CHAPTER 10

STUDIES ON THE PECTORAL MUSCLES OF THE ROSY PASTOR AND THE HOUSE SPARROW

It is now well established that the fat is the chief source of energy for sustained muscular activity and for quick contractions glycogen is utilized (George and Berger,1966). A greater efficiency is achieved by those muscles which possess specialized fibres to utilize one of these fuels more than the other. Thus, morphologically and biochemically different types of fibres are met with, which are termed as broad, white, glycogen utilizing and narrow, red, fat utilizing fibres (George and Jyoti,1955; George and Naik,1957; 1959; George and Talesara,1960; 1962a; 1962b; Vallyathan and George, 1963). A third type namely the intermediate type was also proposed by George and Susheela (1961) and Stein and Padykula (1962). The composition of the pectoral muscles of birds, in general, varies with the presence of one or more of these types of fibres and these differences are evolutionarily and functionally of great significance (George and Berger,1966).

Recently, Chandra-Bose and George (1964) have shown that the pectoralis muscle of the House Sparrow (Passer domesticus) consists of only the red type, whereas in the Rosy Pastor (Sturnus roseus), although the fibres are of the same diameter, the red and white types could be distinguished. Inspite of the fact that such specialization of fibre types was not encounterd

within the breast muscle of Sparrow, George et al.(1964) observed a biochemical differentiation between its different layers. Histochemical studies by these authors revealed that the superficial layer contained more glycogen and phosphorylase while the dehydrogenase like SDH and fat enriches the deeper layer. In Rosy Pastor, the white (most precisely intermediate) fibres were seen predominantly in the superficial part of the muscle. A similar aggregation of white fibres in this region was also reported in the pigeon pectoralis (Pishawikar, 1961). A study on the metabolic implications and the adaptive significance of this characteristic feature of the pectoral muscles of Rosy Pastor and House Sparrow would be useful to establish the mechanical or functional advantages of having the glycogen utilizing fibres in the periphery of the muscle or having a reduced number of white fibres in the deeper or central part of the muscle.

As ambient temperature falls suddenly, the birds rely on shivering for thermogenesis. Non-shivering thermogenesis occurs only when they are exposed to hypothermia for a very long duration and then they are said to be acclimatized. Shivering action is sporadic quick contractions of muscle fibres which are not effective for movement of any part of the body. Moreover, the shivering birds are known to utilize glycogen, sparing fats (Depocas,1961). Determinations of glycogen and some enzymes were carried out on the pectoral muscle of Sparrows which were exposed to low temperature for a short duration and

also of those which were not exposed (controls) but kept for the same duration in darkness, in order to find out the extent of activation of the fibres in the superficial layer which contributes to the shivering actions.

MATERIALS AND METHODS

Quantitative estimations of glycogen in the different layers were carried out in the muscles (pectoralis) of Rosy Pastor, Brahminy Myna and House Sparrow which were shot in the fields.

Only the House Sparrows were utilized for cold exposure studies because of the following reasons.

(1) The body size is small and hence they have a high metabolic rate.

(2) The pectoralis muscle of this bird consists of only one type of fibre with the biochemical differences between the layers. Hence, the erroneous readings due to the presence of an admixture of red and white fibres, which would otherwise happen when Rosy Pastors are used, could be avoided.

(3) The insulating mechanism in Sparrow is rather ineffective in maintaining the body temperature. They could only avail themselves on the shivering or non-shivering thermogenesis for regulating the body temperature.

The Sparrows were trapped in Japanese mist nets, reared in cages and kept until they were accustomed to the laboratory conditions. In winter, when the temperature drops to 5-10 °C in this tropical region, the birds were found acclimated to such

low temperatures. The experiments, therefore, were carried out in both cold and hot seasons (in summer the temperature ranges from 40-45°C). The birds were subjected individually to cold stress at 0-5°C, in complete darkness, for 5 hours. The control birds were of the same sex and body weight as the experimentals and were kept in darkess at room temperature for the same duration. The experimental birds were either defeathered or wetted to prevent any interference of insulative mechanism, in maintaining the body temperature.

After 5 hours of cold exposure, the experimental as well as the controls birds were decapitated and the two muscle layers and the liver were removed for various estimations.

The glycogen was estimated colorimetrically with anthrone reagent using the method of Seifter et al. (1950). Readings were taken on a Bausch and Lomb, "Spectronic 20" colorimeter at 620mμ. Values are expressed as g/100 g fresh tissue.

Succinic dehydrogenase activity was assayed by the method of Kun and Abood (1949) using tetrazolium chloride (TTC) to trap the hydrogen ions. The readings were taken at 420 mμ in a Klett-Summerson photoelectric colorimeter. The protein was estimated by the Biuret method (Colowick and Kaplan, 1957). The enzyme activity is expressed as μg formazan formed per mg protein per hour.

The activity of phosphorylase in the muscle was determined by the method of Cori et al. (1943) as modified by Cahill

et al.(1957). Controls were run with each experiments in which 1 ml of 10% TCA was added before the homogenates were added. All samples and controls were incubated at 37°C for 15 minutes and the reaction was stopped by adding 1 ml of 10% TCA to the samples. Both samples and controls were filtered to 10 ml graduated test tubes and the organic phosphate liberated from the (G-1-P) by the enzyme was assayed by the method of Fiske and Subbarow (1925) and the optical density was measured at 660 mμ on a Klett-Summerson photoelectric colorimeter. Enzyme activity is expressed as μg phosphorus liberated per mg protein per 15 minutes.

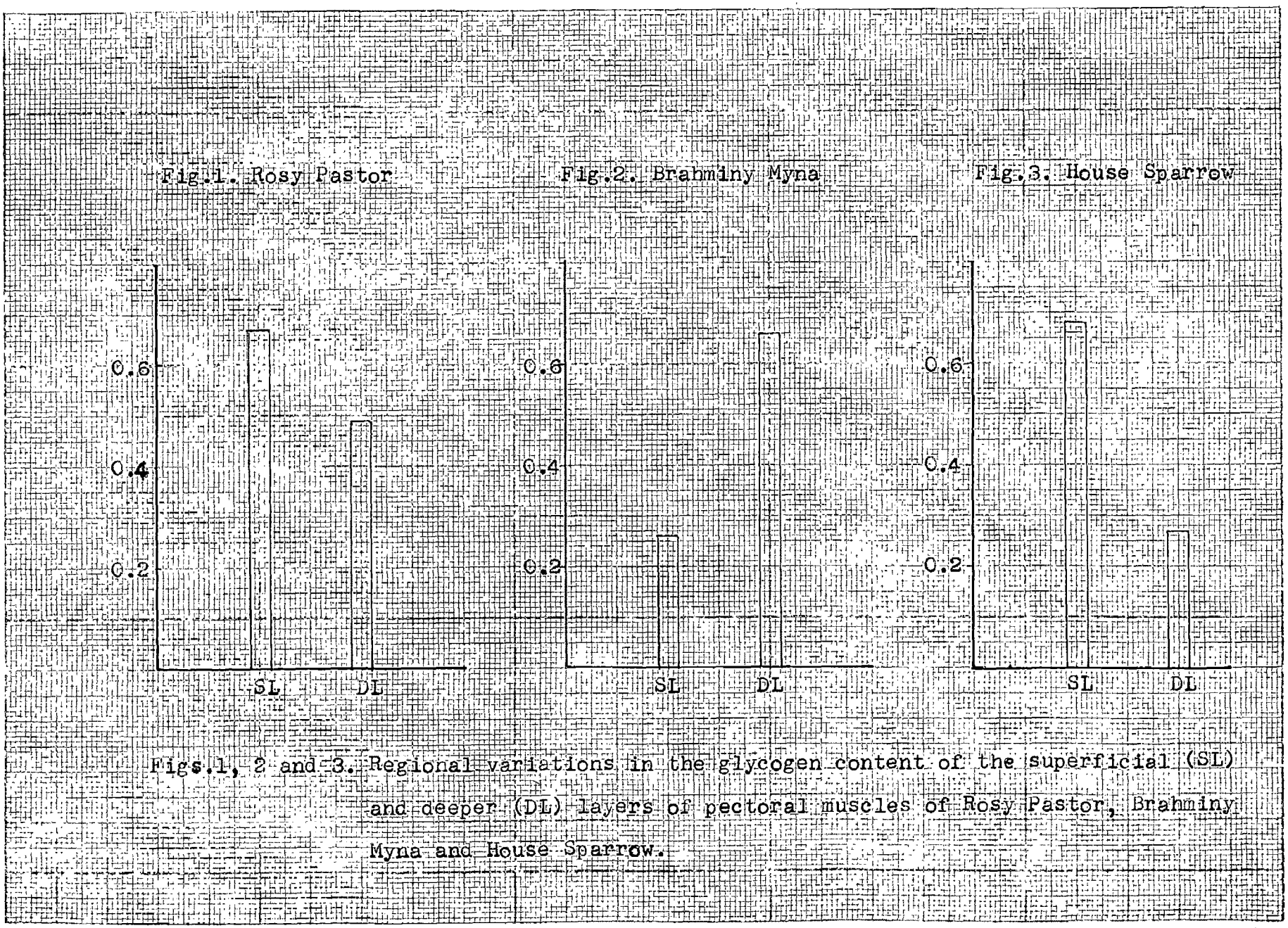
Quantitative determination of ATPase (myosine) was done employing the method of Umbreit et al.(1957), The readings were taken at 660 mμ on a Klett- Summerson. The values are expressed as μg phosphorus liberated per mg protein per 10 minutes.

Demonstration of acetylcholinesterase activity was carried out histochemically in the muscles using the method described in chapter 9. The intensity of activity was judged by the time of incubation necessary for the maximum reaction. The more active the enzyme, the lesser is the period of incubation required for the development of the colour.

RESULTS

Differences in glycogen content in the two layers of the muscles of wild birds :-

In the Rosy Pastor the superficial layer contained more glycogen than the deeper one (Fig.1). But in the related



Figs.1, 2 and 3. Regional variations in the glycogen content of the superficial (SL) and deeper (DL) layers of pectoral muscles of Rosy Pastor, Brahminy Myna and House Sparrow.

Fig.4. Amount of glycogen in the superficial (SL) and deeper (DL) layers of pectoral muscle of cold exposed anc control sparrows during cold and hot seasons.

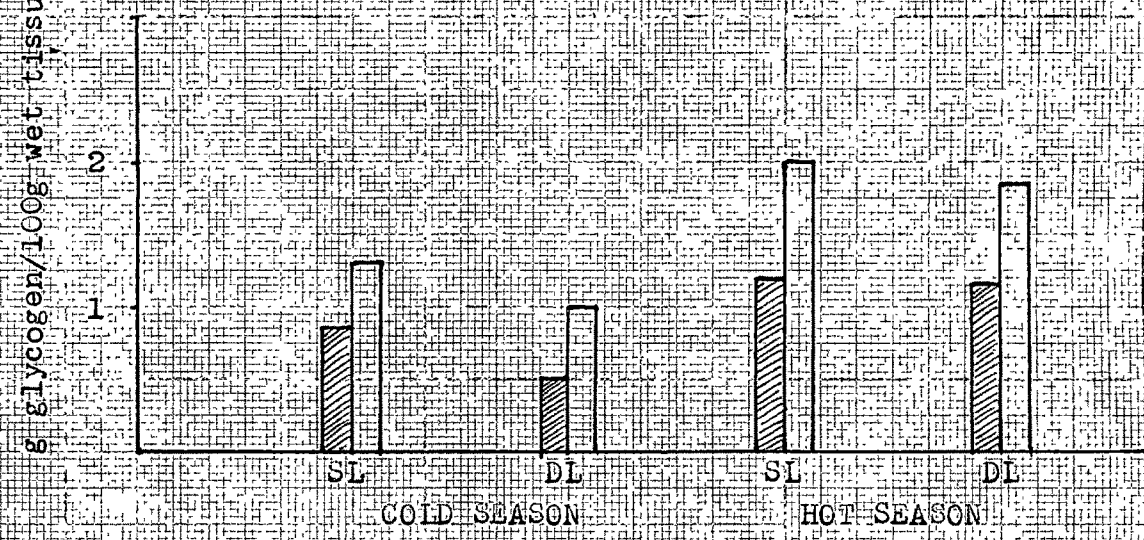


Fig.5. Amount of glycogen in the liver of cold exposed and control sparrows during cold and hot seasons.

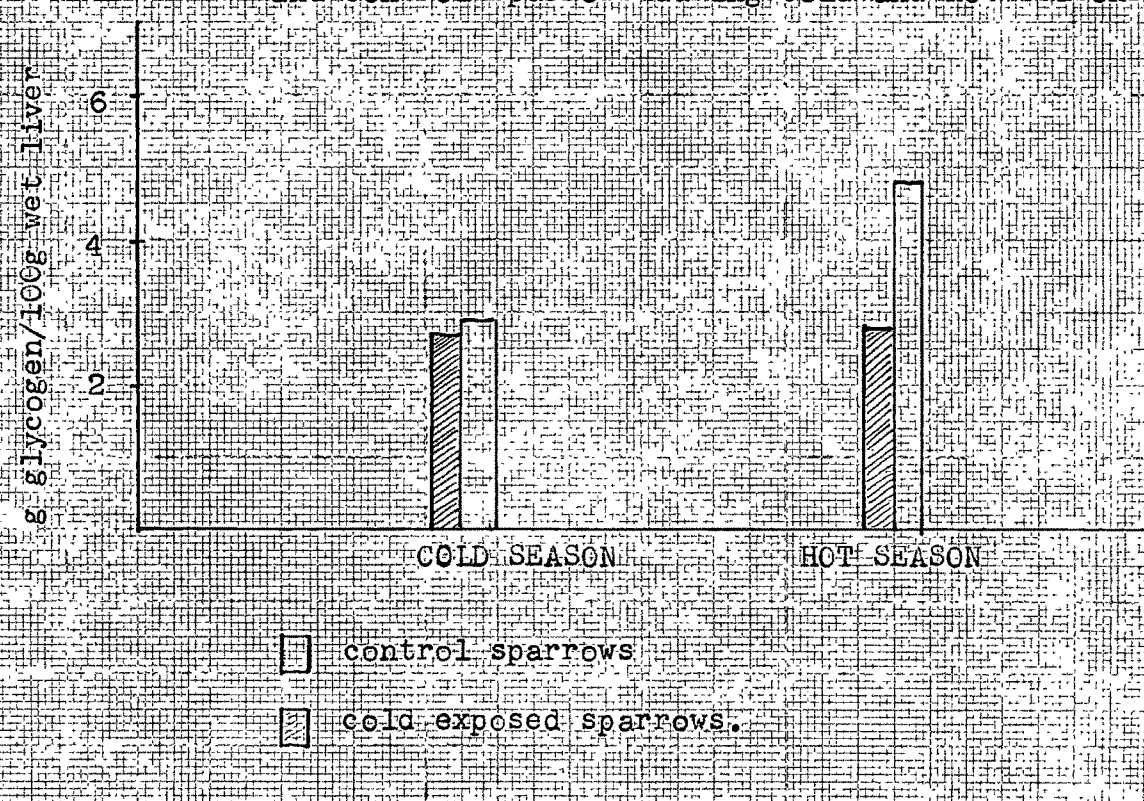


Fig.6. Phosphorylase activity in the superficial (SL) and deeper (DL) layers of the pectoral muscle of the cold exposed and control sparrows in cold and hot seasons.

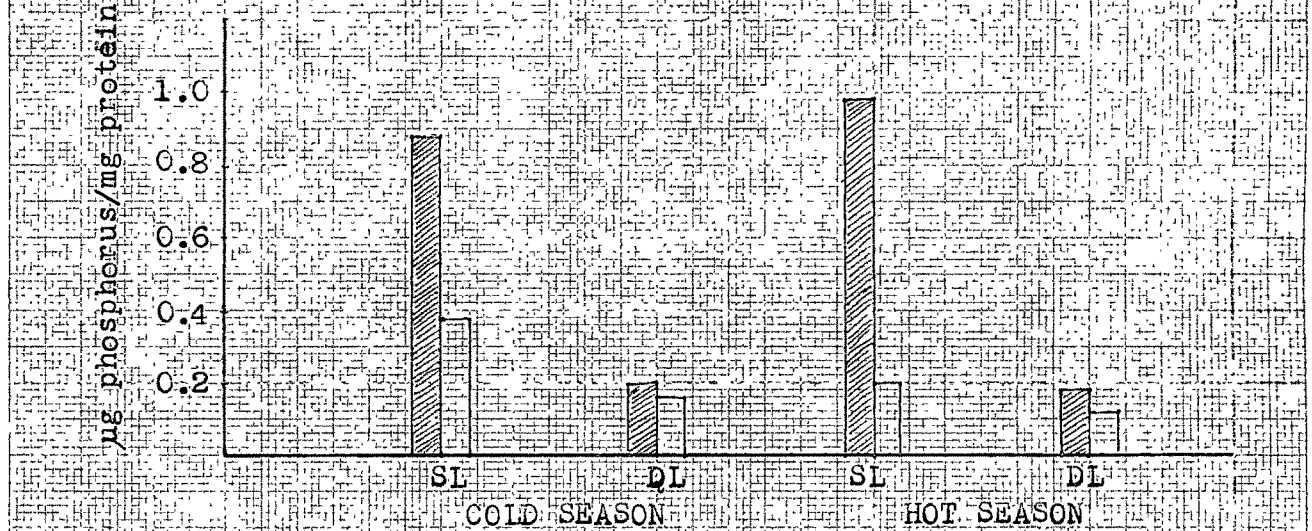


Fig.7. ATPase activity in the SL and DL of the pectoral muscle of the cold exposed and control sparrows in cold and hot seasons.

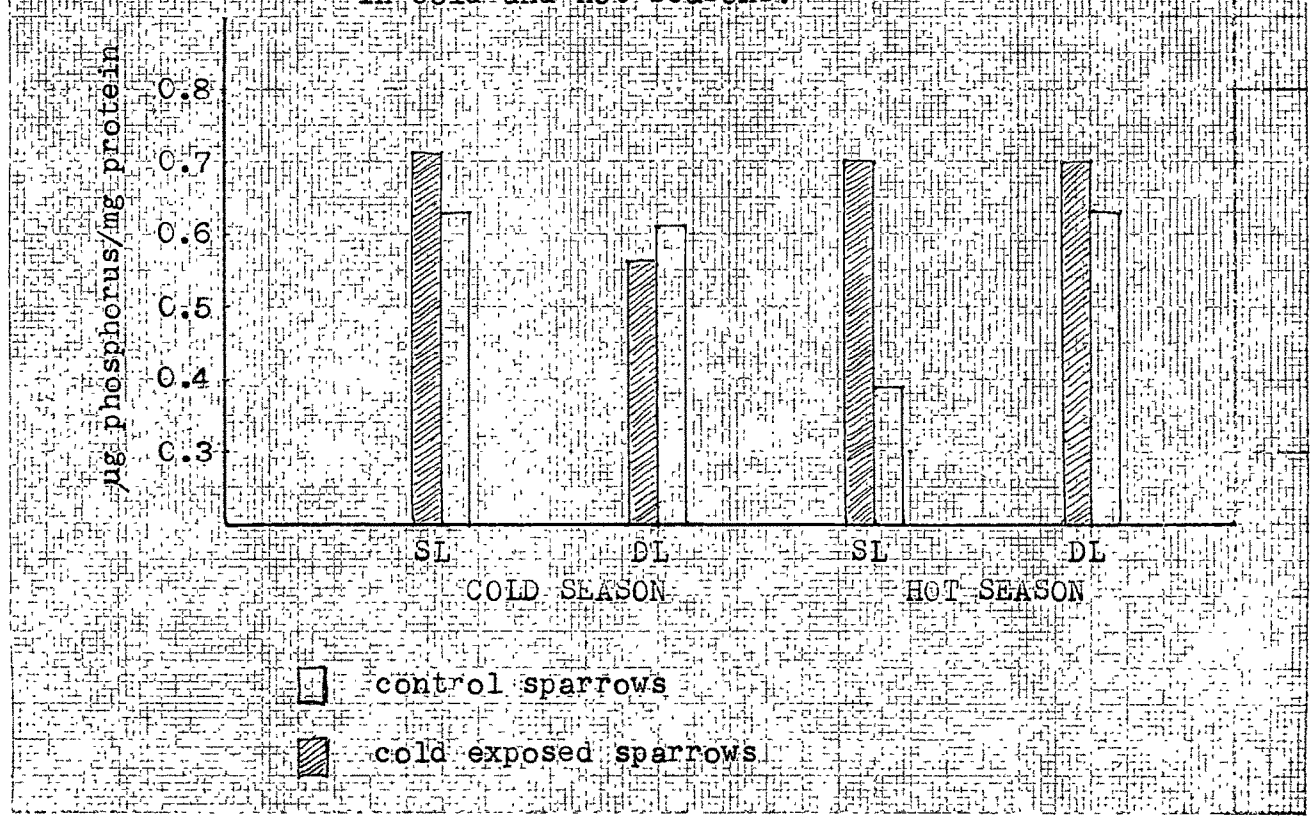


Fig.8. SDH activity in the superficial (SL) and deeper (DL) layers of pectoral muscle of the cold exposed and control sparrows.

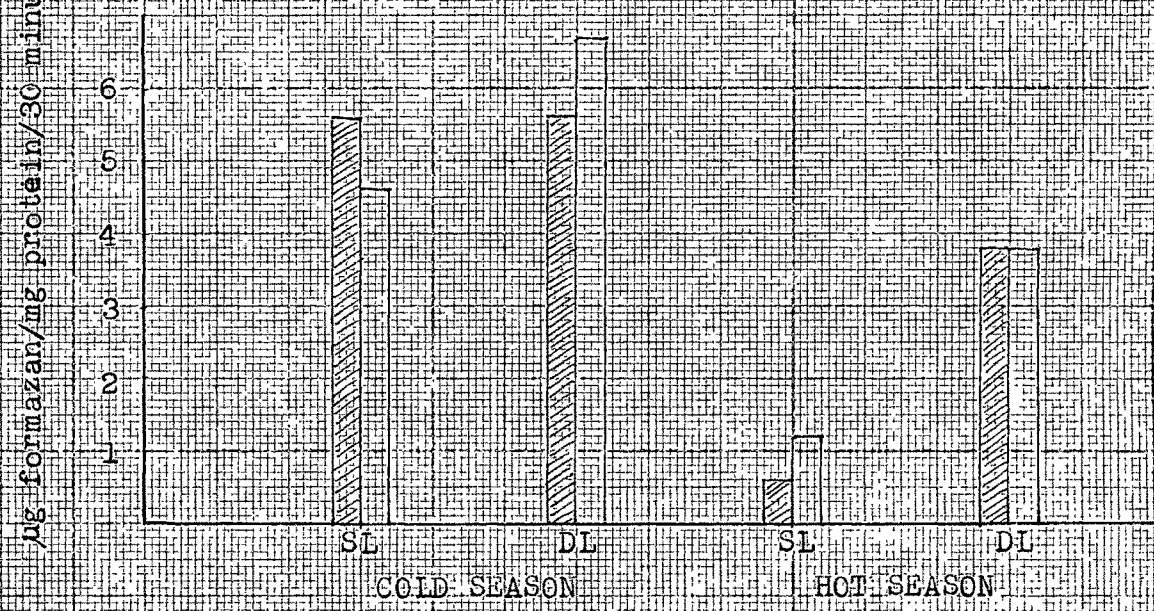
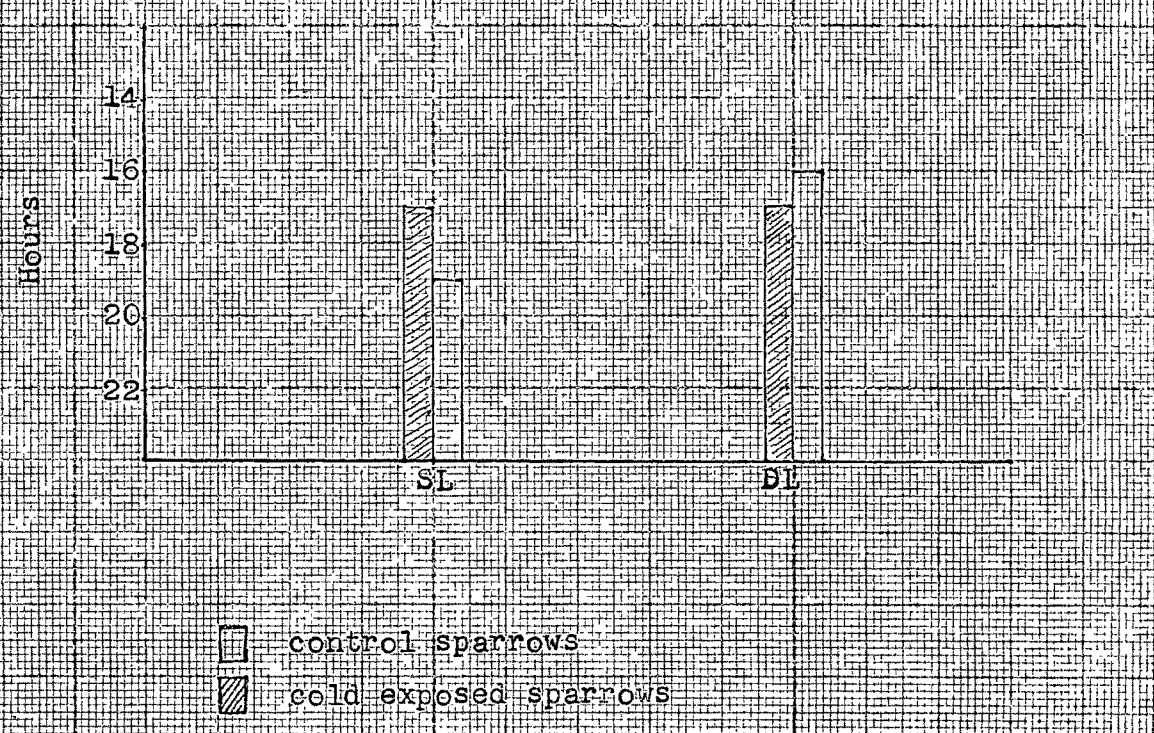


Fig.9. AChE activity in the Sl and DL of the sparrow muscle due to cold exposure in hot season.



species, Brahminy Myna (Sturnus pagodarum), it was just the reverse (Fig.2). In the House Sparrow (Passer domesticus), the condition was similar to that of Rosy Pastor (Fig.3).

Sparrow muscle glycogen after cold exposure (Fig.4):-

Cold season:- The reduction of glycogen in the superficial layer (SL) was lesser than in the deeper layer (DL) in the experimental birds in comparison with the levels in the controls.

Hot season:- The control birds had approximately equal amounts of glycogen in both layers and its reduction after cold exposure was also more or less same in these layers.

Liver glycogen after cold exposure (Fig. 5):-

The experimental birds showed only a slight reduction during cold season while in hot season more glycogen was being utilized, as controls had 4.5 g% and experimentals only 2.5 g%. The liver glycogen percentage was by and large greater in summer than in winter.

Phosphorylase in the muscle layers after cold exposure (Fig.6):-

Cold season:- Superficial layer of cold exposed sparrows possessed more phosphorylase activity than that of controls. The deeper layer on the contrary, had comparatively lesser activity, so much so that even after cold exposure very little increase was recorded in this layer.

Summer season:-The enzyme activity in both layers of the muscle in this season was less than in those of the winter, as seen in the controls. In the exposed Sparrows, however, the enzyme activity in the SL increased considerably whereas in DL an

insignificant increase was noticed.

Adenosine Triphosphatase activity upon cold exposure (Fig.7):-
Cold season:- ATPase activity was found to be greater in DL than in SL of control birds. But in cold exposed birds, it increased significantly in SL and decreased in the DL.
Hot season:- Though cold exposure increased the activity of ATPase in both the layers, the SL showed a marked rise as compared to the DL.

SDH activity in different layers of cold exposed birds (Fig.8):-
Cold season:- SDH activity was consistently higher in the deeper layer and in experimental birds it increased slightly in SL and was reduced in DL.
Summer season:- The cold exposure failed to activate the SDH in both the layers of the muscle. While a slight decline was noticed in SL, there was no change in DL.

Acetylcholinesterase activity in cold exposed sparrows (Fig.9):-

AChE activity was studied in summer season . Cold exposed sparrows had an increased AChE activity in the SL, since it took only 16 hours to demonstrate maximum intensity of staining, whereas in the controls, it took 19 hours. In the deeper layer a slight fall in AChE activity was noticed.

DISCUSSION

The quantitative estimation of glycogen clearly showed that there exists a biochemical difference between the superficial and deeper layers of fibres in the pectoral muscle of House Sparrow. This observation agrees with that of George et al.

(1964) who showed such differences through histochemical methods. The estimation of glycogen in the muscle layers of Rosy Pastor also revealed such regional differentiation with regard to the content of this metabolite. As mentioned earlier, the Rosy Pastor muscle consists of two types of fibres, the red and intermediate. Therefore, the presence of more glycogen in the SL could be attributed to the fact that the glycogen utilizing fibres are predominant in this layer. However, in Brahminy Myna, a non-migratory related species, having similar type of fibre differences as that of Rosy Pastor, such regional aggregation of fibres was not observed since the amount of glycogen was less in SL than in the DL (Fig.2). Thus it could be stated that a regional distribution of one type of fibre is not of common occurrence among birds, but confined to certain species only. This could be due to some characteristic movement in flight manoeuvres exibited by these birds. The white fibres are known for their quick tetanic contraction, whereas, the red ones show a slow tonic activity. Hence, grouping together of one type of fibres to one side and others to another side of the muscle certainly could give some kind of mechanical advantage. In order to decide what is the real significance, more detailed and exacting studies are required. Perhaps this fibre arrangement could be a mere physiological adjustment.

A white or intermediate fibre, utilizing glycogen could function in anaerobic conditions, a red one on the other hand, needs more oxygen supply. The vascular supply is compara-

tively poorer in the superficial part. So that the white ones which do not require much oxygen are placed in the superficial part, while the red fibres occupy the deeper part. But this explanation is rather inadequate, because, if it is so, then almost all birds should have the white fibres grouped in the superficial part. One could state then, that this kind of fibre distribution has something to do with functional advantages. The evolution of muscles has proceeded in the line of maximizing the efficiency for sustained work. It is suggested that the more evolved muscles possess a majority of red fibres and less advanced ones, white fibres (George and Berger,1966). The flight muscles of Hummingbird and Sparrow are far advanced as they possess only red fibres (George and Berger,1966). The passerine muscles have a mixed fibre distribution where the white ones are usually of the size of the red ones. In this migratory bird the muscle shows a tendency to evolve further by reducing the number of white fibres from the deeper part and thereby increasing its capacity to withstand sustained flight for which the red ones are most suitable.

If one assumes that such fibre distribution has some functional significance, then the different fibres are to contract selectively to bring about certain type of contractions (movements) where only tonic or tetanic contractions are involved. Then during shivering actions, which are sporadic, quick contractions utilizing mainly glycogen, the white fibres or the glycogen utilizing fibres (in Sparrows those of superficial

part) have to be more active. When exposed to low ambient temperature Sparrows showed only shivering movements as they were kept in darkness inorder to prevent other movements due to excitement.

It is already known that SL has more phosphorylase activity while the DL has greater SDH activity (George et al., 1964). If the glycogen utilizing fibres become more active, during shivering, the enzymes concerned with its metabolism (such as phosphorylase) should also be more. Since, the phosphorylase in the SL increased considerably when the birds are exposed to cold, it can be considered that the fibres in the SL were more active. The increased activity of AChE in the same layer also confirms this.

In Sparrows that were already acclimated to low temperatures during cold season, the activities of phosphorylase, ATPase and SDH were more in both the layers than in hot season. When exposed, however, only phosphorylase showed a greater response in the SL than the other enzymes during this period. In hot season, when the birds are subjected to low temperature, phosphorylase as well as ATPase showed an increased activity in the SL and very slightly in the DL too. SDH did not increase at all in both the layers, on the contrary it decreased in SL. Thus, it is clear that during thermogenesis with shivering, it is phosphorylase and not SDH which is greatly increased, pointing thereby a higher utilization of glycogen as well as increased activity of glycogen utilizing fibres. However, a marked

reduction of this metabolite was not observed in the SL. This could be due to continuous turnover of glucose as supplied by the liver via blood stream. Since SDH was not activated, it could be mentioned that oxidative metabolism was not favoured at this period or that the fat utilizing fibres were inactive during shivering. The higher concentration of SDH in the cold season could be taken as that the long duration of low temperature exposure leads to metabolic adaptations where fat is greately used for the heat production. Although the muscle usually abide by the all or none rule, it may be further emphasized in the light of these observations, that during quick contractions it is the white fibres which provide the force to the entire muscle. At the same time the red fibres might be passively contracting. On the other hand, during long sustained flights, the white fibres are passive, while the red ones contract actively using fat as fuel. Hence, in a muscle where both types of fibres are seen and is engaged in long distance flight such as that of Rosy Pastor predominance of red fibres over that of white ones are explainable.

The observation that AChE was more active in SL in the cold exposed sparrows also shows that the fibres in the SL received more stimulation from the nerves. It is now known that different axons innervate different fibres and the excitory junctional potential (e.j.p) evoked by different motor axons differs in amplitude, rate of spikes and their capacity to facilitate repetitive stimulation. This variance in producing

different e.j.p by different axons decides the kind of contraction in the same muscle (Kennedy and Takeda,1965). Recent studies have shown that muscle fibres themselves may differ in the "electrical constants of their membranes" and evidences show that some fibres produce twitches because their membrane is such that it could withstand electrogenic response, while others contract tonically as they lack this (Atwood,1963; Dorai Raj and Cohen,1964; Dorai Raj,1964; Atwood and Dorai Raj, 1964; Jasper and Pezard,1934; Cohen,1963). Thus, when fibres themselves possess the capacity to respond differently to stimulus the aggregation of tetanic fibres to SL and tonic fibres to DL may have great mechanical advantage in bringing about quick movements using only tetanic or twitch fibres, whereas tonic fibres are for sustained flight, without much loss of energy due to the resistance of other fibre when one type contracts. Recently Rayner and Keenan (1967) suggested that the white muscle of the fish (Skipjack Tuna) is more or less like a reservoir of power for short bursts of high activity. Hence, the separation of muscle fibres though incomplete, increases the efficiency of the pectoralis of this migratory bird for the sustained activities during migratory flight.