CHANGES IN URINARY 17-HYDROXYCORTICOSTEROIDS BY FIVE YOUNG ADULT MALES DURING AMBULATION AND HORIZONTAL BED REST RECUMBENCY AND AT DIFFERENT PERIODS OF THE DAY

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We hereby recommend that the thesis prepared under our supervision by Catherine Wei-Shan Hsu entitled CHANGES IN URINARY 17-HYDROXYCORTICOSTEROIDS BY FIVE YOUNG ADULT MALES DURING AMBULATION AND HORIZONTAL BED REST RECUMBENCY AND AT DIFFERENT PERIODS OF THE DAY

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

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INTRODUCTION

It frequently has been observed that, when man leaves his normal daily routine of upright physical activity and assumes the inactive recumbency of bed rest, multiple physiologic and metabolic changes take place. In the past, bed rest has been studied to ascertain its effects on a person inactivated by illness or injury. In recent years, however, a renewed interest in experimental immobilization has been prompted by the apparent similarity between this state and the prolonged inactivity and weightlessness of space flight.

Many studies have been done on the effects of immobilization upon various metabolic and physiologic functions of normal men. Miller et al (31) did a study on the effects of bed rest on circulatory functions in man. Both Deitrick, Whedon, and Shorr (9) and Wolenstenholme et al (59) observed a variable increase in urinary corticosteroid excretion during bed rest immobilization.

The present study was undertaken to measure possible changes and diurnal variations in the secretion of the adrenal hormones by assessing urinary levels of 17-hydroxycorticosteroids in man under conditions of bed rest immobilization.

The so-called Reddy Method for the determination of urinary 17-hydroxycorticosteroids was used in this study. This determination employs the phenylhydrazine-sulfuric acid reaction of the Porter and Silber Method. The daily urine excretions of five normal, healthy male subjects who participated in two 14-day periods of the bed rest study were analyzed.

The major objectives of this study have been the following:

1. To make daily urinary analyses throughout the entire study in order to find the excretion of 17-hydroxy-corticosteroids by five normal healthy subjects under conditions of bed rest immobilization;

2. To repeat (1) on the same subjects under conditions of ambulation; and

3. To find possible diurnal patterns of urinary excretion of 17-hydroxycorticosteroids under the conditions described.

REVIEW OF LITERATURE

CHEMISTRY

No less than 46 steroids have been isolated from the adrenal cortex so far, chiefly by research groups led by Soffer et al (47), Kendall (23), Wintersteiner and Pfiffner (58), and Heftmann and Mosettig (20). Most of these steroids probably represent precursors or catabolites of the adrenocortical hormones. The 17-hydroxycorticosteroids are often referred to as gluco-corticoids, Porter-Silber chromogens, and cortisol. They include hydrocortisone (cortisol, 17-hydroxycorticosterone, Kendall's Compound F), cortisone, (17-hydroxy-11-dehydrocorticosterone, Kendall's Compound E), desoxycortisol (11-desoxycortisol, Reichstein's Compound S) and their tetrahydro derivatives. The principal product elaborated by the adrenal cortex is cortisol.

Essential structures of 17-hydroxycorticosteroids are: (a) a double valence bond at C-4-5; (b) a ketonic group at carbon 3 and at carbon 20; (c) a hydroxyl group at carbon 17; and (d) a hydroxyl group at carbon 21 which enhances the capacity of the compound to increase sodium retention, and which must be present for an effect on carbohydrate metabolism. The presence of a hydroxyl group at carbon 17 also increases action on carbohydrate metabolism. The corticosteroids

having oxygen at carbon 11, either as hydroxyl or ketonic group, exert major activity in carbohydrate metabolism and generally decrease sodium retention. Those without O at the 11-C position, with virtually no activity in carbohydrate or protein metabolism and major effect on electrolyte and water metabolism, such as 17-hydroxy-11-deoxycorticosterone (Compound S; 11-deoxycortisol). (See Figure 1.) (7).

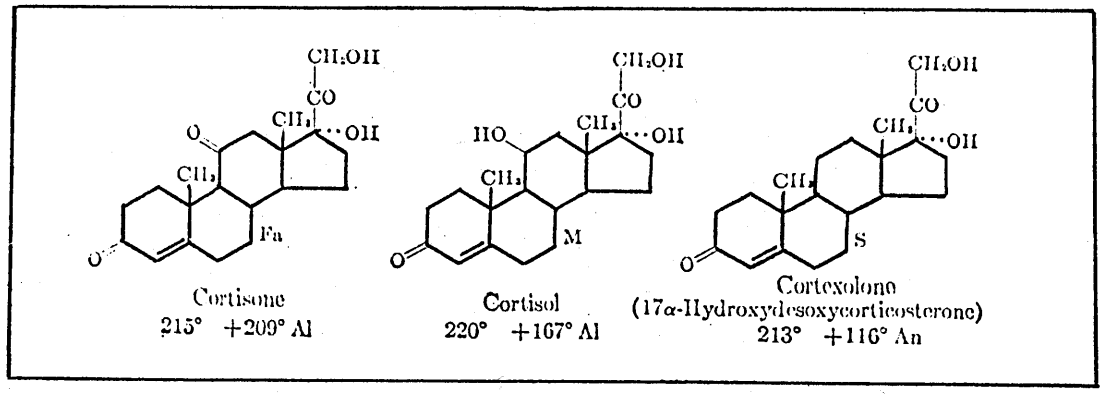

FIGURE 1. BIOLOGICALLY ACTIVE CORTICOIDS

(From: Fieser, L. F., and M. Fieser. Steroids, Reinhold Publishing Corporation, New York, 1959)

BIOSYNTHESIS

The primary compound formed by the adrenal, either from acetate or cholesterol, apparently is pregnenolone. Pregnenolone then is oxidized to progesterone. In the biosynthetic route progesterone is hydroxylated to cortisol in the 17α-position to yield 17α-hydroxyprogesterone. Hydroxylation of 17-hydroxyprogesterone produces 11-desoxy-

cortisol, which can be enzymatically hydroxylated at 11-C to yield cortisol. The 11β-hydroxyl group in cortisol can be reversibly oxidized to a keto group giving cortisone. Possible pathways of synthesis of these hormones are indicated in Figure 2 (20).

METABOLISM

The main secretory products of the adrenal gland are corticosterone and cortisol. The relative amounts of these two hormones depend on the species. In man cortisol secretion predominates (47).

The corticosteroids are metabolized, primarily in the liver, and excreted almost exclusively through the kidneys in man. Very small amounts of adrenocortical hormones are normally formed in the urine. They are largely reduced and conjugated with glucuronic acid in the liver (20). Conjugation is effected by the enzyme glucuronosyl transferase, which couples the 3α-hydroxyl group of the metabolites with uridine diphosphate glucuronic acid to yield 3α-(β-D-glucosiduronates) (7).

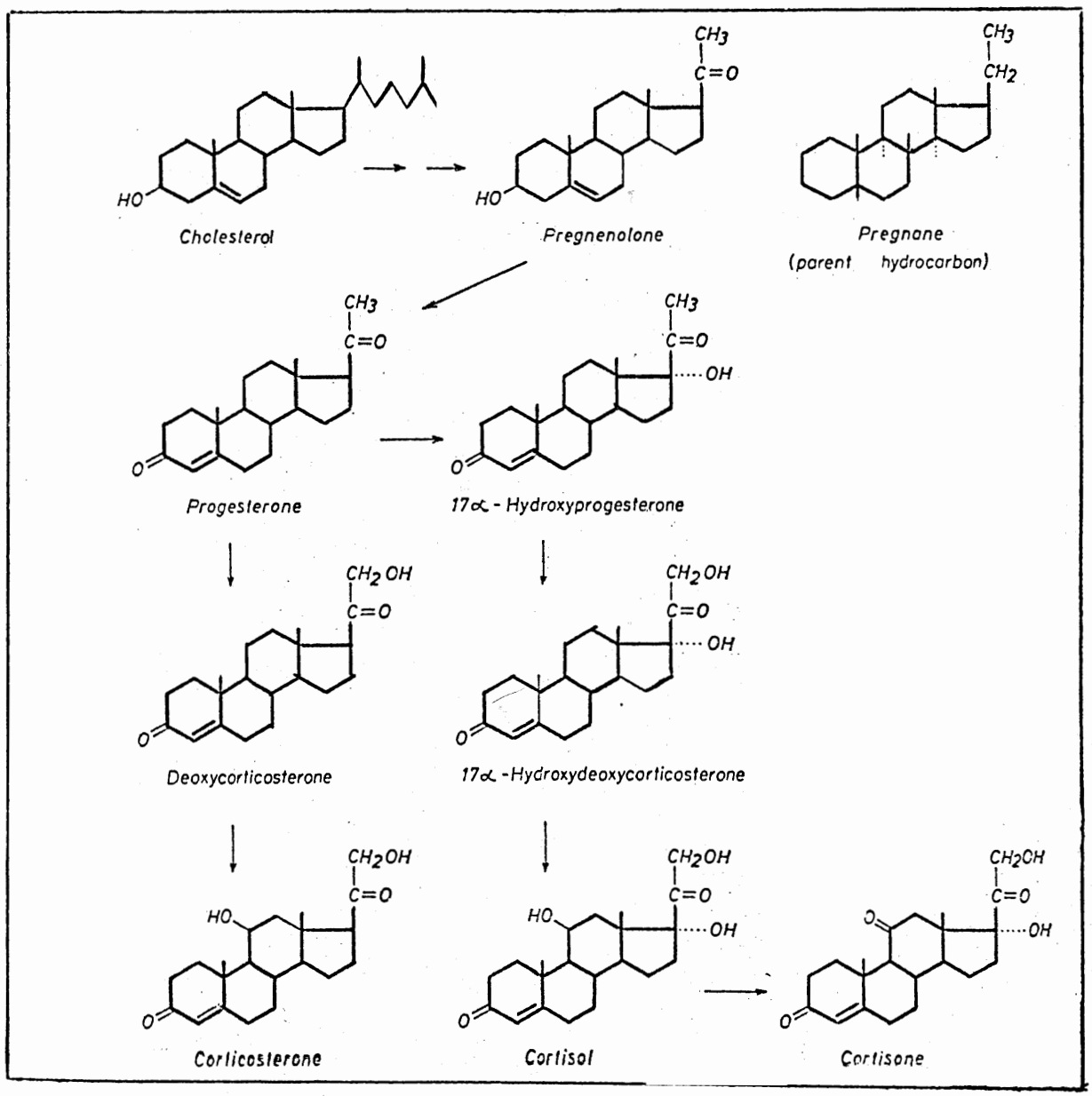

FIGURE 2. POSSIBLE PATHWAYS OF SYNTHESIS OF ACTIVE CORTICOIDS

(From: Karlson, P. Introduction to Modern Biochemistry, Academic Press, New York, 1963)

The metabolism of desoxycortisol (Figure 3) involves not only the reductions in ring A and in the side chain, but also cleavage of the side chain to 17-ketosteroids. The reaction is mediated by an enzyme, 17-ketodesmolase, which converts 17α-hydroxy 21-C steroids to the 17-ketosteroids of the 19-C series. It is an important metabolic reaction, leading from the corticosteroid series to the androgen series.

The metabolism of cortisol and cortisone is shown in Figure 4. Cortisol and cortisone are interchangeable in the organism. Removal of the side chain yields the two adrenal 17-ketosteroids Δ^4-androsten-11-01-3, 17-dione and Δ^4-androstene-3,11,17-trione (adrenosterone). Reduction of the Δ^4-3-keto group leads via DHE and DHF (the 3-keto-pregnane or dihydro derivatives) to the main urinary metabolites THE (urocortisone) and THF (urocortisol). The 20-keto group of the THE and THF may be reduced to the 20α-or 20β-hydroxy derivatives, the cortols and cortolones.

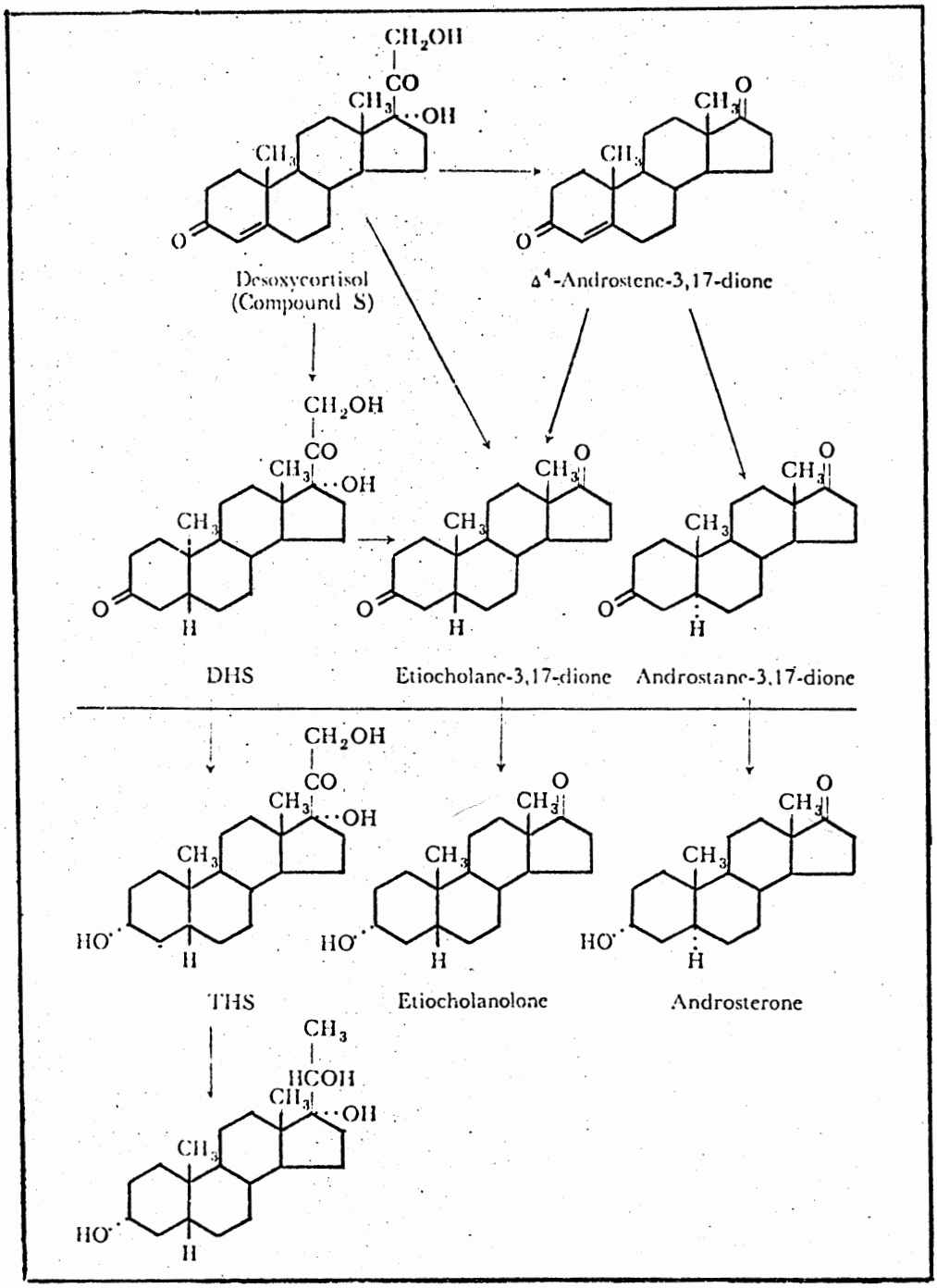

FIGURE 3. METABOLISM OF DESOXYCORTISOL

(From: Heftmann, E., and E. Mosettig. Biochemistry of Stero

Reinhold Publishing Co., New York, 1960)

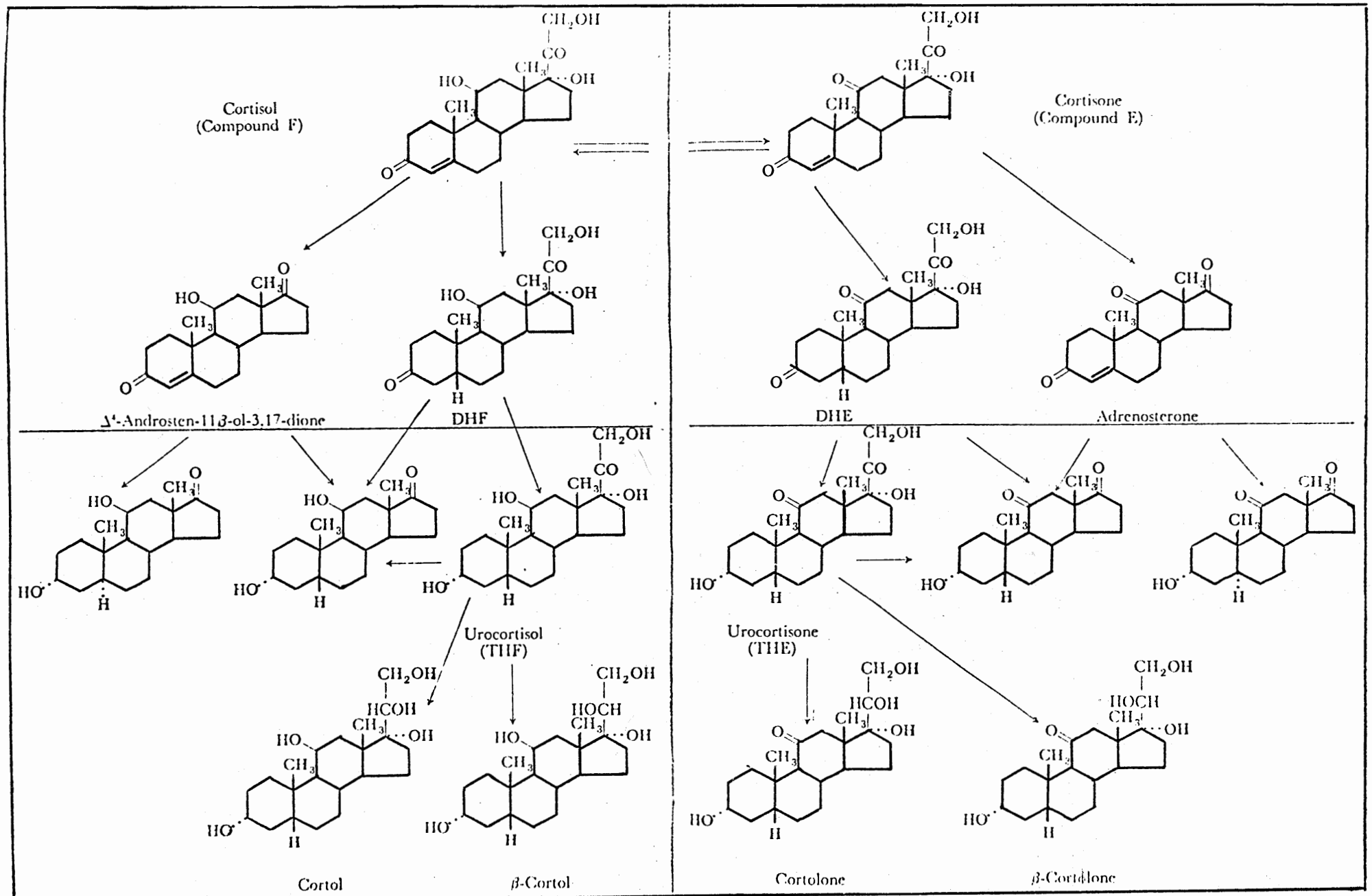

FIGURE 4. METABOLISM OF CORTISOL AND CORTISONE

(From: Heftmann, E., and E. Mosettig. Biochemistry of Steroids, Reinhold Publishing Co., N.Y., 1960)

URINARY EXCRETION

Under Normal Conditions

Current developments in the field of adrenal physiology necessitate standard methods for the measurement of adrenal cortical steroids and their transformation products. Many different methods have been utilized in numerous studies during past years, which have provided normal values for the urinary excretion of 17-hydroxycorticosteroids.

Reddy, Jenkins, and Thorn (43) used the Porter-Silber reaction to detect corticosteroids that have a 17α-21-dihydroxy-20-one side chain namely, cortisol, cortisone, tetrahydrocortisol (THF) and tetrahydrocortisone (THE). These investigators showed that the range of 24-hour total urinary 17-hydroxycorticosteroids in normal adults was 2.9 to 12 mg., with a mean of 5.8 mg. per 24-hour for males and 1.1 to 8.6 mg. with a mean of 3.8 mg. per 24-hour for females. Baylink and Hurxthal (3) reported a normal range of 17-hydroxycorticosteroids at different ages.

Normal Urinary Output of 17-OHCS
(milligrams per 24 hours)

Age (years)	Male	Female
0- 2	2- 4	2- 4
2- 6	3- 6	3- 6
6-10	4- 8	4- 8
10-14	4-10	4-10
Adult	4-12	4- 8

Metcalf (28) published a simplified method for the estimation of the urinary 17-hydroxycorticosteroids which contain a keto- or a hydroxyl-group in the 20 position. Seventeen normal men had a mean urinary excretion of 17-hydroxycorticosteroids of 16.7 mg. per day, with a standard deviation of 3.7 mg. per day; and 22 normal women had a mean excretion of 11.9 mg. per day, with a standard deviation of 3.6 mg. per day.

Uete (54) studied 144 healthy individuals (95 adults and 49 children of both sexes). The daily output of 17-hydroxycorticosteroids increased with age up to 20 to 30 years and, thereafter, decreased gradually. This variation with age was more marked in the conjugated fraction. For 50 healthy males 16-60 years of age the mean values ± standard deviations were: unconjugated, 0.20 ± 0.12; conjugated, 5.07 ± 1.65; total, 5.27 ± 1.84 mg. per day. For 45 healthy females in the

same age range the values were respectively 0.17 ± 0.12, 4.12 ± 1.60, and 4.29 ± 1.69 mg. per day.

Niskikaze and Furuya (35) introduced an improved method which showed a normal value of 17-hydroxycorticosteroids for 20 men (age, 23-31) and women (age, 24-32) as 6.3 ± 1.34 and 4.4 ± 0.98 mg. per day; 1.04 ± 0.175 and 0.94 ± 0.171 mg. per day per 10 kilograms of body weight; or 3.8 ± 0.75 and 3.2 ± 0.69 mg. per day per square meter body surface, respectively.

Ulstrom et al (56) studied the urinary Porter-Silber chromogens of 27 normal full-term new born infants. The values for total steroid were generally lower than would be predicted on the basis of small body size alone. The free steroid fraction accounted for a higher percentage of the total steroids than is commonly found in adult man. Unlike the urinary pattern in the adult, unmetabolized cortisol was not the major steroid component of the free fraction. Excretion values showed a trend toward increasing with each day of life.

Influence of Body Size, Sex,
and of Age

It has been well confirmed that the rate of excretion of 17-hydroxycorticosteroid increases with age and shows a relation to body size and sex.

Abbo (1) determined the rate of excretion of 17-hydroxycorticosteroids in 284 ambulatory healthy men ranging in age from 17 through 88 years. It was found that the ratio of this group of hormones significantly declined with increasing age. This ratio apparently can be used as a measure of the physiological age of the human adrenal cortex. Later, Minick (33) confirmed Abbo's work in a study of 43 healthy infants, children, and young adults. The excretion rate was expressed in μg. per kilogram of body weight per 24 hours. The correlation between cortisol and age was good ($r = +0.532$). Cortisone excretion and age do not correlate ($r = +0.022$). The excretion of cortisol by infants, on a body weight basis, is also significantly less than any other group. Children below age 10 excrete significantly less cortisol than do adults (21 - 30 years). No significant differences exist between any of the groups with respect to cortisone excretion. The children below age 6 were found to have excretory ratios which are different from those of age 11 and above, because they excrete relatively more cortisone than cortisol. Kajunura (22) found that the composition of 17-hydroxycorticosteroids of the male newborn's urine was characterized by the absence of cortisol and tetrahydrocortisol, and by a markedly lower proportion of the conjugated glucuronide when compared with male adult's urine.

Migeon (29) demonstrated a progressive increase in 17-hydroxycorticosteroid levels from childhood to adulthood. When the data were

expressed in mg. per surface area per 24 hours, the excretion at all ages was 3.1 ± 1.1. Cortisol production rate in 15 normal adult males was 20.37 ± 3.14 mg. per 24 hours. This average was significantly higher than that of 15 normal adult females who had a production rate of 17.64 ± 1.94. When the production rates were corrected for surface area, males and females had similar values, the average for the 30 control subjects being 11.3 ± 1.6 mg. per surface area per 24 hours. De Moor et al (10) also studied the influence of body size and of sex on urinary corticoid excretion in two homogeneous groups consisting of 27 male and 19 female students. The significant difference in hormone excretion which existed between these two groups of subjects disappeared when the hormonologic data were recalculated per unit of body weight, height or surface. Difference in body weight explained most of this sex difference; it is therefore suggested that data concerning urinary corticosteroid excretion be expressed not in mg. per 24 hours, but in mg. per 100 kilogram of body weight per 24 hours.

Under Abnormal Conditions

During Immobilization and During Ambulation

Over the past decade, there has accumulated considerable evidence that the pituitary-adrenocortical system is responsive to a wide variety of environmental stimuli both for man and for laboratory animals

(19) (27) (40). Although several studies have been done concerning the physiological effects of immobilization (4) (48) (49), only a few studies have been made in an attempt to investigate the urinary 17-hydroxycorticosteroid excretion during bed rest immobilization and during ambulation.

Deitrick, Whedon, and Shorr (9) found that there was a variable increase in excretion of urinary glucocorticoid steroid during immobilization.

A recent study was carried out by Vallbona, Vogt, Cardus, Spencer, Lipscomb, and Eik-Nes (57) on the daily excretion of total urinary 17-hydroxycorticosteroids while the subjects were ambulatory and while they were at bed rest. Urinary 17-hydroxycorticosteroids were determined with a modification of the Peterson method, applying the Porter-Silber colorimetric reaction. The average daily excretion of total 17-hydroxycorticosteroids, while the subjects were ambulatory, ranged from 2.799 mg. to 8.146 mg. with an average value of 4.842 mg. per 24 hours; and during immobilization, the value ranged from 3.045 mg. to 8.461 mg. with an average of 4.657 mg. per 24 hours. A higher excretion of urinary 17-hydroxycorticosteroids occurred during immobilization than during the ambulatory period, although the difference between the two periods was not statistically significant.

Corticosteroids Activity in

Rheumatoid Arthritis

Bailey, Greaves, Murphy, and West (2) attempted to detect abnormal extrahepatic metabolism of cortisol in the synovial fluid of rheumatoid arthritic patients. They found no evidence of abnormal pattern of cortisol metabolites, however, in the urine of patients with uncomplicated rheumatoid arthritis.

Rothermich (44) showed that a continuous steroid therapy consisting of small maintenance doses has an important place in the management of rheumatoid arthritis. That corticosteroids do alter the basic course of this disease by inhibiting the destructive inflammatory changes and retarding development of deformities and ankyloses. In rachitic infants (4 - 11 months old), Hornik (21) found that the urinary output of 17-hydroxycorticosteroids decreased from 0.99 to 0.79 mg. per day. Treatment with large doses of vitamin D_3 increased both values, especially, in the third to fourth week of the therapy.

Thyroid Diseases

Recently it has been shown that the metabolic state produced either by hyper- or hypothyroidism may influence the metabolism of corticosteroid. According to Uete (55), the excretion of total 17-hydroxycorticosteroids, particularly the conjugated fractions, was high in hyperthyroidism and was low in hypothyroidism. These findings agree

with previous studies in vitro and in vivo that thyroxine administration increases the metabolism of corticosteroids in the liver. In thyroid diseases the thyroid hormones might act directly on the pituitary or adrenal to control adrenal secretion.

Obesity

Krotkiewshi et al (25) compared obese patients with subjects of normal body weight. They found an increased urinary excretion of 17-hydroxycorticosteroids in obese patients, with 8.04 mg. per day compared with 3.94 mg. per day for normal weight persons. Differences with reference to body surface were 4.089 vs. 2.517, and with reference to body weight, 0.0799 vs. 0.0703 mg. per kilogram. Migeon (29) also confirmed that the values of urinary 17-hydroxycorticosteroids in obese subjects were significantly higher than normal subjects. When the values were related to surface area, the mean remained significantly higher (4.35 ± 1.87).

Prezio, Carreon, Clerkin, Meloni, Kyle, and Canary (42) did a study of urinary steroid excretion and body composition in 40 obese patients. Eleven patients were found to be hyperexcretors on the basis of urinary 17-hydroxycorticosteroid levels greater than 10 mg. per 24 hours. Total body weight, lean body mass, and body surface area were significantly different in the two groups of obese patients, but no difference of statistical significance between the groups in height, excess

fat, or creatinine excretion was demonstrated. Resting urinary 17-hydroxycorticosteroid levels correlated best with the lean body mass, whereas following ACTH stimulation the best correlation of 17-hydroxy-excretion was between excess fat and total body weight.

Cushing's Syndrome

A characteristic finding in Cushing's syndrome is increased urinary 17-hydroxycorticosteroid output. Increased 17-hydroxycorticosteroid output also is found in cushingoid states associated with extra-adrenal tumor. Narbutt, Buntner, and Japa (34) confirmed in their studies that a markedly elevated excretion of urinary 17-hydroxycorticosteroid in Cushing's disease from 9.5 mg. to 23 mg. per 24 hours. Uete (55) found that in Cushing's syndrome levels of both conjugated and unconjugated 17-hydroxycorticosteroids and the ratio of unconjugated to total corticosteroids varied markedly from day to day.

Cancer

Trapezontseva (50) made a study of the daily excretion of 17-hydroxycorticosteroids in the urine in patients with stomach cancer. In the early stages of stomach cancer the 17-hydroxycorticosteroid levels in the urine were elevated and decreased during the development of malignant tumors. In stomach cancer the ratio of free 17-hydroxycorticosteroids to total urinary corticosteroids was significantly higher than in normal subjects. Tsomaya (52), using the method of Porter and

Silber, estimated the value in patients with cancer of the uterine cervix to be 3.8 ± 0.55 mg. per 24 hours.

Gastric and Duodenal Ulcers

Dubrovina (14) stated that gastric and duodenal ulcers are accompanied by increased urinary output of 17-hydroxycorticosteroids (free and in a proportion of patients also conjugated, respectively, 0.42 mg. and 4.5 mg. per day). He also noted that the increase in the production and excretion of steroid hormones was correlated to the severity of the disease and the intensity of pain. These indicated that peptic ulcer is associated with cortical hyperfunction which evidently should be considered a humoral factor in the pathogenesis of the disease.

Hypertension

Kornel and Takeda (24) studied urinary 17-hydroxycorticosteroids in essential hypertension. They estimated the excretion of free, glucuronide and sulfate conjugated 17-hydroxycorticosteroids in 20 normotensive subjects and in 20 patients with uncomplicated essential hypertension. There was no statistically significant difference between normotensives and hypertensives in the sums of the urinary excretion of sulfate and glucuronides, nor in the excretion of total conjugated 17-hydroxycorticosteroids measured by the technique of extracting and estimating all conjugates. However, there was a highly significant difference in the excretion of the fraction of conjugated 17-hydroxycortico-

steroid which is measured by estimating all but very polar conjugates (e.g. 6α-hydroxycortisol-21-sulfate, tetrahydrocortisol-3,21-disulfate). They concluded that an altered metabolism of corticosteroids exists in patients with essential hypertension.

Under Stress

Bunney, Mason, and Hamburg (6) attempted to determine whether or not urinary corticosteroid values would correlate positively with specific behavioral variable during the course of a depressive illness. Seventeen patients who showed severe depressive features were selected for study. Measurements of urinary 17-hydroxycorticosteroids were used as an index of pituitary adrenal cortical activity. In the subgroup of nine patients with marked behavioral fluctuations, the 17-hydroxycorticosteroid values fluctuated also, and correlated positively ($P < 0.001$) with changes in ratings of depression and anxiety. Elevations in corticosteroids up to five times the normal were found in this subgroup of depressed patients. Gibbon (17) also supported the view that the increased adrenocortical activity in depressive illness is due to increase secretion of pituitary corticotrophin.

Infectious Diseases

Trinus (51) studied the excretion of 17-hydroxycorticosteroids in some infectious disease. He found that humans suffering from abdominal typhus or paratyphus excreted 4.0 mg. per day; patients with

influenza excreted 4.1 mg. per day; patients with parainfluenza infection excreted 4.3 mg. per day; and those with neuroinfectious disease 9.0 mg. per day, in comparison with 17 healthy human beings who excreted 4.7 mg. of 17-hydroxycorticosteroids per day. Also, in serious typhus, the glucocorticotropic function of the adrenal cortex was reduced.

Liver Disease

Current studies indicate that liver diseases result in profound alteration of adrenal cortical steroid metabolism (5) (15). It also has been demonstrated that patients with liver cirrhosis snythesized hydrocortisone and cortisone at a decreased rate (39). These patients thus have a form of hypofunction of the adrenal cortex. The primary defect in cirrhosis may be due to a decreased rate of hepatic enzymatic transformation of steroids. Uete (55) stated in his study that the patients with liver cirrhosis excreted normal amounts of unconjugated 17-hydroxycorticosteroid and less of conjugated 17-hydroxycorticosteroid.

Hypoxemia

Fujita (16) studied on the effect of acute induced hypoxemia on urinary 17-hydroxycorticosteroid level. In normal subjects, the urinary 17-hydroxycorticosteroid level increased clearly when they were subjected to moderate hypoxemia. In patients with hypopituitarism, not only cases with severe hypoadrenocorticoidism, but also in a mild one,

there were no significant variations of urinary 17-hydroxycorticosteroid level in spite of moderate or more severe hypoxemia. From these facts he concluded that there is no doubt that the increase of urinary 17-hydroxycorticosteroid level by acute induced hypoxemia in normal subjects is due to the reactionary hypofunction of the anterior pituitary lobe.

Under Basal Conditions and

Exhaustive Exercise

To find the pituitary-adrenal activity in the normal subject living under basal conditions, Sachar, Mason, Fishman, Hamburg, and Handlon (45) studied 62 normal subjects living for periods of many weeks in hospital wards. The mean excretions of 17-hydroxycorticosteroids for normal males and females 18 to 25 years old were 8.5 mg. (13.3-5.3) and 5.1 mg. per day (8.6-3.0), respectively. Weight "correction" did not abolish this significant sex difference, although it did alter somewhat the rank-order of "high" to "low" excretors of 17-hydroxycorticosteroids. Values for males were about 50 per cent higher than those reported in outpatient studies. Subjects tended to be relatively consistent in 17-hydroxycorticosteroids excretion, although individuals varied. On coed wards, girls excreted approximately 30 per cent more 17-hydroxycorticosteroids per day than on all-girl wards, a significant difference probably related to psychological tension.

Diczfalusy, Cassmer, and Ullmark (11) studied on the functional reserve capacity of the adrenal cortex in 22 healthy volunteers aged 19 to 23 after a period of normal exercise and after a period of exhaustive exercise. In healthy subjects prolonged exercise to the point of exhaustion does not interfere with the functional reserve capacity of the adrenal cortex, but it significantly modifies the urinary steroid response to a subsequent infusion of ACTH.

Diurnal Pattern of Urinary 17-Hydroxycorticosteroids Excretion

Diurnal rhythm in the excretion of urinary 17-ketosteroids was first described in a group of young men by Pincus (41) in 1943. Since then Laidlaw, Jenkins and Reddy (26) have confirmed from their studies this diurnal variation in the urinary excretion of adrenal cortical steroids. In 1954, Tyler and his associates (53) found changes in plasma 17-hydroxycorticosteroids as well.

The hourly urinary excretion of 17-hydroxycorticosteroid is usually maximal between 8 and 10 A.M. and minimal from 12 midnight to 2 A.M. The peak values at about 9 A.M. is two to three times greater than the minimal values close to 1 A.M. (32). In Laidlaw, Jenkins, and Reddy's study (26) on one or more days in 75 normal subjects, they found the pattern was almost uniformly characterized by a higher level of excretion between 7 A.M. and 7 P.M. than during the overnight period.

The fall in 17-hydroxycorticosteroid excretion in the early evening does not usually persist until the following morning but is frequently succeeded by a raise in urinary levels around 3 A.M.

Using successive hourly urinary 17-hydroxycorticosteroids excretion, however, it has been found possible to calculate approximations of hourly adrenal secretions of cortisol (12). It was found that the adrenal secretes approximately 70 per cent of the daily secretion of cortisol under basal conditions from 12 midnight to 9 A.M. It would thus appear that the major portion of adrenal activity occurs in the early morning hours and during sleep.

The diurnal rhythm of adrenocortical secretion, as well as other cyclic biologic phenomena in man, is an intrinsic functional mechanism which is not easily altered by environmental change or voluntary disruption of basic habit patterns (18) (32). This diurnal cycle appears fixed for any given subject in any species, subject only to variations superimposed by changes in the external environment. These are mediated through the central nervous system and anterior pituitary adrenocorticotropin (26). The cycle might conceivably be based upon cyclic dischanges from the central nervous system or on rhythmic changes in tne activity of the cells of the adrenal cortex and their enzyme systems. It is apparently abolished by hypophysectomy (8).

Since approximately 80 per cent of the glucocorticoids secretion of the adrenal gland is cortisol and since 17-hydroxycorticoids are a measure of cortisol and its metabolites, they are the preferred metameter for following changes in adrenal cortical secretory activity. However, one cannot relate urinary total 17-hydroxycortical levels to adrenal activity without considering the temporal and quantitative factors which characterize the disposal of cortisol.

The characteristic cycle persists despite enforced inactivity in bed (12), a reversal of the normal working schedule (night workers) (30), a 24-hour period of starvation (26), a 24-hour period in which feedings were given every four hours and a reversal of the normal daytime predominance in urine output (8).

In the studies of Perkoff, Eik-Nes and their associates (38) significant loss of diurnal variation of plasma free 17-hydroxycorticosteroid levels was observed "only in clinical situations associated with alterations of consciousness or sleep patterns." The group having "irregular" diurnal variation was comprised of patients with cerebral vascular accidents, cerebral trauma, miscellaneous neurological disorders, or toxic delirium.

Sholiton, Werk, and Marnell (46) studied the diurnal variation in three types of adrenocortical function in non-endocrine disease states. They concluded that in chronically ill patients, without primary liver or

kidney disease, demonstrated normal diurnal variation of urinary 17-hydroxycorticosteroids. Comparable variation occurred in patients with advanced, but stabilized, hepatic and renal disease. Alert, ambulatory lung cancer patients exhibited a slightly less marked diurnal change, with generally higher plasma 17-hydroxycorticosteroid levels, whereas variation was absent in advanced cancer patients. The diurnal rhythm in alert, acutely ill patients was comparable to that in the ambulatory lung cancer patients. In mentally confused, acutely ill patients, the diurnal pattern was disrupted.

In the studies of both Doe (13) and Nugent (36) and their associates, marked alterations in diurnal adrenocortical function have been noted in primary pituitary and adrenal disorders, such as Cushing's syndrome and Addison's disease.

Pena et al (37) clearly demonstrated that deficits as well as excessive levels of glucocorticoids inhibit the normal rhythms of urinary excretion of 17-hydroxycorticosteroids.

PLAN OF PROCEDURE

PERIODS OF STUDY

Under the auspices of the National Aeronautics and Space Administration, the Texas Woman's University Research Institute has been conducting a series of bed rest studies. These studies are part of a vast research program being conducted in an effort to examine the response of subjects to conditions which simulate those which will be encountered in participation in the space flights.

This particular study lasted 97 days and included the following periods:

Equilibration Period, 29 days, June 19 - July 18, 1967.

Bed Rest Number One, 14 days, July 18 - August 1, 1967.

Interim Ambulatory Period, 14 days, August 1 - August 15, 1967.

Bed Rest Number Two, 14 days, August 15 - August 29, 1967.

Post-Bed Rest Period, 26 days, August 29 - September 23, 1967.

SUBJECTS OF THE STUDY

Chosen for this study were five male university students. Before their selection for participation in the study, these young men underwent extensive examinations, both physical and psychological. The following table shows their respective ages, heights and weights upon entering the study.

Subject	Age	Weight (lbs.)	Height (inches)
AA	24	155	70 1/4
BB	21	151	71 1/4
CC	21	138	66
DD	22	163	67
EE	21	182	73 1/2

DIET AND REGIMEN OF THE SUBJECTS

During the entire study, the subjects were housed and fed at the metabolic ward of the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute. Specially trained dietitians planned and supervised the preparation of the meals which were nutritionally adequate in all nutrients, calcium being the nutrient which was variable. A careful record was kept of the food intake of the individual subjects throughout the study.

This study was conducted under close medical supervision. Periodic x-rays were made as well as various clinical laboratory tests. A record was made of height and weight changes throughout the study. Specially trained orderlies attended to the hygienic needs of the subjects when immobilized.

Equilibration Period

During this span of 29 days, the subjects led a normal life. They were engaged in conducting various tasks in the laboratory eight hours daily. Their meals were planned to contain 800 mg. calcium/day during this period.

Bed Rest Number One

For a period of 14 days, the subjects were immobilized. They assumed a horizontal position on a single bed equipped with one pillow. They were encouraged not to lift their heads. Only limited arm and leg movement was allowed. They were provided with hospital type television sets and given glasses equipped with prismatic lenses for reading. During this period the orderlies cared for the hygienic needs of the subjects. The young men were spoon fed, and a careful record was kept of their individual intake of food. Ca^{47} was incorporated into their milk intake the first morning of this period. Diets were planned to contain 800 mg. calcium, including the calcium isotope. The purpose of the $calcium^{47}$ was to aid in following the metabolic pathways of the calcium, which

constituted the basis for a collateral unit of the study not related to the work of the author of this thesis.

Interim Ambulatory Period

During this period, the young men again were ambulatory. Four hours daily again were spent in performing tasks assigned in the laboratories. Supervised physical activity was compulsory in the afternoons. Again, meals were served under the dietitian's supervision in the metabolic ward and were planned to contain 800 mg. calcium daily.

Bed Rest Number Two

The same conditions prevailed during this 14-day period as described under Bed Rest Number One, with the exception of the calcium content of the diet. In this phase of the study, the daily intake of calcium was lowered to 300 mg. As in the previous bed rest, Ca^{47} was incorporated into the milk the first morning of recumbency.

Post-Bed Rest Period

Conditions during this period were similar to those during the interim ambulatory period with regard to work and physical activity. During this time, the calcium intake was varied as follows: August 29-September 13 (1500 mg.), September 13-16 (300 mg.) (Bed Rest 2), September 16-23 (1500 mg.).

PROCEDURE ADOPTED IN THE TWU LABORATORIES

1. Urine Collection and Storage

A 24-hour urine specimen is collected in a plastic bottle. Record the volume and keep in the frozen state until ready for extraction. No chemical preservative is added to the samples during this study. However, DiRaimondo et al (12) found no significant loss of 17-hydroxycorticosteroids when the sample with two milliliters of 0.5 per cent thymol in glacial acetic acid as preservative was added.

2. Hydrolysis

If the specimen is frozen, this is removed from the deep freeze and permitted to thaw. The specimen is mixed thoroughly and adjusted to pH 2.4 with 2N sulfuric acid by means of a pH meter.

3. Extraction and Purification

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.

The sample is shaken for 10 minutes on a mechanical shaker, and then is centrifuged at 2000 RPM for 10 minutes. The supernatant (butanol) layer is transferred to a set of the clean screw cap test tubes

by means of "serum lifter." A second similar extraction is made, and the butanol extracts are combined. The aqueous phase may be discarded. This butanol extract then is shaken with 1 ml. or 10 per cent potassium carbonate on a mechanical shaker for 30 minutes and is centrifuged for 10 minutes at 2000 RPM. The butanol extract again is transferred to a new set of screw cap test tubes by means of "serum lifter." Immediately one gram of anhydrous sodium sulfurate 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. Finally, the butanol extract is decanted into a clean centrifuge tube, and is stored in the refrigerator until ready to continue.

4. Colorimetry

Tubes are prepared for color development. For each butanol extract, two tubes are set up as indicated below. Then they are placed in the refrigerator until they are needed.

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

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

An addition of 2 ml. of butanol extract is added both to the A tube (sample) and the B tube (sample blank). The tubes are stoppered with a plastic stopper, and are mixed by inversion and placed in a water bath at a temperature of 60° C. for exactly 30 minutes. Then they are

removed and cooled for five minutes in an ice-water bath. The optical density is read at 410 millimicrons on a spectrophotometer (Coleman Junior, Model 6A) set at zero with a distilled water blank.

5. Calculations

(1) $\frac{\text{OD Unknown - OD Reagent Blank}}{\text{OD Standard - OD Reagent Blank}} \times 10 = \mu g./ml.$

(2) $\frac{\text{Total Volume of Urine} \times \mu g./ml.}{1000} = \text{mg. per 24 hrs.}$

6. Normal Excretions

Male -- 5-14 mg./24 hrs.

Female -- 4-9 mg./24 hrs.

Children

0 - 2 years -- 2- 4 mg./24 hrs.
2 - 6 years -- 3- 6 mg./24 hrs.
6 - 10 years -- 6- 8 mg./24 hrs.
10 - 14 years -- 8-10 mg./24 hrs.

7. Standardization

Stock Hydrocortisone Standard (200 µg./ml.)

Exactly 20 mg. of hydrocortisone (free alcohol) are dissolved in about 1/2 ml. of alcohol and are diluted exactly to 100 ml. with distilled water.

Working Hydrocortisone Standard (10 µg./ml.)

The stock standard (5 ml.) was diluted to 100 ml. with distilled water.

8. Reagents

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

(2) 18N Sulfuric Acid -- To this is added 127 ml. of concentrated sulfuric acid (with cooling) into 100 ml. of distilled water.

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

Recrystallized Phenylhydrazine -- Phenylhydrazine hydrochloride is dissolved in a minimal amount of absolute alcohol by heating. This is allowed to cool at room temperature, and then is placed in a refrigerator at least for one hour. This is filtered through a sintered glass filter. The crystals, which should be peach colored, are transferred to a clean container and placed in a dessicator.

(4) 10% Potassium Carbonate -- This is dissolved (10 grams) 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 DATA WITH DISCUSSION

Table I (Appendix) gives the data concerning urinary excretion of 17-hydroxycorticosteroids for Bed Rest 1 and Bed Rest 2, and the related Ambulatory Periods. Table II presents the data concerning the diurnal pattern of daily urinary excretion of 17-hydroxycorticosteroids during the period from 8:00 A.M. to 12:00 noon and that from the 12:00 noon to 8:00 A.M. period. In Table III the statistical findings are summarized when the different periods of the study were compared for the individual subjects, with pooled data from all subjects. Table IV gives the statistical findings of urinary excretion of 17-hydroxycorticosteroids during different periods of the day.

COMPARISON OF EXCRETION OF URINARY 17-HYDROXY-CORTICOSTEROIDS DURING BED REST 1 WITH THE PRE-BED REST AMBULATORY EQUILIBRATION PERIOD

The data showed that the excretion of urinary 17-hydroxycorticosteroids varied for the five subjects when Bed Rest 1 was compared with the Pre-Bed Rest Period. More 17-hydroxycorticosteroids were excreted in the urine during Bed Rest 1 than during the Pre-Bed Rest Period for Subjects AA, CC, and EE, but the differences were not statistically

significant. When the data for all five subjects were pooled, again the difference was not statistically significant.

COMPARISON OF FINDINGS FROM BED REST 2 AND THE INTERIM AMBULATORY PERIOD

The quantity of 17-hydroxycorticosteroids excreted in the urine was greater during the Interim Equilibration Period than during Bed Rest 2 for Subjects BB, CC, and EE. The difference was statistically significant for Subject EE. When the data for the five subjects were pooled, there was no statistically significant difference between the two periods.

COMPARISON OF URINARY EXCRETION OF 17-HYDROXY-CORTICOSTEROIDS DURING THE TWO BED REST PERIODS

When the amounts of 17-hydroxycorticosteroids excreted in the urine during Bed Rest 2, during which period only 300 mg. calcium were fed, were compared with excretions during Bed Rest 1 when 800 mg. were provided, there were no statistically significant differences between the two levels when data from all subjects were pooled. Nor were there any statistically significant differences between the two bed rest periods for four of the individual subjects. In the case of Subject DD, the amounts of urinary 17-hydroxycorticosteroids excreted during Bed Rest 2 slightly surpassed that of Bed Rest 1 ($P < 0.10$).

COMPARISON OF AMBULATORY PERIODS

The quantity of 17-hydroxycorticosteroids excreted in the urine was greater during the Interim Ambulatory Period than during both the Pre-Bed Rest Period and the Post-Bed Rest Period for all subjects. The differences, however, were not statistically significant for three subjects. When the data for all five subjects were pooled, however, the excretions of 17-hydroxycorticosteroids in urine were higher for the Interim Ambulatory Period than for the Pre-Bed Rest Period and the Post-Bed Rest Period by a highly significant difference ($P < 0.01$ and $P < 0.02$ in the two cases). There were no statistically significant differences between the Pre-Bed Rest and the Post-Bed Rest Periods, on the other hand.

COMPARISON OF BED REST PERIODS WITH THE FINAL AMBULATORY RECONDITIONING PERIOD

Both the first and second bed rest periods showed increases in excretion of urinary 17-hydroxycorticosteroids greater than the final Ambulatory Period for all subjects except Subjects AA and BB. The differences were statistically significant for two subjects, but when the data for all five subjects were pooled, the differences in 17-hydroxycorticosteroid in the urine were not statistically significant.

COMPARISON OF EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING THE DAY WITH THE NIGHT PERIOD

The excretion of 17-hydroxycorticosteroids in the urine during the 8:00 A.M. to 12:00 noon period was higher than that during the 12:00 noon to 8:00 A.M. period for all five subjects. The differences were statistically significant for all subjects but one. When the data for all five subjects were pooled, the urinary excretion of 17-hydroxycorticosteroids during the 8:00 A.M. to 12:00 noon period surpassed that of the 12:00 noon to 8:00 A.M. period by a difference which was highly significant ($P < 0.001$).

SUMMARY AND CONCLUSIONS

A study designed to test the daily excretion of total 17-hydroxycorticosteroids in urine under immobilization and ambulatory conditions on five healthy males was carried out. This study was composed of two 14-day Bed Rest Periods. A Pre-Bed Rest Period, an Interim Ambulatory Period, and a Post-Bed Rest Period with ambulation were used for comparison with the Bed Rest Periods. The two Bed Rest Periods were compared with each other. In this study, it was observed that there were irregular variations in the urinary excretion of total 17-hydroxycorticosteroids for individual subjects during each designated test period.

During the Pre-Bed Rest Period, the overall average excretion of total urinary 17-hydroxycorticosteroids with the data pooled for all five subjects was 7.27 mg. per 24 hours which was within the normal range. The mean value tended to decrease during both bed rest periods. When the excretion values for all subjects were pooled for each period, both Bed Rest Periods provided differences which were not statistically significant from the values of the Pre-Bed Rest Period.

An increase in urinary 17-hydroxycorticosteroids was noted during the Interim Ambulatory Period for all five subjects. The overall average for this period was 8.45 mg. per 24 hours as compared to 7.25 mg.

per 24 hours during the Pre-Bed Rest Period, 7.57 mg. per 24 hours during Bed Rest 1, 7.69 mg. per 24 hours during Bed Rest 2, and 7.34 mg. per 24 hours during the Post-Bed Rest Period. The increase during this final period was slightly significant in comparison with Bed Rest 1 when the excretion values of urinary 17-hydroxycorticosteroids of all five subjects were pooled. During Bed Rest 2 the overall value for all subjects was lower than during the previous Ambulatory Period by a difference which was not statistically significant.

During the Post-Bed Rest Period three of the five subjects showed a decreased level of 17-hydroxycorticosteroids excretion as compared with the excretion values during either Bed Rest Period. The decrease during this period was not statistically significant when compared with both Bed Rest Periods for all subjects.

All subjects showed a higher excretion in urinary 17-hydroxycorticosteroids during the morning than during the remainder of the day. When the excretion values for all five subjects were pooled, this increase was highly significant.

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A P P E N D I X

TABLE I

EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS

(gm. per 24 hours)

PART A. SUBJECT AA

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 8.09	(1) 3.72	(1) 7.90	(1) 5.43	(1) 10.43
(2) 7.40	(2) 4.48	(2) 9.10	(2) 6.38	(2) 3.30
(3) 5.41	(3) 9.94	(3) 6.80	(3) 4.87	(3) 8.02
(4) 3.41	(4) 7.00	(4) 9.09	(4) 12.67	(4) 14.65
(5) 2.82	(5) 6.90	(5) 9.04	(5) 3.39	(5) 5.11
(6) 3.33	(6) 11.32	(6) 5.97	(6) 9.75	(6) 8.11
(7) 12.00	(7) 5.79	(7) 7.44	(7) 7.05	(7) 9.85
(8) 6.41	(8) 6.09	(8) 8.27	(8) 8.51	(8) 7.27
(9) 15.59	(9) 4.40	(9) 7.23	(9) 9.00	(9) 6.24
(10) 7.97	(10) 6.37	(10) 8.44	(10) 6.89	(10) 9.60
(11) 4.72	(11) 4.82	(11) 8.89	(11) 8.70	(11) 7.78
(12) 8.01	(12) 14.32	(12) 5.69	(12) 14.03	(12) 6.19
(13) 4.50	(13) 3.93	(13) 7.60	(13) 6.15	(13) 5.97
(14) 7.24	(14) 8.34	(14) 8.64	(14) 9.95	(14) 8.89
(15) 12.47	X	X	X	X
(16) 4.46	X	X	X	X
(17) 5.15	X	X	X	X
(18) 4.50	X	X	X	X
(19) 3.32	X	X	X	X
(20) 8.87	X	X	X	X
(21) 5.07	X	X	X	X
(22) 8.05	X	X	X	X
(23) 4.32	X	X	X	X
(24) 8.11	X	X	X	X
(25) 6.69	X	X	X	X
(26) 5.42	X	X	X	X
(27) 6.29	X	X	X	X
(28) 7.10	X	X	X	X
(29) 3.36	X	X	X	X

TABLE I, CONTINUED

EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS

(gm. per 24 hours)

PART B. SUBJECT BB

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 5.48	(1) 4.39	(1) 5.47	(1) 4.69	(1) 2.85
(2) 8.60	(2) 4.68	(2) 7.15	(2) 5.20	(2) 8.20
(3) 13.55	(3) 8.36	(3) 8.11	(3) 3.40	(3) 6.97
(4) 11.08	(4) 5.28	(4) 6.33	(4) 8.87	(4) 10.80
(5) 5.99	(5) 3.77	(5) 7.86	(5) 4.64	(5) 4.80
(6) 3.26	(6) 5.24	(6) 12.36	(6) 8.36	(6) 4.54
(7) 9.20	(7) 5.02	(7) 5.29	(7) 2.85	(7) 3.76
(8) 6.74	(8) 4.29	(8) 9.32	(8) 5.15	(8) 7.87
(9) 13.65	(9) 6.22	(9) 7.46	(9) 9.27	(9) 12.12
(10) 3.36	(10) 8.40	(10) 7.57	(10) 7.88	(10) 7.55
(11) 6.17	(11) 6.53	(11) 6.18	(11) 5.87	(11) 2.97
(12) 4.28	(12) 7.49	(12) 5.00	(12) 5.33	(12) 6.85
(13) 13.89	(13) 8.24	(13) 6.98	(13) 7.53	(13) 5.81
(14) 4.46	(14) 5.12	(14) 6.37	(14) 4.78	(14) 5.09
(15) 6.00	X	X	X	X
(16) 4.59	X	X	X	X
(17) 5.93	X	X	X	X
(18) 4.74	X	X	X	X
(19) 4.16	X	X	X	X
(20) 5.54	X	X	X	X
(21) 5.46	X	X	X	X
(22) 5.38	X	X	X	X
(23) 7.55	X	X	X	X
(24) 7.73	X	X	X	X
(25) 6.90	X	X	X	X
(26) 3.55	X	X	X	X
(27) 4.95	X	X	X	X
(28) 8.14	X	X	X	X
(29) 6.23	X	X	X	X

TABLE I, CONTINUED

EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS

(gm. per 24 hours)

PART C. SUBJECT CC

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 3.75	(1) 3.21	(1) 11.20	(1) 5.65	(1) 8.74
(2) 6.94	(2) 7.26	(2) 9.03	(2) 3.79	(2) 8.18
(3) 7.94	(3) 3.23	(3) 9.41	(3) 4.09	(3) 15.05
(4) 7.50	(4) 10.96	(4) 3.85	(4) 6.79	(4) 9.58
(5) 7.50	(5) 9.37	(5) 11.57	(5) 8.61	(5) 5.61
(6) 14.74	(6) 7.61	(6) 7.49	(6) 7.17	(6) 7.71
(7) 2.90	(7) 3.77	(7) 4.82	(7) 3.92	(7) 5.64
(8) 10.05	(8) 8.20	(8) 8.66	(8) 11.61	(8) 10.25
(9) 8.86	(9) 8.62	(9) 10.40	(9) 9.75	(9) 7.15
(10) 8.64	(10) 10.03	(10) 9.03	(10) 6.54	(10) 9.33
(11) 6.05	(11) 3.19	(11) 6.72	(11) 5.47	(11) 4.76
(12) 5.32	(12) 6.10	(12) 2.73	(12) 12.45	(12) 10.31
(13) 7.96	(13) 10.20	(13) 5.49	(13) 3.64	(13) 4.75
(14) 6.18	(14) 11.55	(14) 5.99	(14) 8.48	(14) 9.32
(15) 5.94	X	X	X	X
(16) 4.79	X	X	X	X
(17) 4.28	X	X	X	X
(18) 4.50	X	X	X	X
(19) 4.32	X	X	X	X
(20) 6.19	X	X	X	X
(21) 11.98	X	X	X	X
(22) 5.31	X	X	X	X
(23) 8.65	X	X	X	X
(24) 5.68	X	X	X	X
(25) 8.10	X	X	X	X
(26) 4.47	X	X	X	X
(27) 8.84	X	X	X	X
(28) 8.16	X	X	X	X
(29) 6.87	X	X	X	X

TABLE I, CONTINUED

EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS

(gm. per 24 hours)

PART D. SUBJECT DD

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 6.97	(1) 7.38	(1) 11.21	(1) 5.55	(1) 5.00
(2) 6.77	(2) 5.60	(2) 5.18	(2) 7.52	(2) 14.23
(3) 5.83	(3) 9.49	(3) 8.15	(3) 7.44	(3) 9.51
(4) 5.95	(4) 11.91	(4) 11.10	(4) 12.51	(4) 6.94
(5) 13.60	(5) 4.95	(5) 11.02	(5) 9.14	(5) 6.43
(6) 7.84	(6) 8.85	(6) 14.93	(6) 12.07	(6) 7.71
(7) 14.02	(7) 3.71	(7) 8.77	(7) 8.61	(7) 5.35
(8) 8.90	(8) 6.83	(8) 7.03	(8) 7.53	(8) 6.50
(9) 14.89	(9) 7.76	(9) 14.41	(9) 13.15	(9) 3.18
(10) 8.99	(10) 7.76	(10) 3.62	(10) 9.83	(10) 8.03
(11) 8.97	(11) 7.70	(11) 10.64	(11) 6.61	(11) 6.76
(12) 11.02	(12) 8.58	(12) 8.65	(12) 13.13	(12) 4.21
(13) 6.98	(13) 10.24	(13) 7.40	(13) 14.77	(13) 8.32
(14) 3.45	(14) 7.80	(14) 9.89	(14) 6.96	(14) 4.67
(15) 5.62	X	X	X	X
(16) 5.33	X	X	X	X
(17) 7.37	X	X	X	X
(18) 4.34	X	X	X	X
(19) 7.56	X	X	X	X
(20) 13.11	X	X	X	X
(21) 5.53	X	X	X	X
(22) 9.63	X	X	X	X
(23) 4.40	X	X	X	X
(24) 6.88	X	X	X	X
(25) 5.23	X	X	X	X
(26) 6.38	X	X	X	X
(27) 8.93	X	X	X	X
(28) 7.88	X	X	X	X
(29) 7.22	X	X	X	X

TABLE I, CONTINUED

EXCRETION OF URINARY 17-HYDROXYCORTICOSTEROIDS

(gm. per 24 hours)

PART E. SUBJECT EE

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 5.25	(1) 5.36	(1) 7.35	(1) 3.56	(1) 7.20
(2) 10.38	(2) 3.02	(2) 8.50	(2) 4.38	(2) 9.44
(3) 9.40	(3) 13.97	(3) 10.60	(3) 4.01	(3) 7.16
(4) 9.15	(4) 13.80	(4) 11.06	(4) 8.55	(4) 9.48
(5) 8.18	(5) 7.53	(5) 11.66	(5) 8.76	(5) 7.30
(6) 9.54	(6) 9.18	(6) 13.20	(6) 11.09	(6) 7.96
(7) 5.03	(7) 7.64	(7) 10.17	(7) 4.16	(7) 4.81
(8) 11.66	(8) 7.22	(8) 9.59	(8) 7.06	(8) 13.30
(9) 12.68	(9) 12.88	(9) 14.51	(9) 13.08	(9) 5.51
(10) 3.86	(10) 13.36	(10) 9.25	(10) 7.33	(10) 9.67
(11) 9.28	(11) 7.03	(11) 14.39	(11) 5.40	(11) 5.40
(12) 13.91	(12) 10.30	(12) 7.52	(12) 12.73	(12) 13.51
(13) 11.43	(13) 16.81	(13) 8.87	(13) 10.96	(13) 4.71
(14) 11.09	(14) 8.96	(14) 4.63	(14) 7.68	(14) 6.13
(15) 4.48	X	X	X	X
(16) 5.25	X	X	X	X
(17) 2.70	X	X	X	X
(18) 2.76	X	X	X	X
(19) 5.61	X	X	X	X
(20) 13.05	X	X	X	X
(21) 13.43	X	X	X	X
(22) 5.70	X	X	X	X
(23) 8.84	X	X	X	X
(24) 4.80	X	X	X	X
(25) 9.00	X	X	X	X
(26) 6.31	X	X	X	X
(27) 7.88	X	X	X	X
(28) 5.84	X	X	X	X
(29) 9.02	X	X	X	X

TABLE II

URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS AT DIFFERENT PERIODS OF THE DAY

PART A. SUBJECT AA

Periods	
12:00 Noon to 8:00 A.M. (mg./ml.)	8:00 A.M. to 12:00 Noon (mg./ml.)
(1) 0.0040	(1) 0.0075
(2) 0.0074	(2) 0.0036
(3) 0.0044	(3) 0.0051
(4) 0.0038	(4) 0.0075
(5) 0.0084	(5) 0.0084
(6) 0.0039	(6) 0.0063
(7) 0.0086	(7) ----
(8) 0.0062	(8) 0.0042
(9) 0.0047	(9) 0.0089
(10) 0.0066	(10) 0.0044
(11) 0.0074	(11) 0.0077
(12) 0.0058	(12) 0.0063

TABLE II, CONTINUED

URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS

AT DIFFERENT PERIODS OF THE DAY

PART B. SUBJECT BB

Periods	
12:00 Noon to 8:00 A.M. (mg./ml.)	8:00 A.M. to 12:00 Noon (mg./ml.)
(1) 0.0051	(1) 0.0042
(2) 0.0043	(2) 0.0058
(3) 0.0045	(3) 0.0022
(4) 0.0042	(4) 0.0120
(5) 0.0069	(5) 0.0064
(6) 0.0039	(6) 0.0075
(7) 0.0053	(7) 0.0160
(8) 0.0070	(8) 0.0096
(9) 0.0044	(9) 0.0091
(10) 0.0051	(10) 0.0086
(11) 0.0075	(11) 0.0022
(12) 0.0037	(12) 0.0067

TABLE II, CONTINUED

URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS

AT DIFFERENT PERIODS OF THE DAY

PART C. SUBJECT CC

Periods	
12:00 Noon to 8:00 A.M. (mg./ml.)	8:00 A.M. to 12:00 Noon (mg./ml.)
(1) 0.0044	(1) 0.0105
(2) 0.0050	(2) 0.0135
(3) 0.0046	(3) 0.0059
(4) 0.0042	(4) 0.0146
(5) 0.0029	(5) 0.0122
(6) 0.0061	(6) 0.0174
(7) 0.0022	(7) 0.0138
(8) 0.0036	(8) 0.0135
(9) 0.0053	(9) 0.0111
(10) 0.0058	(10) 0.0106
(11) 0.0071	(11) 0.0058
(12) 0.0043	(12) 0.0069

TABLE II, CONTINUED

URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS

AT DIFFERENT PERIODS OF THE DAY

PART D. SUBJECT DD

Periods	
12:00 Noon to 8:00 A.M. (mg./ml.)	8:00 A.M. to 12:00 Noon (mg./ml.)
(1) 0.0073	(1) 0.0072
(2) 0.0059	(2) 0.0058
(3) 0.0041	(3) 0.0081
(4) 0.0051	(4) 0.0078
(5) 0.0069	(5) 0.0113
(6) 0.0067	(6) 0.0080
(7) 0.0082	(7) 0.0115
(8) 0.0069	(8) 0.0059
(9) 0.0046	(9) 0.0051
(10) 0.0065	(10) 0.0076
(11) 0.0081	(11) 0.0055
(12) 0.0034	(12) 0.0116

TABLE II, CONTINUED

URINARY EXCRETION OF 17-HYDROXYCORTICOSTEROIDS

AT DIFFERENT PERIODS OF THE DAY

PART E. SUBJECT EE

Periods	
12:00 Noon to 8:00 A.M. (mg./ml.)	8:00 A.M. to 12:00 Noon (mg./ml.)
(1) 0.0096	(1) 0.0080
(2) 0.0059	(2) 0.0121
(3) 0.0032	(3) 0.0054
(4) 0.0063	(4) 0.0145
(5) 0.0060	(5) 0.0085
(6) 0.0066	(6) 0.0078
(7) 0.0063	(7) 0.0084
(8) 0.0067	(8) 0.0088
(9) 0.0053	(9) 0.0049
(10) 0.0060	(10) 0.0089
(11) 0.0076	(11) 0.0054
(12) 0.0047	(12) 0.0065

TABLE III

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

PART A. SUBJECT AA

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	6.55 6.96*	2.92 2.97	0.4031	N.S.
Pre-Bed Rest Period Interim Ambulatory	6.55 7.86*	2.92 1.09	1.5568	N.S.
Pre-Bed Rest Period Bed Rest No. 2	6.55 8.06*	2.92 2.84	1.5199	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	6.55 7.51*	2.92 2.45	1.2388	N.S.
Bed Rest No. 1 Interim Ambulatory	6.96 7.86*	2.97 1.09	0.9949	N.S.
Bed Rest No. 1 Bed Rest No. 2	6.96 8.06*	2.97 2.84	0.9279	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	6.96 7.51*	2.97 2.45	0.5872	N.S.
Interim Ambulatory Bed Rest No. 2	7.86 8.06*	1.09 2.84	0.2181	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	7.86* 7.51	1.09 2.45	0.4943	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	8.06* 7.51	2.84 2.45	0.6002	N.S.

*Period which is greater

TABLE III, CONTINUED

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

PART B. SUBJECT BB

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	6.78* 5.93	2.93 1.56	0.9707	N.S.
Pre-Bed Rest Period Interim Ambulatory	6.78 7.25*	2.93 1.82	0.5248	N.S.
Pre-Bed Rest Period Bed Rest No. 2	6.78* 5.99	2.93 1.96	0.8746	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	6.78* 6.15	2.93 2.96	0.7462	N.S.
Bed Rest No. 1 Interim Ambulatory	5.93 7.25*	1.56 1.82	1.9068	$P < 0.10$
Bed Rest No. 1 Bed Rest No. 2	5.93 5.99*	1.56 1.96	0.0783	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	5.93 6.15*	1.56 2.96	0.2417	N.S.
Interim Ambulatory Bed Rest No. 2	7.25* 5.99	1.82 1.96	1.6329	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	7.25* 6.15	1.82 2.96	1.1938	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	5.99 6.15*	1.96 2.96	0.1717	N.S.

*Period which is greater

TABLE III, CONTINUED

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

PART C. SUBJECT CC

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	6.98 7.44*	2.50 2.97	0.5013	N.S.
Pre-Bed Rest Period Interim Ambulatory	6.98 7.60*	2.50 2.65	0.7112	N.S.
Pre-Bed Rest Period Bed Rest No. 2	6.98 7.00*	2.50 2.77	0.0198	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	6.98 6.99*	2.50 2.66	0.0180	N.S.
Bed Rest No. 1 Interim Ambulatory	7.44 7.60*	2.97 2.65	0.1429	N.S.
Bed Rest No. 1 Bed Rest No. 2	7.44* 7.00	2.97 2.77	0.3756	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	7.44* 6.99	2.97 2.66	0.4530	N.S.
Interim Ambulatory Bed Rest No. 2	7.60* 7.00	2.65 2.77	0.5458	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	7.60* 6.99	2.65 2.66	0.6481	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	7.00* 6.99	2.77 2.66	0.0046	N.S.

*Period which is greater

TABLE III, CONTINUED

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

PART D. SUBJECT DD

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	7.92* 7.75	2.92 2.04	0.1791	N.S.
Pre-Bed Rest Period Interim Ambulatory	7.92 9.43*	2.92 3.04	1.4946	N.S.
Pre-Bed Rest Period Bed Rest No. 2	7.92 9.63*	2.92 2.84	1.7328	$P < 0.10$
Pre-Bed Rest Period Post-Bed Rest Ambulatory	7.92* 7.01	2.92 2.83	1.1164	N.S.
Bed Rest No. 1 Interim Ambulatory	7.75 9.43*	2.04 3.04	1.5883	N.S.
Bed Rest No. 1 Bed Rest No. 2	7.75 9.63*	2.04 2.84	1.8626	$P < 0.10$
Bed Rest No. 1 Post-Bed Rest Ambulatory	7.75* 7.01	2.04 2.83	0.8279	N.S.
Interim Ambulatory Bed Rest No. 2	9.43 9.63*	3.04 2.84	0.1681	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	9.43* 7.01	3.04 2.83	2.3647	$P < 0.02$
Bed Rest No. 2 Post-Bed Rest Ambulatory	9.63* 7.01	2.84 2.83	2.6288	$P < 0.01$

*Period which is greater

TABLE III, CONTINUED

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

PART E. SUBJECT EE

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	8.12 9.79*	3.21 3.74	1.4383	N.S.
Pre-Bed Rest Period Interim Ambulatory	8.12 10.09*	3.21 2.68	1.8969	$P < 0.10$
Pre-Bed Rest Period Bed Rest No. 2	8.12* 7.77	3.21 3.14	0.3242	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	8.12* 7.18	3.21 2.66	1.1218	N.S.
Bed Rest No. 1 Interim Ambulatory	9.79 10.09*	3.74 2.68	0.2288	N.S.
Bed Rest No. 1 Bed Rest No. 2	9.79* 7.77	3.74 3.14	1.4381	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	9.79* 7.18	3.74 2.66	2.3944	$P < 0.02$
Interim Ambulatory Bed Rest No. 2	10.09* 7.77	2.68 3.14	1.9569	$P < 0.10$
Interim Ambulatory Post-Bed Rest Ambulatory	10.09* 7.18	2.68 2.66	3.1063	$P < 0.01$
Bed Rest No. 2 Post-Bed Rest Ambulatory	7.77* 7.18	3.14 2.66	0.5900	N.S.

*Period which is greater

TABLE III, CONTINUED

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

PART F. ALL SUBJECTS

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	7.27 7.57*	2.97 3.04	0.6903	N.S.
Pre-Bed Rest Period Interim Ambulatory	7.27 8.45*	2.97 2.61	2.7985	$P < 0.01$
Pre-Bed Rest Period Bed Rest No. 2	7.27 7.69*	2.97 2.99	0.9533	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	7.27 7.34*	2.97 2.75	0.1758	N.S.
Bed Rest No. 1 Interim Ambulatory	7.57 8.45*	3.04 2.61	1.7948	$P < 0.10$
Bed Rest No. 1 Bed Rest No. 2	7.57 7.69*	3.04 2.99	0.2195	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	7.57* 7.34	3.04 2.75	0.4726	N.S.
Interim Ambulatory Bed Rest No. 2	8.45* 7.69	2.61 2.99	1.5751	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	8.45* 7.34	2.61 2.75	2.4362	$P < 0.02$
Bed Rest No. 2 Post-Bed Rest Ambulatory	7.69* 7.34	2.99 2.75	0.7112	N.S.

*Period which is greater

TABLE IV

STATISTICAL COMPARISON OF URINARY 17-HYDROXYCORTICOSTEROID EXCRETION BETWEEN DAY AND NIGHT FOR FIVE BED REST SUBJECTS

Populations Compared	Means (mg./ml.)	Standard Deviation	"t" Value	Probability
Subject AA				
12:00 Noon to 8:00 A.M.	0.0059	0.0017	0.5396	N.S.
8:00 A.M. to 12:00 Noon	0.0064	0.0017		
Subject BB				
12:00 Noon to 8:00 A.M.	0.0052	0.0012	1.8847	$P < 0.10$
8:00 A.M. to 12:00 Noon	0.0075	0.0038		
Subject CC				
12:00 Noon to 8:00 A.M.	0.0046	0.0013	6.1158	$P < 0.001$
8:00 A.M. to 12:00 Noon	0.0109	0.0030		
Subject DD				
12:00 Noon to 8:00 A.M.	0.0061	0.0015	2.1321	$P < 0.05$
8:00 A.M. to 12:00 Noon	0.0080	0.0023		
Subject EE				
12:00 Noon to 8:00 A.M.	0.0062	0.0015	2.1687	$P < 0.05$
8:00 A.M. to 12:00 Noon	0.0083	0.0027		
All Subjects				
12:00 Noon to 8:00 A.M.	0.0056	0.0016	5.6471	$P < 0.001$
8:00 A.M. to 12:00 Noon	0.0082	0.0032		