In the year 1798, an English Clergyman, Thomas Malthus, put forward the famous theory that human population can grow much more rapidly than the agricultural means available to feed them. According to him, the growth of population may be exponential, or logarithmic, while the production of food could only be arithmetic. Malthus postulated that vice and misery were caused to a greater extent by the uncontrolled growth of population, which have been proved beyond doubt in today's world.

The contraceptive technology has been available commercially since 1960 in the United States, thanks to Dr. Gregory Pincus. Once Pincus started his experiments, still ten years had lapsed to develop the marketable drug for contraception.

According to World Health Organization (WHO) reports (1980), the world population is increasing by 60-70 million people each year, even with an estimated 54 million women using oral contraceptives and many more using intra-uterine devices and other mechanical means. The WHO believes that the world population is growing at about 1.8% a year and will double within the next 40 years.

Although a large number of female contraceptives are available at present, a simple easy to use, economical, cent
percent effective and non toxic male contraceptive is still a far cry due to various biological, and socio-economic reasons. For example an attitude prevalent in many societies that contraception is the responsibility of women; and also it is due to an incomplete knowledge of male reproductive functions. The 'male pill' should: have a quick onset of action; be reversible; free from toxic side effects; should be acceptable to both partners; have an efficacy similar to vasectomy or the female pill.

The development of such a male contraceptive drug is of great scientific importance for andrologists all over the world. Many drugs affecting male fertility have been tried in laboratory animals and clinical trials with some have been very successful. A practical classification of male contraceptive drugs may be: (1) Drugs producing oligo or azoospermia, (2) Drugs decreasing sperm motility or in some manner affecting 'fertilizing capacity'. This category includes the drugs affecting the composition of the seminal fluid (Schaffenburg et al., 1981).

There are at least seven separate possible sites of action of contraceptive products via spermatocytes. Four prospective sites of action would require administration of contraceptives to women; these include the inhibition of spermatocoidal transport and/or interference with capacitation.

The possible male methods of fertility control that may be used are: vasectomy, various vas occlusive devices (some-
times certain forms of spermicidal agents such as copper of zinc are used), disruption or inhibition of spermatogenesis; administration of various hormones such as androgens and estrogens; several antiandrogens; ultrasonic vibration and irradiation of testis; inhibition of steroidogenesis; post testicular sperm maturation; interference with sperm transport; inactivation of sperm enzymes; alteration in the composition of seminal plasma and several immunological approaches to male contraception. (Briggs and Briggs, 1976; Zatuchni et al., 1980; Sciarra et al., 1978; Haies, 1980; Hawkins and Elder, 1979; Shain and Pauerstein, 1980; Talwar, 1979; Mann and Lutwak-Mann, 1981; Cunningham et al., 1980; Bain et al., 1978).

Vasectomy and vas occlusive devices will be discussed in detail in the latter part of this chapter.

1. Inhibition or disruption of spermatogenesis:

Several pharmacologic compounds have been used to inhibit spermatogenesis without producing irreversible infertility or abnormal gametes. The most desirable antispermatogenic agent may have a high specificity of action for the later stages of spermatogenesis (transformation of spermatids into spermatozoa). Colchicine, fluorooacetamide, several alkylating agents and heterocyclic antispermatogenic agents are capable of interrupting spermatogenesis without interferring with the endocrine function of the testis.
Administration of androgens and estrogens induce testicular changes similar to those produced by hypophysectomy, i.e., atrophy of the interstitial tissue and arrest of spermatogenesis. Estrogens and progestins were first used to interfere with spermatogenesis by inhibiting pituitary gonadotrophin secretion and thus lowering testosterone levels. However, this resulted in gynecomastia and loss of libido.

Testosterone was substituted for the female hormones which inhibits spermatogenesis via the pituitary with the inhibition of pituitary FSH secretion. Withdrawal of testosterone resulted in normal spermatogenesis and sperm count. A progestin-testosterone combination was also used to inhibit spermatogenesis. Progestins act as inhibitors of spera production, whereas, exogenous testosterone compensates for the androgen loss (decrease of libido and potency) resulting from Leydig cell suppression (Frick, 1976).

Extensive research activities are going on to isolate and characterize testicular inhibin, a naturally occurring testicular protein which selectively inhibits FSH secretion.

Subcutaneous implants of polydimethylsiloxane (PDS) capsules filled with dry crystalline hormone have been used to ensure a constant hormonal supply over longer periods of time. The hypothesis that steroids might pass directly from the nasal cavity to the brain have led to the development of
nasal sprays through intranasal route of administration of hormonal steroids in male and female primates including humans (Anand Kumar et al., 1979).

Administration of other agents:

Several alkylating agents produce "functional sterility" without significant change in spermatogenesis, sperm concentration, morphology, motility and libido. However, these alkylating agents were shown to cause damage to genetic material and overall toxicity (Jackson, 1966). The nitrofurans evaluated on several mammals (Rogers et al., 1956) are effective in man.

Ultrasonic vibration and irradiation of testis and epididymis:

Sertoli cells and spermatogonial cells of testis have been shown to be highly radiosensitive to ionizing radiation, whereas, spermatocytes and spermatids are more resistant to radiation. Ultrasonic treatment interferes with cell membrane relationships, tubular membrane functions and electrolyte transport. Chinoy and Buch (197-a) have shown that radiation affects some enzymes and metabolites of sperm suspensions from testis and epididymis in rats. The results were obtained by whole body exposure to low dose of gamma irradiation. Testicular spermatozoa were found to be more radiosensitive than the epididymal sperms. However, partial recovery occurred
within three days after irradiation. Ascorbic acid feeding prior to irradiation acted as a radioprotective agent (Chinoy et al., 1980).

Inhibition of Sertoli cells:

Certain pharmacologic agents may selectively impair the function of Sertoli cells without direct effects on the germ cells. However, at present there are no such drugs available which could exert their preferential effects on Sertoli cells alone.

Inhibition of Steroidogenesis:

Two techniques have been employed in an attempt to inhibit steroidogenesis as a means to disrupt spermatogenesis. (1) antiserum to testosterone developed either passively or actively, (ii) anti-androgens to interfere with testosterone binding with specific receptorsites. Several steroids exhibit varying degrees of inhibition of 5 a-dihydrotestosterone (5 a-DHT) but their usefulness as antiandrogenic agents is limited.

2. Inactivation of Sperm enzymes:

The inactivation of the enzymes of the sperm acrosome, hyaluronidase and acrosin may be an effective approach to the development of male contraceptives. Antifertility agents include hyaluronidase inhibitors and nitrated hyaluronic acid derivatives. Other inhibitors highly specific for acrosin,
may prevent fertilization in vitro and/or in vivo. Synthetic inhibitors may be applied as vaginal contraceptives, such as creams and jellies (Misz, 1980).

Motility of spermatozoa is regulated by the activity of their mid-piece enzymes. Inhibition of these enzymes lead to immobilization of spermatozoa. Not all sperm immobilizing agents act by the direct inhibition of enzymes, e.g., most of the spermicidal agents that are commercially used as contraceptives function by increasing the permeability of the lipid rich sperm cell surface (Zaneveld, 1976). This results in the leakage of enzymes and other cell components from the mid-piece. Enzyme inhibitors may affect two groups of midpiece enzymes: the cytochrome oxidase (respiratory) system and the glycolytic enzyme system. Several advantages are associated with the use of enzyme inhibitors as antifertility agents. The most striking is their possible high specificity, so that they are directed only against spermatozoa (Zaneveld, 1976).

3. Immunological contraception for the male:

The use of heterologous sources of LH for active immunization of males causes disruption of spermatogenesis but may reduce sexual libido. The immunization of rabbits with bovine LH caused exfoliation of immature germ cells from the seminiferous epithelium (Tallat and Laurence, 1971).
Testicular antigens to produce specific immunizations have been successful in animals and clinical trials are going on at present.

The effects of treatment of male rats with hCG-antiserum on their reproductive organs and fertility were studied by Chinoy et al. (1982a). The treatment caused significant variation in the sperm morphology, motility and fertilizability of spermatozoa as compared to the control sperms. The blood testosterone levels decreased indicating the antiandrogenic and antifertility effects of the treatment. Several other immunological fertility regulation methods are available (Jones, 1982; Shulman et al., 1982; Semm and Hettler, 1981; Brindza and Schumacher, 1980).

Labrie et al. (1981) have found that treatment with LH-RH agonists can cause both gonadal and pituitary desensitization in adult male albino rats. The discovery of LH-RH, the availability of synthetic neurohormone and a large number of its highly potent agonistic analogues has helped in the understanding of the control of pituitary gonadotrophin secretion in both experimental animals and human beings. Burgus et al. (1979) and Labrie et al. (1981) have suggested that the antifertility effects manifested after administration of LH-RH and its agonist result from the simultaneous inhibitory effects of the synthetic peptides at the pituitary and gonadal levels. Their studies under in vivo conditions
suggest that gonadal desensitization induced by LHRH agonist in turn induced endogenous LH release which is the main factor involved in causing antifertility effects.

4. The most recent advances in male contraceptive research deal with the post-testicular target site; interference with the acquisition of the fertilizing capacity of the spermatozoa as well as with the factors regulating sperm maturation in the epididymis. This approach has been extensively explored by andrologists all over the world, chiefly because the spermatozoa undergo maturation in the epididymis and several biophysicochemical parameters are involved in this process which could be selectively interfered with, by a suitable chemical contraceptive.

Several important reports and reviews have been published in recent years regarding the importance of epididymis in the development of fertilizing capacity of mammalian spermatozoa, but the reports are too numerous to be quoted here. The secretions of epididymis affect the sperm motility, their metabolic patterns, morphology and the surface properties of the sperm membranes.

The epididymal sperms in situ are non motile but they develop progressive motility as they traverse along the epididymis. The sperms removed from the cauda show disoriented circular movement, whereas, those of cauda have the
characteristic progressive movement of ejaculated sperms. The significance of epididymis with regard to sperm saturation is clearly demonstrated by the lack of response of the motility of sperms of testicular and proximal epididymal origins to chloroquine stimulation in contrast to the response of ejaculated sperms (Norman and Gombe, 1975).

The gross divisions of the mammalian epididymis comprises the head (caput), the middle region (Corpus) and the tail (Cauda region) respectively. However, various species differences make it difficult to equate the segments from species to species accurately (Hamilton, 1972; Glover, 1980; Grignon et al., 1981). The epididymis and the vas deferens are portions derived from the mesonephric duct (Wolffian duct).

Histology and Ultrastructure:

Several investigators have studied the epididymal histology in detail, however, ultrastructural studies are comparatively lesser. The rat epididymal epithelium is stabilized from 30 day old rats and is characterized by the presence of tall, columnar pseudostratified cells consisting of principal, pale or clear, basal cells and wandering population of intraepithelial leucocytes and macrophages (Chinoy and Asok Kumar, 1983). Similar cell types were shown in other mammals (Grignon et al., 1981) and in monkey (Anand Kumar et al., 1980; Prakash et al., 1979 a,b, 1980; Prakash, 1980) and in the human adult.

Besides the two cell types, i.e., the principal and basal cells which have been observed in all species so far investigated, additional cell types were reported to be present in many species. Fine structural studies carried out in rat cauda epididymal epithelium indicated the occurrence of two spatially separate and functionally distinct compartments which are sealed off from the epididymal lumen by occluding tight junctions joining the adluminal ends of the epithelial cells even in 5 day old animals (Chinoy and Asok Kumar, 1983). Gignon et al. (1961) have demonstrated the presence of tight junctions and desmosomes. In the four month old human foetus, the epididymis was well developed and the principal cells possessed junctional complexes. The blood epididymal barrier exists due to these junctional complexes (Hinton, 1980; Turner, 1981 a,b).

In the rat epididymis, intraepithelial leucocytes were observed which are known to have a spermophagic function (Chinoy and Asok Kumar, 1983). These had similar ultrastructural features as reported in monkeys (Anand Kumar et al., 1980; Prakash, 1980; Prakash et al., 1979 a,b, 1980).

The interstitial tissue of the epididymis consists of fibroblasts, mast cells, macrophages and eosinophils. The epididymis receives sympathetic and para-sympathetic innervations from the abundant nerve plexus surrounding both the
vascular tree and the duct, but the distribution of the axons vary from species to species. In rat, rabbit and guinea-pig adrenergic fibres 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. The sperm transport along the epididymal duct is facilitated by the surrounding smooth musculature along its entire length.

Blood supply to the caput and corpus are the superior and inferior epididymal arteries which are branches of testicular artery. The cauda is supplied by the vasal artery and also by an anastomosing loop of inferior epididymal artery. The microvasculature serving the epididymal tubule varies from zone to zone and provides a varying amount of capillary blood flow (Turner, 1979; Clavert et al., 1981).

It is known since long that epididymis is metabolically a very active organ chiefly involved in absorption and secretion of various substances. The principal cells of the epididymis are mainly involved in absorptive and synthetic activity (Mann and Lutwak-Hann, 1961). Fluid from the epididymal lumen is removed along the entire length of the duct (Turner, 1979). However, the resorption of the fluid mainly takes place in the ductuli efferentes and first part of the epididymis. The mechanism by which water resorption takes place in the epididymal epithelium is not exactly known.
although Hamilton (1977) suggested that micropinocytosis at the luminal plasmalemma may initially be involved. Ultrastructural studies on rat epididymis (Chinoy and Weiss, 1983) strongly support this observation. The standing osmotic gradient model (Diamond and Bossert, 1968) must be considered as a process by which water may be reabsorbed along the epididymis. In caput and corpus epididymis, chloride transport followed by passive movement of sodium ions is the probable drawing force whereas, in cauda epididymis, it is the active transport of sodium ion across the epithelium (Wong et al., 1978).

Several workers (see review by Hamilton, 1977) suggest that the absorption of particulate material takes place in the lumen of the duct in a variety of species. Hoffer et al. (1975) suggested that under some experimental conditions principal epithelial cells in various parts of the epididymis are capable of absorbing portions of degenerating spermatozoa. Under normal conditions epididymal epithelial spermophagy is a rare occurrence, although in bulls, rabbits and monkeys it has been reported (Roussel et al., 1967).

In the rat epididymis, sodium is reabsorbed from the caput to cauda epididymis, and the concentration of potassium in the luminal fluid increases. In the cauda epididymis, sodium and potassium movements are coupled. In the rat caput epididymis, hydrogen ions are also secreted into the luminal fluid of the rat.
caput epididymis and the pH of the luminal fluid changes from 7.31 in seminiferous tubules to 6.4 in caput epididymis and rises to 6.85 in the cauda epididymis, which is a site for hydrogen ion resorption. A shift in pH optimum of epididymal milieu from acidic to alkaline range has been suggested (Mag et al., 1975; Chinoy, 1984) which influences sperm motility. The presence of calcium ions in the luminal fluid of rat epididymis may also be important in the development of sperm motility (Horton et al., 1978). It has been reported by Chinoy et al. (1963 a) that epididymal sperm motility in vitro was affected by a shift in the calcium ions in the buffer medium. The maximum motility was attained only on addition of $10^{-5} \text{M}$ calcium chloride in the buffer, whereas, a higher or lower concentration than the above in the medium had an inhibitory effect on sperm motility of rats.

The rate of sodium and water resorption could be altered under treatment with antiandrogens and efferent duct ligation which also caused loss of sperm motility (Wong et al., 1978). The fluid resorption in rat epididymis is dependent on the presence of circulating androgens and is also influenced by other hormones such as adrenaline and noradrenaline (in the presence of phenoxybenzamine) and gonadotrophins (Wong and Yeung, 1977).

Turner (1979) has investigated the osmolality of fluid
obtained by in vivo micro puncture of hamster epididymis which revealed that rete testis fluid was iso-osmolar with blood serum, seminiferous tubule fluid and all epididymal fluid were very hyperosmolar. It is presumed that a similar situation probably exists in human epididymal fluid, although in vivo micro puncture samples have not been obtained so far. Moreover, it is not clear as to which constituents of seminiferous tubule fluid and epididymal fluids are responsible for hyperosmolality. It is still an enigma as to why the osmolality subsequently declined from caput to corpus epididymis.

It is a well known fact that maturing spermatozoa can receive androgens bound to ABP (androgen binding protein) from the circulation or via synthesis by the epididymis. Steroids and their metabolites are found in epididymal luminal fluid and in the bull, their concentration progressively declines from testis to epididymis. The epididymal epithelium is responsible for transporting steroids into and across it, but their entry and passage into the lumen, is influenced by luminal protein contents (Hinton, 1980).

Secretory products of epididymis:

Recently considerable information has been accumulated regarding the major secretory and/or synthetic products of the epididymis (Etchell and Hinton, 1981; Courrot, 1981;
These biomolecules are mainly secreted by the principal cells of the epididymal epithelium although other cell types might also be responsible for the same. They include: (i) Glyceryl-phosphoryl-choline (GPC), (ii) phospholipids, (iii) carbohydrates, (iv) sialic acids, (v) proteins, enzymes, (vi) steroid binding proteins, (vii) carnitine, (viii) steroids, (ix) vitamins and (x) various metal ions.

Recent studies by Hinton (1980) have suggested that GPC, phosphocholine and inorganic phosphate were secreted in the caput epididymis of rat during sperm maturation and some amounts of these compounds were lost along the epididymis. GPC exercises a stabilizing influence on spermatozoa and maintenance of osmotic pressure balance in the luminal fluid of cauda epididymis (Hinton, 1980). Higher amounts of lipids and phospholipids were observed in caput than in cauda epididymis of rat, sheep and monkey (Rajalakshmi et al., 1976; Prasad and Rajalakshmi, 1976a,b; 1977). However, in human beings little variation was seen in the lipid levels in the two regions of the epididymis (Rajalakshmi et al., 1975).

Prasad and his collaborators showed that the major phospholipid present in caput and cauda epididymides of hamster, monkey and human beings were phosphatidylcholine and phosphatidyl-ethanolamines (Prasad and Rajalakshmi, 1977;
Pajalakshmi and Prasad, 1979), and the high concentrations of phosphatidylcholine in the caput might be related to synthesis of GPC (Voglmayr, 1975). The phospholipid concentration was high in caput epididymal spermatozoa and decreased during their transit through corpus and cauda. Although, the final steps in maturation of sperm have been associated with the acquisition of choline plasmalogens during their transit through the epididymis, the levels of choline plasmalogens either remained constant or else decreased during their transit (Prasad and Pajalakshmi, 1977).

Numerous histochemical, quantitative and autoradiographic studies have been carried out to demonstrate a variety of carbohydrates and enzymes involved in their metabolism in epididymis (Hamilton, 1972). Epididymal spermatozoa utilize glucose via Embden-Meyerhof pathway in vitro or via pentose shunt pathway, or they utilize lactate as the main energy substrate (see Hamilton, 1977).

Sialic acids, free or bound to proteins as glycosamino-proteins are secreted by the epididymis of a number of mammals including human beings (Prasad and Pajalakshmi, 1976 a,b; 1977; Pajalakshmi et al., 1976; Turner, 1979; Hinton, 1980). They probably help in sperm maturation and this has been investigated in rat, hamster and monkey and have been reviewed in detail by the above authors.

The ability of different segments of the epididymis to
synthesize protein in vivo was demonstrated for the first time by Rajalakshmi and Prasad (1976) and this event is an androgen dependent one. 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. Similar findings have been reported by others (Turner, 1979; Hinton, 1980). Flickinger (1983) showed that mouse epididymal epithelium synthesizes and secretes glycoproteins.

Microelectrophoresis of rat reproductive tract fluid proteins (Turner, 1979) have revealed that fewer proteins are present in these fluids than in the serum. The caput and cauda epididymis secrete some proteins and reabsorb others. The proteins of the epididymis are important in sperm saturation, motility and fertilizability (Orgebin-Crist and Jahad, 1978) and the glycoprotein of epididymal luminal fluid of various species induces forward motility of caput epididymal spermatozoa (Hinton, 1980). Acidic epididymal glyco-protein, another protein secreted by epididymal epithelium seems to coat maturing spermatozoa as they pass along the duct (Lea et al., 1978) and thereby affects their surface characteristics, but its exact function is not known. Therefore, prevention of protein synthesis, secretion and absorption would be important targets for male contraception.

Several authors have reported the presence of various
hydrolytic and oxidative enzymes which are androgen sensitive (Ambadkar, 1969; 1973; Iqbal et al., 1973; several papers from Prasad and his group; Chinoy, 1976; Chinoy et al., 1982; and Chinoy, 1984 and several others). These enzymes help in numerous metabolic functions of epididymis.

Two steroid binding proteins have been characterized in rat epididymis (Hansson et al., 1975). In their properties they resemble the rat ventral prostate receptor, in that it is specific for 5α dihydrotestosterone (5α-DHT) (Hansson et al., 1976). This protein is involved in the translocation of the steroid to the nuclear binding sites. The other protein, androgen binding protein (ABP) has high affinity for both 5α-DHT and testosterone. ABP concentration is known to rise significantly from seminiferous tubule fluid to a peak activity in the caput fluid which indicates its selective reabsorption in the ductuli efferentes and epididymal epithelium. This was shown for the first time by Turner (1979) in epididymal fluid. Fobaire (1979) and Fobaire et al. (1981) suggested that epididymal Δ⁴-5α-reductase activity was regulated by a substance directly secreted into the epididymis by the testis and is most likely, ABP. Other testicular proteins (enzyme inhibitors, decapitation factor, inhibin and glycoproteins etc) could be present in epididymal fluid and influence its physiology.
Marquis and Fritz (1965 a, b, c) reported very high concentrations of carnitine and carnitine acetyl-transferase in the epididymis. Zehner et al. (1978) have reported that the highest activity of the enzyme was in the spermatozoa. The concentration of carnitine increased in the epididymal luminal fluid from caput to cauda epididymis as a result of active transport (Setchell and Hinton, 1981) in the middle segment of the rat epididymis.

Several functions have been attributed to carnitine, (1) in maintaining the osmotic pressure of the fluid in the cauda epididymis as a sequel to absorption of ions; (2) as a cofactor in the oxidation of fatty acids by the epididymal spermatozoa; (3) as an acetyl transfer system in the epididymis and the sperm as a buffer preventing rapid changes in the ratio of acetyl-Co-A; (4) in generating the acetyl groups as "energy reservoir" for sperms in a medium poor in substrates and (5) in development of spermatozoal motility in the caput. Carnitine has also been found in human epididymis (Turner, 1979). If carnitine is involved in human sperm motility, then any interference with its active transport across the epididymal epithelium may lead to male infertility. But this is not a feasible approach since heart and several smooth muscle tissues also have carnitine active transport carrier.

The principal cells of the epididymis have been known to
be associated with steroid synthesis in view of their structure, wherein, the Golgi complex is involved in steroid conjugation in epididymis (Hamilton, 1972; Chinoy and Asok Kumar, 1983). The localization of $3\beta$ and $17\beta$ steroid dehydrogenases was demonstrated in rat epididymis (Chinoy et al., 1979 a; 1980 b; Chinoy and Rao, 1982).

**Micropuncture**

Micropuncture is a new technique employed for collecting fluid from the lumen without interfering with the whole tissue and its surroundings. Tuck et al. (1970) first adopted this technique for studying the male reproductive physiology, which was later modified by Hinton et al. (1979) and Hinton and Howards (1982). Chinoy et al. (1983 b) have developed it recently in our laboratory, which will help to study the micro-environment of epididymis and the luminal contents. The obvious advantage of this technique over the others is that, samples could be collected free from contamination of blood and other extra cellular fluid. It will help in the long run to isolate and purify the secretory products of epididymis and to measure the intraluminal steroid hormone titres, transport studies by injection of radioisotopes and the effect of contraceptive agents on the epididymal microenvironment.

Thus it is clear from a reproductive standpoint that the
epididymal fluid is of primary interest because it controls or mediates epididymal function and as such any interference with epididymal physiology can hopefully result in the successful development of a post-testicular male contraceptive. However, care ought to be taken so as not to bring about adverse effects in the genetic make up of the spermatosoa. Hence at present intense research activities are going on to introduce a new male contraceptive agent which selectively interferes with the acquisition of fertilizing capacity of spermatosoa as well as the major factors regulating their maturation in the epididymis. Here, the possibility of a mutagenic effect is reduced and as is the case when using a hormonal therapy. The likelihood of interfering with the normal hormonal status is much less than for agents acting at the testicular level. However, the most beneficial point of the epididymal approach is its speed of action. Any effect on stored spermatosoa could produce infertility within days whereas, an effect on the testis would require several weeks. Therefore, success in any effort to develop new male contraceptive will depend chiefly upon epididymal function, the role of its secretions and absorption on sperm maturation, viability and survival.

Taking into consideration all the factors discussed earlier, efforts have been made to study the epididymal and vas deferens physiology of rat under normal and physiologically altered conditions.
The need to study the mechanism of sperm transport through the vas deferens stems from the need to develop convenient and harmless male contraceptive devices and procedures at the post testicular level (Hulka and Davis, 1972; Bräschke et al., 1974 a, b).

Morphologically, the vas deferens can be differentiated into two regions. One near to the cauda which is called the proximal vas deferens (VDP) and the part adjacent to the accessory sex complex known as the distal vas deferens (VD). Some investigators termed them as scrotal/testicular vas and abdominal vas respectively. Both these regions are significantly different from each other, because of the less thick muscular coat at the proximal end and the well developed muscle coat of the distal end, besides a difference in their luminal shape. Each region of the vas deferens can be differentiated from another on the basis of the characteristic epithelium, lamina propria, vascular supply and the thickness of the muscle coat (Hamilton and Cooper, 1978; Alexander, 1978; Chinoy and Chinoy, 1982). Histologically, the vas deferens consists of mucosa, muscularis, and fibrosa from inside to outer side. The innermost layer, the mucosa has tall columnar and pseudostratified epithelial cells which may or may not possess stereocilia. The epithelial cell height increases gradually from the proximal to the distal vas deferens, and the lamina propria as well as the
longitudinal mucosal infoldings appear more prominent. These folds give the irregular stellate or astral shape of the lumen in the distal vas. The proximal vas deferens has a thin mucosal layer and hence, no infoldings are seen and the lumen appears oval. The infoldings first appear at the junctional region of proximal and distal vas deferens (Orlandini and Pacini, 1977; Hamilton and Cooper, 1978). The difference in the shape of the lumen have been found to be true in the two regions of the ductus deferens of rat, guinea pig as well as mouse (Chinoy and Chinoy, 1982).

The major blood supply of the vas deferens is through the internal spermatic arteries and veins (Hamilton and Cooper, 1978; Alexander, 1978) and a more intense venous plexus is observed around the distal vas deferens as compared to the proximal one (Orlandini and Pacini, 1977; Hamilton and Cooper, 1978).

Flickinger (1973, 1975) and Flickinger et al. (1978) have carried out extensive work on the ultrastructural characteristics of the two regions of the ductus deferens of rat and hamster.

Flickinger (1973) demonstrated by ultramicroscopic studies that the cells of the proximal vas deferens (VDP) in rat and hamster have extensive basal and perinuclear Golgi complexes. Numerous apical microvilli, vacuoles, vesicles, lysosomes and scattered mitochondria occur all
Throughout the cytoplasm. However, the epithelial cells of distal vas deferens (VDD) showed the presence of extensive smooth endoplasmic reticulum (SER) and smaller rough endoplasmic reticulum (RER). The SER existed in fenestrated whorls, whose development in the distal vas varied from cell to cell. The large supranuclear golgi complex, microvilli and smaller but numerous mitochondria were observed which had more sinuous elongate profile than those in the proximal segment. The cytological features of the middle segment shared the characteristics of both proximal and distal regions.

According to Chinoy and Asok Kumar (1989) the rat vas deferens was anatomically and structurally demarcated into two zones, the proximal and distal regions. The columnar epithelium of the vas deferens was pseudostratified from the 5th day old animals and in the distal region it was folded. The epithelium consisted of Type I and Type II or mitochondria-rich cell and basal cells. In older group of rats 60-65 days old, the pencil cell was observed. The Type I cell was the predominant cell type, similar to the principal cell of cauda epididymis. The ultrastructural evidence of absorption and secretion in Type I cell was also similar or more than the principal cells. However, they are structurally distinguishable. viz., the Type I cell mitochondria possessed thicker transverse cristae and a more electron dense matrix than their counterparts in epididymis.
The Type II cell of mitochondria-rich cell in the distal region of the vas deferens showed apical protruberance probably involved in apocrine secretion. The occurrence of this cell in both the regions justified its probable role in acidification of seminal fluid. Basal cells were characteristically equipped with all secretory and absorptive functions. Microfilaments were also observed in the distal region. These facts and the earlier biochemical studies (Chinoy, M.J. and Chinoy, N.J. 1979; Chinoy and Chinoy, 1982; 1983a; Chinoy and Geetha Ranga, 1984) indicate that proximal and distal vas deferens have both secretory and absorptive functions to a variable degree.

Primate vas deferens:

The histology and ultrastructure of monkey vas deferens have been studied by a number of workers. Ultrastructural studies on monkey vas deferens has shown the presence of apical blebs containing tubular profiles of smooth endoplasmic reticulum in the principal cells (Prakash, 1980). The blebs are believed to be a form of apocrine secretion (Ferrall, 1969; Prakash, 1980).

The histology and ultrastructure of adult, fertile human vas deferens revealed (Flickinger et al., 1978) pseudostratified epithelial cells in both regions which could be classified into tall, thin principal cells studded with long stereocilia, small round pyramidal basal cells and osmophilic pencil cells. In addition, mitochondria rich cells were observed
only in monkeys and man (Hoffer, 1970). The occurrence of
different cell types varies in different mammals. The so
called 'pencil cells' of the human vas deferens appears to
be dead or dying columnar epithelial cells. In such case,
these cells do not have any important functions. The basal
cells have poorly developed cytoplasmic organelles. The most
prominent feature being the accumulation of 100 Å cytoplasmic
filaments that resemble filaments seen in muscle cells
(Ishikawa et al., 1969; Cooke and Ray, 1972). Since the
mucosa of the human vas deferens undergoes significant and
rapid changes in diameter during ejaculation, the filaments
may help to stabilize the epithelium when it is stretched.

The above mentioned structural features suggested
that the proximal vas deferens functions in the synthesis
of proteins/glycoproteins and in absorption of material from
the lumen. The protein is absorbed from the lumen in large
vesicles and the hydrolytic enzymes are synthesized by the
proximal vas. The observations of Hamilton and his
associates (Hamilton et al., 1969; Hamilton and Fawcett,
1970; Hamilton, 1971) on the fine structure and biosynthesis
of steroids in the epididymis and vas deferens of intact and
castrated mice and rat suggest that, (1) the epididymis and
vas deferens can synthesize testosterone and other steroids
from acetate, (2) these steroids may depress the respiratory
metabolism of spermatozoa; and (3) the concentric membranes/
whorls of agranular endoplasmic reticulum within the vas deferens of mice may convert acetate into polar compounds beyond cholesterol at a faster rate than the randomly organized tubular agranular endoplasmic reticulum within the epididymis of this species.

From the above discussion it is clear that both epididymis and vas deferens are potential sites for male contraception, since these two organs are actively concerned with sperm maturation, metabolism, viability, transport and survival. Recent studies in several laboratories have shown that the epididymis and vas deferens their structural components, sperms and biochemical constituents manifest differential sensitivity to different androgens and their metabolites in the adult and prepubertal animals (Prasad and Rajalakshmi, 1977; Chinoy et al., 1978 a; Chinoy and Chinoy, 1983 a).

Chinoy and Chinoy (1983) have reported differential response of the vas deferens of the two sides from the same animal to adrenaline, which has been correlated with the greater concentrations of calcium and sodium in the right vas deferens of rats and guinea-pigs. It is known that the high levels of membrane-bound calcium renders the cells less excitable (Westfall et al., 1975). The differential response of the left and right vas deferens might also be related to the levels of intracellular calcium, which is bound to calmodulin, a polypeptide (Cheung, 1960; Slater, 1981), which
acts as an activator for norepinephrine release and muscle contraction as well as other cellular processes, only when bound to calcium. It is presumed by the above authors that the left vas deferens might possess more calmodulin-bound calcium than the right, which has instead, more membrane-bound calcium. Hormonal homeostasis for the maintenance of the functions of epididymis may involve not only free testosterone and its metabolites but also testosterone bound to androgen binding proteins (Rajalakshmi and Prasad, 1979 and several others) and that circulating androgen concentrations is not sufficient for maintenance of epididymal epithelium, but intraluminal androgens are required at least in the ductuli efferentes and proximal epididymal tubules (Turner, 1979). The epididymis and its sperms are also capable of metabolizing the androgens (Prasad and Rajalakshmi, 1976 a, b) and the interaction between spermatosores and the epididymis in maintaining homeostasis of the epididymal tubules is also important (Glover, 1980).

**EPIDIDYMIS AND VAS DEFERENS AS POTENTIAL SITES FOR REGULATION**

An antiandrogen cyproterone acetate has been tried extensively in male laboratory animals for selective inhibition of epididymal functions (Prasad et al., 1970) without affecting accessory sex gland functions or libido.
However, clinical trials by Neumann and his coworkers in Germany and by Prasad and his group in India revealed conflicting results. Studies by Chinoy and Sheth (1977a; Chinoy et al., 1979a; Chinoy and Chinoy, 1979) and Chinoy and Haithili (1980) revealed that CA manifested antianabolic and antifertility effects in agreement with the work of Prasad and his group, but unlike their selective action on epididymis, CA manifested effects at multiple sites. Pajalakshmi et al. (1976) suggested that in the male genital tract, the epididymis is the organ having most sensitivity to antiandrogens. In studies of Chinoy and her coworkers (quoted above) the antianabolic effects were significantly reversible by discontinuation of treatment as well as by ascorbic acid feeding without interfering with its antifertility effects. The beneficial effects of ascorbic acid were found to be mediated via the paramagnetic electron flow from the ascorbate free radical and testosterone-ascorbate charge transfer complex formation which potentiates the anabolic action of endogenous testosterone (Chinoy, 1979; Chinoy et al., 1979b; 1977a). However, CA affected the adrenal cortical functions and therefore its effect as a potent male contraceptive is questioned (Chinoy, unpublished observations).

Another non-steroidal compound Flutamide (a,a,a-trifluoro-2-methyl-4-nitro-m-propionotoluidine or SCII 13521) has been shown by Neumann and Schenck (1980) to have potent antiandro-
genic effects. Dhar and Setty (1978) have also investigated its androgen antagonistic effects on epididymal functions.

It has been suggested that treatment with estrogen in vivo decreases testosterone production by direct inhibitory action on testicular steroidogenesis. Synthetic and natural estrogens inhibit spermatogenesis and accessory gland function. The effects manifested are antinatalotrophic, antifertility and antagonizing the action of testosterone in males (Albert, 1970; Jones, 1970; Patanelli, 1975; Duckett and Lacey, 1975; Johnson and Jones, 1977; Dufau et al., 1979; Taiwar, 1977, 1980; Chinoy and Fao, 1982).

Similarly, studies with estradiol benzoate (E2B) to intact male rats impaired spermatogenesis and reduced the circulating levels of testosterone by inhibiting activities of 3β and 17β hydroxy steroid dehydrogenase of testis and also by affecting Leydig cell function. The treatment reduced sperm density, motility and morphology resulting in zero fertility rate (Chinoy and Fao, 1982; Fao and Chinoy 1983 a,b; Chinoy H.F. et al., 1983 a; Chinoy H.J. et al., 1983 d). The mode of action of estrogens is controversial, some workers believe that they act directly on the steroidogenic enzymes involved in testosterone production in testis by affecting Leydig cell function (Fartas et al., 1977; Purvis et al., 1978; Chinoy et al., 1983 c; Fao and Chinoy, 1983 a,b). The other view is that
they exert their influence indirectly at the pituitary level by a feed back mechanism and thereby causes a decrease in testosterone production and release followed by a significant alteration in secondary sex characteristics and testicular weights (Kalra and Prasad, 1969; Hunt et al., 1979). The supression of postcastration gonadotropin rise by estrogens in immature and adult rats including human beings have been reported (Peterson et al., 1968).

A number of studies have revealed that copper inhibits motility and metabolic activity of human and rat spermatozoa in vitro and in vivo (Bernstein, 1955; Saito et al., 1967; Oster, 1972; Ullman and Hammerstein, 1972; Chinoy and Sanjeevan, 1980 a). Chinoy and Chinoy, 1983 c; Jacht and Bernstein, 1973). In vitro motility of the cauda epididymal spermatozoa of rat decreased more rapidly with increasing concentrations of copper sulphate solutions (Chinoy and Sanjeevan, 1980 a). The copper ions affect sperm survival and penetrability and exert more toxic effects on spermatozoa than the copper salts (Oster and Salgo, 1977), as the former bind with the sulphydryl groups of sperms.

Louzko and Kinel (1971) demonstrated that copper-loop device placed in the ductus deferens of rats produced transient reduction in fertility, without any effect on the sex accessory glands, whereas, local toxic effects of copper-wire devices or salts in silastic tubes/implanted in vas
deferens (Ahsan et al., 1976; Shakoon, 1976), testis (Zoukova and Ninci, 1971), seminal vesicles (Gilmore et al., 1973), epididymis, and scrotum (Chinoy and Sanjeevan, 1980 a) as well as intravasally (Chinoy and Chinoy, 1983 c) have been reported in rat, rabbit, and hamster. Intravasal copper implantation was postulated to be a promising non-occlusive device for long term control of fertility in animals (Ahsan et al., 1976; Shakoon, 1978). Chinoy and Chinoy (1983 c) have reported that intra-epididymal copper device (1ED) and intravasal copper device (IVCD) were more effective than intrascrotal copper device (ISCD) in reducing the rate of fertility of adult male rats.

The above mentioned authors (Chinoy and Sanjeevan, 1980 a) have reported an accumulation of copper in several reproductive tissues in 1ED and ISCD bearing rats and they suggested that such high concentrations of copper caused cytotoxic effects which in turn led to changes in the histophysiolo of these organs. However, in rats which were simultaneously fed with ascorbic acid, the copper levels were restored to almost normal levels thus suggesting that ascorbic acid is beneficial in this case also. It was suggested by these authors, that the contraceptive efficacy of 1ED could probably be enhanced by implanting a larger and/or thicker copper device, since it is known that the surface area exposed to the tissue environment (epididymal,
scrotal and vasal) is a crucial factor in determining the contraceptive efficacy of the device and is related to the amount of copper ions released into the milieu of the organ (Ahsan et al., 1976; Chinyo, 1984).

The effects of testosterone treatment to bring about antifertility effects in the male reproductive functions have been mentioned earlier. On the basis of various physiological and biochemical studies dealing with the relationship in the gonado-pituitary feed back mechanism, in vitro testosterone production, and the role of testosterone in the spermatogenic process (Steinberger, 1970, 1976 a; Steinberger and Steinberger, 1972; Steinberger et al., 1973, 1974). Steinberger et al. (1978) suggested that it should be possible to induce and maintain spermatogenic arrest in man by administrating testosterone enanthate at a dose which would suppress pituitary gonadotropins but would not raise the plasma testosterone levels above the normal range (Steinberger, 1973). The studies of Steinberger et al. (1973) on the effects of testosterone enanthate (TE) as a possible male contraceptive on 20 male human volunteers do not make this testosterone preparation an ideal contraceptive. From their studies on normal adult men, Swerdloff et al. (1978, 1979) have reported that testosterone enanthate (TE) can be given in doses that will suppress sperm counts to below 5 million/mL in most subjects (35 of 39
overall and 16 of 17 with weekly treatment) and produces azoospermia in 36-58% of the subjects. This was accomplished with minimal adverse effects and was entirely reversible.

Neumann et al. (1978) have reported new steroid androgens, perhaps out of the D-homo-Δ^16-series—which are approximately 10 times more active than testosterone. The interesting phenomena they observed was that, in comparison to subcutaneously injected testosterone propionate, the testosterone capsules they used were about 10 times more effective. The explanation for this is the possibility that the plasma levels of testosterone propionate, when injected daily, undergo major fluctuations, whereas, the continuous release by implants maintains a constant plasma level.

Testosterone, in the proper dosage has been shown to cause suppression of spermatogenesis while maintaining the other male characteristics. Sharma et al. (1978) reported that different doses of testosterone caused alterations in the histophysiology of testis, epididymis, vas deferens and other accessory sex glands thus altering the normal milieu of epididymis.

Prostaglandins (PG) E, and F, a have also been reported to bring about alterations in the structure and metabolism of the reproductive tissues. Reduced sperm density, motility, and alterations in their morphology occurred which caused partial infertility (Chinoy et al., 1980 b; Chinoy and
Chinoy, 1981). The latter authors have also reported that the prostaglandins function as α-adrenoceptor blocking agents in adult male rats in agreement with the observations of Stajanche (1973). Their results indicated a probable decline in target organ response to androgen and/or in conversion of testosterone to its metabolites (Chinoy et al., 1980 b).

PGF has been shown to have an inhibitory effect upon testicular steroidogenesis in laboratory animals, whereas, the role of PGZ has not been determined (Bartke et al., 1972; Ericsson, 1973; Ek-Nes, 1971). PGF<sub>2</sub>α markedly inhibited the motility of human spermatozoa but no ultrastructural differences could be ascertained (Cohen et al., 1977). Temporary sterility was achieved by intrascrotal implantation of PGF<sub>2</sub>α (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, 5α-DHT and progesterone after 3-7 days of intrascrotal insertion of silastic IVR tubes containing PGF<sub>2</sub>α and PGF<sub>2</sub>α in the male rats.

Cadmium chloride (CdCl<sub>2</sub>), an antifertility agent was found to have adverse effects on testicular and epididymal metabolism as well as those on the spermatozoa (Gunn and
Ascorbate feeding during the treatment had beneficial effects in maintaining the redox milieu in testis, epididymal spermatozoa (Chinoy and Sethalakshmi, 1976a) and accessory sex organs (Chinoy and Seth, 1977b).

Ford and Whites (1976) investigated the biochemical aspects of control of male fertility by chlorosugars (6-chloro-6-deoxy glucose; 6-chloro-6-deoxy fructose and 6-chloro-6-deoxy sucrose). The treatment brought about reversible antifertility effects. The chloro sugars reduced the oxidation capacity of glucose by the sperms and a decline in the levels of ATP and total adenine nucleotides (Hinton, 1980). However, they are found to have toxic side effects when used as a contraceptive.

a-Chlorohydrin (3-Chlor-1,2-propanediol; U-8597), a derivative of glycerol, has been reported to possess selective effect on epididymis and induces functional sterility in the rat, guinea-pigs, hamster, ram, boar and monkey. Prasad and Rajalakshmi (1976b) and Glover (1976, 1980) have reviewed the work on a-Chlorohydrin and its antifertility effects in detail.etty et al. (1976) have reported severe toxic effects of a-Chlorohydrin. Its effects are mainly on sperms in epididymis and vas deferens.

Effects of nutritional deficiencies (Vitamin C deficiency and Total protein deprivation):

Vitamin C deficiency in guinea pigs have been shown to
affect the androgen-sensitive parameters and structure of
the testis, epididymis, vas deferens and accessory sex glands
and caused an 'androgen-deprived effect' to these target
organs. This inturn resulted in changes in the metal ion
profile and in the morphology, motility and density of cauda
epididymal and vas deferens spermatozoa. The sensitivity
of vas deferens to different doses of adrenaline was also
reduced in such animals and decreased their fertility rate.
However, the primary action seems to be at the testis level.
These studies manifested that AA is essential for maintaining
the physiological integrity of the androgen target reproductive
organs in guinea pigs (Chinoy et al., 1983a; 1984a).

It is a misbelief that malnourished populations have
high fertility rates, but on the contrary, it limits fertility
(Gopalan and Haidu, 1972). Studies from our laboratory (Chinoy,
1984) have revealed that under protein-caloric malnutrition
conditions, the metabolism of reproductive organs were affected
decreasing the fertilizability of spermatozoa due to their
decapitation. In such cases, oral feeding of AA helped in
restoring the metabolic activities. However, fertility rate
remained the same.

Vasectomy and vasocclusion:

Although, epididymis is a very feasible site for male
contraception, vasectomy enjoys the maximum popularity in
the world today, because of its effectiveness, speed and
simplicity. In recent years vas deferens has received a
great deal of attention since vasectomy (Hulka and Davis,
1972); reversible vas occlusive devices (Hooker and
Gilmore, 1972); Johnson, 1972) and intravasal devices which
could be turned "on" or "off" with or without minor
surgical procedures (Brueschke et al., 1975; Clark and Prager
1972) could be used. Since the production of testosterone is
not altered under vasectomy, this surgical technique has
not been known to interfere with the libido of the individual.
However, a large scale, long term statistical study comparing
various vasectomy techniques with their respective failure
rates and complications is still warranted. Individual
studies demonstrated that the complication rates for vasecto-
 mies are extremely low. They can include epididymiditis,
sperm granuloma, haematoma, and infection in some cases.
Although present techniques of vasectomy have proved safe,
effective and acceptable, efforts are being made to make
future male sterilization techniques more simple and totally
reversible. Future procedural prospects for male steriliza-
tion are: (1) Non-surgical transcutaneous vas occlusion,
(2) pharmacological sterilizing agents, (3) irrigation of
vas for immediate post vasectomy sterility, (4) vas occlusive
plugs and prosthetics and (5) intravasal reversible devices
and valves. Thus it is clear that although vasectomy has
many advantages over numerous other contraceptive devices,
Research is still going on to develop a more sophisticated post testicular method for male contraception.

Biochemical and electron spin resonance (ESR) spectroscopy studies carried out by Chinoy et al. (1978 c) to study the effects of short term administration of AA to vasectomised rats on the metabolism of testis, and epididymides indicate that vasectomy causes alterations in the physiology of these organs. However, the adverse effects were overcome by extraneous AA feeding to vasectomised animals. The biochemical and ESR data suggest that the beneficial effects of AA and its mechanism of action have important implications in prophylactic treatment following vasectomy. Similarly, ESR studies on Mn++ free radical in the cauda epididymal sperm suspension of normal, vasectomised, and vasectomised + AA treated rats were carried out. The characteristic hyperfine line spectrum of $g = 2.003$ at a magnetic field of 3390 gauss was obtained in each case. The analysis of the spectra revealed that the spin concentrations of the free radical of the experimental groups were not significantly different from those of control. Taking into consideration the importance of Mn++ in various reproductive functions, it is evident that vasectomy does not affect the utilization of Mn++ by epididymis (Chinoy and Seethalakshmi, 1978 ; Chinoy et al., 1983 f). Other long term studies (Chinoy and Sanjeevan, 1980 b; Chinoy and Chinoy,
1984 a) revealed that vasectomy altered the androgenic metabolism and histology of the reproductive organs. Ascorbic acid metabolism was significantly augmented and oral feeding of ascorbate to those animals was beneficial. Based on these studies ascorbate feeding is recommended for clinical trials to prevent atherosclerosis (Alexander and Clark, 1978) and other side effects in human males (Chinoy, 1978; Chinoy et al., 1982 b).

The metabolism of testis, caput and cauda epididymidis were significantly altered in vas deferens ligated (VDL), vasa efferentia (VBL) and total ligated (VDL + VBL) rats, although their weights were found to be the same as in control. Here again the ascorbic acid metabolism was found to be the same as in control. Here again the ascorbic acid metabolism was found to be enhanced indicating the response of these organs to overcome the stress due to ligation (Chinoy and Seethalakshmi, 1978 b).

Vas occluding agents such as ethanol (Sharma et al., 1983), ascorbic acid, prostaglandins, copper sulphate and ferric nitrate (Chinoy and Chinoy, 1984 b) have been carried out by these authors. However, the practical applicability of this technique has to be studied in detail.

The studies elucidated in the present thesis also stresses the importance of ascorbic acid, an important biologically active reductant, playing a dynamic role in
several oxido-reduction reactions in reproductive tissues, organs and functions of several mammals (Chinoy, 1978). The human beings, other primates, guinea-pigs, armadillo, Indian giant fruit-bat, some birds and insects are incapable of synthesizing Vitamin C in their bodies (Chinoy, 1978). The loss of capacity to synthesize AA is an example of evolutionary loss of function and is due to the absence of an enzyme, gulonolactone oxidase from the liver microsomes which converts L-gulono-γ-lactone to ascorbic acid.

Ascorbic acid occurs in free and bound form or ascorbigen in animal tissues (Halakar, 1963; Chinoy, 1978, 1984; Chinoy et al., 1982 b). The various storage sites of the vitamin in the body are liver, adrenals, gonads, brain, kidneys, and accessory sex glands. The ascorbigen content and storage capacity show distinct variations. According to Dieter (1969), Hazarudar and Chatterjee (1974), Chinoy and Seethalakshmi (1978 a), Chinoy and Rao (1979), Chinoy et al. (1979 b), the storage, tissue distribution and also synthesis of ascorbic acid are under the control of sex hormones in rats and cockerels. A profound sex difference in the synthesis and concentration of ascorbate in avian and mammalian tissues has also been demonstrated, wherein, the males had more AA than the females (Stubbs and Mc Kernen, 1967; Chinoy et al., 1974 a, b).

It is a well established fact that ascorbic acid
participates in several oxido-reduction processes. The oxidation of ascorbic acid (AA) involves two important steps. In the first instance, its oxidation is catalyzed by a special peroxidase (Gurevich, 1963; Gorbunova, 1966; Chinoy, 1970; 1973), which monovalently oxidizes ascorbic acid to its free radical (monodehydroascorbic acid (MDHA), which is a highly unstable, semi-quinone like radical (Yamazaki et al., 1959; Lewin, 1976; Chinoy, 1978). 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 completes the whole cycle (Sebrell and Harris, 1967; Chinoy, 1973).

Extensive studies carried out from our laboratory have revealed for the first time that the tissue metabolism is energized not only by the high energy phosphate (ATP), but also via the paramagnetic electron flow from MDHA which participates in several oxido-reduction reactions as a source of electron energy. Ascorbic acid also forms charge transfer complexes (CTC) with macromolecules via., proteins, nucleic acids and steroids (Elizain, 1971; Swartz et al., 1972; Chinoy et al., 1978 b). 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, the mechanism of action of ascorbic acid in animal tissues is via the formation of its free radical and CTC formation with macromolecules. Ascorbate also has an indirect involvement, since it inhibits the activity of the enzyme, phosphodiesterase (PDE) and hence, increases the level of cyclic - AMP. c-AMP, the second messenger is known to activate many enzymes.

The storage and the concentration of the vitamin can be altered under several stress conditions, viz.: treatments with contraceptives, vasectomy and other surgical stress, changes in temperature, nutritional deficiencies, radiations etc., some of which have been already discussed earlier (Chinoy, 1976, 1984; Chinoy et al., 1982 b). They have shown that the concentration of the vitamin is more in actively growing and metabolically active tissues. The concentrations of AA were higher in the testis and epididymis than in the liver, where the synthesis of the vitamin is known to take place (Chinoy, 1976).

All reproductive organs as well spermatocytes are capable of utilizing ascorbic acid, since they possess the necessary enzymes for its metabolism. The rate of utilization as is shown in the present studies also increased under any imposed stress condition and involves the mobilization of the bound form of ascorbate to its free form. The utilization of ascorbic acid in various oxido-reduction reactions,
enzyme activation, electron transport, metabolism of proteins, nucleic acids, carbohydrates, lipids, minerals as well as in brain and muscles are well documented by Chinoy (1978). Ascorbic acid has an important relationship with various hormones such as testosterone, norepinephrine (Levin, 1976; Chinoy and Rao, 1979). It has a synergistic action with testosterone and potentiates its anabolic action for germ cell saturation, increasing activity of androgen-sensitive enzymes and metabolism in target tissues (Matsky, 1973; Chinoy and Seethalakshmi, 1970 a, b, 1979 a, b; Chinoy, 1978; 1984; Chinoy et al., 1962 b; Chinoy and Chinoy, 1963 a; Chinoy et al., 1964 a) under normal and altered physiological conditions.

PLANTS AND PLANT PRODUCTS AS FERTILITY REGULATING AGENTS:

Promoting the use of plant products for the contraceptive purpose have been done by various organizations including WHO in India and several other countries. Throughout history, peoples in all parts of the world have used different parts of plants for fertility regulation. The Paraguayan Hatto Grosso Indian tribes still use a weed extract to prevent conception. The women prepare a solution using dry weeds and water and drink this orally. This preparation has been tested in the laboratory on rats and does reduce fertility by 59-79%.
There are many natural remedies found throughout the world in folk medicines passed from one generation to the next. Some of these are (Himes, 1963):

The Apache Indians used a solution of a yellow flowering plant, a lithosperm, that did have some ability to block ovulation. In Morocco, Castor beans were given to prevent pregnancy; one bean for each year for the woman to prevent pregnancy. Castor beans have been known to be highly toxic and can cause severe cramps (4 to 5 beans taken orally causes death of the individual). In Martinique, both lemon juice and a concoction of mahogany bark (the bark has tannic acid) was used as a douche. Since both these substances are acidic in nature they act as potent spermicides. A South African tribe is known to use tampons of Acacia twigs. These may act as mechanical barrier, but acacia has gum arabic in it, which forms lactic acid and it is metabolized. For many years, lactic acid was used as a spermicide.

The first written record of contraceptives from plants was found in the Petri papyrus dating to 1850 B.C. This Egyptian papyrus listed a number of contraceptives, such as crocodile dung paste, and a mixture of honey and natron (Sodium bicarbonate) both used as suppositories. But neither honey nor natron could have been chemically effective, but would have definitely acted as mechanical barriers. Although, honey is a popular substance even today in several remedies
still there is no evidence concerning its chemical effect on spermatozoa.

The Greeks used cedar oil and lead salts as well as alum, oils and fruit juices etc., for contraception. It is presumed that these agents may have diluted the semen and acted as barriers, but probably no other chemical action could be induced with them (Unless the juices were acidic). Since lead can accumulate in the body and can act as a cell poison it could have been dangerous over a period of time. Later the Romans used a number of extracts for contraception including myrtle and other plants. But there is no evidence to state that these extracts had any effects on sperm, but myrtle does have a drug called vincristine (which is similar to that found in **verwinkle, Vinca rosea** plant), which is used to block cancer of the chorion in pregnant women. These substances may not have been active directly, but they could possibly have reduced the libido of both men and women.

In recent times, it is believed that a contraceptive from a plant(s) would be more acceptable for economic reasons in terms of self reliance and the possible practicability of a male pill approach. For several other reasons and their widespread use they deserve a proper scientific assessment, since they may have toxic properties, even if some would seem to be effective.
Recently extensive efforts have been made to study the antifertility drugs from plants. Many of these drugs have been screened for antifertility activity in laboratory animals and some for clinical trials (Chaudhury, 1966; Dhawan et al., 1977; Garg et al., 1971; 1978; Schwartzman et al., 1977; Nikhat Begum, 1979; Guerra and Andrade, 1978; Setty et al., 1977; Lantum, 1980; Syed I. A. and Shaamuddin, 1980; Chaudhury and Haq, 1980; Osamchieska et al., 1980; Chaudhury et al., 1980; Talal, 1981; Karimov and Hata, 1981; Popli, 1981; Khakami, 1981; WHO Annual Reports, 1979, 1980, 1981, 1982).

The possible contraceptive and/or abortifacient value of some herbs and other plants used by preindustrial people is attested to by World Health Organizations (WHO) Task Force on Indigenous Plants for Fertility Regulation (Chain and Lane, 1980). WHO is pursuing other projects in the same area specifically in isolating and characterizing the active agents in substances where preliminary pharmacological data already exists (WHO 9th Annual Report, 1980). Plants used as contraceptive agents are currently being studied in Mexico, Paraguay, Hong Kong, Bangladesh, China, and India.

Here a brief review is given on the plants and plant products used in females for contraception and later those few compounds which are useful as male fertility agents will be dealt with.
**Montana tomentosa** (Cerv); s.s.p. *tomentosa* (Spn), commonly known as *zoapatle*, is a well-known plant in Mexico. Its name was given by Vicente Cervantes in honour of the outstanding Mexican physician of the 18th century, Luis Montana. The plant which belongs to the family, Compositae has been in use since the past 500 years as a crude aqueous extract after boiling its leaves in water for 10 to 20 minutes. Oral ingestion of this aqueous extract is associated with an increase in uterine contractions in pregnant and non-pregnant women; with no side-effects being reported (Gallegos and Cortes-Gallegos, 1974). Recently, two uterotonic compounds of novel structure, Zoapatanol and Montanol have been isolated from zoapatle plant (Levine et al., 1979). These compounds have abortifacient action (Farnsworth et al., 1981).

Similarly, *Mentha arvensis* family Labiatae, was a folklore remedy used to terminate pregnancy. Kanjanapothi et al. (1980) have reported that its aqueous extract exhibited a uterine stimulant (uterotonic) effect when tested on the rat uterus in situ (Kanjanapothi and Taeositikul, 1978). The fraction revealing uterotonic activity was isolated and it was found to be active on the nonpregnant as well as the pregnant rat uterus (Kanjanapothi et al., 1980).

The effects of a number of plant products have been
studied on reproductive processes and fertility rates of male and female animals. A large number of these possess abortifacient properties, viz., alcoholic extract of carrot seeds (Sharma et al., 1976; Garg and Garg, 1971; Jacob and Morris, 1969; Jacob and Kaul, 1973), bitter roots of Aristolochia indica L., and indigenous shrub (Biswas and Ghosh, 1973; Pakrash et al., 1976; Pakrash and Pakrasi, 1977), antiestrogenic potency (Pakrash and Shaha, 1977).

Aphyranthes squamata Linn. (Amaranthaceae) is an abundant indigenous medicinal herb, where the extracts showed significant abortifacient effects in mice and rabbits (Pakrash et al., 1975) but not in rats (Pakrash and Bhattacharya, 1977). These workers have suggested that the abortifacient action might be due to deficiencies of prolactin, GH or pituitary gonadotrophins. A number of other workers (Batta and Einthakumari, 1971; Kholkute et al., 1972; Kholkute and Udupa, 1974; 1976; Alami, 1976; Dixit, 1977; Biegel and Farnsworth, 1980) have reported that the flowers of Hibiscus rosa-sinensis (Linn. Malvaceae) possess antifertility, antiestrogenic and anti-implantation properties and also cause degenerative changes in the ovarian tissue.

However, daturacystone, (DQ₁) isolated from Datura quereifolia was the most effective antifertility agent, but possessed no anti-estrogenic activity (Chandhoke, 1978; Char and Halla, 1976).
Some other plant extracts such as methanol extract of whole plant of *Luna carinifolia* Linn. and chloroform extract of leaves of *Cudocarpus previfolius* altered normal estrus cycle in rats and prevented pregnancy (Khokute et al., 1978).

The aqueous extract of dry berries of *Ribes rubrum* Linn. was reported to cause antifertility and anti-implantation effects in mice and rats (Hunshi and Rao, 1972; Hunshi, 1974; Radhakrishnan and Anjum, 1975; Prakash and Hathur, 1975; Arora and Chatak, 1971). However, Khokute et al. (1978) failed to reveal any antifertility effect of Embelin isolated from *Ribes rubrum*. Similarly, the antifertility and antigestational activities of fresh green leaves of *Artobotrys odoratissimus* Linn. have been confirmed in rats (Chakrabarti et al., 1968; Prakash and Hathur, 1977 a, b; Prakash, 1978 a, b).

The antifertility effects of dried fruits of *Piper longum* (Piperaceae) and its various extracts are controversial as some authors report antifertility effect in female rats (Khokute et al., 1975; 1979), but others (Hunshi et al., 1977) failed to demonstrate the same.

Similar antifertility effects were noted in Unani drugs (Mukhat Begum, 1979); aqueous and alcoholic extracts of *Lycopodium flexorum* (Tailande and Mahajan, 1980) and indigenous drugs (Suganthan and Santhakumari, 1979). Antigonadotrophic activity of *Lithospermum ruderale* and *Lycopodium flexorum*
Two diterpenoid ortho-esters, Yuanhuacine and Yuanhuadine, isolated from the root of *Daphne genkwa* in 1971 and 1979 by Chinese workers manifested abortifacient effects in monkeys and these two components are considered to be relatively safe for clinical trials (Lin Thong-Min et al., 1981). Their mechanism of action is release of endogenous prostaglandins and necrosis of decidual cells.

Recently, Chinoy and Trivedi (1980; 1983) from our laboratory showed that aqueous and alcoholic plant extracts of *Carica papaya* seeds and *Vigna rossa* leaves possessed anti-estrogenic and/or estrogenic effects on rats depending on the dose of the treatment. The extracts also manifested anti-implantation and antifertility effects. The uterine contractility was enhanced and the cycle was disturbed. Aqueous extract had more potent effects than alcoholic and more so on the ovary than the uterus. The withdrawal of the treatment caused recovery in structure and metabolism of the ovary and uterus and also restored the fertility. Therefore, functional sterility could be induced in female rats by short term plant alkaloid treatment (Chinoy and Trivedi, 1980; 1983; Trivedi, 1982).

Studies on the effects of plant products on male reproductive system and fertility are comparatively few and
far fetched. However, recently many laboratories are engaged in developing a male contraceptive from the plants. Extracts of about 1600 Indian plants were tested in vitro on rat and/or human spermatosaa (Setty et al., 1977). Thirty extracts showed spermiocidal activity in rat and 16 of them caused instantaneous immobilization of human spermatosaa. Singvi and Lall (1980, 1981 a, b) observed the contraceptive properties of the flower extracts of *Hibiscus rosa-sinensis*. They also studied the isoenzyme, lactate dehydrogenase (LDH) activity in spermatogenic and androgenic cells of normal and treated testis of a non-scrotal rat, *Hippobosca kinneri*. Seshadri and Venkataraaghavan (1980) and Seshadri et al. (1980) investigated the effects of aqueous and alcoholic extracts of *Euphorbia rubra* on reproductive organs in male rats. Fabelin exhibited 57.9 and 55.5% antifertility activity in albino rats (Munshi et al., 1972; Krishnaswamy and Purushothaman, 1980) and an anti-spermatogenic action (Geth et al., 1982).

The effects of *Punarnava tuberosa* on the male reproductive system of rats (Daftari et al., 1980), of Plumbagin on spermatogenesis and accessory sex organs in adult rats (Santhakumar et al., 1980) were also investigated. The crude steroidal extract of *Anura bactatorium* Linn. caused testicular lesions marked by the cessation of spermatogenesis and a significant reduction in the diameter of the
seminiferous tubules of testis of albino rats (Bajul et al., 1981). They also found an increase in cholesterol levels but decrease in sialic acid, total proteins and glycogen contents of the treated epididymal tissue.

The extracts of Azadirachta indica manifest antifertility effects in male mice (Deshpande et al., 1980) and Cryptolepine possess α-adrenoreceptor blocking properties in the isolated vas deferens of rats (Noséski and Bangbose, 1980). The effects of Alfalfa seeds on cholesterol metabolism have also been reported (Malinow et al., 1980). Speanan, an indigenous herbal drug produced testicular regeneration particularly in the germinal epithelium, the number of spermatocytes, their size and structure in rats (Sethi and Chaturvedi, 1980).

Kauwolfia alkaloids have been known to produce gynaeco-mastia and gynaecomastia. This effect was first reported by Wilkins (1954) and the site of action is thought to be at the hypothalamic level.

Over the years a number of anti-spermatogenic compounds have been found and characterized (Jackson, 1966; Patanelli, 1975; Shandilya et al., 1979; National Coordinating Group on Male Infertility Agents, 1979).

In the Peoples' Republic of China, which is aiming for zero population growth by the year 2,000 A.D., a priority programme for achieving control of fertility in the male has...
been developed. Considerable interest has been aroused around the world in gossypol and its use for fertility regulation in men (Zou et al., 1981). Gossypol is a yellow coloured polyphenolic asymmetrical dinaphthyl dialdehyde (2,2'-binaphthalene-8,8'-dicarboxaldehyde-14',6,6',7,7'-hexahydroxy-5,5'-disopropyl-3,3'-dimethyl) compound present in cotton seed and stem and root of plant *Theophrasaps populnea* (Family, Malvaceae). It is optically active and strongly dextro-rotatory (+) (Hing and Silva, 1968), possesses reducing properties and is very susceptible to oxidation. The six phenolic groups are also very active, readily forming esters (Jackson, 1962). A large number of derivatives of gossypol including imino-compounds, esters, acetate, formate, and metallic complexes have been prepared, but none have shown antifertility activity better than gossypol acetic acid (Wong et al., 1979).

Animal studies performed in and outside China since 1978 revealed major species differences in the antifertility effects of gossypol, while male rats, hamsters, monkeys and human beings are sensitive, mice, guinea-pig, rabbits, pigs, goat, sheep and cow are relatively resistant (Chang et al., 1980). However, Wong et al. (1983) have concluded that in rats gossypol acetic acid had no direct effect on the transport processes of cauda epididymides and that the change in the epididymal sodium concentration and sperm
function could be attributed to an effect of gossypol at the more proximal sites. According to Chang et al. (1980) the rats and monkeys seem to be the most sensitive species. However, there appears to be strain differences in rats to antifertility action (Prasad and Diosfaiusy, 1981). Hajhn et al. (1981) have reported reversible male antifertility activity with orally administered gossypol acetic acid in rats at 20 mg/kg/day and in hamster at dosages at 10 mg/kg/day but not in mice at dosages up to 40 mg/kg/day. They observed increased number of degenerating spermatocytes in the testicular tubules and several pigment laden cells containing an intracytoplasmic lipofuscin like material in the testis and epididymal interstitium in rats and mice. Male rats became infertile in 3-5 weeks after daily administration of 15-30 mg/kg of gossypol acetic acid. The onset of infertility seems to be dose related (Xue, 1981; Xue et al., 1980), and the antifertility effects persisted for 3-5 weeks following withdrawal of treatment, and thereafter fertility gradually returned. The reversibility dependent upon whether the spermatogonia were damaged or not. Long term treatment increased the possibility of damage to the spermatogonia thus leading to sterility (Xue, 1981; Zhou Lan- Fang and Lei Hui - Peng, 1981).

The antispermatogetic effect of gossypol has been tested in experimental animals and human beings in China and has
been found to be effective in inhibiting spermatogenesis with only minimal side effects. The Leydig cells did not appear to be damaged by gossypol treatment, the serum testosterone and LH levels were unchanged and libido was not affected. Studies by Nadakavukaren et al. (1979) on rats revealed a marked decrease in the number of sperms in the epididymis after gossypol treatment. However, studies by Lin et al. (1980) showed that 30 mg/kg/day dosage caused significant reduction in sperm production and the degree of reduction was related to the duration of treatment. They also observed a decrease in the serum testosterone levels. At a low dosage (7.5 mg/kg/day) no differences in the morphologic effects of gossypol monoacetate on the testis or epididymal spera were observed. However, chronic administration of gossypol to rats led to mitochondrial and flagellar damage in testicular and epididymal spermatonia (Hoffer, 1980; 1982; Oka and Hruda, 1982) and to a decrease in sperm ATP content with a concomitant loss of motility (Ke and Iso, 1982). Mitochondrial involvement was also implicated by studies suggesting that gossypol may act as an uncoupler of mitochondrial oxidative phosphorylation (Abou-Donia and Dieckert, 1974), and inhibit lactate dehydrogenase – X, an enzyme postulated to participate in a shuttle system transferring H⁺ from cytosol to mitochondria (Gerez de Burgos et al., 1973).
Bardin and associates (1980) found that the male rhesus monkey was resistant to the antifertility effects of gossypol (20 mg/animal/day for 3 months). No changes were observed in testosterone or gonadotropin levels. Although, sperm counts were normal, decapitated sperms were frequently observed.

Hoffer (1983) has reported light and electron microscopic observations on rat testis treated with gossypol at a dosage of 10, 20 and 30 mg/kg per day for 2-11 weeks. At the light microscopic level, the author has observed the presence of severely damaged and entirely normal seminiferous tubules adjacent to one another in the same section. Affected tubules exhibited intratesticular vacuoles of varying sizes, exfoliation, and atrophy. In electron microscopic studies, severely affected Bertoli cells were observed with many large vacuoles as well as an overall decrease in the cytoplasmic ground substance, rough and smooth endoplasmic reticulum and Golgi apparatus. At the MI level the most striking effect of gossypol treatment was the production of ultrastructural defects exclusively in the mitochondrial sheath of stage 18 and 19 spermatids. These changes were obtained in rats treated with gossypol at a dosage of 20 mg/kg body weight/day for 2 weeks and increased with the dose and the duration of treatment (Hoffer, 1983). However, Shandilya et al. (1982) did not observe any ultrastructural changes of
a consistent nature either in the mitochondria or the plasma membranes with low doses of gossypol. However, with higher dosages, total disruption of the integrity of axial filaments occurred and it resulted in a spermicidal effect. Koffer (1982) indicated that the deleterious effects of gossypol in vivo are specific to spermatozoa, since no changes were observed in the epididymal and vasal epithelium.

At present several findings point towards significant toxic effects of gossypol in experimental animals at the required antifertility dosages. These included body weight reduction, damage to lungs, liver, kidneys and viscera and caused even mortality in male rats, mice, rabbits and hamsters (Wang et al., 1979; Saksena et al., 1981; Patuohni and Osborn, 1981). Studies by Heinbaur et al. (1983) confirmed the above observations.

The first clinical trial of gossypol was performed in China in 1972 and three types of tablets containing gossypol, gossypol acetic acid and gossypol formic acid have been prepared for clinical studies in 14 provincial and municipal districts of China since 1974. No serious side effects were observed provided the dosage was kept at the antifertility level.

A recent report indicates that more than 9,000 human volunteers have been treated with gossypol in China over a period of three years without any overt toxicity. The drug has
been reported to be 98.4% effective (Liou, 1981). A dosage of 60-70 mg/day for 35-42 days caused a gradual increase in the percentage of non motile spermatozoa followed by oligospermia, necrosperrmia and azoospermia. Recovery occurred around three months after discontinuation of the drug (Liu Zheng-que et al., 1981). Most of the side effects could be reduced by lowering the dosage to 24-35 mg/day (Qian et al., 1980).

Richmann et al. (1983) have reported that gossypol brings about a dose related inhibitory effect on human sperm motility. The drug also inhibited fructolysis and glycolysis by human spermatozoa. Both lactate and CO₂ formation from the 14C-labelled sugars was inhibited, and the prevention of CO₂ formation from [1-14C]pyruvate and [2-14C]pyruvate by gossypol indicated a direct effect on the tricarboxylic acid cycle. The significant disturbances on the sperm energy metabolism induced by gossypol treatment were also reflected by a striking fall of the sperm ATP content. However, Richmann et al. (1983) observed that gossypol was also relatively inert in human vaginal mucosa cells. This specificity of gossypol towards spermatozoa, could lead to gossypol-based vaginal contraceptives.

Gossypol did not interfere with the hormonal secretory system of hypothalamo-pituitary-gonadal axis and libido in human beings as well as animals (Xue, 1981). Of all the side
Gossypol can induce renal potassium loss which may be the result of its stimulatory effect on renal prostaglandin biosynthesis and/or its inhibitory effects on its renal Na-K-ATPase activity. The influence of gossypol on K metabolism is conditional. Clinically, the prior use of potassium salt can prevent the occurrence of gossypol-induced hypokalemic paralysis.

Chi-Yu Lee and Halling (1981) of NIBS, USA have discovered that gossypol is a preferential inhibitor of an important enzyme, lactate dehydrogenase-X (LDH-X) crucial in the function of spermatogonia, and has an effect only on certain sperm generating cells and spermatagonial itself. Gossypol inhibits sperm motility and metabolism (Ridley and Masco, 1981; Poso et al., 1980) as well as mitochondrial respiration (Xu, Esha Pu, 1981).

The present rapid increase in research aimed at clarifying the mode of action of gossypol and the synthesis of more active and/or less toxic analogues was recently discussed by Prasad and Liczfeldy (1982); Johnson et al. (1982) have shown that gossypol is a potent inhibitor of
human sperm acrosin and that this inhibition is effected by a strong attachment to and/or penetration of the membranes of the sperm head. The mode of action is not fully understood. However, several possibilities have been considered, e.g., gossypol penetrates the sperm head membranes and reacts with acrosin when the enzyme is released from the spermatozoa, or gossypol stabilizes the sperm structures preventing the release of acrosin. However, the results obtained by Tso and Lee (1960b) on sonicated boar spermatozoa indicate that gossypol is able to interact directly with the acrosin molecule.

The investigations of several others and the ones mentioned above enable them to work towards redesigning or restructuring the drug to enhance its antifertility potential and decrease or eliminate any undesirable side effects.

Although several investigators have substantiated the fact that gossypol is a very effective and reversible antifertility agent and has provided a new lead in drugs for male fertility regulation from natural sources, further research and continuing studies are needed to reduce the side effects, especially hypokalemia, and to increase the reversibility rate after cessation of gossypol treatment. If this could be achieved, gossypol may eventually become a practical and potential answer for male contraception and the synthesis of gossypol analogues might lead to the
Another plant product which has evoked some interest for its antifertility effects is the berries of *Solanum xanthocarpum* (Family, Solanaceae Linn.). Recently the antispermaticogenic/antiandrogenic properties of solasodine (C_{27}H_{43}O_{2}) obtained from the berries of *Solanum xanthocarpum* has been studied on the male genital tract of dog (*Canis familiaris*) by Dixit and Gupta (1982). Their results indicated that chronic administration of solasodine (20 mg/kg body weight every alternate day for 30 days) caused testicular lesions leading to impairment of spermatogenic elements, lack of sperms in epididymal tubules, significant reduction in total protein, sialic acid and glycogen content of testis and epididymis as well as elevation of testicular cholesterol. A significant decrease in the acid phosphatase levels were also observed in testis. The reduced number of spermatogenic elements, degenerating seminiferous tubules, shrunken Leydig cell nuclei etc., indicated lower gonadotrophin plasma levels (Dixit et al., 1975). However, the authors were not sure whether solasodine caused a reduction in testosterone synthesis by enzyme inhibition or competed with androgens for binding sites in androgen-dependent tissues.
Carica papaya L. (Papaya, Papaw, Melon tree, Maamoa, Fruta de Bomba, Lechosa, Melon Zapote) is found in Tropical South America, West Indies and India. The plant is cultivated throughout Hawaii. The plant produces a latex which is extracted by cutting the bark. The latex contains a proteolytic enzyme papain which resembles pepsin in the physiological action. The latex is coagulated in earthenware vessels. It is a commercial source of enzyme which is used in the production of digestive medicines, canned meat, leather tanning, and shrink resisting woolen fabrics. The enzyme is also used to prevent cloudiness in chilled wine. The plants are propagated by seeds. Other important parts used are milky juice, seeds and pulp.

**Constituents:** In the early stages, the fruit secretes a white milky viscid juice which contains an albuminoid digestive enzyme or milk curdling ferment - papain or papainotin. A milky juice comes from the rind, which becomes yellow or orange when ripe. The pulp of fresh fruit is known to contain a caoutchouc like substance, a soft yellow resin, fat and albuminoids, sugar, pectin, citric, tartaric and malic acids, dextrin etc. Dried fruit contains a large amount of ash 0.4 per cent, which contains soda, potash and
phosphoric acid. Seeds contain an oil or caricin, an oil like substance of disagreeable taste and smell and several acids similar to palmitic acids, carica fat acid and a crystalline acid called papayaic acid, also a resin acid and a soft resin.

Leaves contain an alkaloid called carpaine and a glucoside named carposide. On examining carpaine, Heron and Van Rijn found that it is a secondary base. The present accepted formula is $C_{14}H_{25}O_4$. The alkaloid can be purified by repeatedly crystallising the base from dilute spirit when it occurs in the form of colourless, lustrous, needle shaped crystals with a melting point of $121^\circ C$. Carpaine hydrochloride soluble in water, used hypodermically as an injection $1/30$ to $1/15$ of a grain as a cardiac tonic in place of digitalis.

Carpaine is said to be not very toxic. A dose of 5 mg when injected intravenously in experimental animals caused only a slight fall of blood pressure which, however, returned to the normal level within a very short time. The action of the heart is depressed and both the ventricles and auricles show evidence of slight depression. The respiration was not depressed to any great extent. The volumes of the different organs were very slightly affected, if at all. The alkaloid is said to have not been used in therapeutics (Nadkarni, 1954).
Another alkaloid pseudocarpaine isolated from the plant showed that it was diastereic. The fragmentation pattern observed for pseudocarpaine was identified with that of carpaine. But for minor differences in the relative intensities of the ions, the two alkaloids had the same gross structure. Pseudocarpaine on acid hydrolysis yielded carpamic and pseudocarpamic acids. The NMR data provided confirmatory evidence for the build up of pseudocarpaine from the two dissimilar halves, one represented by carpamic acid moiety and the other by pseudocarpamic acid residue (Govindaohari et al., 1965).

From the seeds of Carica papaya a substance with m.p. 165°C, and molecular formula C₁₇ H₁₀ N₂ S was isolated and named carpasamine. Chemical properties of this compound together with its degradation products have been studied and some new derivatives have been prepared from it. Carpasamine, has been identified as benzylthiourea or benzylthiocarbaamide.

The ripe fruit of Carica papaya is digestive and the green fruit is laxative and diuretic. It is most efficacious in dyspepsia. Juice of green fruit if applied locally in the shape of a dressing to the os uteri induces abortion; it dissolves in coagulated albumin. Fresh milky juice and seeds are used as emmenagogue, in cases of scorpion bites; are the best vermifuge especially for tape, round and ring worms in children (Bose et al., 1961). The dark coloured seeds taste like water-cress and have varied uses (Nadkarni, 1954).
The aqueous extract obtained from the latex of *C. papaya* exhibited anti-coagulant properties both against plasma and whole blood. No clotting was observed even after 24 hr (Pillai et al., 1951; Chandrashekar et al., 1961 a, b).

The latex of green fruit has been known to possess oxytocin-like activity of a higher order. The seeds decreased fertility of albino mice, but were found to be highly toxic (Saha et al., 1961; Larsen et al., 1961).

The petroleum ether extract of the pulp of *Carica papaya* exerted significant antifertility activity in the female albino rat (Garg and Garg, 1971). The aqueous and alcoholic extract of *C. papaya* seeds have been shown to exert anti-implantation, abortifacient and antifertility effects which were reversible in adult female albino rats (Chinoy and Trivedi, 1980, 1984, 1983).

Keeping the above facts in mind, studies embodied in the present thesis has been carried out in our laboratory to elucidate the effects of *Carica papaya* seed extracts on adult male albino rats, effects of combined extract + ascorbic acid (oral feeding) treatment and cessation of treatment for one and 2-2½ months respectively.

Studies were also carried out to investigate the antiandrogenic and antifertility effects of another important plant, *Vinca rosea*, whose antimitotic and subsequent anticarcinogenic effects have been well established in the last
two decades. The Madagascan periwinkle, *Catharanthus roseus*, G. Don. has been variously designated *Vinca rosea* L. and *Lochnera rosea* (L.) Hechenbach; it is probably indigenous to Madagascar but is now widely distributed throughout warm regions and is much cultivated as an ornamental plant. It grows profusely in Southern Florida. Commercial supplies of the drug are obtained from both wild and cultivated sources in various parts including Africa, India, West Europe and Australia.

*Catharanthus roseus* has been well known among the natives of Africa for many years as a cure for diabetes. It is said to be more efficacious than insulin. Although the plant has a certain reputation in folk medicine for the treatment of diabetes, modern investigators have been unable to confirm this property (Trease and Evans, 1976). Instead, Canadian workers, during 1955-1960, discovered that extracts of leaves produced leukopenic action in rats. These observations led researchers at Eli Lilly & Company to undertake an intensive phytochemical investigation of the plant with a view to isolate the constituents of value in cancer therapy. Six alkaloids proved active in this respect and two are now available commercially.

At least 70 alkaloids have now been isolated from *C. roseus*, of particular interest is a group of about 20 dimeric alkaloids which contain those having antineoplastic
activity, including vincristine, and vinblastine. Vinblastine is composed of the indole alkaloid Catharanthine and the dihydroindole alkaloid vindoline both of which are present free in the plant.

Vinblastine sulphate is used mainly for the treatment of generalized Hodgkin's disease and chorionepithelioma. Vincristine sulphate is also a cytotoxic agent and is used principally in the treatment of leukaemia in children; short remissions of Hodgkin's disease and reticulum cell sarcoma have been achieved with it.

It is also known that the mammalian nerve and its subcellular components take up vinca alkaloids (Zafar Lybal and Uchii, 1980), and Vinblastine affects glucagon, and various states of the adenyl cyclase from rat liver plasma membranes (Whetton and Honsley, 1980). Vincaamines, an alkaloid obtained from Vinca minor leaves is metabolized in rat both in vivo and in vitro (Vereeckey et al., 1980). However, very few reports clearly demonstrate the effects of vinca alkaloids on male reproductive system.

In the light of the above studies, the present investigations were undertaken to elucidate the effects of aqueous extracts of Carica papaya seeds and Vinca rosea leaves on the reproductive organs of adult male albino rats. Short term administration of the extract treatment (7 and 15 days), combined extract + ascorbic acid feeding (AA) for 7 and 15 days, castration + testosterone (T) treatment and
castration + extract + T treatment (In the case of Vino rossata) treatment, and discontinuation of treatment for one and 2½ month periods were studied on the histophysiology of testis, epididymides, vas deferens, and seminal vesicles besides carrying out scanning electron microscopic (SEM) studies, recordings of isolated vas deferens and measuring the hormonal levels (T, FSH and LH) of control and treated animals by RIA.