CHAPTER – 1

NEONATAL HYPERMELATONEMIA IN THE PRE-WEANING PERIOD DECREASES INSULIN LEVELS BUT POTENTIATES INSULIN MEDIATED SYSTEMIC CARBOHYDRATE METABOLISM.

INTRODUCTION:

Melatonin, the hormone secreted by the pineal gland is now firmly established to be involved in various physiological functions such as seasonal reproduction (Tamarkin *et al.*, 1985), thermoregulation (Saarela and Reiter, 1994) and moulting (Allain and Rougeot, 1980). Apart from these seasonal and circadian functions, melatonin is also being implicated in various routine physiological functions as, melatonin binding sites have been localized in many peripheral tissues like the gastro-intestinal tract (Martin *et al*, 1998), liver (Acuna-Castroviejo *et al*, 1994), kidneys (Song *et al.*, 1995) and pancreas (Williams *et al.*, 1997). The hormone is also known to participate in neuro-immuno modulation in rodents as well as humans (Maestroni, 1993), display analgesic effect (Ebadi *et al.*, 1998), decrease both the incidence and growth of spontaneous and induced tumors in animals (Hill and Blask, 1988), act as an efficient anti-oxidant *in vitro* as well as *in vivo* (Tan *et al.*, 1993a,b) and serve as a potent cardio protective

agent (Lagneux *et al.*, 2000). Melatonin influences the functions of liver and muscle through specific receptors (Pang *et al.*, 1993). It influences the activities of a number of enzymes (Frehn *et al.*, 1974; Becham *et al.*, 1989; Popov *et al.*, 1990; Walsh *et al.*, 1994; Woodward and Fisher, 1976) and affects carbohydrate metabolism (Ramachandran, 2002).

With regard to carbohydrate metabolism, both hyper and hypoglycemic effects of melatonin has been reported in a variety of animals (Ramachandran, 2002) Melatonin administration has been reported to increase (Delahaunty *et al.*, 1978; Dhar *et al.*, 1983; Mahata *et al.*, 1988, Zemen *et al.*, 1993) or decrease (Mahata *et al.*, 1988) or have no effects on blood glucose level (John *et al.*, 1990; Ramachandran, 2002). The mechanism by which melatonin modulates glycemic status is not clear. It is suggested that the hormone may act by interactions with other metabolic hormones like insulin, glucagon, growth hormone, corticosterone or catecholamines (Ramachandran, 2002). Melatonin has been shown to influence the plasma insulin level (Diaz and Blazquez, 1986), insulin secretion (Bailey *et al.*, 1974; Peschke *et al.*, 1997) and even possibly insulin action (Frankel and Trandberg, 1991) It is also known to modulate the liver insulin and glucagon receptor concentrations (Rodriguez *et al* , 1989) and increase the catecholamine content (Mahata *et al.*, 1988; Maitra *et al.*, 2000). A central site of action in the brain, possibly the supra chiasmatic nucleus has also been considered (Shima *et al.*, 1997; Van Cauter, 1998). Though melatonin is known to effect body weight, adiposity and food

intake in seasonal animals (Wade and Bartness, 1984; Valtonen *et al.*, 1995, Le Gouic *et al.*, 1996), the mechanism of melatonin action on energy metabolism in mammals is not well known. As a focal centre of intermediary metabolism, it is possible that melatonin might interfere with lipid metabolism Melatonin is assumed to act directly on hepatocytes and pancreatic B cells (Acuna-Castroviejo *et al.*, 1994; Peschke *et al.*, 2000) or even indirectly through the supra-chiasmatic nucleus (Fleur *et al.*, 2001). The effects of melatonin administration to laboratory animals are at best controversial with differing effects on insulin secretion and glucose metabolism (Bailey *et al.*, 1974; Frankel and Trandberg, 1991; Iizuka, 1996; Bizot-Espiard *et al.*, 1998). The apparently contradictory results of melatonin action on carbohydrate metabolism are likely to be due to the various treatment schedules, doses, time and duration of administration as well as the animal species used and the maintenance conditions. All the above factors are crucial in deciphering melatonin action and as such the various experimental studies have employed different paradigms. The season of study as well as the age of the animal are also factors of relevance and a survey of the literature on melatonin and metabolism clearly reveals the age dependent differential effects on various species including the laboratory rat (Unpublished observations).

It is in this context that the present study has been designed with the specific objective of understanding the impact of continuous melatonin administration for the entire duration of pre-weaning period of rat neonates on various facets of carbohydrate metabolism. To this

end rat neonates have been treated with melatonin from day 1 to day 21 and assessed on the 22nd day.

MATERIAL AND METHODS: See page Nos. 16 to 37.

RESULTS:

- **Body and Organ weights:** The body weight of melatonin treated animals showed significant decrease. The relative weights of pancreas, kidney and testes decreased significantly while those of liver, spleen and adrenals increased significantly (Figure and Table, 1.1, 1.3 and 1 4).
- **Serum Glucose and Insulin levels:** Both serum insulin and glucose levels showed a significant decrease in the experimental neonates compared to controls (Figure and Table; 1 5).
- **Hepatic glycogen content and activities of Glycogen Synthetase, Glycogen Phosphorylase and Glucose-6-Phosphatase:** Whereas hepatic glycogen content, glucose-6-phosphatase, and glycogen synthetase activity were significantly elevated, the glycogen phosphorylase activity was significantly decreased in the melatonin treated animals (Figure and Table; 1.6, 1.7, 1.8, 1.9).
- **Muscle glycogen content and activities of Glycogen Synthetase and Glycogen Phosphorylase:** The muscle glycogen content and glycogen synthetase activity were also significantly increased while the glycogen phosphorylase activity

was significantly decreased in the melatonin treated animals (Figure and Table; 1.6, 1.8, 1.9).

- **Hepatic and Muscle Protein content:** The muscle protein content was not altered while the hepatic protein content was significantly decreased in melatonin treated animals (Figure and Table; 1.10).
- **Histological observations:** The sections of pancreas show distinct hypertrophy of acinar and islet cells in hypermelatonemic rats compared to the controls. Alongwith the hypertrophy of the islet cells the islet size also shows hypertrophy. There are also empty spaces between cells suggesting loss of cells. Apparently, there appear to be both an increase in cell number as well as cell loss The number of A cells seems still lesser than the B cells.

DISCUSSION:

This is the only study which reports on chronic neonatal hypermelatonemia on glycemic and insulin status and on hepatic and muscle carbohydrate metabolism. Most of the reports on the influence of melatonin on carbohydrate metabolism are restricted to adult or peripubertal rats.

Figure 1.1: Body weight and absolute liver weight of weaning rats on 22nd day subjected to neonatal melatonin treatment:

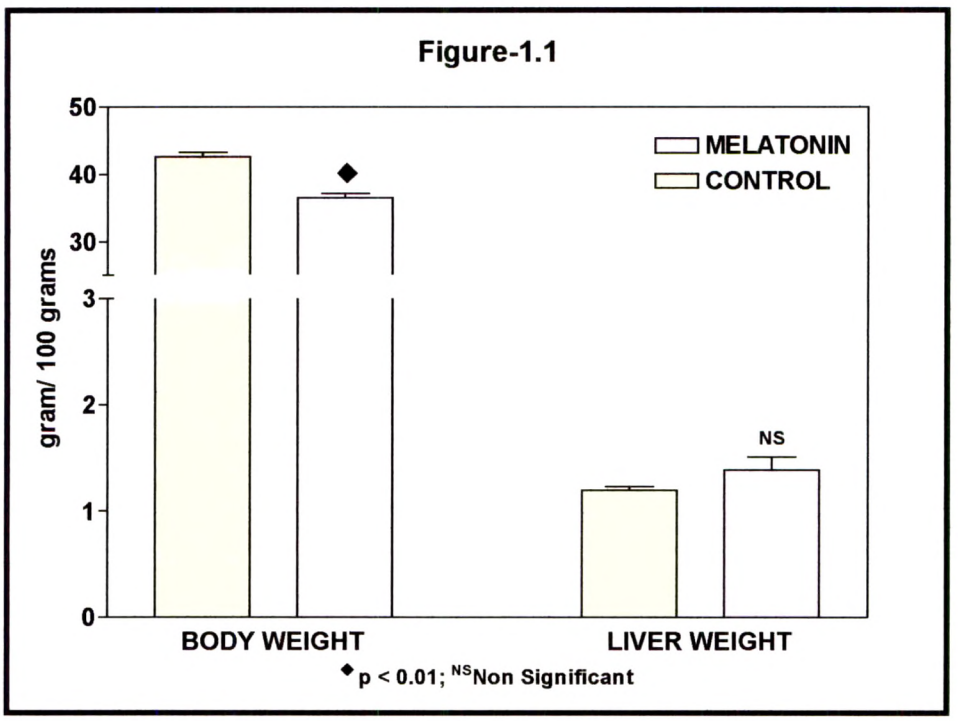


Table 1.1: Body weight and absolute liver weight of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
BODY WEIGHT	42.66 ±0.66	36.5◆ ±0.66
LIVER WEIGHT	1.20 ±0.034	1.39 NS ±0.12

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

Figure 1.2: Absolute weights pancreas, spleen, kidney, testes and adrenals of weaning rats on 22nd day subjected to neonatal melatonin treatment:

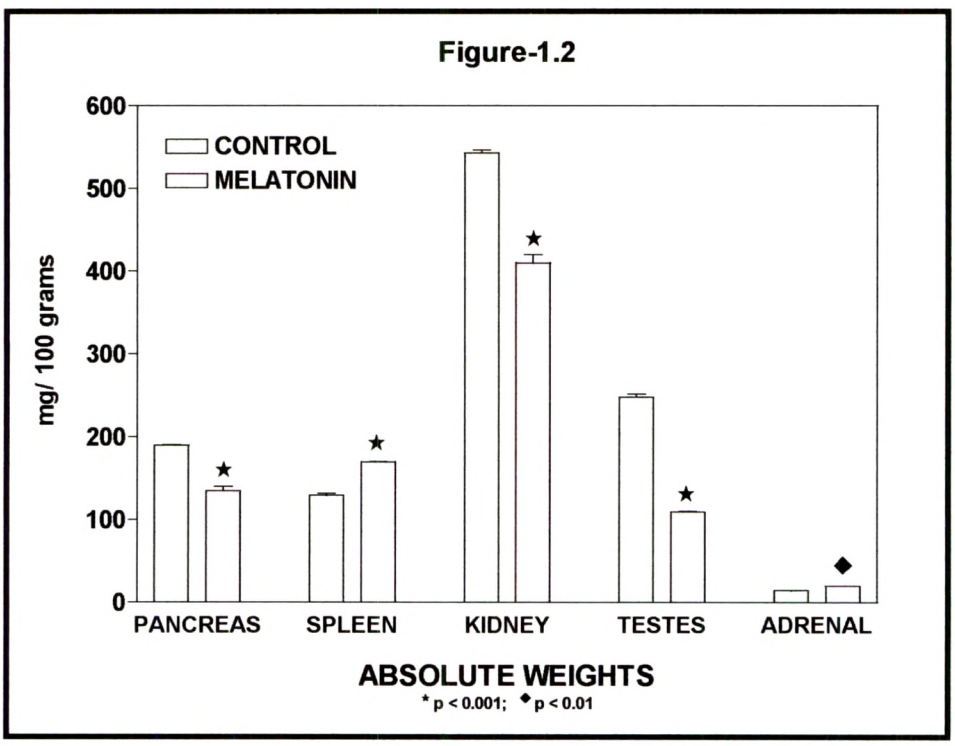


Table 1.2: Absolute weights of pancreas, spleen, kidney, testes and adrenals of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	PANCREAS	SPLEEN	KIDNEY	TESTES	ADRENAL
CONTROL	189.66 ±0.66	129.66 ±2.33	543.00 ±3.53	248.33 ±3.71	15.00 ±0.04
MELATONIN	135.00* ±5.01	170.00* ±0.80	410.00* ±10.02	110.00* ±0.00	20.00◆ ±0.08

Values are expressed as mean ± SEM, *p < 0.001; ◆ p < 0.01

Figure 1.3: Relative weights of pancreas, spleen, testes and adrenal of weaning rats on 22nd day subjected to neonatal melatonin treatment:

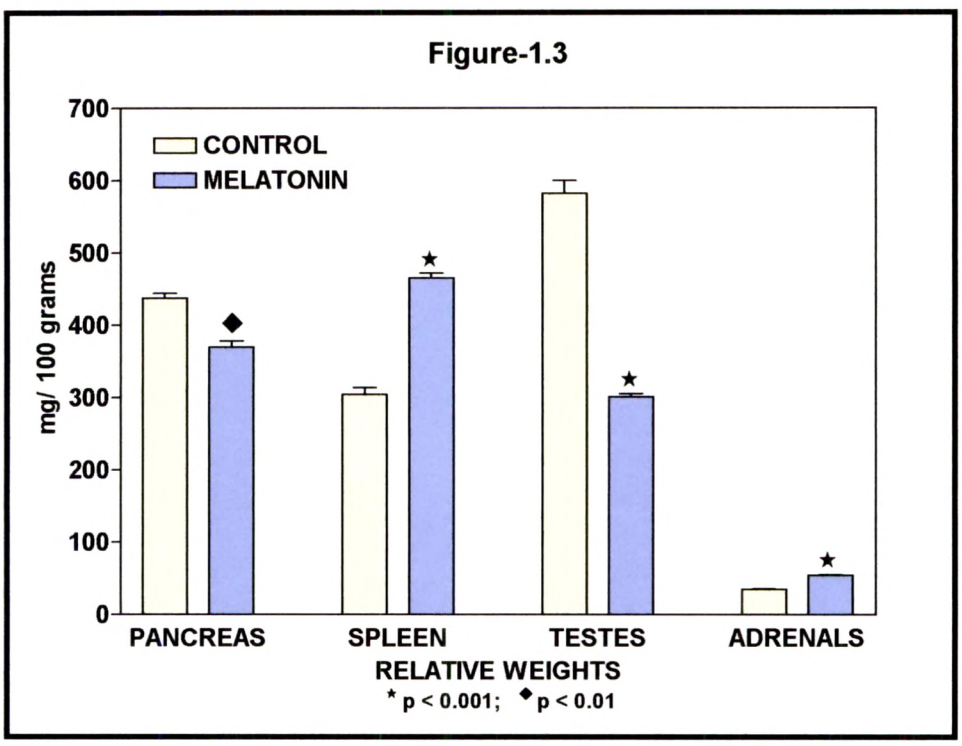


Table 1.3: Relative weight of pancreas, spleen, testes and adrenal of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	PANCREAS	SPLEEN	TESTES	ADRENAL
CONTROL	437.87 ±6.20	304.18 ±9.55	582.57 ±17.49	35.17 ±0.53
MELATONIN	369.74◆ ±8.65	465.83* ±6.39	301.42* ±4.14	54.80* ±0.75

Values are expressed as mean ± SEM, *p < 0.001; ◆ p < 0.01

Figure 1.4: Relative weight of liver and kidney of weaning rats on 22nd day subjected to neonatal melatonin treatment:

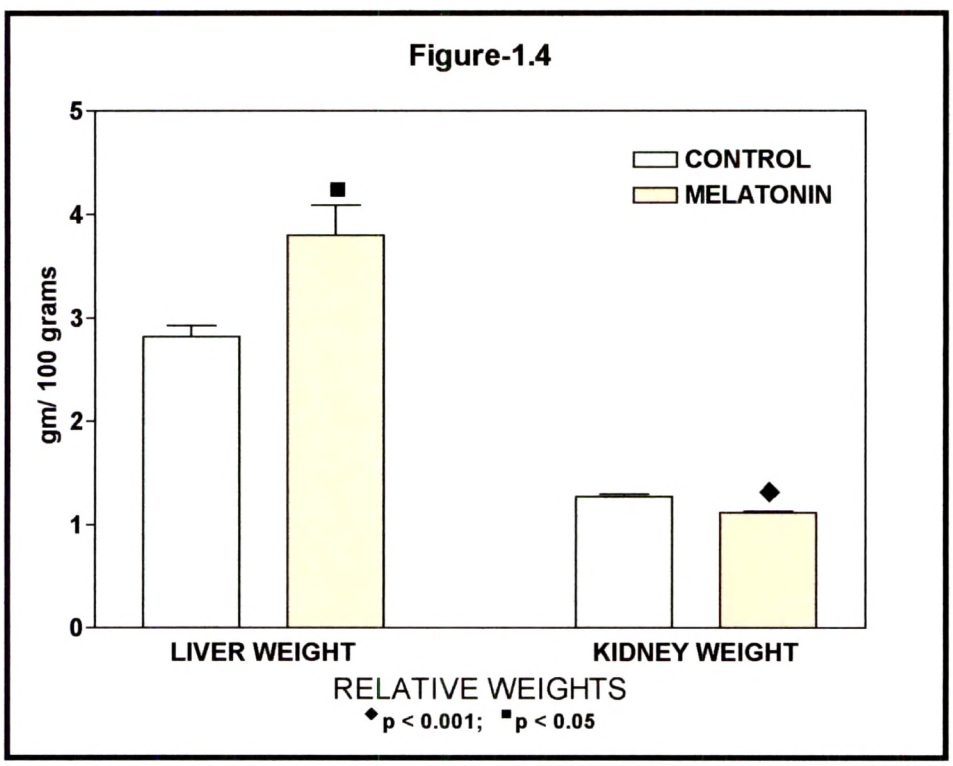


Table 1.4: Relative weight of liver and kidney of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
LIVER WEIGHT	2.82 ±0.11	3.80■ ±0.29
KIDNEY WEIGHT	1.27 ±0.024	1.12◆ ±0.0099

Values are expressed as mean ± SEM, ◆p < 0.001; ■ p < 0.05

Figure 1.5: Serum glucose and insulin levels of weaning rats on 22nd day subjected to neonatal melatonin treatment:

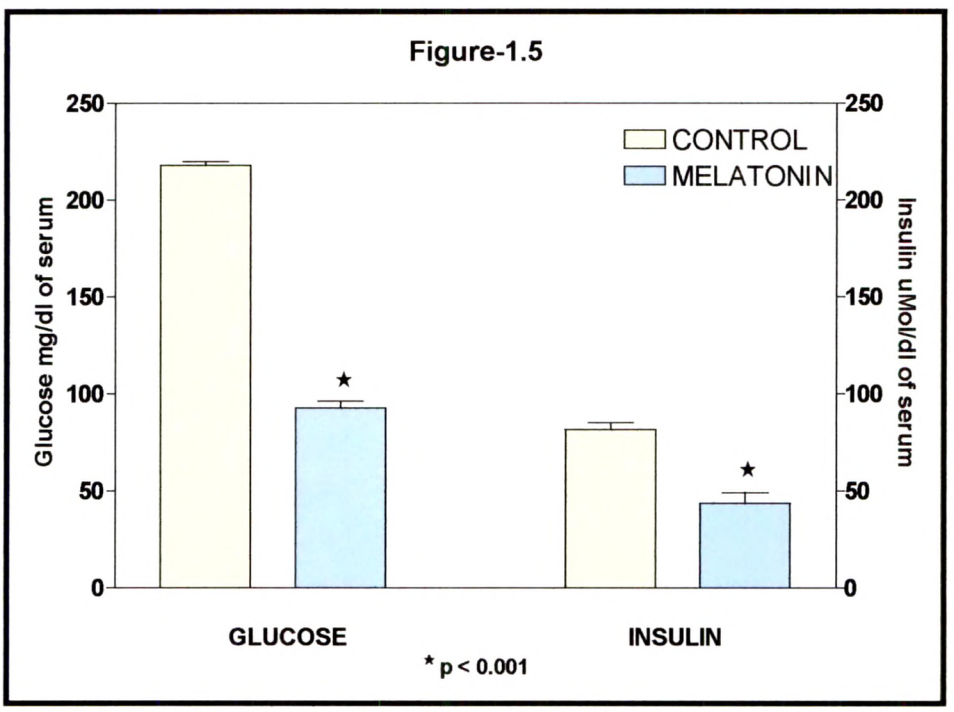


Table 1.5: Serum insulin and glucose levels of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	GLUCOSE	INSULIN
CONTROL	218.09 ±1.76	81.61 ±3.58
MELATONIN	92.76* ±3.53	43.54* ±5.60

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

Figure 1.6: Activities of glycogen phosphorylase in liver and muscle of weaning rats on 22nd day subjected to neonatal melatonin treatment:

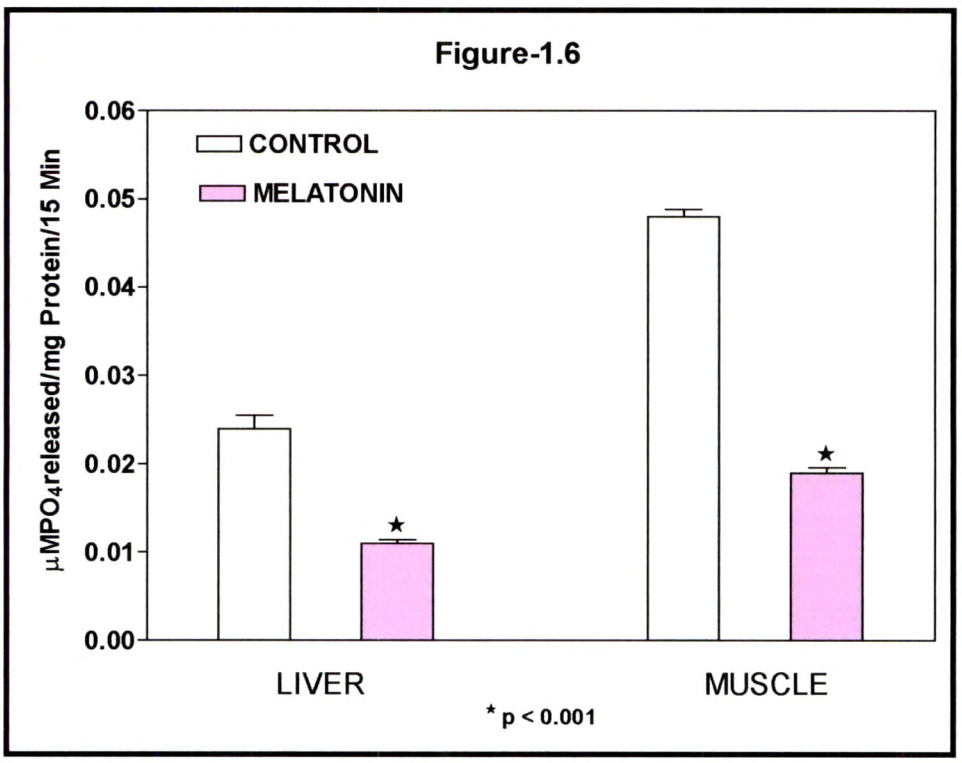


Table 1.6: Activities of glycogen phosphorylase in liver and muscle of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.024 ±0.15	0.048 ±0.00085
MELATONIN	0.011* ±0.00041	0.019* ±0.0006

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

Figure 1.7: Glucose-6-phosphatase activity in the liver of weaning rats on 22nd day subjected to neonatal melatonin treatment:

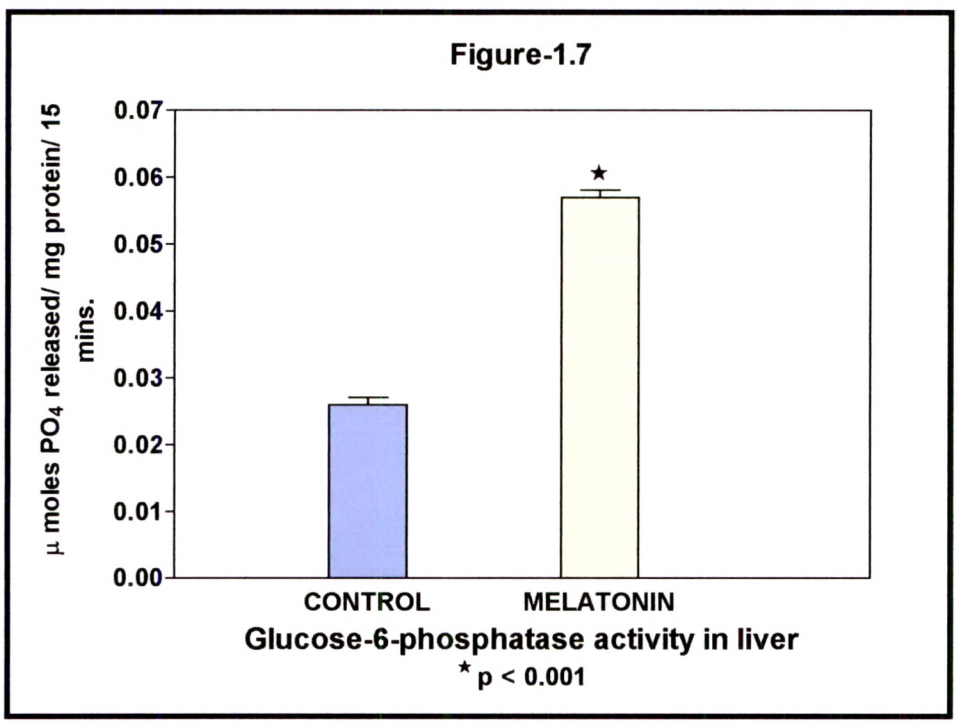


Table 1.7: Glucose-6-phosphatase activity in the liver of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	CONTROL	MELATONIN
GLUCOSE-6-PHOPHATASE	0.026 ±0.0011	0.057* ±0.0011

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

Figure 1.8: Glycogen synthetase activity in liver and muscle of weaning rats on 22nd day subjected to neonatal melatonin treatment:

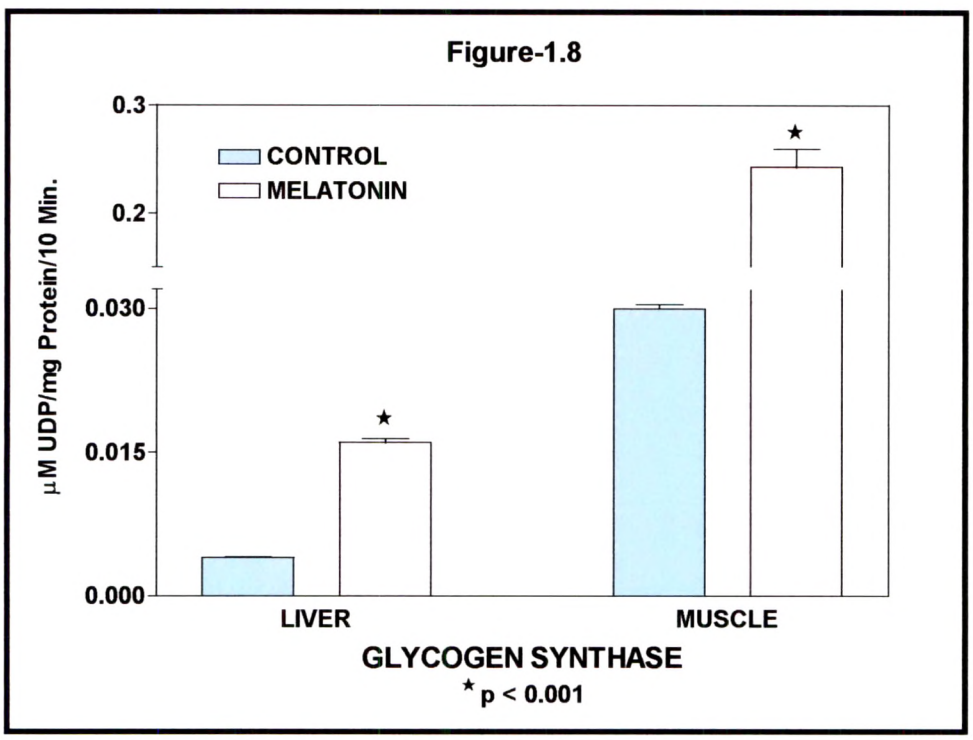


Table 1.8: Glycogen synthetase activity in liver and muscle of weaning rats on 22nd day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.004 ±0.0001	0.030 ±0.0004
MELATONIN	0.016* ±0.00047	0.24* ±0.016

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

Figure 1.9: Hepatic and muscle glycogen content of the weaning rats on 22nd day subjected to neonatal melatonin treatment:

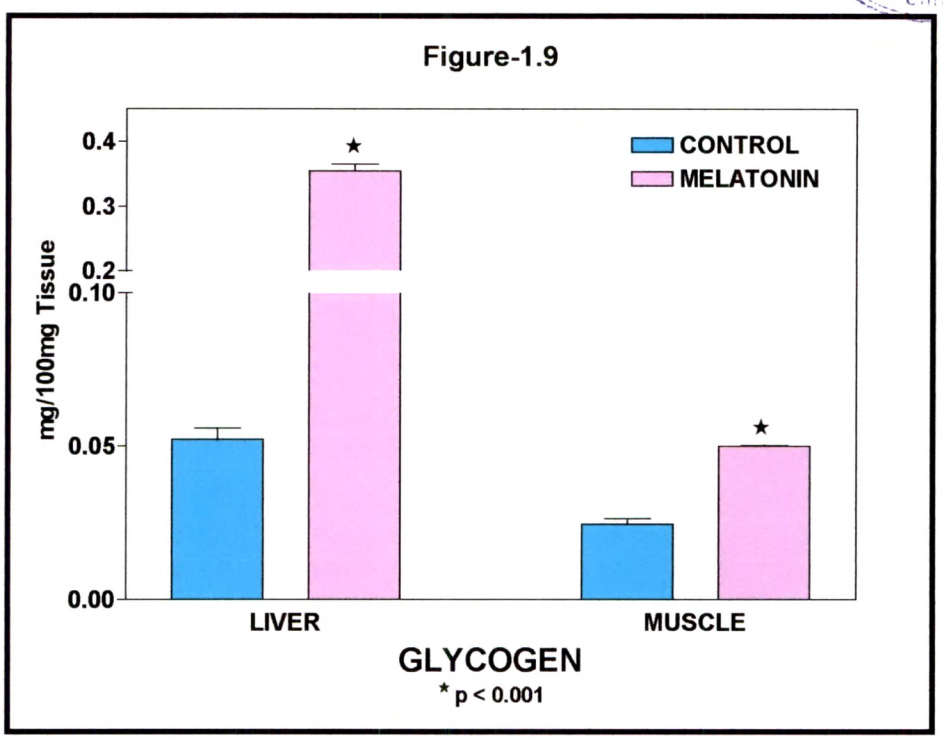


Table 1.9: Hepatic and muscle glycogen content of the weaning rats on 22nd day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	0.052 ±0.0037	0.024 ±0.0017
MELATONIN	0.35* ±0.01	0.05* ±0.0003

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

Figure 1.10: Hepatic and muscle protein content of the weaning rats on 22nd day subjected to neonatal melatonin treatment:

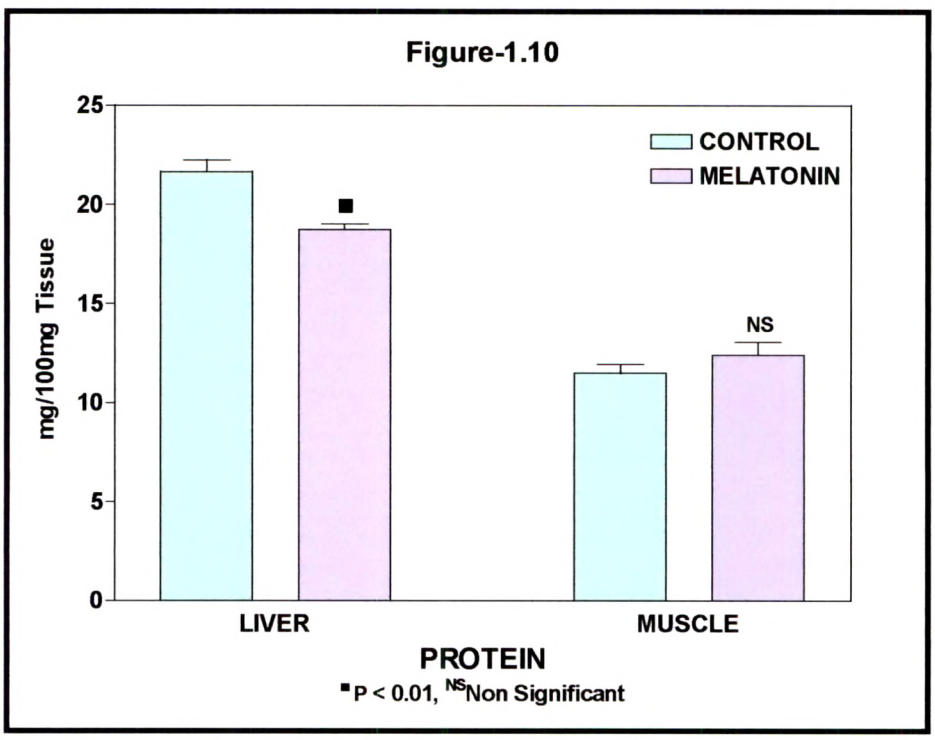


Table 1.10: Hepatic and muscle protein content of the weaning rats on 22nd day subjected to neonatal melatonin treatment:

	LIVER	MUSCLE
CONTROL	21.66 ±0.60	11.49 ±0.44
MELATONIN	18.74▪ ±0.28	12.41NS ±0.64

Values are expressed as mean ± SEM, ▪p < 0.01; NS Non Significant

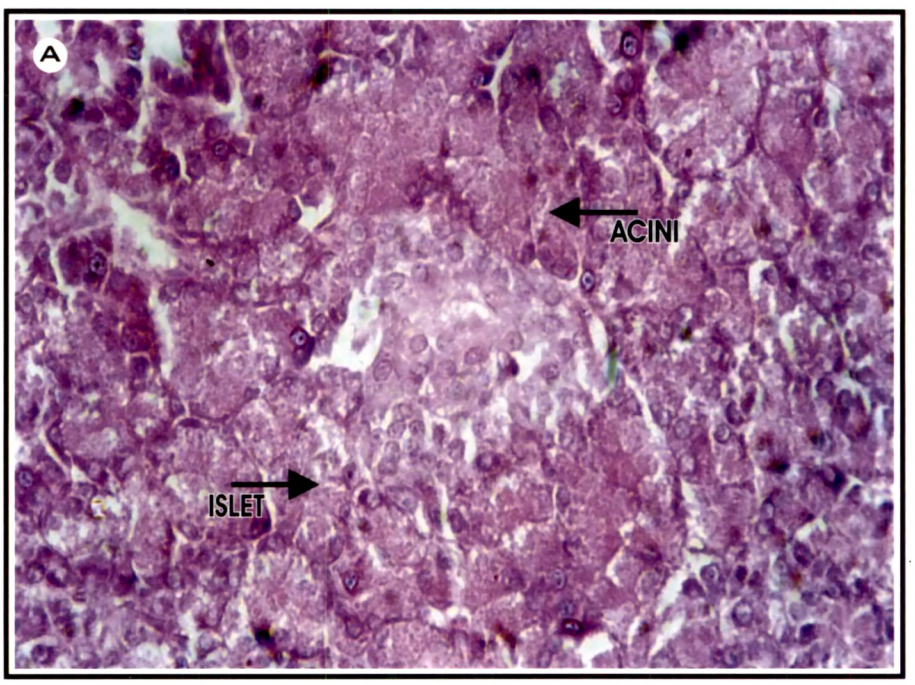
PLATE – 1

Photomicrographs of sections of pancreas – 450 X

FIGURE (A): Transverse section of the pancreas of male control weaning (22nd 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 22nd day showing islet and pancreatic acini The islet and acinar cells are loosely packed with empty spaces (dotted arrow) in between. There is an increase in the islet and acinar cell number, with approximately same number of A and B cells

PLATE - 1



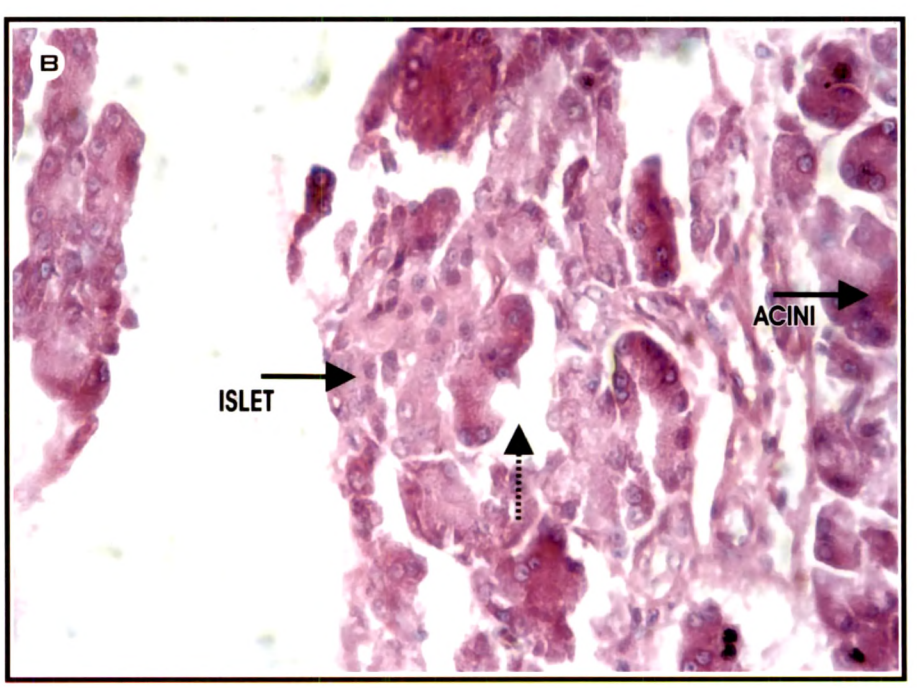


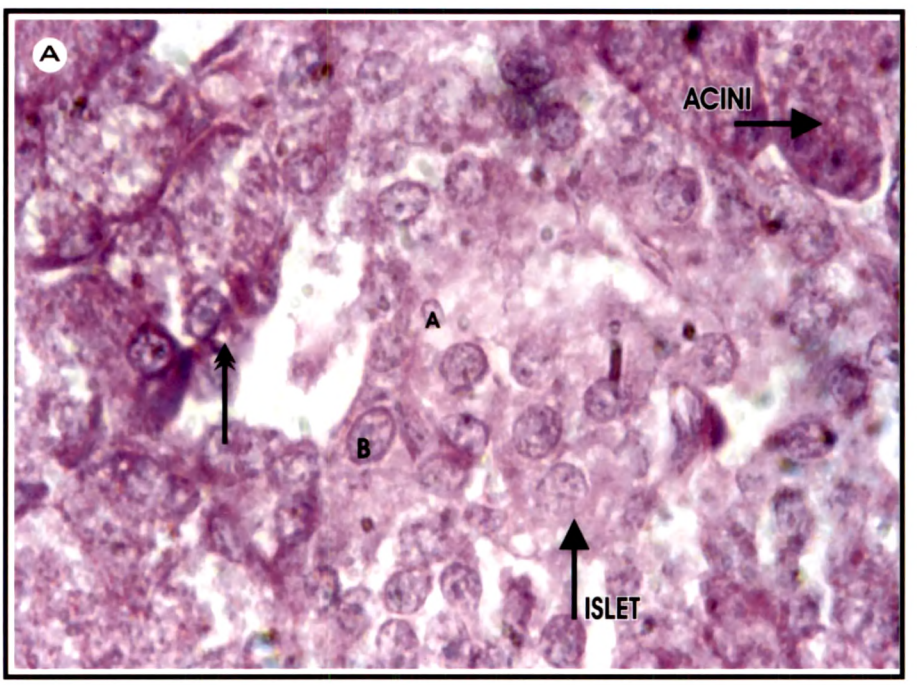
PLATE – 2

Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control weaning (22nd day) rats showing islet and pancreatic acini. Note the centrally distributed A cells, peripherally distributed B cells and the transdifferentiating cell (double headed arrow).

FIGURE (B): Transverse section of the pancreas of male hypermelatonemic rats on the 22nd day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between. There is an increase in the islet and acinar cell number some transdifferentiating cells are also visible (double headed arrow)

PLATE - 2



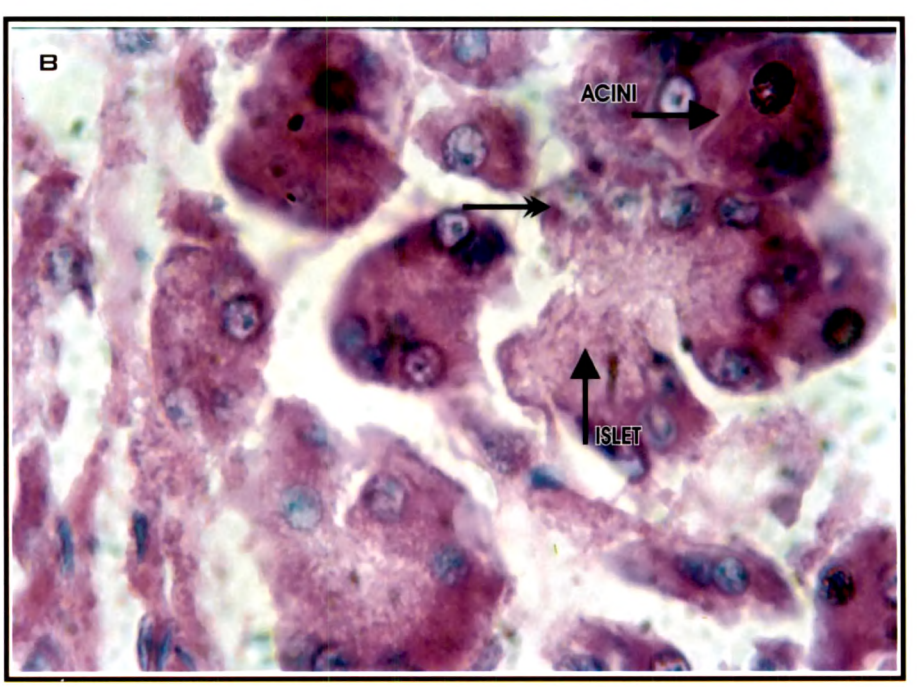


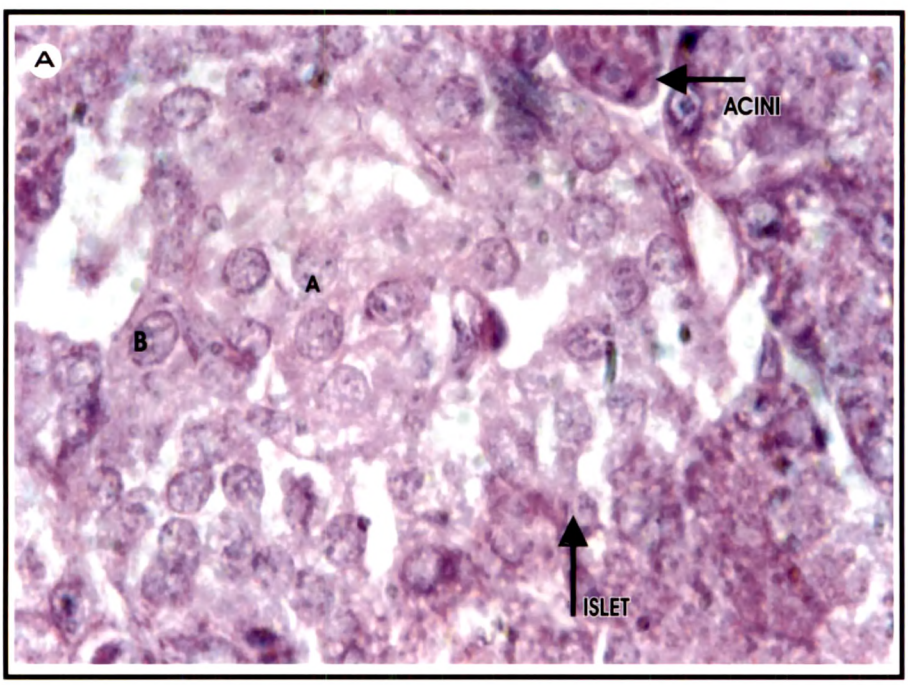
PLATE – 3

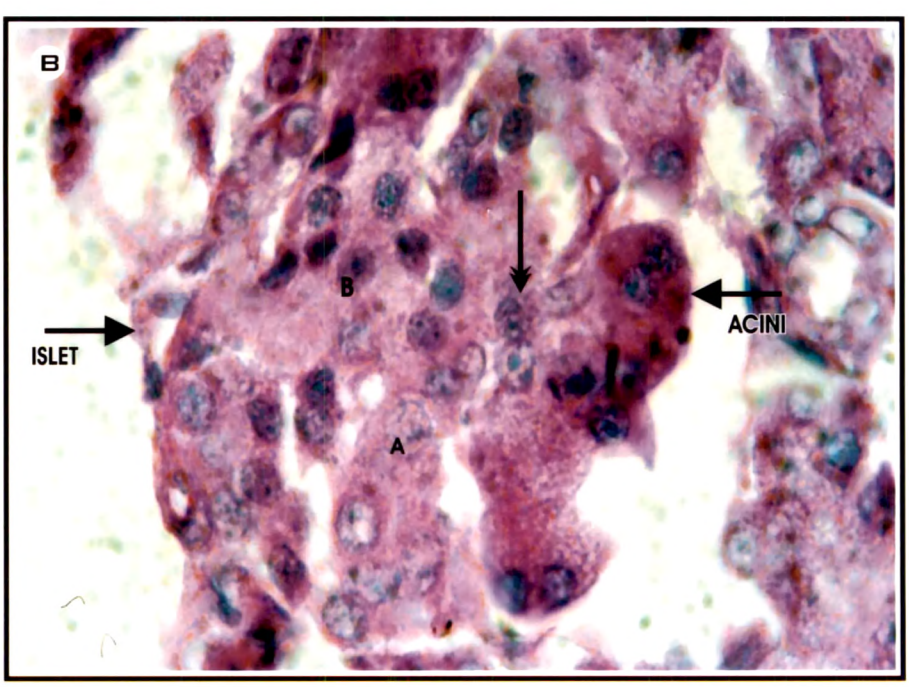
Photomicrographs of sections of pancreas – 1000 X

FIGURE (A): Transverse section of the pancreas of male control weaning (22^{nd} 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 22^{nd} day showing islet and pancreatic acini. The islet and acinar cells are loosely packed with empty spaces in between There is an increase in the islet and acinar cell number, transdifferentiating cells are also visible (double headed arrow). The A and B cells can be viewed at the core and periphery of the islet area

PLATE - 3





It is interesting that pre-weaning hypermelatonemia has induced significant increase in hepatic and muscle glycogen content and hypoglycemia and hypoinsulinemia (Table and Figure; 1 9, 1.5). The increased tissue glycogen content is well supported by the significantly increased glycogen synthetase activity and reduced phosphorylase activity (Figure and Table; 1 6, 1.8) with increased synthetase to phosphorylase activity ratio 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. The presently observed increased hepatic and muscle glycogen contents and hypoglycemia in melatonin treated neonates are fully corroborated by the previous report of increased hepatic glycogen content and hypoglycemia in rat neonates treated from day 10 to day 25 (Patel and Ramachandran, 1992). The increased hepatic and muscle glycogen contents in the hypermelatonemic rat neonates is essentially due to an

increased glycogen synthetase activity and reduced glycogen phosphorylase activity. Obviously the high glycogen synthetase : glycogen phosphorylase activity ratio over that of controls is a significant contributory factor.

Similar to tissue glycogen contents even serum glucose level reveals contradictory effects of melatonin. Whereas Dhar *et al.* (1983), Fabis *et al.* (2002) and John *et al* (1990) have recorded hyperglycemic response to single injection of melatonin, the present study involving chronic administration of melatonin has recorded significant hypoglycemia. Similar hypoglycemic response was also obtained due to melatonin administration in the neonatal period (Patel and Ramachandran, 1992). Based on studies involving short duration as well as long duration melatonin administration in different vertebrate species, a hypoglycemic action of chronic melatonin administration has been inferred (Ramachandran, 2002). The presently observed significant hypoinsulinemia in melatonin treated neonates is however not in accordance with the concomitantly recorded hypoglycemia and increased hepatic and muscle glycogen contents. Adi (2004) in his study on anti-melatonin action of luzindole during the neonatal period has recorded hyperinsulinemia and hypoglycemia. The presently observed hypoinsulinemic and the increased B cell count with a higher B:A ratio in M22 neonates appear contradictory (Plate; 1, 2 and 3). In the light of reported ability of melatonin to induce insulin secretion it is likely that chronic melatonin treatment from post natal day 1 itself might have brought about over secretion from B cells and ultimately secretary

exhaustion and B cell loss. Apparently, the picture available at postnatal day 22 is one of increased new immature B cells generated by regeneration and /or transdifferentiation (Lipsett and Finegood, 2002). Obviously the immature B cells are still in the process of synthesizing and storing insulin secretary granules and hence the low serum insulin levels despite the increased number of B cells. Further, the increased hepatic glucose-6-phosphatase (Fig. and Tab.; 1.7) activity in melatonin treated rats is also at variance. The increased hepatic glucose-6-phosphatase activity seems to be a stimulatory effect on glucose-6-phosphatase synthesis by genetic activation either directly by melatonin or indirectly by corticosterone (Patel *et al.*, 2004). Though the serum insulin level is significantly lower, the increased glycogen synthetase activity and tissue glycogen contents are suggestive of a pronounced or potentiated insulin action. Clearly chronic melatonin treatment increases insulin sensitivity by a probable direct action of melatonin on insulin receptor kinetics or by way of GLUT gene expression. Interestingly acute effect of a single melatonin injection in rats, mice and post menopausal women is manifested in the form of reduced insulin sensitivity (Fabis *et al.*, 2002; Poon *et al.*, 2001; Cagnacci *et al.*, 2001) and lack of melatonin has also been shown to decrease insulin sensitivity as well as GLUT-4 gene expression (Zanquetta *et al.*, 2003) Viewed in this context, chronic melatonin treatment in the neonatal period seems to have potentiated insulin sensitivity as well as GLUT-4 gene expression, which are changes readily relatable with the presently recorded hypoglycemia and

increased tissue glycogen content. Conversely, luzindole induced blockage of melatonin action has also been shown to induce hypoglycemia and increase tissue glycogen content by some alternate mechanisms (Adi, 2004).

The purported increased insulin sensitivity as well as the increased tissue glycogen contents are supported by the observed higher glucose uptake by liver and muscle slices of melatonin treated neonates (Chapter 2). Melatonin treatment has also shown retarded body weight gain and significantly decreased pancreas, kidneys, testes and adrenal weights and significantly increased liver and spleen weights (Fig. and Tab. 1 1, 1.3, 1.4) Obviously melatonin has differential effects on organ growth kinetics and the relation between the altered organ weights and their functional implications need to be studied in detail.

It can be concluded from the present observations that chronic neonatal hypermelatonemia has significant effect on tissue carbohydrate homeostasis, glycemic status as well as serum insulin level and tissue sensitivity to insulin.

SUMMARY:

Though there are many studies with regard to melatonin and glucoregulation/carbohydrate metabolism in adult rats, there are no studies related to neonatal melatonin status. It is in this context, that the present study has been designed with the specific objective of understanding the impact of continuous melatonin administration for the entire duration of pre-weaning period of rat neonates on various facets of carbohydrate metabolism. 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. Melatonin treatment resulted in decreased body weight as well as the weights of pancreas, kidneys, and testes Both, serum insulin and, glucose levels were decreased significantly in the experimental animals. Whereas the hepatic and muscle glycogen contents and the activities of glycogen synthetase and glucose-6-phosphatase increased significantly, the activity of glycogen phosphorylase decreased significantly in the melatonin treated animals. The sections of pancreas showed distinct hypertrophy of both acinar and islet cells in the experimental animals. Though the serum insulin level is significantly lower, the increased glycogen synthetase activity and tissue glycogen contents are suggestive of a pronounced or potentiated insulin action. Clearly, chronic melatonin treatment increases insulin sensitivity by a probable direct action of melatonin on insulin receptor kinetics or by way of GLUT gene expression, which are changes readily relatable with the presently recorded hypoglycemia and increased tissue glycogen content. It can be concluded from the present study that chronic neonatal hypermelatonemia has significant effect on tissue carbohydrate homeostasis, glycemic status as well as serum insulin level and tissue sensitivity to insulin.