DISCUSSION

In the present investigation, studies were carried out biochemically with $\Delta^5-3\beta$-hydroxysteroid dehydrogenase ($\Delta^5-3\beta$-HSD) which is related to the oxido-reduction between hydroxysteroids and ketosteroids and requires NAD as a cofactor (217). This enzyme together with $\Delta^4$-isomerase converts pregnenolone to progesterone and dehydroepiandrosterone to androstenedione in presence of NAD.

Studies were also done on testicular $17\beta$-hydroxysteroid dehydrogenase ($17\beta$-HSD) which is responsible for the conversion of androstenedione to testosterone in the presence of reduced NADP (NADPH) (218). This study was proposed mainly to have an idea about the rate of androgen synthesis in the testes under different experimental conditions. In the present studies serum level of gonadotrophins and testosterone were measured, which are intimately related with both steroidogenic (55, 65-67, 219) and gametogenic functions (128).

As $\Delta^5-3\beta$-HSD is one of the key enzymes responsible for the synthesis of adrenal corticoids from pregnenolone or $17\beta$-hydroxypregnenolone (155), the study of the adrenal $\Delta^5-3\beta$-HSD indicates the adrenocortical steroidogenic function. Measurement of serum levels of corticosterone clearly indicates the adrenocortical status in the synthesis of corticosteroids which is controlled by pituitary trophic hormone, ACTH (159, 160).
Previous studies regarding the role of prostate gland on pituitary prolactin secretion have demonstrated that prostatectomy is associated with increased release and content of pituitary prolactin in mature rats (36). On the other hand, in immature condition prostatectomy leads to inhibition of prolactin secretion (220). But, no approach has been done further in evaluating the role of the prostate gland on testicular and adrenocortical functions.

In experiment I, we are in attempt to show the effect of prostatectomy on testicular functions after 3, 7 and 14 days of the operation. It appears that there is no alteration of testicular activities, both steroidogenic and gametogenic, after 3 and 7 days of surgery when compared to sham-operated controls. But, following 14 days of prostatectomy both the testicular steroidogenic enzymes, $\Delta^5$ -3$\beta$ -HSD and 17$\beta$ -HSD showed increased activities. Radioimmunoassay of different hormones indicate that serum level of FSH, PRL and testosterone are elevated subsequent to 14 days of prostatectomy, while no significant change in the above parameters are observed after 3 and 7 days of operation. The results also show that serum level of LH remains more or less similar to that of control values.

The stimulation of testicular $\Delta^5$ -3$\beta$ -HSD and 17$\beta$ -HSD activities along with increased serum level of FSH, PRL and testosterone after 14 days of prostatectomy indicate that the ventral prostate can exert certain influence on testicular...
steroidogenesis. However, the observed effects raise a number of questions like, whether the ventral prostate has any direct inhibitory effect on testicular steroidogenesis or has an indirect effect through modulation of pituitary gonadotrophin secretion. The elevation of serum level of PRL after 14 days is in agreement with the findings of Asano (36). But, the mechanism of stimulation of PRL secretion after prostatectomy is still not known. This may be due to the removal of the prostate gland which is said to contain a prolactin-regulating factor (220). The stimulation of testicular $\Delta^5$-3$\beta$-HSD and 17$\beta$-HSD activities following 14 days of prostatectomy are the probable effect of accentuation of PRL secretion because PRL can increase the synthesis of testosterone by stimulating the activities of $\Delta^5$-3$\beta$-HSD and 17$\beta$-HSD in hereditary dwarf mice (106, 107). The insignificant alterations of serum level of PRL following 3 and 7 days of the operation are the cause of ineffective changes in testicular steroidogenic enzymes. The increased level of serum testosterone is the probable effect of stimulation of testicular $\Delta^5$-3$\beta$-HSD and 17$\beta$-HSD activities, as these enzymes play a key role in testicular androgenesis. The rise in serum androgen level subsequent to 14 days of prostatectomy may possibly be the effect of PRL, as Hafiez et al. (221) have observed, that PRL can significantly increase plasma androgen levels in hypophysectomized rats.

The elevation of serum level of FSH after 14 days of prostatectomy is perhaps due to the stimulation of PRL.
secretion, as elevation of PRL level causes a pronounced increase in FSH synthesis and release by the pituitary (222). This hypersecretion of FSH may also be responsible for stimulation of testicular $\Delta-3\beta$-HSD and $17\beta$-HSD activities because in hypophysectomized immature rats FSH can stimulate both the steroidogenic enzymes (55). Moreover, FSH can augment the testosterone secretion in normal rats (96, 223). So, the stimulation of testicular androgenesis after 14 days of prostatectomy may be partly due to hypersecretion of FSH.

Quantitative analysis of the spermatogenesis at stage VII of the seminiferous epithelium revealed that the spermatid count is below the control value after 14 days of prostatectomy, but no change is observed after 3 and 7 days of operation. Theoretically, the pachytene spermatocyte, spermatid ratio should be 1:4 (115) (1:3.55 in our control value). This ratio became 1:3.13 after 14 days of the operation, indicating that during the spermatocyte to spermatid conversion process 21.75% of cells degenerated. This effect is most probably indirect and is caused by the changes in pituitary gonadotrophin secretion. Although, the normal process of spermatogenesis depends mainly on the secretion of LH and to a lesser extent on FSH, it seems not unreasonable to speculate from the present experiment, that hypersecretion of PRL affects the gametogenesis, as PRL can exert an inhibitory influence on the gametogenic function of testis (224). Moreover, there
are evidences to show that the damage to the germinal epithelium may be due to elevated levels of FSH. High dose of FSH can produce injury to seminiferous epithelium in rat testis (225). Baker et al. (226) have shown that patients with elevated FSH levels showed a more severe reduction in germ cell numbers than those with normal levels of FSH. However, our observation of reduction of the number of spermatids in rat to some extent supports the findings of these workers. Nevertheless, it appears to be justified if the degeneration of the spermatids is ascribed to the high circulating levels of FSH.

The results of experiment II indicates that prostatectomy causes no alteration in testicular acid and alkaline phosphatase activities, adrenal $\Delta^{5}\Delta-3\beta$-HSD activity and serum level of corticosterone after 3 and 7 days of surgery. While after 14 days both testicular acid and alkaline phosphatase activities are increased. The adrenal weight, $\Delta^{5}\Delta-3\beta$-HSD activity and serum level of corticosterone are also increased after 14 days of prostatectomy. The stimulation of testicular acid and alkaline phosphatase activities are probably due to the injury to the seminiferous epithelium (227) caused by excessive secretion of PRL and FSH (224, 225). Further, certain mammalian tissues are known to respond to glucocorticoids by increase in the alkaline phosphatase activity (228). In our experiment the high circulating levels of corticosterone perhaps resulted in the augmentation of alkaline phosphatase activity. The mechanism of increase of
adrenal weight and stimulation of $\Delta^5$-3$\beta$-HSD activity subsequent to 14 days of prostatectomy is not very clear from the present experiment. It has been reported by some workers that hyperprolactinemia is associated with increase in adrenal weight and in the concentration of corticosterone in peripheral plasma (181). So, the rise in serum PRL level after 14 days of prostatectomy in our experiment (experiment I) is the probable cause of hyperactivity of the adrenal gland. Moreover, Kitay et al. in 1966 (188) and Kitay in 1968 (189) have shown that testosterone can enhance corticosterone production by adrenal gland. Thus, the increased testosterone formation (as observed in experiment I) may also be a probable cause of adrenal hyperactivity.

Since only a single cycle of seminiferous epithelium was completed within 14 days (229), it was our endeavour to examine whether the prostatectomy could alter the spermatogenic process as a whole. Moreover, as no change in the serum levels of gonadotropins and testosterone was observed 7 days after the operation, it is possible that a period of 14 days may not cover for complete manifestation of all the changes in spermatogenesis. In view of this, further changes could be expected to be revealed in the subsequent period. Consequently, in experiment III the animals were sacrificed after 21 days of prostatectomy. The tables of experiment III
clearly show that prostatectomy causes no change in testicular weight, while testicular steroidogenesis is augmented along with elevation of serum levels of FSH and PRL. The stimulation of $\Delta^5$-3\(\beta\)-HSD activity and 17\(\beta\)-HSD activities after prostatectomy are in agreement with the previous findings described in experiment I. The increased activities of these two enzymes along with elevation of serum level of testosterone are possibly the effect of hypersecretion of FSH and PRL (55, 106, 107), which further confirms the findings of experiment I.

The quantitative analysis of seminiferous epithelium at stage VII shows that the number of spermatids are decreased significantly after 21 days of prostatectomy. Theoretically, the pachytene spermatocyte/spermatid ratio should be 1:4 (115) (1:3.44 in our sham-operated controls). This ratio becomes 1:2.46 in 21 days following prostatectomy in rats. It indicates that 38.5% of germ cells degenerated during the conversion from spermatocyte to spermatid. The inhibition of spermatogenesis in prostatectomized animals is possibly due to the effect of excessive secretion of PRL as it can exert an inhibitory influence on spermatogenesis (224). The reduction in spermatid number may also be due to hypersecretion of pituitary FSH as findings of Baker et al. (226) indicate that elevated levels of FSH is associated with damage of seminiferous epithelium. Quantitative evaluation of spermatogenesis shows that
the percentage of spermatid degeneration after 21 days was more pronounced than those after 15 days (experiment I). Hence in all further experiments the duration of prostatectomized condition was fixed at 21 days.

In experiment IV we are in attempt to show the effect of prostatectomy on testicular acid and alkaline phosphatase activities along with adrenocortical activity following 21 days of surgery in mature rats. It appears that both acid and alkaline phosphatase activities in testis are increased significantly. The adrenal weight increases along with augmentation of $\Delta^5\text{-}3\beta$-HSD activity and elevation of serum level of corticosterone.

The increased activities of alkaline and acid phosphatase in testis can be attributed to the destruction of cell membranes and lysosomes (227) owing to the damage of the seminiferous tubules caused by increased FSH and PRL secretion. Although, the effect of testosterone on seminiferous tubules are controversial, Hotchkiss (230) and Heller et al. (231) have shown that testosterone can cause the damage of seminiferous epithelium in association with pronounced changes in the basement membrane of the seminiferous tubules which further confirms our findings related to the stimulation of the above hydrolytic enzymes in the testis. Acid phosphatase is known to be a testosterone dependent enzyme (39). So, the increase in serum
level of testosterone may also have been responsible for increase in acid phosphatase activity. The increase in alkaline phosphatase activity may also be ascribed to elevated serum level of corticosterone which is known to stimulate the activity of the enzyme (228).

The aetiology of the increase in adrenocortical functions cannot be ascertained from this experiment. Some workers have reported that chronically elevated circulating levels of PRL are associated with increases in adrenal weight and in the concentration of corticosterone in peripheral plasma (181). So, the increase in serum levels of PRL, 21 days after prostatectomy in our experiment (experiment III), is the probable cause of hyperactivity of adrenal gland. Elevation of serum level of testosterone may also be the probable cause for increased corticosterone secretion by the adrenal cortex as testosterone is known to enhance corticosterone production by the adrenal gland (188, 189).

For further understanding the influence of ventral prostate on testicular and adrenocortical functions, studies have been carried out with supplementation of aqueous extract of ventral prostate in prostatectomized rats. The results in experiment V show that supplementation of aqueous extract of ventral prostate to prostatectomized animals can prevent both the gametogenic and steroidogenic alterations of
testis caused by prostatectomy. The observed fall in testicular \( \triangle -3\beta \) -HSD and \( 17\beta \) -HSD activities along with fall in serum testosterone levels, after supplementation of aqueous extract to prostatectomized animals, can be attributed to the changes in serum levels of FSH and PRL. The cause for inhibition of FSH and PRL secretion after administration of aqueous prostatic extract is not clear. Perhaps the reason behind this effect is the presence of a water-soluble prolactin-regulating factor as well as FSH-suppressing peptide in the rat prostate (220).

Quantitative evaluation of spermatogenesis revealed that prostatectomy causes reduction in the number of spermatids as observed in experiment III. This spermatogenic inhibition is also prevented by supplementation of aqueous extract. The mechanism by which this spermatid number is restored is yet to be elucidated. This may be due to the restoration of serum level of PRL, FSH and testosterone, as the exposure of the seminiferous epithelium to the optimal hormonal milieu is critical in maintaining the normal gametogenic function. Thus, the seminiferous tubular damage caused by hypersecretion of PRL and FSH in prostatectomized animals is reversed by supplementation of aqueous prostatic extract owing to the restoration of the normal hormonal status.

For correlating the effect on spermatogenesis with testicular hydrolytic enzymes following supplementation of
aqueous prostatic extract to prostatectomized rats, testicular acid and alkaline phosphatase activities have been determined in experiment VI. It appears that supplementation of aqueous prostatic extract causes significant recovery of testicular acid phosphatase activity without any change in alkaline phosphatase activity. This recovery in acid phosphatase activity is possibly due to the restoration of germ cell within the seminiferous tubules and decrease of testosterone. The mechanism for unalteration of alkaline phosphatase activity following supplementation of aqueous extract of prostate in prostatectomized rats is not clear from this experiment. The results also indicate that supplementation of aqueous prostatic extract to prostatectomized animals caused reduction in the weight of adrenal gland, adrenal $\Delta^5$-3$\beta$-HSD activity and serum level of corticosterone in comparison to prostatectomized animals. The protection of adrenal $\Delta^5$-3$\beta$-HSD activity and serum level of corticosterone after treatment with prostatic extract is probably the effect of reduction of pituitary PRL secretion (described in experiment V). This is due to the fact that PRL has got a stimulatory effect on adrenal steroidogenesis (181). This experiment clearly demonstrates that the prostatic factor involved in regulation of testicular and adrenocortical functions are water-soluble.

To explore whether the solvent extract of prostate is able to rectify the testicular and adrenocortical
alterations in prostatectomized animals, studies have been carried out in experiment VII. The results of this experiment show that there is no significant variation in testicular $\Delta^5-3\beta$-HSD and 17$\beta$-HSD activities, serum levels of FSH, PRL and testosterone following supplementation of solvent extract of prostate to prostatectomized animals. It clearly indicates that prostatic factors involved in the regulation of testicular functions are not lipid in nature.

In experiment VIII, we have observed that there was no change in testicular acid and alkaline phosphatase activities, adrenal $\Delta^5-3\beta$-HSD activity and serum level of corticosterone after supplementation of solvent extract of ventral prostate to prostatectomized animals when compared to the parameters of prostatectomized rats. This experiment also supports the findings of experiment V and VI that the prostatic principles responsible for the testicular and adrenocortical alterations have the characteristics of non-lipid substances.

In spite of variations in pituitary secretion of PRL and FSH resulting in alterations in the testicular and adrenocortical functions, a possibility remained of a direct effect of prostatic principle on these organs. To explore this possibility, ammonium sulphate fractionated prostatic peptides were used in in vitro study of testicular steroidogenesis in experiment IX. Three fractions (0-30% saturation: fraction-I, 30-60% saturation: fraction-II, 60-90% saturation:...
fraction-III) were used, of which fractions-I & II had no effect on testicular $\Delta^5$-3$\beta$-HSD and 17$\beta$-HSD activities. It also appeared that fraction-III at a concentration of 2 $\mu$g/ml of incubation medium was without effect on testicular steroidogenic enzymes in normal rats. When the testes of normal rats are incubated with fraction-III at a concentration of 4 & 8 $\mu$g/ml, both the $\Delta^5$-3$\beta$-HSD and 17$\beta$-HSD activities were decreased in comparison to control rat testis with or without BSA in the incubation medium. This experiment clearly indicates that fraction-III of prostatic peptides act directly on testicular steroidogenesis by inhibiting the activity of steroidogenic enzymes. So, the stimulation of testicular steroidogenesis following prostatectomy, as observed in experiment III, is possibly due to the effect of removal of prostatic inhibitory principles. In another part of the experiment, the activity of the adrenal $\Delta^5$-3$\beta$-HSD was studied in vitro. The results of this experiment show that the enzyme activity remains unaltered after incubation of adrenal gland in presence of fractions-I,II & III at the same concentration used in in vitro study of testis. It indicates that prostatic principle has no direct effect on adrenal steroidogenesis.

To confirm the effects of fraction-III of ventral prostatic peptide on testicular activities, studies have been carried out in mature prostatectomized rats after administration of fraction-III. The findings of experiment X
demonstrate that administration of fraction -III to prostatectomized animals result in decreased activities of both testicular $5\Delta^3\beta$-HSD and $17\beta$-HSD in association with decreased serum levels of FSH, PRL and testosterone when compared to those of prostatectomised rats. This reduction in PRL and FSH secretion may be due to the existence of PRL and FSH controlling factors in fraction-III of the prostatic peptide. The suppression both $5\Delta^3\beta$-HSD and $17\beta$-HSD activities after administration of fraction-III to prostatectomised animals may be due to either the effect of reduced PRL and FSH secretion or due to the direct action of this fraction at the testicular site or due to both direct or indirect effect. The reduction in serum level of testosterone may be the effect of inhibition of those steroidogenic enzymes.

The quantitative analysis of spermatogenesis at stage VII of seminiferous cycle indicates the number of spermatid is more or less similar to that of control value after administration of fraction-III to prostatectomized animals. This protection of spermatogenesis can be ascribed to the rectification of the serum levels of FSH, PRL and testosterone as well as restoration of testicular androgenesis.

To study the nature of action of fraction-III of ventral prostatic principle on adrenocortical activity in prostatectomized animals experiment XI was performed. The
results of this experiment show that administration of fraction-III to prostatectomized animals causes decrease in adrenal $\Delta^5-3\beta$-HSD activity with reduction in serum level of corticosterone when compared to prostatectomized animals. It indicates that the fraction-III can rectify the adrenal hyperactivity caused by removal of the ventral prostate. It has already been established in experiment IX that the fraction-III has no direct influence on adrenal $\Delta^5-3\beta$-HSD activity. So, the inhibition of the enzyme activity and reduction of serum corticosterone level after administration of fraction-III of prostatic peptide is possibly due to the indirect effect through inhibition of pituitary PRL secretion (as described in experiment X).

To evaluate the influence of prostate gland in sexual maturation, studies have been carried out on immature rats in experiment XII. Immature animals were prostatectomized on 35 days of age. They were sacrificed on 50, 55 & 60 days of age. It appears that prostatectomy in immature condition results in inhibition of both testicular $\Delta^5-3\beta$-HSD and $17\beta$-HSD activities at 50, 55 & 60 days of age. Serum levels of FSH, LH, PRL and testosterone also showed significant reduction at all age groups. The mechanism of inhibition of FSH and LH after prostatectomy in immature state is not known but the inhibition of PRL at all age groups is consistent with the findings of Hurkadli et al. (220). The testicular inhibition of $\Delta^5-3\beta$-HSD and $17\beta$-HSD activities and decreased level of testosterone are
due to the fall in the serum levels of pituitary gonadotrophins. Although, the prostate has opposite effects on testicular functions in mature and immature rats, the mechanism of probable stimulating influence in immature condition is yet to be established. The sensitivity of the pituitary gland to prostatic principles may be the important criterion in the reversal of effects of the ventral prostate on testicular functions. Several suggestions have been made by Odell and Swerdloff (139) regarding the physiological mechanisms responsible for the sexual maturation. They have observed that following hypophysectomy in immature rats, if LH is supplemented alone after 5 days, the animals did not respond to the treatment. But, when the supplementation is preceded by either FSH or GH and PRL treatment, there was marked enhancement of acute testosterone response to LH. These workers have therefore assumed that the effectiveness of the hormone or hormone combination lies in their ability to induce LH receptor formation that is essential for the action of LH. At the same time the alteration of the pattern of steroid metabolism in the maturing testis was also noted. Thus, it appears that these two factors may be the crucial steps in the sexual maturation. In our experiments LH, FSH and PRL levels in the serum have decreased. Therefore, in the light of these observations, it does not seem to be unjustified if the reduction in testicular function is attributed to the fall in the serum levels of LH, FSH, and PRL.
The quantitative analysis of germ cells at stage VII of seminiferous cycle reveals that all the germ cells are reduced after removal of the ventral prostate in immature condition. Spermatozoa appears in the seminiferous tubules of control testis at the age of 55 days. But, in prostatectomized rats very few spermatozoa appear within the seminiferous tubule at 60 days of age. It also clearly indicates that prostatectomy in immature condition leads to inhibition of spermatogenesis. This spermatogenic arrest is probably due to the reduction in gonadotrophin and testosterone secretion (128).

In experiment XIII, we have shown that prostatectomy at 35 days of age causes increase in adrenal weight, stimulation of adrenal $\Delta^-3\beta$-HSD activity and rise in serum level of corticosterone at 50, 55 and 60 days of age. The results also indicate that testicular alkaline phosphatase activity increases at 50, 55 and 60 days of age following prostatectomy at 35 days of age. But, no change is observed in testicular acid phosphatase activity. The increased activity of alkaline phosphatase in the testis subsequent to prostatectomy may be the effect of hypersecretion of corticosterone, as it is well established that glucocorticoids are responsible for stimulating alkaline phosphatase activity (228). The mechanism of stimulation of adrenal $\Delta^-3\beta$-HSD activity after removal of the ventral prostate in immature rats is not clear from this experiment. As adrenal $\Delta^-3\beta$-HSD activity is under the influence of pituitary ACTH secretion, there is a probability of
hypersecretion of ACTH following removal of the prostate gland in immature animals. In the previous experiment, we have shown that serum level of gonadotrophins are reduced following prostatectomy in immature rats. As the pituitary ACTH secretion seems to be at the expense of gonadotrophins (232), the stimulation of adrenal 5\(\Delta-3\beta\)-HSD activity and elevation of serum corticosterone level may be due to increased secretion of ACTH. This is supported by the fact that there is an increase in adrenal weight.

The correlation between adrenal function and PRL in immature condition cannot be drawn from this experiment. The question remains as to why the adrenocortical functions increased in response to the reduction in pituitary PRL secretion. However, the responsiveness of the adrenal gland to PRL in maturing rats are yet to be elucidated.