CHAPTER 5

STUDIES ON DEVELOPING AVIAN LIVER 5. ACTIVITIES OF NON-SPECIFIC ACID AND ALKALINE PHOSPHATASES IN LIVER AND SERUM OF PIGEON DURING POST-HATCHING DEVELOPMENT

The liver is an organ where almost all types of metabolic pathways are in an operative state and consequently most of the metabolic enzymes are also found to be active in the organ. However, one or several metabolic reactions could be in a hyperactive state at a particular moment, depending upon several factors such as nutritional condition of animal, degree of organ's development (both structural and functional) and neuroendocrine control.

Certain developmental changes observed in the liver of pigeon during post-hatching period have provided quite an informative hint about its pattern of growth and development of functional maturity (Chapters 1, 2, 3, and 4). During early post-hatching development (1 to 10 days), liver is in an active phase of growth when a protein rich diet *i.e.*, crop milk is available. However, 10th day onwards, there occurs in the liver a gradual increase in the metabolic reactions such as glycogen deposition and lipogenesis probably under the influence of carbohydrate rich diet and hyperactive thyroid gland (Chapter 4).

Non-specific acid and alkaline phosphatases are a group of enzymes which not only hydrolyses phosphate esters but also helps in transport of metabolites across cell membrane. Omnipresent distribution of one or the other phosphatase in different tissues is suggestive of their role in several cellular activities, *viz.*, absorption, secretion, cellular phagocytosis, protein synthesis and many phosphorylating reactions.

Shah *et al.* (1972a) in their histochemical study, have observed high acid phosphatase reactivity in the liver of graminivorous birds, while reactivity of alkaline phosphatase was more intense in the liver of insectivorous birds. In the liver of two migratory birds, Rosy Pastor and Wagtail, Shah *et al.* (1976) have reported a high acid phosphatase activity in Rosy Pastor than that found in the liver of Wagtail. A progressive increase in the activity of acid phosphatase and gradual decrease of alkaline phosphatase activity is found to be related to glycogen accumulating capacity of embryonic liver of chick (Moog, 1965).

In the present study, quantitative estimations of non-specific acid and alkaline phosphatases in the liver and serum of pigeon during post-hatching period have been carried out with a view to understand the changes in the pattern of enzymes in relation to the organ's functional development, changes in diet and possible hormonal regulation. A further preliminary attempt has been made here to characterize the type of alkaline phosphatase present in the liver during post-hatching development.

MATERIALS AND METHODS

Young ones of pigeon ~~of~~ 1, 5, 10, 15, 20, 25 and 30 days old as well as adults were obtained from an open aviary maintained by the department. The birds were sacrificed by decapitation immediately after collecting blood from the jugular vein for serum. A piece of liver was excised from the sacrificed bird and was processed for quantitative estimations of non-specific acid and alkaline phosphatases according to the method described in Sigma Technical Bulletin~~s~~, No. 104 and the protein content was estimated using Biuret reagent (Layne, 1957). The liver is believed to have both intestinal ~~type~~ and liver types of alkaline phosphatase~~s~~. To distinguish the types of alkaline phosphatase~~s~~, the liver homogenate was incubated with an organ-specific inhibitor (L-Phenylalanine) of intestinal type (I-type) of isozyme. For this, substrate (P-Nitrophenyl phosphoric acid) was dissolved in 0.05 M L-Phenylalanine instead of glass distilled water. The total alkaline phosphatase activity and the activity with the inhibitor were measured in the liver homogenates from the young ones of various ages as well as adult pigeons. The intestinal type of alkaline phosphatase was calculated by substracting the values of samples treated with L-Phenylalanine from the total enzyme activity measured from samples incubated without inhibitor. The acid and alkaline phosphatases are expressed as μ mole P-Nitrophenol released/ mg protein or ml serum/30 minutes.

RESULTS

Table 1 presents the data on the activities of the non-specific acid and alkaline phosphatases in the liver and serum of growing and adult pigeons. While Table 2 shows degree of inhibition by L-Phenylalanine and the calculated values of L-type and I-type of alkaline phosphatase in liver.

LIVER (Fig.1)

Activity of acid phosphatase is minimum (0.5372 unit/mg protein) in the liver of one day old pigeon, which gradually increased and reached the maximum level (0.9438 unit/mg protein) at the age of 20 days after hatching. 20th day onwards the activity of acid phosphatase declined to reach 0.5977 unit/mg protein by 25th day and remained at a more or less same level on 30th day. In the liver of adult pigeon, there was a remarkable high activity of acid phosphatase.

The activity of alkaline phosphatase, on the other hand, showed a different pattern; its concentration being maximum (0.2156 unit/mg protein) in the liver of one day old pigeon, ~~and~~ which gradually declined by 20th day, reaching 0.1032 unit/mg protein. During further development it showed only a minimum activity (0.0402 unit/mg protein) at 25 and 30 days. But the activity of alkaline phosphatase was found to be high in the liver of adult pigeons.

THE TYPES OF ALKALINE PHOSPHATASE

It was observed that I-type of alkaline phosphatase (0.0451 unit/mg protein) was maximum in the one day old pigeon. As the development proceeded I-type decreased gradually and by 20th day it altogether disappeared (judged from the fact that no inhibition of alkaline phosphatase occurred in the presence of L-Phenylalanine). Likewise, the L-type of enzyme was also maximum (0.1703 unit/mg protein) in the liver of one day old pigeon, which decreased gradually till 25th day. The liver of adult pigeon, however, showed a slightly higher L-type of alkaline phosphatase which was still only half the value as that observed in the liver of one day old pigeon.

SERUM (Fig.1)

In the serum the activity of acid phosphatase was low in the early days of post-hatching period and remained low till 20th day. Thereafter it increased during 25th and 30th days and reached the level which was more than double the level registered by the serum of one day pigeon. And again serum of adult pigeon showed quite a high level of acid phosphatase activity.

The alkaline phosphatase activity in the serum on the contrary showed a gradual increase from first day to 15th

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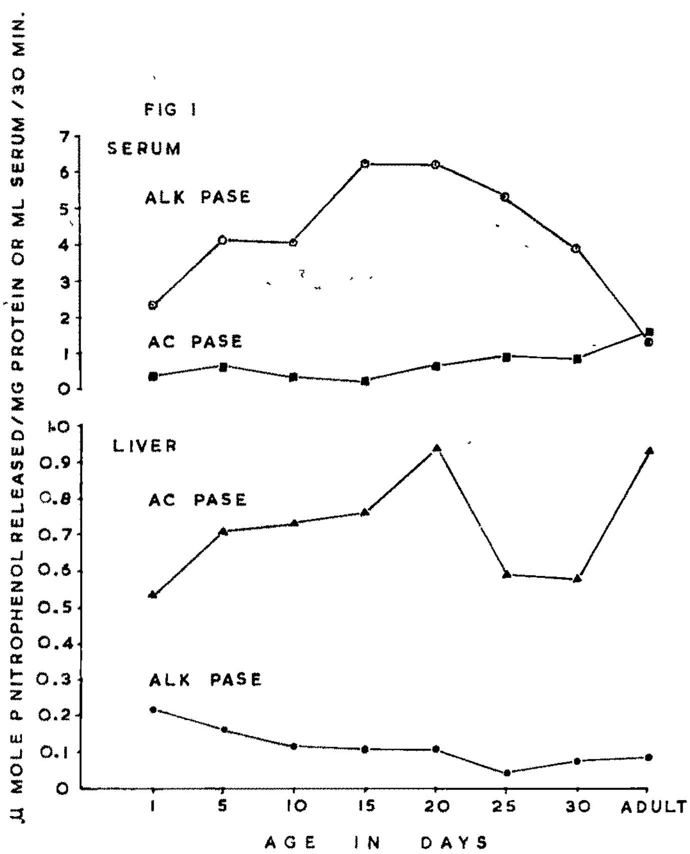
EXPLANATION FOR FIGURE

Fig. 1. Graph showing quantitative analyses of activities of non-specific acid and alkaline phosphatases in the liver and serum of pigeon during post-hatching development.

FIG I


µ MOLE P NITROPHENOL RELEASED/MG PROTEIN OR ML SERUM/30 MIN.
SERUM
ALK PASE
AC PASE
LIVER
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ALK PASE
7
6
5
4
3
2
1
0
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
5
10
15
20
25
30
ADULT
AGE IN DAYS

TABLE 1

Activities of non-specific acid and alkaline phosphatases in the liver and serum of pigeon during post-hatching development. Expressed as μ mole P-Nitrophenol released/mg protein or ml serum/30 minutes.

Mean value ± S.D.

Age in days	LIVER		SERUM	
	Acid phosphatase	Alkaline phosphatase	Acid phosphatase	Alkaline phosphatase
1	0.5373 ±0.0131	0.2156 ±0.0130	0.417 ±0.101	2.342 ±0.262
5	0.7155 ±0.0249	0.1683 ±0.0202	0.668 ±0.218	4.135 ±0.970
10	0.7319 ±0.0282	0.1130 ±0.0100	0.3880 ±0.0970	4.079 ±0.965
15	0.7699 ±0.0214	0.1002 ±0.0103	0.2826 ±0.0850	6.244 ±0.410
20	0.9438 ±0.0298	0.1032 ±0.0090	0.6710 ±0.1020	6.144 ±0.513
25	0.5977 ±0.0209	0.0402 ±0.0027	0.9440 ±0.1030	5.329 ±1.073
30	0.5849 ±0.0189	0.0794 ±0.0123	0.928 ±0.107	3.973 ±0.901
Adult	0.9309 ±0.0301	0.0812 ±0.0139	1.529 ±0.104	1.308 ±0.431
*Significant at the level	$p < 0.05$	$p < 0.02$	$p < 0.02$	$p < 0.001$

*p values refer to differences between 1-day and 20-day stages. The Student's 't' test was used to analyze differences in means.

TABLE 2

Percentage of inhibition of total non-specific alkaline phosphatase and corresponding values of I-type and L-type isoenzymes of alkaline phosphatase in the liver of pigeon during post-hatching development. Expressed as μ mole P-Nitrophenol released/mg protein/30 minutes.

Mean $\pm$ S.D.

Age in days	Percentage of inhibition of total ALK PASE	Intestinal type ALK PASE	Liver type ALK PASE
1	20.92	0.0451 $\pm$0.0027	0.1703 $\pm$0.0051
5	12.48	0.0210 $\pm$0.0012	0.1572 $\pm$0.0039
10	8.23	0.0093 $\pm$0.0006	0.1032 $\pm$0.0020
15	4.09	0.0041 $\pm$0.0001	0.0961 $\pm$0.0041
20	Nil	Nil	0.1032 $\pm$0.0090
25	Nil	Nil	0.0402 $\pm$0.0027
30	Nil	Nil	0.0794 $\pm$0.0123
Adult	Nil	Nil	0.0812 $\pm$0.0139

day after hatching (2.342 units/ml serum of one day to 6.244 units/ml serum of 15 days), and maintained this level on the 20th day also. However, during further development it declined gradually attaining the lowest level (1.308 units/ml serum) in the adult pigeon.

DISCUSSION

LIVER

ACID PHOSPHATASE: Progressive increase of non-specific acid phosphatase from the lowest value (0.5373 unit/mg protein) on the 20th day after hatching could be correlated with the progressive increase of carbohydrate rich diet and the resultant increasing capacity of the liver for glycogen deposition and lipogenesis. Some phosphatases are known to be active at an acidic pH, and have certain supplementary functions in carbohydrate metabolism in the liver cells. Glucose-6-phosphate is hydrolysed by Glucose-6-phosphatase at an acidic pH releasing glucose. Another function of the same enzyme at an acidic pH, i.e., synthesis of glucose-6-phosphate via PPi glucose phosphotransferase is also reported by Stetten (1964). Thus, the utilization of glucose-6-phosphate produced by these phosphatases in HMP shunt and EMP pathways for the purpose of $NADPH_2$ and energy production could be expected. In fact, the gradual increase in lipogenesis in the liver of pigeon upto 20th days after hatching (Chapter 4), which needs more $NADPH_2$ and energy could be fulfilled by sufficient supply of glucose-6-phosphate. Hence one could expect high acid phosphatase activity in the liver of developing pigeon. Phosphatidic acid phosphatase is another enzyme, which is also active at an acidic pH and plays an important role in the synthesis of triglycerides and phospholipids. Thus, high activity of non-specific acid phosphatase could be actually due to total activities of these phosphatases described above. From the parallel pattern in the activity of acid phosphatase and the degree of lipogenesis, one could easily suggest that this group of enzymes, by one way or the other is involved in lipogenic activity of the liver of pigeon during post-hatching development. It is also interesting here to note that acid phosphatase activity of the liver is low in the early days of post-hatching development when young ones are fed with crop milk containing more protein and fat. However, it increases in the course of further development and reaches the peak level on 20th day after hatching when the young ones are maximally provided with grains rich in carbohydrate. Shah et al. (1972a) have observed a comparatively higher activity of acid phosphatase in the liver of adult graminivorous birds such as pigeon and dove. Attainment of maximum acid phosphatase activity in the liver by 20th day of post-hatching development suggests that the metabolic maturity is attained by the liver by this time to effectively deal with grains.

ALKALINE PHOSPHATASE: The alkaline phosphatase activity in the liver showed entirely a different pattern from that of acid phosphatase. It declined gradually from the highest value (0.2156 unit/mg protein) in the liver of one day old pigeon touching the lowest level (0.1002 unit/mg protein) by 15th day, then remained at an even lower level during further development and adult age. High activity of alkaline phosphatase in the liver during early days of post-hatching development is suggestive of its active participation in the absorption of amino acids and lipids, which is actually the major food component of the crop milk on which nestlings subsist at that period.

Shah et al. (1972a) have observed a comparatively intense alkaline phosphatase activity in the hepatic lobules of insectivorous and omnivorous birds than that of graminivorous birds. Recently Shah et al. (1976) in their histochemical and quantitative study of these two phosphatases in migratory birds, Rosy Pastor (Sturnus roseus) and Wagtail (Motacilla alba) have also observed more intense histochemical reactivity and quantitatively higher values of alkaline phosphatase in the liver of Wagtail than in that of Rosy Pastor (the former consumes lipid and protein rich diet i.e., insects, while latter eats more of carbohydrate rich diet). The relationship between these two enzymes and glycogen deposition in chick embryonic liver is reported by Moog (1965), where it is suggested that as the glycogen deposition commences, alkaline phosphatase decreases while acid phosphatase starts accumulating.

Thus in the liver of developing pigeon, high alkaline phosphatase activity in the early phase of development could be explained in relation to its active participation in the uptake of fat and protein while gradual increase of acid phosphatase could be correlated with its active involvement in the uptake of glucose by hepatic cells and the resultant progressive increase of glycogenic and lipogenic capacity of parenchymal cells of developing liver during post-hatching period.

The increase of acid phosphatase and the decrease of alkaline phosphatase in the developing liver of pigeon, could be correlated not only with the change in the diet but also with the hormonal control. It is shown that in the migratory starling (_Sturnus roseus_), which changes the diet from the mixed type in the post migratory period to a carbohydrate rich diet (fruits) in the premigratory period, the acid phosphatase increased significantly in the liver (Shah _et al_., 1976). This increase in the acid phosphatase was found to be lower in the thyroidectomized starling (Patel, S.T., personal communication). Thus, the increase in the activity of acid phosphatase noticed in the developing liver could also be brought about by the thyroid gland, the follicles of which showed maximum development on 20th day (Chapter 4). Whereas, the high alkaline phosphatase in the liver during early phase of post-hatching development is probably due to higher concentration of corticosteroid hormones, because in one day old pigeon 80-90% of the total adrenal gland is composed of interrenal (cortical) cells, which in the adult age is reduced to 50% of the total gland (Chapter 7). Boernig _et al_. (1970) have reported the inducing effect of adreno-corticosteroid hormones, particularly cortisol and corti-costerone on alkaline phosphatase of liver and intestine of rat.

THE TYPES OF LIVER ALKALINE PHOSPHATASE

From the results given in Table 2, it could be seen that alkaline phosphatase was sensitive to inhibitory effect of L-Phenylalanine, and it was maximum in the one day old pigeon, which gradually decreased till 15 days (minimum level 0.004) and finally disappeared from the liver of 20 day old and adult pigeons.

It is suggested that there is no heterogeneity or isoenzymes of alkaline phosphatase within a single tissue except human placenta (Moss, 1970). However, liver is known for having isoenzymes of several enzymes such as LDH (Zinkham et al., 1966), MDH (Thorne, 1960), hexokinase (Ureta, 1972) etc.; some of which are known to show certain developmental changes also. ~~and~~ This type of isozymic study of alkaline phosphatase is almost fragmentary in liver during development. But very recently, Simon and Sutherland (1977) have reported that there exist~~s~~ two forms of alkaline phosphatase in rat liver (1) membrane bound and (2) cytosolic, having different chemical properties and showing different responses to various treatments viz: heat inactivation, pH optima, Km value and chemical inhibition and also suggested that both of which are regulated by different control mechanisms.

The activity of alkaline phosphatase in the primordial cells of newly developed hepatic diverticulum is quite high like that of intestine (its origin). So one could easily be enticed to suspect the presence of not only similar high concentration but also similar (intestinal) type of alkaline phosphatase, though not in mature or adult liver but atleast in immature or developing liver. In fact there occurred a maximum (0.0451 unit) inhibition in the activity of total non-specific alkaline phosphatase, which gradually decreased upto 0.0041 unit on 15th day and disappeared totally in the liver of 20th day old and adult pigeons. So it could be suggested that part of the alkaline phosphatase is sensitive to L-Phenylalanine, which is a specific inhibitor of intestinal type alkaline phosphatase and high alkaline phosphatase activity in the early age of development may be due to presence of L-Phenylalanine sensitive alkaline phosphatase in addition to the high native (L-type) alkaline phosphatase.

SERUM

The acid phosphatase activity in the serum was low (0.417 unit/ml) on one day and it remained more or less constant till 15th day. However, it reached a considerably high level on 20th day (0.671 unit/ml). Thereafter increase in acid phosphatase activity was quite high, reaching the highest value in serum of adult pigeon (1.529 units/ml).

Similarly, the activity of alkaline phosphatase of serum too increased along with age till 20th day (2.342 units/ml on 1st day to 6.144 units/ml on 20th day) and then registered a fall, marking the lowest level (1.308 units/ml) in the serum of adult pigeon. Thus alkaline phosphatase activity in serum of all the stages of post-hatching developing pigeons was higher than that found in serum of adult pigeon. Tanabe and Wilcox (1960) have reported that hyperthyroidic condition is responsible for high alkaline phosphatase activity in serum of developing chick. In fact,in case of pigeon also, we have observed maximum secretory activity of thyroid gland at the age of 20 days after hatching (Chapter 4). Part of this enzyme which is higher in the serum of developing pigeon may have originated from the intestine because, Lan and Mistilis (1973) have reported an increase in the intestinal type alkaline phosphatase in lymph of rat during active absorption. At the same time it is pertinent to mention that alkaline phosphatase in the serum of growing birds is high because of its active release from developing bone cells. Thus, the high alkaline phosphatase activity in the serum of pigeon in the early phase of development could be regarded as due to the influence of hyperactive thyroid gland, presence of some enzyme from intestine and developing oestoblasts.