INTRODUCTION

Evaluation and understanding of the endocrine disorders have greatly been facilitated by the ever increasing knowledge of intermediary metabolism in the biosynthesis of various hormones. This is particularly so in the field of biochemistry of steroid hormones which has developed considerably during the past few years. Biosynthesis of cholesterol from 2 carbon units and its subsequent transformation into various physiologically active steroid hormones forms the basis of studies on various endocrine diseases. Several review articles and original papers have been published in recent years\textsuperscript{1,2,3,4,5,6,7} which cover the various aspects of steroidogenesis. Because of the relevance and importance of this subject to the present thesis the biochemistry underlying such pathological states as hirsutism, Cushing's syndrome, Klinefelter's syndrome and hermaphroditism, the mode of action of hormones and their abnormal production etc. have been briefly discussed in the introduction.

Biosynthesis of Cholesterol: Bloch and Rittenberg\textsuperscript{8} were the first to demonstrate that acetate is a major precursor of Cholesterol. This was further confirmed by showing the incorporation of acetate $1^-C^{14}$ and acetate $2^-C^{14}$ into cholesterol\textsuperscript{9}. Longdon and Bloch\textsuperscript{10} isolated labelled
BIOSYNTHESIS OF CHOLESTEROL

\[ \text{CH}_3\text{C}=\text{CH}_2 \xrightarrow{\text{CH}_3\text{C}=\text{CH}_2-\text{CO}-\text{P}_{2}\text{O}_6} \text{FARNESYL PYROPHOSPHATE} \]

\[ \text{CH}_3\text{C}=\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{CO}-\text{P}_{2}\text{O}_6 \xrightarrow{\text{CH}_3\text{C}=\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-\text{CO}-\text{P}_{2}\text{O}_6} \text{SQUALENE (C}_{30}\text{H}_{50}) \]

Fig. 1

BIOSYNTHESIS OF STEROIDS IN ADRENAL CORTEX

\[ \text{ACETATE} \xrightarrow{\text{ACTH}} \text{CHOLESEROL} \]

\[ \text{PREGNENOLONE} \xrightarrow{\text{PREGESTERONE}} \text{PROGESTERONE} \xrightarrow{\text{11-DESOXYCORTICOSTERONE}} \text{CORTICOSTERONE} \]

\[ \text{17}-\text{HYDROXYPREGNENOLONE} \xrightarrow{\text{17}-\text{HYDROXYPROGESTERONE}} \text{11-DESOXYCORTISOL} \xrightarrow{\text{18}-\text{HYDROXYCORTICOSTERONE}} \text{ALDOSTERONE} \]

\[ \text{DEHYDROEPIANDROSTERONE} \xrightarrow{\Delta-\text{ANDROSTENEDIONE}} \text{ANDROSTENEDIONE} \]

\[ \text{HYDROXYLASE ENZYMES} \]

Fig. 2
squalene from the rats given labelled acetate and using this squalene proved the \textit{in vivo} conversion of squalene to cholesterol. Tavormina and co-workers demonstrated that mevalonic acid is an intermediate between acetate and squalene and that mevalonic acid is an efficient precursor of cholesterol. It was further shown that the transformation of mevalonic acid to squalene is through its phosphorylated derivatives\textsuperscript{11,12}. The synthetic farnesyl pyrophosphates were effective substrates for the biosynthesis of squalene by liver microsomes\textsuperscript{13}. The various biosynthetic pathways and the enzymes participating in these transformations have been briefly summarized in Fig. 1.

Transformation of cholesterol to various steroid hormones:

Pregnenolone seems to play a central role in the biosynthesis of steroid hormones. 20\textsuperscript{\alpha}-Hydroxylation followed by 22-hydroxylation of cholesterol side-chain followed by cleavage between carbon atoms 20 and 22 have been shown to be successive steps in the formation of pregnenolone\textsuperscript{14,15}. Chaudhari \textit{et al.}\textsuperscript{16} have shown the possibility that the reaction sequence, 22\textsuperscript{\alpha}-hydroxycholesterol 20\textsuperscript{\alpha}, 22\textsuperscript{\alpha}-dihydroxycholesterol and hence to pregnenolone may exist in the adrenal in addition to the previously observed 20\textsuperscript{\alpha}-hydroxycholesterol\textsuperscript{20\alpha}, 22\textsuperscript{\alpha}-dihydroxycholesterol sequence.

A clear sequence of enzymatic reaction involved in the synthesis of biologically potent C\textsubscript{21}, C\textsubscript{19} and C\textsubscript{18} steroids
BIOSYNTHESIS OF STEROIDS IN TESTIS

PREGNENOLONE

PROGESTERONE

17\(\alpha\) - HYDROXY-PREGNENOLONE

DEHYDROEPIANDROSTERONE

\(\Delta^4\) - ANDROSTENE-3,17-DIONE

19-HYDROXY-\(\Delta^4\)
ANDROSTENE-3,17-DIONE

ESTRONE

ESTRADIOL - 17\(\beta\)

\(a\) - 3\(\beta\) - HYDROXYDEHYDROGENASE
\(b\) - ISOMERASE
\(c\) - 17\(\alpha\) - HYDROXYLASE
\(d\) - DESMOLESE

Fig. 3
BIOSYNTHESIS OF STEROIDS IN OVARY

PREGNENOLONE \(\xrightarrow{a+b}\)

PROGESTERONE \(\xrightarrow{c}\) 17\(\alpha\)-HYDROXY-PREGNENOLONE

17\(\alpha\)-HYDROXY-PROGESTERONE \(\xrightarrow{c}\)

DEHYDROEPIANDROSTERONE \(\xrightarrow{d}\)

\(\Delta^4\)-ANDROSTENE-3,17-DIONE \(\xrightarrow{a+b}\)

TESTOSTERONE

19-HYDROXY-\(\Delta^4\)-ANDROSTENE-3,17-DIONE

ESTRONE

ESTRADIOL-17\(\beta\)

\(a\) - 3,\(\beta\)-HYDROXYDEHYDROGENASE
\(b\) - ISOMERASE
\(c\) - 17-HYDROXYLASE
\(d\) - DESMOlASE

Fig. 4
from pregn-5-en-3-ol-20-one has been demonstrated\textsuperscript{17,3,18,19,20,21}. Fig 2 shows the synthetic pathways of corticosteroids in the adrenal cortex, and the biosynthesis of androgens and estrogens are illustrated in Fig. 3,4. All the tissues which produce steroid hormones contain \( \Delta^7 \)-hydroxydehydrogenase and \( \Delta^5 \)-hydroxysteroid isomerase enzymes which convert pregnenolone to progesterone\textsuperscript{17}. Progesterone gets hydroxylated at position 17 and the \( \Delta^7 \)-hydroxyprogesterone thus formed is converted to \( \Delta \)-desoxycortisol and finally to cortisol by the action of 21-hydroxylase and then 11-hydroxylase in the adrenal cortex. Progesterone also undergoes 21-hydroxylation giving rise to \( \Delta \)-desoxycorticosterone. \( \Delta \)-Desoxycorticosterone is a poor substrate for corticosterone or other corticosteroids\textsuperscript{22}. 17-Hydroxyprogesterone is readily converted into cortisone\textsuperscript{23,18,24,25,26}.

In testicular and ovarian tissues as well as in adrenal cortex the side chain of \( \Delta^7 \)-hydroxyprogesterone splits off by the action of 17-desmolase to form androstenedione which is further transformed into testosterone by the hydroxylating action of 17\( \beta \)- (testosterone) dehydrogenase\textsuperscript{3}. The carbon atom at position 19 is hydroxylated and finally androstenedione is converted to its nor-compound and aromatized to form estrogens prominently in the ovary. The other pathway of formation of androstenedione is also shown in Fig. 4.
Biochemical Basis for Endocrine Disorders

(a) Steroidogenesis in adrenal cortex: When the sequence of reactions in the biosynthesis of biologically potent steroids gets disturbed, formation of abnormal steroids may take place. For example, if there is a partial or complete block of 21-hydroxylation or 11-hydroxylation in the adrenal cortex no cortisol will be produced. On the contrary, in the absence of 21-hydroxylation more pregnanetriol may be found. If both these enzymes are blocked, pregnanetriol-11-one will be formed which is characteristic of abnormal function of adrenal cortex. In this situation 17α-hydroxyprogesterone in the adrenal cortex may also produce more androstenedione and possibly testosterone. In the absence of cortisol the carbohydrate metabolism will be impaired and there will not be a "feed back mechanism" to control the secretion of ACTH. More and more ACTH will be produced and the abnormal steroid production in adrenal cortex will be stimulated. There will be enhanced production of androgens by the adrenal cortex in the absence of 21-hydroxylase action. This increased production of androgens in a woman will lead to masculinization and hirsutism in addition to other pathological complications due to absence of cortisol.

The abnormal production of steroids may also occur due to a change in the sequence of various hydroxylating enzymes. If hydroxylation of position 11 of
progesterone occurs prior to hydroxylation at position 21, 11β-hydroxyprogesterone serves as a poor precursor for the cortical steroids. These normal and abnormal pathways of steroidogenesis have been discussed in detail in relevant chapters.

(b) Steroidogenesis in gonads: In testis and ovary progesterone is first hydroxylated irreversibly at position 17 and a cleavage between carbon 17 and 20 takes place by the action of 17-desmolase to form 4-androstenedione and testosterone (see Fig. 3). In ovary 4-androstenedione is hydroxylated at position 19 to form 19-hydroxy-4-androstenedione. The cleavage of carbon 19 and aromatization of ring A take place to form estrone and estradiol. In this sequence of reactions if there is partial or complete stimulation or inhibition of enzymes, abnormal quantities of androgens and estrogens will be produced which will cause several pathological conditions in the human being. There is sufficient evidence to suggest the existence of more than one pathway for the synthesis of biologically potent steroids. A disturbance in the balance of these pathways may also lead to abnormal production of such steroids.

Interrelationship between Gonads, Adrenal Cortex and Anterior Pituitary: The anterior pituitary regulates steroid production by adrenal cortex or gonads on the "feed back" principle and any irregularity in this mechanism results in
hypo- or hyper- secretion of adrenocorticotropic and gonadotropic hormones. Abnormal secretion of tropic hormones due to failure of the feed back mechanism or due to adenoma of basophils of the pituitary, causes high or low rates of secretion of adrenocortical and gonadal steroids resulting in various endocrine diseases such as Cushing's syndrome, Addison's disease, Salt loosing syndrome, sterility, hirsutism in women etc. Similarly hyper-responsiveness of the target gland to the normal amount of tropic hormones or malignant growth of target gland also produces such diseases.

**Excretion of Steroids:** The steroids are excreted, both in the urine and in the bile as glucuronides or sulphates. Glucuronic acid transfer to the steroids is mediated through uridine diphosphoglucuronic acid and specific enzymes with the formation of uridine diphosphate. The sulphate is transferred from adenosine-3'-phosphate-5'-phosphosulphate. Most of the steroids are excreted as the glucuronides, with the exception of dehydroepiandrosterone which is excreted mainly as the sulphate. The glucuronides are readily filtered by the kidney but sulphates are cleared much more slowly.

In the present investigation pathological conditions due to defective biosynthesis of steroids by adrenal cortex and gonads were assessed by the measurements of 17-KS and 17-KGS, pregnanetriol and pregnanetriol-11-one
in the urine. High excretory values of 17-KS and 17-KGS would indicate higher secretion of androgens and cortisol respectively. Similarly high excretion of pregnanetriol and presence of pregnanetriol-11-one would suggest a partial or complete block of 11 and 21 hydroxylation which would result in low or insignificant production of cortisol by the adrenal cortex. The abnormal function of liver and kidney was excluded by the measurement of the 17-OHCS in peripheral blood plasma. These measurements of 17-KS, 17-KGS, pregnanetriol and detection of pregnanetriol-11-one in urine and 17-OHCS in plasma were carried out after a standard test dose of intravenous injection of ACTH to assess the adrenocortical function.

Investigations of Endocrine Disorders: Since 1955, an extensive research programme to evaluate the various endocrine disorders has been in progress at the Department of Endocrinology, Indian Cancer Research Centre, Bombay. In order to evaluate and appreciate the significance of the tests for the diagnosis and prognosis of endocrine diseases, it is important to determine the secretory and excretory rates of steroid hormones in normal human beings. This was necessitated by the fact that the endocrine methodology has been progressing rapidly, making more precise methods of assay available from time to time. Therefore, in many instances the average range for the normal values differ from laboratory to laboratory. As
regards establishing the normal range for Indian population, our studies appear to be the first of its kind. Besides establishing a normal range in Indian population these studies have helped to formulate racial comparison of secretory and excretory steroid levels. Thus, these studies reveal that although the excretion of 17-KS and 17-KGS are lower in Indian subjects than in the white population of the United States, the levels of plasma 17-OHCS were similar in both races living under different environmental conditions. This finding would indicate that a difference in steroid clearance exists between Indian population and the U.S. Whites. Finally, it also appeared that normal Indian women had higher concentration of plasma 17-OHCS in the post-ovulatory phase than in the pre-ovulatory phase of the menstrual cycle.

Secretion and excretion of steroids in hirsutism: A major problem to be discussed in the present investigation is the biochemical basis for the etiological causes of the idiopathic hirsutism. A group of 22 women having a male type of hair growth were carefully selected for this study. This group did not show apparent lesion such as hyperplasia or tumour of adrenals, gonads or anterior pituitary gland. The excretion of 17-KS and 17-KGS during the different phases of menstrual cycle was slightly higher than the excretion of these steroids in
normal women. In some of the patients investigated, an abnormal ACTH response indicated presence of a mild form of adrenocortical hyperplasia. No correlation was found between the excretory levels of 17-KS and 17-KGS and the number of abnormal regions of hair growth on the body. Furthermore, in some cases of idiopathic hirsutism higher excretion of pregnanetriol and the presence of pregnanetriol-11-one in the urine was noted indicating that the enzymes 11-hydroxylase and 21-hydroxylase may be inhibited or blocked. Another possible reason could be that some abnormality in the biosynthetic pathways leading to estrogens may be associated with ovary. The isolation and identification of steroids have been carried out in the cyst fluid obtained directly from the ovaries of normal and hirsute women to obtain more direct evidence. On laparotomy most of the patients showed sclerotic ovaries with multiple cysts. The steroids in the cyst fluid had high concentration of androstenedione which may give rise to increased formation of testosterone. This highly potent androgen and the high concentration of androstenedione may be responsible for the male type of the hair growth.

Attempts were also made to study the biosynthesis of estrogens by the ovaries of the patients suffering from idiopathic hirsutism. The ovarian tissues were obtained from patients suffering from Stein–Leventhal syndrome and
homogenised. The homogenate was then incubated with carbon 14 labelled progesterone, 17β-hydroxyprogesterone, androstenedione and testosterone. Our observation suggests that these tissues are capable of producing small amounts of estrone and estradiol indicating only a partial block of 19-hydroxylation of androstenedione rather than a complete block previously suggested by Short and London based on cyst fluid analysis of polycystic ovaries.

Secretion and excretion of steroids in Cushing's syndrome and Klinefelter syndrome: Cushing's syndrome has been shown to be a clinical entity with different etiological dysfunctions of the adrenal cortex and the anterior pituitary gland. The hypersecretion of cortisol which causes this disease may be due to basophilic adenoma of pituitary gland causing high secretion of ACTH or hyper-responsiveness of the adrenal cortex to normal amount of ACTH or ACTH-independent tumour of the adrenal cortex. A detailed investigation has been carried out by studying secretory and excretory levels of corticosteroids, adrenocortical response to a standard test dose of exogenous ACTH in normal and in patients suffering from Cushing's syndrome to locate the site of the lesion for differential diagnosis. In addition four cases of Klinefelter's syndrome with normal adrenal function were also investigated on similar lines to hirsutism and Cushing's syndrome. Another interesting case of true hermaphroditism having testis and ovary which
was followed for a period of about seven years, showed normal adrenocortical function. This patient had XX sex chromosomes and after surgical removal of female gonad and uterus, the production of androgenic steroids was not stimulated by the intramuscular administration of HCG. This patient thus proved to be basically a female with an abnormal development in intrauterine life.

In conclusion, the estimations of steroids in urine and plasma before and after administration of ACTH in Indian population have provided the basis for comparison and evaluation of endocrine diseases. An investigation has been carried out on similar lines in patients suffering from various endocrine disorders. Attempts have been made to locate the defects in steroidogenesis in adrenal cortex and ovarian tissues of patients suffering from Cushing's syndrome and idiopathic hirsutism respectively.