Discussion
DISCUSSION

Caution needs to be exercised in experiment with stress. While under stress is ineffective, over stress results in exhaustion and may leads to mortality. Experiments on chronic stress has chances of acclimatisation. Pilot study in this project resulted in greater mortality when stress presentation was attempted from day one (D1) but from D5 it was free from this problem. Kuhn et al (1978) stated that in general maternal deprivation (MD) or in other words interruption of mother-infant interaction is a "stressful" experience that has adverse biochemical, physiological and behavioural consequences for the offsprings. The authors have shown that ornithine decarboxylase (ODC) activity, a sensitive index of organ growth and differentiation, decreases in brain and heart of rat pups after just 1 hour of MD and increase rapidly when the pups were returned to the mother. The decline in ODC appears to be mediated by a metabolic or endocrine signal rather than from a direct neural stimulus. The decline in ODC is independent of adrenal steroid but several suggestions points the involvement of other hormones responsible for decline in ODC in maternally deprived pups.

The pre-weaning (PW) period of this study (D5 to D20) closely corroborate with that of Lau et al (1996) who experimented with MD for 6 hours a day between D4 to D21 PN and reported that it delays vagina opening and estrous cycle in females. MD also affects the production of sperms and changes in reproductive
tissue in male rats. In the present study, during PW period adrenal weight of control rats increased by 68.7% where as in the stressed rats it increased by 98.8% indicating hypertrophy of adrenals due to stress. MD enhances cold induced corticosterone levels indicating basal and stress induced corticosterone after 24 hours of maternal separation (Avishai-epherner et al., 1995). Lau et al (1996) was of the opinion that when other stressors are taken into consideration maternal separation stress itself does not appear to alter the growth parameters.

As for the reproductive organs the testis weight of normal rats in PW period increased 630.11% where as in stressed rats it increased 589.95% only. In a similar experiment, Shaligram (1998) reported significant reduction in the weight of epididymis and histological parameter of epididymis in stress induced Wistar rats. In the present work histological parameters of the testis in pre-weaning stage were not markedly affected.

From D21 the dams were removed from all the litters, no untoward effect was observed; this may be because, the young rats had already started nibbling solid food in addition to breast feeding. The same food with which the young rats were accustomed and other environmental conditions maintained same as before, the only change from D21 was in the mode of stress. Nevertheless, to be on guard no sudden prolonged foot shock (FS) was presented. The intensity and duration of FS and change of Light-Dark period was tested in pilot study. Out of the various types of studies, e.g. crowding (Armario et al., 1984), swimming (Desai et al., 1998), starvation (Nelson et al., 1997) and chemical agents (Tohei et al., 1997) etc.
electrical foot-shock appeared to be one that provide accuracy of measurement of quantum of stress. Looking at the results of the study confirms that foot-shock of this magnitude produced an effect on the adrenals and testis of the rats during post-weaning, that vital period which can be called as transitional at least for some of the events of male reproductive system in rats. Necessary physiological and functional sexual maturity in male rats reaches after 7th week (Lane-Petter, 1976). In an earlier study (Mukerjee, 1987) reported that on D56 the epididymal tubules possess scanty number of spermatozoa but by D63 the epididymal tubules were full of spermatozoa. This can be taken as an indication for complete sexual maturity and accordingly in the present study the foot-shock treatment was terminated from D70; thereafter the period of quiescence was allowed to the chronically stressed male rats. That was the reason that this study was divided into three periods namely (a) pre-weaning MD stress period, (b) post-weaning FS stress period and (c) quiescent period.

Normally, adrenals enlarge in the postnatal period. The increase in size was conspicuous upto 10th week PN, thereafter, it slowed down. Effect of stress on the size of adrenal glands both at pre and post-weaning periods continued in post-stress period of quiescence till D100. Under various conditions of stress on adult rats in the post-weaning period, adrenal hypertrophy has been reported by various authors (Selye, 1936, 1937, 1940; Tepperman, 1943 a & b; Mikhail, 1961; Mikhail and Mahran, 1965).
Hypertrophy can result due to exposure to cold and unilateral adrenalectomy (Selye, 1937). On the contrary, Malendowickz (1985) observed that unilateral adrenalectomy does not change the weight of the remaining adrenal gland. There are clear sex differences in the compensatory hypertrophy of the adrenal cortex. Adrenalectomy evoke an increase in the average volume of Zona fasciculata cells in male and Zona reticularis cells in female hamsters. Environmental condition play a role in maintaining the size of adrenals. Rats bred for 8 generations under tropical conditions of temperature and humidity show a relative increase in the adrenal size. Intact animals forced to exercise show a considerable hypertrophy of adrenals within a short time as 12 hours (Selye, 1936).

Shock treatment can be observed in two phases. During the first phase the animal can imagine the impending shock (pre-shock). It is longer than the characteristic shock which come in second stage of shock (Counter shock). Hypertrophy is the resultant of combined effect of the two phases. Selye et al (1940) stated that prolonged shock brings about protein destruction that is often caused by the shock precipitating episode, the peripheral circulatory failure might result in very extensive tissue breakdown. Events occurring during catabolism of protein may serve as a stimulus for the hypertrophy of the adrenal cortex (Tepperman et al, 1943b). Hypertrophy of adrenals was also reported to be a consequence of sodium pentobarbital anaesthesia (Ludewig and Chanutin, 1947). The present study cannot substantiate the same reports as in this study sodium pentobarbital was used as anaesthesia for sacrifice of the rats.
Contrary to many of the reports stating hypertrophy of adrenals, Deane and Greep (1947) have shown on two strains of rats, thyroidectomy results in shrinkage of adrenals from normal 19 to 13 mg%. Histologically, the adrenal atrophy was noticed in Zona fasciculata. In agreement, stress in the present work registered significant increase in the absolute volume of Zona fasciculata. Deane and Greep (1947) also reported increased activity of Zona glomerulosa in several conditions which they attribute to alterations in salt balance resulting from different treatments. In the present study, the type and quantum of stress presented to rats did not express noteworthy effect on the other two adrenocortical zones. The results of Mikhail (1973) indicated that the different cortical zones do not develop altogether, some of the areas appear and reach full development in sequential manner.

Present findings shows that absolute volume of Zona fasciculata in normal control rats increase with age of the rat. It ranged from 2.68 ± 0.44 on D28 to 7.81 ± 0.41 mm³ on D100. Similar increase was not found in Zona glomerulosa and in Zona reticularis. Gallo-Payet and Escher (1985) reported 7 fold greater number of Zona glomerulosa than that of Zona fasciculata.

The same species of animal living under different conditions may have different reactions to stress. In non-domesticated animals stress may not activate the pituitary-adrenal cortical system (Woods, 1954). Severe stresses may not result in metabolic depletion of the adrenal cortex in wild rats. Furthermore, Woods (1957) stated that undoubtedly adrenocortical hypertrophy results due to continued exposure to stress in domesticated animals. The rats used in the present study
were very similar to any domesticated animals as they lived for generations in environment and diet controlled vivarium.

Amount of ascorbic acid in adrenals increase as the gland increase in weight (Woods, 1957). This was substantiated by Mukerjee (1987), besides the latter reported elevation of ascorbic acid with each episode of foot-shock stress finally leading to exhaustion.

Stress has been tested by various means such as crowding that decreased food consumption and increased water intake resulting in decreased body weight and significantly increased relative testicular weight (Armario et al, 1984). Variety of stresses, e.g. noise, swimming and restrain has shown mixed reactions to stresses (Armario et al, 1985a and 1985b) and Gamello et al (1986a and 1986b). None of these authors have reported adaptation to stress although varied reaction was observed in all these experiments. The present study fully substantiates the past reports by various authors.

In the present study, as the control and stressed groups of rats were maintained in similar ambient conditions and dietetic regime, retardation in increase in body weight of rats during MD as well as FS stress periods can be attributed to effect of stress alone. Although maternally deprived rats did not show much effect on the BW, the foot shock stress showed immediate, significant and persistent effects. Gamello et al (1986b) have shown that crowd reared rats have lower body weight compared to control rats. After 200 days of being reared in normal
condition, the body weight of crowd reared rats were still significantly lower than those of control rats. Present study showed that there was no respite from the stressful effect even 4 weeks after withdrawal of stress.

Short period of MD does not have persistent effect instead there is a critical length of deprivation, only beyond which persistent changes in adrenocortical responsivity ensue (Rosenfeld et al, 1992). Hennessy et al (1989) have shown PW guineapigs subjected to MD, exhibited higher levels of ACTH. It is proved from this that brief periods of maternal separation can serve as potent stimulus for activation of hypothalamo-pituitary adrenal (HPA) axis.

Study of the histo-architecture of testis can be taken as mirror image of a part of the activities going in the hypothalamo-hypophysio-gonadal axis. Its involvement in stress is well noted. The long term effects of stress on testis was carried out in the present study in the neonatal, early postnatal and adult rats. Thus, the complete analysis of structural changes in testis can be correlated with functional properties through the entire period of growth.

The weight and volume of testis as well as BW are progressive and positively correlated. The weight of testis showed a rapid increase from D42 to D70. Thereafter, it slowed down. MD as well as FS has profound effect on testicular weight. Gayton et al (1986) reported that the increase in testicular volume was more rapid between 20 and 70 days of age. Testis appears to recover from the effect of stress. Percentage increase in the testicular weight shows that after
cessation of stress, weight of testis increased more than double than that of control rats.

Stress induced testicular weight reduction by various stressors has been reported by many authors. Abnormal temperatures such as cold (Nazian and Piacsek, 1977) and heat (Sailor et al, 1997) have reduced testicular weight and delayed sexual maturation. Withdrawal of gonadotropin by GnRH immunization have greatly reduced the testicular weight (McLachlan et al, 1995). Edmonds and Stetson (1995) stated that exposure to short or long photoperiod during the period of lactation (D1-D14) affect testicular maturation.

In the present work increase in testicular weight can be attributed to the increase in length and diameter of seminiferous tubules. Contrary to the present findings Lczkowski et al (1991) reported that there was no significant increase in the tubular diameter in the prepubertal period; in fact it decreased until week 4. Where as in the present study in the rat testis, diameter of seminiferous tubules increased steadily in both pre-weaning and post-weaning period. Tubular development nearly cease at D70 of rats age. From D80 there was negligible increase in the diameter of the tubule. The tubules of stressed rats had reduced diameter. The MD stress did not alter the diameter noticeably. The significant differences in the diameter of the tubules were seen throughout the post-weaning period. The diameter of tubules in post-stressed rats did not recover to normalcy.
Absolute volume of seminiferous tubules of normal rats increased markedly from D0 to D100. In pre-weaning male rats, the seminiferous tubules appeared as a solid cellular cords. Different cell types in the seminiferous epithelium were not clearly distinguishable. Mendez and Emery (1979) reported that in humans the seminiferous tubules are solid cord or cells until time of puberty. Present observation shows that stress retards the increase in absolute volume of seminiferous tubules from D21. The chronic effect of stress persist even in 30 days period of quiescence. Similar observations were made when rats were treated with GnRH antagonist but when it was replaced with FSH the seminiferous tubular volume increased (Sinha-Hikim and Swerdloff, 1995).

In the normal rats absolute volume of seminiferous epithelium increased at faster rate from D49. Seminiferous epithelial volume continues to increase even till D100. Such sharp increase is not found in stressed group of rats where epithelial volume is significantly retarded. After withdrawal of chronic stress the epithelial volume definitely increased at a faster rate but could not improve to normal in 4 weeks of quiescence.

In this study, the lumen appeared in the seminiferous tubules of 35 day old rats. The luminal volume of the stressed rats did not increase probably due to relative reduction in tubular volume and diameter of seminiferous tubules. McLachlan et al (1995) suggested that in GnRH immunized rat, the luminal volume decreased possibly due to reduction in fluid production by Sertoli cells.
In normal control rats the absolute volume of the testicular stroma did not change much till D42, then there was a steady increase till D100. FS produced significant reduction in stromal volume in the testis of stressed rats showing less compactness but on withdrawal of FS stress, testicular stroma rapidly tends to be denser and compact.

The cell types in the seminiferous epithelium of rat testis presented in tables appears to be in small numbers. In fact, the crude counts of each type of cells are in large numbers but the corrected counts calculated by using Abererombie's formula presents only small number.

In growing rats population of spermatogonial cells are in progressive order. The type A spermatogonia normally being in smaller number, their differences between the normal control rats and rats under stress is not conspicuous. As for the type B spermatogonia its population increase with age. In young adult rats, after they attain maturity the number of type B spermatogonia rotates around 10 per tubular cross section. The figures are markedly reduced in FS stressed rats but restoration to normal number soon begins after cessation of chronic stress. However, the number of type A spermatogonia are about ten times lesser than the type B spermatogonia. This result confirms the earlier reports of Clermont and Perey (1957). Early after birth (about 4th day) the gonocytes proliferate and give rise to type A spermatogonia. Successive generation proliferates. By 15th day the yield of spermatocytes per type A spermatogonial stem cell becomes identical to that found in the normal adult. Present observations are in agreement with
Clermont and Perey (1957) for both type A and type B spermatogonia in first 4 weeks PN. In the ensuing weeks, FS stress drastically suppress the normal proliferation of spermatogonial cell types. On withdrawal of stress, first to normalise are the type A spermatogonia followed by type B spermatogonia.

In the present study, the population of germ cells and Sertoli cells at Stage VII of the cycle of seminiferous epithelium were assessed, because the hormone sensitive cell degeneration occurs only in stage VII (Russell et al, 1993). Authors also reported that after hypophysectomy, four types of germ cells (pre-leptotene, pachytene, step 7 and step 19 spermatids) degenerate at Stage VII. Due to this reason enumeration of viable number of type A spermatogonia, preleptotene and pachytene spermatocytes and step 7 (round) spermatids at stage VII are best recorded.

Type B spermatogonia were counted at Stage V. This parameter effectively reflects the successful completion of the proliferative phase of spermatogenic process and would allow to determine the number of differentiating spermatogonia (Type B) available to enter meiosis at Stage VII (Sinhahikim and Swerdloff, 1995).

Spermatocytes appear to be very sensitive to stress. On D21 PN, both preleptotene and pachytene spermatocytes population was suppressed in stressed rats. Possibly, this may be due to prolonged MD stress since D5. Its severity was relentless. After withdrawal of stress, the population of preleptotene spermatocytes showed little sign of recovery from stressful effect where as the pachytene
Spermatocytes did not show any sign of recovery. Stress causes reduction of the nuclear diameter of spermatocytes. This effect is late to appear in preleptotene spermatocytes but also found a week earlier in pachytene spermatocytes; withdrawal effect appeared after 10 days in preleptotene spermatocytes where as in pachytene spermatocytes it did not appear even after 30 days. Similar findings were observed in hamsters in which short photoperiod caused a regression in spermatogenesis, where the most affected cells were pachytene spermatocytes which did not progress to meiosis I while in the same period the controls reached meiosis II stage (Breckon and Cawood, 1985).

The population of round spermatids were much affected by stress. The numbers were greatly reduced and could not be restored in post-stressed rats. The nuclear diameter of round spermatids also altered in persistent chronic stress. On withdrawal of stress the nuclei tend to restore the normal size.

The present study has shown degeneration or reduced population of germ cells in stressed rats. Withdrawal of stress did not restore the normal population of spermatogenic cells. Restoration of spermatogenesis after GnRH antagonist treatment with recombinant FSH, increases the germ cell numbers (Sinha Hikim and Swerdloff, 1995).

In normal or stressed rats Sertoli cell population is at peak in two week old rats. Thereafter the number declines gradually to about one-third. This confirms Clermont and Perey (1957) who have stated that the supporting cells proliferate
actively at and soon after birth but stop dividing in 15 day old rats. By D45 the Sertoli cells assume their typical shape and place in the tubular epithelium. Nistal et al (1982) also reported decline in the Sertoli cell number in human testis from 3 year onwards. Remarkable to note in this study, from the age of two weeks till D100, under stress the Sertoli cells of rats are always greater than the normally growing rats, this is in contrary to Mc Lachlan et al (1995) report that Sertoli cell number does not alter by any treatment. However, the authors stated that Sertoli cell nuclear volume significantly decrease from control value by GnRH immunization and increase after FSH treatment.

Leydig cells featured very low proliferative activity up to day 21. Thereafter the population and volume density of the Leydig cells increase in control rats. Consequent upon, the total cytoplasmic volume of Leydig cells increase greatly. FS stress reduced the volume density and cytoplasmic volume of the Leydig cells. However, the effect on the nuclear volume of Leydig cells is inconsistent. Setchell et al (1990) reported that the number and the total volume of Leydig cells are related to testosterone. In monkeys, immature Leydig cells increased 7 fold from neonatal to early pubertal and increased significantly during later pubertal development (Rey et al, 1996). In control rats, 2.5% of the testicular volume is occupied by Leydig cells. Mori et al (1982) reported this as 3.8%. Kaler and Neaves (1978) stated that decline in testosterone production reflects on the Leydig cell population.
Pellagrini et al (1998) have stated that repeated stress proved to bring about morphological and histochemical changes both in adrenal cortex and testis of rats. Consequent upon the stress, corticosterone and progesterone in plasma levels increase in consonant with the lipid depletion observed in Zona fasciculata, while testosterone decreased. This proves that repeated stress even for a temporary period, is able to influence directly or indirectly the morphofunctional linkage between the two organs.

The above study seems to have thoroughly recorded morphometric changes in adrenal and testis of albino rats from D0 to D100 PN. The study was confined to histomorphometry because other methods of investigations in the field of stress research may have led to a separate direction and distracted the specific aim of this study. However, the results of this study has been amply helpful to confirm the past reports and has been able to produce some numerical data as a proof of effect of stress regarding the adrenal gland and testis of albino rat. It is interesting to find that stress has profound effects on the rat adrenal and testis, some of which are reversible to certain extent and others are not reversible at least within certain limited period.