GENERAL CONSIDERATIONS

Investigations on ascertaining the avian breeding seasons can be tackled by histophysiological studies or ecological means or sometimes both the approaches are employed. In the Columbiformes for example, Lofts et al. (1966, 1967) and Murton (1958, 1960) have considered eco-physiological criteria in understanding the breeding cycles whereas, Frith et al. (1974, 1976) and Crome (1975 a & b, 1976) while assessing the breeding biology of different species of Australian pigeons in different environmental conditions, have employed ecological parameters. Recently, Murton and Westwood (1977) have extensively dealt with allround ecological as well as physiological observations on the avian reproductive cycles to bring out very clearly various themes of rhythmicity of gonadal development under tropical and temperate climates. They have even suggested some evolutionary trends in the adaptation of species to different climatic conditions. In this context, it is worth pointing out that in the case of pigeons, marked periodicities of annual gonadal developmental phases have been self-suggestive. Whatever the means, most of the studies on pigeons indicate that these birds do have a capacity to breed throughout the year. Such observations tend to favour catagorization of the Columbiformes birds along with some of the domestic

species of birds that have more or less a continous reproductive activity.

In the present project, chiefly histoenzymological and endocrinological parameters were chosen to assess the reproductive cycles of feral pigeons. These experiments were carried out for a period of 5 years commencing from the year 1973. These parameters were chosen because of the fact that with respect to reproductive cycles in wild species of birds, comparatively fewer attempts have been made from the physiological and biochemical point of view. For all the experiments that were carried out, the birds (Indian feral blue rock pigeon : Columba livia G.) were shot down in the University campus with an air rifle during certain morning and evening hours as specified in each chapter. Both the sexes were studied.

Preliminary investigations were chiefly concerned with estimation of cholesterol and lipid contents in the liver, gonads and blood plasma of the birds (Chapter I). There are ample evidences that indicate, that in birds, lipids synthesized in the liver are utilized for egg-yolk synthesis and these are transported via the blood plasma (Ranney et al., 1951; Husbands and Brown, 1965). Synthesis and release is known to be under the influence of female sex hormones. In the male birds too, cholesterol positive lipids get utilized or accumulated depending upon the

functional or non-functional state of gonads; with respect to hormonogenesis (Lofts and Murton, 1973). Thus, fluctuations in the levels of lipids and cholesterol could broadly give an idea of the probable breeding times in a year. It was found that during certain phases of the yearly cycle, cholesterol and lipids revealed well marked declining or increasing trends in the tissues studied. In the case of the male birds hepatic cholesterol exhibited a steadily decreasing trend from February to September which was indicative of its sustained rate of release in the general circulation. In the testes, a remarkable increase in the levels of cholesterol and lipids was discernible in the months of June and July indicating that these constituents remained metabolically unutilized leading to their accumulation. Such non-utilization and storage of cholesterol in the testes of vertebrates reflects a regressive phase (Lofts and Marshall, 1959). Comparatively lower levels, particularly that of cholesterol, were evident in the months preceeding and succeeding to June and July. So was also true for ovarian cholesterol. From August onwards, cholesterol in the ovaries exhibited a steady decrease which indicated an acceleration of steroidogenesis by the ovarian tissue. That cholesterol serves as a precursor for the synthesis of sex steroid hormones need hardly be stressed. The blood plasma cholesterol levels

never quite faithfully reflected the changes associated with the rates of either release from the liver or uptake by the gonads. Fluctuations of plasma cholesterol were very wide and sometimes erratic. In the early wet summer (June, July) when all the other indications were aiming at a halt in the gonadal steroidogenesis, plasma cholesterol did show an increase. Except for a small rise in October, plasma lipids of males revealed more or less a constant state of levels all throughout the year. A steep rise in blood lipids in the case of female birds was however strikingly noticeable in the months of March when hepatic lipids were decreasing. Liver lipids, perhaps under the active influence of estrogens, were getting mobilized for egg-yolk synthesis. Yellow yolk laden ova were commonly seen in March-April and September-October and an egg trapped in oviduct was a frequent feature. Another peak of plasma lipids in October in the females was observable that could be due to the similar situation prevailing in gross ovarian structure noted during September-October. From the data obtained and taking aid of some behavioural observations (pairing, mating, nest building), it was possible to ascertain the probable breeding times in the year when gonadal steroidogenesis would be most active. These were the functional stages of gonads : one seen in Summer (March to May) and the other in wet summer and early

winter (late August to October). During the months of January, February, June and July and November, comparatively less active gonadal phases were observed. It was also concluded that a minor simulation to breeding could possibly occur in December.

To pinpoint the reproductive cycles more precisely, further assessment of the physiological state of gonads was thought essential. Keeping this in mind, and to focus attention on gonadal steroidogenic pathways, total and neutral lipids and activities of two key enzymes of sex stroid metabolism viz. Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSDH) (oxidoreductase) and 17β-hydroxysteroid dehydrogenase (17β-HSDH) (oxidoreductase) were studied histochemically throughout the year in both the sexes (Chapters II & III) during the years 1975-1976. These observations helped locating the steroidogenic cellular sites and also permitted a study of fluctuations in the activities of the enzymes so as to judge either increase or decrease in the rate of sex hormone production. A distinct non-functional gonadal state in both sexes in the months of June and July was evident; when the total weight of gonads was minimum (Chapter VII) and the intensities of both the hydroxysteroid dehydrogenases were most feeble. The latter observation showed that gonadal sex hormone synthesis in the months of June and July was

the least. This regressive phase was highlighted by accumulated lipids and cholesterol in the gonads. On the other hand, two distinct peak functional stages were observable before and after this regressive state. Activities of enzymes were maximally intense during March-April and September-October. Moderate enzyme activities were discernible in the months of January, February, November and December. Interstitial cells of Leydig exhibited maximum intensities of enzymes under study indicating that those were the main sites of androgen production in testes as has amply been proved in many avian species (Deane and Rubin, 1965, Garnier et al., 1973, Gorman, 1974). Moderate intensities of 3β-HSDH and 17β-HSDH in the lining of the seminiferous epithelium were probably due to participation of the Sertoli cells in the production of sex hormones. The endocrine role of these supporting cells in androgen production has been documented by Lofts (1972) on the basis of ultrastructural, histochemical and in vitro tracer studies in the snake (Naja naja).

In the case of the female birds, both the granulosa as well as the thecal cells of the pre-ovulatory follicles exhibited strong enzyme activities suggesting that both the types of cells may be actively involved in estrogen production. Over and above this, it also appeared that

the post-ovulatory follicles on a short time basis and atretic follicles on long term basis partake in steroid hormone production, particulary in the synthesis of progesterone. The above deductions were based on observing strong activities of 3β-HSDH(P) particulary in the atretic follicles. Recent investigation (Dick et al., 1978) reveals that progesterone levels in the post-ovulatory follicles of fowl drop within 15 hours after ovulation and continue to decline upto 52 hours. It is therefore possible that post-ovulatory follicles are short lived endocrine tissues.

In order to gain a wider insight of the metabolism of sex steroids, it was felt desirable to study localization of steroid catabolizing enzymes in extra-gonadal tissues that may probably involved in steroid hormone breakdown and excretion. That both the liver and kidney are actively involved in steroid hormone catabolism and excretion has been shown by biochemical and clinical investigations (Baillie et al., 1966). Biochemical and histochemical studies in this regard in avian species are rare. Two enzymes were studied : 3 α-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase in the liver, kidney and intestine of pigeons during the breeding and non-breeding phases (Chapter IV). Results revealed very weak intensities of these enzymes in all the three tissues

during the June-July phase (non-breeding) increasing gradually through August and reaching maximum intensities during September and October (breeding). A possible correlation has been attempted between the feeble enzyme activities encountered in June-July and lower sex hormone output during this phase (Chapters I, II & III). On the other hand, during the peak breeding times (September - October), higher circulating levels of sex hormones due to increased synthetic rate would need enhanced rate of breakdown and consequent excretion. This was noted particularly in the liver and the kidney wherein the enzyme activities were high in the breeding season. In the kidneys, the convoluted tubules exhibited maximum intensities of the enzymes whereas the medulla remained unreactive. Glomeruli showed moderate activities. With respect to liver, the hepatocytes demonstrated a uniform pattern of distribution and apparently no periportal, peribiliary or sinusoidal localization was discernible. The convoluted tubules and hepatocytes (in the kidney and liver respectively) are the major sites where alterations and inactivation of steroid compounds at 3 α- and 17β-positions were effected. The gastrointestinal tract does not seem to partake in the interconversions of sex hormones because very weak intensities of both the enzymes were encountered even during the breeding season. Sex hormone catabolism thus

may occur in the liver but fat soluble pathway via the bile into the g.i. tract, where further metabolism may possibly occur, does not seem to be operative at least in the case of pigeon. The metabolites may be converted to watersoluble forms and after such suitable modifications in the liver and kidney, may be excreted via urine.

Commenting on the phylogenetic trends with regard to synthesis of ascorbic acid (AA), Chatterjee (1973) suggested that the capacity to synthesize AA commences with amphibian kidney, resides in the reptilian kidney and gets transferred to the liver in mammals. According to him, kidney of the non-passerines is the site of synthesis (e.g. in pigeon and chicken : graminivores) and in the higher passerines (e.g. crow and myna : omnivores) both the liver as well as the kidney posses this capacity. Besides the conventional roles played by AA in assisting the functions like resisting cold stress, haemotopoeisis, synthesis and maturation of collagen, reticulin, dentine and bone matrix and metabolism of glycosaminoglycans (GAG), recent investigations point out that AA may also assist in hormonogenesis (Biswas and Deb, 1970). To elucidate the possible role played by AA in steroidogenesis, estimations of the same were carried out in the liver, gonads, kidney and blood serum during the breeding and non-breeding phases in both the sexes of birds. Generally,

low levels were encountered in the breeding phase and higher in the non-breeding phase in all the three organs except the blood serum, that is when Δ^5-3β-HSDH activity in the gonads was high and low respectively. In toads, AA in conjunction with leutinizing hormone may increase testicular Δ^5-3β-HSDH activity (Biswas, 1969). In the present study, high AA concentrations in the non-breeding stages were observed when gonadal Δ^5-3β-HSDH was lowest. AA concentrations could thus not be correlated with Δ^5-3β-HSDH activity and consequently with steroid hormone production. On the other hand, lower AA concentrations in all the tissues were noted when testicular 3β-HSDH was at its peak (March-April). This was suggestive probably of active utilization of AA in enhancing testicular sex hormone output. The increased output of androgens may regulate tissue concentrations of AA since Dieter (1969) and Majumdar and Chatterjee (1974) have shown that in the cockerels and the rats, tissue concentrations of AA are under the control of testosterone. High AA levels in non-breeding phases perhaps indicated the postnuptial non-utilization leading to its subsequent storage in tissues (Malakar, 1963).

Classical reports by Goodridge and Ball (1966, 1967 : in silver king pigeons) and by Goodridge (1968 a and b) and O'Hea and Leveille (1969 : in the domestic fowl) stress

that, unlike the adipose tissue of mammals, (Faverger, 1965), it is the liver which plays a major role in lipogenesis. Involvement of 'malic' enzyme in this process plays a predominant role (Duncan and Common, 1967) in supplying the reduced NADP. Balnave (1968, 1969) has suggested that over and above estrogens, androgens and progesterone influence markedly the overall rates of lipid synthesis. With enhanced lipogenesis, the levels of 'malic' enzyme are greater in the laying hen as compared to the non-laying hen and cockerels (Pearce, 1971, 1973) and this may be estrogen mediated.

With a view to investigate on the interrelationship between gonadal hormones and lipogenesis, another project was taken up. The lipogenic rates during the breeding (March-April) and non-breeding (June-July) phases were studied in both the sexes. Levels of 'malic' enzyme and glucose-6-phosphate dehydrogenase were assayed quantitatively in the liver and gonads. It was found that hepatic 'malic' enzyme plays a prominant role in fatty acid synthesis as was apparent from the comparatively higher levels (about 5 fold higher than G-6-PDH levels). The shunt enzyme seemed to play a rather minor role in this respect as the levels of this enzyme in the liver were very low. This is in conformity with the investigations (Goodridge and Ball, 1966, 1967) that G-6-PDH contributes negligibly

to fatty acid synthesis. Also, the levels of these two enzymes were higher in the female birds which goes to show that fatty acid synthesis is in all probability higher in the females than in males, particulary during the breeding season. This is not quite surprising keeping in mind the fact that lipid precursors are needed in great quantities for egg-yolk synthesis. The levels of both enzymes in both sexes during the breeding peak (March-April) were perhaps under the influence of estrogens and androgens. Comparatively high levels of G-6-PDH in the testes (as compared to those found in liver) have been correlated more with the demands for supply of precursors of nucleic acids for spermatogenesis rather than with lipogenesis.

Finally, the body weights and gonadal weights were recorded and the gonads were studied histologically. Body weights during the wet summer (June to September) appeared to be highest in both the sexes i.e. during the non-breeding season; and during the preceeding and succeeding breeding phases, body weights recorded low values. Males were significantly heavier than the females all throughout the year which is in conformity with early investigation by Wexelsen (1936) on pigeons. Higher body weights during June and July have been correlated with the easy availability of abundant food. Moderately

high winter body weights could possibly be due to winter fattening and enlarging reproductive organs. Two clear cut peaks with respect to gonadal weights were seen during March-April and September-October phases. Minimum gonadal weights were observed in June-July. Active spermatogenesis was evident in the months of March-April and late August to October. January, February and to a certain extent December too, revealed spermatogenesis but hydroxysteroid dehydrogenase activities in the gonads were moderate and behavioural observations did not imply any breeding activity. Lofts et al. (1966) have commented that the testes of feral pigeons im temperate climate are capable of remaining in the active spermatogenic condition well after the breeding phase for a considerable period time. ~~(upto 6 months)~~. In the present context, it could be said that after the breeding phase (October), testes did not exhibit complete supression of spermatogenic activity. June and July showed typically regressed gonads. Right testis was always heavier than its left counterpart. All these observation clearly indicated that the feral pigeons breed twice a year and the two relatively long periods (approximately 3 months each) are separated by a distinct regressive phase in June and July. Some individuals of different populations of pigeons may breed upto December. The broad spectrum of reproductive

activity is probably the result of abundantly available food supply as was remarked by Lofts et al. (1966) in case of British Columbidae, and perhaps also, because of the good deal of shelter and protection that these birds enjoy inhabiting the niches in and around the towns and cities.