

CHAPTER 6
A COMPARATIVE STUDY OF THE LIPASE ACTIVITY IN THE BLOOD SERA
OF THREE REPRESENTATIVE BIRDS

The recent studies conducted on the structure and metabolism of the breast muscle of birds, have shown that in birds indulging in sustained flight, fat is the chief energy fuel and this muscle in such birds is structurally and functionally adapted for a high rate of aerobic metabolism of fat. It has also been shown that ⁱⁿ the pectoralis of these birds there is high concentration of lipase (George and Scaria, 1956). Even in the same muscle where exist fibres of two types, one loaded with glycogen adapted for a glycolytic metabolism and the other loaded with fat adapted for an oxidative metabolism of fat, as are found in the pigeon breast muscle, it has been demonstrated that there is a high concentration of lipase in the fat-loaded fibre while the enzyme could not be detected in the glycogen-loaded one (George and Scaria, 1958a; George and Iype, 1960). These studies have been recently reviewed by Drummond and Black (1960).

Similarly the heart which is an organ capable of continuous activity, is also known to use fat for energy (Bing et al, 1956). This organ has also been shown to possess a high lipase activity (George and Scaria, 1957; George and Iype, 1959). Bing et al, (1956) have also shown that the myocardium extracts fatty acids from the coronary circulation and utilizes as the major fuel for its energy needs.

In the light of the above mentioned studies it was thought desirable to search the possibility of a lipase in the blood of these birds playing an important part in the mobilization of fat and the active transport of fatty acids to the muscles and other organs. It was therefore felt necessary to conduct the present study on the lipase activity in the sera of three representative birds, e.g. a non-flying bird, a good flier but non-migratory and a migratory bird. The domestic fowl (Gallus domesticus), the blue rock pigeon (Columba livia) and the Rosy pastor (Sturnus roseus) were chosen as the three representative types.

The occurrence of a lipolytic enzyme in the blood of vertebrates is well known. Harriot (1896) noted the presence of an enzyme in the serum and tissues of vertebrates which hydrolyzed monobutylin. He called it lipase. Lipolytic activity of the serum with tributyrin has been noted by Alper (1953). In recent years, however, many authors have expressed doubt as to the occurrence of a true lipase in the blood. Korn (1955) Hahn (1943) and Engelberg (1958) described a heparin activated lipoprotein lipase in the blood of mammals. This lipoprotein lipase does not split tributyrin, nor does it enhance the tributyrin splitting capacity of rat or dog plasma. It could clear lipemic plasma in the almost complete absence of both an aliesterase and a lipase. In view of these findings the action of clearing factor seems to be in every respect different from an aliesterase or a lipase (Overbeek and Van Der Vies, 1955).

In this chapter is reported the results of a quantitative study of the lipolytic activity in the sera of three representative birds using tributyrin as the substrate for the enzyme assay.

Materials and Method

The fowls and pigeons used in this study were domesticated laboratory birds. In the case of the former the hens used were in the period of egg laying. The Rosy pastors were captured by trapping them by means of a mist net during the period of March and April (pre-migratory period) and were sacrificed within 12 hours of captivity. These birds were in their pre-migratory state and would have started on their return journey within a few weeks time.

In all the cases the blood was directly collected from the heart in clean centrifuge tubes and allowed to clot at room temperature for 5 to 7 minutes and then centrifuged at a speed of 2500 r.p.m. for 5 minutes. The blood was not kept in the refrigerator because it takes longer time for clotting and moreover, it was found that the enzyme activity was not reduced during the time it was kept for clotting. The clear supernatant serum was transferred to another tube and stored in cold till used. The serum was measured and diluted in cold distilled water and was used as the enzyme material in all the experiments. In the case of Rosy pastor 2 to 3 birds were sacrificed at a time and the blood was pooled for each experiment.

The lipolytic activity was determined by a manometric

method adapted from Martin and Peers (1953) using a bicarbonate carbon dioxide buffer system of pH 7.4 at 37°C in the Warburg apparatus (George, Vallyathan and Scaria, 1958). The reaction flask contained 1.5 ml. of 0.025 M bicarbonate buffer solution and 1 ml. of the enzyme in the main chamber and in the side arm 0.5 ml. of tributyrin, emulsified by shaking 4% (v/v) tributyrin in 0.0148 M bicarbonate with a drop of "Tween 80". The flasks and the manometers were gassed with a mixture of 5% carbon dioxide and 95% nitrogen for three minutes. After 10 minutes equilibration in the Warburg bath the substrate was tipped in and after another equilibration of 3 minutes the levels were adjusted and the readings were taken at regular intervals for one hour. The lipase activity is expressed as the number of $\mu\text{l CO}_2$ evolved/ hour/ ml. of serum.

Results

Table 1

Showing the lipase values in the sera of three birds studied. Figures in the parenthesis indicate the minimum number of experiments in each set.

Sex	Fowl	S. D.	Pigeon	S. D.	Rosy pastor	S. D
Male	1189.87 \pm 112.39		6824.25 \pm 261.74		10699.00 \pm 1207.81(4)	
Female	996.50 \pm 65.78		5733.40 \pm 849.32		12091.50 \pm 504.40(4)	

The lipase activity in the blood sera of fowl, pigeon and Rosy pastor are shown in Table 1. The results obtained clearly indicate that there is a very high activity of the enzyme in the blood of Rosy pastor. The lowest enzyme activity was found in the blood sera of fowl. In the case of fowl a difference between the males and females was also noted.

Discussion

George and Jyoti (1955; 1957) showed that when the muscle of the pigeon is subjected to prolonged activity, fat is the chief energy source for the contraction of the muscle. George and Naik (1960) have estimated the intramuscular store of fat in the breast muscle of a number of different birds and have shown that those birds indulging in sustained flight have a much higher fat store in their breast muscle than the others. The lowest figure is of course for the domestic fowl which is a non-flying bird. George and Scaria (1958a) showed the presence of a high concentration of a fat synthesizing and hydrolyzing enzyme, lipase in the breast muscles of flying birds. During prolonged flight as in migration, the fat store in the muscle cannot be expected to supply all the energy required and so additional supplies should come, from the liver and adipose tissue. That migratory birds store up fat in the body prior to migration (Odum and Perkinson, 1951; MaGreal and Farner, 1955; Odum and Connell, 1956; Merkel, 1958) and that fat is reduced during migration (Williamson, 1952; 1955) are well known. George and Jyoti (1953; 1955) have shown that in the

pigeon, there is a reduction in the liver fat also when the breast muscle is subjected to prolonged activity. In the light of these observations it is logical to expect a considerably higher fat content and lipase activity in the blood of flying birds than in the non-flying ones. George and Menon (1954) reported the fat content of the fowl and the pigeon bloods as 0.38 and 1.76 gm. respectively for 100 ml. of whole blood. It was observed that in the Rosy pastor too the blood has a high fat content.

For a definite correlation of these differences in the serum enzyme concentrations and fat content it is essential to know in detail their modes of flight, structure of the muscle and its various enzyme concentrations. The Rosy pastor is a flapping flier indulging in prolonged flight and its breast muscle is composed of narrow red type of fibres with high concentration of oxidative enzymes, (George and Talesara, 1961) lipase and fat (Chapter 8). The pigeon though a good flier its pectoralis consists of both narrow and red fibres with high concentrations of lipase and oxidative enzymes in the narrow red fibres. The fowl on the other hand is practically a non-flier and its breast muscle consists of light larger fibres with more glycogen and glycolytic enzymes. Thus in these three different birds the muscle is adapted for different types of metabolism. For example the Rosy pastor muscle is well equipped for an oxidative metabolism of fat than the breast muscle of fowl.

I In the present study also on the lipase activity in the blood sera of these three birds one sees the same trend in that the values obtained for the Rosy pastor and pigeon are very much higher than that for the fowl. The considerable higher lipase activity in the Rosy pastor blood is interesting and significant in the light of the fact that the Rosy pastor is a migratory bird.

 However, if one looks at the figures obtained for the two sexes in the three birds studied one sees a difference. This difference cannot be considered as a distinct sex difference which is statistically significant since the range of variation is rather wide as could be seen from the standard deviation.