INTRODUCTION

Avian adrenal glands are discrete encapsulated organs composed of an aggregation of chromaffin and adrenocortical cells, corresponding cytologically to the mammalian medullary and cortical tissues respectively. This arrangement in birds is the culmination of an evolutionary trend through the vertebrate series from complete separation in agnathans, elasmobranchs and dipnoi through partial association in teleosts and lower amphibians to increasingly intimate mingling of the two tissue types in anurans, reptiles and birds. Mammals other than the prototherians show an additional development with the consolidation of chromaffin and adrenocortical tissues into precise concentric masses (Chester Jones, 1957; Deane, 1962).

The basic unit of avian interrenal tissue is a cord composed of double row of parenchymal cells. These cords radiating from the centre of the gland, branch and anastomose frequently (Haack *et al.*, 1972). The nucleus of the parenchymal cells is mostly situated towards the basal lamina on the outer margin of the cord. Between the cords of parenchymal cells and ensheathed within a connective tissue reticulum, lie groups of chromaffin cells; these display an accumulation of granules believed to reflect lowered catecholamine release.

In the recent years much attention has been diverted to the interrenal tissue which has been noted to undergo marked season specific alterations in its histomorphology and biochemistry with respect to the breeding cycle of an animal. Jallageas et al. (1978) in their study on male ducks reported that during the reproductive season the plasma corticosterone titres were increased by 50% while the total binding capacity of CBG (corticosterone binding globulin) rose by 68%. Early studies on adrenal cortex with respect to reproductive cycle in birds had been more or less directed to the histomorphological alterations of the cortical cells. Höhn (1947) reported an increased activity of the adrenal cortex during the breeding season in ducks. Fromme-Bouman (1962) found in the black bird, Turdus merula, an increase to a maximum in adrenal fractional cortical volume during the breeding season which fell to a minimum in the autumn. Bhattacharya and Ghosh (1965) found in the house sparrow, Passer domesticus adrenal hypertrophy during breeding alongwith active spermatogenesis and then regressed post-breeding. These specific alterations pertaining to adrenals (Cortex) have been noted to occur in lower vertebrates as well as mammals. Munshi et al. (1978) reported near parallelism in the activity of interrenal tissue, with water, temperature, photoperiod and gonadal cycle in the air breathing fish, Heteropneustes fossilis. Bhatta and Nadkarni (1984) reported an increase in the interrenal activity

twice a year corresponding to the preparatory and breeding periods in an apodan amphibian, Ichthyophis beddomi. Rana (1982) in his study on rats has also reported seasonal fluctuations in adrenal glands in relation to body weight, reproductive activity and population density. However, reciprocal relationship of adrenal to gonads too has been reported in the pied flycatcher and the Indian palm squirrel by Bengt (1979) and Kanwar and Veena (1977) respectively.

Ghosh (1962) differentiated on histochemical grounds, a subscapular zone (SCZ) from an inner zone (IZ) of interrenal tissue in several species of birds. Studies conducted by various workers suggest that the inner zone is sensitive to ACTH and is associated with glucocorticoid production. Miller and Riddle (1942) reported the cytological reactions of the adrenal cortex to a variety of experimental conditions. Thus injection of ACTH, formaldehyde and unilateral adrenalectomy stimulated the activity of adrenocortical cells as judged by mitochondrial proliferation, hypertrophy of golgi apparatus and decrease in lipids and cholesterol contents. Apart from these, hypophysectomy, photoperiod, environmental factors, gonadotropins, gonadal steroids and other endocrine principles have all been known to alter adrenal function. (Kar, 1947; Holmes et al., 1970; Chan and Phillips, 1970; Chan et al., 1972; Daugherty and Callard, 1972; Chan and Phillips, 1973; Jallageas et al., 1978; Patel et al.,

1985; Ramachandran and Patel, 1986).

Steroid substances, elaborated by the adrenal cortex throughout the vertebrate series, and synthetic products having similar structure and function as the native ones (homologues), are defined as corticosteroids. Birds are primarily corticosterone producers which has been reported by several authors based on their work on different species of birds including White King Pigeon, Columba livia (Phillips and Chester Jones, 1957; Urist and Deutsch, 1960; Sandor, 1972). Corticosterone has been known to have both glucocorticoid and mineralocorticoid effects. Corticosterone has been known to exhibit seasonal, circannual as well as circadian variations, the importance of which is being increasingly realised in recent years. On diurnal basis, some species of birds exhibit one peak of plasma corticosterone (Dusseau and Meier, 1971) while some others exhibit two peaks (Boissin and Assenmacher, 1968; Chan and Phillips, 1973). The relationship of fluctuating corticosteroids with that of the reproductive cycle is now becoming increasingly clear. Both stimulatory as well as suppressive effect of corticosteroids on gonadotrophin release have been realised in mammals. Nazian (1982) reported that adrenalectomy decreased FSH release and also lowered the set point for FSH secretion. David et al. (1982) found that ACTH treatment reduced pituitary sensitivity to GnRH but LH synthesis was not affected. Further, injection of ACTH has been noted to bring

about increase in hydrocortisone, 17 - hydroxy pregnenalone, 17-hydroxy progesterone and DHEA two hours after injection followed by 160% increase in testosterone secretion in the sacred baboons (Katsya and Goncharov, 1982). In contrast, ACTH has been shown to suppress LH and testosterone production in bulls (Johnson et al., 1982). Ducharme et al. (1979) in their studies on female rats reported that adrenals are not directly involved in pubertal development. Bittman and Zucker (1979) too reported, based on their study on hamster that adrenal hormones play no major role in external or internal coincidence mechanism for seasonal suspension of reproductive activity. Contrary to the above mentioned reports, ovulation inducing effect of highly purified ACTH has been worked out by Etches et al. (1982) in the laying hens, the most appropriate dose ranging between 6.25 IU/hen to 100 IU/hen. Jacobs and Peppler (1979) based on their study reported that rats adrenalectomised for 30 days ovulated fewer eggs than intact controls and the adrenal was found to be involved in pituitary - gonadal activities. Etches (1979) reported a two fold surge in Corticosterone level prior to progesterone rise during ovulation. These studies revealed the importance and the role of Corticosteroids in reproductive biology of animals. Apart from this, adrenalectomy or Corticosteriod treatment have also been known to play important roles in fat, protein and Carbohydrate metabolisms in birds. Adrenal insufficiency has been

known to affect alkaline phosphatase, ATPase, hexokinase, and G6Pase activities in the intestine (Schraier et al., 1963; Pfeiffer and Debro, 1966; Rodners et al., 1967; Kilkowska and Kozlowska, 1969). Chicks treated with cortisol or corticosterone showed reduced growth rate and stimulation of increased adrenocortical activity in adult drakes causing them to lose body weight (Dulin, 1956; Baum and Meyer, 1960). Corticosteroids modify the metabolism of growing chicks in favour of fat deposition (Nagra and Meyer, 1963). These multifaceted role of corticosteroids and their importance in sustaining life, need to be re-investigated, since age, sexual maturity, pituitary activity and experimental procedure can all significantly affect the response and results. Some of the physiological effects of low circulating concentration of corticosteroids have sometimes been deduced from adenohypophysectomy. Corticotrophin replacement therapy must be examined in order to discriminate between hypoadrenocorticalism and those resulting from birds being deprived of pituitary hormones other than corticotrophin. Further, as corticosteroids are rapidly metabolised, care must be exercised in interpreting the experimental results. From the many reports available, it is becoming increasingly clear that adrenal steroids do affect other endocrine glands (Rudneva et al., 1980; Mitsuma et al., 1982). Interactions between testicular hormones, thyroid and adrenal glands have

also been reported by Peczely and Daniel (1979). Jacobson et al. (1978) have reported the influence of pineal and adrenal glands on proestrous release of hormones in rats and concluded that pituitary hormone release was depressed. Previous work carried out in our laboratory clearly underscored the differential seasonal responses of adrenal gland vis a vis the breeding seasons in the pigeon, Columba livia (Patel, 1982). However, these studies were conducted with a different bias, and the direct involvement of adrenal corticosteroids in reproductive physiology as well as general body physiology needs to be evaluated in depth. To further the knowledge on the importance of adreno-cortical functioning with respect to breeding season, the present study was undertaken in the feral blue rock pigeons, Columba livia.

To have a better insight into the complex adrenal-gonad relationship in this species, the season specific variations of adrenals with respect to the cyclic changes of gonads were closely followed. More light could be thrown on the importance of adrenal steroids on gonadal physiology by manipulating the adrenal function, and so chemical adrenal-ectomy and activation of adrenals were used as the tools to understand the importance of adreno-cortical secretions on gonadal physiology. The response of experimental manipulation on certain parameters that reflect the functional status and

physiological state of gonads was studied and compared with the normal controls on a seasonal basis. As the alteration in general body metabolism and physiology could lead to disturbances in gonadal functioning, the study was extended to liver too which is known to be a centre for metabolic turnover. Parameters studied include organ weights (absolute and relative weights of gonads, adrenals, thyroid, spleen and Uropygium), metabolites (blood glucose, tissue glycogen, protein, ascorbic acid, lipid fractions), enzymes (phosphatases, lactate and succinate dehydrogenases, transaminases, glucose-6-phosphatase, phosphodiesterase and adenosine tri-phosphatases) and ions in the gonads and liver, which yielded convincing results implicating the relative importance of adrenal secretions on gonadal functioning.

Changes in organ weights and histological observations after experimental manipulation of adrenals underscore the definite involvement and importance of adrenal corticosteroids in normal gonadal functioning and physiology. The changes observed indicate a parallel adrenal-gonad axis to be operative in this species. Patel et al.(1985) and Ramachandran and Patel (1986) have also made similar observations. In contrast to domestic pigeons, where a parallel thyroid-gonad axis seems to be operative (Ramachandran and Patel, 1986), inverse thyroid-gonad relation seems to be involved in wild pigeons

as revealed by the increased thyroid weight during the non-breeding phase and its decrease during the breeding phase. Adrenals seem to depict a parallel relationship with that of gonads as revealed by the progressive increase in their weight from regressive phase through recrudescence to breeding (Chapters II and III). Chemical adrenalectomy (Adx) as revealed by decreased cortical activity brought about regression of gonads with accompanied increase in thyroid weight, which further gives credence to the observed parallel adrenal-gonad and inverse adrenal-thyroid relationships. Spleen and Uropygium too exhibited season specific changes and alterations under experimental conditions. Increased spleenic weight during the non-breeding phase and decreased weight during the breeding phase were also affected by chemical Adx which further lowered its weight. Similarly, increased Uropygium weight during breeding and its reduction during non-breeding were reversed by experimental manipulations of adrenocortical activity. These changes revealed the involvement of adrenal steroids in several but specific homeostatic mechanisms.

It is now a universally accepted fact that tissue carbohydrate stores together with the blood glucose form an important and immediate source of energy. Several hormones have been known to influence carbohydrate metabolism including corticosteroids. To assess the involvement of adrenals in

carbohydrate metabolism in terms of blood glucose level and tissue glycogen contents, the values of experimental animals were compared with those of controls on a seasonal basis (Chapter IV). Heralding of breeding activities is accompanied by increased blood glucose level and reduced glycogen content in the liver and muscle. Contrary to this, the non-breeding season depicted low blood glucose level and high hepatic as well as muscle glycogen contents. Gonadal glycogen store was more during breeding and less during non-breeding. Adrenal suppression brought about increased glycogen deposition in liver and muscle during the recrudescent and breeding phases. Gonadal glycogen content was found to increase during recrudescence and deplete during breeding in adrenal suppressed birds. Adrenal activation led to a reverse set of changes in liver and muscle, and an increased glycogen content in the gonads. G-6-Pase, an enzyrne involved in gluconeogenesis, also exhibited season specific alterations in the form of increased levels during breeding and reduced activity during non-breeding. Adrenal suppression and activation induced, decreased and increased activity levels respectively, corresponding to the circulating levels of glucose.

Role of phosphomonoesterases in several cellular metabolic functions has been reported by many workers. Hormones have also been known to affect the activity levels of phosphatases (Manwell and Betz, 1966; McWhinne and Thommes, 1973;

Yeh and Moog, 1977; Wilfred and Rao, 1977). To have a better understanding of the role of nonspecific phosphomonoesterases in gonadal physiology and in general body metabolism, these enzymes were estimated in the gonads and liver of experimental and control birds on a seasonal basis (Chapter V). Acid phosphatase was found to be low during non-breeding and high during recrudescence and breeding in both the tissues. Alkaline phosphatase too depicted a similar pattern of changes. Adrenal suppression led to decreased acid phosphatase level in liver and gonads during recrudescence and breeding, and reverse set of changes under adrenal activation during non-breeding. Gonads depicted decreased alkaline phosphatase activity upon adrenal suppression which was reversed on adrenal activation. These changes are correlated with the observed shrinkage of gonads and induced activation of gonads in the respective seasons.

Of the various vitamins, ascorbic acid (AA) has caught the attention of scientists more, because of its involvement in steroidogenesis, and its role as an indicator of metabolic status of tissues (Chinoy, 1970; Chinoy et al., 1973, 1978). Disturbance in vitamin C metabolism is known to alter the metabolism of carbohydrates, proteins, lipids, and iron. (Rusch & Kline, 1941; Mazur et al., 1961; Banerjee and Ganguli, 1962). As adrenals and gonads are the main steroidogenic centres in vertebrates, and liver being the storage

organ of AcAc in birds, the quantitative content of the vitamin was estimated in these three tissues (Chapter VI). Ascorbic acid content of adrenals and its depletion is one of the criterion of adrenal function. The prevailing level in the gonads could reveal the functional status of gonads and the content in liver could reveal the rates of mobilization and utilization. These reasons prompted the present study on ascorbic acid content of liver, gonads and adrenals in the control and experimental pigeons. Low ascorbic acid content during breeding and high content during non-breeding were the feature in liver and gonads. Adrenals exhibited an opposite pattern. Adrenal suppression led to stockpiling of this vitamin in gonads and a significant decrease in adrenals, while liver exhibited a marked decrease in its vitamin content. Adrenal activation led to depletion of the vitamin from all the three organs.

Any alteration or disturbance in the metabolic pathways could exert a consequent effect on the functional ability of the tissues. Thus to have a generalised idea of the various metabolic pathways and also the total energy flux and the influence of adrenocortical activity on these pathways, evaluation of certain key enzymes like LDH and SDH was undertaken in liver and gonads. For understanding the energy flux mechanisms, quantitative evaluation of ATPase too was carried out (Chapter VII). Results accrued indicate definite but subtle involvement of adreno-cortical steroids

in modulating the metabolic reactions to the optimum, to meet the adaptive needs of breeding.

Role of cAMP in reproductive processes has been shown by Menon and Gunaga (1974). The physiologic responses that occur at different levels of regulation of the reproductive processes appear to be mediated by a common intracellular intermediate such as cAMP. Obviously, changes in the level of cAMP as exemplified by phosphodiesterase activity could reveal the response of target tissues to specific hormones. Hence this part of the study was taken up to get an idea about the prevailing level of cAMP in gonads and liver which could throw some light on the possible actions of hormones (Chapter VIII). Phosphodiesterase activity in the gonads exhibited seasonal variation, while liver phosphodiesterase activity exhibited only marginal seasonal fluctuations. Adrenal suppression during the active phases led to differential response in the two tissues with increased activity in liver and decreased level in gonads, a situation parallel to that found during the regressive phase of gonads. Contrary to this, adrenal activation during the inactive phase led to increased phosphodiesterase activity in both the tissues; the levels of enzyme activity being comparable to those found in liver and gonads during the breeding period.

Lipids have been shown to undergo cyclic changes in relation to gonadal activity. Reciprocal relationship between

gonadal activity and sudanophilic cholesterol positive lipids has been reported by several workers in different vertebrates (Lofts & Marshall, 1959; Lofts, 1968; Lofts and Murton, 1973). As the adrenals too depicted cyclic alterations in relation to gonadal cyclicity, quantitative study of total lipids and various lipid fractions was undertaken on a seasonal basis, in the adrenal and gonads of normal and experimental birds (Chapter IX). Low lipid content during breeding and increased content during non-breeding in the gonads were the prominent feature in the normal birds. Lipid fractions - Cholesterol, cholesterol esters, Phospholipids, triglycerides - all tended to be high during the non-breeding and low during the breeding phase. Adrenals exhibited high lipid content during breeding and low content during non-breeding. Although Cholesterol (total) did not exhibit season specific variations, Cholesterol ester tended to be high during non-breeding and low during breeding.

Both adrenal suppression and activation led to increased lipid content in the adrenals. Cholesterol depicted reduction during suppression and increase during activation. Adrenal suppression led to increased lipid content in the gonads; the various fractions too revealing a similar trend, a picture similar to the inactive gonads. Adrenal activation reversed the situation thus revealing a condition similar to that of active gonads.

After the general study on carbohydrates and lipids, protein too attracted the attention and was deemed fit to study especially as corticosteroids are gluconeogenic in nature bringing about conversion of glucogenic amino acids. Total protein content together with the activity levels of alanine and aspartate transaminases (GOT and GPT) were also assayed in liver and gonads (Chapter X). Both the transaminases exhibited seasonal alterations showing highest activity levels during recrudescence and least during regression. Adrenal suppression induced reduction in the activity level of GOT in liver and gonads and increase in the activity level of GPT in liver which were similar to the condition characteristic of the non-breeding phase. Conversely, adrenal activation brought about increased activity of both GOT and GPT in gonads and only that of GOT in liver with a reduction in GPT level which again resembled the picture characteristic of breeding season.

Protein content did not reveal any marked change on a seasonal basis but adrenal suppression did bring about significant increase in total protein content in all the tissues studied. However, adrenal activation failed to bring out any significant response.

Of late, tissue ion and water contents are gaining attention because of their involvement in tissue functions

and moresoever the content of water and ions is considered to reflect the functional status of tissues. Ions especially Na^+ and K^+ alongwith water content have been reported to undergo season specific alterations in the plasma of birds (Kapur and Toor 1978; Rzasa et al., 1982). As avian corticosteroids have been known to exert mineralocorticoid action, it was thought feasible to estimate the Na^+ and K^+ contents of serum and gonads alongwith the water content of gonads (Chapter XI). Both Na^+ and K^+ were found to exhibit season specific alterations in the gonads and the serum. Both the monovalent ions were found to be high during the breeding phase as compared to the non-breeding phase. However, gonadal ionic content revealed a different picture in the sense that high K^+ and low Na^+ content were the feature during the breeding phase, while low K^+ and high Na^+ content were found during the non-breeding phase. Adrenal suppression and activation led to significant alterations in the tissue and serum ionic contents as well as in the tissue water content. The results obtained are related in terms of gonadal activity and functional status.

problem and the animals would have to be maintained on saline water and compensated for the absence of medullary hormones.

To avoid all the above complications, chemical adrenalectomy (Adx) was preferred as the technique for studying the effect of ablation of adrenal cortex on the morpho-physiology and functioning of the gonads. Adrenal suppression was brought about by using the synthetic glucocorticoid, Dexamethasone disodium phosphate (dxm) which is commercially available in the market. This drug is known to act at the level of adenohypophysis blocking the endogenous synthesis and release of ACTH. This was observed by Kjaerheim (1968 a,b,c) in the fowl and found that under the influence of this drug the interrenal tissue atrophied. Similar observations on other vertebrates have been reported by several workers. Donaldson and McBride (1967) found that dexamethasone (dxm) interferes with the normal interrenal - hypophysial interactions. Dxm in doses that had no direct effect on the interrenals reduced the level of plasma cortisol from 8.6 ± 5.9 µg/100 ml to 1.6 ± 1.2 µg, a level characteristic of hypophysectomised fish. In the bull frog, Ulick and Feinholtz (1968) found that dxm reduced aldosterone secretion which suggested that the drug acts by depressing the secretion of ACTH, perhaps by interfering with the hypothalamic control of ACTH release. Similar observation of dxm reducing plasma corticosterone ( ) to insignificant levels has been reported by Licht and Bradshaw

(1969) in Anolis. Inability of dxm to reduce secretion of corticosterone from adrenal tissue in Vitro is a further proof of its action at the hypophysial level (Holmes and Phillips, 1976). Thus dxm causes adrenal inactivity and atrophy presumably due to inhibited ACTH secretion; the drug exerting its inhibitory action either directly on the corticotrophic cells or indirectly via inhibition of CRF release (Holmes and Phillips, 1976).

Activation of adrenal gland was brought about by injecting adrenocorticotropic hormone (ACTH) obtained from Sigma Chemicals U.S.A. This in turn is known to act on adrenals resulting in hypertrophy of adrenals. Treatment of Myxine glutinosa with large doses of mammalian ACTH, raised the level of plasma corticosteroids (Idler et al., 1971). Hypertrophy of interrenal cells under the influence of mammalian ACTH in Elasmobranchii was reported by Dittus (1941). Injection of 10 or 50 mμ ACTH increased Corticosterone equivalent to 4.2 ± 0.9 & 4.7 ± 0.6 μg/100 ml plasma respectively in Bufo. Adrenal activity in reptiles too depends on pituitary corticotrophic stimulation. Treatment with 0.2 IU ACTH daily for 12 days caused hypertrophy of adrenals in the lizard, Dipsosaurus. Effects of ACTH on the adrenocortical cells of pigeons as judged by mitochondrial

proliferation, Golgi hypertrophy and decreased lipid and cholesterol contents have been reported by Miller and Riddle (1942).

Another aspect of the experimental set up was injection of corticosterone to induce hyperadrenocorticalism. Bouille et al. (1969) reported that the corticosterone level in intact pigeon varied from 2.2 to 2.5 µg/100 ml. The exact corticosterone level during the nonbreeding months has not been reported although there are several reports indicating reduced activity of adrenal cortex during the nonbreeding phase in this species (Chapter II). The dose chosen was therefore in a range equivalent to the circulating level characteristic of recrudescent and breeding months. Dose selection was also based on evaluation of many parameters such as absolute and relative weights of adrenals, ascorbic acid, cholesterol (free, total & esterified) and lipid contents and also the histology of the gland; criteria suitable to judge adrenal functioning.

Dose, Time and Route of Administration

The bioassay for hypothalamus CRF content of pigeons maintained on a 12 hour light and 12 hour dark regimen in terms of the ability of the hypothalamic extracts to stimulate the release of ACTH from rat pituitaries revealed a diurnal pattern with the increase in hypothalamic CRF

content always preceding that of plasma corticosterone concentration during the diurnal cycle (Sato and George, 1973). Diurnal fluctuations in plasma corticosterone have been reported by several workers (Chan and Phillips, 1970, 1973; Assenmacher and Boissin, 1970, 1972; Dusseau and Meier, 1971). At constant light and temperature, the corticosterone level was reportedly minimum at the end of the light period and maximum during the dark phase.

Dexamethasone injections were given at 9.00 hrs during both recrudescent and breeding periods. This drug has been reported to depress ACTH secretion probably by interfering with hypothalamic control of ACTH release (Ulick and Feinholtz, 1968). Thus the drug would inhibit the release of the next surge of ACTH. As dose specific response of the drug administration was noted, 3 doses of the drug were tried. Maximum dose selected (based on mortality rate) was 160 µg/0.1 ml/bird/day, while low and intermediate doses equivalent to 80 µg/0.1 ml/bird/day and 120 µg/0.1 ml/bird/day respectively were also employed. The drug was diluted to the required concentration in chilled redistilled water.

Mammalian ACTH (Sigma Chemicals) was dissolved in 0.9% Nacl to yield 1 I.U. soln/0.1 ml. Birds were injected with 0.5 I.U/0.5 ml/bird/day at 9.00 hrs.

Corticosterone (Sigma Chemicals) was used at two doses termed as low and high doses equivalent to 1 µg/0.1 ml/bird/day and 3 µg/0.1 ml/bird/day respectively. The hormone was dissolved in chilled freshly redistilled water with a few drops of ethanol for completely dissolving the drug. Two time periods of injection of corticosterone were selected. One at 9.00 hrs. and the other at 18.00 hrs. for both the doses. This was done for the evaluation of specificity of time as a factor contributing to the hormonal response and also because of the reports of two peaks of corticosterone in recent years (Boissin and Assenmacher, 1968; Chan and Phillips, 1973 c).

Control animals were injected with the respective vehicles. Administration of the drug/hormones was done by a 1 ml syringe which was sterilized by boiling in hot water prior to use. Both dxm and the hormones were introduced intraperitoneally. Injections were continued for ten consecutive days in all experimental birds and were sacrificed for evaluation of various parameters on the 11th day.

Experimental Set-up

Birds procured from the local animal dealer were maintained in an aviary for about 10-15 days for acclimation before bringing them into the experimental set-up.

After this, the birds were divided into 4 experimental groups during the recrudescent and breeding periods extending from January-March and March-May respectively. Each experimental group consisted of 12 birds of both sexes, consisting of at least 5 males and 5 females. Three groups were injected with the 3 different doses of dxm and the 4th group served as the control which was injected with the vehicle.

During the regression phase (June-August) the pigeons were divided into seven experimental groups. One group was injected with ACTH, second with low dose of corticosterone (LCM) at 9.00 hrs and the third with high dose of corticosterone (HCM) at 9.00 hrs while the fourth and fifth groups of birds were injected with low and high doses of corticosterone respectively (LCE and HCE) at 18.00 hrs. The sixth and seventh groups served as controls and were injected with the respective vehicles corresponding to the ACTH and corticosterone experimental groups.

All the birds were maintained in the aviary under natural photoperiodic regimen and were fed on grains and water ad libitum. At the end of the experimental period, the birds were brought from the aviary and sacrificed under mild anaesthesia by decapitation. Individual body weights were recorded prior to injections as well as prior to

sacrifice. The methodology employed for the assay of enzymes and metabolites, and, the histological procedure are given below :

Methods -

Organ Weights (Absolute and Relative)

After the aminal was sacrificed, the abdominal cavity was opened and the desired organs were quickly excised, blotted free off blood and tissue fluids and weighed to record the absolute weight. The absolute weights were then converted to relative weights in terms of percentage body weight.

Estimation of Metabolites -

Glucose

Prior to sacrifice of the birds, blood was drawn from the brachial vein by a small puncture and was processed for blood glucose estimation according to the micro-method of Folin and Malmros (1929). Blood glucose concentration is expressed as mg/100 ml blood.

Glycogen (Liver, Muscle and Gonads)

Tissue glycogen content was estimated according to the method of Seifter et al. (1950). After blotting the tissues, they were digested in 2 ml of hot 30% KOH and the glycogen was precipitated with absolute alcohol. Anthrone

reagent was used for developing colour and the optical density was measured at 620 mμ using a "spectronic 20" photoelectric colorimeter. The glycogen content is expressed as mg/100 mg wet tissue weight.

Protein - (Liver, Muscle and Gonads)

After preparing the requisite concentration of tissue homogenates in chilled redistilled water, protein content was estimated by the method of Lowry et al. (1951) and expressed as percentage of fresh tissue weight.

Ascorbic Acid - (Liver, Adrenals and Gonads)

Estimation of ascorbic acid content in the tissues was carried out by the method of Roe (1954). The ascorbic acid was first extracted with 6% Trichloroacetic acid (TCA) which reduces pH, stabilizes the vitamin and also prevents its catalytic oxidation. Ascorbic acid was then oxidised to dehydroascorbic acid by shaking the extract with activated charcoal for 15 minutes. From the filterate 4 ml of aliquot was incubated with 2,4, dinitrophemyl-hydrazine for 3 hrs at 37°C which yielded an osazone. This was allowed to react with 85% H_2SO_4 forming a reddish brown colour, which was read colorimetrically at 540 mμ. Ascorbic acid content is expressed as mg/100 gm tissue weight.

Lipids (Liver, Adrenals and Gonads)

Total lipid content was estimated employing the method of Folch et al. (1957) using a mixture of chloroform and methanol (in 2:1 V/V) as the extractant and measured gravimetrically and expressed as mg/100 gm dry tissue wt.

Total cholesterol content was measured employing the method as described by Crawford (1950). The concentration of total cholesterol was read at 540 mμ in a photoelectric calorimeter and is expressed as mg cholesterol/100 mg wet tissue weight in the case of gonads and as mg percentage of total lipid content in the case of adrenals.

The various lipid fractions were separated by thin layer chromatography (TLC). Glass plates were coated with silical gel G (about 500 μ thick) and activated at 100°C for one hour (Stahl, 1965). The plates were predeveloped overnight in ether and after drying, each plate was marked in six-3 cm lanes and was reactivated. Alternatively, pre-coated and activated glass plates were also used. Known quantity of lipid extracts was spotted in 4 lanes with a mixture of authentic standards in the last lane (one was left as the blank). The plates were then developed unidirectionally using two solvent systems as described by Freeman and West (1966). The plates were allowed to run

first in solvent-1 containing diethyl ether, Benzene, Ethanol and acetic acid in a ratio of 40:50:2:0.2 (V/V) respectively. The plates were dried in air and were developed in the second solvent system containing a mixture of diethyl and Hexane in the ratio of 6:94 (V/V). The plates were then dried in the oven for 30 minutes at 60°C and were kept in a Chamber saturated with iodine vapour to render the spots visible. Corresponding areas marking the spots of different lipid fractions from sample and standard lanes were separated and taken in test tubes and processed for quantitative estimations employing the method described by Marzo et al. (1971). The concentration of different lipid fractions are expressed as percentage of total lipid content.

Enzymes

Glucose - 6 Phosphatase - G-6-Pase (liver)

Homogenate for estimation of this enzyme was prepared in cold citrate buffer at pH 6.5 and the method of Harper (1965) was employed. Glucose - 6 - phosphate (disodium salt, Sigma Chemicals) was used as the substrate. Inorganic phosphate released was measured employing the method of Fiske and Subbarow (1925) and was read at 660 mμ on Klett Summerson calorimeter. Activity of the enzyme is expressed as μg phosphorous released/mg protein/minute.

Acid and Alkaline Phosphatases (Liver and Gonads)

Biochemical assay of both the phosphatases was carried out by employing the method as described in the Sigma technical Bulletin No.104 using p-nitrophenyl phosphate as the substrate. Enzyme activities are expressed as μ moles p-nitrophenol released/100 mg protein/30 minutes.

Lactate Dehydrogenase (LDH - Liver and Gonads).

Activity level of LDH was assayed by the calorimetric method of king as described by Varley (1975). The optical density of the colour developed was read at 440 mμ on a Bausch and Lomb spectronic - 20 Calorimeter. The activity is expressed as μ moles lactate oxidised/mg protein/15 minutes.

Succinate Dehydrogenase (SDH - Liver and Gonads)

Activity of succinate dehydrogenase was assayed according to the method of Pennington (1961). INT (Iodo nitro tetrazolium, Sigma Chemicals) was used as the hydrogen acceptor. The formazan formed was extracted in 4 ml of ethylacetate and the colour intensity was read at 490 mμ on a Bausch and Lomb spectromic - 20 calorimeter. Enzyme activity is expressed as μ moles formazan formed/mg protein/15 minutes.

Adenosine triphosphatase (ATPase - Liver and Gonads)

Both Na^+ - K^+ - ATPase and Mg^{++} ATPase were estimated, Na^+ - K^+ - activated ATPase was estimated according to

the method described by Stansmy (1971). Adenosine - 5 - triphosphate disodium salt (Sigma Co., USA) was used as the substrate. Mg^{++} - activated ATPase was measured quantitatively adopting the method of Umbreit et al. (1957) with adenosine triphosphate as the substrate, and ouabain was used as the inhibitor of Na^{+} - K^{+} - activated ATPase. Inorganic phosphorous released was estimated according to the method of Fiske and Subbarow (1925). Readings were taken at 660 mμ on a Klett-Summerson photoelectric colorimeter and activity of the enzymes is expressed as μg phosphorus released/ mg protein/10 minutes.

cAMP Phosphodiesterase (Liver and Gonads)

Phosphodiesterase activity was measured according to the method of Butcher and Sutherland (1962). Inorganic phosphorus released was estimated according to the method of Fiske and Subbarow (1925). Enzyme activity is expressed as μ moles inorganic phosphorus released/mg protein/20 minutes.

Transaminases - GOT and GPT were estimated according to the method described in Sigma Technical Bulletin No.104. Homogenates were prepared in 0.14 M KCl. Activities of glutamate-pyruvate and glutamate-oxaloacetate transaminases (GPT and GOT) were assayed by making use of dl-alanine or L+-aspartate and α-ketoglutarate as substrates. Enzyme activity was expressed as Karpman units/mg protein.

Ions (Serum, Gonads)

Blood for estimation of ions in serum was collected from the Brachial Vein prior to the sacrific of the birds. Serum was separated and the content of Na^+ and K^+ was estimated on an Eel-flame photometer. Lipid free tissue was taken for estimation of inorganic cations using Eel-flame photometer (Methods as described in the Eel manual). Ionic concentrations in blood and tissue are expressed in terms of mg/ml serum and mg/ g dry, lipid free tissue respectively.

Histological Technique

Tissues to be processed for histological study were fixed in Bouin's fluid and processed in the routine fashion. Adrenals and thyroid were fixed in Bouin's fluid for 24 hours and the gonads for 48 hours respectively. Paraffin sections of 5 u thickness were cut and stained with haematoxyline eosin. The ratio of cortex to medulla was determined by projecting areas at random on a 6 inch diameter screen using a 40x objective. The outlines of both cortex and medulla were drawn on thin paper, which were then cut and weighed. The percentage of both cortex and medulla was determined and the approximate cortex: medulla ratio was calculated.

Evaluation of Statistical Significance

For every estimation done, six to eight observations were made. The mean and standard deviation were obtained and student's 't' test was used to determine statistical significance.