CHAPTER 9

EFFECT OF STIMULATION ON ACID ATPase IN SKELETAL MUSCLE

The problem of ATP serving as the immediate source of energy in the contracting muscle, had not been satisfactorily tackled until recently. A number of earlier investigators had suggested that muscle can do work under certain well defined conditions without the decomposition of an energetically equivalent amount of ATP or creatine phosphate.

Fleckenstein et al. (1954) reported that when the rectus abdominis of frog, which is a slow muscle, was electrically stimulated, no change in ATP or ADP took place at 20°C, but the concentration of creatine phosphate fell. They further stated that the inorganic phosphate liberated during contraction, was from an unidentified precursor, in just sufficient quantities to supply the required energy, provided the phosphate undergoing fission was energy rich. Mommaerts (1955) obtained similar results for single twitch contractions of turtle muscles. Working with the sartorius muscle of the frog, a diminution of ATP in a single contraction could not be directly demonstrated by Carlson and Siger (1960). Gould and Rawlinson (1959) while studying the effect of exercise on ATPase and creatine phosphokinase in the presence and absence of added calcium found no marked changes in the concentrations of either of the two enzymes. Their results are in agreement with those of Hearn and Wainio (1956, 1957) who found no marked change in the concentrations

of succinic dehydrogenase and aldolase activities in the heart as well as the skeletal muscle of rats subjected to strenuous exercise. No detectable differences were obtained in the levels of lactic and malic dehydrogenases and phosphorylase in the hindlegs of exercised rats (Gould and Rawlinson, 1959). Hearn and Gollnick (1961) observed significant changes in the calcium activated ATPase activity and Gollnick and Hearn (1961) in the lactic dehydrogenase activity of the heart muscle after exercise, but not in the gastrocnemius muscle. Nevertheless, Yakovlev (1950), Yampolskaya and Yakovlev (1951) and Yampolskaya (1952) claimed to have obtained increases in the concentrations of glycogen, phosphagen, ascorbic acid, glutathione as well as in "phosphorolytic activity" and in the activity of hexokinase, lactic and succinic dehydrogenases in exercised muscle.

However, the direct experimental proof of a prior breakdown of ATP in physiologically induced contractile activity was provided by the works of Mommaerts et al. (1962) and Cain et al. (1962). They demonstrated that in a brief tetanus of the frog sartorius muscle, phosphocreatine is being split from the first moment of contraction onwards.

The studies to date with respect to the effect of stimulation on ATPase activity have been concerned mainly with biochemical changes in the skeletal muscle. To our knowledge, no reports have appeared concerning similar effects on the histochemical localization of any of the ATPases. Recently an acid ATPase having pH optimum of 2.5, which is present in

high concentrations in white muscles and white fibres of mixed muscles, has been histochemically demonstrated (Chapter 8). The effect of electrical stimulation of the frog gastrocnemius muscle on this enzyme has been studied in the present investigation.

MATERIAL AND METHOD

The gastrocnemius muscle of the frog (Rana tigrina) was used as the experimental material. The animal was tied to a plank of wood, so as to prevent any struggle during the process of electrical stimulation. The gastrocnemius muscle of one side was stimulated directly and also through its nerve by means of an electronic stimulator (Arthur Thomas Co., Philadelphia Pa., Model 751-A) at a rate of 5 stimulations per second, of 2 milliseconds duration, of 60 to 70 voltage, for intervals of 10, 30 and 60 minutes. The gastrocnemius muscles of frogs which were not subjected to stimulation were used as a control. Small pieces of muscle from the experimental and control animals were removed and dropped directly into liquid oxygen at -182°C. Sections 10 to 15 u thick were cut directly into the incubation medium prepared according to the method of Wachstein and Meisel (1957) and adjusted to pH 2.5. The rest of the procedure was as described in Chapter 8.

RESULTS

A comparison of the intensity of ATPase activity at an acid pH of 2.5 in the stimulated and control muscles, revealed

a significant increase of the enzyme activity in the stimulated muscle. In all cases, this ATPase activity appeared to be located mainly in the sarcoplasmic reticulum of the red as well as the white fibres, the enzyme level being higher in the latter. The enzyme activity was also concentrated in sites corresponding to mitochondria and nuclei. The nuclei in the frog muscle cells were present within the substance of both the red and the white fibres unlike that in the pigeon, pectoralis muscle where such disposition of the nuclei were seen only in the white fibres (Chapter 8).

In sections of muscles stimulated for 10 minutes, (Fig. 1), marked acid ATPase activity was seen more prominently in the white fibres within 1 to 1½ hours of incubation. After 30 minutes of stimulation (Figs. 2 & 3), the enzyme showed still greater activity, as could be judged by the incubation time which was considerably less (½ to ¾ hour), but with 60 minutes of stimulation (Fig. 4) no appreciable difference in the enzyme level from that obtained with 30 minutes stimulation, was seen.

In the control sections (Fig. 5), this acid ATPase activity was distinctly low and could be located only after prolonged incubation for 3 to 3½ hours.

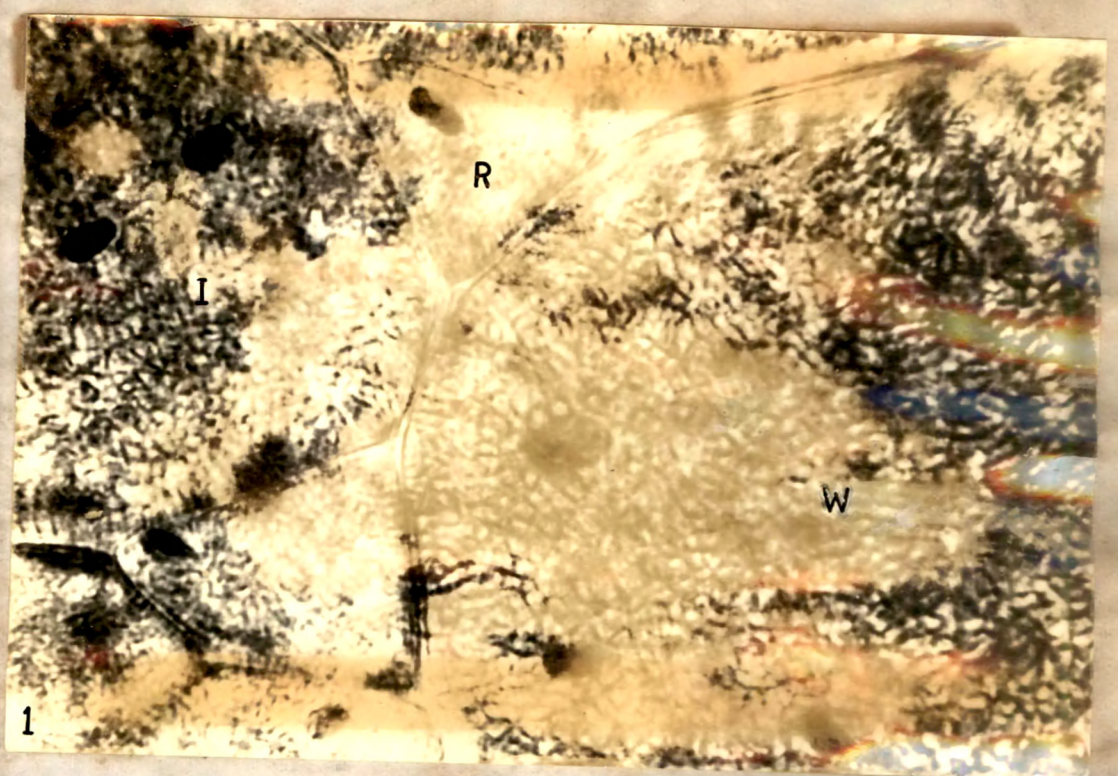

Fig. 1 Transverse section of the frog gastrocnemius showing ATPase activity at pH 2.5 after 10 minutes of electrical stimulation. Enzyme activity is seen to be located at sites corresponding to the sarcoplasmic reticulum in the red (R), intermediate (I) and white (W) fibres. Note the nuclei deeply stained in all the 3 types of fibres. X 1130.

Fig. 2 Transverse section of the frog gastrocnemius stimulated for 30 minutes. Increase in sarcotubular ATPase activity (pH 2.5) was noted in the red (R), intermediate (I) and white (W) fibres. Note the high enzymatic activity in the sarcolemmal regions of the fibres. Nuclei are also stained. X 1130

Fig. 3 A portion of the white fibre from Fig. 2 magnified to show more clearly the localization of the enzyme in the sarcoplasmic reticulum and nuclei. X 1800

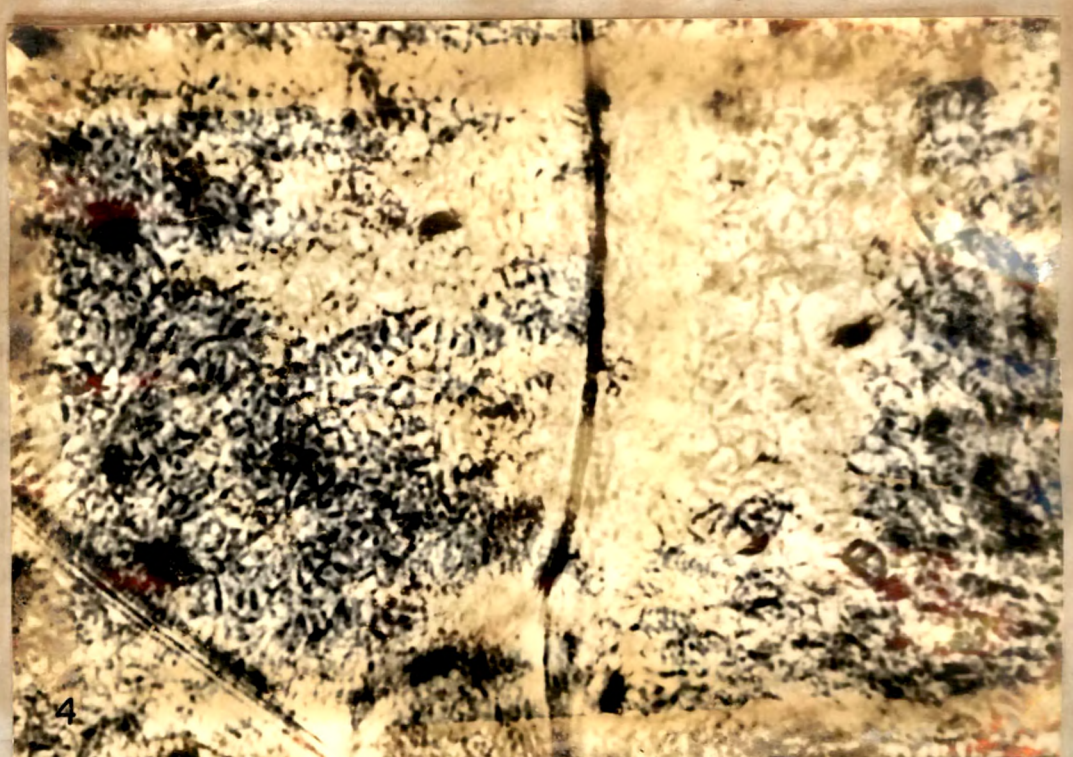

Fig. 4 Transverse section of the frog gastrocnemius demonstrating ATPase activity (sarcotubular) at pH 2.5, after 60 minutes of electrical stimulation of the muscle. No appreciable difference in the enzymatic activity from that seen in Fig. 2 is discernible. Sarcolemmal region is not stained but the nuclei are well stained. X 1130

Fig. 5 Transverse section of the frog gastrocnemius used as control. ATPase activity at pH 2.5 is relatively low as compared to the stimulated muscles. The traces of enzymatic activity in the sarcoplasmic reticulum seen in this figure was obtained only after prolonged incubation. The nuclei are stained. X 1800

DISCUSSION

A well defined and highly organized system of tubular elements called the sarcoplasmic reticulum is known to be located in the sarcoplasm between the myofibrils in the skeletal muscle cells of frog (Muscatello et al. 1961). They further demonstrated that the sarcotubular fraction of frog skeletal muscle homogenate has a Mg^{++} stimulated ATPase activity which is considerably higher as compared to mitochondrial ATPase. Andersson-Cedergren (1959) pointed out that this sarcoplasmic component varies in different skeletal muscle cells of the frog.

The results obtained in the present study show that in the unstimulated frog gastrocnemius, the acid ATPase activity is low, but with electrical stimulation of the muscle for 30 minutes, the enzyme activity increased considerably and began to decline from after 60 minutes of stimulation. It has been suggested earlier (Chapter 8) that the occurrence of a higher concentration of acid ATPase in the glycogen utilizing fibres is to be regarded as an adaptation for the enzyme to function at a lower pH range produced by a possible accumulation of lactic acid. The fact that this enzyme is activated considerably on exercising the muscle, as seen from the present study, supports the above suggestion. Such accumulation of large quantities of lactic acid as a result of exercise has been demonstrated in fish muscle (Black et al. 1959). It has also been suggested that this happens as a result of the slow rate of diffusion of lactate at the low body temperature encountered

in the fish, which would tend to delay the transfer of lactate from muscle to blood, or because the circulation is adversely affected as a result of severe activity (Black et al. 1962). The frog being a poikilothermic animal like the fish, similar accumulation of lactic acid is to be expected. Moreover, the frog is a less active animal than the fish. Therefore the fact that the enzyme activity could not be demonstrated in the resting frog muscle shows that there is a relationship between the activity of the enzyme and the activity of the muscle.