In recent years, much research effort has been directed towards understanding the basic mammalian reproductive processes and their control. The reproductive tract of animals is under the influence of pituitary-gonadal axis hormones. It is known that testis is the site of sperm production and steroidogenesis. The role of androgens in the initiation of spermatogenesis is known (Steinberger et al., 1973; Ritzen et al., 1978). The testis also produces a factor called "inhibin". Other sources of inhibin include rete testis fluid, interstitial testis fluid (Krause, 1978) and semen, but exact site of production is not known. It is a polypeptide and testicular inhibin has molecular weight ranging from 15,000 - 17,000 daltons (Baker et al., 1976). The functions of inhibin in the male are not fully elucidated, since the exact role of FSH in the control of spermatogenesis is still not clear (Setchell et al., 1977). However, Franchimont et al.
(1975 a) have shown that antisera can be prepared in the rabbits against an inhibin rich fraction of bull semen. These antisera when injected into rats caused significant increase in serum FSH, suggesting that inhibin has feedback control on pituitary FSH. Franchimont et al. (1975 b, c) have purified and characterized inhibin from bull seminal plasma and also reported the presence of such a substance in human seminal plasma.

Several studies by Steinberger and Steinberger (1977) and others have clearly demonstrated that Sertoli cells secrete an androgen binding protein (ABP) and that this secretory activity is directly stimulated by FSH. Recently, more attention has been paid to ABP. These are believed to be glycoproteins of molecular weight 86,000 - 91,000 (Ritzen et al., 1973). ABP has been demonstrated in testicular fluid collected from adult rats (French and Ritzen, 1973 a, b), rabbit (Ritzen and French, 1974; Danzo et al., 1975), ram (French et al., unpublished data) and bull (Hansson et al., unpublished data). Androgen binding activity in the human testis and epididymis has also been demonstrated (Lipshultz et al., 1977). Similarly, a species distribution of testosterone-binding globulin was reported in 21 different species of animals (Corvol and Bardin, 1973). ABP binds testosterone and 5α-dihydrotestosterone (5α-DHT) with high affinity and a close
correlation exists between the concentration of testosterone, DHT and those of ABP in the testicular fluid of rabbits (Guerrero et al., 1975). ABP presumably assists the transport of androgens locally to the germ cells and distally with the testicular fluid to the epididymis (Hansson et al., 1975). It is known that testosterone is metabolised to several 5α-reduced androgens by testicular tissue of adult rats (Rivarola and Podesta, 1972; Folman et al., 1972a, Dorrington and Fritz, 1973), mice (Kassai et al., 1973), human beings (Rose et al., 1973; Payne et al., 1973), dogs (Folman et al., 1972b), frogs (Ozon and Pouche, 1972) and rabbits (Ewing and Brown, 1975; Ewing et al., 1975).

These reduced forms of androgens exert their major effects on target cells, where, physiological action is initiated (O'Malley and Means, 1973; King and Mainwaring, 1974; Sluyser, 1975).

Rat seminiferous tubules secrete a fluid (Tuck et al., 1970; Cheng et al., 1977) which is reabsorbed by the epididymis (Craw and Gustafsson, 1964; Levine and Marsh, 1971). The testicular fluid may be important in providing oxidizable substances for the spermatozoa during maturation and testicular spermatozoa can draw on their own reserve of lipid (Scott et al., 1967).

The epididymis is a highly vulnerable organ, where spermatozoa undergo the process of maturation during their
sojourn and attain motility and fertilizability. The most marked morphological change is the migration of the cytoplasmic droplet (Bedford, 1965; Orgebin-Crist, 1967; Fawcett and Philips, 1969). Other features of the maturation process are changes in the membrane characteristics (Glover, 1962; Bedford, 1972), increase in specific gravity (Lavon et al., 1968), and fertilizing capacity (Bedford, 1965, 1975; Orgebin-Crist, 1967; Hamilton, 1975; Orgebin-Crist et al., 1975) and changes in the pattern of motility of spermatozoa (Blandau and Rumery, 1964; Gaddum, 1968; Chinoy and Sharma, unpublished observations). Chulyatánatol and Yindepit (1976) revealed that rat spermatozoa from the cauda epididymis were found to have lower activity of the surface ATPase than the sperm from the caput region. The extent to which this maturation is inherent in the sperm as they age or subject to influence by the epididymal epithelium has been debated (reviewed by Bedford, 1975; Orgebin-Crist, et al., 1975), but it appears that both factors are involved (Hamilton, 1975). The precise epididymal region at which sperm normally acquire fertilizing ability varies with the species but is generally in the distal corpus or proximal cauda (Dyson and Orgebin-Crist, 1973; Blandau and Rumery, 1964; Horan and Bedford, 1972; Bedford, 1966; Orgebin-Crist,
1967; Paufler and Foote, 1968). It has been also shown that sperms can mature without epididymal transit (Young, 1931; Gaddum and Glover, 1965; Bedford, 1967), but as a consequence of this, large numbers of abnormal spermatozoa are found (Glover, 1969; Gaddum and Glover, 1965) and significant post-fertilization embryonic mortality occurs (see review of Orgebin-Crist, 1969). The rabbit spermatozoa retained in the caput or corpus epididymides by ligature acquire normal morphological and motility characteristics but do not fertilize the ova. Hence, the passage of spermatozoa through the cauda epididymides appears to be essential for the final process of maturation (Bedford, 1965; Gaddum and Glover, 1965; Orgebin-Crist, 1969).

According to Glover and Nicander (1971) the epididymis is divided broadly into initial or caput, middle or corpus and terminal or cauda segments. These divisions hold generally, although numerous species differences make it difficult to equate the segments from species to species accurately (Hamilton, 1972). Reid and Cleland (1957) described six zones and three subzones of the rat epididymis and have delimited these zones solely on variations in height of the principal cells. Similarly, Nicander (1957 a, b), subdivided the rabbit epididymis into eight zones and dealt similarly with stallion, boar and ram epididymis.
The histology and ultrastructure of the adult epididymis have been described in a number of mammalian species (Nicander, 1957a, b; 1958; Reid and Cleland, 1957; Allen et al., 1957; Allen and Slater, 1961a, b; Abdel-Raouf, 1960; Nicander and Glover, 1973; Suzuki and Racey, 1976; Ambedkar, 1973; Prasad and Rajalakshmi, 1976; Chinoy et al., 1979a; Ramos and Dym, 1977; Leeson and Leeson, 1970; Alexander, 1972; Riar et al., 1973; Flickinger et al., 1978). The various cell types and regional differences in structure have been analyzed in detail by Hamilton (1975) in rat and hamster epididymides by Anand Kumar et al. (1980) and Flickinger et al. (1972, 1978) and rhesus monkeys (Prakash et al., 1979a, b; 1980). In all species so far investigated, two cells are invariably present, the principal and basal cells, although in many species additional cell types are reported. The principal cell extends from basal lamina of the duct epithelium to the lumen. Its luminal surface bears stereocilia, its cytoplasm has a lobated nucleus, lysosomes, multivesicular bodies, mitochondria, smooth endoplasmic reticulum and rough endoplasmic reticulum, free ribosomes and extensive Golgi complex. The principal cell is involved in the synthetic activity and resorption of substances. The basal cells are found at the base of
the epididymal pseudostratified epithelium. They are variable in shape and their cytoplasm is remarkable for the paucity of organelles and inclusions as they contain few mitochondria, scattered profiles of rough-endoplasmic reticulum, attenuated segments of smooth endoplasmic reticulum and small Golgi complex. But lipid inclusions are found (Hamilton, 1972). Their functions are obscure but Martan and Risely (1963) and Martan (1969) have implicated them in holocrine secretory cycle, although convincing evidence is lacking. Other cell types with obscure functions also occur, viz., halo cells, apical cells, clear cells etc., in rat epididymis (Reid and Cleland, 1957; Hamilton, 1972).

The initial segment or caput epididymis is characterized by very tall epithelium with long stereocilia, which extends into a narrow lumen that contains only a small number of sperms. The middle segment or corpus epididymis has a lower epithelium with highly vacuolated apical cytoplasm and shorter stereocilia, and a wide lumen containing many more sperms than in the caput epididymis. The terminal segment or cauda epididymis has the lowest epithelium of all three segments and a very wide lumen that is filled with spermatozoa. The epithelium of epididymis is pseudostratified with fine stereocilia
and the interstitial regions are filled with the normal components of loose alveolar connective tissue.

The epididymal tubules are surrounded by smooth muscle along their whole length. In the initial segment and head, only one or two circular layers surround the tubules and are continuous with the complex muscular ensheathment of the vas deferens. The contraction of this smooth musculature helps in sperm transport. The detailed study on rabbit epididymal musculature was carried out by Holstein (1967).

Rhythmic peristaltic contractions of the tubules have been observed by many workers (Muratori and Contro, 1951; Muratori, 1953, 1956; Risley and Turbyfill, 1959; Cross, 1959) and the independence of these contractions from nervous stimulation has been demonstrated in excised, cultured specimens (Battaglia, 1958; Risley and Vandevelda, 1959; Hib, 1974). Battaglia (1958) recorded spontaneous movements in the caput, corpus epididymis and segmentation movements of the tubules of the cauda. Cross (1955) did not find clear effect of oxytocin on epididymal contractility in rabbits.

Studies have been conducted by Bielanski and Evi (1964), who have recorded spontaneous contractions in the rabbit epididymis in vitro, however, oxytocin reduced the
frequency of the contractions. Hib (1974) found spontaneous contractions of mouse epididymis and an increase in the intensity and frequency of contractions in the cauda epididymis by oxytocin concentrations ranging from 125 to 1000 μU/ml. Hib and Fonzie (1977) have shown that androgen maintains the activity of the contractile cells in the epididymis. It seems possible that testosterone maintains the contractility of the smooth musculature of the epididymis indirectly by an effect on the short adrenergic nerves. The observations of Holstein (1967); Cross (1959) and Gaddum (1969), have shown that the caput region contracts faster than the tail region.

The epididymis receives both sympathetic and parasympathetic innervation (Kuntz and Morris, 1946) from one abundant nerve plexus surrounding both the vascular tree and the duct, but the distribution of the axons vary from species to species (El-Badawi and Schenck, 1967; Norberg et al., 1966). However, Norberg et al. (1966) have shown that, in rat, guinea pig and rabbit, adrenergic fibers do not end along the efferent ductules or in relationship to the tubules of the head of the epididymis, whereas, in cat and dog all portions of the duct contain both types of endings (Norberg et al., 1966; El-Badawi and Schenck, 1967).
Differential threshold of androgen requirements by epididymis and accessory sex glands have been well documented by many workers. The threshold requirement of androgen for maintenance of the epididymis is higher than that required by the accessory sex glands (Dinakar et al., 1974 a,b; 1977 a; Karkun et al., 1976; Gupta et al., 1974 a; Rajalakshmi and Prasad, 1971; Prasad et al., 1977; Chinoy et al., 1978 a). The cellular integrity of epididymis is androgen dependent (Alexander, 1972; Jehan et al., 1973; Lubicz-Hawrocki, 1974; Dyson and Orgebin-Crist, 1973; Das et al., 1973; Gupta et al., 1974 a,b; Karkun et al., 1974; Orgebin-Crist et al., 1975), as has been demonstrated in the rabbit (Orgebin-Crist, 1973; Orgebin-Crist and Davis, 1974) and in the other mammals (see reviews by Hamilton, 1972; Orgebin-Crist et al., 1975). Amongst the two regions of epididymis, regional threshold requirement of androgen varies, i.e. caput requires less androgen, as compared to other regions of epididymis for maintaining its normal integrity and function (Dinakar et al., 1977 a; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978 a; Prasad and Rajalakshmi, 1976 a, 1977).

The epididymis, and its structural components, sperms and biochemical constituents also manifest differential sensitivity to androgens and their metabolites (Dinakar
et al., 1974 a, b; Gupta et al., 1976; Bose et al., 1977).
In adult and prepuberal animals also, a difference in androgen sensitivity was found (Singh et al., 1977 a, b; Arora-Dinakar et al., 1977 a). It is evident that spermatozoa of epididymis are capable of metabolizing the androgens (Djoseeland et al., 1973; 1974; Dinakar et al., 1975; Arora-Dinakar et al., 1977 b; Prasad and Rajalakshmi, 1976). Even among accessory sex glands, differential sensitivity of androgens is noticed. Seminal vesicle is more sensitive to circulating androgens than other accessory sex glands (Rajalakshmi and Prasad, 1971; Spring-Mills, 1980), for maintaining normal physiological integrity, hence accessory sex organ weights have been taken as a measure of normal functioning of these glands.

Epididymis as a whole has two main functions: (1) Absorptive, (2) secretory, which play an important role in the maintenance of the normal epididymal milieu which is conducive for sperm maturation and motility. The fluid secreted by testis is mainly resorbed by the first part of the rat epididymis (caput) and the cells there are taller than those found elsewhere in the epididymal duct (Reid and Cleland, 1957; Nicander, 1957 a,b). The epithelium of the epididymis has the function of transferring fluid from the lumen into the interstitium and of absorbing and digesting the particulate material. The absorption
of particulate material from the lumen of the duct has been studied by numerous workers in a variety of species (see review by Hamilton, 1977). Hoffer et al. (1975) have shown that under some experimental conditions, the principal epithelial cells in various parts of the epididymis are capable of absorbing portions of degenerating spermatozoa, whereas, under normal condition the above phenomena rarely occurs (Nicander, 1955), but was observed in epididymis of bulls, rabbits and monkeys (Roussel et al., 1967). The fluid movement across the epithelium also takes place. The fluid is moved out of the luminal and epithelial compartment into interstitial vessels (Crabo, 1965; Setchell et al., 1969; Waites and Setchell, 1969). Fluid resorption mainly occurs in the ductuli efferentes and first part of epididymis but no conclusive evidences exist. A peculiar feature of the absorptive function is that water transport occurs only in certain places along the epididymis, while particulate absorption occurs all along. The initial stages of fluid movement probably involve micropinocytosis at luminal plasmalemma (Hamilton, 1977). The possible role of electrolyte and water transport in sperm maturation in rat epididymis has been suggested by Wong et al. (1978). The
absorption of sodium chloride and water and secretion of potassium occurs in the normal caput and proximal corpus epididymides at a lower rate than in the cauda epididymis. In proximal regions of the rat epididymis, water reabsorption may be secondary to an active transport of chloride, while in the cauda, a net transepithelial transport of sodium ions is the driving force for water reabsorption. The rate of water and sodium resorption can be altered under treatment with antiandrogens and efferent duct ligation. This change was accompanied by loss of sperm motility (Wong et al., 1978). Wong and Yeung (1977) also showed that fluid resorption in the rat epididymis is dependent on the presence of circulating androgens and is also influenced by other hormones such as epinephrine, norepinephrine (in presence of phenoxybenzamine) and gonadotropins. The cauda epididymis (previously held to be a storage organ) was shown to have an active transporting role. Water was reabsorbed down the osmotic gradient generated by active Na⁺ transport and K⁺ was secreted into the ductal lumen, proteins were also secreted into the lumen (Wong and Yeung, 1978). It appeared that the epididymal epithelium actively maintained a particular environment within the tubule in which the spermatozoa were maturing.
Scanning electron microscopy (SEM) has wide application in reproductive biology (Gould, 1973). Alterations in sperm morphology render them nonmotile, nonviable and loss of their fertilizing ability. The first SEM observations on mammalian spermatozoa were done by Dott (1969) and later by other (Fujita et al., 1970; Gould et al., 1971; Lung and Bahr, 1972; Yanagimachi and Noda, 1972; Hafez and Kanagawa, 1973; Flechon and Bustos-Obregon, 1974; Chinoy et al., 1979 b; 1980 a; e; Chinoy and Sanjeevan, 1980; Chinoy, M.R. et al., 1980 a; Chinoy, N.J. and Chinoy, M.R. 1980 a, b, 1981; Chinoy and Rao, 1981). Thus SEM is a useful tool for the easy observation of fine superficial details of spermatozoa in three dimensions; it is especially suited for analysis of sperm populations inorder to detect natural or induced abnormalities in the fields of animal husbandry and in medical or basic biological research. The sickle shaped head of rat spermatozoa (Chinoy et al., 1979 b; Chinoy and Sanjeevan, 1980; Chinoy et al., 1980 a, e; Chinoy, M.R. et al., 1980 a, b; Chinoy and Chinoy, 1980 a, b; 1981; Rao, 1981), paddle shaped of guinea pig (Koehler, 1975; Fawcett, 1977; Sanjeevan, 1979; Chinoy, 1981), scimitar shaped in mouse (Chinoy et al., 1980 b) and pyriform in human beings (Chinoy and Rao, 1981; Fujita, 1975; Zaneveld, 1975) have been observed. In guinea pig only, the sperms appeared in rouleaux formation. The presence of cytoplasmic droplet in caput epididymal or immature spermatozoa has also been documented (Mann, 1964; Flickinger, et al., 1978; Chinoy, M.R. et al., 1980 a).
It is evident that epididymal tissue can synthesize and/or secrete and metabolize numerous macromolecules which are important in maintaining its structural and functional integrity as well as the metabolism and integrity of their contained spermatozoa. The comparative biochemistry of mammalian epididymal plasma was studied by Jones (1978). Dawson et al. (1957) first noted that the epididymis contained large amount of glycerylphosphorylcholine (GPC), and their observations have been confirmed and extended by numerous investigators (Scott and Dawson, 1968; Scott et al., 1963; Wallace et al., 1966). Scott et al. (1963) provided convincing evidence that the epididymis is the major source of this compound in semen, and not the testis. It may have stabilizing effect on spermatozoa, and it may play a role in maintaining osmotic pressure balance in the lumen as NaCl is absorbed (Scott et al., 1963).

Higher amounts of total and phospholipids are observed in caput than in cauda epididymis of rat, sheep and monkey (Rajalakshmi et al., 1976; Prasad and Rajalakshmi, 1977). In human beings little variation is seen in the lipid levels in the two regions of the epididymis (Rajalakshmi et al., 1975). Sudanophilic lipids and lipase activity in epithelial cells of caput and cauda
epididymides of rat and rabbits have also been demonstrated (Ambadkar, 1969, 1973). Prasad and his collaborators have shown that the major phospholipid present in caput and cauda epididymides of hamster, monkey and human beings are phosphatidylcholine and phosphatidylethanolamine (Arora et al., 1975; Prasad and Rajalakshmi, 1977; Rajalakshmi and Prasad, 1979) the high concentrations of the phosphatidylcholine in the caput may be related to synthesis of GPC (Voglmayr, 1975). In caput spermatozoa, the phospholipid concentration is high and decreases during their transit through corpus and cauda epididymides.

Carbohydrates may be produced by the epididymis, since the epididymis contains a number of enzymes for carbohydrate metabolism and synthesis, and many of these have been localized in the epithelium histochemically (Gustafsson, 1966). Maneely (1955), Cavazos (1958), Malone and Bower (1962) and Hamilton (1972) have shown histochemically the presence of carbohydrates in rat and Hamster epididymis. Turner and Johnson (1973 a, b) found a significantly higher utilization of glucose and Krebs-cycle intermediates by the intact mouse epididymis. The epididymal spermatozoa utilize glucose via Embden-Meyerhof pathway in vitro (Wu et al., 1959) or via pentose shunt pathway (Johnson and Turner, 1971; Turner and
Johnson, 1972, 1973a, b) or they utilize lactate as the main energy substrate (Cross and Silver, 1962; Wales et al., 1966). Histochemically, presence of glycogen was described by Montagna and Hamilton (1952) and Nicander (1957a, b). Sialic acid has an important role in epididymal function (Prasad and Rajalakshmi, 1976, 1977) but the histochemical localization pattern is not available.

The ability of the different segments of the epididymis to synthesize protein in vivo was demonstrated for the first time by Rajalakshmi and Prasad (1976) and was observed to be an androgen dependent event. This is an anabolic effect of testosterone leading to new synthesis of proteins associated with growth and secretory activity of epididymal cells during post-natal development (Singh et al., 1977a, b). Flickinger (1979) also showed evidence of protein synthesis in mouse epididymis. Sedlakova et al. (1968) identified albumin in the epididymal fluid and assumed that it was originated from serum. But presence of several proteins unique to epididymis in different species, suggest that the tissue of the epididymis can secrete specific proteins into the lumen of the epididymal duct (Hung and Johnson, 1975; Crabo and Hunter, 1975). Some of these proteins are sperm associated antigens of epididymal origin (Barker and Amann, 1969; Johnson and Hunter, 1970). The latter authors and Crabo and Hunter
(1975) suggested that these antigens coat the sperm during its passage through the epididymis.

Several oxidative and hydrolytic enzymes are present in epididymis and these are androgen sensitive (Allen and Slater, 1957, 1958, 1959, 1961 a, b; Cavazos, 1958; Martan and Risley, 1963; Prasad et al., 1972; Pinero and Roussel, 1973; Ambedkar, 1969, 1973; Riar et al., 1973; Nag et al., 1975; Einarsson, 1976; Brooks, 1976; Sud, 1977; Nikkanen and Vanha-Perttula, 1977; Arora-Dinakar et al., 1977 c, d; Dinakar et al., 1977 b; Chinoy and Seethalakshmi, 1978 a; Majumder and Biswas, 1979). The role of these enzymes have been implicated in various epididymal functions and metabolism of different substances.

The high concentration of carnitine was first reported by Marquis and Fritz (1965 a, b, c), and later by Pearson and Tubbs (1967). Numerous contributions on carnitine were made by Brooks et al. (1973, 1974), Casillas (1972, 1973) and others.

The presence of steroids in seminal fluid is reported (Breuer, 1955; Dirschl and Breuer, 1963; Dirschl and Knuchel, 1950; Steeno et al., 1966; Mullen et al., 1968; White and Hudson, 1968; Oertel and Tribber, 1967); and it is known that the epididymis actively synthesizes steroids as it possesses the enzymic machinery for steroid biosynthesis from some precursors (Hamilton, 1977;
Hamilton et al., 1968, 1969; Hamilton and Fawcett, 1970; Frankel and Elk-Nes, 1968, 1970), and thus probably contributes to the steroid content of the seminal fluid. The metabolism of androgens by cauda epididymal spermatozoa in vitro was demonstrated by Rajalakshmi et al. (1978). Histochemical localization of 3β- and 17β-hydroxy steroid dehydrogenases were demonstrated in epididymis of rats (Chinoy et al., 1979 b; 1980 a; Rao, 1981, unpublished observations). Two steroid binding proteins have been characterized in rat epididymis (Hansson et al., 1974; 1975, 1976; Denzo et al., 1973). One of these binds specifically to 5α-DHT and the other is ABP (Purvis and Hansson, 1978), which has affinity for both 5α-DHT and testosterone. It is suggested that ABP is synthesized in testis on stimulation by FSH and carried to epididymis via rete testis and ductuli.

There is virtually no information on the role of vitamins in epididymal physiology, although it is known that vitamins A and E are important for reproduction. Histochemical and ultrastructural localization of ascorbic acid has been carried out in our laboratory on epididymis, testis, vas deferens and other reproductive organs of rats, guinea pigs and mice for the first time. Higher contents of ascorbic acid were observed to be localized in the nuclei than in the cytoplasm of all the
reproductive tissues investigated (Chinoy, 1978, 1979; Chinoy and Sanjeevan, 1978 a; Seethalakshmi and Chinoy, 1976; Chinoy and Seethalakshmi, 1978 b; Chinoy et al., 1979 c; Chinoy and Chinoy, unpublished observations; Chinoy and Sharma, unpublished observations). A higher content of ascorbic acid was present in tissues with a corresponding higher tempo of metabolic activity. It was also observed that the concentration of ascorbic acid was higher in reproductive tissues than in the liver, wherein it is synthesized (Chinoy and Seethalakshmi, 1978 b; Chinoy, 1978, 1980). The epididymal tissue and sperm are capable of metabolizing ascorbic acid and a depletion of ascorbic acid occurs in rat testis and epididymis on day 45 with the onset of puberty (Chinoy et al., 1980 b, c; Chinoy and Asok Kumar, 1980).

The structure and physiology of the accessory sex glands of the male have been known for some time, but the details and the exact mechanisms by which they interact in the reproductive process is still not clear. Evidences suggest that the effects of male sex hormones on accessory reproductive organs are mediated partly or entirely by intracellular metabolites of the circulating androgens (Williams-Ashman and Reddi, 1971; Ofner, 1971). Baulieu (1973) has reviewed the mode of action of steroid hormones. The studies elucidating the action of androgens on the seminal
vesicles, prostate gland and the bulbourethral glands have involved chiefly biochemical, histochemical and fine structural techniques (Mann, 1954, 1956, 1964; Cavazos and Melampy, 1954). A number of reviews have appeared on sex accessory glands of rat, mouse, dog and man (Mann and Lutwak-Mann, 1951; Price and Williams-Ashman, 1961; Cavazos, 1975; Brandes, 1966; Brandes and Growth, 1963; Flickinger, 1971; Spring-Mills and Hafez, 1980).

Two types of cells are observed in epithelium of seminal vesicle, the principal or superficial, glandular cells and basal cells. The epithelium is pseudostratified. The principal cells within active glands are usually columnar in shape. Their apical surfaces are either smooth or are covered with short microvilli. The basal plasmalemnae cover interdigitating cytoplasmic processes which interlock these cells at the base with comparable processes on neighbouring cells (Riva, 1967). The arrangement of mucosa varies from one area of the gland to another in normal individuals and showed additional alterations with advancing age or disturbances in hormonal status (Spring-Mills, 1980). Mucosal ridges and folds vary considerably even within the same cross-section. The internal walls contain interconnecting ridges and folds supported by thin vascularized cores of lamina propria covered by simple or pseudostratified epithelium.
Surrounding the central lumen, both circular and longitudinal folds are present in the mucosa. The thickness of the ridges and folds also varies considerably. The recesses between the ridges and folds vary in size as well as in the amount of contained secretion which is a secretory product of the gland. In active glands of young, intact rats and mice, the nuclei are centrally or basally placed and are approximately 40% as tall as the entire cell (Spring-Mills, 1980).

Moderate reaction of periodic acid-Schiffs in the cytoplasm of the epithelium of the hamster seminal vesicle was observed by Feagans et al. (1961), but only a slight colouration in that of rat (Leblond, 1950; Melampy and Cavazos, 1953). Other workers (Cavazos, 1963; Belt and Cavazos, 1967; Kovacs, 1968) have shown electron microscopically that electron dense structures of variable sizes, are the lysosomes, which are the sites of focal cytoplasmic degradation and have a considerable reaction to acid phosphatase. The secretion of the seminal vesicles are viscid, white or slightly yellowish in colour (Spring-Mills and Hafez, 1980). In most species, the seminal vesicles are capable of intense synthetic and secretory activity. The seminal vesicle contributes a substantial
portion to the whole ejaculate and has a remarkable concentration of reducing substances. The reducing substances belong to two groups; one is made up of molecules such as ergothioneine and ascorbic acid; the other is chiefly sugars. The most important free sugar secreted by the gland is fructose (Mann, 1964). This sugar can serve as a good index to evaluate the secretory activity of the seminal vesicle. Other reducing sugars are free carbohydrates; inositol, glucose, ribose, fucose and sorbitol which are present in small amounts (Brandes, 1974). The seminal vesicles maintain a peculiarly high concentration of potassium, whereas Na⁺ is much lower than K⁺ and Cl⁻ is surprisingly almost absent (Brandes, 1974). Von Euler and Eliasson (1967) have shown that seminal vesicle secretion contains the largest concentration of prostaglandins (PG).

Prostate as a whole is a complex gland, divided into three parts in rats, as ventral prostate, dorsolateral prostate and coagulating gland (Price, 1963; Cavazos, 1977). It is a tubulo-alveolar gland, produces a colourless fluid, pH about 6.5, rich in proteolytic enzymes and almost lacking in reducing sugars. The heterogenous nature of the developing human prostate gland was noted by Lowsley (1912). The epithelium of the prostate forms multiple compound tubulo-alveolar glands that appear in
sections as irregular circular, oval and curving alveoli or saucules lined with a columnar epithelium. The epithelium is pseudostratified and contains two types of cells: (1) a secretory or glandular cell, and (2) a non-secretory basal cell. The interstitial tissue fills the space between the epithelial elements and forms sheets or septa that separate and invest the alveoli. The two most numerous cell types in the interstitial tissue are smooth muscle cells and fibroblasts.

The prostate gland of male mammals contributes numerous components to semen (Price and Williams-Ashman, 1961; Mann, 1964). The most characteristic constituents of prostate are acid phosphatase, citric acid, zinc and spermine. Protein synthesis occurs in prostate in the rough endoplasmic reticulum and is transported to the Golgi, wherein, it gets packed in vacuoles and is released into the lumen (Flickinger, 1974). The fine structure of the interstitial tissue of the rat prostate has been studied by Flickinger (1972). 

Ascorbic acid is an important biologically active reductant, that plays a dynamic role in several oxidoreduction reactions. The occurrence of ascorbic acid in the testis and the semen of several animals including human beings is known. Guinea pigs, primates and human
beings are unable to synthesize ascorbic acid and they have to depend on dietary source (Burns et al., 1956; Hassan and Lehninger, 1956; Chatterjee et al., 1960). The loss of capacity to synthesize ascorbic acid is an example of evolutionary loss of function and is related to the genetic absence of the enzyme, gulonolactone oxidase from the liver microsomes, which converts L-gulono-δ-lactone to ascorbic acid. Gulono-lactone oxidase was observed to be androgen-sensitive in rat liver (Majumder and Chatterjee, 1974). Rosenberg (1945) and Kutsky (1973) have shown that the presence of traces of Mn++ is necessary for the successful synthesis of ascorbic acid from sugars.

Ascorbic acid occurs in the free and the bound form or ascorbigen in animal tissues (Malakar, 1963; Chinoy, 1971 a, b; 1972 a, b, c; Chinoy and Seethalakshmi, 1975/1977; Chinoy, 1978; several other papers from this laboratory). The ascorbigen content and storage capacity of tissues vary considerably. There are various storage sites of the vitamin in the body, viz., liver, adrenals, gonads, hepatopancreas, brain, kidney, accessory glands of reproduction. The storage, tissue distribution and also synthesis of ascorbic acid is under the control of sex hormones in cockerels and rats (Dieter, 1969; Majumder and Chatterjee, 1974; Chinoy and Rao, 1979; Chinoy and Seethalakshmi, 1978 b; Chinoy et al., 1979 c). A profound
sex difference in synthesis and concentration of ascorbic acid in tissues of rats has also been demonstrated (Stubbs and McKernan, 1967).

It is known that ascorbic acid participates in several oxido-reductive processes. The oxidation of ascorbic acid involves a two-step mechanism. (1) In the first step, its oxidation is catalyzed by a special peroxidase (Chinoy, 1970; 1973; Gurevich, 1963; Gorbunova, 1966); which monovalently oxidizes ascorbic acid to its free radical, monodehydroascorbic acid (MDHA), which is a highly unstable semi-quinone like radical (Staudinger et al., 1961; Yamasaki et al., 1959). (2) MDHA is finally converted to the oxidized form, dehydroascorbic acid (DHA). Glutathione is responsible for reducing DHA to ascorbic acid in animal tissues, and thus oxidation and reduction are two separate phenomena which complete the whole cycle (Rosenberg, 1945; Bicknell and Prescott, 1953; Sebrell and Harris, 1967).

Extensive studies carried out from this laboratory have revealed for the first time that the tissue metabolism is energized not only by the high energy phosphate (ATP), but also directly by the paramagnetic electron flow from MDHA which functions as an electron donor and participates in several oxido-reduction reactions as a source of electron energy. The spin concentration of MDHA can be determined
by electron spin resonance (ESR) spectrometry. It revealed that ascorbic acid yields a characteristic ESR spectrum which is a doublet with a g-value of 2.004 and a line width of 1.8 gauss. It was recorded in cauda epididymal sperm suspensions of rats and in normospermic semen of human volunteers for the first time by Chinoy and her associates (Chinoy and Buch, 1977 a; Chinoy et al., 1978 b, 1979 b).

Further, it is evident that ascorbic acid forms charge transfer complexes (CTC) with macromolecules viz., proteins, nucleic acids, and steroids (Slifkin, 1971; Swartz et al., 1972; Chinoy et al., 1978 c). The formation of CTC helps in establishing a direct electron energy flow for synthesis of cell constituents. In CTC only one electron is transferred as opposed to oxido-reduction reactions wherein two electrons are transferred. Therefore, vitamin C functions as biologically active reductant in animal tissues via the formation of its free radical and CTC formation with macromolecules. The third important mechanism of ascorbic acid is its indirect involvement, since it inhibits the activity of the enzyme, phosphodiesterase, and hence, increases the levels of cyclic-AMP. C-AMP is a second messenger and known to activate many enzymes and thus ascorbic acid indirectly activates several enzymes by increasing the levels of cyclic-AMP.
Several workers have shown that ascorbic acid activates numerous hydroxylating enzymes and those involved in oxido-reduction reactions in various tissues (Bourne and Kidder, 1953; Sebrell and Harris, 1967; Kutsky, 1973). Recently, it has also been reported that AA activates androgen dependent enzymes in androgen target tissues and sperm (Chinoy and Seethalakshmi, 1975/1977; Chinoy et al., 1977; Chinoy, 1978). Ettlinger et al. (1961) had suggested that ascorbic acid has coenzyme like activity, however Lewin (1976) observed that adenyl cyclase, which is the key enzyme necessary for the formation of C-AMP is also activated by ascorbate.

Several studies have revealed that metabolism of ascorbic acid and testosterone are inter-related, as ascorbic acid is involved in the synthesis of androgen and likewise ascorbic acid synthesizing enzymes in the liver are androgen dependent (Dieter, 1969; Majumder and Chatterjee, 1974; Chinoy and Seethalakshmi, 1978 b; Chinoy et al., 1979 c). Moreover, ascorbic acid augments the anabolic actions of testosterone in androgen target organs, so that their structural and functional integrity are maintained in animals under treatment of various kinds (Chinoy and Buch, 1977 b; Buch et al., 1978; Chinoy, 1978; Chinoy and Chinoy, 1979 a; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978 b, c; 1979 b).
Ascorbic acid has a synergistic action with testosterone and potentiates its anabolic action for germ cell maturation, increasing activity of androgen-sensitive enzymes/metabolites in target tissues (Kutsky, 1973; Chinoy and Seethalakshmi, 1977 a-c; 1978 a,b; Chinoy, 1978; Chinoy et al., 1977 a-c; 1978 b) under normal and altered physiological conditions.

The depletion of ascorbic acid in ovary under influence of LH is known (Parlow, 1958, 1961). Agrawal and Laloraya (1977) have also shown that ascorbic acid undergoes rapid oxidation in the presence of peroxidase producing an intermediate free radical, which, when coupled with pregnenolone produces progesterone in rat corpora lutea. A dose dependent ascorbic acid depletion by LH in immature rat testis (Chinoy et al., 1980 b, c) and in Leydig cells (Greep, 1976) have also been reported.

Antiandrogens, like cyproterone acetate inhibit the action of 3 β and 17 β hydroxy-steroid dehydrogenases of the testis and cauda epididymis, but their activity was restored to control level after ascorbic acid feeding to rats along with antiandrogen treatment (Chinoy et al., 1979 b). Ascorbic acid also activates the hydroxylation of dehydro-corticosterone to hydroxy-corticoids in adrenal cortex (Fisher, 1962; Kutsky, 1973). Datta and Sanyal (1978) have shown that trophic hormones, PGs, c-AMP and
ascorbic acid play a combined role in the regulation of adrenal and gonad steroidogenesis acting at different levels of hypothalamo-hypophyseal-adrenal-gonadal axis. Therefore, the above data suggests that ascorbic acid is involved in a hitherto unknown manner in steroidogenesis in gonads as well as in adrenal, but its precise mechanism of action still remains an enigma.

The metabolic significance of ascorbic acid has been recently reviewed by Chinoy (1978). Chinoy et al. (1974 a, b; Chinoy and Parmar, 1975 a, b) have correlated the higher concentration of ascorbic acid in tissues having a correspondingly high tempo of metabolic activity as well as the actively growing embryonic and developing tissues. High concentration of ascorbic acid in wound healing process and regenerating tissues are well documented (Gould, 1963; Shah et al., 1971).

Ascorbic acid also prevents the oxidation induced by radiation and acts as a radio-protective agent in several tissues including reproductive organs (Ellis, 1970; Chinoy and Buch, 1977 b; Chinoy et al., 1980 d; Chinoy and Garg, 1977).

For the first time it has been suggested that ascorbic acid has an important role in human sperm motility and metabolism via the formation of MDHA (Chinoy and Buch, 1977 a)
and similar observations have been made on rat spermatozoa 
(Chinoy et al., 1978 b, d, e; 1979 b; Chinoy and Sanjeevan, 
1978 b; Rao et al., 1978).

Ascorbic acid plays an important role in overcoming 
the induced stress conditions (Selye, 1950), and it is an 
anti-stress factor (Kutsky, 1973). During various stress 
conditions, enhanced utilization of ascorbic acid was 
observed in testis, epididymides, accessory glands and 
other organs (Chinoy, 1978; Chinoy and Parmar, 1975 a, b; 
Chinoy and Sheth, 1976; 1977 a, b, c; 1978; 1979; Chinoy 
and Seethalakshmi, 1977 a-c; 1978 a-d; Sheth and Chinoy, 
1977). The increased rate of vitamin synthesis is related 
to the increase in the levels of stress induced histamine 
and its detoxification by ascorbic acid (Nandi et al., 1974). 
The studies carried out from this laboratory on the effect 
of vasectomy, cyproterone acetate, cadmium chloride and 
several antifertility drugs have suggested that ascorbic 
acid and its mechanism of action have important implica-
tions in the prophylactic treatment following vasectomy, 
administration of cyproterone acetate and other 
antifertility drugs without interfering with the contrace-
ptive purpose of the treatment. Ascorbic acid synergises 
with the endogenous testosterone and potentiates its 
anabolic action for maintenance of the structural and
functional integrity of androgen sensitive organs. This potentiation effect is brought about by the formation of charge transfer complex of ascorbate and testosterone, which upon breaking would yield the free radicals of both the interacting substances (Chinoy, 1978; Chinoy et al., 1978 c; Seethalakshmi and Chinoy, 1978). Thus, these studies have important implications on human fertility control especially those studies involving use of antifertility drugs and steroids, as lower doses of steroids would be needed if coupled with ascorbate and with other contraceptive drugs, the stress induced alterations in reproductive and non-reproductive tissues would be overcome by ascorbate without interference with the effects of the contraceptive. Our results have added significance since Alexander and Clarkson (1978) reported that vasectomized monkeys were more prone to develop atherosclerosis than the control ones. Therefore, ascorbic acid feeding to vasectomized individuals would have a beneficial effect in reducing cholesterol levels in blood, as it is known to oxidize it (Ginter et al., 1970; 1971; 1972; Ginter, 1975). This would be in addition to the other prophylactic effects of ascorbate during vasectomy as reported earlier (Chinoy and Sheth, 1977 c; Chinoy et al., 1978 b).
Ascorbic acid also has an important role in metabolism of drugs by the liver, detoxification of drug induced histamine and has a prophylactic influence on tissue metabolism following drug administrations (Zannoni et al., 1972; Subramanian et al., 1973; 1974; Nandi et al., 1974; Chinoy and Sheth, 1976, 1977 a; 1978; 1979; Buch et al., 1978; Chinoy and Kshatriya, 1976 a, b, 1978; Chinoy and Seethalakshmi, 1978 c, d). The turnover of ascorbic acid is enhanced by androgen deprivation.

On the basis of the above studies it was postulated that tissue metabolism is energized not only by high energy phosphate (ATP) but also via the paramagnetic electron flow from ascorbate free radical. Hence, ascorbic acid and its mechanism of action have important implications in the prophylactic treatment of volunteers during and following a contraceptive treatment.

It is well known that the metals and electrolytes play an important role in reproduction. Although copper has long been known to be involved in reproductive processes, interest has increased considerably due to the recent clinical application of the copper intrauterine devices. It has been suggested that the copper intrauterine device has its principal contraceptive effect by inactivation of spermatozoa (Ullman and Hammerstein, 1972; Elstein and Ferrer, 1973; Heinawi et al., 1975). Copper is
a powerful catalyst for the auto-oxidation of glutathione, an -SH containing compound and other reducing substances. The participation of membrane -SH groups in epididymal maturation of human and rabbit spermatozoa has been implicated (Reyes et al., 1976). The S-S bonds are important for the structural stability of spermatozoa since reduction of these compounds with dithiothreitol followed by the action of the detergent, SDS, causes the head to swell and tail to dissolve (Calvin and Bedford, 1971; Calvin et al., 1975). Bedford and Calvin (1974) have shown that as the spermatozoa mature in the epididymis, there is an increasing amount of disulphide bond formation. The rigidification of the head by normal disulphide bond formation, could play a crucial role in the fertilization process (Bedford and Calvin, 1974). Only copper in the mid-piece region was positively correlated with motility when high and low fertility groups were compared (Battersby and Chandler, 1977). Gilmore et al. (1973) have found that copper wires implanted into animals have limited effect on subsequent fertility. Copper might act synergistically with the releasing hormone to cause the release of LH and FSH (Fevold et al., 1936; Suzuki et al., 1970; Labella et al., 1973). Copper might be involved in steroid metabolism in the aromatization of androgens, and inhibits PGE1 synthesis (Oster and Salgo, 1977).
Zinc like copper, affects practically all phases of mammalian reproductive processes in both males and females (Underwood, 1971). Copper and zinc are often antagonists (Van Campen, 1969; Murthy et al., 1975; Underwood, 1974), but zinc is essential for spermatogenesis in mammals (Gunn and Gould, 1970). Zinc is found in high concentration in spermatozoa. Spermatids, Sertoli cells and spermatozoa in the testis and other components of testis have less amounts of zinc (Gunn and Gould, 1970). Initially, spermatozoa obtain zinc during their development in the seminiferous tubules and carry with them during their transit through the epididymis. According to Lindholmer and Eliasson (1972), the highest zinc concentration was found in the dead and immotile spermatozoa and among the tissues, the prostate gland (dorsolateral prostate), wherein, the Zn$^{++}$ concentration of seminal plasma seems to originate (Gunn and Gould, 1970; Greep and Koblinsky, 1977). It is also found to be an essential component of a number of androgen-sensitive enzymes, such as, succinate dehydrogenase, lactic dehydrogenase, malic dehydrogenase, alkaline phosphatase etc., which are Zn-metalloproteins.

Zn$^{++}$ has a possible inhibitory effect on the succinate oxidase system by an interaction with the plasma membrane and might be an important constituent of the sperm membrane making it less permeable to various substances.
These results support the hypothesis that zinc removal from the spermatozoa is essential for their fertilizing ability (Johnsen and Eliasson, 1978). Zn\(^{++}\) in human spermatozoa is loosely bound and can be easily washed out (Lindholmer and Eliasson, 1972), whereas, in rat spermatozoa it is firmly bound and no loss was detected after repeated washings (Birnbaum et al., 1961). Zinc is also related to the morphology of sperm tails (Eliasson, 1976) and direct relationship of seminal plasma Zn\(^{++}\) with sperm motility has also been elucidated by Skandhan et al. (1978).

Fair et al. (1976) identified a Zn\(^{++}\) salt as an antibacterial factor. They suggest that zinc may serve in defense mechanism for the prostate against bacterial invasion and subsequent urinary tract infection. Influence of Zn\(^{++}\) on protein synthesis by polyribosomes from the dog prostate and the dorsolateral lobes of the rat, has also been demonstrated by Webb et al. (1973).

Zinc is concentrated in the flagellar accessory fibers (outer dense fibers) in most of the mammals and cephalopods (Baccetti et al., 1976), which also contain the highest percentage of sulphurhydryl groups. Evidently, the sulphurhydryl groups are involved in the binding of zinc (Baccetti et al., 1976).
Manganese is also one of the important trace elements for reproduction. Its deficiency causes testicular degeneration, atrophy of accessory sex glands, lack of sexual interest and sterility. There are few reports available to elucidate the importance of manganese in reproductive functions. It has been reported that the greatest storage of manganese in rat occurs at the time of sexual maturity. Mn\textsuperscript{++} is capable of being incorporated into the interstitial tissue of testis and some phase of development of spermatozoa which are transported into the epididymis (Gunn and Gould, 1970). It helps in spermatogenesis and is a potent protector against cadmium toxicity (Gunn et al., 1970). Manganese is associated with spermatozoa as it is necessary for the activity of sperm specific adenyl cyclase. Most of the Mn\textsuperscript{++} is also known to be localized within the mitochondria as well as it binds at the major grooves of the DNA helix (Gunn and Gould, 1970; Reuben and Gabbay, 1975). Seethalakshmi et al., (1980) found that the human seminal plasma Mn\textsuperscript{++} concentrations were lowered by vasectomy as compared to those of normal subjects and suggested that the higher Mn\textsuperscript{++} concentration was related to greater sperm density and motility. It was also found that the Mn\textsuperscript{++} free radical signal was unaltered by vasectomy in rats (Chinoy and Seethalakshmi, 1978 e) and in human semen (Seethalakshmi
et al., 1980), showing thereby that utilization of Mn\(^{++}\) by epididymis was not altered by vasectomy.

Reports on comparative aspects of reproductive organs are obscure. In the light of above data, the present investigation was undertaken, to elucidate in detail the comparative aspects of testis, epididymis, seminal vesicle and ventral prostate of rats and guinea pigs. Emphasis has been laid on studying sperm motility pattern, sperm density and fertilizing ability; histophysiology, histochemistry, scanning electron microscopy of spermatozoa from different regions of epididymis, metal ion profile and histochemical localization of ascorbic acid. The contractile pattern of isolated rat epididymis was also recorded by using different doses of adrenaline and noradrenalin.

PART II

STUDIES ON EFFECT OF ANDROGEN DEPRIVATION AND ANDROGEN REPLACEMENT:

1. EFFECTS OF ANDROGEN DEPRIVATION BY CYPROTERONE ACETATE (CA), ASCORBIC ACID THERAPY AND EFFECTS OF CA WITHDRAWAL:

Cyproterone acetate (1,2-a-methylene-6-chloro-pregn-4, 6-diene-17 a-ol-3, 20-diene-17a-acetate), a synthetic steroid, is a potent androgen antagonist which has been explored in greater detail than any other antiandrogen.
Neumann and his associates have made numerous contributions on the action of cyproterone acetate (Neumann et al., 1970; 1976; Neumann and Steinbeck, 1974; Neumann and Schenck, 1976; Neumann and Van Berswordt-Wallrabe, 1966). Cyproterone acetate (CA) prevents the differentiation of the male reproductive tract by foetal and post-natal androgens (Neumann, et al., 1966). The steroid also inhibits the action of testosterone on the hypothalamic cells that regulate gonadotropin secretion (Bloch and Davidson, 1967). The steroid neither alters the sexual behaviour of male rats or guinea pigs (Zucker, 1966; Beach and Westbrook, 1968; Whalen and Edwards, 1969; Prasad et al., 1971/72 nor the androgen-dependent aggressive behaviour of male mice and gerbils (Edwards, 1970; Sayler, 1970).

All antiandrogens compete with androgens for their specific binding sites in androgen target cells (Fang and Liao, 1969; 1971; Coffey, 1974). "Pure" antiandrogens also block the effect of androgens on the pituitary-hypothalamic centers, which are responsible for the feedback regulation of gonadotrophin secretion. This means, that "Pure" antiandrogens simulate an androgen deficit, comparable to the effects of castration, on the pituitary-hypothalamic system (Neumann, 1966). Hohlweg (1956) has reported that hypothalamic centers controlling gonado-
trophin secretion change their sensitivity in response to long-term gonadal steroid deprivation or excess. Cyproterone has only antiandrogenic effects, whereas, cyproterone acetate has both antiandrogenic as well as antigonadotrophic activity.

Accessory sexual gland functions as well as sexual behaviour are among the processes which are maintained by the action of testicular androgens in male mammals including man. Prevention of access by androgens to these targets, either surgically or chemically results in impairment or even abolition of their normal function. Cyproterone or its acetate has been claimed to inhibit any effects of endogenous or exogenous androgens (Neumann et al., 1970), presumably in the target organs by competitive antagonism. Conversion of testosterone to 5α-dihydrotestosterone is now known to be an essential prerequisite to androgen action in prostate gland and skin (Raspe, 1971), though not muscle (Hansson et al., 1971). CA competitively inhibits binding of dihydrotestosterone (DHT) by the epididymal cytosol (Ritzen et al., 1971) and the nuclear DHT receptor complex from rat ventral prostate (Smith et al., 1978). CA partially decreases uptake of radioactive testosterone in the seminal vesicle and ventral prostate which are most susceptible to antiandrogens (Belham et al., 1971; Geller et al., 1968; 1969; Stern and Eisenfeld,
1969; 1971; Whalen et al., 1969), but it does not reduce
the conversion of testosterone to DHT (Belham et al.,
1971).

Schenck and Neumann (1978) reported the effects of
CA on male rats treated with daily doses ranging from
0.625 to 30 mg/day S.C. for six weeks. The highest dose
of CA inhibited spermatogenesis and reduced testicular
weights as well as ABP content and concentration in the
caput epididymis of treated rats, but the serum FSH,
LH and testosterone levels were unaffected. Their data
revealed that CA acts directly upon the testis and inhibits
Sertoli cell function as reflected by the decreased ABP
production. These authors also reported that CA does not
compete with androgens for binding to ABP and, therefore,
has no other effect on androgen transport in the testis
than inhibition of ABP production. Accumulation of lipids
in Sertoli cells and mitochondrial changes in all stages
of the cycle of the seminiferous epithelium in 30 day
CA (10 μg/day/rat) treated animals was found by Hovatta
and Koskimies (1978). The phosphatases and dehydrogenases
of rat testis were also affected by CA (Kalla and Ehasin,
1977). CA may also have a direct effect on testicular
biosynthesis of testosterone (Mergins and Stitch, 1974).
By impairing the Δ4-pathway of androgen synthesis, the
transformation of \( ^{3}H \)-pregnenolone to \( ^{3}H \)-testosterone and \( ^{3}H \)-androstenedione is reduced. CA may have an inhibitory effect on 3\( \beta \)-hydroxysteroid dehydrogenase, a key enzyme in androgen synthesis (Purvis et al., 1978; Chinoy and Seethalakshmi, 1978a), but has no direct influence on 5\( \alpha \)-reductase activity (Neumann and Steinbeck, 1974), whereas, Rajalakshmi and Prasad (1976) have found that by long term CA treatment, the conversion of testosterone to DHT is inhibited in the prostate.

Spermatozoa have to undergo their final morphological and physiological maturation during their transport through the epididymis to acquire motility and fertilization capability (Gaddum, 1968; Orgebin-Crist, 1967; Prasad et al., 1971/1972). This maturation process depends on the proper epididymal milieu (Orgebin-Crist, 1967) which is in turn maintained by the presence of sufficient androgenic activity (Cavazos and Melampy, 1956; Martan, 1969; Prasad et al., 1971/1972), and to some extent also, by the normal flow of testicular fluid containing spermatozoa (Gustafsson, 1966; Rajalakshmi and Prasad, 1971). It has been demonstrated that the epididymis and ductus deferens are sites of androgen uptake and accumulation by cytosol and nuclear receptors (Blaquier, 1971; Hansson and Tveter, 1971; Ritzen et al., 1971). There is evidence that, like the seminal vesicles and prostate,
Dihydrotestosterone (DHT) is the active androgenic principle necessary for maintaining epididymal function (Blaquier, 1971). The androgen requirement to maintain the morphological and functional integrity of the epididymis is relatively high, compared with other androgen-dependent organs (Cavazos and Melampy, 1956; Prasad et al., 1971/1972). The epididymal need for high levels of androgen is provided from two sources, (i) absorption from the blood circulation and (ii) uptake of androgens which are contained in the testicular fluid (Voglmayr, 1966).

These conditions for maintenance of the functional and morphological integrity of the epididymis make it understandable that this organ is highly vulnerable to the action of antiandrogens. In rats, CA affects all genital organs directly. In respect to the susceptibility of these organs to CA, a hierarchy exists (Schenck and Neumann, 1978). Use of silastic implants with low-dose release of CA led to infertility without apparent loss of libido or spermatogenesis in rats (Prasad et al., 1970), epididymal sperm appeared to have lost normal function after long-term exposure to the drug (Rajalakshmi et al., 1971). Schenck et al. (1975) and Chatterjee et al. (1977), however, were unable to confirm these results. Histological and ultrastructural studies of the epididymis treated
with CA (Rajalakshmi and Prasad, 1975; Rajalakshmi et al., 1976; Prakash et al., 1979; Sharma et al., 1980) have shown that the absorptive and secretory functions are impaired following CA treatment. However, Flickinger and Loving (1976) did not obtain any change in the principal cells of the rat epididymis after CA treatment. Rajalakshmi et al. (1976) have reported loss of integrity of acrosome of cauda epididymal spermatozoa in CA treated rats. Back et al. (1977) have suggested that CA may interfere with the lining cells of epididymis. However, Glover and co-workers (1976) did not find any alteration in the concentration of sodium or potassium ions in the epididymal plasma in rats treated with CA. But Wong et al. (1978) reported a reduction in Na$^{+}$ and water reabsorption by cauda epididymis in CA treated rats.

CA administration has been reported to reduce testosterone levels (Brotherton and Barnard, 1974) and spermatogenesis (Brotherton, 1974) at higher doses in men and to decrease spermatozoa density and libido (Morse et al., 1973), but no consistent decrease was noted in FSH, LH levels or Leydig cell morphology (Brotherton, 1974; Morse et al., 1973). However, Briggs and Briggs (1971) observed that CA administration to human volunteers blocked spermatogenesis and severely reduced libido. Similarly, depression of libido by CA treatment was found
almost invariably in men, particularly in cases of aberrant sexuality (Laschet and Laschet, 1967 a, b; Laschet et al., 1967; Giese et al., 1968; Hoffet, 1968; Ott, 1968; Seebandt, 1968). Continuous administration for several weeks leads to a gradual reduction in testicular size, though this is reversible on withdrawing the compound. Spermatozoa from CA treated men were less motile, large number were deformed especially with deformities of head, and they were unable to penetrate the cervical mucus in Kremer test indicating their decreased penetrability (Neumann and Schenck, 1976; Roy and Prasad, 1978; Roy and Chatterjee, 1979).

In laboratory animals, CA suppresses $^3$H testosterone uptake (rat) (Sar and Stumpf, 1973), but not conversion of DHT (rat) (Peets et al., 1974). Supression of androgen production in rabbits (Grants and Stjäch, 1973), as well as gonadotropin inhibition, leading to depressed sex activity occurred (rabbit) (Agmo, 1975). Inhibition of libido insexually mature rats (Steinbeck, et al., 1967) as well as complete lack of influence in sexually experienced, cyproterone and cyproterone acetate treated rats and guinea pigs (Zucker, 1966), have also been reported.

Roy et al. (1976) and Roy and Chatterjee (1979) have administered mesterolone, a weak androgen along with CA to minimize the side effects of antiandrogen and to maintain libido in men. From our laboratory it has been
reported that CA manifests antianabolic and antifertility effects in rats and guinea pigs. The latter effects are probably related to the changes in the epididymal and vas deferens physiology which affect sperm maturation and result in abnormal spermatogenesis (Chinoy et al., 1979 b; Chinoy, M.R. and Chinoy, N.J., 1979 a; Chinoy and Maithili, 1980; Sharmas et al., 1980; Chinoy, N.J. and Chinoy, M.R., 1980 b; Chinoy, N.J. et al., 1980 e). The antianabolic effects were transient and almost reversible by combined CA + ascorbic acid administration and CA withdrawal. Ascorbic acid synergizes with testosterone to augment its anabolic functions in androgen target organs and is known to potentiate the anabolic action of endogenous androgen in CA treated rats, so that the structural and functional integrity of their reproductive organs are maintained (Chinoy and Sheth, 1977 d; Chinoy and Seethalakshmi, 1978 a; Chinoy et al., 1979 b; Chinoy, M.R. and Chinoy, N.J., 1979 a; Seethalakshmi and Chinoy, 1978; Chinoy and Maithili, 1980). The beneficial role of ascorbic acid in restoration of status quo of reproductive tissues under several altered physiological conditions including CA treatment, has led to the suggestion, that ascorbic acid be administered for prophylactic treatment of volunteers during and after CA treatment (Chinoy, 1978).
In the light of the above findings, the present study was undertaken, to carry out detailed investigations on long term CA administration, effects of its withdrawal and the combined treatment of rats with CA plus ascorbic acid. As such, the effects of CA are still controversial and the present findings would be a significant contribution to the pre-existing knowledge in this field. Therefore, histophysiological, histocytometric, atomic absorption spectrometric studies were carried out in epididymis, accessory glands and testis of male rats given the above mentioned treatments. Radio-immunocassay of serum testosterone under different treatments was also carried out.

2. **EFFECTS OF ANDROGEN DEPRIVATION BY CASTRATION: REPLACEMENT THERAPY WITH ANDROGENS AND WITH ASCORBIC ACID ALONE; AND COMBINED TREATMENTS WITH ANDROGEN+ASCORBIC ACID:**

In normal men, over 95% of the testosterone comes from the Leydig cells in the testis (Lipsett, 1975). The remaining 5% arises from peripheral metabolism of other testicular and adrenal steroids. Evidence is accumulating that DHT may come principally from peripheral 5α-reduction of the available testosterone and that the prostate may be the primary site of this conversion (Ito and Horton, 1971).
It is now well established that the epididymis is the site where sperm maturation takes place and which is an androgen dependent phenomenon (Rajalakshmi et al., 1976). In rat, the upper cauda epididymis which requires the highest levels of androgen for maintenance of its functions, is the region where sperm maturation is achieved. During the passage of the spermatozoa through the epididymis, they encounter a series of changes in lumen of the duct, and as a result they gradually gain motility and fertilizability. The acquisition of the fertilizing capacity is accompanied by a number of morphological and biochemical changes in the spermatozoa (Voglmayer, 1975; Hamilton, 1975; Chulavatnatol and Yindepit, 1976; Chulavatnatol et al., 1978; Orgebin-Crist et al., 1976; Greep and Koblinsky, 1977; Rajalakshmi and Prasad, 1979; Chinoy, 1980).

Rat seminiferous tubules secrete a fluid (Tuck et al., 1970; Cheung et al., 1977) which is reabsorbed by the epididymis (Crabo and Gustafsson, 1964; Levine and Marsh, 1971). Micropuncture experiments have shown that the concentration of Na\(^+\) decreased and that of K\(^+\) increased as the epididymal fluid flows down the epididymis. This observation suggests that both Na\(^+\) and water are reabsorbed by the epididymal epithelium (Levine and Marsh,
The results of Wong et al. (1978) on the ionic basis of water reabsorption in the rat cauda epididymis in vitro and in vivo have revealed that fluid reabsorption is a passive process and secondary to active Na\(^+\) transport (Wong and Yeung, 1976, 1977 a; 1978). This process is abolished by castration, suggesting that fluid reabsorption, similar to many epididymal functions, is dependent upon the presence of circulating androgens in the blood (Wong and Yeung, 1977 b, 1978).

The epididymis and accessory sex organs manifest differential sensitivity to androgen. The epididymis has a much higher threshold requirement of androgen than the other accessory sex glands for maintenance of its weight and secretory activity (Prasad et al., 1971/1972; Prasad and Rajalakshmi, 1976; 1977; Rajalakshmi and Prasad, 1971; Rajalakshmi et al., 1976; Chinoy and Sheth, 1977 c,d; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978a). A number of hormonal replacement studies have indicated:

(1) the differential androgenic potency of various metabolites of testosterone (Gloyna and Wilson, 1969; Paulieu et al., 1971; Robel et al., 1971), (ii) the higher threshold requirement of androgens by the epididymides than the accessory sex glands for their growth and secretory function in rats, hamster and rhesus monkeys (Djoseland et al., 1973; Prasad et al., 1973; Gupta et al.,
1974 a; Karkun et al., 1974; Dinakar et al., 1974 a, b; 1974; Sheth, 1976; Chinoy and Seethalakshmi, 1978 f; Chinoy et al., 1978 a), (iii) Rajalakshmi et al., (1971) have also shown that, lower threshold of androgen is needed for maintenance of the structural integrity of stereocilia than that required for maintenance of the secretory activity of epididymal epithelial cells, (iv) the cauda epididymal spermatozoa motility and metabolism too, have differential androgen sensitivity and (v) epididymal biochemical constituents, their spermatozoa and accessory sex glands also manifest a varied sensitivity to testosterone and its metabolites (Das et al., 1973; Chinoy et al., 1973 a, b, 1974 e; Gupta et al., 1974 a; Karkun et al., 1974; Dinakar et al., 1974 a, b; Sheth, 1976; Buch, 1976; Seethalakshmi, 1976; Rajalakshmi and Prasad, 1971; Chinoy et al., 1978 a). Therefore, the effects of castration and androgen deprivation are also varied.

The relative amount of receptors in the cytoplasm and nucleus of the prostatic cell is determined by the hormonal status of the animal. In the prostatic tissue of the non-castrated animal, most of the receptors are in the nucleus, whereas, 24 hours after castration, the receptor is detected mainly in the cytoplasm (Van Doorn
et al., 1976; Van Doorn and Bruchovsky, 1978). After long term castration, the total concentration of receptors in the cell declines, but is quickly resynthesized following injections of DHT (Bruchovsky et al., 1978). Thus androgenic effects on the cell are controlled through both the formation of new receptors and the binding of DHT to the receptor molecule.

The seminal vesicles secrete substances which affect sperm viability and sperm motility (Spring-Mills and Hafez, 1980). In species such as the guinea pig and rat, ligation or removal of the seminal vesicles either renders the animals sterile or depresses fertility by as much as 80%. Castration of men and other mammals cause the principal cells of seminal vesicle to atrophy, and the secretion to greatly diminish or cease altogether. In addition, the administration of the appropriate amounts of testosterone usually completely reverses these changes (Morley and Wright, 1972; Spring-Mills and Hafez, 1980).

Cell renewal in the male accessory sex organ is dependent on a permanent stimulation by testicular androgens and by removal of testis, the accessory sex organs shrink to their minimum size. A similar slope of involution has been found in the rat and mouse (Saunders, 1963; Tuohimaa and Niemi, 1974; Bruchovsky and Craven, 1975; Alison et al., 1976). Seminal vesicles undergo the most rapid and
extensive involution after castration, whereas, epididymis
and preputial glands are the least responsive to
castration (Saunders, 1963; Tuohimaa et al., 1973;
Tuohimaa and Niemi, 1974; Tuohimaa and Soderstrom, 1974).

The administration of androgen to castrated animals
promotes a dramatic increase in weight, intra- and extracellu-
lar secretions after a protracted delay or latent
period of 2 to 3 days (Tuohimaa et al., 1973; Tuohimaa
and Niemi, 1974; Tuohimaa and Soderstrom, 1974; Chinoy
et al., 1978 a). The effects of castration, testosterone
treatment and vasoligation on accessory gland function and
Levetor ani muscle was studied in rats and guinea pigs
(Chinoy et al., 1973 a, b; 1974 c). Similar studies have
been carried out on epididymis of several mammals, their
spermatozoa and hormonal levels in blood (Jehan et al.,
1973; Karkun et al., 1974; Dinakar et al., 1974 a, b;
Lubicz-Nawrocki, 1974; Dyson and Orgebin-Crist, 1973;
Jones and Glover, 1973; Orgebin-Crist, 1973; Shaar et al.,
1975; Croix, 1977; Setty et al., 1977; Barkley and Goldman,
1977; Veneziale et al., 1977; Tenniswood et al., 1978;
Moore and Bedford, 1979 a,b; Seethalakshmi and Chinoy,
1978; Morris and Jackson, 1978; Fischer and Swain, 1978).

The role of ascorbic acid in enzyme activation,
steroidogenesis, metabolism of spermatozoa, reproductive
processes and in stress mechanism, as a source of
Biosynthesis of ascorbic acid, its distribution in tissues is under the control of testosterone (Dieter, 1969; Majumder and Chatterjee, 1974) and in turn, ascorbic acid is involved in the biosynthesis of testosterone via the formation of its free radical monodehydroascorbic acid (Chinoy, 1978; Agrawal and Laloraya, 1978) as well as by formation of charge transfer complexes with steroids (Chinoy et al., 1978 c; 1980 c).

In castrated rats administration of testosterone helped to restore the secretory functions of epididymis and accessory glands differentially. Moreover, combined treatment with testosterone plus ascorbic acid manifested a synergistic or potentiation effect on anabolic action of testosterone in target organs (Chinoy and Sheth, 1977 c,d; Seethalakshmi and Chinoy, 1978).
The present study was undertaken to elucidate the metabolic role of ascorbic acid in epididymis, and accessory sex glands of male rats during castration, androgen replacement, ascorbate treatment and in combined testosterone and ascorbic acid treatments respectively in the light of recent data. A histophysiological, histocytometric and atomic absorption spectrophotometric study was undertaken.

3. STUDIES ON TESTOSTERONE (T) TREATMENT TO INTACT MALE RATS:

The problem of hormonal contraception for men, acting by inhibition of spermatogenesis is known. Aspermia could be induced by administration of sufficient amounts of any sex hormone, so that gonadotrophin suppression leads to failure of spermatogenesis.

Considerable attention has been paid to suppression of spermatogenesis by a variety of steroids and non-steroidal agents. These have been ably reviewed by Nelson and Patanelli (1965); MacLeod (1965); Jackson (1966, 1969; 1971); Jackson and Schnieden (1968); Fox and Fox (1967) and Gomes (1970).

Many investigators have considered the use of testosterone either alone or in combination with progestogens or estrogens as a male contraceptive agent (Frick, 1973; Johansson and Nygren, 1973; Skogland and
Paulsen, 1973; deKretser, 1974; Brenner et al., 1975; 1977; Maheesh et al., 1975; Ulstein et al., 1975; Alvarez-Sanchez et al., 1977; Frick et al., 1977 a, b; Steinberger and Smith, 1977; Melo and Coutinho, 1977; Bressler and Lustbader, 1978; Flickinger, 1978; Neumann et al., 1978; Scheerer et al., 1978; Falvo et al., 1979; WHO reports, 1979; 1980), because estrogens, androgens and progestins suppress gonadotropin secretion and spermatogenesis in both animals and human beings.

The effects of testosterone on the testis are dose dependent; small doses inhibit testicular function through suppression of pituitary gonadotropins, while larger doses maintain spermatogenesis by direct stimulatory effects on the seminiferous epithelium (Albert, 1961; Sharma et al., 1978). A number of workers (Heller et al., 1950; MacLeod and Heller, 1965; Nieschlag et al., 1978; Swerdloff et al., 1978; Flickinger; 1978; Steinberger et al., 1978) have shown that testosterone or testosterone enanthate cause suppression of spermatogenesis at the spermatocyte stage in 2 months without impairing accessory gland function or mating behaviour. These effects of testosterone were transient (Reddy and Rao, 1972; Reddy and Prasad, 1973; Cervantes et al., 1978; Melo and Coutinho, 1978).

Celotti et al. (1979) have reported that different
androgens may exert a separate control on the activities of the three enzymes, viz., 3β-, 17β-ol-dehydrogenase and 5α-reductase, which intervene in the metabolism of testosterone in the rat ventral prostate and seminal vesicles.

Preliminary studies by a number of investigators have suggested that a long acting testosterone ester can be administered in a dose regimen that reversibly suppresses both gonadotrophins and spermatogenesis, without significant side effects. These investigations have led to increased interest in the development of an effective and safe male contraceptive agent. Hence, in the light of the above data and the recent reports from our laboratory (Chinoy, M.J. and Chinoy, M.R., unpublished data), that administration of 1, 10 and 100 μg testosterone (T) to intact male rats caused alterations in the histophysiology of their vas deferens and reduced the fertility rate, the present study was undertaken to investigate the effects of administration of 1, 10 and 100 μg testosterone on histophysiology of testis, epididymides and accessory reproductive glands and fertility rate of intact male rats treated for 5, 10 and 30 days.
4. **EFFECTS OF ESTRADIOL BENZOATE (E$_2$) TREATMENT TO INTACT MALE ALBINO RATS:**

Synthetic and natural estrogens are known to inhibit spermatogenesis, steroidogenesis and accessory sex gland functions. They also manifest antigonadotropic, antifertility effects and antagonize the action of testosterone in males (Duckett and Racey, 1975; Johnson and Gomes, 1977; Dufau *et al.*, 1978; Talwar, 1979; 1980).

The other view is that estrogen, exert their influence indirectly at the pituitary level by a feedback mechanism and thereby cause a decrease in testosterone production and release, followed by a significant alteration in secondary sex characteristics and testicular weights (Bacon and Kirkman, 1955; Feagans et al., 1961; Allanson and Parkes, 1966; Kalra and Prasad, 1969; Kalra et al., 1969; Verjans et al., 1974; deJong et al., 1974, 1975; Johnson and Gomes, 1977; Hunt et al., 1979; Thompson, 1979).

The antifertility potential of estrogenic compounds in the male is attributed to their inhibitory action on gonadotropin release (de Jong et al., 1975; Tcholakian et al., 1974), resulting in suppression of steroidogenesis and spermatogenesis. Short term treatment with estrogens did not manifest antifertility effects in rats (Hunt et al., 1976). Lubicz-Nawrocki et al., (1973) reported that short term treatment of male hamsters with $E_2B$ had no effect on libido but sterility resulted in 12 days, corroborating with earlier studies on rats and mice (Deansey, 1939; Emmens, 1950). Estradiol 17B treatment to adult male rats induced a dose-related decline in sperm density and eventually azoospermia occurred and hence reduced the number of implantation sites, but mating behaviour was not affected (Hunt et al., 1979). It has
been shown that hamster epididymal spermatozoa lose their fertilizing ability, if deprived of testosterone (Lubicz-Nawrocki and Glover, 1973) for a period of 12 days, and the same effect can be induced by estradiol benzoate treatment (Lubicz-Nawrocki et al., 1973; Lubicz-Nawrocki, 1974), which has a direct local effect upon epididymal tissue and thereby affects the fertilizing ability of epididymal spermatozoa. Similarly, Chinoy, M.R. et al. (1980) have reported very scanty vas deferens sperms possessing varied head and tail anomalies in E2B treated rats. Many sperms were decapitated. The antifertility effects of E2B have been attributed to the alterations in sperm morphology and in histophysiology of the vas deferens, by these authors.

However, the exact mechanism of estrogen action is still a controversial issue. Although it is known that estradiol and testosterone have a synergistic effect in stimulating glandular epithelium and interstitial stroma in rat ventral prostate (Feyi-Cabanes et al., 1977). Hence the present study was undertaken to highlight the effects of E2B treatment on testis, epididymis and accessory sex organs of intact male rats. The effects of the treatment on fertility rate, histophysiology, metal ion profile of the above tissues were studied.
1. STUDIES ON VASECTOMY AND VASECTOMY + ASCORBIC ACID (AA)

FEEDING:

Vasectomy is one of the leading methods of male contraception. Three main advantages offered by this technique are: (1) that it is a simple outpatient surgical procedure requiring one time motivation of the patient and (2) that a permanent sterilization is achieved (3) Vasovasostomy is now possible for its reversal. No conclusive evidence exists at present to show that vasectomy affects libido. A number of new modifications have been introduced, in the surgical operation of vasectomy to make it more effective and safe which minimizes adverse side effects or complications (Moss, 1976; Pardanani et al., 1976; Albert and Seebode, 1977; Schmidt and Free, 1978; Shapiro and Silber, 1979; Escho and Cass, 1978; Rhodes et al., 1980). There are various opinions regarding the effects of vasectomy on male reproductive organs. Many scientists believe that it does not have any side effects and others believe the contrary. Hence this still remains a controversial issue.

Vasectomy does result in changes in the histology of the testis and epididymis. Alexander (1971, 1974) observed accumulation of sperm and dilation of the efferent ductules,
along with a decline in the number of ciliated cells, a loss of lipid complexes from the cells and thickening of the epithelial basal lamina. The disposal of sperm by macrophages in the lumen of the tubules was also observed. A spermiophage cell was observed within the lumen of epididymal duct of vasectomized Japanese monkeys (*Macacus fuscatus*) (Murakami et al., 1977). Changes in structure and resorptive activity of the epididymis have been detected after vasectomy (Phadke, 1964; Alexander, 1971; 1973; Flickinger, 1972 a). Kobota (1969) reported that sperm maturation was abnormal in men. Hagedoom and Davis (1974) found that after vasectomy, the spermatozoa appeared close to the basement membrane rather than occupying their normal location close to the lumen. On the other hand, the studies of Jones (1973) on vasectomized rabbits, suggest that the procedure does not seriously impair the capacity of the cauda epididymides to maintain a stable milieu in the lumen of the duct, and it seems unlikely that the normal maturation and survival of spermatozoa are affected. The epithelium continued to be functionally active after vasectomy (Flickinger, 1975 a), although the epididymis was distended. But Alexander and Tung (1980 in press) have shown an increased influx of macrophages in the adjacent connective tissue as well as in the epididymal lumen of vasectomized rabbits. Changes in the
histology of the rat vas deferens proximal and distal as well as morphological alterations in their contained spermatozoa were reported (Chinoy, M.R. et al., 1980; Chinoy, M.R. 1981).

Kubota (1969) observed the presence of many vacuoles in Sertoli cells and fine structural alterations in human spermatids. In the dog and rabbit, vasectomy appeared to give rise to a temporary impairment of spermatogenesis, followed by a regeneration of the germinal tissue (Var and Bansal, 1973). Kothari and Mishra (1972) recorded significant changes in Leydig cell volume in the dog testis after vasectomy and suppressed spermatogenesis in rabbit testis (Flickinger, 1975 b). Similarly, Kanwar et al. (1978) found severe histological damage in rat and dog testis. But in rat, vasectomy did not alter the structure of testis (Flickinger, 1972 b).

Several workers have shown that a decline in organ weights, transitory degenerative changes in testis, structural changes in spermatozoa and epididymis, disturbances in spermatogenesis, reduced sperm counts, a decline in androgen levels concomitant with the reduction of spermatic granuloma in the epididymis/vas deferens at the site of operation occur and in the secretory function of epididymis and accessory sex organs of a variety of animals (Laumas and Uniyal, 1967; Kubota, 1969; Igboeli
and Rakha, 1970; Macmillan et al., 1968; Alexander, 1972; 1973; Kothari et al., 1973; Sacher and Schilling, 1973; Vare and Bansal, 1973; Bruschke et al., 1974; Deerick et al., 1974; Kinson and Layberry, 1975; Chatterjee et al., 1976; Jahan and Setty, 1977; Dixit et al., 1975; Lohiya and Dixit, 1975; Thakur et al., 1975). The endocrine and biochemical consequences of vasectomy in mammalian systems has been reviewed by Singhal et al. (1977) and Kinson et al. (1977).

On the other hand, in general, vasectomy does not appear to result in histological changes in the testis, although part of the epididymis may undergo dilation (Amann, 1962; Smith, 1962; Kar et al., 1962; Paufler and Foote, 1969). Studies on rodents, dogs, bulls and human beings have revealed that vasectomy has no adverse effects on pituitary weight, gonadotropin activity, histology of genital organs, spermatogenesis, sperm metabolism, physiology of testis, epididymis and secretory functions of accessory reproductive organs and fertility (Kar et al., 1965; Flickinger, 1972; 1973; 1975; Kwartz and Coffey, 1973; Easterday et al., 1973; Wieland et al., 1972; Lohiya and Dixit, 1974; Johnsonbaugh et al., 1975; Neaves, 1974; 1975; Meenakshi et al., 1976 a, b; Bedford, 1976; Chinoy and Sheth, 1977 c; Jhunjhunwala et al., 1977; Chinoy et al., 1978 b). The circulating levels of testo-
sterone did not alter even after 6-36 months of vasectomy in human volunteers (Seethalakshmi et al., 1980). Similarly, electron spin resonance spectra of Mn⁺⁺ free radical were not significantly different in the cauda epididymal sperm suspensions of normal and vasectomized rats nor in normospermic, oligo, azoospermic and vasectomized men (Chinoy and Seethalakshmi, 1978 e; Seethalakshmi et al., 1980). Likewise, Davis and Alexander (1978) reported that there are no adverse endocrine changes and no psychological problems in sexually well adjusted men. However, vasectomy results in an immune response and more than half the men develop antisperm antibodies. Changes may also occur in cellular immunity after vasectomy but further studies are necessary to define and describe these alterations. The sexual behaviour in long-term vasectomized male rhesus monkeys was also not different than in sham vasectomized controls (Phoenix and Alexander, 1979).

Vasectomy did not alter the weight of seminal vesicle in rams and bulls (Skinner and Rawson, 1968 a, b; Rakha and Igobeli, 1971). Similarly, long term studies on rats showed that vasectomy failed to produce a significant alteration in weight of the accessory sex glands (Lee, 1967; Collins et al., 1972).
A study conducted by Rothman and Friedman (1977) revealed that sperm antibodies following vasectomy may account for infertility despite a subsequent successful vasovasostomy. Vasectomy caused sperm antibody production and autoagglutination. Antibody, autoagglutination and motility and no autoagglutination lead to pregnancy. This study suggests that immunological factor may be related to infertility after vasovasostomy. Alexander (1975) and Alexander and Schmidt (1977) also found the presence of antisperm antibodies, in sperm granulomas on one or both sides of the operation site, wherein, they developed sperm-immobilizing and sperm agglutinating bodies. In 1964, Phadke and Padukone, found sperm agglutinins in the serum of patients with obstruction of the efferent ducts. Vasoligation and vasectomy in bull, ram, rabbit (Bratanov et al., 1964), rat, mice (Rumke and Titus, 1970; Kosuda and Bigazzi, 1978), rhesus monkey (Alexander, 1972), guinea pigs (Alexander, 1973) and human males stimulate the production of sperm agglutinins (Ansbacher et al., 1972; Shulman et al., 1972; Ansbacher, 1973; Van Lis et al., 1974).

The cause of persistent azoospermia after an accurate microscopic vasectomy is due to the secondary epididymal obstruction induced by rupture of the epididymal ducts related to the pressure increase after vasectomy. Hence
the failure of vasectomy occurs (Silber, 1978). It is evident that sperm granuloma is often a painful lesion of post vasectomy (Schmidt, 1979), but the formation of sperm granuloma appears to be effectively controlled by the fulguration technique (Albert et al., 1975) and suggest that vas deferens repair by microsurgical technique is the best method for vasovasostomy (Belker et al., 1977). The various criteria for evaluation of success after vasovasostomy have been reported by Wicklund and Alexander (1979).

Several reports from our laboratory, have shown that ascorbic acid feeding to post vasectomized rats, has beneficial effects for maintaining the status quo of reproductive organs without affecting the contraceptive purpose of vasectomy. This is aided by the greater utilization of ascorbic acid by the reproductive tissues following vasectomy (Chinoy and Sheth, 1977 c; Chinoy et al., 1978 b). The metabolic significance of ascorbic acid turnover pattern in rats, guinea pigs and human beings for maintaining the reproductive functions via the involvement of its free radical, monodehydroascorbic acid and charge transfer complex formation (AA-MM), has been reported elsewhere (Chinoy and Sheth, 1977 c; Chinoy and Buch, 1977 a; Seethalakshmi and Chinoy, 1978; Chinoy et al., 1978 a-f). It is also known that ascorbic acid
potentiates the anabolic action of endogenous testosterone (Chinoy and Seethalakshmi, 1980; Chinoy et al., 1979 b). Therefore, ascorbic acid and its mechanism of action have important implications in the prophylactic treatment during the following vasectomy. These findings have added significance in the light of the observations of Alexander and Clarkson (1978), that vasectomized monkeys developed an increased tendency to suffer from atherosclerosis in comparison to the control. Ascorbic acid feeding to vasectomized animals/men would therefore have beneficial effects, since ascorbic acid is known to decrease the blood cholesterol levels (Ginter et al., 1970; 1971; 1972).

The present study was undertaken to elucidate the long term (30, 60 and 90 days) effects of vasectomy and vasectomy + ascorbic acid feeding on reproductive functions of male rats, in the light of the recent data. The histology, the metal ion concentrations and ascorbic acid metabolism of testis, epididymis and accessory sex organs were investigated in normal and treated rats as mentioned above.

2. STUDIES ON VASOCLUSION BY ETHANOL AND ASCORBIC ACID THERAPY:

The first attempt was made by Freeman and Coffey (1973 a, b) to develop a simple, nonsurgical technique for male sterilization by occluding the vas with 95% ethanol injected through the skin of the scrotum directly into the
vas. Thus this technique involves chemical induction of obstruction of the vas deferens by injection of small quantities of sclerosing agents directly into the vas deferens. A wide variety of sclerosing agents have been tested and 95% ethanol appears to produce complete blockage of the vas and the occurrence of sterility in a large variety of animals (Freeman and Coffey, 1973a, b). The ejaculates were found to be completely free of spermatozoa following treatment which also produced physiological changes in the vas.

Setty et al. (1972), reported occlusion of vas with camphor, potash alum or quinaerine and found diminished sperm in the distal portion of the vas lumen. Urea-ethanol mixture or urea alone treatment was used by Geeta Raman et al. (1976) who found sperm granuloma in epididymis, whereas the testis showed empty tubules and Leydig cell hyperplasia. A gradual decrease in the fertility of rats was also observed. However, no pathological changes were observed in the testes and epididymides of gerbils following vas injections of \( \text{KMnO}_4 \) for 3 weeks (Dixit et al., 1976). The seminiferous tubules and Leydig cell function appeared to be normal. The lumen of epididymides showed the presence of normal spermatozoa, but sterility was achieved after two weeks. Dixit (1976) has also reported that a single injection of a-chlorohydrin and/or
cadmium chloride into the vas deferens of dogs produced an effective block in the lumen, caused severe degenerative changes in the testis and altered its biochemical composition. On the other hand, Poddar et al., (1975) used intravasal nylon thread in rats and observed no significant change in the weight of testis, epididymis and accessory glands and in the alkaline phosphatase activity of ventral prostate. However, epididymal sialic acid and spermatozoa motility were markedly reduced following intravasal nylon thread implantation in distal vas. A single injection of 95\% ethanol (50 μl) directly into the vas deferens of fertile albino rats was found to result in adverse effects on the structure and metabolism of testicular and epididymal spermatozoa after 30 days post injection. The lumen of the vas was occluded due to sperm granuloma formation which also occurred in the epididymis (Chinoy and Sanjeevan, 1979 a). The alcohol treatment caused loss of fertility, alteration in sperm morphology, reduced activity of some spermatozoal enzymes as well as decline in their redox milieu (Chinoy and Sanjeevan, 1979 a; Chinoy, N.J. and Chinoy, M.R., 1980 a, b). Changes were also observed in the histophysiology of the vas deferens and their spermatozoa (Chinoy, M.R., 1981).

The beneficial role of ascorbic acid during and
following stress condition is well known (Selye, 1950; Chinoy and Seethalakshmi, 1977 a-c; 1978 a-f; Sheth, 1976; Sanjeevan, 1979; Chinoy et al., 1978 b, e; 1979 a-c; Chinoy, 1978). In the light of above data, the present investigation was undertaken. The role of ascorbic acid during ethanol treatment is obscure, hence detailed study on testis and epididymal physiology of rats, during ethanol treatment and ethanol + ascorbic acid treatment was undertaken. These studies are relevant since it is known that ethanol treatment significantly reduced the plasma testosterone in mice (Badr and Bartke, 1974; Badr et al., 1977). These authors concluded that the decrease in plasma testosterone levels observed after administration of ethyl alcohol in vivo may be related to a direct inhibition of testicular testosterone production by acetaldehyde derived from the metabolism of alcohol. The reduced testosterone might be indirectly responsible for causing decline in androgen dependent metabolism of vas deferens, epididymis and contribute to alteration in their milieu and thus render them hostile for sperm survival. The combined ethanol + ascorbic acid treatment was carried out in view of the potentiation action of ascorbate with endogenous testosterone to enhance its anabolic action in target tissues (Seethalakshmi and Chinoy, 1978).
PART IV

EFFECTS OF COPPER AND PROSTAGLANDINS ON MALE REPRODUCTIVE FUNCTIONS:

1. EFFECTS OF COPPER:

A number of studies have revealed that copper inhibits motility and metabolic activity of human and rat spermatozoa in vitro and in vivo (Bernstein, 1958; Saito et al., 1967; Kesseru and Cazmacha-Ortega, 1972; Loewit, 1971; Oster, 1972; Ullmann and Hammerstein, 1972; White, 1955; Chinoy and Sanjeevan, 1980). It has long been appreciated that spermatozoa are rich in sulphur, and that S-S bonds are important for maintaining the structural stability of sperms and their motility. The spermicidal activity of copper ions has been attributed to the binding of these ions to the sulphydryl groups of compounds present in spermatozoa (MacLeod, 1951; Mann, 1964; Oster and Salgo, 1977). The stability of spermatozoa is brought about by increase in disulfide bond formation as spermatozoa mature in epididymis (Bedford and Calvin, 1974). Zinc and Copper are antagonistic in action. Copper plays a crucial role in sperm motility and in the fertilization process. Eliasson (1976) suggested that the morphology of sperm tail is related to the zinc concentration as zinc protects the sulphydryl groups from oxidation by copper (Calvin et al., 1973).
The antifertility effects of copper wire devices or copper salts in silastic tube implanted in the vas deferens and seminal vesicles of rodents and monkeys has also been reported (Gilmore et al., 1973; Ahsan et al., 1976; Khatoon, 1978; Chinoy and Sanjeevan, 1980). According to Ahsan et al. (1976) and Khatoon (1978), the intravasal copper appeared to be a promising technique for reversible control of fertility in the male rats and monkeys. The contraceptive effectiveness of the method is dependent on the proper placement of the device as well as on the surface area exposed to the vasal environment in the vasal lumen, while the reversibility depends on its careful removal with least damage to the vas deferens. Khatoon (1978) did not find any deleterious effects on the testis and accessory organs of the male reproductive system by the presence of the intravasal copper device. On the other hand, Dixit and Jain (1977) implanted copper wire in the vas deferens of gerbils which resulted in severe damage to the seminiferous tubules. The contraceptive effectiveness of the copper device has been attributed to either a direct toxic effect of copper on the spermatozoa present in the vas or to the involvement of copper ions in the binding of -SH groups of spermatozoa. The copper may also create a hostile environment for the sperm maturation in the epididymis and/or survival in the vas (Khatoon, 1978; Chinoy, M.R. et al., 1980).
The in vitro studies carried out from this laboratory (Chinoy and Sanjeevan, 1980) to investigate the effects of different concentrations of analar copper sulphate, solutions as well as copper solution + ascorbic acid on motility of cauda epididymal spermatozoa of rats revealed, that the decrease in motility was more rapid with increasing concentrations of copper solution. The addition of ascorbic acid to the copper sulphate solutions inhibited the motility of the spermatozoa much more rapidly than the copper sulphate solution alone. The decreased activity of some androgen-sensitive parameters was observed after intra-epididymal and intra-scrotal copper device (IECD, ISCD) implantations in rats. The increased level of copper ion were found in reproductive tissues. However, after feeding ascorbic acid, the levels were restored to almost control values (Chinoy and Sanjeevan, 1980), suggesting that ascorbic acid has beneficial effects in copper toxicity i.e., in animals bearing the copper device.

Jecht and Bernstein (1973) have shown that copper ions released from the copper device have more effective toxic effects on the spermatozoa than copper salts. The possible displacement of zinc by copper in the spermatozoa may be important for affecting the sperm motility.

A copper containing enzyme spermine oxidase can limit
the survival of spermatozoa (Malmstrom et al., 1975). This enzyme acts on the substrate spermine, a polyamine, normally found in the seminal plasma, and produces a volatile product, which is toxic to spermatozoa (Tabor and Rosenthal, 1956).

Copper ions injected into the laboratory animals produce testicular damage which is histologically similar to that produced by cadmium but to a lesser degree (Hoey, 1966). However, copper does not produce the severe necrosis which is characteristic of cadmium although there is some necrosis at the head of the epididymis by copper administration (Hoey, 1966).

The fact that copper is the most powerful catalyst for auto-oxidation of ascorbic acid and glutathione has implications in reproductive physiology. The cupric ion catalysed auto-oxidation of ascorbic acid generates free radicals which can bring about tissue damage (Oster and Salgo, 1977). This has a practical implication in terms of vitamin C deficiency for women taking oral contraceptives. The increased ceruloplasmin associated with exogenous estrogen is accompanied by reduced plasma levels of ascorbic acid. Therefore, a woman taking oral contraceptive must take about 500 mg vitamin C per day i.e., ten times the normal recommended daily level to compensate the increased loss (Oster and Salgo, 1977).
Vitamin G also has effect on copper metabolism. In copper deficient animals, the additional vitamin C makes the deficiency more severe. On the other hand, in copper toxicity the vitamin has a protective effect (Oster and Salgo, 1977; Prasad and Oberleas, 1976). Copper could also play an important role in reproduction via its destructive action on vitamin E. It acts synergistically with releasing hormone to cause the release of gonadotropins (Suzuki et al., 1970; LaBella et al., 1973). Copper might also be involved in steroid metabolism, in the aromatization of steroid to estrogens, in the sulphonation of steroids and it inhibits the biosynthesis of prostaglandin E1 (Oster and Salgo, 1977).

In the light of the above data an attempt was made to study the effects of an intra-epididymally and intra-scrotally implanted copper wire device on male reproductive functions of rats, with a view to developing a suitable and feasible male contraceptive method. A detailed study on testis and epididymal physiology including accessory sex organs was carried out with respect to sperm morphology, several androgen-sensitive biochemical parameters and ascorbic acid metabolism. A histological study on reproductive tissue viz., testis, epididymis, seminal vesicle and ventral prostate was also carried out.
2. STUDIES ON PROSTAGLANDINS (PGs):

1) Subcutaneous injections of PGs:

The use of prostaglandins (PGs) in female contraception is well documented, whereas, reports on effects of PGs in male contraception are limited. In males prostaglandins are implicated in the process of penile erection and contractions, ejaculation, sperm motility and morphology, testicular contractions and in steroidogenesis (Karim, 1975; Curtis-Prior, 1976; Hargrove et al., 1971). Moreover, prostaglandins have been considered as intracellular messengers (Silver and Smith, 1975); and have a role in LH secretion in rats and monkeys (Carlson et al., 1977; Tressenders et al., 1977). Amongst the sex accessory organs, the seminal vesicles produce the highest amount of prostaglandins and thus contribute it to the seminal plasma and these PGs might help in sperm transport in reproductive tract of female (Eliasson, 1959; Karim, 1975). Prostaglandin is localized on plasmalemma of the rabbit sperm (Bartozewicz et al., 1975). No correlation was observed between PG content of seminal fluid and the number and degree of motility of spermatozoa, nor does addition of PGE\textsubscript{1} to sperms affect their motility or metabolism (Eliasson, 1959). On the other hand, Nicholson (1972) found that exogenously added PGs may accelerate sperm transport. However, there are indications of a
correlation between the amounts of prostaglandins of the E type and male fertility (Bygdeman et al., 1978). PGF₂α markedly inhibited the motility of human spermatozoa, but no ultrastructural differences could be ascertained (Cohen et al., 1977). However, scanning electron microscopic studies made by Chinoy et al., (1980a); Chinoy, N.J. and Chinoy, M.R. (1980 b) have shown that treatment of rats with PGF₂α and E₁ for one month, caused decapitation of spermatozoa, furrow formation in head as well as abnormalities in mid-piece and tail regions. Tso and Lacy (1979) have carried out ultrastructural studies on sperms of rats treated with PGE₂ and F₂α. Thus PGs have an influence on male potency and fertility (Pharris and Shaw, 1972; Karim, 1975; Ellis et al., 1975; Batta, 1975; Hinman, 1972; Shaw and Tillson, 1973; Davis et al., 1970; Hafs et al., 1974; Curtis-Prior, 1976). The chronic subcutaneous injection of either PGE₁ or PGE₂ significantly decreased the testis and accessory gland weights and suppressed spermatogenesis and also decreased testosterone production in rats (Ericsson, 1972; Saksena et al., 1973). Similar results were obtained in mice by Abbatiello et al., (1975, 1976) wherein, degeneration of spermatocytes and decrease in number of spermatids were noted. The other reports on the parenteral administration of PGF₂α on various parameters of male reproductive functions were inconclusive (Bartke et al., 1973; Tso
and Lacy, 1975). However, Bartke et al. (1973, 1976) reported lowered plasma testosterone levels and inhibition of testicular steroidogenesis in male mice after PGF$_2\alpha$, PGA$_1$, PGA$_2$ or PGF$_1$ treatments \textit{in vivo} or \textit{in vitro}.

The synthesis of PGF$_2\alpha$ in the reproductive system of the male rat is under androgenic control (Bartke and Koerna, 1974). Barcikowski et al. (1973) demonstrated decreased PGF levels after castration and their restoration by testosterone administration. Moreover, maximum quantities of PG and C-AMP were found in the same most actively functioning organs such as brain, secretory glands (Komissarenko et al., 1977). Johnson and Ellis (1977) have demonstrated prostaglandin synthetase activity histochemically in various reproductive tissues of the male rat. Moderate activity was observed in tails of spermatozoa within the corpus and cauda epididymis but there was no activity in caput epididymis or seminiferous tubules. The sperm tail activity was maximal for cells within the vas deferens.

Recently, Kimball et al. (1978 a) implanted silicone rubber discs containing 15(s)-15-methyl prostaglandin F$_2\alpha$ methyl ester (15-Me. PGF$_2\alpha$) in the left side of the scrotum of Sprague - Dawley rats and observed reduced testicular and accessory gland weights. The histology of testis and epididymis showed decreased or absence of
spermatogenic elements and hypertrophy of the interstitial
cell masses. The PG also caused significantly depressed
serum testosterone, luteinizing hormone and follicle-
stimulating hormone (FSH) concentrations, and as drug
concentration declined, the hormone concentrations returned
to the control level, suggesting that PGF$_2$ may act
directly on the testes to suppress testosterone production
or may suppress gonadotropin secretion, resulting in
depressed testosterone. Further, they suggested that
PGF$_2$ reduced the potency and fertility of treated rats
which was restored 2 weeks after implantation (Kimball
et al., 1978 a).

Temporary sterility was achieved by intrascrotal
implantation of PGF$_2$ (Saksena et al., 1978 a) and
significant reduction in epididymal sperm density and
testicular weight also occurred, but libido and ejaculate
volume were not affected. Saksena et al. (1978 b) have
also reported a significant reduction in the levels of
serum testosterone, androstenedione, $5'$-dihydrotesto-
sterone and progesterone after 3-7 days of intrascrotal
insertion of silastic PVP tubes containing PGE$_2$ and PGF$_2$ in
the male rats. Similar results were found in mice and
rats treated with PGA$_1$ and A$_2$ (Saksena et al., 1974).

Role of PGs in human fertility have been suggested
(Das and Padma, 1978). Antifertility effects of PGE$_2$ and
F$_2$ have been reported by Aref et al. (1974) and studies
from our laboratory have revealed that S.C. injections of PGE$_1$ and PGF$_{2\alpha}$ exert antiandrogenic and antifertility effects as well as they antagonize the adrenergic response of the vas by blocking the $\alpha$-adrenergic receptors and cause histological and metabolic changes in the vas deferens. The two prostaglandins also exhibited a growth promoting effect particularly in seminal vesicle and the ventral prostate (Chinoy et al., 1980a; Chinoy, N.J. and Chinoy, M.R. 1981). Likewise, the possible involvement of prostaglandins in affecting the fertilizing capacity of rat epididymal spermatozoa have been suggested (Chatterjee and Rej, 1978). Chinoy and Sanjeevan (1980) have also observed that percent motility of cauda epididymal spermatozoa and their density diminished after both PG (E$_1$ and F$_{2\alpha}$) treatments. Scanning electron microscopic study revealed that the two types of prostaglandins altered the morphology of cauda epididymal as well as vas deferens spermatozoa causing decapitation and severe deformities in the head, mid-piece and tail regions (Chinoy and Sanjeevan, 1980; Chinoy et al., 1980a; Chinoy, N.J. and Chinoy, M.R. 1980b; 1981).

The physiological role of prostaglandins in the male is not adequately understood. The present investigation was undertaken in the light of the above data, to highlight the effects of PGE$_1$ and PGF$_{2\alpha}$ treatments on the physiology of testis, epididymis and accessory glands.
Studies on their histology, several androgen-sensitive biochemical parameters, including ascorbic acid turnover pattern and Cu\textsuperscript{++}, Mn\textsuperscript{++} and Zn\textsuperscript{++} concentrations were carried out in normal and PG treated rats.

ii) Studies on intravasal injection of prostaglandins:

For developing a simple, non-surgical technique for male sterilization and its reversal, many scientists have tried different chemicals, copper devices and nylon threads, but so far none of them are safe and 100% effective (see part III, No. 2, Part IV, No. 1, for details).

For the first time, an attempt was made in this direction by injecting a single dose of prostaglandin E\textsubscript{1} or F\textsubscript{2a} into distal vas in the retrograde direction.

The effect of intravasal PGE\textsubscript{1} and PGF\textsubscript{2a} were studied on several androgen sensitive parameters, percent sperm motility, surface morphology and fertility rate. Furthermore, study was carried out on histology of testis, epididymis, seminal vesicle and ventral prostate of treated rats.

These studies were undertaken in the light of recent work from our laboratory (Chinoy, N.J. and Chinoy, M.R., 1980a) wherein it was reported that intravasal injection of PGE\textsubscript{1} and F\textsubscript{2a} in rats affected the morphology of vas
deferens spermatozoa causing deformities in head, mid-piece and tail as well as decapitation leading to absence of fertility. Histologically too, severe changes occurred in vas deferens so that its lumen was occluded. PGE₁ caused more severe changes than PGF₂α.

PART V
EFFECTS OF NUTRITIONAL DEFICIENCY

VITAMIN C DEFICIENCY IN GUINEA PIGS:

The functional integrity of the endocrine system is to some extent dependent upon the proper nutritional status of the body. Experimental and clinical studies have shown that chronic and acute starvation, caloric restriction, quantity and quality of protein intake and also vitamin and mineral deficiencies could alter the endocrine functions (Meites and Nelson, 1960; Leathem, 1961).

The relationship of vitamin C to male fertility in animals and human beings is not clear. However, chronic lack of ascorbic acid in the laboratory rodent causes atrophy of testis, degeneration of both Leydig cells and seminiferous tubules, impaired androgen synthesis, spermatogenic arrest, decreased spermatozoa motility and metabolism, accumulation of lipids in testis and affects levels of gonadal cholesterol and plasma testosterone.

Furthermore, studies from our laboratory (Seethalakshmi and Chinoy, 1976; Chinoy, N.J. and Chinoy, M.R., 1979 b; Chinoy and Sanjeevan, 1979 b) have shown that vitamin C deficiency in guinea pigs resulted in alterations in morphology of epididymal and vasal spermatozoa/sperm abnormalities, especially in the acrosomes and head, thereby reducing their motility and fertilizing capacity (Chinoy and Sanjeevan, 1979 b; Chinoy, N.J. and Chinoy, M.R. 1979 b). The fertility rate of scorbutic guinea pigs was also significantly reduced. The vas deferens contractile pattern and its androgen dependent metabolism was also altered together with severe changes in its histology. The vasal lumen was occluded with sperm granuloma.

In the light of above data, the present study was undertaken to understand further the metabolic significance of ascorbic acid and its role in reproduction by studying the alterations in reproductive functions, if any, under scorbutic conditions. The physiology of testis,
epididymis and sex accessory glands with respect to histophysiology, biochemical estimations of several androgen-sensitive components of these organs, sperm morphology, their density and motility were studied after inducing the scorbutic condition in guinea pigs. The fertility rate was also determined.