CHAPTER 9

TRANSIENT NEONATAL HYPERTHYROIDISM ALSO FAVOURS EARLY ATTAINMENT OF ADULT TYPE CARBOHYDRATE HOMEOSTASIS IN MALE RATS

Importance of carbohydrate metabolism in the postnatal period of mammals has been established (Hers, 1976; McCormick *et al.*, 1979; Freinkel, 1980). Alterations in hepatic glycogen stores and enzymes concerned with glycogen metabolism have also been reported to occur in the postnatal periods till adulthood (Kawai and Arinze, 1981; Margolis, 1983). Testis carbohydrate metabolism has been shown to exhibit a distinct difference in the prepubertal and postpubertal periods in the rat (Free, 1970). The metabolic affects of thyroid hormones, especially on carbohydrate metabolism, are well established. Previous studies have shown that, both transient neonatal hypothyroidism and neonatal pinealectomy (Px) affect the maturation of the male reproductive system and also induce perturbations in systemic and testis carbohydrate metabolism (chapters 1 and 4). Besides, transient neonatal hyperthyroidism (HPRT) also results in perturbations in neonatal maturation of the male reproductive system.

In this context, the present investigation aims to evaluate the possible consequences of transient neonatal hyperthyroidism on systemic and testis carbohydrate metabolism and possibly relate the same with the observed effects on the male reproductive system.

RESULTS

BLOOD GLUCOSE (Table 9.1; 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.

Hyperthyroidism: The HPRT animals showed significant hypoglycemic status at 35 days. Thereafter, the blood glucose level increased at 45 days, then decreased at 60 days and again increased to significant hyperglycemic level at 90 days.

HEPATIC GLUCOSE-6-PHOSPHATASE (Table 9.3; 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.

Hyperthyroidism: The enzyme activity was slightly high in the HPRT rats at 35 days compared to the controls. Thereafter, there was a significant and consistent decrease in the enzyme activity through 45 days to reach the lowest level at 60 days. However, at 90 days the enzyme activity increased significantly compared to the control levels.

HEPATIC GLYCOGEN (Table 9.3; 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.

Hyperthyroidism: The HPRT rats showed significantly higher glycogen content at 35 days. There was significant depletion in the hepatic glycogen content at 45 days; thereafter, there was

Table. 9.1 Chronological alterations in Blood Glucose (mg/dL) level in intact and hyperthyroid (HPRT) 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
HPRT	83.31 ± 5.56^{d}	98.98 ± 5.76^{b}	88.47 ± 5.76^{c}	116.06 ± 10.87^{ns}

@ Values expressed as Mean ± SD of five experiments

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

Table. 9.2 Chronological alterations in Testis Glycogen (μg/100 mg) and Phosphorylase activity (μmoles of P released/mg protein/10 min.) in intact and hyperthyroid (HPRT) 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
HPRT	15.30 ± 3.70^{d}	137.60 ± 8.50^{d}	12.80 ± 4.10^{ns}	3.70 ± 0.30^{d}	232.63 ± 13.24^{d}	171.02 ± 10.06^{d}	142.53 ± 9.83^{d}	228.38 ± 11.67^{ns}

@ Values expressed as Mean ± SD of five experiments

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

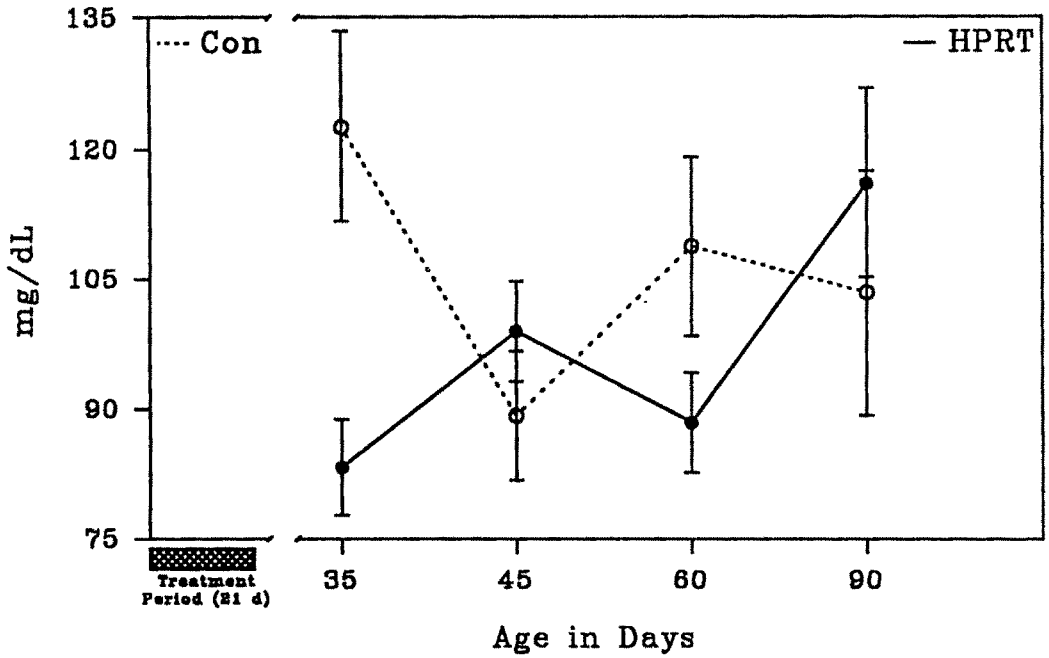

Fig. 1 Chronological alterations in blood glucose level in neonatal rats subjected to transient hyperthyroidism (HPRT)

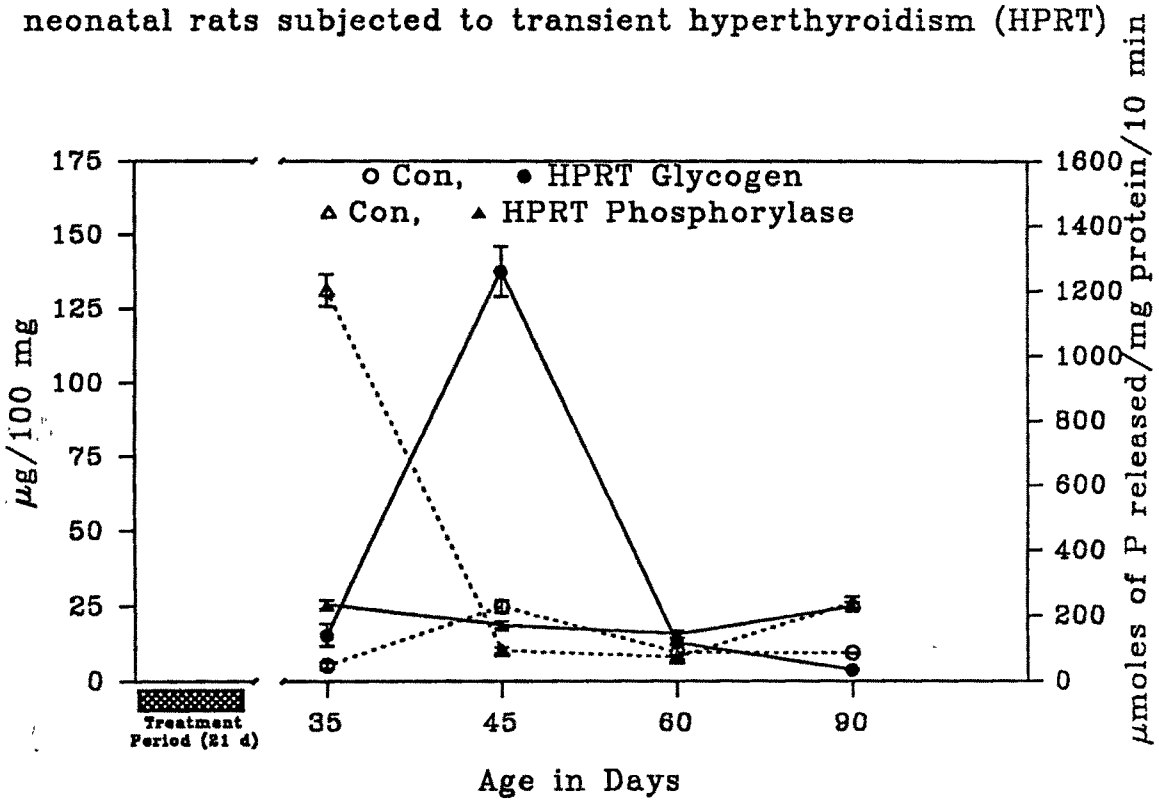

Fig. 2 Chronological alterations in testis glycogen and phosphorylase activity in intact and hyperthyroid (HPRT) rats

Table 9.3 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 hyperthyroid (HPRT) 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
HPRT	2.47 ± 0.29^d	1.52 ± 0.18^d	4.89 ± 0.42^d	1.36 ± 0.21^d	200.29 ± 23.37^d	131.09 ± 12.51^d	46.29 ± 8.26^d	125.98 ± 12.73^d	15.09 ± 2.74^{ns}	11.78 ± 1.92^c	8.17 ± 0.95^d	16.65 ± 2.11^{ns}

@ Values expressed as Mean ± SD of five experiments

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

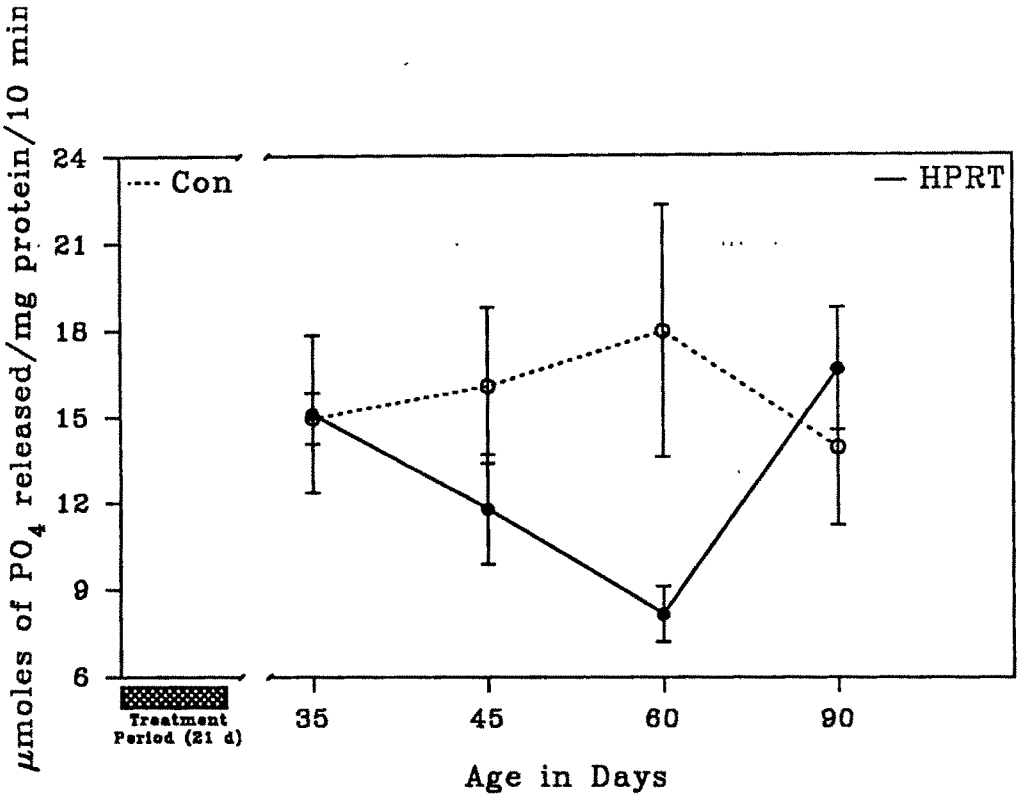

Fig. 3 Chronological alterations in liver G-6-Pase activity in neonatal rats subjected to transient hyperthyroidism (HPRT)

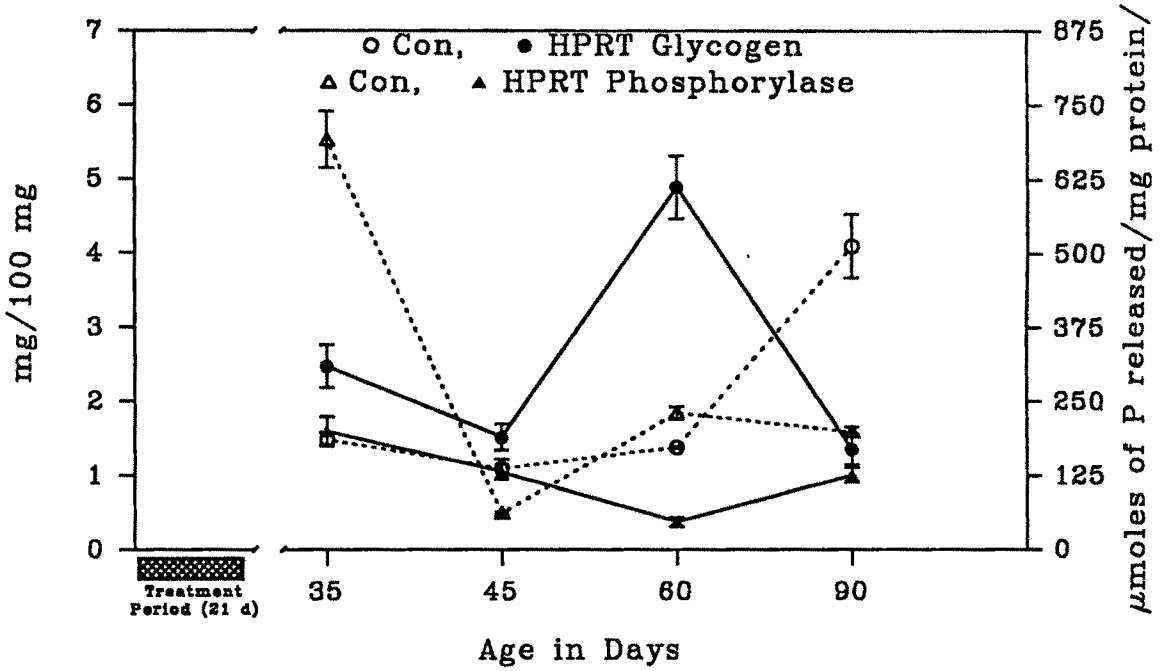

Fig. 4 Chronological alterations in hepatic glycogen and phosphorylase activity in intact and hyperthyroid (HPRT) rats

a significant increase at 60 days, which was comparable to the level of control rats at 90 days. However, the glycogen content again decreased significantly at 90 days.

HEPATIC PHOSPHORYLASE (Table 9.3; Fig. 4)

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

Hyperthyroidism: The HPRT animals showed significantly reduced hepatic phosphorylase activity at 35 days. Further, these rats continued to show a steady and continuous decline in enzyme activity thereafter, to reach a steady level at 60 days. The enzyme activity then increased to adult levels at 90 days.

TESTIS GLYCOGEN (Table 9.2; 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.

Hyperthyroidism: The HPRT animals had significantly higher testis glycogen content at 35 days. The glycogen content increased significantly to the highest level at 45 days. Thereafter, the glycogen content decreased, significantly and steadily, to attain the lowest level at 90 days.

TESTIS PHOSPHORYLASE (Table 9.2; Fig. 2)

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

Hyperthyroidism: The HPRT animals showed a significantly decreased testis phosphorylase activity at 35 days. The enzyme activity decreased significantly and steadily thereafter to attain the lowest level at 60 days which, again showed an increase to reach the adult level at 90 days.

DISCUSSION

The present study on transient neonatal hyperthyroidism shows a hypoglycemic state till 60 days and increased hepatic glycogen contents at 35 and 60 days and decreased contents at 45 and 90 days. The tendency for increasing hepatic glycogen content is similar to the increased contents noted under neonatal hypothyroidism, Px or HPOT + Px (chapters 6-8). Obviously, a similar influence prevails under all these experimental conditions and, in the above studies, the early increase in hepatic glycogen content was correlated with an early attainment of adult type insulin:glucagon molar ratio. A common manifestation attendant to the experimental studies in the previous studies, as well as in the present study, is the apparent hypothyroidic state in the postweanling period. In the above studies, while PTU induced hypothyroidism was a natural consequence, the Px induced hypothyroidism was attributed to a delayed maturation of the hypothalamic-pituitary-thyroid axis (chapter 2). In the present case, neonatal hyperthyroidism also reduced thyroid hormone levels subsequent to the withdrawal of thyroxine treatment. This is due to the purported action of the higher thyroid hormone levels on the pituitary-thyroid axis resulting in lowering of the set-point of pituitary-thyroid axis (Dussault *et al.*, 1982; chapter 4). An inferred action, of reduced thyroid hormone levels in the weanling and postweanling periods, was to increase the insulin:glucagon molar ratio leading to precocious attainment of the adult type carbohydrate homeostasis (chapters 6-8). The presently observed increased glycogen content at 35 days is the natural consequence of reduced thyroid hormone levels. However, the moderate increase in the glycogen content, compared to the HPOT and Px rats, is due to the antagonistic action of melatonin on elevation/sensitivity of insulin as, an earlier maturation of the

pineal rhythmicity and increased melatonin secretion due to neonatal hyperthyroidism was envisaged (chapter 4).

The greatly increased glycogen content at 60 days is related to the above purported scheme of hormonal dynamics as, melatonin secretion decreases in the postpubertal period (Ebling and Foster, 1989). Experimental evidence are forthcoming, in this context, from some of the past and, ongoing studies on, the pigeon and the rat. Pinealectomy in the pigeon and the rat have been to shown to increase tissue glycogen contents and manifest increased glucose tolerance to a glucose challenge and, increased insulin sensitivity (Ramachandran and Patel, 1987; 1989; Patel and Ramachandran, 1989; 1996; Patel and Ramachandran, unpublished; Patel *et al.*, unpublished) conversely, melatonin administration resulted in reversed set of changes (Patel and Ramachandran, unpublished; Patel *et al.*, unpublished). The decrease in glycogen content at 45 and 90 days seems to be related to the overall body growth kinetics as, the HPRT rats, whose growth was suppressed, showed increased growth rates during these two periods (chapter 4). The overall inferred increased insulin secretion/sensitivity in the HPRT rats is confirmed by the noted hypoglycemic state till 60 days. It is evident from the data that, the observed changes in the glycogen content bear no causal relationship with phosphorylase activity. Presumably, the dynamics of carbohydrate metabolism during these period are essentially a consequence of the overall synthetase to phosphorylase ratio, as was inferred even in the earlier studies. Another contributory factor for the increased insulin sensitivity, is the reduced GH level in the HPRT rats. This is due to the effect of neonatal hyperthyroidism in lowering the GH set-point permanently (chapter 5). The reduced G-6-Pase activity till 60 days also attests to a reduced glucagon/corticosterone tone (Joseph and Ramachandran, 1992; chapter 7), again favouring the concept of increased insulin:glucagon molar ratio.

Like in the case of liver , in the testis too, except for 35 and 90 days, there was no correlation between the glycogen content and phosphorylase activity in HPRT rats. Apparently,

the ratio between the synthetase to phosphorylase activity dictates the net glycogen content as presumed. The initial glycogen content at 35 days is significantly greater compared to the controls. This is again similar to that observed in HPOT and Px animals (chapters 6 and 7); however, the increase was moderate, due to the antagonistic effects of reduced thyroid hormones and melatonin as discussed above. The significant depletion in testis glycogen content seen between 60 and 90 days coincides with the establishment of spermiogenesis as marked by the appearance of elongated spermatids (chapter 4). This coincidence in glycogen depletion and progression through spermiogenesis/full establishment of spermatogenesis observed in the present case, as well as in the previous experimental paradigms like neonatal hypothyroidism and, neonatal hypothyroidism cum pinealectomy and, also in the controls, further embellish the importance of testis glycogen store in the molecular ecology of the germ cells during the first wave of spermatogenesis. The post-meiotic germ cells in the adult testis are dependant on the lactate produced by the Sertoli cells and, the Sertoli cells principally rely on blood glucose for its metabolite requirement (Leiderman and Mancini, 1969; Fouquet and Guha, 1969; Gunaga *et al.*, 1972; Grootegoed and Den Boer, 1970). The ability of lactate production by the Sertoli cells, is one of the many differentiated functions that are expressed by these cells. In other strains of rat, differentiation of the immature Sertoli cells has been shown to be induced by triiodothyronine (Jannini *et al.*, 1990; Palmero *et al.*, 1993; 1995), while, in the Charles foster strain, FSH is found to be the principal hormone but, dependent on the permissive influence of triiodothyronine (chapter 1). It is likely, that the induction of glucose transporter (GLUT 1) and, the expression of glucose transporting mechanisms, may not have attained their full functional competence during the initial wave of spermatogenesis and hence, the glycogen reserve meets the sudden and heavy demand of glucose moieties. Besides the glycolytic breakdown to lactate, much needed for spermatocytes and spermatids (Jutte *et al.*, 1981; 1983; Grootegoed *et al.*, 1984; Grootegoed and Den Boer, 1990). Carbohydrate moieties might also be needed for synthesis of amino acids, as they are needed in adequate amounts for synthesis of various proteins, all

of which appear at this time (Jegou, 1993). The persisting low glycogen content subsequent to the first wave of spermatogenesis, indicates that, the glucose transporting mechanism is functioning to the full potential and, with the attainment of a steady state of spermatogenesis as well as synthetic activities of the Sertoli cells, the blood glucose alone suffices to meet the requirements.

In conclusion, the present study re-emphasizes the fact that transient hypothyroidic state in the immediate postweanling period favours augmented temporal expression of adult type carbohydrate homeostasis and that, both thyroxine and melatonin delay the same. Further, the testis glycogen stores are important during the first wave of spermatogenesis for meeting the exigencies at this period.