CHAPTER 9

LIPASE ACTIVITY IN THE NORMAL AND DYSTROPHIC HUMAN MUSCLE

The importance of the hydrolytic enzyme lipase and its physiological role in tissue metabolism particularly in the muscle has been the subject of intense research in this laboratory in the past decade. A high concentration of this enzyme was reported in active muscles such as the flight muscles of insects, red muscle of fish, pigeon pectoralis, vertebrate heart, and rat diaphragm (George, 1964). Its occurrence in high concentrations in the red narrow fat loaded muscle fibres is convincing enough to suggest its role in the hydrolysis of fat into fatty acids as the initial step in the liberation of fat bound energy through oxidative metabolism. The red, narrow fibres are also well equipped with oxidative enzymes, such as succinic dehydrogenase, cytochrome oxidase, malic dehydrogenase, and lactic dehydrogenase (George and Talesara, 1961).

If the above mentioned characteristics are indices of a muscle in active state, a decline in the enzyme levels is to be expected when the muscle is inactive. Vallyathan (1963) has shown that when the pigeon breast muscle was subjected to plaster cast thereby causing immobilization of the wings, there occurred an initial increase of lipase activity in the muscle followed by a decrease in the later stage. The studies conducted on the denervated pigeon breast muscle also showed a decline in the activity of this enzyme in the

later period of atrophy (Chapter 6).

It has already been shown histochemically that the activity of this enzyme diminishes in the muscles of muscular dystrophy patients (Chapter 8). The present study was therefore carried out with a view to compare the abnormalities resulting from experimental atrophy with the myopathies of human muscle.

Materials and Method

Dystrophic human gastrocnemius muscles and normal human gluteus muscles were studied. The human material was obtained from the S.S.G.Hospital, Baroda. After proper diagnosis, muscle biopsies were done. The excised piece of muscle was frozen in liquid oxygen, brought to this laboratory and kept in a cryostat at -20°C. A preliminary histological and histochemical observation was carried out in order to note the degree of degeneration of the fibres prior to the quantitative study. The remaining piece of the muscle was thoroughly ground in a chilled mortar and a homogenate was prepared in precooled distilled water.

The method employed for the estimation of lipase activity was one adopted from Martin and Peers (1953) employing a bicarbonate carbondioxide buffer system of pH 7.4 at 37°C. using the Warburg apparatus as followed in this laboratory (George, Vallyathan and Scaria, 1958). The side arm contained 0.5 ml. of 4% (v/v) tributyrin in 0.0148 M. bicarbonate, emul-

sified by shaking with a drop of Tween 80. Each reaction flask contained 1.5 ml. of 0.025 M. bicarbonate buffer and 1 ml. of enzyme solution being tested, in the main chamber. The flask and the manometers were gassed for 3 minutes with a mixture of 95% nitrogen and 5% carbon dioxide . After an equilibration of 10 minutes in the Warburg constant temperature water bath, the substrate was tipped in and allowed to equilibrate for 3 minutes to ensure complete mixing of the contents. The manometers were shaken at about 120 oscillations/minute allowing an amplitude of 4 to 5 cms. per oscillation. The readings were taken at regular intervals for 1 hour. The enzyme activity in the muscle is expressed as micro liters of CO_2 / milligram protein / hour. The protein content of the enzyme solution was determined according to the method of Gornall et al. (1949).

Results

Studies were conducted on 7 dystrophic samples. Depending on their histological and histochemical characteristics they were divided into two groups, viz. (1) initial stage and (2) later stage of dystrophy. Three of the cases studied were seen to be at the onset of dystrophy as is revealed by histology. Degenerating fibres were very few in number, the connective tissue growth was limited to the endomysial regions and there was comparatively less fat deposition in the intra cellular spaces. On the other hand the remaining four cases were of a later stage of atrophy. There was considerable derangement of the muscle fibres and an enormous

deposition of fat and connective tissue.

As regards the lipase level a variation was noticed between these two groups (initial and later stages) of muscular dystrophy patients (Table 1). A high level of enzyme activity was noticed in the muscles of group one, which is at the onset of dystrophy as is indicated only by the occurrence of a biochemical lesion and not by any morphological evidence for derangement.

The other four cases, in which degenerative changes were more severe and seemed to be at a later stage of dystrophy, showed a very low lipase level.

In all the cases of normal gluteus samples, the enzyme level appeared to be rather constant from 10 µl.to 15 µl. CO_2 evolved / milligram protein / hour.

Discussion

The interpretation of the results in the present study is confronted with many limitations as the study is made at random due to the difficulty in getting the samples in the increasing order of the disease. Moreover, all the diseased muscles availed of were gastrocnemius and no normal gastrocnemius muscle samples was available during the period under investigation. Hence the author is left with no other choice but to compare the results with those of normal gluteus muscle samples. Such a comparison may be justified because of the fact that the histological and histochemical

TABLE 1

HUMAN MUSCLE LIPASE

Stages of atrophy	Muscle	Lipase activity in muscle µl CO_2 /mg. protein/hr	No. of experiments
Initial	Gastrocnemius	26.38 ± 7.81	4
Later	"	5.83 ± 1.49	4
Normal	Glúteus	17.23 ± 6.08	4

observations did not show any significant difference in the fibre composition of both the muscles.

The observations made in the present study reveal some interesting facts. In the acute cases of muscular dystrophy as was observed in four cases studied, a depletion of lipase was noticed, whereas in the initial stage of dystrophy as three of the cases indicated, the enzyme level seemed to increase. These results are well in accordance with those of experimental atrophy as reported by Vallyathan (1963). The histochemical observation on lipase in the denervated pigeon breast muscle also revealed a similar decrease in lipase activity (Chapter 6).

George and Talesara (1962) suggested a reversal of the hydrolytic action of lipase during fat synthesis in the muscle. This suggestion finds support in the work of George and Vallyathan (1964) who observed an increase in the level of lipase with a corresponding increase of fat in the breast muscle of Rosy Pastor, the migratory starling, during the pre-migratory period. It is well known that during the pre-migratory period there is a rapid accumulation of fat in the body resulting also in an increase in the level of fat in the muscles. The increase in lipase however, is not to that extent as it occurs during fat utilization (Vallyathan, 1963).

The high lipase level noticed in the muscle of dystrophic patients during the initial stages of dystrophy,

thus indicates synthesis of fat. Once the fat is accumulated, the lipase level decreases since there is no utilization of fat. The low level of lipase in the acute cases may thus be accounted for.

One interesting phenomenon emerging from the present study is that, the degree of dystrophy can be correlated with the lipase level after the initial stage. Though the present evidences are not sufficient to form such generalizations, such a relationship may well be suggested.

The inability of the dystrophic muscle to utilize fat is further revealed by the depletion of the enzymes concerned with aerobic metabolism (Chapter 8). Whether the inability of the muscle especially in the later stages of dystrophy is due to the lack of lipase for the initial hydrolysis of fat or due to the total depletion of oxidative enzymes is not known. The high level of lipase during the onset of dystrophy however, shows that, there is no derangement in the synthesis of this enzyme in the initial stage. On the other hand, during the later stage some factors responsible for the synthesis of lipase or oxidative enzymes may be lacking in the muscle. Probably an investigation in this direction would be rewarding.