CHAPTER II

ALTERATIONS IN CARBOHYDRATE METABOLISM AND PANCREATIC ISLET FUNCTIONS IN PIGEONS TREATED WITH PINEAL INDOLES AND pCPA.

A large part of avian metabolism is geared towards provision of energy for reproduction (see Ralph,1981). Carbohydrates are by far the immediate and the most important metabolic reserve utilized by animals for various activities. Seasonal reproductive activities involving gonadal development and regression can be expected to bring about altered metabolic activities and energy flux. Metabolic contents and activity levels of enzymes have been shown to undergo adaptive changes during seasonal breeding activites in pigeons (Ayyar,1987; Patel and Ramachandran,1987; Ramachandran and Patel,1987; Patel et al.,1988). Quay (1970) opined that "one of the areas of pineal endocrinology most difficult to evaluate is that relating to associated phenomena in liver, tissue metabolism and blood chemistry". Some of the earliest reports had suggested "Pinealin" a pineal polypeptide to be a potent and specific hypoglycemic factor in mammals (Milcu et al.,1957,1963). The workers of this school believed the pineal polypeptide to be acting synergistically with insulin and to have protective action on the pancreatic β cells of animals treated with alloxan (see Damian,1989). Some workers reported hypertrophy of pancreatic islets

following chronic injection of pineal extract (Notario,1956; Petronio and Tavazza,1958). Related reports in this regard are PX induced hyperinsulinemia (Milcu et al.,1971; Gorray et al.,1979) and the decreased glucose tolerance (Milcu et al.,1971) in rats. The above reports indicate a role for pineal in glucose homeostasis. Besides, a season specific influence of pineal on carbohydrate metabolism and glycemic status has also been shown in the goldfish, Carassius auratus (Delahunty et al.,1978,1980; Delahunty and Tomlinson,1984). Influence of pineal on glycemic status of rat, rabbit and pigeon has also been reported (Mihail and Giurgea,1979; Murlidhar et al.,1983; Diaz and Blazguez,1986; John et al.,1980).

Previous studies from this laboratory have shown the involvement of pineal in the seasonal reproductive activities of pigeons (Patel et al.,1985; Ramachandran and Patel,1986) and associated alterations in carbohydrate metabolism (cited above). Moreover, altered glycemic response to exogenous glucose, insulin, glucagon and adrenalin has also been reported to occur in PX pigeons (Patel and Ramachandran,1989; Ramachandran and Patel,1989). These, as well as the previous observations showing the influence of pineal indoles (M, ML & MT) and pCPA-induced chemical pinealectomy on testicular recrudescence (Chapter I), prompted the present investigations on the possible

impact of pineal indoles and pCPA on carbohydrate metabolism and pancreatic islet functions in the pigeon.

MATERIALS AND METHODS :

Procurement and maintenance of pigeons, experimental setups and preparation of solutions are as outlined in chapter I.

Parameters and methods of evaluation:

Blood glucose : Prior to decapitation of pigeons, 0.1 ml of blood was drawn from the brachial vein by a needle prick. Blood glucose level was estimated by the method of Winckers and Jacobs. (1971). The glucose concentration was expressed as mg/dl.

Hepatic and Muscle Glycogen Content : The glycogen content was estimated employing the method of Seifter et. al. (1950). Small pieces of tissues were dropped in preweighed test-tubes containing 2 ml of 30% KOH. Glycogen was precipitated with 95% alcohol. The diluted precipitates were treated with anthrone reagent and colour intensity was read colorimetrically at 620 nm.Glycogen content was expressed as mg/100mg tissue weight.

Hepatic Glucose-6-phosphatase (G-6-P'ase/E.C.3.1.3.9) : Liver tissue homogenate was prepared in cold citrate buffer. Enzyme activity was assayed by the method of Harper(1960). Glucose-6-phosphate disodium salt (Sigma chemicals, USA) was used as the substrate. Inorganic phosphate released was estimated as per the method of Fiske and Subbaraw (1925) and the colour intensity was read at 660 nm on a Klet Summerson colorimeter. Enzyme activity was expressed as μ moles phosphate released/mg protein/15 min.

Hepatic phosphorylase (E.C.2.4.1.1) : Total phosphorylase activity was assayed by the method of Cahill et. al. (1957) using glucose-1-phosphate dipotassium salt (Sigma chemicals, USA) as the substrate. The inorganic phosphate released was measured by the method of Fiske and Subbaraw (1925). Enzyme activity was expressed as μg phosphate released/mg protein/15 min.

Differential staining of Pancreas : It was performed using chrome alum haematoxylin-phloxine stain as described by Bancroft and Stevens (1977).

RESULTS :

Blood Glucose : The normal blood glucose level in pigeons

Treatments	Blood Glucose (mg/dl)	Tissue Glycogen (mg/100 mg tissue)		Hepatic G-6-P'ase (μ moles PO_4 released/mg protein/15 min.)	Hepatic Phosphorylase (μg PO_4 released/mg protein/15 min.)
		Liver	Muscle		
CONTROL	166.55 ± 15.54	1.82 ± 0.18	0.73 ± 0.11	1.82 ± 0.19	12.41 ± 2.48
MM	126.23* ± 16.63	0.46* ± 0.05	0.35* ± 0.06	2.98* ± 0.34	35.08* ± 6.41
ME	135.45* ± 18.25	0.51* ± 0.05	0.30* ± 0.07	2.84* ± 0.30	36.83* ± 6.57
ML	217.64* ± 19.23	2.35* ± 0.44	0.82 ± 0.08	1.24* ± 0.14	9.33* ± 1.45
MT	208.11* ± 18.10	3.55* ± 0.57	1.27* ± 0.07	1.07* ± 0.11	8.19* ± 0.97
pCPA	86.41* ± 10.49	1.03* ± 0.48	0.40* ± 0.04	1.01* ± 0.14	11.27 ± 1.38

Table 2.1 :Alterations in blood glucose, tissue glycogen content and activity levels of G-6-P'ase and phosphorylase in liver of pigeons treated with methoxyindoles and pCPA in the recrudescant phase .

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

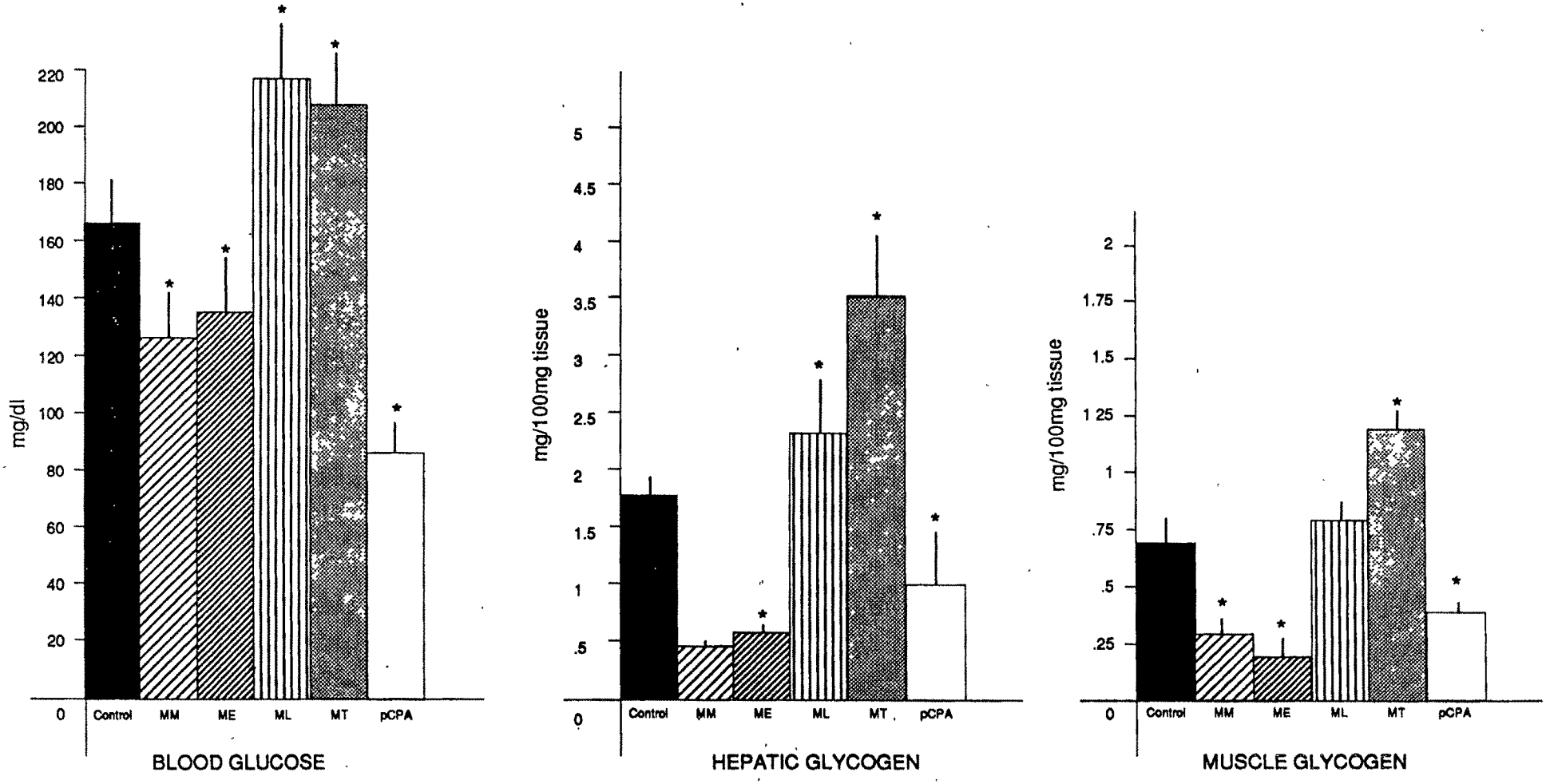

Fig. : 2 A Alterations in blood glucose and tissue (Hepatic and Muscle) glycogen content of pigeons treated with methoxyindoles and pCPA in the recrudescent phase.
(* Significant at $p < 0.05$; values are $\bar{x} \pm SD$)

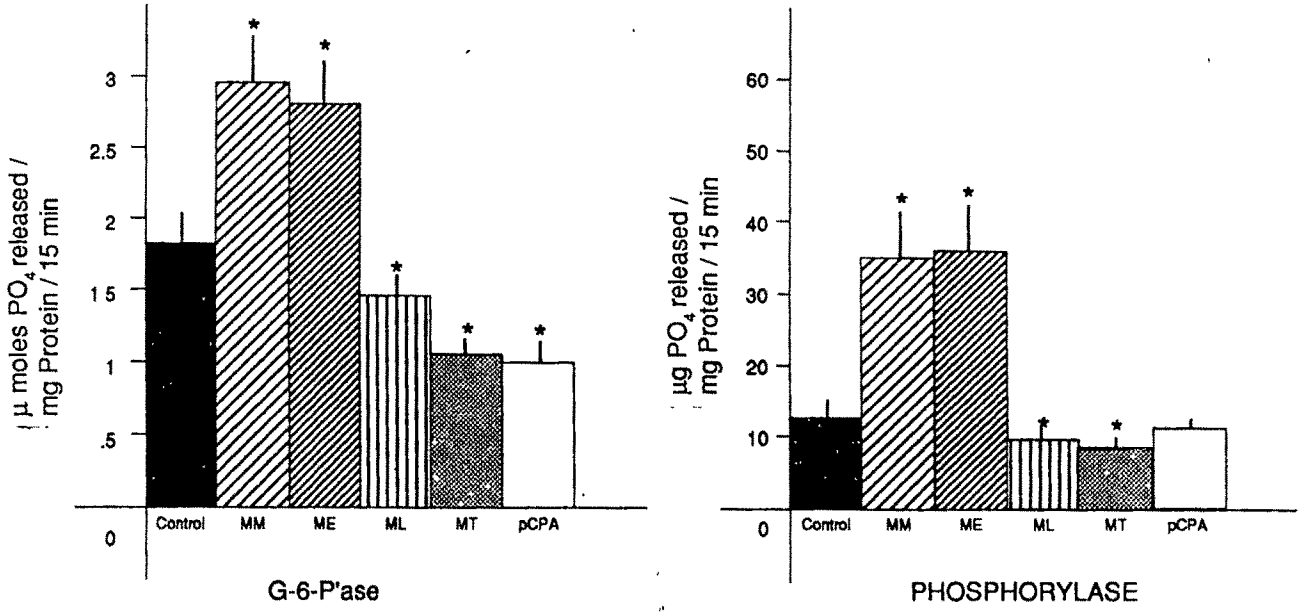

Fig. : 2B Changes in activity levels of hepatic G-6 P'ase and phosphorylase in pigeons treated with methoxyindoles and pCPA during the recrudescent phase.
(* = Significant at $P < .05$; values are $\bar{x} \pm SD$)

during the recrudescent phase was 166.55±11.76 mg/dl. Treatments with M and pCPA induced significant hypoglycemia. At an average M treatments caused about 20% and pCPA about 50% decrement in glycemic level. In contrast, treatments with ML and MT brought about hyperglycemia and the increase in glycemic level was more than 20% (Table- 2.I Fig.-2A).

Hepatic and Muscle Glycogen contents : The glycogen content of liver and muscle were significantly decreased in MM, ME and pCPA treated groups of birds. In contrast, ML and MT treatments increased the glycogen content of liver and muscle (Table -2.I ; Fig.-2A).

Hepatic Phosphorylase and G-6-P'ase : Both, hepatic phosphorylase and G-6-P'ase activity was significantly increased in M treated birds. Activity of both the enzymes was decreased in pCPA as well as ML and MT treated birds.(Table-2.I Fig.-2B).

Histological observations on Pancreatic Islets :

Differential staining for A and B cells of islets revealed significant degranulation of A cells in M treated pigeons and B cell degranulation in pCPA treated birds. However, ML and MT treatments showed increased granulation in both A and

B cells suggesting decreased secretion of both the hormones.

DISCUSSION :

The present findings add many novel features with regard to pineal and carbohydrate metabolism in birds specially and vertebrates in general. It is clear that chronic M treatment as well as long term PX induce hypoglycemia and glycogen depletion from liver and muscle. Such changes in pigeons subjected to chemical pinealectomy by pCPA are in agreement with previous observations from this laboratory on surgically pinealectomised pigeons (Patel et al.,1983,1988; Patel and Ramachandran,1989; Ramachandran and Patel,1989). A survey of available literature on this topic projects an apparently confusing and contradictory picture. The reports of hyperglycemia in both PX and M treated mammals (Murlidhar et al.,1983; Diaz and Blazquez,1986), PX-induced hyperglycemia and, hypoglycemia due to injection of pineal extracts in mammals (See Damian,1989) and PX-induced hyperglycemia and hepatic glycogen depletion and M-induced hypoglycemia and glycogen deposition in the gold fish (Delahaunty et al.,1978; Delahunty and Tomlinson,1984) adequately illustrate the point. Apparently, pineal cannot be accredited a pan-vertebrate role in carbohydrate metabolism and the role of the pineal seems to be dependent on the species as well as on the thyroid and adrenal

secretions (Chapter I). A clue to the phase relationship between pineal secretions and pancreatic hormones is provided by the currently observed pronounced A cell degranulation under M treatments suggesting an increased glucagon secretion. This inference is strengthened by the recorded increase in the activity levels of hepatic phosphorylase and G-6-P'ase. The observed decrement in the hepatic and muscle glycogen contents is in complete harmony with the above purported changes. However, the attendant hypoglycemia strikes a discordant note. But based on the unpublished observations emanating from the many on going investigations in this laboratory indicating increased lipogenesis and ascorbic acid biogenesis, it can be assumed that the M induced hypoglycemia is essentially due to withdrawal of glucose for the above processes. Support to our contention comes from the report of Muller et al.,(1988) suggesting a glucagon synergistic effect of M based on their in vitro studied on liver slices.

In this context, pCPA treatment (chemical pinealectomy) induced decrease in hepatic and muscle glycogen content and hypoglycemia is paradoxical. A careful consideration of the observations indicate decreased hepatic phosphorylase and G-6-P'ase activity and pronounced B cell degranulation in the pancreas. Apparently, these changes tantamount to decreased glucagon secretion and increased insulin

secretion. This is fully confirmed by the previous observation of hypoglycemia and increased glucose tolerance and increased insulin sensitivity in the PX pigeons (Patel et al.,1983; Ramachandran and Patel,1989). The decreased hepatic glycogen content is due to the basal rate of glycogenolysis in the absence of glycogenesis. The latter aspect is due to the suppressive effect of PX on the hepatic glucose uptake (Patel and Ramachandran,1992). However, the hypoglycemic status is due to insulin induced increased peripheral utilization as denoted by a three fold increase in glucose uptake by the muscle of PX pigeons (Patel and Ramachandran,1992). In the present context, the avian system seems to be opposite to that of mammals as, some reports available indicate reduced insulin secretion and reduced insulin receptor concentration in PX rats (Victoria et al.,1989).

Except the report of Vaughan et al. (1983), showing hyperglycemic response to both ML and MT in the syrian hamster, there are no other studies related to these indoles and carbohydrate metabolism. The present study is the first in this respect and has provided evidences for physiological effects of these indoles in the pigeon. Incidently, both ML and MT induced hyperglycemia and glycogen deposition in liver and muscle. Histologically, the pancreatic islets of ML and MT treated pigeons depicted a picture indicating

reduced secretion of both glucagon and insulin. Concomitantly observed activity levels of hepatic phosphorylase and G-6-P'ase are in agreement with the purported reduction in the glucagon level. The observed increase in the glycogen stores seems to be a consequence of reduced /decreased glycogenolysis. The hyperglycemic status is related to the decreased hepatic and peripheral utilization of glucose in the wake of purported simultaneous decrease in the insulin level. A unique aspect of ML and MT action seems to be to lower the overall glucagon : insulin ratio, a crucial aspect of avian carbohydrate metabolism (Hazelwood,1984), probably by activating D cell secretion of somatostatin (SRIF) as, SRIF has been shown to decrease the release of both glucagon and insulin in birds (Hazelwood,1986).

The highlights of the present findings are :

1. Melatonin increases glucagon:insulin molar ratio,
2. Pinealectomy decreases glucagon:insulin molar ratio,
3. Methoxytryptophol and Methoxytryptamine decrease the overall glucagon: insulin molar ratio.

Apparently, pineal seems to have an intricate relationship with pancreatic functions and thereby on carbohydrate metabolism.