

## GENERAL CONSIDERATIONS

About two decades back, pineal was for all purposes in scientific parlour referred to as a vestigial organ. This designation, to a greater extent dampened the curiosity of biologists and precluded any serious attempt to study the gland in its own merit. Since then, some of those adventurous workers interested in exploring the non-entites did however keep probing this enigmatic structure. As a result of such investigations into a supposedly dark blind alley, many faint, poorly illuminated illusionary exits took shape. It is these experimental forays which have by now established quite a bit of the phylogeny, morphology, cytology, chemistry and pharmacology of the pineal organ. Phylogenetically, pineal appears to be represented in all vertebrate groups except in the hagfish (*Myxine*), crocodileans, edentates and perhaps dugongs (Wurtman *et al.*, 1968). According to Hulsemann (1967), Machado (1963) and Di Dio (1963) even in these species some sort of diffuse pineal tissue is suspected. In some,

like procavians, elephants, opossums and whales very much reduced pineal is the feature. Such widespread occurrence of pineal bespeaks of a constancy like that of pituitary rather than an evolutionarily regressive structure. Despite this constancy, no common function could be ascribed to the pineal in vertebrates as a whole.

However, the many experimental excavations into the elusive and illusionary outlets of the alley have crystallised scientific insight into three reasonably convincing channels. These three channels represent the three commonly implicated functions of pineal: reproduction, rhythmicity and thermoregulation. Anatomical and cytological distinction into a saccular and parenchymal pineal characteristic of cold blooded and warm blooded vertebrates respectively have given rise to the notion of a functional dichotomy into photoreception and secretion. Though such a functional dichotomy is inherent, it is likely that both functions are integral to both types of pineal with only a difference in the degree of specialisation. Accordingly, predominant photosensitivity in lower vertebrates, and secretion in higher vertebrates may represent only a necessary evolutionary adaptation, with both, nevertheless, retaining at the functional level the ability to influence reproduction, rhythmicity and thermoregulation/thermal

adaptation. With the renewed interest in pineal structure and function, the study of this gland is currently in an exciting phase; however many pitfalls and temporary frustrations have also been experienced. During the last ten years or so, accretion of evidence has slowly occurred to implicate the pineal in many pivotal physiological functions. Nevertheless, ignorance rules the roost as far as the mechanisms of its functions are concerned.

Influence of pineal in reproductive functioning is better documented in mammals. The bulk of the evidences at hand appears to indicate an antigonadal role for this gland. Most persuasive literature comes from the golden hamster in which this structure is considered to modulate seasonal reproductive rhythms (Reiter, 1974; 1975; 1978). In lower vertebrates like fishes, amphibians and reptiles, the function of photoreception is better established. Though less emphasized, the thermoregulatory role of pineal has been more readily demonstrated in more classes of vertebrates. The present chasm between our intuition of the importance of pineal, and our ignorance of what the importance is, and how is it mediated, is more pertinent to the avian pineal. It is quite likely that the avian pineal represents a conglomerate of all the above functions as scattered evidences are available for each.

Good evidence has been accumulated for the involvement of avian pineal in circadian rhythmicity ( Gaston and Menaker, 1968; Binkley <sup>e</sup> et al., 1971; McMillan, 1972; McBride and Ralph, 1972; Cogburn et al., 1976; ~~McBride and Ralph, 1972; Cogburn et al., 1976;~~ Rutledge and Angle, 1977; Gwinner, 1978). A general scan of the relevant literature on the possible role of pineal in reproduction among aves fails to yield a common case. In fact, a progonadal, an anti-gonadal and even no relation with the gonads whatsoever have all been suggested in various species of birds. Quite likely, negative results might occur due to the fact that appropriate experimental designs have not been made or proper challenges have not been presented especially with regard to temporal features (Ralph et al., 1979).

It is this temporal aspect which gains credence, as seasonal cycling of the state of reproductive structures is of greater significance in avian than in mammalian species. Besides, the occurrence of pineal in almost all vertebrates also implies the possibility of pineal participating in subtle modulations and regulations of various physiological processes, if not directly (Halbeitz ~~1979~~) indirectly. Speculations on these lines have been sounded by other workers also. "There is now considerable documentation that the mammalian pineal participates in the affairs of the gonads, the thyroid, the brain and several

other organs and organ systems. One can also find evidence to support the argument that sub-mammalian pineal organs influence pigmentation and behaviour. However, it has not yet been demonstrated that any physiological process in the body is controlled primarily by pineal organs. And presently, no one knows what pineal organs, be they photo-receptive or secretory, are for. This situation is not unique to the pineal, and certainly should not lead potential students of pineal physiology to conclude that the pineal has no function. An alternative explanation is that the appropriate questions about pineal function remain to be asked" (Wurtman et al., 1968). Recently, Ralph et al. (1979) have stated "There has been much well founded speculations about special physiological roles of pineal organs in a few species, but it has proven difficult to formulate a pan vertebrate role for pineal organs. This situation may indicate that either pineal bodies do not have identical functions in different kinds of animals or that they participate in some central, fundamental, regulatory mechanism that underlines the operation of several different organ systems. If the latter is true, one might expect to discover subtle relationship between pineal organs and a number of physiological functions. Such appear to be the case, especially in regard to endocrine organs". Wurtman and Tay (1969) have conjectured pineal gland to be a neuro-endocrine device, receiving

exogenous and endogenous input and having outputs to several peripheral systems. Further, Quay (1969) thought that pineal organs are primarily concerned with adaptation to the environment.

The present studies are an offshoot of these prevailing facts and speculations and have primarily attempted to establish the possible relationship if any, between pineal and gonads together with other endocrines and also between pineal and metabolic physiology on a temporal basis in relation to seasonal reproductive cyclicity of wild feral blue rock pigeon, Columba livia. Pinealectomy has served as the tool for the above evaluations and besides, intact controls have been used to establish the normal seasonal alterations based on which deviations occurring in the absence of pineal would enable in establishing the subtle seasonal involvement of this neuro-endocrine transducer in the economy of an avian body.

Results obtained in the present study indicate definite alterations in body physiology and total body economy in relation to reproductive cyclicity. Such seasonal fluctuations essentially involved progressive synthesis and utilization of metabolic reserves during late regression through recrudescence and, late

recrudescence through breeding respectively. Late breeding-early regression was marked by a general exhaustion of energy stores which got stabilised to a basal level by mid regression, only to enter into a new wave of synthesis and utilization during the next active phases of breeding. Gonadal activation and ensuing breeding processes involve highly geared up metabolic participation as denoted by the changes in carbohydrate and lipid reserves of the body. Lipid utilization seems very prominent during both recrudescence and breeding. This is well exemplified by the increased activities of both SDH and ATPase in various organs. The immediate energy supply for gonadal recrudescence appears to be provided by the muscle as noted by the decreased muscle glycogen content during the recrudescence period. However, with the commencement of gonadal functioning, hepatic glycogen store does take over the responsibility of supplying the carbohydrate moities. Increased metabolic efficiency would entail active metabolite transfer, characteristic metabolic transformations etc. Herein observed alterations in ascorbic acid content as well as  $\text{Na}^+$  and  $\text{K}^+$  ions seem to be favourable changes in this respect. Increased  $\text{K}^+$  ion content paralleled by decreased  $\text{Na}^+$  ion content in serum and tissues portend conditions conducive to efficient metabolic utilization of energy store of the body (Chapter 6). High stores of ascorbic acid have been correlated with increased metabolic

activity (Chinoy, 1969; 1970; Chinoy et al., 1973; 1978). In this light, the build up of ascorbic acid content by late regression/early recrudescence and its depletion during recrudescence and breeding would again indicate metabolic activation in response to the breeding requirements (Chapter 7). Phosphatases are reported to show increased activity in conjunction with gonadal activation and breeding (Patel, 1976). Phosphatases by their multifarious involvement in many metabolic and allied processes can be purported to play useful roles in recrudescence and breeding associated adjustments in metabolic strategies. Corresponding to the decreased hepatic glycogen content, blood glucose level was also lowered during breeding (Chapter 1). This would indicate accelerated movement of glucose from the blood into the peripheral organs. Plasticity towards higher levels of blood glucose appears to be a feature of glucogen oriented metabolism as in pigeon and lizards, and the attainment of elevated blood glucose during recrudescence may have to be looked upon as a build up of a ready store for utilization during breeding. Since the hepatic glycogen content is also noted to show a parallel increase, hepatic glycogen as the source of blood glucose becomes untenable and in this wake, reduced peripheral utilization coupled with increased dietary uptake should be responsible for the recrudescence build up

of blood glucose noted in the present study. Though carbohydrates, in general, might be considered to play a negligible role during gonadal activation occurring in the recrudescence period, the observed decrease in lipids (Chapter 5) suggests a lipid involvement in the initial phases of gonadal recrudescence. In fact, lipids appear to be utilized during both recrudescence and breeding and the principal lipid component that has been calculated to be responsible for the observed fall in total lipids of liver and muscle is the triglycerides (Chapter 5). Liver being the principal centre for lipid esterification in birds, it is quite likely that the reduced hepatic lipid content during breeding might also denote decreased flow of glucose into lipogenic pathways which would once again emphasize the utility value of carbohydrates during the breeding months. Importance of cholesterol in breeding activities cannot be denied and in this connection, the increased cholesterol content of liver and muscle during recrudescence and breeding in feral pigeons is self explanatory. The most interesting observation is the constancy of adipose tissue lipid content which is maintained steady in all the three reproductive phases. Predictably, adipose tissue lipids remain insensitive to the alterations in mileu inter<sup>n</sup>ae or the internal environment occurring in conjunction with annual gonadal cycle.

In this context, it would be worth pondering whether adipose tissue lipids would become sensitive in the free living natural conditions of feral pigeons with the full expression of their breeding activities ? It is an established fact that most of the wild species of animals do not give full vent to their breeding potentialities and that they do not breed under captivity. Could it be possible that such species may have different physiological and metabolic responses under the free natural conditions ? : in which case the pigeons under captivity might be expressing an altered metabolic response in contrast to the one naturally expected to occur. Or else, it is also possible that the responses instead of being altered, are only partially expressed under captivity, and that full expression is brought forth only under the natural conditions. Such a state bespeaks of a prevailing inhibitory subtle stress factor, which in turn speaks of an inbuilt mechanism aimed at body economy by preventing wasteful expenditure of energy reserves.

Metabolic adaptations and alterations in metabolic strategies in response to either changing environmental conditions or seasonal requirements are known to be under the purview of neuro-endocrine regulation. Endocrines like pituitary, thyroid, pancreas, adrenals and gonads are implicated (either singly or even in

concert) in various physiological modulations in almost all vertebrate species. Seasonal shifts in metabolic patterns as seen presently in wild pigeons necessitated by the cyclic changes in their reproductive status should also be, no doubt, under the influence of neuro-endocrine regulation. Hormones of the pituitary, thyroid, adrenal and pancreas can all be considered to exert both positive and negative effects in the observed seasonal responses in feral pigeons in conjunction with gonadal hormones. Since it is strongly suspected that environmental inputs into the central nervous system acting via certain neuro-endocrine transducers are responsible for the endocrine alterations and resulting physiological responses observed during the reproductive cyclicity of seasonal breeders, pineal gland, which is attaining prominence as the fourth neuro-endocrine transducer was deemed an ideal topic of investigation in the seasonal periodicity of feral blue rock pigeons, a wild species. Pinealectomy (PX) was used as the mode of study and the effects that occur due to PX at various time intervals post-pinealectomy were recorded and compared with the corresponding intact and sham-operated birds.

The study has proved profitable in so far as it has demonstrated inhibitory influence in the normal pattern of changes occurring in response to changes in the

reproductive functioning. Moreover, PX has also induced unfavourable modulations in various physiological parameters investigated. Interestingly, PX induced alterations appear to be more pronounced during recrudescence and breeding as compared to regression. The first observation in terms of reproductive activity that has responded to PX is the size of the gonads (both testes and ovary) evaluated in terms of morphologically visible size and the weight. Both indicate a reduction in size of the gonads due to pinealectomy in all the three seasons with statistically significant decrease being obtained during recrudescence and breeding periods. This would suggest a pro-gonadal role for the pineal in wild pigeons and seems to be in marked contrast to the anti-gonadal role purported for it in mammals. Though an anti-gonadotropic action has been ascribed to the anti-gonadal role of pineal in mammals (Motta et al., 1967; Goldman et al., 1979; Chen, 1981), it is premature in the present case to suggest a pro-gonadotropic action. There could be any number of levels at which pineal can act to exert its pro-gonadal influence. Interestingly, decreasing thyroid weight and increasing adrenal weight characteristic of normal intact birds had been reversed in the PX pigeons during recrudescence and breeding (Chapter 1). This would indicate reduced thyroid secretion <sup>and</sup> increased adrenal functioning in the active

reproductive phases which was altered by pinealectomy to increased thyroid activity and reduced adrenal functioning. This may have relevance in the light of the known antagonism between thyroxine and gonadal hormones and the reported inhibitory action of thyroxine on reproductive functioning in some birds (Jallageas and Assenmacher, 1974; Jallageas et al., 1978). Again, adrenal is supposed to have a favourable influence on gonadal activation and breeding activities. In this wake, the increased thyroid output and decreased adrenal activity might together contribute to the gonadal regression induced by pinealectomy. In almost all vertebrate species investigated so far i.e. amphibians, reptiles and mammals, pineal tends to be anti-thyroid in action with its action seemingly being in the form of an inhibitory one at the level of TRH. (Ralph et al., 1979). In addition, either the direct action of pineal substances on gonads or a permissive property of melatonin or other pineal principle for trophic hormones action on gonads also need to be explored fully.

At the physiological level too, deviations from the normal were induced by pineal ablation. Both carbohydrate and lipid metabolisms appear to be sufficiently deviated to suppress gonadal functioning. In general, the glycogen content of liver, and glucose level of blood were both lowered during recrudescence and breeding. Decreased

hepatic glycogen together with hypoglycemia overrule a glucagon influence. However, in the light of the known role of avian pancreatic polypeptide (APP) in inducing hepatic glycogenolysis without the resulting hyperglycemia (Hazelwood et al., 1973), increased secretion of this pancreatic hormone in PX condition can be presumed, thus, signifying an anti-APP role for avian pineal. Since no explanation is available for this seemingly contradictory response of hepatic glycogenolysis with unaltered glycemic level, it is quite likely that the glycogenolytic products might be either oxidised or else channelised into lipogenic pathways within the hepatocytes and as such needs experimental confirmation. More than that, PX birds tended to depict significant hypoglycemic condition which could indicate either decreased absorption from the alimentary canal or increased peripheral utilization or even both. Insulin action can easily be suspected in this context which would again lead us to the conclusion that the avian pineal is anti-insulinic. Pertinently, Gorray et al. (1979) have demonstrated hypersecretion of insulin from the pancreatic islets of pinealectomized rats. Moreover, Delahunty et al. (1980) also reported lowered hepatic glycogen content in pinealectomized fish, Carassius auratus in the month of May, which was not repeatable in the month of January. All these tend to indicate not only some pineal-pancreas axis but also season specific

influence of pineal. Both, the observed alterations in weight of pancreas post-pinelectomy, and the reversed trend of changes during regression (Chapters 1, 2 and 3) are self explanatory. The differential action of pineal is further emphasized by the observed increase in muscle glycogen content in PX condition as compared to the decreased content in intact birds during recrudescence.

One of the principal actions of insulin is to resist mobilization of lipids, and in this context the observed insensitivity of hepatic and muscle triglycerides in PX pigeons once again emphasizes the anti-insulinic role of avian pineal. In contrast, the intact controls were marked by depletion of triglycerides during recrudescence and breeding (Chapter 5). Again, the influence of pineal on lipid metabolism is shown by the loss of triglycerides from the adipose tissue (merely a storage structure in birds) in PX condition as opposed to the insensitivity in intact controls. Most significantly pinelectomy seems to have a notable effect on phospholipid loss from the organs. Since the loss of phospholipids could induce structural and functional instability in tissues, an intact pineal could be of favourable disposition in this context.

Adaptive seasonal shifts in metabolic patterns and reactions should also involve coordinated changes in the activities of various enzymes, which might be either those directly concerned with metabolism and energetics, or those indirectly influencing the metabolic transformations by their allied effects. Seasonal breeding periodicity in wild pigeons was marked by alterations in the activity levels of SDH, ATPase, G6Pase and acid and alkaline phosphatases in the various organs of the bird (Chapters 4 and 8). These alterations in enzyme levels and patterns can be conceived to be under the regulative influence of humoral factors. In PX pigeons, the activity levels of ATPase, SDH and G6Pase were found to be distorted either on the higher side or lower side; moreover, the deviations from the normal varied not only with respect to the three reproductive phases in the same organ but also from organ to organ in the same phase (Chapters 4 and 8). This suggests irregular, non-specific and inconsistent changes (temporal and spatial) which could ultimately lead to upsets in adaptive metabolic patterns involving unwarranted, wasteful expenditure of metabolites and energy sources or even the non-occurrence of warranted and necessary reactions. Some subtle alteration in the integrated endocrine milieu in the absence of pineal could be the explanation for such a state of affairs. What turns out to be relevant in this context is the suggestive importance of pineal and its principle(s) in



the normal temporal physiological adaptations characteristic of seasonally breeding aves. In this respect, phosphatases have depicted more specific changes in the form of a general reduction in activity in gonads of PX birds (Chapter 8). Both these enzymes (which were noted to register an increasing trend during recrudescence and breeding in normal birds) by their versatile actions are known to favour various metabolic and other processes. Decreased activity in the absence of pineal could therefore definitely affect the metabolic efficiency usually associated with seasonal cycles. Unlike the gonads, phosphatases of liver, muscle and kidney, nevertheless, showed a variable response like SDH; ATPase and G6Pase, once again emphasizing the subtle disruption of the normal homeostatic mechanisms in the absence of pineal.

Aside these changes, the present study has also succeeded in demonstrating PX induced alterations in free ionic content ( $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$ ) of blood, liver, muscle and adipose tissue (Chapter 6). Such deviations from the normal, apart from affecting the metabolic abilities can also alter many cellular processes. The relationship of endocrine secretions (known to have a regulative influence on various ions in the body) with the pineal principles will need to be better understood. Comments on observation of altered thyroid and adrenal weights in the pinealectomized

condition have already been made; however the histological and functional correlates of these morphological changes need to be established and as such are under investigation. It is also interesting that some of the haematological parameters like Hb, ESR, hemostasis etc. also responded to pinealectomy, and is substantiated by the observed alteration in spleen weight. Though it is premature to offer any suitable explanations, it is, nevertheless, evident that such effects are due to subtle alterations at many levels of physiological and endocrinological hierarchy and homeostatic mechanisms. More evidences can be drawn from the observed alterations in the pattern of body weight changes as well as urogygeal gland weight, which are again indicative of the diverse effects of the simple but elusive structure - the pineal.

On the whole, it may be safely concluded (from the experimental observations made herein) that the breeding associated metabolic activation and occurrence of adaptive biochemical strategies are suppressed to a greater extent in the pinealectomized condition. A very valid substantiation, in this respect is provided by the increased ascorbic acid content of various organs in recrudescence and breeding phases of pinealectomized pigeons unlike in the intact ones where these two phases are characterised by depleting ascorbic acid content denoting active metabolic

transformations. Since the present study was restricted to observations post-pinealectomy conducted in individual reproductive phases, it may be profitable to conduct similar studies on a still longer temporal basis i.e. the effect of pinealectomy performed in one season on morphology, metabolic alterations and physiology of such individuals in the other two seasons of the same year or even in the next year. Though such studies are warranted, the present study conducted on three different time periods post-pinealectomy (i.e. 30, 45 and 60 days) in each reproductive phase, has indicated a tendency of the various physiological alteration to return towards the normal state in PX 60 group. Apparently, on a long time basis, corrective measures are being initiated by the organism. This is indicative of a situation where nature with great fore-sightedness has stacked up many regulative mechanisms within the architecture of vertebrate organism of which pineal is one as yet poorly understood one, whereby when one is lost another one slides into the slot resulting in the rectification of the imbalances. This would ultimately set back the skewed physiology back to normal, essentially by slight manipulations in the existing neural and humoral micro-circuitry.