CHAPTER VI

VARIATIONS IN THE LEVELS OF LIPOGENIC ENZYMES IN THE LIVER AND GONADS OF FERAL BLUE ROCK PIGEON COLUMBA LIVIA G.

For the availability of reduced NADP which is essential for lipogenesis (Duncan and Common, 1967), cytoplasmic source can serve as an important donor of hydrogen ions. Participation of the lipogenic enzyme like 'malic' enzyme (NADP-malate dehydrogenase, TPN-MDH) or even HMP-shunt enzyme like Glucose-6-phosphate dehydrogenase (G-6-PDH), would ensure the supply of NADPH for lipid biosynthesis. Involvement of other enzymes like citrate cleaving enzyme (ATP-citrate lyase) or Acetyl-CoA-Carboxylase would make available the precursors needed for fatty acid synthesis. In the chick, for example, it has been shown that 'malic' enzyme plays a predominant role in providing NADPH (O'Hea and Leveille, 1968).

It is of great interest and significance to note that unlike the adipose tissue of mammals (Faverger, 1965, Leveille, 1967) liver is the major site of de novo lipogenesis in the pigeon (Goodridge and Ball, 1966, 1967) and also the fowl (Goodridge, 1968a; O'Hea and Leveille, 1969). This could probably hold true also for the other avian species. The lipogenic activity in the above reports has been demonstrated both by in vitro and in vivo

experiments based on the rate of uptake of glucose-U-^{14}C by the liver and depot fat in mammals as well as birds. According to Faverger (1965) adipose tissue contributes as much as 50% of the total lipogenic activity in mammals (and liver only 3-5%). Whereas Goodridge (1967) and O'Hea and Leveille, (1969) have shown that in the case of pigeons and fowls respectively, the hepatic tissue is the major site for fatty acid synthesis and accounts for more than 90% of the total body lipogenesis.

Various workers have studied the nutritional or dietary (Hill et al. 1958; Butterworth et al. 1966) and hormonal factors (eg. thyroxin, insulin, glucagon, gonadal hormones) that control the activities of the lipogenic enzymes in birds; particularly in immature and mature fowls and laying and non-laying hens (Chandrabose and Bensadoun, 1971 a & b., Pearce, 1971 a & b, Goodridge, 1978).

However, seasonal studies with respect to variations of lipogenic enzymes in birds are scarce. Recently, DeGraw (1975) has reported on seasonal (pre- and post-migratory) variations of 'malic' enzyme in the white crowned sparrows (Zonotrichia leucophrys). Similarly, pre- and post-migratory changes in the levels of 'malic' enzyme and G-6-PDH have been demonstrated in the liver and fat depot of the Wagtail (Motacilla alba)

and the Rosy pastor (Sturnus roseus) (Patel, 1976). Both these studies have recorded an increase of the enzyme levels during the period of hyperlipogenesis (the premigratory period).

The present project was undertaken to see whether there were any significant alterations in the levels of 'malic' enzyme and G-6-PDH in the liver and gonads of feral pigeons during breeding and non-breeding months. Enzyme levels of male birds were compared to those of the females in order to point out possible sex differences.

MATERIAL AND METHODS

Adult feral blue rock pigeons (Columba livia G.) inhabiting in and around the University campus, Baroda were utilized for the present work. They were shot down with an air gun (.22) between 9.00 a.m. and 10.00 a.m. in the months of March, April (breeding) and June, July (non-breeding) (Chapters, I, II and III) in the year 1977. Birds were promptly brought to the laboratory, liver and gonads were taken out, blotted and employed for the estimation of the enzyme activities. Both, the males as well as the females were employed. Part of right liver lobe and left gonad were homogenized in chilled 0.15M KCl solution. Homogenates were then quickly subjected to high speed centrifugation in cold

and vacuum (20,000 x g at 0-2°C) for 20 minutes. Supernatants were carefully decanted and keeping them chilled, enzyme essays were carried out using U.V. Spectrophotometer (Carl Zeiss). Glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49) was measured as per the method of Kornberg and Horecker (1955) with the modifications suggested by Marks (1960). 'Malic' enzyme i.e. TPN-MDH (E.C. 1.1.1.40) was assayed employing the method of Hsu and Lardy (1969). Protein content of the homogenates was estimated using biuret reaction (Layne, 1957). Enzyme activities are expressed as µmole NADPH formed/mg. protein/minute at 37°C.

RESULTS

Variations in the levels of both the enzymes in liver and gonads during breeding phase (March, April) and non-breeding phase (June, July) are presented in table I and Figs. 1 & 2. It is evident that hepatic 'malic' enzyme activity was found to be 5 fold more than the G-6-PDH levels during both the breeding and non-breeding phases. Further, the 'malic' enzyme activity in the liver was extremely high (more than 5 times) in both the sexes than that of the gonads during either of the phases. Regarding the G-6-PDH levels, it could be said that the hepatic activity was higher than that of the ovaries

MALIC ENZYME LEVELS

(μmole NADPH formed/mg protein/minute)

	MALE BIRDS		FEMALE BIRDS	
MONTHS	LIVER	TESTIS	LIVER	OVARY
MARCH	284.37 ± 22.92	54.46 ± 5.24	539.85 ± 33.40	22.29 ± 2.64
APRIL	402.73 ± 41.40	52.05 ± 7.22	347.70 ± 40.70	26.79 ± 4.14
JUNE	360.13 ± 25.76	30.10 ± 7.40	383.96 ± 25.77	19.58 ± 5.54
JULY	273.92 ± 20.12	20.34 ± 5.62	438.38 ± 30.80	13.41 ± 1.33

G-6-PDH LEVELS

(μmole NADPH formed/mg protein/minute)

MARCH	36.39 ± 4.02	72.32 ± 5.51	43.97 ± 4.46	20.64 ± 3.06
APRIL	46.72 ± 6.14	63.78 ± 7.08	50.13 ± 7.51	25.41 ± 2.88
JUNE	23.84 ± 4.89	29.32 ± 4.42	29.72 ± 5.56	15.36 ± 2.86
JULY	20.01 ± 5.23	26.38 ± 2.44	24.90 ± 3.89	16.32 ± 2.70

whereas in the males, testicular enzyme levels exceeded that of the liver. This was true for both the breeding as well as non-breeding phases.

SEX DIFFERENCE:

Liver of female birds depicted an overall high activity of 'malic' enzyme. This was particularly clear in the months of March and July. On the other hand hepatic 'malic' enzyme activity in the males showed the opposite pattern. Again, liver G-6-PDH of female birds was significantly higher than those of males. In contrast to this, both 'malic' enzyme and G-6-PDH levels were significantly higher in the testes than the ovaries at any time.

SEASONAL CHANGES:

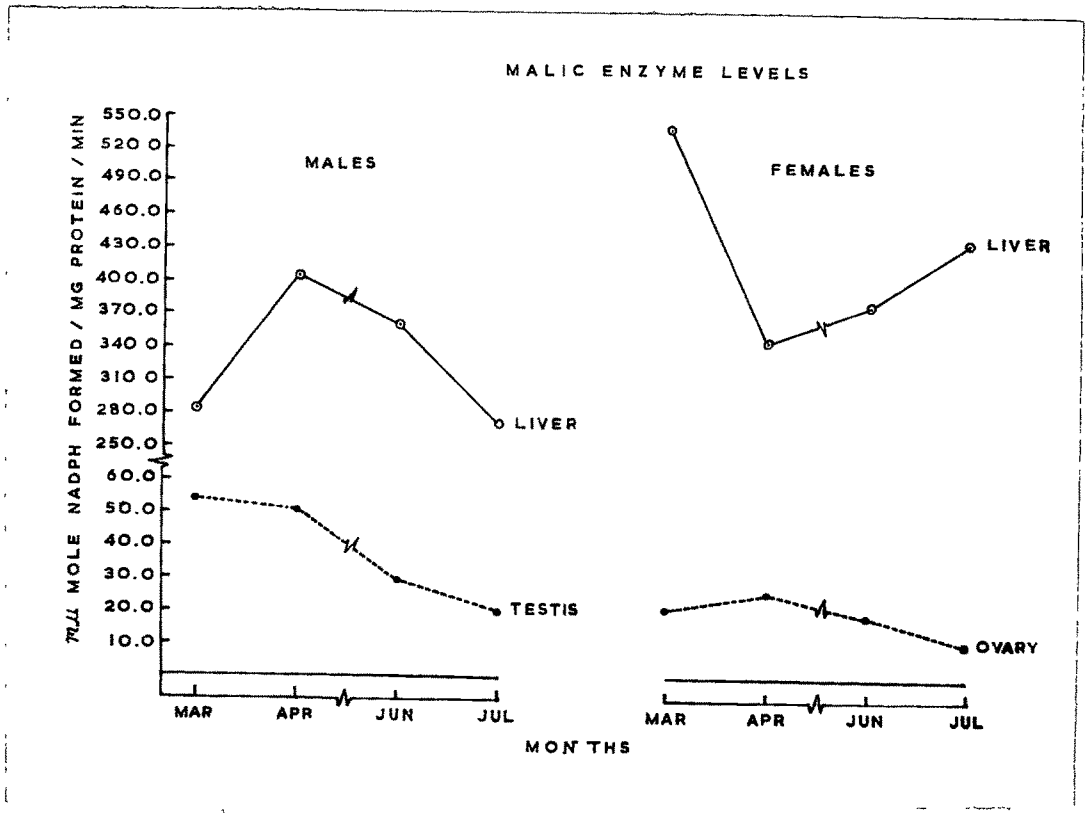
'MALIC' ENZYME: Liver of males showed a sharp increase in the activity of this enzyme from March to April, reaching to the peak levels and then dropping down in June and July. Highest values for the females were recorded in the month of March. These fell down in April and rose significantly through June and July. Testicular enzyme activity was high in the month of March and remained more or less at that levels during April, declined sharply in June and fell further in July.

EXPLANATION TO FIGURES

Fig. 1. Graph showing variations in the levels of 'malic' enzyme in liver and gonads of both sexes during breeding (March-April) and non-breeding (June-July) phases.

Fig. 2. Graphic representation of fluctuations of Glucose-6-phosphate dehydrogenase levels in liver and gonads of both sexes during breeding (March-April) and non-breeding (June-July) phases.

FIG. 1


MALIC ENZYME LEVELS
mμ MOLE NADPH FORMED / MG PROTEIN / MIN
550.0
520 0
490.0
460.0
430.0
400.0
370.0
340 0
310 0
280.0
250.0
60.0
50.0
40.0
30.0
20.0
10.0
MALES
FEMALES
LIVER
LIVER
TESTIS
OVARY
MAR
APR
JUN
JUL
MAR
APR
JUN
JUL
MONTHS

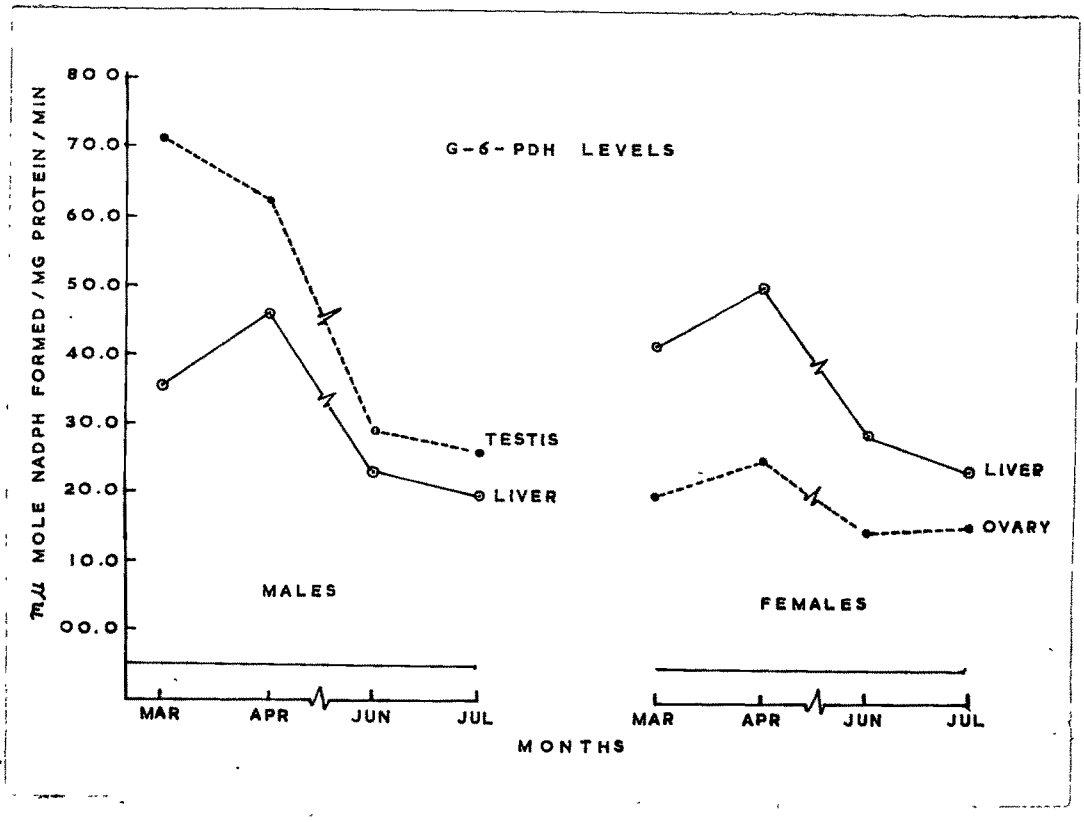

mμ MOLE NADPH FORMED / MG PROTEIN / MIN
80 0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
00.0
G-6-PDH LEVELS
TESTIS
LIVER
LIVER
OVARY
MALES
FEMALES
MAR
APR
JUN
JUL
MAR
APR
JUN
JUL
MONTHS

FIG. 2

Ovarian enzyme level exhibited a slight increase in April but thereafter it showed a decreasing tendency upto the month of July.

G-6-PDH: In both the sexes hepatic enzyme contents increased perceptibly from March to April. There was a marked decrease in June reaching the minimum level in July. Testicular enzyme concentrations exhibited highest values in March falling slightly in April. There was however a significant drop towards the non-breeding phase. Ovarian enzyme level increased in April and decreased thereafter.

DISCUSSION

There are certain noteworthy studies being carried out in the livers of sexually immature and mature and laying and non laying birds implying the relative contributions of lipogenic enzymes in fatty acid biosynthesis and their comparisions to that of mammals. Duncan and Common (1967) while investigating the $^{14}CO_2$ production from 1-^{14}C-glucose and 6-^{14}C-glucose uptake by chicken liver slices concluded that the pentose phosphate pathway is not operative to any great extent in the fowls and that this pathway is not an important source of reduced pyridine nucleotide required for lipogenesis.

Other studies on pigeon (Goodridge and Ball, 1966, 1967) and on chick (Goodridge, 1968 a & b; O'Hea and Leveille, 1968; Chandrabose and Bensadoun, 1971 a & b) lend support to the above observation suggesting that the HMP-shunt pathway plays a minor role in hepatic lipogenesis. The data obtained during the course of the present investigation are in conformity with the above observations. Concentrations of hepatic G-6-PDH were at least 5 times less than the 'malic' enzyme levels during both the phases. This was perhaps indicative of the major contribution of 'malic' enzyme (as compared to G-6-PDH) in supplying NADPH for lipogenesis. In Chicks, O'Hea and Leveille (1968) have demonstrated that it is the 'malic' enzyme which plays a chief role in making the redued pyridine nucleotide available that is needed for fatty acid synthesis. Once again, hapatic 'malic' enzyme levels were 5 fold more than the gonadal levels during both the breeding and non-breeding months which goes to emphasize that liver is the main organ involved in lipogenesis. That in the birds, liver is the main site of de novo fatty acid synthesis has often been proved in past. Between 90-95% of fatty acid synthesis in the chick (Gallus domesticus) appears to take place in the liver (as compared to depot fat). This has been shown by administering I-C^{14} and glucose-U-C^{14} and isolating the C^{14}- labelled lipids from the body tissues (O'Héa and Leveille, 1969). Noteworthy reports in pigeons

(Goodridge and Ball, 1966, 1967) reveal that liver is the main site of fatty acid synthesis and accounts for as much as 96% of total body lipogenesis.

Specific activities of citrate cleavage enzyme and 'malic' enzyme have been found to be significantly greater and lipogenesis increases in the laying hen than in the non-laying pullet and cockerels (Pearce, 1971a, 1972). Husbands and Brown (1965) too have shown that hepatic lipogenesis is greater in the laying hen than in the cockerels. In the liver of White Leghorn chicken, activity (histochemical) of NADP-dependent MDH ('malic' enzyme) increased by administration of estrogen but not by androgens (Akaishi et al., 1974). In the present study, almost two times higher concentrations of liver 'malic' enzyme in the month of March (breeding) in female birds as compared to males indicated a clear sex difference. Even in June and July, liver of females exhibited significantly higher enzyme levels. Male birds at any time did not reveal as high values to that seen in the females (peak in March: 539 µmole NADPH formed /mg protein/min). In all probability, therefore, hepatic lipid synthesis was greater in the month of March (breeding) in the females than in males. That, in birds, egg-yolk precursors are synthesized in the liver, and under hormonal mediation, are mobilized for the egg yolk synthesis, has amply been proved. Blood plasma lipid

level was highest in the month of March (1.67g/100ml) and the concentration of total lipids in the liver exhibited a declining trend at this time of the year indicating its steady utilization (Chapter I). Apparantly the rate of lipogenesis decelerated in April in the case of females as there was a significant drop in the 'malic' enzyme activity as compared to the level observed in the month of March. The increase in 'malic' enzyme in non-breeding phase suggested moderately high rate of lipid synthesis. It was observed that most of the birds during the months of June and July (non-breeding) were in the process of moulting. A possible explanation for increased lipid synthesis in the post breeding phase could be to meet the energy demands of post nuptial moult.

It has been demonstrated in the toad (Bufo arenarum) that liver G-6-PDH levels (which are necessary for cell growth and hepatic vitellogenesis) get stimulated and increased on administration of a single dose (100µg/100g. body wt.) of estradiol-17β (Sanchez and Alicia, 1977). Comparitively high levels of hepatic G-6-PDH levels (as compared to gonadal levels) were high during March-April in the females indicated that this enzyme contributed though in a minor way, to the fatty acid synthesis occuring during the breeding phase. This rise of liver G-6-PDH concentrations in females could perhaps be under the influence of estrogens.

Balnave (1968; 1969) has suggested that in pullets, androgens and progesterone also exert a marked influence on the overall rate of hepatic lipid synthesis and its degradation emphasizing that the total lipid contents may however, remain unaffected. Later, Balnave and Pearce (1974) carrying out certain experiments on immature pullets studying the levels of hepatic lipogenic enzymes after administration of gonadal hormones reported that testosterone treated birds showed a significant increase in the specific activities of both ATP-citrate lyase and TPN-malate-dehydrogenase. Rats treated with DHEA (dehydroepiandrosterone) led to a small but significant increase in the liver TPN-malic enzyme (Tepperman, *et al.*, 1968). It appears from the above studies that androgens may affect the lipogenic enzyme system by increasing the activity levels. During the present investigation, a significant rise of liver 'malic' enzyme was noticeable form March-April (breeding). Our previous studies have shown that androgen production during this phase could be high. (Chapter I and II). Increasing circulating concentrations of androgens were perhaps responsible for enhancing the lipogenic rate in the birds during breeding months.

In general, the hepatic enzyme levels exceeded those of the gonads in both the sexes. An interesting exception being that in the case of male birds,

testicular G-6-PDH levels exceeded those of the liver. This difference was very obvious in the breeding phase. Testicular weight was very high and the testes revealed active spermatogenesis at this time of the year. (Chapter VII). It is, therefore, logical to suggest that hightened G-6-PDH activity of the testes could involve a greater metabolic flux of the pentose sugars (ribose, deoxyribose) through the HMP shunt which would facilitate enhanced nucleic acid synthesis underlying accelerated spermatogenesis (i.e. supporting hyperplasia). On the other hand, when the testes were spermatogenically (Chapter VII) as well as endocrinologically inactive in the non-breeding phase (Chapter II), they should show low G-6-PDH levels too, and this has been well borne by the present observations.

From the above discussion, it appears that:

1. Hepatic 'malic' enzyme activity plays chief role in lipogenesis.
2. Glucose-6-phosphate dehydrogenase seems to contribute negligibly to fatty acid synthesis.
3. The rate of fatty acid synthesis was apparantly higher in females than in males.
4. In both sexes, the enzyme activities were found to be higher during the breeding phase and low in the

non-breeding phase. It appears, therefore, that these enzymic activities are influenced by the levels of circulating sex hormones.