URINARY EXCRETION OF TESTOSTERONE IN IDIOPATHIC HIRSUTISM

by

Karoon Mansuwan, M.D.

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Department of Investigative Medicine,
McGill University,
Montreal, Canada.

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I. INTRODUCTION

There is a lack of understanding of simple or idiopathic hirsutism concerning both the etiology and the treatment. Even the nature of normal hair growth itself is not completely clarified. Hirsutism has been reported in a variety of conditions. It may be associated with defects of the pituitary gland, the adrenal gland, the ovary, and the thyroid gland, with genetic defects, central nervous system defects, and other miscellaneous conditions, e.g. pregnancy, burns, anorexia nervosa, dermatomyositis, treatment with certain drugs, thymic tumor, mumps, teratomata (Brookbank review, 1961).

The occurrence of hirsutism may be purely coincidental in some of these cases. Very little is known about the relation of the nervous system to hair growth. Genetic factors seem to be important for the growth of body hair, but whether their influence is a direct one on the hair follicles or mediated by control over the metabolism of hormones which in turn affect hair growth is still a question. The frequent finding of hirsutism with a defect in endocrine glands has led to the study of the hormonal status in this condition, particularly with regard to androgenic hormones. However, the indefinite pattern of metabolites of androgenic steroid in both urine and blood in this condition makes it necessary to search for a more reliable index of effective circulating androgen. Testosterone, which is the most potent, naturally occurring androgen, has become the centre of investigation in recent years. Few methods have been developed for measurement of testosterone in blood and

in urine. The following work deals with methodology and results of measurement of testosterone excretion in idiopathic hirsutism, as well as in normal subjects.

II. HISTORICAL INTRODUCTION

A. DEFINITION

Hirsutism is an excessive growth of body hair with characteristic masculine distribution. It is one of several signs of virilism which occur in the case of overactivity of androgen-secreting cells. A condition in which hirsutism appears without any demonstrable etiology is called idiopathic or simple hirsutism. This may sometimes be associated with an increase of body hair in general, menstrual disturbances, obesity, and acne. Women with idiopathic hirsutism are usually fertile.

James et al (1962) selected their patients for the study of steroid excretion patterns by the abnormal growth of facial hair, especially in the chin and moustache areas.

An excessive hair growth means an increase in the number of terminal hairs; the actual number of hair follicles in the skin still remains the same. The transformation of small vellus hairs to big terminal hairs which are easily recognized, results in the impression of excessive hairs.

There is no definite dividing line between normal and abnormal hair growth. Attempts have been made to assess body hair by dividing the body into different areas and making a "hair score" of each area (Garn, 1951, Shah, 1957, Ferriman and Gallwey, 1961, McKnight, 1964). The principal of these methods is based on the method of Garn. Shah (1957) modified it by taking into account not only the number and density of hairs, but also the quality on palpation. By this evaluation, it is

possible to exclude the patients who have generalized hairiness or hypertrichosis from those who are hirsute. Nevertheless, these methods are not generally used in actual practice. The hirsutism of women is usually judged by examining the hair growth in characteristic male areas, e.g. face, chest, back.

B. NATURE OF HAIR

1. CLASSIFICATION

Danforth (1939) classified body hair into 3 types:

(1) Vellus hair or soft hair in general.

(2) Ambosexual hair, similar in the two sexes and dependent on endocrine factors, i.e. axillary and pubic hair.

(3) Hair which constitutes a secondary sexual characteristic, present only in the male, i.e. beard, chest, abdomen, shoulder.

Another hormonal classification was offered by Garn (1951) in his study of the type and distribution of the hairs in man:

(1) Hair not dependent upon steroid hormones, but which can be influenced by growth hormone and sometimes is inhibited by androgenic hormone, i.e. head hair, eyelash, eyebrow, mid-phalangeal hair, hair on the distal segment of limbs, and to some extent, hair on the lumbodorsal triangle.

(2) Hair which is dependent on female steroid hormones, i.e. pubic, axillary, limb, and to a lesser extent, hypogastric area.

(3) Hair which is dependent on male steroid hormones, i.e. beard, moustache, ear, body hair, especially chest and back.

There is no definite evidence of the control of hair growth except in some areas, and even so, there may be more than one factor involved. Hamilton (1958) stated that although growth of axillary hair and beard was dependent upon testicular and ovarian secretion, genetic factors also had some influence.

The best classification for body hair at the present time is a morphological classification. The hair is divided into two types:

(1) Terminal hair: big, coarse, and containing much melanin pigment.

(2) Vellus hair: small, soft, and colorless.

The length alone cannot be used to classify the types of hair because there is variation in different areas. The vellus hair of the scalp may be longer than terminal hair of the back, for example.

2. HAIR FOLLICLE

The total number of hair follicles in the epidermis is constant from childhood on. However, "dilution" occurs with the expansion of body surface area. Moreover, there is no significant difference in the number of hair follicles in the skin of men and women (Szábo, 1958).

Kligman and Strauss (1956) reported that the vellus hair follicles can be newly formed on the face of an adult human. This situation has also been reported in mice (Lacassagne and Latarjet, 1946), in rats (Taylor, 1949, Butcher, 1959), and in rabbits (Breedis, 1954, Billingham and Russel, 1956, Billingham, 1958). Straile (1959) and Montagna (1962), however, do not accept this concept.

During its life cycle, a hair follicle can produce more than one type of hair. In the human, the follicles which in preadolescent year give rise to small vellus hairs, will produce terminal hairs after puberty. A change in the type of hair produced by given follicles has also been shown in castrated male and female hamsters. After giving testosterone, both locally and systemically, the hairs in the pigmented costo-vertebral spot, which is a secondary male characteristic in the hamster, change from the vellus type to the large melanin-containing type as in the adult male hamster. After the treatment is stopped, the growth declines (Hamilton and Montagna, 1950).

3. HAIR GROWTH

Hair growth is a cyclic phenomenon. There is a distinct histological change in the hair follicle during the growing and resting periods. In the human, each hair follicle has its own cycle independent of that of nearby follicles (Montagna, 1962). This is also true in the guinea pig (Butcher, 1934, 1951, Chase and Eaton, 1959). The periods of activity and quiescence differ in different parts of the body, different individuals, at different ages, and in the two sexes. During the growing stage, there will be numerous mitosis in the matrix of hair follicles and growth of hair is the result of this proliferation. Mitotic activity stops in the resting stage; the follicle becomes smaller and shorter. When a new hair is formed, it either dislodges the full grown hair, which is called a club hair, or grows alongside it. Sometimes, a single follicle may form more than one hair (Montagna, 1962).

In the rat and mouse, there is a spontaneous hair growth wave

pattern which usually starts from the ventral side and moves upward to the dorsum. The hair follicles in one area are therefore in the same phase (Butcher, 1934, 1951, Chase and Eaton, 1959). In the growing period in these animals, the subcutaneous fat is clearly 2-3 times thicker than in the resting stage (Durward and Rudall, 1949, Montagna, 1962).

4. RATE OF HAIR GROWTH

The rate of normal hair growth differs in each area in the human (Butcher, 1951, Myer and Hamilton, 1951, Storey and Leblond, 1951).

Chin	0.38 mm./day	Myer and Hamilton, 1951
Axilla	0.30 mm./day	
Thigh	0.20 mm./day	
Eyebrow	0.16 mm./day	
Crown of scalp	0.35 mm./day	

In the mouse, the total growing period is about 19 days and in rat, 21-26 days. Hair grows in both animals at a rate of approximately 1 mm./day (Montagna, 1962, Davis, 1963).

In the guinea pig, the growing stage is 4 weeks; the resting stage is 12 weeks in males and non-pregnant females, and 15 weeks in pregnant females (Dawson, 1930).

C. FACTORS INFLUENCING HAIR GROWTH

1. GENETIC

It is a well known fact that both men and women of certain racial origin have more body hair generally than others. The incidence

of hirsutism in the Caucasian women is much higher than in Mongolian women.

Comparing beard and axillary hair in male twins, related males, Caucasian and Japanese males and females, and in eunuchs, Hamilton (1958) concluded that genetic factors had an important role in controlling hair growth in these two areas. There was no relationship between geographic, climatic, or dietetic factors and the growth of hair.

Genetic factors may determine the steroid pattern in each individual (Gallagher et al, 1958, Bush and Mahesh, 1959). It seems that the urinary level of steroids normally excreted by a particular person will persist through life, whether the level be high, low, or average, relative to others (Savard, 1961).

Recently, evidence has been brought forward that there may be racial differences in "normal" steroid excretion patterns (Bulbrook et al, 1964). Total 17-ketosteroids (17 KS) excretion in Japanese women is about half that of British women, but the difference disappears if the excretion is expressed in terms of creatinine excretion. This has been attributed to differences in body build. The ratio of androsterone to etiocholanolone, however, is significantly higher in Japanese than in English women.

From these evidences, it cannot be concluded at the present time that genetic factors determine activity in the hair follicle locally, or determine the steroid pattern which will then control the hair growth. Androgenic steroid patterns of production and excretion in various races have never been studied. Perhaps differences in these patterns may explain the prevalence of hirsutism in certain groups.

2. PHYSICAL FACTORS

(a) Mechanical

Clipping and shaving have no effect on hair growth in the human being or in animals (Montagna, 1962). Plucking of club hair in rat and mouse initiates new hair growth, but a certain minimal number of hairs is required to produce this response (Durward and Rudall, 1958, Chase and Eaton, 1959). This is true only if the plucking is done in resting phase; otherwise, there will be no acceleration or retardation of hair growth (Butcher, 1959). In the guinea pig, hair growth increases after clipping or plucking, in comparison with the non-traumatized area, but plucking is more effective (Kim et al, 1962). Plucking is also effective in the human (Montagna, 1962).

(b) Chemical

In man, a therapeutic dose of amethopterin causes a transient but reversible injury to the hair bulb of the scalp (Van Scott and Ekel, 1958, Van Scott, 1959). The hair formed during the period of drug administration has a smaller diameter than normal.

In the rat, some antimitotic agents and thallium are able to destroy the hair follicle. On the other hand, some irritants, e.g. benzoic acid, tinc. capsicum, tinc. cantharides, and xylene, can stimulate quiescent hair bud (Butcher, 1940, 1959).

(c) Radiation

In the human scalp, a dose of x-ray enough to cause severe damage to growing follicles, actually initiates growth in quiescent ones (Chase, 1958).

3. NERVOUS CONTROL

The role of the nervous system in hair growth is not known. It has been observed that in children with brain damage, there is a growth of fine hair over the back and arms. The rate of hair growth decreases as the child responds to physiotherapy and retraining (Dratman, 1963).

In the rat, denervation by destruction of segmental nerves to the skin has no effect on hair growth (Durward and Rudall, 1949); but after sectioning of segmental nerves and resectioning of the cervical sympathetic chain in male and female rabbits, the hair grows faster than in the control (Balus, 1961).

4. ENDOCRINE FACTORS

There is evidence, both direct and indirect, that in the human, androgen has an influence on the hair which is characteristic of the male. Most of the studies were done with the facial hair.

Treatment of elderly men with testosterone increases the rate of beard growth (Chieffi, 1949). In eunuchs who had been castrated prior to puberty, the beard fails to grow. After orchidectomy in mature man, there is a regression of beard growth. The growth of facial hair in these two groups can be induced again by giving testosterone systemically (Hamilton, 1958).

In women, hirsutism is one of the features of overactivity of androgen-secreting cells either in the ovary or the adrenal gland, and of treatment with large doses of testosterone.

Maguire (1964) injected testosterone repeatedly, locally over

the chin of white females aged 50-65 and found that 4 out of 5 subjects had terminal hair growth at the injection site after a period of six months to one year.

The pattern of hair loss on the limbs in old people is similar in both sexes, but the female tends to lose more axillary hair than the male and at the same time, there is an increase in hair growth on the upper lip and chin. The dissociation between loss of pubic and axillary hair and the growth of facial hair suggests different determining factors. The decline in hair growth can be due to decreasing hormone levels or decreasing sensitivity of hair follicles (Melick and Taft, 1959). Since the growth of facial hair can be induced by testosterone in both old men and women, it is more likely that a change in hormone level is responsible for the declination of hair growth.

Hirsutism which is associated with pregnancy and disappears post partum has been reported in the past (Stoddard, 1945). Recently, Turunen et al (1964) presented three cases of recurrent hirsutism in pregnancy. The hairs began to fall out a few days after delivery, completely disappeared within a few months, and recurred in successive pregnancies. There was also a high incidence of congenital anomaly among the children born. The steroid excretion pattern was studied in detail in 2 cases. There was an increase of total 17 KS, epiandrosterone and androsterone in both, and of dehydroepiandrosterone (DHA) in one. Etiocholanolone and 11 oxy 17 KS, 17-hydroxycorticosteroids (17 OHCS) and pregnanediol were within normal limits. 17-Ketogenic steroids (17 KGS) were low in one. The author suggested the possibility that a block in 11 hydroxylation resulted in an increased

production of 17αOH progesterone. Furthermore, in one case in which pregnancy was terminated, and the placenta incubated with testosterone, there was evidence of lower 17β dehydrogenase activity than in normal placenta.

Trotter (1935) found no difference in rate of hair growth in pubic, perineal, and lumbar areas in seven women studied during pregnancy.

Hormones other than androgen may have an influence on hair growth. Loss of eyebrow and body hair is commonly observed in severe myxoedema (Segre et al, 1964). Some cases of Addison's disease and panhypopituitarism have absent body hair and patients with anterior pituitary hyperfunction are frequently hairy (Bissel and Williams, 1945).

Hormonal influences on hair growth in animals have been studied mostly in rats and mice. Steroids which show an inhibition of hair growth are oestrogen (Whitaker, 1956, Mohn, 1958, Ebling and Johnson, 1964), cortisone (Baker, 1951, Fukuyama and Baker, 1958, Mohn, 1958, Davis, 1963, Ebling and Johnson, 1964), and epinephrine (Mohn, 1958, Davis, 1963).

There is controversy regarding the effect of androgens. Houssay et al (1959) and Davis (1963) reported an inhibition of hair growth whereas Mohn (1958) and Rennel and Callahan (1959) found no effect except that androgen promoted the sexual difference in hair texture.

Thyroxin stimulates hair growth (Mohn, 1958, Davis, 1963, Ebling and Johnson, 1964). Insulin restores the spontaneous hair

replacement in alloxan diabetic rats (Mohn, 1958, Davis, 1963). Pregnancy and lactation retard hair growth (Mohn, 1958). Gonadectomy, hypophysectomy (Mohn, 1958, Ebling and Johnson, 1964), and adrenalectomy (Whitaker, 1956, Mohn, 1958, Ebling and Johnson, 1964) stimulate hair growth.

Rats which are hypophysectomized before puberty have an infantile type of hair. Oestrogen and androgen will cause an alteration in hair texture only in the presence of growth hormone (Mohn, 1958). For the pubertal maturation of hair, luteotrophic hormone is more important than growth hormone (Rennel and Callahan, 1959).

D. EXCRETION OF HORMONES IN IDIOPATHIC HIRSUTISM

1. TOTAL 17-KETOSTEROIDS

As metabolic products of androgen, secreted from adrenal gland and ovary, 17-ketosteroids (17 KS) have been widely investigated. Twenty five per cent of total 17 KS are products of the ovaries in normal female; 35% of the total are derived from the testes in normal males. In both sexes, the remainder comes from the adrenals (Williams, 1962). Only some of the 17 KS are androgenic. The reported normal values for total 17 KS excretion are very variable; therefore, the comparison between normal and hirsute subjects should be made from the same laboratory.

Dorfman and Shipley (1956) collected the data on 17 KS excretion in idiopathic hirsutism. Although the values ranged from normal to high, the mean was higher than the normal subjects.

Other papers, not quoted by these authors, support this conclusion (Bissel and Williams, 1945, Salter et al, 1946, Escamilla, 1949, Butt et al, 1950, Perloff et al, 1957, 1958, Gilbert-Dreyfus et al, 1958, Prunty et al, 1958, Lipsett and Riter, 1960, Brooksbank, 1961, Martin et al, 1961, James et al, 1962, Mauvais-Jarvis and Baulieu, 1962, Tropea et al, 1962, Gold and Goldberg, 1963, Julesz et al, 1963, Lloyd et al, 1963).

In some cases, total 17 KS are lower than in normal subjects (Bissel and Williams, 1945).

There are reports of distinct variations in total 17 KS in hirsute patients in relation to the menstrual cycle (Koets, 1949, Merivale, 1951, Argüelles et al, 1959, Brooksbank, 1961). In normal subjects, day-to-day variation is small (Kappas and Gallagher, 1955, Vestergaard and Leverett, 1957, Fotherby, 1960), but some show great variability (Vestergaard and Leverett, 1957). There is no clear evidence of variation during the menstrual cycle (Mason and Engstrom, 1950, Dorfman and Shipley, 1956, Gold and Goldberg, 1963).

2. FRACTIONS OF 17-KETOSTEROIDS

To get more information about the androgenic status, different fractions of 17 KS have been studied. Total 17 KS can be divided into 2 groups:

A. 11 Deoxy 17 KS or $C_{19}O_2$ which consists of:

1. Dehydroepiandrosterone or DHA (3β-OH-Androst-5-en-17 one)
2. Epiandrosterone (3β-OH-5α Androstan -17 one)
3. Androsterone (3α-OH-5α Androstan -17 one)
4. Etiocholanolone (3α-OH-5β Androstan -17 one)

B. 11 Oxy 17 KS or $C_{19}O_3$ consists of:

1. 11β-OH Androsterone (3α,11β dihydroxy-5α Androstan -17 one)
2. 11β-OH Etiocholanolone (3α,11β dihydroxy-5β Androstan -17 one)
3. 11 Keto Androsterone (3α-OH-5α Androstane-11,17-dione)
4. 11 Keto Etiocholanolone (3α-OH-5β Androstane 11,17-dione).

Only DHA, androsterone, and 11β-OH androsterone have some androgenic activity, but they are weak in comparison with testosterone (Dorfman and Shipley, 1956). There is no sign of virilism after DHA administration at a dose of 100 mg., 3 times a week for a period of 100 days, whereas testosterone, 100-200 mg. per month, induces signs of masculinization (Dorfman, 1963).

(a) Free fraction of 17 KS

In idiopathic hirsutism, the free or unconjugated fraction of total 17 KS is in the normal range, while in hirsutism associated with the adrenogenital syndrome or with the Stein-Leventhal syndrome, it is found to be high (Gold and Goldberg, 1963).

(b) Dehydroepiandrosterone (DHA)

DHA is secreted mainly by the adrenals, but it can also be produced by testes or ovaries (Dorfman, 1963, Macdonald et al, 1963). Part of DHA is excreted in urine as a sulphate conjugate. Another part, which is free, forms Δ^4 androstenedione and is excreted as androsterone and etiocholanolone glucuronide and sulphate. A very small portion is excreted as DHA glucuronide (Siiteri et al, 1963).

In some of the earlier methods for measurement of 17 KS, a strong acid was used to hydrolyse the steroid conjugates, by which DHA could be converted to another compound (Venning et al, 1942). Therefore, the value of DHA might not be true. Using a mild hydrolysis procedure, DHA is found to be elevated only in some subjects with idiopathic hirsutism (Johnsen, 1956, Gilbert-Dreyfus et al, 1958, Prunty et al, 1958, Bush and Mahesh, 1959, Gemzell et al, 1959, Jayle et al, 1959, Brooks and Prunty, 1960, Lipsett and Riter, 1960, Zerssen, 1960, Goldzieher and Axelrod, 1962, James et al, 1962, Mauvais-Jarvis and Baulieu, 1962, Lanthier, 1963). Pesonen (1963) found normal or slightly decreased values for DHA.

In normal subjects, the DHA concentration in plasma and urine varies over a wide range (Kellie and Wade, 1956, Kellie and Smith, 1957, Brooks, 1958, Gallagher et al, 1958, Brooksbank and Salokangas, 1959, Jayle et al, 1959, James, 1961).

(c) Epiandrosterone

Epiandrosterone, a minor urinary metabolic product of testosterone, has less androgenic activity than androsterone (Dorfman and Shipley, 1956). It is elevated in idiopathic hirsutism and in the Stein-Leventhal syndrome (Pesonen, 1963). An increase was also reported in two cases of recurrent hirsutism associated with pregnancy (Turunen, 1964). Pesonen (1963) stressed that an increase in epiandrosterone was a typical sign of "male phenomenon." However, when the "inactivation coefficient" $\frac{\text{epiandrosterone + etiocholanolone}}{\text{epiandrosterone + etiocholanolone + androsterone}} \times 100$ was estimated, there was no difference between normal subjects and those with

idiopathic hirsutism. This coefficient is slightly lower than normal in cases of hirsutism associated with pregnancy (Turunen, 1964).

(d) Androsterone and etiocholanolone

Androsterone and etiocholanolone are metabolic products of the weak androgens DHA and Δ^4 androstenedione, of the strong androgen testosterone, and to a limited extent, of the non-androgens 17α-OH progesterone and 11 deoxycortisol (Dorfman, 1963). They form the major portion of 17 KS and are present as both glucuronide and sulphate conjugates. The normal excretion values of these two steroids also vary widely (James, 1961).

To interprete the results of 17 KS excretion measurements, the method of analysis has to be taken into consideration. If fractionation is made after hot acid hydrolysis, the result may be artificially raised by artifacts derived from 11β-OH androsterone and 11β-OH etiocholanolone (Brooksbank, 1961).

Some women with idiopathic hirsutism show an elevation of androsterone, or etiocholanolone, or both (Pond, 1954, Johnsen, 1956, Perloff et al, 1957, 1958, Gallagher et al, 1958, Gilbert-Dreyfus et al, 1958, Prunty et al, 1958, Herrmann et al, 1960, Lipsett and Riter, 1960, Zerssen, 1960, Brooksbank, 1961, James et al, 1962, Mauvais-Jarvis and Baulieu, 1962, Tropea et al, 1962, Julesz et al, 1963, Lanthier, 1963).

Pesonen (1963) reported normal values of androsterone and etiocholanolone in all of his patients. There are also reports of deficiency of these 2 steroids (Brooksbank, 1961).

The high excretion of total 17 KS in this type of patient is due largely to increases in androsterone and etiocholanolone (Kappas et al, 1956, Gallagher et al, 1958, Brooks and Prunty, 1960, Lipsett and Riter, 1960, James et al, 1962).

(e) 11 Oxy 17 KS

The precursors of this group are 11β-OH androstenedione and 11 oxy C-21 compounds from adrenal gland. 11β-OH androstenedione, which is androgenic, is mainly excreted as 11β-OH androsterone, which also has androgenic activity (Dorfman and Shipley, 1956, Bradlow and Gallagher, 1959, Brooksbank, 1961).

The ratio $\frac{\text{11β-OH androsterone}}{\text{11β-OH etiocholanolone + 11 ketoetiocholanolone}}$ may offer an index for the proportion of androgen and cortisol production from adrenal gland (James et al, 1962). It has been considered to be significant in indicating an androgenic influence of the adrenal gland in hirsutism (Bush and Mahesh, 1959, Brooks and Prunty, 1960). However, the elevated ratio is found only in some subjects with idiopathic hirsutism (James et al, 1962).

Prunty et al, 1958, reported a high ratio of $\frac{\text{11β-OH androsterone}}{\text{11β-OH etiocholanolone}}$ in some cases of idiopathic hirsutism.

In general, the excretion of total 11 oxy 17 KS, and in particular that of 11β-OH androsterone, is higher than normal (Gallagher et al, 1958, Bradlow and Gallagher, 1959, Bush and Mahesh, 1959, Rodrique et al, 1959, Brooks and Prunty, 1960, Lipsett and Riter, 1960, Zerssen, 1960, Brooksbank, 1961, James et al, 1962, Mauvais-Jarvis and Baulieu, 1962).

However, some authors have reported normal values (Perloff et al, 1957, 1958, Goldzieher and Axelrod, 1962, Julesz et al, 1963, Pesonen, 1963).

3. ANDROSTENOL (5α ANDROST-16-EN-3α-OL)

Dorfman (1961, 1963) suggested that androstenol might be a unique metabolite of testosterone:

a. There was an excessive secretion of androstenol after injection of labeled cholesterol and pregnenolone intravenously to a woman suffering from virilizing adrenal adenoma.

b. Incubation of testosterone with rat testes yielded androstenol.

c. In virilized women, whether the adrenal gland or ovary is involved, there is an elevation of urinary androstenol.

Androstenol was also high in 2 hirsute women (Richter and Arnold, 1962).

Richter and Arnold (1962), Wilson et al (1962) and Bulbrook et al (1963), however, presented evidence that androstenol was not a peripheral metabolic product of either DHA or testosterone. There is a high correlation between androstenol, DHA, androsterone, and etiocholanolone, in urine. As a means of evaluating androgenic status, it does not appear to be more useful than 11 deoxy 17 KS (Bulbrook et al, 1963).

4. 17-HYDROXY CORTICOSTEROIDS (17 OHCS) AND 17-KETOGENIC STEROIDS (17 KGS)

17 OHCS and 17 KGS are both used as indices of adrenal function. 17 OHCS or Porter-Silber chromogens, include all the steroids with a dihydroxyketone side chain, while 17 KGS include those with the dihydroxy-

ketone side chain and also pregnanetriol, cortol, and cortolone. In terms of cortisol and cortisone, 17 OHCS measurement is more specific than 17 KGS (Williams, 1962).

In many studies, no distinctive abnormality was found in 17 OHCS or 17 KGS in idiopathic hirsutism; the values range from normal (Levell et al, 1957, Prunty et al, 1958, Bradlow and Gallagher, 1959, Gemzell et al, 1959, Rodrique et al, 1959, Lipsett and Riter, 1960, Brooksbank, 1961, Martin et al, 1961, Goldzieher and Axelrod, 1962, Pesonen, 1963) to high (Brooksbank, 1961, James et al, 1962, Mauvais-Jarvis and Baulieu, 1962). Other reports indicate that the values are high in some patients only (Gemzell et al, 1959, Zerssen, 1960, Julesz et al, 1963).

However, Kent et al (1963), who measured both 17 KGS and 17 OHCS in the same patients, reported high values for the 17 KGS and normal values for the Porter-Silber chromogen. These authors also measured cortisol secretion rate in one patient and found it to be abnormally high. Urinary pregnanetriol was normal in this patient. On the basis of this patient, it was postulated that idiopathic hirsutism metabolizes excessive cortisol through the pathway leading to the excretion of steroids which are measured as 17 KGS, possibly cortolone.

Lloyd et al (1963) measured urinary 17 OHCS every 3 hours and found no difference between hirsute and normal women. After ACTH stimulation, the patients excreted more than control subjects in the first 3 hours, but later, there was no difference.

Pasargiklian (1961) gave ACTH infusions to 9 patients with post-pubertal idiopathic hirsutism. The ratio $\frac{\text{17 KGS } (NaBH_4 + NaBiO_3)}{\text{Porter-Silber chromogen}}$

increased, with a marked constant increase of androsterone. The author suggested an adrenal defect of 11 and 21 hydroxylase activity, causing hypersecretion of 17α-OH progesterone with a resulting overproduction of C-19 androgens.

5. PREGNANETRIOL (5β PREGNANE-3α,17α,20α-TRIOL)

Pregnanetriol is a urinary metabolic product of 17α-OH progesterone. A defect in adrenal cortical hormone synthesis will result in retention of 17α-OH progesterone and high level of pregnanetriol will be expected.

Pregnanetriol is in the normal range in most cases of hirsutism (Prunty et al, 1958, Rodrique et al, 1959, Herrmann et al, 1960, Lipsett and Riter, 1960, Martin et al, 1961, Pasargiklian, 1961, Goldzieher and Axelrod, 1962, Kent et al, 1963, Lloyd et al, 1963). Only one patient out of five has an elevation of pregnanetriol (Bradlow and Gallagher, 1959).

6. PREGNANEDIOL (5β PREGNANE-3α,20α-DIOL)

Pregnanediol, a urinary metabolite of progesterone, is used as an index for progestational activity of the ovaries. In idiopathic hirsutism, it is normal (Merivale, 1951, Gemzell et al, 1959, Rodrique et al, 1959, Martin et al, 1961, Julesz et al, 1963).

7. PREGNANETRIOLONE (5β PREGNANE-3α,17α,20α TRIOL-11 ONE)

Finkelstein (1959) claimed that the presence of this steroid in the urine shows the deficiency of 21 hydroxylase activity; however,

it was not found in cases of idiopathic hirsutism.

8. OESTROGEN

Post-menopausal women tend to develop hair on the upper lip and chin. Normally, the urinary levels of DHA, androsterone, and etiocholanolone are lower than in young women (Kappas and Gallagher, 1955, Pincus et al, 1955, Brooksbank and Salokangas, 1959). There are reports of high 17 KS excretion in post-menopausal hirsute women and DHA was high in one case (Brooksbank, 1961). Because of the possibility that a change in androgen-:oestrogen ratio may be responsible for hirsutism in these cases and in idiopathic hirsutism, oestrogen has been investigated. Urinary oestrogen was found to be low, normal, or high (Brooksbank, 1961). Cruz Ferreira et al (1960) observed that only 3 out of 22 hirsute patients had high oestrogen levels; two of these had adrenal hyperplasia which was confirmed surgically. Two additional subjects had subnormal urinary oestrogen and the rest were within normal limits. Rodrique et al (1959) reported normal values.

An abnormal increase in oestrogen level is found in hirsute patients with menstrual disorders, after a dexamethasone suppression test (Jayle et al, 1961).

In a study of 16 women with idiopathic hirsutism, oestrone excretion was less than normal, oestradiol-17β was at the lower limit of normal, and oestriol was in the normal range (Julesz et al, 1963).

9. CONCLUSIONS

a. In idiopathic hirsutism, the mean total 17 KS is higher than normal, but the range is very wide.

b. There is no distinctive pattern of various components of 17 KS. As a group, $C_{19}O_2$ or 11 deoxy 17 KS seem to be higher than normal (Bradlow and Gallagher, 1959, Bush and Mahesh, 1959, Goldzieher and Axelrod, 1962). DHA is elevated only in some patients. There is sometimes an increase in androsterone, or etiocholanolone, or both. 11 Oxy 17 KS, especially 11β-OH androsterone, are high only in some cases.

c. Androstenol - There is not enough evidence to make any conclusion, but it seems no better than total 17 KS in demonstrating androgenic status.

d. 17 OHCS and 17 KGS range from normal to high.

e. Pregnanediol, pregnanetriol, and pregnanetriolone, are within normal limits.

f. Oestrogen excretion varies from low to high.

E. RELATION OF HAIR GROWTH TO 17-KETOSTEROIDS

The relationship between hair growth and 17 KS is controversial. Hamblen et al (1941) and Bayer and Koets (1951) found a tendency for the elevation of 17 KS values to parallel the degree of hirsutism. Perloff et al (1958) showed that they could correlate the degree of hirsutism with the level of $C_{19}O_2$ 17 KS. There is a significant correlation between degree of hairiness, 17 KS excretion, incidence of oligomenorrhea (Ferriman et al, 1957), and between hair growth and biacromial width (Ferriman et al, 1957, Ferriman et al, 1962). However, there was no difference in the oestrogen excretion in urine of hirsute and non-hirsute women. The non-hirsute ones had large biiliac diameter (and were therefore distinctly feminine in body build) (Ferriman et al, 1962). Pedersen (1942) found a correlation between the hair along the linea alba and the level (using bioassay) of androgen in urine.

Lipsett and Riter (1960) and Zerssen (1960) found no significant correlation between the intensity and progress of hair growth and 17 KS excretion.

In normal male and female, there is no relationship among the axillary hair growth, total 17 KS (Hamilton, 1951, Kinsell et al, 1954, Hamilton et al, 1962), the urinary content of androgenic activity (bioassay) (Hamilton, 1951, Hamilton et al, 1962), and any fraction of 17 KS (Hamilton et al, 1962). There is also no correlation among beard growth, 17 KS, urinary content of androgen (bioassay), and any fraction of 17 KS in male (Hamilton et al, 1962). Tanner et al (1959) found definite correlation between 17 KS, hair growth generally, and muscle mass in young men.

Dorfman (1960, 1963) has pointed out that 17 KS, either in plasma or urine, cannot be used as a reliable index of androgen production in the body because only a small portion of 17 KS (approximately 1 mg.) comes from the most potent androgen, testosterone, while the bulk of 17 KS (approximately 9 mg.) is derived directly or indirectly from weak androgens (DHA and Δ^4 androstenedione). This is supported by the evidence that in cases where plasma testosterone is high, 17 KS may be normal. Direct measuring of testosterone should give more information about the effective circulating androgen.

F. TESTOSTERONE (17β-OH ANDROST-4-EN-3-ONE)

Testosterone was isolated from testicular tissue in 1935 (David et al). It is mainly a secretory product of the testes (Savard et al, 1952, West et al, 1952, Slaunwhite and Samuels, 1956, Lucas et al, 1957), but also may be produced by the adrenal glands and ovaries, and perhaps the placenta (Slaunwhite and Samuels, 1956, Solomon et al, 1958, Kase et al, 1961, Ichii et al, 1962, Kase and Kowal, 1962, Dorfman, 1963, Dorfman et al, 1963, Vande Wiele et al, 1963).

In fact, the pattern of steroidogenesis of adrenal glands, testes, and ovaries is similar. Differences in local enzymes will determine which steroids are produced. Alteration in normal enzyme patterns may change the steroid production. It is well accepted that such defects occur in congenital adrenal hyperplasia. In Stein-Leventhal or polycystic ovary syndrome, it becomes more and more evident that the ovary fails to synthesize oestrogen and there is an accumulation of

testosterone, DHA, or Δ^4 androstenedione in the circulation, which may be responsible for the hirsutism and virilism (Sandor and Lanthier, 1960, Warren and Salhanick, 1961, Axelrod and Goldzieher, 1962, Mahesh and Greenblatt, 1962, Giorgi, 1963, Dorfman, 1963).

The major urinary metabolites of testosterone are androsterone and etiocholanolone. After the administration of labeled testosterone, 40-50% is recovered as androsterone and etiocholanolone (Fukushima et al, 1954, a, b, Horton et al, 1963, Korenman et al, 1963, Camacho and Migeon, 1964). Only 1% appears as testosterone glucuronide and very small amounts in free form and sulphate conjugate (Horton et al, 1963, Camacho and Migeon, 1964). The presence of testosterone in urine as glucuronide conjugate was supported by the works of Schubert and Wehrberger (1960) Camacho and Migeon (1963) Korenman et al (1963).

Testosterone, DHA, and Δ^4 androstenedione, apart from being secreted by gonad and adrenal glands, are interconvertible to some extent in the periphery (Mahesh et al, 1962, Vande Wiele et al, 1963).

DHA----> Δ^4 androstenedione<----> testosterone. This view was confirmed by Camacho and Migeon (1964), who showed that after intravenous injection of Δ^4 androstenedione, a significant amount of radioactivity is recovered as testosterone glucuronide, while DHA and 17α-OH progesterone do not contribute to urinary testosterone.

Testosterone will produce signs of masculinization in women at a dosage of 200 mg. per month. It is roughly estimated from this result that endogenous production of testosterone of 6 mg. per day over a period of one month will cause hirsutism (Dorfman, 1963). In normal men, the daily production rate of testosterone has been estimated at 4-9

mg. (Hudson et al, 1962), 4-11.8 mg. (Korenman et al, 1963), and 6.9 mg. (Vande Wiele et al, 1963). In normal women, the production rate has been reported to be 0.9-2.8 mg. per day (Korenman et al, 1963) and 1.8 mg. per day (Vande Wiele et al, 1963). The testosterone production rate in idiopathic hirsutism has not yet been studied. Macdonald et al (1963) found that the secretion of ovarian androgens (DHA and Δ^4 androstenedione plus testosterone) increased in patients who had hirsutism with abnormal ovarian function.

The metabolism of exogenous testosterone in 2 hirsute and 4 normal women has been studied. After injection of testosterone intramuscularly, total 17 KS excretion increased and then returned to the pre-injection level in both groups; DHA plus epiandrosterone increased slightly, androsterone moderately increased, and etiocholanolone increased to a greater extent than both. Oestrogen rose slightly in one hirsute subject. Androstenol was high in hirsutism, but independent of testosterone. It was concluded that hirsute women metabolize testoserone in the same manner as normal women, and that the excretory metabolic products appear in urine in the known forms (Richter and Arnold, 1962).

Forchielli et al (1963), using the modified method which had been developed by Finkelstein et al (1961), found plasma testosterone to be high in idiopathic hirsutism. This method involves the conversion of testosterone to oestrogen and measurement of oestrogen by fluorimetry. In this particular group of patients, the mean total 17 KS were also slightly elevated.

Hudson et al (1963), using a double isotope dilution technique, reported high plasma testosterone only in some hirsute patients (etiology not mentioned).

Burger et al (1964) and Casey and Kellie (1964) suggested that testosterone from the adrenal glands was involved in some cases of idiopathic hirsutism because the testosterone level in adrenal vein blood was significantly higher than in peripheral blood (double isotope dilution technique). Only some patients had high plasma testosterone.

Lloyd et al (1964), using the double isotope dilution technique of Riondel et al (1963), also found high plasma testosterone only in some cases of hirsutism (etiology not mentioned).

There is a report of urinary testosterone in only one hirsute subject (etiology not mentioned), using the technique of oxidation of testosterone to Δ^4 androstenedione, and measuring the latter as 17 KS. The level was found to be higher than in normal (Vermeulen and Verplancke, 1963).

III. METHODOLOGY

The method of measurement of urinary steroids in general consists of three main steps: extraction, purification, and quantitation. Most of the extraction procedures are similar in principle and are based on partition between immiscible solvents. Purification is achieved by means of several types of chromatography and the method of quantitation depends on the individual steroid under study.

A. MEASUREMENT OF URINARY TESTOSTERONE

1. EXTRACTION

The method of Goldzieher and Axelrod (1962) was followed with slight modification. Although it is reported that testosterone is present in urine mainly as glucuronide conjugate, both enzyme hydrolysis and solvolysis were performed so that the subsequent measurement included three forms of testosterone: free, glucuronide, and sulphate conjugates. Total 17 KS were measured on the same extract.

The total 24-hr. urine was adjusted to pH 4.5-4.6, roughly with 1/20 volume of acetate buffer pH 4.5, and then more exactly with acetic acid. It was then incubated at 37°C for 60 hrs. with enzyme β-glucuronidase (Ketodase), 300 unit/ml. Testosterone-4-C^{14}, 0.4 μc. (specific activity 22 millicurie per millimole) was added at this point to serve as an indicator for recovery of endogenous testosterone.

The urine was neutralized to pH 7 with 50% NaOH. NaCl, 17.4 gm. per 100 ml., was added. The urine was extracted 3 times with

redistilled ethyl acetate, using an equal volume once and 4/5 volume twice. The extract was dried by the addition of sodium sulphate, 6 gm./100 ml. Solvolysis (Segal et al, 1960) was carried out for 16-20 hrs. at 30°C. The extract was washed once with saturated sodium carbonate, and twice with distilled water (1/10 volume). The pooled washings were re-extracted with ethyl acetate which was then added to the original ethyl acetate extract. This was dried under reduced pressure at a temperature below 40°C.

2. SEPARATION AND PURIFICATION

(a) Hexane-methanol partition

Partition between two immiscible solvents such as hexane or petroleum ether and 70% methanol or ethanol, is used as the first step of purification in the determination of plasma and tissue testosterone (Samuels, 1947, Hollander and Hollander, 1958, Oertel and Eik-Nes, 1959, Oertel, 1961, Finkelstein et al, 1961). Hexane extract is supposed to remove cholesterol and neutral fat (Gallagher and Koch, 1929). In urine, the concentration of fat and protein is lower than in blood; therefore, this step may not be necessary. Because of poor initial results with subsequent stages in the measurement and recovery of testosterone, hexane partitioning was introduced along with other steps to be described. No attempt was made to determine the contribution of this procedure to the overall improvement in results, but it was continued on an empirical basis.

The dried residue was dissolved in 2 ml. of 70% methanol and partitioned against 0.4 ml. of hexane (Horton et al, 1963). The methanol

layer was removed; the hexane was re-extracted twice with 1 ml. 70% methanol which was added to the original methanol fraction. This was evaporated to dryness under N_2.

(b) Thin-layer chromatography

The separation of testosterone was obtained by thin-layer chromatography (TLC). The advantages of TLC over paper and column chromatography are the greater simplicity and the shorter time required for development. The separation of steroids is as good as with the other types of chromatography.

1. Preparation of plates

In trial experiments to determine the best solvent systems, microscopic slides were used as plates. They were prepared by mixing about 1 gm. of silica gel G. or aluminum oxide G. with 2 ml. of distilled water. The material was then poured on the slide and rolled by hand until it was evenly distributed. This amount was enough for 2 slides. These were dried in the oven at 110°C for 15-30 minutes. A covered beaker was used as a chromatographic jar. The time for preparation and development was short, making it possible to screen a great number of solvent systems. The solvents which showed promising results were further tried on the usual glass plates, 20 x 20 cm.

Glass plates, 5 x 20 cm., 20 x 20 cm., and 20 x 40 cm. were prepared by Desaga-Brinckman apparatus. The same resolution was achieved with plates of 0.5 mm. and 1.0 mm. thickness. Since a large volume of urine extract would be applied, a thickness of 1 mm. was selected as the standard preparation.

Silica gel G., 20 gm. + 40 ml. of distilled water, or aluminum oxide G., 35 gm. + 45 ml. of distilled water (Ritter and Meyer, 1962) were sufficient for one 20 x 20 cm. glass plate. The plates were left air-dried for 10-15 minutes and activated in the oven at 110°C for at least 1 hr. They were stored in a desiccator until used.

Solvents for developing were mixed about 16 hours before use and normally replaced at intervals of about 2 weeks. The depth of the solvents in the chromatographic jars was kept at approximately 1 cm. Development was allowed to proceed until the solvent front was about 16.5 cm. from the starting line on 20 x 20 cm. plates and approximately 35 cm. on 20 x 40 cm. plates.

2. Location of steroids

Steroids are located on the plate by 3 techniques:

1. 10% phosphomolybdic acid in alcohol (Kritchevsky and Kirk, 1952).
2. U.V. lamp, short wave (254 mμ) for the detection of testosterone and Δ^4 androstenedione (Bush, 1952).
3. Scanner for radioactivity.

The first method was used during development of the chromatographic procedure, when it was not necessary to elute the steroids. Subsequently, when the separated compounds were to be recovered, method 2 and 3 were used.

As mentioned above, testosterone-4 C^{14}, specific activity 22 millicurie/millimole (New England Nuclear Corporation), was used as a marker. An end window Geiger counter with a window 3 mm. wide was used as a scanner.

Several experiments were performed to insure that the standard non-labeled testosterone traveled with T.-4 C^{14} standard on the thin-layer plate, using both aluminum oxide G. and silica gel G. as supporting media.

In the final method, the standard non-labeled testosterone was located approximately with the U.V. lamp, then the appropriate area of the plate was scanned for the peak of radioactivity in a stepwise manner, each step being equal to the width of the detector window. The amount of radioactivity in counts per minute (CPM) was recorded on a graph paper and the peak area thus determined. This area was marked and eluted.

For final measurement of radioactivity, to calculate recovery, a liquid scintillation counter was used. Samples were transferred to counting vials and evaporated to dryness. The residue was then dissolved in 10 ml. of counting solution which consisted of 6.5 gm. of 2,5 diphenyl-oxazole (PPO) and 0.39 gm. of 1,4-di-2,5,phenyloxazolyl-benzene (POPOP) dissolved in 1 litre of redistilled toluene. Counting efficiency was calculated from a quench-correction curve and was always in the range of 78-80%.

3. Technique of elution

Glass pipettes were prepared and used according to Beroza and McGovern (1963). The steroids were eluted with 3 portions of 1-2 ml. of ethanol. Each portion was shaken with the steroid-loaded powder and allowed to remain in contact with it for 10-15 minutes before separation by filtration through glass wool. (See Figure I.)

4. Standard compounds

At the beginning of the separation experiments, only standard DHA, androsterone, etiocholanolone (Sigma) and testosterone (Sigma and CIBA) were used. Subsequently 11-OH androsterone, 11-OH etiocholanolone (gift from Prof. W. Klyne), 11 keto androsterone, 11 ketoetiocholanolone (Southeastern Biochemicals, Inc.), and Δ^4 androstenedione (Sigma) were tried. All the standard were dissolved in methanol or ethanol.

5. Solvent systems

A. Silica gel G. as supporting medium

The solvent systems which were tried with the microscopic slides are as follows:

1. Toluene:ethyl acetate 1:1, 1:2.5, 1:3, 1:4, 4:1 (Shen et al, 1962), 3.5:1, 3:1.
2. Hexane:ethyl acetate 1:1 (Matthews et al, 1962, Smith and Foell, 1962), 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4.
3. Benzene:ethyl acetate 1:1, 1:1.5, 1:2, 4:1, 3.5:1, 3:1, 2.5:1, 2:1, 3:2 (Dorfman, 1962).
4. Hexane:ether 1:1, 1.5:1, 1:3, 1:4.
5. Benzen:ether 2:1, 1.5:1, 1:1 (Matthews et al, 1962).
6. Hexane:toluene 1:3 no movement.
7. Benzene:chloroform 1:1, 1.5:1, 2:1.
8. Hexane:methyl acetate 1:1.
9. Ethyl acetate:cyclohexane 1:1, 3:7.

The solvent systems which were chosen for the trial on 20 x 20 and 20 x 40 cm. glass plates, and the Rf. of the standard steroids are given in Table I, A and B.

On silica gel G., in all of the solvents used, DHA and androsterone had similar Rf; etiocholanolone and testosterone also traveled close to each other. 11 Oxy 17 KS and Δ^4 androstenedione were well separated from testosterone.

B. Aluminum oxide G. as supporting medium

With microscopic slides, the following solvents were investigated:

1. Hexane:ethyl acetate 1:1 (Matthews et al, 1962), 1:2.
2. Toluene:ethyl acetate 1:2.5, 1:4.
3. Benzene:ether 1:1 (Matthews et al, 1962).
4. Hexane:methyl acetate 1:1, 1:1.5, 1:2, 1.5:1, 2:1, 2.5:1, 3:1.
5. Benzene:methyl acetate 1.5:1, 2:1, 2.5:1.
6. Benzene:ethyl acetate 2:3.
7. 6% ethanol in chloroform:isooctane 1:1 (Chiang and Schweppe, 1963), 1:2.5, 1:5, 1.5:1.
8. Ethyl acetate:cyclohexane:toluene 10:10:1 (Chiang and Schweppe, 1963), 3:3:1.
9. Ethyl acetate:cyclohexane 1:1 (Golab and Layne, 1962).

For 20 x 20 and 5 x 20 cm. glass plates, the results are shown in Table II, A and B.

Generally, on an aluminum oxide G. plate, etiocholanolone could be separated from DHA, androsterone, and testosterone. Androsterone and testosterone tended to move together. 11 Oxy 17 KS were well separated from testosterone. System benzene:ether 1:1 also permitted the separation of testosterone and epitestosterone (Matthews et al, 1962). From

these studies, it appeared that by two-dimensional chromatography, testosterone could be adequately separated from the other steroids. Hexane:ethyl acetate 1:1 as a first solvent, and benzene:ether 1:1 as the second; did give good separation of testosterone from DHA, androsterone, and etiocholanolone on an aluminum oxide G. plate (see Table III).

Because of the large volume of urine extract to be used, it was not possible to apply the extract in one spot, consequently, the two-dimensional system on a single plate was not feasible. Two plates were then substituted. An aluminum oxide G. plate, using solvent benzene: ether 1:1, allowed the separation of testosterone from DHA, etiocholanolone, epitestosterone, and 11 Oxy 17 KS. For the separation of androsterone and testosterone, either hexane:ethyl acetate 1:1 or benzene: ethyl acetate 3:2 could be used. The degree of separation, however, was better on silica gel G. than on aluminum oxide G., employing the same solvent systems. Moreover, silica gel G. was easier to handle than aluminum oxide G. Δ^4 Androstenedione and 11 Oxy 17 KS were also well separated from testosterone. The original sequence of plates was therefore as follows:

1. Aluminum oxide G. plate, solvent benzene:ether 1:1
2. Silica gel G. plate, solvent hexane:ethyl acetate 1:1 or benzene:ethyl acetate 3:2.

During one experiment with urine extract, the first plate was developed in solvent hexane:ethyl acetate 1:1, by mistake. However, the resolution was found to be better because the distance from the starting point was greater than in solvent benzene:ether 1:1. It was followed

by another two plates which had been planned earlier. Testosterone added to urine extract and isolated by these three plates, however, did not have a characteristic U.V. absorption spectrum in methanol. The isolated material was then re-applied on another silica gel G. plate, using benzene:ethyl acetate 3:2 as a solvent. The isolated testosterone now gave the expected absorption spectrum. Subsequently, a series of trials with urine extract was performed, using a sequence of these four chromatographs (i.e. aluminum oxide G. hexane:ethyl acetate 1:1, aluminum oxide G. benzene:ether 1:1, silica gel G. hexane:ethyl acetate 1:1, and silica gel G. benzene:ethyl acetate 3:2). Testosterone occasionally showed a spectrum identical to that of standard testosterone. Usually, however, the absorption peak shifted slightly, indicating some contamination. The order of the plates and solvents was then re-arranged on a trial and error basis. Furthermore, since it was easier to get an even application of crude urine extract on silica gel G. than on aluminum oxide G, the final sequence used was as follows:

1. Silica gel G. plate — benzene:ethyl acetate 3:2
2. Silica gel G. plate — hexane:ethyl acetate 1:1
3. Aluminum oxide G. plate — benzene:ether 1:1
4. Silica gel G. plate — benzene:ethyl acetate 3:2

This gave better reproducibility of the absorption spectrum, though the peak still was shifted slightly at times. However, changes made simultaneously in the method of quantitation (see next section) made it unnecessary to refine the chromatographic procedures further.
(See Figures II, III, IV.)

3. QUANTITATIVE MEASUREMENT

The techniques that have been used for the determination of testosterone in biological fluid are as follows:

1. An isotope dilution technique (Hollander and Hollander, 1958).

2. Modified Koenig et al reaction (Oertel and Eiknes, 1959, Oertel, 1961).

3. Conversion to oestrogen and measurement by fluorimetry (Finkelstein et al, 1961, Forchielli et al, 1963).

4. Fluorimetry (Korenman et al, 1963).

5. Double isotope dilution techniques (Hudson et al, 1963, Riondel et al, 1963, Burger et al, 1964).

6. Gas chromatography (Futterweit et al, 1963, Guerra-Garcia et al, 1963).

7. Conc. H_2SO_4 reagent (Sachs, 1964).

8. Absorption in conc. H_2SO_4 (Camacho and Migeon, 1963).

9. Absorption at 240 mμ in methanol or ethanol (Horton et al, 1963).

10. Oxidation to form Δ^4 androstenedione and measurement of the latter by Zimmermann reaction (Horton et al, 1963, Vermeulen and Verplancke, 1963).

There were four possibilities available for the present work: color reaction, absorption spectrometry in conc. H_2SO_4, absorption spectrometry in methanol, and gas chromatography.

(a) Gas chromatography

Since it has been shown that gas chromatography can measure a minute amount of steroids, of the order of nanograms (Horning et al, 1963), and since it is claimed to be fast, simple, and reproducible (Kirschner and Lipsett, 1962), this technique was tried first.

The gas chromatograph was from Research Specialties, model 600, with an argon ionization detector. Two types of column were tested. A column of 1% Q.F. 1 was unsatisfactory with the standard compounds (DHA, androsterone, etiocholanolone, testosterone), their trimethylsilyl ether (Kirschner and Lipsett, 1963), or their acetate derivatives (Wotiz and Martin, 1961).

Trimethylsilyl ether derivatives of these steroids were well separated with a column of 3% X.E. 60 under the following conditions:

Column: 6 ft., 21 inches, stainless steel.

Column temp. 232°C.

Detector temp. 248°C.

Vaporizer temp. 250°C.

Argon gas flow 60 ml/min.

The reproducibility was rather poor even after a priming injection. The sensitivity of this particular detector for testosterone was only about 1 µg. Another difficulty was the type of injector employed which made it impossible to analyse the small quantity of testosterone in urine. The reproducibility and the sensitivity could probably have been improved by altering temperature, by the use of a flame ionization detector and by the use of a solid injector. Unfortunately, the last two items were not available. Therefore, gas chromato-

graphy might have been suitable for qualitative analysis, but not for quantitative measurement in the present work.

(b) Modified Koenig et al reaction (Oertel, 1961) and conc. H_2SO_4 reaction (Sachs, 1964)

These two color reactions are specific for testosterone, Δ^4 androstenedione, and isotestosterone. Δ^4 Androstenedione and isotestosterone are removed by the previous TLC; therefore, it appeared feasible to use these reactions for quantitation of testosterone. The sensitivity was found to be approximately 1 µg. per sample for both reactions. When these color reactions were applied to testosterone isolated from urine extract after four TLC, the persistent presence of an interfering substance which reacted with conc. H_2SO_4 in both reactions, made it impossible to measure testosterone accurately. Moreover, the Koenig et al reaction showed a great deal of variability even with standard testosterone.

(c) Absorption spectra

The absorption spectrum with conc. H_2SO_4 was discarded for the same reason as above.

Absorption spectroscopy in methanol or ethanol presented the same problem. The presence of contaminants often shifted the spectral peaks and quantitation could not be made on this basis. The sensitivity was in the same range as that of the Koenig et al and conc. H_2SO_4 reagents.

(d) Oxidation and Zimmermann reaction (Vermeulen and Verplancke, 1963)

This method involves oxidation of testosterone to Δ^4 androstenedione and measurement of the latter as 17 KS. It was thought that the oxidation may well be an effective way to get rid of the non-specific contaminants. After separation on the first three successive chromatographs as mentioned earlier, the testosterone area was eluted and taken to dryness under N_2.

To the dried residue were added 0.2 ml. of 2% chromic trioxide and 0.3 ml. acetic acid. These were mixed and left overnight at room temperature. The solution was then diluted with 2 ml. of distilled water and extracted with an equal volume of ethyl acetate once, and with a half volume twice. The ethyl acetate extracts were combined and evaporated to dryness. The residue was applied to the fourth TLC for final purification of the Δ^4 androstenedione obtained.

Two modifications of the Zimmermann reaction were then tried for quantitation of the Δ^4 androstenedione:

1. Modified Masuda and Thuline method (1953)

The volumes of all the reagents were cut in half in order to increase sensitivity. Δ^4 Androstenedione from the last TLC was eluted and evaporated to dryness, together with 2, 5, and 10 µg. aliquots of standard Δ^4 androstenedione. 0.1 ml. of absolute ethanol, 0.15 ml. of 5 N KOH (freshly prepared) and 0.1 ml. of 2% m-dinitrobenzene in ethanol were added to the dried residue, mixed, and incubated for 90 min., at 27°C, in a covered water bath. 0.5 ml. of 70% ethanol was added and mixed; the color was then extracted into redistilled amyl acetate (0.5 ml.). The biphasic mixture was left in the dark for 8 min. The optical density

of the amyl acetate-ethanol layer was then read in a Beckman spectrophotometer at 510 mμ. A reagent blank and a plate blank were used as references for the standard Δ^4 androstenedione and unknown samples, respectively. A correction was said to be unnecessary (Masuda and Thuline, 1953).

However, sometimes there was an interfering color which could not be controlled and the color was unstable.

2. Zimmermann reaction (Corker et al, 1962)

This method employs benzyl trimethyl ammonium hydroxide instead of KOH which has been used widely in Zimmermann reaction. It has advantages over alcoholic KOH or aqueous KOH in that it is stable and the optical density of the blank is found to be lower, but some batches of this reagent may also give high blank. The sensitivity of the method is in the range of 0.5-4.0 μg. per sample for the micro method, and 3-20 μg. for the ordinary method. The stability of color is better than in the first method used.

To the dried Δ^4 androstenedione were added 0.1 ml. of 1% m-dinitrobenzene in ethanol and 0.1 ml. of 40% aq. benzyl trimethyl ammonium hydroxide. The solution was incubated in a covered water bath at 25°C for 90 min. 0.5 ml. absolute ethanol was added and an absorption spectrum was then made in a recording spectrophotometer (Spectronic 505). A "true" optical density was obtained from the O.D. values at wavelength 460, 520, and 580 mμ by the Allen correction (1950).

$$\text{Corrected O.D.} = \text{O.D. } 520 - \frac{\text{O.D. } 460 + \text{O.D. } 580}{2}$$

With this procedure, the standard curve was reproducible, and duplicate determinations agreed well.

B. MEASUREMENT OF TOTAL 17-KETOSTEROIDS

Measurement was made on an aliquot of the urine extract prepared for measurement of testosterone. After extraction, the dried residue was dissolved in 10 ml. of ethanol, of which 0.1-0.2 ml. were taken for 17 KS determination and the remainder for testosterone. The former aliquot was diluted with ethanol and duplicate aliquots containing approximately 10 µg. of total 17 KS were taken to dryness.

To this dried residue and to DHA standards (5, 10, and 20 µg.) were added 0.1 ml. of 0.5% m-dinitrobenzene in absolute ethanol and 0.05 ml. of 40% aq. benzyl trimethyl ammonium hydroxide. This was incubated for 90 min. at 25°C in covered water bath. 3 ml. of absolute ethanol were added and the optical density was read in the Beckman spectrophotometer at wavelength 460, 520, and 580 mµ. Allen correction was applied as above.

C. SUMMARY

The final method for determination of urinary testosterone was as follows:

1. Enzyme hydrolysis, solvolysis, extraction
2. Purification and separation
   a. Hexane-methanol partition
   b. Thin-layer chromatography

      Silica gel G. benzene:ethyl acetate 3:2

      Silica gel G. hexane:ethyl acetate 1:1

      Aluminum oxide G. benzene:ether 1:1

c. Oxidation with chromic trioxide

d. Thin-layer chromatography

Silica gel G. benzene:ethyl acetate 3:2

3. Quantitative measurement by the modification of Zimmermann reaction.

D. MATERIALS USED AND PURIFICATION

1. Ethyl acetate (Segal et al, 1960).

2. Ethanol (Peterson et al, 1955).

3. Benzene, analytical grade, redistilled.

4. N-hexane, spectrograde, otherwise purified according to Wilson et al (1958).

5. Ether (Wilson et al, 1961).

6. Methanol redistilled.

7. Amyl acetate redistilled.

8. Water for mixing with silica gel G. and aluminum oxide G. was glass-redistilled (Korenman et al, 1963).

9. Washed silica gel G. (Research Specialties Co.)(Korenman et al, 1963).

10. Aluminum oxide G. (Research Specialties Co.), no further purification.

11. Test tubes for oxidation were cleaned with 2% chromic trioxide and acetic acid overnight before use, then rinsed with tap water and distilled water.

12. Pipettes for elution were put in chromic acid overnight. They were then rinsed, washed with detergent, rinsed with tap water, distilled water, and purified ethanol.

13. All test tubes and pipettes used in Zimmermann reaction were cleaned with hot alcoholic KOH for 2-3 hrs., then rinsed with tap water and redistilled water.

IV. EXPERIMENT

A. EFFECT OF TESTOSTERONE ON HAIR GROWTH IN RATS

Testosterone may have an influence on sexual hair growth, but there is no proof of a direct effect of testosterone on the hair follicle. In mice, inhibitors of carbohydrate metabolism (in vitro) and severe starvation and shock (in vivo) are able to inhibit the mitotic activity of the hair bulbs (Bullough and Laurence, 1958). Since the proliferation of cells in the hair follicle can be modified by various factors, the action of testosterone on cell proliferation in the hair follicles in the facial area of normal women was considered worthy of investigation. By using the approach of Bullough and Laurence, pieces of skin could be incubated with testosterone and the effect of testosterone on the mitotic activity studied.

Another approach was considered. It was not feasible to apply testosterone locally on the face of women in active sexual life because of the possibility of inducing irreversible cosmetic changes. Yet it is with this age group that the studies on idiopathic hirsutism are mainly concerned. (Recently this approach was used by Maguire (1964), but in older subjects, 50-65 years of age, as reviewed in the historical introduction.) However, the effect of local application of testosterone on the size of hair follicles or hairs in female rats might be studied and the response would be at least suggestive of the situation in the human.

For these purposes, two preliminary experiments were performed.

EXPERIMENT I

In order to develop a technique for examination of human skin in vitro, pieces of skin from the rat were taken by biopsy and fixed on a cork board to prevent curling. Sections were made both horizontally and vertically and stained with hemalum-phloxine-saffran. In horizontal sections, measurement of the diameter of hair follicles or estimation of mitotic activity was impossible because the sections of different pieces of skin were never in the same plane and there was no definite landmark to rely on. Both measurements could be done on sections made on a plane perpendicular to the surface, but even in rats which possessed a great number of hairs, the number of follicles per section was very low. The experiment was thus not feasible with human skin because of limitations in size of skin biopsy and because of the low density of hair follicles. This approach was therefore discontinued.

EXPERIMENT II

In idiopathic hirsutism, facial hair in the female takes on the characteristics of male facial hair, in regard to thickness, rapidity of growth, pigmentation, and distribution. In order to determine whether testosterone would have such a masculinizing effect on the hair of the female rat, it was first necessary to establish that differences in fact exist between the body hairs of males and females in this species. An experiment was therefore undertaken to compare the length and thickness of body hairs in male and female white rats.

(a) Experimental animals

Ten male and ten female white rats, four months old.

(b) Procedure

The hair was clipped with small scissors close to the skin, from a constant site on the bak. Thirty terminal hairs were taken from each animal for measurement. A microscope with a scale in the eyepiece was used for measuring the largest diameter of the hairs, and a ruler under a magnifying lens was used for measurement of the length.

(c) Results

The results are shown in Table IV.

(d) Discussion

Statistical "t test" analysis of the results for the difference in length and diameter gave a P value of 0.05 and 0.1, respectively. It was concluded that there was no significant difference in either the width, or the length of terminal hairs of male and female white rats. The sex difference in hair texture (Mohn, 1958, Rennel and Callaghan, 1959) is therefore probably due to the ratio of vellus hairs and terminal hairs. The proposed experiment with testosterone was therefore not feasible.

B. URINARY EXCRETION OF TESTOSTERONE

At the time this work was started, the first efficient method of measurement of plasma testosterone had been developed (Finkelstein et al, 1961). However, there was no published report concerning the level of plasma or urinary testosterone in idiopathic hirsutism. Testosterone had been shown to induce hirsutism in normal women, but the analysis of androgenic steroid metabolites in idiopathic hirsutism showed controversial results. The evidence also indicated that measurement of such metabolites (17 KS) could not be used as an index for evaluating the effective androgen in the circulation. There was a possibility that these patients might have high production of testosterone either by the abnormal activity of glands in connection with testosterone secretion, or by an abnormal metabolic pattern in the peripheral circulation. Furthermore, since genetic factors play an important role in controlling the hair growth pattern in human beings, idiopathic hirsutism could also be due to the mediation of genetic factors through the production of testosterone. In order to determine whether females with idiopathic hirsutism do have an increased production of testosterone, the measurement of urinary testosterone excretion was undertaken. Since it was also evident that women of Oriental origin do not suffer from hirsutism, a study was undertaken to compare urinary testosterone excretion in normal women of Occidental and Oriental origin, with the hope that this might contribute evidence regarding the role of testosterone in hirsutism and the mechanism by which the genetic factor is manifested.

1. PURPOSE

There were 3 specific purposes to the experiment:

a. Comparison of urinary testosterone in normal women and in subjects with idiopathic hirsutism.

b. Comparison of urinary testosterone excretion in normal women of Occidental and Oriental origin.

c. Correlation of total urinary 17 KS and urinary testosterone excretion.

2. SUBJECTS

a. Six idiopathic hirsute women, aged 18 to 55, whose physical examination, including pelvic examination and other investigations, revealed no pathological findings to account for hirsutism.

b. Fifteen normal healthy women aged 20 to 39 of Oriental origin (Chinese, Thai, Phillipino), and Occidental origin (Canadian, Swiss), who had a normal working life.

3. PROCEDURE

Urine was collected for 24 hours, without preservative, at the beginning of the luteal phase of the menstrual cycle; collections made at other times in the cycle were indicated. The specimen, if not processed immediately, was kept in a frozen state under N_2.

The whole 24-hr. sample was extracted with ethyl acetate, dried, and purified by hexane methanol partition, as previously described. After removing 0.1-0.2 ml. for measurement of total 17 KS, the remainder was applied to TLC plates for testosterone isolation. Running time at room temperature for the various solvent systems was as follows:

1. Solvent benzene:ethyl acetate 3:2, 55-65 min.

2. Solvent hexane:ethyl acetate 1:1, 50-60 min.

3. Solvent benzene:ether 1:1, 35-45 min.

For the first 3 plates (silica gel G. solvent 1, silica gel G. solvent 2, and aluminum oxide G. solvent 3), standard testosterone was applied on one side of the plate and was located by U.V. lamp to assist in localizing the area to be scanned for radioactivity. The testosterone zone was eluted according to the peak of radioactivity. It was then oxidized to Δ^4 androstenedione and repurified by the fourth TLC (silica gel G. solvent 1). After localization on the TLC plate by U.V. fluorescence and radioactivity, the Δ^4 androstenedione was eluted in the usual manner. The eluate was made up to 3 ml. in calibrated test tube. 0.5 ml. was transferred to a counting vial and taken to dryness. Radioactivity was measured in a liquid scintillation counter.

Duplicate aliquots of 0.5 or 1 ml. were evaporated to dryness and Zimmermann reaction was performed as described before. Modified Masuda-Thuline method was employed for samples 2 and 3 in the normal Occidental group, and the method of Corker et al for the rest. The results were expressed as µg. of Δ^4 androstenedione per 24-hr. urine.

Total 17 KS in all samples were measured by the method of Corker et al, as previously described. The result was expressed in mg. of standard DHA per 24-hr. urine.

The average recovery through the entire procedure ranged from 45% to 75%. The recovery could be improved, but in order to avoid non-specific contaminants, only the peak area of radioactivity was taken. All the values reported had been corrected for loss.

4. RESULTS

The results of urinary testosterone and total 17 KS are shown in Table V.

From an analysis of variance, there was found to be no significant difference for the testosterone and 17 KS excretion between the 3 groups studied ($P > 0.1$). Because the creatinine excretion in one sample (No. 6 Hirsute) was low, the results of the testosterone and 17 KS determination were not included in the statistical analysis.

There was no significant correlation between urinary testosterone and 17 KS in the normal Occidental group or the hirsute group ($P > 0.1$). The regression coefficient was also not significant ($P > 0.1$). In the normal Oriental group, both the correlation between testosterone and 17 KS ($P < 0.02$), and the regression coefficient ($P < 0.05$) were significant. However, there was no significant correlation between those 2 determinations, and the regression coefficient was not significant ($P > 0.1$) when determined for the 3 groups combined (Statistics: Snedecor, 1956). (See Figure V.)

5. DISCUSSION

During the course of this study, there appeared reports on the measurement of urinary testosterone, using a variety of methods for purification and quantitation (Camacho and Migeon, 1963, Horton et al, 1963, Futterweit et al, 1963, Vermeulen and Verplancke, 1963, Brooks and Giuliani, 1964).

At the outset of the experiment in which the urine extracts were applied to aluminum oxide G. plate and developed in the solvent,

benzene:ether 1:1, the testosterone areas which had been identified by equating them to the corresponding standard non-labeled testosterone area, gave a recovery for testosterone,as measured by testosterone-4 C^{14}, as low as 1-4%. It was found through sampling of successive ascending areas of the plate that most of the labeled testosterone traveled much faster than the non-labeled one. The experiments previously described (see Methodology) were then carried out to determine whether the labeled and non-labeled testosterone standards moved at the same rate. The discrepancy in the two rates was removed through the introduction of the hexane-methanol partition, as a first step of purification, plus the change in the solvent system, made in error, as mentioned in the methodological part.

The use of 4 TLC plates in place of paper or column chromatography as used by others, permitted a good separation of the testosterone in much less time than that required by the other methods. This method, however, did not remove all of the non-specific urinary chromogens which interfere with the spectrophotometric quantitative method most commonly employed. It was therefore necessary to find a method for testosterone quantitation not affected by such chromogens. Oxidation of testosterone to Δ^4 androstenedione and measurement of the latter by the Zimmermann reaction proved suitable. This method had a lower limit of sensitivity of approximately 0.4 µg. and was highly reproducible. In future work, it may therefore be feasible to reduce the volume of urine to be extracted to 1/3 or 1/4 that used in the present study. It is necessary, however, to take the noted precautions concerning cleaning of glasswares, purification of solvents and reagents, and use of freshly-prepared m-dinitrobenzene reagent.

While localization of the testosterone on TLC plates could be made by U.V. fluorescence, the identification of testosterone through the scanning of testosterone radioactivity provided a more exact localization and a correspondingly lower contamination by non-specific materials. In some cases where the amount of testosterone was below the level of detection with U.V. light, the scanner was most helpful in locating the area. In addition, the use of labeled testosterone permitted calculation of recovery of endogenous testosterone.

Urinary testosterone excretion by normal women in this study was within the previously reported range (Camacho and Migeon, 1963, Horton et al, 1963, Vermeulen and Verplancke, 1963, Brooks and Giuliani, 1964), although the variation was greater (1.03-18.5 μg./24 hrs.). The level of urinary testosterone has been studied in only one case of hirsutism (etiology not mentioned) and was found to be high (Vermeulen and Verplancke, 1963). In the present study, only one patient with marked hirsutism showed an exceptionally high value. Two patients had values at the upper limit of normal and the hirsute subject No. 6 would have had a value in the upper normal range as well, if the recorded testosterone value had been corrected to 24 hrs. by creatinine estimation. Plasma testosterone in idiopathic hirsutism was found to be high only in some patients (Forchielli et al, 1963, Hudson et al, 1963, Burger et al, 1964, Casey and Kellie, 1964, Lloyd et al, 1964). The level of urinary testosterone in this study supports the findings for testosterone in blood. The degree of hirsutism seems to be related to the blood testosterone concentration (Segre et al, 1964). Based on the one hirsute patient who had a high value of urinary testosterone, it would appear that the degree

of hirsutism is related to the urinary testosterone concentration as well.

Since there was no difference in urinary testosterone and total 17 KS concentration between normal Occidental and Oriental women, it is unlikely that genetic factors related to racial differences have a role in controlling steroid production. The prevalence of hirsutism in Occidental women is therefore probably due to genetically deterined differences in the hair follicles.

Values for total 17 KS excretion in the normal group were within the range of those described in published reports, in which similar extraction procedures were employed, but were on the rather low side of the range (Jailer et al, 1959, Lanthier, 1960, James, 1961, James et al, 1962). They were similarly in agreement with the reported data which had been collected by Dorfman and Shipley (1956). In idiopathic hirsutism, total 17 KS values overlapped the normal values in agreement with the other investigators previously reviewed. There was, however, no significant difference in the mean excretion of total 17 KS between the idiopathic hirsute and normal groups. In the majority of cases, the high total 17 KS excretion in idiopathic hirsutism was accounted for by an elevation of androsterone and etiocholanolone fractions (Kappas et al, 1956, Gallagher et al, 1958, Brooks and Prunty, 1960, Lipsett and Riter, 1960, James et al, 1962). Jayle et al (1959) found that patients with high levels of 17 KS had correspondingly elevated levels of DHA. The urinary 11 deoxy 17 KS (DHA, androsterone, and etiocholanolone) are derived mainly from DHA, Δ^4 androstenedione, and testosterone, all of which have varying degress of androgenicity. The contribution of testosterone to androsterone and etiocholanolone is much less than the other precursors. Moreover, from

the present study, testosterone excretion is not always elevated in idiopathic hirsutism. The elevation of urinary androsterone and etiocholanolone in such cases could therefore be due to an increased production of either DHA or Δ^4 androstenedione. The finding that the production rate of either DHA or Δ^4 androstenedione plus testosterone, or both, was increased in hirsute women with oligomenorrhea (Macdonald et al, 1963) seems to support this view.

There was no significant correlation between urinary testosterone and total 17 KS for the whole group of subjects studied. It is probable therefore that the apparent correlation in the case of the Oriental group was fortuitous and related to the small sample size. The lack of correlation re-emphasizes the view that the total 17 KS value is not a reliable index of testosterone or androgen excretion.

V. C O N C L U S I O N S

1. There is no significant difference in urinary testoserone and total 17 KS concentration among idiopathic hirsutism, normal Occidental and normal Oriental women. Only some women with idiopathic hirsutism showed high testosterone levels. It is concluded that there is no indication for a mediation of the urinary testosterone or total 17 KS level by genetic factors.

2. There is no correlation between urinary testosterone and total 17 KS excretion in the subjects studied. These findings confirmed the view that urinary 17 KS are not a reliable index for androgenic status.

VI. SUMMARY

A method for the measurement of urinary testosterone was developed. A urine extract, after enzyme hydrolysis and solvolysis, was purified by hexane-methanol partition. Testosterone was isolated and purified by 3 TLC, oxidized with chromic trioxide to form Δ^4 androstenedione. Δ^4 Androstenedione was further purified by 1 TLC and the quantitation was made by a modified Zimmermann reaction.

Measurement of total 17 KS was made on an aliquot removed prior to the hexane-methanol partition.

The urinary testosterone and total 17 KS excretions were measured in 6 idiopathic hirsute, 7 normal Occidental, and 8 normal Oriental women. In these 3 groups, there was no significant difference in both urinary testosterone and total 17 KS. Only one patient with idiopathic hirsutism showed an exceptionally high testosterone value, while 3 had values in the upper normal range, and 2 were within the normal limit. Since there was no difference in testosterone and total 17 KS in urine of normal Oriental and Occidental women, it was concluded that the prevalence of hirsutism in the latter was not due to the mediation of a genetic control of steroid production, but probably due to a genetically determined difference in sensitivity of hair follicles.

The correlation between urinary testosterone and total 17 KS excretion was significant in the normal Oriental group but not in the idiopathic hirsute group, normal Occidental group, or all 3 groups combined. The regression coefficient was also significant in the normal Oriental group. The findings with the latter group were perhaps fortuitous due

to the small number of cases studied. It is therefore concluded, in agreement with previous results, that urinary total 17 KS are not a reliable index of the androgenic status.

BIBLIOGRAPHY

1. Allen, W.M. J. Clin. Endocrinol. 10: 71, 1950.

2. Argüelles, A.E., Salaber, J., Pomes Ottone, J.P.A., Chekherdemian, M., and Ricca, A. J. Clin. Endocrinol. and Metab. 19: 252, 1959.

3. Axelrod, L.R. and Goldzieher, J.W. J. Clin. Endocrinol and Metab. 22: 431, 1962.

4. Baker, B.L. Ann. N.Y. Acad. Sci. 53: 690, 1951.

5. Balus, L. Brit. J. Dermatol. 73: 69, 1961.

6. Bayer, L.M. and Koets, P. Am. J. Med. Sci. 222: 13, 1951.

7. Beroza, M. and McGovern, T.P. Chemist-Analyst 52: 82, 1963.

8. Billingham, R.E. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc., 451, 1958.

9. Billingham, R.E. and Russel, P.S. Nature 177: 791, 1956.

10. Bissel, G.W. and Williams, R.H. Ann. Internal Med. 22: 773, 1945.

11. Bradlow, H.L. and Gallagher, T.F. J. Clin. Endocrinol. and Metab. 19: 1575, 1959.

12. Breedis, C. Cancer Research 14: 575, 1954.

13. Brooks, R.V. Biochem. J. 68: 50, 1958.

14. Brooks, R.V. and Prunty, F.T.G. J. Endocrin. 21: 263, 1960.

15. Brooks, R.V. and Giuliani, G. Proc. Soc. for Endocrinology. J. Endocrin. 29: iv, 1964.

16. Brooksbank, B.W.L. and Salokangas, A. Acta Endocrin. 30: 231, 1959.

17. Brooksbank, B.W.L. Physiological Reviews 41: 623, 1961.

18. Bulbrook, R.D., Thomas, B.S., and Brooksbank, B.W.L. J. Endocrin. 26: 149, 1963.

19. Bulbrook, R.D., Thomas, B.S., and Utsunomiya, J. Nature 201: 189, 1964.

20. Bullough, W.S. and Laurence, E.B. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc., 171, 1958.

21. Burger, H.G., Kellie, A.E., and Kent, J.R. J. Clin. Endocrinol. and Metab. 24: 432, 1964.

22. Bush, I.E. and Mahesh, V.B. J. Endocrin. 18: 1, 1959.

23. Bush, I.E. Biochem. J. 50: 370, 1952.

24. Butcher, E.A. Anat. Record 61: 5, 1934-35.

25. Butcher, E.A. Am. J. Physiol. 129: 553, 1940.

26. Butcher, E.A. Ann. N.Y. Acad. Sci. 53: 508, 1951.

27. Butcher, E.A. Ann. N.Y. Acad. Sci. 83: 369, 1959.

28. Butt, W.R., Mason, A.S., and Morris, C.J.O.R. Lancet 259: 894, 1950.

29. Camacho, A.M. and Migeon, C.J. J. Clin. Endocrinol. and Metab. 23: 301, 1963.

30. Camacho, A.M. and Migeon, C.J. J. Clin. Invest. 43: 1083, 1964.

31. Casey, J.H. and Kellie, A.E. Proc. Soc. for Endocrinology. J. Endocrin. 29: vi, 1964.

32. Chase, H.B. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc. 435, 1958.

33. Chase, H.B. and Eaton, G.J. Ann. N.Y. Acad. Sci. 83: 365, 1959.

34. Chiang, S.P. and Schweppe, J.S. Federation Proceedings 22: 270, 1963. No. 652.

35. Chieffi, M. J. Gerontology 4: 200, 1949.

36. Corker, C.S., Norymberski, J.K, and Thow, R. Biochem. J. 83: 583, 1962.

37. Cruz Ferreira, A., Olympio da Fonseca, H., and da Costa Pinto, C. An. Bras. Ginecol. 47: 85, 1959 (Excerpta Medica Section x No. 325, 1960).

38. Danforth, C.H. Physiological Reviews 19: 99, 1939.

39. David, K., Dingemanse, E., Freud, J., and Laquere, E. Z. Physiol. Chim. 233: 281, 1935.

40. Davis, B.K. Acta Endocrin. 44 Suppl. 85: 9, 1963.

41. Dawson, H.L. Am. J. Anatomy 45: 461, 1930.

42. Dorfman, R.I. and Shipley, R.A. "Androgen", New York: John Wiley and Sons Inc., 1956.

43. Dorfman, R.I. Acta Endocrin. 34 Suppl. 50: 211, 1960.

44. Dorfman, R.I. Metabolism 10: 902, 1961.

45. Dorfman, R.I. A lecture on "Androgen and Oestrogen in Blood" presented at the Royal Victoria Hospital, Montreal, December 10, 1962.

46. Dorfman, R.I. Obst. Gyn. Survey 18: 65, 1963.

47. Dorfman, R.I., Forchielli, E., and Gut, M. Recent Progress in Hormone Research 19: 251, 1963.

48. Dratman, M. Personal communication to C.W. Lloyd, in "Advances in Neuroendocrinology," 478 (Univ. Illinois Press, Urbana, Ill., 525 pp., 1963).

49. Durward, A. and Rudall, K.M. J. of Anatomy 83: 325, 1949.

50. Durward, A. and Rudall, K.M. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc., 189, 1958.

51. Ebling. F.J. and Johnson, E. J. Endocrin. 29: 193, 1964.

52. Escamilla, R.F. Ann. Internal Med. 30: 249, 1949.

53. Ferriman, D. and Gallwey, J.D. J. Clin. Endocrinol. and Metab. 21: 1440, 1961.

54. Ferriman, D., Thomas, P.K., and Purdie, A.W. Brit. Med. J. 2: 1410, 1957.

55. Ferriman, D., Page, B.H., and Newnham, R. J. Endocrin. 25: 351, 1962.

56. Finkelstein, M. Acta Endocrin. 30: 489, 1959.

57. Finkelstein, M., Forchielli, E., and Dorfman R.I. J. Clin. Endocrinol. and Metab. 21: 98, 1961.

58. Forchielli, E., Sorcini, G., Nightingale, M., Brust, N., Dorfman, R.I., Perloff, W.H., and Jacobson, G. Anal. Biochem. 5: 416, 1963.

59. Fotherby, K. Brit. Med. J. 1: 1545, 1960.

60. Fukushima, D.K., Dobriner, K., and Gallagher, T.F. J. Biol. Chem. 206: 845, 1954a.

61. Fukushima, D.K., Bradlow, H.L., Dobriner, K., and Gallagher, T.F. J. Biol. Chem. 206: 863, 1954a.

62. Fukuyama, K. and Baker, B.L. J. Invest. Derm. 31: 327, 1958.

63. Futterweit, W., McNiven, N.L., Narcus, L., Lantos, C., Drosdowsky, M., and Dorfman, R.I. Steroids 1: 628, 1963.

64. Gallagher, T.F. and Koch, F.C. J. Biol. Chem. 84: 495, 1929.

65. Gallagher, T.F., Kappas, A., Hellman, L., Lipsett, M.B., Pearson, O.H., and West, C.D. J. Clin. Invest. 37: 794, 1958.

66. Garn, S.M. Ann. N.Y. Acad. Sci. 53: 498, 1951.

67. Gemzell, C.A., Tillinger, K.G., and Westman, A. Acta Endocrin. 30: 387, 1959.

68. Gilbert-Dreyfus, Zara, M., and Sebaoun, J. Semaine Hôp. 34: 1291, 1958 (Excerpta Medica 13III, No. 545, 1959).

69. Giorgi, E.P. J. Endocrin. 27: 225, 1963.

70. Golab, T. and Layne, D.S. J. Chromatog. 9: 321, 1962.

71. Gold, J.J. and Goldberg, S. Fertility and sterility 14: 73, 1963.

72. Goldzieher, J.W. and Axelrod, L.R. J. Clin. Endocrinol. and Metab. 22: 425, 1962.

73. Guerra-Garcia, R., Chattoraj, S.C., Gabrilove, L.J., and Wotiz, H.H. Steroids 2: 605, 1963.

74. Hamblen, E.C., Cuyler, W.K., and Baptist, M. J. Clin. Endocrinol. 1: 763, 1941.

75. Hamilton, J.B. and Montagna, W. Am. J. Anatomy 86: 191, 1950.

76. Hamilton, J.B. Ann. N.Y. Acad. Sci. 53: 585, 1951.

77. Hamilton, J.B. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc., 399, 1958.

78. Hamilton, J.B., Bunch, L.D., and Mestler, G.E. J. Clin. Endocrinol. and Metab. 22: 1103, 1962.

79. Herrmann, W., Buckner, F., and Morris, J.M. Fertility and Sterility 11: 74, 1960.

80. Hollander, N. and Hollander, V.P. J. Clin. Endocrinol. and Metab. 18: 966, 1958.

81. Horning, E.C., Luukkainen, T., Haahti, E.O.A., Creech, B.G., and Vanden Heuvel, W.J.A. Recent Progress in Hormone Research 19: 57, 1963.

82. Horton, R., Rosner, J.M., and Forsham, P.H. Proc. Soc. Expt. Biol. and Med. 114: 400, 1963.

83. Houssay, A.B., Naller, R., and Saurer, E. Acta Physiol. Lat. Amer. 9: 35, 1959.

84. Hudson, B., Coghlan, J., Dulmanis, A., Wintour, M., and Ekkel, I. Austral. J. Expt. Biol. 41: 235, 1963.

85. Ichii, S., Forchielli, E., Cassidy, C.E., Rosoff, C.B., and Dorfman, R.I. Biochem. Biophys. Res. Com. 9: 344, 1962.

86. Jailer, J.W., Vande Wiele, R., Christy, N.P., and Lieberman, S. J. Clin. Invest. 38: 357, 1959.

87. James, V.H.T. J. Endocrin. 22: 195, 1961.

88. James, V.H.T., Peart, W.S., and Iles, S.D. J. Endocrin. 24: 463, 1962.

89. Jayle, M.F., Malassis, D., and Pinaud, H. Acta Endocrin. 31: 1, 1959.

90. Jayle, M.F., Scholler, R., Mauvais-Jarvis, P., and Metáy, S. Acta Endocrin. 36: 375, 1961.

91. Johnsen, S.G. Acta Endocrin. 21: 127, 1956.

92. Julesz, M., Faredin, I., Tôth, I., Dávid, M.A., and Kovács, K. Acta Medica Hung. Tomus XIX, Fasc. 4: 311, 1963.

93. Kappas, A. and Gallagher, T.F. J. Clin. Invest. 34: 1566, 1955.

94. Kappas, A., Pearson, O.H., West, C.D., and Gallagher, T.F. J. Clin. Endocrinol. and Metab. 16: 517, 1956.

95. Kase, N., Forchielli, E., and Dorfman, R.I. Acta Endocrin. 37: 19, 1961.

96. Kase, N. and Kowal, J. J. Clin. Endocrinol. and Metab. 22: 925, 1962.

97. Kent, J.R., Gold, E.M., and Forsham, P.H. J. Clin. Endocrinol. and Metab. 23: 828, 1963.

98. Kellie, A.E. and Wade, A.P. Acta Endocrin. 23: 357, 1956.

99. Kellie, A.E. and Smith, E.R. Biochem. J. 66: 490, 1957.

100. Kim, J.H., Herrmann, F., and Sulzberger, M.B. J. Invest. Derm. 38: 351, 1962.

101. Kinsell, L., Bryant, D., and Albright, F. J. Clin. Endocrinol. and Metab. 14: 897, 1954.

102. Kirschner, M.A. and Lipsett, M.B. J. Clin. Endocrinol. and Metab. 23: 255, 1963.

103. Kligman, A.M. and Strauss, J.S. J. Invest. Derm. 27: 19, 1956.

104. Koets, P.J. J. Clin. Endocrinol. 9: 795, 1949.

105. Korenman, S.G., Wilson, H., and Lipsett, M.B. J. Clin. Invest. 42: 1753, 1963.

106. Kritchevsky, D. and Kirk, M.R. Arch. Biochem. Biophys. 35: 346, 1952.

107. Lacassagne, A. and Latarjet, B. Cancer Research 6: 183, 1946.

108. Lanthier, A. J. Clin. Endocrinol. and Metab. 20: 1587, 1960.

109. Lanthier, A. Un. Med. Canada 92: 32, 1963.

110. Levell, M.J., Mitchell, F.L., Paine, C.G., and Jordan, A. J. Clin. Pathol. 10: 72, 1957.

111. Lipsett, M.B. and Riter, B. J. Clin. Endocrinol. and Metab. 20: 180, 1960.

112. Lloyd, C.W., Moses, A.M., Lobotsky, J., Klaiber, E.L., Marshall, L.D., and Jacobs, R.D. J. Clin. Endocrinol. and Metab. 23: 413, 1963.

113. Lloyd, C.W., Segre, E.J., Wyss, H.I., and Lobotsky, J. Unpublished data as quoted by Segre et al, 1964.

114. Lucas, W.M., Whitman, W.F., Jr., and West, C.D. J. Clin. Endocrinol. and Metab. 17: 465, 1957.

115. Macdonald, P.C., Vande Wiele, R.L., and Lieberman, S. Am. J. Obst. Gynec. 86: 1, 1963.

116. Maguire, H.C., Jr. Lancet 1: 864, 1964.

117. Mahesh, V.B. and Greenblatt, R.B. J. Clin. Endocrinol. and Metab. 22: 441, 1962.

118. Mahesh, V.B., Greenblatt, R.B., Aydar, C.K., and Roy, S. Fertility and Sterility 13: 513, 1962.

119. Martin, M.M., Reddy, W.J., and Thorn, G.W. J. Clin. Endocrinol and Metab. 21: 923, 1961.

120. Mason, H.L. and Engstrom, W.W. Physiol. Reviews 30: 321, 1950.

121. Masuda, M. and Thuline, H.C. J. Clin. Endocrinol. and Metab. 13: 581, 1953.

122. Matthews, J.S., Pereda V., A.L., and Aguillera, A. J. Chromatog. 9: 331, 1962.

123. Mauvais-Jarvis, P. and Baulieu, E.E. J. Clin. Invest. 41: 1691, 1962.

124. McKnight, E. Lancet 1: 410, 1964.

125. Melick, R. and Pincus Taft, H. J. Clin. Endocrinol. and Metab. 19: 1597, 1959.

126. Merivale, W.H.H. J. Clin. Pathol. 4: 78, 1951.

127. Mohn, M.P. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York: Academic Press Inc., 398, 1958.

128. Montagna, W. "Structure and Function of the Skin", Academic Press Inc., New York, 1962.

129. Myer, R.J. and Hamilton, J.B. Ann. N.Y. Acad. Sci. 53: 562, 1951.

130. Shen, Nai-Hsuan C, Francis, F.E., and Kinsella, R.A., Jr. J. of Lab. and Clin. Med. 60: 1017, 1962.

131. Oertel, G.W. and Eik-Nes, K.B. Proc. Soc. Expt. Biol. Med. 102: 553, 1959.

132. Oertel, G.W. Acta Endocrin. 37: 237, 1961.

133. Pasargiklian, E. Studi. Sassaresi 39: 22, 1961 (Excerpta Medica Endocrinology 16, No. 1424, 1962).

134. Pedersen, J. Acta Obstet. Gynaecol. Scand. 22: 381, 1942-3.

135. Perloff, W.H., Hadd, H.E., Channick, B.J., and Nodine, J.H. Arch. Internal Med. 100: 981, 1957.

136. Perloff, W.H., Channick, B.J., Suplick, B., and Carrington, E.R. J. Am. Med. Assoc. 167: 2041, 1958.

137. Pesonen, S. Acta Endocrin. 43: 220, 1963.

138. Peterson, R.E., Brodie, B.B., Wyngaarden, J.B., Bunim, J.J., and Guerra, S.L. J. Clin. Invest. 34: 1779, 1955.

139. Pincus, G., Dorfman, R.I., Romanoff, L.P., Rubin, B.L., Bloch, E., Carlo, J., and Freeman, H. Recent Progress in Hormone Research 11: 307, 1955.

140. Pond, M.H. J. Endocrin. 10: 202, 1954.

141. Prunty, F.T.G., Brooks, R.V., and Mattingly, D. Brit. Med. J. 2: 1554, 1958.

142. Rennel, E.G. and Callahan, W.P. Anat. Rec. 135: 21, 1959.

143. Richter, R.H.H. and Arnold, M. Helv. Physiol. et Pharmacol. Acta 20(4): c76, 1962.

144. Riondel, A., Tait, J.F., Gut, M., Tait, S.A.S., Joachim, E., and Little, B. J. Clin. Endocrinol. and Metab. 23: 620, 1963.

145. Ritter, F.J. and Meyer, G.M. Nature 193: 941, 1962.

146. Rodrique, J., Cugolo, E., Cruz Ferreira, A., Olympio da Fonseca, H., and Pinto, C.C. Arq. Brasil Endocrinol. Metabologia 8(1): 5, 1959. Biol. Abstracts No. 18859, 1962.

147. Sachs, L. Nature 201: 296, 1964.

148. Salter, W.T., Cahen, R.L., and Sappington, T.S. J. Clin. Endocrinol. 6: 52, 1946.

149. Sandor, T. and Lanthier, A. Rev. Canad. Biol. 19: 445, 1960.

150. Samuels, L.T. J. Biol. Chem. 168: 471, 1947.

151. Savard, K., Dorfman, R.I., and Poutass, E. J. Clin. Endocrinol. and Metab. 12: 935, 1952.

152. Savard, K. Clin. Chem. 7: 328, 1961.

153. Schubert, K. and Wehrberger, K. Naturwissenschaften 47: 281, 1960.

154. Segal, L., Segal, B., and Nes, W.R. J. Biol. Chem. 235: 3108, 1960.

155. Segre, E.J., Klaiber, E.L., Lobotsky, J., and Lloyd, C.W. Ann. Rev. Med. 15: 315, 1964.

156. Shah, P.N. Am. J. Obst. Gynec. 73: 1255, 1957.

157. Siiteri, P.K., Vande Wiele, R.L., and Lieberman, S. J. Clin. Endocrinol. and Metab. 23: 588, 1963.

158. Slaunwhite, W.R. and Samuels, L.T. J. Biol. Chem. 220: 341, 1956.

159. Smith, L.L. and Foell, T. J. Chromatog. 9: 339, 1962.

160. Snedecor, G.W. "Statistical Methods", The Iowa State College Press, Amos, Iowa, 1956.

161. Solomon, S., Vande Wiele, R., and Lieberman, S. J. Am. Chem. Soc. 78: 5453, 1956.

162. Stoddard, F.J. Am. J. Obst. Gynec. 49: 417, 1945.

163. Storey, W.F. and Leblond, C.P. Ann. N.Y. Acad. Sci. 53: 537, 1951.

164. Straile, W.E. Ann. N.Y. Acad. Sci. 83: 499, 1959.

165. Szabo, G. "The Biology of Hair Growth", edited by W. Montagna and R.A. Ellis, New York Academic Press Inc., 33, 1958.

166. Tanner, J.M., Healy, M.J.R., Whitehouse, R.H., and Edgeson, A.C. J. Endocrin. 19: 87, 1959.

167. Taylor, A.C. J. Expt. Zoology 110: 77, 1949.

168. Tropea, P.P., Coghi, I., and Sibilla, A. Riv. Ostet c Ginecol. 17(11): 753, 1962 (Biol. Abstracts No. 17971, 1963).

169. Trotter, M. Surgery, Gynecology, and Obsetetrics 60: 1092, 1935.

170. Turunen, A., Pesonen, S., and Zilliacus, H. Acta Endocrin. 45: 447, 1964.

171. Vande Wiele, R.L., Macdonald, P.C., Gurpide, E., and Lieberman, S. Recent Progress in Hormone Research 19: 275, 1963.

172. Van Scott, E.J. and Ekel, T.M. J. Invest. Derm. 31: 281, 1958.

173. Van Scott, E.J. Ann. N.Y. Acad. Sci. 83: 480, 1959.

174. Venning, E.H., Hoffman, M.M., and Browne, J.S.L. J. Biol. Chem. 146: 369, 1942.

175. Vermeulen, A. and Verplancke, J.C.M. Steroids 2: 453, 1963.

176. Vestergaard, P. and Leverett, R. Acta Endocrin. 25: 45, 1957.

177. Warren, J.C. and Salhanick, A.A. J. Clin. Endocrinol. and Metab. 21: 1218, 1961.

178. West, C.D., Hollander, V.P., Kritchevsky, T.H., and Dobriner, K. J. Clin. Endocrinol. and Metab. 12: 915, 1952.

179. Whitaker, W.L. Anat. Rec. 124: 447, 1956.

180. Williams, R.H. "Text Book of Endocrinology", W.B. Saunders Co., Philadelphia, 1962.

181. Wilson, H., Borris, J.J., and Garrison, M.M. J. Clin. Endocrinol. and Metab. 18: 643, 1958.

182. Wilson, H., Lipsett, M.B., and Ryan, D.W. J. Clin. Endocrinol. and Metab. 21: 1304, 1961.

183. Wilson, H., Lipsett, M.B., and Korenman, S.G. J. Clin. Endocrinol. and Metab. 23: 491, 1962.

184. Wotiz, H.H. and Martin, H.F. J. Biol. Chem. 236: 1312, 1961.

185. Zerssen, D.V. Schweiz. Med. Wchnschr. 90: 1333, 1960.

ABBREVIATIONS USED IN THE TABLES AND FIGURES

T = Testosterone

A = Androsterone

11-OH A = 11-OH Androsterone

11 Keto A = 11 Keto Androsterone

Δ^4 Andro = Δ^4 Androstenedione

DHA = Dehydroepiandrosterone

E = Etiocholanolone

11-OH E = 11-OH Etiocholanolone

11 Keto E = 11 Keto Etiocholanolone.

TABLE I

Movement (Rf.) of standard steroids in various solvent systems by TLC, using silica gel G. as supporting medium

A. Plate size 20 x 20 cm.

B. Plate size 20 x 40 cm.

A.

Standards	Toluene:Ethyl acetate		Hexane : Ethyl acetate			Benzene: Ethyl acetate	Hexane: Ether	Benzene: Ether
	1:2.5	1:4	1.1	1:1.5	1:2	3:2	1:1	1:1
T	0.49	0.53	0.42	0.45	0.48	0.41	0.07	0.28
DHA	0.58	0.62	0.51	0.54	0.62	0.52	0.14	0.37
A	0.6	0.64	0.55	0.57	0.64		0.15	0.39
E	0.5	0.62	0.46	0.47	0.51	0.46	0.07	0.30
11-OH A			0.33			0.29		
11 Keto E			0.23			0.27		
11 Keto A			0.27			0.29		
11-OH E						0.27		
Δ^4 andro.			0.51			0.60		

B.

Standards	Hexane:Ethyl acetate			Benzene:Ethyl acetate		Toluene: Ethyl acetate
	1:1	1:1.5	1:2	3:2 0.5 mm. thick	3:2 1 mm. thick	1:4
T	0.23	0.34	0.37	0.25	0.26	0.50
DHA	0.4	0.46	0.49	0.39	0.41	0.6
A	0.4	0.48	0.49	0.36	0.41	0.6
E	0.28	0.37	0.40	0.30	0.31	0.52

TABLE II

Movement (Rf.) of standard steroids in various solvent systems by TLC, using aluminum oxide G. as supporting medium.

A. Plate size 20 x 20 cm.

B. Plate size 5 x 20 cm.

A.

Standards	6% Ethanol in chloroform:Isooctane			Hexane:Ethyl acetate		Benzene: Ethyl acetate	Toluene: Ethyl acetate	Benzene: Ether	Ethyl acetate: Cyclohexane: Toluene
	1:1	1:2.5	2.5:1	1:1	1:2	3:2	1:2.5	1:1	10:10:1
T	0.62	0.11	0.65	0.42	0.50	0.37	0.51	0.36	.51
DHA	0.54	0.13	0.61	0.46	0.50	0.42	0.51	0.44	.50
A	0.54	0.13	0.70	0.48	0.47	0.40	0.53	0.36	.37
E	0.63	0.08	0.61	0.36	0.35	0.27	0.39	0.25	.54
11-OH A								0.10	
11 Keto A									
11 Keto E								0.07	
11-OH E									
Δ^4 andro								0.61	

B.

Standards	Hexane:Methyl acetate				Benzene: Methyl acetate
	1:1	1.5:1	2:1	2.5:1	2:1
DHA	0.49	0.39	0.29	0.30	0.47
A	0.52	0.39	0.30	0.31	0.48

TABLE III

Measurement (Rf.) of standard steroids in two-dimensional system, aluminum oxide G. plate 20 x 20 cm.

Standards	Rf. of 1st dimension Hexane:Ethyl acetate 1:1	Rf. of 2nd dimension Benzene:Ether 1:1
DHA	0.55	0.21
Androsterone	0.56	0.14
Etiocholanolone	0.41	0.09
Testosterone	0.49	0.15

TABLE IV

Mean length (cm.) and diameter (mm.) ± S.D. of 10 male and 10 female white rats.

Sex	Length (cm.)	Diameter (mm.)
male	1.66 ± .163	.0687 ± .007
female	1.48 ± .179	.0652 ± .004

TABLE V

Urinary excretion of testosterone (μg./24 hrs.), total 17 KS (mg./24 hrs.), and mean + S.D. in Occidental idiopathic hirsute, Occidental normal, and Oriental normal women.

Occidental Hirsute				Occidental Normal				Oriental Normal			
no.	age	testosterone	total 17 KS	no.	age	testosterone	total 17 KS	no.	age	testosterone	total 17 KS
1. J.A.	34	2.21	3.77	1. T.R. (3)	22	15.46	2.25	1. B.J.	35	8.49	3.88
2. L.M.	18	13.8	6.82	2. L.K.	39	11.58	3.57	2. P.T.	31	3.82	2.19
3. A.V.	55	71.09	4.99	3. M.K.	26	18.5	4.08	3. M.J.	20	13.57	3.98
4. A.H.	44	18.31	8.89	4. G.T.	28	16.66	9.59	4. P.R.	28	11.8	4.18
5. Po. (1)	21	6.03	8.8	5. Y.R. (4)	21	3.26	3.47	5. M.B. (6)	30	6.21	3.9
6. Ak. (2)	20	7.4	5.14	6. G.N. (5)	25	3.45	3.72	6. P.	26	1.03	2.49
				7. E.K.	21	8.45	3.22	7. R.T.	26	7.25	3.12
								8. S.B.	34	8.30	2.80
Mean		22.29	6.65	Mean		11.05	4.27	Mean		7.65	3.32
S.D.		±28.04	±2.28	S.D.		±6.22	±2.41	S.D.		±4.03	±0.08

1. Urine was collected on Day 7.
2. Creatinine excretion = 0.32 gm./24 hrs.
3. Urine was collected on Day 10.
4. Urine was collected on Day 25.
5. Urine was collected on first day of menstrual period.
6. Urine was collected on Day 27.

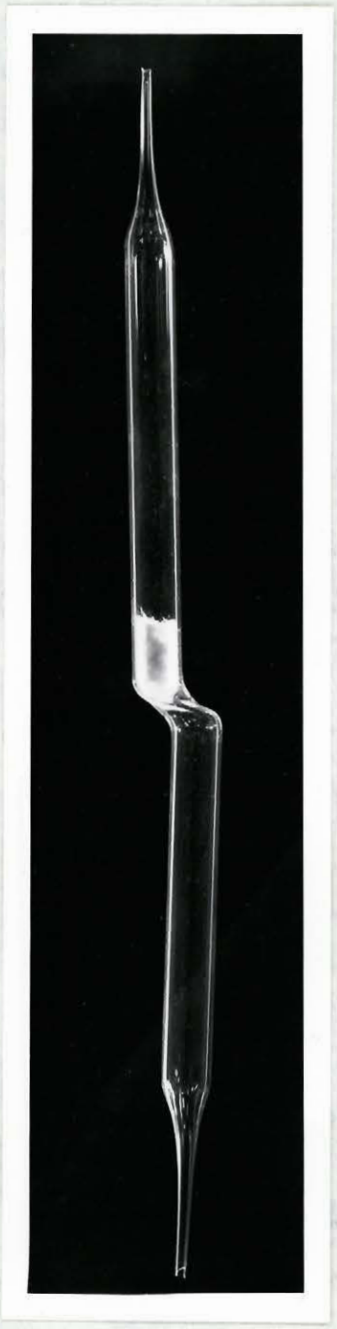

Figure I:

Pipette for elution.

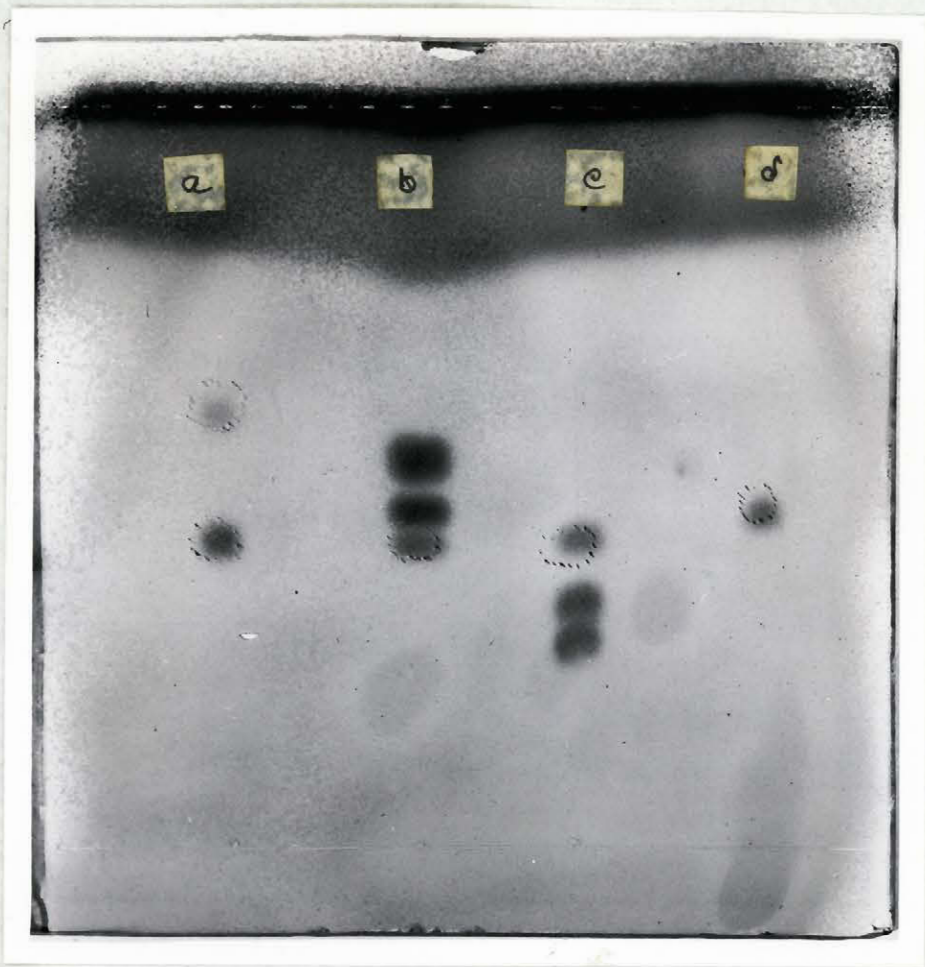

Figure II:

Standard steroids on silica gel G. plate,
solvent benzene:ethyl acetate 3:2

From top:

(a) Δ^4 andro, T

(b) DHA and A, E, T

(c) T, 11-OH A and 11 keto A, 11-OH E and 11 keto E

(d) T

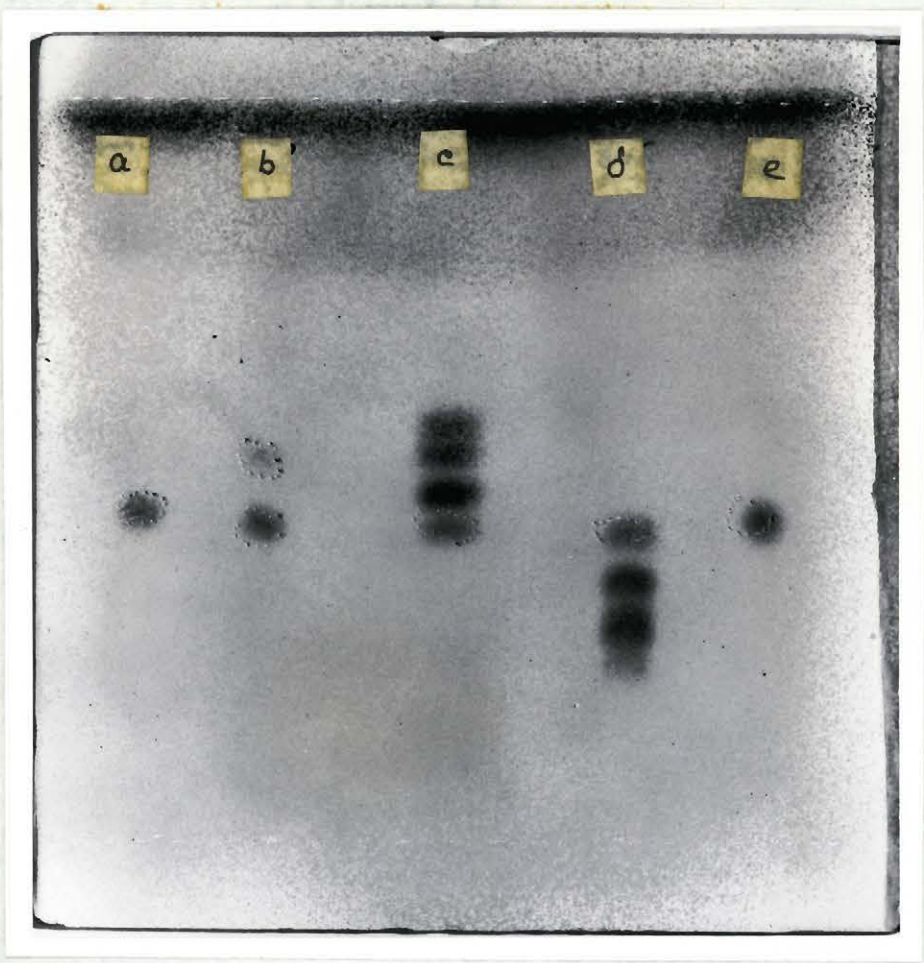

Figure III:

Standard steroids on silica gel G. plate,
solvent hexane:ethyl acetate 1:1

From top:

(a) T

(b) Δ^4 andro, T

(c) A, DHA, E, T

(d) T, 11-OH A, 11 keto A and 11-OH E, 11 keto E

(e) T

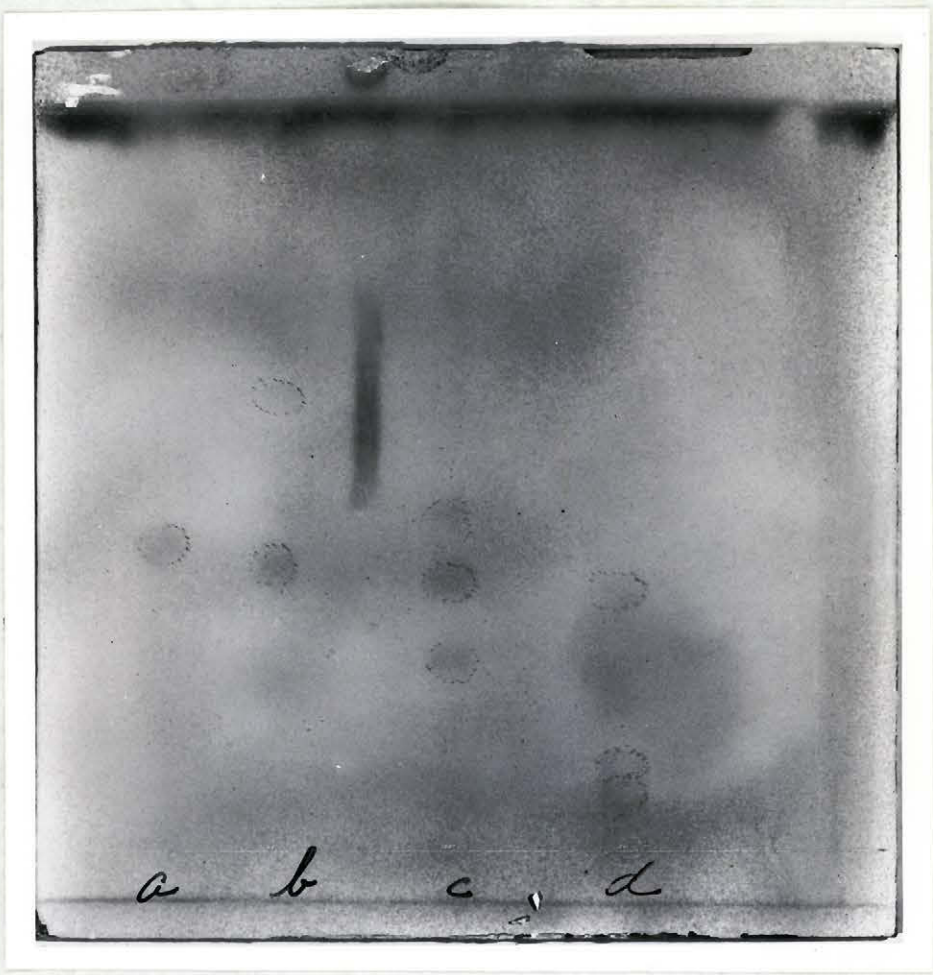

Figure IV:

Standard steroids on aluminum oxide G. plate,
solvent benzene:ether 1:1

From top:

(a) T

(b) Δ^4 andro, T

(c) DHA, A and T, E

(d) T, 11-OH A and 11 keto A, 11-OH E and 11 keto E

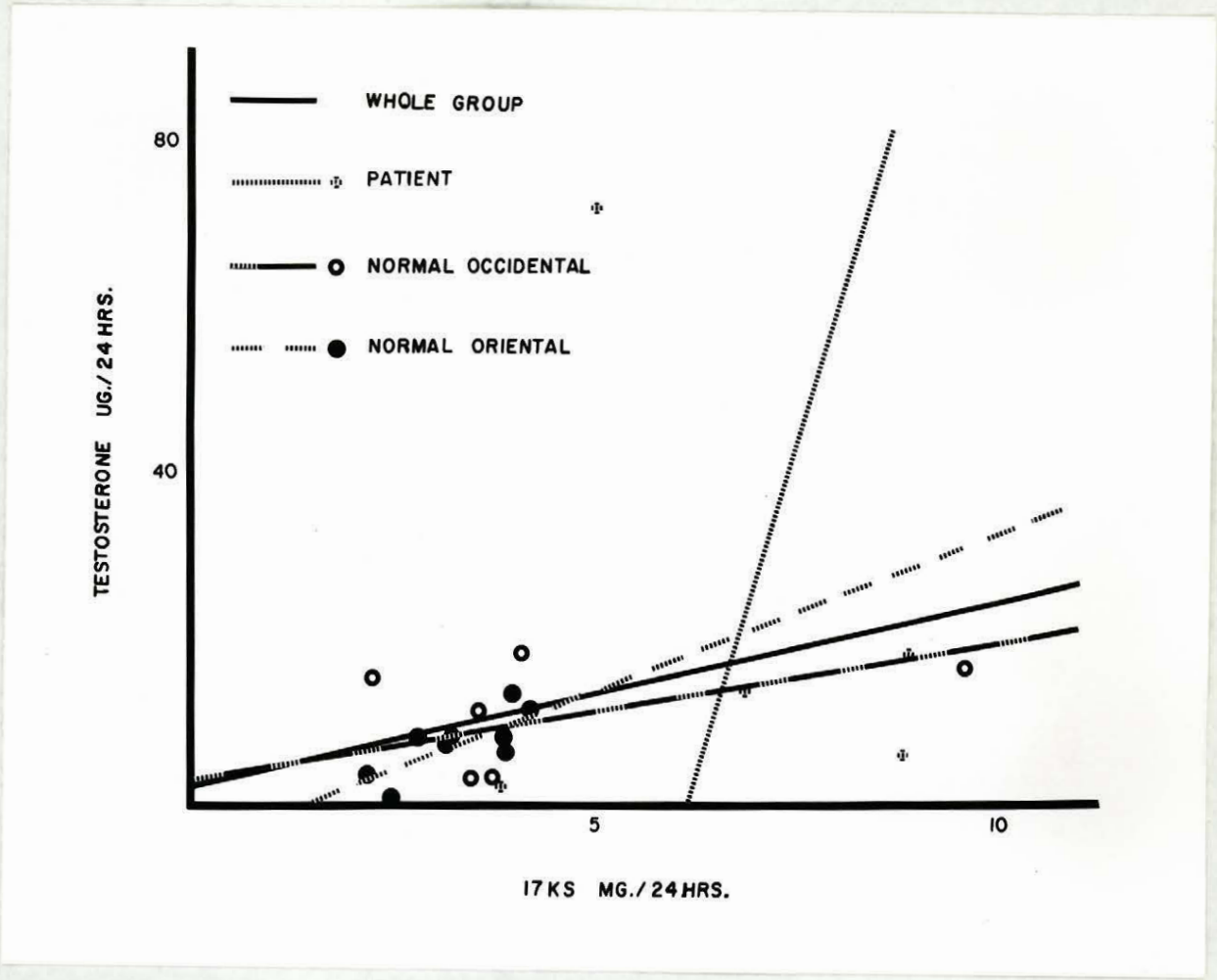

Figure V:

Correlation of urinary testosterone and total 17 KS