CHAPTER I
SECRETION AND EXCRETION OF STEROIDS IN NORMAL POPULATION

Several endocrine disorders arise from abnormal function of adrenal cortex and gonads. Their etiological causes are however complex. Complete understanding of these disorders requires a detailed knowledge of the chemical nature of steroids elaborated by endocrine glands, their physiological effects on various metabolic processes, and their excretory patterns. Quantitative measurement of various steroids in blood and their excretory products in the urine in normal population forms the base line with which to compare the excretory pattern of steroids in various endocrine diseases. This helps to evaluate the causes and the degree of manifestation of the disease.

Since Indian population consists of different groups, differing greatly in their nutrition, habits and standards, the determination of certain steroids in blood and urine was carried out with following objectives:

1) To establish the excretory pattern of steroids in normal Indian population and compare it with other races of the world.

2) To establish a base line to evaluate the abnormal function of adrenal cortex or gonads in patients suffering from certain endocrine disorders.

3) To ascertain whether the excretory pattern of 17-KS in children differs in various endogamous groups of
Indians having different nutritional status,

4) To determine the levels of plasma 17-OHCS differ in two phases of menstrual cycle in women,

5) Whether pregnanetriol-11-one is an excretory product in normal women before and after stimulation with ACTH,

and 6) To study the adrenocortical response to standard dose of ACTH in normals and to compare it with endocrine disorders of adrenocortical origin.

In recent years, the knowledge of biosynthesis of steroids in adrenal cortex and gonads has greatly advanced. A short resume of biosynthetic pathways of steroids in adrenal cortex and gonads has been given along with the development of ACTH stimulation test. This would enable to discuss the abnormal pathways of steroidogenesis in pathological conditions.

Resume of Literature

A. Biosynthesis of steroids in adrenal cortex:

Corticosteroids: The importance of acetate as a precursor of cholesterol and other physiologically active steroids has been briefly discussed (see Introduction, page-...). One of the direct evidence on the adrenocortical enzymes in human being has been provided by the work of Lombardo and Hudson. Using radioactive pregnenolone, progesterone, 17α-hydroxyprogesterone, desoxycorticosterone and 11-
desoxycorticisol as substrates incubated with human adrenal slices and homogenates, these authors confirmed the presence of 11β-, 17- and 21-hydroxylase enzyme activity. They observed that 11-desoxycorticisol or desoxycorticosterone were not hydroxylated at position 17, indicating that 21-hydroxysteroids do not undergo 17-hydroxylation. Hechter and co-workers investigated in detail the action of various hydroxylating enzymes by adrenal perfusion experiments and postulated that a definite sequence exists for the various hydroxylations in the conversion of progesterone to the cortical steroids as shown in Fig. 2. Progesterone could undergo either 17α-hydroxylation or 21-hydroxylation. If however 21-hydroxylation occurred first, 17α-hydroxylation is inhibited since 21-hydroxylated steroid does not act as a precursor. But desoxycorticosterone could get hydroxylated at position 11α. If progesterone undergoes 17-hydroxylation first, followed by 21-hydroxylation to give 11-desoxycorticisol subsequent formation of cortisol is possible by the action of 11-hydroxylase. It has further been demonstrated that corticosterone was not converted to cortisol or vice versa and 11β-hydroxyprogesterone was a poor precursor for the cortical steroids. Hence it was postulated that a compound hydroxylated in either position 21 or 11 could not be further hydroxylated by 17-hydroxylating enzyme. In brief, progesterone or
17α-hydroxyprogesterone could serve as the substrate for the 21-hydroxylase but if the compounds have been hydroxylated at carbon 11 it would not serve as substrate for either the 17- or the 21-hydroxylase. Samuels suggested that these distinctions could not be so rigid as originally stated but this appeared to be the preferred sequence. Any gross change in this sequence or increased or decreased enzyme activity could lead to a defective biosynthesis of steroids in the adrenal cortex.

Adrenal androgens and estrogens: The androgen production by the adrenal cortex and the gonadal tissue essentially follows the same pathways as shown in Fig. 3. 17α-hydroxyprogesterone is an intermediary product in the synthesis of cortisol which is a major secretory product of adrenal cortex. Part of 17α-hydroxyprogesterone gets converted to androstenedione by 17-desmolase action. Bligh et al. and Heard et al. demonstrated that acetate and cholesterol when incubated in a cell-free preparation of hog adrenals could produce androstenedione as one of the end products. Solomon et al. separated human fetal adrenals into two fractions: one consists of a 'fetal zone' and the other is the mainly adult tissue contaminated with some fetal zone. Both these fractions transformed progesterone to 17α-hydroxyprogesterone and to androstenedione. Bloch and Benirschke demonstrated the formation of dehydroepiandrosterone, androstenedione and
11β-hydroxyandrostenedione when slices of human fetal adrenals were incubated with acetate-1-C\textsuperscript{14}. It has been observed that administration of ACTH results in an increase in androgens. Bloch et al\textsuperscript{4} incubated human adrenal slices with acetate and have shown that when ACTH is added to the incubation mixture, the synthesis of C\textsubscript{19} steroids was increased. When pregnenolone-H\textsuperscript{3} and cholesterol-C\textsuperscript{14} were incubated with human adrenal adenoma homogenate tritiated dehydroepiandrosterone was formed\textsuperscript{6}. This indicated the existence of the pathway pregnenolone→ 17α-hydroxyprogrenolone→dehydroepiandrosterone. Dehydroepiandrosterone can be easily converted to androstenedione by the action of 3 β-hydroxydehydrogenase and isomerase enzyme action and then to 11β-hydroxyandrostenedione. Thus dehydroepiandrosterone, androstenedione and 11β-hydroxyandrostenedione appear to be the main C\textsubscript{19} steroids normally produced by the human adrenal cortex.

Adrenal estrogens: The conversion of androgens i.e., androstenedione and testosterone to estrogens appears to be the same as in the ovary. Androstenedione gets hydroxylated at C\textsubscript{19} prior to the formation of estrone. The steps after hydroxylation at carbon 19 are not well established yet. Baggett et al\textsuperscript{36} incubated labelled testosterone with slices from adrenal tumour and gave direct evidence that testosterone is converted to estrone and estradiol-17β by adrenal tissue,
Biosynthesis of steroids in testicular tissue: Testosterone is the chief androgenic steroid secreted by the testis. Testosterone and androstenedione have been isolated from human testicular tissue and from spermatic vein blood of humans and dogs. The sequence of biosynthetic reactions from acetate and cholesterol to progesterone in testicular tissue appears to be the same as in adrenal cortex. Mason and Samuels perfused dog testis with acetate-$\text{C}^{14}$ and measured androstenedione and testosterone produced by the testes before and after the stimulation of human chorionic gonadotrophins. When progesterone was incubated with homogenates or slices of rat testes, $1\alpha$-hydroxyprogesterone, androstenedione and testosterone were synthesised and when $1\alpha$-hydroxyprogesterone was incubated with testicular tissue no progesterone was formed but $1\alpha$-hydroxyprogesterone readily converted to androstenedione and testosterone.

The splitting of the side chain as acetic acid by $1\beta$-desmolase enzyme requires oxygen and TPNH as cofactor and the first product is androstenedione which is then reduced to testosterone by the action of $1\beta$ (testosterone) dehydrogenase enzyme. This conversion of androstenedione to testosterone is a reversible reaction (Fig. 3). The presence of $3\beta$-hydroxysteroid dehydrogenase, $\Delta^4-5$ steroid isomerase, $1\alpha$-hydroxylase, $1\beta$-desmolase and $1\beta$ (testosterone) dehydrogenase in testicular tissue has been
demonstrated\(^3\). Neher and Wettstein\(^43\) suggested the possibility of the presence of another pathway viz. \(\Delta^5\)-pregnenolone\(\rightarrow\)17\(\alpha\)-hydroxy \(\Delta^5\)-pregnenolone\(\rightarrow\)dehydroepiandrosterone to androstenedione and finally to testosterone. Subsequently, Eik-Nes and co-workers\(^44,45\) have demonstrated that the latter pathway is a major pathway of steroidogenesis in dog testes. This pathway essentially utilises the same enzymes as required in the pathway suggested by Samuels and co-workers i.e. progesterone\(\rightarrow\)17\(\alpha\)-hydroxyprogesterone\(\rightarrow\)androstenedione\(\rightarrow\)testosterone but in a different order of sequence as shown in Fig. 3.

**Biosynthesis of estrogens in the ovary:** Biosynthetic pattern of \(C_{19}\) steroids i.e. androstenedione and testosterone follows the same sequence of reaction as in testis (Fig. 4).

The incorporation of acetate-\(1-C^{14}\) to progesterone has been demonstrated by Sweat et al.\(^46\). In subsequent incubation of progesterone-\(4-C^{14}\) and androstenedione-\(4-C^{14}\) with ovarian luteal tissue, it was observed that these steroids converted to estrone and estradiol. Recently, Ryan and Smith\(^47,48,49,50\) in their series of publications demonstrated clearly the sequence of reactions in the biosynthesis of estrogens from acetate-\(1-C^{14}\) and cholesterol and other intermediary steroids to estrogens. Neher and Wettstein\(^43\) isolated DHEA and proposed an alternative pathway from pregnenolone\(\rightarrow\)17\(\alpha\)-hydroxy-
pregnenolone→DHEA→androstenedione. West and Naville\textsuperscript{51} demonstrated the conversion of dehydroepiandrosterone to estrogen by ovarian tissue.

From all the above evidence it is established that androgens are converted to estrogens in the ovarian tissue. This conversion required TPNH or TPNH-generating system. Prior to the aromatization of ring A, androstenedione is converted to 19-hydroxy-androstenedione. Thus carbon-19 is detached to form $C_{18}$ steroids i.e. estrogens.

B. Development of ACTH Test and its Significance in Endocrine Disorder:

Tests like the salt deprivation test and the water loading test were poor in judging adrenal cortical function. These tests were indirect and dependent on general metabolic alterations. Furthermore, in the early years of working with steroids in blood and urine the techniques used failed to differentiate subjects with adrenal insufficiency from those with intact adrenals. By stimulating the adrenal cortex with ACTH, diagnostic difficulties could be overcome. The response of the adrenal cortex to ACTH provides an excellent guide for evaluating the functional status of the adrenal under most circumstances\textsuperscript{52}. Earlier the fall in circulating eosinophils and the rise in urinary 17-KS were chosen as indicators of adrenal response. A linear relationship was observed between the increase of 17-KS and the duration of ACTH
infusion and 17-KS increased proportionately to the dose of ACTH and reached a maximum when 15 to 20 I.U. were administered\(^{53,54,55,56,57}\). Furthermore, Renold \textit{et al.}\(^{58}\) observed that in normal subjects the daily repeated ACTH infusion gave a lower 17-KS excretion on the first day compared to the excretion on the next day. After giving ACTH for two or more days a maximum excretion was apparently obtained.

The adrenocortical response to intravenous ACTH was three times higher than that observed when ACTH was given intramuscularly. When ACTH was injected intramuscularly following termination of intravenous ACTH the response was one and a half times greater than that of intramuscular ACTH injected without preceding intravenous ACTH. These authors also observed that impure preparations of ACTH were inactivated by the body tissues when injected intramuscularly. Thorn \textit{et al.}\(^{59}\) added one more criterion to the previously discussed ACTH test, viz. the estimation of 17-OHCS in urine. These workers used urinary 17-OHCS as a sensitive index of adrenal function on ACTH administration. Bliss \textit{et al.}\(^{60}\) estimated the adrenocortical response by rapid intravenous injection of various doses of ACTH and measurements of 17-OHCS in plasma. They observed that 15 I.U. ACTH induced an almost maximum adrenal response and suggested that a more prolonged stimulation such as intravenous administration of ACTH over a period of many hours
would test this aspect of glandular function more effectively. Eik-Nes et al.\textsuperscript{61} evaluated extensively the relation of time through which ACTH should be infused and the amount of ACTH to be used. The ACTH was given by continuous intravenous infusion in 500 ml of normal saline or 5 per cent glucose in water. They observed that as little as 1 I.U. of ACTH had an effect in raising the levels of 17-OHCS in the blood plasma when infused over a period of 6 hours and the maximal stimulation of the adrenal cortex was induced by 15 to 25 I.U. of ACTH. No further increase of 17-OHCS in blood plasma was observed during higher rates of ACTH infusion. The 17-OHCS were increased significantly when 25 I.U. of ACTH was infused over 2, 4, 6 and 8 hours, and when the dose was spread over 8 hours, the level at the end of infusion did not differ significantly from the one observed at 6 hours. Similarly, continuous intravenous administration every six hours with the optimal dose of ACTH - 25 I.U. did not raise significantly the level of 17-OHCS above that which was achieved after the first six hours of infusion. In conclusion, it was stated that the intravenous administration of 25 I.U. ACTH over six hours could serve as a test for adrenocortical capacity. This was further confirmed in a case showing the clinical picture of Addison's disease, but with normal levels of 17-OHCS in blood plasma. When ACTH was infused over a period of
six hours a decreased adrenocortical response was found.

Simultaneously, Gordon et al.\(^{62}\) used a continuous stimulation of the adrenal by injecting 10 I.U. (10 mg) of ACTH every 6 hours in seven doses and measured the response of adrenal cortex to ACTH by urinary 17-KS, formaldehydogenic steroids, eosinophil counts and the uric acid/creatinine ratio. In panhypopituitarism the results of a single dose of ACTH were negative, whereas the results of the 48 hour test in such cases were positive. In two patients with thyroid myxedema, the adrenal responsiveness was similar to that seen in pituitary failure rather than pituitary adrenal failure. These authors concluded that the continuous stimulation gave a more accurate index of adrenal response to ACTH than a single injection of 25 I.U. over a period of 6 hours.

Jenkins et al.\(^{63}\) have elucidated the use of ACTH in the diagnosis of adrenocortical function. They administered pure ACTH in three ways: (i) a single intramuscular injection (ii) intravenous infusion over a period of 8 hours and (iii) intramuscular or subcutaneous injection of ACTH in a long acting gelatine vehicle and concluded that for the evaluation of adrenocortical function, an 8 hours infusion of ACTH is the method of choice.

The response of the plasma 17-OHCS to a standard ACTH test in various clinical conditions has been described by Eik-Nes et al.\(^{64}\). Addison's disease seemed to fall into
two categories. In three of these patients, no measurable concentrations of 17-OHCS were found at rest or after administrations of ACTH. In the other three patients low but normal amounts of plasma 17-OHCS were observed at rest which did not increase after ACTH administration. On the other hand, in a case of Cushing's syndrome due to adrenal hyperplasia and with elevated resting levels of 17-OHCS a marked rise in plasma level of 17-OHCS occurred when a standard ACTH test was performed. A normal response of plasma 17-OHCS to ACTH was observed in patients suffering from diabetes and hypertension due to malignant adrenals, rheumatoid arthritis, sprue, dermatitis, eosinophilia and also in Turner's syndrome.

Christy et al.\textsuperscript{65} did not believe in the maximum stimulation of the adrenal gland due to ACTH, since there were contradictory reports in the literature about the maximal dose of ACTH and period of stimulation prior to the classical work of Eik-Nes et al.\textsuperscript{64}. The work of Christy et al.\textsuperscript{65} was carried out simultaneously to the work of the Eik-Nes's\textsuperscript{64} group. The response of the plasma 17-OHCS by administering 25 I.U. of ACTH intravenously over a period of 4 hours was standardized in a control group of normal persons and this ACTH response was compared to that of patients with disturbed adrenal function. The control range of plasma 17-OHCS in normal subjects of both sexes was from 4 to 23 \textmu g per 100 ml which was raised to the
range of 35 to 54 µg per cent after administration of ACTH intravenously for 4 hours. No correlation was found between the initial values of plasma 17-OHCS and the values after stimulation with ACTH. Two subjects belonging to the control group and having widely different resting plasma 17-OHCS values (4 to 21 µg per cent) showed identical values after ACTH administration. In patients suffering from Addison's disease the resting levels of plasma 17-OHCS was definitely low in all the cases. These values were either the same or slightly elevated after ACTH administration. This ACTH test helped differentiating patients suffering from hypopituitarism from those with primary adrenal insufficiency. In patients with hypopituitarism the resting values of plasma 17-OHCS were 0 to 12 µg per cent which were definitely increased in all the cases with ACTH in contrast to the complete lack of response to ACTH found in patients with primary adrenal insufficiency. In Cushing's syndrome due to adrenal hyperplasia as well as adrenal carcinoma these authors observed that the elevated resting values were increased to a very high level, (61 to 118 µg per cent) similar to that found in normal women during the third trimester of pregnancy. On the other hand, in a pregnant Addisonian patient the elevated plasma 17-OHCS did not increase by ACTH. This observation was in support of the work by Hills et al. indicating that the source of 17-OHCS in
pregnancy is the adrenal and not the placenta or the ovary. Furthermore, during pregnancy, the adrenal cortex might be hyperresponsive to ACTH. This interpretation has now become obsolete. It is known that a specific protein 'transcortin' binds the 17-OHCS in peripheral blood. This binding principle is increased in the blood of pregnant women thus accounting for high levels of plasma 17-OHCS, high ACTH responses and delayed clearance of cortisol from the plasma. This latter factor is of paramount importance in interpreting plasma 17-OHCS during administration of ACTH, a fact already pointed out by Eik-Nes et al.

In conclusion, the administration of ACTH stimulates the adrenal cortex and the measurement of plasma 17-OHCS before and after ACTH provides a functional index for this gland. The maximum stimulation of the adrenal cortex can be achieved by administering 15 to 25 I.U. ACTH intramuscularly in a long acting gelatin vehicle or by continuous infusion over a period of 6 hours. This ACTH test essentially differentiates patients with primary adrenal insufficiency from hypopituitarism and adrenal hyperplasia from adenoma or carcinoma.
MATERIAL

Normal Population

A. Children:

In order to study the excretory pattern of 17-KS in children belonging to different communities who differ from each other in nutritional status, children from Deocani, Sindhi and Parsi communities were selected. Thirty three apparently healthy children, 2 to 12 years of age, were investigated. The parents of these children were made to understand the importance of collecting a complete 24 hour urine specimen. Toluene was used as a preservative and the samples were stored at 4°C until analysed. Six subjects, who were already in the hospital due to some illness not pertaining to the endocrine system and who at the time of study were completely free from their ailments, were selected for studying day to day variation in urinary 17-KS excretion. In these subjects 24 hour urine samples were collected every day for one week.

B. Normal men:

Fifteen normal men, ranging in age from 19 to 39 were selected for a study of normal excretion of 17-KS and 17-KGS. These subjects were research workers from the Indian Cancer Research Centre, Bombay and the Tata Memorial Hospital, Bombay. All of them were apparently healthy.

Twenty four hour urine samples were collected in polyethylene bottles without preservative. During collection
the urine bottles were kept in an ice box at 0 to 4°C. The urine samples were measured and stored at 4°C until subjected to analysis.

Free plasma 17-OHCS were estimated in three out of these fifteen subjects.

C. Normal women:

Twenty-seven normal women were selected for a study of 17-OHCS in plasma, ten were in the pre-ovulatory phase while the rest were in the post-ovulatory phase of the menstrual cycle.

The excretion of 17-KS, 17-KGS, pregnanetriol and pregnanetriol-11-one were also studied in these women.

The criteria for normalcy were:

1) Age ranging from 16 to 39 years.
2) Satisfactory general healthy state by physical examination and normal amount of body hair for a woman as assessed by the technique of Shah.
3) Cyclic and quantitative normal menses.
4) Evidence of ovulatory cycle as judged by basal body temperature (BBT) graphs for three consecutive cycles or histological evidence of secretory phase in a endometrial biopsy studied 24 to 48 hours before the expected menstrual period.
5) Absence of any signs and symptoms of endocrine diseases.

ACTH infusion:

The adrenal response to the adrenocorticotropic hormone (ACTH) was studied by infusing 25 I.U. ACTH dissolved in 500 ml of physiological glucose saline solution over a
period of four hours. In each case the infusion was started between 8.00 and 8.30 a.m. At the end of the infusion, blood was drawn for the estimation of plasma 17-OHCS. Two urine samples of 12 hours each starting at the time of infusion (total 24 hours), were collected from every subject and the excretion of 17-KS, 17-KGS, pregnanetriol and pregnanetriol-11-one was studied after the ACTH infusion.
METHODS

Urinary 17-KS: The main steps involved in the chemical estimation of 17-KS in urine are hydrolysis of steroid conjugates, extraction of steroids from the hydrolysed urine, removal of acidic compounds and interfering pigments from this extract and colour development with m-dinitrobenzene. The major portion of the urinary 17-KS is DHEA, androsterone and etiocholanolone of which the former is excreted as sulphates while the latter two are excreted mainly as glucuronides. These all conjugates can be hydrolysed by strong inorganic acids at elevated temperatures. The glucuronides are hydrolysed by \( \beta \)-glucuronidase. More recently, DePaoli et al. and Jacobson and Lieberman showed complete hydrolysis of 17-KS conjugates by extracting urine saturated with ammonium sulphate at pH 4 and using 0.01 M perchloric acid for hydrolysis. Peterson and Pierce reviewed the methods of hydrolysis, purification and estimation of 17-KS in the urine and suggested a suitable procedure. In the present investigation, which was started before 1955, two methods, described by Landau and later the one presented by Drekter et al. are used. The details of the procedure is given in Appendix.

Urinary 17-KGS: The chemical estimation of corticosteroids are based on (A) the reducing properties of these steroids. (B) Formaldehydogenic steroids. (C) Acetaldehydogenic steroids. (D) Porter-Silber reaction, Lorain has reviewed these...
methods extensively and discussed their specificity. In the present investigation a method based on the oxidation of corticosteroids having 17, 21-diol-20-one, 17, 20, 21 triol and 17, 20 glycol side chains with sodium bismuthate and the liberated 17-KS are measured along with the 17-KS present in the urine. By subtracting the value of 17-KS present in the urine prior to bismuthate oxidation, the value of 17-KGS can be obtained. The detailed procedure is described in Appendix.

Plasma 17-OHCS: The bioassay procedures are not sensitive enough to detect small quantities of these substances in the peripheral blood. Nelson and Samuels reported more precise methods in which the free corticosteroids are extracted from the plasma and purified. The purified extract was treated with Porter-Silber reagent and measured in spectrophotometer. The various modifications of this technique have been suggested by Eik-Nes et al. The procedure used in the present investigation is described in Appendix.

Urinary pregnanetriol and pregnanetriol-11-one: Various methods have been described for the estimation of pregnanetriol in urine. Bongiovanni and Clayton, Stern and Bongiovanni and Eberlein used column chromatography on alumina to separate pregnanetriol in extracts of urine. The purified product thus formed gave a colour complex with concentrated sulphuric acid which could be estimated spectro-
photometrically. Hermann and Silvermann\textsuperscript{94} and Cox\textsuperscript{95} combined column and paper chromatography respectively and estimated the oxidised products of pregnanetriol side chain. These methods are time consuming and less specific. In the present study the method described by Stern\textsuperscript{92} as modified by Fotherby and Love\textsuperscript{96} was used.

**Pregnanetriol-11-one:** Finkelstein \textit{et al}\textsuperscript{97} and Fukushima \textit{et al}\textsuperscript{98} isolated pregnanetriol-11-one from the urine of patients with adrenocortical hyperplasia. Cox and Finkelstein\textsuperscript{99} and Cox\textsuperscript{95} have studied the excretion of this steroid in urine in normal subjects of both sexes and observed that it is not excreted by normal persons in detectable quantities. It was, however, found in considerable amounts in patients with adrenal hyperplasia, but the diagnostic significance is attached to the detection, rather than the exact estimation of this steroid in the urine. In the present investigation the technique of Cox and Finkelstein\textsuperscript{99} has been employed for the detection of pregnanetriol-11-one.

**Assay of gonadotrophins in urine:** Two extraction procedures were used for urinary gonadotrophins during this work. The investigation was started with the extraction method of Loraine and Brown\textsuperscript{100}. During the course of the investigation, Butt\textsuperscript{101} published a method for extraction of gonadotrophins from urine by the combined use of benzoic acid and tungstic acid. This method was found to be more efficient than the original method of Loraine and Brown\textsuperscript{100}. The
bioassays were carried at two levels 6.6 and 50 I.U., on 24 hours urine specimens from patients suffering from Klinefelter's syndrome.
RESULTS

Normal Population

Excretion of 17-KS in Indian Children: This investigation was done on male subjects. It had been reported previously that the excretion of 17-KS does not differ in boys and girls up to the age of 8 and 9 years \(^{102}\).

The Indian population is composed of various communities which differ in food habits and nutritional status. Children, ranging from 7 to 12 years of age and belonging to Deccani, Sindhi and Parsi communities, were selected to study the effect of diet on the urinary excretion of 17-KS. The output of these steroids per 24 hours was 0.99±0.45, 1.12±0.15, and 1.18±0.53 mg in Deccani, Sindhi and Parsi communities respectively. These values do not differ significantly from each other and the average value of all subjects was 1.18±0.11 mg per 24 hours.

Thirty three children from this study were divided into five groups depending on age. Their excretion of 17-KS is shown in Table I.

The study on excretion of 17-KS was repeated in six children to study the day to day variability in the excretory pattern of an individual subject. The coefficient of variation ranged from 9.8 to 41.1 which indicates that the individual child does show variation in day to day excretion of 17-KS (Table II).
Table I

Excretion of 17-KS in normal children.
mg / 24 hours

<table>
<thead>
<tr>
<th>Age in years</th>
<th>2 to 4</th>
<th>5 to 6</th>
<th>7 to 8</th>
<th>9 to 10</th>
<th>11 to 12</th>
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<tbody>
<tr>
<td>0.35</td>
<td>0.54</td>
<td>0.85</td>
<td>0.94</td>
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<tr>
<td>0.30</td>
<td>0.76</td>
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<td>1.15</td>
<td>1.04</td>
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<tr>
<td>0.32</td>
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<td>0.41</td>
<td>0.55</td>
<td>2.30</td>
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</tr>
<tr>
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<td>0.34</td>
<td>1.08</td>
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<td>0.78</td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>0.89</td>
<td>1.24</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>0.84</td>
<td>1.38</td>
<td>1.69</td>
<td></td>
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</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>1.63</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

Mean  
0.26 ± 0.05*  0.59 ± 0.08  0.73 ± 0.10  1.21 ± 0.09  1.36 ±0.1

* - Standard error of the mean.
### Table II

**Evaluation of the Day to day excretion of 17-KS in six normal boys.**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age</th>
<th>No. of 24 hrs. urine samples processed</th>
<th>Mean of 17-KS mg / 24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN</td>
<td>3</td>
<td>6</td>
<td>0.38 ±0.05* 13.27**</td>
</tr>
<tr>
<td>VS</td>
<td>7</td>
<td>6</td>
<td>0.48 ±0.20 41.10</td>
</tr>
<tr>
<td>DY</td>
<td>7</td>
<td>6</td>
<td>0.86 ±0.22 25.61</td>
</tr>
<tr>
<td>KM</td>
<td>7</td>
<td>6</td>
<td>0.79 ±0.08 9.77</td>
</tr>
<tr>
<td>HM</td>
<td>11</td>
<td>4</td>
<td>1.61 ±0.52 32.06</td>
</tr>
<tr>
<td>CX</td>
<td>6</td>
<td>2</td>
<td>0.41 ... ...</td>
</tr>
</tbody>
</table>

* - Standard deviation.

** - Coefficient of variation in percent.
Excretion of 17-KS in normal women: The urinary excretion of 17-KS was estimated in 25 women of active reproductive age. The values of 17-KS in the pre-ovulatory phase ranged from 4.9 to 12.5 mg per 24 hours (mean value 8.03 ± 0.86), while in the post-ovulatory phase the value ranged between 3.9 and 11.9 (mean value 8.09 ± 0.58). These results clearly show that there is no difference in the excretion of 17-KS in the two phases of the menstrual cycle. In the total group of 25 women the excretion of 17-KS per 24 hours ranged from 3.9 to 12.5 mg (mean 8.06 ± 0.48) (Table III). Dorfman and Shipley have summarized the results of numerous studies of 17-KS excretion in urine of normal women. In this review the mean, uncorrected, concentration varied from 10.1 to 13.2 mg per 24 hours with an average mean of 11.8 mg by the method of Callow et al. Using the method of Haltorff and Koch the overall average found was 12.6 mg per 24 hours; whereas with the polarographic method (Butt et al) the average found was 14.1 mg per 24 hours.

Our studies indicate that the excretion of 17-KS in normal young Indian women is lower than reported for normal women in the United States.

Urinary excretion of 17-KS in normal women after ACTH administration: In normal women the excretion of 17-KS in the control state ranged from 7.0 to 11.4 mg per 24 hours with a mean value of 8.9 ± 1.46. After ACTH administration the 17-KS excretion varied from 10.7 to 16.6 mg per 24
Table III

Excretion of 17-KS and 17-KGS in normal women

<table>
<thead>
<tr>
<th>Subjects</th>
<th>17-KS mg/24 hours</th>
<th>17-KGS mg/24 hours</th>
<th>Subjects</th>
<th>17-KS mg/24 hours</th>
<th>17-KGS mg/24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP</td>
<td>11.4</td>
<td>6.8</td>
<td>SU</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>ST</td>
<td>4.9</td>
<td>5.9</td>
<td>MD</td>
<td>9.0</td>
<td>5.7</td>
</tr>
<tr>
<td>VL</td>
<td>6.8</td>
<td>4.8</td>
<td>SV</td>
<td>9.5</td>
<td>7.8</td>
</tr>
<tr>
<td>MK</td>
<td>7.3</td>
<td>6.5</td>
<td>AM</td>
<td>7.7</td>
<td>6.1</td>
</tr>
<tr>
<td>HG</td>
<td>4.5</td>
<td>7.2</td>
<td>MK</td>
<td>5.3</td>
<td>5.3</td>
</tr>
<tr>
<td>RV</td>
<td>7.1</td>
<td>4.6</td>
<td>GR</td>
<td>7.2</td>
<td>3.6</td>
</tr>
<tr>
<td>SD</td>
<td>6.2</td>
<td>4.1</td>
<td>JT</td>
<td>6.2</td>
<td>7.9</td>
</tr>
<tr>
<td>UP</td>
<td>10.3</td>
<td>6.1</td>
<td>AK</td>
<td>10.3</td>
<td>5.9</td>
</tr>
<tr>
<td>SS</td>
<td>12.5</td>
<td>9.0</td>
<td>WD</td>
<td>8.3</td>
<td>5.8</td>
</tr>
<tr>
<td>KJ</td>
<td>9.3</td>
<td>6.4</td>
<td>PW</td>
<td>10.7</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT</td>
<td>11.9</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RM</td>
<td>7.0</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SN</td>
<td>3.9</td>
<td>4.9</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>SB</td>
<td>8.0</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GT</td>
<td>9.9</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Range 4.5 to 12.5 4.1 to 9.0 3.9 to 11.9 3.6 to 10.3

Mean 8.03 ± 0.5* 6.14 ± 0.4 8.09 ± 0.58 6.31 ± 0.38

* - Standard error of the mean.
hours. The mean value was $13.7 \pm 2.0$. The individual values are shown in Table IV.

**Excretion of 17-KS in normal young men:** The urinary 17-KS were estimated in fifteen normal young men. The value ranged from 5.38 to 14.8 mg per 24 hours with a mean value of $9.47 \pm 0.64$ (Table V). Kenigsberg et al.\(^{107}\), using the method of Drekter et al.\(^{108}\), found that the 17-KS in a group of normal men ranging in age between 17 and 34 years, varied from 9.0 to 30.0 mg per 24 hours with a mean value of 18.0 mg. The values obtained by Drekter et al.\(^{108}\) in 35 normal subjects from 20 to 30 years old, ranged between 10.0 and 28.9 mg per 24 hours with a mean value of 16.9 mg. The excretion of 17-KS in the present study in a group of Indian men is nearly half that measured by Kenigsberg et al.\(^{107}\) and by Drekter et al.\(^{108}\) in normal young men living in the United States.

**Urinary excretion of 17-KGS in normal women:** Urinary 17-KGS were estimated in normal women of active reproductive age (16 to 39 years). The values of 17-KGS ranged from 3.6 to 10.3 mg per 24 hours (Table III). The values of 17-KGS in the pre-ovulatory phase ranged from 4.1 to 9.0 mg per 24 hours (mean value, $6.14 \pm 0.45$); while in the post-ovulatory phase the values ranged between 3.6 to 10.3 (mean value, $6.31 \pm 0.38$) (Table III). These results show that there is no difference in the excretion of 17-KGS between the two phases of the menstrual cycle.
Table IV
Excretion of 17-KS and 17-KGS in normal women in control state and after administration of ACTH

<table>
<thead>
<tr>
<th>No. Subjects</th>
<th>17-KS mg/24 hrs.</th>
<th>17-KGS mg/24 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After ACTH</td>
</tr>
<tr>
<td>1.</td>
<td>9.5</td>
<td>16.3</td>
</tr>
<tr>
<td>2.</td>
<td>9.0</td>
<td>11.1</td>
</tr>
<tr>
<td>3.</td>
<td>11.4</td>
<td>16.6</td>
</tr>
<tr>
<td>4.</td>
<td>7.0</td>
<td>13.4</td>
</tr>
<tr>
<td>5.</td>
<td>7.2</td>
<td>14.1</td>
</tr>
<tr>
<td>6.</td>
<td>7.7</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Range .. 7.0 to 11.4 10.7 to 16.6 5.5 to 7.8 9.1 to 23.4

Mean ... 8.7 +1.46 13.7 +2.0 6.2 +0.3 15.2 +6.2
### Table V

**Excretion of 17-KS and 17-KGS in normal men**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>17-KS mg/24 hours</th>
<th>17-KGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV</td>
<td>20</td>
<td>14.57</td>
<td>9.30</td>
</tr>
<tr>
<td>MR</td>
<td>31</td>
<td>13.79</td>
<td>12.83</td>
</tr>
<tr>
<td>PT</td>
<td>19</td>
<td>7.60</td>
<td>5.15</td>
</tr>
<tr>
<td>TR</td>
<td>35</td>
<td>8.78</td>
<td>7.32</td>
</tr>
<tr>
<td>MM</td>
<td>22</td>
<td>11.04</td>
<td>8.68</td>
</tr>
<tr>
<td>DS</td>
<td>26</td>
<td>9.30</td>
<td>10.03</td>
</tr>
<tr>
<td>DN</td>
<td>29</td>
<td>9.73</td>
<td>9.88</td>
</tr>
<tr>
<td>RG</td>
<td>20</td>
<td>8.58</td>
<td>9.68</td>
</tr>
<tr>
<td>JM</td>
<td>28</td>
<td>11.20</td>
<td>8.90</td>
</tr>
<tr>
<td>PW</td>
<td>21</td>
<td>9.72</td>
<td>5.36</td>
</tr>
<tr>
<td>RC</td>
<td>23</td>
<td>8.75</td>
<td>7.38</td>
</tr>
<tr>
<td>LG</td>
<td>20</td>
<td>9.83</td>
<td>8.30</td>
</tr>
<tr>
<td>AB</td>
<td>25</td>
<td>7.78</td>
<td>6.30</td>
</tr>
<tr>
<td>SP</td>
<td>37</td>
<td>5.38</td>
<td>4.48</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>5.49</td>
<td>4.37</td>
</tr>
<tr>
<td>TT</td>
<td>22</td>
<td>6.06</td>
<td>6.36</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>6.75</td>
<td>6.13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Range</th>
<th>15</th>
<th>19-37</th>
<th>5.38 - 14.80</th>
<th>4.50 - 12.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>...</td>
<td>...</td>
<td>9.47 ± 0.64</td>
<td>8.03 ± 0.50</td>
</tr>
</tbody>
</table>
The urinary excretion of 17-KGS in normal females has been studied by Norymberski et al.\textsuperscript{109} They reported that in normal women the values ranged between 4.6 to 13.4 mg per 24 hours with a mean of 8.9 mg.

An ACTH response test was carried out in six women. The control excretion of 17-KGS in these subjects ranged between 5.5 and 7.8 mg per 24 hours with a mean of 6.2±0.31. After ACTH administration these values ranged from 9.1 to 23.4 mg per 24 hours with a mean of 15.2±6.2 (Table IV).

**Urinary excretion of 17-KGS in normal men:** An estimation of 17-KGS was carried out in fifteen young men ranging in age from 19 to 37 years. The values of these steroids varied from 4.43 to 12.33 mg per 24 hours and the mean value was 8.03±0.5 (Table V). Norymberski et al.\textsuperscript{109} reported that in normal men the excretion ranged from 9.6 to 19.2 mg per 24 hours with a mean value 13.2 mg. Moreover, similar figures were reported by Diczfalasy et al.\textsuperscript{110}, Prunty\textsuperscript{111} and Moxham and Naberroll\textsuperscript{112}.

**Urinary pregnanetriol in normal women:** This steroid was determined in the urine of 13 normal women and ranged from 0.21 to 2.78 mg per 24 hours, the mean being 0.82 (Table VI). A study of the variations in excretion of this particular steroid was carried out in 4 normal women who were given 25 I.U. of ACTH over a period of four hours. A 24 hour urine specimen was collected from the starting period of ACTH infusion. The excretion of this steroid increased with
### Table VI

**Pregnanetriol Excretion in Normal Women.**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Pregnanetriol: mg/24 hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control level</td>
</tr>
<tr>
<td>VS</td>
<td>0.73</td>
</tr>
<tr>
<td>VL</td>
<td>0.35</td>
</tr>
<tr>
<td>RV</td>
<td>0.08</td>
</tr>
<tr>
<td>GI</td>
<td>0.96</td>
</tr>
<tr>
<td>SA</td>
<td>0.21</td>
</tr>
<tr>
<td>AD</td>
<td>0.54</td>
</tr>
<tr>
<td>PA</td>
<td>1.17</td>
</tr>
<tr>
<td>SA</td>
<td>0.25</td>
</tr>
<tr>
<td>TT</td>
<td>1.22</td>
</tr>
<tr>
<td>MR</td>
<td>0.48</td>
</tr>
<tr>
<td>VN</td>
<td>2.75</td>
</tr>
<tr>
<td>SH</td>
<td>0.84</td>
</tr>
<tr>
<td>RG</td>
<td>1.06</td>
</tr>
<tr>
<td>Mean...</td>
<td>0.82</td>
</tr>
</tbody>
</table>
Table VII

Pregnanetriol and pregnanetriol-11-one in patients suffering from Cushing's syndrome and the adrenogenital syndrome.

<table>
<thead>
<tr>
<th>Diagnosis and Subjects</th>
<th>Pregnanetriol mg/24 hours</th>
<th>Pregnanetriol-11-one in 24 hours.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control level</td>
<td>After ACTH</td>
</tr>
<tr>
<td>Cushing's syndrome ... .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KK</td>
<td>1.85</td>
<td>4.37</td>
</tr>
<tr>
<td>SD</td>
<td>2.12</td>
<td>1.88</td>
</tr>
<tr>
<td>TN</td>
<td>3.17</td>
<td>3.50</td>
</tr>
<tr>
<td>Adrenogenital syndrome . . .</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>3.99</td>
<td>5.88</td>
</tr>
<tr>
<td>SR</td>
<td>4.0</td>
<td>7.35</td>
</tr>
</tbody>
</table>
ACTH administration (Table VII) and appeared to be similar to that found in pregnant women by Fotherby and Love. The pregnanetriol excretion in normal women obtained by these authors ranged between 0.1 and 3.0 mg per 24 hours with a mean value of 0.9 mg.

Urine pregnanetriol-11-one excretion in normal subjects:
No pregnanetriol-11-one was found in the urine of four normal women and two men. The negative results were confirmed by processing larger volumes (100 to 200 ml) of urine for assay.

Furthermore, pregnanetriol-11-one did not appear in the urine after the infusion of 25 I.U. ACTH in normal women.

17-OHCS in plasma: Normal subjects:
Plasma 17-OHCS were estimated on 34 occasions in twenty-seven women and three men. The range of values was from 5 to 22.5 µg per 100 ml of plasma (Fig. 5). The variation in these levels represents the normal range of both sexes, since it has been established that there are no differences in the levels of plasma 17-OHCS between human males and females (Wallace et al.113). Our values are in agreement with the results of Wallace et al.113 for American subjects, where the upper limit of normal subjects was 23.5 µg per 100 ml of plasma. The methods for determining plasma 17-OHCS are similar in both investigations. Thus, the data on the Indian subjects are comparable with those
NORMAL VALUES OF 17-HYDROXYCORTICOSTEROIDS

![Graph showing normal values of 17-hydroxy corticosteroids.]

PLASMA 17-HYDROXYCORTICOSTEROIDS DURING TWO PHASES OF MENSTRUAL CYCLES

- **Pre-Ovulatory**: 
  - 11.01 ± 1.12

- **Post-Ovulatory**: 
  - 15.21 ± 0.85

![Graph showing plasma 17-hydroxy corticosteroids during two phases of menstrual cycles.]

**Fig. 5**

**Fig. 6**
on American subjects. During the last decade various reports have been published on the concentration of 17-OHCS in peripheral blood plasma of normal subjects using the Porter-Silber reaction. The overall values are ranging between 0.0 to 28.5 μg per 100 ml of plasma. Substances other than 17-OHCS interfere with the Porter-Silber reaction; purified plasma extracts partitioned between water and benzene gave a range of 17-OHCS from 6 to 18 μg per 100 ml. Sandberg et al. have reported an elevation in the urinary 17-OHCS at the time of ovulation. An attempt was therefore made to find whether the concentration of plasma 17-OHCS varies significantly during the two phases of the menstrual cycle. Of the 27 normal subjects having regular ovulatory bleeding, 17-OHCS were estimated in the pre-ovulatory phase in 12 cases and in the post-ovulatory phase in 15 cases. The results are given in Fig 6. The average value in the pre-ovulatory phase was 11.01±1.12 and in post-ovulatory phase 15.21±0.85. The results indicate that the average value for the post-ovulatory phase is higher than that of the pre-ovulatory and the difference is statistically significant (P < 0.02).

Adrenal response to ACTH: The individual variations of adrenocortical response in 6 healthy adult women were studied by measuring the change in plasma 17-OHCS before and after giving 25 I.U. of ACTH intravenously over a period of 4 hours. The results are shown in Table VII. In these
RESPONSE TO ACTH (I.V.)

NORMAL WOMEN
(6)

PLASMA 17-HYDROXYCORTICOSTEROIDS
µg/100 ml

TIME IN HOURS

Fig. 7
subjects the 17-OHCS per 10^6 ml of plasma varied from 8.8 to 22.5 µg (mean 14.77 µg) before ACTH. The plasma 17-OHCS increased to a range of 18.4 - 45.4 µg (mean 33.4 µg) after ACTH administration. This response in Indian subjects is comparable to that observed in a comparatively larger group studied by Christy et al.65 in the United States (Fig. 7). Their values were 35 to 55 µg per 10^6 ml of plasma. Eik-Nes et al.64 gave 25 I.U. ACTH by infusion over a period of 6 hours and found values ranging between 25 to 50 µg per 100 ml of plasma. 25 I.U. ACTH given by intravenous infusion stimulated the adrenals maximally and further increases in the dose of ACTH did not increase the plasma concentration of 17-OHCS (Eik-Nes et al.64).
DISCUSSION

Urinary 17-KS in children:

The excretory level of 17-KS in children shows a gradual increase with age. Our observation, in contrast to that of Ramchandran et al.\textsuperscript{115} which is based on a smaller series of cases, indicates that the excretion pattern of 17-KS in Indian children is similar to that of children in the United States\textsuperscript{74,116}. It appears however, that the output of 17-KS in Indian children above 8 years is much lower than seen in the children of a similar age group of U.S. Whites (Fig. 8). The sharp rise in excretion of 17-KS in U.S. White children particularly at the age of 11 - 12 years may probably be due to testicular production of these steroids or their precursors during onset of puberty\textsuperscript{117}.

It has been observed that children suffering from nutritional edema excreted a smaller amount of 17-KS than did normal children\textsuperscript{115}. We have investigated the relationship between consumption of foods with different nutritional value, the urinary excretion of creatine and 17-KS in pre-pubertal Indian boys. With an increased consumption of meat, the urinary excretion of creatine increased but no correlation could be established between the excretion of 17-KS and consumption of meat products (high protein diet).

In order to determine the dependability of measuring excretion of 17-KS per day, estimation of these steroids was repeated from day to day in six children. The
EXCRETION OF 17-KETOSTEROIDS IN NORMAL CHILDREN

- INDIANS
- U.S. WHITES

Fig. 8
results of these experiments showed that the individual child does show a day to day variation. Yet, abnormally high values can be distinguished for their diagnostic importance even though analysis of 17-KS may be made on a single 24 hour sample. In this prepubertal age especially below 8 years the 17-KS are probably derived almost entirely from the adrenal cortex. The increment in urinary excretion of 17-KS before the onset of puberty therefore, reflects the growth and development of the adrenal gland.

Urinary 17-KS in normal men and women:

In our investigations young men and women below the age of 40 were selected. The values reported in the present investigation are slightly higher than those reported for Indians\textsuperscript{119,120} which may be due to use of different methods but are lower than those reported for U.S. Whites\textsuperscript{79}. The factor or factors which are responsible for such a wide difference in these values are as yet completely unknown. It is generally observed that women tend to excrete smaller amounts of 17-KS than normal men of a comparable age group. However, a considerable overlap exists in the urinary values of 17-KS in either sex.

The urinary 17-KS in women arise mainly from precursors secreted by the adrenal cortex and the fluctuation of these steroids in the urine could therefore represent variations in adrenocortical function. It appears that the urinary 17-KS do not vary according to the ovarian cycle in
women since the values in pre- and post-ovulatory phases are approximately the same as shown in Table III. These findings confirm the previous observations.\textsuperscript{121}

\textbf{Urinary 17-KGS in normal men and women:}

The method employed for the estimation of 17-KGS in the present work is adequate to determine the adrenocortical function. The excretory values of 17-KGS in normal men and women of the present investigation are slightly lower than those reported by Norymberski\textsuperscript{109} and Sobel \textit{et al.}\textsuperscript{122} Bondy \textit{et al.}\textsuperscript{123} found a statistical correlation between weight, size, age and sex and the quantity of 17-KGS excreted in the urine. If the excretion of 17-KGS depends on the weight and size of the individual and subjects with higher body weight excrete more 17-KGS than subjects with low body weight, this may explain why the Indian subjects have slightly lower excretion of 17-KGS than those reported in American subjects. The excretion of 17-KGS in women is significantly lower than those of the men. This confirms the findings of Borth \textit{et al.}\textsuperscript{121}. Men excrete more 17-KGS than women (Table III and V) and these authors have attributed this sex difference entirely to the fact that men are taller and heavier than women. The urinary excretion of 17-KGS increased two to three fold by administration of 25 I.U. of ACTH in all the Indian women studied as shown in Table IV.
Concentration of plasma 17-OHCS in normal subjects:

It is well established that there is no difference in plasma 17-OHCS between men and women. The variation in concentrations of plasma 17-OHCS found in the present investigation may well represent the normal range for either sex in a group of normal subjects. In contrast to the findings of Poinsnick and D'Amondo, no correlation could be found between the levels of plasma 17-OHCS and the weight of the individual. This is in conformity with the observation of Szenas and Patte. Similarly, no significant difference exists in the concentration of plasma 17-OHCS between normal Indian subjects and U.S. Whites though living under quite different environmental conditions.

Fig. 6 shows the changes in plasma 17-OHCS in the pre- and post-ovulatory phases of menstrual cycle. The results indicate that the average value for the post-ovulatory phase is significantly higher (P < 0.02) than the average plasma values of 17-OHCS in the pre-ovulatory phase. It is unlikely that the 21-hydroxylase enzyme system is present in the human ovary, but larger amount of progesterone, which serves as one of the precursors for the synthesis of corticosteroids and which is known to be present during post-ovulatory phase of menstrual cycle produced by the ovary and that could have been used by the adrenal cortex for the production of 17-OHCS. Alternatively, the secretion of ACTH may fluctuate with the menstrual cycle and may be responsible
for the elevation of 17-OHCS seen in the post-ovulatory phase. Such a small increase in ACTH secretion may not affect the excretion of 17-KS and 17-KGS in the post-ovulatory phase, since plasma values of 17-OHCS are more sensitive to exogenous ACTH than that of urinary 17-KS and 17-KGS.

The observation that elevated levels of plasma 17-OHCS can be demonstrated in Cushing's syndrome and in the third trimester of pregnancy\textsuperscript{64,126,127} and also after the administration of ACTH, seems to suggest that the measurement of plasma 17-OHCS is important in evaluating adrenal function. Furthermore, a vast amount of data have now accumulated showing low or no concentration of plasma 17-OHCS when the adrenal function is known to be nil, such as in Addison's disease. Since free 17-OHCS are physiologically active, the free plasma 17-OHCS and not conjugated 17-OHCS were determined.

Urine pregnanetriol and pregnanetriol-11-one:

The results from this investigation of pregnanetriol in normal women indicate that formation of pregnanetriol is a normal feature of steroid metabolism since all normal women excrete pregnanetriol in the urine\textsuperscript{92,95,96}. The excretion of this steroid in the individual subject varied within a wide range (from 0.03 to 2.73 mg per 24 hours) and increased after the administration of ACTH. Such a rise following the administration of ACTH indicates that
the adrenal cortex is the main source of pregnanetriol or its precursors and a high excretion of this steroid suggests either hyperactivity or defective steroidogenesis by the adrenal cortex, reflecting a lack of 21-hydroxylase and/or stimulation of 20-dehydrogenase activity. This could thus explain why there is a high excretion of pregnanetriol in cases of congenital adrenal hyperplasia and Cushing's syndrome (Table VII). Patient SR (Table VII) with classical clinical picture of adrenogenital syndrome, excreted about 4 mg per 24 hours at rest which increased to about 7.4 mg per 24 hours after administration of ACTH.

Pregnanetriol-11-one is an abnormal steroid metabolite and it has not been detected in the urine of normal men and women, confirming the previous findings. With the present methods for the estimation of pregnanetriol-11-one, it was possible to detect this steroid in the urine of patients with congenital adrenal hyperplasia and in Cushing's disease with adrenal hyperplasia. Hence, the detection of this steroid in the urine signifies the defective steroidogenesis in adrenal cortex.
SUMMARY

The urinary levels of 17-KS, 17-KGS, pregnanetriol, pregnanetriol-11-one and plasma-17-OHCS in normal Indian population have been established. These steroids were also determined after the administration of a standard test dose of ACTH.

(1) The excretory levels of 17-KS in Indian children were similar to that of U.S. White children below the age of 8 years but the Indian children of 10 to 12 years excreted less 17-KS than the U.S. White children of the same age.

(2) The excretion of 17-KS in children varies from day to day.

(3) No correlation was observed in excretory levels of 17-KS and consumption of food with different nutritional values.

(4) The urinary levels of 17-KS and 17-KGS in normal Indian men and women were lower than those of U.S. Whites. These values did not change in different phases of menstrual cycle in normal women. The urinary 17-KS and 17-KGS increased after administration of ACTH.

(5) The plasma content of 17-OHCS of Indian population were similar to that of U.S. Whites. In post-ovulatory phase of menstrual cycle in women, the plasma 17-OHCS levels were slightly higher than those observed in the pre-ovulatory phase. The plasma 17-OHCS levels increased after
administration of ACTH and provide an important test in evaluating the adrenal function.

(6) The excretion of pregnanetriol varied from 0.8 to 2.78 mg per 24 hours in normal women and increased after the administration of ACTH. No pregnanetriol-ll-one was detected in the urine of normal men and women. High amounts of pregnanetriol or presence of pregnanetriol-ll-one in urine may reflect defective steroidogenesis in the adrenal cortex.
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