CHAPTER - V

SEASONAL ALTERATIONS IN THE VARIOUS LIPID FRACTIONS OF GONAD IN NORMAL AND PINEALECTOMISED DOMESTIC PIGEONS, *COLUMBA LIVIA*

Many of the animals including birds designated as seasonal breeders have a well defined hectic period of breeding activities restricted to a small part of a year. Most of the regions of this planet depict a regular circannual periodicity in terms of climate and nutritional factors. It is a part of this circannual rhythmicity which is chosen by a species for its procreational activities which is best suited for begetting and rearing the young ones in terms of appropriate environmental conditions and abundance of choicest food. Once this annual best period is fixed, it is somehow entrained and imprinted within the species as an endogenous biological clock which times the annual variations by making use of varieties of external cues such as light, temperature, humidity, etc. In most of the temperate species of birds, light is the important cue (accordingly there are long day breeders and short day breeders) while for the tropical species, temperature and humidity appear to be more eventful. Whatever be the external cue, it has to act on a specific inducible phase of the endogenous clock which in turn leads to the synchronisation of many internal cues and rhythms. Light or temperature as the case may be by acting on the photo or thermo-inducible phase of the endogenous clock activates the hypothalamo-hypophysio-gonadal axis through appropriate neuro-endocrine transducers which are as yet poorly understood. Concomitant to the hypothalamo-hypophysio-gonadal axis, even other endocrine axes are activated thereby setting up the right internal environment suitable for reproductive functioning. Undeniably, these alterations in the neuroendocrine axis have to be collated with definite metabolic changes in the body in general and the gonads in particular. Of the many metabolic adjustments, changes in the so called lipid cycle of the gonads have gained wider attention and significance. Lipids apart from their structural and energy yielding properties are also important precursors of gonadal steroids. Accordingly, fluctuations in the gonadal content of various lipid fractions in relation to breeding activities have been documented for many vertebrates (Lofts and Marshall, 1959; Cavazos and Feagans, 1960; Hilton, 1961; Hoffman, 1968; Johnson, 1970). Many endocrine factors are known to affect the quantitative and qualitative content of lipids in gonads and influence their functioning (see Johnson, 1970). This fact has kindled the idea of studying the influence of pinealectomy on gonadal lipid content of domestic pigeons, Columba livia, as pineal is now well recognised as an integrator or modulator of reproductive activities through its link with the hypothalamo-hypophysial axis. Since the study is conducted during both the breeding as well as post-breeding phases, it could provide us with information on pineal involvement in the two seasons as well as the normal seasonal variation in this species.

MATERIAL AND METHODS

Domestic pigeons purchased from the local animal dealer were used for experimentation. Maintainence, operative procedure and the detailed experimental protocol were as described in the previous chapters. Pigeons of the three groups (C, PN and PX) during the two reproductive phases and at the specified time periods post-surgery (ie. 30, 45 and 60 days) were sacrificed under mild anaesthesia, the gonads removed, blotted free of blood and tissue fluids and then used for the quantitative estimations.

Total lipids were extracted from the dry tissue using a 2:1 mixture of chloroform : methanol (v/v). Extracted total lipids were eventually measured gravimetrically. Total cholesterol was measured employing the method described by Crawford (1958). Concentration of total cholesterol was read at 540μ in a photoelectric colorimeter. Phospholipid content was estimated according to the method of Dittmer and Wells (1969). Inorganic phosphate was determined at 660μ calorimetrically, adopting the classical method of Fiske and Subbarao (1925). Total glyceride content was calcuated by the method adopted by Reddy _et al_. (1972).

RESULTS

The quantitative content of various lipid fractions in the control birds during the two seasons as well as the alterations induced by pinealectomy are depicted in table-1 and Figs.1-4.

NORMAL SEASONAL CHANGES

It is obvious from the table and figures that the changes in the various lipid fractions during the two phases of gonadal activity in intact control birds are not that dramatic. Total lipids, phospholipids and total glycerides have shown more or less similar pattern of changes during the two periods. In all the three cases, the lowest level was recorded during the early breeding period which increased through mid breeding to a maximal level during the late breeding period. In the non-breeding period, the contents settled down to a moderately high steady level. Cholesterol content too depicted the progressively increasing trend through the breeding season. However, the average total cholesterol content tended to be higher in the non-breeding season than in the breeding season.

CHANGES DUE TO PINEALECTOMY

Pinealectomy induced changes are in contrast to normal seasonal changes more dramatic during both the seasons. Drastic alterations in the quantitative content of all the lipid fractions studied, could be easily discerned. Clearly, tremendous increase of all lipid fractions was the feature during 30 and 45 days post-pinealectomy which correspond to early and mid breeding phases. During all the three periods during

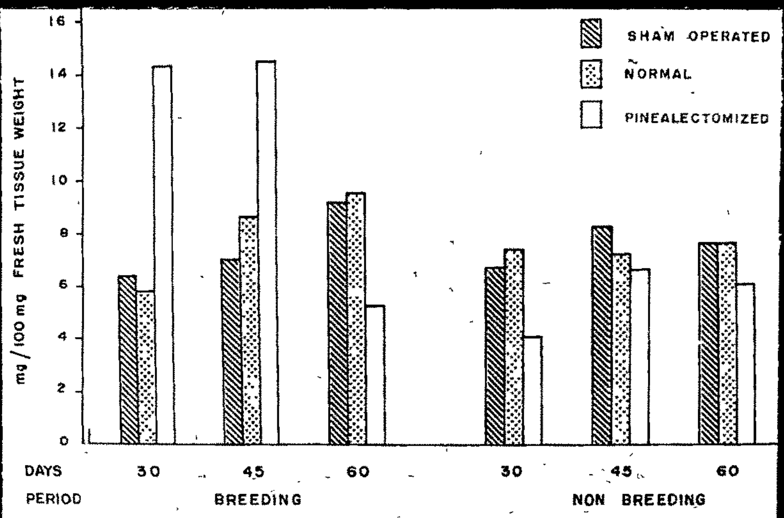

FIG. 1 CHANGES IN THE TOTAL LIPID CONTENT POST PINEALECTOMY IN DOMESTIC PIGEON

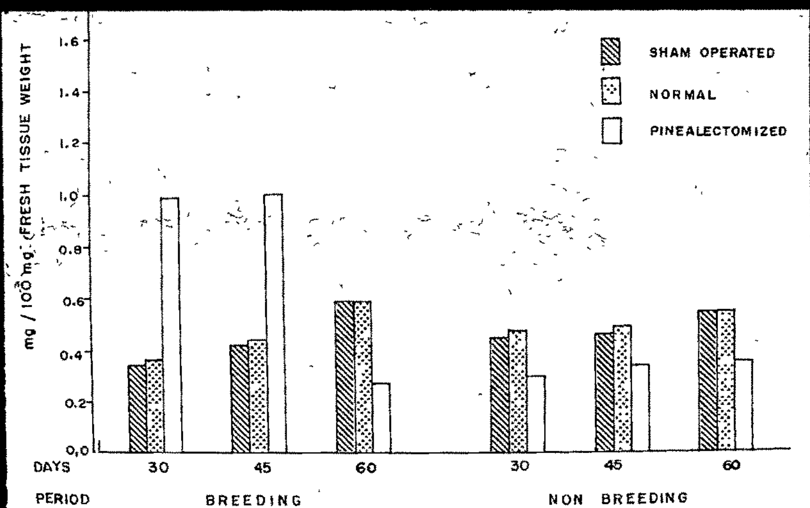

FIG. 2 ALTERATION IN CHOLESTEROL CONTENT OF GONAD POST PINEALECTOMY IN DOMESTIC PIGEON

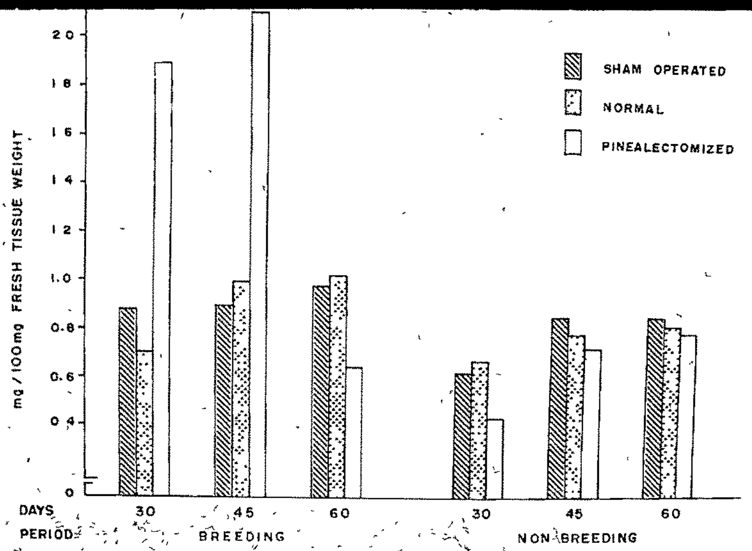

FIG 3 ALTERATIONS IN PHOSPHOLIPID CONTENT OF GONAD POST PINEALECTOMY IN DOMESTIC PIGEON

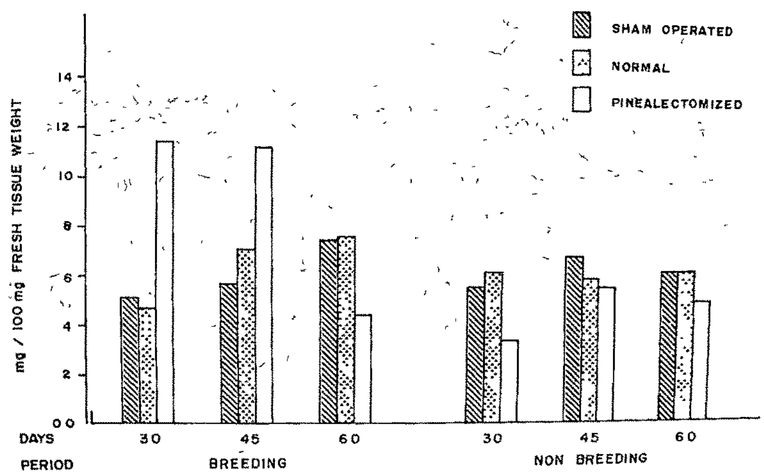

FIG. 4 ALTERATIONS IN TOTAL GLYCERIDE CONTENT OF GONAD POST PINEALECTOMY IN DOMESTIC PIGEON

non-breeding, the gonadal lipid fractions were significantly lower than those of control birds. Such a reduction in the content of lipid fractions was evident from the late breeding itself (i.e. 60 days post-pinealectomy during breeding). On an average, more than 100 % increase was registered in all the lipid components during 30 and 45 days post-pinealectomy in the breeding season. During 30, 45 and 60 days post-pinealectomy in the non-breeding season as well as 60 days post-pinealectomy in the breeding season the average decrement in the lipid fractions was about 25-40 % . Whereas the increase during the breeding season was maximal with respect to phospholipids and cholesterol, the decrease during the non-breeding season was more pronounced with respect to cholesterol and glycerides.

DISCUSSION

A general survey of the literature on gonad and lipids indicates a preponderance of studies biased towards the male gonad. Evidently, studies on ovarian lipids and their seasonal alterations are scanty. Presence of lipid in the male gonad was recognised as early as the beginning of the 20th century (Loisel, 1903). More interest was however generated only by the middle of the 20th century. With greater knowledge of lipids in gonads, the importance of lipids to gonadal function was realised. Higher content of cholesterol in the male gonad and its ability to synthesize cholesterol (Srere et al., 1950; Johnson, 1970) were suggestive of the utility of lipids to gonads. It is now known that majority of the biologically occurring compounds which can be classified under the term "lipid" are qualitatively present in the testis; and the quantitatively high content of lipids in the gonads bespeak of the important metabolic role that they have (Johnson, 1970). Unlike the continuous breeders, the seasonal breeders show periodic gonadal activation and regression which in turn should produce alterations in the lipid content (both qualitatively and quantitatively) due to its functional association with the gonads. Accordingly, almost all the seasonally breeding vertebrates depict a seasonal lipid-cholesterol cycle in the form of decrease during active spermatogenesis and steroidogenesis and increase during the steroidogenically and spermatogenically inactive periods (see review by Johnson, 1970; Lofts and Lam, 1973; Skinner et al., 1973; Mcpherson and Marion, 1982). The present results on domestic pigeons do corroborate the above as lower lipid levels were obtained in the early phases of breeding. The increase in all the lipid classes by the late breeding phase is indicative of the reduced utilization of lipids concomitant to the diminishing activity of the gonads. The moderately high but steady level of lipids in the non-breeding season can suggest both decreased utilization as well as decreased synthesis.

Since the annual reproductive events of a restricted breeder are controlled by the endocrine secretions elicited at the appropriate period by the interaction of many factors, hormonal influences on lipid chemistry of gonads can be considered a certainty. In this respect, the review of Johnson (1970) on hormonal influences of testicular lipids is a ready reference. Though there is no comparable compilation of available data on ovarian lipids, presumably, influence of hormones on lipid chemistry of both the gonads can be considered to be more or less similar. Pertinent in this context is the observation of Kotak (1979) of comparatively higher content of lipids and cholesterol in the ovary as compared to that of the testis of wild pigeons. Apparently, processes like vitellogenesis and egg laying place heavy demand on the female gonad, and the ovary is in this respect equally adept in meeting the lipid requirements. Pineal ablation in the present study resulted in increased content of all lipid components in the breeding season in the regressed gonads. The average increase being about 98 % for glycerides, 105 % for total lipids, 135 % for phospholipids and 150 % for cholesterol. Apparently, maximal elevation was shown by cholesterol and phospholipids indicating their non-utilization and accumulation in the wake of the progonadotropic role of pineal (Chapter 1). This is in contrast to the reports of Elena et al. (1979a,b) and Elena (1980) who have shown increased cholesterol content post-pinealectomy and decreased cholesterol after administration of melatonin free extract in the testis of rat. The raison d'être for this is the fact that rat being a continuous breeder a steady synthesis and utilization would be a feature which could lead to increased lipid contents under enhanced gonadal function and decreased contents during suppressed gonadal activity. In this light of the progonadotropic role of pineal during the breeding season in domestic pigeons (Chapter 1), the reports of increased cholesterol and other lipid fractions in the gonads after hypophysectomy (Johnson, 1970; Hafiez and Bartke 1972) are relevant and agree well with the present observations.

Further confirmation to the dictum of inverse relationship between gonadal cycle and lipid cycle is provided by the herein noted decreased contents of various lipid classes in the gonads of pinealectomised birds during the non-breeding season whence the gonads were enlarged and functionally active in keeping with the suggested antigonadal role of pineal in this season (Chapter 1). The average decrement in lipid fractions in the pinealectomised birds range from 25-40 % with the maximal decrease of 40 % being shown by the cholesterol fraction, while the other three lipid classes (total lipids, phospholipids and glycerides) showed a more or less identical percentage depletion of 25-28 % . The pronounced depletion of cholesterol is understandable due to its importance as the steroidogenic precursor. With the purported antigonadotropic role of the pineal, its removal can be considered to elicit output of gonadotropic hormones which may be responsible for the observed gonadal activation. The changes in the gonadal lipid fractions observed in this context are adequately corroborated by the reports of gonadal lipid depletion under the influence of gonadotropic and gonadal hormones (Johnson, 1970; Barysaw, 1980; Sinha, 1982; Mukerjee and Bhattacharya, 1982). It is also worth noting that Manimekalal _et al._ (1980) had observed striking changes in the lipid classes in harmony with the rhythm of endogenous gonadal and gonadotropic hormones during oestrous cycle of female rats. In addition, metabolic hormones such as epinephrine and thyroxine have also been shown to increase and decrease respectively the content of cholesterol and other lipid fractions in the gonads of rabbit and rats (Ewing _et al._, 1964; Massie, 1968). These observations seem to tally with the observed seasonal and pinealectomy induced alterations in the cortico-medullary ratio of the adrenal gland and thyroid histology of the domestic pigeons (Chapter 1). It is however difficult to evaluate and predict the relative importance of the above metabolic hormones and gonadotropic and gonadal hormones in the lipid cycle of the gonads associated with seasonal reproductive periodicity. It is likely that there might be some sort of permissive and/or synergistic/antagonistic interactions which might ultimately control the metabolic modulations of gonads.