EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING A PROLONGED HUMAN BED REST STUDY WITH AND WITHOUT EXERCISE

A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE IN NUTRITION

IN THE GRADUATE DIVISION OF THE

TEXAS WOMAN'S UNIVERSITY

COLLEGE OF HOUSEHOLD ARTS AND SCIENCES

BY

MEI-SHENG CHUU, B.S.

DENTON, TEXAS

MAY, 1971

Texas Woman's University

Denton, Texas

May 19 71

We hereby recommend that the thesis prepared under our supervision by Mei-Sheng Chuu

entitled EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING A PROLONGED HUMAN BED REST STUDY WITH AND WITHOUT EXERCISE

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

Committee:

Pauline Beery Mack
Chairman

Betty B. Alford

Ralph E. Pyke

Walter T. Greenwit

Accepted: I. S. Morrison

Graduate Studies

ACKNOWLEDGMENTS

The author expresses her thanksgiving to the Mighty God for His comfort and guidance during this period of studying and conducting the research which led to the writing of her thesis and to all who have helped her to accomplish this goal.

To Dr. Pauline Beery Mack, Director of the Texas Woman's University Research Institute, for organizing the nutritional bed rest study of which the author's graduate assistantship was a part, for her genuine guidance during the author's entire graduate program, and for editing this thesis.

To the National Aeronautics and Space Administration for sponsoring the research program which made this study possible.

To Dr. Florence Langford, Acting Dean of the College of Household Arts and Sciences, for her instruction and support in the author's undergraduate and graduate studies.

To Dr. Betty Bohon Alford and Dr. Kathryn Montgomery for their guidance, discipline, and leadership during the author's program of study.

To Dr. Ralph E. Pyke and Mr. Walter Gilchrist for their generous assistance and guidance while the author's research program was in progress and in the preparation of this thesis.

To Dr. Roman J. Kutsky, for his special instruction and guidance toward the understanding of the biochemical processes involved in this study and for his patient criticism and encouragement of the author which led in completing this thesis.

To Mrs. Jessie Ashby and Mrs. Lenoir Norwood O'Rear, for their coding and statistical analysis of the data for this report.

To Mrs. Reba Fry and Mr. Martin Cudd, for their kind assistance during the author's graduate study.

To Mr. Bill Stover, for the drafting of figures included in this report.

To Mrs. Dorothy Jones, for the typing of this thesis with precision.

To Mrs. Jeanette Johnson, for her copy reading and encouragement in preparing this report.

To the author's sister, Mrs. Mei-Ling Sur, for her love and guidance toward choosing this meaningful career and for her financial assistance during the author's study in the United States.

Finally, the author would like to dedicate this thesis to her parents, Mr. and Mrs. Chen-Chi Chuu, for they have taught their children to reach toward higher goals in life and have given their love and encouragement to them in pursuing a higher education.

TABLE OF CONTENTS

page

LIST OF TABLES

page

LIST OF FIGURES

INTRODUCTION

The phenomenon of weightlessness during space travel produces physiologic changes which can be simulated experimentally by horizontal bed rest. Under the auspices of the National Aeronautics and Space Administration, the Nelda Childers Stark Laboratory for Human Nutrition Research of the Texas Woman's University has participated in research in the realm of aerospace problems by conducting a series of bed rest studies designed to determine metabolic changes experienced because of the condition of recumbency.

The present study is one of a series of investigations conducted at the Texas Woman's University Research Institute Laboratories designed to delve into the possible existence of circadian periodicities for various metabolites by observing the reactions of experimental subjects who were maintained under controlled recumbency conditions.

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

This study was undertaken to observe the effect of exercise on both the total 24 hour excretion and the periodicity of adrenal hormone secretions by measuring the urinary levels of 17-hydroxycorticosteroids of immobilized human subjects.

The specific objectives of this research project have been the following:

1. To analyze the urinary 17-hydroxycorticosteroid excretion values of eight men in a recumbency study;

2. To compare statistically the daily 17-hydroxycorticosteroid excretion values of the three different periods of the study which included an Equilibration Period, a Bed Rest Period, and a Recovery Period;

3. To determine whether or not a circadian rhythm in urinary 17-hydroxycorticosteroid excretions during a 56-day bed rest period could be established;

4. To investigate the effect of exercise on the excretory levels of urinary 17-hydroxycorticosteroids.

REVIEW OF LITERATURE

MODE OF ACTION OF 17-HYDROXYCORTICOSTEROIDS

According to Turner[1], the main 17-hydroxycorticosteroid secreted by the human fascicular-reticular layers of the adrenal cortex are cortisol and cortisone. Together with corticosterone, these steroids are 11-oxygenated, and they exert their main function on carbohydrate, protein, and fat metabolism. They are called glucocorticoids. Corticosterone is important in rats and other lower animals, but not in human beings. The presence of hydroxyl groups at carbon 17 and 21 enhances this organic effect. The presence of a hydroxyl group at position 21 also is a requirement for electrolytic-water activity. Therefore these hormones possess a slight electrolytic-water effect, although they are 11-oxygenated, which decreases the electrolytic-water balance greatly.

In the body, the metabolism of carbohydrate, protein, and fat is integrated through the actions of many hormones and enzymes. Exton[2] stated that glucocorticoids play a significant role in catabolism of protein and amino acids, gluconeogenesis, and glycogenesis. They have a permissive role in lipolysis. The mobilization of protein is within the peripheral tissues. According to Betheil et al.[3], the catabolic activity of free amino acids and gluconeogenesis occurs mostly in the liver, and

in the kidney to a lesser degree. Ray[4] defines gluconeogenesis as a process by which syntheses of glucose and other hexose derivatives proceed via synthesis with molecule-containing substances which have five or fewer carbon atoms. Weber[5] showed that the overall effect of glucocorticoids was to increase the carbohydrate stores, such as blood glucose and liver glycogen in the absence of dietary glucose, so that the homeostasis of glucose could be maintained.

According to Tepperman[6], adrenalectomized animals show a rapid fall in blood glucose and liver and tissue glycogen levels on fasting. The intestinal absorption of glucose also is decreased. Patients with Addison's disease or cortisol insufficiency often show a hypoglycemia, suggesting that the intact individual uses his adrenal hormones as a brake on the reduction of blood glucose concentrations.

ACTION OF GLUCOCORTICOIDS ON THE PERIPHERAL TISSUES

Nucleic Acid and Protein Metabolism

Glucocorticoids have a net protein catabolic effect on the peripheral tissues, such as muscle, thymus, and adipose tissue.

Skeletal Muscle. Goldberg et al.[7] and Kaplan et al.[8] have tested injection of glucocorticoids into animals causing a rapid decrease in body weight and marked atrophy of skeletal muscle, followed by increased

free amino acid levels in the plasma. They also have found that cortisone decreased protein half-life. Goldberg[9] showed a greater total loss of radioactive ^{3}H-leucine from the labeled muscle of cortisone-treated rats as compared with controls, indicating that there was an increased protein degradation in the former group. Cortisone-treated rats also showed decreased protein synthesis, as indicated by the unchanged specific activity of muscle proteins.

Thymus Tissue. In Makman's experiments[10], animal lymphoid cells treated with glucocorticoids showed a diminished degree of incorporating radioactive precursors into DNA, RNA, and proteins. Intact or adrenalectomized animals receiving glucocorticoids also demonstrated similar results. The following changes were observed after glucocorticoid injections:

(a) There has been shown to be a reduced incorporation of ^{3}H-thymidine or ^{3}H-dioxycytidine into lymphatic DNA as found by Hofert and White[11].

(b) Makman et al.[10] have reported that the intracellular phosphorylated nucleosides and guanine are accumulated.

(c) Feigelson[12] has stated that incorporation of glycine into adenine, RNA, and protein concentrations are decreased in the thymus cells.

(d) Mosher et al.[13] have indicated that the incorporation of uridine into RNA is reduced by this treatment.

(e) Mosher et al.[13] also have shown that the steroids inhibited the transport of amino acids to the RNA where proteins are synthesized.

That the biosynthesis of protein depends upon DNA and RNA is evident. If the formation of these nucleic acids and the transport of amino acids are impaired, protein production thus also must be impaired.

Nakagawa and White[14] demonstrated that rats treated with cortisol had decreased rates of RNA polymerase activity of the thymic nuclei. However, Young[15] has indicated that the inhibitory effect of cortisol in the labeling of both RNA and protein probably is not on the RNA polymerase, but rather on the carbohydrate metabolism. Cortisol first showed an inhibitory effect on glucose oxidation, then it gave a slow depressing effect on uridine incorporation into RNA and on amino acid incorporation into protein.

As indicated by Orten and Neuhaus[16], phosphorylation of glucose by ways of anaerobic glycolysis and oxidative phosphorylation in the mitochondria produces energy in the form of ATP. Glenn et al.[17] and Munck[18] have shown that ATP must be present in order for the syntheses of RNA and protein to proceed. Cortisol inhibits this type of glucose oxidation.

Adipose Tissue. Epstein et al.[19] have shown that cortisone had an inhibitory effect on the incorporation of thymidine into DNA stimulated by the growth hormone in the adipose tissue. The growth hormone-stimulated thymidine kinase activity also was reduced by the action of cortisone.

Carbohydrate and Lipid Metabolism

Levine and Haft[20] found that glucocorticoids caused a decreased sensitivity of the muscle membrane to insulin in the peripheral tissues. Schaeffer[21] reported that the steroids promoted epinephrine and adenosine 3',5'-monophosphate to activate muscle glycogen phosphorylase. Cortisol has a direct, although delayed, inhibitory effect on basal glucose uptake by the adipose tissue. Turner[1] stated that the conversion of carbohydrate to fat was markedly increased in the adrenalectomized animals. Exton[2] reported that glucocorticoids exerted a permissive effect on epinephrine induced lipolysis in adipose tissue. Weber[5] found that there was a net increase of free fatty acids in the blood stream and in the liver under a gluconeogenic condition.

MODE OF ACTION OF GLUCOCORTICOIDS IN THE LIVER AND KIDNEY

Nucleic Acid and Protein Metabolism

Feigelson[12] has revealed in his studies that, while glucocorticoids have a catabolic effect on nucleic acids and protein of the peripheral tissues, they exert an anabolic effect on the macromolecules of the liver. Evidences shown by West et al.[22] indicate that the liver is the main site of enzyme syntheses. Betheil et al.[3] showed that glucocorticoids in the liver stimulated the formations of many enzymes to catabolize amino acids and to form glucose from the breakdown of these precursors. Young[15] concluded that glucocorticoids induced enzyme syntheses through changes in RNA synthesis. Amaral et al.[23] showed that administration of cortisone increased the volume and total protein content of animal hepatic nuclei. Beato et al.[24] demonstrated that cortisol interacted with isolated rat-liver nuclei and led to a stimulation of DNA-dependent RNA synthesis. Kidson and Kirby[25] have found that messenger RNA and microsomal RNA are increased by treating liver cells with cortisol. Gilder et al.[26] have found that there is a reduced rate of microsomal RNA hydrolysis by the action of glucocorticoids, indicating a stabilizing effect of the hormone on microsomal RNA. Ottolenghi and Ottavio[27] also found similar results. Cortisone is transported from the cytoplasm to the nuclear macromolecular DNA, which results in an

increased template capacity of the chromatins for RNA synthesis. Experiments done by Ottolenghi and Ottavio[27] have shown clearly that cortisol increases the nuclear, DNA dependent RNA polymerase which is an essential enzyme in RNA synthesis. At the same time, cortisol has an inhibitory effect on microsomal ribonuclease which is an enzyme responsible for the degradation of RNA. The net effect of the hormone is to synthesize more hepatic RNA. Betheil et al.[3] concluded that many of the amino acids accumulated in the liver first were used to synthesize enzymes by the RNA in the cells. The excess amino acids then underwent gluconeogenesis by the actions of the formed enzymes.

ENZYME REGULATION ON AMINO ACID CATABOLISM, GLUCONEOGENESES AND GLYCOGENESIS

With fasting or any stressful condition, the secretions of glucocorticoids do increase as revealed by Bouille and Assenmacher[28]. According to Lehninger[29], glucocorticoids increase the hepatic induction of amino acid catabolic enzymes and gluconeogenic enzymes. These amino acid catabolic enzymes are tyrosine-α-ketoglutarate transaminase as tested by Kupfer and Partridge[30]; glutamate-pyruvate transaminase, tryptophan pyrrolase, serine dehydratase as tested by Nakajima et al.[31]; and glutamic-alanine transaminase as worked by Segal and Kim[32]. The gluconeogenic enzymes are pyruvate carboxylase, phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, and fructose-1,6-diphosphatase

according to experiments conducted by Nakajima et al.[31] and Weber[5]. Glycogen synthetase and enzymes in the urea cycle are tested by Szepesi et al.[33], and Weber[5]. All of these enzymes are induced by glucocorticoids in the liver and in the kidneys. Figure 1 shows all of the possible enzymes that are affected or induced by glucocorticoids in the complex metabolic system.

Amino Acid Catabolism. According to Greenberg[34], tyrosine is catabolized to acetoacetate by the action of tyrosine-α-ketoglutarate transaminase. Glutamate-pyruvate transaminase converts glutamic acid to α-ketoglutarate. Tryptophan pyrrolase catalyzes the oxidative conversion of tryptophan to kynurenine which subsequently is degradated to form acetyl CoA. Orten and Neuhaus[16] indicated that serine dehydratase deaminated serine to form pyruvate. Marliso et al.[35] showed that amino acids were increased in the liver, particularly alanine, glutamate, and asparate. By means of transamination and deamination these substrates are catabolized to form acetoacetate, α-ketoglutarate, pyruvate, acetyl CoA, and possibly many other intermediary metabolites of the tricarboxylic acid cycle. These all can be quickly converted to pyruvate, and then to glucose.

Studies done by Pagliara[36], Churchill[37], Kamm[38], and their coworkers have indicated that the degradations of protein and amino acids

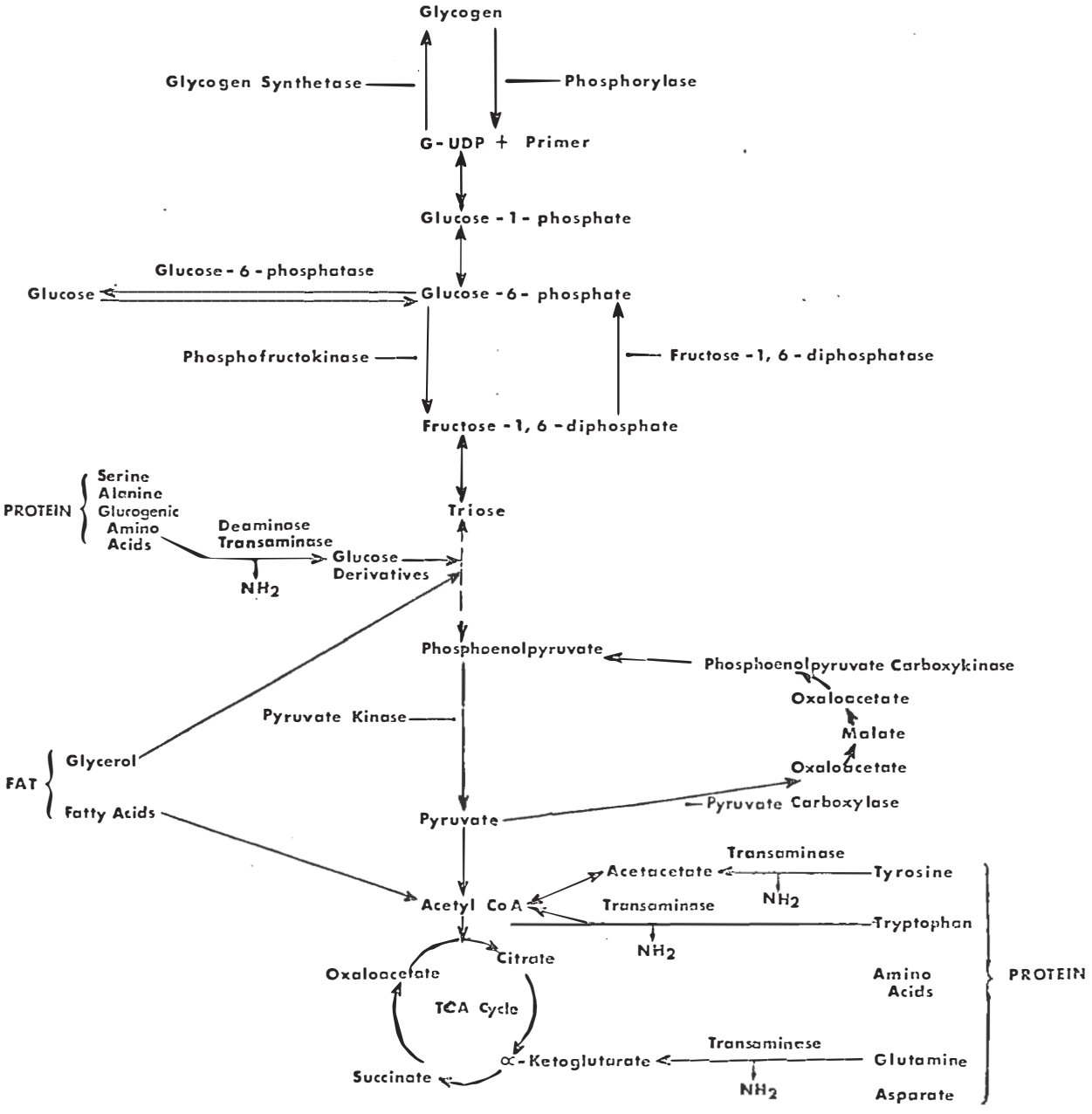
lead to increased ammonia and urea excretions. Under stressful conditions, negative nitrogen balance is evident.

Gluconeogenesis. Uete[39] has found that glucocorticoids can stimulate the $^{14}CO_2$ into glucose. All of the glucose precursors can be converted to glucose by the action of gluconeogenic enzymes. Szepesi et al.[33] are able to indicate that liver is capable of catabolizing amino acids and synthesizing glucose. The liver is the most important site for these two actions, whereas kidneys are less responsible for gluconeogenesis.

The body does not synthesize glucose by the simple reverse way of glycolysis. Gluconeogenesis has its own unique enzyme system for this purpose. In converting pyruvate to phosphoenolpyruvate, two enzymes are involved. According to Lehninger[29], pyruvate carboxylase converts pyruvate to oxaloacetate, which then is reduced to malate in the mitochondria. Malate is dehydrogentated and forms oxaloacetate again. Oxaloacetate leaves the mitochondria and is converted to phosphoenolpyruvate by the action of phosphoenolpyruvate carboxykinase (Figure 1).

FIGURE 1. Integration of Carbohydrate, Protein, and Fat Metabolism. The scheme indicates the enzymes and substrates affected by the action of 17-hydroxycorticosteroids. This chart is a combined form prepared from Orten[16], Ray[4], and Schoner[40].

CARBOHYDRATE



Schoner et al.[40] also have found that the enzyme pyruvatekinase, which catalyzes the reverse reaction, is inhibited by cortisol-induced alanine. Cortisol-induced fructose-1,6-diphosphatase takes off one phosphate group from the substrate and forms fructose-6-phosphate. As shown by Weber[5], glucocorticoids also inhibit the reverse catalytic phosphofructokinase activity by increasing glucose-6-phosphate level. Finally glucose is formed from glucose-6-phosphate by the action of glucose-6-phosphatase.

Glycogenesis. As experiments by Glenn et al.[17] show, glucose or glucose-6-phosphate can be further converted to glycogen by the presence of glycogen synthetase induced by cortisol. At this stage the blood glucose levels are raised, and the energy requirement thus is met in presences of adrenalin, noradrenalin, or other hormones. Glenn et al.[17] also indicated that excessive administration of cortisol caused a marked increase of liver glycogen and a loss of body weight.

MODE OF ACTIONS OF GLUCOCORTICOIDS ON ELECTROLYTE - WATER METABOLISM

Tepperman[6] states that the 21-hydroxylated 17-hydroxycorticosteroids possess some properties of mineralocorticoids secreted by the glomerulosa layer of the adrenal cortex. The effect of 17-hydroxycorticosteroids on electrolyte-water metabolism, however, is much smaller than aldosterone. Sodium and water retention and potassium excretion

by the kidney are promoted by the glucocorticoids as shown by Nocenti and Cizek[41]. Certain Addisonian patients without a serious salt loss can manage well on cortisol alone. By assisting water excretion, cortisol prevents water intoxication. The hormones influence the distribution of water between the intracellular and extracellular fluid compartments and prevent the intracellular increase of water that occurs in the untreated adrenal insufficient animals.

REGULATION OF 17-HYDROXYCORTICOSTEROID SECRETION

Biosynthesis

According to Turner[1], hydroxycorticosteroids are synthesized from acetate and/or cholesterol in the adrenal cortex. The side chain of cholesterol first is broken to form pregnenolone, a 21 carbon steroid. McCune et al[42] indicated that progesterone is formed from pregnenolone by the actions of 3β-hydroxydehydrogenase and Δ^5-3-ketosteroid isomerase. This compound then undergoes a series of hydroxylations of carbon 17, 21, and 11 catalyzed by three respective hydroxylases to form cortisol and cortisone. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) is required in each hydroxylation step for the supply of energy. An extensive review of literature on this subject is done by Qureshi[43].

As indicated by Orten and Neuhaus[16], the hydroxylation of the steroids is an oxidation-reduction reaction. The adrenal cortex has very high concentrations of ascorbic acid and cholesterol in proportion to its small body mass. Adrenocorticotropic hormone (ACTH) stimulates the corticosteroid secretion with simultaneous depletions of ascorbic acid and cholesterol. Therefore, ascorbic acid may be a necessary component of the oxidation-reduction system that produces an oxy-type of adrenal hormones such as cortisone.

Effects of Adrenocorticotropin and Adenosine 3',5'-Cyclic Monophosphate on Corticosteroidogenesis

According to Tepperman[6], cells of the adrenal fasciculata zone are trophically controlled by ACTH which stimulates the cells to produce glucocorticoids. In fact, the level of cortisol output in the adrenal venous blood is almost entirely determined by the intensity of the ACTH stimulus.

Recently many investigators including Earp et al.[44] and Grahame-Smith et al.[45] have demonstrated that the stimulatory effect of ACTH on adrenal cortex is mediated through its activation on adenosine 3',5'-cyclic monophosphate (cyclic AMP). In Earp's studies[44], infusion of dibutyryl cyclic AMP resulted in depletion of adrenal ascorbic acid concentrations and increased corticosterone production in hypophysectomized rats.

Grahame-Smith et al.[45] and Schulster et al.[46] found ACTH increased the concentration of adrenal cyclic AMP which in turn was capable of stimulating steroidogenesis in vivo.

Bransome et al.[47] state that adenyl cyclase is present in the adrenal cell membranes. Taunton et al[48] and Grahame-Smith et al.[45] suggested that ACTH acting at the adrenal cortical cell membrane, activates adenyl cyclase and causes an increased conversion of ATP to 3',5'-cyclic AMP. Indeed as Roberts[49] and McCune et al.[42] indicated, 3',5'-cyclic AMP is a second messenger in the stimulation of adrenal corticosteroidogenesis by ACTH.

According to Tepperman[6] and Halkerston[50] the ACTH-induced cyclic AMP elevation in the adrenal cortex, not in the liver, is a very specific action of ACTH. The cyclic AMP can activate phosphorylase to break down glycogen and cause a rise in glucose-6-phosphate in the adrenal cortex. Halkerston[50] reported that ACTH and stress caused a decrease of glycogen from the fascicular and reticular regions of the rat adrenal. The adrenal cell has an extremely active direct oxidative pathway for glucose. The glycogenolysis promoted by the phosphorylation activation insures that much of the glucose-6-phosphate will go through the direct oxidative pathway. The oxidation of glucose by this route results in the generation of NADPH which is required at many of the intermediate steps of the hormonal synthesis.

McCune et al.[42] stated that ACTH and cyclic 3',5'-AMP may have several effects in the adrenocortical cell which serve to provide a fine control over corticosteroidogenesis under widely varying conditions of stimulation.

Both Kowal[51] and Earp et al.[44] have suggested that ACTH or cyclic 3',5'-AMP may stimulate glycolysis to produce glucose-6-phosphate from glycogen. McKerns[52] pointed out that ACTH in the adrenal cortex can activate glucose-6-phosphate dehydrogenase. This enzyme leads to an increased rate of reduction of $NADP^+$ by a transfer of H^+ and an electron from glucose-6-phosphate. The rate of reduction of $NADP^+$ by this enzyme is correlated with the rate of synthesis of corticoids.

According to Halkerston[50], ACTH via cyclic 3',5'-AMP increases the oxidation of ^{14}C-glucose and causes a four- to six-fold increase in the corticosteroid output. Experiments done by Tsang and Péron[53] suggest that NADPH is produced by the oxidation of pyruvate in isolated rat adrenal mitochondria. They have concluded that glucose-6-phosphate is a good precursor of rat adrenal cell cytoplasmic pyruvate which can be utilized for intramitochondrial NADPH production. When pyruvate was substantially oxidized, there occurred a significant conversion of 11-deoxycorticosterone into corticosterone. The authors believed that pyruvate synthesized by a breakdown of glucose via glucose-6-phosphate

might form an important link between an effect of ACTH or cyclic 3',5' AMP on glycolysis and steroidogenesis.

Halkerston[50] states that the adrenal cortex tissue is heterogeneous with its response to ACTH and NADPH. That ACTH first stimulates cyclic 3',5'-AMP which in turn stimulates phosphorylase may not be true. It is because, as indicated by Bransone[47], cyclic 3',5'-AMP can stimulate steroid hydroxylations regardless of the level of NADPH and in the absence of glycogen phosphorylation. It was reported by Roberts and Creange[54], that ACTH mediated through cyclic 3',5'-AMP exerts a direct effect on mitochondrial steroid transformation.

ACTH stimulates cholesterol side chain cleavage and C-11 steroid hydroxylase activity in the adrenal mitochondria. Jaanus et al.[55], Tsang and Pĕron[53], and Roberts et al.[54] also have indicated that ACTH enhances the production of pregnenolone from cholesterol by activating mitochondrial enzymes. These enzymes are rate-limiting in the utilization of cholesterol.

Hirshfield and Kortz[56] and Roberts et al.[49] show that ACTH enhances the permeability of mitochondria to cholesterol or to pregnenolone. Bransome[47] and Nay et al.[57] found that the ACTH or cyclic 3',5'-AMP had a significant stimulatory effect in increasing amino acid incorporation into protein in the rat adrenal cells. Farese[58] showed that ACTH activated or induced the specific rate-limiting protein produced

by adrenocortical cells. This protein is essential for corticosteroidogenesis. Schulster et al.[46] indicated that the mechanism for ACTH in stimulating the protein synthesis or rapid protein turnover rate was mediated by cyclic 3',5'-AMP.

ACTH increases the synthesis of cholesterol from acetate as indicated by Kowal[51]. ACTH or cyclic 3',5'-AMP also increases the lipase activity in the rat adrenal homogenates. Bransome[47] and Halkerston[50] indicated that lipolysis was correlated to the steroid production after ACTH administration. They concluded that acetoacetyl CoA and Acyl CoA, the breakdown products of lipolysis, are important sources for steroid synthesis.

ACTH-Cortisol Feedback Control

As indicated by Orten and Neuhaus[16], the synthesized glucocorticoids are transported by the blood α-globulins, called transcortins, to the tissues and to the pituitary gland which regulated the release rate of ACTH. Kraicer and Milligan[59] have found that high concentrations of glucocorticoids act directly in the adenohypophysis to inhibit ACTH secretion, perhaps by decreasing the response of adenohypophysis to corticotrophin-releasing factors. Glucocorticoids in physiological concentrations also have this suppressing effect. Tepperman[6] states that ACTH is released in response to a lowering of cortisol levels in the blood.

STRESS AND PITUITARY - ADRENAL RESPONSE

Stress cause increased secretion of ACTH. Wedemeyer[60] has shown that the earliest detectable changes following stress are very similar to that of ACTH administration: a striking depletion of ascorbic acid and cholesterol in the adrenal cortex which is associated with an increased output of blood glucocorticoids. Gilder et al.[26] has indicated that the response to stress of all kinds is characterized by the activation of the sympathetic nervous and pituitary-adrenal systems. The adrenal cortex responds promptly to environmental influences. Turner[1] states that the Zona fasciculata shrinks in size following hypophysectomy suggesting the close relationship between pituitary and adrenal glands. According to Krieger and Krieger[61] and Arthur[62], signals coming either from chemical, sensory, or stressful stimuli cause the hypothalamus to produce substances called corticotrophin-releasing factors. As indicated by Tepperman[6], these factors are transported through the hypophysial portal blood vessels to the adenohypophysis of the pituitary gland, and activate the gland to produce ACTH. Liu[63] and Qureshi[43] have reviewed the relationship between adrenocortical activity and psychological state of individuals. That the central nervous system participates in ACTH release is an established fact. Tepperman[6] has stated that very striking increase in 17-hydroxycorticosteroid concentrations of the blood can be provoked by the technique of traumatic interview in man which consists of deliberately insulting or

embarrassing subjects in conversation, and by frightening monkeys in various ways. Fear, or anticipation of physical injury or pain, can trigger precisely the same sequence of endocrine responses as pain itself or actual physical injury. Functional relationships exist between higher cerebral processes and autonomic responses, whether of the sympathetic nervous system or the endocrine.

THE ABILITY OF 17-HYDROXYCORTICOSTEROIDS TO WITHSTAND STRESS

Glucocorticoids have the ability to enable organism to withstand stress. According to Orten and Neuhaus[16], Tepperman[6], and Turner[1], adrenalectomized animals have very little ability to tolerate stresses. These stresses can be hunger, temperature extremes, hypoxia, burns, hemorrhage, prolonged muscular activity, trauma, infection, intoxication, or severe psychological trauma. The effects of adrenalectomy are weakness, hypotension, and gastrointestinal disturbances, including anorexia, nausea, vomiting, abdominal pain, and diarrhea. Adrenal cortical hormone insufficiency influences mood and adjustment to reality as seen in depressed Addisonian patients. If the patients are given cortisol treatment, they become euphoric with marked increase in appetite and weight gain. It appears that as a result of the above physiological observations that cortisol has a direct effect on the cells of the central nervous system.

As indicated by Turner[1], the response of the organism to non-specific stress is called the "General Adaptation Syndrome." The endocrine adjustments that occur during stress must be of utility to the organism in its attempt to maintain homeostasis. However, Tepperman[6] has pointed out that adrenocorticoids are not direct nor the only causative agents to the responses. Paul et al.[64] show that other hormones such as adrenalin, noradrenalin, and thyroxine are also involved. Different stresses lead to different responses to these hormones.

IMMOBILIZATION STRESS

Numerous investigations on human beings and animals have shown that prolonged immobilization causes increased 17-hydroxycorticosteroid secretions. Liu[63], Burton and Beljan[65], Ryzhendov[66], and Burstein et al.[67] are a few of these investigators. Paul et al.[64] and Burton and Beljan[65] have demonstrated that the hormonal elevation during immobilization is initiated via the hypothalamic-pituitary-adrenal axis. Immobilization caused highly significant rises in ACTH, in adrenal cortex tissue, adrenal cortical cyclic AMP, and plasma glucocorticoids. Simonov and Fedorov[68] found that under the influence of prolonged immobilization it was impossible to synthesize new protein in the peripheral level.

Mental Stress

Immobilization causes mental stress. Burton and Beljan[65] found chronic restraint of adult domestic fowl produced a typical environmental stress. The most commonly observed change in the fowl appeared to be behavioral, which included initially a period of anxiety and hyperactivity, followed by a rapidly progressive lethargie state. This condition was accompanied usually by a reduced food intake and loss of body mass which resulted in death if the restraint continued.

BED REST

Skeletal Muscles under State of Bed Rest

Bourne[69] states that in normal conditions muscles of the body are concerned with movement and maintenance of posture as they work against gravity. Skeletal muscles maintain a balanced muscle tone which means a state of balanced reflex condition. The postural muscles work constantly against gravitational force. Birge and Whedon[70] state that physiological weightlessness has been simulated experimentally by strict horizontal bed rest and continuous water immersion. In the weightless state, muscles and bones are not subjected to the force of gravity; therefore, the muscles are relaxed and do not initiate sensory impulses. When a man is lying on his back under conditions of normal gravity, the muscles and bones involved in maintaining his erect posture

have no antigravitational work to do. Experiments done by Wells[71], Bykov and Smirnov[72], Kakurin et al.[73], Malm[74], and Mikhaleva et al.[75] have shown that in prolonged bed rest there is significant muscle atrophy with increased excretions of muscle breakdown products such as sulfur, nitrogen, and phosphorus and calcium from bones. Mack and LaChance[76] have found that immobilization cause a significant decrease in bone density.

Hormone Secretion and
Orthostatic Hypotension

Bourne et al.[77] state that the muscle fibers are well supplied with blood and nerves. Rodahl[78] showed the most striking changes in healthy young men confined to bed for prolonged periods had a reduced tilt-table tolerance (orthostatic hypotension), increased urinary calcium excretion, and reduced physical work capacity. Malm[74] found prolonged bed rest led inevitably to a series of debilitating changes, including reduction of blood volume, disuse atrophy of muscles and bones, vasomotor instability, and increased extracellular free fluid. The hypotension and reduction of blood volume lead to secretions of epinephrine and norepinephrine by the adrenal medulla. Paul et al.[64] found that immobilization caused an increased secretion of epinephrine. These hormones stimulate the production of ACTH, which in turn stimulates the secretion of 17-hydroxycorticosteroids. Krieger and Krieger[61] found that norepinephrine was a potent agent in stimulating the hypothalamus

to produce ACTH and corticosterone in rats. The concentrations on norepinephrine was found very high in the hypothalamus. These results indicate that hypotension and increased hypothalamus stimulating agents during prolonged horizontal bed rest may be the causative factors in raising 17-hydroxycorticosteroid secretions.

Hormonal Effects on

Muscular Fibrosis

Browse[79] indicates that complete muscular atrophy took many months, but the process began after a few days in bed. Zov'Yalov et al[80] in their 100-day bed rest study found that the exposure by hypokinetic conditions was shown to deteriorate significantly the efficiency of muscle movement. Browse[79] stated that Thompson in 1934 indicated that, after the muscles underwent a series of histological changes, areas of fibrosis began to appear in the muscle after six weeks of being in bed. According to Briggs and Brotherton[81], glucocorticoids have the properties of both preventing the formation of new fibroblasts and also promoting the removal of already formed collagen.

Hormonal Effects on Bones

Rodahl[78] suggests that the highly significant negative calcium balance during prolonged bed rest can be caused by lack of longitudinal pressure on the long bones and by the increased secretion on 17-hydroxycorticosteroids which act catabolically on the protein in the bone

matrix as indicated by Orten and Neuhaus[16] and Bourne et al.[77]. Birge and Whedon[70] believed that the corticosteroid hormones might have primarily a causative role in the pathogenesis of osteoporosis and might cause the demineralization of bone during immobilization.

PLAN OF PROCEDURE

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

A detailed account of the 17-hydroxycorticosteroid excretions of the subjects participating throughout this investigation is presented in this report.

Periods of the Study

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

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

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

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

Subjects of the Study

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

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

GENERAL PROCEDURE USED IN THE STUDY

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

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

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

EQUILIBRATION PERIOD

During this period, which lasted for 17 days, the eight subjects led a normal life while engaging in various tasks in the laboratories. They were encouraged to participate in moderate exercise each

day and were required to be in bed at 10:30 P.M. with the lights out and the bed cubicles darkened from 11:00 P.M. until 7:00 A.M. The meals which were prepared and consumed in the metabolic ward were planned to contain 2600 calories daily, including 1.0 gram of calcium and 100 grams of protein.

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

BED REST PERIOD

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

This phase of the study was designed to investigate many physiological changes, including the phenomenon of circadian rhythm. A strict day-night regimen was maintained which provided 14 hours of

daytime and 10 hours of night. All outdoor and hall windows in the metabolic ward were screened with opaque black paper and heavy drapes, thereby allowing no light whatsoever to penetrate the parts of the ward in which the experimental subjects were kept in bed. The light intensity in the 14 daytime hours in the various parts of the metabolic ward in which the men were in bed exhibited a mean of 30 foot candles when measured with a General Electric Type DW-68 Light Meter.

At 9:00 A.M. the day began by turning on the lights. This lasted until 11:00 P.M. when all lights including television lamps were turned off. A thermometer was placed on the wall above the head of each subject and the temperature was maintained carefully within a range of $78^{o} \pm 2^{o}F$.

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

Four subjects were selected to take part in the Exercise Program with the other four subjects not exercising. The subjects selected to exercise were 2A, 3A, 6A, and 7A. One subject, however, (3A), pleaded fatigue when the exercise program was one-half completed and

was replaced by Subject 1A who followed the program the remainder of the 56-day period.

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

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

POST-BED REST PERIOD

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

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

SUMMARY A

EXERCISE SCHEDULE

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

Step	Activity	Time
1	Isometric (EXER-GENIE)	10 seconds
2	Leg Exercise (EXER-GENIE)	6 minutes
3	Rest	2 minutes
4	Hand - Fingers (Gripper)	1 minute
5	Rest	2 minutes
6	Isometric (EXER-GENIE)	10 seconds
7	Arm Exercise (EXER-GENIE)	6 minutes
8	Rest	2 minutes
9	Hand - Fingers (Gripper)	1 minute
10	Rest	2 minutes
11	Isometric (EXER-GENIE)	10 seconds
12	Leg Exercise (EXER-GENIE)	6 minutes
Total	Isometric	30 seconds
	Isotonic	20 minutes

SUMMARY B

SUMMARY OF EXERCISE ACCOMPLISHMENT

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

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

PROCEDURE FOR THE DETERMINATION OF URINARY 17-HYDROXYCORTICOSTEROIDS

The method employed at the TWU Laboratories for the analysis of urinary 17-hydroxycorticosteroids is adapted from the procedure outlined by Porter and Silber[82].

1. Urine collection and storage. A urine specimen is collected in a plastic bottle. Record the volume and keep in the frozen state until ready for extraction.

2. If the specimen is frozen, remove this from the deep freeze and permit it to thaw. Thoroughly mix specimen and adjust pH to 2.4 with sulfuric acid by means of a pH meter.

3. For extraction, the screw cap test tubes are set up as follows:

	Unknown	Standard	Reagent Blank
Urine, pH adjusted	8 ml.	----	----
Working Standard, 10 µg/ml.	----	8 ml.	----
Distilled Water	----	----	8 ml.
n-Butanol	4 ml.	4 ml.	4 ml.

4. The sample is shaken for 10 minutes on a mechanical shaker, and then is centrifuged at 3000 RPM for 10 minutes.

5. The supernatant (butanol) layer is transferred to a set of the clean screw cap test tubes by means of a "serum lifter".

6. A second similar extraction is made, and the butanol extracts are combined. The aqueous phase may be discarded.

7. This butanol extract then is shaken with 1 ml. of 10 per cent potassium carbonate on a mechanical shaker for 30 seconds and is centrifuged for 10 minutes at 3000 RPM.

8. The butanol extract again is transferred to a new set of screw cap test tubes by means of a "serum lifter".

9. Immediately one gram of anhydrous sodium sulfate is added to remove any water reserved. The mixture then is shaken on a mechanical shaker for 30 seconds and is centrifuged for 10 minutes at 3000 RPM.

10. Finally, the butanol extract is decanted into a clean centrifuge tube, and is stored in the refrigerator until ready to continue.

11. Prepare tubes for color development. For each butanol extract, set up two tubes as indicated below. Then place in refrigerator until needed.

A. tube (sample) --- 4 ml. phenylhydrazine sulfuric acid reagent.

B. tube (sample blank) --- 4 ml. 18N sulfuric acid.

12. Add 2 ml. of butanol alcohol extract both to the A tube and B tube.

13. The tubes are mixed with a mixer. Incubate in a water bath at a temperature of 60° C for exactly 30 minutes.

14. At end of incubation transfer to ice-water bath for 5 minutes.

15. Read in spectrophotometer at 410 mμ wave length. Set zero OD with tube B (sample blank) and read tube A (sample) against it.

CALCULATION:

1. $\frac{\text{OD Unknown - OD Reagent Blank}}{\text{OD Standard - OD Reagent Blank}} \times 10 = \text{mcg./ml.}$

2. $\frac{\text{Total Volume X mcg./ml.}}{1000} = \text{mg./24 hours}$

STANDARDIZATION:

Stock Hydrocortisone Standard (200 μg./ml.)

Dissolve exactly 20 mg. of hydrocortisone (free alcohol) in about 1/2 ml. of alcohol and dilute to exactly 100 ml. with distilled water.

Working Hydrocortisone Standard (10 μg./ml.)

Dilute 5 ml. of stock standard to 100 ml. with distilled water.

REAGENTS:

(1) n-Butanol --- Reagent grade, obtainable from City Chemical Corporation, New York, or Distilled Industrial Products, Eastman Kodak. The reagent must be checked with the phenylhydrazine and sulfuric acid reagent to give a low blank reading.

(2) 18N Sulfuric Acid --- Carefully add 127 ml. of concentrated sulfuric acid (with cooling) into 100 ml. of distilled water.

(3) Phenylhydrazine-Sulfuric Reagent --- Dissolve 49 mg. of recrystallized phenylhydrazine in 75 ml. of 18N sulfuric acid.

Recrystallized Phenylhydrazine --- Dissolve phenylhydrazine hydrochloride in a minimal amount of absolute alcohol by heating. Allow to cool at room temperature. Then place in a refrigerator at least for one hour. Filter through a sintered glass filter. Transfer crystals, which should be peach colored, to a clean container and place in a dessicator.

(4) 10% Potassium Carbonate --- Dissolve 10 gm. of potassium carbonate in 100 ml. of distilled water.

(5) 2N Sulfuric Acid --- An approximate dilution of 1 to 10 may be made of the 18N sulfuric acid.

(6) Sodium Sulfate, Anhydrous --- Reagent grade.

PRESENTATION OF FINDINGS WITH DISCUSSION

Eight healthy adult male subjects participated in this immobilization study which covered a span of 85 days, including Pre-Bed Rest, Bed Rest, and Post-Bed Rest Periods. The daily urinary excretion data on the 17-hydroxycorticosteroid hormone for the three periods are presented in Table I (Appendix). Table II contains the data concerning the diurnal pattern of urinary 17-hydroxycorticosteroid excretion during the Bed Rest Period. The statistical comparisons of circadian rhythm are recorded in Table III. The statistical data pertaining to exercise are shown in Tables IV, V, VI, VII, and VIII. The statistical comparisons of excretion between pairs of different periods are presented in Tables IX, X, and XI.

CIRCADIAN PATTERN OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING BED REST PERIOD

In order to study the variation of the 17-hydroxycorticosteroid, the urine excreted during the 24-hour period was collected into four aliquots at different time periods which ended respectively at 8 a.m., 12 noon, 8 p.m., and 12 midnight. The statistical data regarding the excretion patterns are given in Table III and in Figures 3 and 4.

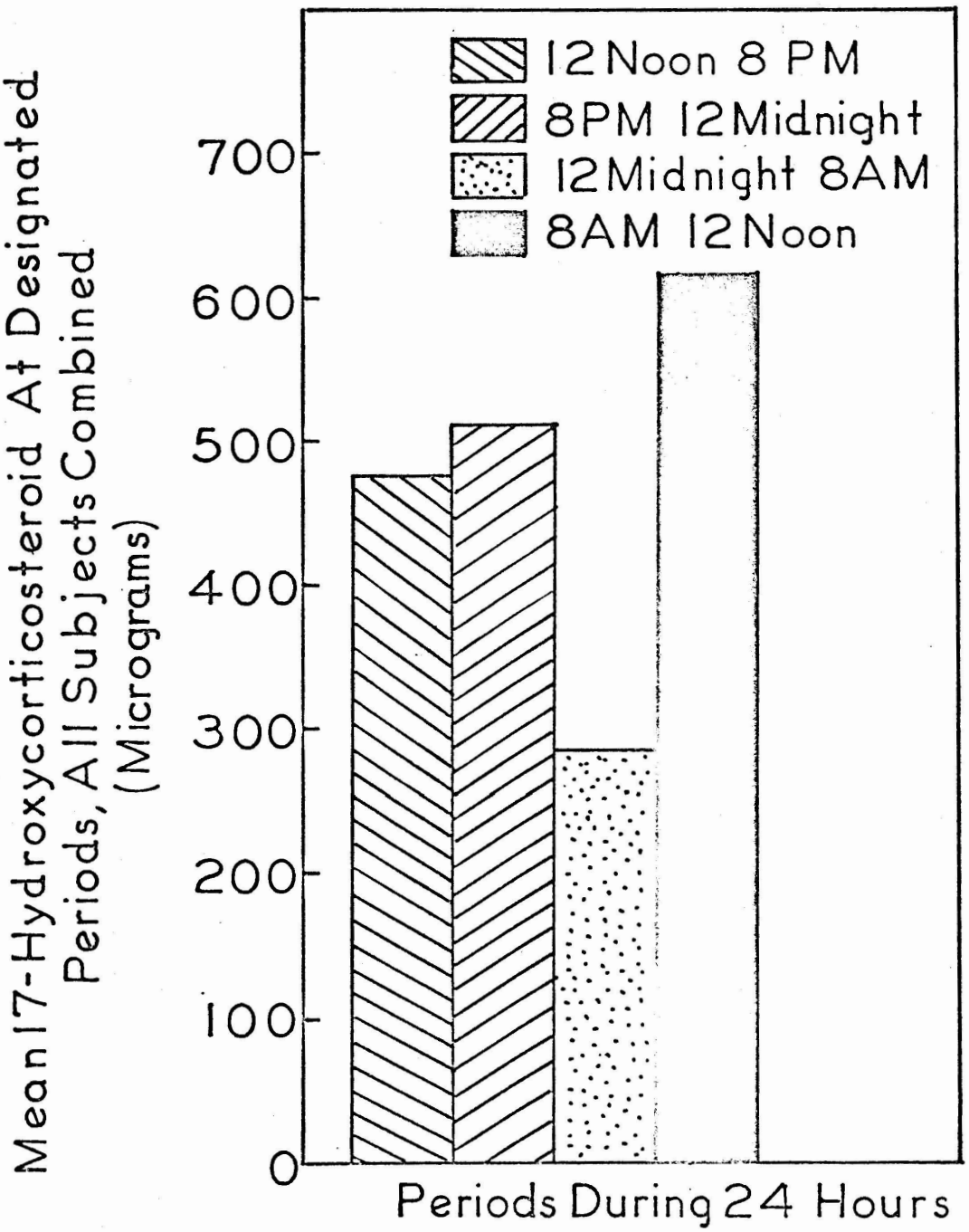


Figure 3. MEAN 17-HYDROXYCORTICOSTEROID EXCRETION AT DESIGNATED PERIODS, ALL SUBJECTS COMBINED

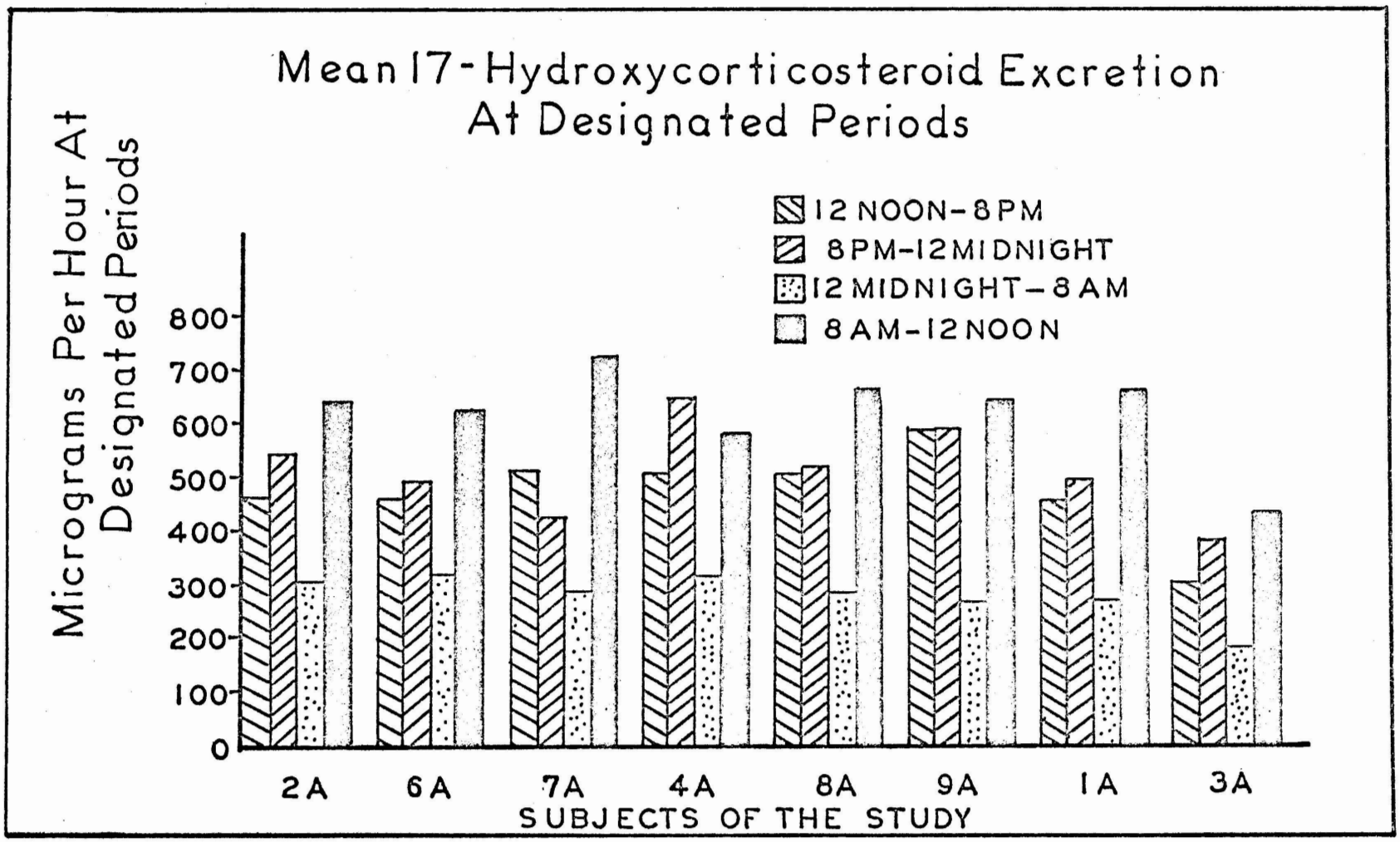


Figure 4. MEAN 17-HYDROXYCORTICOSTEROID EXCRETION AT DESIGNATED PERIODS, OF INDIVIDUAL SUBJECTS

SUBJECT 2A

Table III, Part A, shows that the quantity of 17-hydroxycorticosteroid excreted by Subject 2A between 12 noon and 8 p.m. was lower than that excreted between 8 p.m. and 12 midnight. There was no statistically significant difference between these two periods. Highly significant differences were found, however, when the 12 noon to 8 p.m. period was compared with the 12 midnight to 8 a.m. and the 8 a.m. to 12 noon periods, and the 8 p.m. to 12 midnight period was compared with the 12 midnight to 8 a.m. period and the 8 a.m. to 12 nood period. No statistically significant difference was found between the 8 p.m. to 12 midnight and the 8 a.m. to 12 noon periods. Among these periods, the hormones excreted during 8 a.m. to 12 noon were the highest, 8 p.m. to 12 midnight ranked next, 12 noon to 8 p.m. was the third highest, and 12 midnight to 8 a.m. was the lowest.

SUBJECT 6A

As shown in Table III, Part B, very similar results were found for Subject 6A as were found for Subject 2A. No significant difference was found when the 12 noon to 8 p.m. excretion was compared to that excreted from 8 p.m. to 12 midnight, as well as that from the 8 p.m. to 12 midnight period; and the 8 a.m. to 12 noon period were compared. Highly significant differences were found when the other periods were compared.

SUBJECT 7A

Table III, Part C, contains the daily urinary hormone excretions for Subject 7A. No significant difference was found between the 12 noon to 8 p.m. period and the 8 p.m. to 12 midnight period. Highly significant differences were found when all other periods were compared. The rank from high to low for the amount of excretion is as follows: 8 p.m. to 12 noon, 12 noon to 8 p.m., 8 p.m. to 12 midnight, and 12 midnight to 8 a.m. With the exception of having no significant difference between the 12 noon to 8 p.m. period and the 8 p.m. to 12 midnight period, highly significant differences were found when all other periods were compared in pairs ($P < 0.001$), Table III, Part D.

SUBJECTS 2A, 6A, and 7A

When data for the three subjects who exercised throughout the study were collected, it was found that the amount of urinary 17-hydroxycorticosteroid excreted from 8 p.m. to 12 noon represented the highest level of excretion, the sample from 8 p.m. to 12 midnight ranking next, the 12 noon to 8 p.m. third, and the 12 midnight to 8 a.m. the lowest. With the exception of having no significant difference between the 12 noon to 8 p.m. period and the 8 p.m. to 12 midnight period, highly significant differences were found when all other periods were compared in pairs ($P < 0.001$). Table III, Part D.

SUBJECT 4A

As shown in Table III, Part E, when pairs of the four collection periods were compared by means of the "t" test for quantities of urinary 17-hydroxycorticosteroid excreted, the amount excreted by Subject 4A during the period from 8 p.m. to 12 midnight was higher than that excreted between 12 noon to 8 p.m. ($P < 0.05$). The hormone excretion from 12 noon to 8 p.m. was highly significantly greater than that from 12 midnight to 8 a.m. No significant differences were found between the 12 noon to 8 p.m. period and the 8 a.m. to 12 noon period, as well as the 8 p.m. to 12 midnight period and the 8 a.m. to 12 noon period. Urinary hormone excretion during the 12 midnight to 8 a.m. period was significantly lower than the 8 p.m. to 12 midnight period or the 8 a.m. to 12 noon period.

SUBJECT 8A

Table III, Part F contains the statistical data pertaining to the periodic comparison of the four periods of 17-hydroxycorticosteroid excretion for Subject 8A. The amount of urinary 17-hydroxycorticosteroid excreted from 12 noon to 8 p.m. was not significantly different from the period from 8 p.m. to 12 midnight. Significant differences were found when all other periods were compared in pairs. The rank of excretion from the highest to the lowest is as follows: 8 a.m. to 12 noon, 8 p.m. to 12 midnight, 12 noon to 8 p.m. and 12 midnight to 8 a.m.

SUBJECT 9A

As shown in Table III, Part G, there were no significant differences found when the 12 noon to 8 p.m. period and the 8 p.m. to 12 midnight period, the 12 noon to 8 p.m. period and the 8 a.m. to 12 noon period, and the 8 p.m. to 12 midnight period and the 8 a.m. to 12 noon period were compared, although the highest amount was found during the period from the 8 a.m. to 12 noon period. The amount of urinary 17-hydroxycorticosteroid excreted during the 12 noon to 8 p.m. period was significantly higher than that excreted from 12 midnight to 8 a.m.; whereas the excretion during the 8 p.m. to 12 midnight period was significantly higher than that period from 12 midnight to 8 a.m. to 12 noon ($P < 0.001$).

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

Table III, Part H, indicates the statistical information compiled from the combined data of the three subjects who did not exercise throughout the study. The sub-group from 8 a.m. to 12 noon showed the highest level of 17-hydroxycorticosteroid excretion. A high level of significance was found when comparing the excretion levels of these hormones during this period with the 12 noon to 8 a.m. and the 12 midnight to 8 a.m. periods. No significant difference was found when the excretion during this period was compared with that from 8 a.m. to 12 midnight. The urinary 17-hydroxycorticosteroid excreted during the 12 noon to 8 p.m. period was significantly higher than that from 12

midnight to 8 a.m. No significant difference was found when comparing this urinary hormone excreted during the 8 p.m. to 12 midnight period with the 8 a.m. to 12 noon period, but the hormone excreted during this same period was significantly higher than that for the period from 12 midnight to 8 a.m.

SUBJECT 1A

As shown in Table IV, Part A, Subject 1A excreted the greatest amount of urinary 17-hydroxycorticosteroids during the period from 8 a.m. to 12 noon. When this amount was compared with the quantities excreted during other periods, the differences were found to be significant. The amount of the excretion from 12 noon to 8 p.m. was significantly higher than that excreted from 12 midnight to 8 a.m. On the other hand, there was no significant difference when compared with the excretion from 8 p.m. to 12 midnight. The amount of the excretion from 12 midnight to 8 a.m. was significantly lower than the amount excreted from 8 p.m. to 12 midnight or from 8 a.m. to 12 noon.

SUBJECT 3A

Table IV, Part B, shows the urinary 17-hydroxycorticosteroid excretions of Subject 3A who exercised the first 28 days of the study. The largest amount of excretion was during the period from 8 a.m. to 12 noon. The quantity of hormones excreted during this period markedly surpassed the amounts excreted from 12 noon to 8 p.m. ($P<0.02$) and

from 12 midnight to 8 a.m. ($P < 0.001$). However, the amount excreted during this period was not significantly higher than the amount excreted from 8 p.m. to 12 midnight. The quantity excreted during the 12 midnight to 8 a.m. period was the lowest, and it was significantly lower than that from 12 noon to 8 p.m., or from 8 p.m. to 12 midnight. There was no significant difference between the 12 noon to 8 p.m. and the 8 p.m. to 12 midnight periods.

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 17-hydroxycorticosteroid was found during the period from 8 a.m. to 12 noon. During this period the 17-hydroxycorticosteroid excretion was significantly higher in comparison with any of the other periods ($P < 0.001$). The urinary hormone excretion from 8 p.m. to 12 midnight was not significantly higher than that from 12 noon to 8 p.m. period, and the excretion from 8 p.m. to 12 midnight was significantly higher than that from 12 midnight to 8 a.m. The 17-hydroxycorticosteroid excretion from 12 noon to 8 p.m. surpassed that from 12 midnight to 8 a.m. by a highly significant difference ($P < 0.001$). See Table V.

Although there was a very slight variation among the individuals, the excretion pattern of the data for all eight subjects during the different periods was very consistent and similar in the amounts of urinary

17-hydroxycorticosteroid excretions. The pooled data for all subjects indicated the existence of a marked characteristic circadian pattern in urinary 17-hydroxycorticosteroid excretion. The peak level of excretion was attained between 8 a.m. to 12 noon. Then the excretions decreased as the day proceeded with a slight rise before midnight. Finally, the excretion level dropped to a very lower level during the overnight sleeping hours, until the next morning when the excretion again was sharply raised. Figures 3 and 4 contain the individual and the combined results of these subjects, indicating the circadian pattern of this study.

Lee[83] has found a diurnal pattern of the urinary 17-hydroxycorticosteroid excretion in her 28-day Bed Rest study analyses. The highest values were found to be during the morning hours (between 8 a.m. to 12 noon), the next higher values were found in the afternoon period, and the lowest excretion values were found during the overnight period. This circadian pattern is based on the clear cyclic secretory activity of the adrenal cortex. According to Browse[79], the plasma 17-hydroxycorticosteroid concentrations increase to a maximum during the latter part of night (6 a.m.) and fall to a minimum between 8 p.m. and 12 midnight. The urinary 17-hydroxycorticosteroid concentration shows a similar variation but the peak is approximately two hours later. These previous findings are consistent with the results of this study.

COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING BED REST AS INFLUENCED BY EXERCISE

Table VI, VII, and VIII contain the data pertaining to the effect of exercise on urinary 17-hydroxycorticosteroid excretion during the Bed Rest Period.

SUBJECT 1A (Exercised Second 28 Days of the Study)

As shown in Table VI, Subject 1A did not have appreciable decrease in urinary 17-hydroxycorticosteroid during the second half of the Bed Rest Period when he was engaged in programmed exercise. No significant difference was reflected in the amounts of urinary 17-hydroxycorticosteroid excreted by Subject 1A under different activity schedules during the four collection periods. Except for the period from 8 a.m. to 12 noon, the urinary 17-hydroxycorticosteroid excretions during inactive periods were slightly higher than the amounts excreted during the active periods. See Table VIII, Part A.

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

The data in Table VI indicate the urinary 17-hydroxycorticosteroid excretion of Subject 3A who exercised during the first 28 days of the Bed Rest Study. No statistical difference was found between urinary 17-hydroxycorticosteroid excretions of the exercised and non-exercised periods. Table VIII, Part B shows when the comparisons were

made for the same subject between exercise and non-exercise periods, no significant difference was found in any of the daily four periods.

Subject 3A complained continuously of fatigue during the time when he was supposed to exercise, and rarely completed an exercise program. With one exception found in the period from 8 p.m. to 12 midnight, the urinary 17-hydroxycorticosteroid excretions during the inactive period were slightly lower than the active period.

GROUP OF SUBJECTS WHO EXERCISED
(2A, 6A, 7A)

VERSUS GROUP WHO DID NOT EXERCISE
(4A, 8A, 9A)

A statistical comparison was made between the group of subjects who exercised (2A, 6A, 7A) and the group of subjects who did not exercise (4A, 8A, 9A) during the Bed Rest Period. As shown in Table VII, no significant difference was found when the amounts of urinary 17-hydroxycorticosteroid excreted by those non-exercisers were compared with those of the exercisers. It was found, however, that the mean excretion among the exercisers was lower than that of the non-exercisers.

Table VIII, Part C shows that when the 17-hydroxycorticosteroid values for the respective times of the day during Bed Rest Period were compared between those of the group who exercised and those of the group who did not exercise, it was found that the mean value for the

non-exercisers was significantly higher than that of the exercisers during the period from 8 p.m. to 12 midnight ($P < 0.01$). The amounts excreted by the non-exercisers also were higher than the exercisers, though there was no significant difference between the mean values. No statistical differences were found when the groups were compared for the periods of 12 midnight to 8 a.m. and of 8 a.m. to 12 noon. The amount of urinary 17-hydroxycorticosteroid excretions was slightly higher for the active group than for the inactive group.

COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING THE BED REST, PRE-BED REST, POST-BED REST PERIODS

The statistical analysis of the urinary 17-hydroxycorticosteroid excretion data for the periods of Bed Rest, Pre-Bed Rest, and Post-Bed Rest are shown in Table IX.

SUBJECT 2A

The urinary 17-hydroxycorticosteroid excretions of Subject 2A have a highly significant difference between Bed Rest and Pre-Bed Rest ($P < 0.01$), or Bed Rest and Post-Bed Rest Periods ($P < 0.001$). There is a significant difference between the two Ambulatory Periods. The value in Pre-Bed Rest was higher than the Post-Bed Rest. See Table IX, Part A.

SUBJECT 6A

Subject 6A did not show any significant difference in his urinary 17-hydroxycorticosteroid secretion in any of the compared periods except for the fact that the amount of hormone secreted during the Bed Rest was higher than the other two periods. See Table IX, Part A.

SUBJECT 7A

Highly significant differences ($P < 0.01$) were found when the urinary excretion during Bed Rest was compared with the other two ambulatory periods. Urinary hormone excretion during Pre-Bed Rest was higher than the Post-Bed Rest excretion ($P < 0.05$). See Table IX, Part A.

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

Table IX, Part B is a presentation of a statistical comparison of the data for all subjects who exercised throughout the Pre-Bed Rest, Bed Rest, and Post-Bed Rest Periods. The amount of 17-hydroxycorticosteroids excreted in the urine during the Bed Rest Period was significantly ($P < 0.001$) higher than that during the Pre- and Post-Bed Rest Periods. There was no significant difference between Pre- and Post-Bed Rest Periods.

SUBJECT 4A

The excretion of urinary 17-hydroxycorticosteroids by Subject 4A during the Bed Rest Period was significantly greater than that during the

Pre-Bed Rest Period ($P < 0.001$). However, in spite of higher value for the Bed Rest Period, there was no statistically significant difference when compared to the ambulatory periods. See Table IX, Part C.

SUBJECT 8A

The urinary 17-hydroxycorticosteroid excretion of Subject 8A during the Bed Rest Period did not differ significantly from the Pre-Bed Rest Period, but the excretion was significantly higher than the Post-Bed Rest Period ($P < 0.001$). The excretion value for the Pre-Bed Rest Period was significantly higher than the Post-Bed Rest Period ($P < 0.05$). See Table IX, Part C.

SUBJECT 9A

As shown in Table IX, Part C, during the Bed Rest Period, Subject 9A excreted significantly higher level of urinary 17-hydroxycorticosteroid than during the Post-Bed Rest Period. There were no significant differences when other periods were compared, though the value for the Bed Rest Period was greater than for the other periods.

SUBJECT 4A, 8A, 9A (No Exercise)

An analysis of the pooled data for the three subjects who did not exercise showed that, the total 17-hydroxycorticosteroid excretion during the Bed Rest Period surpassed that of the Pre- and Post-Bed Rest Periods with the difference being highly significant ($P < 0.01$) in both

cases. There was no significant difference in the excretions between the two ambulatory periods of this group. See Table IX, Part D.

17-HYDROXYCORTICOSTEROID URINARY EXCRETION BY SUBJECTS WHO EXERCISED ONLY ONE-HALF OF THE BED REST PERIOD

Table X contains the urinary 17-hydroxycorticosteroid statistical data for Subject 1A, who exercised during the second half of the Bed Rest Period, and for Subject 3A, who exercised during the first half of the Bed Rest Period.

Similar results were found in both subjects. There was no significant difference between Pre-Bed Rest and Bed Rest Periods in both cases. Significantly higher values were found when Pre-Bed Rest and Post-Bed Rest ($P < 0.05$), or Bed Rest and Post-Bed Rest ($P < 0.001$) were compared.

ALL SUBJECTS

As shown in Table XI, when statistical comparisons were made of the pooled data for all eight subjects, it was found that the highest level of urinary 17-hydroxycorticosteroid excretion occurred during the Bed Rest Period of the study. When this period was compared, both with the Pre- and Post-Bed Rest Periods, a highly significant difference was evident ($P < 0.001$). The results of this study support previous numerous

reports, as cited in the review of literature, that prolonged immobilization is a stress leading to increased 17-hydroxycorticosteroid excretions. However, there also was a highly significant difference between Pre- and Post-Bed Rest Periods, in which Pre-Bed Rest was higher than the Post-Bed Rest Period.

Figure 5 shows the excretion pattern for all eight subjects during the entire three periods of the study. The excretion pattern during Pre-Bed Rest fluctuated greatly. Although the mean level of the 17-hydroxycorticosteroid excretion was higher during the Bed Rest Period, the amount of excretion dropped greatly soon after the beginning of the Bed Rest study. The excretions increased steadily and reached to a plateau after the eighth or ninth day of the Bed Rest Period. The excretions remained high, although the level fluctuated, until approximately 42 days of the in-bed period, where it started to decline. The excretions continued to decrease throughout the Post-Bed Rest Period.

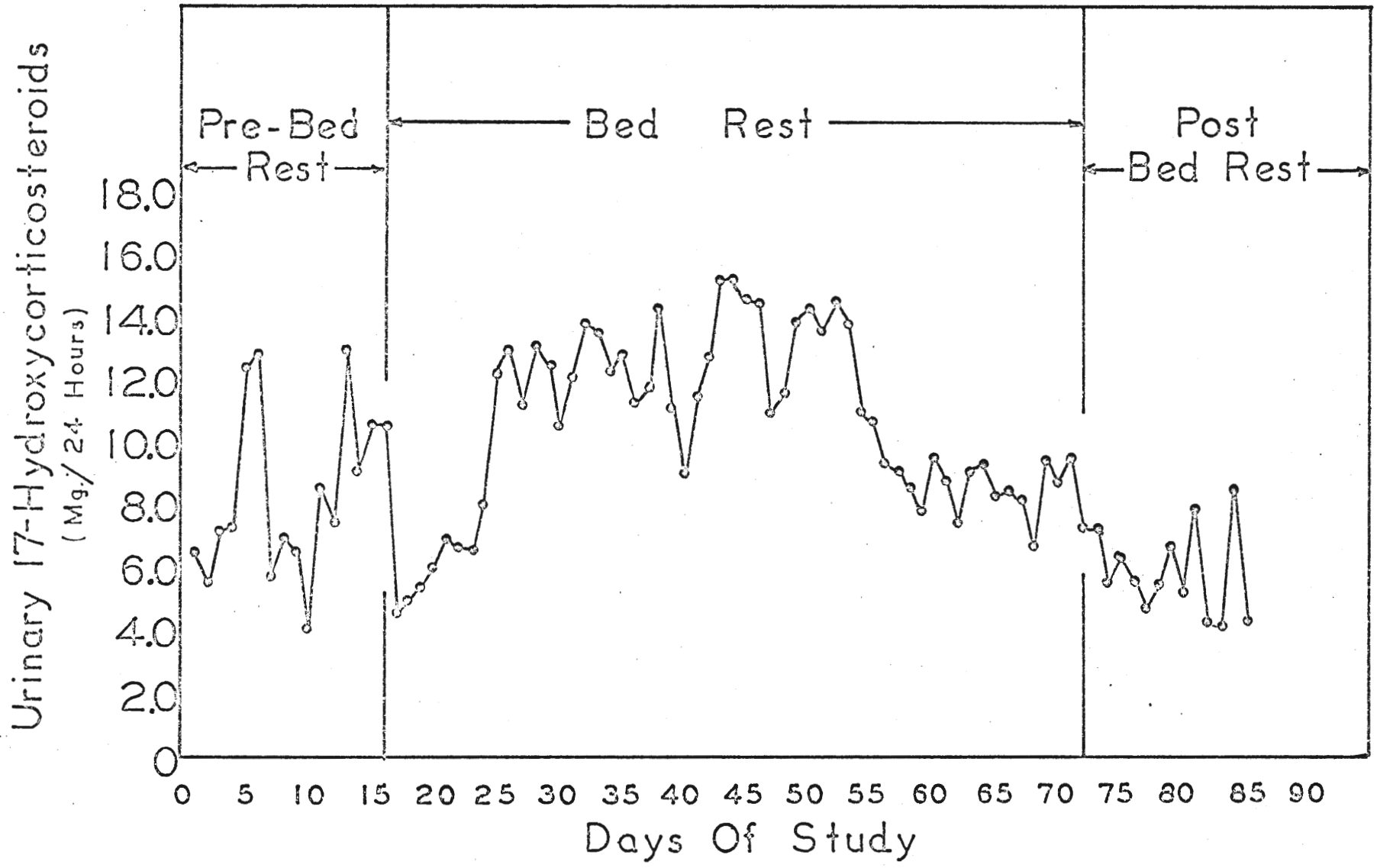


Figure 5. MEAN OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION

FOR ALL EIGHT SUBJECTS

Deitrick et al.[84] studied the metabolic aspects of young men in an immobilized condition for six weeks. These young men were in positive nitrogen balance while active. After going to bed, this positive balance persisted for four days, but it was then quickly reversed and by the sixth and seventh day there was a negative balance. This increased to reach a peak by the tenth day, then gradually returned to an equal balance. The subjects remained in balance from the fourth to the tenth week of bed rest. The return to full activity produced a sudden large swing into positive balance. The source of this nitrogen was from atrophying muscles. Burton and Beljan[65] point out that under immobilization stress muscle protein catabolism and gluconeogenesis are accelerated, suggesting increased levels of 17-hydroxycorticosteroid in the plasma and in the urine. These findings agree with the results of the author's study at Nelda Childers Stark Laboratory for Human Nutrition Research Institute.

EFFECTS OF EXERCISE DURING PROLONGED BED REST

Rose et al.[85] stated that exercise appeared to exert an influence on the capacity of the adrenal cortex. According to Turner[1] and West et al.[22], immobilization leads to an increased gluconeogenesis. During this stage excessive glucose is accumulated in the blood and muscle tissue. Exercise helps to stimulate the nerve endings on arteriols in

the muscles to produce epinephrine and norepinephrine. These hormones promote the oxidation of glucose. During immobilization the excessive muscle glucose can not be oxidized by adequate amounts of epinephrine and norepinephrine because of the lack of exercise of the muscles. Thus, there is a disturbed glucose metabolism in the muscle which causes muscle weakness.

As shown by Malm[74], all the pathophysiological changes during prolonged bed rest were counteracted by exercise. A 100-day bed rest study done by Zav'yalov et al.[80] indicated that the decline of muscular coordination was much less in the operators who performed physical exercise during the experiment. Bourne et al.[77] state that only a small amount of exercise is necessary to maintain muscle strength during bed rest. Experiments done by Fuller et al.[86] showed that two 30-minute exercise periods daily during a nine-day bed rest study revealed significantly less losses of lean body mass, potassium, and water than the controls who had no exercise. The results suggested a decreased secretion of 17-hydroxycorticosteroids in the exercised group, since these hormones caused potassium excretion and sodium retention. Nocenti and Cizek[87] indicate that the presence of adrenal cortical tissue or glucocorticosteroids is a necessary requisite for the induction of polyuria in food restricted rabbits. The loss of body water and protein by the astronauts during immobilization is again indicated in

Berry's report[88] on Apollo 7 through 11 manned spaceflights space missions.

A total daily exercise which lasted 90 seconds in an isometric manner and 60 minutes in an isotonic manner was done by the four subjects during the Bed Rest Period in this Laboratory. As shown in Table VII, the non-exercisers did excrete more urinary 17-hydroxycorticosteroids during the Bed Rest Period than the exercisers, but the difference was not significant. Rose et al.[85] showed that plasma 17-hydroxycorticosteroids did not increase in moderate exercise as seen in one-mile running experiments performed by nine healthy men. Suzuki et al.[89] indicated that it was only when animals were slightly or completely exhausted that the adrenal cortex secreted increased or markedly increased 17-hydroxycorticosteroids. The same principle seems also applied to immobilization. As shown in Figure 5, urinary 17-hydroxycorticosteroid excretions for all eight subjects increased almost linearly after the subjects were in bed. The peak excretion was reached after eight or nine days of immobilization. The determining factor is the degree of stress from exercise or immobilization.

These findings indicate that exercise is a release for the stressful condition during prolonged immobilization. Optimal muscle tone and hormonal homeostasis can be maintained only when the body

exerts moderate amount of exercise or movement and enjoys adequate relaxation.

SUMMARY AND CONCLUSIONS

Eight male university students participated in an immobilization study which covered a span of 85 days and consisted of a 16-day Pre-Bed Rest Period, a 56-day Bed Rest Period, and a 14-day Post-Bed Rest Period. This study was conducted during the summer of 1969 at the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute, and was sponsored by the National Aeronautics and Space Administration. This investigation was designed to observe any changes occurring in bone density and various metabolic functions, including circadian response, in human subjects experiencing prolonged immobilization under carefully controlled environmental conditions. This report deals with the urinary 17-hydroxycorticosteroids which were analyzed by the method of Porter and Silber[82].

The circadian variation of urinary 17-hydroxycorticosteroids was studied during the 56-day Bed Rest Period. Throughout this period of the study, a strict regimen of 14 hours daytime and 10 hours nighttime was maintained, and the urinary samples were collected four times daily. A circadian rhythm of these excreted hormonal metabolites of all subjects was clearly revealed. The peak level of excretions was

attained between 8 a.m. to 12 noon. Then the excretions decreased as the day proceeded (12 noon to 8 p.m.), with a slight rise before midnight (8 p.m. to 12 midnight). Finally, the excretion level dropped to a very low level during the overnight sleeping hours (12 midnight to 8 a.m.), until the next morning when the excretion again was sharply raised. The rise of excretion during 8 p.m. to 12 midnight was not significantly greater than the period from 12 noon to 8 p.m., but highly significant differences were found when all other periods were compared.

During the immobilization period the subjects were divided into two groups of those who did and did not participate in the daily programmed 90-second isometric and 60-minute isotonic exercise. Urinary 17-hydroxycorticosteroids excreted by the exercised group was lower than the non-exercised group, but the difference was not significant. The small amount of exercise seemed to relieve the stressful condition during this prolonged immobilization period. Two individuals exercised for 28 days each, one during the first half of the Bed Rest and the other during the second part. No statistical difference was found between urinary 17-hydroxycorticosteroid excretions of the exercise and non-exercise periods in both cases.

Total 24 hour urinary 17-hydroxycorticosteroid excretion was found to be significantly higher ($P < 0.001$) for all subjects during the

Bed Rest Period as compared to the two ambulatory periods. Immobilization stress appears to increase the excretion levels of 17-hydroxycorticosteroid, but this stress did not affect the basic physiological diurnal pattern of the adrenal cortex hormone secretion.

BIBLIOGRAPHY

1. Turner, C. D., General Endocrinology, Fourth Edition, W. B. Saunders Company, Philadelphia (1966)

2. Exton, J. H., Hormonal Control of Hepatic Gluconeogenesis, Recent Progress in Hormone Research, 26:411 (1970)

3. Betheil, J. J., M. Feigelson, and P. Feigelson, The Differential Effects of Glucocorticoid on Tissue and Plasma Amino Acid Levels, Biochimica Et Biophysica Acta, 104:92 (1965)

4. Ray, P. D., "Adrenal Glucocorticoids and Gluconeogenesis", in Function of the Adrenal Cortex, Volume 2, Edited by K. W. McKerns, Appleton-Century-Crofts, New York, pp. 1137 (1968)

5. Weber, G., "Action of Glucocorticoid Hormone at the Molecular Level", in Function of the Adrenal Cortex, Volume 2, Edited by K. W. McKerns, Appleton-Century-Crofts, New York, pp. 1059 (1968)

6. Tepperman, J., Metabolic and Endocrine Physiology, Second Edition, Year Book Medical Publishers Incorporated, Chicago (1968)

7. Goldberg, A. L., and H. M. Goodman, Relationship Between Cortisone and Muscle Work in Determining Muscle Size, Journal of Physiology, 200:667 (1969)

8. Kaplan, S. A., and C. S. N. Shimizee, Effects of Cortisol on Amino Acids in Skeletal Muscle and Plasma, Endocrinology, 72: 267 (1963)

9. Goldberg, A. L., Protein Turnover in Skeletal Muscle: II. Effects of Denervation and Cortisone on Protein Catabolism in Skeletal Muscle, Journal of Biological Chemistry, 244:3223 (1969)

10. Makman, M. H., B. Drorkin, and A. White, Influence of Cortisol on the Utilization of Precursors of Nucleic Acid and Protein by Lymphoid Cells in Vitro, Journal of Biological Chemistry, 243: 1485 (1968)

11. Hofert, J., and A. White, Inhibitory Effect of a Liver Extract on the Incorporation of ^{3}H-Deoxycytidine into Thymus DNA of Adrenalectomized and Adrenalectomized-Hepalectomized Rats, Endocrinology, 82:777 (1968)

12. Feigelson, M., Cortisone Induced Alterations in Purine and RNA Metabolism in Liver and Lymphoid Tissues and Their Dependence on Protein Synthesis, Federation Proceedings, 23:481 (1964)

13. Mosher, K. M., D. A. Young, and A. Munck, Evidence for Irreversible Actinomycin D-Sensitive, and Temperature-Sensitive Steps Following Binding of Cortisol to Glucocorticoid Receptors and Preceding Effects on Glucose Metabolism in Rat Thymus Cells, Journal of Biological Chemistry, 246:654 (1971)

14. Nakagawa, S., and A. White, Response of Rat Thymic Nuclear RNA Polymerase to Cortisol Injection, Endocrinology, 81:861 (1967)

15. Young, D. A., Glucocorticoid Action on Rat Thymus Cells, Journal of Biological Chemistry, 245:2747 (1970)

16. Orten, J. M., and O. W. Neuhaus, Biochemistry, Eighth Edition, The C. V. Mosby Company, Saint Louis (1970)

17. Glenn, E. M., B. J. Bowman, R. B. Bayer, and C. E. Meyer, Hydrocortisone and Some of Its Effects in Intermediary Metabolism in Vitro Studies, Endocrinology, 68:386 (1961)

18. Munck, A., Metabolic Site and Time Course of Cortisol Action on Glucose Uptake, Lactic Acid Output, and Glucose-6-Phosphate Levels of Rat Thymus Cells in Vitro, Journal of Biological Chemistry, 243:1039 (1968)

19. Epstein, S., C. Esanee, and M. S. Raben, The Effect of Growth Hormone and Cortisone on Thymidine Kinase Activity in Rat Adipose Tissue, Biochimica Et Biophysica Acta, 186:280 (1969)

20. Levine, R., and D. E. Haft, Carbohydrate Homeostasis, New England Journal of Medicine, 283:237 (1970)

21. Schaeffer, L. D., Adrenal Corticosteroid Involvement in the Control of Phosphorylase in Muscle, Biochimica Et Biophysica Acta, 192:304 (1969)

22. West, E. S., W. R. Todd, H. S. Mason, and J. T. Van Bruggen, Textbook of Biochemistry, Fourth Edition, The Macmillan Company, New York, pp. 468 (1966)

23. Amaral, L., L. G. Moriber, and M. Himer, The Effect of Cortisone on the Volume and Total Protein Content of Mouse Liver Nuclei, Journal of Cell Biology, 42:835 (1969)

24. Beato, M., W. Brandle, D. Biestewig, and C. E. Sekeris, On the Mechanics of Hormone Action: XIV. Transfer of (1,2-$^3H_2$) Cortisol from the Cytoplasm to the Nucleus of Rat Liver Cells, Biochimica Et Biophysica Acta, 208:125 (1970)

25. Kidson, C., and K. S. Kirby, Selective Alterations of Mammalian Messenger-RNA Synthesis: Evidence of Differential Action of Hormones on Gene Transcription, Nature, 203:599 (1964)

26. Gilder, H., M. Valicic, V. Delcon, and D. Slernberg, Comparative Effect of Experimental Injury and Cortisol Injection on Liver and Plasma Composition of Rats, Metabolism, 19:562 (1970)

27. Ottolenghi, C., and B. Ottavio, Reduced Breakdown in Vivo of Liver Microsomal Ribonucleic Acid and Protein in Rats Treated with Cortisone, Endocrinology, 86:949 (1970)

28. Bouille, C., and I. Assenmacher, Effects of Starvation on Adrenal Cortical Function in Rabbits, Endocrinology, 87:1390 (1970)

29. Lehninger, H. L., Biochemistry, Worth Publisher, Inc., New York (1970)

30. Kupfer, D., and R. Partridge, Corticoid Mediated Increase in Tyrosine-α-Ketoglutorate Transaminase in the Rat, A Sensitive Glucocorticoid Assay, Endocrinology, 87:1198 (1970)

31. Nakajima, K., H. Matsutaka, and E. Ishikawa, Hormonal and Dietary Control of Enzymes in the Rat: II. Effect of Pancreatectomy and Adrenalectomy on Some Liver Enzymes Unique to Amino Acid Metabolism and Gluconeogenesis, Journal of Biochemistry, 67:779 (1970)

32. Segal, H. L., and Y. S. Kim, Environmental Control of Enzyme Synthesis and Degradation, Journal of Cell and Comparative Physiology, 66:11 (1965)

33. Szepesi, B., E. H. Avery, and R. A. Freedland, Role of Kidney in Gluconeogenesis and Amino Acid Catabolism, American Journal of Physiology, 219:1627 (1970)

34. Greenberg, D. M., Metabolic Pathways, Volume III. Amino Acids and Tetrapyrroles, Third Edition, Academic Press, New York (1969)

35. Marliso, E., T. T. Aoki, P. Felig, T. Pozefsky, and G. F. Cahill, Hormones and Substrates in the Regulation of Gluconeogensis in Fasting Man, Advances in Enzyme Regulation, 8:3 (1970)

36. Pagliara, A. S., and A. D. Goodman, Relation of Renal Cortical Gluconeogenesis, Glutamate Content, and Production of Ammonia, The Journal of Clinical Investigation, 49:1967 (1970)

37. Churchill, P. C., and R. L. Malvin, Relation of Renal Gluconeogenesis to Ammonia Production in the Rat, American Journal of Physiology, 218:353 (1970)

38. Kamm, D. E., and R. R. Asher, Relation Between Glucose and Ammonia Production in Renal Cortical Slices, American Journal of Physiology, 218:1161 (1970)

39. Uete, T., Mode of Action of Adrenal Cortical Hormones: IV. Stimulation of ^{14}C Incorporation from $^{14}CO_2$ and ^{14}C-Pyruvate into Glucose by Triamcinolone in Presence of Ethanol in Rat Liver Slices, Journal of Biochemistry, 66:799 (1969)

40. Schoner, W., U. Haag, and W. Seubert, Regulation of Carbohydrate Metabolism by Cortisol Independent of the De Novo Synthesis of Enzymes in Rat Liver, Hoppe-Seyleis Z. Physiological Chemistry, 351:1071 (1970)

41. Nocenti, M. R., and L. J. Cizek, Effects of Hydrocortisone Acetate and Estrodiol in Normal and Adrenalectomized Salt Deficiency Rabbits, Endocrinology, 87:1140 (1970)

42. McCune, B. W., S. Roberts, and P. L. Young, Competitive Inhibition of Adrenal Δ^5-3β-Hydroxysteroid Dehydrogenase and Δ^5-3-Ketosteroid Isomerase Activities by Adenosine 3',5'-Monophosphate, Journal of Biological Chemistry, 245:3859 (1970)

43. Qureshi, Sanober, Study Involving Metabolism of 17-Ketosteroids and 17-Hydroxycorticosteroids of Healthy Young Men During Ambulation and Recumbency, Doctoral Dissertation, Texas Woman's University, Denton, Texas (1970)

44. Earp, H. S., B. S. Watson, and R. L. Ney, Adenosine 3',5'-Monophosphate as the Mediator of ACTH-Induced Ascorbic Acid Depletion in the Rat Adrenal, Endocrinology, 87:118 (1970)

45. Grahame-Smith, G. D., R. W. Butcher, R. L. Ney, and E. W. Sutherland, Adenosine 3',5'-Monophosphate as the Intracellular of the Action of Adrenocorticotropic Hormone on the Adrenal Cortex, The Journal of Biological Chemistry, 242:5535 (1967)

46. Schulster, D., S. A. S. Tait, J. F. Tait, and S. Miotek, Production of Steroids by in Vitro Superfusion of Endocrine Tissue: III. Corticosterone Output from Rat Adrenals Stimulated by Adrenocorticotropin or Cyclic 3',5'-Adenosine Monophosphate and the Inhibitory Effect of Cycloheximide, Endocrinology, 86: 487 (1970)

47. Bransome, E. D., "Adrenal Cortex", in the Annual Review of Physiology, Edited by V. E. Hall, Annual Reviews, Inc., California, p. 1005 (1968)

48. Taunton, O. D., J. Roth, and I. Pastan, Studies on the Adrenocorticotropic Hormone-Activated Adenyl Cyclase of a Functional Adrenal Tumor, Journal of Biological Chemistry, 244:247 (1969)

49. Roberts, S., R. W. McCune, J. E. Creange, and P. L. Young, Adenosine 3',5'-Cyclic Phosphate: Stimulation of Steroidogenesis in Sonically Disrupted Adrenal Mitochondria, Science, 158:372 (1967)

50. Halkerston, I. D. K., "Heterogeneity of the Response of Adrenal Cortex Tissue Slices to Adrenocorticotropin", in the Functions of the Adrenal Cortex, Volume 1, Edited by K. W. McKerns, Appleton-Century-Crofts, New York, p. 399 (1968)

51. Kowal, J., Adrenal Cells in Tissue Culture: IV. Use of an Inhibitor of Steroid Synthesis for the Study of ACTH Action, Endocrinology, 85:270 (1969)

52. McKerns, K. W., Mechanism of Action of Adrenocorticotropic Hormone through Activation of Glucose-6-Phosphate Dehydrogenase, Biochimica Et Biophysica Acta, 90:357 (1964)

53. Tsang, C. P. W., and F. G. Péron, Further Studies on Steroidogenesis: VIII. Glycolytic Breakdown of Glucose-6-Phosphate and 11β - Hydroxylation of 11-Deoxycorticosterone in a Cell Free Preparation of the Rat Adrenal Gland, Steroids, 16:41 (1970)

54. Roberts, S., and J. E. Creange, "The Role of 3',5'-Adenosine Phosphate in the Subcellular Localization of Regulatory Processes in Corticosteroidogenesis", in Function of the Adrenal Cortex, Volume I, Edited by K. W. McKerns, Appleton-Century-Crofts, New York, p. 339 (1968)

55. Jaanus, S. D., M. J. Rosenstein, and R. P. Pubin, On the Mode of Action of ACTH on the Isolated Adrenal, Journal of Physiology, 209:539 (1970)

56. Hirshfield, I. N., and S. B. Koritz, The Stimulation of Pregnenolone Synthesis in the Large Particles from Rat Adrenals by Some Agents Which Cause Mitochondrial Swelling, Biochemistry, 3:1994 (1964)

57. Nay, R. L., W. W. Davis, and L. D. Garren, Heterogeneity of Template RNA in Adrenal Glands, Science, 153:896 (1966)

58. Farese, R. V., Inhibition of the Steroidogenic Effect of ACTH and Incorporation of Amino Acid into Rat Adrenal Protein in Vitro by Chloramphenoicol, Biochimic Et Biophysica Acta, 87:701 (1964)

59. Kraicer, J., and J. V. Milligan, Suppression of ACTH Release from Adenohypophysis by Corticosterone: An in Vitro Study, Endocrinology, 87:371 (1970)

60. Wedemeyer, G., Stress-Induced Ascorbic Acid Depletion and Cortisol Production in Two Salmonid Fishes, Comparative Biochemistry, 29:1247 (1969)

61. Krieger, H. P., and D. T. Krieger, Chemical Stimulation of the Brain: Effect on Adrenal Corticoid Release, American Journal of Physiology, 218:1632 (1970)

62. Arthur, M. J., Seasonal Changes in Glucocorticoid Production and Response During Physiological Dehydration in the Frog Rana Pipirens, American Society of Zoologist, p. 1084, December, 1969

63. Liu, C. L., A Study on 17-Hydroxycorticosteroid Excretion During Horizontal Bed Rest, Master's Thesis, Texas Woman's University, Denton, Texas (1970)

64. Paul, M. I., R. Kvetnansky, H. Cramer, S. Silbergeld, and I. J. Kopin, Immobilization Stress-Induced Changes in Adrenocortical and Medullary Cyclic AMP Content in the Rat, Endocrinology, 88:338 (1971)

65. Burton, R. R., and J. R. Beljan, Animal Restraint: Application in Space (Weightless) Environment, Aerospace Medicine, 41:1060 (1970)

66. Ryzhenkov, V. E., "Effect of Central Neurotropic Substances on the Hypophysis Adrenal Cortex System During the Immobilization of Animals", Farmakol. Toksikol, 31:545 (1968), in Biological Abstracts, No. 96284, September 1, 1970

67. Burstein, S., B. R. Bhavnani, and H. L. Kimball, Observations on Urinary Corticosteroid Excretion Patterns in Individual Guinea Pigs, Endocrinology, 75:226 (1964)

68. Simonov, E. E., and I. V. Fedorov, "Dynamics of Changes in Protein Metabolism in Rats During Prolonged Hypokinesis", Kosmicheskaia Biologica Meditsina, 4:18 (1970), in Biological Abstracts, No. 118221, November 1, 1970

69. Bourne, G. H., "Neuromuscular Aspects of Space Travel", in Physiology of Man in Space, Edited by J. H. U. Brown, Academic Press, New York, p. 1 (1963)

70. Birge, S. J., and G. D. Whedon, "Bone", in Hypodynamics and Hypogravics, Edited by M. McCally, Academic Press, New York, p. 213 (1968)

71. Wells, J. B., Functional Integrity of Rat Muscle after Isometric Immobilization, Experimental Neurology, 24:514 (1969)

72. Bykov, G. P., and V. P. Smirnov, "Morphological Changes in Bone and Muscle Tissue under Hypokinetic Conditions", Kosmicheskaia Biologica Meditsina, 4:46 (1970) in Biological Abstracts, No. 112887, October 15, 1970

73. Kakurin, L. I., B. S. Kamkovskii, V. S. Georgievskii, Y. N. Purakhin, M. A. Cherepakhin, V. M. Mikhaclov, B. M. Petukhov, and E. N. Biryukov, "Functional Disorders in Human Hypokinesia", Vop Kurortol Fizcoter Lech Fizkult, 35:19 (1970), in Biological Abstracts, No. 123395, November 15, 1970

74. Malm, O. J., "Pathophysical Changes During Prolonged Bed Rest", Tidsskn Nor Laegeforen, 89:478 (1969), in Biological Abstracts, No. 88270, August 15, 1970

75. Mikhaleva, N. P., I. I. Ivanov, I. V. Federov, and E. M. Amdii, "Study of the Fractional Composition of Proteins of Skeletal Muscle under Hypokinetic Conditions", Kosmicheskaia Biologica Meditsina, 4:42 (1970), in Biological Abstracts, No. 112881, October 15, 1970

76. Mack, P. B., and P. A. LaChance, Effects of Recumbency and Space Flight on Bone Density, American Journal of Clinical Nutrition, 20:1194 (1967)

77. Bourne, G. H., K. Nancy, and M. N. Golarz de Bourne, "Muscle and Weightlessness State", in Hypodynamics and Hypogravics: the Physiology of Inactivity and Weightless, Edited by McCally, Academic Press, New York, p. 187 (1968)

78. Rodahl, K., "Physiological Effects of Prolonged Bed Rest", Tidsskn Nor Laegeforen, 89:469 (1969), in Biological Abstracts, August, 1970

79. Browse, N. L., The Physiology and Pathology of Bed Rest, Charles C. Thomas, Springfield, Illinois (1965)

80. Zav'Yalov, E. S., S. G. Mel'nik, G. Y. Chugunov, and A. A. Vorona, "Performance of Operators During Prolonged Bed Rest", Kosm. Biologica Meditsina, 4:61, (1970), in Biological Abstracts, No. 88259, August 15, 1970

81. Briggs, M. H., and J. Brotherton, Steroid Biochemistry and Pharmacology, Academic Press, New York, p. 150 (1970)

82. Porter, C. C., and R. H. Silber, A Quantitative Color Reaction for Cortisone and Related 17, 21-Dihydroxy-20-Ketosteroids, Journal of Biochemistry, 185:201 (1950)

83. Lee, Lil Ha, Circadian Rhythm Pattern of 17-Hydroxycorticosteroid Urinary Excretion in Healthy Adult Human Males, Master's Thesis, Texas Woman's University, Denton, Texas (1970)

84. Deitrick, J. E., G. D. Whedon, and E. Shorr, Effect of Immobilization upon Various Metabolic and Physiologic Functions of Normal Men, American Journal of Medicine, 4:3 (1948)

85. Rose, L. I., H. S. Friedman, S. C. Beering, and K. H. Cooper, Plasma Cortisol Changes Following a Mile Run in Conditioned Subjects, Journal of Clinical Endocrinology and Metabolism, 31:339 (1970)

86. Fuller, J. H., E. M. Bernaur, and W. C. Adams, Renal Function, Water and Electrolyte Exchange During Bed Rest with Daily Exercise, Aerospace Medicine, 41:60 (1970)

87. Nocenti, M. R., and L. J. Cizek, Effects of Hydrocortisone Acetate and Estradiol in Normal and Adrenalectomized Salt Deficient Rabbits, Endocrinology, 87:1140 (1970)

88. Berry, C. A., Summary of Medical Experience in the Apollo 7 Through 11 Manned Spaceflights, Aerospace Medicine, 41:500 (1970)

89. Suzuki, T., K. Otsuka, H. Matusui, S. Ohukuzi, K. Sakai, and Y. Harada, Effects of Muscular Exercise on Adrenal 17-Hydroxycorticosteroid Secretion in Dogs, Endocrinology, 80:1148 (1967)

A P P E N D I X

TABLE I

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION

DURING BED REST

(Milligrams per 24 Hours)

SUBJECT 1A				SUBJECT 2A			
Day		Day		Day		Day	
1	5.08	29	18.23	1	4.29	29	14.75
2	7.36	30	13.49	2	4.26	30	13.02
3	6.67	31	10.71	3	5.37	31	16.02
4	5.14	32	13.39	4	4.56	32	10.05
5	11.23	33	15.98	5	6.16	33	11.96
6	5.13	34	12.37	6	7.79	34	12.36
7	7.10	35	18.21	7	7.91	35	14.92
8	6.55	36	15.68	8	9.00	36	17.34
9	13.30	37	11.10	9	14.31	37	13.34
10	17.28	38	8.44	10	13.52	38	12.48
11	5.51	39	8.68	11	17.97	39	10.90
12	17.63	40	8.89	12	8.85	40	7.93
13	9.44	41	6.66	13	11.12	41	11.19
14	12.46	42	8.91	14	8.63	42	9.09
15	14.36	43	8.05	15	13.37	43	11.39
16	19.24	44	8.46	16	14.75	44	8.19
17	16.30	45	9.30	17	14.77	45	9.23
18	13.22	46	4.46	18	13.02	46	9.32
19	14.51	47	7.60	19	11.60	47	10.47
20	14.87	48	9.26	20	10.24	48	7.94
21	12.57	49	8.45	21	11.69	49	9.98
22	11.91	50	8.12	22	12.63	50	7.80
23	9.71	51	9.92	23	14.56	51	8.98
24	10.95	52	3.99	24	11.65	52	7.46
25	4.47	53	7.76	25	13.57	53	8.48
26	11.06	54	7.10	26	14.70	54	8.22
27	10.49	55	12.88	27	11.54	55	10.51
28	12.39	56	5.69	28	16.01	56	4.48
Mean			10.67	Mean			10.81

TABLE I, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION

DURING BED REST

(Milligrams per 24 Hours)

SUBJECT 3A

Day		Day	
1	7.40	29	11.92
2	2.29	30	4.87
3	3.06	31	6.46
4	3.26	32	7.84
5	6.51	33	9.39
6	3.85	34	10.08
7	5.04	35	4.89
8	3.88	36	10.87
9	7.91	37	11.20
10	6.21	38	9.63
11	10.10	39	6.35
12	5.65	40	7.55
13	10.04	41	6.16
14	4.07	42	5.62
15	10.00	43	3.02
16	10.47	44	11.83
17	10.91	45	5.07
18	7.58	46	3.25
19	10.26	47	7.62
20	9.17	48	8.88
21	8.84	49	5.54
22	8.53	50	8.47
23	8.74	51	7.16
24	5.78	52	3.80
25	11.61	53	3.93
26	6.53	54	5.34
27	12.64	55	13.81
28	11.61	56	5.04
Mean			7.46

SUBJECT 4A

Day		Day	
1	4.74	29	16.33
2	6.02	30	15.21
3	6.88	31	13.87
4	8.31	32	11.87
5	3.99	33	14.68
6	7.80	34	15.89
7	11.88	35	14.07
8	10.68	36	10.51
9	11.01	37	13.98
10	15.23	38	9.27
11	7.70	39	12.04
12	14.89	40	10.44
13	11.69	41	9.37
14	14.89	42	9.87
15	13.98	43	16.09
16	12.65	44	6.03
17	8.43	45	11.29
18	13.54	46	9.97
19	14.71	47	11.16
20	6.41	48	10.91
21	13.51	49	9.07
22	11.25	50	8.80
23	13.87	51	10.69
24	8.47	52	9.38
25	15.63	53	12.59
26	11.30	54	12.44
27	16.69	55	7.23
28	13.89	56	8.61
Mean			11.16

TABLE I, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION

DURING BED REST

(Milligrams per 24 Hours)

SUBJECT 6A

Day		Day	
1	3.26	29	15.30
2	4.64	30	24.33
3	3.69	31	8.83
4	3.79	32	13.35
5	4.16	33	12.55
6	8.68	34	19.31
7	3.45	35	14.00
8	9.47	36	18.79
9	13.14	37	15.72
10	15.01	38	9.53
11	13.00	39	13.42
12	10.34	40	10.95
13	9.26	41	10.21
14	10.16	42	X
15	7.35	43	X
16	10.58	44	6.79
17	19.09	45	6.57
18	8.08	46	7.78
19	7.97	47	6.47
20	14.46	48	9.39
21	11.38	49	7.51
22	16.96	50	7.41
23	8.28	51	5.79
24	5.48	52	7.05
25	10.02	53	18.07
26	18.83	54	8.20
27	17.36	55	5.31
28	16.05	56	13.25
Mean			10.74

SUBJECT 7A

Day		Day	
1	2.77	29	10.17
2	2.22	30	17.72
3	4.15	31	10.14
4	5.12	32	13.00
5	5.78	33	13.95
6	7.04	34	15.90
7	6.32	35	13.19
8	9.56	36	15.55
9	11.84	37	12.40
10	14.15	38	7.53
11	8.83	39	12.34
12	18.86	40	7.21
13	16.06	41	7.88
14	11.34	42	12.78
15	11.02	43	6.45
16	15.86	44	11.08
17	14.88	45	11.16
18	14.79	46	10.55
19	12.88	47	10.37
20	12.35	48	8.94
21	13.65	49	8.78
22	14.80	50	6.67
23	14.26	51	7.19
24	7.79	52	6.39
25	12.76	53	7.43
26	12.09	54	8.72
27	13.32	55	9.63
28	16.70	56	6.88
Mean			10.74

TABLE I, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION

DURING BED REST

(Milligrams per 24 Hours)

SUBJECT 8A

Day		Day	
1	7.95	29	15.86
2	5.44	30	15.89
3	4.48	31	11.89
4	7.28	32	11.80
5	4.50	33	18.74
6	7.58	34	13.01
7	7.52	35	13.58
8	8.84	36	12.49
9	9.50	37	16.38
10	12.10	38	16.83
11	11.75	39	12.78
12	12.15	40	13.41
13	16.92	41	10.00
14	11.32	42	15.42
15	15.61	43	8.00
16	11.52	44	14.25
17	9.31	45	9.36
18	17.93	46	9.76
19	13.42	47	9.45
20	6.95	48	10.45
21	12.96	49	9.46
22	12.88	50	11.54
23	14.22	51	6.00
24	11.59	52	5.53
25	13.91	53	8.60
26	12.18	54	8.59
27	12.74	55	7.04
28	22.04	56	9.16
Mean	11.43		

SUBJECT 9A

Day		Day	
1	2.12	29	14.87
2	8.91	30	11.80
3	8.92	31	8.95
4	10.61	32	10.41
5	14.80	33	13.31
6	6.71	34	15.73
7	3.57	35	15.41
8	6.55	36	15.92
9	16.55	37	21.66
10	11.07	38	14.33
11	14.94	39	9.61
12	17.80	40	7.72
13	16.88	41	11.41
14	10.75	42	7.18
15	10.59	43	8.18
16	15.57	44	10.12
17	13.83	45	8.20
18	9.64	46	9.20
19	16.74	47	10.54
20	16.22	48	8.28
21	8.11	49	7.55
22	25.99	50	8.91
23	5.81	51	10.20
24	9.15	52	10.15
25	9.69	53	9.40
26	15.02	54	10.97
27	28.00	55	9.20
28	13.80	56	5.62
Mean	11.66		

TABLE II

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms per Hour)

PART A. SUBJECT 1A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	190	190	160	543	29	583	793	985	630
2	374	320	136	500	30	1075	778	148	148
3	351	283	144	395	31	235	63	208	1730
4	218	225	80	465	32	166	955	524	1013
5	126	1715	183	475	33	826	638	568	568
6	304	113	90	383	34	886	95	408	408
7	425	328	219	160	35	1060	535	633	633
8	151	55	196	888	36	633	1610	273	500
9	515	598	273	1152	37	603	518	351	351
10	950	540	504	873	38	214	490	397	397
11	220	513	186	53	39	549	178	298	300
12	1265	585	304	685	40	88	45	59	1885
13	525	500	183	445	41	286	193	3	895
14	698	315	261	883	42	518	378	65	685
15	319	1153	529	743	43	365	173	29	1053
16	425	1890	461	1148	44	198	180	304	104
17	891	770	216	1090	45	103	400	501	715
18	790	350	325	725	46	140	135	74	553
19	714	1225	325	325	47	164	393	61	1058
20	768	578	523	563	48	259	710	60	968
21	673	420	498	383	49	531	325	43	640
22	758	140	501	320	50	599	298	25	485
23	115	588	39	1533	51	711	103	61	833
24	298	225	360	1198	52	73	213	236	168
25	120	320	18	523	53	501	433	168	168
26	205	508	616	615	54	241	320	230	513
27	430	688	408	260	55	51	1003	504	1108
28	1125	578	87	87	56	298	X	X	X
					Mean	462	503	273	653

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms per Hour)

PART B. SUBJECT 2A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	119	130	130	445	29	574	1090	463	465
2	70	100	250	575	30	503	730	360	800
3	385	50	129	265	31	624	973	209	1368
4	144	230	241	140	32	444	888	75	588
5	225	405	115	455	33	521	463	388	710
6	334	328	310	333	34	715	663	184	630
7	441	303	135	523	35	978	355	253	915
8	243	620	174	708	36	915	805	513	675
9	1006	653	121	670	37	650	575	324	813
10	509	1003	340	680	38	681	338	335	750
11	895	973	579	573	39	515	395	378	545
12	191	1078	138	478	40	493	513	211	63
13	309	648	271	973	41	376	370	433	810
14	313	438	185	725	42	351	568	270	461
15	661	365	346	963	43	461	625	310	680
16	546	500	670	755	44	470	225	253	378
17	776	538	695	213	45	310	485	270	663
18	333	850	358	1025	46	524	190	108	878
19	351	798	369	663	47	455	348	374	613
20	355	833	276	465	48	455	73	200	603
21	280	560	254	640	49	700	163	225	483
22	330	305	863	468	50	291	270	211	675
23	706	663	371	823	51	436	365	264	480
24	511	58	461	910	52	261	325	171	675
25	625	963	230	720	53	270	613	166	635
26	250	2178	416	415	54	253	463	236	615
27	655	305	338	595	55	535	180	335	708
28	646	958	355	1043	56	31	X	X	X
					Mean	464	559	303	635

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(Micrograms per Hour)

PART C. SUBJECT 3A

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
1	88	70	40	1525	29	948	545	121	298
2	154	13	46	160	30	53	658	166	123
3	184	90	121	66	31	194	430	163	473
4	66	285	99	200	32	0	328	538	558
5	380	193	191	293	33	558	258	223	528
6	171	78	190	163	34	630	360	31	838
7	54	250	216	495	35	31	73	219	650
8	133	175	169	193	36	650	230	391	402
9	456	215	245	360	37	402	455	243	1058
10	16	275	100	1045	38	610	235	175	603
11	524	30	481	485	39	151	588	59	580
12	318	148	210	210	40	133	855	284	199
13	318	428	356	375	41	199	463	146	388
14	178	343	115	90	42	160	733	160	33
15	979	98	109	228	43	234	63	75	75
16	453	180	115	130	44	101	2155	150	301
17	666	433	68	828	45	301	298	101	166
18	365	765	60	280	46	166	58	6	410
19	280	1273	153	428	47	259	23	183	1000
20	453	725	19	625	48	349	743	106	568
21	309	648	138	670	49	515	73	69	145
22	466	400	250	300	50	630	333	54	418
23	306	643	313	305	51	374	150	221	450
24	81	370	304	304	52	68	433	118	148
25	254	850	251	1043	53	148	280	135	135
26	46	322	322	575	54	74	500	315	58
27	678	215	219	1153	55	58	445	171	255
28	603	153	515	515	56	29	X	X	X
					Mean	304	390	182	435

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms per Hour)

PART D. SUBJECT 4A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	68	196	196	393	29	466	1063	355	1378
2	295	328	181	225	30	756	1295	223	550
3	309	213	200	490	31	725	625	364	665
4	400	410	161	545	32	653	505	310	563
5	346	123	29	125	33	1008	400	346	563
6	498	303	160	333	34	298	1623	506	743
7	596	370	144	1120	35	924	710	355	249
8	108	310	610	1025	36	249	1003	414	298
9	560	1003	210	210	37	520	653	410	983
10	958	270	489	645	38	343	518	288	540
11	356	356	119	620	39	639	365	385	598
12	270	1688	498	498	40	399	338	473	530
13	468	415	290	992	41	479	338	169	710
14	992	710	340	348	42	293	755	275	577
15	326	1495	395	558	43	577	1053	748	320
16	708	788	309	343	44	304	680	93	35
17	149	1490	23	275	45	675	438	281	473
18	700	422	422	718	46	743	135	81	710
19	718	665	358	860	47	412	412	421	710
20	40	630	84	724	48	641	338	261	585
21	724	928	231	540	49	513	500	85	573
22	208	1108	423	445	50	490	515	235	235
23	693	650	329	775	51	412	412	360	715
24	254	270	375	590	52	178	1160	174	483
25	1094	435	475	335	53	342	342	608	905
26	466	765	329	470	54	453	453	470	810
27	920	768	434	698	55	428	355	199	199
28	483	1180	219	890	56	489	X	X	X
					Mean	502	641	307	573

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms 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
1	174	127	127	85	29	628	550	464	1093
2	171	103	106	478	30	178	1678	1260	1530
3	93	423	70	175	31	318	665	108	693
4	180	27	171	213	32	554	883	165	978
5	180	168	124	265	33	656	630	80	1035
6	669	220	259	95	34	123	635	495	738
7	43	185	31	530	35	675	398	326	1099
8	141	153	375	1183	36	1099	555	405	1135
9	875	355	433	315	37	315	518	403	1978
10	979	320	388	700	38	470	438	290	425
11	610	530	495	510	39	664	890	268	603
12	444	265	435	563	40	374	530	331	798
13	243	545	428	428	41	301	455	491	491
14	576	93	166	963	42	113	360	346	X
15	241	203	340	473	43	X	X	X	540
16	371	1105	205	388	44	461	185	118	355
17	1146	1035	223	1000	45	135	615	185	388
18	104	378	335	770	46	599	248	84	323
19	198	350	275	698	47	550	658	273	415
20	741	738	293	810	48	568	568	125	393
21	776	595	186	325	49	466	215	248	163
22	1096	783	354	558	50	276	575	183	360
23	360	148	453	298	51	76	158	304	530
24	129	135	378	223	52	194	445	301	328
25	268	990	229	523	53	855	535	740	793
26	894	530	689	1013	54	253	530	264	488
27	808	668	813	433	55	110	305	174	455
28	703	995	309	995	56	729	X	X	X
					Mean	453	484	314	613

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS THROUGHOUT THE BED REST

(Micrograms per Hour)

PART F. SUBJECT 7A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	126	63	63	125	29	601	525	9	798
2	118	13	104	100	30	940	620	619	693
3	125	135	261	130	31	323	675	395	425
4	118	163	394	95	32	525	625	378	820
5	160	198	236	455	33	788	550	414	535
6	333	740	153	50	34	703	450	391	1338
7	273	373	48	568	35	658	493	369	751
8	354	130	118	1318	36	751	798	315	958
9	590	635	251	643	37	563	653	113	1098
10	574	933	245	1143	38	249	433	230	493
11	519	238	185	575	39	745	553	6	1030
12	1423	300	403	765	40	439	215	63	585
13	538	95	1103	640	41	256	535	250	423
14	529	730	288	473	42	284	213	456	1503
15	743	50	498	225	43	273	250	20	778
16	950	435	529	573	44	754	220	218	608
17	790	193	369	1210	45	690	293	86	945
18	250	1363	185	1460	46	644	230	93	935
19	490	748	349	795	47	598	303	129	838
20	534	370	579	493	48	366	545	268	423
21	786	483	319	720	49	354	328	288	585
22	745	455	324	1108	50	279	45	121	823
23	1053	683	235	308	51	146	418	301	485
24	81	453	380	573	52	166	153	293	526
25	565	288	124	1525	53	119	485	400	335
26	670	250	279	875	54	281	535	355	373
27	619	95	400	1198	55	291	520	43	1220
28	689	508	594	1103	56	478	X	X	X
					Mean	500	415	284	720

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms per Hour)

PART G. SUBJECT 8A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	210	337	337	345	29	215	868	641	1385
2	313	135	138	325	30	459	1180	535	805
3	118	135	130	490	31	400	468	150	1405
4	170	315	468	225	32	408	723	261	890
5	149	145	180	323	33	1334	918	320	460
6	490	170	253	240	34	445	333	504	1023
7	448	355	174	285	35	613	508	455	753
8	391	168	219	823	36	753	680	146	643
9	456	765	228	243	37	811	925	380	788
10	433	468	499	695	38	939	463	479	918
11	324	195	873	350	39	849	700	101	595
12	590	193	360	945	40	779	113	513	658
13	815	495	673	760	41	534	428	183	640
14	430	853	253	613	42	490	1858	196	625
15	968	725	295	628	43	211	630	154	640
16	364	455	515	668	44	954	505	263	620
17	146	890	196	753	45	301	473	253	760
18	988	895	473	667	46	543	158	225	748
19	667	505	479	558	47	439	613	158	558
20	55	425	394	415	48	515	720	178	508
21	495	603	410	828	49	419	438	264	563
22	625	125	343	1160	50	500	743	309	525
23	1234	308	270	240	51	203	100	183	630
24	154	635	776	403	52	119	235	138	635
25	830	1003	20	775	53	459	410	169	485
26	199	380	511	995	54	284	418	268	628
27	543	218	495	893	55	104	625	234	460
28	1226	548	706	1098	56	448	X	X	X
					Mean	506	521	333	656

TABLE II, CONTINUED

URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING FOUR DAILY PERIODS

THROUGHOUT THE BED REST

(Micrograms per Hour)

PART H. SUBJECT 9A

Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon	Day	12 Noon-8 P.M.	8 P.M.-12 Mid-night	12 Mid-night-8 A.M.	8 A.M.-12 Noon
1	191	19	19	90	29	1034	315	411	513
2	704	198	36	550	30	421	1143	149	668
3	670	170	144	433	31	515	273	69	798
4	366	888	313	393	32	799	168	141	555
5	681	280	770	518	33	829	508	181	800
6	405	50	186	445	34	751	348	609	865
7	104	320	146	73	35	1069	193	338	847
8	318	140	250	113	36	847	1015	275	720
9	621	980	18	1880	37	1040	540	619	1558
10	493	490	484	325	38	728	1175	130	693
11	540	1093	196	1170	39	385	295	320	698
12	1165	873	538	423	40	270	450	268	405
13	1104	860	61	1030	41	520	468	261	823
14	479	945	241	553	42	248	103	313	573
15	440	438	251	828	43	250	658	300	288
16	886	833	239	810	44	563	590	219	378
17	500	988	470	530	45	259	485	335	378
18	508	303	254	385	46	418	428	85	868
19	526	1700	318	798	47	576	410	140	793
20	768	1140	276	828	48	603	225	140	360
21	51	1203	228	268	49	275	243	256	583
22	1310	1683	351	1493	50	535	535	28	568
23	203	508	230	80	51	648	333	158	608
24	331	540	178	730	52	421	603	245	603
25	285	778	146	783	53	450	460	146	698
26	531	1373	408	505	54	740	333	243	445
27	1854	608	1055	575	55	670	220	118	505
28	603	425	374	1073	56	261	X	X	X
					Mean	587	589	267	641

TABLE III

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART A. SUBJECT 2A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	464	218	1.3854	N.S.
8 P.M. - 12 Midnight	543	357		
12 Noon - 8 P.M.	464	218	4.4481	$P < 0.001$
12 Midnight - 8 A.M.	303	152		
12 Noon - 8 P.M.	464	218	3.9551	$P < 0.001$
8 A.M. - 12 Noon	635	228		
8 P.M. - 12 Midnight	543	357	4.5185	$P < 0.001$
12 Midnight - 8 A.M.	303	152		
8 P.M. - 12 Midnight	543	357	1.5730	N.S.
8 A.M. - 12 Noon	635	228		
12 Midnight - 8 A.M.	303	152	8.8195	$P < 0.001$
8 A.M. - 12 Noon	635	228		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART B. SUBJECT 6A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	454	298	0.5913	N.S.
8 P.M. - 12 Midnight	489	309		
12 Noon - 8 P.M.	454	298	2.7215	$P < 0.01$
12 Midnight - 8 A.M.	317	208		
12 Noon - 8 P.M.	454	298	2.4629	$P < 0.02$
8 A.M. - 12 Noon	614	365		
8 P.M. - 12 Midnight	489	309	3.3260	$P < 0.001$
12 Midnight - 8 A.M.	317	208		
8 P.M. - 12 Midnight	489	309	1.8856	$P < 0.10$
8 A.M. - 12 Noon	614	365		
12 Midnight - 8 A.M.	317	208	5.0921	$P < 0.001$
8 A.M. - 12 Noon	614	365		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART C. SUBJECT 7A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	504	278	1.7422	P<0.10
8 P.M. - 12 Midnight	414	254		
12 Noon - 8 P.M.	504	278	4.7722	P<0.001
12 Midnight - 8 A.M.	284	189		
12 Noon - 8 P.M.	504	278	3.4097	P<0.001
8 A.M. - 12 Noon	720	371		
8 P.M. - 12 Midnight	414	254	2.9852	P<0.01
12 Midnight - 8 A.M.	284	189		
8 P.M. - 12 Midnight	414	254	4.9437	P<0.001
8 A.M. - 12 Noon	720	371		
12 Midnight - 8 A.M.	284	189	7.6054	P<0.001
8 A.M. - 12 Noon	720	371		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

PART D. SUBJECTS 2A, 6A, 7A
(Exercised throughout the study, combined data)

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	474	267	0.2470	N.S.
8 P.M. - 12 Midnight	482	314		
12 Noon - 8 P.M.	474	267	6.7904	$P < 0.001$
12 Midnight - 8 A.M.	301	185		
12 Noon - 8 P.M.	474	267	5.4811	$P < 0.001$
8 A.M. - 12 Noon	656	331		
8 P.M. - 12 Midnight	482	314	6.3167	$P < 0.001$
12 Midnight - 8 A.M.	301	185		
8 P.M. - 12 Midnight	482	314	4.8616	$P < 0.001$
8 A.M. - 12 Noon	656	331		
12 Midnight - 8 A.M.	301	185	11.9156	$P < 0.001$
8 A.M. - 12 Noon	656	331		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART E. SUBJECT 4A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	502	244	2.2370	$P < 0.05$
8 P.M. - 12 Midnight	641	385		
12 Noon - 8 P.M.	502	244	4.9369	$P < 0.001$
12 Midnight - 8 A.M.	308	152		
12 Noon - 8 P.M.	502	244	1.4466	N.S.
8 A.M - 12 Noon	573	260		
8 P.M. - 12 Midnight	641	385	5.8645	$P < 0.001$
12 Midnight - 8 A.M.	308	152		
8 P.M. - 12 Midnight	641	385	1.0775	N.S.
8 A.M. - 12 Noon	573	260		
12 Midnight - 8 A.M.	308	152	6.4100	$P < 0.001$
8 A.M. - 12 Noon	573	260		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART F. SUBJECT 8A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	506	300	0.2505	N.S.
8 P.M. - 12 Midnight	521	319		
12 Noon - 8 P.M.	506	300	3.5989	$P < 0.001$
12 Midnight - 8 A.M.	333	183		
12 Noon - 8 P.M.	506	300	2.7457	$P < 0.01$
8 A.M. - 12 Noon	656	263		
8 P.M. - 12 Midnight	521	319	3.7261	$P < 0.001$
12 Midnight - 8 A.M.	333	183		
8 P.M. - 12 Midnight	521	319	2.3744	$P < 0.02$
8 A.M. - 12 Noon	656	263		
12 Midnight - 8 A.M.	333	183	7.3436	$P < 0.001$
8 A.M. - 12 Noon	656	263		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

(Data for Individual Subjects)

PART G. SUBJECT 9A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	587	321	0.0152	N.S.
8 P.M. - 12 Midnight	588	395		
12 Noon - 8 P.M.	587	321	6.2973	$P < 0.001$
12 Midnight - 8 A.M.	267	187		
12 Noon - 8 P.M.	587	321	0.8485	N.S.
8 A.M. - 12 Noon	641	341		
8 P.M. - 12 Midnight	588	395	5.3493	$P < 0.001$
12 Midnight - 8 A.M.	267	187		
8 P.M. - 12 Midnight	588	395	0.7425	N.S.
8 A.M. - 12 Noon	641	341		
12 Midnight - 8 A.M.	267	187	7.0075	$P < 0.001$
8 A.M. - 12 Noon	641	341		

TABLE III, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

PART H. SUBJECTS 4A, 8A, 9A
(Did not exercise, combined data)

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	532	293	1.4054	N.S.
8 P.M. - 12 Midnight	584	371		
12 Noon - 8 P.M.	532	293	8.5827	$P < 0.001$
12 Midnight - 8 A.M.	303	177		
12 Noon - 8 P.M.	532	293	2.8355	$P < 0.01$
8 A.M. - 12 Noon	623	293		
8 P.M. - 12 Midnight	584	371	8.7234	$P < 0.001$
12 Midnight - 8 A.M.	303	177		
8 P.M. - 12 Midnight	584	371	1.0734	N.S.
8 A.M. - 12 Noon	623	293		
12 Midnight - 8 A.M.	303	177	11.9780	$P < 0.001$
8 A.M. - 12 Noon	623	293		

TABLE IV

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

PART A. SUBJECT 1A (Exercised second half of study)

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	462	304	0.4794	N.S.
8 P.M. - 12 Midnight	494	377		
12 Noon - 8 P.M.	462	304	3.7765	$P < 0.001$
12 Midnight - 8 A.M.	273	203		
12 Noon - 8 P.M.	462	304	2.7985	$P < 0.01$
8 A.M. - 12 Noon	653	395		
8 P.M. - 12 Midnight	494	377	3.7606	$P < 0.001$
12 Midnight - 8 A.M.	273	203		
8 P.M. - 12 Midnight	494	377	2.1295	$P < 0.05$
8 A.M. - 12 Noon	653	395		
12 Midnight - 8 A.M.	273	203	6.2223	$P < 0.001$
8 A.M. - 12 Noon	653	395		

TABLE IV, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

PART B. SUBJECT 3A (Exercised first 28 days of study)

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	303	232	1.5034	N.S.
8 P.M. - 12 Midnight	390	352		
12 Noon - 8 P.M.	303	232	3.3735	$P < 0.001$
12 Midnight - 8 A.M.	183	118		
12 Noon - 8 P.M.	303	232	2.4499	$P < 0.02$
8 A.M. - 12 Noon	435	317		
8 P.M. - 12 Midnight	390	352	4.0601	$P < 0.001$
12 Midnight - 8 A.M.	183	118		
8 P.M. - 12 Midnight	390	352	0.6902	N.S.
8 A.M. - 12 Noon	435	317		
12 Midnight - 8 A.M.	183	118	5.4239	$P < 0.001$
8 A.M. - 12 Noon	435	317		

TABLE V

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION AT DIFFERENT TIMES OF THE DAY

ALL EIGHT SUBJECTS

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.	473	287	1.7134	$P < 0.10$
8 P.M. - 12 Midnight	510	355		
12 Noon - 8 P.M.	473	287	11.7078	$P < 0.001$
12 Midnight - 8 A.M.	283	181		
12 Noon - 8 P.M.	473	287	6.8419	$P < 0.001$
8 A.M. - 12 Noon	616	332		
8 P.M. - 12 Midnight	510	355	11.8826	$P < 0.001$
12 Midnight - 8 A.M.	283	181		
8 P.M. - 12 Midnight	510	355	4.5436	$P < 0.001$
8 A.M. - 12 Noon	616	332		
12 Midnight - 8 A.M.	283	181	18.3587	$P < 0.001$
8 A.M. - 12 Noon	616	332		

TABLE VI

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 hr)	Standard Deviation	"t" Value	Probability
Subject 1A				
No Exercise	10.93	4.16	0.7935	N.S.
Exercise	10.06	3.67		
Subject 3A				
No Exercise	7.34	2.85	0.2852	N.S.
Exercise	7.57	2.89		

TABLE VII

STATISTICAL COMPARISON OF THOSE WHO EXERCISED DURING THE FULL STUDY AND THOSE WHO DID NOT EXERCISE THROUGHOUT THE STUDY

Populations Compared	Means (mg/hr)	Standard Deviation	"t" Value	Probability
Exercisers	10.76	4.03	1.5798	N.S.
Non-Exercisers	11.46	3.95		

TABLE VIII

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 1A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	501	307	0.9525	N.S.
Exercise	421	295		
8 P.M. - 12 Midnight				
No Exercise	561	436	1.3044	N.S.
Exercise	427	292		
12 Midnight - 8 A.M.				
No Exercise	279	167	0.2153	N.S.
Exercise	267	234		
8 A.M. - 12 Noon				
No Exercise	622	358	0.5767	N.S.
Exercise	686	429		

TABLE VIII, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 3A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
No Exercise	286	238	0.5469	N.S.
Exercise	321	226		
8 P.M. - 12 Midnight				
No Exercise	436	403	0.9250	N.S.
Exercise	345	288		
12 Midnight - 8 A.M.				
No Exercise	171	112	0.6729	N.S.
Exercise	193	123		
8 A.M. - 12 Noon				
No Exercise	402	270	0.7206	N.S.
Exercise	466	354		

TABLE VIII, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 C. SUBJECTS 1A AND 3A

Populations Compared	Means (mcg/hr)	Standard Deviation	"t" Value	Probability
12 Noon - 8 P.M.				
Exercisers	474	267	1.8736	$P < 0.10$
Non-Exercisers	532	293		
8 P.M. - 12 Midnight				
Exercisers	482	314	2.6609	$P < 0.01$
Non-Exercisers	584	371		
12 Midnight - 8 A.M.				
Exercisers	301	185	0.0685	N.S.
Non-Exercisers	303	177		
8 A.M. - 12 Noon				
Exercisers	656	331	0.9524	N.S.
Non-Exercisers	623	293		

TABLE IX

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 hr)	Standard Deviation	"t" Value	Probability
Subject 2A				
Pre-Bed Rest	7.57	3.74	3.2536	$P < 0.01$
Bed Rest	10.82	3.32		
Pre-Bed Rest	7.57	3.74	2.5502	$P < 0.02$
Post-Bed Rest	4.35	2.24		
Bed Rest	10.82	3.32	6.5032	$P < 0.001$
Post-Bed Rest	4.35	2.24		
Subject 6A				
Pre-Bed Rest	10.03	4.16	0.5140	N.S.
Bed Rest	10.74	4.84		
Pre-Bed Rest	10.03	4.16	1.3656	N.S.
Post-Bed Rest	8.10	2.85		
Bed Rest	10.74	4.84	1.9004	$P < 0.10$
Post-Bed Rest	8.10	2.85		
Subject 7A				
Pre-Bed Rest	4.43	3.40	5.8203	$P < 0.001$
Bed Rest	10.74	3.80		
Pre-Bed Rest	4.43	3.40	2.0596	$P \leq 0.05$
Post-Bed Rest	7.08	3.14		
Bed Rest	10.74	3.80	3.2310	$P < 0.01$
Post-Bed Rest	7.08	3.14		

TABLE IX, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 hr)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	7.34	4.42	5.0166	$P < 0.001$
Bed Rest	10.76	4.03		
Pre-Bed Rest	7.34	4.42	0.9204	N.S.
Post-Bed Rest	6.56	3.19		
Bed Rest	10.76	4.03	6.1560	$P < 0.001$
Post-Bed Rest	6.56	3.19		

TABLE IX, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 hr)	Standard Deviation	"t" Value	Probability
Subject 4A				
Pre-Bed Rest	7.58	4.93	3.4911	$P < 0.001$
Bed Rest	11.28	3.14		
Pre-Bed Rest	7.58	4.93	0.5636	N.S.
Post-Bed Rest	9.49	10.97		
Bed Rest	11.28	3.14	0.9742	N.S.
Post-Bed Rest	9.49	10.97		
Subject 8A				
Pre-Bed Rest	10.21	7.06	0.8863	N.S.
Bed Rest	11.43	3.72		
Pre-Bed Rest	10.21	7.06	2.0732	$P < 0.05$
Post-Bed Rest	5.65	2.46		
Bed Rest	11.43	3.72	5.1927	$P < 0.001$
Post-Bed Rest	5.65	2.46		
Subject 9A				
Pre-Bed Rest	9.85	7.13	1.1488	N.S.
Bed Rest	11.66	4.80		
Pre-Bed Rest	9.85	7.13	1.7042	N.S.
Post-Bed Rest	5.55	2.53		
Bed Rest	11.66	4.80	3.8280	$P < 0.001$
Post-Bed Rest	5.55	2.53		

TABLE IX, CONTINUED

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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 hr)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	9.21	6.56	2.9118	$P < 0.01$
Bed Rest	11.46	3.95		
Pre-Bed Rest	9.21	6.56	1.5337	N.S.
Post-Bed Rest	6.86	6.81		
Bed Rest	11.46	3.95	5.2895	$P < 0.001$
Post-Bed Rest	6.86	6.81		

TABLE X

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID 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)

SUBJECTS 1A, 3A

Populations Compared	Means (mg/24 hr)	Standard Deviation	"t" Value	Probability
Subject 1A				
Pre-Bed Rest	9.36	6.34	0.8483	N.S.
Bed Rest	10.49	3.95		
Pre-Bed Rest	9.36	6.34	2.1422	$P < 0.05$
Post-Bed Rest	4.32	3.43		
Bed Rest	10.49	3.95	4.5093	$P < 0.001$
Post-Bed Rest	4.32	3.43		
Subject 3A				
Pre-Bed Rest	8.77	5.79	1.2013	N.S.
Bed Rest	7.46	2.87		
Pre-Bed Rest	8.77	5.79	2.5199	$P < 0.02$
Post-Bed Rest	4.34	2.23		
Bed Rest	7.46	2.87	3.6885	$P < 0.001$
Post-Bed Rest	4.34	2.23		

TABLE XI

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

ALL SUBJECTS

Populations Compared	Means (mg/24 hrs)	Standard Deviation	"t" Value	Probability
Pre-Bed Rest	8.47	5.78	4.6404	$P < 0.001$
Bed Rest	10.58	4.05		
Pre-Bed Rest	8.47	5.78	3.2350	$P < 0.01$
Post-Bed Rest	6.12	4.81		
Bed Rest	10.58	4.05	9.4991	$P < 0.001$
Post-Bed Rest	6.12	4.81		