CHAPTER 8

TRANSIENT NEONATAL HYPOTHYROIDISM AND NEONATAL PINEALECTOMY TEND TO NULLIFY EACH OTHER'S INFLUENCE ON HEPATIC AND TESTIS CARBOHYDRATE METABOLISM

Perinatal period of rat development is marked by heavy increment in hepatic glycogen content to be used as an energy source during the first 72 hours postnatally (Margolis, 1983). During the suckling period, when the young ones are being nourished by a fat rich milk diet, the hepatic glycogen store remains low. However, towards the end of suckling, in preparation for the carbohydrate rich diet, hepatic glycogen starts increasing (Turkenkopf *et al.*, 1982). Subsequently, in the prepubertal to pubertal periods, the glycogen content remains decreased and then, attains a higher adult level by 90 days (chapter 6). Previous studies on transient neonatal hypothyroidism or neonatal Px revealed an earlier attainment of adult level hepatic glycogen content due to temporal advancement of the establishment of high insulin ; glucagon ratio, earliest in the latter case (chapters 6 and 7). The testis glycogen content was marked by an initial deposition, followed by a depletion with the establishment of full spermatogenic activity. The concurrence or nonconcurrence of the depletion was related with the full establishment of spermatogenesis or its delay, respectively (chapters 6 and 7). In this behest, it was thought pertinent to study the effect of transient hypothyroidism in neonatally pinealectomised rats on carbohydrate metabolism and relate with the earlier observed effects on reproductive system.

The present study in this context deals with the changes in carbohydrate metabolism that occurs from prepubertal to adult stage due to a combination of transient hypothyroidism and neonatal Px.

RESULTS

BLOOD GLUCOSE (Table 8.1; 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.

HPOT + Px: The HPOT + Px rats showed significant hyperglycemia at 35 days. Thereafter, there was a steady decline through 45 and 60 days to reach significant hypoglycemic status; the level again increased significantly to attain a hyperglycemic status at 90 days.

HEPATIC GLUCOSE-6-PHOSPHATASE (Table 8.3; 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.

HPOT + Px: The enzyme activity at 35 days was significantly high in HPOT + Px 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, though slightly high.

HEPATIC GLYCOGEN (Table 8.3; Fig. 4)

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

Table. 8.1 Chronological alterations in Blood Glucose (mg/dL) level in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

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 + Px	137.32 ± 11.07[a]	103.74 ± 10.38[b]	83.74 ± 5.44[d]	111.23 ± 11.45[ns]

@ Values expressed as Mean ± SD of five experiments

[a] $p < 0.05$; [b] $p < 0.025$; [d] $p < 0.001$; [ns] Not Significant

Table. 8.2 Chronological alterations in Tests Glycogen (μg/100 mg) and Phosphorylase activity (μmoles of P released/mg protein/10 min.) in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

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 + Px	17.10 ± 1.5[d]	135.10 ± 29.8[d]	13.50 ± 2.40[b]	11.90 ± 1.20[d]	380.17 ± 13.31[d]	33.09 ± 4.69[d]	121.87 ± 9.07[d]	255.71 ± 10.82[ns]

@ Values expressed as Mean ± SD of five experiments

[b] $p < 0.025$; [d] $p < 0.001$; [ns] Not Significant

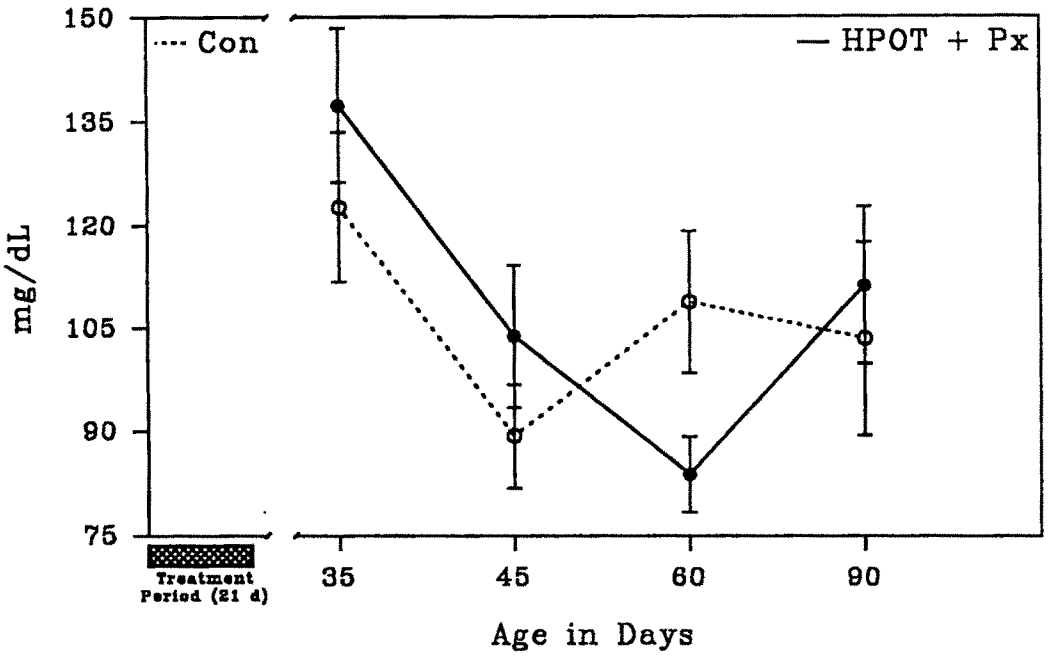

Fig. 1 Chronological alterations in blood glucose level in hypothyroid rats subjected to pinealectomy (HPOT + Px)

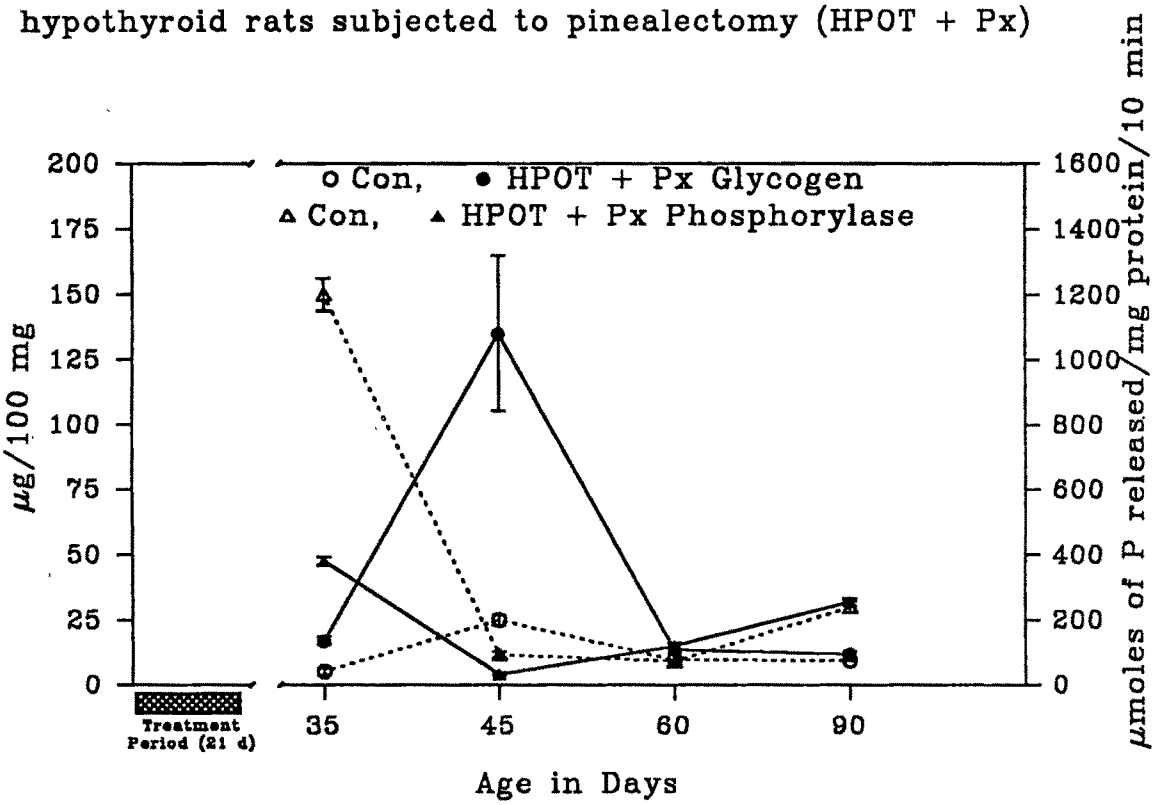

Fig. 2 Chronological alterations in testis glycogen and phosphorylase activity in intact and HPOT + Px rats

Table 8.3 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 rats subjected to pinealectomy (HPOT + Px)

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 + Px	2.57 ± 0.30^{d}	2.07 ± 0.26^{d}	2.12 ± 0.06^{d}	3.58 ± 0.27^{b}	241.49 ± 23.23^{d}	32.45 ± 1.27^{d}	143.62 ± 11.30^{d}	204.94 ± 20.19^{ns}	19.06 ± 2.87^{c}	19.74 ± 2.37^{b}	28.32 ± 4.78^{c}	17.10 ± 3.81^{ns}

@ Values expressed as Mean ± SD of five experiments

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

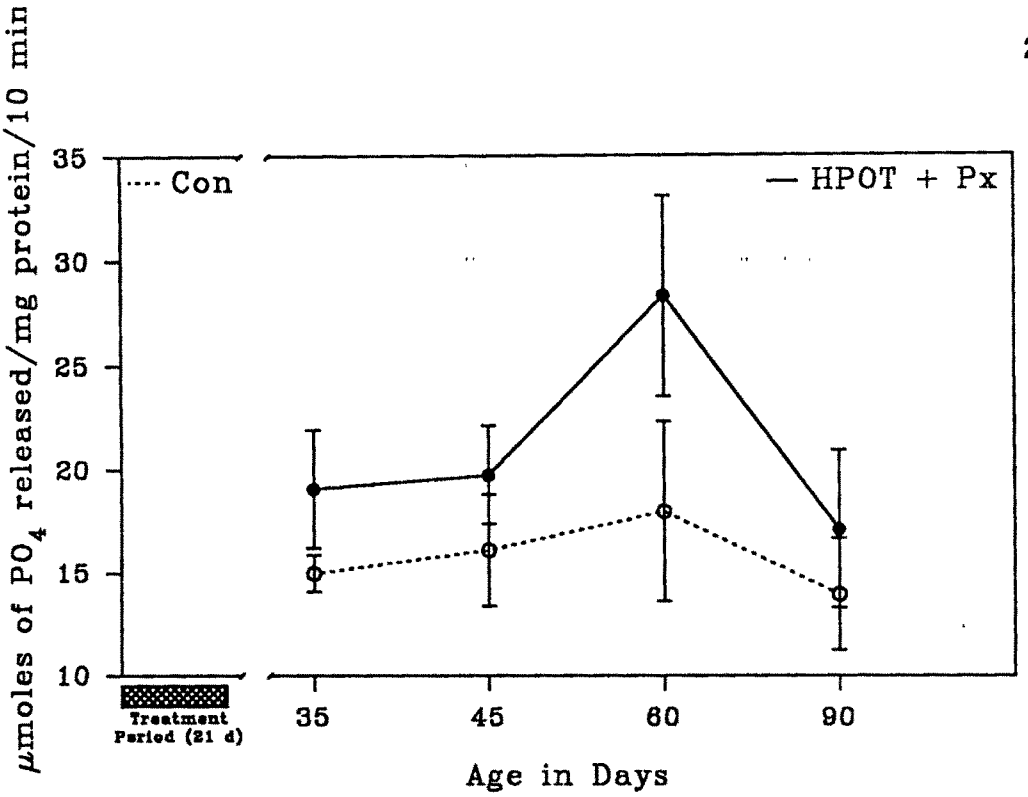

Fig. 3 Chronological alterations in liver G-6-Pase activity in hypothyroid rats subjected to pinealectomy (HPOT + Px)

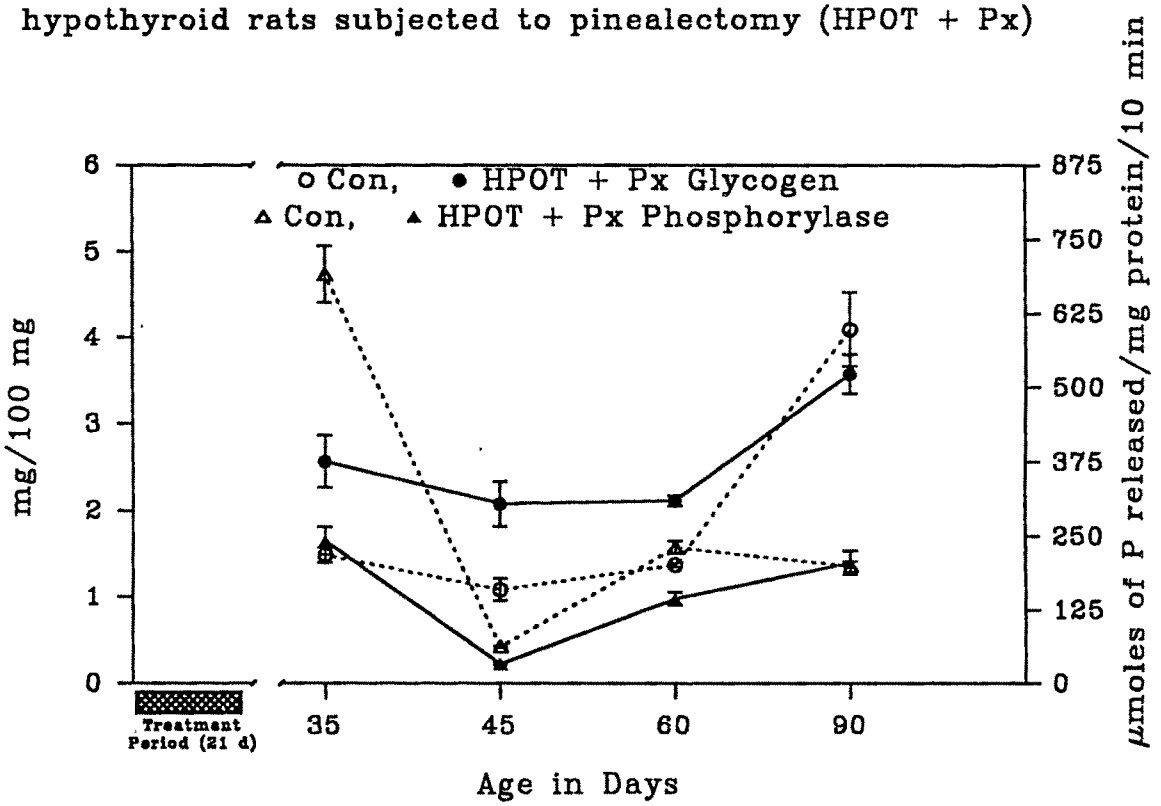

Fig. 4 Chronological alterations in hepatic glycogen and phosphorylase activity in intact and HPOT + Px rats

HPOT + Px: The HPOT + Px animals showed a greater glycogen content than the controls at 35 days. Thereafter, the glycogen content was more or less maintained steady till 60 days, though there was a slight decrease. At 90 days, the hepatic glycogen content increased significantly but was still lower than that of the control levels.

HEPATIC PHOSPHORYLASE (Table 8.3; Fig. 4)

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

HPOT + Px: The enzyme activity was about 50% less than the control animals at 35 days. At 45 days, there was a fall in the enzyme activity, to less than the control levels. Thereafter, the enzyme activity increased through 60 days to attain a steady adult level at 90 days.

TESTIS GLYCOGEN (Table 8.2; 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.

HPOT + Px: In the testis of HPOT + Px animals, the glycogen content was significantly higher than the controls at 35 days. The glycogen content increased to a significant maximal level at 45 days. Thereafter, there was a significant depletion at 60 days, to attain a level, comparable to the control animals, though higher. There was further decrease at 90 days and the level reached the values close to those of controls.

TESTIS PHOSPHORYLASE (Table 8.2; Fig. 2)

Control: There was significantly higher testis phosphorylase activity at 35 days in the control animals. 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.

HPOT + Px: The enzyme activity was significantly decreased in HPOT + Px animals at 35 days compared to the controls. The enzyme activity decreased significantly at 45 days to reach the lowest level. Thereafter, the enzyme activity increased continuously to attain the characteristic adult levels at 90 days.

DISCUSSION

Characteristic adult hepatic glycogen content and the appropriate levels of activity of glycogen synthetase and phosphorylase are attributed to an increased insulin:glucagon molar ratio (Wakelam and Walker, 1981; Margolis, 1983). Temporal advancement of these changes from 90 to, 45 days or even 35 days, have been probably shown to occur by transient neonatal hypothyroidism or Px respectively (chapters 6 and 7). The present study involving combined experimental manipulation of neonatal hypothyroidism and Px has also apparently shown the probable temporal advancement in the attainment of increased insulin:glucagon ratio, as noted by the increased hepatic glycogen content right from 35 days. In this respect, this finds identity with Px. However, the glycogen content is lower than that of Px . There is hypoglycemia at 35 days much like that in neonatal hypothyroidism, but attenuated. Like in the hypothyroid rats, the glycemic level decreases steadily thereafter to attain normal glycemia by 90 days. Apparently the alterations represent mixed effects of hypothyroidism and pinealectomy. The increased blood glucose level was related with increased G-6-Pase activity and associated gluconeogenic effect due to stimulated adrenocortical activity in an earlier study (chapter 7). In the present study, G-6-Pase activity is significantly elevated at 35 days and increases further by 60 days and there is concomitant stimulated adrenocortical activity (unpublished observations). Interestingly, the increased G-6-Pase activity is parallelled by decrease in blood glucose level and increased protein content (unpublished data), which are paradoxical to the purported gluconeogenic activity. As a whole, the changes observed in the present study seem to be a consequence of independent and unrelated perturbations involving hormones of the pancreas and the adrenal.

Obviously, there are altered titres of hormones and their interactions, resulting in modified differential tissue sensitivities and establishment of a new metabolic homeostasis as compared to the controls. The differential unrelated effects and the degree of responses seen in the present study attest to the above.

The glycogen content of the testis was higher than the controls at all time periods except at 45 days. Similar increase in glycogen content was also seen in hypothyroid as well as pinealectomised rats. However, the degree of increase in the present case is significantly attenuated. Moreover, unlike the pinealectomised animals, which showed significant increase in glycogen content at 60 and 90 days, the HPOT + Px rats depicted a tendency for decrease, like in the HPOT animals. Though the decreased phosphorylase activity at 35 days compared to the control, and the increased activity at 90 days as compared to 60 days, bear causal relationship with the observed glycogen content. The levels of enzyme activity at 45 and 60 days are unrelated with the glycogen contents. These could be due to the temporal alterations in synthetase:phosphorylase ratio. The significant depletion in the testis glycogen content noted earlier at 60 days in the control animals and at 90 days in the HPOT rats, were correlated with the full expression of the first wave of spermatogenesis (chapter 6). The HPOT + Px rats also depicted a late decrease in glycogen content between 60 and 90 days, lesser in degree mainly due to the low glycogen load. This delayed decrement finds correspondence with the earlier observed delay in spermatogenesis (chapter 3). Though the delay in the establishment of full spermatogenic activity observed in HPOT + Px rats, was similar to that observed in Px rats, a notable feature was that while sperms could hardly be seen in the latter, sperms appeared in some tubules in the former by 90 days (chapters 2 and 3). Obviously, in the Px rats, there was some functional impediment in the completion of spermiogenesis, which finds explicable correlation with the undepleted glycogen content (chapters 2 and 6). But in the HPOT + PX rats, the observed delay in the completion of spermiogenesis and appearance of sperms in most of the tubules seems to be more due to the inadequate availability of energy due to the reduced

glycogen stores. Undoubtedly, consequent to the initial glycogen depletion associated with the first wave of spermatogenesis, blood glucose becomes a source of energy, with the acquisition of mechanisms of glucose transport by the differentiating Sertoli cells. The delay in differentiation of Sertoli cells inferred earlier, in both Px and HPOT + Px rats, could also result in delayed induction of glucose transporter (GLUT 1), a reported transporter in testis (see, Grootegoed and Den Boer, 1990). In this context it may be inferred that of the various differentiated functions of Sertoli cells, induction of GLUT 1 could be one of the last.

Viewed in the entire context, a combination of neonatal Px and hypothyroidism seems to mimic mostly the Px effects. However, the degree of changes is much attenuated by hypothyroidism. It is also apparent that, the combination of these two manipulations tend to nullify the independent effects of each and make it more like the control animals.