The ability of androgens to influence adrenocortical activity is currently accepted as not beyond the realm of facts. The precise nature and the pathways through which this influence is exerted, however, still awaits proper elucidation, though this problem has been the subject of intensive research for the past so many years. The pertinent literature, unfortunately, teems with reports of an equivocal nature largely because of the lack of recognition of variable factors like the dosage and duration of androgen treatment in the final evaluation of experimental results. Further, while considering the probable mechanisms through which the androgens might influence the adrenal cortex, the almost dogmatic adherence to the "ACTH-adrenocortical" pathway has contributed in no small measure to the creation of this general melee. The net result of this, has been a failure to arrive at some broad-based principles which might explain the diverse nature of responses so often reported to be evoked by the androgens in the adrenal cortex.

With regard to the effect of androgens on thyroidal activity the approach so far made has been equally one-sided. No consideration has been given to the possibility that the thyroid and the adrenal cortex might be bound together in an intimate relationship for mediating the effect of androgens on each other in a reciprocal manner. The primacy of the intertwined nature of endocrine functioning does not
sway this possibility beyond the limits of expectation. The present work has, therefore, been undertaken with full awareness of these drawbacks and possibilities. The data presented and the conclusions arrived at, have tended to make the picture more cohesive and the available controversial knowledge more meaningful.

REVIEW OF LITERATURE

The evidence concerning the influence of gonadal hormones on the adrenal cortex is primarily derived from the studies on sex dimorphism in adrenal size, the changes in the adrenal associated with reproductive cycle, the effects of gonadectomy and the administration of sex hormones. The literature on each of these aspects, therefore, will be reviewed a seriatum.

Sex dimorphism in adrenal size:

Sex dimorphism in the adrenals is found in a number of species. It usually takes the form of a size difference which may or may not be associated with obvious histological distinctions. The adrenals in the female are usually heavier than those of the male. In rare instances the cortex in one sex or other possesses a special zone which may be either evanescant or permanent. How far this species difference in size reflects actual difference in secretory activity of the adrenal cortex is, however, not definitely known.
The best known instance of sex dimorphism of the adrenals is to be found in the mouse. The gland is much larger in the female than in the male and this difference is ascribed to the much greater development of zona reticularis in the female (Masui and Tamura, 1926). Carlson et al. (1937) noted that the percentage of cortex was greater in the adrenals of the female than that in the male. Howard-Miller (1927) and Deanesly (1928) made a detailed study and reported that there was a much greater development of the inner cortical zone in the young female mouse from the age of 5 weeks onwards. The former investigator introduced the term "X-zone" for this region. This zone consists of small darkly staining cells, to distinguish it from the narrower and less sharply defined zona reticularis common to males and older females.

In the rats too, similar sex difference in the size of the adrenals has been noted. Jackson (1913) and Hatai (1913) reported that the adrenal gland in the female was larger than that of the male. The latter worker remarked that the difference became greater with the increasing body weight and he subsequently recorded (Hatai, 1914) that up to the body weight of 50 gms., the adrenals of the rat are of equal in size in the two sexes. With the increasing body weight the sex dimorphism appears and at 300 gms. body weight the adrenals are almost twice as large in the female rat as in the male. Donaldson (1924) also mentions that sex dimorphism in adrenal weight increases with the increase.
in body weight of rats. Donaldson (1919) studied the relative contribution of the cortex and medulla to this sex dimorphism. According to Howard (1938) the zona reticularis in the adult rats is somewhat more distinct in the female than in the male. In more recent studies (Greep and Jones, 1950; Jones, 1955), it has been confirmed that the adrenals in female rats are larger than those of the male. Greep and Jones (1950) have further observed that in the adrenal cortex of adult rats there is a sex difference in the staining reaction with sudan stain. There occurs a prominent sudanophobic transition zone in between the zona glomerulosa and the outer fasciculata in intact males. This zone is absent in intact females.

Similarly, sex dimorphism in the adrenal has also been observed in other mammalian and non-mammalian species. Thus, Deanesly and Rowland (1936) and Zalesky (1936) in guinea-pig, Baker (1937) in dog, Materna (1923) and Freeman (1934) in the humans, Sauer and Latimer (1931) and Kar (1947) in the fowl, have noted that either the whole adrenal or the amount of cortical tissue is larger in female than in the male.

Adrenal changes during reproductive cycle:

In the mouse the X-zone develops equally in the two sexes until the age of 5 weeks. In the male, degeneration of this zone takes place at puberty. In the female, this zone continues to grow until puberty when it occupies more than half the cortex. It gradually degenerates in the
unmated female. Masui and Tamura (1926) reported hypertrophy of the cortex of mouse during oestrus. The other investigators (Howard-Miller, 1927; Deanesly, 1928), however, failed to note any change. Tamura (1926) reported a decrease in the gross size of the adrenal during pregnancy, although there was some hypertrophy of the zona glomerulosa and zona fasciculata. Howard (1927) noted that the first pregnancy in a young adult mouse caused a disappearance of the X-zone by the 14th day of pregnancy. Similar observation was made by Jones (1952).

Anderson and Kennedy (1932) observed that in rats also there occurs enlargement of the adrenals during oestrus. The absolute as well as relative weight of the adrenals are increased and this is due purely to cortical enlargement affecting the zona fasciculata. Zonde and Zuckerman (1941) obtained somewhat similar results.

Adrenal hypertrophy has been noted during pregnancy in guinea-pigs by several workers (Guieysse, 1899; Kolmer, 1912; Kolde, 1913; Castaldi, 1922; Verdozzi, 1914; Hewitt and Liere, 1941). Similar observation has been made in the case of rabbit also (Randall and Graubard, 1940; Courrier, 1953). Foster (1934) noted enlargement of the adrenal gland of thirteen-lined ground-squirrel during the breeding season. The hypertrophy lasts throughout the oestrus and pregnancy. Zalesky (1934) also found adrenal enlargement in both male and female ground squirrels during the breeding season. The changes could be reproduced in these...
animals in the non-breeding season by the injection of gonadotrophic hormone.

Effect of gonadectomy on the adrenals:

Altenburger (1924) observed that castration increased the thickness of the adrenal cortex of male mouse. Similar observation was made by other investigators (Masui and Tamura, 1926; Howard-Miller, 1927; Deanesly, 1928), who noted that this was due to enlargement of the X-zone, which follows this operation. This effect could be reversed by grafting of testis tissue into the castrated male (Takewaki, 1938). Prepubertal castration causes the adrenal of the male to approximate to that of the female both in size and histological appearance. Post-pubertal castration also shows similar reaction, but the X-zone is less well developed (Howard, 1939; Jones, 1949b). Delost (1952) noted that castration of field mouse caused neoformation of the "inner zone" of fasciculata, which disappears in male adult animals in correlation with the testicular development. In some special strains of mice gonadectomy often leads to nodular hyperplasia of the adrenal gland (Ferguson and Wisscher, 1953).

Following an extensive study on rats, Hatai (1915) reported that the effects of gonadectomy on the adrenals are opposite in two sexes, there being an increase in males and a decrease in females. Hatai's work had, however, been criticized by Anderson and Kennedy (1933), who remarked that the results were not so definite as suggested.
latter investigators concluded that the degree of the effect was dependent upon the sex of the animal, the age at which the operation was performed and the interval after operation. They noted that nine weeks after castration of the immature male, the adrenals were hypertrophied and contained more lipoids than the controls. Castration of the adult male rats produced no change in weight and there occurred only transient histological changes. Ovariectomy of adult female caused a transient hypertrophy followed by atrophy. The atrophy was characterized by a decrease of lipoid in the zona fasciculata and degenerative changes in the reticularis. Winter and Emery (1936) also found hypertrophy of the adrenal in the male and hypotrophy in the female rats. They used sexually mature but not fully grown rats. In an earlier study, Minowada (1928) noted a slight increase in the size of adrenals in the castrated male rats. Korenchevsky (1930) and Korenchevsky and Dennison (1934) found hypertrophy of the adrenals in the castrated male rats. This adrenal hypertrophy was associated with an increase in width and cellular size of zona fasciculata and zona reticularis (Hall and Korenchevsky, 1938). Post-castration hypertrophy of the adrenal gland was also reported by Chiodi (1938) and Pinto (1941). Hall (1940) observed that these changes were absent in the adrenals of ovariectomized female rats.

Ellison and Burch (1936) ovariectomized adult female rats and noted adrenal atrophy after 25 days. Lauson et al. (1937), however, failed to find any change in the adrenal
weight in adult rats spayed for 20 days. Blumenfield (1939) ovariectomized rats shortly after weaning and sacrificed them when they were 3 months old. He found that the adrenals were smaller than those of the controls, due to a decrease in the cortex. Such changes were more marked in the rats killed at the age of 6 months. These changes were associated histologically with cell shrinkage and cell destruction. Waterman (1939) noted that ovariectomy for one week caused a reduction in the weight of the adrenal. He further observed that this decrease did not influence the survival time when the rats were subsequently adrenalectomized. Bourne and Zuckerman (1941), however, stated that cellular enlargement took place in the zona fasciculata and zona reticularis in spayed rats. In a more recent study Greep and Jones (1950) confirmed that castration causes adrenal hypertrophy and spaying causes a reduction in the size of the adrenals of rats.

These findings indicate in an unequivocal manner that response of the adrenals of rats to gonadectomy is hypertrophy in males and hypotrophy in females.

Post-bastration hypertrophy of the adrenals, either transient or permanent, had also been reported in other species of animals. This effect had been reported in guinea-pig (Marrasini, 1906; Rigano-Irera, 1929; Leinati, 1929; Maqsood, 1951), in rabbit (Koldi, 1913; Livingston, 1916; Maqsood, 1954), in pigs, sheep, cows and horses (Giroud and Santa, 1940). Moore (1922) reported a decrease in the
size of the adrenals in guinea-pigs following ovariectomy.

From a critical consideration of the material laid down in these sections it is clear that there is an intimate relationship between the adrenal and the sex glands; and any changes in the gonadal activity are invariably reflected on the adrenals. If it be assumed at this stage that the connection is entirely endocrinic, then it could be expected that the changes could be reproduced or abolished by the injection of appropriate gonadal hormones. It will be seen from the findings presented in the next few pages that this expectation is indeed a reality.

Effect of gonadal hormones on the adrenal:

As the present work is solely concerned with the effects of androgens on the adrenal cortex, greater emphasis will naturally be paid to this hormone rather than on the other gonadal hormones—the oestrogens and progesterone.

The effect of oestrogens. — The effects of oestrogens on the adrenal had been studied by various investigators and the works were reviewed by Tepperman et al. (1943) and Parkes (1945). In almost all cases, a stimulating influence had been observed. In very recent studies (Greep and Jones, 1950; Jones, 1955; Carter, 1956) this was confirmed. Jones (1955) attributed this to the stimulating influence of oestrogen on the LH and ACTH secretion of the pituitary.

The effect of progesterone. — Progesterone had been reported to cause atrophy of the adrenal cortex in rats.
The effect of androgens. — As the development of X-zone in mice is associated with the absence of the testis, it could be expected that the administration of testicular extract or androgens to the castrated male or the female would prevent the development of the zone or cause it to disappear. Martin (1930) failed to find any effect of injecting "testicular hormone" for a few days, but observed that prolonged injections caused disappearance of the zone in castrated male and females. Poll (1933), using an androgenic urine extract obtained similar results. Deanesly and Parkes (1937) noted the same, following injections of various androgenic substances. The effectiveness of testosterone and testosterone propionate in suppressing the X-zone was shown by different investigators (Cramer and Hörning, 1937; Starkey and Schmidt, 1938; Tolenaar, 1939; Howard, 1940), who obtained the effect in castrated male mice, young-mature females, immature males and females, adult females and/or adult ovariectomized females. Selye (1939) noted that testosterone decreased the weight of the adrenal in normal female mice. It is interesting to note, however, that Selye (1940a) in his experiments on castrated and spayed mice observed that daily administration of 3 mg. of
testosterone caused no change in the average adrenal weight in males though it caused a decrease in the females. Microscopic examination, on the other hand, showed that irrespective of the adrenal weight X-zone disappeared under the influence of androgen in both sexes. In a more recent study, Jones (1949a) observed that the persistent X-zone of the adrenal of prepubertally castrated male mice was rapidly destroyed by the injection of testosterone. This effect was also noted in hypophysectomized animals.

Sex dimorphism in the size of the adrenals in rats and other animals has been referred to before, the gland being smaller in the case of male than in female. The observations on the effect of castration of male animals suggest that the size difference in the adrenal gland may be largely due to the suppression of the male adrenal by the testicular activity. There are observations which bear out such a viewpoint.

Korenchevsky et al. (1937c) administered testosterone propionate to normal adult female rats and noted a decrease in the size and weight of the adrenal gland with a daily dose of 0.5 mg. However, with a larger dose (1.5 mg.) of the hormone this effect was not seen. Wolfe and Hamilton (1939) treated female rats chronically with different doses of testosterone propionate and the littermate brothers and sisters served as controls. It was noted that the relative weights of the adrenals of injected females were more comparable to those of their untreated
brothers rather than those of the untreated sisters. Mazer and Mazer (1939) noted a decrease in adrenal weight of immature and mature female rats following administration of testosterone propionate. The fact that testosterone decreases the adrenal weight in females, in which these glands are normally larger than in males, had been reported by other investigators as well (McEuen et al., 1937a, 1937b; Selye, 1940b; Hall, 1940). A decrease in the adrenal weight of spayed rats by the administration of testosterone or other androgens was reported (Korenchevsky et al., 1936, 1937a, 1937b). Schilling and Laqueur (1942) had shown that the decrease in the adrenal size in female rats following androgen treatment was greater at dioestrus than at oestrus and that body weight increase was greater at the former period. Wilkins et al. (1949), using vinyl and methyl testosterone and testosterone propionate obtained a significant decrease in adrenal weight in female rats. With the latter two hormones, there was depletion of the cholesterol content of the gland. Later on, Lewis et al. (1949) reported similar findings. Results obtained in their study with potent androgenic steroids, e.g., testosterone propionate and methyl testosterone, confirmed those in male rats reported earlier by Selye (1941). It was observed that small doses produced greater atrophy of adrenals than larger doses. With the highest dose of methyl testosterone there was practically no change in the adrenal weight. The effect of vinyl testosterone, which has weak androgenic properties
(Kochakian, 1944) differed from that of the potent androgens in that large doses had more effect than small doses. They further noted that the adrenal cholesterol depleting effect of methyl testosterone, contrary to its adrenal atrophic action, increased with the doses of the hormone. Vinyl testosterone on the other hand, caused no reduction in the lower dose levels, while in larger doses it caused slight rise in the cholesterol concentration of the gland. In a very recent study, Carter (1956), using testosterone propionate had confirmed that the androgen causes a decrease in the weight of the adrenal gland in female rats.

In keeping with the above result it had been shown that in castrated males, the hypertrophy of the adrenals which followed removal of testes could be reduced or abolished by the administration of androgens. Korenchevsky et al. (1936) noted that administration of testosterone to castrated rats caused the increased weight of the adrenals to return to normal level. This effect was more manifest in the higher dose levels of the hormone, they had used. Reduction in the weight of hypertrophied adrenals of the castrated rats by various androgens, administered either for shorter or prolonged periods had been reported by many workers (Hall and Korenchevsky, 1937, 1938; Korenchevsky et al., 1937a, 1937b; Korenchevsky and Dennison, 1937 and Korenchevsky et al., 1939). It was remarkable, however, to note that longer period of injections in the last experiment (Korenchevsky et al., 1939) did not, as one
would theoretically expect, cause greater involution of the adrenals. On the contrary, it produced less pronounced changes "as though some kind of neutralizing mechanism had developed during the prolonged administration of male sex hormone." It was further noticed that large doses of testosterone propionate caused abnormal depletion of lipoid from some parts of the adrenal cortex. Michael and Segal (1948) also noted that testosterone and testosterone propionate caused a decrease in the cholesterol esters and total cholesterol contents of the adrenals of castrated rats. In very recent studies (Sforzini, 1953; Carter, 1956), it had been confirmed that testosterone reduces the hypertrophied adrenal glands of castrated rats. Similar action of testosterone was reported in the case of fowl also (Breneman, 1942; Kar, 1947).

The adrenal atrophic effect of male sex hormone had also been demonstrated in normal animals including rats. Selye (1940b) in his study on the masculization effect of testosterone in extremely young rats noticed that testosterone decreased the adrenal weight not only in the female rats but also in the normal males and remarked that the effect should not be regarded as a mere transformation of the female type of adrenal into male type but as an actual atrophy. The histological changes underlying this atrophy were quite characteristic. The cells of zona glomerulosa were atrophic while those of the other zones presented a normal appearance.
Testoids had been claimed to be more effective than DOCA in inhibiting the adrenal enlargement normally produced by alarming stimuli (Selye, 1940c). The intensity of the anticorticotrophic effect of androgens was demonstrated in rats in which systemic stress was produced either by formaldehyde injections (Selye, 1940c) or by very high doses of thyroxine (Korenchevsky and Hall, 1941; and Selye et al., 1945). Testosterone had also been shown to inhibit the adrenal enlargement caused by the folliculoids in prepubertal and post-pubertal rats (Selye, 1940c; Albert, 1942).

Selye (1941) studying the effect of dosage (varying from 0.1 mg. to 25 mg.) on the morphogenic action of testosterone in rats observed that the inhibitory action of testosterone on the adrenal had definite optimum at 0.5 to 1.0 mg. daily dose level in as much as smaller doses did not cause atrophy and the larger amounts (10 mg. and 25 mg. per day) might actually stimulate the growth of the gland. Atrophy was manifested as more or less uniform decrease in the cells of all layers. In the larger dose levels where adrenal enlargement was observed an adverse effect on the body weight of the animals was seen. So he attributed this adrenal stimulation to the non-specific damaging action of the hormone. Later on, Selye et al. (1943) observed that chronic treatment with large doses of methyl testosterone elicited changes in the adrenal cortex which were characterised by an involution of the glomerulosa, marked thickening
of the connective tissue capsule and deposition of coarse fat granules in the cells of fasciculata and reticularis. In many cases these fat granules fused into a single voluminous lipid globule which distended the cytoplasm and pushed the nucleus to one side, giving the cells the typical signet-ring appearance of ordinary fat cells. The cells of the latter two zones did not show any atrophic change.

The absolute weight of the adrenals in the hormone treated animals (though presents a lower value) did not show any significant difference from that of the controls. In one subsequent study of Selye and coworkers (1950), however, the period of treatment with the same high dose of methyl testosterone was extended further and there occurred a significant decrease in the weight of the adrenals. Histological examination revealed atrophy in all the layers of the cortex along with pronounced fatty metaplasia of the cortical cells.

Lewis et al. (1949) studying with vinyl testosterone reported adrenal atrophy in male rats. It is noteworthy that the effect was less striking in males than in females, but the effect was not diminished by ovarietomy or orchidectomy. Creep and Jones (1950) reported that testosterone tended to lower the weight of the adrenal of rats in both sexes, but the effect was more marked in females. During the course of histochemical studies they observed a constant sudanophobin transition zone in between the glomerular and the fascicular zones in the adrenal cortex of male.
rats. The adrenals of intact females and castrated males had no sudanophobic zone. Treatment with testosterone caused the sudanophobic zone to appear in them. The hormone maintained this transition zone in intact males.

Anzer and Gonzalez (1950) administered testosterone propionate to young male rats in physiologically low and heavy doses for prolonged periods. They noted that lower dose of testosterone tended to decrease the water content, prior to an eventual decrease in protoplasmic solids of the adrenal glands. Later on, Sforzini (1953) and Vallecorsì and Checchia (1953) reported adrenal atrophy in normal male rats caused by testosterone and testosterone propionate respectively. The latter group of workers noted lipid discharge from the adenosuprarenals. Vascoucelos (1953), on the other hand, noted selective deposition of lipoids in an intermediate zone between the glomerular and fascicular zones following treatment of rats of both sexes with testosterone propionate. This was more apparent in males than females. Very recently Contopoulos et al. (1955) observed that testosterone decreased the weight of the adrenals in new-born rats.

Bergstrand (1948) studied the distribution of lipids in the adrenal cortex of very young rabbits after injection of testosterone propionate. It was noted that after hormone treatment the location of sudanophil and birefringent materials had shifted from reticular and glomerular zones. Riviere and Combescot (1953) reported that testosterone
had some degenerative effect on the zona fasciculata of the adrenal of the monkey. Krueger et al. (1954) noted that methyl testosterone caused some reduction in the weight of the adrenals of beef cattle.

Observations from clinical investigations also provide evidences that testosterone probably exerts an inhibitory effect on the adrenocortical function. Reinstein and coworkers (1945) reported that administration of methyl testosterone to humans caused a decrease in the excretion of 17-ketosteroid of adrenal origin. Venning and Brown (1947) studied excretion of urinary glycogenic corticoids and 17-ketosteroids in individuals before and after administration of either testosterone propionate or methyl testosterone. In all the cases with the exception of one male with Cushing's syndrome, testosterone therapy caused a reduction in the rate of excretion of glycogenic corticoids. In the cases with methyl testosterone therapy a decrease in the urinary 17-ketosteroid was also noted. It was suggested that this inhibition caused by testosterone therapy is mediated through pituitary by reduction in the production of adrenocorticotropic hormone. Later on Bartter et al. (1949) carried out metabolic studies on humans treated with methyl testosterone and concluded that the testoid acted by inhibiting production of ACTH by the pituitary, as it could not prevent the action of exogenous ACTH. Subsequent observations by Falloon et al. (1950, 1951) on the eosinopenic response to ACTH and adrenaline in women receiving testos-
terone treatment for breast cancer, also bear out the view point expressed by Bartter et al. (1949). Daughaday et al. (1950) reported that in hyper-adrenal corticism in a case of acromegaly with insulin resistant diabetes, methyl testosterone treatment brought down the adrenal steroid excretion to normal level with remission in the severity of diabetes.

From the above it would appear that the male sex hormone has got a depressing influence on the adrenocortical activity. As a matter of fact these observations have, to a great extent, contributed to the formation of the general belief that the androgen inhibits the ACTH secretion of the pituitary (see Burrows, 1949; Selye, 1950; Williams, 1950). But it will be seen in the next few paragraphs that there are observations which cannot be explained by such an apparently simple mechanism.

It is not clear why a larger dose of testosterone could not produce adrenal atrophy in female rats, though a reduction in the weight of the gland was noted with a lower dose (Korenchevsky et al., 1937c). Comparable observations, as have already been mentioned, were reported with testosterone in male rats by Selye (1941) and with testosterone propionate and methyl testosterone in female rats by Lewis et al. (1949). Selye (1940a) reported that in castrated mice daily administration of 3 mg. of testosterone caused no decrease in the weight of the adrenal though it caused disappearance of the X-zone.
Similarly, it is difficult to understand why in the castrated rats longer period of treatment with testosterone propionate did not, as one would theoretically expect, cause greater rather less pronounced, involution in the adrenal gland (Korenchevsky et al., 1939).

It is equally confusing to note that the testoids caused a depletion of lipids and cholesterol from the adrenal gland (Korenchevsky et al., 1939; Wilkins et al., 1949; Lewis et al., 1949; Vallecorsi and Chécchia, 1953). Because, following hypophysectomy of shorter duration there occurs an elevation in the concentration of these substances (Sayers, 1948). Moreover, it is interesting to note that, although the adrenal atrophic action of methyl testosterone (Lewis et al., 1949) decreases with the increase in dosage, the cholesterol depleting ability increases with the dose. In the highest dose level, they have employed, there occurred no significant change in the weight of the adrenal gland but there occurred maximum depletion of the cholesterol content. This effect cannot possibly be explained by any direct action of the male sex hormone. On the contrary, certain workers noted that the depletion of adrenal lipids was lessened by testosterone in animals hypophysectomized for prolonged period (Zizine et al., 1950; Montanari and Gualandi, 1952). Michael and Segal (1948) also noted a decrease in the cholesterol esters and total cholesterol contents of the adrenals following administration of testosterone propionate. They, however, explained this to be possibly due to augmenta-
tion of the formation of corticosteroids.

Besides, contrary to the cortico-inhibitory action of the androgen, it has been claimed by certain investigators that testosterone exerts a stimulating influence on the adrenal cortex of hypophysectomized rats. In 1938, Cutuly et al. noted that in hypophysectomized rats an increase in the weight of the adrenals resulted from injections of testosterone propionate. They thought that the unexpected high weight of these glands was probably due to variable quantities of the lipoidal material. Histological examination of these glands, however, showed them to be readily distinguishable from sections of normal adrenals, since they possessed typically shrunken cortices. Leathem and Brent (1943) also reported a decreased rate of atrophy of the cortex in hypophysectomized rats treated with androgen. In 1942, Leonard studying the effects of various androgenic substances on the adrenal cortex of hypophysectomized rats, obtained same kind of results as that of the previous authors and he attributed this to the unchanged state of the cell size. Zizine et al. (1950) noted that testosterone propionate caused a partial repair of the atrophied adrenal cortex of hypophysectomized female rats and maintained partially the adrenal weight and cortical lipid distribution in hypophysectomized male rats. In 1953, Rennels et al. reported that testosterone propionate had no significant effect on the relative adrenal weight of hypophysectomized rats, although there appears to be slight maintenance effect.
only the absolute adrenal weight is considered. Lewis et al. (1949), however, reported a further diminution in the adrenal weight of hypophysectomized female rats as a result of androgen treatment. Very recently, Brummel et al. (1954) during their studies on the influence of steroid hormones on the energy metabolism of the adrenal cortex in vitro, noted that testosterone and dehydro-iso-androsterone stimulated the oxygen uptake rate.

In addition to the above, it has also been reported that the androgens can act prophylactically against the well-known atrophic action of cortisone on the adrenal cortex. Winter and Holling (1951) noted that testosterone propionate and MAD counteracted markedly the adrenal inhibiting effect of cortisone. Subsequently, similar observations with various androgens have been reported by different groups of investigators (Gaunt et al., 1952; Zizine, 1952; Winter et al., 1953; Turiaf et al., 1953). Gaunt et al. (1953) observed that MAD, given alone to hypophysectomized rats partially prevented the adrenal atrophy. When given with cortisone, relative but not the absolute adrenal weights were enhanced. They further observed that the adrenal maintaining ability of combination of steroids was found to be more effective in intact than in hypophysectomized animals and hence remarked that any pituitary mediated action of the testoid could not be ruled out totally.

Added to these, there are observations which are directly contradictory in nature to the reports on the
adrenal atrophic action of testosterone. Thus, Nathanson and Brues (1941) studying the effect of testosterone propionate on the mitotic activity of the adrenals in intact immature female rats noted stimulation of the adrenal cortex. Mitoses were almost strictly confined to the capsule, zona glomerulosa and the outer fasciculata. Mitoses were occasionally seen elsewhere in fascicular and reticular zones. In case of longer duration, however, mitoses were found in the deeper layers of zona fasciculata and zona reticularis also. As already mentioned, Selye (1941) observed adrenal enlargement in male rats following administration of massive dose of testosterone propionate. Hecht-Lucari (1952) reported that testosterone propionate increased the weight of intact female rats which was not seen in spayed ones. He failed also to demonstrate any inhibiting effect of testosterone on the ACTH formation and storage. Castellani (1952b) studied the effect of increasing doses of testosterone propionate on the adrenal gland of male guinea-pigs. A slight hypertrophy of the glomerular and fascicular zone was observed in the group treated with larger doses of the hormone while the mean weight of the adrenals also showed a slight increase. This adrenocortical stimulation was attributed to the metabolic effects of testosterone, like retention of potassium and the general anabolic effect of this hormone. Similar stimulation of the adrenal gland by testosterone propionate was reported in rats by Rennels et al. (1953). This was associated with a
depletion of the ascorbic acid content of the gland.

To make the issue more complicated, a number of workers failed to obtain any significant change whatsoever in the adrenal weight after testosterone treatment. McEuen et al. (1937b) observed no change in the adrenal weight following administration of testosterone to young male rats. Korenchevsky et al. (1937d) administered testosterone and testosterone propionate to adult male rats and noted that neither of these hormones had any definite effect on the weight of the adrenal gland. Later on, Korenchevsky and Hall (1939) treated adult male rats with various androgens (androsterone, testosterone and testosterone propionate) in small and large doses for prolonged periods (90-118 days). In no case except in the animals treated with androsterone there occurred any decrease in the adrenal weight. Histological examination showed that lipoid content and vacuolation of the cortical cells were decreased. Testosterone propionate, in large doses, was the only hormone which caused a considerable lipoid depletion. Clausen and Frudenberger (1939) in immature female rats and Bottomley and Folley (1938b) in guinea-pigs noted no influence of crystalline androgens on the adrenal gland. In a very recent study, Carter (1956) also failed to note any change in the adrenal gland of normal male rats following administration of testosterone propionate.

It is sufficiently clear from the above that the reports on the response of the adrenal gland to androgens
are full of contradictions. Besides, the true nature and mechanism of action of the testoid do not seem to be as simple as it is currently held to be. In appraising these issues, the following considerations must be constantly kept in view.

Reference has already been made to the changes in the adrenal cortex during the different stages of the reproductive cycle. Moore et al. (1934) and Zalesky (1934) noted adrenal enlargement in the ground squirrel of both sexes in the breeding season. Interestingly, they could reproduce this change in the adrenal of squirrels during the non-breeding season by the administration of gonadotrophin. Apart from this there are evidences to suggest that gonadotrophic hormone may have a regulatory influence on the adrenal cortex. Many workers have considered that gonadotrophins have a direct effect upon the adrenal cortex (Miller and Riddle, 1939; Golla and Reiss, 1942; Greep and Jones, 1950b; Zuckerman, 1953). Martin (1930) reported that in mice the administration of pituitary gonadotrophin caused enlargement of the X-zone in female mice and its persistence in male mice. Jones (1948, 1949b, 1949c) has shown that the X-zone of mouse which partially involutes after hypophysectomy, is not restored by ACTH although the other zones become normal. Conversely, hypophyseal gonadotrophin (principally LH) maintains only the X-zone but chorionic gonadotrophin fails to do so (Jones, 1949b, 1949c). It has further been observed (Cattaneo and Hecht-Lucari,
1952) that injection of human chorionic gonadotrophin (HCG) caused much more increase in the weight of uterus in spayed mice than in spayed-adrenalectomized ones. The uterine weight of the former group did not differ much from that of a second control group of intact treated mice. From this it was concluded that treatment with HCG stimulated the adrenal cortex to secrete hormones with oestrogenic function.

In rats, chorionic gonadotrophin (which principally contains LH) has been noted to enhance the compensatory hypertrophy of the adrenals, which occurs following unilateral adrenalectomy (Hortobayi and Agoston, 1949). Fuchsinophilia of the adrenal cortex is usually considered to be associated with increased sex hormone production. Under the influence of HCG, which has been shown to stimulate the adrenal of normal rats, fuchsinophilic granules develop not only in reticularis thus transforming it into a sexual zone as it is found physiologically but also in fasciculata (Cano and Bottela, 1950a). This demonstrates that the last mentioned zone although generally concerned with the secretion of corticoids, can also be induced to exert a sexual action (Selye, 1951). Cano and Bottela (1950b) further observed that in ovariectomized rats chorionic gonadotrophin stimulated uterine proliferation by causing oestrogenic secretion as a result of stimulation of the adrenal cortex.

In 1951, Opsahl and Long observed that a human placental preparation is effective in stimulating the adrenal as
judged by the inhibition of the hyaluronidase spreading effect. Interestingly, it would appear from their data that this preparation is deficient in Sayer's test. Later on, Stack-Dunne (1953) has examined human chorionic gonadotrophin and the placental preparation made by the method of Opsahl and Long, and found that they are effective in adrenal weight test but have little activity in Sayer's test.

In addition to the above, there are indirect evidences to suggest that gonadotrophic hormone may cause stimulation of the adrenal cortex. Houssay et al. (1953) observed that in particular strain of rats, bilateral castration caused the subsequent appearance of adrenal hyperplasia followed by adenomas. After several months these adenomas began to secrete oestrogens as evidenced by uterine hypertrophy and the appearance of vaginal oestrous. The oestrous was suppressed by removal of adrenals. They remarked that these adrenal tumors were under pituitary control and were sensitive to gonadotrophins.

Observations from the clinical field also lend support to the above view. Albright and his colleagues (1942) considered that pituitary leuteinizing hormone stimulates the androgen production by the adrenal gland. In their view, the occurrence of hirsutism in certain women with cystic ovaries is due to stimulation of leutinizing hormone in association with the arrested ovarian follicular phase. Similarly, Botella (1951) mentioned that there might
be an increase in the androgenic activity (virilism, increase of urinary androgens and 17-ketosteroids) in menopausal women, corresponding to androgen production by the adrenal cortex. All this hormonal activity was due chiefly to the adrenal cortex, which acts as the third gonad under the influence gonadotrophin, the secretion of which is enhanced in this condition. It had also been demonstrated that injection of chorionic gonadotrophin to surgically castrated women causes an increased excretion of 17-ketosteroids in the urine (Botella et al., 1952; Plate, 1952). The latter author also observed an increased excretion of 17-ketosteroids following administration of large doses of chorionic gonadotrophin to an eunuchoid with marked atrophy of the genital organs. As in these cases the only source of 17-ketosteroid is the adrenal cortex, it is most probable that these changes are due to stimulation of the adrenal cortex by chorionic gonadotrophin.

From the above observations it would appear that the gonadotrophic hormone (LH) of the pituitary may have a regulatory influence on some aspects of adrenal cortical activity. As the testostoids are known to decrease the elaboration and secretion of this pituitary hormone (see Neiburgs, 1949; Greep and Zones, 1950a), an influence of testosterone on the adrenal cortex mediated through LH may be envisaged.

The influence of androgens on the thyroid. — It also seems pertinent to note that some investigators have reported in different species of animals that testosterone stimu-
lates the thyroid gland (Selye, 1939; Nathanson et al., 1940; Money, 1950; Money et al., 1950, 1951; Castellani, 1952; Burris et al., 1953; Krüeger et al., 1955). Thyroid hormone in its turn, is capable of stimulating the adrenal cortex (McQueen-Williams, 1934; Hartman and Brownell, 1949; Preston, 1928; Schmidt and Schmidt, 1938; Abelin, 1944; Prina, 1946; Deane and Greep, 1947; Balty and Leblond, 1954; Kar et al., 1955b). It is not known whether such action of testosterone play any part in determining the influence of the testoid on the adrenal gland. It is thus worthwhile to investigate into the role of thyroid gland in the influence of testosterone on the adrenal cortex.

It is to be noted, however, that reports in contradiction to the thyroid stimulating action of testosterone are equally abundant. Thus a number of workers reported, in various species of animals, to have observed no influence of testosterone on the thyroid gland (Korenchevsky et al., 1937d; Lacassagne and Raynaud, 1939; Leathem, 1947, 1951; Aron and Marescaux, 1952; Combescot and Riviere, 1953; Zingg and Perry, 1953). Another group of workers noted a depression of the thyroid gland following administration of testosterone (Selye, 1941; Contopoulos et al., 1955; Compsa, 1954).

From the above it would appear that the action of testosterone on the thyroid gland is still a controversial issue. It is, therefore, desirable to ascertain the precise nature and mechanism of influence of testosterone on the
thyroid gland before making any attempt to explore the role of thyroid, if any, in mediating the action of testosterone on the adrenal cortex.

A SYNOPSIS OF CURRENTLY KNOWN SALIENT FACTS REGARDING THE INFLUENCE OF ANDROGENS ON ADRENALS & THYROID.

(1) Sex dimorphism in the adrenals is found in a number of species. It usually takes the form of a size difference, which may or may not be associated with overt histological distinctions. The adrenals in the males are smaller than those of the females.

(2) Changes occur in the adrenals in correlation with the reproductive cycle. Adrenal enlargement, which occurs in the breeding season in the ground squirrel could be induced in the non-breeding season by the administration of gonadotrophin.

(3) Castration causes hypertrophy of the adrenals in the male and the disparity in size of the gland with those of females is eventually neutralized. In the female, spaying causes a reduction in the size and weight of the gland. Replacement therapy with sex hormones brings them back to the original states.

(4) Adrenal weight and function may vary with the fluctuating levels of the sex hormone in the body. It would appear that the presence of male sex hormone exerts an
inhibitory influence on the adrenal cortex.

(5) Reports regarding the changes in the adrenal following administration of male sex hormone to normal animals are, however, conflicting. Thus,

(a) Testosterone is reported to cause adrenocortical atrophy. It has also been shown to prevent the adrenal stimulating effect of stressor agents. Chronic overdosage with testosterone has been reported to cause adrenocortical atrophy along with fatty metaplasia of the cortical cells.

(b) Some investigators, on the contrary, reported that it prevents the adrenal atrophic effect of cortisone. Such maintenance effect of testosterone is manifested in a more pronounced way in the presence of pituitary.

(c) There are also some direct evidence to show that the adrenal cortex is stimulated by the male sex hormone. This adrenal stimulating action of androgen has been attributed to various factors by different workers.

(d) To complicate the issue still further, a group of workers noted no change in the weight of the gland following androgen treatment.

No adequate explanation is available for these discrepancies in the response of the adrenal gland towards androgen influence.

(6) There are evidences to show that gonadotrophic
hormone (LH) stimulates the adrenal cortex in various species of animals including human beings.

(7) Testosterone depresses the elaboration and secretion of LH from the pituitary.

(8) It is reported by some investigators that testosterone stimulates the thyroid gland. Thyroid hormone in its turn, is known to stimulate the adrenal cortex through augmentation of pituitary ACTH secretion. It is not known whether such action of testosterone plays any part in shaping the response of the adrenal cortex to androgens.

(9) The influence of androgens on the thyroid gland is still subjudice. A group of investigators observed stimulation of the gland while others failed to note any change. Another group noted a depression of the thyroid gland following testosterone treatment. Further investigation is thus indicated to elucidate the nature of influence of testosterone on the thyroid. The precise mechanism of action of the androgen on the thyroid is virtually unknown.

**OBJECT AND SCOPE OF THE PRESENT STUDY**

Part I deals with the elucidation of the precise nature and mechanism of influence of androgen on the thyroid gland of rats. Testosterone propionate has been shown to stimulate the thyroid, both in high and low doses, presum-
ably by augmenting the TTH secretion of the pituitary. This stimulating action is independent of the hypophyseal gonadotrophin secretion. Testosterone seems to have no direct stimulating action on the thyroid. This part of the work is presented in Chapter I.

Influence of testosterone propionate on the thyroid of goiterogen treated rats has also been studied (Chapter II). It is noted that testosterone exerts its effect on the thyroid of such rats mainly through an augmentation of pituitary TTH secretion and may thereby indirectly stimulate the peroxidase activity of the gland. It seems to have no direct stimulating influence on this enzyme activity in the thyroid. The ultimate response of the thyroid of thiourea treated rats to testosterone may be different depending upon various factors.

Part II deals with the elucidation of precise nature and mechanism of action of testosterone propionate on the adrenal gland of rats and mice and with the evaluation of the role of thyroid gland in such testoid action.

Testosterone propionate in a dose which stimulates the thyroid gland also provokes stimulation of the adrenal cortex (Chapter I). This adrenal stimulating action of androgen cannot be attributed to the potassium retaining property or general anabolic action of the hormone. It cannot also be attributed to the non-specific damaging action of the hormone. Such influence of testosterone seems to be independent of any thyroid mediation.
Administering testosterone propionate into rats in gradually increasing doses three ranges of dosage are worked out, at which the responses of the adrenal cortex to testosterone treatment are distinctly different (Chapter II). The lowest dosage range is associated with an atrophy of the adrenal cortex. This is followed by the "intermediate" range where no change in the adrenal is observed. With a still higher range there is stimulation of the adrenal cortex. A tentative theory has been proposed to account for these differential responses of the adrenal to androgen treatment. According to this theory the influence of androgen on the adrenal cortex is exerted through several pathways; the principle effects being mediated through LH and ACTH secretions of the pituitary.

Chronic administration of a high dose of testosterone propionate is seen to cause adrenocortical stimulation (Chapter III). This finding is contrary to that observed by Selye and coworkers (1950) who noted an atrophy of the adrenal cortex following chronic overdosage with methyl testosterone. It is argued that the crucial effect of testosterone at the higher dose levels is stimulation of the adrenal cortex by an increase of the pituitary ACTH secretion and the adrenocortical atrophy observed by Selye et al. (1950) may actually arise from exhaustion consequent to prolonged over-stimulation of the gland by the androgen. The occurrence of pronounced "fatty metaplasia" of the cortical cells, which is a characteristic feature under
such conditions is more concordant with this viewpoint.

A study of the influence of graded doses of testosterone propionate on the adrenal cortex of mice is taken up (Chapter IV) with a view to adduce some support to the theory proposed about 'the mechanism of action of androgen on the adrenal cortex.'

In the adrenal cortex of immature and prepubertally castrated mice there are two distinct zones upon which LH and ACTH exert separate trophic effects—LH maintains the X-zone whereas ACTH influences the fascicular zone. It is seen that at the lower dose levels testosterone decreases the weight of the adrenals of prepubertally castrated male mice by causing disappearance of the X-zone. At the higher dose levels also there is disappearance of the X-zone but the weight of the adrenal is not much diminished owing to marked hypertrophy of the fascicular zone. These observations clearly show how at different dose levels the androgen may exert differential influences on the adrenal cortex acting through different pathways.