GENERAL CONCLUSIONS

The present study was undertaken essentially to understand the impact of altered melatonin status in the pre-weaning period on weaning, pubertal and adult carbohydrate and lipid metabolism, pancreatic function and alloxan induced diabetes. The influence of pre-weaning melatonin status on *in* vitro glucose uptake in response to different secretogogues was also studied. Experiments were essentially carried out under a photoperiod of LD 8:16 and a constant temperature regimen of 21±2° C. Individual effects of melatonin excess and alloxan induced diabetes along with melatonin excess have all been assessed within the framework of the above cited objectives.

Altered melatonin status has been reported to increase, decrease or have no effect on blood glucose level and is known to influence plasma insulin level, insulin secretion and even insulin action. The modulatory influence of melatonin on intermediary metabolism is known. The present study clearly indicates that pre-weaning melatonin treatment influences weaning, pubertal and adult carbohydrate and lipid homeostasis as well as weaning alloxanisation status on pubertal and adult carbohydrate and lipid homeostasis with or without melatonin treatment.

The realized alterations in weaning stage as immediate post-treatment effects are:

1. The body weight is decreased.
2. Relative weight of pancreas is decreased while, that of liver is increased.
3. Both serum insulin and glucose levels are decreased.
4. The hepatic and muscle glycogen contents are increased.
5. Hepatic and muscle glycogen synthetase activity are increased.
6 Glycogen phosphorylase activity is decreased in both liver and muscle.
7. The activity of glucose-6-phosphatase is increased.
8. Hepatic protein content is decreased.
9. The sections of pancreas show distinct hypertrophy of acinar and islet cells.
10. The liver slices showed increased glucose uptake with melatonin and acetylcholine.
11. The muscle slices showed increased glucose uptake with insulin.
12. Luzindole promoted glucose uptake in both liver and muscle slices.
13. The hepatic and muscle total lipid and cholesterol contents are decreased.
14. The adipose tissue total lipid and cholesterol contents are decreased.

15 Serum total lipid, phospholipid and free fatty acid levels are increased.

The possible explanations for the above recorded observations are:

1. The increased tissue glycogen content is well supported by the increased glycogen synthetase activity and reduced glycogen phosphorylase activity with increased GS:GP ratio.
2. The increased hepatic glucose-6-phosphatase activity seems to be a stimulatory effect on glucose-6-phosphatase synthesis by genetic activation either directly or indirectly by corticosterone.
3. 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.
4. 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.
5. Melatonin is a potent stimulator of glucose uptake along with insulin and acetylcholine. Also melatonin treatment potentiates the glucose uptake by all the three stimulants as well as their combinations
6. The presently observed increased serum free fatty acid level and decreased tissue total lipid content could suggest an adaptive increased lipid utilization to counteract the carbohydrate sparing effect of neonatal hypermelatonemia.
7. The low tissue lipid stores could be easily correlated with the prevailing hypoinsulinemia.

8. Neonatal hypermelatonemia decreases lipid synthesis and increases lipid utilization as against increased lipid synthesis and decreased utilization in control weanings.

The alterations in the pubertal period due to pre-weaning melatonin treatment are:

1. The body weight is decreased.
2. Relative weight of pancreas and liver are decreased.
3. The serum insulin level is increased significantly while, the serum glucose level is decreased.
4. The hepatic and muscle glycogen content are increased.
5. The muscle glycogen synthetase activity is increased.
6. The hepatic glucose-6-phosphatase activity is increased.
7. The glycogen phosphorylase activity is decreased in muscle while, it is increased in the liver.
8. The hepatic and muscle protein contents are increased.
9. Islet size and cell number are reduced with relatively less B cells.
10. The liver slices showed increased uptake with insulin and melatonin.
11. The uptake promoted by L+I was maximum as compared to any other stimulant alone or by their combination.
12. In the muscle slices, M+Ac+I induced maximum glucose uptake while, that induced by L is minimal.

13. The C^{14} glucose oxidation in the liver slices was decreased in the basal state as well as with I, Ac and I+Ac.
14. Melatonin and luzindole and their combinations increased the C^{14} glucose oxidation by the liver slices.
15. The C^{14} glucose oxidation induced by luzindole and its combinations was decreased while, that with melatonin increased.
16. The C^{14} glucose oxidation in the basal state is decreased in the muscle slices.
17. The hepatic and muscle total lipid contents are increased.
18. The hepatic cholesterol content is decreased.
19. The adipose tissue cholesterol content is decreased.
20. The serum free fatty acid level is increased.
21. The serum triglyceride and cholesterol levels are decreased.

The possible explanations for the above recorded observations are:

1. Chronic pre-weaning hypermelatonemia has persistent glycogenic effect and increased glycogen synthetase activity.
2. Apparently, neonatal hypermelatonemia has a protein anabolic influence as a long term effect in the pubertal period, it may suggest a potentiated influence of testosterone
3. Melatonin treatment in the neonatal period seems to hasten the postpartum development of insulin sensitivity.
4. The relatively greater uptake capacity coupled with reduced glucose oxidation suggests an overall anabolic *milieu* in the neonatal hypermelatonemic rats.

5. The reduction in serum levels of various lipid fractions other than free fatty acids suggests a hypolipidemic effect of chronic melatonin treatment.
6. Cholesterol lowering effect of melatonin seems to be a characteristic feature as at both weaning and in the pubertal stage the cholesterol content of the body is significantly less in neonatal hypermelatonemic rats

The alterations in the adult period due to pre-weaning melatonin treatment are·

1. The body weight is decreased.
2. Relative weight of pancreas, liver, testes and kidneys are increased.
3. The serum insulin level is increased while, the serum glucose level is decreased.
4. The hepatic and muscle glycogen contents are increased.
5. The hepatic and muscle glycogen synthetase activities are increased
6. The glycogen phosphorylase activity is increased in liver while the muscle glycogen phosphorylase activity remained unaltered.
7. The hepatic glucose-6-phosphatase activity is decreased.
8. The muscle protein content is increased.
9. The islets seem to be larger in size with less compactly packed cells. B cell neogenesis by transdifferentiation from acinar cells seems to be a distinct feature.

10. The glucose uptake promoted by I+Ac in the liver slices was maximum as compared to any other stimulant or by their combinations.
11. The glucose uptake promoted by M+Ac and M+Ac+I in the liver slices is decreased.
12. In the liver slices glucose uptake induced by luzindole as well as its combinations is decreased.
13. The uptake induced by I, Ac and M along with their combinations is decreased in the muscle slices.
14. The glucose uptake induced by L+Ac is increased in the muscle slices while, that by L, L+I and L+Ac+I is decreased.
15. The hepatic cholesterol content is decreased.
16 The muscle lipid content is decreased while the, muscle cholesterol content is increased.
17. The adipose tissue total lipid content is decreased while the, adipose tissue cholesterol content is increased.
18. The serum triglyceride, cholesterol, phospholipid and free fatty acid levels are decreased.

The possible explanations for the above recorded observations are:

1. The significantly higher muscle glycogen and protein content of the hypermelatonemic rats is a consequence of the higher levels attained at the pubertal age itself.
2. There is a similar degree of glycogenolysis in both control and experimental animals indicating utilization for energy purposes and the hypermelatonemic animals show a higher tissue load of

metabolites essentially due to the increase recorded in the pubertal age

3. The higher insulin level in hypermelatonemic rats is substantiated by the relatively higher number of B cells, many of which are generated by transdifferentiation of acinar cells and increased B:A cell ratio.
4. The hypermelatonemic liver slices show significantly reduced acetylcholine sensitivity as marked by low and unchanged uptake at 10 or 90 minutes suggesting a reduced parasympathetic tone.
5. A comparison of the glucose uptake by tissues of weaning rats (Chapter 2) and young rats (present chapter) indicates a higher glucose uptake at 10 minutes with lower intake at 90 minutes in the former and reversed lower intake at 10 minutes and higher intake at 90 minutes at later. This may be considered as an indication of an age related increased resistance to insulin as well as other uptake agents.
6. The decreased glucose uptake shown by hypermelatonemic tissues at both 10 and 90 minutes with concurrent glycogenolysis (Chapter 7) may be in this context related to decreased lipid contents.
7. Apparently there could be an age dependent difference in the partitioning of carbohydrates among tissues and intro cellular pathways.

8. The depletion in adipose tissue, hepatic and muscle lipid contents seen in hypermelatonemic rats may suggest increased activity of lipolytic enzymes.
9. A generalized long duration protective action of neonatal hypermelatonemia on adiposity is clearly suggested by the herein observed significant decrement in all serum lipid fractions.

The observed alterations in the pubertal period due to neonatal melatonin treatment and weaning alloxanisation on the 22nd day are:

1. The body weight is decreased in all the alloxanised rats.
2. Relative weight of pancreas, liver and spleen is increased in the nMT alloxanised rats.
3. The hepatic glycogen content is decreased while the, muscle glycogen content is increased in all the alloxanised rats.
4. Both serum insulin and glucose levels are decreased.
5. The hepatic glycogen synthetase and glycogen phosphorylase activities are decreased in all the alloxanised rats.
6. The muscle glycogen synthetase activity is increased in the control alloxanised rats.
7. The muscle protein content is increased in all the alloxanised rats.
8. The muscle total lipid content is decreased in all the alloxanised rats.

9. Whereas the, the adipose tissue total lipid content is decreased, the adipose tissue cholesterol content is increased in hypermelatonemic alloxanised rats.
10. The serum triglyceride level is decreased in all the alloxanised rats.
11. The serum cholesterol level is increased significantly in the hypermelatonemic alloxanised rats.
12. The serum phospholipid level is decreased in all the alloxanised rats.
13. The serum free fatty acid level is increased in all the alloxanised rats.
14. Both the control and nMT rat islets show alloxan induced damage marked by loss of cells and formation of large spaces.
15. Both the control and nMT rat islets show B cell neogenesis from acinar cells with the result that the areas between the islet and acinar area has got befudged.

The possible explanations for the above recorded observations are:

1. The significant hypoglycemia seen in all alloxan treated rats, relatively more potent in nMT rats, suggests increased insulin action probably due to regeneration of the B cells.
2. The purported regeneration of B cells is adequately supported by the histologically observable B cell regeneration and B cell density in the islets of alloxan treated control rats and increased islet density with regeneration of B cells in alloxan treated nMT rats.

3. In the present study, nMT rats seem to resist the diabetogenic effect of alloxan, as noted by the relative healthy condition of the islet tissue compared to the non-nMT rats. This suggests a protective action of prior melatonin treatment in resisting the effects of B cell damaging agents
4. The body lipid profile shows a generalized ability of the weaning animals to resist diabetes related increase in lipid content. The reduced tissue lipid and serum lipid fractions in both nMT and non-nMT rats relative to non alloxanised controls is probably indicative of reduced insulin level/action in the post alloxan treatment period. This is substantiated by the observed insulin status in these rats.

The observed alterations in the adult period due to neonatal melatonin treatment and weaning alloxanisation on the 22nd day are:

1. The body weight of MA(100) and CA(100) rats is decreased.
2. Relative weight of pancreas, liver, kidney and adrenals is increased in the MA(100) rats.
3. The hepatic and muscle glycogen contents are decreased significantly in all the alloxanised rats.
4 The serum insulin and glucose levels are increased in all the alloxanised rats.
5 The activity of hepatic and muscle glycogen synthetase is decreased in all the alloxanised rats as compared to nMT rats.
6. The hepatic glycogen phosphorylase activity is decreased.

7. The hepatic glucose-6-phosphatase activity is increased in the MA(150) rats.
8. The muscle protein content is increased in the MA(100) rats.
9. The hepatic cholesterol content of MA(100) rats is increased.
10. The adipose tissue cholesterol content of all the alloxanised rats is decreased as compared to nMT rats.
11. The serum triglyceride level is decreased in all the alloxanised rats.
12. The serum cholesterol level of MA(100) rats is increased.
13. The serum total lipid level of all the alloxanised rats is decreased.
14. The serum phospholipid level of MA(100) rats is decreased.
15. The serum free fatty acid level of MA(100) rats is decreased.
16. The islets show pronounced recovery from alloxan induced damage. Islets of both alloxan treated control and nMT rats showed prominent cells with a higher B:A ratio.
17. Sections of pancreas clearly showed transdifferentiation of acinar cells to B cell at the interjunction between islet and acinar tissue.

The possible explanations for the above recorded observations are:

1. The increase in body lipid content is marked by a reciprocal decrement in serum lipid fractions. Since the nMT rats show relatively higher serum lipid profiles as well as tissue lipid loads, it is likely that a progressive insulin resistance is manifesting in these rats.

2. The present study on weaning alloxan treatment seems to suggest development of insulin resistance and hyperinsulinemia as, long term effects, probably, more potentiated by a higher melatonin level in the neonatal period.
3. A protein anabolic influence related to insulin resistance is also evident as the tissue protein contents are significantly increased in the rats.
4. Protective action of neonatal melatonin on alloxan induced B cell damage is clearly seen from the histologically observable structural features of pancreatic islets.
5. However, as a long term effect of weaning alloxan induced diabetes, neonatal melatonin treatment tends to potentiate hyperinsulinemia and insulin resistance and thereby predisposes these animals to NIDDM.