CHAPTER 2

HISTOCHEMICAL OBSERVATIONS ON SUCCINIC DEHYDROGENASE IN PIGEON PECTORALIS DURING DISUSE ATROPHY

It has been reported by earlier investigators (George and Naik, 1960; George and Vallyathan, 1962) that the pigeon breast muscle under disuse atrophy shows considerble structural and physiological changes with the progress of atrophy (Chapter 1). Studies conducted on the fat content, lipase activity and water content of the muscle (George and Vallyathan, 1962) have shown an altered metabolic state of the muscle. Histochemical investigations on glycogen and phosphorylase (Chapter 1) have indicated a shift in the normal metabolic pattern of the muscle from oxidative to glycolytic. These observations have stimulated the present histochemical study on succinic dehydrogenase in atrophic breast muscle. Succinic dehydrogenase was chosen for the studies because of its significant role in the metabolism of carbohydrates and fats. Moreover the data on the activity of succinic dehydrogenase in muscular atrophy are controversial. A reduction in the enzyme activity was reported in the rat muscle after 14 days of denervation (Humoller, 1956; Schmidt and Schlief, 1956; Nachmias and Padykula, 1958; Romanul *et al.*, 1965; Smith, 1965). On the other hand Dreyfus *et al.* (1955), Baker (1958), Sibrik and O'Doherty (1965) did not observe any change in the succinic dehydrogenase activity during muscular atrophy in humans and mice.

The present investigation was carried out with a view to obtain a better understanding of the metabolic shift reported in the previous chapter.

Material and Method

Fully grown pigeons weighing 300-320 g. were used. A plaster cast was applied at the middle of the humerus with the wings kept in a back to back position(George and Vallyathan, 1962). Pigeons were killed by decapitation after 1 day to 60 days of immobilization and a piece of breast muscle was immediately cut out and blotted free of blood. The piece was always taken from the same region and through the entire depth of the muscle, since it has been shown that the relative distribution of white fibres (George and Naik, 1959), and consequently enzyme levels (George and Talesara, 1960) differ in different parts of the muscle.

For the histochemical demonstration of succinic dehydrogenase the muscle was allowed to freeze completely on the stage of a freezing microtome . Sections of 15 μ thick were put in cold phosphate buffer, pH 7.6. After 15 minutes, they were transferred to freshly prepared incubation medium containing the following ingredients.

Cold phosphate buffer(0.1M.pH 7.6) ----------2.6 ml
Sodium succinate (0.5M. neutralized)--------0.6 ml
Aluminium chloride(4mM)----------0.5 ml
Magnesium sulphate(5mM)----------0.05 ml
Sodium bicarbonate(0.6M)----------0.3 ml
Sodium cyanide (0.03M.neutralized)----------0.5 ml
Methylene blue (2mg/ml)----------1.0 ml

After incubation at 37°C. under anaerobic conditions for 10 minutes, sections were rinsed in phosphate buffer and fixed in cold neutral 10% formalin for 15 minutes, then rinsed in distilled water and mounted in glycerine jelly.

Results

The succinic dehydrogenase activity in the red (narrow) and white(broad) fibres of the normal pigeon breast muscle is shown in fig.1 . The red fibres show considerably high succinic dehydrogenase activity than the white fibres. No conspicuous change could be detected in the histochemical preparations during the early days of atrophy. However, by 7th day after immobilization of the wings the red fibres generally showed a reduction in enzyme activity, though a few fibres exhibited an activity even greater than the normal. As regards the white fibres, there was a slight increase in enzyme activity when compared with controls. It is possible that this increase in enzyme activity was due to an increase in the number of mitochondria (Fig.3). The narrow fibres around such broad fibres were always found to possess high enzyme activity. By the 14th day of atrophy histochemical diversity among narrow fibres with regard to the enzyme activity became sharper. The enzyme level, however, continued to increase still further in the broad fibres(Fig.2). By the 3rd week , the difference observed among the narrow fibres began to disappear and the darkly stained narrow fibres were few, while the broad fibres still showed increased

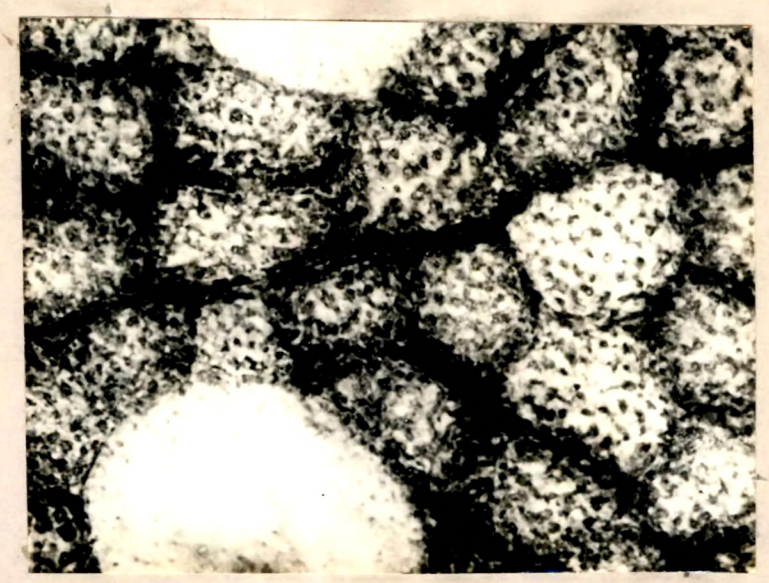

Fig. 1. Succinic dehydrogenase activity in normal pigeon breast muscle (cross section). Note marked deposition of diformazan in narrow, red fibres. X 720.

Fig. 2. Succinic dehydrogenase activity in 14 day atrophied muscle (cross section). Note differences among narrow, red fibres; some are darkly stained while others are as in normal muscle. Broad fibres also show higher enzyme ativity than in the normal muscle. X 720.

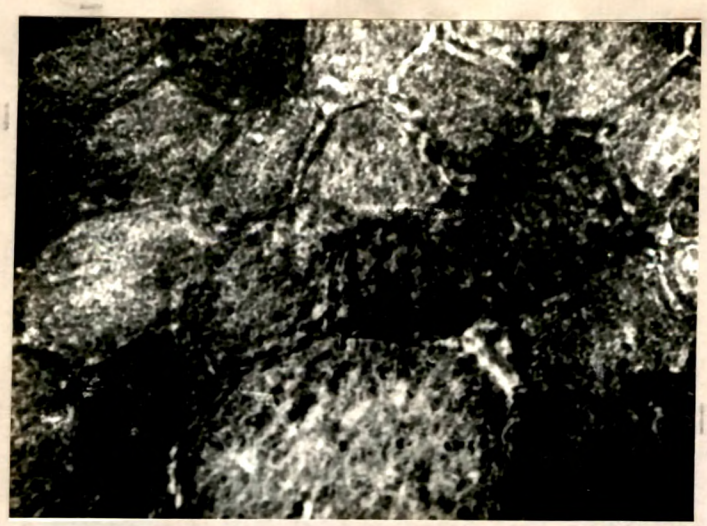

Fig. 3. Succinic dehydrogenase activity in muscle after 21 days atrophy. Note broad, white fibres with increased diformazan deposition . X 720.

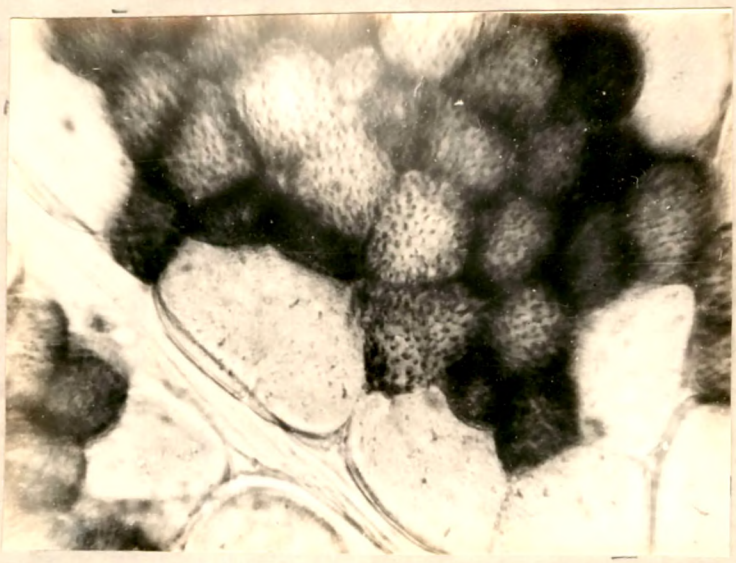

Fig. 4. Succinic dehydrogenase activity in muscle after 30 days atrophy. Differences among fibres are less. X 400.

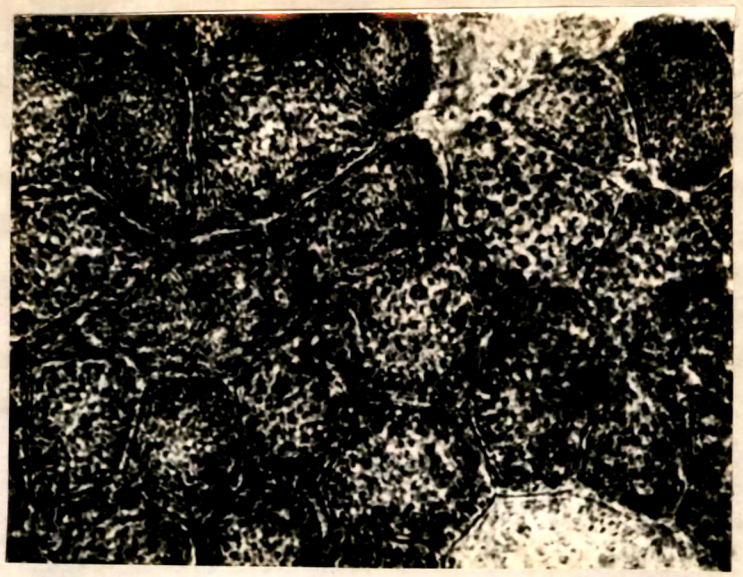

Fig. 5. Succinic dehydrogenase activity in 60-day atrophied muscle(cross section). Differences among narrow, red fibres have disappeared. Note the degenerated broad white fibre. X 720. Incubation time for the demonstration of succinic dehydrogenase activity in all cases 10 minutes.

deposits of formazan (Fig. 3). However, on the 30th day the number of the narrow fibres showing increased enzyme activity was less when compared to the early days of atrophy, and the intensity of the enzyme reaction on the whole had also declined(Fig.4). In broad fibres too by this time a decline in the enzyme activity in contrast to the early days of atrophy was seen, and some began to change their shape, indicative of degeneration which occurs after 14 days of atrophy. Changes in the shape of broad fibres were much more pronounced after 60 days of atrophy(Fig.5 and 6). Narrow fibres at this time presented uniform staining,but of less intensity. It should be mentioned that, during the incubation period, no succinic dehydrogenase activity was perceptible in the connective tissue, which increased during later stages of atrophy.

Discussion

The high concentration of oxidizing enzymes and lipase in the red fibres of the normal pigeon breast muscle suggest the presence of highly organized cyclophorase system including all the enzymes of the Krebs cycle , well equipped for fat metabolism(George and Talesara, 1961,1962a,1962b). On the other hand the white fibres have a highly organized glycolytic system for the metabolism of carbohydrates(George and Talesara, 1962; Vallyathan and George, 1963).The existence of these contrasting systems side by side is not congenial for quantitative biochemical studies on these fibres separately so as to acertain the characteristic changes taking place in them. Therefore histochemical techniques were found to

be very useful in assessing the characteristic nature of the different biochemical and physiological changes that occur in the two types of fibres during atrophy. In a recent study, George and Vallyathan (1962)observed during atrophy of the pigeon breast muscle a great increase in lipase activity, an increase in the fat and water contents and a corresponding low lipase level in blood during the first week after immobilization of the wings. From this they concluded that there is a severe disturbance in lipid metabolism ~~in the metabolism~~ in the muscle during atrophy . One possible explanation for the increase in lipid in the muscle could be that it is due to a lowered level of oxidative metabolism. Evidence for this is provided in the present study by the sharp fall in succinic dehydrogenase activity in the muscle during this period. An explanation for this might be that the muscle metabolizes carbohydrates in preference to fat . It is well known that succinic dehydrogenase plays an important role in the oxidative metabolism of fats, and the level of this enzyme is enhanced when the muscle is electrically stimulated (Vallyathan and George, 1964).

Studies on the capacity for fatty acid (butyrate) oxidation and on the respiratory quotient of the homogenate of the pigeon breast muscle undergoing atrophy, have shown that the muscle is incapable of oxidizing fatty acid <u>in vitro</u> (Chapter 5).These studies have also shown that the respiratory quotient of the muscle during atrophy is above 1, indicating a high conversion rate of carbohydrates into fats. These

observations lend further support to the hypothesis that fat accumulates in the muscle since it is not utilized and suggested the possibility of a shift in the metabolism. It is likely that the normal pigeon breast muscle, with its high capacity for oxidative metabolism utilizing predominantly fat, shifts to glycolytic metabolism during atrophy. Since evidence for this suggestion is also available from the previous study (Chapter 1) where it was shown that the narrow, red, fat utilizing fibres in the pigeon breast muscle acquire increased concentrations of phosphorylase and glycogen during atrophy. According to Villar-Palasi and Larner (1960) energy could be obtained from the degradation of glycogen to hexose phosphate by phosphorylase. On the other hand the broad, white, glycogen utilizing fibres of normal pigeon breast muscle were found to be almost devoid of glycogen and phophorylase during atrophy(Chapter 1). But not all red fibres and all white fibres were so. In the same fasciculus, while some fibres showed high enzyme activity, in others of the same type practically no enzyme activity could be detected (Chapter 1). The same was true in the case of succinic dehydrogenase in the present investigation. It would be of interest to know what actually is responsible for such differentiation among fibres of which otherwise are of the same type. One reason might be the altered blood flow resulting from immobilization.

With regard to succinic dehydrogenase, the broad glycogen utilizing fibres showed an increase in activity, and the number of the mitochondria also seemed to increase .

Recent studies by Smith (1965) on rat denervated muscle also have shown an increase in the mitochondrial content of the white fibres. It is possible that this increase in succinic dehydrogenase activity in the broad, white fibres is to meet the additional demands of the oxidative phase of carbohydrate metabolism in such fibres during atrophy. This might also explain the shivering action of the muscle seen in the experimental pigeons after the immobilization of the wings.