DISCUSSION
During the present studies, a gradual decrease was recorded in the weight of the testis, which was more pronounced following clomiphene treatment than after treatment with Net-en. The weight of the testis was reduced to 36.2% following clomiphene treatment whereas Net-en treatment brought the weight down to 56.2% of the control. Similar decrease in weight has also been reported by Srivastava and Malaviya (1980), Anand Kumar et al. (1980) and Moudgal et al. (1985) after treatment with Net-en, and also by Kalra and Prasad (1967), and Flickinger (1977c) following treatment with clomiphene.

Decrease in testicular weights after treatment with various antispermatogenic agents has also been reported by Kar et al. (1967), Setty and Kar (1969), Lacy and Collins (1973), Walsh and Swerdloff (1973), Neumann et al. (1976), Flickinger (1977a, 1978), Hunt et al. (1978), Jayaraman and Sheth (1980), Sharma and Kanwar (1980) and Kanwar et al. (1985). Alvarez-Sanchez et al. (1977), Frick et al. (1977 a,b) and Melo and Coutinho (1977), however, reported no change in size and weight of testis following steroid administration.

The loss of weight of the testis could be attributed to the overall shrinkage of the testis,
particularly of the seminiferous tubules which was indicative of the desquamation of the germinal epithelium. The decrease in testicular weight also indicated decreased FSH secretion which could be achieved by steroidal/non-steroidal treatment (Nelson, 1965).

The basement membrane (i.e. tunica propria) plays a vital role in transporting nutrients across the tissue (Steinberger and Steinberger, 1972). The thickening of tunica propria as witnessed following the present treatments was also observed and related to a change in its permeability by Kanwar et al. (1974) and Kalla and Chohan (1980).

Following drug treatment the spermatogenic process initially seemed to be stalled at the spermatid stage. This was indicated by the depletion/desquamation of the elongated spermatids followed by loss of the younger round spermatids. Further prolongation of the treatment also affected spermatocytes, the number of which was reduced considerably.

Quantitative histological changes have revealed significant alterations in the frequencies of occurrence and ratios of the various types of germinal cells after drug administration. For this purpose, cell types were
counted in the tubules at stage VII of the seminiferous epithelial cycle as described by Bansal and Davies (1986).

It was noted that the frequency of occurrence of stage VII-VIII tubules decreased with prolongation of treatments. Patanelli and Nelson (1964) also reported a decline in the frequency of stage VII-VIII after administration of the antispermatogenic compound, diethylcarbamylmethyl-2,4-dinitropyroll. Clermont and Morgentaler (1955) on the other hand, observed an increase in VII-VIII stages after hypophysectomy.

Azoospermia, following the administration of the progestins - Norlutin and Enovid in adult males, has been reported by Heller et al. (1959). Norethisterone enanthate reportedly caused complete depletion of spermatocytes, spermatids and spermatozoa, whether by intranasal administration (Anand Kumar et al., 1980) or when released from implants (Srivastava and Malaviya, 1980). Similar effects have also been recorded following treatment with the progestins, i.e. norgestrel and methyltestosterone, cyproterone acetate and a combination of cyproterone acetate and testosterone enanthate (Singh et al., 1972; Sharma and Kanwar, 1980 and Lohiya and Sharma, 1983).
Clomiphene treatment, too arrested spermatogenesis at the early spermatid stage (Nelson and Patanelli, 1962; Kalra and Prasad, 1967; Flickinger, 1977c). According to Flickinger (1977c), these changes resembled those following hypophysectomy. The histological damage suffered by the seminiferous epithelium following treatment with clomiphene was akin to that described as a consequence of insufficient supply of testosterone because of suppressed gonadotrophin levels (Nelson and Patanelli, 1962; Kalra and Prasad, 1967).

The first cells to disintegrate and disappear from the seminiferous tubules after treatment of both the drugs were the elongated spermatids, followed by the round spermatids, thereby revealing their increased susceptibility to the treatments. Decrease in stage 19 elongated spermatids indicated an increased permeability of stage VII tubules in addition to a greater vulnerability of germ cells themselves to the drugs.

Pycnosis, cytolysis and desquamation of spermatids could be observed during early stages of treatment. By the end of both treatments, elongated and round spermatids were completely absent while resting and pachytene
spermatocytes were reduced to 31.88% and 22.12% respectively following Net-en treatment and 49% and 42% respectively following clomiphene treatment. These observations suggest that due to treatment of the drugs, some spermatids did develop up to step 7 of spermiogenesis and then underwent degeneration. Flickinger (1977 a,c; 1978) made similar observations on the limited development of spermatids up to 7-8 spermateleotic stages after combined steroid administration, clomiphene treatment and after testosterone enanthate treatment in rats. Clermont and Morgentaler (1955) and Russell and Clermont (1977) have also observed degeneration of the elongating spermatids after hypophysectomy in rats.

In normal animals, spermatogonia are known to occur in a definite number (Clermont and Leblond, 1953; Steinberger and Steinberger, 1975). During spermatogenesis, type A spermatogonia proliferate to produce intermediate stages which undergo mitotic division in stage IV to produce type B spermatogonia, which undergo a second mitotic division to form resting spermatocytes in stage VII. Some spermatogonia stop dividing at an early stage and are kept as reserve stem A cells for the next wave of spermatogenesis to begin in stage VII. Clermont and Leblond (1953) have reported the ratio of type A
spermatogonia to resting spermatocytes of stage VII to be 1:24. According to them any deviation would reflect impairment of spermatogenesis.

Initiation of drug treatments did not alter much the number of spermatogonia per tubule, though the number of resting spermatocytes was reduced, thereby altering the spermatogonia resting spermatocyte ratio. This decreased ratio is suggestive of either degeneration of some spermatogonia or a fall in mitotic index. Clermont and Morgentaler (1955) reported similar results and they recorded a decline in the number of spermatogonia after hypophysectomy in rats and conjectured degeneration of certain spermatogonia which however has not been observed in the present studies.

During the present studies some degenerating pachytene and step 7 spermatids were observed in the seminiferous tubules of the experimental animals. Degeneration of meiotic spermatocytes after hypophysectomy or after steroid treatment has also been reported by Clermont and Morgentaler (1955), Russell and Clermont (1977) and Flickinger (1977a, 1978). Degenerating spermatocytes are characterised by their intense staining and pycnotic nuclei. The ratio of resting spermatocytes to pachytene spermatocytes was also reduced as compared to
the controls thus indicating a decrease in the number of spermatocytes. Of all the spermatocytes, pachytene spermatocytes seemed to be more susceptible to hormonal alterations due to treatment.

Varying amounts of cell debris were noticeable in the seminiferous tubules of the drug treated animals. The presence of this cell debris was indicative of excessive exfoliation of immature cells which were unable to survive in the altered milieu of the tubules.

Singh et al. (1972) reported that after norgestrel treatment, tubular lumina were filled with cell debris of desquamated germinal elements.

Cell debris in the lumen predominantly contained pachytene spermatocytes and step 7 and 19 spermatids. It is possible that step 7 spermatids were retained even after the normal time of sperm release and phagocytosed by the Sertoli cells (Waller et al., 1983).

Hypertypic and multinucleated giant cells were to be seen in the testis of treated animals. Bishop and Walton (1960) described such abnormal cells in normal rodent testis, but in the present study, such cells were not seen in the testis of control animals. Presence of multinucleated and uninucleated giant cells in the
lumen of seminiferous tubules was indicative of germ cell degeneration and probably resulted because of liquefaction of the cytoplasm. Giant cell formation has also been described by Amroso (1963), Rao and Srivastava (1967), Kanwar et al. (1971), Singh et al. (1972), Kaya and Harrison (1975), Sullivan et al. (1979), Kalla and Chohan (1980), Dixit et al. (1978 a,b), Dixit and Gupta (1982 a,b) and Veeraragavan and Ramakrishnan (1984) after treatment with different antispermatogenic agents. There were however, different opinions about the formation of such cells.

Multinucleated giant cells were reportedly formed as a result of coalescence of the spermatids that failed to metamorphose beyond cap stage i.e. step VII (Kanwar et al., 1971). These workers observed multinucleated giant cells seven days after testicular hyperthermia and conjectured that their formation was a result of coalescence of identical spermatids. Coalescence of spermatids was probably aided by the liquefaction or dissolution of cell membrane of neighbouring cells. Cytoplasmic liquefaction and dissolution of testicular cells had also been described by Patanelli and Nelson (1964) and Neumann et al. (1976) under different experimental conditions.
Anroso (1963) on the other hand, believed that they represented macrophages with phagocytosed spermatids. According to him, regression initiated formation of such cells. The presence of macrophages had been reported by Srivastava and Rao (1968) in Ca\(^{45}\) treated gerbil testis. Theories of cell fusion or these being macrophages could not explain presence of similar nuclei - the presence of such cells in normally regressing hedgehog testis suggest nuclear division proceeding up to a variable number of divisions, but cytokinesis to follow was inhibited thus resulting in multinucleated giant cells. Rao and Srivastava (1967) and Bidwal and Bawa (1977) have suggested that giant cells were formed by multiple nuclear divisions unaccompanied by cytoplasmic cleavage.

Hypertypic/uninucleated giant cells probably represented pachytene spermatocytes/failed to differentiate further and became hypertrophied by liquefaction thus transforming into uninucleated giant cells.

It has been postulated that formation of giant cells in the testis could be the result of direct effect of a physical or chemical agent on seminiferous epithelium. This hypothesis obtained support from other observations.
demonstrating direct effect of non-steroidal gossypol on germinal epithelium (Zhou and Lei, 1980).

Kanwar et al. (1981) observed giant cells in seasonally degenerated testis of hedgehog and had commented upon the physiological significance of giant cells formation. According to them: (1) emergence of giant cells suggested testicular malfunctioning - due to seasonal regression which could also be produced by experimental intervention. These abnormal cells could be markers for determining testicular malfunctioning in general. (2) Formation of giant cells was a device by which the testis got rid of cells which failed to differentiate further and had to be eliminated. Mononucleate hypertypic giant cells were individual desquamated and degenerating spermatocytes whose differentiation was irreversibly arrested. Multinucleated giant cells, offered a mechanism for bulk elimination of degenerating spermatids which failed to metamorphose into viable sperm.

Vacuolization was observed in the seminiferous epithelium after 15 days of treatment. This could be a result of the sloughing off of the primary spermatocytes,
leaving empty spaces behind or a result of disintegration of the cellular contents of these cells. Vacuolization has also been reported by Heller et al. (1959), Patanelli and Nelson (1964), Neumann et al. (1966), Kalla and Chohan (1980), Srivastava and Malaviya (1980), Hoffer (1983), Rovan et al. (1983) and Bansal and Davies (1986) after various chemical treatments.

The mechanism of action of Net-en and clomiphene has not yet been established. But it seems that both acted by suppression of gonadotrophins and thereby inhibited spermatogenesis. This view was supported by the fact that the testicular changes observed presently resembled those described after hypophysectomy (Clermont and Morgentaler, 1955; Russell and Clermont, 1977). Earlier workers have given different views on the mechanism of action of Net-en, while that of clomiphene was reported to be via pituitary.

Anand Kumar et al. (1980) reported an impairment of spermatogenesis and a significant reduction in levels of circulating serum testosterone following intranasal spray of Net-en in monkeys. Srivastava and Malaviya (1980) described that implants of Net-en caused a selective and local testicular damage since the contralateral testis and epididymis were not affected. So they thought that a
systemic effect mediated via hypothalamic-pituitary-gonadal axis did not seem to be probable. Moudgal et al. (1985) noted that Net-en, intranasal administration, reduced significantly the serum testosterone level, probably by blocking the secretion of both FSH and LH.

Schurmeyer et al. (1984) reported a long lasting suppression in the level of serum LH, FSH and testosterone after treatment with 19-nortestosterone, which could be responsible for the azoospermic effect. Similar observations were made by Knuth et al. (1985) after administration of 19-nortestosterone hexoxyphenyl-propionate.

Earlier studies on the influence of progestational compounds on testis had revealed different results. Delalutin (17α-hydroxyprogesterone caproate) and Prodox (17α-hydroxyprogesterone acetate) produced no effect, whereas 17-ethinyl-5(10)estræenedone (Enovid) and 17 ethyl-19-nortestosterone (Norlutin) produced reversible inhibition of spermatogenesis by suppression of pituitary gonadotrophins (Patanelli and Nelson, 1959). Progesterone, 17α-hydroxypregnenolone and Δ11-pregnen-3, 20-dione, have also been shown to induce atrophy of seminiferous epithelium in rat by Kar et al. (1967).
Steinberger (1971), on the contrary, did not report suppression of gonadotrophins and suggested that these agents either exerted their effect by antagonising the peripheral action of androgens or by direct suppression of spermatogenesis. Antigonadotrophic activity of norgestrel had been well documented by Edgren et al. (1968), Setty and Kar (1969) and Singh et al. (1972). Singh et al. (1971) had attributed the antispermatogenic effect of Provera to its inhibitory effect either on the pituitary or its antiandrogenic activity.

Clomiphene, a non-steroidal triphenyl derivative of chlorotrianisene had been found to inhibit testicular function (Nelson and Patanelli, 1962; Nelson, 1965), but in oligospermic men it elevated sperm count (Jungck et al., 1964; Mellinger and Thompson, 1966). Kalra and Prasad (1967) conjectured that the antispermatogenic activity of clomiphene might be due (a) to inhibition of synthesis and/or release of gonadotrophins from the pituitary (b) to a direct inhibitory effect on the testis and accessory reproductive organs and (c) to the inhibition of circulating gonadotrophins. These workers observed that at low levels, clomiphene facilitated and/or enhanced the release of ICSH from pituitary while at higher dose levels, pituitary
gonadotrophins were inhibited. So, the arrest of spermatogenesis, in clomiphene treated rats might be due to inhibition of synthesis and/or release of gonadotrophins from pituitary. They concluded that clomiphene had an estrogenic effect on some tissues and antiandrogenic effect on other target sites depending upon the threshold of the different target organs.

Singh and Prasad (1973) reported that cis- and trans-isomers of clomiphene reduced pituitary FSH and LH. They concluded that the arrest of spermatogenesis and involution of accessory glands in clomiphene treated rats could be due to androgen deprivation as a sequel to inhibition of pituitary gonadotrophins.

Flickinger (1977c), reported the antifertility effects of clomiphene to be a result of decreased androgen stimulation of the target tissues. They found the effects of clomiphene treatment to be comparable to castration, or treatment with estrogens/progestagens.

The loss of epididymal weight following treatment with both Net-en and clomiphene has been noted. These results are in accordance with those of Srivastava (1983), Rajalakshmi et al. (1970) following treatment with

Some workers have attributed this loss in epididymal weight to decreased gonadotrophin secretion which in turn had reduced androgen stimulation of the reproductive organs. Weight loss was also attributable to the decrease in the luminal contents of the epididymis which formed part of the weight of the organ. Fall in spermatozoal density in extreme cases, and the absence of spermatozoa in the epididymal lumen was indicative of spermatogenic arrest of the testis as has been testified histologically. Fall in the spermatozoa in the epididymis has also been reported by Rajalakshmi et al. (1970), Flickinger (1977 b,c, 1978), Sharma and Kanwar (1981), Srivastava (1983) and Kanwar and Hazuria (1985) after treatment with clomiphene,
testosterone enanthate, methyltestosterone + norgestrel and norethisterone enanthate. Also responsible for the depletion of spermatozoa from the epididymal lumen, could be the accelerated rate of transport of spermatozoa from the epididymis to the vas deferens or their resorption in the epididymis.

Supply of androgen to the epididymis is mainly from the seminal fluid entering via rete testis or directly from blood (Waite and Setchell, 1969; Hansson et al., 1975; Gupta et al., 1976). According to Das et al. (1973) and Dyson and Orgebin-Crist (1973), the fall in the testosterone levels enhanced the rate of epididymal sperm transport. Meistrich et al. (1975) reported that the inhibitors of androgen production, tended to accelerate sperm transport through the epididymis. Furthermore, androgen binding protein (ABP) produced by Sertoli cells in the testis, had an important role in the transport of testosterone in the luminal fluid (Hansson et al., 1974; 1975). If the production of ABP in the testis decreased after treatment with progestins and/or androgens (c.f. Flickinger, 1977a) it might be conjectured that normal accumulation of androgen in the lumen of the epididymis and its availability to the epithelium would be adversely affected.
Histologically, the various epididymal regions were affected differently in response to the drug treatments. Such differential responses to changing androgen levels had been reported earlier by a number of workers (Dinsker et al., 1974 a, b; Gupta et al., 1974a; Karkun et al., 1974; Rajalakshmi and Prasad, 1975 and Prasad and Rajalakshmi, 1977).

Following administration of both drugs, the epithelial cell height in the caput tended to decrease marginally, as against a pronounced increase in the cellular height in the cauda epididymis. Similar observations had been reported by Flickinger (1977c), Sharma and Kanwar (1981) and Kanwar and Hazurie (1985) following various treatments.

On the other hand, Rajalakshmi et al. (1970) and Srivastava (1983) had reported a reduction in the epithelial cell height in both caput and cauda epididymis following treatment with clomiphene citrate and norethisterone enanthate respectively. Glover (1976) reported increased epithelial height in the cauda region after castration and correlated it to the diminished luminal contents. Moore and Bedford (1979a), on the other hand, reported decreased epithelial cell height in the cauda after castration.
Prasad et al. (1973) further observed that the changed hormonal milieu tended to diminish cell height in the caput but increased the same in the cauda epididymis.

Clomiphene treatment for 30 days revealed highly shrunken tubules of the cauda with greatly reduced and irregularly shaped lumina. These observations were in accordance with those of Flickinger (1977c) following clomiphene treatment. According to Flickinger (1977b) alterations in the epididymis epithelium reflected the response of different parts of the organ to changes in the spermatogenic activity and to luminal sperm content rather than being the result of decreased testosterone stimulation.

Along with reduction in the concentration of luminal sperm, there was observed in the epididymal lumen, some cell debris which consisted of degenerating germinal cells of testicular origin, globular cytoplasmic masses and parts of degenerating spermatozoa. This was more pronounced after clomiphene than Net-en treatment. Higher incidence of degenerating spermatozoa might be due to their inability to survive in the altered hormonal milieu in the epididymis. The inability of exogenous androgen supply to replace the diminished endogenous androgen supply could be the cause.
of the altered hormonal milieu. The degenerating cells in the lumen were identifiable as spermatocytes or spermatids, probably as a result of early desquamation of germinal epithelium. According to Abdi and Hasan (1973) exfoliation of cells, especially spermatids, during and after steroid administration was of common occurrence.

There seemed to be an increase in the number of clear cells in cauda epididymis after drug treatment. Though their function was not well defined as revealed by Clermont and Flannery (1971) and Hamilton (1972), they were considered to be of absorptive nature by Moore and Bedford (1979a). These cells had further been found to possess a large number of lysosomes (Flickinger, 1972; Moore and Bedford, 1979a) which suggested their phagocytic role, facilitating clearance of epididymal luminal contents. Alternatively, the sperm could be lysed by the lysosomal enzymes released by these cells. Increased number of clear cells could be correlated to the enhanced acid phosphatase activity. Miller (1972) also reported the dissolution of spermatozoa by lysis in the lumen of epididymis of seasonally breeding animals. Moore and Bedford (1979a,b) had reported an increase in the lysosomal content of epithelial cells when spermatozoal degeneration took place.
During the present investigations there has been observed a fall in weights of both seminal vesicle and ventral prostate. Such weight reductions had also been reported by Singh et al. (1971, 1972), Singh and Prasad (1973) and Flickinger (1977c) following administration of progestins, like Norgestrel and Depo-Provera and the non-steroidal compound, clomiphene citrate. Cavazos and Melampy (1956), Wli and Chang (1973), Pakrashi and Pakrashi (1977) and Dixit (1977a) had also reported a fall in the weights of accessory organs after gonadectomy following administration of estradiol and plant extracts. Decrease in the weight of ventral prostate had also been reported by Coulson (1983) following gossypol treatment. Das et al. (1976) and Jackson et al. (1977) however reported an increase in the weights of these organs after estradiol-testosterone administration. Flickinger (1977b) could maintain accessory organs at normal level by administering testosterone along with Provera. Concomitant with the loss of weight in accessory organs, was observed a reduction in the epithelial height of the accessory glands. Patanelli and Nelson (1959), Setty and Kar (1967), Terner and MacLaughlin (1973), Rao and Chinoy (1983) and Chinoy et al. (1984) had all reported a decline in the weight and alterations in the functions of accessory sex
organs following treatment with progestins including 19-norsteroids and estradiol benzoate.

Androgen dependence of the seminal vesicle and prostate for the maintenance of their structural and functional integrity has been well established (William-Ashman et al., 1964; William-Ashman and Reddy, 1971; Cavazos, 1975; Mann and Mann, 1981). Decreased androgen supply following hypophysectomy (Cavazos and Melampy, 1956) or castration or antiandrogen treatment (Jones, 1977; Bose et al., 1977), led to dysfunction and involution of accessory sex organs. Exogenous androgen administration prevented or reversed these changes (Brandes, 1974). According to Neumann (1966) and Loving and Flickinger (1976), in the rat, seminal vesicle was more sensitive to antiandrogens than ventral prostate. Threshold androgen requirement of the seminal vesicle seemed to be higher than that of ventral prostate, according to Callow and Deanesly (1935) but Surfin and Coffey (1974) and Flickinger (1977b) had reported to the contrary. Different doses of androgen varying from 10-150 μg/day were found sufficient for maintenance of accessory sex organs after castration or treatment with Provera or ethynodiol acetate (Kragt et al., 1975; Terner and MacLaughlin, 1973; Cavazos and Melampy, 1956).
However, according to Hunt et al. (1978), 100 µg testosterone enanthate was insufficient to maintain accessory sex organs when 1 mg DMPA was administered. Similarly during the course of the present studies, 100 µg of androgen seemed insufficient for the maintenance of accessory sex organs, more so during clomiphene treatment than during Net-en treatment because the former seemed to cause more pronounced antiandrogenic effects.

There seemed to be reduced availability of androgens to target organs in the presence of progestins. Altman et al. (1972) and Briggs and Briggs (1976) had described rapid metabolic clearance of androgen due to stimulation of hepatic steroid oxido-reductase system by the progestagens. Thus retention time of testosterone in the body was reduced. Kragt et al. (1975) have proposed that in the presence of Provera, the amount of testosterone converted to estradiol was more than usual thus reducing the availability of testosterone to the target organs.

The role of lipids in the reproductive tissues has become increasingly important (Lacy, 1964). Therefore, the lipid composition in various reproductive organs seems essential to understand precisely their role in reproductive biology (Gonglio et al., 1974, 1975).
In the testis, in particular, the role of lipids has been found to be complex. Apart from serving as a general source of energy and as structural material for membranes, lipids have certain specific functions to perform (e.g., the role of essential fatty acids as prostaglandin precursors and the role that glycolipids might play in the cell surface phenomena - Coniglio, 1977). Testicular cholesterol is precursor for androgen synthesis (Dorfman et al., 1963; Eik-Nes, 1975).

The presence of sufficiently high levels of lipids in the normal testis and marked changes during impaired spermatogenesis suggested their vital role in testicular physiology (Johnson, 1970). Significant alterations in the level of testicular lipids following exposure to irradiation (Pokrovskii, 1963), during cryptorchidism (Perlman, 1950a,b; Johnson et al., 1968), following administration of various antispermatogenic agents (Gomes et al., 1968; Kar et al., 1968; Johnson, 1970; Coniglio, 1977; Kanwar et al., 1977a,b, 1986) and during the sexually quiescent period in the seasonal breeders (Short and Mann, 1966; Kanwar and Rahil, 1972 and Kanwar and Sheikher, 1975) have been observed.
The treatment with both Nete-en and clomiphene citrate during the course of the present studies significantly elevated the levels of lipids in the testis. Some workers (Fritz, 1975; Means, 1975; Steinberger and Steinberger, 1975; Coniglio, 1977), had related the fluctuations in total lipids with alterations in gonadotrophin levels which in turn regulated the fatty acid and phospholipid metabolism in the testis. Johnson (1970) and Fritz (1975) had conjectured that low androgen production resulted in lipid accumulation in the testis in the absence of spermatogenesis. Gambal and Ackerman (1967) and Nakamura et al. (1968) reported that hypophysectomy tended to increase testicular lipids. Gambal and Ackerman (1967) further related this increase with the suppressed steroidogenesis and spermatogenesis. According to Jehan et al. (1970) and Garg (1979), administration of progesterone to rats and flower extract of Calotropis procera to male gerbils, caused higher lipid concentration in the testis. Sheriff and Rijulu (1975), on the other hand, observed no difference in total lipid concentration after progesterone administration.

Nakamura et al. (1968), have described that some classes of lipids such as long-chain fatty acids were
preferentially increased during spermatogenic arrest. Increase in testicular lipids with the onset of the sexually quiescent phase in birds and seasonally breeding mammals, further suggested their role in the hormonal regulation of reproduction (Johnson, 1970; Kenwar et al., 1977b). According to Lofts (1965) an increase in the testicular lipids in the sexually quiescent phase was caused as a consequence of their accumulation due to non-metabolization into the androgens.

Phospholipids have been the major components of cell membranes and appear to be involved in the initial response of many cell types to the specific regulatory agents.

Melampy and Cavazos (1954) have shown that phospholipids were present in almost all cell types present in the testis, the Leydig cells being rich in them and Sertoli cells containing moderate amounts.

According to Gambal and Ackerman (1967), gonadotrophins regulated the phospholipid metabolism in the testis. Nakamura et al. (1968) had reported that although the extent of hormonal involvement in the control of phospholipid biosynthesis was not known, the enzymes involved were hormone-sensitive. The above workers had
observed a decline in the phospholipid content after hypophysectomy. According to Kingsley-Smith and Lacy (1959) and Posalaky et al. (1961), suppression of spermatogenesis was accompanied by a decrease in the phospholipid content of the seminiferous tubules. Sheriff and Rijulu (1975), did not find any change in the phospholipid content of the testis of rats treated with progesterone and progesterone + estrogen. They, however, found marked alterations in the level of individual phospholipid types. According to Lowitt et al. (1982), phospholipids were involved in steroidogenesis.

Cholesterol requirement for normal activity of the testis is well established (Eik-Nes, 1971). It is the main substrate for androgen biosynthesis (Dorfan et al., 1963; Eik-Nes, 1975). Localization of cholesterol in the testis was mainly in the Leydig cells (Perlman, 1950a,b; Montagna and Hamilton, 1951; Christensen, 1975), though traces were also found in the seminiferous tubules (Perlman, 1950b; Montagna and Hamilton, 1951; Lofts et al., 1966; Johnson, 1970). With regard to other possible roles of testicular cholesterol, it was proposed that it served a trophic function for the developing sperm (Leupold, 1921; Kunze, 1922), or was related to the 'Spermiogenetischen
Prozesses' (Kunze, 1922). Cholesterol might also be related to the total lipid metabolism of the organism (Berberich and Jaffe, 1932), or to the hormone secretions of the testis (Lipschulz, 1924; Perlman, 1950 a,b). Any alteration, viz. an increase or decrease of testicular cholesterol has been considered important, since it was implicated in the inhibition/stimulation of sperm formation in the testis (Lacy, 1967). It is well established that impairment of testicular function/spermatogenesis has always been accompanied by an increase in cholesterol level (Perlman, 1950 a,b; Johnson, 1970; Singh et al., 1971), and this occurred regardless of the functional state of the interstitial cells (Kar and Roy, 1955; Nakamura et al., 1968).

Long and Engle (1952) and Dorfman et al. (1963) have intimately related the levels of cholesterol to fertility and sperm output. Increase in testicular cholesterol, as observed in the present studies, could be attributed to its decreased utilization for steroidogenesis which might either be due to the pituitary inhibition or due to a direct inhibitory action of drugs on target tissue. The increased cholesterol level was also suggestive of a decreased androgen level. Similar increase had been
reported by Lacy and Lofts (1965), Dixit et al. (1978 a,b), Garg (1979), Dixit (1979) and Dixit and Gupta (1982 a,b) after treatment with various chemical compounds.

It is probably the neutral lipids on the whole mainly, which caused an increase in total lipids, as the phospholipids tend to decrease and the increase in cholesterol alone could not account for the rise as it constituted only a small fraction. Nakamura et al. (1968) have attributed the higher level of the total lipids after hypophysectomy, to long-chain fatty acids of cholesteryl esters and glycerol esters-diesters. Hormonal control of fatty acid metabolism in the maturing rat testis had been documented by Davis et al. (1966), Ewing et al. (1966), Goswami et al. (1968), and Steinberger and Steinberger (1974).

The present investigations have revealed an accumulation of total lipids and cholesterol with a simultaneous depletion of phospholipids in the epididymis as it became devoid of spermatozoa as a result of drug treatments. These observations are in conformity with those of Cavazos and Melampy (1956) and Gohari et al. (1962) who had observed extra accumulation of lipids in
atrophied epididymal epithelium of castrated rats and
hamsters. They had confirmed this by injecting testo-
sterone propionate to castrated hamsters and noted the
restoration of carbohydrates, total lipids and phospholipids
in the epithelial cells. Turner and Johnson (1971)
reported increased amounts of total lipids in the
epididymis of unilaterally castrated rats. The work of
Allen and Slaster (1957) helped to explain the extra
accumulation of lipids. They observed an inhibition of
esterase enzyme activity in castrated animals, which
impaired the breakdown of fat into simple fatty acids,
resulting in the accumulation of fatty material in the
cells. These effects in castrated animals were reversible
after testosterone propionate therapy. Increased
epididymal lipids could also be attributed to decreased
androgen supply in view of atrophy of Leydig cells which
supplied androgen to epididymis through blood and
testicular fluid (Waites and Setchell, 1969). Dinaker
et al. (1974 a,b) reported, on the other hand, a decrease
in lipid content following castration in monkeys. They had
also reported that synthesis of phospholipids was androgen
dependent. The decreased phospholipid content at present
might also be attributed to decreased androgen supply.
Decrease in phospholipids in rat epididymis after ligation of efferent duct had been reported by Turner and Johnson (1971) and Prasad et al. (1972). Treatment with various antiandrogens caused a depletion in epididymal phospholipids as reported by Umapathy et al. (1979).

The role of cellular lipids being diverse, these were associated with the maintenance of cellular structure and function (Locke, 1969; Neill and Masters, 1973) and also reported to increase cellular resistance to stressful conditions (Martin, 1963). Hafez and Prasad (1976) had conjectured that phospholipids might help to serve as a substrate for epididymal maturation of spermatozoa or as a source of glycercyl phosphoryl choline. An overall disturbance in cellular lipid metabolism was suggested by the alterations in lipids after drug treatments.

High cholesterol levels have been observed in the epididymis during the present studies. Similar observations had been made by Umapathy et al. (1979) and Kanwar and Hazuria (1985) after treatment with medroxy progesterone acetate and a combination of ethinyl estradiol and testosterone enanthate. Administration of various plant extracts had also revealed similar elevation in cholesterol content (Dixit and Gupta, 1982 a,b; Dixit and Bhargava, 1983; Bhargava, 1984; Gupta et al., 1986). According to
Hamilton and Fawcett (1970), the rat epididymis was capable of synthesizing cholesterol and testosterone from acetate. They had correlated the high level of cholesterol with possible impairment in androgen production.

Increased total lipid and cholesterol contents and decreased phospholipid content has been observed in the seminal vesicle and prostate during present studies. Androgen-withdrawal methods such as hypophysectomy, castration and antiandrogen administration had reportedly increased lipid content in accessory sex glands (Cavezos and Melampy, 1954; Gohari et al., 1962). Increased sudanophilia and aggregation of lipids in atrophied epithelium of seminal vesicle, coagulating gland and prostate of castrated rats and hamsters had been observed by the above workers.

Reduced level of proteins could be presumed to be due to loss of nucleic acids as a result of atrophy of the organs. Tyagi et al. (1979) observed low levels of proteins after cyclohexanol treatment and attributed it to the inhibition of spermatogenesis and suppressed Leydig cell function. In the epididymis this decrease has been attributed to the absence of spermatozoa (Dixit, 1977a).
Acid phosphatase, essentially a lysosomal enzyme (Katash et al., 1970; Males and Turkington, 1971) had been reported to help in the synthesis of phospholipids (Rouiller, 1963). This enzyme had been localized in all cell types of the testis, as well as in the residual bodies and dense bodies in Sertoli cells (Blackshaw, 1970; Males and Turkington, 1971; Elkington et al., 1973; Gomes and Van Demark, 1974). The present studies revealed an increased activity of this enzyme which could be related to the histological lesions suffered by the testis after both drug treatments. Elkington et al. (1973) and Blackshaw (1973) have related the increase in acid phosphatase activity with the decrease in the number of sperm in the testis. Such an increase has also been associated with the disposal of the desquamating and dead germinal elements and spermatozoa (Nicander, 1966) and also with cell disintegration (De Duve, 1969). The lysosomes had been related with the germinal epithelial cell breakdown following drug treatment. Hugon and Borgers (1966) similarly related the breakdown of germinal elements in the mouse testis following X-irradiation with the increased phagocytotic activity of the Sertoli cells. Gupta (1973) too, attributed the transitory rise in testicular acid phosphatase following X-irradiation to lysosomal breakdown and increased phagocytotic activity of
Sertoli cells. Elkington and Blackshaw (1971) and Elkington et al. (1973) have attributed the increase in acid phosphatase activity in rat testis after estrogen treatment and following hypophysectomy, respectively, to the fragility of testicular lysosomes which changed under altered hormonal milieu. The increased acid phosphatase activity of the testis could be related to the degeneration of germinal elements of the testis. Increase in the activity of the said enzyme has been reported following testicular damage by a host of workers - Kasinathan et al. (1972), Kalla and Bhasin (1977), Garg (1979), Kaur and Mangat (1979), Chauhan et al. (1979), Kalla and Chohan (1980). On the other hand, decrease in acid phosphatase activity has been reported by Bedwal and Mathur (1980) and Balasubramanian et al. (1980) after treatment with chlorpromazine and aspirin, respectively.

Increase in acid phosphatase activity in the epididymis could similarly be related to the degeneration of the luminal contents. Glover (1976) and Rastogi et al. (1979) reported a transitory rise in the enzyme activity of the epididymis of castrated and cyproterone acetate-treated rats and mice. According to Kasinathan et al. (1972), Chauhan et al. (1979), Garg (1979) and Bhargava (1984), treatment with many plant extracts possessing antifertility
effects also caused an increase in acid phosphatase activity. Linnett and Amann (1968), had correlated increase in enzyme activity with epididymal sperm disposal. Loss of sperm viability due to androgen deficiency coincided with increased lysosomal activity of the epithelial cells (Moore and Bedford, 1979a).

Alkaline phosphatase is an ubiquitous enzyme, usually associated with the plasma membrane and helping in the transport of metabolites across the membrane, differentiation of cells and with the synthesis of testicular hormones (Allen and Slater, 1957; Lobel and Levey, 1968). According to Goyal and Mathur (1974), and Brandes (1974), this enzyme was a histochemical marker for primordial germ cells in various species including rats. Alkaline phosphatase was very conspicuous in particular, in the membrane propria (Kochar and Harrison, 1971). Alkaline phosphatase activity was also considered to be androgen dependent (Dempsey et al., 1949). Chowdhury and Mukherjee (1977) demonstrated that administration of exogenous androgen increased the alkaline phosphatase activity whereas administration of progesterone brought about a decrease in the activity of this enzyme. These workers also related decreased enzyme activity with the desquamation of germinal
epithelium which was rich in this enzyme. Decreased enzyme activity has been reported by Tyagi et al. (1979), Balasubramanian et al. (1980) and Bedwal and Mathur (1980) after various treatments.

Bedwal and Mathur (1980) reported a reduction in the activities of acid and alkaline phosphatases and ATPase after chlorpromazine treatment. They concluded that the decrease in phosphatase activity may be because of the impairment of spermatogenesis since these enzymes had been found to be associated with the differentiation of spermatozoa and with the change of substances between Sertoli cells and spermatogenic cells or because of suppression of gonadotrophins and testicular androgens which were actively associated with the activities of these enzymes in the testis and accessory sex glands.

Alterations in the activity of phosphatases of accessory glands as revealed in the present study were probably due to androgen deficiency, as these organs were highly androgen dependent.

Carbohydrate metabolism is an important aspect to study in order to determine the extent and nature of glucose involvement in testicular function. Glucose is
known to be the major nutrient that could supply energy rapidly in the testis (Free, 1970), as well as in other tissues (Rouiller, 1963). The involvement of carbohydrate metabolism during maturation of the male gonad was obvious from studies on the prepubertal and adult rats which showed the influence of glucose on protein synthesis (Davis and Morris, 1963; Davis and Firlit, 1965; Hollinger and Davis, 1966; Means and Hall, 1968). According to Hollinger (1971), there seemed to be faster utilization of ATP in rat testis compared to that in the liver and kidney. Spermatozoal metabolism almost exclusively involved ATP generation for motility as well as for maintenance of osmotic balance (Sidhu and Gureya, 1985). The present investigations revealed an increased amount of glycogen and reduced activities of most of the enzymes of the Embden-Meyerhoff glycolytic pathway following both drug treatments, thereby indicating an inhibitory effect of the drugs on glycolysis.

According to Chatterjee (1966), spermatogenic arrest led to accumulation of non-utilized sugar in the unaffected Sertoli cells, thereby causing the testicular glycogen to increase. Sertoli cells stored glycogen for the nourishment of germinal cells, especially spermatozoa.
Kar et al. (1968), reported an increase in testicular glycogen during degeneration of testis following administration of bis(dichloroacetyl)diamine as also did Jehan et al. (1970) following progesterone treatment. Similar observations had been made by Gupta and Bawa (1977) and Shah and Bhatavdekar (1980) following irradiation induced testicular atrophy. Malvaviscus consatti flower extract treatment to mice also resulted in elevated testicular glycogen (Verma et al., 1980; Joshi et al., 1981). Altered glycogen metabolism seemed related to inhibited glycolysis in the presence of normal glycogenesis and inhibited Kreb's cycle in the cells of the germinal epithelium (Ord and Stocken, 1961). Two additional pathways might also be acting indirectly to cause the accumulation of testicular glycogen: (i) the hydrolysis of glucose-6-phosphate to glucose by the action of glucose-6-phosphatase and (ii) the control of activities of phosphorylase and UDPG glycogen transferase by glucose 6-phosphate (Trant and Lipmann, 1964). Increased concentration of glucose-6-phosphate tended to activate UDPG · glycogen transferase leading to elevated glycogen levels. Jehan et al. (1970) attributed the rise in glycogen level following progesterone administration, to denote an enhanced rate of glycogenesis and accelerated
pace of carbohydrate oxidation via the Embden-Meyerhoff pathway, particularly since no change in oxygen uptake of tubules was observed.

Glycogen phosphorylase was an important enzyme involved in glycolysis as it propagated the breakdown of glycogen to glucose-6-phosphate (Harper et al., 1977). The reduced activity of this enzyme indicated decreased availability of glucose-6-phosphate for further oxidation in the glycolytic pathway due to inhibition of glycogen degradation, thus resulting in the accumulation of glycogen. Glycogen accumulation was also observed in the present studies which could be attributed to decrease in the metabolic activity of tissues. Similar results had been reported by Gupta and Bawa (1977) in radiation induced testicular atrophy and also by Veeraragavan and Ramakrishnan (1984) while working on germinal cells in 5-thio-D-glucose treated mice testis.

Glucose-6-phosphatase was an essential terminal hydrolytic enzyme for both the glycogenolytic and gluconeogenetic pathways. This enzyme was highly concentrated in the rat testis (Eisenberg, 1967). As this enzyme helped in the removal of phosphate from glucose-6-phosphate, an inhibition of the activity of this enzyme could lead to an
accumulation of glucose-6-phosphate instead of its further breakdown into glucose and phosphate moieties. Increased accumulation of glucose-6-phosphate can shift the equilibrium towards production of fructose 1,6-diphosphate or triose phosphate via hexose monophosphate shunt and ultimately result in increased concentration of glycolytic intermediates such as pyruvate. The inhibition of glucose-6-phosphatase activity could probably block the production of glucose from other sources, such as amino acids and oxaloacetate. Increased level of glucose-6-phosphate might consequently lead to increased production of glucose-1-phosphate by the action of the enzyme of phosphoglucomutase which in turn might lead to glycogen production by glycogen synthetase. The glycogen primer and uridine diphosphate glucose catalysed the above reaction. Therefore formation and storage of glycogen in the intracellular reservoir, along with its reduced degradation due to suppressed glycogen phosphorylase activity, suggested that sufficient energy from carbohydrate source may not be available to the cells including spermatozoa for their metabolic activities and so spermatozoa might lose motility and thus also the capability of fertilizing the ovum. Gupta and Bawa (1977) had thus related the accumulation of glycogen in radiation-induced atrophic
testis to the decreased activities of glycogen phosphorylase and glucose-6-phosphatase accompanied by the loss of germinal cells.

Glucose-6-phosphate isomerase catalysed the isomerization of glucose-6-phosphate to fructose 6-phosphate. This reaction might proceed readily in either direction and was reversible in glycolysis and gluconeogenesis (Lehninger, 1978). Thus an inhibition of isomerase activity as was observed in the present studies, would lead to a reversal of glycolysis by blocking the formation of fructose-6-phosphate and then to fructose-1,6-diphosphate.

Fructose-1,6-diphosphatase is another important rate limiting enzyme like glucose-6-phosphatase, that maintained equilibrium between glycolysis and gluconeogenesis. Inhibition of fructose-1,6-diphosphatase enzyme activity thus might lead to the decreased breakdown of fructose-1,6-diphosphate and consequently to the lesser generation of glucose by gluconeogenic pathway. The accumulated fructose 1,6-diphosphate could lead to the production of triose phosphate such as glyceraldehyde-3-phosphate and dihydroxy acetone-phosphate. The latter in particular, could be utilized in glycerol, glycerol-3-phosphate and phospholipid metabolism.
The presence of the disaccharide-maltose as a glycogen intermediate and consequent amylase activity had been confirmed by Panse and Sheth (1981) in mammalian spermatozoa. Enhanced activity of amylase, as in the present observations, indicated increased formation of simple carbohydrates. Inhibition of glycolytic intermediates and enzymes lowered the rate of utilization of these monosaccharides, resulting in more synthesis and accumulation of glycogen in the tissues.

Thus it could be said that drug treatments affect glycolysis and oxidative phosphorylation by potential inhibition of the key enzymes of the Embden-Meyerhoff pathway.

ATPase hydrolyzed ATP to ADP and inorganic phosphorus (Pi), the latter being used for various cellular activities. Dawson (1958) reported that ATP is probably abundant during late stages of spermatogenesis. Considerable amount of ATP is associated with spermatozoa and was related to sperm motility (Mann, 1964; Mann and Mann, 1981). Present studies have revealed a significant decrease in the activity of this enzyme. Administration of Thimet to desert gerbil (Saxena and Sarin, 1979), 5-thio-D-glucose to mice (Veeraragavan and Ramakrishnan, 1984) and
cholorpromazine to bats (Bedwal and Mathur, 1980) had revealed similar results. Decrease in ATPase activity could be related to the preservation of ATP to meet the impaired energy supply to the tissue due to slow cellular metabolism or due to loss of spermatids. Shah and Bhatavdeker (1980) conjectured, that probably the function of ATPase in testis was related to energy linked transport of glucose which could either be oxidised to produce energy or get converted to glycogen in non-germ cells.

ATPase has been associated with the energy yielding systems in the reproductive organs (Sood and Kakaria, 1983). According to Singh and Mathur (1968), ATPase activity was confined to the germinal elements where it was generally associated with spermatids.

Lactate dehydrogenase catalysed the reversible conversion of lactate to pyruvate in the presence of NAD. Kaur and Guraya (1981) have described a very high LDH activity in testicular interstitial tissue as in epididymal epithelial cells, but could find only moderate activity within seminiferous tubule. They observed reduced enzyme activity after treatment with \( \alpha \)-chlorohydrin. Balasubramanian et al. (1980) had revealed similar effects after treatment with aspirin and Veeraragavan and
Ramakrishnan (1984) after 5-thio-D-glucose treatment. Reduced activity of the enzyme has also been noted in the present observations. Reduced LDH activity lowered the amount of lactate converted to pyruvate. The latter was converted to oxaloacetate by the action of pyruvate carboxylase (in mitochondria) in the presence of ATP, biotin and CO$_2$. This reaction was a part of the citric acid cycle. So a decrease in LDH activity would ultimately inhibit the citric acid cycle.

Succinate dehydrogenase, localized on the inner mitochondrial membrane, was an index of cellular oxidative activity. This enzyme is an iron sulphur (Fe:S) protein, containing FAD and catalyzes the conversion of succinate to fumarate. This enzyme was involved in a direct transfer of hydrogen from the substrate to a flavoprotein without the participation of NAD$^+$. Fumarate was formed as a result of the dehydrogenation. Decreased SDH activity following drug treatment might be due to androgen deprivation (Prasad et al., 1972) or due to a reduced number of spermatozoa or testicular fluid in the epididymis. Decline in the epididymal SDH activity after $\alpha$-Chlorohydrin treatment has been correlated by Kalle and Chohan (1976), to the disintegration of the entire
cellular system. According to Kaur and Mangat (1979) there was a decrease in SDH activity of the testis after treatment with progestational antiandrogenic chloromadinone acetate. They had explained it to be due to the inactive state of the testis, caused by inhibited spermatogenesis and decreased metabolism.

Since SDH was indicative of the functioning of Kreb's cycle, the changes in its activity reflected the general metabolic alterations in the tissues.

Sialic acids formed a major group of carbohydrates in the glycoproteins of the epididymis of rat, hamster, monkey and human (Prasad and Rajalakshmi, 1976; Rajalakshmi and Prasad, 1979). An optimal level of sialic acid seemed to be essential for the maintenance of the functional integrity of spermatozoa (Gupta et al., 1974b; Prasad and Rajalakshmi, 1976). Androgen deprivation led to a concomitant loss of epididymal sialic acids followed by a loss of integrity of the acrosome and loss of motility and viability of spermatozoa (Mann, 1964; Prasad et al., 1970; Rajalakshmi et al., 1971; Gupta et al., 1974b).

Apart from their possible role in sperm maturation, sialoproteins have been implicated in antigenic interaction between sperm and epididymal fluid and were
also important in maintaining an ionic balance of the epididymal fluid (Prasad and Rajalakshmi, 1976, 1977). Sialic acid present in the spermatozoa might also act as a decapacitation factor which had been identified as a sialic acid containing protein (Rajalakshmi and Prasad, 1979).

Since spermatozoa are known to be rich in sialoproteins (Hartree and Srivastava, 1965), the low levels of sialic acid, as in the present studies, could be attributed to decreased sperm population in the epididymal lumen. Nag et al. (1977), attributed the decrease in sialic acid and increased neuraminidase activity in rat testis and epididymis following treatment with anti-fertility agents, to the degeneration and depopulation of sperm cell. Prasad et al. (1973) have correlated decrease in sialic acid level to the antiandrogenicity of cyproterone acetate as did Goyal et al. (1979) after treatment with methallibure. Mann (1964) reported reduction in sialic acid contents in a number of species after castration or hypophysectomy.

The citric acid content of seminal plasma and accessory glands was an androgen dependent factor (Humphrey and Mann, 1949; Mann and Parson, 1947; Mann, 1964).
The exact physiological role of citric acid had not been clearly understood. Huggins and Neal (1942) had correlated it with the clotting-lysis mechanism of the ejaculate by its binding with calcium ions. Salisbury and Lodge (1962) have reported citric acid to play an important role in the stabilization of extracellular milieu essential for the normal viability and functioning of spermatozoa. Price and Williams-Ashman (1961) had reported a disappearance of citric acid from seminal fluids after castration and a reappearance following testosterone treatment. Baumberger and Fried (1948) were of the view that action of citric acid was linked to hyaluronidase activity.

Dondero and D'Ottavio (1971) had however observed no correlation between citric acid levels and the number and motility of spermatozoa. A decrease in citric acid levels has been observed during the present investigation. Similar decreases have been observed after hypophysectomy (Ortiz et al., 1956), following administration of progesterone and Δ11-pregnen-3,20 dione (Kar et al., 1967), megestrol acetate (Setty and Kar, 1968) and the isomers of clomiphene citrate (Singh and Prasad, 1973). The decrease might be due to decrease in plasma testosterone level as reported by Polakoski et al. (1976).
The occurrence of ascorbic acid in the seminal fluid of guinea pig, bull, man, ram and rat has been well documented (Mann, 1964; Mann and Mann, 1981) but its exact role was not established (Mukkadan, 1980), although it was known to be a reducing agent. Phillips *et al.* (1940) reported a direct relationship between fertility and ascorbic acid content of semen. Reduction in the ascorbic acid content of seminal vesicle could be due to decreased androgen production as this was an androgen dependent parameter.