INTRODUCTION

Early biologists and philosophers had referred to pineal as a vestigeal organ, third eye, or seat of soul, and had received only a passing reference in scientific literature till recently. Basically, pineal is a simple evagination of the diencephalonic roof and shows quite a bit of variation in its morphology and anatomy in the various vertebrate groups. In general, this structure is of common occurence in all the vertebrates except in the hagfishes, crocodilians, edentates and dugongs (Adam, 1957; Ariens-Kappers, 1965; Oksche, 1965). The microanatomy of the pineal has been variously described as saccular and parenchymal or follicular. Saccular appearance is characteristic of lower vertebrates where photoreception appears to be the primary function. Parenchymal or follicular appearance of the pineal is characteristic of higher vertebrates where photoreception is secondarily subjugated to the primary function of secretion. Apparently during the course of evolution, an essentially sensory neural apparatus has been transformed into a neuroendocrine transducer serving as a pivotal link between the neural information generated in the central nervous system and the chemical endocrine orchestra of the body. Factors such as hypophysectomy, testosterone, dihydrotestosterone, estradiol, cryptorchidism and stress have all in fact been shown to affect or modify the activity of pineal (Urry et al., 1976). Given the complexity of net work of interconnected circuitry of fiber systems in the central nervous system, the pineal can be visualised to be provided with a variety of sensory input of information generated in the central nervous system in response to the multiple external and internal cues. These diverse sensory information reaching the pineal after being transduced into chemical signals can be thought to interact with various systemic factors, thus exerting subtle influence on various physiological and metabolic features of the body. Ralph (1976) has suggested hypothalamus to be a probable site of action of melatonin as accumulation of melatonin could be observed in hypothalamus as well as brain of chickens. One of the earliest functional significance of pineal detected was with reference to reproduction in mammals (Reiter, 1974 a; 1975 a; 1978). Since its accidental discovery, this idea has been put to experimental tests in various vertebrate groups and a general antigonadal role has been assumed as least in mammals. In other vertebrate groups too, though its influence on reproduction is evident, the effects appear to be highly varied, with progonadal, antigonadal and even no relation to gonads being all suggested at one time or another by different investigators. Worth mentioning is the report of Ralph (1970) that though pinealectomy has some controlling influence on gonadal function in birds, the evidence is not consistent. With the renewed interest on this once considered vestigeal organ and the flurry of experimental investigations launched in the last two decades, many other roles such as photoreception, thermoregulation, circadian activity, time keeping activity etc., have all been assigned to pineal (Wurtman and Axelrod, 1965 a; Gaston and Menakar, 1968; Quay, 1974; Menakar and Zimmerman, 1976; Quay, 1976; Binkley, 1979; Ralph et al., 1979; Ralph, 1983). In due course, its relation with other endocrine organs has also become a matter of debate and discussion. It was in this wake of a new found functional status for pineal in a vertebrate body, that certain investigations to understand the possible involvement of pineal in general body metabolism and reproductive activities on a seasonal basis in the wild pigeon, Columba livia were undertaken in this laboratory. These investigations have yielded convincing evidences in favour of definite but subtle involvement of pineal in general body metabolism as well as reproductive activities. An interesting revelation was the seasonal variation in pineal involvement in both general body physiology and reproductive activities (Patel, 1982). The above studies clearly underscored the differential seasonal interaction of pineal principles with the other endocrines in the body so as to regulate the total body economy according to the needs of the animal in different seasons. Since it is assumable (from the many reports available) that domestic and wild forms of avian species have different pineal-gonad relationship, it was thought pertinent to conduct comparable studies on domestic pigeon Columba livia so that more light could be thrown on this aspect. Further, with the preliminary results obtained from the previous studies mentioned above, it was apparent that pineal has some influence on carbohydrate metabolism in wild pegions and hence this aspect was also thought worth probing further.

As a sequel to the above thoughts, studies were initiated to establish the role of pineal in general body physiology with a bias towards reproductive activities in the domestic pigeon, Columba livia. To this end, pinealectomy was used as the tool and the response of the pinealectomised birds in terms of organ weights, metabolite levels (glycogem, lipids, proteins, ascorbic acid and cholesterol) and levels of enzyme activity (phosphatases and steroid dehydrogenases) in the gonads have been compared with the corresponding responses of intact and sham operated controls during both the breeding and non-breeding periods. For the purpose of furthering our information on the involvement of pineal in carbohydrate metabolism in wild pigeons, glucose tolerance test, and insulin, glucagon and adrenalin responses in terms of glycemic level at different time intervals on a seasonal basis have been carried out. In continuation, to understand the possible alterations if any in the rate and mechanism of uptake and conversion of glucose (glycogenesis) and also break-down and release (glycogenolysis), *in vitro* experiments on liver and muscle slices under the influence of insulin, glucagon and adrenalin have also been undertaken in intact, sham operated and pinealectomised pigeons. In all the above studies, 30, 45 and 60 days post-pinealectomy were taken as the ideal time to understand the effects of pineal ablation as these protracted periods give sufficient time for all the non-specific disturbances to settle down.

Changes in organ weights and histological alterations recorded post-pinealectomy during breeding and non-breeding periods (Chapters 1 & 2) have shown definite influence of pineal on the various endocrine and glandular structures. The gonads, adrenals and thyroid show parallel seasonal changes in intact animals in the form of increase in weights during breeding and decrease during non-breeding. These changes are offset by pinealectomy and operated animals recorded shrinkage during breeding and enlargement during non-breeding. The above set of changes shown by the intact and pinealectomised birds apparently suggest a parallel axis between gonad, adrenal and thyroid in the seasonal changes associated with breeding. The reduced gonadal size and activity during breeding and enlarged active ones during non-breeding post-pinealectomy are suggestive of two different pineal principles to be operative in tropical domestic pigeons: one inducing gonadal activation during breeding and the other bringing about regression during non-breeding. This is very much unlike the results obtained for the wild pigeons where it was shown to be progonadal in nature only (Ramchandran <u>et al</u>., 1984a). The other three organs <u>i.e.</u>, pancreas, spleen and uropygeal gland have shown alterations in their normal seasonal changes due to pinealectomy. Increasing weights of pancreas and uropygeal gland and decreasing weight of spleen during non-breeding in normal intact birds have been reversed by pinealectomy. Similarly during breeding, pinealectomy brought about increase in weight of pancreas and uropygium and reduction in weight during non-breeding. These changes also indicate subtle involvement of pineal in endocrine homeostatic mechanisms.

Carbohydrate reserves of the body together with the circulating glucose are known to be the primary energy sources for various physiological activities of the body. Breeding activities do place heavy demands on the energy resources of the body and the necessary adaptive regulatory mechanisms are well entrenched in seasonally breeding animals. Though the domestic pigeons are capable of breeding all throughout the year, they do have nevertheless a well defined period of breeding during the summer months and a relatively slack breeding phase during the fall and winter months. In this light, the relative importance of carbohydrate reserves of the body in breeding and the effects of pinealectomy on the adaptive responses have been studied by evaluating the content of glycogen in liver, muscle and gonad and glycemic level in intact, sham operated and pinealectomised pigeons during both breeding and non-breeding phases (Chapter 3). In the control birds, the glycogen content of both liver and gonads was found to be low during the breeding months while it was high during non-breeding months. Correspondingly, the glycemic level was slightly high during breeding and low during non-breeding. In contrast, the muscle glycogen content tended to be high during breeding and reduced during non-breeding. These changes indicate the definite involvement of carbohydrate reserves in the breeding activities and their replenishment in the quiscent phase. These adaptive alterations were found to be altered and reversed in pinealectomised birds keeping in with the changes in the gonads noted earlier. Apparently these changes are indicative of interaction of pineal principles with other systemic factors in bringing about the adaptive seasonal metabolic alterations.

Ascorbic acid is implicated in steroidogenesis in adrenals and gonads and is also shown to be indicative of metabolic status of tissues (Chinoy, 1969; 1970; Chinoy et al., 1973; 1978). Accordingly, the changes in ascorbic acid content of liver, muscle and gonads of pinealectomised birds have been estimated and compared with those of control birds on a seasonal basis (Chapter 4). Comparatively higher levels of ascorbic acid in all the three organs during breeding is indicative of the metabolic activation associated with breeding. Pinealectomised birds too have depicted parallel changes in the content of gonadal ascorbic acid with relatively higher levels in comparison to the controls. Hepatic and muscle ascorbic acid contents seem to be not much affected by pinealectomy as they are very much comparable with those abserved in the controls. Pinealectomy induced alterations in mobilization and utilization of ascorbic acid by the gonads are discussed in this context.

Seasonal breeding activities have been shown to influence the lipid content of gonads. Reciprocal relationship between gonadal activity and lipids has now beem well established for many vertebrate species with increasing appearance of lipids in the seminiferous tubules and ovarian follicles heralding the quiscence of gonads during the non-breeding phase (Lofts and Marshall, 1959; Lofts, 1968; Lofts and Murton, 1973). This prompted studies on quantitative alterations in the content of various lipid fractions in the gonad of normal domestic pigeons during their breeding and non-breeding periods. Similar studies were also carried out in birds pinealectomised during the two phases (Chapter 5). All the lipid fractions (total lipids, cholesterol, phospholipids and glycerides) tended to be low during the early breeding period which progressively increased and reached a maximum level towards the late breeding period. The non-breeding months were marked by an intermediary level of the lipids. Again, pinealectomy led to increased lipid contents during breeding and reduced contents during non-breeding which are in keeping with the changes in gonadal weight and structure observed.

Having studied the changes in carbohydrates and lipids with regard to pinealectomy, it was thought desirable to look at the possible alterations if any in protein content and activity of phosphatases in the gonads of control and pinealectomised pigeons (Chapter 6). Unlike the glycogen and lipid contents, protein content of the gonads had a tendency to remain unaltered during both the periods except for a slight fall towards the terminal phase of breeding. Pinealectomy in general did not influence the protein content of the gonads during early and mid breeding phases. However during late breeding and non-breeding phases pinealectomised birds depicted a reduced protein content. There appear to be not much dramatic changes in the activity levels of acid and alkaline phosphatases in the gonads of intact birds on a seasonal basis. Acid phosphatase activity was found to be slightly higher during the breeding phase as compared to non-breeding, while alkaline phosphatase activity was noticeably higher during the late breeding/early non-breeding periods. A temporal increase in acid and alkaline phosphatases activity post pinealectomy was the feature in breeding period. This trend was not observable during the non-breeding period. However, the activity of the enzymes were comparatively higher in pinealectomised birds during non-breeding than in the intact birds. This is being correlated with the pinealectomy induced activation of gonads observable during the non-breeding months.

Tissue content of sodium and pottasium ions and water content.. are usually reflective of the functional status. Since the gonads show cyclic variations in activity, the quantitative alterations in the water content and sodium and potassium ions were thought pertinent to study not only to reveal the normal seasonal changes but also to gain some idea about the possible influence of pineal ablation on the adaptive changes (Chapter 7). Normal birds were characterised by increased water and potassium contents and reduced sodium content during breeding and reverse changes during non-breeding. Pinealectomy had reverse effects with increased water and potassium contents and decreased sodium content during non-breeding which are in correlation with the changes in gonadal condition. The above changes are being discussed, in terms of increased potassium content coupled with sodium content being indicative of ennanced gonadal activity.

Finally, as gonadal recrudescence and quiscence are paralleled by changes in steroid dehydrogenases, histochemical distribution and localization of 3β, 3α and 17β hydroxysteroid dehydrogenases together with neutral lipids and total lipids were undertaken in normal, sham operated and pinealectomised domestic pigeons (Chapter 8). All these dehydrogenases are linked with steroidogenic pathways and accordingly their activity and localization were very much pronounced during the breeding periods. Concurrently the lipid distribution too was found to be low. Reduced intensity of dehydrogenases and increased lipid content were the feature during the non-breeding period. In comparison, pinealectomy induced gonadal regression during breeding was marked by low steroid dehydrogenase activity and high lipid content and the reverse during the non-breeding period whence pinealectomy induced gonadal activation.

The second part of this work deals with the pinealectomy induced effects on carbohydrate metabolism in wild pigeons which is a continuation of the previous preliminary studies carried out in this laboratory. These investigations had shown altered glycogen content of liver and muscle and reduced glycemic level in pinealectomised birds especially during the breeding season thereby suggesting a possible pineal-pancreas axis and even probably a pineal-adrenal axis involved in regulation of carbohydrate metabolism. In order to throw more light on these aspects the response of pinealectomised pigeons to a single injection of insulin, glucagon and adrenalin as evaluated by the glycemic level at different time intervals after injection and glucose tolerence test after glucose loading have been studied during both breeding and non-breeding phases (Chapters 9-10). These responses have been compared with those obtained in intact and sham operated groups of birds. From a comparison of the time taken for attaining maximum and minimum glycemic levels after glucose loading and insulin injection respectively, and the time taken for returning to the normal glycemic level, it has become apparent that even among normal birds, the insulin responsiveness is more during non-breeding period than during breeding period. In contrast, pinealectomy brought about highly pronounced insulin sensitivity during breeding and much reduced sensitivity during non-breeding. Using similar criteria of evaluation, even glucagon and adrenalin injections too have produced differential responses during breeding and non-breeding by normal as well as pinealectomised birds. These results are being discussed in detail in the light of possible hormonal interactions occurring with and without pineal and gonadal hormones.

Patel (1982) had shown altered glycogen content in the liver and muscle and hypoglycemia in pinealectomised wild pigeons, thereby indicating a pineal-pancreas axis in regulation of carbohydrate metabolism. Moreover the glycemic responses of pinealectomised birds to single injections of insulin, glucagon and adrenalin have also yielded results suggestive of definite alterations in regulation of carbohydrate metabolism. From the results obtained by Patel (1982) it was suggested that increased utilization, decreased absorption and/or increased peripheral utilization may contribute to the decreased hepatic glycogen store and hypoglycemia in pinealectomised birds. In this context an attempt is made to study the ability of liver and muscle of pinealectomised birds for uptake and release of glucose by *in vitro* tissue slice technique in presence of insulin, acetyl choline, glucagon and adrenalin (Chapter 11). The results obtained are discussed in terms of altered tissue responses to hormonal factors post-ponealectomy.