

GENERAL CONSIDERATION

Pineal has been implicated in thermoregulation, reproduction and many cyclic processes in mammals. However, in the case of birds, except for locomotory and perching activities, ^{an understanding of} its role in other functions like reproduction and endocrine activities has by far remained inadequate. Available literature on pineal and reproduction in birds preclude evolving any uniform concept, let alone, suggest a pan-avian role. Due to the lack of concerted and comprehensive studies, the information base has remained fragmented and inconsistent. It is in this context that studies were initiated in this laboratory to understand pineal functions in a single avian species, the pigeons, Columba livia. Earlier studies revealed a progonadal role for the pineal as pinealectomy (PX) in the reproductively active phases led to testicular collapse (Ramachandran et al., 1987; Ramachandran and Patel, 1988). It was also shown that PX brings about alterations in the levels of metabolites and activities in enzymes thereby implicating the pineal in subtle modulations of metabolic functions also (Patel et al., 1983; Ramachandran and Patel, 1987). Though PX-induced alterations have been studied, the direct actions of pineal principles needed evaluation. Hence in the present study, the actions of pineal indoles (melatonin-M, methoxytryptamine-MT and methoxytryptophol-ML) as well as

that of chemical pinealectomy by pCPA have been evaluated.

A uniform anti-testicular role has been noted for all the three indoles used as well as for pCPA. Moreover, a time independent action of melatonin (M) also emerged, as exogenous M given either in the morning or evening brought about testicular regression. Obviously, two concepts became established i.e. 1. all pineal indoles are antigonadal and 2. the action of M is time independent unlike in mammals. However, the studies also threw up a paradox as both PX as well as treatment with pineal indoles induced testicular involution. Involvement of differential modus operandi appeared to be the only answer to this paradox. An understanding of the modus operandi necessitated looking into other changes, especially the functions of other endocrine glands known to have effects on reproductive organs. Concurrent evaluations of adrenal and thyroid functions revealed differential effects of PX and pineal indoles. It is observed that whereas MT and PX-induced adrenocortical regression and lowering of plasma corticosterone (B) level coupled with accelerated colloid depletion from thyroid follicles and elevated plasma thyroid hormone level, M and ML caused diametrically opposite effects. Obviously, pineal has influences on HHT and HHA axes apart from the HHG axis. Inferably, M has a stimulatory action on HHA axis and inhibitory action on HHG axis and

absence of M or lowered levels of M has opposite effects.

In the light of the many studies reporting thyroid-induced testicular regression in birds (Chandola and Bhatt,1982; Pathak and Chandola,1983;see Thapliyal,1993), the PX-induced testicular involution observed in the pigeon appears to be a consequence of the hyperactive HHT axis. This was confirmed by the unpublished observation of thyroxine (T₄)-induced testicular regression in the breeding season. Since M produced opposite effects of activation of HHA axis and inhibition of HHT axis, changes which are favourable for gonadal function, the testicular involution induced by M appears not to be mediated through its action on HHT or HHA axis. A definite antigonadotropic action of M has been clearly established (Ralph,1978; Reiter,1980,1981; Blask,1981; Volrath,1981; Chanda and Biswas,1988). This antigonadotropic influence of M has been related to its inhibitory effects on the hypothalamic GnRH neurons in mammals (Silman,1991). Even in birds, there is some indication that M affects the GnRH neurons (Ohta et al.,1989). In this background,it is safe to assume that higher levels of M can bring about testicular regression in birds through its inhibitory action on the HHG axis.

To test the concepts evolved above, further studies were conducted in the form of M replacement to PX

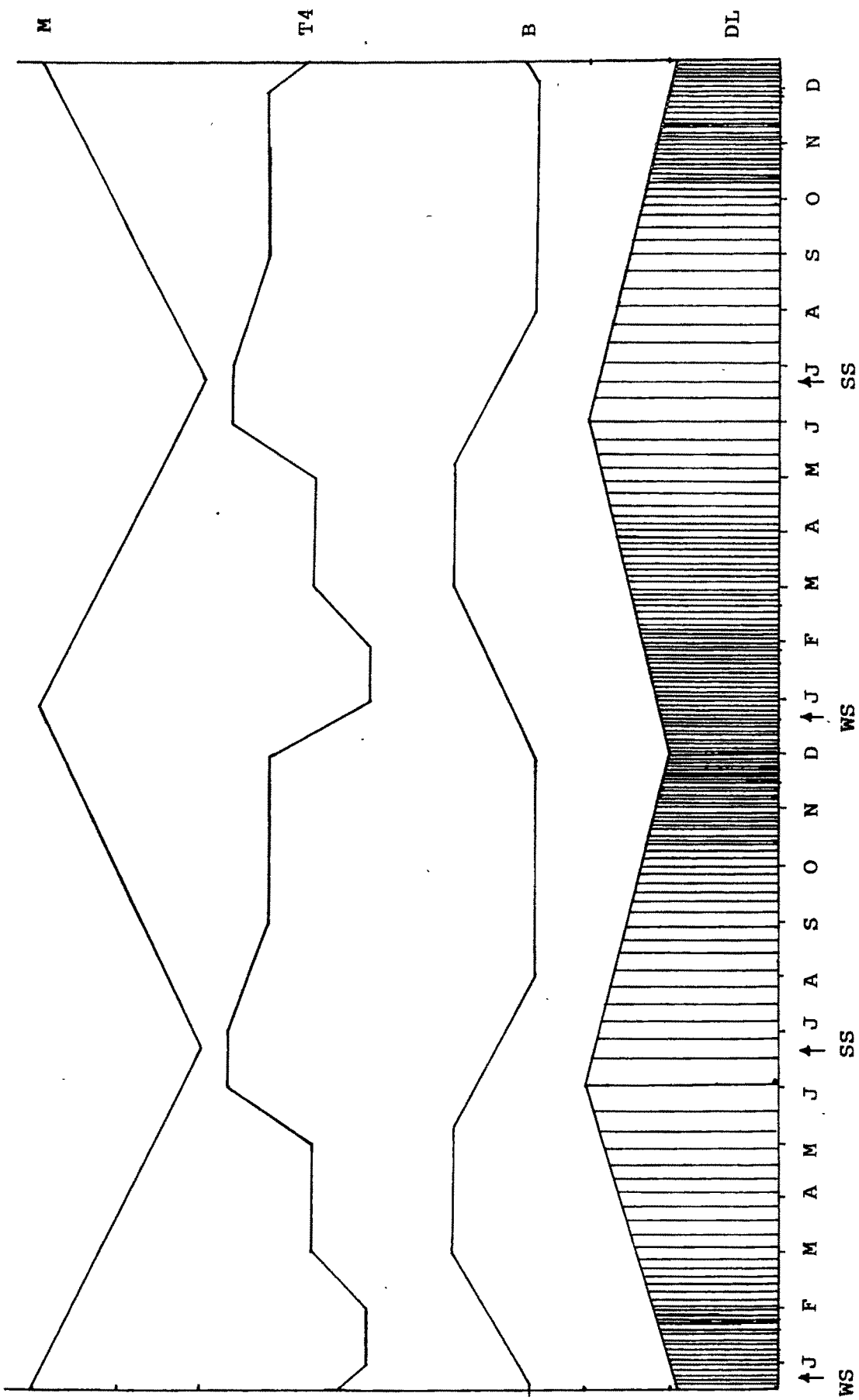
pigeons. Of the two dosages employed, whereas the lower dose (50 ug) did not prevent the occurrence of PX effects, the higher dose (100 ug) was successful in preventing the effects on adrenal and thyroid functions though testicular involution could not be arrested. These observations confirmed the earlier idea that higher M levels can inhibit the HHT axis and activate HHA axis. Since the treatment was in the form of injections, it also became evident that, the effects on HHT and HHA axis could be exerted by a high amplitude even of low duration M elevation. However, an ongoing study showed that low dose M implantation in PX birds prevented the testicular involution. This tends to suggest the requirement of a low amplitude but long duration M for keeping the testis in active phase. The suppressive action of M on thyroid function coupled with its stimulatory effects on adrenocortical activity were manifested even during the non-breeding phase. This was shown by the decrease in the prevailing plasma T4 level and an increase in B level in birds administered with M.

Based on the observations made throughout the year, four clear cut phases in terms of testicular activity could be made out. These phases are 1. Recrudescent or the phase of gonadal activation during Jan-Feb. 2. Breeding phase or fully active phase during March-May 3. Regression or phase of gonadal involution during May-June and 4.

Non-breeding or quiescent phase during July-December. It can be deduced from these cyclic changes that gonadal recrudescence occurs immediately after ^{the} winter solstice, while gonadal regression occurs prior to ^{the} summer solstice. Based on this fact and the observations on changes in the profile of plasma thyroxine and B levels as well as the experimental observations involving PX and pineal indoles, a tentative hypothesis on the neuroendocrine regulation of reproductive cyclicity in the pigeon can be fashioned out. Apparently, prior to gonadal recrudescence the high threshold level of M attained closer to ^{the} winter solstice effectively suppresses the HHT axis leading to lower titres of plasma hormones. Concurrently, M also activates the HHA axis. Under such pervading permissive environment, gradually increasing daylengths subsequent to ^{the} winter solstice activates the HHG axis due to the decreasing M level, thereby inducing testicular recrudescence. It is also likely that the HHG axis becomes refractory to the inhibitory influence of M by this time. Later, as the day length during the summer months keeps increasing, the reducing M level gradually loses its inhibitory effect on the HHT axis leading to increasing thyroid hormone output. Upon reaching an effective threshold level of circulating thyroid hormone level prior to ^{the} summer solstice (May-June), it exerts an inhibitory action on both the HHG axis as well as on the gonads leading to sudden testicular collapse. Soon the increasing M level subsequent

to the summer solstice keeps the HHG axis effectively suppressed, (though the thyroid hormone starts decreasing) and the testis in a long quiescent state. Since the B level is higher during the active phases and lower during the inactive phase, it is presumable that this steroid has a permissive influence on gonadal activation and its function. This is confirmed by the previous observations from this laboratory showing testicular involution following adrenocortical suppression in the breeding season (Ayyar, 1987; Ayyar et al., 1992). However, the exact role of corticosteroid is not very clear and needs further experimentation. A synthesis of the above concept is depicted in fig.1.

On the metabolic front, carbohydrate metabolism was noted to undergo seasonal alterations and these alterations were affected by pinealectomy (Patel et al., 1988; Ramachandran and Patel, 1987). Pinealectomy was also shown to induce increased glucose tolerance and insulin sensitivity (Ramachandran and Patel, 1989). Besides, altered glucose uptake by liver and muscle was also noted in PX pigeons. Since these observations suggested a definite role for pineal in regulating carbohydrate metabolism in birds, the possible effects of pineal indoles and pCPA on parameters related to carbohydrate metabolism were assessed in the present study. Moreover, as various factors of



M = Melatonin (Presumed) WS = Winter Solstice. SS = Summer Solstice.
 T4 = Thyroxine B = Corticosterone DL = Day length
 Fig 1: Schematic presentation of circannual variation in day-lengths and plasma levels of
 M, T4 & B in relation to gonadal cyclicity.

carbohydrate metabolism are principally influenced by pancreatic hormones, changes in islet cell function were also noted. It became evident that though all the pineal indoles tested showed antigonadal effects, their effects on carbohydrate metabolism were differential. Though M and pCPA induced hypoglycemia and tissue glycogen depletion as was the case in PX (Patel et al., 1983; 1988), ML and MT produced hyperglycemia and increased tissue glycogen content. As in the case of gonadal effects, M seemed to apparently simulate the changes induced by PX. A careful scrutiny however revealed a differential modus operandi in inducing similar effects. Histological observations of pancreatic islets showed increased B cell degranulation in PX pigeons and increased A cell degranulation in M treated birds. In contrast, ML and MT treatments depicted increased granulation of both A and B cells. These observations suggest a functional relationship between pineal principles and pancreatic hormones. The increased insulin : glucagon molar ratio due to PX was deemed responsible for the observed hypoglycemia by way of increased peripheral utilisation of glucose. The decreased hepatic glycogen content was related to the decreased hepatic glucose uptake due to PX. In contrast, increased glucagon : insulin molar ratio in M treated birds was responsible for the decreased hepatic glycogen content. This

was confirmed by the increased hepatic phosphorylase and G-6-p'ase activity. However, the hypoglycemic status was accredited to increased steatogenesis and ascorbic acid biogenesis. Methoxytryptophol and methoxytryptamine showed a differential relationship with pancreatic functions and seemed to inhibit the release of both the pancreatic hormones. The observations and conclusions derived foretell pineal-pancreatic inter-relationship. In fact, possible relationship between pineal secretions and pancreatic hormones have also been hinted by other workers on other mammals and birds (Notario, 1956; Petromio and Tavazza, 1958; John et al., 1980). The possible influence of pineal, in modulating the release of pancreatic hormones, ^{in inducing} alterations in tissue sensitivity to pancreatic hormones as well as ^{in the} commissioning of other metabolic pathways, all need to be ascertained to provide a clear picture regarding pineal-pancreatic inter-relationship and attendant effects on carbohydrate metabolism. The ability of M to cause increased glucagon secretion had been confirmed by the observations made in experiments involving M replacement to PX birds. This action of M to induce glucagon release has proved to be independent of any seasonal influence.

Photoperiod as an effective environmental clue in synchronising reproductive cyclicity has been very clearly established in temperate species of birds over the years (see Mu^hton and Westwood, 1977; Follet and Robinson, 1980; Follet, 1984; Nicholls, 1988). However, its role in annual gonadal cyclicity of tropical and sub-tropical species of birds was not considered very important but, some studies of late have shown the importance of photoperiod as a synchroniser in some of the tropical species of birds (see Thapliyal and Gupta, 1989). Even some of the species which were considered not dependent on light for their reproductive activities have shown photosensitivity in terms of gonadal activation under experimental conditions (Thapliyal, 1993). Since the pigeons showed gonadal activation and inactivation coinciding with winter and summer solstice respectively, it is likely that subtle changes in daylength may modulate the neuroendocrine reproductive axis on a seasonal basis. The second part of the thesis is a preliminary attempt to throw light on this line of thinking. The experimental design involved testing the photosensitivity of intact and PX pigeons for gonadal activation by exposing the birds to a long photoperiod of LD18:6 at two different time periods closer to the normally expected time of recrudescence. From the results obtained, two conclusions could be easily drawn 1. PX birds are

incapable of showing the changes induced by photostimulation in intact birds, 2. Long photic schedule has a stimulatory influence on testicular recrudescence but this is expressed only towards the fag end of the quiescent phase. Obviously absence of pineal prevents the expression of gonadostimulatory as well as other associated changes of photoperiod atleast in the immediately ensuing breeding cycle. Whether a normal gonadal activation would occur in the next cycle is a matter of conjecture. It is also apparent that some sort of photorefractoriness may exist during the quiescent phase and the dessipation of this refractoriness occurs only towards the end of the quiescent phase just prior to winter solstice. Further detailed experimentation are required to cement the concept of photorefractoriness in pigeons and to clearly elucidate the underlying factors or mechanisms.

The earlier mentioned parallel adrenal-gonad relationship and the observation that long photoperiod stimulates adreno-cortical activity, served as a basis for evaluating the effects of induced adreno-cortical excess or insufficiency under long photoschedule. Both adreno-cortical excess as well as insufficiency showed inhibitory influences on testicular recrudescence occurring under normal light dark (NLD) schedule. Further, they also proved retardatory on long photoperiod-induced gonadal activation. Though a

parallel-adrenal-gonad relationship was established and long photoperiod was recorded to show adreno-cortical activation, the retardatory influence exerted by ~~CORT~~ appears a bit enigmatic but the similar effects in the form of increased thyroid output under both ~~CORT~~ and DXM treatment suggested a common effect on the HHT axis as marked by the decreased colloid content in the thyroid follicles. In the already discussed antigonadal effects of T4, the inhibitory influences exerted by both ~~CORT~~ and DXM on testicular activation appears to be due to a common locus of action related to HHT axis. It is likely that exogenous ~~CORT~~ administration to birds which have already increased their adreno-cortical output under the long photic schedule may have created a hypercortical situation leading to suppression of HHA axis. This would also suggest that both hypo-and hypercorticalism may have inhibitory effects on testicular recrudescence. Further experimentations are required to clarify these points. It is likely that corticosteroids may exhibit a low degree of HHG axis activation which is by far nullified by either adreno-cortical excess or insufficiency. Finally, it can be concluded that whereas long photoperiod and increased adreno-cortical activity may be permissive for testicular activation, an optimal level of corticosteroids seems to be necessary for gonadal activation in the pigeon. Observations made with reference to photoperiod and

carbohydrate metabolism and the effect of altered functional status of the adrenal on the same have highlighted the importance of effective phase relationships between various hormones in regulating carbohydrate metabolism. An important change in carbohydrate metabolism associated with gonadal recrudescence is glycogen utilization. This change is photo-inducible and occurs only towards the end of quiescent phase. Earlier to that, there seems to be some sort of photorefractoriness in this respect and may have some causal relationship with the observed photorefractoriness of the HHG axis. Further, adreno-cortical excess or insufficiency altered the photoperiod induced glycemic status differentially suggesting intricate interrelationship between hormones like corticosterone, insulin, glucagon and prolactin. Possibility of altered phase relationships between these hormones on the purported hypothalamic glucoregulatory centre also cannot be overruled. The observations made herein tend to provide validity to the concept of hormonal phase relationship in regulating carbohydrate metabolism as has been shown with respect to lipid metabolism (Meier and Cincotta,1993). Future experimentations on this line would prove fruitful in understanding the significance of hormonal phase relationships in the many intricate variations in carbohydrate metabolism not only between various classes or species of animals but even between individuals.