CHAPTER II
Steroid Metabolism in Idiopathic Hirsutism

'Idiopathic hirsutism' represents a typical case of endocrine disorder which has been a socio-clinical problem. It has so far defied all attempts of understanding the etiological factors. Measurements of androgen secretion or the ratio of circulating androgen to estrogens or hyper responsiveness of hair follicles to the normal amounts of androgens have been the subject of several clinical and biochemical reports.1,2.

Resume of Literature

A. Definition and clinical symptoms of hirsutism:

According to Bishop3, hirsutism in women consists of excessive growth of body hair with characteristic masculine hair distribution, and may or may not be accompanied by oligomenorrhea or amenorrhea and acne. The condition is unrelated to adrenal hypertrophy, androgen producing tumour of the adrenal or the ovary.3,4. This type of hirsutism is often designated as 'simple' or Idiopathic and is distinct from 'virilism' ('defeminization' and 'masculinization'). Hirsutism may be one of the characteristics of virilism along with amenorrhea, diminished libido, atrophy of mammary glands, loss of gynecological fat, increased masculinity, enlargement of clitoris and deepening of voice and acne. In normal women the pattern of hair distribution varies from individual to individual. However, genetic
Influences may produce marked deviation in hair distribution in otherwise normal females. In other cases, hirsutism is a reflection of some distinct endocrine disorders.

**Androgen secretion in hirsutism:**

The early investigations showed that in simple hirsutism as well as in hirsutism resulting from endocrine disorders, there is a higher excretion of androgens.\(^1\),\(^2\) There were however cases showing normal excretion of androgens. These cases may have a higher concentration of circulating androgenic material than normal subjects but this material may be excreted in the urine in such a form that it could not have been measured by the bioassays. It may be that the hair follicles in some cases may be more sensitive to the normal amount of androgens and may give rise to a distribution of hair of a male type.

**Excretion of 17-KS:**

Increased urinary 17-KS excretion associated with simple hirsutism is frequently observed by various investigators. Dorfman and Shipley\(^5\) compiled the data on the excretion of 17-KS in simple hirsutism and on hirsutism due to endocrine disorders. There is a distinct higher mean value for urinary 17-KS in simple hirsutism though there is a considerable overlap of individual values. Determination of urinary 17-KS on several 24 hour specimens rather than a single sample, is recognised as the best way of evaluating androgen excretion in hirsute women\(^6\).
Kotes observed that there is a cyclic variation in the excretion of 17-KS in hirsute patients with maximum excretion at the time of ovulation. Bissel and Williams found that the urinary 17-KS in simple hirsutism were not significantly elevated and in some cases they were even subnormal. Salter et al. observed normal distribution of 3-α and 3-β hydroxy-17-KS in the urine of five hirsute women, but the ratio of androgen to estrogen in the urine was higher than that seen in normal women. Brooksbank quoted that Moranzi and co-workers observed a higher excretion of 17-KS (22.4 mg/24 hours) in hirsute patients with polycystic ovaries (normal women excreted 14.2 mg/24 hrs). However, hirsute patients with a normal menstrual cycle or having oligomenorrhea or secondary amenorrhea did not show significantly higher values of urinary 17-KS than normal women.

Fractionation of urinary 17-KS:

Many investigators have attempted to fractionate urinary 17-KS in order to evaluate the differential function of endocrine glands producing steroids. 3-β-hydroxy-17KS, mainly DHEA is generally recognised as an adrenal product in man. Gardner using the Pettenkofer reaction for DHEA estimation observed that in seven hirsute patients, three excreted normal amounts of 17-KS (10.3-12.2 mg/24 hrs), four excreted increased amounts of 17-KS (19.6-35.1 mg/24 hrs) and all of the seven patients excreted larger amounts of DHEA (1.1-4.2 mg/24 hrs). Four of these patients, when
treated with cortisone acetate, showed a diminution of total 17-KS and DHEA excretion. A diminution of DHEA of the same order could also be produced when normal women were given cortisone acetate. The reduction in the excretion of DHEA was however, not observed in two of the patients studied. More recently, Lipsett and Riter, using paper chromatographic technique, found that five out of seven hirsute patients excreted higher than normal amounts of DHEA but there was no correlation between excretion of DHEA and body hair. High urinary concentrations of DHEA in hirsutism have been found in the work of Jailer and Vande Wiele. Jayle et al. observed that although 80 hirsute patients out of a total of 180 showed normal excretion of 17-KS the ratio of DHEA to total 17-KS was higher in most of the patients. Twenty nine per cent of the hirsute patients excreted higher amounts of DHEA than normal women.

Lipsett and Riter found that the ratio androsterone to etiocholanolone was close to one in all the idiopathic hirsute patients except one. The average being 1.3 and the normal control ratio was 1.1. The mean values for the urinary concentration of 11-hydroxy-etiocholanolone, 11-hydroxy-androsterone and 11-oxy-etiocholanolone were 0.63, 0.70 and 0.68 mg/24 hours, respectively, against the normal values of 0.54, 0.57 and 0.60 mg/24 hrs. Many individual values of the patients overlapped with values
corresponding to those of normal women. They concluded from these data that hirsutism is associated with higher urinary excretion of etiocholanolone and androsterone confirming the observation of Gallagher et al. On the other hand, the studies of Jailer and Vande Wiele, Johnsen and Gemzell et al. show normal urinary excretion of 17-KS in patients with hirsutism.

**Production of C_{19} steroids by adrenal cortex:**

The concept that the underlying cause of simple hirsutism might be abnormal functioning of adrenal glands has received considerable support. The most consistent finding is that administration of the corticosteroids suppresses the 17-KS excretion and often alleviates the clinical signs of hyperandrogenism and may even restore the urinary excretion pattern of steroids to normal. The dynamic approach to ascertain adrenal function is to measure the adrenal response following the administration of ACTH. Gardner administered cortisone to seven patients with simple hirsutism and noticed that the urinary 17-KS and DHEA excretion fell to normal. Similar effects of cortisone administration were also observed in some of the patients of Greenblatt who were classified as suffering from hirsutism of adrenal origin.

Jailer and Vande Wiele found that the levels of excretion of all the fractions of urinary 17-KS except 11-keto-etiocholanolone were brought to normal values when
subjected to cortisone therapy in a group of hirsute women having a normal ovulatory menstrual cycle. They further observed that in 27 patients with polycystic ovaries, but with normal pattern of urinary 17-KS, neither cortisone therapy nor wedge resection of the ovary affected the 17-KS excretion although wedge resection corrected the menstrual abnormalities. It is noteworthy, however, that none of these treatments could make any significant difference as to the state of hirsutism. Mattingly et al.\textsuperscript{21} were able to diminish hirsutism in only a few cases by administering prednisone. Perloff et al.\textsuperscript{22} on the other hand, believed that hirsutism, with or without disorders of menstruation is an adrenal disease and observed a reduction of hirsutism with prednisone therapy. Kappas et al.\textsuperscript{23} studied one case of severe hirsutism and found high urinary 17-KS, normal DHEA and a higher than normal ratio of 11-deoxy-17-KS to 11-oxy-17-KS. Cortisone suppressed the 11-oxy-17-KS to the normal range but ACTH administration increased the excretion of these steroids. They postulated a theory that hirsutism can be a mild form of the adrenogenital syndrome, and is basically a disorder of the adrenal gland. This theory was further supported by the work of Perloff\textsuperscript{24}, Green et al.\textsuperscript{25} and Gallagher\textsuperscript{17}. Prunty\textsuperscript{26} put forward another hypothesis involving the existence of an accessory pituitary or hypothalamic factor appearing normally only at puberty to explain the phenomena.
The secretion of this factor is maintained at an increased rate in hirsute women. There exists considerable discrepancy among the various investigators as to the steroid response of hirsute patients to the administration of ACTH. Nabarro et al. noted that urinary 17-KS and 17-OHCS were abnormally elevated by ACTH, whereas Jayle et al. and Gemzell et al. failed to observe an abnormally high response. They observed a normal ACTH response in hirsute and normal women. Other workers found only a slightly higher response than normal in urinary 17-KS excretions following ACTH. In some hirsute patients there is a higher response of urinary 17-OHCS during ACTH administration than normal women and greater fall of control 17-KS during prednisolone treatment.

**Production of ovarian androgens:**

Amongst the characteristics of Stein-Leventhal syndrome are hirsutism and amenorrhea. Shippel suggested that this was because the hypertrophied ovarian theca tissue found in Stein-Leventhal patients produces androgens. Gemzell et al. could not correlate hirsutism and excretion of 17-KS with the histological changes occurring in the polycystic ovaries but observed that in some cases the excretion of 17-KS could be reduced by wedge resection of the ovaries. This is also evident in the work of Greenblatt who found 17-KS excretion lowered after panhysterectomy in Stein-Leventhal patients.
Benson et al. differentiated his hirsute patients into an ovarian and an adrenal type. The ovarian type patients did not show suppression of 17-KS by cortisol treatment but excreted excessive amounts of urinary 17-KS following HCG administration. Such an effect of chorionic gonadotrophin was also observed by Sternberg et al. in normal and pregnant women and they suggested that the hilus cells might be producing androgens under the influence of chorionic gonadotrophin. Another theory or explanation regarding the pathogenesis of simple hirsutism stated that higher amount of LH are secreted in this condition. It was suggested that LH acts on theca interna cells and thus inhibits the production of androgens. Androgens in turn inhibit the secretion of the FSH and hence lower the output of estrogens.

Normal ovarian tissue is capable of synthesizing androstenedione from progesterone. Baggett et al. isolated C14-estradiol-17/β and androstenedione following the incubation of testosterone-3-C14 with human ovarian slices. In the same year Wotiz and co-workers showed that estrone, estradiol-17/β and estriol could be synthesised by the human ovary from testosterone. 17α-hydroxyprogesterone and androstenedione have been found in human follicles obtained one day before ovulation and in corpora lutea.

Short and London detected progesterone,
17α-hydroxyprogesterone and androstenedione in cyst fluid from polycystic ovaries of Stein-Leventhal patients. In addition to these steroids, estrone and estradiol-17β were found in cyst fluid from normal women, but not in the cyst fluid of the Stein-Leventhal patients investigated. Recently, Michael et al\textsuperscript{38} found higher concentration of plasma testosterone in a woman with hilus cell adenoma than those found in normal male subjects. They were also able to detect testosterone in the blood of normal women. Dorfman and co-workers\textsuperscript{39} estimated testosterone in plasma of normal and idiopathic hirsute women by isotope derivative technique and observed a higher concentration of testosterone in the plasma.

B. Defective biosynthesis of steroids in hirsutism:

Adrenal cortex: The differences in the excretory levels of steroids in idiopathic hirsutism and in normals are not sufficiently large so as to aid the diagnosis. Number of investigators have reported normal or slightly elevated excretion of 17-KS\textsuperscript{5}. Kovacic et al\textsuperscript{40} and Gallagher et al\textsuperscript{17} fractionated the urinary 17-KS and found that 11-oxy-17-KS are increased in majority of their cases of idiopathic hirsutism. These authors postulated that this kind of hirsutism may be associated with a mild form of adrenogenital syndrome, and suggested a block at 21-hydroxylating enzyme action. This would lead to the decreased production of cortisol and corticosterone (Fig.9). Since the plasma content
PROBABLE PATHWAYS OF ANDROGEN-SYNTHESIS IN
ADRENAL CORTEX
(SIMPLE HIRSUTISM)
(SOFFER et al 1961)

ACETATE \rightarrow CHOLESTEROL

\[ \text{ACTH} \]

\[ \text{PREGNENOLONE} \]

\[ \text{17\alpha-HYDROXY-PREGNENOLONE} \]

\[ \text{PROGESTERONE} \]

\[ \text{11-DESOXYCORTICOSTERONE} \]

\[ \text{CORTICOSTERONE} \]

\[ \text{DEHYDROEPI-ANDROSTERONE} \]

\[ \text{17\alpha-HYDROXYPROGESTERONE} \]

\[ \text{11-DESOXYCORTISOL} \]

\[ \text{CORTISOL} \]

\[ \text{ANDROSTENEDIONE} \]

\[ \text{TESTOSTERONE} \]

\[ \text{11\beta-HYDROXY-ANDROSTENEDIONE} \]

Fig. 9
of 17-OHCS and the excretion of 17-KGS are normal or slightly elevated in simple hirsutism, it was postulated that the adrenal cortex may have become hyper sensitive to the normal amount of ACTH secreted by the anterior pituitary. Similarly, the 11- as well as the 21-hydroxylase activity may also be normal but the formation of pregnenolone and progesterone and their subsequent transformation to their 17-hydroxy compounds might be stimulated. Thus, the increased amounts of 17-hydroxypregnenolone and 17-α-hydroxyprogesterone would get converted to DHEA and androstenedione respectively by the action of 17-desmolase enzyme. These steroids are fairly androgenic in human and higher secretion of these compounds may induce male type of hair growth with or without causing menstrual disturbances. This has been supported by the fact that in a number of hirsute patients there is an increased excretion of DHEA, androstenedione and etiocholanolone. Soffer et al. believed that the production of testosterone in adrenal cortex may be the underlying cause of this disease.

Ovary: It has been reported that polycystic ovary particularly of the Stein-Leventhal type produces androgenic steroids i.e., androstenedione which is not further converted to estrone and estradiol. Short and London postulated that there is a complete enzymatic block at 19-hydroxylation of androstenedione. The increased accumulation of this compound may be responsible for producing male type of hair growth in women. Sandor and Lanthier showed that the conversion of testosterone to androstenedione
occurs in polycystic ovary to a small extent. Further, Axelrod and Goldziehar\textsuperscript{42} and Lanthier and Sandor\textsuperscript{44} suggested that the enzymatic equilibrium is in favour of reduction of the 17-keto group to 17-hydroxyl group. As shown in Fig. 3, polycystic ovary thus converts androstenedione to testosterone before it is hydroxylated at position 19, thereby limiting the synthesis of estrogens.
MATERIAL

A. Hirsutism:

Twenty two women having abnormal hair growth were selected for this investigation. All these subjects were of the active reproductive age (16-37 years). Nine of these had a normal menstrual cycle while eleven had only a scanty excretion of blood during menstruation with a wide variability in duration of the cycle. Twenty four hour urine samples were collected from these women between the 11th to 13th and 21st to 23rd day of the menstrual cycle to determine the basal excretion of 17-KS, 17-KGS, pregnanetriol and pregnanetriol-11-one. It was not possible in all the cases particularly those with irregular menstrual cycles to state that the sample collected between the 21st and 23rd day of the cycle was from the post-ovulatory phase of the particular subject.

The basal level of 17-OHCS in plasma was studied in all these subjects. ACTH tests were carried out in 10 of them. 17-OHCS in plasma were determined after the infusion of 25 I.U. ACTH and two urine samples of twelve hours each (total of 24 hours' including the infusion period) were collected in all these cases and 17-KS, 17-KGS, pregnanetriol and pregnanetriol-11-one estimated.

B. 17-KS in ovarian cyst fluid:

This investigation was carried out in ten cases, ranging from 18 to 39 years in age. Four of these were
hirsute women. Three of the hirsute patients had normal menstrual cycles while the fourth subject had scanty flow of blood during menstruation and the cycle showed wide variability pertaining to time intervals. The rest of the subjects were non-hirsute and were surgically operated for other gynecological disorders as mentioned in Table VIII. The ovaries in all ten cases were sclerotic and contained multiple cysts. After the collection of fluid from the cyst in each case, the ovaries were bisected in order to exclude neoplastic conditions. No histological evidence of neoplasm was found in any of the sections. It may be pertinent to mention here that nine of the ten cases were not treated with any hormone at least two months prior to surgery. Patient (SD) was, however, given 100 mg testosterone every second day for two months prior to operation. Testosterone therapy was, however, stopped two weeks before surgery.

Cyst fluid obtained from the polycystic ovaries for isolation and identification of steroids: Five hirsute women from 18 to 23 years old suffering from the Stein-Leventhal syndrome were selected from a large group of hirsute patients. They had started to menstruate at the age of 12 to 14 years and two of them had regular menstrual cycles while the third one had irregular and scanty menstrual bleeding. All five patients had marked hirsutism and none of them were obese. Their urinary excretion of 17-KS, 17-KGS and pregnanetriol
## Table VIII

17-KS in the ovarian cyst fluid of hirsute and non-hirsute women.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Clinical diagnosis</th>
<th>Amount of cyst fluid in ml</th>
<th>17-KS µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP</td>
<td>39</td>
<td>Hirsutism</td>
<td>5.5</td>
<td>45.4</td>
</tr>
<tr>
<td>DS</td>
<td>21</td>
<td>Hirsutism</td>
<td>16.5</td>
<td>9.1</td>
</tr>
<tr>
<td>VV</td>
<td>34</td>
<td>Hirsutism</td>
<td>1.5</td>
<td>30.0</td>
</tr>
<tr>
<td>KM</td>
<td>18</td>
<td>Hirsutism</td>
<td>2.8</td>
<td>21.4</td>
</tr>
<tr>
<td>KC</td>
<td>23</td>
<td>Habitual abortion</td>
<td>6.0</td>
<td>3.8</td>
</tr>
<tr>
<td>RP</td>
<td>34</td>
<td>Secondary sterility with prolapse of uterus</td>
<td>2.5</td>
<td>non detectable</td>
</tr>
<tr>
<td>FA</td>
<td>18</td>
<td>Primary sterility</td>
<td>4.7</td>
<td>non detectable</td>
</tr>
<tr>
<td>HP</td>
<td>20</td>
<td>Primary sterility</td>
<td>7.0</td>
<td>5.4</td>
</tr>
<tr>
<td>SD</td>
<td>34</td>
<td>Dysfunctional uterine bleeding</td>
<td>6.5</td>
<td>8.5</td>
</tr>
<tr>
<td>CK</td>
<td>46</td>
<td>Dysfunctional uterine bleeding</td>
<td>6.5</td>
<td>non detectable</td>
</tr>
</tbody>
</table>
were within normal limits. Exploratory laparotomy was done in all five cases. No abnormalities of the adrenals could be found in any of these patients. The ovaries were enlarged, sclerotic and had multiple cysts. Bilateral wedge resection was carried out. Histologically, the ovarian tissues showed follicular cysts and large amount of theca interna cells. The right ovary of one patient (RP) was much enlarged and had a big cyst. Forty ml of fluid was removed from this ovary. The cystic fluid from the four other patients as well as from the left ovary of patient RP was pooled giving a total of 12.5 ml.

**Ovarian tissue for biosynthesis of steroids:**

The ovarian tissues were removed surgically as a wedge resection from the ovaries of patients IK and RK. Both these patients had marked hirsutism, irregular menstruation cycles. The endometrium biopsy was suggestive of ovulatory menstrual cycle. The morphological changes in the ovaries of both these patients were typical of those described in the ovaries of Stein-Leventhal syndrome with the only exception that there was a presence of a corpus luteum. In patient RK, the ovarian tissue was surgically removed on 107th day after menstruation and she started menstruating following surgery. The tissues were frozen in dry ice till they were taken for incubation.
METHOD

Isolation and identification of steroids in cyst fluid:

The pooled sample of cyst fluid A and the fluid from a solitary cyst B were extracted continuously for 24 hours with 200 ml of ether in a liquid-liquid extraction apparatus. The ether extract was evaporated at 40°C in air. The extracted fluid was then hydrolysed with 20,000 I. U. β-glucuronidase (beef liver preparation, Warner-Chilcott) for 24 hours at pH 4.7 and 37°C and extracted again continuously for 24 hours. The extracts were evaporated as indicated above.

The original extracts from samples A and B as well as the extracts following hydrolysis were dissolved in 25 ml of toluene and extracted twice with 10 ml and 5 ml aliquots of 1.0 N NaOH and then with water. The combined alkaline and water extracts were adjusted to pH 7 with 1.0 N HCl and extracted with three portions of 25 ml of methylene dichloride. The extracts were washed with water and dried at 40°C under nitrogen. The toluene fraction which contained neutral steroids was also washed with water till neutral and dried under nitrogen. These two extracts were saved for subsequent characterization.

Paper chromatography: (a) The methylene dichloride extracts were chromatographed on paper irrigated with benzene/formamide solvent system and C14-labelled estrone, estradiol and estriol were used as indicators. The chromatographs were scanned with Westenskow C14 paper scanner.
after chromatography. A small strip of the chromatogram was cut and the unknown compounds were detected as blue coloured spots following Barton's reaction\textsuperscript{46} on the strip. However, the free fraction of sample A gave only one spot corresponding to $R_f$ value of estrone-$C^{14}$. Attempts were made to identify estradiol and estriol by (i) Kober reaction\textsuperscript{47} which is specific for estrogens and (ii) gas chromatography. For this purpose the areas on the paper strips corresponding to $C^{14}$ labelled estrone, estradiol and estriol were eluted and dried under nitrogen.

The colour reaction of Kober\textsuperscript{47} as modified by Ittrich\textsuperscript{48} was carried out on the total estrone fractions and on aliquots of the estradiol and estriol fractions eluted from paper strips. The estrone fraction of sample A, when scanned on a recording spectrophotometer (Beckman DK-2) gave a curve identical to that of authentic estrone. Sample B as well as the hydrolysed samples from A and B however, did not give any colour with the Kober reagent. Further, aliquots gave negative Kober reaction. Aliquots taken for gas chromatography on a QF-1 column at $205^\circ C$\textsuperscript{49} showed a small peak corresponding to estrone. Quantitatively this peak accounted for only $0.15 \mu g$ of estrone, indicating complete absence of estradiol and estriol.

The toluene layer from the original alkali extracts was evaporated to dryness under nitrogen and this neutral fraction chromatographed on paper using hexane-
benzene/formamide solvent system. An ultra-violet absorbing spot was observed on the chromatograms of the free fractions for samples A and B with a $R_f$ value of 0.6 to 0.65. The Zimmermann reaction was carried out on thin strips cut from these chromatograms and the U.V absorbing region of the chromatograms gave a characteristic Zimmermann colour. A pinkish brown colour also appeared with this reagent at the origin of each chromatogram of the free fraction of Samples A and B. This spot fluoresced in U.V. light and was designated as X. Another spot giving similar reactions had a $R_f$ value of 0.9 and was designated as $X_1$ (Fig. 10).

The ultra-violet absorbing areas from chromatograms A and B were eluted and chromatographed in hexane-benzene/formamide solvent system. In sample A, two compounds, both U.V. absorbing and having $R_f$ value corresponding to androstenedione and progesterone were separated. In sample B, however, only one spot with a $R_f$ value similar to androstenedione was found by the Hain's scanner. These U.V. absorbing areas of the chromatograms were eluted and chromatographed on a small column of alumina.

**Column chromatography for characterization of androstenedione:**

Woelm alumina grade I was dry packed in a micro column (5 mm diameter) and washed with 20 ml of hexane. The sample was applied with three 1 ml portions of hexane. The column was washed with 10 ml of hexane followed by 5 ml of a
FIRST PAPER CHROMATOGRAM OF NEUTRAL EXTRACT OF
OVARIAN CYST FLUID

A - POOLED CYST FLUID SAMPLE
B - FLUID FROM ONE CYST
* CONJUGATED STEROIDS FROM SAMPLE A & B

Fig. 10
mixture of hexane and methylene dichloride (10:90 v/v) and finally treated with 1 per cent methanol in methylene dichloride. The last fraction was collected, dried, dissolved in methanol and read in a Beckman DK-2 spectrophotometer between 220 and 260 mµ.

**Column chromatography for progesterone:** The column was prepared in the same manner as in the case of androstenedione column. The sample was applied with three 1 ml portions of hexane, the column was washed with 5 ml of hexane followed by 15 ml of 30% hexane in methylene dichloride. Progesterone was eluted with 20 ml of 1% methanol in methylene dichloride. The sample was dried, dissolved in vacuum distilled methanol and estimated spectrophotometrically.

All the eluates observed for spectral characters which showed a peak at 240 mµ. Allen's correction\(^{52}\) was used for quantitative estimation.

**Androstenedione:** The eluates of androstenedione were divided into approximately three equal parts. To two of these portions approximately 3,000 cpm of androstenedione-4-C\(^{14}\) were added and the mixtures chromatographed on paper in methylcyclohexane/propylene glycol. The paper chromatograms were counted in a strip counter, the radioactive portions eluted, chromatographed in alumina, and the U.V. spectra measured and quantitated as described. The samples were then dried and dissolved in 1 ml of methanol. A 0.1 ml
Aliquot in duplicate of each sample was plated and counted. The ratio of cpm to total amount was calculated. The remaining 0.8 ml of each sample was then processed by the same procedure except that the paper chromatography was done by Bush's A system\textsuperscript{53}. This procedure permitted an evaluation of cpm per total amount of steroids following repeated steps of purification.

**Progesterone**: The constant specific activity procedure used for androstenedione fraction was also employed for progesterone fraction using the solvent system of chromatography described.

**Preparation of derivatives**: Two derivatives were obtained (a) by reduction of androstenedione to testosterone and (b) acetylation of testosterone.

(a) **Reduction of androstenedione to testosterone**: The remaining one-third of the combined androstenedione sample isolated from cyst fluid was dissolved in 0.4 ml methanol, 2 mg of sodium borohydride were added and the mixture incubated at 0°C for 1 hour. A few drops of glacial acetic acid were added and the sample was dried under nitrogen at 40°C. The residue was applied to a paper chromatogram which was developed in hexane-benzene/formamide, and scanned in U.V. light. An area absorbing U.V. light with mobility characteristic of authentic testosterone was found. The authentic androstenedione-4-C\textsuperscript{14} was reduced in the same manner (yield of testosterone, approximately 70%).
(b) Acetylation of testosterone: The U.V. light absorbing area was eluted, dried and acetylated with acetic anhydride and pyridine (1:5) for 24 hours at room temperature in the dark. The acetylated compound was chromatographed on paper in methylcyclohexane/propylene glycol solvent system. The compound showed R_f value identical with authentic testosterone acetate. This acetyl derivative was then eluted and chromatographed on the alumina column. It was eluted from this column with 3% methanol in methylene dichloride. This fraction was collected, evaporated and dissolved in 1 ml of vacuum distilled methanol and U.V. absorption spectral characteristics determined using recording spectrophotometer (Beckman model DK-2) between 220 and 260 mp. This compound also showed characteristic peak of 240 mp.

Identification of spots X and X_1 observed on the chromatogram of toluene fraction: The compounds at areas X and X_1 gave Zimmermann and Barton's reaction on paper indicating that they may be 17-KS or estrogens. These compounds were eluted, dried and dissolved in 2 ml of methanol. One ml of each sample was dried and the Zimmermann reaction was carried out according to Haltorff and Koch. However, the pink colour which turned brown during this reaction did not give characteristic peak at 510 mp. Similarly, another aliquot of the same material did not give positive Kober reaction. This rules out the suggestion that the spots X and X_1 were either due to presence of 17-KS or estrogens.
Biosynthesis of Steroids

Preparation of homogenates:

The frozen specimens were powdered in a glass mortar with dry ice and then one of the samples was homogenized with 0.15 M Potassium phosphate buffer, pH 7.4, and other was homogenized with 0.15 M phosphate buffer, pH 7.0. Both buffer solutions contained 0.03 M nicotinamide. Four ml of buffer were used for each gram of tissue. The homogenates were then centrifuged at 700 Xg for 10 minutes and the supernatant used as enzyme source. One ml of this supernatant was added to 4 ml of the buffer for incubation. The incubation medium contained following cofactors:

1) 0.4 mM NADP
2) 0.4 mM NAD
3) 3.0 mM Dipotassium glucose-6-phosphate
4) 1.0 mM sodium fumarate
5) few crystals of glucose-6-phosphate dehydrogenase enzyme.

Substrate: When the substrate steroids were used in μmol concentration, unlebelled steroids were mixed to the corresponding radioactive steroids having 0.1 μc radioactivity to make a total amount of 100 μmol.

Incubation: The incubations were carried out in a Dubnoff incubator at 37°C with oxygen as gas phase. An aliquot of 5 ml was removed from each reaction flask after 30 minute incubation and the rest was incubated for 60 minutes.

One of the reaction flasks containing 800 mg of tissue was incubated with 1 μc androstenedione (sp. activity) as substrate for 4 hours.
The reaction was stopped with 0.5 ml of 1 N hydrochloric acid and the incubation mixture extracted 5 times with ether and chloroform mixture (4:1). The extraction procedure procedure in brief is given below. The radioactivity remaining in the aqueous medium was found to vary between 0.05 to 1 percent.

**Extraction Procedure**

**Incubation mixture**

Extracted 5 times with 4 volumes of ether : chloroform (4:1 v/v)

Extract evaporated under nitrogen

Dissolved in 20 ml of toluene

Extracted 3 times with 15 ml of 1 N sodium hydroxide

**Toluene layer**

Dissolved in 20 ml of toluene

Extracted 3 times with 15 ml of 1 N sodium hydroxide

Ethy extract was washed with water twice

Residue subjected to paper chromatography

**Alkaline extract brought to pH 7.0 with 5 N hydrochloric acid**

Extracted with ether 5 times with 3 volumes of ether

Ether extract was washed with water twice

Residue subjected to paper chromatography

**Neutral extract**

Residue subjected to paper chromatography.
**Paper chromatography:** The Zaffaroni system⁴⁵ of paper chromatography was used for the initial separation of steroids. To the residues obtained from both neutral and phenolic extracts about 50 μg each of the carrier steroids were added to facilitate identification and estimation of the metabolites formed.

The neutral extract with the carrier steroids, was chromatographed on a paper with the hexane-benzene/formamide solvent system⁴⁵ to the end of the strip. The positions of all the steroids containing the unsaturated ketonic structure in ring A were marked on the paper chromatogram by their absorption of ultraviolet light in the region of 240 μm by the Haines Scanner⁵¹. The paper chromatogram strips were first scanned in a gas flow, thin window Westenskow strip counter. The quantitation of the radioactive area was confirmed by counting the eluate of the individual area in Packard liquid scintillation counter. The radioactivity was found to be proportional to the area obtained in the strip scanner.

**Identification of individual steroids:**

**Separation of 17α-hydroxyprogesterone and testosterone:**

In the Zaffaroni system⁴⁵ of hexane-benzene/formamide, 17α-hydroxyprogesterone and testosterone ran together. The areas of these steroids on the chromatogram were marked (under ultraviolet light) and were cut and eluted with 10 ml of chloroform : methanol (1:1 v/v) mixture.
The eluted strips were checked under Westenskow strip scanner to confirm that the radioactive compounds were completely eluted from the paper chromatogram. The eluate was evaporated and the residue treated with a freshly prepared 0.2 ml mixture of acetic anhydride and pyridine (1:5) for 24 hours at room temperature in dark. The acetylation reaction was stopped by adding one ml of methanol and dried under nitrogen. The residue was chromatographed in the same system of hexane-benzene/formamide to the end of the chromatogram strips when testosterone acetate was widely separated from 17\(-\)hydroxyprogesterone. The paper chromatogram was scanned under a Westenskow strip counter. No testosterone was found in the incubations when progesterone-4-\( \text{C}^{14}\) and 17\(-\)hydroxyprogesterone-4-\( \text{C}^{14}\) were used as substrates. 17\(-\)hydroxyprogesterone was further identified by running in hexane/formamide solvent system and finally by crystallization of the compound to constantly specific activity.

\( \Delta^4\)-Pregnene-3-Keto-17\( \alpha \), 20\( \alpha \)-diol:

This steroid was separated from the extract on the chromatogram developed in hexane-benzene/formamide solvent system. In Zaffaroni's system\(^{45}\), its \( R_f \) value was 0.15. It was then eluted and re-chromatographed in benzene/formamide solvent system. Further identification was carried out by acetylating the material with 0.2 ml of acetic anhydride and pyridine and rechromatographing on paper hexane/formamide solvent system.
$\Delta^4$-pregnene-3-keto-20-ol:

This steroid was separated on the paper chromatogram developed in hexane-benzene/formamide solvent system ($R_f$ 0.22) from the neutral extract when progesterone was used as substrate. It was further identified by the same procedure adopted for $\Delta^4$-pregnene-3-keto-17$\alpha$, 20$\alpha$-diol.

Androstenedione:

This steroid was separated on the chromatogram from other neutral steroids with hexane-benzene/formamide solvent system. It was eluted and submitted to acetylation and the product chromatographed in Zafforoni system$^{45}$ but no acetylated product could be detected.

When androstenedione was a metabolic product either from progesterone or from 17$\alpha$-hydroxyprogesterone, its identity was confirmed by reducing it to testosterone.

19-Nor-testosterone:

This compound was present in the neutral extract when androstenedione with 1$\mu$C radioactivity was used as substrate and the incubation was carried out for 4 hours. The $R_f$ value of this steroid was 0.17 when the neutral extract was applied on paper chromatogram which was developed in hexane-benzene/formamide. On rechromatographing in benzene/formamide solvent system the $R_f$ was found to be 0.41. It was eluted, acetylated and again chromatographed in a solvent system of hexane/formamide for further identification.
The labelled steroids which were used as substrate and remained partly unchanged in the incubation mixture were also chromatographed in different chromatographic systems. They were acetylated and their identity was confirmed in order to confirm that no other compound of the same Rf value was left undetected.

**Estrone and Estradiol:**

Unlabelled estrone, estradiol and estriol were added as carriers to the phenolic extracts before chromatographing on paper. The chromatogram was developed in a solvent system of benzene/formamide. The phenolic extracts did not contain any compound with Rf value similar to that of estrone or estradiol or estriol, when progesterone and 17α-hydroxyprogesterone was used as substrates. Small amount of radioactivity remained at the point of application of the chromatogram which could not be acetylated with a mixture of acetic anhydride and pyridine.

When androstenedione was substrate and the incubation was carried out for 4 hours, phenolic extracts gave two spots on chromatogram (benzene/formamide system) with Rf values 0.15 and 0.57 corresponding to authentic estrone and estradiol respectively. These spots gave positive Barton's reaction which is characteristic of estrogens. Their identity was confirmed by running these and their acetylated compounds in different solvent systems and by constant specific activity on recrystallization.
Crystallization of steroids:

Crystallizations of androstenedione, estrone and estradiol were carried out with added authentic 10 mg samples, first in acetone, then in methanol and finally in acetone. The crystals were washed with hexane and dried in vacuum desiccator. The samples of crystals weighed on Cahn electrobalance were counted in 10 ml of scintillating fluid in Tricarb scintillation counter.
RESULTS

Urinary excretion of 17-KS and 17-KGS in hirsute women:

In a group of 22 hirsute women estimations of 17-KS were performed repeatedly. The values ranged from 5.2 to 17.2 mg per 24 hours, the overall mean being 10.1 mg. These values appear to be higher than those found in normal women (8.06 ± 0.44) but corresponded to the values of 17-KS in normal men (10.24 ± 0.64). Out of these hirsute women, 13 had fairly regular menstrual cycles. In these the excretion of 17-KS during the ovulatory phase ranged from 6.5 to 17.2 mg per 24 hours with a mean value of 10.58 and in the luteal phase the values ranged between 5.3 and 16.7 mg per 24 hours with a mean of 9.34. The values in luteal phase are not significantly different than those seen in the ovulatory phase (Table IX).

The excretion of 17-KGS in the hirsute women ranged from 4.0 to 12.1 mg per 24 hours, the mean being 7.1 mg. When the 17-KGS were estimated in the pre- and post-ovulatory phases in thirteen hirsute women, the values were 7.6 and 8.1 respectively. The difference between these values is not significant.

17-KS in ovarian cyst fluid:

Three out of six non-hirsute women did not show any 17-KS in cyst fluid. The concentration ranged from 3.8 to 8.5 μg per ml in the remaining three non-hirsute patients. On the other hand, in four hirsute women the amount was higher and ranged from 9.1 to 45.4 μg per ml. In hirsute subjects the
Table IX
Excretion of 17-KS and 17-KGS in hirsute women.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Phase of menstrual cycle</th>
<th>17-KS mg/24 hours</th>
<th>17-KGS mg/24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>RP</td>
<td>19</td>
<td>70/84*</td>
<td>13.8</td>
<td>4.2</td>
</tr>
<tr>
<td>UJ</td>
<td>23</td>
<td>43/64/</td>
<td>9.4</td>
<td>7.9</td>
</tr>
<tr>
<td>MK</td>
<td>25</td>
<td>12/25 22/25</td>
<td>12.8</td>
<td>9.4</td>
</tr>
<tr>
<td>MG</td>
<td>23</td>
<td>11/26 22/26</td>
<td>6.5</td>
<td>6.8</td>
</tr>
<tr>
<td>MC</td>
<td>30</td>
<td>13/32 23/32</td>
<td>12.9</td>
<td>11.2</td>
</tr>
<tr>
<td>KJ</td>
<td>25</td>
<td>17/29 22/23</td>
<td>17.2</td>
<td>16.7</td>
</tr>
<tr>
<td>SK</td>
<td>30</td>
<td>11/24 20/24</td>
<td>16.1</td>
<td>16.2</td>
</tr>
<tr>
<td>CB</td>
<td>34</td>
<td>Oligomenorrhia 12.5</td>
<td>14.0</td>
<td>5.1</td>
</tr>
<tr>
<td>AJ</td>
<td>30</td>
<td>36.78</td>
<td>11.9</td>
<td>7.1</td>
</tr>
<tr>
<td>SK</td>
<td>25</td>
<td>17/27 22/28</td>
<td>7.9</td>
<td>6.9</td>
</tr>
<tr>
<td>NS</td>
<td>22</td>
<td>16/27 23/27</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>SG</td>
<td>19</td>
<td>Amenorrhia 13.6/14.8, 16.9</td>
<td>10.4, 8.8, 8.7</td>
<td></td>
</tr>
<tr>
<td>UF</td>
<td>30</td>
<td>6/28</td>
<td>7.3</td>
<td>6.6</td>
</tr>
<tr>
<td>DS</td>
<td>30</td>
<td>13/23 22/23</td>
<td>6.9</td>
<td>8.7</td>
</tr>
<tr>
<td>NM</td>
<td>32</td>
<td>14/30</td>
<td>11.4</td>
<td>8.9</td>
</tr>
<tr>
<td>AN</td>
<td>34</td>
<td>12/30 22/30</td>
<td>7.4</td>
<td>5.3</td>
</tr>
<tr>
<td>SB</td>
<td>20</td>
<td>11/30 22/30</td>
<td>10.3</td>
<td>5.4</td>
</tr>
<tr>
<td>NR</td>
<td>30</td>
<td>Amenorrhia 11.2</td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>22</td>
<td>11/23 22.29</td>
<td>11.7</td>
<td>11.3</td>
</tr>
<tr>
<td>KG</td>
<td>24</td>
<td>12/27 24/27</td>
<td>6.6</td>
<td>9.4</td>
</tr>
<tr>
<td>PP</td>
<td>25</td>
<td>15/29 27/40</td>
<td>9.4</td>
<td>6.6</td>
</tr>
<tr>
<td>VT</td>
<td>22</td>
<td>23/27</td>
<td>14.3</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Range... 6.5 - 17.2  5.3 - 16.7  4.0 - 12.1  3.0 - 12.7
Mean.... 10.3    9.3     7.1     8.1

* day of determination/menstruation period in days.
amount of fluid was also comparatively large. The results are shown in Table X.

The urinary 17-KS and abnormal regions, and amount of body hair in the hirsute females:

In a group of 22 hirsute patients the body regions with abnormal hair growth varied from 2 to 5 and the total counts of body hair in these regions ranged from 7 to 25 as shown in Table X. There was however, no correlation between excretion of 17-KS and the degree of hirsutism.

Influence of ACTH infusion on the concentration of 17-OHCS in plasma and on the excretion of 17-KS and 17-KGS in hirsute women:

The ACTH infusion test was carried out in 13 hirsute women. The adrenal cortical response to 25 I. U. ACTH measured by the concentration of 17-OHCS in the peripheral blood plasma is shown in Fig. 11. The percentage increase in the values of 17-OHCS varied from 144.1 to 352.6.

The effect of ACTH infusion on the excretion of 17-KGS and 17-KS was determined by measuring the urinary 17-KGS and 17-KS before and after ACTH infusion. These data showed that there is a large variation in the increments of these steroids from individual to individual. The percentage increase in the excretion of 17-KS varied from 98.8 to 219.6 and in 17-KGS, it ranged from 129.0 to 489.6. The detailed values are given in Table XI.
### Table X

**Urinary 17-KS and 17-KGS in relation to body hair growth**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>17-KS mg/24 hr.</th>
<th>17-KGS mg/24 hr.</th>
<th>Unusual hairy regions</th>
<th>Total amount of hair on unusual regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>RP</td>
<td>19</td>
<td>11.0</td>
<td>4.5</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>UJ</td>
<td>23</td>
<td>8.6</td>
<td>6.6</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>MK</td>
<td>25</td>
<td>11.3</td>
<td>10.5</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>MG</td>
<td>23</td>
<td>7.2</td>
<td>5.5</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>MC</td>
<td>30</td>
<td>11.9</td>
<td>12.8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>KJ</td>
<td>25</td>
<td>13.7</td>
<td>10.5</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>SK</td>
<td>30</td>
<td>16.2</td>
<td>8.7</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>CB</td>
<td>34</td>
<td>13.3</td>
<td>6.6</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>AJ</td>
<td>30</td>
<td>9.2</td>
<td>6.3</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>SKu</td>
<td>25</td>
<td>7.3</td>
<td>4.5</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>SG</td>
<td>19</td>
<td>15.1</td>
<td>9.3</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>NS</td>
<td>22</td>
<td>7.9</td>
<td>4.9</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>UF</td>
<td>30</td>
<td>7.3</td>
<td>6.6</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>DS</td>
<td>30</td>
<td>7.8</td>
<td>6.8</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>NM</td>
<td>32</td>
<td>10.9</td>
<td>8.9</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>AN</td>
<td>34</td>
<td>6.4</td>
<td>5.9</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>SB</td>
<td>20</td>
<td>7.9</td>
<td>8.4</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>MR</td>
<td>30</td>
<td>10.0</td>
<td>9.7</td>
<td>3</td>
<td>18</td>
</tr>
<tr>
<td>LS</td>
<td>22</td>
<td>11.5</td>
<td>6.6</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>KG</td>
<td>24</td>
<td>8.0</td>
<td>7.6</td>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>PP</td>
<td>25</td>
<td>5.2</td>
<td>6.1</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>VT</td>
<td>22</td>
<td>14.3</td>
<td>4.4</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

See text for explanation of units in columns 5 & 6.
Table XI
Influence of ACTH infusion on plasma 17-OHCS and on the excretion of
17-KS and 17-KGS in hirsute women

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>Phase of menstrual cycle</th>
<th>Plasma 17-OHCS mg/100 ml</th>
<th>17-KS mg/24 hrs.</th>
<th>17-KGS mg/24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>After ACTH</td>
<td>Control</td>
</tr>
<tr>
<td>RP</td>
<td>19</td>
<td>35/90*</td>
<td>22.0</td>
<td>31.7</td>
<td>8.2</td>
</tr>
<tr>
<td>UJ</td>
<td>23</td>
<td>-</td>
<td>34.2</td>
<td>52.6</td>
<td>8.3</td>
</tr>
<tr>
<td>MK</td>
<td>25</td>
<td>9/18</td>
<td>16.6</td>
<td>50.6</td>
<td>11.7</td>
</tr>
<tr>
<td>MG</td>
<td>23</td>
<td>9/26</td>
<td>25.0</td>
<td>52.6</td>
<td>8.2</td>
</tr>
<tr>
<td>MC</td>
<td>30</td>
<td>18/32</td>
<td>10.9</td>
<td>30.4</td>
<td>11.7</td>
</tr>
<tr>
<td>KJ</td>
<td>25</td>
<td>12/28</td>
<td>15.0</td>
<td>52.5</td>
<td>7.1</td>
</tr>
<tr>
<td>AJ</td>
<td>30</td>
<td>-</td>
<td>28.5</td>
<td>61.5</td>
<td>6.5</td>
</tr>
<tr>
<td>CB</td>
<td>34</td>
<td>Oligomenorrhia</td>
<td>25.0</td>
<td>33.3</td>
<td>-</td>
</tr>
<tr>
<td>SK</td>
<td>25</td>
<td>34/36</td>
<td>27.5</td>
<td>42.5</td>
<td>6.6</td>
</tr>
<tr>
<td>NM</td>
<td>32</td>
<td>14/30</td>
<td>26.9</td>
<td>49.0</td>
<td>9.9</td>
</tr>
<tr>
<td>SB</td>
<td>20</td>
<td>-</td>
<td>15.3</td>
<td>32.4</td>
<td>-</td>
</tr>
<tr>
<td>SM</td>
<td>32</td>
<td>-</td>
<td>11.9</td>
<td>31.0</td>
<td>-</td>
</tr>
<tr>
<td>SK</td>
<td>30</td>
<td>21/24</td>
<td>9.7</td>
<td>34.2</td>
<td>16.2</td>
</tr>
</tbody>
</table>

* Irregular menstrual period.
RESPONSE TO ACTH (I.V.)

Fig. 11
Excretion of Pregnanetriol and Pregnanetriol-11-one in hirsute women:

The determination of urinary pregnanetriol was carried out in 11 women having marked idiopathic hirsutism. The control values ranged from 0.06 mg to 2.07 mg per 24 hours with a mean value of 1.07 (Table XIII). None of the hirsute patients had signs of Cushing's syndrome, adrenogenital syndrome or any kind of tumour of the adrenal or the ovarian origin. The excretion following ACTH infusion, ranged from 0.074 to 1.9 mg per 24 hours in seven hirsute women. When the control excretion and the excretion after ACTH are compared in individual subjects the values in five patients after ACTH are slightly increased. On the other hand, in patients MC and SK the values are lower after ACTH (Table XIII).

The excretion of pregnanetriol-11-one was studied in 10 hirsute women, out of which four showed a positive test for pregnanetriol-11-one. Patient MC who had a positive test on control urine did not show pregnanetriol-11-one after ACTH infusion, while patient KG did not excrete this steroid normally and gave distinct positive test for this steroid after ACTH infusion (Table XIII). Patient KJ excreted higher amounts of this steroid than the other patients, both in the control state and after ACTH infusion.

Steroids in ovarian cyst fluid obtained from hirsute women:

The steroids isolated and measured in follicular
# Table XII

Plasma 17-OHCS in idiopathic hirsutism

<table>
<thead>
<tr>
<th>Menstrual pattern</th>
<th>No. of cases</th>
<th>17-OHCS μg/100 ml plasma (Range of values)</th>
<th>Mean value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pre-ovulatory</td>
<td>4</td>
<td>4.2 - 25.0</td>
<td>16.25 ± 4.05</td>
</tr>
<tr>
<td>post-ovulatory</td>
<td>6</td>
<td>9.8 - 25.0</td>
<td>16.65 ± 2.50</td>
</tr>
<tr>
<td>Irregular</td>
<td>7</td>
<td>10.3 - 34.2</td>
<td>18.90 ± 2.50</td>
</tr>
</tbody>
</table>
### Table XIII

**Pregnanetriol and Pregnanetriol-11-one Excretion in Hirsute Women.**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Pregnanetriol mg/24 hours</th>
<th>Pregnanetriol-11-one mg/24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control level</td>
<td>After ACTH</td>
</tr>
<tr>
<td>UJ</td>
<td>1.68</td>
<td>1.82</td>
</tr>
<tr>
<td>MK</td>
<td>0.46</td>
<td>0.58</td>
</tr>
<tr>
<td>RP</td>
<td>0.43</td>
<td>0.89</td>
</tr>
<tr>
<td>XJ</td>
<td>1.82</td>
<td>1.90</td>
</tr>
<tr>
<td>MG</td>
<td>0.06</td>
<td>0.10</td>
</tr>
<tr>
<td>MC</td>
<td>1.50</td>
<td>0.074</td>
</tr>
<tr>
<td>SK</td>
<td>2.07</td>
<td>1.72</td>
</tr>
<tr>
<td>PA</td>
<td>0.62</td>
<td>not done</td>
</tr>
<tr>
<td>SB</td>
<td>1.23</td>
<td>1.38</td>
</tr>
<tr>
<td>SG</td>
<td>1.12</td>
<td>not done</td>
</tr>
<tr>
<td>NS</td>
<td>0.81</td>
<td>not done</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>1.07</strong></td>
<td><strong>1.20</strong></td>
</tr>
</tbody>
</table>
cystic fluid obtained from hirsute women having Stein-Leventhal type ovaries are listed in Table XIV. In the pooled sample of 12.5 ml of fluid, progesterone and androstenedione were isolated and identified as free steroids. Estrone also appeared to be present in small amount. The 40 ml cystic fluid from a single ovary contained only 53.0 μg of androstenedione. After the extraction of free steroids, when the fluid was hydrolysed and again extracted, no detectable steroids were found. Thus, conjugated steroids seem to be absent from this biological fluid.

The unknown compounds X and X₂, though giving positive Zimmermann⁵⁰ and Barton's reactions⁴⁶ on paper, were found to be not 17-KS upon more detailed analysis. The other steroids known to occur in the pathway of the ovarian biosynthesis of steroids were not present in detectable quantities in any of the samples investigated.

Table XV shows the constant specific activity of androstenedione and progesterone. The ratios of cpm of the added authentic androstenedione and progesterone obtained after the first and second chromatographs differ very little indicating that androstenedione and progesterone found in the cystic fluid, indeed, are these steroids.

**Biosynthesis of steroids in ovarian tissue of hirsute women:**

(A) The homogenate of the ovarian tissue obtained from patient (IK) was incubated with labelled progesterone (0.2 μc)
Table XIV

Free steroids in the cyst fluid of hirsute women suffering from Stein-Leventhal syndrome.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount (ml)</th>
<th>Steroids: µg per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Androstenedione</td>
</tr>
<tr>
<td>A</td>
<td>12.5</td>
<td>40.4</td>
</tr>
<tr>
<td>B</td>
<td>40.0</td>
<td>53.0</td>
</tr>
</tbody>
</table>
**Table XV**

**Constant specific activity of isolated androstenedione and progesterone**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Steroid</th>
<th>After 1st Chromatography</th>
<th>After 2nd Chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Androstenedione</td>
<td>166</td>
<td>171</td>
</tr>
<tr>
<td>B</td>
<td><strong>N</strong></td>
<td>112</td>
<td>106</td>
</tr>
<tr>
<td>C</td>
<td>Progesterone</td>
<td>178</td>
<td>187</td>
</tr>
</tbody>
</table>
for 30 to 60 minutes. The extract of steroids after the incubation for 60 minutes was fractionated into neutral fraction and phenolic fraction. Only small amount of radioactivity was found in phenolic fraction which stayed at the starting point of the paper chromatogram (benzene/formamide solvent system). The radioactive spot did not move from the origin point even when the chromatogram was developed in chloroform/formamide solvent system for 6 hours. Further, this compound could not be confirmed as either estrone, estradiol or estriol. Since, after 60 minutes incubation the phenolic fraction did not contain any estrogenic steroids, the extract obtained after 30 minutes incubation of the same ingredients was not washed in 0.1 N sodium hydroxide solution. The percentage of conversion of progesterone into its metabolites is listed in Table XVI. Testosterone fraction when separated from 17α-hydroxyprogesterone as testosterone acetate had Rf value as 0.8. It was not possible to confirm because the radioactivity of this fraction was too low to process further to confirm its identity. The neutral fraction after 60 minutes incubation, large amount of radioactivity stayed between the point of application and a position of Rf 0.15 on the paper chromatogram. The fraction might have contained the Δ^5-pregnen-20α, 3-one, but this compound was not separated from other radioactive unknown compound.

(B) In this experiment the homogenate of the same tissue used in A, incubated with 200, 400 and 200 μmol of progeste-
### Table XVI

**Conversion of progesterone into its metabolites by polycystic ovary obtained from hirsute woman**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Percentage conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 minutes</td>
</tr>
<tr>
<td></td>
<td>incubation</td>
</tr>
<tr>
<td>Unconverted substrate</td>
<td>72.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>5.8</td>
</tr>
<tr>
<td>(\Delta^4)-Pregnenol-20-ol-3-one</td>
<td>4.4</td>
</tr>
<tr>
<td>(\Delta^4)-Pregnen-17\alpha, 20\alpha-ol-3-one</td>
<td>2.3</td>
</tr>
<tr>
<td>17\alpha-Hydroxyprogesterone</td>
<td>12.0</td>
</tr>
<tr>
<td>(\Delta^4)-Androstenedione</td>
<td>3.4</td>
</tr>
<tr>
<td>Testosterone*</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* This was not further confirmed.
rone-4-C$^{14}$, 17-hydroxyprogesterone-4-C$^{14}$ and androstenedione-4-C$^{14}$ respectively. The absolute conversion of these substrates into their different metabolites are given in Table XVII. A pooled sample of androstenedione was crystallized to its constant specific activity as shown in Table XVIII.

(C) Androstenedione-4-C$^{14}$ (1 μc) was incubated with 800 mg of ovarian tissue used in (A) and (B) and the incubation was carried out for 4 hours. The percentage conversion of androstenedione into neutral and phenolic fractions has been shown in Table XIX. Estrone and estradiol were found in the phenolic fraction. Both these steroids were crystallized to the constant specific activity. (The results of counts at each crystallization have been given in Table XVIII).

19-Nor-testosterone was found in the neutral fraction. The ovarian tissue obtained from patient (HK) was incubated for 4 hours at 37 °C with androstenedione-4-C$^{14}$, testosterone-4-C$^{14}$ with radioactivity of 1 μc per sample substrate. One gram of tissue was used in each incubation and the phosphate buffer of pH 7.2 contained 25 mM sucrose. In the neutral fraction only androstenedione and testosterone were found. The percentage conversion of these substrates into neutral and phenolic fractions is shown in Table XIX.
Table XVII

Biosynthesis of steroids in polycystic ovarian tissue obtained from a hirsute woman

(Unidentified compounds are not included).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incubation period in minutes</th>
<th>Steroid formed - µmol per gm. tissue.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Progestrone-4-Cl4 (500 µM/gm.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>215.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>17α-hydroxy-progesterone-4-Cl4 (500 µM/gm.)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>227.0 (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>4-Cl4 (500 µM/gm)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>196.5 (S)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.7</td>
</tr>
</tbody>
</table>

(S) Unconverted substrate.
Table XVIII

Recrystallization of steroids to constant specific activity

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Solvent</th>
<th>cpm/mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{4}$-Androstenedione-$^{4}$-C$^{14}$</td>
<td>Acetone</td>
<td>867*</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>858</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>870</td>
</tr>
<tr>
<td>Estrone-$^{4}$-C$^{14}$</td>
<td>Acetone</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>98</td>
</tr>
<tr>
<td>Estradiol-$^{17}$-$^{3}$-$^{4}$-C$^{14}$</td>
<td>Acetone</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>187</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>192</td>
</tr>
</tbody>
</table>

* Mean of duplicate values.
Table XIX

Biogenesis of steroids in polycystic ovary using androstenedione and testosterone as substrates

(Percent conversion)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Time of incubation - hrs</th>
<th>Steroids formed</th>
<th>Neutral fraction</th>
<th>Phenolic fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Un-</td>
<td>19-nor-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>known</td>
<td>testosterone</td>
</tr>
<tr>
<td>Δ-Androstenedione-4-C14</td>
<td>4</td>
<td></td>
<td>15.0</td>
<td>0.8</td>
</tr>
<tr>
<td>Δ-Androstenedione-4-C14</td>
<td>4</td>
<td>36.5</td>
<td>-</td>
<td>5.1</td>
</tr>
<tr>
<td>Testosterone-4-C14</td>
<td>4</td>
<td>65.5</td>
<td>-</td>
<td>25.1</td>
</tr>
</tbody>
</table>

* Unknown.
DISCUSSION

Urinary excretion of 17-KS and 17-KGS in hirsute women:

The excretory values of 17-KS in hirsute women which are similar to that of normal men, are conspicuously higher than those observed with normal women of the same age group. The ovulatory phase and luteal phase of the menstrual cycle had no significant influence on the excretion of 17-KS in simple hirsutism.

17-KGS levels in hirsute women showed in our investigation were slightly higher than that of normal women with considerable overlap of the levels. This overlapping in the levels did not permit the conclusion that the adrenal cortex is hyperactive but it does not exclude a mild form of adrenal overactivity in some cases. In attempts to find a mild form of adrenal hyperplasia in 13 hirsute women urinary 17-KS and 17-KGS were determined before and after administration of ACTH. The values of 17-KGS were found to be more sensitive index of the adrenal cortex stimulation by exogenous ACTH than the urinary 17-KS values. These however, failed to point out any case apparently suffering from any kind of major adrenal abnormality.

Concentration of plasma 17-OHCS in hirsute women:

In the group of hirsute women, having regular cyclic bleeding and biphasic basic body temperature graph (B.B.T.),
the average value of plasma 17-OHCS was 16.25 ± 4.05 μg/100 ml during the pre-ovulatory phase and 16.65 ± 2.50 μg/100 ml plasma in the post-ovulatory phase (Table XII). The difference in these values is statistically insignificant. Furthermore, the average value of plasma 17-OHCS in hirsute women, having regular, ovulatory bleeding does not differ significantly from that of the hirsute women having regular, anovulatory bleeding.

The individual values of plasma 17-OHCS in a group of hirsute women having menstrual inequalities and monophasic B.B.T. are higher than normal subjects (Table XII). This high level of plasma 17-OHCS may partly be due to a direct action of high estrogen concentration either on ACTH secretion or the effect of estrogen on the ability of the plasma to bind cortisol. It may be presumed that the menstrual irregularities are due to the improper estrogen/progesterone balance resulting in high circulating estrogens. The excretion of 17-KS, in this group of patients was within normal range and if ACTH production was higher than normal, excess amount of 17-KS would have been excreted. It is therefore, likely that these patients had an increased concentration of 'transcortin' in plasma and have normal adrenocortical function. This may also be the explanation for the high control levels of plasma 17-OHCS found in hirsute women with amenorrhea. In two hirsute patients having irregular anovulatory bleeding the values of plasma
17-OHCS are definitely elevated (29.4 and 34.2 μg per 100 ml of plasma) and are comparable to those seen in Cushing's patients. The absence of signs and symptoms of Cushing's syndrome in these two patients as well as lack of hyperresponsiveness of adrenal cortices to a standard test dose of ACTH would exclude the possibility that an adrenal hyperplasia, belonging to the classical symptomatic Cushing's syndrome which is responsible for the hirsutism seen.

It appears that plasma 17-OHCS in normal Indian subjects and in normal U.S. whites fall within the same range. The data presented on plasma 17-OHCS at two phases of the menstrual cycle are in agreement with Sandberg et al. showing high concentration of urinary 17-OHCS in the post-ovulatory phase. The reason why the 17-OHCS are fluctuating in this fashion during the menstrual cycle is still unknown but a higher ACTH secretion in the post-ovulatory phase may be one of the possible reasons.

The average value of plasma 17-OHCS in all the patients investigated was normal or slightly elevated than the control level. Also the response of these levels of plasma 17-OHCS to ACTH administration is of normal magnitude. The control plasma 17-OHCS levels may occasionally be high in this group of patients which could be ascribed to the 'stress' of the disease itself.

Different reports 19, 23, 26, 37, 39 suggest that
various etiological factors may be at play in simple hirsutism. These reports are frequently contradictory to each other. However, the present data on urinary 17-KS, 17-KGS and plasma 17-OHCS in the control state and after the administration of ACTH indicate that grossly two types of lesions may be causing simple hirsutism. One lesion may arise from defective adrenal steroidogenesis resulting in the adrenal type of hirsutism and the other may be due to abnormal ovarian steroidogenesis. Six out of thirteen hirsute women showed higher than normal response of plasma 17-OHCS to ACTH administration indicating the presence of hyperresponsive adrenal cortex. Such adrenals might also be producing more androgens than normals by the activation of endogenous ACTH. In the rest of the patients studied, the ovary might have defective steroidogenesis, producing highly potent androgens.

**Urinary pregnanetriol and pregnanetriol-11-one:**

The women with idiopathic hirsutism excreted higher amounts of pregnanetriol than normal women but the range of output of urinary pregnanetriol had a considerable overlap with that observed in normal subjects confirming previous findings. The mean value of urinary pregnanetriol in a group of simple hirsutism falls at the higher limits of the normal excretory range of this steroid and is increased by the administration of ACTH. Patients SK and MC were the
exception to this statement as they did not excrete increased amounts of pregnanetriol after ACTH administration (Table XIII). Though this steroid is mainly of adrenal origin, it may also originate from the gonads. In five cases which were definitely diagnosed as Stein-Leventhal syndrome, the excretion of pregnanetriol was within the limits of the normal range. Fotherby and Love demonstrated that about 52 percent of 17-hydroxyprogesterone injected into human subjects was converted to pregnanetriol and this percentage conversion was similar in both adrenalectomized and normal persons. According to these authors 17-hydroxyprogesterone is a main precursor for pregnanetriol synthesis. The conversion of 17α-hydroxyprogesterone to pregnanetriol was noticed in adrenalectomised persons which indicates that the adrenals are not the only tissues able to convert 17α-hydroxyprogesterone to pregnanetriol. Recently Cox and Shearmann observed normal urinary excretion of pregnanetriol in patients suffering from Stein-Leventhal disease. These patients excreted higher amounts of 5-pregnene-3α, 17α, 20α-triol (5-pregnanetriol) than did normal subjects. However, the range of normal excretion of this steroid has not been well established.

Four patients with simple hirsutism excreted pregnanetriol-11-one before and after ACTH administration. Out of these, patient J had a polycystic ovary characteristic of the Stein-Leventhal syndrome. In patient MG with
simple hirsutism and polycystic ovary, this steroid was only detected after the administration of ACTH. From these observations it can be concluded that some patients with simple hirsutism and polycystic ovaries might be exhibiting a defective adrenal steroidogenesis pertaining to pregnanetriol-11-one formation. Such a condition may also exist in some cases of simple hirsutism with normal ovarian function. Cox and Shearman have shown the presence of pregnanetriol-11-one in the urine of six Stein-Leventhal patients. This observation is in agreement with the present investigation.

The question whether wedge resection of the polycystic ovary has any effect on the production and excretion of this steroid is still debatable.

Isolation and identification of steroids in the cyst fluid obtained from hirsute women:

Recently, Ryan and Smith in a series of articles have shown that acetate-1-C¹⁴ and cholesterol-4-C¹⁴ are converted to estrogens by human ovarian tissue stimulated with FSH in vitro. In this reaction progesterone, 17α-hydroxyprogesterone, pregnenolone, DHEA and androstenedione were also found to contain radioactivity. This finding may be in support of two pathways of steroidogenesis in the ovary leading to the formation of androstenedione which is further converted to estrone and estradiol through 19-hydroxylation. In the present study, progesterone, androstenedione and estrone were isolated from a pool of ovarian cyst fluid obtained from
patients suffering from the Stein-Leventhal syndrome. The relative amounts of these steroids in the cyst fluid may, however, not indicate the rate of their formation or secretion. The rate of steroidogenesis leading to estrogens by either pathway (Fig. 4) might be normal or interrupted by an unknown inhibitor at a particular stage of biogenesis of steroids in the Stein-Leventhal ovary. Also, the steroids produced by the follicular cells and accumulating in the cystic fluid may not be equally free to escape from this fluid and the absolute presence or absence from the cyst fluid of any of these steroids will be of limited value for the evaluation of biosynthetic disturbances underlying the Stein-Leventhal syndrome.

Simmer and Voss speculated that in the Stein-Leventhal syndrome the ratio of testosterone to androstenedione is of the order of 1:11.5. This concentration of testosterone may be too low for detection by the present methods. If this ratio was correct one should have expected about 4 µg testosterone in sample A and approximately 5 µg in sample B. These are borderline concentrations of U.V. positive material using the Haines scanner for detection and could thus have been ignored in the present investigation. Furthermore, testosterone may never accumulate in the cyst fluid to such concentrations that it can be detected. Still testosterone could be present in the systemic circulation of these patients giving rise to some of the disturbances
seen in Stein-Leventhal syndrome.

Sandor and Lanthier observed that tissue from polycystic ovaries synthesised more androstenedione from 17α-hydroxyprogesterone than ovaries of normal women. In the following year Warren et al also found more androgenic steroids when the polycystic ovarian tissue was incubated with progesterone and correlated that the capsular and stromal connective tissue forms considerable amount of 20-hydroxylated derivatives. Lanthier and Sandor further reported that Stein-Leventhal type of ovarian slices showed accelerated rate of production in all intermediary reactions in the production of androstenedione and testosterone from pregnenolone, progesterone and 17α-hydroxyprogesterone. More recently, Axelrod and Goldzieher observed deficiencies of aromatization, 17-hydroxylation and 3β-hydroxydehydrogenase activities in polycystic ovarian tissues when incubated with pregnenolone, progesterone, testosterone and 19-hydroxyandrostenedione. All these in vitro studies indicate that there is a higher rate of biogenesis of androstenedione and testosterone in polycystic ovaries, but Mahesh and Greenblatt in a similar investigation observed that such conclusions based on the in vitro studies are misleading in some of the cases of Stein-Leventhal syndromes, because the ability of steroidogenesis of these ovarian tissues in in vitro conditions may be different. They postulated a failure of 3β-hydroxy-
dehydrogenase activity in the ovaries of some of their patients having Stein-Leventhal syndrome.

Short and London in their work on steroids present in the cyst fluid from Stein-Leventhal patients postulated a complete block of 19-hydroxylation in such ovaries. This was evident by the absence of estrone, estriol or estriol in ovarian cyst fluid from Stein-Leventhal patients and the presence of these steroids in the cystic fluid from the normal ovary. In our pooled sample (A) estrone was detected. Thus, a considerable controversial interpretation exists and it is too early to state that complete absence of 19-hydroxylation is alone responsible for the Stein-Leventhal syndrome. On the other hand, in the fluid obtained from a large ovarian cyst from a Stein-Leventhal patient no estrogenic hormones were present supporting the theory of Short and London. It is interesting to speculate that different degrees of severity of the Stein-Leventhal syndrome may be associated with complete or partial block of 19-hydroxylation and aromatisation.

Biosynthesis of steroids in the ovarian tissues obtained from hirsute women:

When progesterone-4-C\(^{14}\) was incubated with polycystic ovarian tissue, no estrogens were synthesised confirming the previous observation of Axelrod and Goldzieher. The percentage conversion of progesterone
into its metabolites by this polycystic ovarian tissue could not be compared with normal ovarian tissue since normal ovarian tissue was not available. This would have enabled us to compare the ability of various enzymatic reactions in the metabolism of progesterone in normal and polycystic ovary. Recently, it has been reported that polycystic ovary could metabolise androstenedione at a faster rate to estrogens than normal ovary.

Since progesterone gets converted to 17α-hydroxyprogesterone and then to androstenedione to form estrogens (Fig. 4) progesterone-4-C\textsuperscript{14}, 17α-hydroxyprogesterone-4-C\textsuperscript{14} and androstenedione-4-C\textsuperscript{14} were used as substrate in 0.1 - 0.2 mM concentrations. The absolute conversions of these steroids were measured. Neither estrone nor estradiol was synthesised by the polycystic ovary during 60 minutes incubation period. As the main interest of this investigation was to observe whether these ovarian tissues produce estrogens, all the intermediate metabolites were not further analysed. Only those intermediates which were confirmed are listed in Table XVII. This experiment also confirmed the previous finding that there is some enzymatic block in the synthesis of estrogens specially, in the conversion of androstenedione to estrone (Fig. 12).

When androstenedione-4-C\textsuperscript{14} with 1 μc radioactivity was used as substrate and the incubation was carried out for
BIOSYNTHESIS OF STEROIDS IN STEIN-LEVENTHAL TYPE OVARY

PREGNENOLONE

↓

PROGESTERONE

↓

17α-HYDROXY-
PROGESTERONE

↓

19-HYDROXY-Δ^4-
ANDROSTENEDIONE

↓

ESTRONE

ESTRADIOL-17β

TESTOSTERONE

DEHYDROEPI-
ANDROSTERONE

Δ^4-ANDROSTENE-
3,17-DIONE

Fig. 12
4 hours, estrone and estradiol were detected in the incubation, supporting our findings that during the conversion of androstenedione to estrone there may be a partial block rather than a complete block as earlier suggested by Short and London 37. This statement was further supported by a subsequent incubation of androstenedione-4-Cl4 and testosterone-4-Cl4 with polycystic ovarian tissue obtained from patient (RK) suffering from hirsutism and who had a Stein-Leventhal type ovary. This tissue could also synthesise estrone and estradiol from androstenedione and testosterone.
SUMMARY

1. The urinary values of 17-KS and 17-KGS, though slightly higher than normal women, failed to point out any major ovarian or adrenocortical dysfunction in hirsute women.

2. The average value of plasma 17-OHCS in hirsute women as well as the response of plasma 17-OHCS to exogenous ACTH were normal. These values are higher in hirsute women having irregular, monophasic menstrual cycle.

3. The mean value of urinary pregnanetriol in hirsute women falls at the higher limits of normal excretory range and is increased by the administration of ACTH. Five of these hirsute women who had Stein-Leventhal type ovaries excreted normal amounts of pregnanetriol.

4. Pregnanetriol-ll-one was detected in the urine of four cases of simple hirsutism, suggesting defective steroidogenesis in adrenal cortex.

5. Most of the women with simple hirsutism had polycystic ovaries. The presence of estrone along with high concentration of androstenedione in the ovarian cyst fluid obtained from Stein-Leventhal type ovary suggested a partial block at 19-hydroxylation of androstenedione during the estrogen synthesis.

6. When progesterone-4-C\(^{14}\), 17\(\alpha\)-hydroxyprogesterone-4-C\(^{14}\) and androstenedione-4-C\(^{14}\) were incubated in 0.1 to 0.2 mM concentration having 6.1 to 0.2 µc radio-
activity with Stein-Leventhal ovarian tissue for 60 minutes, no estrogen were synthesised. This suggests some enzymatic block specially in the conversion of androstenedione to estrone.

7. When androstenedione-$^{14}$C and testosterone-$^{14}$C with high specific activity were incubated with Stein-Leventhal type tissue for 4 hours, estrone and estradiol were synthesised in small amounts. This supports the statement of partial block of 19-hydroxylation and not a complete block during the conversion of androstenedione to estrogens in the Stein-Leventhal type ovaries.
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