24	X	(25) 57.48	X
(26) 29.40	(26) 102.50	X	(26) 57.48	X
(27) 98.34	(27) 74.51	X	(27) 75.26	X
(28) 30.00	(28) 72.30	X	(28) 30.55	X
(29) 29.70	X	X	X	X

TABLE I--Continued

EXCRETION OF URINARY HYPROXYPROLINE

(mg. per 24 hours)

PART B. SUBJECT FF (Exercised Regularly During Bed Rest II)

Equilibration Period	Bed Rest Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 44.40	(1) 36.00	(1) 35.58	(1) 26.62	(1) 51.40
(2) 35.10	(2) 32.49	(2) 30.87	(2) 52.86	(2) 92.22
(3) 79.23	(3) 29.81	(3) 39.21	(3) 40.00	(3) 37.52
(4) 59.80	(4) 42.80	(4) 46.77	(4) 86.51	(4) 43.61
(5) 55.68	(5) 28.30	(5) 18.03	(5) 122.58	(5) 70.46
(6) 50.85	(6) 31.40	(6) 35.70	(6) 69.74	(6) 50.25
(7) 26.97	(7) 40.75	(7) 26.91	(7) 51.61	(7) 45.39
(8) 51.30	(8) 76.35	(8) 32.24	(8) 48.82	(8) 39.69
(9) 30.71	(9) 64.00	(9) 33.66	(9) 89.73	(9) 22.96
(10) 17.64	(10) 38.79	(10) 29.84	(10) 47.79	(10) 31.72
(11) 35.60	(11) 86.00	(11) 53.80	(11) 73.75	(11) 37.76
(12) 10.50	(12) 56.72	(12) 45.36	(12) 44.75	(12) 23.62
(13) 18.27	(13) 25.06	(13) 32.52	(13) 43.17	(13) 30.07
(14) 40.31	(14) 77.98	(14) 44.31	(14) 45.89	(14) 24.11
(15) 16.06	(15) 43.82	X	(15) 48.82	X
(16) 28.40	(16) 49.00	X	(16) 46.76	X
(17) 22.88	(17) 43.22	X	(17) 41.50	X
(18) 39.44	(18) 46.63	X	(18) 51.35	X
(19) 30.08	(19) 17.50	X	(19) 49.97	X
(20) 14.97	(20) 54.90	X	(20) 49.97	X
(21) 48.50	(21) 55.60	X	(21) 46.97	X
(22) 28.00	(22) 87.00	X	(22) 60.90	X
(23) 12.90	(23) 49.08	X	(23) 64.54	X
(24) 21.00	(24) 76.80	X	(24) 67.57	X
(25) 15.58	(25) 122.00	X	(25) 30.77	X
(26) 42.64	(26) 102.64	X	(26) 49.58	X
(27) 19.71	(27) 67.11	X	(27) 49.97	X
(28) 21.40	(28) 44.90	X	(28) 32.28	X
(29) 32.12	X	X	X	X

TABLE I--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per 24 hours)

PART C. SUBJECT HH (Exercised Regularly During Bed Rest II)

Equilibration Period	Bed Rest - Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 92.00	(1) 49.80	(1) 36.77	(1) 73.86	(1) 49.98
(2) 14.76	(2) 34.68	(2) 48.07	(2) 41.40	(2) 50.02
(3) 62.40	(3) 23.44	(3) 26.08	(3) 47.43	(3) 50.99
(4) 13.20	(4) 26.11	(4) 61.36	(4) 36.48	(4) 44.92
(5) 28.73	(5) 30.24	(5) 23.27	(5) 66.50	(5) 86.12
(6) 41.76	(6) 25.15	(6) 42.15	(6) 59.94	(6) 44.28
(7) 25.08	(7) 52.97	(7) 33.04	(7) 79.39	(7) 55.53
(8) 55.76	(8) 105.81	(8) 79.65	(8) 70.30	(8) 24.20
(9) 24.60	(9) 57.02	(9) 26.13	(9) 66.22	(9) 34.96
(10) 24.05	(10) 53.38	(10) 25.18	(10) 24.84	(10) 46.18
(11) 29.90	(11) 60.84	(11) 62.23	(11) 63.44	(11) 32.21
(12) 15.68	(12) 55.97	(12) 83.68	(12) 63.20	(12) 38.02
(13) 12.20	(13) 76.31	(13) 19.30	(13) 51.09	(13) 48.56
(14) 32.50	(14) 58.70	(14) 23.13	(14) 45.31	(14) 23.74
(15) 39.20	(15) 49.56	X	(15) 58.11	X
(16) 60.00	(16) 56.94	X	(16) 45.35	X
(17) 18.88	(17) 44.70	X	(17) 63.81	X
(18) 26.60	(18) 60.04	X	(18) 53.62	X
(19) 32.33	(19) 33.78	X	(19) 17.05	X
(20) 46.00	(20) 61.08	X	(20) 46.10	X
(21) 14.80	(21) 79.00	X	(21) 65.61	X
(22) 37.40	(22) 48.40	X	(22) 61.57	X
(23) 17.60	(23) 65.04	X	(23) 42.70	X
(24) 33.06	(24) 64.80	X	(24) 63.10	X
(25) 21.12	(25) 49.65	X	(25) 50.55	X
(26) 35.34	(26) 70.88	X	(26) 65.76	X
(27) 43.89	(27) 86.21	X	(27) 48.82	X
(28) 30.00	(28) 39.60	X	(28) 26.80	X
(29) 11.52	X	X	X	X

TABLE I--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per 24 hours)

PART D. SUBJECT AA (Exercised At Will During Bed Rest II)

Equilibration Period	Bed Rest Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 17.46	(1) 28.20	(1) 21.33	(1) 31.26	(1) 57.92
(2) 33.25	(2) 20.13	(2) 57.60	(2) 70.30	(2) 78.59
(3) 46.80	(3) 34.57	(3) 57.60	(3) 70.51	(3) 67.66
(4) 74.34	(4) 31.70	(4) 44.10	(4) 40.28	(4) 56.24
(5) 49.00	(5) 36.80	(5) 28.60	(5) 107.46	(5) 63.10
(6) 43.60	(6) 47.54	(6) 33.90	(6) 55.98	(6) 54.78
(7) 27.83	(7) 96.22	(7) 34.43	(7) 43.64	(7) 53.13
(8) 71.68	(8) 76.46	(8) 54.53	(8) 35.63	(8) 43.97
(9) 49.60	(9) 48.41	(9) 36.53	(9) 40.68	(9) 40.00
(10) 9.92	(10) 50.84	(10) 46.66	(10) 45.50	(10) 41.73
(11) 18.48	(11) 41.19	(11) 31.68	(11) 77.42	(11) 47.90
(12) 28.60	(12) 57.78	(12) 39.95	(12) 42.72	(12) 27.41
(13) 14.74	(13) 80.56	(13) 21.84	(13) 50.55	(13) 52.94
(14) 17.48	(14) 45.43	(14) 17.47	(14) 60.38	(14) 22.75
(15) 14.90	(15) 52.86	X	(15) 54.06	X
(16) 11.00	(16) 51.94	X	(16) 42.12	X
(17) 31.29	(17) 27.24	X	(17) 66.27	X
(18) 16.68	(18) 58.62	X	(18) 62.12	X
(19) 9.66	(19) 82.81	X	(19) 44.47	X
(20) 14.24	(20) 48.60	X	(20) 36.94	X
(21) 9.87	(21) 47.40	X	(21) 54.30	X
(22) 37.40	(22) 83.50	X	(22) 47.04	X
(23) 12.46	(23) 85.68	X	(23) 56.27	X
(24) 29.90	(24) 52.82	X	(24) 76.73	X
(25) 37.60	(25) 105.30	X	(25) 54.30	X
(26) 21.28	(26) 139.78	X	(26) 72.10	X
(27) 47.25	(27) 42.68	X	(27) 45.68	X
(28) 49.40	(28) 37.40	X	(28) 45.60	X
(29) 37.50	X	X	X	X

TABLE I--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per 24 hours)

PART E. SUBJECT EE (Exercised At Will During Bed Rest II)

Equilibration Period	Bed Rest Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 11.90	(1) 35.70	(1) 57.80	(1) 42.14	(1) 54.47
(2) 32.63	(2) 26.54	(2) 94.05	(2) 46.61	(2) 69.08
(3) 54.34	(3) 30.78	(3) 26.40	(3) 21.77	(3) 72.70
(4) 35.91	(4) 32.77	(4) 45.53	(4) 80.92	(4) 71.30
(5) 69.54	(5) 40.29	(5) 36.22	(5) 124.40	(5) 70.13
(6) 64.00	(6) 33.61	(6) 52.14	(6) 86.40	(6) 60.84
(7) 16.50	(7) 52.50	(7) 36.85	(7) 19.03	(7) 42.14
(8) 82.46	(8) 66.50	(8) 28.54	(8) 44.06	(8) 41.67
(9) 17.80	(9) 50.54	(9) 21.95	(9) 37.31	(9) 36.14
(10) 15.40	(10) 44.97	(10) 39.20	(10) 75.46	(10) 84.66
(11) 12.24	(11) 44.55	(11) 38.77	(11) 104.38	(11) 38.68
(12) 22.10	(12) 74.20	(12) 83.68	(12) 71.43	(12) 55.58
(13) 34.56	(13) 51.67	(13) 30.46	(13) 74.93	(13) 69.16
(14) 22.00	(14) 51.88	(14) 32.64	(14) 55.34	(14) 61.51
(15) 27.72	(15) 73.55	X	(15) 67.04	X
(16) 21.42	(16) 79.53	X	(16) 62.53	X
(17) 10.64	(17) 107.45	X	(17) 70.88	X
(18) 85.50	(18) 83.04	X	(18) 33.28	X
(19) 23.56	(19) 44.00	X	(19) 60.76	X
(20) 62.00	(20) 67.70	X	(20) 53.30	X
(21) 14.75	(21) 76.84	X	(21) 93.09	X
(22) 26.00	(22) 114.40	X	(22) 74.26	X
(23) 24.20	(23) 54.54	X	(23) 63.51	X
(24) 35.70	(24) 47.20	X	(24) 84.83	X
(25) 15.16	(25) 59.70	X	(25) 42.74	X
(26) 29.48	(26) 87.00	X	(26) 60.76	X
(27) 19.38	(27) 70.40	X	(27) 9.61	X
(28) 33.60	(28) 72.57	X	(28) 40.37	X
(29) 24.80	X	X	X	X

TABLE I--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per 24 hours)

PART F. SUBJECT GG (Exercised At Will During Bed Rest II)

Equilibration Period	Bed Rest Number I	Interim Ambulatory	Bed Rest Number II	Post-Bed Rest
(1) 24.44	(1) 46.80	(1) 22.23	(1) 46.56	(1) 40.18
(2) 12.00	(2) 32.90	(2) 37.62	(2) 33.93	(2) 48.79
(3) 26.72	(3) 41.88	(3) 27.25	(3) 40.77	(3) 34.10
(4) 72.90	(4) 34.40	(4) 27.10	(4) 48.28	(4) 39.14
(5) 37.05	(5) 40.01	(5) 20.41	(5) 74.98	(5) 52.13
(6) 58.20	(6) 37.48	(6) 34.54	(6) 56.05	(6) 64.48
(7) 30.36	(7) 40.10	(7) 31.49	(7) 34.86	(7) 38.48
(8) 69.52	(8) 42.60	(8) 41.98	(8) 66.90	(8) 33.37
(9) 22.20	(9) 75.37	(9) 28.64	(9) 60.31	(9) 35.09
(10) 24.96	(10) 78.60	(10) 32.64	(10) 46.59	(10) 53.29
(11) 18.72	(11) 27.16	(11) 24.61	(11) 44.47	(11) 47.46
(12) 11.10	(12) 47.75	(12) 20.33	(12) 41.67	(12) 40.11
(13) 8.82	(13) 39.74	(13) 26.26	(13) 47.00	(13) 32.24
(14) 14.24	(14) 72.73	(14) 32.56	(14) 43.65	(14) 54.81
(15) 27.50	(15) 61.90	X	(15) 52.62	X
(16) 9.45	(16) 47.10	X	(16) 43.96	X
(17) 18.20	(17) 45.66	X	(17) 67.72	X
(18) 36.11	(18) 52.84	X	(18) 50.59	X
(19) 22.40	(19) 20.40	X	(19) 50.88	X
(20) 17.34	(20) 55.35	X	(20) 46.31	X
(21) 7.28	(21) 62.12	X	(21) 54.52	X
(22) 22.10	(22) 180.20	X	(22) 49.92	X
(23) 3.77	(23) 65.50	X	(23) 61.50	X
(24) 47.40	(24) 57.30	X	(24) 59.94	X
(25) 28.20	(25) 37.17	X	(25) 38.63	X
(26) 35.28	(26) 69.40	X	(26) 50.59	X
(27) 42.00	(27) 73.70	X	(27) 65.11	X
(28) 38.20	(28) 75.44	X	(28) 43.85	X
(29) 30.80	X	X	X	X

TABLE II

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART A. SUBJECT AA

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	3.63	1.90	5.92
10	2.04	1.16	1.12
11	2.21	3.23	3.48
12	1.24	2.05	1.88
13	1.56	1.84	3.52
14	0.92	2.14	2.55
15	3.95	3.20	2.85
16	0.95	2.82	3.22
17	1.43	2.48	2.35
18	2.42	2.62	1.18
19	0.86	1.86	1.35
20	1.44	7.65	2.44
21	3.02	6.75	2.33
22	1.83	1.92	1.91
23	2.00	2.02	2.55
24	3.62	4.90	6.38
25	2.21	2.04	1.72
26	2.18	3.23	1.95
27	5.27	6.62	10.24
28	2.56	6.75	1.72

TABLE II--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART B. SUBJECT BB

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	2.12	2.79	3.87
10	6.24	2.50	4.76
11	3.27	2.75	6.60
12	1.19	2.25	2.80
13	1.69	1.89	3.18
14	1.45	2.92	1.46
15	0.98	3.18	2.27
16	2.21	2.30	3.67
17	0.57	1.50	4.50
18	4.07	2.79	3.57
19	2.00	2.79	5.40
20	2.71	2.55	0.98
21	0.08	3.44	2.40
22	2.64	2.79	4.46
23	3.70	2.79	3.95
24	5.00	1.42	1.72
25	1.75	1.80	2.55
26	1.70	1.80	2.20
27	1.20	4.80	4.45
28	3.82	5.25	5.12

TABLE II--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART C. SUBJECT EE

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	0.75	4.86	1.50
10	1.86	8.06	2.38
11	1.22	4.20	2.18
12	0.92	4.12	1.86
13	1.05	4.28	4.12
14	0.84	7.80	2.02
15	1.20	5.29	4.02
16	0.58	3.19	4.31
17	1.49	5.29	3.51
18	1.49	8.40	5.88
19	2.54	7.50	7.19
20	1.82	0.91	1.76
21	0.97	4.58	1.70
22	2.55	5.88	3.51
23	2.30	5.29	5.96
24	3.96	4.80	1.76
25	1.33	6.12	3.05
26	0.68	3.65	3.42
27	0.61	6.25	4.90
28	1.28	8.12	5.10

TABLE II--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART D. SUBJECT FF

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	1.37	5.31	4.54
10	1.38	5.88	4.37
11	1.63	2.38	2.14
12	1.10	2.10	6.48
13	2.28	1.72	2.38
14	1.23	3.22	0.62
15	0.60	3.22	2.29
16	3.90	3.22	3.02
17	0.82	2.45	2.55
18	1.57	2.45	0.32
19	1.88	4.58	2.78
20	1.93	0.31	1.10
21	0.37	1.08	1.38
22	2.59	3.22	3.50
23	1.90	3.15	6.20
24	1.25	2.60	3.86
25	1.28	3.22	5.55
26	1.97	1.20	4.35
27	2.82	5.85	0.70
28	5.84	6.75	2.97

TABLE II--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART E. SUBJECT GG

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	1.16	1.54	2.16
10	1.08	3.08	5.65
11	1.84	2.02	2.70
12	4.12	1.88	0.56
13	1.48	1.22	2.44
14	1.60	2.25	1.30
15	1.20	3.74	4.13
16	2.37	2.80	4.84
17	1.47	1.40	2.10
18	1.78	2.25	1.28
19	2.00	2.85	2.03
20	2.83	0.66	0.41
21	0.32	3.32	1.42
22	2.90	2.28	2.18
23	3.00	2.18	3.42
24	11.59	3.40	0.84
25	4.30	1.80	2.78
26	2.58	1.05	1.41
27	1.43	2.18	2.60
28	3.33	2.15	4.70

TABLE II--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

BED REST I

PART F. SUBJECT HH

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
9	1.42	4.29	2.21
10	6.70	1.94	1.60
11	2.89	2.40	2.46
12	2.37	1.30	3.51
13	1.66	3.26	2.62
14	1.85	3.26	3.78
15	1.06	8.32	2.09
16	2.66	2.50	2.34
17	1.50	3.26	1.52
18	2.29	2.80	2.20
19	1.46	2.40	2.61
20	2.08	3.56	0.60
21	0.24	6.52	2.34
22	2.37	3.48	2.15
23	4.55	1.80	1.90
24	2.03	2.20	1.82
25	3.13	3.26	1.88
26	3.05	3.30	2.03
27	1.50	3.85	3.70
28	2.37	3.26	4.14

TABLE III

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART A. SUBJECT AA

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	1.47	2.18	2.96
2	3.21	4.17	2.80
3	2.40	4.17	1.38
4	0.78	14.70	1.56
5	1.11	5.57	1.67
6	0.72	4.12	6.22
7	1.37	2.84	0.37
8	1.76	1.32	2.05
9	1.28	5.17	1.29
10	0.58	7.15	1.33
11	1.18	1.82	0.58
12	0.25	1.16	1.67
13	1.37	2.34	1.00
14	1.32	1.94	0.93

TABLE III--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART B. SUBJECT BB

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	2.15	4.22	3.49
2	1.87	4.38	2.45
3	3.06	4.03	0.80
4	0.57	2.74	2.64
5	1.78	4.03	1.30
6	1.78	4.03	2.62
7	1.78	2.19	2.48
8	0.88	1.45	5.76
9	3.27	4.07	1.19
10	2.36	2.88	2.62
11	1.13	2.66	2.53
12	0.94	5.79	2.18
13	0.86	5.46	2.61
14	1.78	7.20	2.26

TABLE III--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART C. SUBJECT EE

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	2.12	4.19	3.51
2	3.44	3.58	1.24
3	1.97	5.25	1.11
4	1.80	4.58	1.82
5	1.80	6.13	7.56
6	1.80	5.12	2.67
7	5.78	5.31	1.25
8	0.86	5.12	6.48
9	0.60	2.50	1.65
10	1.23	5.12	2.67
11	0.79	15.84	1.26
12	1.80	5.10	3.69
13	0.37	1.76	0.70
14	1.47	1.16	2.86

TABLE III--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART D. SUBJECT FF

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	2.17	2.10	2.97
2	1.54	2.80	1.44
3	1.62	2.35	1.25
4	1.58	2.80	0.12
5	1.93	4.25	1.44
6	0.76	10.81	1.44
7	1.04	3.26	3.28
8	1.44	1.73	1.86
9	0.82	0.75	1.03
10	1.08	1.62	1.21
11	1.30	1.92	1.36
12	1.72	2.21	1.28
13	1.73	0.58	1.32
14	1.67	2.20	1.69

TABLE III--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART E. SUBJECT GG

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	3.12	2.00	2.51
2	1.25	4.20	1.92
3	1.59	7.12	2.66
4	1.82	3.07	3.76
5	0.22	2.70	1.30
6	1.59	6.69	1.51
7	2.79	1.04	2.12
8	2.00	3.07	1.46
9	1.25	4.44	1.79
10	2.20	1.44	1.00
11	1.61	2.81	1.35
12	0.55	1.44	1.11
13	1.16	3.07	0.98
14	2.23	1.62	1.34

TABLE III--Continued

EXCRETION OF URINARY HYDROXYPROLINE

(mg. per hour)

INTERIM

PART F. SUBJECT HH

Day	8 P.M. - 8 A.M.	8 A.M. - 12 Noon	12 Noon - 8 P.M.
1	1.65	1.58	2.34
2	4.80	3.84	0.79
3	2.66	3.84	0.90
4	2.61	5.36	0.16
5	2.58	5.89	2.70
6	2.61	3.84	6.00
7	2.61	2.65	2.97
8	1.84	1.52	5.33
9	1.50	1.06	2.49
10	1.30	4.72	2.54
11	1.08	5.51	2.49
12	1.20	3.84	2.90
13	8.64	3.84	1.38
14	2.61	4.16	1.22

TABLE IV

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART A. SUBJECT BB (Exercised Regularly During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	39.971	21.555	4.445	P< 0.001
Bed Rest Immobilization No Exercise	68.570	23.310		
Pre-Bed Rest Ambulatory	39.971	21.555	0.098	N.S.
Interim Ambulatory	40.636	15.991		
Pre-Bed Rest Ambulatory	39.971	21.555	4.078	P< 0.001
Bed Rest Immobilization and Exercise	60.939	15.551		
Pre-Bed Rest Ambulatory	39.971	21.555	0.685	N.S.
Post-Bed Rest Ambulatory	44.584	15.761		
Bed Rest Immobilization No Exercise	68.570	23.310	3.748	P< 0.001
Interim Ambulatory	40.636	15.991		
Bed Rest Immobilization No Exercise	68.570	23.310	1.339	P< 0.20
Bed Rest Immobilization and Exercise	60.939	15.551		
Bed Rest Immobilization No Exercise	68.570	23.310	3.229	P< 0.010
Post-Bed Rest Ambulatory	44.584	15.761		
Interim Ambulatory	40.636	15.991	3.761	P< 0.001
Bed Rest Immobilization and Exercise	60.939	15.551		
Interim Ambulatory	40.636	15.991	0.611	N.S.
Post-Bed Rest Ambulatory	44.584	15.761		
Bed Rest Immobilization and Exercise	60.939	15.551	3.045	P< 0.010
Post-Bed Rest Ambulatory	44.584	15.761		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART B. SUBJECT FF (Exercised Regularly During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	32.867	15.849	4.764	P< 0.001
Bed Rest Immobilization No Exercise	59.995	23.898		
Pre-Bed Rest Ambulatory	32.867	15.849	0.674	N.S.
Interim Ambulatory	36.057	8.870		
Pre-Bed Rest Ambulatory	32.867	15.849	4.471	P< 0.001
Bed Rest Immobilization and Exercise	53.798	18.539		
Pre-Bed Rest Ambulatory	32.867	15.849	1.766	P< 0.100
Post-Bed Rest Ambulatory	42.913	18.521		
Bed Rest Immobilization No Exercise	59.995	23.898	3.420	P< 0.001
Interim Ambulatory	36.057	8.870		
Bed Rest Immobilization No Exercise	59.995	23.898	1.001	N.S.
Bed Rest Immobilization and Exercise	53.798	18.539		
Bed Rest Immobilization No Exercise	59.995	23.898	2.168	P< 0.050
Post-Bed Rest Ambulatory	42.913	18.521		
Interim Ambulatory	36.057	8.870	3.244	P< 0.010
Bed Rest Immobilization and Exercise	53.798	18.539		
Interim Ambulatory	36.057	8.870	1.150	N.S.
Post-Bed Rest Ambulatory	42.913	18.521		
Bed Rest Immobilization and Exercise	53.798	18.539	1.709	P< 0.100
Post-Bed Rest Ambulatory	42.913	18.521		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART C. SUBJECT HH (Exercised Regularly During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	33.005	17.722	5.817	P < 0.001
Bed Rest Immobilization No Exercise	61.517	16.059		
Pre-Bed Rest Ambulatory	33.005	17.722	1.237	N.S.
Interim Ambulatory	40.538	18.436		
Pre-Bed Rest Ambulatory	33.005	17.722	4.592	P < 0.001
Bed Rest Immobilization and Exercise	53.509	14.870		
Pre-Bed Rest Ambulatory	33.005	17.722	2.097	P < 0.050
Post-Bed Rest Ambulatory	44.984	14.880		
Bed Rest Immobilization No Exercise	61.517	16.059	3.440	P < 0.001
Interim Ambulatory	40.538	18.436		
Bed Rest Immobilization No Exercise	61.517	16.059	1.773	P < 0.100
Bed Rest Immobilization and Exercise	53.509	14.870		
Bed Rest Immobilization No Exercise	61.517	16.059	2.954	P < 0.010
Post-Bed Rest Ambulatory	44.984	14.880		
Interim Ambulatory	40.538	18.436	2.332	P < 0.020
Bed Rest Immobilization and Exercise	53.509	14.870		
Interim Ambulatory	40.538	18.436	0.652	N.S.
Post-Bed Rest Ambulatory	44.984	14.880		
Bed Rest Immobilization and Exercise	53.509	14.870	1.667	P < 0.100
Post-Bed Rest Ambulatory	44.984	14.880		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART D. SUBJECT AA (Exercised At Will During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	30.380	17.394	5.588	P< 0.001
Bed Rest Immobilization No Exercise	64.722	25.539		
Pre-Bed Rest Ambulatory	30.380	17.394	1.326	P< 0.200
Interim Ambulatory	37.587	12.750		
Pre-Bed Rest Ambulatory	30.380	17.394	5.315	P< 0.001
Bed Rest Immobilization and Exercise	54.681	16.139		
Pre-Bed Rest Ambulatory	30.380	17.394	3.616	P< 0.001
Post-Bed Rest Ambulatory	50.581	14.389		
Bed Rest Immobilization No Exercise	64.722	25.539	3.517	P< 0.001
Interim Ambulatory	37.587	12.750		
Bed Rest Immobilization No Exercise	64.722	25.539	1.638	P< 0.200
Bed Rest Immobilization and Exercise	54.681	16.139		
Bed Rest Immobilization No Exercise	64.722	25.539	1.800	P< 0.100
Post-Bed Rest Ambulatory	50.581	14.389		
Interim Ambulatory	37.587	12.750	3.301	P< 0.001
Bed Rest Immobilization and Exercise	54.681	16.139		
Interim Ambulatory	37.587	12.750	2.348	P< 0.050
Post-Bed Rest Ambulatory	50.581	14.389		
Bed Rest Immobilization and Exercise	54.681	16.139	0.766	N.S.
Post-Bed Rest Ambulatory	50.581	14.389		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART E. SUBJECT EE (Exercised At Will During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	32.633	20.484	6.150	P< 0.001
Bed Rest Immobilization No Exercise	67.757	18.952		
Pre-Bed Rest Ambulatory	32.633	20.484	1.722	P< 0.100
Interim Ambulatory	44.588	20.429		
Pre-Bed Rest Ambulatory	32.633	20.484	5.138	P< 0.001
Bed Rest Immobilization and Exercise	63.909	24.215		
Pre-Bed Rest Ambulatory	32.633	20.484	4.180	P< 0.001
Post-Bed Rest Ambulatory	59.147	14.362		
Bed Rest Immobilization No Exercise	67.757	18.952	3.309	P< 0.001
Interim Ambulatory	44.588	20.429		
Bed Rest Immobilization No Exercise	67.757	18.952	0.598	N.S.
Bed Rest Immobilization and Exercise	63.909	24.215		
Bed Rest Immobilization No Exercise	67.757	18.952	1.387	P< 0.200
Post-Bed Rest Ambulatory	59.147	14.362		
Interim Ambulatory	44.588	20.429	2.445	P< 0.020
Bed Rest Immobilization and Exercise	63.909	24.215		
Interim Ambulatory	44.588	20.429	2.026	P< 0.100
Post-Bed Rest Ambulatory	59.147	14.362		
Bed Rest Immobilization and Exercise	63.909	24.215	0.648	N.S.
Post-Bed Rest Ambulatory	59.147	14.362		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART F. SUBJECT GG (Exercised At Will During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	28.802	17.083	4.589	P< 0.001
Bed Rest Immobilization No Exercise	60.000	29.993		
Pre-Bed Rest Ambulatory	28.802	17.083	0.065	N.S.
Interim Ambulatory	29.119	6.175		
Pre-Bed Rest Ambulatory	28.802	17.083	5.730	P< 0.001
Bed Rest Immobilization and Exercise	50.808	10.005		
Pre-Bed Rest Ambulatory	28.802	17.083	2.953	P< 0.010
Post-Bed Rest Ambulatory	43.834	9.395		
Bed Rest Immobilization No Exercise	60.000	29.993	3.617	P< 0.001
Interim Ambulatory	29.119	6.175		
Bed Rest Immobilization No Exercise	60.000	29.993	1.460	P< 0.200
Bed Rest Immobilization and Exercise	50.808	10.005		
Bed Rest Immobilization No Exercise	60.000	29.993	1.862	P< 0.100
Post-Bed Rest Ambulatory	43.834	9.395		
Interim Ambulatory	29.119	6.175	7.103	P< 0.001
Bed Rest Immobilization and Exercise	50.808	10.005		
Interim Ambulatory	29.119	6.175	4.548	N.S.
Post-Bed Rest Ambulatory	43.834	9.395		
Bed Rest Immobilization and Exercise	50.808	10.005	2.070	P< 0.050
Post-Bed Rest Ambulatory	43.834	9.395		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART G. SUBJECTS BB, FF, AND HH (Exercised Regularly During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory Bed Rest Immobilization No Exercise	35.281 63.361	18.823 21.712	8.608	P< 0.001
Pre-Bed Rest Ambulatory Interim Ambulatory	35.281 39.077	18.823 15.143	1.129	N.S.
Pre-Bed Rest Ambulatory Bed Rest Immobilization and Exercise	35.281 56.082	18.823 16.754	7.591	P< 0.001
Pre-Bed Rest Ambulatory Post-Bed Rest Ambulatory	35.281 44.160	18.823 16.485	2.585	P< 0.010
Bed Rest Immobilization No Exercise Interim Ambulatory	63.361 39.077	21.712 15.143	6.256	P< 0.001
Bed Rest Immobilization No Exercise Bed Rest Immobilization and Exercise	63.361 56.082	21.712 16.754	2.309	P< 0.050
Bed Rest Immobilization No Exercise Post-Bed Rest Ambulatory	63.361 44.160	21.712 16.485	4.845	P< 0.001
Interim Ambulatory Bed Rest Immobilization and Exercise	39.077 56.082	15.143 16.754	5.456	P< 0.001
Interim Ambulatory Post-Bed Rest Ambulatory	39.077 44.160	15.143 16.485	1.437	P< 0.200
Bed Rest Immobilization and Exercise Post-Bed Rest Ambulatory	56.082 44.160	16.754 16.485	3.725	P< 0.001

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART H. SUBJECTS AA, EE, AND GG (Exercised At Will During Bed Rest II)

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	30.605	18.452	9.512	P< 0.001
Bed Rest Immobilization No Exercise	64.160	25.440		
Pre-Bed Rest Ambulatory	30.605	18.452	1.943	P< 0.100
Interim Ambulatory	37.098	15.685		
Pre-Bed Rest Ambulatory	30.605	18.452	9.097	P< 0.001
Bed Rest Immobilization and Exercise	56.466	18.597		
Pre-Bed Rest Ambulatory	30.605	18.452	6.289	P< 0.001
Post-Bed Rest Ambulatory	51.187	14.369		
Bed Rest Immobilization No Exercise	64.160	25.440	6.106	P< 0.001
Interim Ambulatory	37.098	15.685		
Bed Rest Immobilization No Exercise	64.160	25.440	2.129	P< 0.050
Bed Rest Immobilization and Exercise	56.466	18.597		
Bed Rest Immobilization No Exercise	64.160	25.440	2.973	P< 0.010
Post-Bed Rest Ambulatory	51.187	14.369		
Interim Ambulatory	37.098	15.685	5.707	P< 0.001
Bed Rest Immobilization and Exercise	56.466	18.597		
Interim Ambulatory	37.098	15.685	4.190	P< 0.001
Post-Bed Rest Ambulatory	51.187	14.369		
Bed Rest Immobilization and Exercise	56.466	18.597	1.590	P< 0.200
Post-Bed Rest Ambulatory	51.187	14.369		

TABLE IV--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART I. ALL SUBJECTS

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Ambulatory	32.943	18.784	12.865	P< 0.001
Bed Rest Immobilization No Exercise	63.760	23.653		
Pre-Bed Rest Ambulatory	32.943	18.784	2.172	P< 0.050
Interim Ambulatory	38.087	15.488		
Pre-Bed Rest Ambulatory	32.943	18.784	11.836	P< 0.001
Bed Rest Immobilization and Exercise	56.274	17.700		
Pre-Bed Rest Ambulatory	32.943	18.784	6.180	P< 0.001
Post-Bed Rest Ambulatory	47.674	15.857		
Bed Rest Immobilization No Exercise	63.760	23.653	8.787	P< 0.001
Interim Ambulatory	38.087	15.448		
Bed Rest Immobilization No Exercise	63.760	23.653	3.143	P< 0.010
Bed Rest Immobilization and Exercise	56.274	17.700		
Bed Rest Immobilization No Exercise	63.760	23.653	5.476	P< 0.001
Post-Bed Rest Ambulatory	47.674	15.857		
Interim Ambulatory	38.087	15.448	7.951	P< 0.001
Bed Rest Immobilization and Exercise	56.274	17.700		
Interim Ambulatory	38.087	15.448	3.921	P< 0.001
Post-Bed Rest Ambulatory	47.674	15.857		
Bed Rest Immobilization and Exercise	56.274	17.700	3.732	P< 0.001
Post-Bed Rest Ambulatory	47.674	15.857		

TABLE V

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART A. SUBJECTS AA, BB, AND EE, BED REST I

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Subject AA				
12 Noon - 8 P.M.	2.957	2.061	1.435	P< 0.20
8 P.M. - 8 A.M.	2.211	1.082		
12 Noon - 8 P.M.	2.957	2.061	0.435	N.S.
8 A.M. - 12 Noon	3.230	1.909		
8 P.M. - 8 A.M.	2.211	1.082	2.078	P< 0.05
8 A.M. - 12 Noon	3.230	1.909		
Subject BB				
12 Noon - 8 P.M.	3.493	1.352	2.520	P< 0.02
8 P.M. - 8 A.M.	2.382	1.438		
12 Noon - 8 P.M.	3.493	1.352	1.916	P< 0.10
8 A.M. - 12 Noon	2.787	0.947		
8 P.M. - 8 A.M.	2.382	1.438	1.052	N.S.
8 A.M. - 12 Noon	2.787	0.947		
Subject EE				
12 Noon - 8 P.M.	3.506	1.545	5.198	P< 0.001
8 P.M. - 8 A.M.	1.488	0.797		
12 Noon - 8 P.M.	3.506	1.545	3.345	P< 0.001
8 A.M. - 12 Noon	5.292	1.824		
8 P.M. - 8 A.M.	1.488	0.797	8.558	P< 0.001
8 A.M. - 12 Noon	5.292	1.824		

TABLE V--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART B. SUBJECTS FF, GG, HH, BED REST I

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Subject FF				
12 Noon - 8 P.M.	2.972	1.719	2.315	P< 0.05
8 P.M. - 8 A.M.	1.904	1.146		
12 Noon - 8 P.M.	2.972	1.719	0.464	N.S.
8 A.M. - 12 Noon	3.219	1.642		
8 P.M. - 8 A.M.	1.904	1.146	2.939	P< 0.01
8 A.M. - 12 Noon	3.219	1.642		
Subject GG				
12 Noon - 8 P.M.	2.510	1.385	0.210	N.S.
8 P.M. - 8 A.M.	2.631	2.187		
12 Noon - 8 P.M.	2.510	1.385	0.935	N.S.
8 A.M. - 12 Noon	2.180	0.762		
8 P.M. - 8 A.M.	2.631	2.187	0.872	N.S.
8 A.M. - 12 Noon	2.180	0.762		
Subject HH				
12 Noon - 8 P.M.	2.342	0.806	0.087	N.S.
8 P.M. - 8 A.M.	2.372	1.294		
12 Noon - 8 P.M.	2.342	0.806	2.382	P< 0.02
8 A.M. - 12 Noon	3.264	1.533		
8 P.M. - 8 A.M.	2.372	1.294	1.990	P< 0.05
8 A.M. - 12 Noon	3.264	1.533		

TABLE V--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART C. ALL SUBJECTS, BED REST I

Populations Compared	Means	Standard Deviation	"t" Value	Probability
All Subjects				
12 Noon - 8 P.M.	2.963	1.589	4.244	P < 0.001
8 P.M. - 8 A.M.	2.165	1.442		
12 Noon - 8 P.M.	2.963	1.589	1.745	P < 0.100
8 A.M. - 12 Noon	3.328	1.779		
8 P.M. - 8 A.M.	2.165	1.442	5.794	P < 0.001
8 A.M. - 12 Noon	3.328	1.779		

TABLE VI

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART A. SUBJECTS AA, BB, AND EE, INTERIM

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Subject AA				
12 Noon - 8 P.M.	1.671	1.357	0.715	N.S.
8 P.M. - 8 A.M.	1.367	0.714		
12 Noon - 8 P.M.	1.671	1.357	2.551	P < 0.02
8 A.M. - 12 Noon	4.171	3.272		
8 P.M. - 8 A.M.	1.367	0.714	3.026	P < 0.01
8 A.M. - 12 Noon	4.171	3.272		
Subject BB				
12 Noon - 8 P.M.	2.621	1.273	1.934	P < 0.10
8 P.M. - 8 A.M.	1.780	0.923		
12 Noon - 8 P.M.	2.621	1.273	2.620	P < 0.02
8 A.M. - 12 Noon	4.031	1.471		
8 P.M. - 8 A.M.	1.780	0.923	4.685	P < 0.001
8 A.M. - 12 Noon	4.031	1.471		
Subject EE				
12 Noon - 8 P.M.	2.671	1.892	1.382	P < 0.20
8 P.M. - 8 A.M.	1.797	1.279		
12 Noon - 8 P.M.	2.671	1.892	2.389	P < 0.05
8 A.M. - 12 Noon	5.121	3.188		
8 P.M. - 8 A.M.	1.797	1.279	3.497	P < 0.01
8 A.M. - 12 Noon	5.121	3.188		

TABLE VI--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART B. SUBJECTS FF, GG, AND HH, INTERIM

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Subject FF				
12 Noon - 8 P.M.	1.440	0.615	0.426	N.S.
8 P.M. - 8 A.M.	1.356	0.362		
12 Noon - 8 P.M.	1.440	0.615	2.054	$P < 0.05$
8 A.M. - 12 Noon	2.802	2.317		
8 P.M. - 8 A.M.	1.356	0.362	2.229	$P < 0.05$
8 A.M. - 12 Noon	2.802	2.317		
Subject GG				
12 Noon - 8 P.M.	1.589	0.635	2.728	$P < 0.02$
8 P.M. - 8 A.M.	3.073	1.861		
12 Noon - 8 P.M.	1.589	0.635	0.731	N.S.
8 A.M. - 12 Noon	1.786	0.737		
8 P.M. - 8 A.M.	3.073	1.861	2.324	$P < 0.05$
8 A.M. - 12 Noon	1.786	0.737		
Subject HH				
12 Noon - 8 P.M.	2.487	1.522	0.183	N.S.
8 P.M. - 8 A.M.	2.609	1.890		
12 Noon - 8 P.M.	2.487	1.522	2.460	$P < 0.05$
8 A.M. - 12 Noon	3.844	1.289		
8 P.M. - 8 A.M.	2.609	1.890	1.951	$P < 0.10$
8 A.M. - 12 Noon	3.844	1.289		

TABLE VI--Continued

STATISTICAL COMPARISON OF URINARY HYDROXYPROLINE BETWEEN PAIRS FOR DESIGNATED PERIODS

PART C. ALL SUBJECTS, INTERIM

Populations Compared	Means	Standard Deviation	"t" Value	Probability
All Subjects				
12 Noon - 8 P.M.	2.080	1.400	0.384	N.S.
8 P.M. - 8 A.M.	2.000	1.449		
12 Noon - 8 P.M.	2.080	1.400	5.066	P< 0.001
8 A.M. - 12 Noon	3.626	2.497		
8 P.M. - 8 A.M.	2.000	1.449	5.292	P< 0.001
8 A.M. - 12 Noon	3.626	2.497		