CHAPTER IV

CARBOHYDRATE METABOLISM AND PANCREATIC ISLET FUNCTIONS IN PINEALECTOMIZED PIGEONS REPLACED WITH MELATONIN IN THE BREEDING SEASON.

Role of pineal gland in carbohydrate metabolism has been studied to a certain extent by different workers. One group of workers using protein extract of pineal demonstrated a hypoglycemic action coupled with increased glucose tolerance and stimulated hepatic and muscular glycogenesis in mammals (Damian,1989). Their experiments on alloxonised diabetic dogs also suggested the ability of the pineal polypeptide to partly substitute for insulin. A season specific influence of pineal on carbohydrate metabolism and glycemic status has also been shown in fishes (Delahunty et al.,1978,1980; Delahunty and Tomlinson,1984). Pinealectomy induced alterations in the glycemic status of rat, rabbit and pigeon have been also recorded (Mihail and Giurgea,1979; Murlidhar et al.,1983; Diaz and Blazguez,1986).

Past works involving pinealectomy from this laboratory suggested influence of pineal in the modulation of carbohydrate metabolism in feral pigeons in relation to annual testicular cycle (Patel et al.,1983,1988; Ramachandran and Patel,1987). Moreover, alterations in

glucose tolerance in response to either exogenous glucose or other hormones influencing carbohydrate metabolism have also been reported in pinealectomised pigeons (Patel and Ramachandran,1989; Ramachandran and Patel,1989). Decreased plasma glucose levels and tissue glycogen contents have been reported to occur in feral pigeons subjected to either surgical or chemical pinealectomy (Patel et al.,1983,1988; Patel and Ramachandran,1989; Ramachandran and Patel,1989; Chapter I). So the present study has been designed to assess the effect of melatonin (M) replacement on carbohydrate metabolism in pinealectomised pigeons.

MATERIALS AND METHODS :

Procurement and maintenance of pigeons and preparation of solution (melatonin) are as outlined in Chapter I;
Experimental set-ups - as outlined in Chapter III.
Parameters and methods of evaluation - as outlined in Chapter II.

RESULTS :

Blood Glucose : The mean blood glucose level in the intact control group was 213.14 mg/dl which was decreased to 179.74 mg/dl in the PX group. Replacement with M showed a differential effect with M50 inducing further decrease in

Treatments	Blood Glucose (mg/dl)	Tissue Glycogen (mg/100mg Tissue)		Hepatic G-6-P'ase (u moles PO_4 released /mg Protein/15 min)	Hepatic Phosphorylase (ug PO_4 released/mg Protein/15 min)
		Liver	Muscle		
C	213.14 ± 25.75	3.90 ± 0.92	1.83 ± 0.19	1.18 ± 0.47	15.04 ± 1.81
PX	179.74 ± 21.97	2.74 ± 0.63	1.07 ± 0.10	0.78 ± 0.08	11.79 ± 1.55
PXM50	151.34* ± 19.45	1.51* ± 0.20	0.84* ± 0.07	2.45* ± 0.61	57.90* ± 2.78
PXM100	197.24* ± 19.33	1.87* ± 0.21	0.92* ± 0.08	2.06* ± 0.57	47.82* ± 2.49

Table 4.1 : Alterations in blood glucose, tissue glycogen contents and activity levels of G-6-P'ase and phosphorylase in liver in pinealectomised pigeons replaced with melatonin.

(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

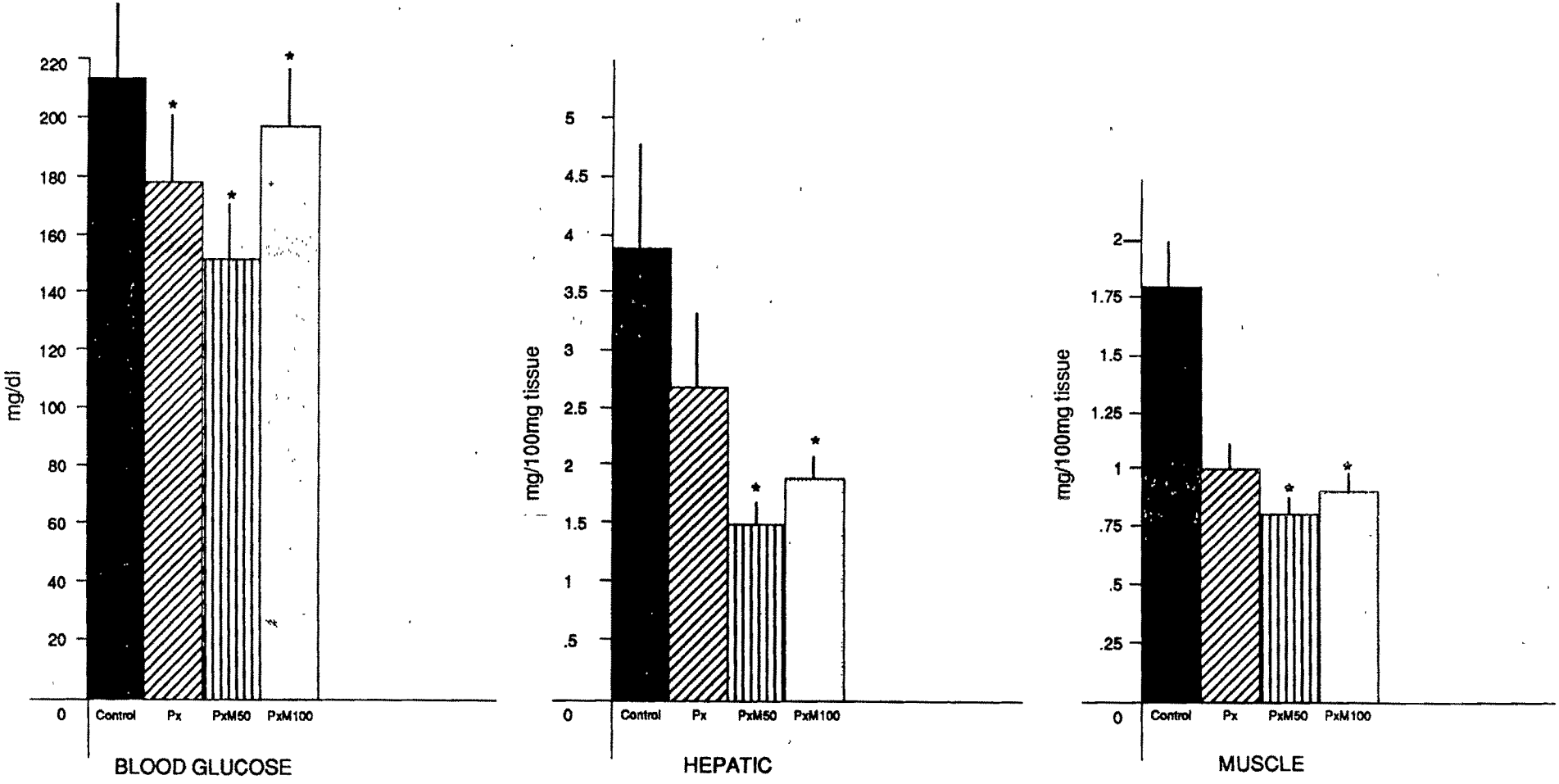

Fig.: 4A : Alterations in blood glucose, tissue (hepatic and muscle)glycogen content and activity levels of G-6-P'ase and phosphorylase in liver of pinealectomised pigeons replaced with melatonin.
(* Significant at $P < 0.05$, values are $\bar{x} \pm SD$).

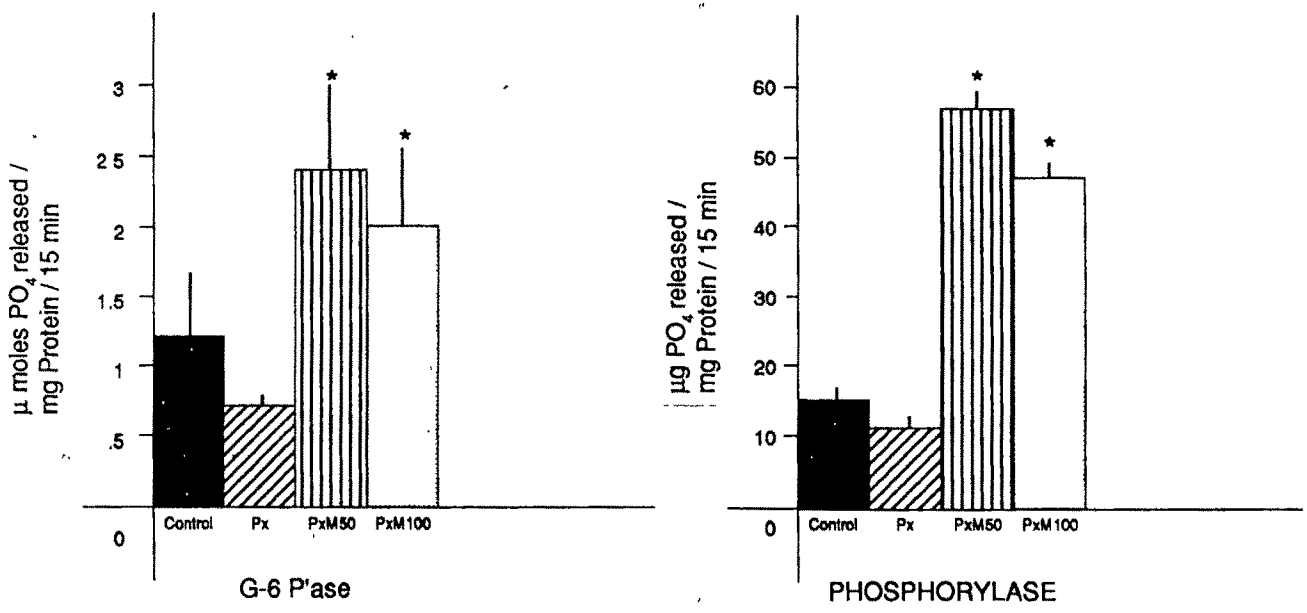

Fig. : 4 B Alterations in the activity levels of hepatic G-6 P' ase and phosphorylase of pinealectomised pigeons replaced with melatonin.
(* Significant at $P < 0.05$, values are $\bar{x} \pm$ SD).

the glycemic level and M100 more or less nullifying the hypoglycemic influence of PX. (Table - 4.1, Fig. - 4A).

Hepatic and Muscle glycogen contents : The glycogen content of both liver and muscle was decreased in the PX birds. Again as in the case of blood glucose, M50 further depleted the hepatic and muscle glycogen contents while M100 showed slightly greater glycogen content though much less than the PX animals (Table - 4.1, Fig. - 4A).

Hepatic Phosphorylase and G-6-P'ase : Both these enzymes registered declined levels of activity in PX birds. On the other hand, both doses of M (50 or 100µg) increased the activity level of the enzymes significantly to above normal levels (Table - 4.1, Fig. - 4B).

Histological observations on Pancreatic islets :
Differential staining for A and B cells of islets depicted significant degranulation of B cells after pinealectomy. Replacement with 50µg induced degranulation of both A and B cells while that with 100µg M showed degranulation of A cells only.

Serum T3 & T4 : (See Chapter III, Table - 3.2).

DISCUSSION :

Previous studies from this laboratory based on the observed changes in carbohydrate metabolism in PX birds suggested an anti-insulinic role for the pineal in feral pigeons (Patel et al.,1983,1988). Subsequently, Ramachandran and Patel (1989) observed increased glucose tolerance and insulin sensitivity in PX pigeons and suggested increased insulin secretion/sensitivity in PX pigeons. These surmises were confirmed by the observation of significant B cell degranulation in the pancreatic islets of PX pigeons (Chapter II). In the present study also, significant hypoglycemia and hepatic and muscle glycogen depletion together with B cell degranulation have been observed after pinealectomy. The predictable decrease in the glucagon : insulin molar ratio is reflected in the decreased hepatic phosphorylase and G-6-P'ase activity. Previous observations of hypoglycemia and increased glucose tolerance and insulin sensitivity in the PX pigeons (Patel et al.,1983; Ramachandran and Patel,1989) corroborate the same. Based on the previous findings of suppressed hepatic glucose uptake coupled with increased glucose uptake by muscle of PX pigeons (Patel and Ramachandran,1992), the decreased hepatic glycogen content and hypoglycemic status have been surmised as due to low rate of glycogenolysis coupled with absence of

glycogenesis in the liver and increased peripheral utilization of glucose (Chapter II).

The present study, intended to test whether replacement with M simulating a normal endogenous dark time increase in intact animals could nullify the PX effects on carbohydrate metabolism has revealed the failure of 50µg M to this intent. Incidentally, the same dose of M failed to prevent the PX induced alterations in adrenal and thyroid functions as well as the attendant testicular regression (Chapter III). However, in the above study, 100µg M was effective in preventing the PX induced alterations in adrenal and thyroid functions. In the present case, neither of the two doses used seemed capable of preventing the PX effects on carbohydrate metabolism, though the higher dose to a certain extent was effective in attenuating the PX induced alterations. Paradoxically, 50µg M caused still more pronounced hypoglycemia and tissue glycogen depletion than in PX animals. This probably suggests independent effects of both, PX and M. The concurrent degranulatory changes in both the A and B cells bear testimony to this. The increased phosphorylase and G-6-P'ase activity provides further evidence to the same. The earlier reported suppressed hepatic glucose uptake in PX pigeons (Patel and Ramachandran,1992) and the presently observed increase in phosphorylase activity could together contribute to decreased

glycogenesis and increased glycogenolysis, leading to greater hepatic glycogen depletion as seen in the present study. Despite the pronounced glycogen depletion, the glycemic level was further lowered than in PX. This is mainly due to PX-induced increased insulin sensitivity and peripheral utilization of glucose (Ramachandran and Patel,1989; Patel and Ramachandran,1992) and M-induced conversion of glucose carbon into more lipids and protein (Unpublished observations). The ability of M to convert glucose into lipids is potentiated due to the presence of higher insulin and thyroxine levels, as B cell degranulation and depletion of colloid content from thyroid follicles have both been observed in PX pigeons treated with 50µg M.

Moreover, increased serum T4 level has been recorded in PX pigeons previously (Chapters I & III). Our unpublished observations indicate that T4 has a lipogenic influence in feral pigeons and a similar tendency has been observed with M as well. It is also well established that insulin is a potent lipogenic hormone in birds (Touchborn et al.,1981; Yanaihara et al.,1983; Griminger,1986). Overall, PX and M (at 50µg dosage) exert independent actions and together contribute a more conducive environment for conversion of more and more glucose carbon into carcass fat. In contrast, 100µg of M was capable of preventing the PX effects on the HHT axis as indicated by the state of thyroid follicles and

serum T4 levels. Moreover, the PX-induced B cell degranulation in pancreas was also not evident. These changes as well as the observed glycogen depletion and hypoglycemia are same as in the case of control birds treated with 50µg (Chapter II). The latter changes related to carbohydrate metabolism are mainly due to M-induced glycogenolysis and utilization of glucose for lipid synthesis as suggested previously (Chapter II).

An overall consideration leads to a surmise that while 50µg M given as single injection is not capable of negating the PX effects, single injections of 100µg M produce symptomatic effects similar to intact birds treated with melatonin. The present obervations as well as the previous observations of the ability of 100µg M to maintain HHT and HHA axes but not of the HHG axis in PX birds together indicate the necessity of both, an optimum level as well as duration of M for normal homeostasis. This view is strengthened by the unpublished observations of the ability of low dose M implants in PX birds to totally prevent all PX effects and low dose M implants in intact birds to mimic the actions of single injections of 50µg M in intact birds or single injections of 100µg M in PX birds. It appears [illegible] that though short lived elevation in M is sufficient to maintain the HHA & HHT axes as well as to negate the PX effects on pancreas, an optimum threshold level of M for some minimum

duration of time is required to maintain the HHG axis and pancreatic functions.