NATURE AND SIGNIFICANCE OF NEUTRAL STEROIDS IN HUMAN URINE IN NORMAL AND IN ABNORMAL STATES: WITH A PRELIMINARY CONSIDERATION OF THE ADRENAL AND GONADAL STEROIDS AND THE FACTORS WHICH INFLUENCE THEIR SECRETION AND BIOLOGICAL ACTION.*

WILLIAM W. ENGSTROM

The development of methods whereby assays of certain groups of steroid substances in urine can be rather easily and accurately carried out has advanced our understanding of the rôle played by the steroid hormones in physiologic processes. Beside the usual procedures of chemical and biological assay, additional information has been afforded by the laborious isolation and characterization of the crystalline steroids that are excreted in human urine. This report will be a survey of data regarding the nature, amounts, and possible significance of the neutral urinary steroids in normal and in abnormal clinical states. With the present state of information in this field it is easy to surround oneself with a jungle of facts but more difficult to emerge with real knowledge; hence, as much emphasis will be placed on the limited applicability of the data and methods as on their value in the diagnosis and interpretation of disease processes. The importance of chemical investigations cannot be depreciated, but one must always return to the biological level for appraisal of the findings. Extensive and valuable animal experimentation has been carried out in an effort to understand the rôle played by the steroids in the organism. Although a review of these data is beyond the scope of this report and can be found elsewhere, certain biological principles derived from this information cannot be disregarded when endeavoring to interpret certain findings in man. Further, it will be necessary to consider briefly the possible origins and nature of those substances which give rise to the excretory products, since assays of the latter are possibly only indirect measures of compounds active in the body. Also, to visualize accurately the various groups of steroid substances that are excreted it is necessary to know the methods which are used to separate one group from another and how the quantitative or qualitative composition of each group is determined. Efforts will be made to summarize and organize the data so that they

*From the Department of Internal Medicine, Yale University School of Medicine.

will be intelligible to those not well acquainted with the subject; however, it is neither wise nor possible to simplify the complexities of the field. This becomes apparent when one is forced to interpret the results of an assay procedure in the light of much uncertain knowledge regarding precise mechanisms of hormone formation, secretion, and action.

Orientation regarding terminology and formulae

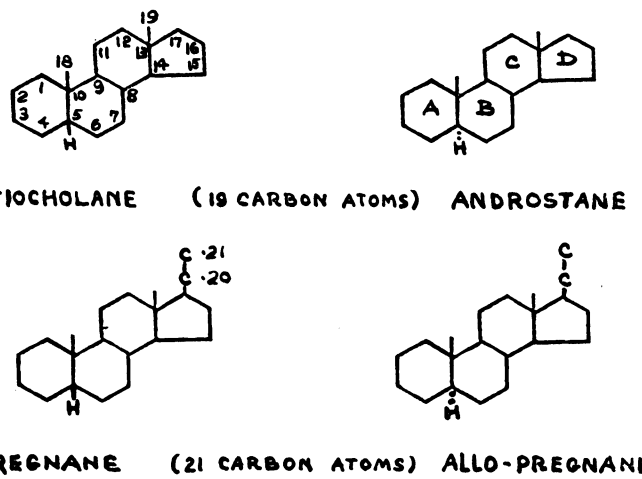


FIG. 1. Structural characteristics of certain fundamental steroids.

The manner in which the carbon atoms and rings of the nucleus are numbered and designated is given in Fig. 1. Many neutral steroids have the basic androstane or etiocholane type of configuration, the difference between the two being the difference in spatial position of the hydrogen atom at C-5. Also, it will be noted that when an etiocholane is substituted with a CH_3CH_2-group at C-17 it is designated as a pregnane; a similarly substituted androstane is known as an allo-pregnane. Therefore when it is stated, for example, that a compound is a "pregnane" or an "androstane," the nuclear structure of the compound or its derivative is indicated and also whether or not the two-carbon side chain is present at C-17. So much for the basic structure.

Examination of the natural steroids reveals, however, that they may have attached hydroxyl or ketone groups and frequently possess a double bond in the nucleus. When a ketone group is present it is sufficient merely to state at which carbon atom the group occurs, as 17-keto-androstane. More frequently, however, it is customary to combine the name of the basic hydrocarbon (such as androstane) with the ending-*one* which designates the presence of a ketone group. The carbon

atom involved in the ketone group is indicated by the appropriate number. Thus, the name androstan-17-one means that the compound has the androstane skeleton and a ketone group at C-17; androstane-3, 17-dione means that there are two ketone groups, one at C-3 and one at C-17. The symbol Δ indicates the presence of a double bond and a superscript shows the position of the double bond, thus Δ^4 indicates the presence of a double bond between C-4 and C-5. Further, the basic hydrocarbon is then designated as being unsaturated by the ending-*ene,* for example, Δ^4 androsten-17-one. If there is any possibility of ambiguity the positions of both ends of the double bond are given, thus $\Delta^{8:9}$ or $\Delta^{8:14}$. If more than one double bond is present, as for instance a double bond between C-3 and C-4 and one between C-5 and C-6, the positions would be designated as $\Delta^{3,5}$ and such a compound would be called a *diene.* An example would be $\Delta^{3,5}$ androstandien-17-one.

The presence of a hydroxyl group is indicated by the ending *-ol;* for example, androstan-3-ol designates the androstane skeleton with a hydroxyl group substituted at C-3. The presence of a ketone and an alcohol group is shown in the name androstan-3-ol-17-one. When a hydroxyl group is substituted for a hydrogen atom two sterioisomers become possible. As an aid in terminology the terms alpha (α) and beta (β)* were suggested by Fieser to denote the steric relation between the rest of the molecule and the hydroxyl group at C-3. This terminology has been extended to include other positions (e.g., C-17, C-11, C-20, etc.). By convention the alpha position is designated by a dotted valency bond, indicating that the attached group is below the plane of the ring, and the beta position by a solid valency bond indicating that the attached group is above the plane of the ring. With these facts in mind it is clear that the compound etiocholan-3(α)-ol-17-one, a so-called 17-ketosteroid, would have the following structural formula:

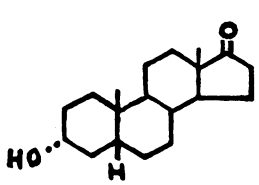


FIG. 2

Precise designations of molecular structure are necessary since isomeric variations within the molecule not only result in altered physical and chemical properties but may account for the presence or absence of biological activity.

The nature of the adrenal and gonadal steroids

A comprehensive review of the various rôles played by the steroid hormones in physiologic

* Although not as convenient, the terms *trans* and *cis* are sometimes used to denote the *alpha* and *beta* positions, respectively.

processes cannot be attempted. For detailed data the reader is referred to several excellent review articles.[26, 108, 109, 143, 214] However, a brief survey of some of this available information must be made before one attempts to interpret the significance of the steroid excretion products. One must consider the possible nature of the steroids as found in the organism before excretion, their major biological actions and transformations, and certain factors which are known to influence their secretion. Since the adrenal cortex and male gonad appear to provide the main source of steroid hormones which give rise to the neutral urinary steroids, the major portion of the discussion must concern itself with the hormones of these glands. The rôle played by the secretions of other endocrine glands will be included only in so far as these appear to affect the adrenal and the testicle.

The steroids of the adrenal cortex

When early studies[91, 115, 143, 213] on adrenal extracts and steroid hormones were being carried out it became difficult to ascribe all the observed effects to one adrenal cortical hormone. Since then a surprisingly large number of crystalline steroids have been isolated from the adrenal, differing qualitatively and quantitatively from one another in their biological actions. In Table 1 are listed the 28 steroids[190] thus far isolated from the adrenal cortex. It will be noted that 11 of these compounds have proved to be biologically active; the remainder are either inactive or their biological activity has not yet been reported. Except for the androgenic compounds and estrone, the physiologically active adrenal steroids contain an α,β unsaturated 3-ketone and the two carbon side chain at C-17 and are therefore Δ^4 pregnenes. The majority

Table 1

ADRENAL STEROIDS GROUPED ACCORDING TO THEIR MAJOR BIOLOGICAL EFFECTS

A. Carbohydrate metabolism (C-21 steroids, 5 oxygen)

I. Δ^4 Pregnene-11(β),17(β),21-triol-3,20-dione
(17-hydroxycorticosterone or Kendall's cpd. F)

II. Δ^4 Pregnene-17(β),21-diol-3,11,20-trione
(17-hydroxy 11-dehydrocorticosterone or Kendall's cpd. E)

Remarks: Cpds. I and II increase the rate of glyconeogenesis from proteins and other precursors when this is necessary and retard the rate of glucose disposal;[111, 148] the site of action and the extent to which fat is involved still is somewhat obscure;[109] they may cause a temporary increase in sodium excretion[226] and appear to be less effec-

(Table 1 Cont.)

tive than Cpd.V to maintain the life of the adrenalectomized animal.[151]

B. Carbohydrate, and salt and water metabolism (C-21 steroids, 4 oxygen)

III. Δ^4 Pregnene-11,21-diol-3,20-dione
(corticosterone or Kendall's cpd. B)

IV. Δ^4 Pregnen-21-ol-3,11,20-trione
(11-dehydrocorticosterone or Kendall's cpd. A)

Remarks: Cpds. III and IV have similar but less intense actions on carbohydrate metabolism[110, 210, 241] and the effects vary in different test animals;[210] they cause moderate retention of sodium, chloride, and water and lengthen the lives of adrenalectomized animals.[109, 151]

C. Salt and water metabolism (C-21 steroids, 3 and 2 oxygen)

V. Δ^4 Pregnen-21-ol-3,20-dione
(11-desoxycorticosterone)

VI. Δ^4 Pregnene-3,20-dione
(progesterone)

Remarks: Cpd. V, isolated in very minute quantities from the adrenal cortex,[189] has powerful effects on sodium, chloride, and water retention and is the most efficient steroid known to prolong the lives of adrenalectomized animals when these are not exposed to certain stresses.[115, 151] Cpd. VI has similar, but much weaker actions; its most prominent feature is its well-known progestational effect.

D. Androgenic (C-19 steroids)

VII. Androstane-3(β),11(β)-diol-17-one

VIII. Δ^4 Androstene-3,11,17-trione
(adrenosterone)

IX. Δ^4 Androstene-3,17-dione

X. Δ^4 Pregnen-17(?)-ol-3,20-dione (C-21 steroid)
(17-hydroxyprogesterone)

Remarks: It is uncertain whether Cpds. VII, VIII, and IX naturally occur in the fresh gland. Although evidence is lacking, these compounds may originate by oxidation of C-21 steroids during the process of isolation. Cpd. X has about the same androgenic activity as androsterone.

E. Estrogenic (C-18 steroid)

XI. Estrone

F. Inactive compounds and compounds whose action has not been determined.

XII. Allo Pregnane-3(β),11(β),17(β),20(?),21-pentol (inactive)

XIII. Allo Pregnane-3(β),11(β),17(β),21-tetrol-20-one

XIV. Allo Pregnane-3(α),11(?),17(β),21-tetrol-20-one

XV. Allo Pregnane-3(β),17(β),21-triol-11,20-dione (inactive)

XVI. Δ^4 Pregnene-11(β),17(β),20(?),21-tetrol-3-one (inactive)

XVII. Δ^4 Pregnene-17(β),20(?),21-triol-3,11-dione

XVIII. Allo Pregnane-3(β),17(β),20(β),21-tetrol (inactive)

(Table 1 Cont.)

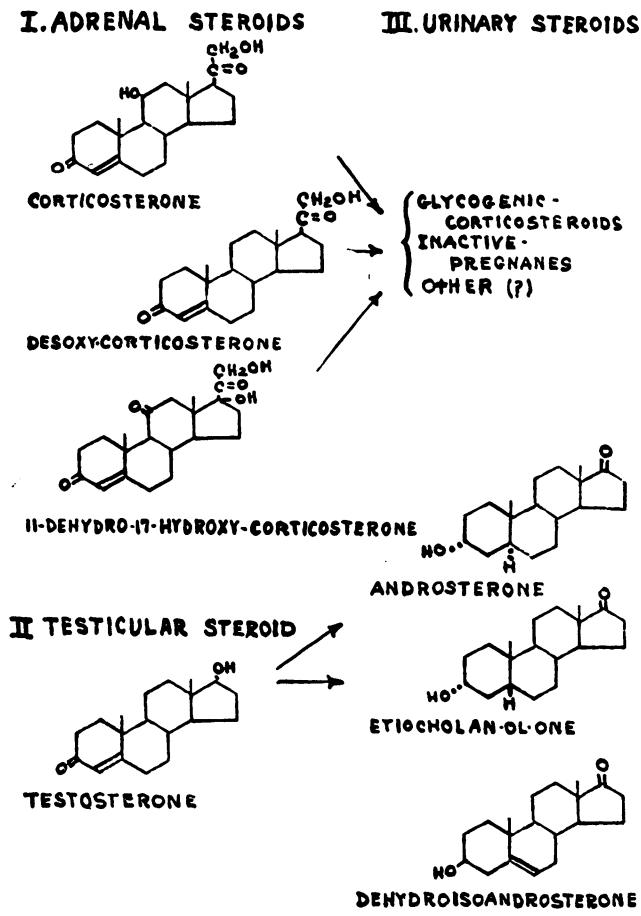
XIX. Allo Pregnane-3(β),17(β),21-triol-20-one
XX. Δ^4 Pregnene-17(β),21-diol-3,20-dione (inactive) (?)
XXI. Allo Pregnane-3(β),11(?),21-triol-20-one
XXII. Allo Pregnane-3(β),21-diol-11,20-dione
XXIII. Δ^4 Pregnene-20(?),21-diol-3,11-dione
XXIV. α,β unsaturated ketone: constitution unknown (inactive)
XXV. Allo Pregnane-3(β),17(β),20(β)-triol
XXVI. Allo Pregnane-3(β),17(β),20(α)-triol
XXVII. Allo Pregnane-3(β),17(β)-diol-20-one
XXVIII. Allo Pregnan-3(β)-ol-20-one (inactive)
G. Amorphous Fraction: nature unknown.

of the inactive compounds are allo-pregnanes. Structurally, these active compounds differ from one another only in the number and position of attached hydroxyl or ketone groups and perhaps can be looked upon as being in different stages of oxidation and reduction. The structural relationships of certain of the adrenal, testicular, and urinary steroids become clearer by examination of Fig. 3.

Except for three androgenic steroids (19 carbon atoms) and estrone (18 carbon atoms) the physiologically active steroids contain 21 carbon atoms (pregnene derivatives). Whether all these different substances are derived by differential degradation of one parent substance and many of the isolated members being intermediate compounds or whether the different hormones are built up separately and directly from smaller fragments, remains to be determined. It has been suggested that the androgenic adrenal steroids are not "true" adrenal hormones but that they may be artifacts derived by degradation of certain of the 21-carbon compounds during the isolation precedures. Evidence for this assumption is lacking. As will be discussed later, certain clinical and laboratory observations in man would indicate that the adrenal indeed does elaborate androgenic substances and it would seem that the quantities elaborated are in no direct way related, at least quantitatively, to those 21-carbon steroids concerned with protein, salt, and water metabolism. However, the exact chemical and biological nature of the "androgens" of adrenal origin is unknown.

The actual quantities of hormone elaborated and discharged into the circulation by the normal adrenal is not known with certainty, since the amounts of cortical hormone require merely for the maintenance of life of adrenalectomized animals may be far different from those required by the normal animal in optimal condition. By cannulizing the adrenal vein Vogt[236] was able to show that the adrenals of a 10 kg. dog secrete an amount of hormone equivalent to at least 230 ml. of

a commercial cortical extract per day (1 ml. of extract equivalent to 75 gm. of gland). If this is the case, its importance both clinically and experimentally cannot be overestimated.



Structural formulae of certain adrenal and testicular hormones and urinary steroids.

It should be noted, further, that after removal of all crystallizable material from the beef or hog adrenal cortex an amorphous fraction remains in the mother liquor which contains the principal part of the original total activity.[116, 132, 158, 247] This fraction is 3 to 5 times as active as desoxycorticosterone acetate and 300 to 500 times as able as compound E (Kendall) to maintain the life of an adrenalectomized dog. The chemical nature of this undoubtedly important fraction is wholly unknown. Its activity does not seem to be due to any of the known biologically active adrenal steroids.

As noted in the table, the various steroids are segregated into groups according to their major biological actions. Although such divisions may be partially artificial it is necessary in view of our ignorance regarding the formation and intermediary metabolism of these substances. Also, since no one steroid from the adrenal can be universally accepted as a

general standard of adrenal activity one cannot, with accuracy, speak of "cortical" activity and convey a precise meaning. It is necessary, therefore, to designate the biological action more specifically. Thus, the adrenal steroids have been grouped, in spite of some overlapping, into those which exert an effect mainly on salt and water exchange, those that act on carbohydrate and protein metabolism, and those which are androgenic, estrogenic, or inactive. Some of the pertinent features of their various actions are summarized in the table. A thorough understanding of these functions is imperative before the manifestations of certain hypo- and hyperfunctioning lesions of the adrenal cortex in man can be interpreted.

It is possible, but of course unknown, that identical substances are elaborated by the normal human adrenal cortex. It is also unknown whether abnormal adrenal cortices (adrenal tumors or hyperplasias) elaborate abnormal steroids or whether disproportionate amounts of normal hormones are produced. Further, since different species occasionally react very differently to the same hormonal substance, it cannot be assumed that the biological effect of a steroid in man will be identical with that produced in a test animal. A substance might promote growth of the capon's comb and be considered a potent "androgen" and yet cause growth of the mammary gland and be a poor "androgen" in man at certain periods of his development.

The steroids of the testicle

In Table 2 are listed those steroids which have been isolated from animal testes. Testosterone is generally considered to be *the* male sex hormone because of its potent androgenic properties and because of its ability to reverse the regressive changes in the secondary sexual

Table 2

STEROIDS ISOLATED FROM THE TESTIS

Compound	*Animal source*
1. Testosterone ($\triangle^4$ androsten-3-one-17(α)-ol)	Bull[45]
Remarks: Testosterone is strongly androgenic;[215] prevents cytologic "castration" changes in pituitary,[5] largely prevents testicular atrophy in hypophysectomized animals and prevents retrogression of secondary sexual characters of the castrated animal;[175] promotes nitrogen retention, apparently independently of other endocrine glands, when nutritive substances are available;[120, 129] particularly metabolized to 17-ketosteroids.[33]	Horse[215]
2. α-Estradiol ($\triangle^{1,3,5:10}$-estratriene-3,17(α)-diol)	Horse[13]

3. Estrone ($\triangle^{1,3,5:10}$-estratrien-3-ol-17-one) Remarks: Compounds 2 and 3 are well known for their estrogenic effects. The actions of the following compounds are largely unsettled.	Horse[13]
4. $\triangle^{5}$ pregnen-3(β)-ol-20-one	Pig[195]
5. Allopregnan-3(β)-ol-20-one	Pig[195]
6. $\triangle^{16:17}$ androsten-3(α)-ol	Pig[188]
7. $\triangle^{16:17}$ androsten-3(β)-ol	Pig[188]
8. Androstane-3,17-dione	Pig[179]
9. Testalolone ($C_{21}H_{32}O_3$) (structure uncertain[196])	Pig[98]
10. Several other crystalline compounds not fully identified.[195]	

characters consequent upon castration. For example, testosterone prevents or repairs the atrophy of the seminal vesicle and prostate in castrate animals and produces a masculine distribution of hair, acne, increased musculature, and growth of the accessory male genital organs in man. Its rôle in the maintenance of spermatogenesis, however, is not entirely clear.

In addition to its well-recognized masculinizing and occasionally defeminizing effects, testosterone is known to bring about other metabolic changes in the organism. Suffice it to state that in a variety of clinical conditions (e.g., normal males and females, aged men and immature boys, hypogonad males and females, patients with adrenal or pituitary insufficiency) administration of testosterone results in nitrogen retention and provides a gain in body weight.[125] Associated with these changes there generally is a slight retention of other elements, such as calcium, phosphate, sulfate, sodium, potassium, and chloride. These observations have been taken to mean that new body tissue is being formed under the influence of the hormone.[2, 119, 220] It would appear that this effect is a rather direct one and not mediated through or entirely dependent upon the secretions of the other endocrine glands.[129] From available evidence it is clear that there are other important factors which modify the effect of testosterone (or its esters) on protein anabolism. The observation that eunuchoid patients and castrate animals retain more nitrogen than do normal men or animals[119, 127, 130] would indicate that the presence of normal endogenous androgen may diminish the response to the hormone. Also, eunuchoid patients retain as much nitrogen when 25 mg. of testosterone propionate per day are administered as when larger amounts are given.[125] Further, an intercurrent illness may diminish the nitrogen retention or even cause a negative nitrogen balance in spite of testosterone adminis-

tration.[23] Kenyon[118] showed a decrease in the effectiveness of testosterone propionate on the excretion of nitrogen when the nitrogen intake was decreased from 13.5 gm. per day to an inadequate intake of 1.7 gm. and when the caloric level was reduced from 2816 calories to 1589 per day. Obviously other factors, nutritional and adaptive in nature, which may or may not be under endocrine control, are instrumental in modifying the response to administered testosterone. From these observations one certainly cannot infer that all anabolic processes are mediated by way of androgenic hormones any more than one can deduce that adjustments to dietary change are entirely under control of the endocrine system as we understand it. Also, from Kochakian's extensive data[128] it is clear that there is no necessary parallelism between "androgenic" activity and "anabolic" activity exhibited by different steroids. Since the precise nature of the "androgens" elaborated by the normal or pathological adrenal cortex is unknown, it has not been possible to demonstrate directly that these are anabolic in man. However, since certain individuals suffering from adrenal cortical neoplasm or hyperplasia exhibit virilism and accelerated growth, it has been suggested[2] that the adrenal, under these circumstances at least, elaborates hormones which favor the deposition of new tissue.

Although estrone, estradiol, and several other steroids have been isolated from testes, their physiological significance in the male organism is entirely obscure. It is well known that the human male excretes estrogen in the urine in appreciable amounts and the observation that castration leads to a fall in estrogen excretion[202] would lend support to the view that at least part of the endogenous estrogen is derived from the male gonad. There is some indirect evidence that this estrogen is elaborated by the Sertoli cells of the testis[107] or possibly by the seminiferous tubular elements.[65, 126] There is rather conclusive evidence that testosterone is elaborated by the interstitial (Leydig) cells.[164] It appears that the tubular and interstitial cell elements are under separate gonadotrophic control. In normal, immature, and hypophysectomized male rats the follicle-stimulating hormone stimulates the tubules to spermatogenic activity without affecting androgen production of the interstitial cells.[64] Androgen secretion appears to be under control of the luteinizing hormone.[80]

Androgenic and estrogenic actions of steroid hormones

An "androgen" can be defined as a substance which, like testosterone,

will stimulate the development of the accessory male organs or characters. The term "estrogen" usually means a compound which, like estrone, will induce cornification of the vaginal mucosa of the adult mouse and which, broadly speaking, assists in the development of organs appropriate to the female. Though the terms "androgen" and "estrogen" sometimes may be useful to convey a general meaning, it must be recognized that the actions of these so-called "male" and "female" sex hormones are not sharply limited to one anatomical location or to one type of response. A compound would be considered to be an "androgen" if it stimulates comb growth in the capon, while the same compound would be called an "estrogen" if it produced vaginal cornification or stimulation of the mammary gland. Even testosterone has been shown directly to elicit vaginal cornification[47, 172] as well as to produce lobule-alveolar growth of the mammary gland[67, 133, 134] when sufficiently large amounts were used. Many substances (e.g., Δ^5 androstene-3,17-diol, ethinyltestosterone, dehydroisoandrosterone) appear to be almost as estrogenic as androgenic in their activity. In male chicks androsterone and dehydroisoandrosterone have strongly feminizing effects;[246] their biological action in the human is uncertain. In view of our clumsy terminology it becomes imperative to specify precisely whenever possible, the various biological actions of each individual substance rather than endeavor to place a substance entirely into one group or another. The nature and intensity of the biological response to a "sex hormone" appear to vary in different species and in different organs and at different ages of a species. Just as there is overlapping of the actions of the biologically active adrenal steroids, there is overlapping of the potentialities of the so-called sex hormones. The duality of action in some instances may be due to the biological conversion in the body of one substance into another;[106] in other instances the duality of effect may reside in an inherent characteristic of the compound.

Factors controlling adrenal cortical and gonadal secretions

The complex factors involved in controlling the formation and secretion of the steroid hormones are only partially understood. Without knowledge or recognition of these factors it is exceedingly difficult, particularly when dealing with clinical conditions in man, to interpret precisely the significance of changes in the excretion of steroids which arise from precursor hormones.

That the anterior pituitary is responsible in some manner for a major portion of the control of the adrenal has long been known. The mechanisms whereby it exerts its effect have been recently only partially and indirectly elucidated. In animals hypophysectomy leads to atrophy and decrease in weight of the adrenal corticies;[108] the atrophy being more marked in the inner than in the outer zones. Also, anterior pituitary insufficiency in man is accompanied by a decrease in adrenal size. Hypophyseal implants or the administration of the adrenocorticotrophic hormone of the pituitary prevents the degenerative changes in the adrenal corticies which otherwise occur in hypophysectomized animals. With increased doses of adrenocorticotrophic hormone gross hypertrophy and hyperplasia of the adrenal cortex result. From this evidence it is apparent that the pituitary influences at least the size and structure of the adrenal cortex. The rôle played by the pituitary in affecting adrenal composition and function has been revealed by more recent data. These later studies have consisted mainly in the measurement of certain phenomena associated with the administration of purified adrenocorticotrophic hormone, or with its activation by other means, in normal and in hypophysectomized animals.

The high concentrations of cholesterol and ascorbic acid in the adrenal cortex have long intrigued the investigator. It has not been directly demonstrated that the cholesterol in the adrenal is transformed to adrenal hormones, nor has the function of the ascorbic acid been clarified. However, when adrenocorticotrophic hormone is administered to certain experimental animals there is a rapid, temporary fall in ascorbic acid content and a slower, temporary fall in cholesterol content of the adrenal without alteration of their concentrations in other tissues.[199, 200] These changes are associated with a rise of liver glycogen and a lymphopenia, both manifestations being considered to be an indication of increased output of certain of the cortical hormones.[56, 199] Similar rapid changes in adrenal cortical concentrations of ascorbic acid and cholesterol (presumably associated with increased secretion of cortical hormones) are observed following the subjection of the animal to a variety of stimuli,[90, 198, 199] e.g., exposure to cold, burns, hemorrhage, sciatic nerve stimulation, and injections of histamine or epinephrine. That this response is mediated through the pituitary is suggested by the fact that the observed changes do not occur in the hypophysectomized animal; in other words, before a stimulus can affect adrenal cortical secretion there must be a preliminary activation of the anterior pituitary to alter its pattern of secretion. The fundamental mechanism involved

in firing the pituitary into activity under such a variety of conditions still remains to be elucidated. A lowering of the level of circulating cortical hormone[198] or activation of the sympathetic nervous system with release of epinephrine,[142] has been suggested as the possible common factor possessed by such stimuli. That similar environmental and possibly nervous influences might affect the gonadotrophic secretions of the pituitary has been indicated, experimentally, in the rabbit, cat, and rat.[10, 65, 66] Certain clinical observations would lead one to believe that analogous mechanisms occur in man.[75] However, it is apparent that the manner in which changes in pituitary secretion are brought about will not be understood until information is available regarding the precise manner in which the adrenal and gonadal hormones are utilized or disposed of in the organism. In any event, at least one effect of environmental change is an altered pituitary secretion with a resultant change in the secretion of the adrenal and possibly other hormones. This fact must be recognized when attempting to interpret the result of hormonal assays in man.

The environmental factors noted above which are known to modify pituitary control of the peripheral endocrine glands do not provide a complete picture of these controlling influences. It appears that the pituitary is very sensitive to various nutritive deficiencies. More is known about the effect of nutrition on the gonadotrophic hormone than on the adrenotrophic hormone. In 1930 Mason and Wolfe[160] showed that the gonadotrophic activity of the pituitaries of female rats was lowered by inanition. Later, Moore and Samuels[167] showed that diets low in thiamin or calories damaged the secondary sex glands and that gonadotrophic hormone was able to repair this damage. Other studies indicate a similar response by the pituitary to a low protein intake. Again gonadotrophic hormone prevented the deleterious effects.[82] That the adrenotrophic hormone is also affected by inanition is suggested by the extensive studies of Mulinos and Pomerantz.[169, 170] It is important to realize that the noted dietary deficiencies did not impair the responsiveness of tissues to stimulating steroid hormones, rather the deficiencies appeared to cause a quantitative reduction in endogenous formation of hormone by the pituitary. An evaluation of the significance of the excretion of hormones in man must, therefore, be considered also in the light of past or present nutritional deficiencies.

Another factor which may govern the activity of any one endocrine gland is the functional status of the others. The literature on this

subject is enormous and reference to this aspect of the problem is included only in an endeavor to complete the picture. Sevringhaus[206] gives a complete survey of data up to 1939 regarding the various cellular changes in the anterior pituitary that occur associated with castration, pregnancy, hyperthyroidism, hypothyroidism, adrenal cortical insufficiency or hyperfunction, and with the administration of androgens and estrogens. Swann[212] has discussed the pituitary-adrenocortical, and Parkes[180] the adrenal-gonadal relationships. Moore[165, 166] and Koch[126] have reviewed the various biological interrelationships concerned with testicular and androgen function, and Allen, Hisaw, and Gardner[7] have discussed the ovary and its hormones in this regard. More recent reviews should also be referred to, particularly those of Fevold[64] and Levin[136] on the gonadotrophins, Long[141] on the metabolic hormones of the anterior pituitary, Marx and Evans[150] on the growth hormone, Ingle[109] on the adrenal cortical hormones, and Lukens[145] on the action of insulin. The close functional relationship between the various members of the endocrine system is clear. At times, however, it is exceedingly difficult to determine whether an observed effect of a hormone is a direct result of its action or whether the effect is brought about in an indirect manner by altering the secretion of other glands. Further, when changes in an endocrine gland occur (e.g., degenerative, hyperplastic, or other) after the administration of a hormone, or even another substance, it is important to know whether the effect is a direct one on the gland itself, an indirect one mediated through another gland, or whether the changes are the result of a secondary response to an extra-endocrine mechanism.

Factors which may condition the response to hormones

When one is confronted with a patient with an endocrinological disorder one naturally would like to know which hormones are being produced in abnormal quantities. One also asks to what extent the clinical picture can be explained on such a quantitative basis. When one endeavors to do so, it soon becomes obvious that in clinical as well as in experimental endocrinology there are certain factors which will modify and condition the response to a hormone or its transformation product. Reference to some of these determinants must be made.

Certain of the problems met with in this phase of medicine involve such complex and poorly understood mechanisms as sex determination, intersexuality, sex differentiation, and maturation phenomena, all of which must be evaluated in any individual case. The factors of sex

determination (the sex of the gametes) appear to be mainly, if not entirely, chromosomal (genetic) in nature and are subject to the usual laws of Mendelian inheritance. The cells of the developing embryo are at least qualitatively different in the two sexes and this original difference subsequently must give rise to the ultimate difference between the sexes. Sex differentiation (sex in bodily structure) appears to be adaptive in nature and is a complicated process. It appears that each sex combines potentialities of both the male and the female type, and the rudiments of the secondary sex characters, male and female in type, are laid down during the development of every individual. Whether the major expression is in one direction, or another may depend on a hormonal level, on an inherited chromosomal level, or on both. It is generally believed that in vertebrates and mammals, the mechanism of sex differentiation is largely taken over by extracellular agents, i.e., the male and female sex hormones, since the first indication of sexual differentiation is in the gonads. This concept grants that the nature of the endocrine cells producing the hormones is first determined by inheritance. Thus, the development of the differences between the sexes would be based on differences in hormone elaboration and the soma indeed could be considered sexually neutral or indifferent. Masculinization and feminization of sex characters could occur with equal ease depending only on hormonal activity. In other words the primordium of any pair of sex characters would be responsive to both male and female hormones in opposite ways.[248] In fish and amphibia the neutral or indifferent phase of the soma appears to persist until puberty. In mammals, however, this indifferent period is very short and sex differentiation is partially achieved by the time of birth. At this time the whole genital tract is differentiated. Moore[166] has raised serious objections to the concept that sex hormones of the developing gonads play an important rôle in the differentiation of the embryonic, reproductive duct system, although he does not deny their probable importance during later development. However, after a critical review of available data he is led to conclude that "the most acceptable evidence for the control of sex differentiation in vertebrates rests upon the conception of the operation of genetic sex differentiating factors unconnected with sex hormone actions." In any event, in man from birth until puberty there is a very gradual, further differentiation, especially as regards body growth, mammary development, and behavior. At puberty voice changes, hair growth, and gonadal activity complete the differentiation. It therefore becomes clear

that when a major developmental anomaly of the genital tract is observed in man the defect and its cause must have originated before birth. Also, when abnormal hormonal influences are developed experimentally or spontaneously in a patient, after birth but before puberty, those characters of sex that develop later (e.g., hair, voice, growth, gonadal function) will be more profoundly affected than those which develop earlier. In fact, by the time of birth certain of the primordia of the heterologous sex have so nearly disappeared that their further development may be impossible, or at least not apparent. Thus the maturity of the individual, as well as the genetic sex, is an extremely important factor in conditioning the response to hormonal influences. The importance of this concept is clear when one endeavors to account for the clinical pictures produced by "masculinizing" tumors of the adrenal cortex in boys, girls, men, and women. Thus, an abnormal masculinizing influence would be apparent in children or women, since at least certain of the male rudiments (hair, voice, penis, or clitoris) are able to react positively to an androgenic effect. However, a similar influence would not be apparent in previously normal males whose masculine characters are already fully developed. An increase in estrogenic influences from any cause would not be readily recognizable in the soma of the previously normal woman, while the effects, if sufficiently great, might be noted in men or children. Even with entirely similar hormonal influences one could not expect to see identical clinical phenomena in individuals of different age and sex.

The importance of sex hormones in governing the development of sex character and function cannot be denied, in fact an entire book has been mainly devoted to this problem.[6] From the standpoint of hormonal control, an organism becomes male in type under the influence of androgenic hormones and female under influence of estrogenic hormones; each primordium would be responsive to both hormones in opposite ways and the degree and nature of the response could be closely correlated with the quantities and nature of hormone elaborated. If this were the whole picture of sexual differentiation in all its subtle aspects, experimental and clinical problems would be relatively simple. Unfortunately, such a "hormone balance" theory, though attractive, leaves much to be desired.[166] There is, however, a broader and probably more truly correct explanation whereby hormones influence the differentiation of sex characters in mammals.

From a variety of clinical and laboratory evidence one must conclude

that certain of the sex primordia are mainly responsive to androgenic influences and others mainly to estrogenic influences. Hence, the male or female form of any pair of sex characters would be a "conditioned" character and the contrasting character would be "unconditioned" or relatively neutral. For example, the penis of the genetic male responds positively and easily to androgenic hormone while the clitoris of the female, being an "unconditioned" character, merely responds as a generally neutral or indifferent character. Normally, the unconditioned sex character is a rudimentary structure and the organs of each sex react to the male or female hormone in their own special way. Thus, one would conclude that inherited, nuclear characteristics of the soma and of the secondary sex characters would influence responses to hormones. In other words, in order to interpret a response one must consider the biological soil on which the hormones act. Further elaboration on this point is necessary since it is likely that the "hormone balance" theory of sexual differentiation may be an oversimplification. The following bits of evidence might be cited. If the excretion of androgenic and estrogenic hormones in urine can be considered as a rough quantitative measure of endogenous elaboration of these hormones, the fact that no significant difference in their excretion can be demonstrated in young boys and girls, in spite of partial sex differentiation, would indicate a fundamental, genetic difference in their response to elaborated hormones. Further, in entirely normal adult men and women, there is great overlapping in estrogen, androgen, and 17-ketosteroid output by individual members of both groups. Admittedly, adult males, on an average, excrete more androgen and less estrogen than do the majority of females, but considered individually, the differences are not usually distinctive of either sex. Experimentally, as pointed out by Moore,[165] "the Mullerian ducts of males and females at the same period of development exhibit entirely different capacities to respond to one and the same substance." These structures must, therefore, have been previously conditioned in some, probably genetic, manner.

Not only will the genetic sex of an organism influence a response, but the same sex in different species or in different strains of the same species may exhibit different responses to the same hormonal influence. In this regard the fact that the male American Indian lacks a beard depends upon a racial characteristic and not on a lack of androgenic hormone. Further, many cases of hirsutism in post-pubertal women appear to be on a hereditary or familial basis, since no deviation from normal is apparent either in hormone excretion or in gonadal or other

endocrine function. In these cases the offending end-organ would seem to be unusually sensitive to masculinizing influences. Danforth has admirably discussed the variations in response to natural and synthetic hormones exhibited by different breeds and species of fowl under a variety of circumstances.[44] From the above remarks, it is evident that an inherited framework on which the hormones act determines the nature of the reaction. However, the extent to which a hormonal influence or an inherited trait may each contribute to the development of the ultimate response is not understood for most reactions. Of necessity both determinants are functioning simultaneously and the line between the action of one and the action of the other is not sharp. Also, it is not necessary to consider an inherited characteristic of a tissue as an immutable state. It is highly probable that when the various cellular functions are better understood certain "inherited" traits or so-called "inborn errors of metabolism" will prove to be alterable.

Finally, one is confronted with the difficult problem of interpreting the effect of hormones in terms of the chemistry of cells. Precisely how do hormones bring about their effects? A ready answer is not available. As has been pointed out,[141] hormones have not been shown to initiate or cause reactions new to a tissue; rather, they appear to affect the rates of certain of those chemical processes already characteristic of that tissue. A good deal is known about the end results of hormone deficiencies and of the overall effects of hormone administration, but little is known of the chemical reactions involved in bringing about the final result. Some of the major actions of the steroid hormones of the adrenal cortex and gonads have been pointed out. Because of the nature of these effects it would seem obvious that all nutritive and other elements essential to the particular reaction need be present and available, whether new tissue is being formed or the constitution of tissue is being otherwise altered. The fact that the response to stilbesterol is impaired in a chick deficient in folic acid[95] might be cited as a single example of a nutritional deficiency leading to a loss of responsiveness on the part of a tissue. However, very little is known regarding those cellular systems which involve the interplay of steroid and protein hormones, vitamins, enzymes, and foodstuffs. Preliminary work along these lines is referred to in recent literature.[96]

Biological transformations of steroid hormones

The biological transformations that steroid hormones undergo have

been studied in four general ways. First, crystalline steroids have been incubated with certain tissues (e.g., liver slices, bacteria, etc.) and the crystalline transformation products isolated. Second, the ability of certain tissues to alter the biological activity of a substance or an extract has been investigated. Third, a steroid may be labeled with an isotope, administered to man or animal, and an excretion product isolated and tested for isotopic activity. Finally, crystalline steroids have been given to man and animals and the nature of the excretory products in urine determined. Although the first three methods are in the preliminary stages of development, certain data deserve mention.

Owing to scarcity of crystalline material it has been impossible to apply precise isolation procedures to an investigation of the transformations of the physiologically active adrenal steroids, e.g., corticosterone. However, using methods of biological assay Vogt[236] has shown that the adrenal veins of dogs contain large quantities of physiologically active material which rapidly disappear during circulation. Since the active hormones are relatively stable in blood and since their excretion by the kidney is small,[185] these substances must have been rapidly inactivated or utilized by the tissues. Further, Vogt[236] concluded from indirect evidence that the liver did not play a predominant rôle in this inactivation. Precise knowledge of the fate of the adrenal steroids is clearly lacking.

It has been shown by implantation experiments in rats that estrogens and testosterone are inactivated by the liver.[17] However, when rats are given a diet deficient in vitamin B complex, testosterone but not estrogen is inactivated.[15, 16] More specific studies would indicate that thiamin, riboflavin, and methionine are necessary for the inactivation of estrogen.[83, 204] How specific these particular substances might be for this reaction is unsettled, since Drill and Pfeiffer[57] conclude that "deficiency of the whole vitamin B complex affects the inactivation of estrogen only through the concomitant inanition produced." Burrill and Green[25] have presented evidence that "androgen" derived from the adrenal is not inactivated by passage through the liver, while "gonadal androgen" appears to be inactivated. The above studies on the rôle played by the liver in these transformations have been carried out in the rat. Since steroid hormones appear to be handled differently by different species of animals, it would seem premature to conclude that entirely similar mechanisms of transformation are present in man.

Using a colorimetric method for determining the presence or absence of testosterone, Samuels et al.[197] showed that livers of rats, mice,

rabbits, and man destroy testosterone, while rabbit uterus and rat prostate do not. The process of inactivation appeared to be an enzymatic, oxidative reaction and did not seem to involve conjugation with other substances. Although the transformation or degradation products were not chemically identified, it was concluded that, at least in the animals tested, the final products were not 17-ketosteroids, Δ^4 androstene-3, 17-dione or the unnatural compound, *cis*-testosterone. However, by isolation and characterization of the transformation products Clark and Kochakian[39] showed that testosterone is metabolized by rabbit liver slices in part to Δ^4 androstene-3,17-dione, *cis*-testosterone, and other partially identified steroids. The fact that none of the urinary steroids (e.g., androsterone or etiocholan-3(α)-ol-17-one) were found might indicate that tissues other than liver may be involved in the formation of urinary steroids from testosterone.

The isotopic labeling of steroid and other molecules has provided interesting and valuable information regarding the origin of steroid substances. For example, Bloch and Rittenberg[21] demonstrated that cholesterol may be formed from small carbon fragments, i.e., from acetate; further, that cholesterol may be degraded in the organism to bile acids.[20] Later Bloch[19] showed that pregnane-3(α),20(α)-diol, a metabolite of progesterone, was derived, at least in part, from exogenous cholesterol during pregnancy. This latter study provided the first direct demonstration that a hormonal product could be derived from cholesterol.

Certain crystalline steroids, more readily available than the active adrenal steroids, have been administered to man and the nature of the excretory products in urine determined. These substances have been given to individuals suffering from anterior pituitary, adrenal cortical, or testicular deficiency inasmuch as the endogenous elaboration of steroids in such cases should be small. This method is particularly valuable since it is applicable directly to man. However, because of technical difficulties inherent in present isolation methods its value has been mainly qualitative. An accurate evaluation of the quantitative conversion of an injected compound to a transformation product must wait further investigations. In Fig. 4 are summarized various transformations known to have occurred in man. Although these transformations occur to a certain limited extent, it must be admitted that the major portion of the injected material does not appear in urine as the usual, known urinary steroids. Apparently this major portion is metabolized and disposed of in some other, unknown manner. From

Known Conversions of Neutral Steroids in Man

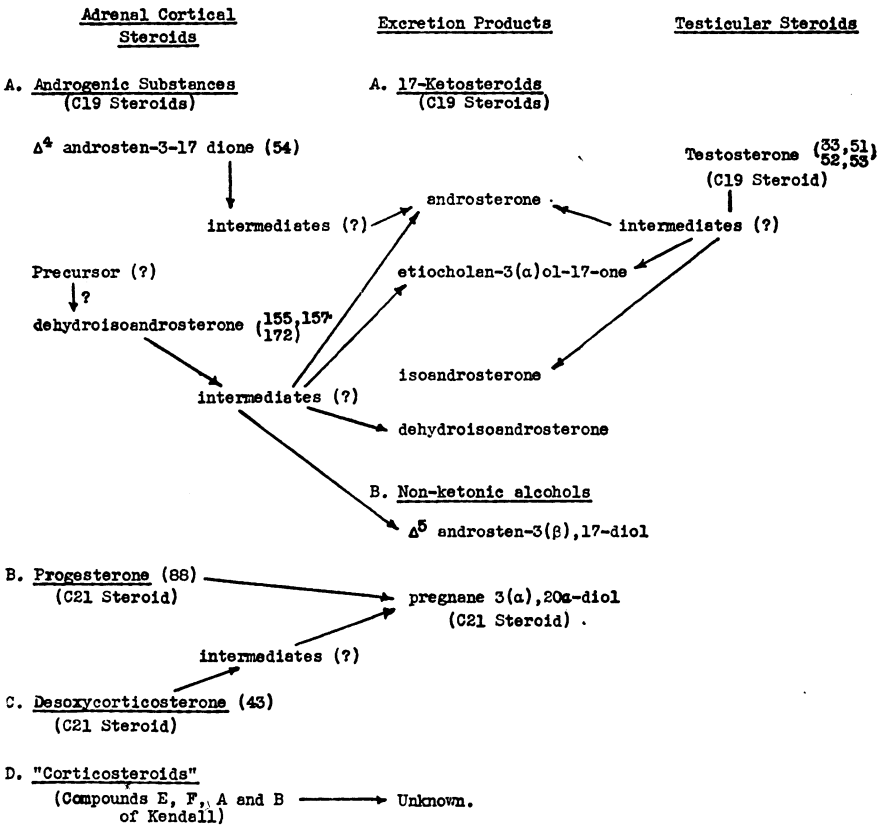


FIG. 4

the data it is clear that the testicular hormone, testosterone (free or the propionate), gives rise to certain of the urinary 17-ketosteroids; mainly the androgen, androsterone, and the biologically inactive compound etiocholan-3(α)-ol-17-one. However, 17-methyl testosterone, a synthetic androgen, is not degraded to 17-keto compounds apparently because the methyl group interferes with the oxidation of the hydroxyl group at carbon 17. From many observations,[38, 73] it is apparent that at least two-thirds of the total 17-ketosteroids which appear in normal male urine and probably all the 17-ketosteroids that appear in female urine are derived from the adrenal cortex. It is clear also that androsterone and etiocholan-3(α)-ol-17-one are the two 17-ketosteroids found in

greatest amounts in normal urine.[33, 34, 35, 48] Both are found in approximately equal quantities and together account for at least 70 per cent of the total.[48] Both are derived from steroids elaborated by the male gonad and the adrenal cortex. Undoubtedly testosterone is a precursor in the male. Although the nature of the adrenal precursors remains obscure, the various possibilities must be considered.

A re-examination of Table 1 wherein the steroids isolated from beef adrenal are listed, reveals that compounds I-IV are 11-oxygenated pregnene derivatives, compounds V and VI are 11-desoxy pregnenes, compounds VIII and IX are androgenic androstenes, and compound X is an androgenic, 17-hydroxyl-pregnene. Thus far there is no convincing evidence that compounds I to VI, the steroids which affect electrolyte and carbohydrate metabolism, provide a major immediate source of the urinary 17-ketosteroids in man. The transformation of these pregnenes to 17-ketosteroids would involve the oxidative removal of the 2-carbon side chain at C-17. The in vitro, chemical removal of this side chain occurs most easily in compounds with a hydroxyl group at C-17 (e.g., compounds I, II, and X). By removal of the 2-carbon alkyl group and reduction at C-11, it is conceivable that adrenal steroids with an oxygen function at C-11 could be metabolized and excreted as the more common 17-ketosteroids. However, the ease whereby biological reduction occurs at C-11 remains unknown. Small amounts of urinary 17-ketosteroids[139, 152, 153, 163] have been isolated which are oxygenated or unsaturated at carbon-11. These substances may have originated from C21 adrenal steroids provided oxygenation of C19 compounds never occurs at carbon-11. The urinary 17-ketosteroids (C19 compounds) which normally are found in greatest amounts do not possess an 11-oxy function. Certain clinical and laboratory observations are pertinent in this regard. In many cases of Cushing's syndrome, not due to adrenal neoplasm, there are evidences of adrenal cortical hyperfunction and of excessive amounts of the "glycogenic corticosteroids" in urine[216, 232] and yet, the total 17-ketosteroid excretion remains within normal limits. Venning has shown that the excretion of "glycogenic corticosteroids"[230] and of pregnane-3(α),20(α)-diol[230] increases progressively during pregnancy. No increase or change in 17-ketosteroid excretion was observed. Also, in cases of pre-pubertal and post-pubertal virilism (not associated with adrenal neoplasm) the 17-ketosteroid excretion may be greatly increased (see below) while the "glycogenic corticosteroid" excretion remains normal[216, 232] and no abnormalities of carbohydrate or salt metabolism are apparent. Mason[154] administered 200 mg. of

11-dehydrocorticosterone (compound A) to three patients with Addison's disease and was unable to detect a rise in 17-ketosteroid excretion with the amounts used. Also patients exposed to chronic stresses (burns, operations) excrete increased amounts of "corticosteroids" while they excrete decreased amounts of 17-ketosteroids.[68, 234] In other words, there appears to be no uniform parallelism or necessary linkage between "corticosteroid" excretion (perhaps a measure of glycogenic and other cortical activities) and 17-ketosteroid excretion (perhaps a measure of androgenic activity). Either these different cortical activities are regulated by different factors or the tissues of the organism do not metabolize or otherwise dispose of the precursor compounds in similar manner under all circumstances. The former concept does not exclude the possibility that the "androgenic" and "corticosteroid" hormones arise from the same parent substance or substances. The fact that purified adrenocorticotropic hormone caused a rise in both 17-ketosteroid and "corticosteroid" excretion in a normal individual[159] and in a person with anterior pituitary failure[227] would favor the latter possibility. The problem is a difficult one and will not be settled until the mechanisms of utilization and transformation of steroid substances by human tissues are better understood.

Since there is no substantial evidence as yet that the adrenal steroids which influence salt water, carbohydrate, and protein metabolism are the immediate precursors of the usual urinary 17-ketosteroids, it is possible that these urinary substances are derived from the androgenic (compounds VII-X, Table 1) adrenal steroids or closely related compounds. In Figure 4 dehydroisoandrosterone (a C19, 3 -beta-hydroxy steroid, unsaturated—as is cholesterol at carbon-5) was placed in such a manner as to indicate that it originated in the adrenal cortex in spite of the fact that it has not been isolated from beef or hog adrenal. However, there can be no reasonable doubt that this compound or more surely its precursor is formed in the human adrenal cortex. Dehydroisoandrosterone may be excreted in enormous quantities in the urine of patients suffering from adrenal cortical tumors;[42] normal males and females excrete small but equal amounts;[35] and eunuchs excrete greater amounts than normal males.[36] Further, dehydroisoandrosterone has not been shown to be a metabolite of testosterone. Normally, dehydroisoandrosterone accounts for about 5 per cent of the total 17-ketosteroid excretion. It is interesting to note that Mason and Kepler[155, 157] have shown that injected dehydroisoandrosterone can give rise to considerable quantities of androsterone and etiocholan-3(α)-ol-17-one. Part of the

dehydroisoandrosterone is excreted unchanged. Since this androgen or more certainly its precursor arises in the human adrenal and since this gland is an important site of androgen elaboration, this observation may provide a clue as to the source of the commonest urinary 17-ketosteroids, namely, androsterone, etiocholan-3(α)-ol-17-one, and dehydroisoandrosterone. These investigations are important also because they indicate that a 3-beta-hydroxy steroid with weakly androgenic and estrogenic properties can give rise to two 3-alpha-hydroxy steroids, one with stronger androgenic properties than the injected material and one biologically inactive. Hence, it is apparent that the biological activity of an excretory product may not invariably reflect the biological activity of its precursor.

It is apparent in Fig. 4 that progesterone and to a lesser extent, desoxycorticosterone are converted to pregnane-3(α),20(α)-diol. This excretory product appears to be the chief end-product of progesterone metabolism and its excretion seems to reflect, in a general manner, the extent of progestational activity. It would appear that, under these circumstances at least, the C21 pregnenes are more easily transformed to C21 pregnanes than to C19 steroids; the removal of the side chain to form a 17-ketosteroid did not readily occur.

These studies in man have provided valuable information which is necessary to an understanding of the significance of the end products of steroid metabolism. However, they do not indicate whether the transformations are mainly related to utilization or to inactivation of hormones. Unfortunately no studies in man are available that might indicate the manner in which a disease might influence these transformations. For example, it is not known whether a low level of steroid excretion invariably indicates a depressed phase of adrenal or gonadal function or whether the decreased value indicates an altered rate of transformation in other tissues of normally elaborated steroids. Similar questions might be raised regarding high levels of excretion. Hence, certain reservations must be kept in mind before levels of excretion can be accepted as absolute measures of the extent of endogenous steroid formation.

The nature, state, and source of the various groups of steroid substances excreted in urine

In Table 3 are outlined the nature and source of the different groups of steroid substances found in human urine. Table 4 summarizes the

principles of the methods used for their individual determination. Since the neutral and phenolic steroids are excreted in conjugated water-soluble form, it is necessary to hydrolyze the urine before extraction with organic solvents. However, pregnane-3(α),20(α)-diol is more frequently determined by direct precipitation and isolation of the conjugate. As has been repeatedly emphasized by workers in this field[77] there is no general method of hydrolysis which will, under all conditions, cleave all of the conjugates without any alteration of the steroid molecule. The urinary steroids are conjugated with several substances; they appear mainly as the sodium salts of glycuronic and sulfuric acids. The individual conjugates differ in their stability. For example, sodium pregnanediol glycuronide is easily hydrolyzed without apparent alteration or destruction of the steroid. However, hydrolysis of sodium androsterone sulfate and sodium dehydroisoandrosterone sulfate has led to varying degrees of alteration and destruction of the steroids. Hydrolysis of androsterone sulfate caused the regeneration of the natural 17-ketosteroid androsterone, as well as the formation of Δ^2 or Δ^3 androsten-17-one, an unsaturated, non-alcoholic 17-ketosteroid.[233] The latter product represents an artifact derived by dehydration of the

Table 3

GENERAL GROUPS OF STEROID SUBSTANCES EXCRETED IN URINE

	Probable origin	
Group	*Gland*	*Major chemical precursors*
1. The 17-ketosteroids (conjugated) (composed of saturated and unsaturated 17-keto-alcohols or non-alcohols)	Testicle Adrenal cortex Ovary (doubtful)	Testosterone Dehydroisoandrosterone (?) Δ^4 Androstene-3-17-dione Others but unknown
2. Pregnanediol (conjugated) (pregnane-3(α),20(α)-diol)	Corpus luteum Placenta Adrenal cortex	Progesterone Desoxycorticosterone Others (?)
3. "Corticosteroids" (unconjugated ?) (Chemically unidentified. Known for its biological activity)	Adrenal cortex	11-oxy adrenal steroids Others (?)
4. A group of non-ketonic alcohols and closely related steroids	Adrenal cortex	Unknown
5. Estrogens (conjugated)	Ovary Adrenal cortex Placenta Testicle	Estrogens Others (?)

hydroxyl compound during the hydrolytic procedure. Several other similar artifacts are known. Although not true excretory products, their presence is not without significance, since they are artificial derivatives of natural substances whose nature is usually known. Nevertheless, it is clear that to interpret the significance of total or individual ketosteroid excretion conditions of hydrolysis must be accurately controlled.

After preliminary hydrolysis and partial purification the neutral "17-ketosteroid fraction" may be assayed biologically for its androgenic activity, or colorimetrically. The m-dinitrobenzene method of Zimmerman[250] or the antimony trichloride method of Pincus[186] may be applied to crude extracts if proper precautions are made[65, 174] or these methods may be applied to the more purified ketonic fraction.[221] Since patients with adrenal cortical tumors frequently excrete large amounts of dehydroisoandrosterone (a 3-beta-hydroxy ketosteroid), separation of the partially purified crude extract into alpha and beta fractions by digitonin precipitation may be carried out.[71, 219] Each fraction then may be assayed by the Zimmerman reaction. Since the antimony trichloride reagent reacts poorly with dehydroisoandrosterone and other unsaturated 17-ketosteroids, it may not be as generally useful as the Zimmerman method, properly employed. Although colorimetric assays are easier to perform, it cannot be assumed categorically that present general chemical methods yield more precise information than do biological methods; each may have its particular virtue depending on the nature of the problem. Biological assays, while reflecting the androgenic activity of the

Table 4

GENERAL OUTLINE FOR THE PREPARATION OF URINARY EXTRACTS FOR ASSAY OF STEROID CONTENT

1. The 17-ketosteroids: Hydrolize the urine with inorganic acid; extract with organic solvent; wash with alkali to remove phenolic substances (estrogens). Dried residue taken up in ethanol and assayed:
   (a) Biologically for androgenic activity and/or
   (b) Colorimetrically by:
      1) Zimmerman reaction: saturated and unsaturated 17-ketosteroids react with m-dinitrobenzene in alkali to form a red color.
      2) Pincus reaction: saturated 17-ketosteroids react with antimony trichloride to yield a blue color.
   (c) References for details (38, 61, 71, 73, 104, 174, 186, 219, 221, 250).

2. Pregnanediol: Extract the conjugated pregnanediol from urine with butyl alcohol and precipitate the conjugate with acetone. The precipitate is

purified and dried. The weight of the precipitate (sodium pregnanediol glucuronide) converted by means of a factor into an equivalent weight of free pregnanediol.

(a) References for details (11,229)

3. Corticosteroids: Extract unhydrolized acidified urine with ethylene dichloride, chloroform or similar solvent; wash with alkali to remove acidic substances; this crude fraction may be separated into ketonic and non-ketonic fractions with Girard's reagent T. The crude fraction or the ketonic fraction assayed:
   (a) Biologically, for ability to protect the adrenalectomized animal against certain stresses ("cold" test, "work" test, "glycogen" test during fasting, etc.).
   (b) Colorimetrically, by reducing or other characteristics.
   (c) References for details (58, 93, 110, 144, 223, 235).
4. Non-ketonic alcohols and related steroids: Identified only by isolation and characterization of the crystalline compounds.

extract, do not detect inactive compounds derived from active androgens. For example, etiocholan-3(α)-ol-17-one, a known metabolite of the androgens testosterone and dehydroisoandrosterone, has no androgenic activity and its presence would not be detected by biological assay. However, it would be detected by colorimetric methods. In this respect chemical assays are distinctly superior since the 17-keto group is quite resistant to vigorous hydrolysis and survives metabolic changes in the rest of the molecule. Although all the urinary steroids with androgenic activity thus far studied, appear to have a 17-keto group, it cannot be assumed that urinary androgens may not exist without this grouping. There may be conditions where biological assay would be more informative than an assay of total 17-ketosteroids; however, neither general method can be expected to yield the precise information afforded by the actual isolation of the individual steroids.

Although pregnane-3(α),20(α)-diol belongs in the general group of non-ketonic alcohols, it was listed in Tables 3 and 4 in a separate category. This compound was first isolated[149] from human pregnancy urine in 1929 and its relationship to progesterone later established. Butenandt was the first to suspect that pregnanediol arose from the in vivo reduction of progesterone. It was found that pregnanediol was not excreted as the free steroid but as a water-soluble, biologically inactive, easily hydrolyzed glycuronide.[178] In spite of some early contradictory reports it is now apparent that up to 46 per cent of injected progesterone can be recovered as pregnanediol.[88] The level of

its excretion appears to be related mainly to the extent of endogenous progesterone elaboration.[231] However, it is possible that pregnenes other than progesterone may be converted to pregnanediol.[43] Progesterone has been isolated from the corpus luteum[8, 30] and small amounts have been found in the adrenal cortex.[189] The elaboration of progesterone by the placenta has been suspected.[114] Normal males, and females in the early phases of the menstrual cycle, excrete very small amounts of pregnanediol. Larger amounts are excreted by women in the luteal phase of the menstrual cycle[231] and very large amounts are encountered in the latter half of pregnancy. Also, occasional cases of adrenal cortical tumor and of adrenal hyperplasia excrete increased amounts of pregnanediol.[156] In these cases it is possible that adrenal cortical pregnenes other than progesterone act as precursors of pregnanediol.

The general principles of the Venning[229] method for the determination of pregnanediol as the glycuronide are given in Table 4. It should be mentioned that since the water-soluble complex is easily hydrolyzed precautions are necessary to prevent the hydrolysis of the conjugate.

Certain extracts of urine appear to have biological actions similar to the 11-oxygenated adrenal steroids. This fraction, for want of a better term, has been designated as the "corticosteroid" fraction since it resembles in its activity those adrenal compounds which affect carbohydrate metabolism, resistance to stress, and life maintenance. Studies on this fraction are still in the preliminary stages of development. The chemical identity of the substances contained in these extracts has not been determined; nor is it known with certainty that they are excreted as free steroids. However, there can be little doubt that the active substances are steroids. It is likely that at least some of the compounds present in this fraction possess an α,β unsaturated ketone in ring A, an oxygenated carbon at position 11, and an α-ketol side chain at C-17. So far as is known, these features appear necessary for glycogen deposition. The α,β unsaturated ketone group and the primary or secondary alpha-ketol group possess reducing characteristics. On the basis of this feature a colorimetric method has been devised for the assay of this fraction.[93, 223] Another chemical method involves the periodate oxidation of the C-17 side chain to yield formaldehyde.[144] Although it would seem rather unique to develop chemical methods for the determination of substances not yet identified, preliminary studies would indicate a fairly good parallelism between the results obtained by "chemical" assay and by biological assay.[93] The colorimetric methods, however, uniformly yield much higher values than do biological methods. Urine undoubtedly

contains biologically inactive pregnenes.

As previously noted the level of the 17-ketosteroid excretion may provide a measure of the androgenic cortical activities but does not necessarily reflect cortical activities concerned with carbohydrate and protein metabolism. It would seem that a measure of the "corticosteroid" fraction would be a better measure of these latter functions. Support for the validity of this interpretation has been gained mainly by rather indirect observations. Certain biochemical and histologic responses indicative of cortical hyperactivity occur in experimental animals[90, 199, 205] when exposed to certain stimuli (see earlier discussion). The fact that human subjects, exposed to similar stresses, excrete increased amounts of "corticosteroids" is generally interpreted to mean an increase in adrenal activity.[205, 234] Also, patients with Cushing's syndrome excrete large amounts, and patients with Addison's disease small amounts of these substances.[218, 232] More direct evidence became available when it was shown that the administration of the adrenocorticotrophic hormone of the anterior pituitary[159, 227] led to an increase in the titer of this fraction.

Except for pregnane-3(α),20(α)-diol little is known of the qualitative and quantitative significance of the conjugated, non-ketonic, alcoholic steroids (group 4, Tables 3 and 4). They probably arise, in the main, from steroids elaborated in the adrenal, and represent excretion products in various stages of reduction and dehydration. The majority of these compounds have been isolated from the urine of patients suffereing from adrenal hyperactivity. The compounds have been identified only by direct isolation; as yet, no chemical method has been developed for their assay. For a detailed discussion of this group of substances the reader is referred to articles by Mason and Kepler.[156] Hirschmann,[102] and Pincus and Pearman.[187]

Any significant reference to the phenolic estrogens is prohibitive in this review. They are included in Tables 3 and 4 merely to complete the picture of the steroid substances found in human urine. Suffice it to state that the estrogens are excreted as conjugates and are weakly acidic (phenolic) in character. They apparently arise from the ovary, the adrenal cortex, the placenta, or testicle, and appear in urine as estrone, estriol, or estradiol. The intermediary metabolism of the estrogens is hardly better understood than is that of the androgens.

Table 5

NEUTRAL 17-KETOSTEROIDS AND CLOSELY RELATED NON-KETONIC ANDROSTANES AND ETIOCHOLANES WHICH HAVE BEEN ISOLATED FROM NORMAL AND PATHOLOGICAL HUMAN URINES (C19 STEROIDS)

A. Ketonic: Alcoholic	
1. 3-Alpha (α), 17-ketosteroids (digitonin non-precipitable)	
(a) androsterone (androstan-3(α)-ol-17-one)	(a) Possesses 1/10 the androgenic activity of testosterone. Isolated from urine of normal[28] and castrate men,[36] normal[34] and castrate women,[99] girl[249] and women[156] with adrenal tumor, pregnant women,[147] girls with adrenal hyperplasia[156] and a man with an interstitial cell testicular tumor.[233] Demonstrated to be a metabolite of testosterone and (g). Source of precursor: adrenal cortex and testicle.
(b) etiocholan-3(α)-ol-17-one	(b) Possesses no androgenic activity. Is an isomer of (a). Isolated from urine of normal and castrate men,[36] normal women,[35] girls and women with adrenal tumor[156] and hyperplasia.[156] Demonstrated to be a metabolite of testosterone and (g). Source of precursor: adrenal cortex and testicle.
(c) Δ^{11} androsten-3(α)-ol-17-one	(c) Possess ¼ the androgenic activity of androsterone.[55] Isolated from urine of girl with adrenal tumor[249] and girl with "virilism."[55] May be the dehydration product of (d).
(d) androstane-3(α), 11-diol-17-one	(d) Possesses ¼ the androgenic activity of androsterone.[163] Isolated from urine of girls and women with adrenal tumor or hyperplasia[156] and from urine of normal men.[153] Also, from girl with "virilism."[163] Likely source of precursor: adrenal cortex.
(e) etiocholan-3(α)-ol-11, 17-dione	(e) Biological action unknown. Isolated from urine of normal men and women;[139] also isolated from urine of certain patients with breast and prostatic carcinoma, with lymphatic leukemia, with "adrenogenital" and Cushing's syndrome.[139] Probably represents a normal metabolic product of one or more of the adrenal steroids.
(f) $\Delta^{9:11}$ etiocholen-3(α)-ol-17-one	(f) Biological action unknown. May be a dehydration product of the hypothetical substance, etiocholane-3(α),11(β)-diol-17-one. Isolated from urine of "a patient with breast

	carcinoma, certain patients with prostatic carcinoma, lymphatic leukemia, essential hypertension, and Cushing's syndrome;" also from two persons, 76 and 72 years of age, without known disease. Has not been detected by the same investigators in individual or pooled collections of urine from normal, younger men and women.[49] The significance of this finding remains to be determined.
2. 3-Beta (β), 17-ketosteroids (digitonin precipitable)	
(g) dehydroisoandrosterone (Δ^5 androsten-3(β)-ol-17-one)	(g) Possesses 1/3 the androgenic activity of androsterone. Castrate men excrete amounts greater than normal men,[36] excreted by normal[34] or castrate women,[100] marked excess usually excreted in adrenal tumor[42] and slight excess in adrenal hyperplasia.[42] Has never been demonstrated to be a metabolite of testosterone. Source undoubtedly the adrenal cortex alone but unknown whether it arises as such, or from some precursor.
(h) isoandrosterone (etiocholan-3(β)-ol-17-one)	(h) A weak androgen. An isomer of (a). Isolated in small amounts from urine of normal women[183] and women with adrenal hyperplasia.[31] Demonstrated to be a minor metabolite of testosterone in man.[52] The compound has not been frequently isolated and, when found, has been present in small amounts.
B. Ketonic:Non-alcoholic (Probably all artifacts derived during acid hydrolysis)	
(i) $\Delta^{3,5}$ androstandien-17-one	(i) A weak androgen. Isolated from urine of "normals,"[48] ovariectomized women,[99] man[27] and girl[249] with adrenal tumor, and women with adrenal hyperplasia.[48] Undoubtedly a dehydration product of (g).
(j) Δ^2 or Δ^3 androsten-17-one	(j) Isolated from urine of ovariectomized women,[100] normal men,[48] and women with adrenal hyperplasia.[48] Shown[233] to be a dehydration product of (a).
(k) 3-chloro Δ^5 androsten-17-one	(k) Isolated from various urines by many investigators.[34] Arises during hydrolysis of (g) with hydrochloric acid.
C. Non-ketonic:Alcoholic	
(l) Δ^5 androstene-3(β),16,17-triol	(l) Isolated from urine of a boy with adrenal carcinoma and liver metastases[101] and

(Table 5 Cont.)

	a women[156] with adrenal tumor. May be a metabolite of dehydroisoandrosterone but, if so, it is remarkable that it has been so rarely isolated.
(*m*) Δ^5 androstene-3(β),17(α)-diol	(*m*) Isolated from urine of women[156, 201] and a boy[102] with adrenal tumor. Shown to be a metabolite of dehydroisoandrosterone in a male with anterior pituitary insufficiency.[155]
(*n*) Etiocholane-3(α),17-diol	(*n*) Isolated from urine of normal men.[29]

Chemical nature of the individual steroids isolated from normal and abnormal urine

In Tables 5, 6, and 7 are listed the various individual steroids which have been isolated from normal and abnormal urines. It is to be expected that further investigations will reveal an even greater variety of compounds. The urinary androstanes and etiocholanes (C19 steroids) are listed in Table 5; the pregnanes (C21 steroids) in Table 6. Because technics of isolation have varied with the investigator, the relative quantities of substances isolated have not been listed. In Table 7 an effort is made to compare steroid excretion with certain clinical states. It is noted that the greatest variety of substances has been found in cases of adrenal tumor and hyperplasia; this may be due to the fact that urines from these patients have been more thoroughly studied than urines from other sources. The pertinent, detailed data relating to the individual steroids are given in the tables. The most striking feature presented by patients suffering from adrenal cortical tumor is

Table 6

KETONIC AND NON-KETONIC ALCOHOLIC PREGNANES (C21 STEROIDS) AND AN ESTRANE WHICH HAVE BEEN ISOLATED FROM NORMAL AND PATHOLOGICAL HUMAN URINES

Compound	*Remarks*
1. Pregnane-3(α),20(α)-diol	1. The main metabolite of progesterone[231] in humans. See discussion elsewhere in text. Frequently found in increased amounts in patients with "adrenal hyperplasia"[156] or adrenal tumor.[156]
2. Pregnane-3(β),20(α)-diol	2. Isolated from urine of patients with "adrenal hyperplasia."[156]
3. Pregnane-3(α),17,20-triol	3. Isolated from urine in one of six patients with adrenal tumor and three of four patients with "adrenal hyperplasia."[156] Also isolated from other cases of "adrenal hyperplasia."[31]

4. Δ^5 Pregnene-3(β),20(α)-diol	4. Isolated from urine of a woman[201] and a boy[102] with adrenal tumor.
5. Δ^5 Pregnene-3(β),17(β)-diol-20-one	5. Isolated from urine of a boy with adrenal tumor.[103] Important because the first Δ^5 compound isolated having an oxygen function at both C-17 and C-20. Relation to the common urinary Δ^5 compound, dehydroisoandrosterone discussed.[103]
6. Pregnane-3(α),17-diol-20-one	6. Isolated from urine of a woman with "adrenal hyperplasia," a woman with an adrenal tumor, a crytorchid male and a eunuchoid male receiving testosterone.[138] Compound not found by same investigators in urine of normal males or females or pregnant females. Can be oxidized in vitro to the common urinary 17-ketosteroid, etiocholan-3(α)-ol-17-one.
7. Estrone (C18 steroid)	7. Of all urines investigated from patients with adrenal tumor or hyperplasia this compound isolated only once.[156]

the large quantities of dehydroisoandrosterone that have been isolated. Also, etiocholan-3(α)-ol-17-one and pregnane-3(α),20(α)-diol are commonly found in increased amounts in these cases. Excessive quantities of these three substances are not as consistently observed in patients with "adrenal cortical hyperplasia."* On the other hand, androsterone and pregnane-3(α),17,20-triol appear to be found more consistently in patients with "hyperplasia." When one examines Table 7, it is apparent that no single steroid can be considered completely characteristic of either tumor or "hyperplasia." Individual cases exhibit great variations in their qualitative and quantitative elaboration and excretion of hormones.[156] When these important studies were undertaken it was hoped that the characterization of the excreted substances would provide a better understanding of the intermediary metabolism of the steroid hormones as well as give helpful information in the difficult clinical task of differentiating cases of adrenal tumor from hyperplasia. Unfortunately, as yet it cannot be said that these hopes have been entirely realized by these methods.

Clinical conditions associated with abnormal amounts of steroids in urine

Before the level of excretion of 17-ketosteroids can be utilized as an

* It should be noted in these cases that the "hyperplasia" was associated with virilism and not with the manifestations of Cushing's syndrome.

index of abnormal endocrine function, normal standards must be established. Normal children before 7 years of age excrete very little 17-ketosteroid (average: 1.3 mg. per 24 hours); from then until puberty there is a gradual increase which is essentially the same in both sexes and which varies directly with chronological age and maturity, especially with the latter.[173, 222] From 11 to 18 years the steroid excretion gradually assumes adult proportions and the slight differences in excretion between the sexes become apparent. Normal adult women excrete from

Table 7

STEROIDS ISOLATED FROM NORMAL AND PATHOLOGICAL HUMAN URINES

Compound	*Normal male*	*Normal female*	*Castrate male*	*Ovariectomized female*	*Adrenal tumor*	*"Adrenal hyperplasia"**
Androsterone	+	+	+	+	+	+
Etiocholan 3(α)-ol-17-one	+	+	+	+	+	+
Δ^{11} androsten 3(α)-ol-17-one					+	+?
Androstane 3(α),11-diol-17-one	+				+	+
Etiocholan 3(α)-ol-11,17-dione	+	+			?	?
$\Delta^{9:11}$ etiocholan 3(α)-ol-17-one[49]						
Dehydroisoandrosterone	+	+	+	+	+	+
Isoandrosterone		+				+
$\Delta^{3,5}$ androstandien-17-one	+	+		+	+	+
Δ^{2} or Δ^{3} androsten-17-one	+			+		+
Δ^{5} androstene 3(β),16,17-triol					+	
Δ^{5} androstene 3(β),17(α)-diol					+	+?
Δ^{5} pregnene 3(β),20(α)-diol					+	
Etiocholan 3(α),17-diol	+					
Pregnane 3(α),20(α)-diol	+	+			+	+
Pregnane 3(α),17,20-triol					+	+
Pregnane 3(β),20(α)-diol						+
Δ^{5} pregnene 3(β),17(β)-diol-20-one					+	
Pregnane 3(α),17-diol-20-one					+	+
Estrone	+	+			+	

* Virilism was present in all of these patients; none had Cushing's syndrome.

5 to 17 mg. of 17-ketosteroids per 24 hours, with an average excretion of about 9 mg. Normal adult men excrete from 6 to 25 mg., with an average excretion of about 13 mg.[62, 73, 76, 104] Although data on elderly individuals are distinctly limited, it would seem that there is a slight decline from these normal levels after the sixth decade of life.[168, 202]

Different investigators, using various modifications of similar technics, do not report identical results for normal levels of excretion. However, the variations in the results from one study to another are small and generally do not differ by more than 2 or 3 mg. On the basis of these normal values it is apparent that males excrete, on an average, about 4 mg. more of these steroids per 24 hours than do females. However, the normal range of excretion is wide and there is great overlapping of the values obtained from normal men and women. Surely on the basis of 17-ketosteroid excretion one cannot classify a urine as male or female in type.

It would appear that the 17-ketosteroid excretion is not absolutely constant from day to day in the same individual;[242] however, the fluctua-

Table 8

CONDITIONS ASSOCIATED WITH ABNORMAL LEVELS OF TOTAL 17-KETOSTEROIDS IN URINE AND CERTAIN ENDOCRINE DISORDERS ASSOCIATED WITH NORMAL LEVELS OF 17-KETOSTEROID

Increased amts. of 17-KS in urine	*Decreased amts. of 17-KS in urine*	*Essentially normal amts. of 17-KS in urine*
1. Functioning Adrenal Cortical Tumor (see Table 10 and text)	1. Chronic stresses of diverse types,[68] (e.g. infections, malnutrition, etc.)	1. Cushing's syndrome, not due to functioning adrenal tumor. (see Table 11 and text)
2. Pre-pubertal virilism (adrenal hyperplasia?) (see Table 12 and text)	2. Extremes in age[217]	2. Ovariectomized women[73]
3. "Adrenal" tumors of ovary[124]	3. Anterior pituitary insufficiency (see Table 15)	3. Most cases of acromegaly (see Table 15)
4. Interstitial cell tumor of testical[233]	4. Addison's disease (see Table 15)	4. Most hypogonad males[243]
5. Some hirsute, post-pubertal females (see Table 13 and text)	5. Myxedema, spontaneous and secondary (see Table 15)	5. Most hirsute, postpubertal females (see text and Table 13)
6. Certain cases of acromegaly (see Table 15)	6. Acute and chronic liver disease[73, 79]	6. Arrhenoblastoma of ovary? (see text)
7. "Constitutional" or "natural" sexual and somatic precosity (slight increase of 17-KS) (see text)	7. Anorexia nervosa (see Table 15)	7. Male pseudohermaphroditism[85, 97]
	8. Some hypogonad males[243]	8. Normal pregnancy[230]

Artificial or Induced Increase by:	*Artificial or Induced Decrease by:*
1. Testosterone or testosterone propionate[38]	1. Methyl testosterone[191]
2. Androsterone[72]	2. Certain other 17-methyl steroids[72]
3. Dehydroisoandrosterone[72]	3. Estrogen[46]
4. Δ^4 androsten-3, 17-dione[72]	
5. Δ^5 androsten-3(β), 17(α) diol diacetate[162]	
6. Androstane-3(α), 17(α) diol diacetate[72]	
7. Castration (?)[202]	
8. Adrenocorticotrophic hormone[159]	

tions are not great and according to Talbot and Butler,[217] "the chances are two to one that a single assay will fall within 15 per cent of the 17-ketosteroid value obtained by averaging 30 or 40 consecutive daily assays." This statement undoubtedly holds true for normal levels of excretion; however, fluctuations greater than 15 per cent are frequently seen when very low levels of excretion, and occasionally when distinctly elevated levels are present.[60]

In Table 8 an effort was made to present in summarized form the various clinical disorders which have been associated with abnormal or normal levels of 17-ketosteroid excretion. Available data on the "corticosteroid" excretion in similar clinical states are summarized in Table 9. Certain but not all, of these conditions will be dealt with in detail and with supplementary data. Since the methods used for the assay of the "corticosteroid" fraction has varied with the investigator, reference should be made to the original articles to obtain the normal levels of excretion.

Table 9

CONDITIONS ASSOCIATED WITH NORMAL AND ABNORMAL LEVELS OF "GLYCOGENIC CORTICOSTEROIDS" IN URINE
(Preliminary survey of existing data)

Increased amounts	*Decreased amounts*	*Essentially normal amounts*
1. Functioning adrenal cortical tumor with symptoms of Cushing's syndrome (2 cases[216])	1. Anterior pituitary insufficiency. Decreased in 11 of 12 cases[216, 232]	1. Pre-pubertal virilism. (? adrenal hyperplasia) 5 cases[216]
2. Cushing's syndrome not due to adrenal cortical tumor. Increased in 14 of 15 cases[216, 232]	2. Addison's disease. Decreased in 15 of 21 cases[216, 232]	2. Hirsute, post-pubertal females. 7 cases[216, 232]
3. Acute and chronic trauma (operations, burns)[216]	3. Myxedema. Decreased in 2 cases[216]	3. Acromegaly; normal in 3 cases, increased in 1[232]
	4. Anorexia nervosa. Slight decrease in 3 cases[232]	

Induced increase by administration of adrenocorticotrophic hormone to a normal female[159] and a male with anterior pituitary insufficiency.[227]

Functioning adrenal cortical neoplasms and disorders producing similar clinical phenomena

Clinical aspects: Detailed descriptions, with different methods of classification, of the varied clinical manifestations of adrenal cortical neoplasm may be found in reviews by Haymaker and Anderson,[92] Kenyon,[117] Parkes,[180] Cahill,[32] Albright,[2] Gross,[81] Kepler and Keating[122] and Kepler and Mason.[123] It would seem that no method of clinical categorization is completely satisfactory when dealing with

certain specific cases. However, the classification proposed by Kenyon would seem to be most generally applicable. For the sake of convenience and clarity, conditions, other than adrenal tumor, which give rise to similar clinical phenomena will be introduced, by notations, directly into the outline. Problems in differential diagnosis then become apparent. Kenyon proposed that the syndromes due to adrenal tumor could be classified roughly as follows: I. The adrenogenital syndrome. II. Cushing's syndrome. III. Intergrades between types I and II. IV. Isolated expressions of the neoplasm. V. Feminization in adult men and rarely in children. VI. Tumors without endocrine manifestations.

I. *The so-called adrenogenital syndrome* is characterized by heterologous sexual precosity in girls, homologous sexual precosity in boys, and somatic precosity in children of both sexes; and by intense masculinization of adult females. "Over-masculinization" has not been described in adult males suffering from adrenal neoplasm. So far as is known Crooke's hyalinization of the basophilic cells of the anterior pituitary is not apparent in the adrenogenital syndrome,[225] nor is contralateral adrenal atrophy commonly observed.[32] Although almost all young girls suffering from adrenal tumor present a picture of marked virilism, there are a few cases which have shown vaginal bleeding preceding or simultaneously with the symptoms of heterosexual precosity,[87, 192, 237]

Remarks: Conditions to be differentiated in girls: (1) Clinical syndromes of precocious development and virilism similar to those produced by adrenal cortical neoplasm have been described, associated with bilateral "adrenal cortical hyperplasia." These cases are sometimes classified as cases of "pre-pubertal virilism" or as "feminine pseudohermaphrodites" if the virilism is antenatal in origin and gross developmental defects of the genital organs exist. Clinical differentiation between these cases and cases of adrenal tumor is difficult or impossible, especially if the virilism develops after birth. (2) Granulosa cell tumors of the ovary may cause slight somatic precosity, but the major manifestations are an enlarging mass in the pelvis; hypertrophy of breasts, nipples, and labia; and estrogen-induced, anovulatory "menstruation." Except for slight genital hirsutism there is no masculinization (large clitoris, masculine contour, etc.). Hence, differentiation from cases of adrenal cortical tumor usually is not difficult.[177] (3) Rarely in girls, certain cerebral lesions (tumors or inflammation) in the region of the hypothalamus and third ventricle may cause accelerated somatic development, precocious maturation of the genitalia (adhering to the sex of the child), breast enlargement, and occasionally "menstrua-

tion."[14, 70] Virilism and heterologous sexual precosity are not seen. (4) Arrhenoblastomas, apparently purely virilizing ovarian tumors, have not been reported in individuals less than 15 years of age.[177] (5) Homologous sexual precosity in girls has been described associated with skin pigmentation and polyostatic fibrous dysplasia of bones.[4] The etiologic interrelationships between these manifestations are not clear.[209, 224] The precosity may be due to hypothalamic lesions.[8] (6) "Natural or constitutional" sexual and somatic precosity in girls is known.[86, 176] In these none of the above abnormal conditions are manifest, development is along sexually homologous lines, and excellent evidence of true ovulation is present in some cases. Even pregnancies at 6 and 9 years of age have been described.[59, 176] A premature awakening of a normal process is likely.

Conditions to be differentiated in boys: (1) Somatic and homologous sexual precosity (macrogenitosomia praecox) is rarely seen in boys with bilateral "adrenal cortical hyperplasia."[218, 245] Clinical differentiation from adrenal cortical tumor is impossible. (2) An interstitial cell tumor of the testicle may produce a similar picture.[193] The palpation of a scrotal mass should make the diagnosis. (3) More commonly than in girls, cerebral lesions (pineal tumors, mid-brain and hypothalamic tumors, and inflammations) may cause accelerated somatic and genital development in boys.[50, 105, 135] (4) "Natural or constitutional" precosity is rare but has been described.[86] In these cases cerebral, testicular, and adrenal cortical lesions must be excluded.

Conditions to be differenetiated in women: (1) Virilism in varying degrees (usually only hirsutism) in post-pubertal women is frequently encountered. In some cases "adrenal cortical hyperplasia" has been described, but in many the adrenals appear grossly and microscopically normal. Certain of these patients have a strong family history of hirsutism and hence the abnormality may be genetically determined. Most of these patients are not defeminized, have essentially normal menses, and bear children. At times, however, differentiation from adrenal cortical tumor may be difficult. (2) Arrhenoblastomas,[177] occurring usually in the third decade of life, are strictly virilizing ovarian tumors which cause defeminization (breast atrophy, amenorrhea, loss of feminine contour) and masculinization (deepening of voice, hirsutism). Apparently these tumors do not cause the development of the stigmata of Cushing's syndrome (see below). Differentiation from the adrenogenital syndrome due to adrenal neoplasm is difficult; the finding of a pelvic mass is necessary for a positive pre-operative diagnosis. (3)

"Adrenal-like" ovarian tumors are known.[124] Histologically they are different from arrhenoblastomas and appear to arise from adrenal cortical rests. Physiologically they behave like adrenal tumors and may give rise to any of the clinical and laboratory phenomena associated with adrenal neoplasm.

II. *Cushing's syndrome,* characterized, in its full-blown aspects, by facial and abdominal obesity, thin arms and legs, purple striae and ecchymoses, plethora, hypertension, osteroporosis, diminished carbohydrate tolerance, and muscular weakness. Polycythemia and a peculiar hypochloremic alkalosis have been described in some cases. Signs of virilism in women and children are mild and are represented by slight or moderate hirsutism. Complete amenorrhea, however, is almost invariably present in women. Adrenal cortical neoplasm associated with Cushing's syndrome is most commonly seen in adult women, and less commonly in children of either sex and in man. Contralateral adrenal atrophy is usually present.[32]

Remarks: Cushing's syndrome in adults frequently is not associated with a unilateral adrenal cortical neoplasm. Of 98 cases of Cushing's syndrome reviewed by Thompson and Eisenhart[225] 76 were not associated with adrenal cortical neoplasm. Pathological lesions reported in such cases have been "adrenal cortical hyperplasia," or adenomas of the pituitary (usually, a basophilic adenoma) with or without evident "adrenal cortical hyperplasia."[225] Occasionally no gross lesions are apparent in any endocrine gland.[228] However, regardless of the nature or even absence of associated lesions, cases of Cushing's syndrome almost invariably exhibit Crooke's hyalinization of the basophils of the anterior pituitary.[40] Also, microscopic lesions in the paraventricular hypothalamic nuclei have been consistently observed by Heinbecker in cases of Cushing's syndrome not associated with adrenal cortical neoplasm.[94]

III. *Intergrades* between Types I (adrenogenital syndrome) and II (Cushing's syndrome). For example, an intensely virilized woman with facial and abdominal obesity and hypertension. Children with adrenal tumors usually tend to fall into this "mixed" group.

IV. *Isolated expressions of the neoplasm.* Although these cases are unusual, either diabetes[211] or amenorrhea[238] has been reported as representing the sole manifestation of the adrenal tumor.

V. *Feminization* of adult men has been observed in a few cases; in these, testicular atrophy, impotence, some loss of body hair, and breast enlargement have been described.[137, 140] As stated earlier, vaginal, and

hence presumably uterine, bleeding has been seen in a few young girls suffering from adrenal cortical neoplasm.

VI. *Adrenal cortical tumors without endocrine or metabolic manifestations* are known.[32]

Steroid excretion in adrenal tumor: In Table 10 are given the total 17-ketosteroid excretions and the per cent of the total represented by the beta fraction (digitonin precipitable) that have been reported in 47 individuals suffering from adrenal cortical neoplasms. Only those cases of tumor which have had chemical assays for steroids are included. For purposes of comparison the levels of excretion are arranged in ascending order and the patients are grouped according to their major

Table 10

THE EXCRETION OF 17-KETOSTEROIDS IN PATIENTS WITH ADRENAL CORTICAL TUMOR (NEOPLASM)

Author and case	*Sex and age in years*	*Total 17-ketosteroids in mgm./24 hours*	*Approx. % of total 17-ketosteroids as β-fraction*
I. Cases of adrenal tumor associated with clinical evidence of intense masculinization but without the major metabolic stigmata of Cushing's syndrome (e.g., those cases without hypertension, osteoporosis, or diabetes).			
1. Callow and Crooke, case 4[37]	Female, 41	76, 78	no data
2. Johnson and Nesbit, case 1[112]	Female, 31	78.5	79
3. Johnson and Nesbit, case 2[112]	Female, 2	75	73
4. Hain, case 5[84]	Male, 10	84	no data
5. Callow and Crooke, case 3[37]	Female, 26	107	no data
6. Talbot et al., case 15a[218]	Female	133	50
7. Talbot et al., case 9[222]	Female, 3	160	65
8. Fraser et al., case 40[73]	Female, 3½	176 (av.)	no data
9. Hain, case 3[84]	Female, 4	320, 166	no data
10. Bauman et al., case K[12]	Female, 25	367	30
11. Engstrom et al., case 8[63]	Female, 45	857	no data
II. Cases of adrenal tumor associated with definite Cushing's syndrome and with minimal virilism.			
12. Fraser et al., case 35[73]	Female, 36	19.9*	no data
13. Kepler and Mason, case 16[123]	Female, 40	30	7.5
14. Crooke and Callow, case 1[42]	Male, 25	40-64	20
15. Hain, case 1[84]	Female, 21	45	no data
16. Callow and Crooke, case 5[37]	Female, 38	54, 52	no data
17. Fraser et al., case 34[73]	Female, 56	72	no data
18. Talbot et al., case 14[218]	Female, 56	74.0	22
19. Wilhelm and Gross[244]	Male, 46	92.7	no data
20. Engstrom et al., case 5[63]	Female, 25	131	no data
21. Patterson et al., case 3[182]	Female, ?	170	no data
22. Crooke and Callow, case 2[42]	Female, 6	126-288	65

23. Patterson et al., case 4[182]	Female, 34	270	no data
24. Kepler and Mason, case 14[123]	Female, 53	800 (av.)	69

III. Cases of adrenal tumor associated with various degrees of masculinization and of the manifestations of Cushing's syndrome and cases where the available data do not warrant categorization.

25. Engstrom et al., case 1[63]	Male, 1¼	2.8	no data
26. Engstrom et al., case 2[63]	Female, 1¼	3.0	no data
27. Kepler and Mason, case 17[123]	Male, 39	4.8**	no data
28. Hain, case 2[84]	Female, 3½	5.9	no data
29. Callow and Crooke, case 1[37]	Female, 61	14.5, 20***	no data
30. Patterson et al., case 2[182]	Male, 5	27	no data
31. Patterson et al., case 1[182]	Male, 1	28 (per l.)	no data
32. Friedgood and Whidden[76]	no data	45	no data
33. Engstrom et al., case 3[63]	Female, 63	45	no data
34. Engstrom et al., case 4[63]	Female, 16	54	no data
35. Kepler and Mason, case 18[123]	Male, 32	68	48
36. Warren, case 66[239]	Female, 54	83	no data
37. Johnson and Nesbit, case 3[112]	Male, 43	86	57
38. Warren, case 99[239]	Female, 40	126	no data
39. Talbot et al., case 10[222]	Female, 13	166	50
40. Engstrom et al., case 6[63]	Female, 3½	170	no data
41. Anderson et al.[9]	Female, 25	215	no data
42. Engstrom et al., case 7[63]	Female, 21	240	no data
43. Warren, case 79[239]	Female, 49	269	Isolated dehydroisoandrosterone but not quant.
44. Friedgood and Whidden[76]	no data	325	no data
45. Hirschmann[101]	Male, 7	275, 420	No quant. data
46. Warren, case 75[239]	Female, 42	690	Isolated dehydroisoandrosterone but not quant.
47. Kepler and Mason, case 15[123]	Female, 55	1005	77

* Urine stored 5 years before assay.
** Probably a non-functioning adrenal cortical tumor.[123]
*** Patient "extremely ill" at time of urine collection.[37]

clinical manifestations, i.e., those with the so-called adrenogenital syndrome, those with Cushing's syndrome, and those that could not be assigned to either group. Only 10 of the 47 patients were males. Thirteen cases of tumor in children are reported; 5 in boys and 8 in girls. Although some of the values for total 17-ketosteroid excretion in young children are below the average range for normal adults, the values are consistently above the average for normal children of comparable age. Difficult problems in differential diagnosis arise when only moderate

elevations of total 17-ketosteroid excretion occur, e.g., when patients with adrenal neoplasm excrete less than 50 mg. per 24 hours. Eight cases of tumor in adults have been reported to exhibit excretions in this range (cases 12, 13, 14, 15, 27, 29, 32, and 33). Although not with certainty, the results in three of these cases (12, 27, and 29) may have been fundamentally erroneous or misleading. In case 12 the urine had been stored for 5 years before assay, case 27 probably had a non-functioning adrenal tumor, and case 29 was moribund at the time of urine collection. Thus, if the results in these three cases can be disregarded then all of the patients with functioning adrenal cortical tumor excreted more than normal amounts of ketosteroids. However, 5 of the adults unequivocally excreted less than 50 mg. per 24 hours (i.e., amounts which are not greatly excessive). Although many patients excreted more than 100 mg., almost an equal number had values between 50 and 100 mg.

In only 14 of the 47 cases was the 3-beta-alcoholic fraction determined. Normally, the beta steroids comprise about 5 per cent of the total 17-ketosteroids. In all but one instance this fraction was distinctly or markedly elevated (e.g., up to 79 per cent). The exception (case 13) is important since it indicates than an adrenal neoplasm may be present in spite of a normal value for the beta fraction. The patient had Cushing's syndrome and a rather low total 17-ketosteroid excretion.

It is interesting that the levels of steroid excretion in patients that are intensely masculinized (adrenogenital syndrome) are not significantly different from the levels seen in those that present the manifestations of Cushing's syndrome. Undoubtedly, factors other than the extent of elaboration (or at least, of excretion) of androgenic steroids are involved in the pathogenesis of these syndromes. Certainly one cannot conclude that in those cases of tumor associated with the adrenogenital syndrome androgen elaboration (anabolic steroids?[2]) was sufficiently great to prevent the development of Cushing's syndrome. The latter patients appeared to elaborate quite as much androgen (as measured by 17-ketosteroid excretion) as the former.

It should be noted that none of the 5 adult males herein reported showed definite evidences of feminization, although case 19 was reported to be impotent and to possess small soft testicles. Two of the adult male patients (cases 14 and 19) presented the picture of Cushing's syndrome; three (cases 27, 35, and 37) showed no evident endocrine or metabolic abnormalities in spite of the fact that cases 35 and 37 showed moderate, but not greatly excessive, increase in 17-ketosteroid output. All of the adult females and all of the children in this series

showed evidences of disturbed endocrine function (see references for details).

Steroid excretion in Cushing's syndrome not due to adrenal tumor: Since certain other pathological states may be associated with clinical syndromes that are similar to or identical with those produced by adrenal cortical tumor, the steroid excretions in some of these states will be considered. The values for the excretion of 17-ketosteroids in 36 patients with Cushing's syndrome proven not to be associated with adrenal neoplasm are given in Table 11. At least half of these cases

Table 11

THE EXCRETION OF 17-KETOSTEROIDS IN PATIENTS WITH CUSHING'S SYNDROME PROVEN NOT TO HAVE ADRENAL CORTICAL TUMOR (NEOPLASM)

Author and case	*Sex and age in years*	*Total 17-keto-steroids in mgm./24 hours*	*Approx. % of total 17-ketosteroids as β-fraction*
1. Callow and Crooke, case 7[37]	Female, 28	7.5	no data
2. Kepler and Mason, case 22[123]	Female, 26	10.0	5
3. Fraser et al., case 37[73]	Female, 26	10.4	no data
4. Venning and Browne, case 17[232]	Female, 33	10.0, 8.1	no data*
5. Talbot et al., case 7[222]	Female, 26	11.2	12
6. Venning and Browne, case 21[232]	Male, 27	11.0	no data
7. Venning and Browne, case 19[232]	Male, 11	11.5	no data
8. Fraser et al., case 38[73]	Female, 43	11.5	no data
9. Crooke and Callow, case 3[42]	Female, 12	11, 18.6	none isolated
10. Deakins et al.,[46]	Female, 15	11, 20	no data
11. Talbot et al., case 6[222]	Female, 43	14.8	7
12. Callow and Crooke, case 8[37]	Female, 30	18.5	no data
13. Fraser et al., case 36[73]	Female, 49	19.7	no data
14. Callow and Crooke, case 6[37]	Female, 39	20.2, 21	no data
15. Kepler and Mason, case 20[123]	Female, 27	24	14
16. Kepler and Mason, case 21[123]	Male, 29	28	2
17. Callow and Crooke, case 9[37]	Female, 43	28.5	no data
18. Pashkis et al.,[181]	Female, 28	31	no data
19. Warren,[239] 17 cases	Females	6-37 (mean: 18.6)	no data
20. Warren, case 122[239]	Male, 34	33.2	no data

* It is not possible to state regarding this patient whether or not the surgically removed "benign adenoma" was a functioning, neoplastic primary adrenal tumor since the opposite adrenal also contained a small cortical adenoma at autopsy. Basophilic adenomas of the anterior pituitary associated with Cushing's syndrome frequently exhibit "cortical adenomas" and/or adrenal hyperplasia.[225]

have total excretions that are within the range of normal. The remainder excrete moderately increased amounts; however, none of the values reported are greater than 50 mg. per 24 hours. When these results are

compared with those obtained in patients with adrenal tumor the following tentative conclusions might be reached concerning adults with Cushing's syndrome: If normal amounts of total 17-ketosteroids are excreted, a unilateral, functioning adrenal cortical neoplasm would not be expected to exist; if the amounts are increased above normal but below 50 mg. per 24 hours, the chances are at least 6 to 1 that the syndrome is not associated with adrenal cortical tumor; if values are consistently above 50 mg., the data would support a diagnosis of tumor. Also, in view of the lack of precise criteria for establishing a diagnosis, it would seem that any individual with Cushing's syndrome who is excreting more than normal amounts of 17-ketosteroids deserves surgical exploration of the adrenal areas for tumor.

The per cent of the total ketosteroids represented by the beta-fraction has been determined in a few cases of Cushing's syndrome not due to adrenal neoplasm. In none of these was the beta-fraction greater than 20 per cent. This finding is in sharp contrast with the results obtained in patients with tumor. Hence, the determination of the beta-fraction may provide additional aid in differential diagnosis. However, more data are necessary before rigid criteria are formulated to differentiate those cases due to tumor from those without it. From the available information, a distinctly elevated beta-fraction (e.g., above 40 per cent) would support a diagnosis of tumor, while a moderate increase (e.g., 10 to 20 per cent) would not necessarily rule out tumor (see cases 13, 14, and 18, Table 10).

Steroid excretion in pre-pubertal virilism: Difficulties in differential diagnosis also arise when dealing with certain cases of pre- and post-pubertal virilism in females. In Table 12 are summarized the 17-ketosteroid excretions that have been recorded in 31 patients with pre-pubertal virilism associated with proven "adrenal cortical hyperplasia," adrenal tumor having been ruled out. The data were arranged according to the chronological age of the patient at time of assay. Some of these patients were categorized by the authors as feminine pseudohermaphrodites, since these children were born with genital abnormalities (e.g., enlargement of the clitoris, external urethra opening at the base of the "penis," a rudimentary vagina, hypoplastic uterus, etc.). In general, these infants do not thrive but if they do, the females become chronically masculinized, never menstruate, and finally become rather stunted in growth. Occasionally these children are reared as boys. In the other cases evidences of virilism developed some time after birth but before puberty. The older individuals had symptoms that dated

back to childhood. In the majority of these latter patients the major clinical manifestations were those of virilism (hirsutism, deep voice, muscular, hypertrophy of the clitoris, and hypoplasia of the uterus), but without other gross genital abnormalities. Hence, it seemed most convenient and logical to classify the entire group as "pre-pubertal virilism." None of these patients had the stigmata of Cushing's syndrome. It is quite apparent that many of these patients excrete more 17-ketosteroids than do many patients with adrenal cortical tumor. Values between 50 and 100 mg. are common, particularly in the older individuals. If one were to use only the 17-ketosteroid excretion as a guide in the differential diagnosis between tumor and hyperplasia in a virilized girl, a value well over 100 mg. would be necessary to support a diagnosis of tumor. When values below 100 mg. are obtained in a girl who develops virilism after birth the differentiation between tumor and hyperplasia becomes exceedingly difficult or impossible. Although the data are limited, it would appear that the determination of the beta-fraction would be helpful in making this differentiation. This fraction is generally higher (usually well over 20 per cent) in the cases associated with tumor than in those with hyperplasia (from zero to 23 per cent) but some slight overlapping may be observed (see cases 13, 14, and 18 in Table 10 and cases 11 and 27 in Table 12). Hence, to diagnose an adrenal tumor unequivocally in this group would necessitate a total 17-ketosteroid excretion above 100 mg. per 24 hours of which at least 30 per cent are beta-steroids.

One gains the impression that the longer the virilism persists the greater the ketosteroid excretion becomes; in the table it is noted that the highest excretions are seen only in the older, and the lowest only in the younger age groups. Although chance distribution and selection of cases may account in part for the phenomenon, cases 17 and 27 would appear to bear out the impression. These cases were studied by the same investigators 1 and 2 years after the initial determinations, and definitely higher values were obtained in the later studies. Similar findings have been reported by Talbot et al.[218] Except as a "maturation phenomenon" no ready explanation is available.

Steroid excretion in post-pubertal virilism: A common "endocrinological" problem is that presented by the post-pubertal, hirsute female. The hirsutism appears at or after the menarche. Almost invariably the question of adrenal or ovarian neoplasm is raised and almost invariably none is found. Hirsutism in varying degrees is the common feature of all these cases; rarely some generalized obesity and/or menstrual dis-

Table 12

THE EXCRETION OF 17-KETOSTEROIDS IN CASES OF PRE-PUBERTAL VIRILISM WITH PROVEN ADRENAL CORTICAL HYPERPLASIA AND IN PATIENTS CLASSIFIED AS FEMININE PSEUDOHERMAPHRODITES IN WHOM VIRILISM AND GENITAL ABNORMALITIES HAVE BEEN PRESENT SINCE BIRTH AND IN WHOM ADRENAL HYPERPLASIA IS HIGHLY PROBABLE OR PROVEN. (ALL THE OLDER PATIENTS HAVE HAD SYMPTOMS DATING BACK TO CHILDHOOD).

Author and case	*Age in years*	*Total 17-keto-steroids in mgm./24 hours*	*Approx. % of total 17-ketosteroids as β fraction*
1. Engstrom et al., case 9[63]	3	13.5	no data
2. Genitis and Bronstein, case 2[78]	4	37	no data
3. Engstrom et al., case 10[63]	5	37	no data
4. Broster, case HA[22]	5	6	no data
5. Talbot et al., case 4[218]	5	19.2	17
6. Broster, case BA[22]	6	12.4	no data
7. Broster, case Eg[22]	7	37	no data
8. Patterson et al., case 4[182]	7	37, 34	no data
9. Talbot et al., case 8[218]	8	11.7	0
10. Talbot et al., case 10[218]	8	23.4	7
11. Talbot et al., case 1,[222] Male	8	17.0	23
12. Patterson et al., case 1[182]	9	52	no data
13. Patterson et al., case 7[182]	9	50	no data
14. Genitis and Bronstein, case 1[78]	10	30	no data
15. Kepler and Mason, case 19[123]	10	31	14
16. Talbot et al., case 2[222]	11	29	0
17. Genitis and Bronstein, case 1[78]*	11	69	no data
18. Broster, case Mot[22]	12	32	no data
19. Talbot et al., case 11[218]	13	23.4	3
20. Patterson et al., case 6[182]	13	64	no data
21. Broster, case Sim[22]	16	43	no data
22. Patterson et al., case 3[182]	17	35	no data
23. Broster et al., case Her[22]	17	54	no data
24. Patterson et al., case 2[182]	18	56	no data
25. Patterson et al., case 5[182]	18	54	no data
26. Engstrom et al., case 11[63]	19	75.2	no data
27. Kepler and Mason, case 11[123]**	21	123	20
28. Hain, case 12[84]	21	35-74	no data
29. Broster, case Ron[22]	22	75	no data
30. Talbot et al., case 4[222]	23	60	9
31. Engstrom[60]***	41	102 (av.)	no data

* Same case as number 14, observed one year later.
** Same case as number 26, observed two years later.
*** New Haven Hospital case B93002.

turbances are observed; in none are definite stigmata of Cushing's syndrome present. In many, the hirsutism appears to be a familial trait. The data on the 17-ketosteroid excretion in over 130 of these cases are presented in Table 13. In slightly more than half of these the total

Table 13

THE EXCRETION OF TOTAL NEUTRAL 17-KETOSTEROIDS IN WOMEN WITH POST-PUBERTAL VIRILISM NOT DUE TO ADRENAL TUMOR*

Reference	*Number of cases*	*Age Range in years*	*Total 17-ketosteroids in milligrams per 24 hours Range*	*Mean*
Callow and Crooke[37]	7	21-44	10.0-30	16.5
Patterson et al.[182]	67	18-34	6.4-33.4†	19.9
Warren[239]				
"adrenal virilism"	20	19-36	5.9-37	22
"hirsutism"	13	20-36	3.4-33	16
Venning and Browne[232]	4	16-54	23.1-37	28
Friedgood and Whidden[76]	21	—	1.0-29§	—

* In these cases adrenal cortical tumor presumably has been excluded but the precise anatomic and functional status of the adrenal cortex remains uncertain. Although the adrenal is incriminated in many of these cases, the possibility that the defect resides in the ovary or elsewhere remains to be considered. Certain of these patients have a strong family history of hirsutism and hence in such individuals this abnormality may be genetically determined. Refinements of subclassification with such terms as "adrenal virilism" seem confusing and unwarranted. Hirsutism is the common feature of all these cases; less commonly irregular menses or obesity is observed, and rarely amenorrhea.

† Less than half of these cases excreted amounts clearly in excess of normal.

§ The clinical data on these patients are extremely fragmentary.

17-ketosteroid excretion is within the range of normal. In the remainder moderate elevations are observed; the highest recorded value being 37 mg. per 24 hours. From what is understood of the origin of the 17-ketosteroids some abnormality in adrenal cortical function is likely in these latter cases. The true nature of the abnormality in those with normal amounts of 17-ketosteroids in urine is obscure. In those cases of hirsutism which seem to be familial in origin an abnormal responsiveness to normal androgenic influences is possible. Also, it is conceivable that under certain circumstances androgens may be elaborated by ovaries or adrenal cortices that are not metabolized to the usual urinary steroids. Until more precise information is available it would seem that this group could be best categorized and characterized by such general terms as "post-pubertal virilism" or "simple hirsutism." The term "adrenal virilism" implies a knowledge of etiology that is not generally warranted.

General remarks regarding "hyperfunctioning" adrenal lesions: Having thus described the patterns of 17-ketosteroid excretion in conditions classified as being representative of adrenal cortical hyperfuction (adrenal tumor and "adrenal cortical hyperplasia") certain general comments are needed. As was pointed out earlier other pathological lesions,

especially in children, may give rise to clinical phenomena identical with or at least superficially similar to those produced by hyperfunctioning adrenal lesions. It is imperative, therefore, that each patient be studied individually. Hormone assays should be utilized only as an adjunct to a careful history and physical examination. The family history, the exact nature of the abnormality, the age at which it became manifest, the evidences of cerebral involvement, etc. are all points to be carefully evaluated when dealing with problems of sex differentiation. A family history of hirsutism in a mildly or moderately virilized woman may be of great importance. Also, certain forms of pseudohermaphroditism (especially of the male type) tend to be familial.[248] Heterologous sexual precosity (sex inversion) in girls is frequently associated with hyperfunctioning adrenal cortical lesions (tumors or hyperplasia) while homologous sexual precosity is not. Homologous precosity in girls is more commonly associated with cerebral or ovarian lesions. Children of either sex that show evidences of Cushing's syndrome in addition to virilism more commonly suffer from adrenal tumor than not,[148] while in adults Cushing's syndrome is more commonly associated with pathological lesions of pituitary or adrenal other than adrenal neoplasm.[225]

If on the basis of clinical evaluation and laboratory studies it appears that a patient suffers from a hyperfunctioning lesion of the adrenal cortex, a study of the steroid excretion in urine may be helpful to differentiate tumor from "hyperplasia." The limitations of this method have already been noted. However, the differentiation is therapeutically important since patients with adrenal tumor may be cured of their condition by the surgical removal of the neoplasm. It is not the purpose of this communication to discuss the theoretical pathogenesis of Cushing's syndrome (see Albright,[2] Kenyon,[117] Kepler,[121] and Crooke[41]) nor to engage in a discussion of the histology of the adrenal cortical lesions associated with Cushing's syndrome and virilism. However, since efforts have been made to compare steroid excretions in different clinical conditions and to view the levels of excretion as expressions of certain aspects of endocrine function, a few general comments are in order. So far as the clinical manifestations of adrenal tumors are concerned it is clear that the age and sex of the patient determines to a certain extent the type of clinical response. As was pointed out in the introductory paragraphs, a given response to a hormone depends on the chemical nature of the administered (or endogenously manufactured) hormone, the dose, the nature of the transformation products, the

effects produced in other glands or tissues, and the biological soil on which the hormone or other products act. The importance of the last factor is borne out by the observation that adrenal neoplasms do not always produce identical clinical syndromes in boys, girls, men, or women. Masculinizing effects of varying degree are one of the most consistent features of adrenal cortical tumors. Hence it would be expected that heterologous sexual and somatic precosity would be observed in girls, homologous precosity in boys, and masculinization in adult women. No distinct evidences of virilism in previously normal adult men would be expected since the conditioned, male secondary sex characters are already fully developed. However, a few cases of adrenal neoplasm in men[137, 140] have been associated with feminization (if testicular atrophy, impotence, and breast enlargement can be considered "feminization"). Also, vaginal bleeding, though unusual, has been observed in girls.[87, 192, 237] There are three possible explanations for these phenomena of feminization. First, it is possible that a true phenolic estrogen is elaborated in excess by certain adrenal cortical tumors. Second, excessive amounts of "androgenic" steroids may be elaborated but these may be partially converted in the body to phenolic estrogens. That this conversion may occur in the human male is suggested by the fact that the administration of testosterone propionate is followed by an appreciable increase in the estrogenic activity of the phenolic fraction of urine.[89, 106] It may be pertinent that Forbes[69] demonstrated that testosterone propionate (an androgen shown to cause lobule-alveolar growth of the mammary glands of monkeys and rats [67, 133]) caused mammary gland enlargement in the adult male rat but not in the pre-pubertal male. However, the biological action of adrenal "androgen" on the human mammary gland and testis is unknown. The fact that an estrogen (estrone) has been isolated in excessive amounts from the urine of a female patient with an adrenal neoplasm[156] can be explained by either of the above two mechanisms. Third, a tumor may elaborate steroids, e.g., dehydroisoandrosterone, Δ^5 androstene-3,17-diol, which are both androgenic and estrogenic in activity. The nature of the response would undoubtedly depend on the age and sex of the patient; feminization or masculinization would appear only when these responses could be recognized (e.g., feminization in an adult male or child and masculinization in an adult female or child). In other words, it would not seem absolutely necessary to believe that excessive amounts of phenolic estrogens are elaborated by the tumors in all of these cases of "feminization" even though the possibility

exists. "Androgenic" and "estrogenic" responses are only relative biological terms.

From the data on total 17-ketosteroid excretions and from data obtained by the isolation of the crystalline compounds it is clear that there probably are great variations in the qualitative and quantitative elaboration, and surely great variation in the output of steroid hormones in patients suffering from adrenal tumor. Equally great variations are observed in the clinical manifestations. However, at least from the data on the conjugated neutral steroids it has not been possible as yet to correlate clinical phenomena with any particular pattern of total or individual steroid excretion. It would seem premature to state that all of the symptoms of adrenal neoplasm are entirely and directly related to the nature and amounts of steroid hormones elaborated. For example, it is not easy to account for all of the manifestations of Cushing's syndrome on the basis of the known actions of the adrenal steroids. Efforts along these lines have not been entirely successful.[117] Although the neoplasm undeniably is the basic cause of the disorder, it is likely that the development of certain of the manifestations depends more on the resultant secondary effects in other endocrine glands or on certain peculiarities of the individual than on the direct actions and effects of the known steroid hormones of the adrenal on biochemical processes.

An adrenal cortical tumor can be considered a pathological entity without much argument; unfortunately, as much cannot be said for "adrenal cortical hyperplasia." Adrenal cortical hyperplasia has been described in several different disorders, e.g., in certain cases of acromegaly, in Cushing's syndrome without adrenal neoplasm, in prepubertal virilism. There has been no unanimity of opinion as to what criteria, besides adrenal weight, define adrenal cortical hyperplasia.[18] In any event, the fact that "hyperplasia" has been described in Cushing's syndrome and again in such a different clinical condition as pre-pubertal virilism can only point to the fallacy of considering the "hyperplasia" a single functional entity. Even though the histology and weights of the adrenals may be described as similar in these two different conditions,[18] the nature of the cortical function or dysfunction must be different in each. On this basis it is likely that the origin of these states also differs. Only by the utilization of a functional approach will the difficult problems of pathogenesis be solved. To use the term "hyperplasia" in a loose sense to denote bilaterally enlarged adrenals, no matter what the associated clinical state may be, may be useful terminology in the differentiation of neoplastic from non-neoplastic

adrenal cortical lesions. However, further than this the term has little meaning at the present time.

Although "adrenal cortical hyperplasia" has been described in functionally and clinically different conditions, differences in steroid excretion might be observed in these dissimilar states in spite of similar pathological lesions. So far as the total- and beta-17-ketosteroids are concerned some differences are present; though these are not striking (compare Tables 11 and 12). When comparable age groups are considered generally much higher total 17-ketosteroid levels are observed in the congenitally virilized cases than in those with Cushing's syndrome not due to adrenal neoplasm. However, no differences in the beta-fraction are noted in the two groups. Unfortunately, a comparison of the nature of the neutral steroids isolated in crystalline form is not possible in these two syndromes. As previously noted data are available for the cases of virilism but the urines of patients with Cushing's syndrome (not associated with adrenal tumor) appear to have been neglected, probably because the neutral 17-ketosteroid excretion is usually not excessive. Thus far mention has not been made of the level of excretion of the "corticosteroid" fraction in states considered to be representative of adrenal cortical hyperfunction. The level of excretion of this fraction is generally considered to provide a measure of the glycogenic and other related cortical activities. Although the data are not extensive, very striking differences are noted between the cases of "hyperplasia" associated with virilism and those associated with Cushing's syndrome.[216, 232] In the latter individuals the "corticosteroid" excretion has been observed to be increased by from 2 to 12 times normal, while in the former essentially normal amounts were noted.[232] Clearly all of the "hyperplastic" adrenal glands do not function similarly. In spite of considerable knowledge concerning the experimental production of enlarged adrenal cortices in animals and the factors involved in the control of cortical activity (see introductory paragraphs), the genesis of "adrenal hyperplasia" in man remains obscure.

Miscellaneous conditions associated with normal and abnormal amounts of steroids in urine

Virilizing ovarian tumors. Data on these few cases (Table 14) are limited to the total 17-ketosteroid excretions. It has not been possible to determine the precise histological picture of several of these reported neoplasms and therefore the entire group was categorized as suffering

from "virilizing ovarian tumors." Those tumors in which the histological data are described or stated were considered to be arrhenoblastomas or adrenal-like tumors. In the past, various names have been used to characterize the adrenal-like ovarian tumors—terms such as luteomas,

Table 14

VIRILIZING OVARIAN TUMORS
(ARRHENOBLASTOMAS AND ADRENAL-LIKE TUMORS OF OVARY)

Author and case	*17-ketosteroid excretion in milligrams per 24 hrs.*	*Remarks*
1. Fraser et al.,[73] case 102	6.9 (av.) 3.2 (post-op.)	Age 30, with hirsutism, obesity, amenorrhea, deep voice, beginning baldness of scalp, enlarged clitoris. Normal glucose tolerance. Recovery after removal of "arrhenoblastoma."
2. Mathiesen (cit. by Pedersen[184])	4.0	Age 24. No further data. Classified as "typical arrhenoblastoma (tubules)."
3. Abarbanel et al.[1]	40.0 (per liter)	Age 22. Amenorrhea developed at 16 followed by hirsutism, deepening of voice, and enlargement of clitoris. No histological data given.
4. Jones and Everett,[113] case 2	56, 36 11 (post-op.)	Age 26. Irregular menses, deep voice, hirsute, enlarged clitoris, female bodily configuration, normal glucose tolerance. No pregnanediol found in urine. Classified as "arrhenoblastoma of intermediate cell type."
5. Kepler et al.[124]	54.6 2.6 (post-op.)	Age 16. Amenorrhea, hirsutism face full and round, headaches, voice rather low pitched, well developed musculature, suggestion of "buffalo hump," acne, and stria present, breasts large and pendulous, blood pressure 160/110. Normal fasting blood sugar. Clinical impression: Cushing's syndrome. Tumor classified as: "adrenal-like ovarian tumor."

6. Hain[84]	116	Age 12. "Virilizing ovarian tumor." Clinical and pathological data not reported.
7. Warren,[239] case 87	158	Age 31. Virilism developed during pregnancy. An "ovarian tumor removed." No further data given.
8. Luft (cit. by Pedersen[184])	23.8	Age 44. Pronounced virilism. "No metabolic changes." Tumor diffuse "adrenal-like cells."
9. Pedersen[184]	12, 17 1.8-8.6 (post-op.)	Age 50. Amenorrhea, hirsutism, baldness, seborrhea, acne, clitoris enlarged, deep voice, plethora of face, breasts normal, questionably impaired glucose tolerance, no osteoporosis or hypertension. Ovarian tumor: "Diffuse, non-lipoid containing adrenal-like tumor."

leuteoblastomas, adrenal cortical carcinomas, and masculinovoblastomas. Three of the neoplasms were classified as arrhenoblastomas (cases 1, 2, and 4); in two, normal amounts of ketosteroids were excreted; in one excessive amounts. Three of the neoplasms were considered to be adrenal-like ovarian tumors (cases 5, 8, and 9); in two excessive amounts and in one, normal amounts of steroids were excreted. What conclusions can be drawn from these few cases? Although arrhenoblastomas appear to be strictly virilizing tumors, adrenal-like ovarian tumors may cause any of the signs, symptoms, and laboratory manifestations of adrenal cortical neoplasms (e.g., very high 17-ketosteroid excretions, Cushing's syndrome, adrenogenital syndrome, etc.). However, these latter cases are rare; Kepler and Dockerty[124] were able to collect only 14 cases up to 1943. Since three patients with virilism and ovarian neoplasm excreted normal amounts of 17-ketosteroids and since the virilism regressed after surgical removal of the tumor, it must be concluded that an abnormal human ovary is capable of elaborating androgen not normally metabolized to 17-ketosteroids. Although these observations are few in number there is no good reason to believe that the results were purely fortuitious. In any event these observations are sufficient to discourage hasty conclusions regarding the adrenal cortical origin of all cases of hirsutism in females.

Male pseudohermaphroditism, true hermaphroditism, and "natural or constitutional" precosity in children. Data thus far reported, though meager because of the rarity of these conditions, would indicate that the excretions of 17-ketosteroids are normal or very slightly increased. A male pseudohermaphrodite can be defined as an individual whose gonads are testes but in whom some of the secondary sex characters and genital organs are female in type. Many of these patients are reared as girls. The direct cause of feminine pseudohermaphroditism is generally believed to be due to excessive androgen production by hyperplastic adrenal corticies, although the cause of the hyperplasia remains unknown; the genesis of male pseudohermaphroditism, on the other hand, is not entirely clear. Witschi[248] has presented statistical evidence to indicate that these patients are genetic males, and he suggests that abnormal maternal hormonal influences (possibly estrogenic) play an important rôle in the development of the anomaly. Total 17-ketosteroid excretions of 10 mg. and 9 mg. were reported by Herweg et al.[58, 97] in two male pseudohermaphrodites aged 15 and 26, respectively; Hain reported 13.3 and 7.4 mg. in a similar patient 14 years of age. If these observations can be supported by further similar studies it is clear that the level of the 17-ketosteroid excretion may be a very helpful diagnostic aid when dealing with cases of doubtful sex. In contrast to the male pseudohermaphrodites it has already been noted that female pseudohermaphrodites excrete excessive quantities of these steroids.

A true hermaphrodite is an individual with both male and female gonads; the ovarian and testicular tissue may be separate or fused (ovotestis). Such patients may be reared either as boys or girls. The condition is rare and has been reported only 40 times.[161] Weed et al.[240] reported a total 17-ketosteroid excretion of 7.6 and 7.5 mg. per 24 hours in a true hermaphrodite 36 years of age.

Hain[86] reported the 17-ketosteroid excretions in 5 girls with "constitutional" precosity* to be slightly above the average for their age in 3 patients and normal in two. In two boys the 17-ketosteroid excretions were also slightly above normal. In these cases primary adrenal cortical and gonadal diseases presumably were ruled out and there was no evidence by history or examination of gross intracranial abnormality.

*After preparation of the manuscript the author discovered that Nathanson and Aub (J. Clin. Endocrinol., 1943, *3*, 321, reported studies on six children (4 to 8 years of age) with "abnormally early adolescence." The levels of 17-ketosteroid excretion (from 3.5 to 14.9 mg. per 24 hours) tended to approach values found in older children and in adults. The amounts excreted appeared to be more directly related to the physical and skeletal maturity of the patients than to their chronological age.

The age of these patients ranged from 3.5 to 11 years, and 17-ketosteroid excretions ranged from 1.4 to 6 mg. per 24 hours. Children with homologous, precocious development associated with intracranial lesions resemble these cases of "constitutional" precosity in many respects. Although precise data are not available, it is likely that the excretion of steroids is quite similar in both groups.

Male hypogonadism. Herein will be considered those patients who show clinical evidences of deficiency of testicular androgen, i.e., those classified as eunuchs or as eunuchoids; patients with failure of gametogenesis only will not be considered. Many difficulties arise when one endeavors to interpret 17-ketosteroid excretion in conditions associated with failure of testicular androgen elaboration. Except in castration or when dealing with distinctly local testicular disease the origin of the failure is frequently in doubt. The genesis of the failure is of utmost importance before interpretation of hormone assay can be made. In individual cases it is frequently difficult to distinguish a "primary" failure (e.g., a genetic defect in the germ plasm or other) from a "secondary" failure (e.g., anterior pituitary failure from any cause; past or present nutritional deficiencies, etc.). Also a deficiency that originates prepubertally would not be expected to produce the same clinical or laboratory picture as would one that originates post-pubertally. Gonadal failure consequent upon pituitary or even other general diseases is frequently associated with altered function of other endocrine glands (e.g., the adrenal cortex or thyroid). In such cases an altered 17-ketosteroid excretion could not accurately reflect the functional status of the interstitial cell elements of the testis, since adrenal and even thyroid disorders may profoundly alter the excretion of steroids (see later discussion). Normally one-third of the 17-ketosteroids is derived from the male gonad and two-thirds from the adrenal cortex. A slight increase in the elaboration of androgen by the adrenal, or in some cases no change in adrenal cortical function, could lead to levels of steroid excretion that are well within the normal range in spite of failure of elaboration of gonadal androgen. Hence, a normal value would not rule out failure of androgen elaboration by the testicle nor would a low value prove a primary gonadal failure. Since puberty is not reached until the sixteenth year in some normal boys, a diagnosis of "hypogonadism" or "eunuchoidism" is difficult before this age. If one determines the 17-ketosteroid excretions in patients with clinically evident testicular failure, regardless of the genesis of the condition, values will be obtained which will range from below normal to high normal. For example, Werner[243] reported values ranging from

2.6 mg. to 14.6 mg. in 16 "eunuchoid" patients; two-thirds of the patients excreted amounts within his range of normal (7 to 17 mg. per 24 hours). These results may be considered to be representative. Clearly, every patient that reveals evidences of testicular failure (regression or lack of development of secondary sexual characters, lack of libido, impotence, testicular atrophy) must be studied individually and no single hormone assay can be relied upon uniformly to indicate the origin of the disorder.

Acromegaly. It is interesting to note in Table 15 that of 15 cases of acromegaly, probably 5 and certainly 3 showed elevated 17-ketosteroid excretions. However, usually the 17-ketosteroid excretion was normal and occasionally even slightly below normal. Eosinophilic adenomas of the pituitary with acromegaly are frequently associated with pathological lesions of other endocrine glands.[131] Nodular or diffuse goiter, hyperplasia of the parathyroids or thymus, degenerations of the pancreatic islet cells, adrenal cortical hyperplasia, and various degrees of degenerative change in the male and female gonads have been described. Unfortunately, clinical and pathological data are not available on the above 15 cases of acromegaly in whom steroid excretions were measured. It is not possible to form any opinion as to the genesis or significance of the reportedly high or low values.

Conditions associated with decreased amounts of steroids in urine

In Tables 8, 9, and 15 are listed various clinical states associated with low 17-ketosteroid and "corticosteroid" excretions. Since these steroids are mainly derived from the adrenal cortex and since normal adrenal cortical function cannot exist in the presence of anterior pituitary failure, it would be expected that the excretion of these substances would be diminished in conditions associated with destruction or atrophy of the adrenal cortex or pituitary. It is noted in Table 15 that consistently low values for the 17-ketosteroid excretion are observed in patients with Addison's disease and also in those with anterior pituitary insufficiency; values below 1 mg. per 24 hours are frequently seen. Since a small portion of these steroids is derived from the male gonad, the lowest values are observed in female patients with Addison's disease and in patients with destruction of the pituitary. There is some discrepancy in the results reported by different investigators, e.g., Fraser et al. consistently obtained lower values than did Callow or Venning et al. These variations are due to slight differences in the technics employed in performing the chemical assay. Fraser and co-workers used the technic of Callow but compensated for the presence of interfering

Table 15

SOME REPRESENTATIVE DATA CONCERNING THE 17-KETOSTEROID EXCRETION IN ACROMEGALY, ANTERIOR PITUITARY INSUFFICIENCY, MYXEDEMA, ADDISON'S DISEASE AND ANOREXIA NERVOSA

Author and case	*17-ketosteroid excretion in mgm. per 24 hours*	*Remarks*
I. Acromegaly (15 cases)		
Fraser et al.,[73] case 59	2.9 (av.)	Female, age 43, symptoms 15 yrs.
Fraser et al.,[73] case 62	3.6 (av.)	Female, age 41
Fraser et al.,[73] case 58	4.5 (av.)	Female, age 28, symptoms 1 yr.
Fraser et al.,[73] case 63	8.3 (av.)	Male, age 31, symptoms 10 yrs.
Fraser et al.,[73] case 61	10.2 (av.)	Female, age 35, symptoms 10 yrs.
Fraser et al.,[73] case 60	10.6 (av.)	Female, age 36, symptoms 3+ yrs.
Venning et al.,[232] case 23	11.8	Female, age 32, symptoms 3 yrs.
Venning et al.,[232] case 22	12.7	Female, age 54, symptoms 15 yrs.
Warren,[239] case 138	15.1	Female, age 26, "early acromegaly."
Warren,[239] case 132	20.4	Female, age 30, "acromegalic."
Venning et al.,[232] case 24	24.0, 26.3	Male, age 39, symptoms 14+ yrs.
Venning et al.,[232] case 25	28.6, 37.6	Male, age 28, symptoms 2 yrs.
Warren,[239] case 110	38.6	Male, age 34, "acromegaly."
Scowen and Warren[203]	70.0	Female, age ?, "acromegaly."
Scowen and Warren[203]	110.0	Male, age ?, "acromegaly."
II. Anterior Pituitary Insufficiency (35 cases)		
Fraser et al.,[73] 15 cases	Range 0.5-2.8	13 of the 15 averaged less than 0.5 mgm.
Fraser and Smith,[74] 10 cases	Range 0.0-1.5	9 of the 10 cases gave zero values.
Venning and Browne,[232] 5 cases	Range 1.4-4.5	
Friedgood,[75] 5 cases	0.5-2.5	
III. Myxedema: Spontaneous (21 cases)		
Fraser et al.,[73] 4 cases	Range 0.6-1.6	All females
Fraser and Smith,[74] 3 cases	Range 0.0-1.7	All females
Engstrom and Mason,[62] 6 cases	Range 1.2-3.3	5 females, 1 male
Friedgood,[75] 8 cases	Range 0.0-2.3	All females
Myxedema: Secondary to thyroidectomy or thyroiditis (9 cases)		
Engstrom and Mason,[62] 4 cases	Range 2.5-6.7	3 females, 1 male (17-KS, 6.7)
Friedgood,[75] 5 cases	Range 1.7-5.8	All females
IV. Addison's Disease: Females (14 cases)		
Fraser et al.,[73] 5 cases	Range 0.5 (all cases)	
Venning et al.,[232] 3 cases	Range 1.9-6.8	
Friedgood,[75] 2 cases	Average 1.0	
Callow et al.,[38]	Range 3.1-7.5	
Addison's Disease: Males (10 cases)		
Fraser et al.,[73] 3 cases	Range 2.1-3.5	
Venning et al.,[232] 1 case	Range 2.3-3.8	
Friedgood,[75] 3 cases	Range 0.0-3.0	
Callow et al.,[38] 3 cases	Range 3.5-7.5	
V. Anorexia Nervosa (severe, chronic malnutrition): all females (12 cases)		
Fraser et al.,[73] 5 cases	Range 2.3- 7.2	(Ages 20-31)
Fraser and Smith,[74] 4 cases	Range 2.7-14.7	(Ages 20-27)
Venning and Browne,[232] 3 cases	Range 3.5- 7.2	(Ages 24-37)

chromogens by using a correction factor. Callow recognized that many of his values were "too high" since atypical colors developed in certain crude extracts. Venning et al. applied a correction equation to the aqueous-alcohol technic of Holtorff and Koch[104] and hence higher values would be expected to result.[62] Engstrom and Mason employed the method of Fraser et al. These differences in technic are not readily apparent when dealing with values that are within or above the normal adult levels but become obvious when dealing with low levels of steroid excretion.

States of severe, chronic malnutrition as seen in anorexia nervosa resemble anterior pituitary insufficiency in many physiological respects, e.g., anorexia, weakness, hypometabolism,[146] cessation of normal gonadal function,[24] diminution of the usual alimentary hyperglycemia induced by ingestion of carbohydrate,[194, 207] etc. There is experimental evidence that inanition leads to lowered gonadotropic and adrenocorticotropic activity of the anterior pituitary.[160, 167, 169] As might be anticipated the 17-ketosteroid excretion is diminished in anorexia nervosa, but in general the observed values are not so low as those seen in patients with grossly destructive pituitary lesions. A functional pituitary insufficiency as a physiologic response to the abnormal nutritional state has been suggested as the most likely cause of many of the observed phenomena in anorexia nervosa.[169, 207]

The observation that myxedema, spontaneous or secondary, is associated with extremely low levels of 17-ketosteroids is a curious finding and the mechanism of this association has not been elucidated. A low "corticosteroid" excretion also has been reported.[216] In Table 15 it is noted that the steroid excretion is very low (frequently below 2 mg.) and comparable in amount to that seen in patients with deficiencies of pituitary or adrenal cortical secretions. The myxedematous state is a profound, generalized metabolic disturbance and it is conceivable that adrenal cortical function could be sufficiently altered, as a part of the general disorder, so that a deficiency of steroid elaboration would result. If this were the case replacement therapy with desiccated thyroid should restore the excretion of steroids to normal levels. This seems to have occurred in a few cases of secondary myxedema (i.e., myxedema following thyroidectomy or thyroiditis) whose symptoms were of short duration;[62] however, replacement therapy has not been shown definitely to alter the low levels of excretion in patients with spontaneous myxedema or with secondary myxedema of long duration.[62, 75] To explain adequately this phenomenon would necessitate an understanding

of the pathogenesis of spontaneous myxedema and an understanding of many subtle effects of chronic deficiency of the thyroid hormone on the pituitary and other tissues. A theoretical consideration of some of these factors is reviewed by Engstrom and Mason,[62] and by Friedgood.[75]

A variety of chronic illnesses (the postoperative state, infections, diabetes, thyrotoxicosis, sprue, etc.) usually leads to a moderate lowering of the 17-ketosteroid excretion.[68, 73] No explanation thus far offered has been entirely satisfactory. The decreased excretion of these steroids may be related to an associated starvation and the mechanisms of its development may be similar to those involved in more severe states of malnutrition (e.g., anorexia nervosa). Albright and his associates[68] have suggested a diminished androgen production by the adrenal cortex as a manifestation of the "adaptation phenomenon" of Selye.[205] However, it is still possible that the precursors of these urinary steroids are elaborated at a normal or even at an increased rate during chronic stresses, but their transformations are diverted to channels other than the urinary 17-ketosteroids. Without more information on the utilization of steroids and on the factors concerned with their transformation and excretion it is premature to imply that increased or decreased steroid excretion, respectively, means increased or decreased elaboration of precursor steroids under all circumstances.

Summary

In this review the nature and amounts of the neutral steroid substances found in urine in certain abnormal clinical states are summarized from the literature through 1947. To appraise the significance of these data necessitated a brief, preliminary consideration of a variety of biochemical data and biological concepts. The chemical nature, biological actions, and possible transformations of the adrenal cortical and testicular hormones were considered so far as this was possible for man. A number of factors (environmental, nutritional, nervous, genetic, and maturity) normally appear to be concerned with the control of the extent of elaboration of hormones. In certain instances the same factors appear to influence the nature of the biological response to hormones. Although data would indicate that the neutral urinary steroids arise from hormones elaborated by the adrenal cortex and gonads, it is apparent that little is known concerning the factors which influence the transformations of precursor steroids to excretory products in man. It is not known whether the transformations that occur are concerned

with hormone utilization or inactivation. In view of the uncertainties involved, little effort was made, except in a few instances, to interpret the genesis of certain clinical states in terms of the known actions of the adrenal or gonadal hormones or in terms of the levels of excretion of steroids. What efforts were made in this regard, were made to point out the variables involved in such considerations. Most emphasis has been placed on the variableness of the quantities of and on the nature of the excreted steroids encountered in different individuals with similar clinical conditions and on the biological factors which might cause differences in results in different clinical states. Only with this knowledge can methods of assay be utilized and applied as diagnostic or investiga tive tools.

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