CHAPTER 6

TRANSIENT NEONATAL HYPOTHYROIDISM FACILITATES EARLY ATTAINMENT OF ADULT CARBOHYDRATE HOMEOSTASIS AND DELAYS TESTIS GLYCOGEN UTILISATION

Glycogen and carbohydrate metabolism are crucial in the postnatal period of mammals. Significant changes in the storage of hepatic glycogen and enzymes concerned with glycogen metabolism have been shown to occur from the neonatal to pubertal periods (Kawai and Arinze, 1981; Margolis, 1983). Carbohydrate metabolism is also very important for testicular functions and, features of carbohydrate metabolism do show specific variations postnatally till the establishment of adult pattern of testicular functions (Free, 1970; Grootegoed and Den Boer, 1990). Though the above changes in carbohydrate metabolism in the neonatal period of the mammals are essentially regulated by the changes in insulin and glucagon and even the gonadotrophic hormones (Free, 1970; Kawai and Arinze, 1981; Margolis, 1983), other hormones can also influence carbohydrate metabolism. Thyroid hormones are well recognised as metabolic hormones and their influence on systemic carbohydrate metabolism in vertebrates is widely recorded (Bargoni *et al.*, 1961; Menhan *et al.*, 1969; Snedecor *et al.*, 1972). Of late, thyroid hormones have been increasingly implicated in testicular development and its functional maturation (Jannini *et al.*, 1985). Both hypo and hyperthyroidism have been shown to affect

testicular carbohydrate metabolism and enzymes involved thereat (Aruldhas *et al.*, 1982a, b; 1984).

Previously it was shown that subjecting rats to transient hypothyroidism from birth up to weaning affects testicular growth, delays its maturation and results in a significantly reduced adult testis size (chapter 1). In this context, the present study is to evaluate the impact of neonatal hypothyroidism on systemic and testicular carbohydrate metabolism and to see whether these alterations could be related to the observed effects on the reproductive system.

RESULTS

BLOOD GLUCOSE (Table 6.1; Fig. 1)

Control: The blood glucose level was significantly high at 35 days which then decreased to a significantly low level at 45 days. The blood glucose level then increased significantly by 60 days (though significantly less than the 35 day level), and this level was maintained thereafter.

Hypothyroid: The HPOT animals showed significant hyperglycemia at 35 days, thereafter there was a steady decline through 45 and 60 days to attain a normal glycemic status by 90 days.

HEPATIC GLUCOSE-6-PHOSPHATASE (Table 6.3; Fig. 3)

Control: The hepatic G-6-Pase activity showed continuous increase from 35 to 60 days to attain the highest level of activity at this period. Thereafter, the activity decreased significantly at 90 days.

Hypothyroid: The enzyme activity at 35 days was significantly low in the HPOT animals. The enzyme activity remained more or less steady at 45 days and then increased significantly at 60 days. Thereafter, the enzyme activity decreased significantly at 90 days to levels very much comparable to the control levels.

Table. 6.1 Chronological alterations in Blood Glucose (mg/dL) level in intact and hypothyroid (HPOT) rats

Treatment	Age in Days			
	35	45	60	90
Control	122.55 ± 10.87@	89.25 ± 7.48	108.77 ± 10.34	103.40 ± 14.06
HPOT	180.96 ± 17.29^{d}	127.48 ± 12.36^{d}	112.98 ± 11.40^{ns}	107.23 ± 11.11^{ns}

@ Values expressed as Mean ± SD of five experiments

^{d} $p < 0.001$; ns Not Significant

Table. 6.2 Chronological alterations in Testis Glycogen (μg/100 mg) and Phosphorylase activity (μmoles of P released/mg protein/10 min.) in intact and hypothyroid (HPOT) rats

Treatment	GLYCOGEN				PHOSPHORYLASE			
	Age in Days				Age in Days			
	35	45	60	90	35	45	60	90
Control	5.10 ± 1.20@	24.90 ± 2.00	9.60 ± 2.50	9.40 ± 0.30	1198.96 ± 49.59	94.07 ± 8.94	72.79 ± 6.34	239.54 ± 17.08
HPOT	50.10 ± 8.00^{d}	45.40 ± 4.00^{d}	44.00 ± 2.70^{d}	7.00 ± 0.30^{d}	133.37 ± 14.21^{d}	204.32 ± 19.43^{d}	192.25 ± 16.82^{d}	230.68 ± 20.08^{ns}

@ Values expressed as Mean ± SD of five experiments

^{d} $p < 0.001$; ns Not Significant

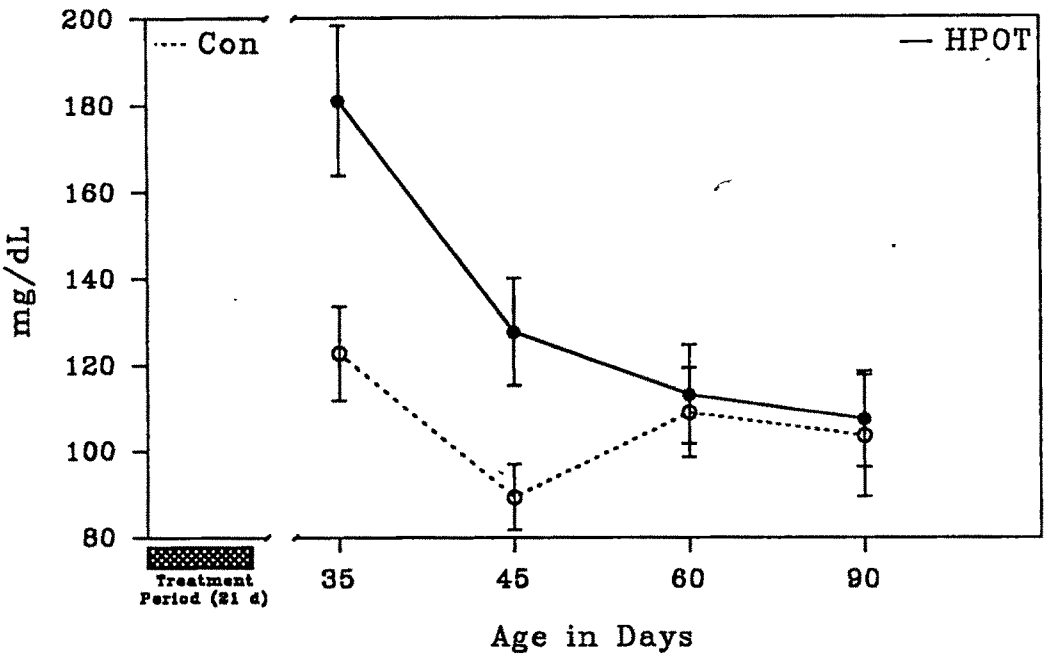

Fig. 1 Chronological alterations in blood glucose level in neonatal rats subjected to transient hypothyroidism (HPOT)

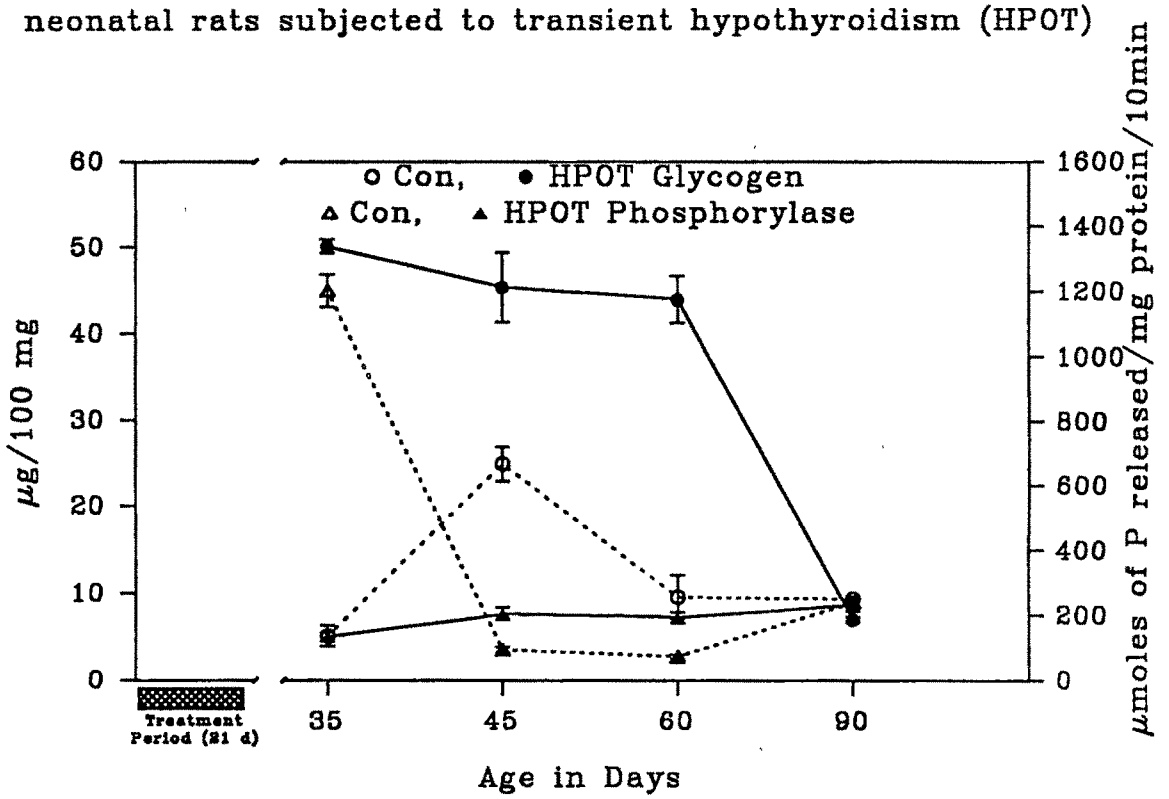

Fig. 2 Chronological alterations in testis glycogen and phosphorylase activity in intact and hypothyroid (HPOT) rats

Table 6.3 Chronological alterations in Hepatic Glycogen (mg/100 mg), Phosphorylase (μmoles of P relased/mg protein/10 min.) and G-6-Pase (μmoles of PO_4 relased/mg protein/10 min.) activities in intact and hypothyroid (HPOT) rats

Treatment	GLYCOGEN				PHOSPHORYLASE				G-6-PASE			
	35	45	60	90	35	45	60	90	35	45	60	90
Control	1.49 ± 0.09@	1.09 ± 0.13	1.38 ± 0.02	4.10 ± 0.43	690.88 ± 47.70	61.54 ± 1.97	230.61 ± 10.62	198.88 ± 8.56	14.96 ± 0.89	16.08 ± 2.70	17.96 ± 4.35	13.94 ± 2.70
HPOT	1.41 ± 0.32^{ns}	4.67 ± 0.08^{d}	3.80 ± 0.36^{d}	4.14 ± 0.33^{ns}	356.32 ± 34.26^{d}	158.32 ± 8.98^{d}	167.93 ± 12.32^{d}	193.73 ± 11.25^{ns}	11.61 ± 2.39^{c}	10.19 ± 2.53^{c}	26 27 ± 6.74^{b}	12.09 ± 3.58^{ns}

@ Values expressed as Mean ± SD of five experiments

^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ns Not Significant

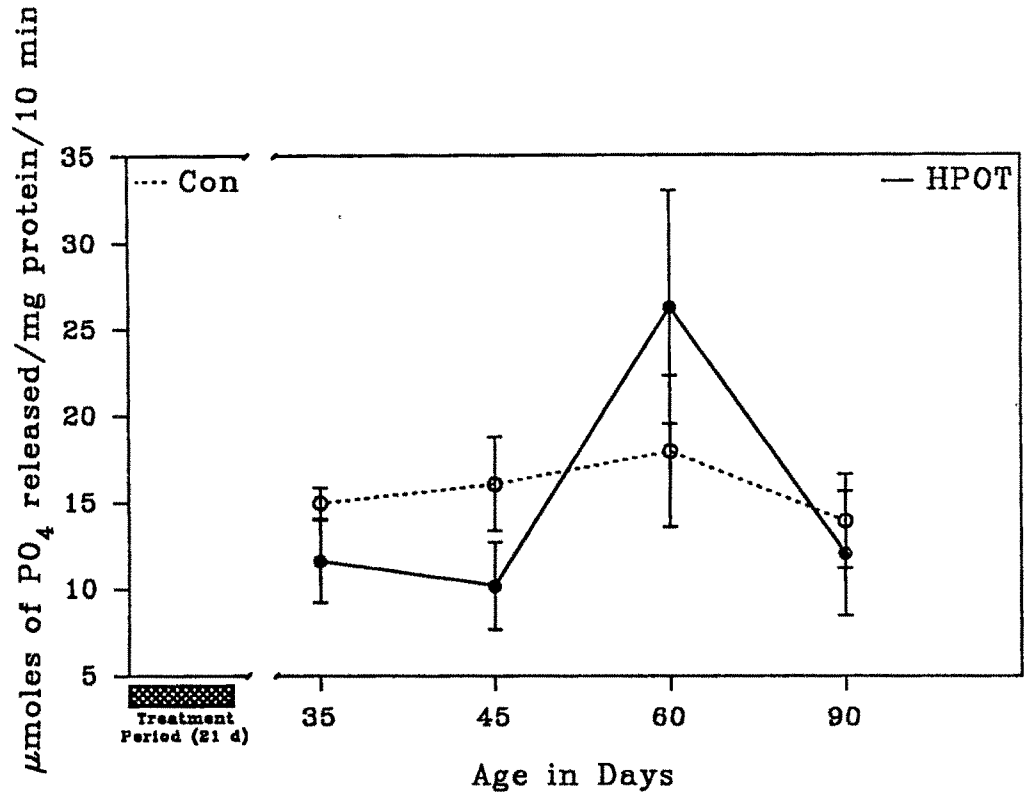

Fig. 3 Chronological alterations in liver G-6-Pase activity in neonatal rats subjected to transient hypothyroidism (HPOT)

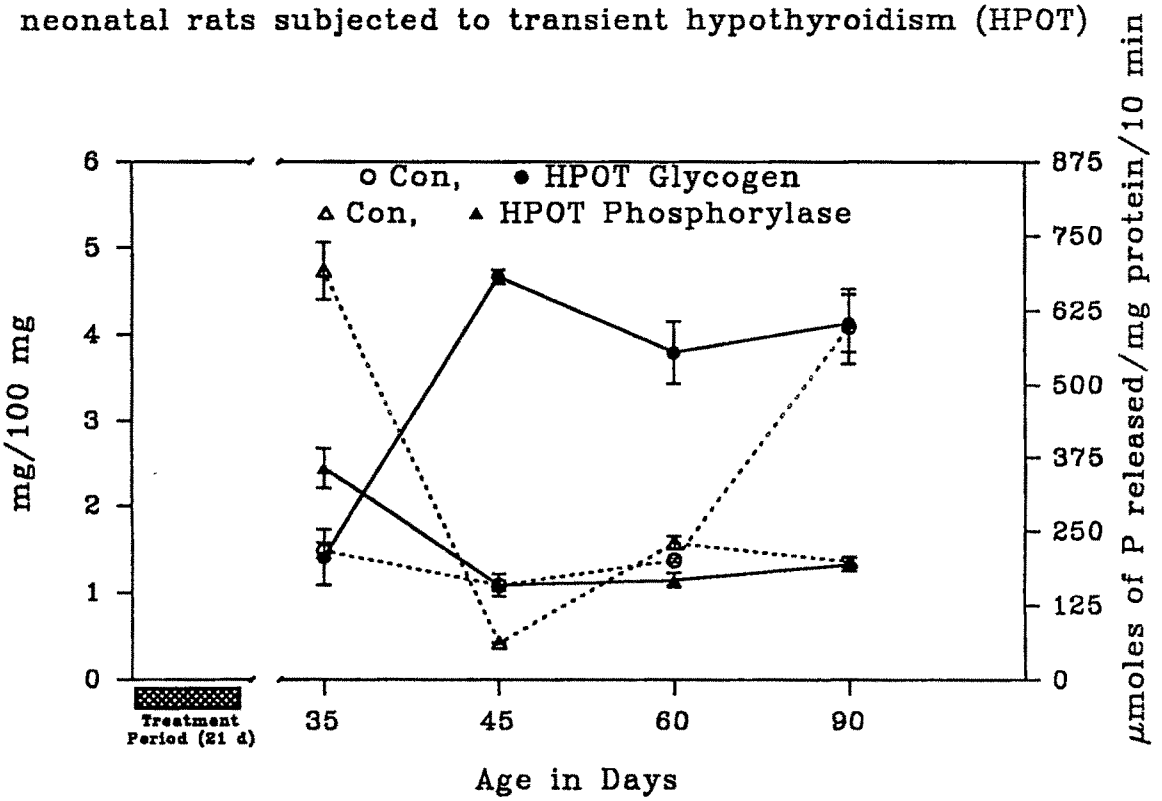

Fig. 4 Chronological alterations in hepatic glycogen and phosphorylase activity in intact and hypothyroid (HPOT) rats

HEPATIC GLYCOGEN (Table 6.3; Fig. 4)

Control: The hepatic glycogen content was more or less steady between 35 and 60 days in control animals with a slight decrement at 45 days. But at 90 days there was a significant increase in hepatic glycogen content.

Hypothyroid: The HPOT animals showed hepatic glycogen content similar to the control animals at 35 days. In the HPOT animals, the hepatic glycogen content increased significantly at 45 days, similar to the levels at 90 days in control animals. This level was more or less maintained thereafter except for a significant decrement at 60 days.

HEPATIC PHOSPHORYLASE (Table 6.3; Fig. 4)

Control: The hepatic phosphorylase activity was significantly high at 35 days in control animals. Thereafter, the enzyme activity showed a drastic decline at 45 days which later on increased to attain the adult levels through 60 to 90 days.

Hypothyroid: In the HPOT rats, the enzyme activity was about 50% of the control levels at 35 days. Thereafter, the enzyme activity showed a decline (less pronounced than the control levels) at 45 days, which showed a gradual steady increase thereafter to attain a level of activity similar to that of the controls by 90 days.

TESTIS GLYCOGEN (Table 6.2; Fig. 2)

Control: The testis glycogen content was low at 35 days, which increased significantly by 45 days. However, at 60 days there was significant depletion and, this level was maintained thereafter.

Hypothyroid: In the HPOT animals, the testis glycogen content was significantly high compared to the controls at 35 days. The glycogen content remained more or less steady at 60 days

though slightly less. But at 90 days the glycogen content was significantly decreased and maintained a level similar in range to the control animals.

TESTIS PHOSPHORYLASE (Table 6.2; Fig. 2)

Control: There was significantly higher testis phosphorylase activity at 35 days. The enzyme activity showed significant decrease thereafter and reached the lowest level at 60 days. At 90 days the enzyme activity reached to an adult level.

Hypothyroid: The HPOT animals had significantly low enzyme activity at 35 days, which then increased slightly but gradually to attain normal levels at 90 days.

DISCUSSION

Carbohydrate metabolism plays a key role in the neonatal period and, the liver in developing mammals stores glycogen abundantly before birth, to maintain euglycemia in the critical prenatal period beginning with cessation of transplacental transport up till the initiation of suckling (Jacqot, 1959; McCormick *et al.*, 1979; Hers, 1976). From the time of suckling till weaning, hepatic glycogen store remains low, which has been attributed to a combination of reduced peripheral glucose utilization (Vernon and Walker, 1972) and, the suppressive effects of the high fat milk diet on pancreatic-insulin release (Turkenkopf *et al.*, 1982). The maintenance of glycemic level at this period is mainly through gluconeogenesis using other substrates like amino acids and glycerol (Vernon and Walker, 1972). The immature rat develops the necessary enzymatic machinery and hormonal modulations for this tremendous accumulation of glycogen that occurs during the third and fourth weeks postnatally (see, Margolis, 1983). Earlier studies have suggested the importance of carbohydrate metabolism and that of glycogen content during the postnatal development and maturation of testis (Leiderman and Mancini, 1969; Fouquet and Guha, 1969; Gunaga *et al.*, 1972).

The present results show that hepatic glycogen content remains at a steady low level between 35 and 60 days and then increases significantly to adult levels by 90 days. A normal glycemic state was attained as early as 35 days and remains so thereafter except for a significant drop between 35 and 45 days. This period of hypoglycemia is also accompanied by a slight decrease in the hepatic glycogen content. This decrease in the carbohydrate moieties between 35 and 45 days could be suspected to have some functional relationship with pubescence as significant changes in the male reproductive system and appearance of spermatids and establishment of spermatogenesis were observed during this period (chapter 1). The changes in the blood glucose levels find positive relationship with the changes in hepatic G-6-Pase activity. However, the changes in the hepatic glycogen content and phosphorylase activity seen as a whole between 35 and 90 days seem to have no meaningful correlation except for significantly greater phosphorylase activity with lower glycogen content at 35 days and reduced phosphorylase activity and increased glycogen content at 90 days. Apparently, the modulations in the hepatic glycogen content between 35 and 90 days are probably more related with glycogen synthetase activity. The only likely explanation is that the peripubertal period is more of a period of establishment of homeostatic balance between glycogen synthetase and phosphorylase.

In contrast, rats which were subjected to neonatal hypothyroidism showed a hyperglycemic state at 35 days. These animals however attained normal glycemia and adult levels of hepatic glycogen content by 45 days itself. Similarly, the hepatic phosphorylase activity which was greater at 35 days decreased to characteristic adult levels by 45 days. The apparent modulations in hepatic glycogen content and phosphorylase activity were correlatable. Thyroid hormones are known to exert marked influence on carbohydrate metabolism (Bargoni *et al*, 1961; Menahan and Wieland, 1969; Tata *et al.*, 1963). Moreover, thyroid hormones are also known to have effects on glycogen synthetase and phosphorylase and on their regulatory enzymes (Malbon and Campbell, 1982; 1984). The very early attainment of adult levels of

hepatic glycogen content, phosphorylase activity and glycemic status in the experimental rats suggest that neonatal hypothyroidism has a favourable influence on the mechanisms leading to adult homeostatic patterns of carbohydrate metabolism. Both metabolic and hormonal factors could contribute to the transition from immature prepubertal to postpubertal adult pattern of carbohydrate metabolism. Amongst the hormonal factors, the insulin:glucagon molar ratio is the major factor involved in the changes in hepatic glycogen content and activities of related enzymes noted during the transition to adulthood (Margolis, 1983). The present observations in hypothyroid rats, provide a strong circumstantial evidence for the early acquisition of an increase in the insulin:glucagon molar ratio due to neonatal hypothyroidism. It is significant to note that the thyroid hormone levels were subnormal till 45 days despite the fact that PTU treatment was discontinued from 21 days. It could be speculated from this that the hypothyroidic condition in the immediate post-weanling period is responsible for the purported early attainment of the increased insulin:glucagon ratio. The role of dietary composition is well documented. High dietary fat content is known to suppress insulin secretion and the low hepatic glycogen content in the first three to four weeks in rats has in this context been attributed to fat rich milk diet (Vernon and Walker, 1972; Turkenkopf *et al.*, 1982). With the change in diet at weaning to a more carbohydrate rich one, the insulin:glucagon ratio is reported to have increased gradually and significantly which in turn leads to induction and activation of enzymes involved in glycogen synthesis and deposition (Wakelam and Walker, 1981; Margolis, 1983). Glucose is also known to be a potent candidate for insulin release and induction of glucokinase activity, leading to increased glucose uptake. Interestingly, the hypothyroid rats had significantly elevated blood glucose level at 35 days. Taking together, all the evidences tend to affirm the contention that the neonatal and/or prepubertal hypothyroidism temporally advances the attainment of the favourable ratio of pancreatic hormones and, sensitivity to these changes, leading to adult pattern of hepatic glycogen content and enzyme activities.

Thyroid hormones are also reported to have definite influence on G-6-Pase activity. Hypothyroidism is shown to decrease hepatic G-6-Pase activity (see, Snedecor *et al.*, 1972). This relates well with the present study as the G-6-Pase activity was significantly lowered at 35 and 45 days, the periods up to which hypothyroidism prevails. The change in G-6-Pase activity at various time periods in the present study also correlates well with the observed modulations in blood glucose levels. It is of interest that G-6-Pase has been implicated in glycogen breakdown and in this context decreased activity of this enzyme in the hypothyroid chick has been correlated with the concomitant increase in hepatic glycogen content (Snedecor *et al.*, 1972; Raheja and Linscheer, 1978). These observations seem to have some implications in the present study in the light of the obtained results. On the one hand, the decreased G-6-Pase activity together with phosphorylase in the early periods of study (35-45 days), could have a co-favourable influence along with the purported change in the insulin:glucagon ratio, in the early attainment of the characteristic adult high hepatic glycogen content in the hypothyroid rats. On the other hand, the relatively higher G-6-Pase activity at these periods in the control rats in the light of the immature state of the pancreatic hormone profile and phosphorylase activity, could be responsible for the decreased hepatic glycogen content.

Earlier studies based on the glycogen content of the testis and enzymes of carbohydrate metabolism had suggested the importance of carbohydrate metabolism during the postnatal development of rat and hamster testis (Leiderman and Mancini, 1969; Fouquet and Guha, 1969; Gunaga *et al.*, 1972). The present observations on testis glycogen content and phosphorylase activity tend to confirm the importance of glycogen related carbohydrate metabolism in the immature stages as, blood glucose and not testis glycogen, is a carbohydrate source for adult testis (Grootegoed and Den Boer, 1990). The present results show a significant increase in testis glycogen at 45 days following which, there is a drastic depletion to attain steady adult level by 60 days in the control rats. Corresponding to the increase in the glycogen content at 45 days, there was a concomitant significant decrease in phosphorylase activity. The attainment of low

adult level of testis glycogen content was again parallelled by the increase in phosphorylase activity at 90 days. The period of increased glycogen content corresponds to appearance of large number of spermatocytes and spermatids and the period of depletion with the establishment of spermatogenesis and appearance of sperms in the seminiferous tubules (chapter 1). Similar increase in glycogen content in the postnatal testis during early periods lasting up to 6 weeks has been observed by Fouquet and Guha (1969), and moreover glycogen has been localised in spermatogonia, spermatocytes and Sertoli cells (Leiderman and Mancini, 1969; Fouquet and Guha, 1969). The depletion noted in the glycogen content between 45 and 60 days in the present study, concomitant to the establishment of spermatogenesis, suggests testis glycogen stores as an important source of energy at least during the establishment of the first wave of spermatogenesis. The steady low level of glycogen observed subsequent to the full establishment of spermatogenesis suggests the dependence of testis on blood glucose in the metabolic scheme of the adult testis, as has been inferred by the many earlier studies on carbohydrate metabolism in the adult testis (see, Grootegoed and Den Boer, 1990). One of the reasons that can be postulated for the dependence of the immature testis on the glycogen store as against blood glucose by the mature testis, could be the absence of glucose transporter (GLUT.1) in the immature undifferentiated Sertoli cells. The induction of GLUT 1 in the Sertoli cells is considered to be one of the features of Sertoli cell differentiation and hence the dependence of the immature testis on glycogen store when the Sertoli cells are not differentiated for adult functions can easily be envisaged.

The glycogen content in the testis of hypothyroid rats remained significantly high till 60 days and then depleted significantly by 90 days to attain similar levels as in the controls. The changes in the glycogen content between 35 to 90 days bear positive relationship with changes in phosphorylase activity. Undoubtedly neonatal hypothyroidism seems to create a conducive hormonal milieu favouring glycogen depletion not only in the liver but even in the testis. The late depletion in the glycogen content (between 60 and 90 days) as against the early depletion in the

control rats (between 45 and 60 days) finds correlation with the earlier observed delay in the establishment of spermatogenic functions in the hypothyroid rats (chapter 1). The phase of glycogen depletion in both cases shows perfect correlation with the phase of establishment of full spermatogenic activity. In the earlier study it was shown that due to the delay in the attainment of normal levels of thyroid hormones, the FSH induced Sertoli cell differentiation along with the permissive influence of thyroid hormones was delayed resulting in a consequent delay in the establishment of spermatogenesis (chapter 1). In this background the presently observed delay in the glycogen depletion is in excellent correspondence and needs no explanation.

Overall, the present study has revealed that neonatal hypothyroidism and/or lowered thyroid hormone levels in the prepubertal period has a favourable influence in advancing the attainment of insulin:glucagon molar ratio leading to early expression of adult type carbohydrate homeostasis. Further, present observations also provide supportive evidence for the earlier observed delay in establishment of full spermatogenic functions by the observed correspondence in glycogen depletion.