CHAPTER – 4

NEONATAL HYPERMELATONEMIA INCREASES SERUM INSULIN LEVEL AND FURTHER POTENTIATES THE GLYCOGENIC AND PROTEIN ANABOLIC INFLUENCE FROM WEANING TO PUBERTAL AGE.

INTRODUCTION:

The pineal indoleamine hormone melatonin is known to influence many physiological activities in mammals like seasonal reproduction, thermoregulation, molting, cell proliferation and, protection against oxidative damage (Saarela and Reiter, 1994; Allain and Rougeot, 1980; Tamarkin *et al.*, 1985; Ebadi *et al.*, 1998; Hill and Blask, 1988; Tan *et al.*, 1993a,b; Lagneux *et al.*, 2000). Due to its greater link with seasonal functions, its role on body mass, adiposity and energy intake of seasonal mammals has been studied (Wade and Bartness, 1984; Valtonen *et al.*, 1995; Le Gouic *et al.*, 1996; Williams *et al.*, 1997). However, its role in energy metabolism of non-seasonal mammals remains largely unknown (Mustonen *et al.*, 2002) Indications for the possible participation of the pineal gland in regulating carbohydrate metabolism had come from some of the early studies (Milcu *et al.*, 1957, 1963; Milcu and Milcu, 1958). Hypertrophy of the pancreatic islets with chronic injection of pineal extracts was also reported

(Notario, 1956, Petronio and Tavazza, 1958). A number of studies have shown the ability of the pineal to modulate glycemic status in the rat, rabbit and pigeon (Mihail and Girguae, 1979; Muralidhar *et al.*, 1983; Diaz and Blasquez, 1986, John *et al.*, 1990; Ramachandran, 2002). The role of exogenous melatonin on blood glucose level has been controversial with, increase, decrease or even no effect have all been reported (Delahaunty *et al* , 1978; Dhar *et al.*, 1983; Mahata *et al.*, 1988; John *et al* , 1990, Zemen *et al.*, 1993) Many other parameters related to carbohydrate metabolism like plasma insulin level (Diaz and Blasquez, 1986), pancreatic insulin secretion (Bailey *et al.*, 1974; Peschke *et al.*, 1997) and insulin action (Frankel and Trandberg, 1991), have all been shown to be influenced by melatonin. Modulation of hepatic insulin and glucagon receptor concentrations (Rodriguez *et al.*, 1989) and increase in catecholamine content (Mahata *et al.*, 1988) have also been shown Most of the disparities in the observed effects of melatonin on carbohydrate metabolism stems from the different experimental protocols involved in the respective studies. Obviously a more systematic evaluation keeping in mind dosage, duration, time of administration and age of the animal only can help clear the disparities and relate the influence of melatonin on carbohydrate metabolism more meaningfully.

Previous study on neonatal hypermelatonemia by subjecting neonates to melatonin administration for the entire 21 day duration of pre-weaning period had shown altered insulin and glycemic status and increased tissue glycogen load as consequences in the immediate

weaning period (Chapter-I). Conversely, blockage of melatonin action for the entire duration of pre-weaning period had revealed reversal of changes indicating the modulatory influence of melatonin at this age (Adi, 2004). The present study is a sequel to the above studies and has tried to evaluate the consequences of neonatal hypermelatonemia on serum insulin and glucose levels, hepatic and muscle glycogen contents and enzymes of carbohydrate metabolism in the pubertal period, as part of long term influence of neonatal hypermelatonemia.

MATERIAL AND METHODS: See page Nos.; 16-37

RESULTS:

- **Body and organ weights:** The body weight of melatonin treated animals was significantly decreased. The relative weights of pancreas and liver decreased while that of kidney and testes increased significantly in the experimental animals. There is no alteration in the relative weight of adrenal in experimental animals (Figure and Table; 4.1, 4.4, and 4.5).
- **Serum glucose and insulin levels:** The serum glucose level showed a significant increase in the melatonin treated animals as compared to control animals, while the serum insulin level was slightly less than the control level though insignificant (Figure and Table; 4 6).
- **Hepatic glycogen content and activities of glycogen synthetase, glycogen phosphorylase and glucose-6-phosphatase:** Hepatic glycogen content and the activities of glycogen phosphorylase and glucose-6-phosphatase were

elevated in the melatonin treated animals as compared to controls however the glycogen synthetase activity did not show any significant alteration (Figure and Table; 4.7, 4.8, 4.9, and 4.10).

- **Muscle glycogen content and activities of glycogen synthetase and glycogen phosphorylase:** The muscle glycogen content and the activity of glycogen synthetase increased significantly while the glycogen phosphorylase activity decreased significantly in the experimental animals as compared to control animals (Figure and Table; 4.7, 4.9, and 4 10).
- **Hepatic and muscle protein content:** The hepatic and muscle protein content increased significantly in the melatonin treated animals (Figure and Table; 4 11)
- **Histological observations:** The section of pancreas from melatonin treated rats show reduced islet size and cell number with relatively lesser B cells compared to control sections (Plate; 4, 5, and 6)

DISCUSSION:

The present results clearly show that chronic pre-weaning hypermelatonemia has persistent glycogenic effect and increased glycogen synthetase activity and altered serum insulin and glucose levels (Fig and Tab.; 4.9, 4.6). A protein anabolic effect is clearly manifested as both hepatic and muscle protein contents were higher than in the control animals (Fig. and Tab.; 4.11).

Figure 4.1: Body weight and absolute liver weight of pubertal rats on 45th day subjected to neonatal melatonin treatment:

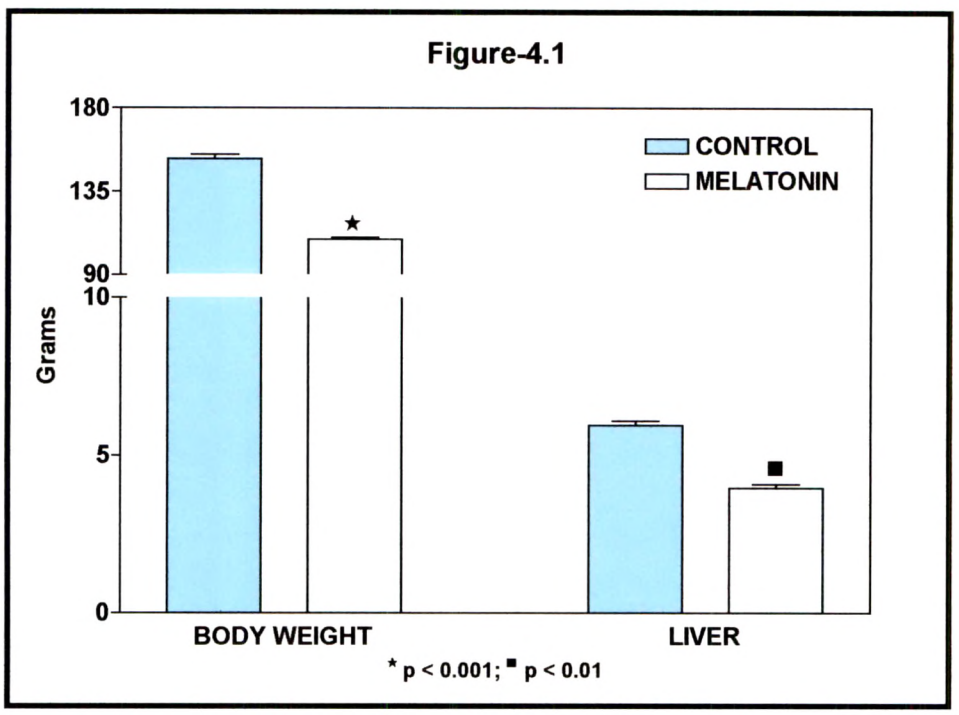


Table 4.1: Body weight and absolute liver weight of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	BODY WEIGHT	LIVER
CONTROL	152.50 ±2.3	5.94 ±0.15
MELATONIN	109.00★ ±1.10	3.79■ ±0.11

Values are expressed as mean ± SEM, ★p < 0.001; ■ p < 0.01

Figure 4.3: Absolute weights of testes and kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment:

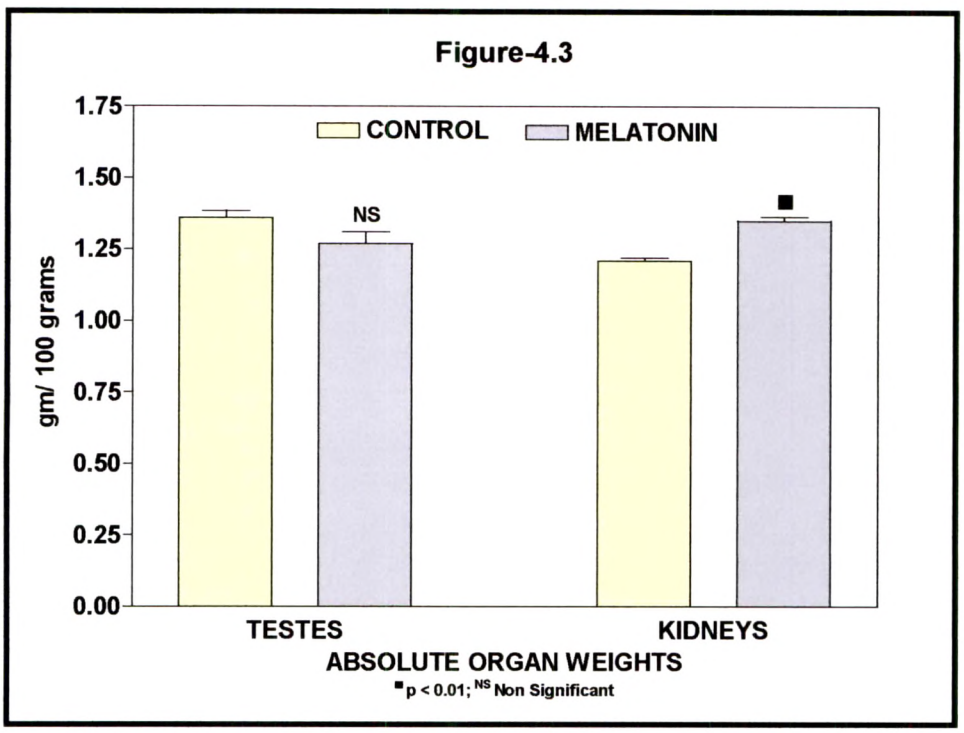


Table 4.3: Absolute weights of testes and kidney of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
TESTES	1.36 ±0.024	1.27[NS] ±0.04
KIDNEYS	1.21 ±0.0099	1.35■ ±0.014

Values are expressed as mean ± SEM, ■ p < 0.01; [NS] Non Significant

Figure 4.4: Relative weights of pancreas, spleen and adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment:

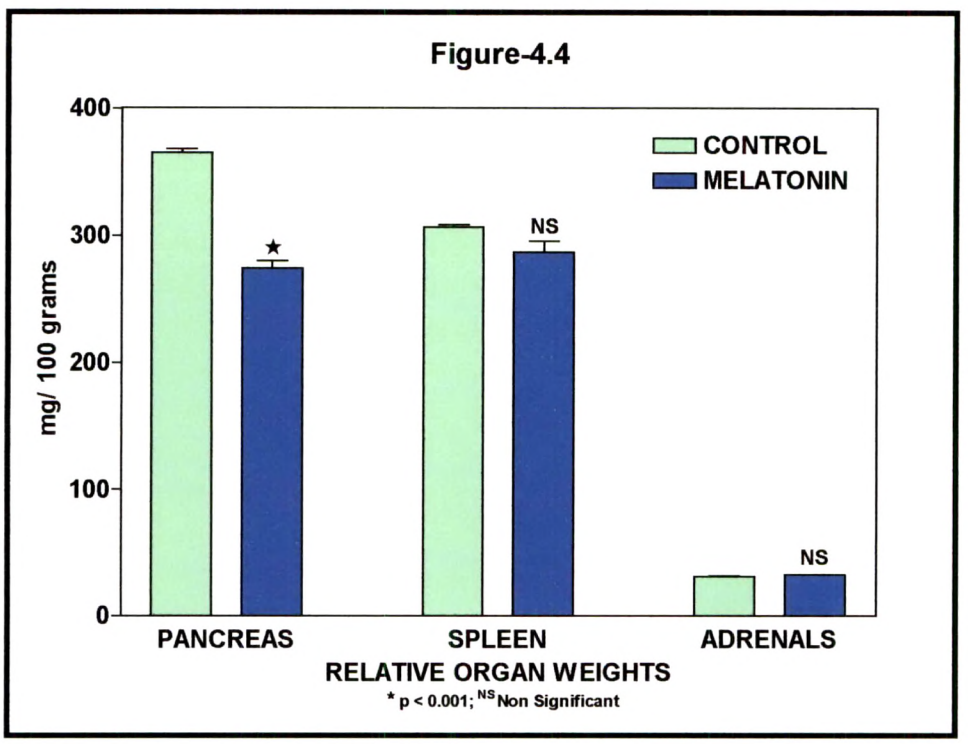


Table 4.4: Relative weights of pancreas, spleen and adrenal of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	PANCREAS	SPLEEN	ADRENALS
CONTROL	365.29 ±2.71	306.58 ±2.07	31.13 0.47
MELATONIN	274.25* ±5.75	286.61NS ±8.86	32.10NS ±0.29

Values are expressed as mean ± SEM, * p < 0.001; NS Non Significant

Figure 4.5: Relative organ weights of pubertal rats on 45th day subjected to neonatal melatonin treatment:

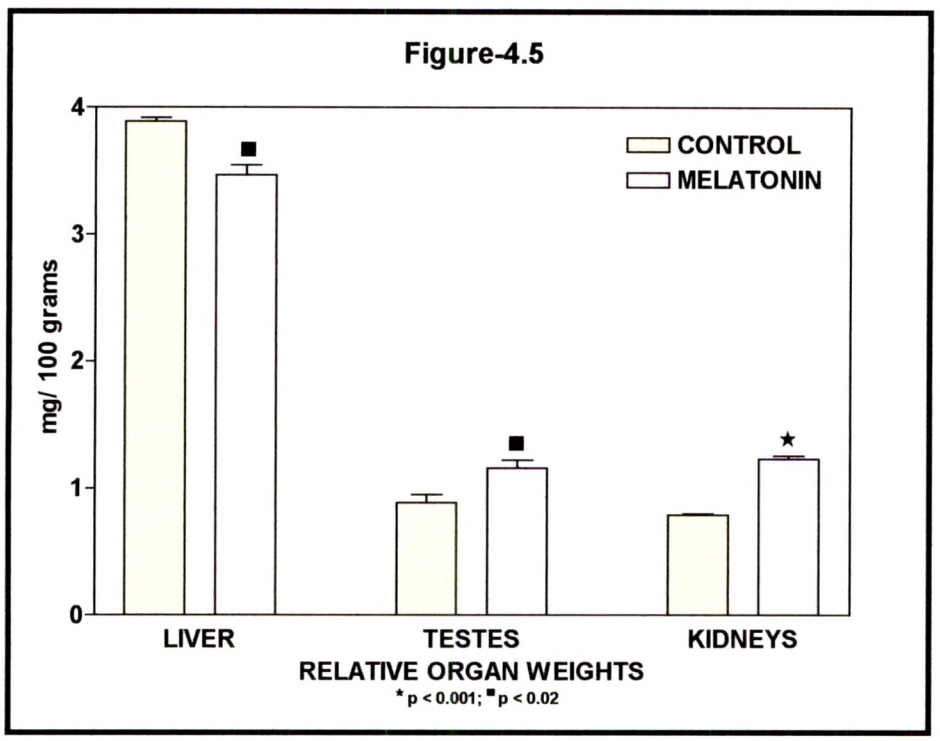


Table 4.5: Relative organ weights of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	LIVER	TESTES	KIDNEYS
CONTROL	3.89 ±0.03	0.89 ±0.00	0.79 0.0099
MELATONIN	3.47■ ±0.078	1.16■ ±0.06	1.23* ±0.024

Values are expressed as mean ± SEM, * p < 0.001; ■ p < 0.02

Figure 4.6: Serum insulin and glucose levels of pubertal rats on 45th day subjected to neonatal melatonin treatment:

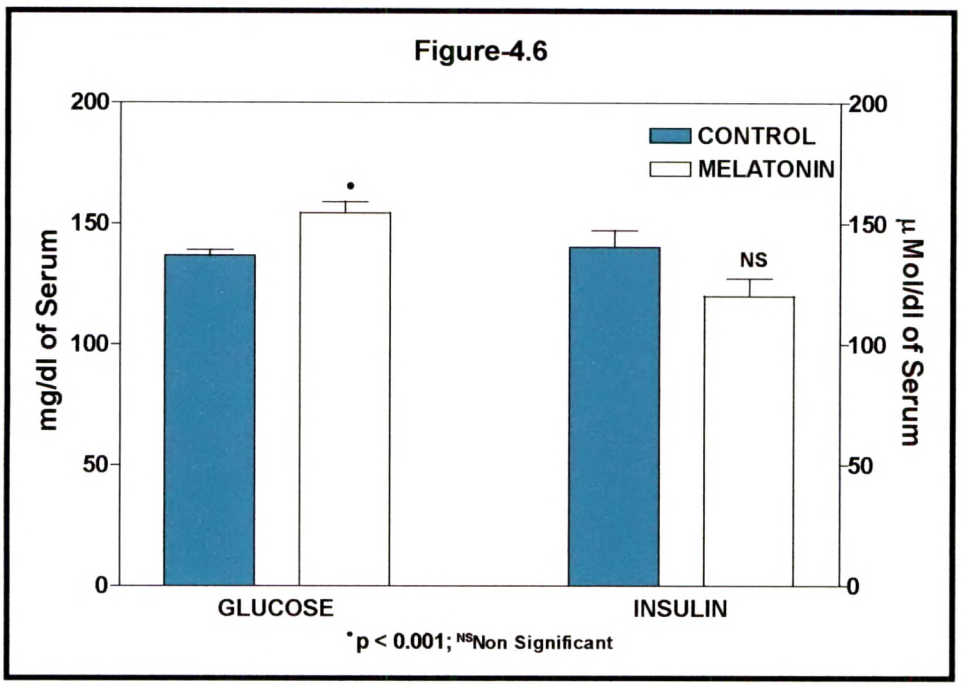


Table 4.6: Serum insulin and glucose levels of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	INSULIN	GLUCOSE
CONTROL	140.27 ±7.068	136.72 ±2.38
MELATONIN	120.22[NS] ±7.25	154.45[■] ±4.58

Values are expressed as mean ± SEM, [■]p < 0.02; [NS] Non Significant

Figure 4.7: Glycogen phosphorylase activity in liver and muscle of pubertal rats on 45th day subjected to neonatal melatonin treatment:

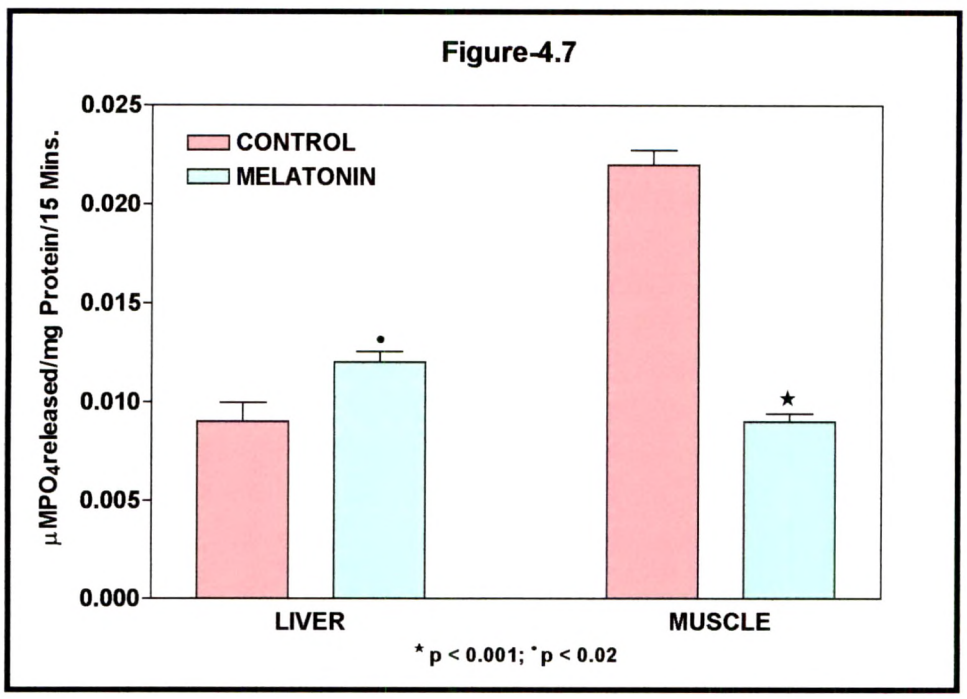


Table 4.7: Glycogen phosphorylase activity in liver and muscle of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.009 ±0.00095	0.022 ±0.00075
MELATONIN	0.012■ ±0.00055	0.009* ±0.000405

Values are expressed as mean ± SEM, *p < 0.001; ■p < 0.02

Figure 4.8: Glucose-6-phosphatase activity in the liver of weaning rats on 45th day subjected to neonatal melatonin treatment:

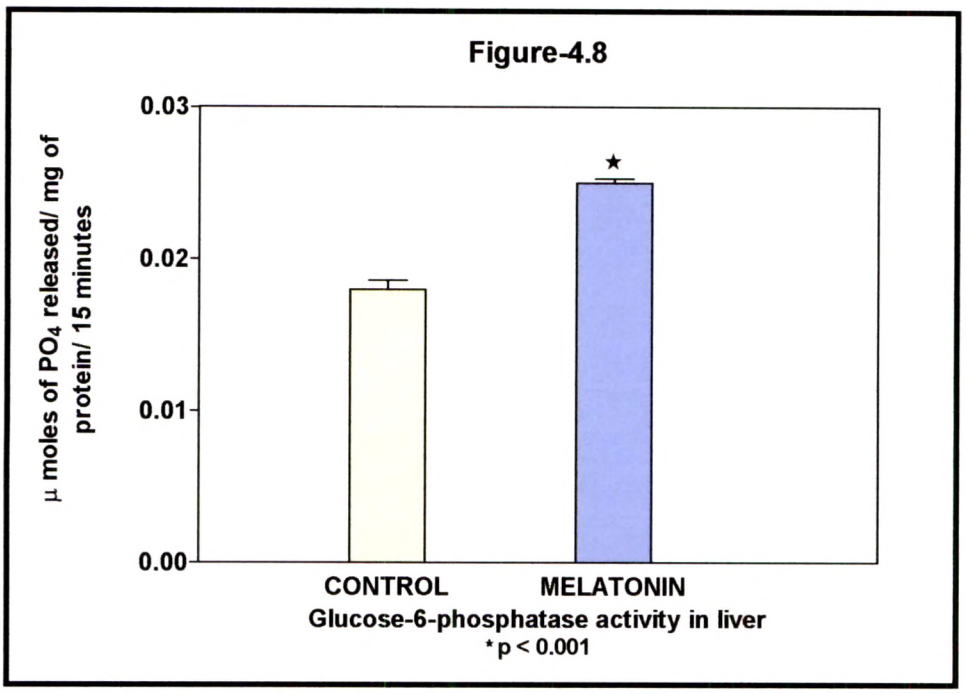


Table 4.8: Glucose-6-phosphatase activity in the liver of weaning rats on 45th day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
GLUCOSE-6-PHOPHATASE	0.018 ±0.0006	0.0025* ±0.00028

Values are expressed as mean ± SEM, *p < 0.001

Figure 4.9: Glycogen synthetase activity in liver and muscle of pubertal rats on 45th day subjected to neonatal melatonin treatment:

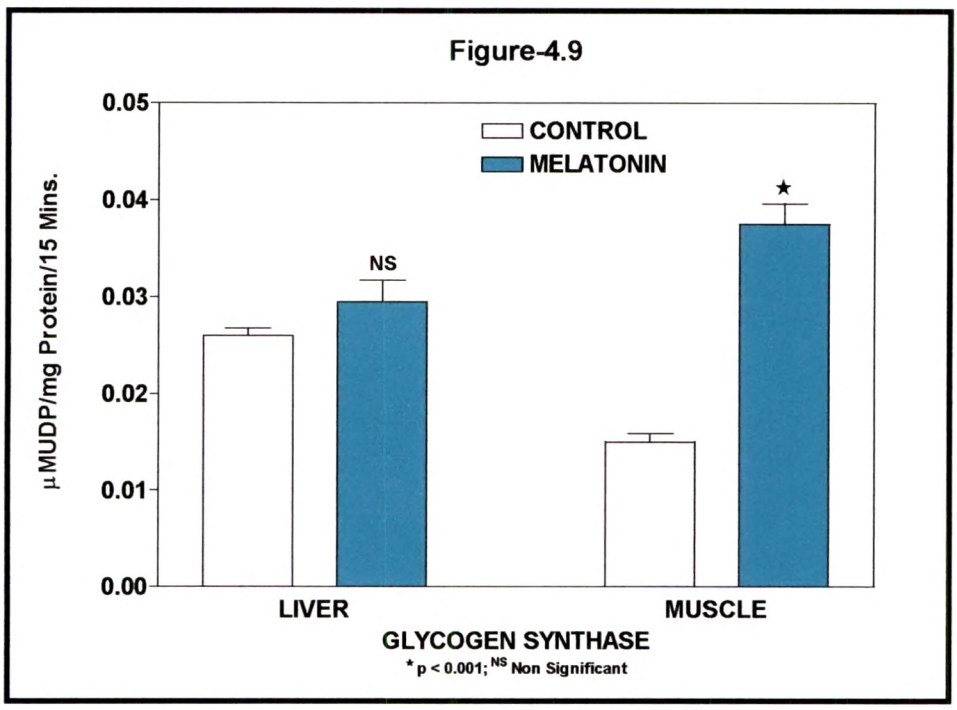


Table 4.9: Glycogen synthetase activity in liver and muscle of pubertal rats on 45th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.026 ±0.00075	0.015 ±0.00085
MELATONIN	0.0295[NS] ±0.0022	0.0375* ±0.0021

Values are expressed as mean ± SEM, *p < 0.001; [NS] Non Significant

Figure 4.10: Hepatic and muscle glycogen content of the pubertal rats on 45th day subjected to neonatal melatonin treatment:

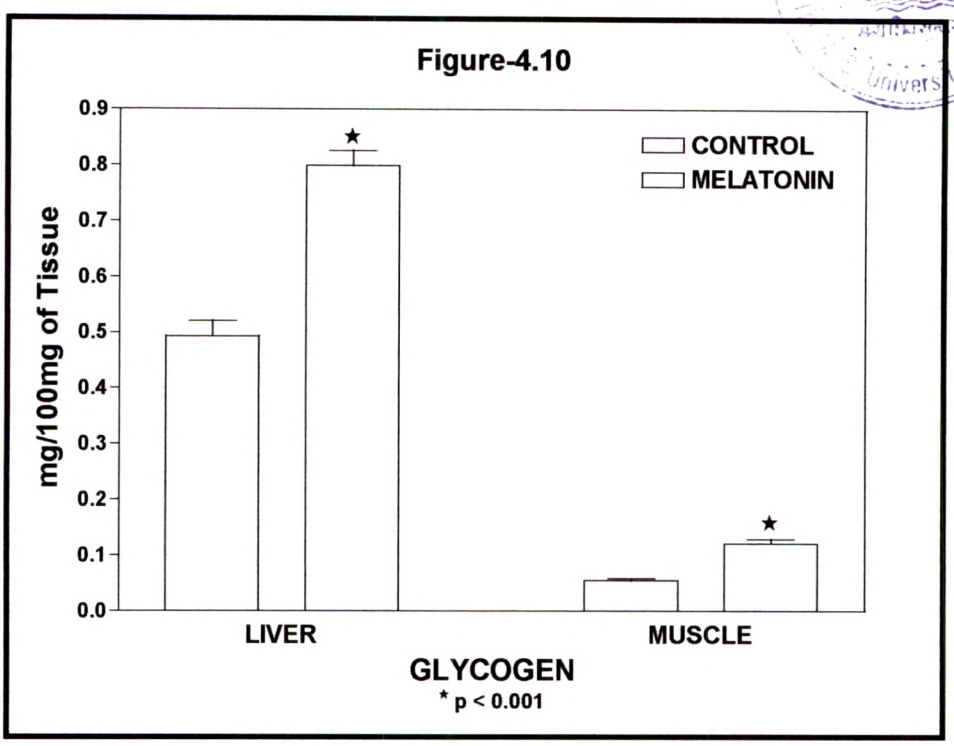


Table 4.10: Hepatic and muscle glycogen content of the pubertal rats on 45th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.49 ±0.027	0.055 ±0.0039
MELATONIN	0.79* ±0.027	0.12* ±0.0079

Values are expressed as mean ± SEM, *p < 0.001

Figure 4.11: Hepatic and muscle protein content of the pubertal rats on 45th day subjected to neonatal melatonin treatment:

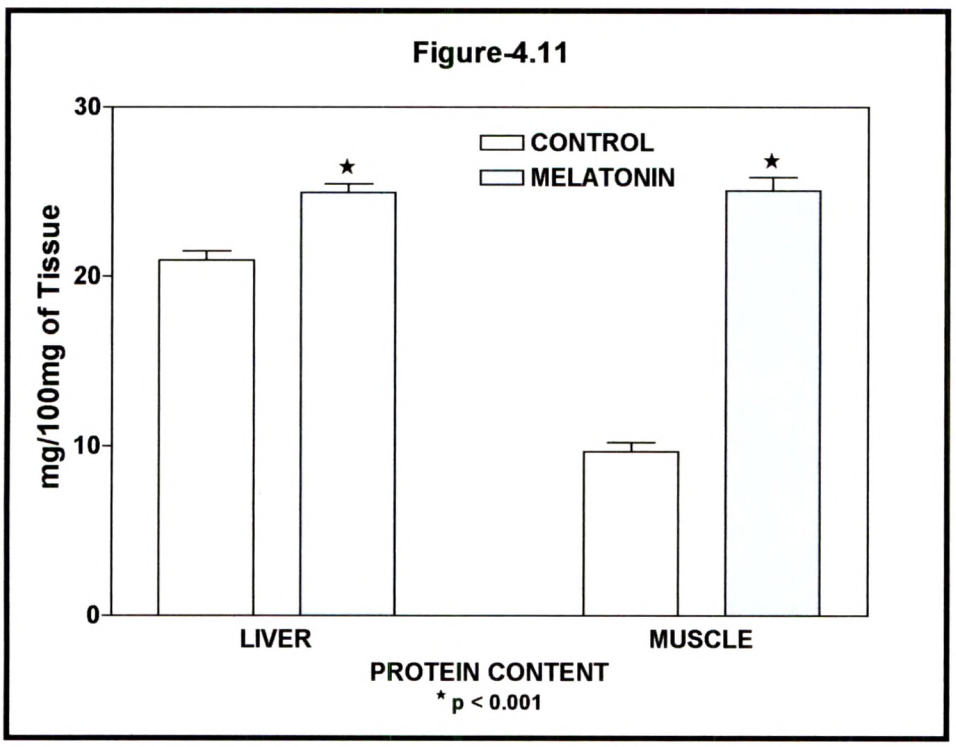


Table 4.11: Hepatic and muscle protein content of the pubertal rats on 45th day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	20.92 ±0.54	9.66 ±0.54
MELATONIN	24.96* ±0.49	25.08* ±0.76

Values are expressed as mean ± SEM, *p < 0.001

PLATE – 4

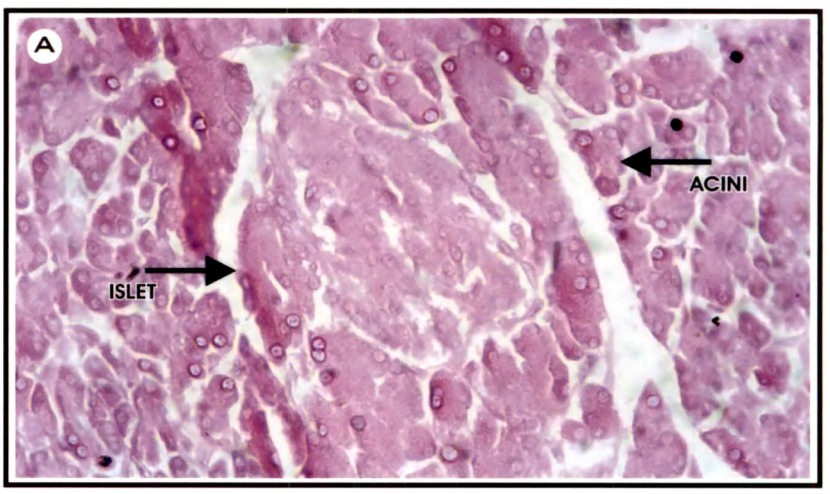
Photomicrographs of sections of pancreas – 450 X

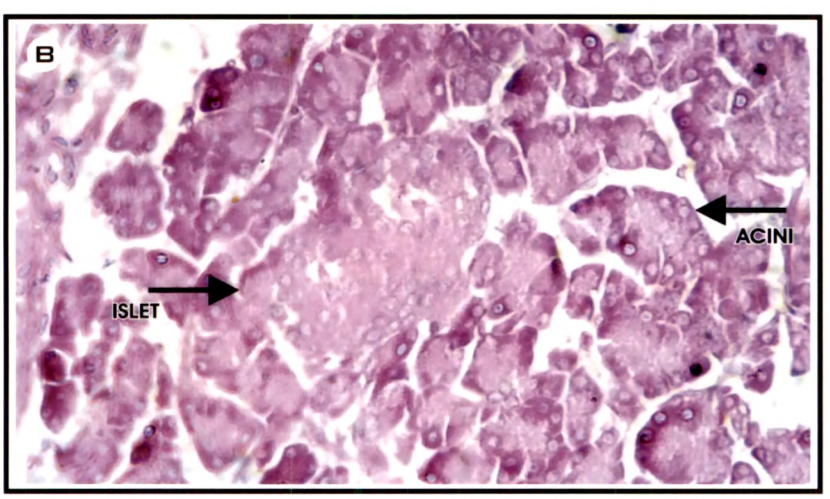
FIGURE (A): Transverse section of the pancreas of male hypermelatonemic rats on the 45th day showing islet and pancreatic acini The islet and acinar cells are loosely packed with empty spaces in between. There is a reduction in the islet and acinar cell number.

FIGURE (B): Transverse section of the pancreas of male control pubertal (45th day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells.

FIGURE (C): Transverse section of the pancreas of male hypermelatonemic rats on the 45th day showing islet and pancreatic acini. The acinar and islet cells are loosely packed. The A cells can be viewed in the core of the islet area whereas the B cells are prominently seen on the periphery of the islet.

PLATE - 4





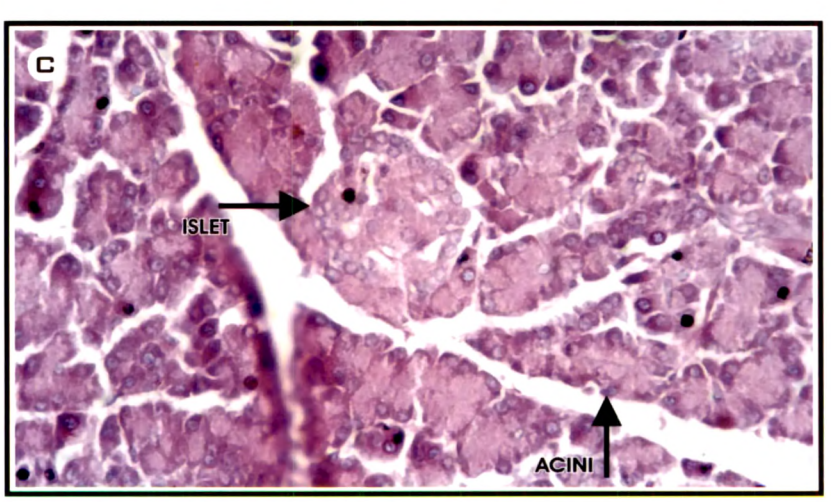


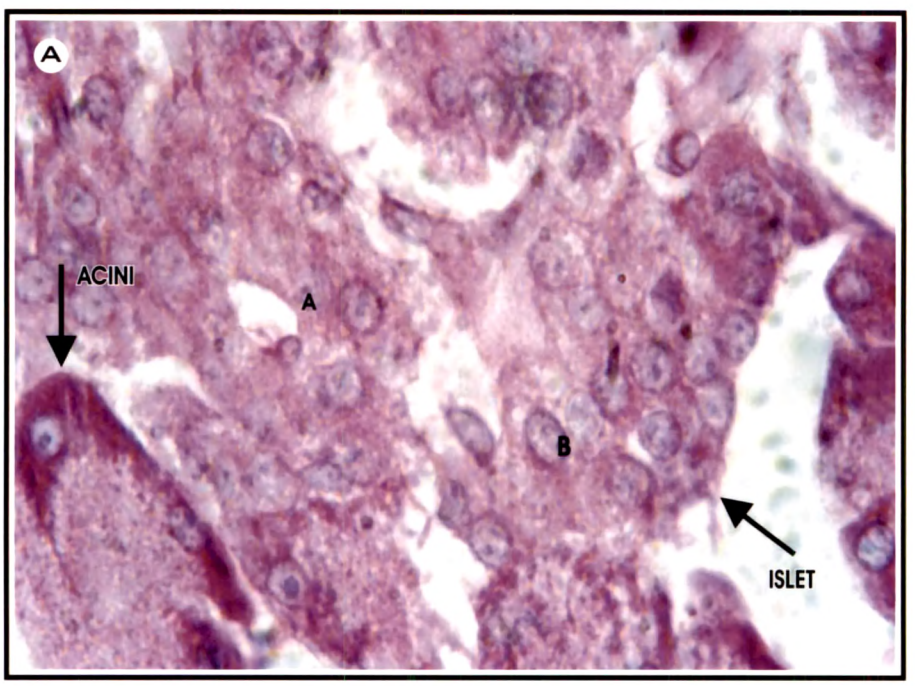
PLATE – 5

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control pubertal (45^{th} day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells.

FIGURE (B): Transverse section of the pancreas of male hypermelatonemic rats on the 45^{th} day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between. There is a reduction in the islet and acinar cell number; also, some transdifferentiating cells are also visible (double headed arrow). The A and B cells are seen distinctly in the islet area.

PLATE - 5



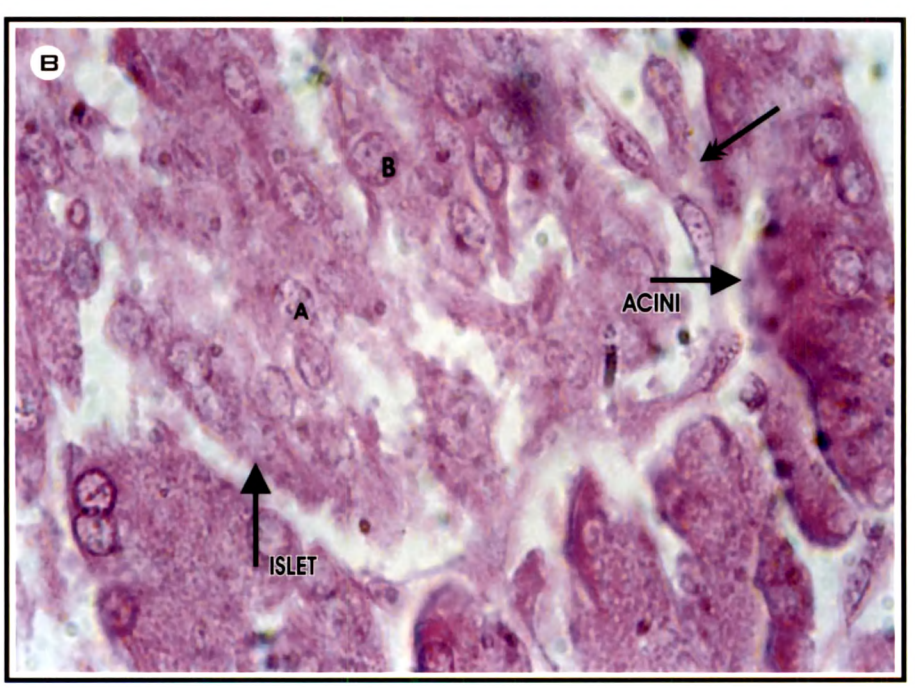


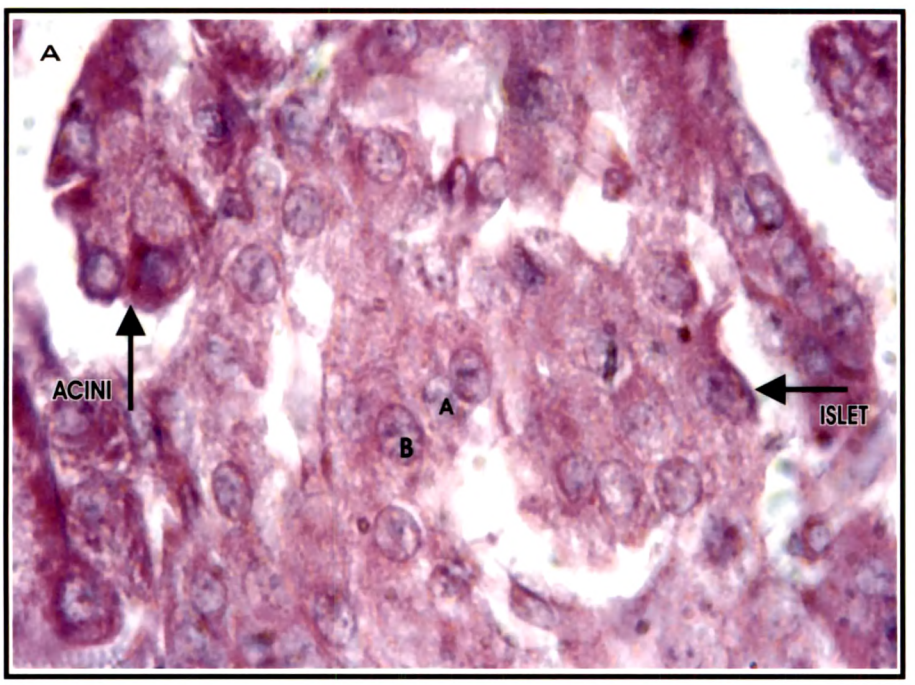
PLATE – 6

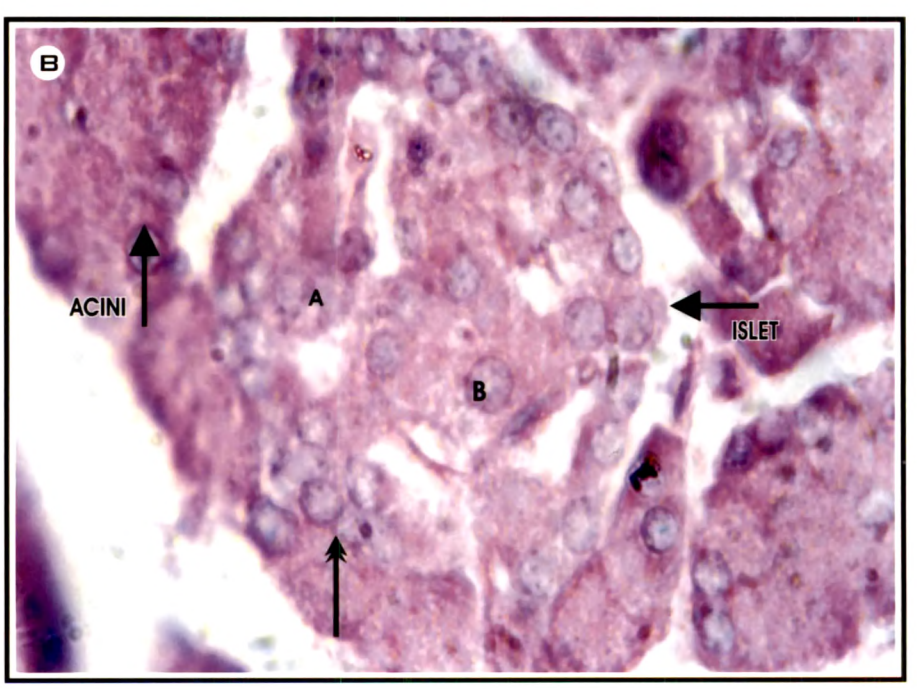
Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control pubertal (45^{th} day) rats showing islet and pancreatic acini Note the centrally distributed A cells, peripherally distributed B cells.

FIGURE (B): Transverse section of the pancreas of male hypermelatonemic rats on the 45^{th} day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between. Note the transdifferentiating cells (double headed arrow). The A and B cells can be viewed at the core and periphery of the islet area.

PLATE - 6





this is in contrast to the observed minimal effect in the weaning period whence the hepatic and muscle protein contents were not significantly altered (Chapter 1). Apparently, neonatal hypermelatonemia has a protein anabolic influence as a long term effect in the pubertal period. Though this protein anabolic influence is not clearly explicable, it may suggest a potentiating influence of testosterone action. A differential effect is also indicated as there is relatively greater increase in muscle protein content than in liver protein content. Obviously the protein anabolic influence is markedly more in the muscle than in the liver. The control animals show increased glycogen synthetase activity coupled with decreased glycogen phosphorylase activity with an increased synthetase: phosphorylase activity ratio and increased hepatic and muscle glycogen contents compared to the weaning period. Hypermelatonemia which had shown similar changes in the weaning period itself, has shown further continued potentiating change resulting in much higher glycogen synthetase activity and glycogen content. Apparently the glycogenic effect persists and gets further potentiated even after cessation of melatonin treatment. Whereas the increased glycogenic effect seen in the control animals from the weaning to pubertal period can be related with increased insulin level and insulin sensitivity, the significantly higher effect seen in the hypermelatonemic animals could not only be seen as an extension of higher insulin sensitivity seen in the weaning period but also an effect of higher insulin level as the serum insulin level is significantly elevated from a hypoinsulinemic status in the weaning period to a near normal

insulinemic status in the pubertal period. Histologically observed reduced number of cells not keeping, the B:A cell ratio is still as high as control rats with a simultaneous reduction in A cells (Plate; 4, 5 and 6). Further it is presumable that the B cells which were regenerated at the time of weaning as inferred earlier (Chapter 1), are now fully mature and fully and maximally secretary contributing to higher insulin level compared to the weaning age. Some of the recent studies involving either single dose administration or implantation or even long term discontinuous treatment of melatonin through drinking water have all failed to register an increment in hepatic glycogen content (Fabis *et al.*, 2002; Mustonen *et al.*, 2002; Markova *et al.*, 2003). Whereas Mustonen *et al.* (2002) showed no change in hepatic glycogen content, Markova *et al.* (2003) showed a decrease in hepatic glycogen content which was accredited to the increased glycogen phosphorylase activity recorded by Mustonen *et al* (2002). Unlike these workers, Mazepa *et al.* (2000) recorded increased liver glycogen content in exercised and non-exercised rats towards a high dose of melatonin. These contradictory results definitely suggest a subtle effect of melatonin on tissue glycogen stores based on the dosage and duration of treatment. A closer scrutiny of the changes reveals lesser difference in muscle glycogen deposition in terms of percentage increase from the contents at weaning period compared between the control animals (121%) and melatonin treated rats (143%). In comparison, the liver of control animals has recorded a much higher increase in glycogen content (845%) than the liver of melatonin treated rats (125%). Whereas this

lesser increase in hepatic glycogen content of hypermelatonemic rats can be related with the increased insulin level from the weaning period (176%), the tremendously increased hepatic glycogen content in the control rats could be related with the relatively lesser insulin level (72%) and greatly enhanced insulin sensitivity. It is likely that the insulin level and insulin sensitivity increase gradually in control rats through weaning to puberty. However, hypermelatonemic rats seem to exhibit higher insulin sensitivity right from weaning and hence, the relatively lesser increase in the hepatic glycogen load from weaning to puberty is essentially a consequence of only increased insulin level. These differences are matched by the recorded 67% increase in serum glucose level in hypermelatonemic rats and 37% decrement in control rats.

The present set of observations clearly suggest prolonged effect of neonatal melatonin treatment on carbohydrate metabolism despite the cessation of treatment. The recorded significant glycogenic effect and insulin sensitivity in the immediate post treatment weaning period is persistent even up to the pubertal stage. Melatonin treatment in the neonatal period seems to hasten the postpartum development of insulin sensitivity. Melatonin treatment also seems to create a protein anabolic influence especially with reference to muscle which may be related with the potentiated action of testosterone. Since there are no studies of this type, the observations cannot be discussed more meaningfully.

SUMMARY:

The present study is designed to observe the long term effects of neonatal hypermelatonemia on serum insulin and glucose levels, hepatic and muscle glycogen contents and enzymes of carbohydrate metabolism in the pubertal period To this end, rat neonates have been treated with melatonin in graded doses of 200 µg/animal from day 1 to day 7, 400 µg/animal from day 8 to day 14 and 600 µg/animal from day 15 to day 21 and assessed on the 22nd day. The melatonin treated rats showed significant decrease in body weight and relative weight of pancreas, adrenal and liver, while the relative weight of testes and kidneys increased significantly. The serum glucose level increased significantly, while the serum insulin level remained unaltered in the experimental rats Whereas the hepatic and muscle glycogen contents increased significantly in the experimental rats, the activity of glycogen synthetase increased significantly in the muscle tissue of experimental rats. The activity of glycogen phosphorylase was also increased significantly in the muscle of melatonin treated rats. Also the hepatic and muscle protein contents increased significantly in the melatonin treated rats. The present set of observations clearly suggests prolonged effect of neonatal melatonin treatment on carbohydrate metabolism despite the cessation of treatment. The recorded significant glycogenic effect and insulin sensitivity in the immediate post treatment weaning period is persistent even up to the pubertal stage. Melatonin treatment in the neonatal period seems to hasten the post partum development of insulin sensitivity. Melatonin treatment

also seems to create a protein anabolic influence especially with reference to muscle, which may be related with the potentiated action of testosterone