CHAPTER I

SEASONAL VARIATIONS OF LIPIDS AND CHOLESTEROL IN LIVER GONADS AND BLOOD PLASMA OF FERAL BLUE ROCK PIGEON COLUMBA LIVIA G.

Generally birds breed at such times of the year when on the average young ones can be profitably raised (Lack, 1950). A number of detailed studies describe seasonal changes in the gonads of male birds (Blanchard, 1941; Wright and Wright, 1944; Blanchard and Erickson, 1949, Threadgold, 1956; Selander and Hauser, 1965; Scott and Middleton, 1968; Silverin, 1973; Gorman, 1974; Lewis, 1975a&b; Mori and George, 1978) whereas parallel cyclic histophysiological studies on female birds are almost lacking. It is well known that gonads of wild birds undergo cyclic changes through the breeding and non-breeding states (recrudescence and regression) of the reproductive cycle (Marshall, 1961; Nalbandov, 1970). The exogenous as well as the endogenous factors control the rhythmicity of the sexual cycle. According to Benoit (1964) light is an extremely important factor stimulating gonadal activity of birds; a retino-hypothalamohypophyseal pathway being involved in this stimulation. However, some temperate zone species, such as the populations of feral pigeons inhabiting the towns and cities, have acquired a capacity to breed throughout the year as a result of availability of continuous food supply. (Lofts et al., 1966), though natural selection favours conservation of an annual breeding cycle. Many workers have investigated the seasonal gonadal cycles of Columbid birds, noteworthy among which are those concerning the dove (Lees, 1946); British Columbidae (Lofts et al., 1966, 1967, 1969); the wood pigeon and feral pigeon (Murton, 1958, 1960; Murton et al., 1973)and frugivorous rain forest and ground feeding pigeon of Australia (Frith et al., 1974). Such observations imply that these species have more or less continuous reproductive activity. In any case, after the spontaneous progression from regeneration to acceleration phase, the sex hormones are known to be liberated (Marshall, 1961). It cannot be doubted that these hormonal changes have varied physiological effects which are of great significance (Nalbandov, 1970). These alterations in the levels of hormones are likely to have significant influence on certain metabolites, particularly those involved in biosynthesis of sex hormones and those involved in egg yolk synthesis. In the vertebrate gonads, depletion of cholesterol-positive lipids during breeding phase and accumulation of the same during regression characterize the two stages.

As a preliminary step therefore, an attempt was made to find out whether there exists anything like a basic annual alteration of the breeding and non-breeding times in feral blue rock pigeon (C. livia). Keeping the above mentioned background in mind, total lipids and cholesterol were estimated in the liver, gonads and blood plasma throughout the year in both sexes of birds. In order to point out the sex difference, if any, monthwise levels obtained in case of males were compared with those of the female birds.

MATERIAL AND METHODS

Indian feral blue rock pigeons (C. livia G.) were studied in the present work. Birds were shot down with an air rifle, around the University campus between 8.00 a.m. to 10.00 a.m. and 4.00 p.m. to 6.00 p.m. Blood was collected from the jugular vein in heparinized tubes immediately after shooting. Birds were then promptly brought to the laboratory. Desired tissues were taken out, blotted free of tissue fluids and then were used for estimations. Yellow yolk-ova were discarded for estimations. Blood was cooled and then it was centrifuged. Plasma was separated and stored at -10°C until analyzed.

Total lipids were estimated employing the method of Folch et al., (1957) which was essentially same as that applied for mamalian tissues. A 2:1 mixture of chloroform : methanol (v/v) was used to extract total lipids which were eventually measured gravimetrically. Total cholesterol in the liver and gonads was extracted with 3:1 alcohol: ether mixture (v/v), which in the presence of ferric chloride reacted with sulphuric acid to yield a stable brown colour(Crawford, 1958). This was read at 540 mμ in a colorimeter against blank and compared with a known standard. A modified method of Rappaport and Eichhorn (1960) was adopted for the estimation of total cholesterol in the blood plasma. Proteins were denatured with sulphosalicylic acid liberating protein bound lipids including cholesterol, which was allowed to react with acetic-anhydride and sulphuric acid. This produced a green colour (Lieberman reaction) which was measured colorimetrically at 540 mμ. Quantitative data were compiled during the year 1973-1975.

RESULTS

Female birds, on an average weighed less than males at any time of the year (Chapter VII). The weight of male bird was usually between 280 to 340 gms. and that of female was between 240 to 280 gms. (Chapter VII). On the basis of certain observations like pairing, nest building, mating activity and presence of yellow yolk studded ova in the ovary of females, it was found that feral pigeons do show some breeding activity at certain periods in all three seasons of the year (Winter : November, December, January and February; Summer: March, April, May and June; Wet Summer (monsoon): July, August, September, October).

No significant difference in the levels of lipids was found to occur in the liver and blood plasma of male and female pigeons. Same was also true for plasma cholesterol. It was interesting to note that testicular lipids and cholesterol always exhibited lower (2-3 times) levels in all the months of the year than those of the ovary (Figs. 1 & 2).

Concentration of liver cholesterol of the female birds generally recorded a higher level than that of male birds.

Monthwise comparison of testicular weight between the right and left testes revealed that the right was comparatively heavier than the left one (Chapter VII).

Lipids: Monthwise variations in the levels of total lipids in the liver, gonads and blood plasma for male as well female pigeons are presented in Table I and Fig. 1. From the table and the figure, it is apparent that the highest recorded level for liver lipids was seen in the month of February in case of males whereas female liver lipids showed two peaks, one in November and the other minor one in February. Liver lipids decreased to the lowest levels in July for males and in May for females.

TABLE I

Seasonal variations of lipid in liver, gonads and blood plasma (Mean ± S.D.)

Month	Male birds g/100g wet weight		g/100 ml	Female birds g/100g wet weight		g/100 ml
	Liver	Testis	Blood Plasma	Liver	Ovary	Blood Plasma
January	4.451 ± 0.565	2.585 ± 0.318	0.816 ± 0.189	4.103 ± 0.597	3.335 ± 0.729	1.050 ± 0.205
February	5.828 ± 1.601	2.815 ± 0.332	0.958 ± 0.233	5.745 ± 0.914	6.178 ± 0.352	0.823 ± 0.123
March	3.935 ± 0.807	2.152 ± 0.600	0.968 ± 0.255	4.412 ± 0.704	4.251 ± 1.333	1.672 ± 0.702
April	4.241 ± 0.530	2.570 ± 0.306	1.060 ± 0.256	3.760 ± 0.587	3.478 ± 0.622	0.980 ± 0.164
May	3.936 ± 0.417	2.596 ± 0.311	1.060 ± 0.243	3.488 ± 1.085	4.290 ± 1.459	0.950 ± 0.125
June	4.296 ± 0.873	3.000 ± 0.302	0.871 ± 0.115	3.925 ± 0.697	5.681 ± 0.862	1.143 ± 0.272
July	3.250 ± 0.738	3.090 ± 0.280	1.050 ± 0.061	3.870 ± 0.368	4.748 ± 1.413	0.810 ± 0.111
August	4.104 ± 0.423	2.499 ± 0.277	0.983 ± 0.133	3.845 ± 0.602	3.648 ± 1.125	0.887 ± 0.186
September	4.026 ± 0.630	2.511 ± 0.372	1.035 ± 0.160	4.026 ± 0.464	3.171 ± 0.451	0.903 ± 0.246
October	4.926 ± 0.647	2.505 ± 0.488	1.223 ± 0.368	4.495 ± 1.161	3.167 ± 0.309	1.157 ± 0.283
November	4.237 ± 0.594	2.605 ± 0.391	1.003 ± 0.149	6.540 ± 0.954	4.152 ± 1.141	1.040 ± 0.207
December	4.801 ± 0.574	2.530 ± 0.257	0.908 ± 0.076	4.960 ± 0.432	5.973 ± 1.497	0.898 ± 0.242

Gonadal lipids were higher in June, July and February in the case of males whereas ovarian lipids showed highest levels in February and December. From July to August, testicular lipids reflected a decline; remaining at more or less same levels in September and October, Similarly, after February, the same again showed marked drop, reaching to lowest concentration in March (2.15g/100g wet weight).

Regarding the plasma lipid levels it could be seen that the variations presented the following pattern : (i) In the case of males, the values showed a small gradual increase from January to May. In these months, highest values were seen in last two months. Thereafter, no particular trend was detectable. Peak concentrations were, however, encountered in the month of October (1.22g/ 100 ml plasma) (ii) Female birds showed variations without any discernible trends except that a peak value could be observed in the month of March (1.67g/100 ml plasma) followed by a minor peak in October.

Cholesterol: Cyclic variations in the levels of total cholesterol of liver, gonads and blood plasma for both sexes are given in Table II and Fig. 2. It is evident from the table and the figure that peak values of liver cholesterol in male pigeons was encountered in the month of February. From February to September, these levels exhibited a steady declining trend reaching to their

TABLE II

Seasonal Variations of cholesterol in Liver, gonads and blood plasma (Mean ± S.D.)

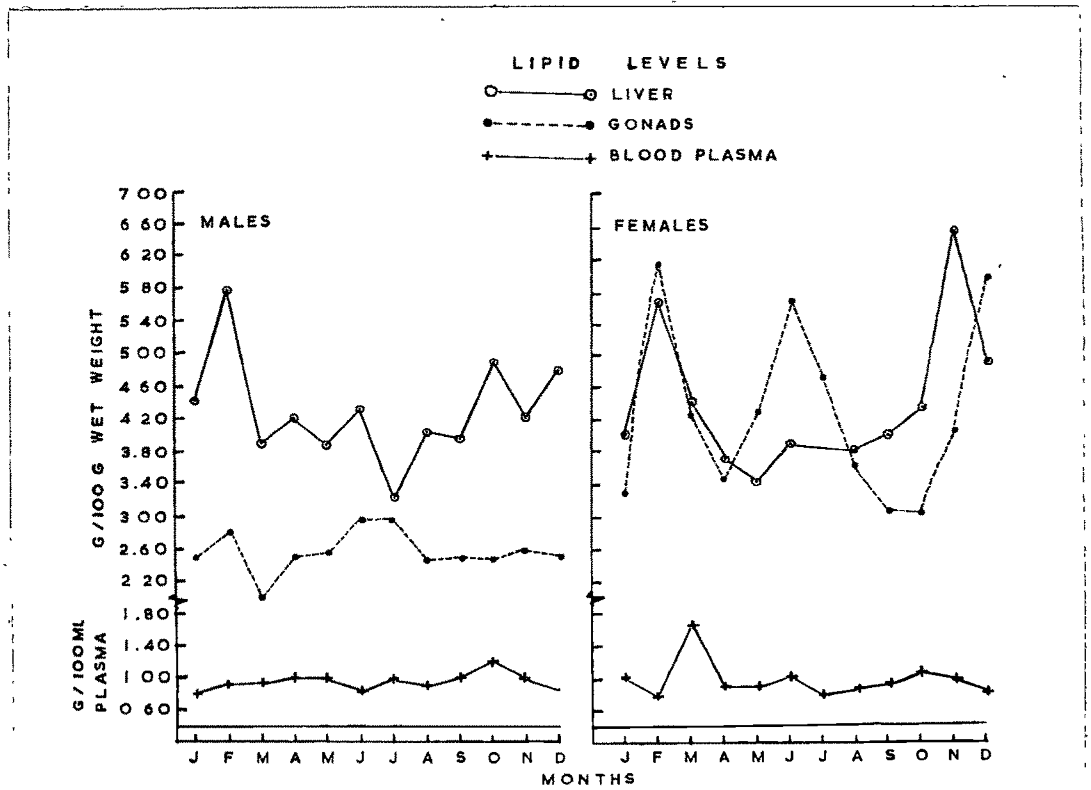
Month	MALE BIRDS g/100g wet weight Liver	Testis	mg/100 ml Plasma	FEMALE BIRDS g/100g wet weight Liver	Ovary	mg/100 ml Plasma
January	0.571 ± 0.149	0.489 ± 0.082	273.75 ± 32.81	0.564 ± 0.038	1.086 ± 0.111	348.37 ± 47.95
February	0.793 ± 0.060	0.570 ± 0.096	333.68 ± 65.27	0.885 ± 0.100	1.089 ± 0.220	343.97 ± 30.45
March	0.706 ± 0.051	0.548 ± 0.038	299.51 ± 41.27	0.797 ± 0.114	1.157 ± 0.211	369.41 ± 23.78
April	0.620 ± 0.050	0.548 ± 0.037	305.93 ± 40.67	0.835 ± 0.122	1.396 ± 0.271	296.96 ± 26.20
May	0.635 ± 0:072	0.525 ± 0.083	377.02 ± 38.25	0.598 ± 0.082	1.487 ± 0.209	362.29 ± 20.82
June	0.600 ± 0.072	0.799 ± 0.131	315.63 ± 38.72	0.552 ± 0.114	1.699 ± 0.397	390.52 ± 78.88
July	0.527 ± 0.037	0.527 ± 0.065	422.27 ± 58.87	0.616 ± 0.078	1.261 ± 0.293	378.72 ± 27.57
August	0.441 ± 0.049	0.415 ± 0.062	380.44 ± 53.85	0.520 ± 0.086	0.794 ± 0.069	362.91 ± 69.53
September	0.426 ± 0.061	0.380 ± 0.055	400.72 ± 52.30	0.454 ± 0.062	0.909 ± 0.112	317.08 ± 74.68
October	0.647 ± 0.024	0.520 ± 0.130	438.50 ± 30.13	0.589 ± 0.051	0.945 ± 0.381	345.14 ± 93.37
November	0.474 ± 0.081	0.431 ± 0.122	326.50 ± 23.49	0.638 ± 0.135	1.310 ± 0.388	397.05 ± 16.87
December	0.553 ± 0.091	0.441 ± 0.044	346.50 ± 24.67	0.653 ± 0.073	1.559 ± 0.290	321.56 ± 53.35

EXPLANATION TO FIGURES

Fig. 1. Graphic representation of seasonal variations of lipids in liver, gonads and blood plasma in both sexes of birds.

Fig. 2. Graph showing cyclic fluctuations of cholesterol levels in liver, gonads and blood plasma in both the sexes.

FIG. 1


LIPID LEVELS
LIVER
GONADS
BLOOD PLASMA
MALES
FEMALES
G/100 G WET WEIGHT
G/100ML PLASMA
7.00
6.60
6.20
5.80
5.40
5.00
4.60
4.20
3.80
3.40
3.00
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2.20
1.80
1.40
1.00
0.60
J F M A M J J A S O N D
MONTHS

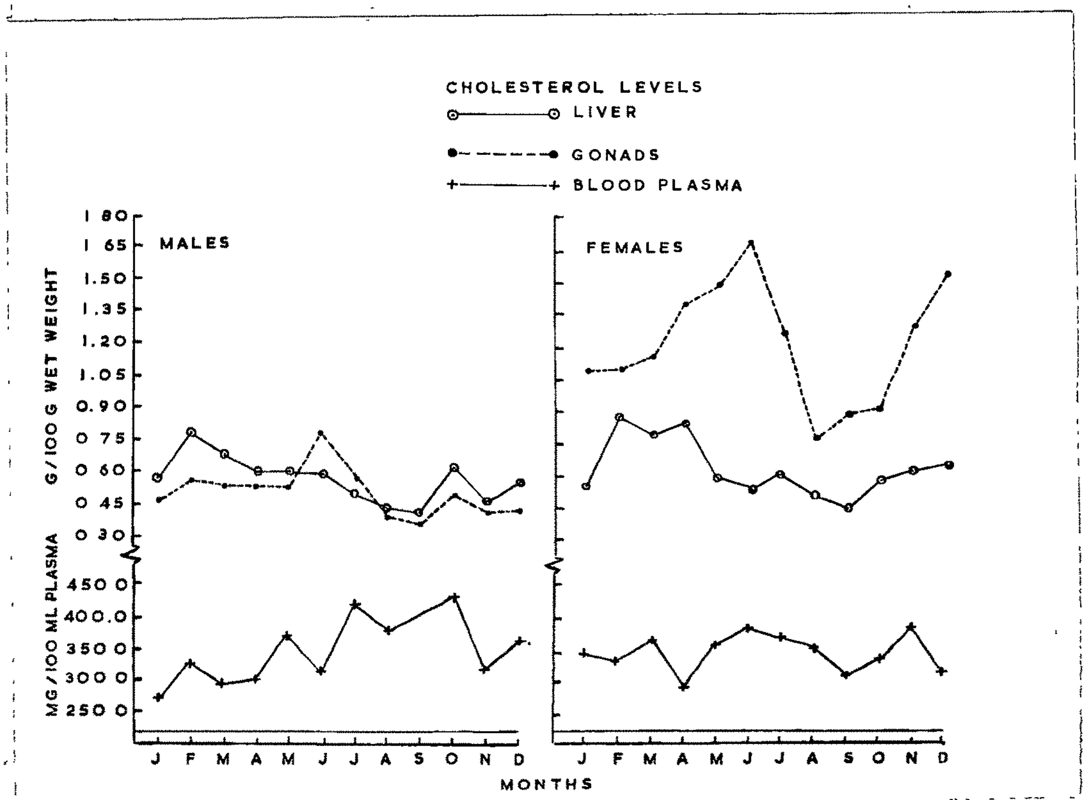
CHOLESTEROL LEVELS
LIVER
GONADS
BLOOD PLASMA
MALES
FEMALES
G/100 G WET WEIGHT
MG/100 ML PLASMA
1.80
1.65
1.50
1.35
1.20
1.05
0.90
0.75
0.60
0.45
0.30
450.0
400.0
350.0
300.0
250.0
J F M A M J J A S O N D
MONTHS

FIG. 2

minimum in September. Female birds too, showed highest liver cholesterol concentrations in February and lowest in September.

Testicular cholesterol on the other hand showed the highest values in June, after which it decreased gradually till September. September revealed the lowest mark for the same. Ovarian cholesterol was at its peak level in June. August, September and October showed low levels of ovarian cholesterol.

Plasma cholesterol in both sexes fluctuated widely. In wet summer, male birds showed highest levels whereas minimum values were observed in January. Higher levels of plasma cholesterol in females were seen in June, July and August. Peak concentrations for the same were obtained in November.

DISCUSSION

The levels of ovarian lipids and cholesterol during any month of the year were significantly higher than those of the testes. This is a clear cut indication of sex difference in gonadal lipids and cholesterol. It shows that, basically the rate of synthesis of gonadal cholesterol in female pigeons is comparatively higher than that obtained in the males. Similarly, the levels of cholesterol in the liver of female birds generally exhibited higher values than those of male pigeons. Though the difference was not as prominant as in the case of gonadal cholesterol, yet it was significant. It may be surmised that hepatic cholesterol synthesis can occur comparatively easily in females than in males.

Watkins (1972) has reported that in female rats, the output of triglycerides by isolated perfused livers exceeded that occurring in males. Husbands and Brown (1965) too, have shown that the liver of the laying hen had more triglycerides, and was more efficient in incorporating 2-C^{14}-acetate into liver lipids than that of cockerels, and that more 2-C^{14}-acetate was incorporated into cholesterol fraction in laying hens than in cockerels. Again, Balnave (1969) has reported that only estrogens affect the liver weight, liver dry matter percentage or total liver lipid content significantly as compared to androgens or progesterone. During the course of the present work, we obtained consistently high values for liver cholesterol during the period February-April and November-December in the female pigeons. This may thus be due to higher rates of synthesis of cholesterol in the liver of female which could be estrogen mediated.

Males: A steadily declining trend in liver cholesterol of male birds beginning from February to September could mean that this was being increasingly released into the blood. If this is so, then the plasma cholesterol levels would be expected to reflect the change, but that could not be seen from the data obtained. This might have been due to uptake and utilization of cholesterol by peripheral tissues in general. However, remarkable interrelationship was very evident in the levels of cholesterol of liver, gonads and plasma during the transition period from the month of May to June. There was a small drop of about 5% in the liver level and interestingly enough plasma level also registered a significant reduction of about 17%. Against this background the testes recorded the highest level of cholesterol of the year with a steep rise of about 52%. Testicular lipids too, reflected highest concentration in June and July. In light of these observations, it could be clearly marked that there appears a sudden change in the metabolic pattern of the testis indicating post-nuptial non-utilization of cholesterol postive lipids and its subsequent accumulation heralding distinctly the regression phase. Histochemical studies (Chapter II) revealed low steroid dehydrogenase enzyme activity and accumulated lipids in interstitium or seminiferous tubules in these months. Such histochemical studies with regard to testicular steatogenesis have been correlated with non-breeding phases or regressive testis (Marshall, 1949; Lofts and Marshall, 1959).

As a corollary to the above observations it could be said that in the preceding months of February to May the gonadal lipids and cholesterol levels being comparatively low, the latter might be getting actively metabolized in the synthesis of androgenic hormones by the testes. That cholesterol is a precursor for steroid hormones has often been proved (Bloch, 1945; Zaffaroni et al., 1951; Hechter, 1953; Hayano et al., 1956; Heard et al., 1956). Bartke (1971) has remarked that cholesterol, particularly its esterified form serves as the precursor for androgenic steroids in mouse testes. The avian testicular tissue also has been shown to synthesize testosterone utilizing cholesterol as a precursor. (Lake and Furr, 1971). With respect to plasma lipids in males, no particular trend was noticeable. In the case of Xenopus laevis, it has been shown that non-estrogenic steroids have no significant effect upon serum lipids (Redshaw et al.,1969) supporting the hypothesis that only estrogens cause vitallogenic response in oviparous vertebrates. This may perhaps be also true in case of feral pigeons where no significant rise in plasma lipids was seen during the probable breeding phases.

Females: A strikingly noticeable fact was that plasma lipids in females reached their peak level in March (1.67g/100 ml plasma) with a concomitant decrease of liver lipids from March to May. Yellow yolk laden ova were present in the ovaries during these months as an indication of the first breeding season of the year. Also, comparatively lower ovarian cholesterol levels in the months of January, February and March revealed that the precursor material was probably getting utilized for female sex hormone synthesis.

All these facts indicated that liver lipids were steadily mobilized, probably under the influence of estrogens, resulting in hyperlipemia in March and the subsequent uptake of the same for egg yolk synthesis. It has amply been proved that yolk precurssors are synthesized in the liver of domestic chicken (Ranney and Chaikoff, 1951; Ranney _et_ _al_., 1951; Lorenz, 1954; Heald and McLachlan, 1964; Husbands and Brown, 1965) and these are then transported to the ovary through the plasma. Estrogen mediated increase in blood lipids at egg laying time has been demonstrated by Entenman _et_ _al_., (1940) in chicken, duck and pigeon. Annison (1971) too, has remarked that the onset of lay in the hen is accompanied by marked increase in the concentrations of plasma lipids. Further, he has suggested that the fat synthesized in the liver, the major site of lipogenesis, is transported as plasma lipoprotein complex. A second minor peak for plasma lipids in females was also observed in October which was probably reflective of a second wave of breeding activity.

The presence of cholesterol (Furr, 1969, 1970), pregnenolone (Furr, 1969, 1970), progesterone (Furr, 1969, 1970), and 3β-hydroxysteroid dehydrogenase activity (Botte, 1963; Chieffi and Botte, 1965; Wyburn and Baillie, 1966) has been reported in fowl ovary, which indicates that conversion of cholesterol to progesterone occurs *in vivo* and the progesterone then may be converted to other sex steroids. From July onwards, ovarian cholesterol showed decreasing levels which could be due to their utilization for the synthesis of estrogens and progesterone. Low ovarian cholesterol concentrations were recorded in August, September and October. Higher levels of the same in June, July and December on the other hand may indicate accumulation of cholesterol due to low biogenesis of female sex hormones. Yellow yolk laden ova were once again obvious in the months of September and October and to a lesser extent in November and December, reflecting the second breeding phase. March, April and May reflected high hepatic cholesterol values whereas late August, September and October showed low levels and during both these periods yellow yolk studded ova were seen in the ovary. It could be said from the above observations that high concentrations of hepatic cholesterol during the first phase could be due to increased rate of synthesis, while its release remaining at a normal rate whereas low liver cholesterol during the second phase was probably a result of increased rate of its release into circulation. These levels would also indicate an alteration in the distribution pattern of cholesterol between liver and other tissues, which may not necessarily be hormonally mediated (Fleischmann and Fried, 1945). The fact that decreased hepatic cholesterol concentrations are also suggestive of increased production of bile salts and bile acid (Bloch *et al.*, 1943; Haslewood, 1955; Ogura, 1971) is less likely in the case of pigeons since they are principally graminivorous birds.

Plasma cholesterol in both sexes did not quite reflect the changes associated with the probable breeding and non-breeding times. In June and July when accumulated lipids and cholesterol in testis gave an indication of cessation of androgen synthesis, plasma cholesterol on the other hand maintained a high level throughout wet summer (Fig. 2). It is known that plasma cholesterol shows wide range of fluctuations depending upon individuals, different months, and more important, the diet that the animal consumes. In mammals, such fluctuations have been reported by Boyd (1935), Schube (1937), Man and Gildea (1937), Turner and Steiner (1949) who have also commented that small and inconsistent variations in plasma cholesterol do occur during the 24 hours of the day. Schube (1937) and Man and Gildea (1937) have also proved that plasma cholesterol fluctuations show individual variations and that maximum cholesterol variation may be as much as 30% in humans.

From the above discussion, it appears that pigeons have two periods of breeding activity in a year. One of the breeding phases commences from March and continues upto May while the second one occurs during the months of late August, September and October and may extend to November and December. January, February and particularly June and July seem to be the chief non-breeding phases. The breeding phases in the pigeon do not seem to be sharply defined and represent rather wider breeding periods. These observations are basically similar to the field observations regarding nidification studies on this bird recently reported by Simwat and Sidhu (1973). This is probably due to readily available food supply and protection that the feral pigeons enjoy by inhabiting niches in and around towns and cities.