The Clinical Significance of Urinary 17-Ketosteroid Assays

GERSON R. BISKIND, M.D., San Francisco

SUMMARY
The urinary 17-ketosteroids are a group of compounds derived from complex steroids produced by the adrenal cortex, testis, or ovary. The method of determining the amount excreted has been simplified so that it is available for routine diagnostic purposes. Usually the amount is increased in diseases in which there is hyperfunction of the adrenal cortex due to tumor or hyperplasia, and decreased in lesions that impair the function of the adrenal cortex.

Other conditions such as myxedema, eunuchism, gout, and arthritis may alter the excretion of the 17-ketosteroids. Low levels are also found in the young and in the aged. Case histories are presented to illustrate the findings in the following diseases: Tumors of the adrenal cortex with (a) masculinization, (b) Cushing's syndrome with virilism and, (c) hirsutism; as well as in gigantism with acromegaly, in gout, eunuchism, Addison's disease, myxedema, and severe panhypopituitarism.

RECENT progress in the understanding of steroid metabolism has made the assay of urinary neutral 17-ketosteroids an important test in the evaluation of certain clinical conditions related to the endocrine system. This review will attempt to analyze briefly the current concepts concerning the origin, nature, and significance of these compounds, and will illustrate by means of case reports most of the conditions in which there is a definite abnormality in the 17-ketosteroid excretion.

The steroids present in the urine can be divided into neutral, phenolic, and acidic compounds (Diagram No. 1). The acidic steroids represent the bile acids; the phenolic are mainly the estrogens. The neutral steroids contain metabolites from the testes or ovaries and from the adrenals. There are four important groups of compounds present in the adrenal cortex that give rise to neutral steroids in the urine. These are: (1) androgenic substances, (2) progesterone, (3) desoxycorticosterone, (4) corticosterone. The testicular hormone, testosterone, is the precursor of some of the neutral 17-ketosteroids in the urine.

The neutral steroids in the urine can be subdivided into the ketonic and the nonketonic fractions. The latter are relatively small in quantity and are usually derivatives of progesterone and desoxycorticosterone. The more important ketonic fraction is composed of the following: Isoandrosterone from the testis, dehydroisoandrosterone from the adrenal cortex, and androsterone and other complex steroids that are metabolites from both adrenal cortex and testis.

The basic principles underlying the assay of neutral urinary 17-ketosteroids are not complex. However, the actual technique is moderately involved and requires careful attention to details. The steroid metabolites in the urine are rapidly converted by hydrolysis to the free compounds by boiling with a strong inorganic acid. The steroids are extracted from the urine by means of an organic solvent. In some techniques, hydrolysis and extraction may be performed simultaneously. The estrogens are removed from the extract by washing with a weak alkali. The remaining extract is dried, dissolved in alcohol, and assayed by reacting it with m-dinitrobenzene. The red color resulting from this reaction is compared with the color produced by a known amount of dehydroisoandrosterone. This determines the total neutral 17-ketosteroid content of the urine. Instead of the chemical reaction, a biologic test for androgenic activity may be substituted; however, the neutral 17-ketosteroids include substances that are not androgens, so that a direct correlation between androgenic activity and 17-ketosteroid content is not always evident. If more accurate determinations are required, the extract is treated with Girard's reagent T to separate the ketonic from the nonketonic fractions. In the normal individual the amount of nonketonic material is not sufficient to require this complex procedure. The total ketonic portion can be measured by means of the m-dinitrobenzene reaction, or it can be treated with digitonin to separate the alpha from the beta compounds. Androsterone represents the major portion of the non-precipitable alpha 17-ketosteroids. The principal component of the digitonin-precipitable beta 17-ketosteroids is dehydroisoandrosterone, and it is derived only from the adrenal cortex. In urine from normal individuals the beta fraction rarely exceeds 10 per cent of the total amount of the ketonic ketosteroids. In males the hormones of the testis give rise to about one-fifth, and the hormones
of the adrenal cortex to most of the rest of the 17-ketosteroid excretion. In females most of the 17-ketosteroids are derived from the hormones of the adrenal cortex.

The values for the 17-ketosteroids assayed in 24-hour urine specimens, obtained from adult normal individuals, fall within the following ranges: Males, 8 to 22 mg.; females, 6 to 13 mg. The amounts in children are variable. Up to two or three years of age none is present; from 3 to 8 years there may be as much as 2 mg.; from 8 to 12 years there are between 1.5 and 5 mg.; from 12 to 16 years the amount varies between 4.5 and 9.5 mg.

Increased amounts of 17-ketosteroids in the urine may be found in certain endocrine diseases. The most important and most common is the functioning tumor of the adrenal cortex. These tumors produce a variety of clinical syndromes that have been classified by Kenyon into: (1) the adenogenital syndrome; (2) Cushing's syndrome; (3) mixed clinical pictures having features common to the first two; (4) isolated expressions of the neoplasm; (5) feminization in adult males and rarely in children; (6) tumors without endocrine manifestations.

In instances of adrenal tumor with clinical evidence of intense masculinization, the 24-hour 17-ketosteroid excretion varied from 76 to 857 mg., and, where measured, the beta fraction varied between 30 and 79 per cent. In the reported cases, ten of the patients were females and one was a male. The following case report lists an additional instance of typical masculinizing tumor of the adrenal cortex with an extremely high 17-ketosteroid excretion in which the beta fraction represented 25 per cent.

CASE REPORT

Case 1: The patient was a white female, 63 years of age, with clinical diagnosis of masculinizing tumor of the right adrenal gland. Virilization had begun 30 years previously, after the last pregnancy, characterized by increase in growth of hair on face and body, baldness, amenorrhea, muscular shoulders and arms, and enlargement of the clitoris to three times normal size. The systolic blood pressure was 200 mm. of mercury, the diastolic, 100 mm. The glucose tolerance test revealed the following blood sugar levels in mg. per 100 cc.: Fasting, 95; 30 minutes, 200; 60 minutes, 258; 120 minutes, 285; and 180 minutes, 219. Intravenous pyelograms showed no abnormality. A large encapsulated adenoma, 17.5 by 12 by 8.5 cm., that had replaced the right adrenal gland was removed on December 8, 1948, after inspection of the normal left adrenal gland and the atrophic pelvic organs. The following assays were performed:

Oct. 27, 1948: Total neutral 17-ketosteroids, 455.7 mg. per 24 hours.

* Courtesy of Roberto F. Escamilla, M.D.
Nov. 5, 1948: Total neutral 17-ketosteroids, 661.0 mg. per 24 hours.
Nov. 5, 1948: Total ketonic 17-ketosteroids, 663.0 mg. per 24 hours.
Alpha fraction, 571.0 mg.
Beta fraction, 170.0 mg. (25%)
Dec. 17, 1948: Total neutral 17-ketosteroids, 15.8 mg. per 24 hours.
Jan. 19, 1949: Total neutral 17-ketosteroids, 9.1 mg. per 24 hours.
Feb. 18, 1949: Total neutral 17-ketosteroids, 10.0 mg. per 24 hours.
Mar. 25, 1949: Total neutral 17-ketosteroids, 5.6 mg. per 24 hours.

In another case of masculinizing tumor of the adrenal cortex, the 17-ketosteroid excretion was elevated but not to such high levels:

**CASE REPORT***

Case 2: The patient, a white female 63 years of age, had a clinical diagnosis of masculinizing tumor of the left adrenal gland. In the preceding five years the patient had become b Ian with a masculine distribution of facial and body hair, and masculine musculature. A carcinoma of the right breast had been removed in 1944. At that time the blood pressure varied between 230 and 260 mm., of mercury systolic, and 125 and 142, diastolic. A 68-gram tumor was removed from the left adrenal gland on May 28, 1948. Subsequently the body hair slowly disappeared. The blood pressure remained elevated and at present there is evidence of hypertensive heart disease, early congestive failure, with fatigue and dyspnea, but no evidence of masculinization. The 24-hour total neutral 17-ketosteroid outputs were: May 15, 1948, 90 mg.; June 14, 1948, 1.5 mg.; April 18, 1949, 11.4 mg.

There are published reports of cases of tumors of the adrenal cortex that produced the Cushing syndrome and practically no virilism. In these cases the ketosteroid excretion paralleled that in the first group, varying from 19.9 to 800 mg., but in seven of the 13 cases reported, it was less than 75 mg. In three of the cases the beta fraction was less than 22 per cent of the total, but in two other cases the percentage was 65 and 69.

There are 23 published reports of cases of tumor of the adrenal cortex in which there were masculinization and manifestations of Cushing’s syndrome, and in these cases the excretion of urinary 17-ketosteroids was extremely variable but always above normal. The following is a case that may be classified in this group:

**CASE REPORT†**

Case 3: The patient was a white female, 39 years of age. The clinical diagnosis was Cushing’s syndrome with virilism due to adrenal tumor. The following signs and symptoms were evident: extreme weakness of legs, moon face, phtiehic countenance, exophthalmos, disproportionately thin extremities without obesity, easy bruising, amenorrhea, hirsutism, acne, hypertension (the blood pressure was 180 mm. of mercury systolic and 120 mm. diastolic), generalized osteoporosis, diabetic reaction to a glucose tolerance test, high 11-oxyestrogens (2.92 mg.), and low cosinophil count. The total neutral 17-ketosteroid excretion was 62.4 mg. in 24 hours. The intravenous pyelograms showed no abnormalities, but perirenal gaseous insufflation revealed a tumor over the left kidney. The patient died on the fifth day of cardiac failure and pulmonary edema after surgical removal of an encapsulated tumor in the left adrenal.

There are instances of Cushings’s syndrome that have been proved not to be due to a tumor of the adrenal cortex, and in those instances the 17-ketosteroid excretion varied between 7 and 37 mg. In at least half of the cases the amount extracted was within normal limits. In a few cases where the beta fraction was determined, it was 14 per cent or less.

The assay of 17-ketosteroids offers some help in the differentiation of tumor from hyperplasia of the adrenal cortex. In the latter condition in females, there may be prepubertal virilism, often associated with pseudohemaphroditism or sex inversion, or postpubertal virilism that is characterized mainly by hirsutism. In the females in whom hirsutism develops after puberty, the 17-ketosteroid excretion is normal or does not exceed 37 mg. in 24 hours. In the prepubertal cases, an increased excretion is more common, but it is usually under 100 mg. and the beta fraction is less than 23 per cent in 24 hours. In cases of tumor of the adrenal cortex with virilizing signs the excretion of 17-ketosteroids is often more than 100 mg. in 24 hours and the beta fraction is over 20 per cent. If in the presence of virilism there are, in addition, elements of the Cushing syndrome, there is usually a definite increase in the cortin content of the urine. In the following case, hirsutism that appeared in a girl at the age of 11 was the single endocrine expression of an adrenal tumor:

**CASE REPORT‡**

Case 4: The patient was an Indian female, 28 years of age. The clinical diagnosis was hirsutism due to malignant adenoma of the left adrenal gland. The patient said that she had had excessive hair on the body since she was 11 years of age. An increased amount of hair over the chest, legs, and face, and a masculine distribution of pubic hair on the abdomen were noted. The voice was normal. The systolic blood pressure was 120 mm. of mercury, the diastolic 84 mm. Roentgenographic studies suggested a mass in the region of the left adrenal gland. The basal metabolic rate was plus 5 per cent. A malignant adenoma of the adrenal gland was removed Aug. 7, 1947. There has been relatively little change in the amount of body hair since the operation. Results of a series of total neutral 17-ketosteroid assays of 24-hour urine specimens were: May 7, 1947, 82.1 mg.; May 10, 62.2 mg.; September 11, 1947, one month after surgical removal of a malignant adenoma of the adrenal gland, 6.3 mg.; November 14, 10.2 mg.; February 4, 1948, 13.5 mg.; April 3, 19.2 mg.; August 10, 8.6 mg.

There are reports of nine cases of virilizing tumors of the ovary. In three of the cases the tumors were arrenoblastomas, in three they were adenal-like tumors, and in the other two tumors were not described. The patients that had arrenoblastomas excreted 4.0 mg., 6.9 mg., and 56 mg. of 17-ketosteroids in 24 hours; the patients with adrenal-like **

*Courtesy of Kendall B. Holmes, M.D., Fresno.
†Courtesy of Minnie Goldberg, M.D.
‡Courtesy of Hana Lissner, M.D.
ovarian tumors excreted 17 mg., 23.8 mg., and 54.6 mg. In the other three cases excretion levels were 40 mg., 116 mg., and 158 mg. The excretion was measured after operation in four cases and in each it dropped to normal or subnormal levels.

There may be a subnormal, normal, or increased excretion of 17-ketosteroids in cases of acromegaly. The following case report illustrates a combination of gigantism with early acromegaly:

CASE REPORT*

Case 5: The patient was a white male, 41 years of age. The clinical diagnosis was eunuchoid gigantism with features of acromegaly. The height of the patient was 87 inches, the span 92 inches, and the weight 342 pounds. Roentgenograms revealed prognathism and slight tufiting of the ends of the phalanges. There was atrophy of the testes. The 24-hour excretion of total neutral 17-ketosteroids was 9.2 mg.

The possible relationship of some diseases of the joints to the endocrine system has been suggested by the 17-ketosteroid excretion levels in gout and in spondylarthritides. A recently published report has brought out the fact that the 17-ketosteroid excretion is diminished in gout. This has been confirmed in one case:

CASE REPORT†

Case 6: The patient, a 31-year-old white male, with known polycythemia, had a first attack of gout on October 6, 1948. The blood uric acid concentration was 12.6 mg. per 100 cc. The patient was treated with colchicine. On October 26, 1948, neutral 17-ketosteroid excretion in the urine in a 24-hour period was 6.9 mg. Several attacks of gout occurred in the succeeding six months, and during one of these urate crystals were demonstrated in tophi.

It has been reported that in 31 cases of spondylarthritides in males the excretion of neutral 17-ketosteroids varied from 10 to 43.5 mg. The average was 26.7 mg. In four females with this disease outputs were 19.5 mg., 40 mg., 23 mg., and 16 mg.†

There is a significantly lowered excretion of 17-ketosteroids in the young and in the aged, as well as in persons with malnutrition, anemia, and chronic debilitating disease. Severe physical stress produces an increased excretion that may be followed by a diminished output. Acute trauma such as is produced by fractures, surgical operation, or the onset of acute febrile disease may be followed for a short time by a rise in 17-ketosteroid excretion. In some instances of chronic hepatic insufficiency the level is decreased. The administration of purified adrenocorticotropic hormone to normal males and females induces a distinct increase in the 17-ketosteroid excretion. In hypogonadal males usually there is a diminished excretion as is shown in the following report:

CASE REPORT‡

Case 7: The patient, a white male, 55 years of age, had been surgically castrated following severe trauma at age 22. Since then he had had decreased libido, diminution in amount of beard, axillary and pubic hair, and loss of strength. Examination revealed recently acquired obesity, gynecomastia, pigmentation of the face with wrinkling of the skin, and normal blood pressure. The basal metabolic rate was zero, the cholesterol content of the blood serum was 222 mg. per 100 cc. The total neutral 17-ketosteroid excretion on two examinations was 5.3 mg. and 4.6 mg. in 24 hours.

There are three outstanding diseases in which there is a decreased excretion, often severe, of 17-ketosteroids. These are anterior pituitary insufficiency, Addison’s disease, and myxedema. The 17-ketosteroid output in the presence of these diseases is usually less than 6 mg., the lowest levels occurring in the first two. In some instances urinary gonadotropin assays are required to distinguish between cases of primary hypophyseal disease with features of adrenal insufficiency and those of primary adrenal failure. Treatment of myxedema with desiccated thyroid usually does not elevate the subnormal excretion of 17-ketosteroids, nor does the maintenance of a patient in good clinical condition with Addison’s disease by means of desoxycorticosterone and adrenal cortex extract.

The following case reports illustrate the clinical and laboratory findings in typical instances of these three diseases.

CASE REPORTS

Case 8:* The patient was a white male, aged 42 years. The clinical diagnosis was panhypopituitarism: “burnt-out” acromegaly with hypogonadism, myxedema and severe Addison’s disease. The patient entered the hospital in Addisonian crisis. Roentgenographic examination showed a large sella, tufiting of the terminal phalanges and prognathism. The testes were atrophic, and biopsy revealed tubular degeneration. The prostate was minute. The skin of the face was fawn-colored. The basal metabolic rate was minus 24 per cent. Urinary excretion of the follicle-stimulating hormone was less than 5 mouse units in 24 hours. The urinary excretion of neutral 17-ketosteroids on March 24, 1948, was 2.6 mg. per 24 hours. The patient was treated for the acute Addisonian crisis and finally stabilized on thyroid extract, testosterone propionate and 4 mg. of desoxycorticosterone acetate daily. On this regimen he returned to lumen-jacking. The total neutral 17-ketosteroid excretion on April 29, 1949, was 2.1 mg per 24 hours.

Case 9:† The patient, a white male, 49 years of age, with a clinical diagnosis of tuberculosis of the adrenal glands, entered the hospital in Addisonian crisis. The Robinson-Power-Kepler tests No. 1 a.d No. 2 showed positive results. The urinary excretion of neutral 17-ketosteroids on Dec. 7, 1948, was 1.2 mg. in 24 hours. The patient was treated in the usual manner and stabilized on salt and desoxycorticosterone acetate. After he had resumed normal physical activity, the 17-ketosteroid assay on February 7, 1949, was 4.2 mg. in 24 hours. He died unexpectedly and autopsy revealed bilateral caseous tuberculosis of the adrenal glands; direct extension of the tuberculous process from the left adrenal to the spleen, with miliary dissemination in the liver.

Case 10:‡ The patient was a white male, 67 years of age, with clinical diagnosis of myxedema. He complained of extreme fatigue for the previous six months, coldness of the hands and feet, pain in the hands associated with stiffness, and a gain in weight of 20 pounds. On examination the skin

* Courtesy of Drs. Sanford B. Leeds and Morton J. Nyda.
† Courtesy of Harris M. Fishbon, M.D.
‡ Courtesy of Myron Arrick, M.D.
* Courtesy Veterans Administration Hospital, Ft. Miley, San Francisco.
† Courtesy of Russell F. Rypins, M.D.
‡ Courtesy of Roberto F. Escamilla, M.D.
was dry, the face was puffy and pale, there was swelling of the hands and feet, and the tongue was thick. The basal metabolic rate was minus 41 per cent. The cholesterol content of the blood serum was 333 mg. per cent, and the iodine level of the blood was 3.2 micrograms per cent. The total neutral 17-ketosteroid excretion was 4.8 mg. in 24 hours.

REFERENCES