CHAPTER 7

NEONATAL PINEALECTOMY HASTENS THE ATTAINMENT OF ADULT CARBOHYDRATE HOMEOSTASIS AND DELAYS TESTIS GLYCOGEN UTILISATION

Earlier studies on prenatal and perinatal rats have shown accumulation of glycogen in the liver of prenatal rats which serves as the ready source of energy in the immediate postnatal days. During the suckling period (0-21 days), the young ones are dependent on fat rich milk diet of the mother and in keeping with this the hepatic glycogen content remains low. It is only towards the end of the suckling period the hepatic glycogen content increases in anticipation of a carbohydrate rich diet from the time of weanling (Turkenkopf *et al.*, 1982). The pineal functions get established between 10 and 15 days in the rat (Balemans *et al.*, 1978). Though the involvement of pineal in carbohydrate metabolism has not been that well studied, some reports do suggest a modulatory influence of pineal and its hormones in carbohydrate metabolism (Patel *et al.*, 1983; Ramachandran and Patel, 1987; Patel *et al.*, 1988; Patel and Ramachandran, 1989; Ramachandran and Patel, 1989; Patel, 1993; Singh, 1993). Besides, chemical pinealectomy by p CPA treatment or melatonin administration in preweanling rats have also been shown to induce alterations in hepatic and gonadal glycogen contents and blood glucose level (Patel and Ramachandran, 1992; 1993). The earlier study on neonatal hypothyroidism also revealed significant effects on systemic and testis carbohydrate metabolism (chapter 6). Since an earlier

study on neonatal Px on the functional maturation of the reproductive system showed some features akin to that of neonatal hypothyroidism and some unique to neonatal Px, the present study was designed to explore the possible consequence of neonatal Px on systemic and testis carbohydrate metabolism and relate them with the earlier observed effects on the maturation and functions of the male reproductive system in the rat.

RESULTS

BLOOD GLUCOSE (Table 7.1; 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.

Pinealectomised: Pinealectomised animals had significant hypoglycemia at 35 days which then increased significantly by 45 days (similar to control animals at 35 days). At 60 days the blood glucose level again showed significant decrease and then again increased at 90 days to maintain hyperglycemic level.

HEPATIC GLUCOSE-6-PHOSPHATASE (Table 7.3; 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.

Pinealectomised: The initial level of activity was higher than the control animals. Thereafter, like the control animals, the enzyme activity increased through 45 days to attain the highest level at 60 days. In general, the pattern of changes of enzyme activity was exactly similar to that of control animals though at all periods the enzyme activity was higher than the control animals.

Table. 7.1 Chronological alterations in Blood Glucose (mg/dL) level in intact and pinealectomised (Px) 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
Px	67.09 ± 4.59^{d}	126.68 ± 12.35^{d}	96.64 ± 8.93^{a}	118.55 ± 11.29^{a}

@ Values expressed as Mean ± SD of five experiments

^{a} $p < 0.05$; ^{d} $p < 0.001$

Table. 7.2 Chronological alterations in Testis Glycogen (μg/100 mg) and Phosphorylase activity (μmoles of P released/mg protein/10 min.) in intact and pinealectomised (Px) 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
Px	12.50 ± 0.70^{d}	14.20 ± 3.10^{d}	32.00 ± 2.80^{d}	65.60 ± 4.00^{d}	634.49 ± 17.59^{d}	76.69 ± 6.87^{c}	207.29 ± 11.07^{d}	252.15 ± 16.39^{ns}

@ Values expressed as Mean ± SD of five experiments

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

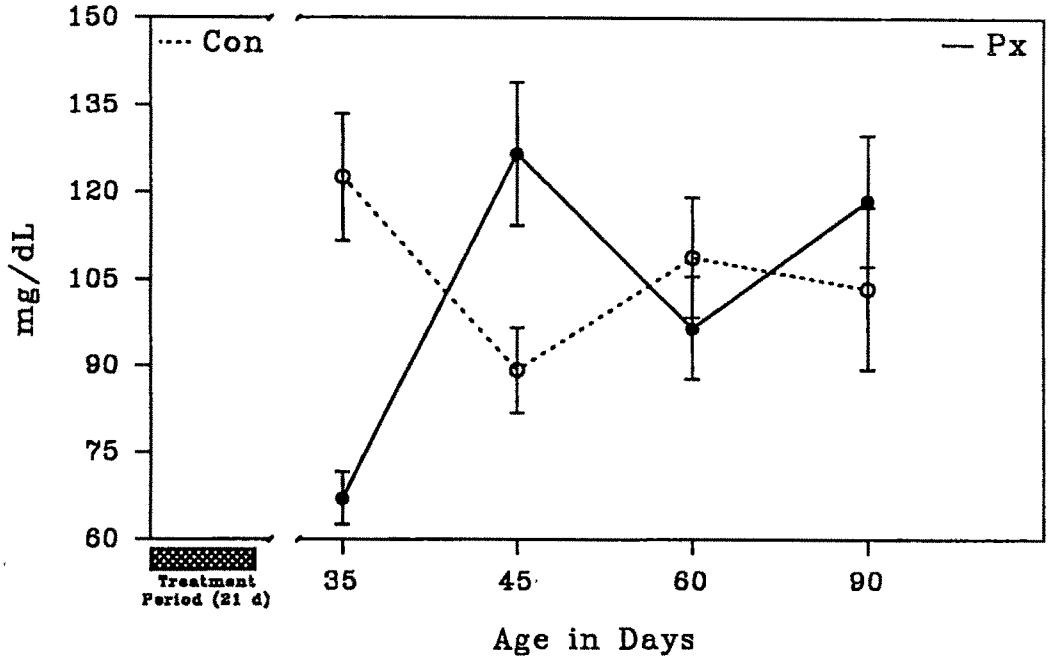

Fig. 1 Chronological alterations in blood glucose level in neonatal rats subjected to pinealectomy (Px)

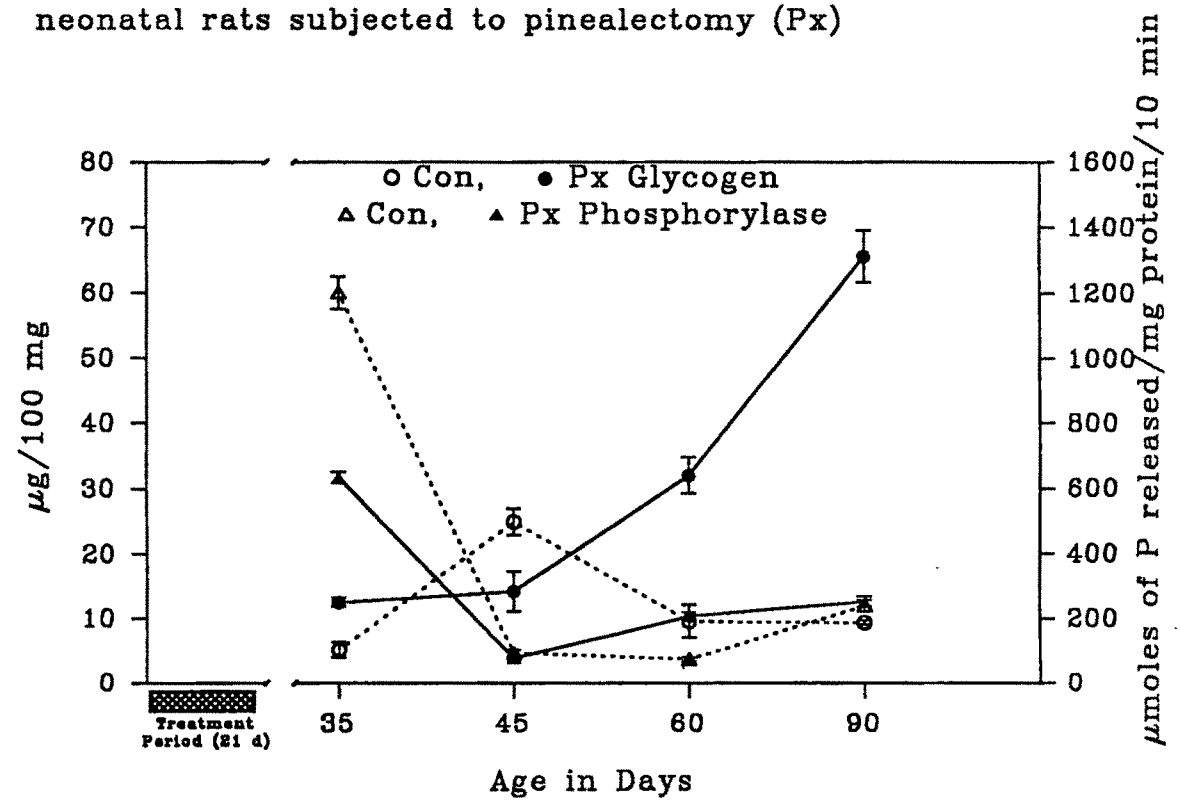

Fig. 2 Chronological alterations in testis glycogen and phosphorylase activity in intact and pinealectomised (Px) rats

Table 7.3 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 pinealectomised (Px) 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
Px	3.16 ± 0.29^{d}	2.32 ± 0.27^{d}	3.68 ± 0.54^{d}	3.06 ± 0.25^{c}	391.71 ± 19.57^{d}	101.22 ± 10.58^{d}	149.48 ± 12.52^{d}	144.20 ± 12.08^{d}	15.98 ± 2.52^{ns}	17.66 ± 3.83^{ns}	28.92 ± 6.71^{c}	16.79 ± 2.95^{ns}

@ Values expressed as Mean ± SD of five experiments

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

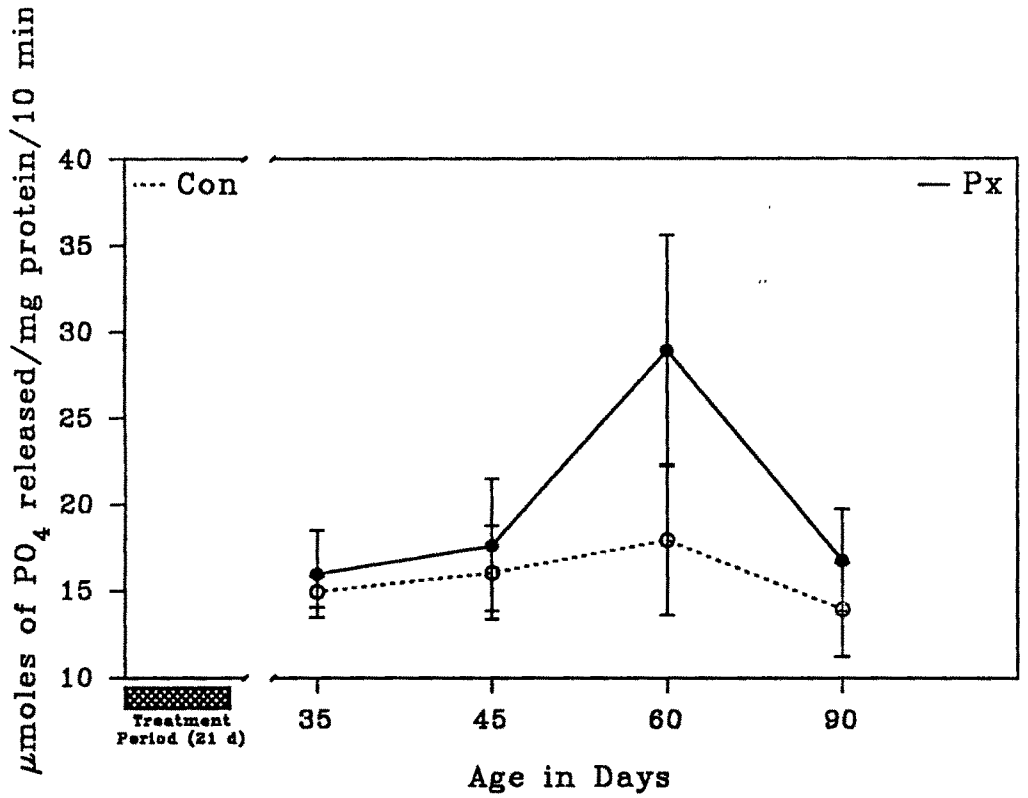

Fig. 3 Chronological alterations in liver G-6-Pase activity in neonatal rats subjected to pinealectomy (Px)

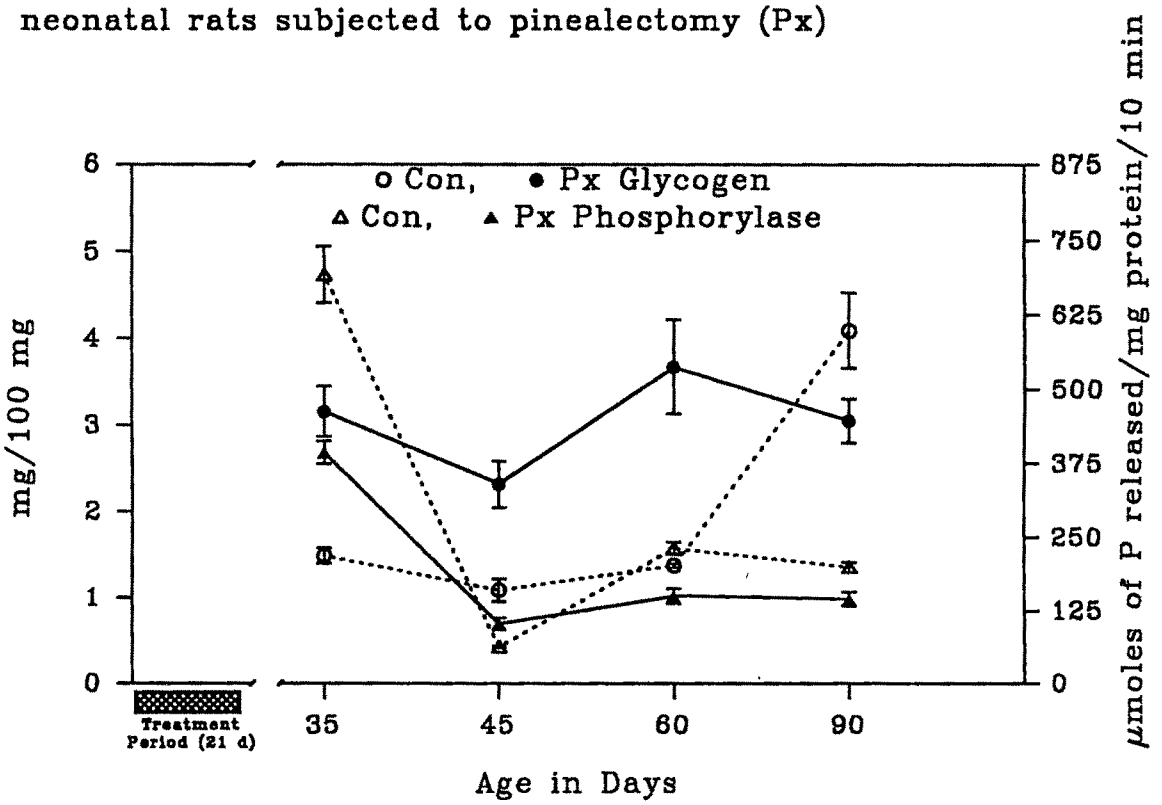

Fig. 4 Chronological alterations in hepatic glycogen and phosphorylase activity in intact and pinealectomised (Px) rats

HEPATIC GLYCOGEN (Table 7.3; 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.

Pinealectomised: The hepatic glycogen content in Px rats was significantly high at 35 days, similar to the control animals at 90 days. The hepatic glycogen content remained more or less steady at all periods except for a significant decrement at 45 days.

HEPATIC PHOSPHORYLASE (Table 7.3; 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.

Pinealectomised: The enzyme activity was significantly reduced at 35 days compared to controls. The activity further showed significant decline at 45 days but less pronounced than the control. At 60 and 90 days the activity of the enzyme showed increase but was significantly less than the control at both time periods.

TESTIS GLYCOGEN (Table 7.2; 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.

Pinealectomised: The testis glycogen content was significantly high at 35 days and the same level was maintained even at 45 days. Thereafter, there was further continuous increase in testis glycogen at 60 and 90 days.

TESTIS PHOSPHORYLASE (Table 7.2; Fig. 2)

Control: There was significantly higher testicular 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 the adult level.

Pinealectomised: The enzyme activity at 35 days was significantly low, almost 50% compared to that observed for the control animals. The enzyme activity decreased at 45 days and then increased gradually to the characteristic adult levels through 60 and 90 days.

DISCUSSION

Results obtained in the present study show that neonatal Px brings about functional alterations resulting in increased tissue glycogen contents. In 35 day old pinealectomised rats the hepatic glycogen content is significantly elevated and is almost similar to the 90 day level in control animals. Correspondingly, there is significant hypoglycemia at 35 days in pinealectomised rats. This is followed by increase to adult level by 45 days and is maintained so thereafter but for a decrease at 60 days. These changes in hepatic glycogen content and blood glucose level indicate that there is an early attainment of adult type carbohydrate homeostasis in pinealectomised rats. Though the overall increase in glycogen content is reflected in the decreased phosphorylase activity, the absolute temporal changes in these parameters do not bear any correlation. Similarly, the absolute levels of hepatic G-6-Pase activity and blood glucose levels also do not reveal any temporal relationship; however, the hepatic G-6-Pase activity is significantly higher in Px rats compared to the controls at all time periods studied. These discordant changes in related parameters of carbohydrate metabolism appear to be due to the independent effects of neonatal Px on secretion/sensitivity of hormones related to carbohydrate metabolism. The increased G-6-Pase activity in Px animals could be correlated with the inductive influence of corticosteroids in the light of the observations made in this laboratory. Pinealectomy

in adult pigeons has been shown to decrease hepatic G-6-Pase activity which is related to decreased adrenocortical activity and reduced serum corticosterone level while, administration of both corticosterone and melatonin increased G-6-Pase activity(Ayyar,1987; Patel, 1993). Corticosterone administration in post-hatched chicks has also been shown to induce hepatic G-6-Pase activity (Joseph and Ramachandran, 1992). Further, some unpublished observations on Px and melatonin administration in adult rats are also in conformity with the tenet of corticosterone induced G-6-Pase activity. Our unpublished histological observations on adrenal of neonatally pinealectomised rats also suggest increased cortical activity. Based on these, the presently observed increased G-6-Pase activity in Px rats appears to be due to a possible hypercorticalism. In this context, while Kaplanski and Ronen (1986) found no difference in the corticosterone levels after neonatal Px, other workers have observed increased adrenocortical activity and corticosterone secretion (Nir *et al.*, 1971; Jacobs, 1974; Kinson *et al.*, 1968; Henzan *et al.*, 1970; Kinson *et al.*, 1967; Ogle and Kitay, 1977) or even a decrease (Niles *et al.*, 1977).These discordant observations seem to be essentially due to the different strains of rats used, a fact which has often been overlooked. This aspect was clearly brought out by the earlier study on transient neonatal hypothyroidism and maturation of the male reproductive system (chapter 1). The inferred hypercorticalism/corticosterone sensitivity is further emphasized by the increased blood glucose level at 45 days which could be accredited to the gluconeogenic action of corticosterone. This is paralleled by decreased hepatic protein content (unpublished observations), conforming to the present assumption.

The increased glycogen content and decreased phosphorylase activity could be accredited to the acquisition of an increased insulin:glucagon molar ratio. It is probable that neonatal Px results in significant temporal advancement in the attainment of characteristic adult ratio of insulin:glucagon. Apparently, this occurs in Px rats by 35 days as against 60 to 90 days in intact rats. The increase in the glycogen content could obviously be related to increased glycogen synthetase activity due to insulin action which is well reflected in the presently noted

phosphorylase activity. Earlier study on neonatal hypothyroidism also showed such a change, though it occurred only by 45 days (chapter 6). Pinealectomy in the present study also showed reduced thyroid hormone levels at 35 days. This could be the factor responsible for the increase in insulin:glucagon molar ratio as inferred in the earlier study on neonatal hypothyroidism. However, the still earlier attainment of insulin: glucagon ratio due to Px (at 35 days) as against neonatal hypothyroidism (at 45 days) suggests a synergistic effect of Px and hypothyroidism in increasing the insulin:glucagon ratio. It can be speculated in this context that both melatonin and thyroid hormone have delaying effect in increasing insulin level and decreasing the glucagon level as the postnatal periods in rats have been shown to have a high glucagon:insulin ratio (Wakelam and Walker, 1981; Margolis, 1983).

The inferred glycogen anabolic influence is also reflected in the increased testis glycogen content and decreased phosphorylase activity. Though the utility value of testis glycogen content is significant in the scheme of metabolic activities of the adult testis (as blood glucose is a preferred source), our previous study had emphasized the importance of testis glycogen store during the first wave of spermatogenesis (chapter 6). This is well evidenced by the gradual accumulation of glycogen by 45 days and the precipitous decrease by 60 days in the control animals coinciding with the full establishment of the spermatogenic functions (chapter 6). However, in the present study the testis glycogen content of the pinealectomised rats showed continuous increase to attain a very high level at 90 days. With the purported importance of testis glycogen during the first wave of spermatogenesis, this appears paradoxical as there was no depletion of glycogen, rather there was continuous increase. It is worth recalling the earlier observations on neonatal Px wherein, elevated serum gonadotropin levels were inferred (chapter 2). In the above study, due to decreased thyroid hormone levels and increased FSH level, hyperproliferation of Sertoli cells and their delayed maturation resulting in increased testis size were recorded. Another significant observation was a delay in completion of spermatogenesis as seen at 90 days though there was prominent increase in germ cell density. Since it is seen

that glycogen depletion coincides with the establishment of terminal phase of spermatogenesis marked by appearance of spermatozoa in all the tubules (chapter 6) and, spermatogonia, primary spermatocytes and Sertoli cells have been reported to be the glycogen storing cells (Leiderman and Mancini, 1969; Fouquet and Guha, 1969), the presently recorded increase in the glycogen content seems quite obvious.

Finally, the present study has on the overall, revealed a probable increased adrenocortical activity, and early attainment of insulin:glucagon molar ratio resulting in increased glycogen deposition due to neonatal Px. The observations in testis glycogen content also corroborate the earlier inferred delay in spermiogenesis.