

CHAPTER 2

SUCCINIC DEHYDROGENASE ACTIVITY IN THE PIGEON BREAST MUSCLE
UNDER DISUSE ATROPHY

It has been shown by many authors that muscle under progressive atrophy shows considerable enzymatic changes. Humoller et al, (1951a); Nachmias and Padykula (1958) and Schmidt and Schlieff (1956) showed that after nerve section, succinic dehydrogenase and cytochrome activity was reduced considerably after a period of 14 days. McCaman (1960) has recently studied certain dehydrogenase systems in the muscle of dystrophic mice and reported that the activity of the TPN-linked enzymes is increased while that of the DPN-linked ones decreased. But Dreyfus et al, (1956) and Baker et al, (1958) reported that the activities of the Krebs cycle enzymes are not markedly altered in muscular dystrophy. In view of these conflicting reports regarding the dehydrogenases in muscular atrophy, it was decided to make a study on the activity of succinic dehydrogenase (SDH) in the pigeon breast muscle under atrophy. The pigeon breast muscle was chosen for these studies since it is an ideal material for studies on disuse muscular atrophy and has also high SDH activity and even small changes in the enzyme level could be easily detected. The present study is a report on the SDH activity in the pectoralis muscle of the pigeon under varying periods of muscular atrophy.

Material and Method

Experiments were carried out on fully grown healthy

pigeons weighing between 290 to 320 gm. A plaster cast was applied on the middle of the upper wings after keeping the wings in a back-to-back position as reported earlier in the first chapter. These pigeons with the wings under immobilization, were used throughout and the pigeons of the same weight were used as controls. They were sacrificed after varying lengths of time from one day to sixty days.

In each experiment the bird was decapitated and a piece of the breast muscle was immediately cut off and blotted free of blood. The piece of muscle was always obtained from the same region and in its entire depth, since it had been shown that the narrow red and broad white fibres differ in their distribution pattern (George and Naik, 1959a) and enzyme levels (George and Talesara, 1960). After weighing quickly, the muscle was homogenized in a chilled mortar with ice cold distilled water. A 5% homogenate was prepared after removing all the connective tissue and other cell debris. The SDH activity of the homogenate was determined according to the method of Kun and Abood (1949) with triphenyl tetrazolium chloride (TTC) as the electron acceptor. The incubation mixture for each experiment contained 0.5 ml. of 0.1 M phosphate buffer of pH 7.4; 0.5 ml. of 0.5 M sodium succinate and 1 ml. of freshly prepared 0.1% TTC in distilled water. 1 ml. of the freshly prepared muscle homogenate was added to this mixture and the tubes were shaken and incubated for 30 minutes at 37°C by occasional shaking. The enzyme activity was terminated at the

end of 30 minutes by the addition of 7 ml. of acetone. The tubes were shaken well and the formazan extracted in the acetone, centrifuged for 5 minutes at 3000 r.p.m. and the clear supernatant drawn off and the intensity of the colour read at 420 mμ on a Klett-Summerson photoelectric colorimeter. The readings were corrected for a blank with zero time incubation and the amount of formazan formed was calculated from a standard graph. The enzyme activity is expressed as μg formazan formed per mg. dry weight of the muscle per 30 minutes at 37°C under aerobic conditions. The dry weight of the muscle was determined by drying 1 ml. duplicate samples of the homogenate in an air oven at 95°C and then kept in a vacuum desiccator until constant weight was obtained.

Results

A decrease in the succinic dehydrogenase activity of the disused breast muscle of the pigeon was immediately observed after the first day of immobilization. The enzyme activity gradually decreased and by the 10th day it was found to be very low (Table 1 and Fig. 1). Later on after this fall, the activity of the enzyme was found to be increasing gradually and reaching almost nearer to the enzyme activity in the normal pigeon muscle at the end of one month. But after 60 days of immobilization it was again observed to be little lower than that in the 30 days atrophied pigeons. It was also observed that there were a few ups and downs in the enzyme activity during the early days of atrophy.

Table 1

Succinic dehydrogenase activity in the pigeon breast muscle under varying lengths of muscular atrophy and the normal values of the enzyme in the control birds.

Atrophy in days	μg Formazan / mg. dry muscle / 30 minutes	S. D.	No. of Expts.
1 Day	6.983	\pm 1.296	6
3 Days	4.114	\pm 0.953	7
7 Days	4.545	\pm 1.276	10
10 Days	2.094	\pm 0.909	9
14 Days	3.989	\pm 1.390	8
21 Days	3.893	\pm 1.452	9
30 Days	5.126	\pm 1.640	12
60 Days	4.579	\pm 1.590	11
Normal (Control)	7.731	\pm 0.734	9

Considerable loss in body weight during the period of atrophy was also noted in the pigeons. The muscle became edematous and swollen. Histological observations of the muscle showed the characteristic reduction in the diameter of the broad white fibres and considerable divergence in their size. In the later periods of atrophy high accumulation of connective tissue was also observed.

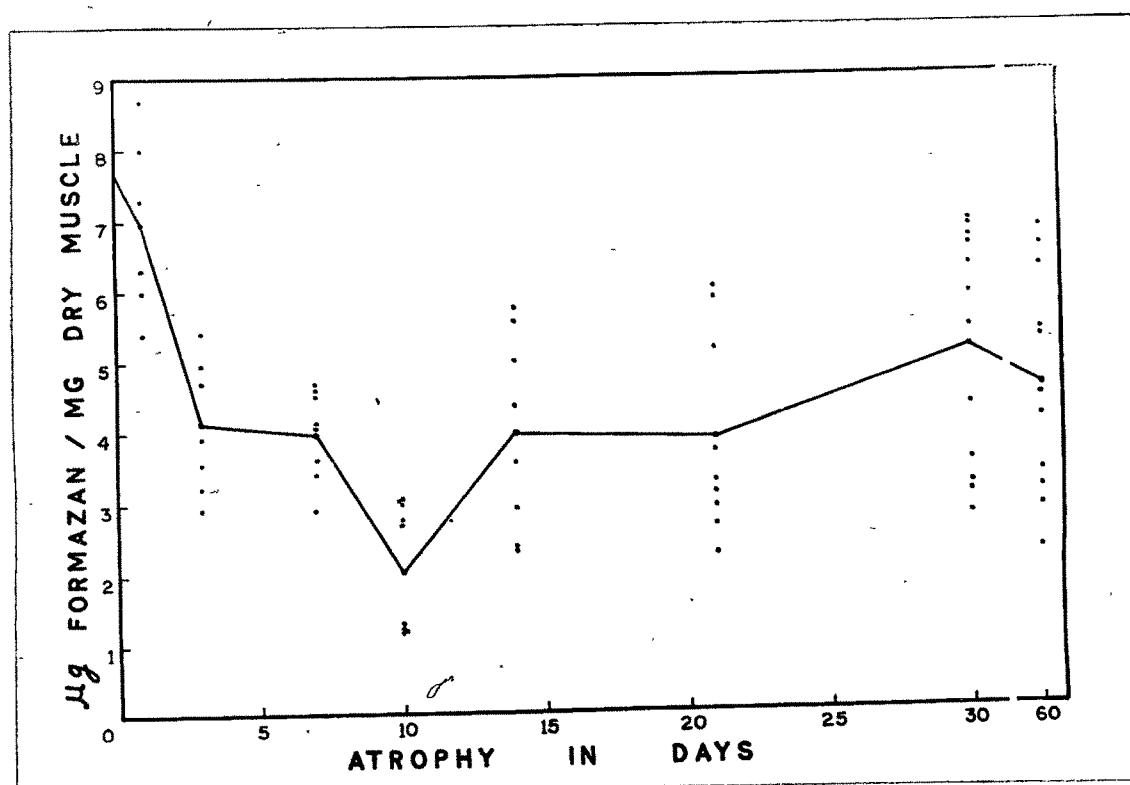


Fig. 1 Changes in succinic dehydrogenase activity in the pigeon breast muscle during induced muscular atrophy. Dots indicate individual readings and the curve the average.

Discussion

The characterization of the features in an atrophied muscle may be regarded as a problem of special significance in comparative biochemistry where numerous biochemical and physiological changes take place in the muscle and blood as a result of the sudden metabolic changes in the muscle. Estimations of different enzyme systems in the atrophied muscle have been extensively studied in order to explore the metabolic alterations during atrophy. Recent studies reported in Chapter 1 on the atrophied pigeon muscle and blood lipolytic activity, have shown that there is a high lipase activity in the muscle and a corresponding low lipase level in the blood during the first week of inactivity of the muscle. The corresponding fluctuations in the muscle fat and water content shows that there is a severe disturbance in the lipid metabolism of the muscle during the first week of atrophy.

The present observations reported in this chapter on the SDH levels of the atrophied breast muscle of the pigeon, also supports the view that there is a disturbance in the lipid metabolism of the muscle during the early stages of atrophy. By the third day of atrophy SDH activity in the muscle was found to be nearly half of that of the normal pigeons and by the 16th and 14th days, it was found to be lower still.

A higher concentration of the oxidizing enzymes and lipase in the normal pigeon breast muscle, suggest the presence of a highly organized cyclophorase system including specially

all the enzymes of the Krebs cycle equipped for fat metabolism, whereas during atrophy of the muscle, these enzymes show striking decrease in activity. Even though, lipase activity was found to be very high during the first week of atrophy, later/on, the enzyme activity decreased gradually. SDH which is one of the major enzymes in the citric acid cycle, showed a decrease in its activity during the early days of atrophy suggesting a low rate of fat metabolism. Based on these findings it could be inferred that the metabolic pattern in the muscle changes due to disuse, probably changing from fat to a glycogen metabolism.