CHAPTER 5

SEASONAL VARIATIONS IN THE LEVELS OF TOTAL LIPIDS, TOTAL CHOLESTEROL AND PHOSPHO-LIPIDS OF NORMAL AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON, *COLUMBA LIVIA*.

Lipids play an important role in diverse physiological processes such as energy production, reproduction, migration, steroidogenesis, tail regeneration and wound healing (George and Berger, 1966; Ramachandren *et al.*, 1978, 1979). The total lipid includes four principal forms: triglycerides, phospholipids, cholesterol and free fatty acids which are widely distributed in different tissues. Phospholipids are present in all cells as constituents of cell membranes, myelin sheath of nerve cells, and also regulate cell membrane permeability. Cholesterol is a sterol, playing vital role in fatty acid transport, production of bile acids, synthesis of various steroid hormones, cell membrane structure, insulation for nerve fibres *etc*.

Liver is the principal site for lipid metabolism and is essentially important in the synthesis of phospholipids, since it produces the greatest fraction of the circulating phospholipids (Reiser et al., 1960; Stein and Shapiro, 1960). Among its lipids, liver contains a large proportion of phospholipids, but it does not differ in this respect from muscle or kidney. Phospholipids are virtually absent in adipose tissue of rodents and only glycerides are deposited in the adipose tissue. Cholesterol is present in various types of cells for various purposes. Hepatic tissue is capable of synthesizing cholesterol in both free and esterfied forms (Swell et al.,1958). Liver is the absolute specific organ for degradation of cholesterol. Pathways of lipid metabolism and carbohydrate metabolism meet at the common intermediate, actyl CoA; and glycerol of fat can join the reversible pathway of carbohydrate metabolism, which indicates close integration of lipid and carbohydrate metabolisms. Various hormones are known to affect mobilization, transport and metabolism of lipids (Fritz and Kaplan, 1960).

Endocrine regulation of lipid metabolism is though by far well documented, the emergence of pineal as a subtle endocrine structure warrants evaluation of its role in the overall metabolic adaptations of a species. Hence in the current chapter quantitative evaluation of various lipid constituents in liver, muscle and adipose tissue of feral blue rock pigeon, Columba livia has been attempted in intact (C) as well as pinealectomized (PX) birds during the three reproductive phases (recrudescence, breeding and regression).

MATERIALS AND METHODS

Maintenance, operative procedure, detailed experimental protocol etc. are as described in chapter 1. Feral pigeons (C, PN, PX) during the three reproductive phases were brought to the laboratory and were sacrificed. A piece of liver, breast muscle and adipose tissue were taken out promptly, blotted free of blood and tissue fluids, and then used for the various quantitative estimations.

Total lipids were extracted from dry tissue using 2:1 mixture of chloroform : methanol (v/v). Extracted total lipids were eventually measured gravimetrically. Total cholesterol in the liver, muscle and adipose tissue was measured employing the method described by Crawford (1958). Concentration of total cholesterol was read at 540 u in a photoelectric colorimeter. Phospholipid was assayed in liver, muscle and adipose tissue according to the method of Dittmer &

Wells (1969). Inorganic phosphate was determined at 660 μ colorimeterically, adapting the classical method of Fiske and Subbarao (1925).

RESULTS

NORMAL SEASONAL CHANGES:

The relative content of total lipids, cholesterol and phospholipids and their seasonal alterations in adipose tissue, liver and muscle are represented in tables 1,2,3 and figures 1,2,3. In general, changes in the content of total lipids and cholesterol were parallel in liver and muscle during the three reproductive phases. Of the three phases, average maximal lipid content was recorded during the regression phase and minimal during the breeding period; while intermediate level was obtained in the recrudescent period. However, early to late recrudescence was marked by a slight increase and early to late regression by tremendous increase in lipid contents in both the tissues. The average adipose tissue lipid content remained constant all throughout. The cholesterol content of liver and muscle though showed a similar changes as that of total lipids, was however, on a percentage basis (in terms of 100 mg lipid) constant during all the three phases in muscle, and only during recrudescent and breeding phases in liver. In the regression phase, hepatic cholesterol content showed

EXPLANATIONS FOR FIGURES

Figs. 1 to 3 : Histogrames showing the levels of total lipid, total cholesterol and phospholipid in liver, muscle and adipose tissue in three different intervals post-pinealectomy.

Fig. 1a - Total lipid in liver

Fig. 1b - Total lipid in muscle

Fig. 1c - Total lipid in adipose tissue

Fig. 2a - Total cholesterol in liver

Fig. 2b - Total cholesterol in muscle

Fig. 2c - Total cholesterol in adipose tissue

Fig. 3a - Phospholipid in liver

Fig. 3b - Phospholipid in muscle

Fig. 3c - Phospholipid in adipose tissue

Abbreviations:

S - Sham operated

N - Normal unoperated

P - Pinealectomized

FIG.1a

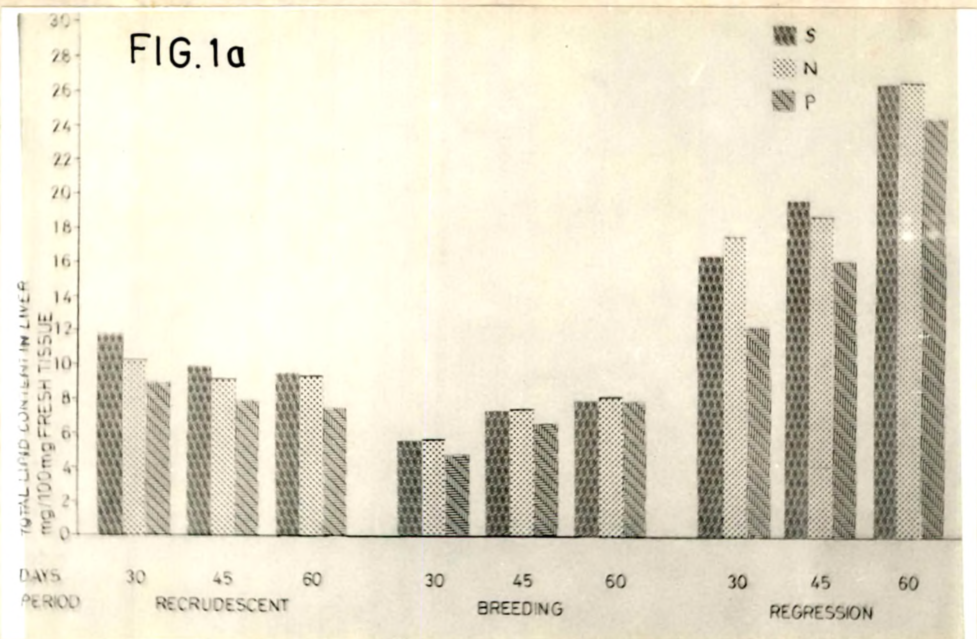
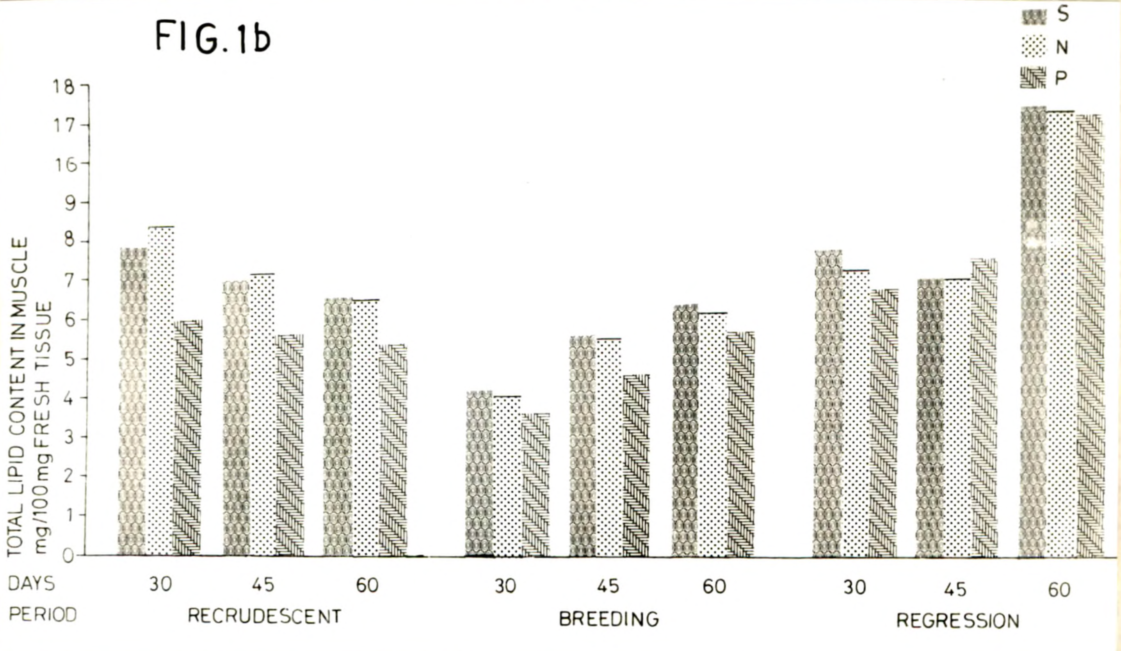

S
N
P
TOTAL LIPID CONTENT IN LIVER
mg/100 mg FRESH TISSUE
DAYS
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG.1b


S
N
P
TOTAL LIPID CONTENT IN MUSCLE
mg/100 mg FRESH TISSUE
DAYS
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

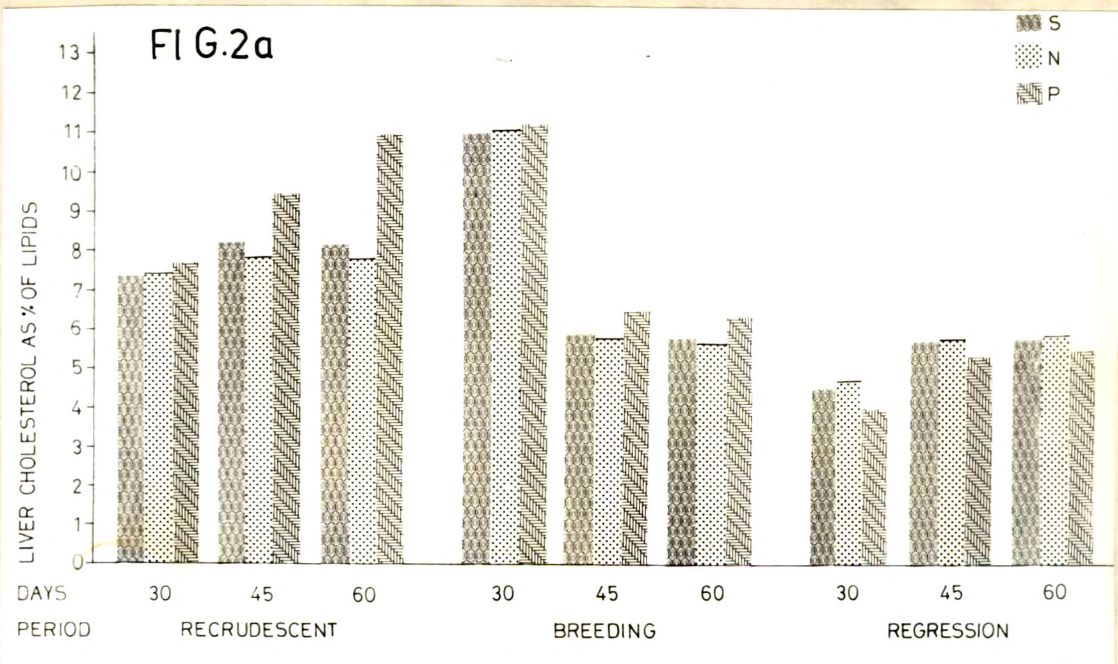


FIG.2a
S
N
P
LIVER CHOLESTEROL AS % OF LIPIDS
0
1
2
3
4
5
6
7
8
9
10
11
12
13
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

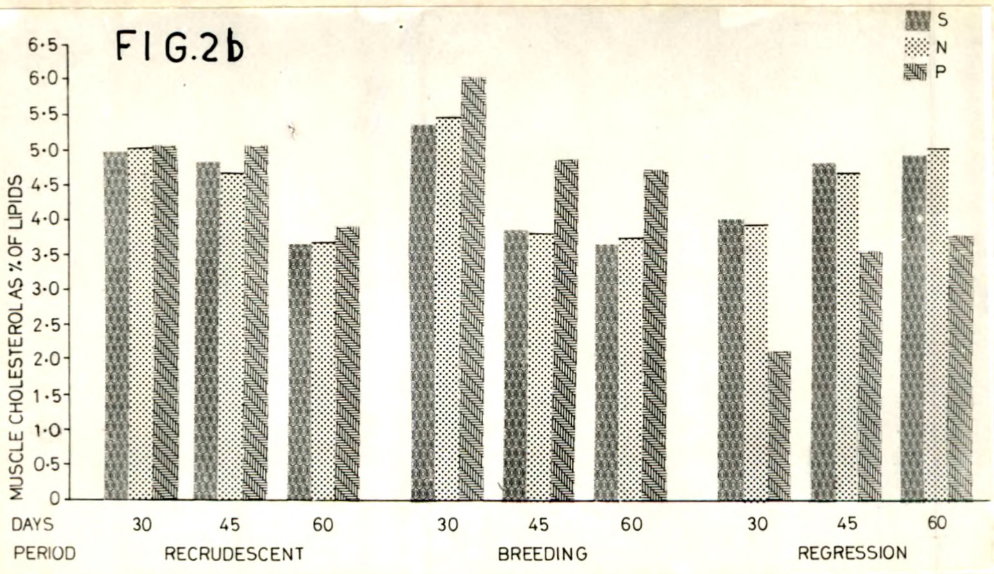


FIG.2b
S
N
P
MUSCLE CHOLESTEROL AS % OF LIPIDS
0
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
4·5
5·0
5·5
6·0
6·5
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3a

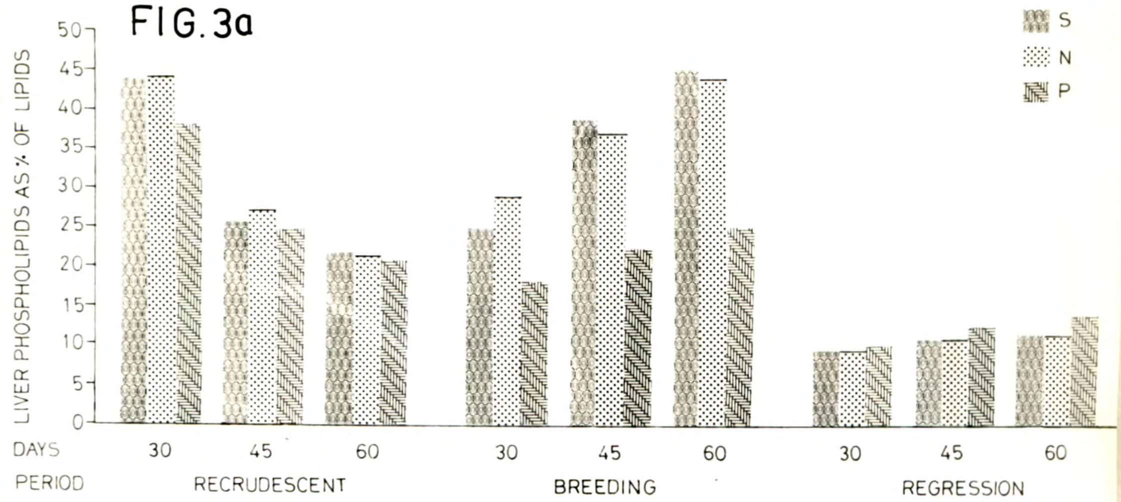
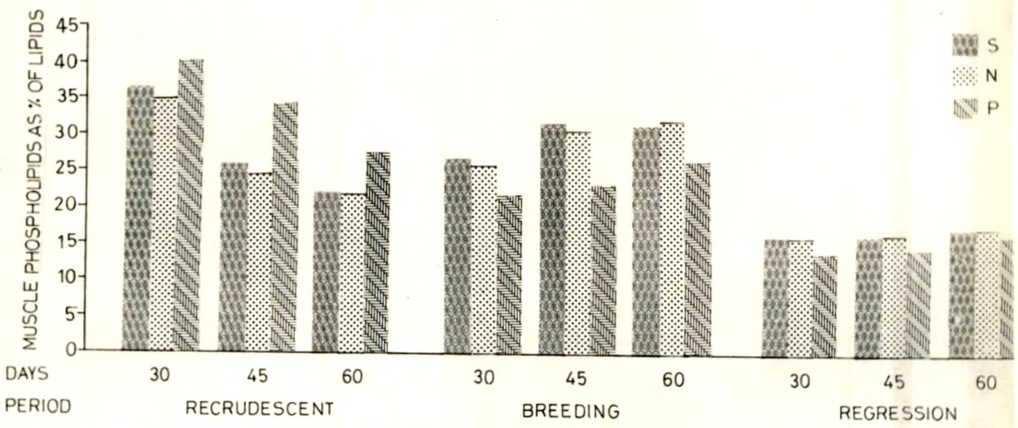

LIVER PHOSPHOLIPIDS AS % OF LIPIDS
0
5
10
15
20
25
30
35
40
45
50
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3b


MUSCLE PHOSPHOLIPIDS AS % OF LIPIDS
0
5
10
15
20
25
30
35
40
45
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

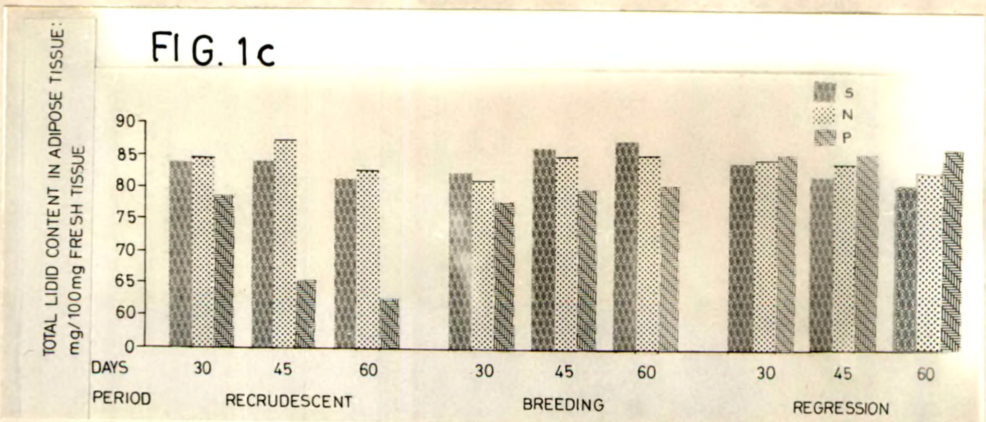


FIG. 1c

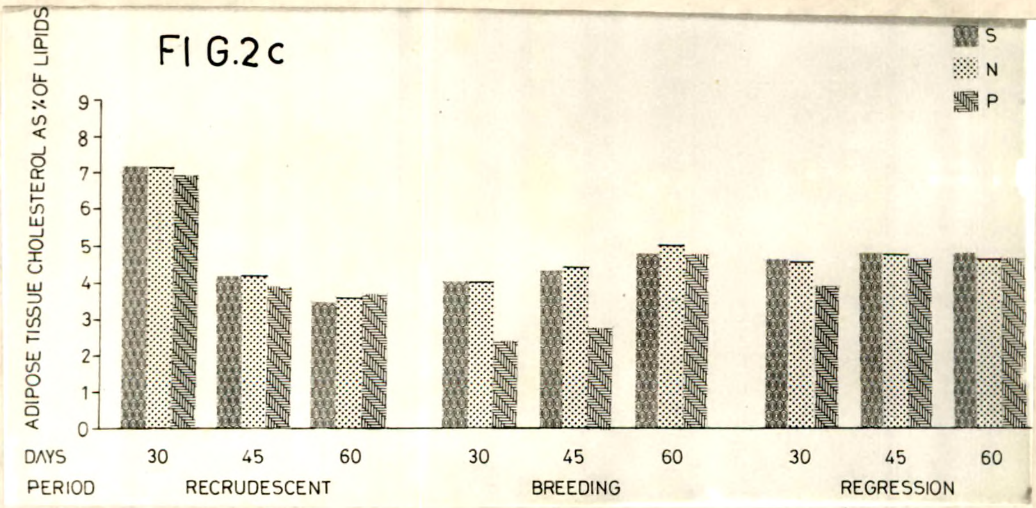


FIG. 2c

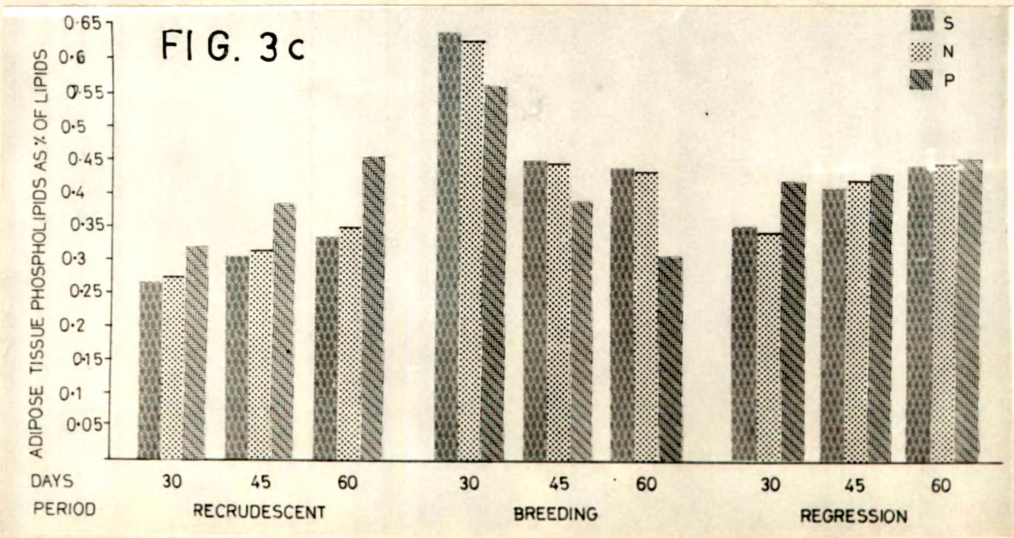


FIG. 3c

a lowered percentage level. The percentage hepatic cholesterol content was high all throughout recrudescence from the regression level and attained a maximal level in the early breeding period. In the case of muscle there was a fall from regression to early recrudescence which then showed continuous increase reaching maximal levels during late recrudescence and early breeding. Though the adipose tissue cholesterol content remained constant throughout, there was a slight fall from regression to early recrudescence and then increased gradually to reach the maximal level during late recrudescence.

Phospholipid content of liver and muscle was comparatively reduced in breeding and regression phases and more in recrudescence on a percentage basis; however, it was maximal during breeding and recrudscence in that order, and minimal during regression. On content wise as well as percentage basis, adipose tissue phospholipid was maximal during breeding, minimal during recrudescence and intermediate during regression. It may, however, be mentioned that adipose tissue phospholipid content is only fractional.

CHANGES DUE TO PINEALECTOMY:

Pinealectomy induced fall in lipid content during all the three phases in liver, and only during recrudescence and breeding in muscle and adipose tissue.

Though there was increased percentage content of cholesterol in liver of PX birds during recrudescence and breeding, the actual tissue concentrations remained more or less same as in the controls. During regression, both the values were lower than the controls. Muscle cholesterol also depicted a similar pattern due to pinealectomy. However, the percentage increase and decrease during breeding and regression respectively were more pronounced. Adipose tissue cholesterol content also showed a tendency to be subnormal in PX birds with a significant reduction being obtained only during breeding.

Pinealectomy induced changes in phospholipid content were of a slightly different pattern in the three tissues. Significant lowering of phospholipid levels was evident in the liver (both content wise and percentage wise) during recrudescence and breeding. Regression period was not affected and, if at all, a tendency to increase was the feature. In the case of muscle, contentwise, there was a lowering effect during all the three phases; however, percentage wise there was an increase during recrudescence and decrease during breeding and regression. Adipose tissue phospholipid depicted increased percentage content during recrudescence (significant) and regression (nonsignificant) and significantly decreased percentage level during breeding.

Approximate content of triglycerides is given in table 4.

DISCUSSION

Among birds with seasonal cyclic breeding activity, reproduction is mainly controlled by photoperiods. Many other factors also influence annual reproductive cycle in wild species of birds like pigeon. Here, natural selection favours conservation of an annual breeding activity. It is noted that artificial selection associated with domestication, is directed at modifying or eliminating by and large the influence of environment on reproduction. According to Benoit (1964), light is an important factor in stimulating gonadal activity of birds through the retino-hypophyseal pathway. Reproduction is one of the main causative factors in bringing about changes in different metabolites. Lipid metabolism is known to be closely connected with reproduction. It is also known that lipid constituents like cholesterol, phospholipids, triglycerides etc. of gonads are affected with changes in reproductive phases. Presently, significant progressive decrease in total lipid content of liver and muscle was observed during recrudescent and breeding periods. This denotes active lipid mobilization from both these tissues to meet the increased energy demands associated with gonadal recrudescence and breeding activities. Gonadal regression is marked by a tremendous rate of lipogenesis and deposition in both these tissues. The component which is involved in these major seasonal alterations appears to be the triglycerides. The maintenance of a steady percentage level of cholesterol in the muscle during these alterations in total lipid content signifies its proportionate loss and gain during recrudescence / breeding and regression respectively. The levels recorded for liver on the other hand denotes either no loss of cholesterol or a loss coupled with proportionate synthesis in the recrudescent and breeding periods. In the adipose tissue though the total lipid and triglyceride levels remained constant, same reciprocal inverse changes appear to occur with regard to cholesterol and phospholipid contents. Minimal cholesterol content during breeding phase and maximal during recrudescence were paralleled by maximal phospholipid content during breeding and minimal during recrudescence. Apparently, triglyceride which is the major constituent of the stored fat in adipose tissue is not influenced by the seasonal breeding activity of wild pigeons. Cholesterol is known to become more and more positive within the regressing testes as the bird enters its post-nuptial refractory phase (Lofts, 1978). Following the release and discharge of the spermatozoa lipids accumulate in the testes and remain in this condition till the cholesterol and lipids become rapidly depleted at the onset of the subsequent spermatogenic recovery phase (Lofts, 1978). Present investigation about changes in the liver and muscle total lipid and cholesterol contents during the recrudescent and regression phases also showed changes similar to those reported above for gonads. From this it could be interpreted that during these phases of reproductive activity, liver, muscle and gonads have same seasonal influence in relation to lipid metabolism. In regression phase lipid accumulation in gona was also observed by Kotak (1979).

The rhythmicity of the seasonal cycle in wild strains of birds is controlled by many exogenous and endogenous factors. Hormonal changes also have varied physiological effects of great significance (Nalbandov, 1970). It is likely, that these factors may have their influence on certain metabolites, particularly those involved in the sex steroid metabolism. Pinealectomy in general, induces a loss of total lipid content during recrudescent and breeding periods. This is evident in the liver even during the regression period. In muscle and adipose tissue on the other hand, during this period, either it was comparable to the controls or slightly higher than the controls. Apparently, it gives an indication of increased mobilization in the PX condition. However, the triglyceride content appears to be increased rather than decreased in the liver during recrudescence and breeding, and in the muscle during breeding and regression. This tends to denote an inhibitory influence on triglyceride mobilization in the PX pigeons. The fall in total lipid content in PX birds appears to be due principally to a loss of phospholipids. The only discordant note is struck by the muscle during the recrudescent period, in which case the fall in lipid content appears to be mainly due to loss of triglyceride. The cholesterol fraction either remains unchanged, or increases, as denoted by the increased percentage contents in both liver and muscle. Pinealectomy tends to have either no effect or an opposite effect in the regression period as is evident from the values obtained. PX has a slightly different effect in the case of adipose tissue. The loss of lipid in this tissue during recrudescence and breeding is due to a proportionate loss of triglycerides as well as a pronounced depletion of cholesterol. During the breeding phase there was a loss of phospholipid fraction also. Like the liver and muscle, adipose tissue also responded in an opposite manner in the regression period. These set of changes lead to the conclusion that pineal has a definite influence in the adaptive lipid metabolism characteristic of recrudescent and breeding periods and that pinealectomy has pronounced and opposite effect from those of the controls. On the other hand, pineal appears to be refractory during the regression period as is shown by the insensitiveness of the various tissues post-pinealectomy and the additive influence exerted thereof on the normal pattern of changes. The only study of a similar nature is that of Damian et al. (1979a,b) who has shown an increase in testicular cholesterol in rat in the absence of pineal, and a decrease of the same after administration of melatonin free pineal extract. Pineal being progonadal in the wild pigeons (chapter 1), it may be safe to surmise that it secretes some principle, which brings about general activation and the resultant metabolic alterations during recrudescence and breeding, and in the absence of which, both are arrested. The absence of this principle during regression is indicated by the similarity between C and PX groups of birds. Possibility of pineal being anti-insulinic was suggested earlier (chapters 2,3). Insulin is moreover, known to inhibit fat mobilization. Herein noted inhibitory action of PX condition on triglyceride mobilization may have some relevance in this context. It appears that explorations on the relation of pineal with lipids and its components could provide enlighting cues for the comprehension of temporal relationship between reproduction and lipid metabolism.