SUMMARY

CHAPTER 1

The effects of transient neonatal hypothyroidism (HPOT) on the development and maturation of male reproductive system till adulthood has been studied in the Charles foster strain of rat. Hypothyroidism was induced in the suckling pups from day 0 to day 21 through mother by providing 0.1% propyl-thiouracil (PTU) in their drinking water. After weanling the impact of hypothyroidism was assessed at 35, 45, 60 and 90 days of age in terms of body, testes and accessory reproductive organ weights, histological features of testis and accessory reproductive organs, steroidogenic potential of the testis as evaluated by histochemical localisation of 3ß, 3α and 17ß-hydroxy steroid-dehydrogenase (HSDH) and the circulating titres of serum T_3, T_4 and T. The HPOT rats showed similar body weights but lesser testes weight at 90 days compared to controls. Histologically, the testis of HPOT rats depicted increased germ cell degeneration in the early periods with ultimate reduced tubular size and number of germ cells and sperm density at 90 days. The HPOT rats also showed reduced T levels and 3ß and 17ß activity. Intra-tubular steroidogenesis and a prominent Δ^5 pathway were also the features of HPOT animals. The sex accessory organs were also smaller in HPOT rats compared to controls and they were at an

average of 65-70% of the control weights at 90 days. These observations are in contrast to what have been reported by other workers. These differences have been discussed in the text in terms of altered gonadotropins, thyroid hormone and gonadal hormone profiles and, on the basis of possible strain specific effects.

CHAPTER 2

It is generally been inferred that pinealectomy (Px) in adult rats is of little consequence in terms of reproductive functions, even Px in the prepubertal rats were reported to show insignificant effects alluding to a concept of no major role for pineal in the rat on reproductive functions (Reiter, 1980; Goldman *et al.*, 1981; Binkly, 1983). Since there had been no study on long term effects of Px, the present study has been carried out to evaluate the long term consequences of early neonatal Px on the male reproductive system of the rat. To this end, rat neonates of Charles foster strain were pinealectomised on day 5 and the impact on the same assessed at 35, 45, 60 and 90 days of age in terms of body, testes and accessory reproductive organ weights, histological features of testis and accessory reproductive organs, steroidogenic potential of the testis, as evaluated by histochemical localisation of 3ß, 3α and 17ß-HSDH and the serum titres of T_3, T_4 and T. The results reveal that Px rats showed a hypersensitive response on a long term basis marked by increased body, testes and accessory organ weights at 90 days compared to age matched controls. Histologically the testis of Px rats depicted increased early tubular diameter followed by increased germ cell population though, with a delayed spermatogenesis ultimately at 90 days. Histochemically there was an early increased induction of 3ß and 17ß-HSDH activities. The hormonal profile showed increased T and delayed normalisation of thyroid hormone levels as Px rats depicted a hypothyroidic state in the postweanling periods. The observed effects on the reproductive system are related to increased Sertoli cell proliferation with delayed maturation and hyperplastic response of the accessory glands as discussed in terms of altered gonadotropins, T, PRL and thyroid hormone profiles.

Overall, it is conclude that neonatal Px in the Charles foster strain of rat can increase adult testes size and germ cell population and also the weights of accessory reproductive organs.

CHAPTER 3

Previous studies showed reduced adult testis size and accessory organ growth by transient neonatal hypothyroidism and increased adult testis size and sex accessory organ weights by neonatal Px. The present study in this context has tried to evaluate the combined effect of neonatal Px and hypothyroidism (HPOT + Px) on the development and maturation of the male reproductive system in the Charles foster strain of rat. The neonates were pinealectomised on day 5 and rendered hypothyroidic from day 0 to day 21 through mother by providing 0.1% propyl-thiouracil (PTU) in their drinking water. The effect of this combined manipulation was assessed chronologically at 35, 45, 60 and 90 days in terms of body, testes and accessory reproductive organ weights, histological features of testis and accessory reproductive organs, histochemical localisation of 3α, 3ß and 17ß-HSDH in the testis and the serum titers of T_3, T_4 and T. The HPOT + Px rats showed persistently reduced body weight, while the paired testis weight which was significantly less at 35 days equalled the control weight by 90 days. The histological and histochemical observations support the delayed compensatory growth response. The HPOT + Px rats remained HPOT till 35 days and the thyroid hormone levels became normal only by 45 days. The weights of sex accessory organs which were similar to controls at 35 days depicted retarded growth till 60 days and then showed a delayed compensatory growth to attain weights similar to the controls by 90 days. The delayed maturation of testis with observable increase in germ cell number have been related to a hyperproliferation of Sertoli cells and their delayed differentiation in the absence of optimal thyroid hormone levels. The similar weights of accessory organs at 35 days in both control and HPOT + Px rats followed by their retarded growth in the peripubertal period and a delayed attainment of optimal PRL and T levels. A growth retardatory influence of melatonin is also inferred by comparing the growth kinetics of the accessory organs

in HPOT + Px animals with those of HPOT and Px animals. These are discussed in detail in the text.

CHAPTER 4

The present study has tried to evaluate the effect of transient neonatal hyperthyroidism (HPRT) on the development and maturation of the male reproductive system in the Charles foster strain of rat as a previous study revealed decreased adult testis size and accessory organ weights due to hypothyroidism in this strain. The neonates were made hyperthyroidic from day 0 to day 21 by i.p administration of 0.09 μg of thyroxine. The effect of the same was then assessed at 35, 45, 60 and 90 days of age in terms of body, testes and accessory organ weights, histological features of testis and accessory reproductive organs, histochemical localisation of 3α, 3ß and 17ß-HSDH in the testis and the serum titres of T_3, T_4 and T. The HPRT rats showed significantly decreased body, testes and accessory organ weights at 90 days. The permanently decreased body weight is related to the effect of neonatal hyperthyroidism in lowering the thyroid and growth hormone set-point and in fact the HPRT animals had persistently low thyroid hormone levels from 35 day onwards. The decreased testis size and germ cell number have been accredited to a delayed synergistic action of thyroid hormone and FSH in inducing Sertoli cell differentiation due to the reduced levels of both the hormones in the early periods. The delayed growth effect manifested by the sex accessory organs seems to be apparently due to the sluggish elevation in T level. Overall, it is concluded from the present study that neonatal hyperthyroidism can retard growth and maturation of the male reproductive system by its effect on thyroid hormone status and gonadotropin and T levels.

CHAPTER 5

In the wake of the observations of retarded growth and development of the male reproductive system by transient neonatal hyperthyroidism and the growth stimulatory influence resulting in increased testis and accessory organ weights by neonatal Px, the present study was envisaged

to understand the possible consequence of neonatal Px and hyperthyroidism (HPRT + Px). The rat neonates of Charles foster strain were pinealectomised on day 5 and rendered hyperthyroidic from day 0 to day 21 by i.p. administration of 0.091 μg of thyroxine. The impact of the combined manipulation was then assessed at 35, 45, 60 and 90 days in terms of body, testes and accessory organ weights, histological features of testis and accessory reproductive organs, histochemical localisation of 3α, 3ß and 17ß-HSDH in the testis and the serum titres of T_3, T_4 and T. The HPRT + Px rats showed reduced body weight, and decreased epididymis weight, normal prostate weight and increased seminal weight at 90 days. The decreased body weight is related with decreased thyroid hormone levels in the early phases and a permanently lowered GH secretion. The delayed hyperplastic growth oh the testis and the increased germ cell number are attributed to prolonged Sertoli cell proliferation coupled with their delayed maturation due to the late elevation of thyroid hormone levels to optimum levels. The observed differential effects on the growth of accessory organs have been correlated with the differential interactions on a temporal scale of melatonin, thyroid and growth hormones, prolactin and testosterone. It is concluded from the present study that transient neonatal hyperthyroidism in pinealectomised rats can increase germ cell production and lowers hypothalamic set-point regulating GH secretion. The possible importance of GH in epididymal growth and a generalised growth retardatory influence of melatonin can also be inferred.

CHAPTER 6

Metabolic alterations including that of carbohydrates in the postnatal phase of development in mammal are of significance in the transition from immature to mature stage. These are essentially modulated by nutritional and endocrine factors in keeping with the changing functional exigencies. In a previous study, transient neonatal hypothyroidism was shown to retard testis and accessory organ development and delay the establishment of spermatogenesis. The present study is an attempt to find the possible correlation if any between perturbations in carbohydrate

metabolism and the above observed changes. To this end, rat neonates of the Charles foster strain were rendered hypothyroid by PTU feeding through mothers' drinking water from day one to 21. Subsequently, the changes in hepatic and testis glycogen content and phosphorylase activity together with that of blood glucose and hepatic G-6-Pase activity was assayed at 35, 45, 60 and 90 days. The results show early attainment of increased hepatic glycogen content and reduced phosphorylase activity and delayed testis glycogen depletion in hypothyroid rats compared to age matched controls. The results are suggestive of temporal advancement in the attainment of adult type systemic carbohydrate metabolism which has been related to the facilitatory influence of reduced thyroid hormone levels on early induction of increased insulin:glucagon molar ratio. The depletion in testis glycogen content has been correlated with the establishment of the first wave of spermatogenesis at the time of puberty and the observed delay in the hypothyroid rats in this respect has been related to the earlier observed delay in the initial pubertal spermatogenesis.

CHAPTER 7

In the present study effect of neonatal pinealectomy (Px) on hepatic and testis carbohydrate metabolism have been assessed at different time periods till the attainment of adulthood. Rat neonates of the Charles foster strain were surgically pinealectomised on day 5 and the levels of hepatic and testis glycogen and phosphorylase activity together with blood glucose and hepatic G-6-Pase activity have been assayed at 35, 45, 60 and 90 days of age. In the control rats the hepatic glycogen content increased gradually to the characteristic adult level by 90 days and the testis glycogen content increased significantly and then decreased at 45 and 60 days to maintain the steady low adult level. Concurrently the blood glucose level and the enzyme activities also attained the characteristic adult levels. The Px rats showed the early attainment of these features by 35 to 45 days. The testis glycogen content showed delay in depletion between 60 and 90 days. The changes in the blood glucose, hepatic glycogen and

phosphorylase activity suggest an early attainment of the adult type carbohydrate homeostasis in pinealectomised rats. This has been related to the early attainment of higher insulin:glucagon molar ratio. The increased G-6-Pase activity in the early stages in pinealectomised rats is correlated with the increased corticosterone action due to Px. The delayed depletion in the testis glycogen is correlated with the earlier observed delay in establishment of full spermatogenic functions. Since, the pinealectomised animals also showed hypothyroidic state in the early stages, it is presumed that both melatonin and T_4 resist the attainment of the higher insulin:glucagon molar ratio and that in the absence of them the attainment of the high insulin:glucagon ratio is hastened.

CHAPTER 8

Transition from the suckling to adult stage in the rat is marked by alteration in the carbohydrate metabolism. Studies on transient neonatal hypothyroidism or neonatal Px had revealed an earlier attainment of adult level hepatic glycogen content and temporal advancement of the establishment of high insulin:glucagon ratio, earliest in the latter case. Similarly, a depletion in testis glycogen was related with the full establishment of spermatogenesis or its delay, respectively. In this context, the present study evaluates the effect of transient hypothyroidism in neonatally pinealectomised rats on carbohydrate metabolism in the pre-pubertal, pubertal and post-pubertal periods. The hepatic glycogen was elevated right from 35 days itself, though lesser than that obtained in Px rats. The glycemic level showed a steady decrease from 35 days till an adult level was attained by 90 days, similar to hypothyroid rats. Concomitantly, the hepatic G-6-Pase activity showed increase from 35 days. The testis glycogen content was higher at all time periods and a depletion occurred only between 60 and 90 days. The hepatic and testis phosphorylase activity did not show any correlation with the tissue glycogen contents. The results suggest that the alterations are a consequence of independent and unrelated changes involving the hormones of the pancreas and the adrenal and that in pinealectomised hypothyroid rats there

is an early induction of the adult type carbohydrate homeostasis and attainment of higher insulin:glucagon molar ratio. The pattern of changes in testis glycogen content is similar to the hypothyroid rats and the depletion in glycogen was delayed probably due to the low glycogen load and also due to the delayed establishment of full spermatogenesis. On the whole, the combination of pinealectomy and hypothyroidism seem to mimic mostly the Px effects but the changes are much attenuated by HPOT. It appears that each of the two treatments tend to nullify the independent effects of each.

CHAPTER 9

Cessation of suckling at weaning is marked by a shift from fat rich milk diet to a carbohydrate diet and is characterised by increasing hepatic glycogen content till adulthood in rats. Since, thyroid hormones are known to influence carbohydrate metabolism, the effect of transient neonatal hyperthyroidism has been tested on the profile of carbohydrate metabolism, specially with reference to testis and liver, in the post-weanling period till adulthood. The neonates were made hyperthyroid by daily injection of 0.091 μg thyroxine from day 1 to 21 and the glycogen content and phosphorylase activity in their testes and liver were assayed together with blood glucose and hepatic G-6-Pase activity. The results show a hypoglycemic state and increased hepatic glycogen content till 60 days in the hyperthyroid rats. The changes in the hepatic phosphorylase activity do not exhibit any correlation with that of the glycogen content. Similar tendency of no correlation between glycogen content and phosphorylase activity of testes was also observed. The testis glycogen content was high till 60 days which then declined significantly between 60 and 90 days. In contrast, the control rats showed decline in glycogen content between 45 and 60 days. The observations indicate an early attainment of adult type carbohydrate homeostasis due to neonatal hyperthyroidism. This is mainly due to the increased insulin:glucagon molar ratio acquired earlier in relation to the controls. This is accredited to the reduced thyroid hormone levels in the immediate post-natal period due to the hypothyroidic state

subsequent to withdrawal of T_4 treatment. The neonatally hyperthyroid rats showed a permanent hypothyroidic state in the adult stage due to a lowering of the set-point of the pituitary-thyroid axis. The characteristic depletion in testis glycogen content occurred later in the hyperthyroid rats related to the controls and is correlated in the delayed expression of spermatogenic activity in hyperthyroid rats.

CHAPTER 10

The transition from immature to mature stage in the rat is marked by significant change in carbohydrate metabolism. Denoted by acquisition of increased tissue glycogen contents and appropriate levels of activity of enzymes concerned with glycogen metabolism. The present study attempts to evaluate the impact of transient hyperthyroidism in neonatally pinealectomised rats as both Px and neonatal hyperthyroidism were shown to alter carbohydrate metabolism significantly to this end rat neonates were pinealectomised on day 5 and treated with 0.091 μg T_4/day from day 1 to 21 and the glycogen content and phosphorylase activity in liver and testis together with blood glucose level and hepatic G-6-Pase activity were assayed at different time periods from post-weanling to adulthood. The results show the attainment of characteristic high adult levels of hepatic and testis glycogen contents by 35 days itself in the HPRT + Px rats. The changes in hepatic and testis phosphorylase activity as well as those of G-6-Pase and blood glucose and tissue glycogen contents tended to show no relation. The characteristic depletion in testis glycogen occurred between 45 and 60 days in both control and HPRT + Px rats. Based on the observations of systemic metabolic profile it is concluded that the attainment of adult type carbohydrate homeostasis and characteristic high insulin:glucagon molar ratio are temporally augmented in HPRT + Px rats. The apparently discordant changes in the various parameters are taken to indicate the expression of individual as well as combined effects of neonatal hyperthyroidism and Px resulting in altered hormonal balances and sensitivities. The depletion in the testis glycogen content noted to occur between 45 and 60 days in both the control and

HPRT + Px animals coinciding with the establishment of full spermatogenic activity has been taken to confirm the importance of testis glycogen as an important source of energy during the initial wave of spermatogenesis. The observations are discussed in detail in relation to the role of thyroid hormone in resisting the early attainment of a high insulin:glucagon molar ratio and sensitivity.