The laboratory rat is known to be a spontaneous ovulator having extremely short estrus cycles of only 4 days in length. This is because the corpora lutea that are formed following ovulation never really become functional; but if mating occurs at estrus then functional corpora lutea develop. In the proestrus rat, estradiol 17β from the Graafian follicle reaches peak values which in turn triggers the ovulatory surge of LH on the afternoon of pro-estrus, and ovulation occurs in the early hours of the following morning. If estrogen is inhibited or absent, the LH peak is abolished and hence no ovulation occurs (Austin and Short, 1972).

Another striking feature of the rat's estrus cycle is the enormous pre-ovulatory surge of progestin secretion (Progesterone + 20α-dihydroprogesterone) that occurs. This is almost coincident with the LH peak. Preovulatory progesterone performs an essential function in the rat; if the animal is to show estrous behaviour, it must be under the influence of a high level of estrogen immediately followed by a high level of progesterone. Since this progesterone
must be secreted in pro-estrus, after the old corpus luteum has regressed but before a new one has formed, the rat has made use of its, "permanent corpus luteum, the interstitial tissue, which secretes large amounts of progesterone and 20β-dihydroprogesterone in response to LH stimulation.

In bilaterally ovariectomized rats, the cyclicity was upset and the cycle consisted of only meta- and diestrus stages which is correlated with the decrease in hormonal levels. The alteration in hormonal levels in bilaterally ovariectomized rats were also accompanied by severe changes in the metabolism and structure of the uterus.

The ascorbic acid metabolism during ovariectomy showed an enhancement in ascorbic acid utilization, free ascorbic acid and special peroxidase activity along with a decrease in ascorbigen (bound form) and rate of ascorbic acid macromolecule complexing which indicates the active mobilization and conversion of the bound to the free form of the vitamin leading to its increased utilization. Similar results have been observed in castrated male rats (Chinoy et al. 1962α; Seethalakshmi and Chinoy, 1973). Increased utilization is probably a physiological response to the stress condition (Ovariectomy), wherein ascorbic acid has a possible protective role in maintaining uterine metabolism. The levels of glutathione were also significantly reduced leading to decreased
conversion of dehydroascorbic acid to TAA. The accumulation in cholesterol levels are also correlated with alteration in ascorbic acid metabolism since hydroxylation and oxidation of cholesterol are dependent on ascorbate (Chinoy, 1973).

The significant decrease in uterine weights, protein levels as well as the changes in the histoarchitecture of the uterus were noted due to the absence of the hormones estrogen and progesterone in ovarioctomized rats. However, the significant increase in glycogen levels are difficult to explain, since estrogens are highly specific hormones for increasing glycogen stores in the rat uterus (Leonard, 1953).

The results revealed that total ovarioectomy leads to cyclicity, alterations in the structural and functional integrity of the uterus which is a target organ for female sex steroids.

In ovarioctomized rats treated with different dose regimens of estrogen and progesterone (Group I to III), the effects observed were related to the dose and type of the hormone. The result revealed that the rats were in estrus stage on the day 5th of the different treatments. As estrus stage involves the presence of both estrogen and progesterone in rats, the results are explainable.

It is shown that the induction of sexual receptivity in ovarioctomized rats also occurs by estradiol and
progesterone (Neder, 1977; Neder and Harrone, 1977; Horin, 1977). The onset of sexual receptivity occurs in close correlation with increasing serum levels of progesterone. Constant low serum levels of oestradiol stimulated sexual behaviour in ovariectomized rats, but progesterone stimulation was required for maximum behavioural responses (Sodersten and Eneroth, 1981). Therefore, in the present study too, using different doses of estrogen and progesterone in three different combinations were found to bring about the estrus stage. It is further known that hypothalamic LH-RH plays a physiological role in the induction of lordosis behaviour during the estrus cycle in ovariectomized oestradiol treated rats (Sakuma and Pfaff, 1980; Pfaff, 1973; Sartar et al., 1976). Speight et al. (1981) reported that oestradiol 17β increases the pituitary responsiveness by a mechanism which involves the release and priming effect of LH-RH.

The estrogens strongly influence the enzymatic regulation of protein, lipid and carbohydrate metabolism in the uterus (Segal and Sencer, 1967). The mechanism is by 1) enzyme induction; estrogens may induce de novo synthesis of various enzymes and they increase the levels of glycolytic enzymes in the uterus (Lee et al., 1970; Sinhala and Valekades, 1970; Sinhala et al., 1967; Valekades et al., 1968); 2) enzyme activation, as estrogen increases phosphorlyase activity in rat or mouse uterus (Fall, 1965; Leonard, 1962); by stimulating
the conversion of an inactive to the active form; 3) enzyme
distribution; 4) enzyme depletion or inhibition; 5) coenzyme
function; 6) molecular conversions and 7) release of enzymes
from subcellular particles (Steinert, 1973).

The estrogens also stimulate growth of the endometrium
and myometrium and the biochemical molecular events accompa­
nying these growth processes have been investigated extensively.
It is therefore probable that the changes observed in the
structure and metabolism of the uterus in rats treated with
different doses of estrogen + progesterone are related to
the effects of estrogen on uterus mentioned above.

The total protein levels were decreased significantly in
the present study with high doses of estradiol, but enhanced
in rats of groups I and II. Similarly, an increase in amino
acid utilization has been reported in the luteal phase of
normal menstrual cycle (Craft and Wise, 1969; Craft et al.,
1970). Analysis of the proteins synthesized by rat uterine
tissue during proestrus or following the administration of
oestradiol $\text{H}^1$ to castrate or immature animals has revealed
a single dominant compound known as induced protein(IP).
Oestrogen is capable of inducing the synthesis of this
protein in vivo and in vitro. The power of induction varies
according to the estrogen used, a close correlation being
observed between induced protein (IP) induction, affinity for a cytoplasmic estrogen receptor and estrogen potency (Aitken, 1979). Progesterone has also been shown to stimulate protein production (specially that of blastocin) in the rabbit uterus (Chilton et al., 1977).

The increase in glycogen levels of uterus occurred in rats of groups I and II in agreement with the observations of Poteat and Bo (1977), who reported that estradiol replacement to ovariectomized rats caused the levels of glycogen to increase in the uterus.

Ovarian steroids, estrogen and progesterone play a definitive role in glycogen deposition in the endometrium during the menstruation cycle (Zondek and Stein, 1940; Jayne and Latour, 1955; Aronnet and Latour, 1977) and the rate of endometrial glycogen synthesis increased gradually from the proliferative phase to secretory phase (Hughes et al., 1969; Matsuo, 1976), showing that estrogen and progesterone have a specific action on glycogen metabolism (Nimori et al., 1981).

Briggs (1976) reported an increase in mean serum cholesterol for women treated with the oral contraceptives. In the present study too, uterine cholesterol accumulated under influence of estrogen and progesterone as in group I and III respectively. Estrogen administration causes a rise in fasting triglyceride levels while the 17-nor testosterone
compounds counteract this effect (Dec., 1973). Both these effects are dose related.

An increase in uterine weights were found during hormone treatments, especially with higher doses of estrogen. Varren and Crist (1973) observed enlargement of the uterus after administration of estrogenic substances and led to the early identification of the uterus as a target organ for estrogens, which are growth-stimulating hormones for uterus.

Thus the results revealed that the estrogen and progesterone dependent parameters of uterus were altered during the various treatments depending on the dose and type of steroid administered. It was also evident that the group I treatment i.e. estrogen (0.5 µg) + progesterone (5.0 µg) was more effective rather than the other treatments, and the combined treatment as given to group IIIA rats i.e. estrogen (5.0 µg) and progesterone (0.5 µg) plus ascorbic acid was likewise more effective.

The ascorbic acid metabolism plays an important role in maintaining the redox milieu of the reproductive tissue (Chinoy, 1978, 1980). During the various hormone treatments, free ascorbic acid, bound AA and ascorbic acid-macromolecule complexing were significantly reduced in uterus. However, the rate of ascorbic acid utilization was increased. This indicates that an active mobilization of bound ascorbate to free form occurs with its enhanced utilization. Moreover,
due to decrease in glutathione levels, the conversion of DHA to TAA does not occur resulting in the accumulation of DHA. The outcome of treatment of rats of different groups with ascorbic acid was more or less similar to the hormone treatments revealing thereby that ascorbate simulates to some extent, the effects especially, the anabolic effects of the steroids. Moreover, the combined treatment of hormones plus ascorbic acid also manifested a synergistic effect particularly for increase in myometrial extent, epithelial cell height, uterine weight (Group I rats) glycogen levels (Group II and IV), cholesterol, TAA, DHA and PAA levels and activity of AA-7R peroxidase.

The role of ascorbic acid in overcoming the induced physiological stress conditions are well known (Selye, 1950), and AA is recognized as the anti-stress factor (Natesan, 1973). The rate of synthesis of the vitamin is also increased. This is related to the increase in the levels of stress induced histamine and its detoxication by ascorbic acid (Vardi et al., 1974). Histamine is also involved in estrogen stimulated uterine hyperemia and edema (McKercher et al., 1973). The increased adrenal cortical steroid output in stress mechanisms also correlated with the high turnover of vitamin A (Selye, 1950; Szreim-Grössy, 1957). In the present study too, the rapid turnover of ascorbic acid leading to its
decrease in the uterus might be related to the stress imposed by various hormone treatments to ovariectomised rats, or else the changes might be related to the estrogen dependency of ascorbate metabolism in female rats (Chinoy, 1973; Chinoy and Rao, 1979). But more or less recovery in all the parameters of ascorbate turnover was found by oral feeding of ascorbic acid along with the hormone treatments.

The beneficial role of ascorbic acid and its mechanism of action in male contraception has been highlighted extensively by Chinoy (1973) and her associates. They postulated that ascorbic acid has an important implication in prophylactic treatment in vasectomy, administration of antiandrogens and other antifertility drugs including plant extracts without interfering with the contraceptive purpose of the treatment. Hence ascorbic acid has a significant bearing in human fertility regulation (Chinoy, 1978; Chinoy and Geetha Ranga, unpublished observation).

The beneficial effects are also manifested by the fact that a woman taking oral contraceptive must take about 500 mg Vitamin C per day i.e. 10 times the normal recommended daily levels to compensate for the increased loss (Wynn, 1975; Rivers and Devine, 1975). It is therefore suggested, that ascorbic acid feeding to the women who are under contraceptive pill treatment may also have a beneficial effect since a lower dose of the steroid need be administered, as ascorbate
simulates the effects of estrogen and progesterone to some extent; hence AA has a significant bearing in female contraception also, as has been suggested earlier for the male (Ohinoy, 1973).

**EFFECTS OF UNILATERAL OVARIECTOMY AND COMPENSATORY HYPERTROPHY:**

The data revealed that unilateral ovariectomy resulted in compensatory hypertrophy of the remaining ovary and associated histophysiological changes in comparison to the control ovary. The compensatory hypertrophy is attributed to increase in weight of the left intact ovary, increase in number of corpora lutea having slightly smaller diameter than in the normal ovary; large numbers of primary and secondary follicles and normal Graafian follicles.

Peters and Breathen (1973) found that a change in the appearance of some of the growing follicles was characteristic in the remaining ovary 12 days after unilateral ovariectomy. A considerable number of growing follicles showed an early accumulation of follicle fluid. The follicles were also characterized by a widened theca layer. Similar changes have been found to occur in ovaries of 14-day old mice whose endogenous gonadotrophin had been blocked continuously from birth with anti-gonadotrophins but who had received simultaneously substitution therapy with follicular stimulating hormone (FSH) and luteinizing hormone (LH) (Eshkol et al.,
1970). Peters and Braathen (1973) found that the increase in ovarian weight after unilateral ovariectomy at birth, as also reported by Gerall and Dunlap (1971), is most likely due to early follicle fluid production and accumulation and is stimulated by the action of LH on the remaining ovary.

Unilateral ovariectomy is followed by compensatory hypertrophy of the remaining ovary which ovulates the same number of ova as do both ovaries of a normal animal (Ingram, 1962). In the unilateral ovariectomized rat, the number of oocytes in the remaining ovary was found to be only about 4 percent less than in the single ovary of a normal rat up to 7 weeks after operation (Arai, 1920). The number of ova ovulated, however, was the same as in both ovaries of the unoperated animal. Mandl and Zuckerman (1951) confirmed Arai’s observations and showed that the number of large follicles in unilaterally spayed rat is about twice that found in the single ovary of controls (Ingram, 1962).

Increased gonadotrophin secretion occurs in unilateral ovariectomized rats and leads to the compensatory hypertrophy of the remaining ovary. Moreover, estradiol or progesterone blocked the compensatory hypertrophy of the remaining ovary and the concomitant increase in serum FSH. These data confirm the increase in serum gonadotrophins following unilateral ovariectomy. It is suggested (Benson et al., 1969) that the
increase in serum FSH after unilateral ovariectomy results from a decrease in circulating ovarian hormones which partially releases the hypothalamo-hypophysial system from feedback inhibition. As the remaining ovary hypertrophies and is stimulated to secrete these hormones, the level of inhibition is restored and serum FSH returns to preoperated levels. Therefore Benson et al. (1969) suggested that an increased FSH secretion was an important component in the mechanism of compensatory hypertrophy.

The left uterus showed significantly thicker myometrium and endometrium and epithelial cell height than the normal control uterus and that of the right side which had the least in comparison (Table IIIB). The rats were found to be undergoing irregular estrus cycles with the predominance of diestrus stage. Hermreck and Greenwald (1964) reported that in hemispayed guinea pigs, the cycle length was not altered and the compensatory response was due to an increase in the rate of proliferation of smaller sized follicles into the larger ones.

The altered ascorbate metabolism, accumulation of cholesterol, decrease in glutathione and protein, uterine glycogen have also been correlated with the changes in ovarian steroids, serum gonadotrophins and their feedback on the hypothalamo-hypophysial gonadal axis.
PART II

A: EFFECTS OF CARICA PAPAYA SEED AQUEOUS AND ALCOHOLIC EXTRACT TREATMENTS AND WITHDRAWAL:

The effects of aqueous and alcoholic extracts of Carica papaya seeds were studied on the histophysiology of ovary and uterus of normal rats, their fertility rates and the possible reversibility of induced effects.

The results revealed that the two extracts had more or less same effects on female reproductive processes and on the whole, the effects on the ovary were more pronounced than in the uterus.

The changes in biochemical profile and histoarchitecture of the ovary and uterus involved changes in levels of protein, ascorbic acid, glutathione, cholesterol, glycogen and those on the histology of both the organs. These effects indicate the antiestrogenic and anabolic effects of the extracts.

Thus ascorbic acid utilization rate was enhanced in ovary and uterus, concurrent with an active mobilization of the bound ascorbigen and its conversion to the free form. The utilization of ascorbic acid occurred by the enhanced formation of charge transfer complex (AA-MM) and ascorbic acid free radical, monodehydroascorbic acid (MDHA) formation, which by virtue of possessing an unpaired electron has stronger reducing properties than ascorbic acid. It is known
to participate in several oxido-reduction reactions in reproductive tissues as a source of electron energy, in addition to the energy obtained through the conventional breakdown of ATP. However, the glutathione levels declined throughout the treatment, except in 15 days alcoholic treatment, which could also contribute to the decrease in free ascorbate. The data of this study substantiates the hypothesis that increased AA utilization via the formation of MDHA has a protective and beneficial influence on metabolism of ovary and uterus by a direct stimulatory effect of ascorbic acid on the activities of several enzymes (Harrer and King, 1941; Sebrell and Harris, 1967; Kutsky, 1973).

The overall enhanced metabolic turnover of ascorbic acid (AA) by ovary of plant extract treated rats was similar to that observed for other stress conditions and suggests that: (i) the higher metabolic turnover of ascorbic acid (AA) is an adaptation to overcome the harmful effects of the drug-induced histamine which is toxic and is inactivated by AA (Subramanian et al., 1974; Nandi et al., 1974) and (ii) ascorbic acid by virtue of formation of its free radical MDHA, plays an important role in maintenance of the physiological integrity of ovary, since an enhanced rate of MDHA formation occurred in plant extract treated rats. That ascorbic acid metabolism is altered in treated rats is also evident by the fact that cholesterol accumulation was noted
especially in the ovary, which in turn indicates a reduced rate of steroidogenesis by the ovary, or else non-functional, atretic corpora lutea and interstitial tissue which revealed vacuolizations.

The decrease in total protein and the insignificant changes in ovarian and uterine weights point to the antianabolic action of the extracts. Similarly, Das (1980) reported reduction in body weight gain in rats by treatment with papaya seed extracts. That the extracts have antianabolic and antiestrogenic effects are further substantiated by the histocytometric data involving significant increase in corpus luteum diameter which were not normal, change in their number, many atretic primary and secondary follicles as well as Graffian follicle in the ovary. The uterine histology was also affected with decrease in endometrial thickness and glands were almost lacking, but increase in the myometrium which should vacuoles. The epithelial cell height of the endometrium was however not affected except by 15 days aqueous extract treatment, but the epithelial and subepithelial nuclear pycnosis was widespread and uterine lumen was very narrow.

The above mentioned severe changes in the uterine structure and thereby its function renders it unsuitable for implantation of the fertilized ovum. Therefore, it is evident that the extracts also manifest anti-implantation effects.
According to Fransworth (1975), the active principle responsible for anti-implantation effects of papaya seed might be 5-hydroxy tryptamine, but it remains to be seen if only this compound or others too, are involved in the process.

The exact mechanism of egg implantation requires a perfect functional equilibrium between estrogen and progesterone inducing a series of irreversible modifications (Psychoylos, 1966; Mayer, 1966) and a slight disturbance in this hormonal balance may lead to the instability of pregnancy. Progesterone independently or together with estrogen inhibits the release of LH and blocks ovulation in rats (Watnick, 1964). It has been suggested that potent antiestrogenic compounds including progesterone, delay the implantation of the egg (Nutting and Sollman, 1967). Moreover, low doses of progesterone also inhibit the implantation in rats (Psychoylos, 1966). Prakash (1978) and Prakash and Mathur (1977a) are of the opinion that the anti-implantation activity of a plant extract is due to their anti-estrogenic mode of action which may, (a) produce a non receptive stage of uterus where implantation will not occur, or (b) delay the implantation of egg, or (c) have direct blastotoxic action. Prakash (1978) found that 50% ethanolic leaf extract of Artobotrys odoratissimus Linn exhibited significant activity at low dose level which decreased as the dose was increased. Though its exact mechanism is not known still the
dose level of this extract may act like an antiestrogen. It is likely that in the present study too, the extracts manifest anti-implantation effect by virtue of possessing anti-estrogenic properties.

The lack of implantation might be related to reduced or absence of mating behaviour in the animals, which in-turn is correlated with irregular estrus cycle with predominance of diestrus. The disruption of cyclicity has been associated with hypothalamic inhibition (Fransworth et al., 1975) and thus followed by the probable disruption of pituitary-gonadal axis, as is evident from the present data.

The above mentioned changes together with absence of implantation, result in 100 percent negative fertility rate in the Carica papaya seed extract treated rats. Similarly, Garg et al. (1970) and Sareen et al. (1961) reported that the antifertility activity of papaya seed in female rats and mice respectively, were due to its anti-implantation activity. The antifertility effects have also been attributed to the estrogen like activity of the extracts in higher doses or on prolonged treatment as was evident from the maintenance of uterine and ovarian weights in treated animals. Estrogens are known to inhibit pregnancy by affecting tubal transport of eggs or endometrial maturation and by causing abortion due to suppression of luteal function of pregnancy (Chandboke, 1978). In the present study also, the disruption of luteal function is likely due to the presence of abnormal corpora lutea.
The antifertility and anti-implantation effects of the extract are further substantiated by the occurrence of the strong abortifacient activity of Carica papaya seed extracts. The abortifacient effect was manifested by increase in myometrial thickness, significant enlargement of endometrial extent with reduced epithelial cell height, reduced or almost absence of glands and acute endometrial nuclear pycnosis. Similar histological changes have been reported by Dixit (1977) by administration of flower extracts of Malva viscus conzattii. These data are further supported by the changes in contractile pattern of uterus in 7 and 15 days treated rats. As is evident from the recordings (Plate C4; Figs. 18-20; Plate C5; Figs. 21, 22), that the contractions of the uterus of treated rats with both aqueous and alcoholic extracts were more in response to different doses of oxytocin in comparison to those of the control. On the whole, the increase was more by the aqueous extract treatment for 15 days than the alcoholic. Since the ability of oxytocin to depolarize the cell membrane and initiate action potential is increased in the presence of elevated concentrations of potassium, sodium, calcium, ions or chloride and vice versa (Kuriyama, 1968; Marshall, 1968; Bentley, 1961), it is evident, that the overall excitatory actions of oxytocin, as observed in the treated rats, may be due to a general increase in the ionic permeability of membranes of uterine cells.
Therefore, the analysis of K, Na, Ca and chloride levels in normal and treated rat uterii would have been more conclusive proof. Many other plant extracts are also known to possess abortifacient effects (Pakrashi, et al., 1975; Pakrashi and Bhattacharya, 1977; Dhawan et al., 1977, 1980), but they are too numerous to be listed here. However, other extracts do have 100% interceptive activity at a single dose of 50 mg/kg but failed to show abortifacient effects (Pakrashi and Pakrashi, 1977).

The mechanism of action of Carica papaya seed extracts (aqueous and alcoholic) seems to be anti-estrogenic in low dose and estrogenic in high doses. The metabolic effects and those on the histology of ovary and uterus, contractility, fertility rate, estrus cycle suggest that the extracts have a direct action on the gonadal structure and functions and thereby affect steroidogenesis and target organs, in addition to acting via the hypothalamo-hypophysial gonadal axis as also suggested by Fransworth et al. (1975). That the extracts favoured prolongation of diestrus stage in treated rats also indicates disruption of the long feedback loop and of the hormonal balance which is needed for maintenance of proper cyclicity. A number of other plant extracts viz. Embelia ribes berries, fruits of Piper longum and flower extracts of Malva viscosa conzattii also caused prolonged diestrus stage in rats and gerbils (Kholkute et al., 1978, 1979;
Dixit, 1977). In this respect, the plant extracts resemble the effects in diestrus prolongation as produced by prostaglandins and sodium barbital (Karim and Amy, 1975; Champak-malini and Rao, 1977).

In general, the aqueous extracts were more potent than the ethanolic, in agreement with the results of others (Kholkute et al., 1979; Prakash, 1978). However, the discrepancies observed by various workers in the effects produced could be attributed to the methodology and duration of screening, the reproductive states of the animals, species of animals as well as due to various ecological factors which are known to affect the medicinal property of plants (Fluck, 1954; Kholkute et al., 1976).

From the above data it is evident that the extracts manifested antiestrogenic, estrogenic, antianabolic, antifertility, anti-implantation and abortifacient effects by 7 and 15 days treatments.

The effects of withdrawal of the treatments for one month and 2½ months respectively were investigated to study the reversibility of the induced effects.

From the results it is evident that on the whole, recovery in most of the parameters occurred by the withdrawal treatment which was more after alcohol extract treatment withdrawal than the aqueous, and also more in the ovary than in the uterus. This corroborates with the findings of the data
on treatments whereby it was shown that the induced effects were more pronounced by the aqueous extract treatment, than the alcoholic, and therefore, the recovery is also faster in the alcoholic extract. Considering the individual effects and parameters; the estrus cycle was more regular especially towards the end of the withdrawal treatment since all the animals on the day of autopsy were in estrus stage. This implies that the hormonal levels and the metabolism of the gonad, uterus and other target organs were being restored. The histology of ovary was completely recovered and also of the uterus to a large extent. The fertility test was 100% positive. The organ weights, levels of protein, cholesterol, glutathione and the complete ascorbate turnover was normalized indicating the restored redox milieu of the organs. The contractile pattern of the uterus also showed significant recovery.

Thus withdrawal treatment overcame the adverse effects of the treatments in ovary and uterus and restored the fertility. These results show that a functional sterility could be induced in rats by treatment with small doses of Carica papaya seed extracts. However, withdrawal period after aqueous treatment ought to be extended till histoarchitecture of uterus is fully recovered.

Further detailed studies should be undertaken to develop a suitable antifertility agent from the extract after purification, since it causes functional sterility in the males also.
The effects of *Vinca rosea* leaf aqueous and alcoholic extract treatments (7 and 15 days) on rat ovary and uterus histophysiology were by and large, similar to those observed by *Carica papaya* seed extracts but comparatively lesser in magnitude or potency than the *Carica papaya* seed extract. The response of the ovary and uterus were also same wherein ovary was more affected and in general, the aqueous extract was more potent as before than the alcoholic.

The effects on fertility rate, estrus cycle, histophysiology of ovary and uterus, its contractility, antiestrogenic, estrogenic effects were also manifested as reported earlier for *Carica papaya* seed extracts. On the other hand, the abortifacient action of *Vinca rosea* leaf extracts were not so marked. Gailili et al. (1979) reported that hydroxylation of progesterone occurred by cell suspension cultures of *Vinca rosea*.

The pattern of recovery also closely paralleled those which were observed earlier after *Carica papaya* seed extract withdrawal.

The data on plant alkaloid treatment reveals that functional sterility could be induced in rats. The monitoring of proper dose regimen, adequate withdrawal period and thorough investigation on the side effects, which seem to be negligible,
could pave the way for the development of a simple, easy to use contraceptive for females whose effects are reversible.

PART III

STUDIES ON EFFECTS OF VARIOUS COPPER-WIRE DEVICES:

The contraceptive use of copper was first demonstrated by Medel and Prager (1969) and Zipper et al. (1969). Some investigators have suggested that an intrauterine device (IUD) encased with copper might effect changes in the biochemical composition of the endometrium to prevent implantation (Medel and Prager, 1969; Chang and Tatum, 1970; Hicks et al., 1975). Copper interacts with free sulphydryl groups and thereby alters the metabolic processes associated with sulphydryl dependent enzyme systems (Naeslund, 1972; Tamaya et al., 1976). Cupric ions can inhibit specific progesterone binding by human myometrial and endometrial cytosol in vitro. This finding suggests that one mechanism of action of copper could be through interference with the action of progesterone at its target sites (Kontula et al., 1974; Young et al., 1977).

Tamaya et al. (1976) reported that the increased contraceptive efficacy of copper has been considered to result from biochemical and morphological reactions of uterine and
oviductal mucosa, direct toxic influence on the blastocyst (Maeslund, 1972), inhibition of implantation (Chang et al., 1970), and spermaticidal effect (MacLeod, 1951). The chemical action of copper involves the degradation of S-S contained protein, inactivation of enzyme, and decreased elasticity of uterine mucus (Oster, 1972). It is known that the steroid hormone receptor has an S-S binding capacity (Jensen et al., 1967; Kontula et al., 1974), therefore, the receptor might be inactivated by copper. Copper was more sensitive to progesterone receptor than to estrogen receptor (Tanaka et al., 1976). The greater stability of the estrogen receptor in the presence of copper suggests an increased estradiol uptake in rat uteri bearing a Cu-IUD (Aedo and Zipper, 1973).

The intrauterine insertion of a foreign body initiates a series of predictable events in the endometrium and surrounding tissues. Tissue reactions result from independent but related stimuli following the insertion of an IUCD (Moyer and Shaw, 1980). In extra- and intrauterine device bearing rats, the effects on structure and metabolism of ovary and uterus were noted to be almost similar in the two organs but more severe in group Y rats (Parallel Intra uterine copper device) than in the others. The protein levels were decreased throughout. Blastokinin, an endometrial protein was also decreased with copper intra uterine device (Johnson, 1972). The organ weights were more than in control. The increase in uterine weights might be due to the inflammatory reaction
or the increase in the thickness of myometrium. In ovary, the increase might be related to the large corpora lutea and increase in their number, and sinuses in the stroma. Since the number of corpora lutea are more than in normal, it signifies that ovulation is not inhibited by copper IUD, but the number of eggs ovulated from adjacent ovary was reduced (Peppler, 1975). In human subjects too, bearing a copper IUD, similar results were obtained (Oster and Salgo, 1977). The ovary contained nevertheless atretic primary, secondary and Graafian follicle, particularly in rats of group IV and V.

The estrus cycle was somewhat altered in copper device bearing rats which showed 60 % negative fertility in group III but 100 % negative fertility in those of groups IV and V respectively. The reduction in fertility is probably due to the blastotoxic, anti-implantation or spermicidal effects of copper ions (Oster and Salgo, 1977). In human volunteers with Copper IUD, the luteal phase was reduced and the shortening of the life span of corpus luteum has been suggested (Paundes et al., 1980).

Numerous endometrial and myometrial changes have been reported to occur in copper device bearing rats and women (Hagenfeldt, 1972). Endometrial sloughing with cell debris in the lumen (present study) or proliferation of the endometrium of rats is seen around the copper wire (Zipper...
suggesting an inflammatory reaction. The subepithelial region of uterus endometrium showed nuclear pycnosis. The estrogen uptake is also greater in uterine copper device bearing rats suggesting that the endometrium in the presence of an intra-uterine copper device requires high levels of ovarian steroids to be maintained. Similar results have been reported by Ghosh and Roy (1977). In the present study, the endometrial thickness and epithelial cell height were reduced in group IV and V rats.

Ultrastructural changes in human endometrial biopsies have also been reported. The mitochondria and lysosomes increased (Gonzalez-Angulo and Aznar-Ramos, 1976) and glycogen containing endometrial protrusions were absent (Nilsson and Hagenfeldt, 1973). But in rats bearing copper devices in the present study, glycogen concentrations were enhanced in all rats except in those of group IV. It is also known that \( \alpha \)-amylase activity and cyclic increase in glycogen synthetase in the endometrium is altered by copper IUD (de la Osa et al., 1972).

The changes in endometrial enzyme levels in both the proliferative and secretory phases in Cu-IUD bearing subjects have been reported by several workers (Middleton and Kennedy, 1975; Johri and Dasgupta, 1980). The loss of mucoproteins accounts for the inhibition of implantation (Prager, 1969).
The effects of copper on myometrium are also varied. In low concentrations, cupric ions cause uterine contractility but in higher concentrations they cause a decrease in contractile force (Oster and Salgo, 1977). Copper may be acting to stimulate the uterus in a variety of ways: Mechanisms of action for copper-induced uterine contraction involving mercaptyl groups, cyclic AMP, or Na⁺-K⁺ ATPase are reported. The copper induced prostaglandin production might also exert a contraceptive effect through increased uterine motility.

The effects of Cu-IUD on ovary and uterus might also be due to a local toxic effect as a result of copper accumulation in situ (Chinoy and Sanjeevan, 1980). Moreover, the effects of Cu-IUD on ascorbate metabolism of ovary and uterus were same as reported earlier in parts I and II i.e. under a stress condition. Cholesterol was however not much affected except in group IV rats; in ovary an increase occurred but a decrease in uterus. This indicates unaltered ovarian steroidogenesis in Cu-IUD bearing rats.

Copper can also cause changes in levels of gonadotrophic hormones (Oster and Salgo, 1977). It has long been known that intravenous injection of copper salts will cause ovulation and pseudopregnancy in the rabbit (Fevold et al., 1936). Copper administration has been shown to produce an increase in LH, and FSH (Suzuki et al., 1970) resulting in ovulation. Copper administration changes levels of gonadotropin in the rat as well as in rabbit (Oster and Salgo, 1977).
The fact that copper is the most powerful catalyst for the autooxidation of ascorbic acid has implications in reproductive physiology (Oster and Salgo, 1977). The cupric-ion-catalyzed autooxidation of ascorbic acid generates free radicals (Staudinger et al., 1964) and this combination can induce mutation and damage DNA (Stich et al., 1976). This has a practical implication in terms of vitamin C deficiency for women taking oral contraceptives. Increased ceruloplasmin levels associated with exogenous estrogens is accompanied by reduced plasma levels of ascorbic acid (Oster and Salgo, 1977).

Vitamin C has effects on tissue metabolism. In copper-deficient animals, additional vitamin C makes the deficiency more severe. On the other hand, in copper toxicity, the vitamin has a protective effect (Spivey Fox, 1975; Hunt et al., 1970) confirming the observations of Chinoy and Sanjeevan (1980).

PART IV

EFFECTS OF PROSTAGLANDINS

The effects of prostaglandins on reproductive system of Laboratory animals are varied. In the present study, the estrogen dependent biochemical parameters were reduced but organ weight were increased as compared to control.
On the whole, ascorbic acid turnover was significantly reduced in ovary and uterus by both PG treatments. The levels of total, dehydro- and reduced ascorbic acid as well as levels of glutathione, bound ascorbic acid, activity of AA-FR special peroxidase were more significantly affected than levels of free AA or the rate of its utilization. These data revealed that both PGs altered the redox milieu of these organs and thereby affect their structure and metabolism.

Ascorbic acid plays an important role in the steroidogenesis (Chinoy et al., 1978a, 1980a,b). Bartke et al. (1973) have indicated that PGs are also directly involved in steroidogenesis. The depletion of adrenal ascorbic acid is induced by PGF1 (Sandow and Babej, 1974), whereas Dasgupta et al. (1976) suggested that PGF2α modified lipid, cholesterol and ascorbic acid content under stress condition by potentiating the action of ACTH. It also appeared that PGF2α complemented the ascorbic acid function in some unknown way, the mechanism of which is still a matter of conjecture.

The histological study revealed that the diameter of corpus luteum and their number were increased in both PG treatments. Some atretic primary and secondary follicles were observed. The stroma contained large sinuses and at places cells resembling adipose tissue were observed (Plate F; Figs. 5,6). The increase in weight of ovary might be related to these changes caused by PG treatment. The presence of
abnormally large corpora lutea, some atretic follicles and changes in stroma suggest that PG treatments have either a direct effect on the ovarian tissue or via the pituitary gonadal axis. The role of prostaglandins in causing ovulation by action at the ovarian level has become apparent from several recent studies. Human ovarian contractility was shown by Coutinho and Maia (1971). It has also been known that the ovary contains prostaglandins (Pickles, 1967), but their precise distribution with respect to various ovarian compartments (corpus luteum, follicle and interstitium), different stages of the reproductive cycle and type of prostaglandins remain to be elucidated.

In uterus, the myometrium, endometrium and epithelial cell height were increased by both PG treatments resulting in increase in the weight of the organ. The increase in weight of the ovary and uterus concomitant with the decrease in protein levels elucidate a growth promoting effect of prostaglandins in female steroid target tissue as reported earlier (Chinoy et al., 1980c) for androgen target tissues of male rats. However, the effect was comparatively less in females.

The synthesis of prostaglandins in the uterus is modulated by the ovarian steroids. The uterine concentration of PGF varied according to the stage of the oestrous cycle, in the rat and hamster, although the pattern was different between
the two species (Labhsetwar, 1975). Oestrogen appears to be a potent stimulant for prostaglandin synthesis in the uterus as the secretion of PGF into the utero-ovarian vein increased significantly following oestrogen treatment of guinea pigs. Labhsetwar (1975) reported that during the terminal part of the luteal phase of the guinea pig oestrous cycle, the uterus is exposed to both hormones thus resulting in an optimal secretion of prostaglandins for luteolysis. He also reported that oestrogen acting in concert with progesterone constitute a physiological trigger for increased prostaglandin synthesis in the uterus under in vivo conditions.

The estrus cycle was not very regular and a prolongation of diestrus stage was observed. This is rather difficult to explain as prostaglandins have a luteolytic effect and promote regression of the corpus luteum and decrease progesterone levels (Labhsetwar, 1975).

The partial antifertility effects of both PGs as seen in the present study which might be due to luteolytic effect of prostaglandins, $\text{PGF}_2\alpha$ and implies that they can exert this effect by creating progesterone deficiency. Such an effect was first described in rat (Gutknecht et al., 1971; Nutting and Cammarata, 1969). $\text{PGF}_1$ also exerts antifertility effects although in general, they are much less potent than PGFs (Labhsetwar, 1975). Prostaglandins may affect fertility by their action on cervical mucus, vaginal secretion or by affecting sperm transport in the uterus and fallopian tubes.
According to morphological, histochemical, and biochemical evidence, PGF₂α has a direct action on the lutein cell. PG exert its action through an effect on steroid synthesis (Goldberg and Ramwell, 1977). The activity of cholesterol ester synthetase and the tissue content of cholesterol ester were depressed in ovaries of gonadotropin-treated immature rats that had been given PGF₂α (Behrman et al., 1971). In the present study too, cholesterol levels were significantly decreased in ovary and uterus of PG treated rats. Ovarian slices from these animals also exhibited depressed progesterone synthesis (Behrman et al., 1971). Another possible mechanism of PG action on steroid synthesis may be the diversion of ovarian steroid production from progesterone synthesis to estrogen synthesis (Goldberg and Ramwell, 1977). This is supported by the fact that the glycogen levels were enhanced in the uterus of PGE₁ and PGF₂α treated rats in the present study.

It was postulated that prostaglandins stimulate LH secretion (Zor et al., 1970; Labbsetwar, 1975; Sato et al., 1974). The stimulatory effects of prostaglandins on gonadotrophin secretion have been amply confirmed in subsequent studies. The mechanism by which prostaglandins exert stimulatory effect on gonadotrophin secretion and whether or not they subserve this function under physiological condition is not known. However they could bring about increased gonado-
trophin secretion and ovulation by acting at various levels in the hypothalamo-pituitary-ovarian axis. Chatterjee (1973) has presented indirect evidence that in rats, PGF₂α acts on the hypothalamus to release LH-releasing hormone.

PART V

EFFECTS OF VITAMIN C DEFICIENCY IN GUINEA PIGS:

The experimental scorbutic condition was created in guinea pigs by feeding them vitamin C deficient diet for 21 days. The physiology, histology, contractile pattern of the uterus under this altered physiological condition were studied and compared with the control. Care was taken to study the tissue ascorbic acid metabolism and the histochemical localization of ascorbic acid in ovary and uterus. The uterus revealed marked reduction in its weight but ovary was not much affected. Similarly, the effect of the deficiency was noted on total body weight, and the significant loss of hair. A decrease in weight and functional integrity of the reproductive organs in male rats (Seethalakshmi and Chinoy, 1976; Chinoy et al., 1982) and reduced body weight (Hank and Weiser, 1973) have been attributed to disturbed physiology under induced stress condition. The changes indicate a probable deficiency of sex hormones due to reduced steroidogenesis in the gonads. The decrease in most of the estrogen-
dependent parameters of vitamin C deficient animals in the present study also suggest that the internal milieu of ovary and uterus are altered corresponding with the probable alteration in the levels of circulating estrogens. These data support the work in scorbutic male guinea pigs (Chinoy et al., 1982; Belavady and Banerjee, 1954; Banerjee et al., 1972), wherein accumulation of lipids in testis and reduced steroidogenesis occurred. Similarly, Banerjee and Deb (1952) also reported reduced 17-keto steroid levels in male guinea pigs suffering from scurvy, indicating reduction in hormonal levels. The probable reduction in circulating estrogen levels and an alteration in ovarian steroidogenesis is associated in the present study too, with the accumulation of cholesterol in the ovary and uterus, decrease in levels of protein and significant reduction in uterine glycogen. The histology of the ovary also showed changes including increase in corpus luteum diameter, and somewhat increase in their number. But primary and secondary follicles were normal and Graffian follicle was not fully mature. The stroma contained large vacuolar areas. In uterus, the myometrial and endometrial thickness were reduced as well as the epithelium. The endometrium contained smaller glands and extensively distorted lumen.

The reduction in fertility rate by 50% in scorbutic guinea pigs is related to the altered, irregular estrus cycle
with prolongation of the meta- and diestrus stages, the reduced contractility of the uterus to different doses of oxytocin, the changes in structure and metabolism of the ovary and uterus and also their ascorbate turnover.

An overall reduction in ascorbate metabolism occurred in both tissues of scorbutic guinea pigs. The bound ascorbic acid was depleted and as the stores could not be replenished due to deficiency, a significant reduction was noted in total, dehydro- and reduced ascorbate as well as glutathione and utilization of the vitamin. This suggests that the redox milieu of the ovary and uterus will be affected since ascorbic acid is known to be involved in their metabolism via the formation of its free radical, monodehydroascorbic acid, which participates in oxidoreduction reactions (Chinoy, 1978). Moreover, in scorbutic guinea pigs the small endometrial glands were devoid of ascorbic acid localization (Plate G, Fig. 13), suggesting that they are not in the active secretory phase as it is known that actively proliferating and metabolic cells have a correspondingly higher concentration and turnover of ascorbate (Chinoy, 1978).

A number of workers have noted the high concentration of ascorbic acid in corpora lutea of several different species (Gough and Zilva, 1933; Ley, 1937), but in only a few studies has an attempt been made to correlate the amount of ascorbic acid at various times with the different phases of the growth.
and waning of the corpora lutea (Giroud et al., 1934; Bourne, 1935). In one study (Pincus and Berkman, 1937) found that the concentration of ascorbic acid rises to a maximum in developing rabbit corpora lutea on the third day of pregnancy, whereupon the concentration diminishes. After the concentration of ascorbic acid begins to fall, the rate of growth of the corpora lutea likewise diminishes. This observation led to the conclusion that vitamin C concentrations are high when active tissue proliferation is occurring (Pincus and Berkman, 1937).

The fact that vitamin C plays some role in the changes which prepare the uterus for implantation and for subsequent accommodation of the products of conception by hyperplasia is clear, although its specific role needs to be elucidated (Reynolds, 1965). It is, however, a reducing substance which appears to bear a relation to the reaction of, and type of metabolism going on within the uterus at certain times. It appears also to be important for cellular proliferation in both the corpus luteum and the uterus. There is evidence that glutathione is utilized directly in proliferation of the endometrial glands (Pincus, 1937), so that, in the present study, with the decrease in glutathione levels in scorbutic guinea pig, the glands were small, non-proliferative and less active in secretion.
Vitamin C deficiency not only resulted in anti-estrogenic and reduced fertilizing capacity but altered the histology and dose dependent response of uterus to oxytocin. The reduced contractility of the uterus in scorbutic animals might be an important factor contributing to the anti-implantation effect in these animals.

Thus vitamin C deficiency caused marked changes in female reproductive processes and as such, the results elucidate the importance of the vitamin for maintenance of structure, metabolism and redox milieu of ovary, uterus conducive to their normal functioning.