CHAPTER 9

ALKALINE PHOSPHATASE ACTIVITY IN THE BREAST MUSCLE OF THE PIGEON AND BAT

In this chapter is reported the result of a histochemical study undertaken to demonstrate the localization of alkaline phosphatase in the pectoralis major muscle of the pigeon and bat.

The alkaline phosphatase activity was studied on transverse sections of the frozen tissue cut according to the method described in chapter 3. The sections were transferred to clean slides and allowed to dry at room temperature (about 1 hour). They were then fixed in 10% cold (4°C) formalin for 2 hours, washed in running water for 30 minutes and used for the study after rinsing in distilled water. The method employed was the revised method of Gomori (Pearse, 1954) using sodium glycerophosphate as substrate. The validity of this method in localizing exactly the alkaline phosphatase activity has been questioned, the literature on this subject is vast and it is thought unnecessary to review them all here. However, it must be mentioned that this method is very widely used and has met with success in the study of this enzyme. The sections were incubated in the substrate medium at 40°C for 24 hours, treated with cobalt nitrate and ammonium sulphide and mounted in glycerine jelly without counterstaining. Two controls were tried; in one the incubation of the sections in the substrate medium was omitted and in the other the sections were incubated after

keeping them in boiling water for 10 minutes.

From previous experience it was found that the pigeon kidney showed very high alkaline phosphatase activity after half an hour of incubation, but in the case of the muscle, satisfactery results could be obtained only on prolonged incubation (24 hours). Figure 1 presents the photomicrograph of a transverse section of the pectoralis major muscle of the pigeon showing localization of the enzyme activity. It is clearly seen that the nuclei are deeply stained indicating high phosphatase activity. Of the two types of fibres, the cytoplasm of the narrow fibre shows a high activity of the enzyme. In the broad fibre on the other hand, the cell border is stained and that too faintly, suggesting that the enzyme is located mainly if not solely in about the sarcolemma.

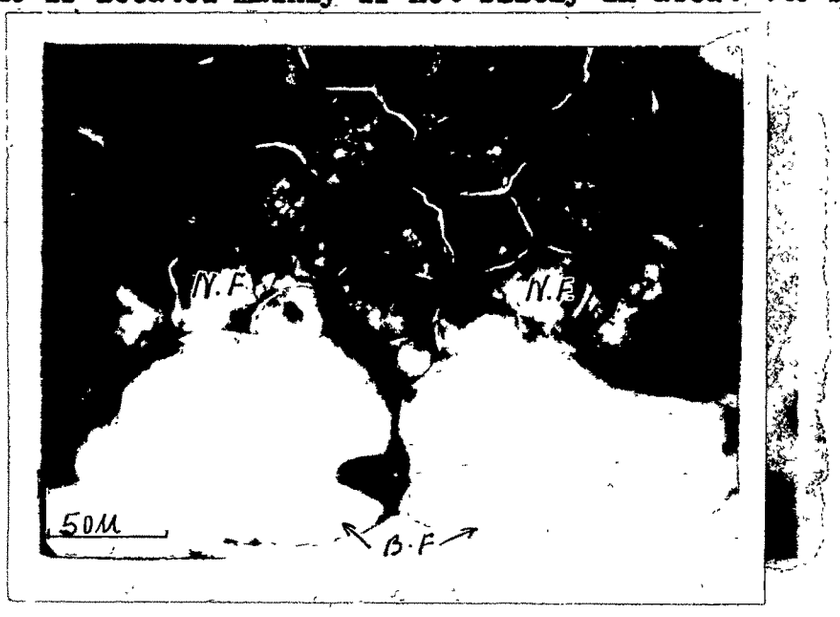

Figure 1. Microphotograph of a transverse section of the pectoralis major muscle of pigeon showing localization of alkaline phosphatase activity (B.F., broad fibre; N.F., narrow fibre)

It would appear that most of the transphosphorylation reactions take place in the narrow fibres and the presence of the enzyme at the border of the glycogen loaded broad fibres is mainly to aid in the transport of glycogen.

In the breast muscle of the bat, alkaline phosphatase was found to be present in all the fibres. But the intensity of staining varied in the different fibres and the distribution pattern closely resembled that of succinic dehydrogenase. Whatever be the function of alkaline phosphatase in the muscle it appears from this study that the concentration of this enzyme in muscle fibres follows the concentration pattern of oxidative enzymes and is related to activity. This study also strengthens the conclusion that the broad glycogen loaded white fibres in the breast muscle of the bat do not resemble those in the pigeon breast muscle as far as their enzymic activity is concerned and the condition in pigeon is an extreme case of specialization.

It should be of interest to point out that I am not aware of any other work reporting alkaline phosphatase activity in skeletal muscles. An attempt by Bourne (1943) to demonstrate alkaline phosphatase activity in voluntary muscle failed. This failure may be due to the comparatively low concentration of the enzyme in the muscle and the insufficiency of the period of incubation.