CHAPTER 8

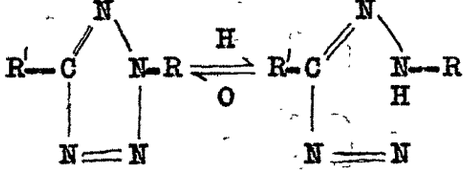
A HISTOCHEMICAL STUDY OF DEHYDROGENASE ACTIVITY IN THE PECTORALIS MAJOR MUSCLE OF THE PIGEON AND BAT AND CERTAIN OTHER VERTEBRATE SKELETAL MUSCLES

Since George and Jyoti (1957) showed that fat is the chief fuel during sustained activity of the breast muscle of flying birds, and George and Naik (1958a) that the white and red fibres in the pectoralis major muscle of the pigeon are respectively loaded with glycogen and fat, this muscle has become the subject of more extensive studies in our laboratories in the hope of discovering the functional significance of these distinct types of fibres existing side by side in one and the same system. George and Naik (1958b) studied the relative distribution of mitochondria in the two types of fibres and observed that the mitochondria occur in large numbers in the narrow red fibres while the broad white ones contain few or none. I have already shown that the pectoralis major muscle of the pigeon contains a lipase and presented evidence to show that the lipase is confined only to the narrow fibres. It was therefore decided to study the distribution of certain other enzymes also in this muscle. Other workers have recently studied the dehydrogenase activity in the pigeon breast muscle by chemical methods (Chappel and Perry, 1953). In this chapter I present the result of a study undertaken to demonstrate the dehydrogenase activity in the pectoralis major muscle

of the pigeon (Columba livia) by histochemical methods. This study was further extended to the pectoralis major muscle of the dove (Streptopelia senegalensis), the fowl (Gallus domesticus) and the bat (Hipposideros speoris) and the gastrocnemius muscle of the fowl and the frog (Rana tigrina) for comparison. In all the muscles except the breast muscle of the dove and bat, the following dehydrogenases were studied: succinic, malic, D-glucose, and glycerophosphate dehydrogenase. In the breast muscle of the dove and bat, only succinic dehydrogenase was studied.

Method

Several authors have used the TTC (2:3:5: triphenyl tetrazolium chloride) method for the study of succinic dehydrogenase in animal tissues (Straus et al, 1948; Black et al, 1950; Black and Kleiner, 1949 and Seligman et al, 1949). We followed the method of Straus et al and Pearse (1954) for succinic dehydrogenase and extended its application to the other dehydrogenases. The principle of the method is that the colourless soluble tetrazolium salt on reduction is converted to an insoluble red compound, formazan, which is deposited at the sites of reduction in the tissues. The reduction is brought about by the enzymic liberation of hydrogen from the substrate. The tetrazolium salt acts as the hydrogen acceptor. The reaction is as follows (Pearse and Scarpelli, 1958).



Tetrazolium Formazan

In biological systems this reaction is not reversible, and the quantity of formazan deposited in tissue sections can be directly related to the amount of succinic dehydr - ogenase (Defendi and Pearson,1955). In the case of all the above enzymes except succinic dehydrogenase the tetrazolium salt cannot act as the hydrogen acceptor without the inter - vention of a cofactor (DPN) which functions as a hydrogen carrier. Reduction of the TTC by reducing substances such as glutathione, cysteine, ascorbic acid, or reducing sugars usually present in tissues does not take place under the conditions of the experiment (Pearse,1954). The incubation medium in each case contained 1.5 ml. 0.1 M phosphate buffer of pH 7.2, 1 ml. 0.1 M solution of the substrate, 7.5 mg. of TTC, and 0.625 mg. of DPN in a total volume of 2.5 ml. in a cuevette. DPN was omitted from the mixture for succinic dehydrogenase, since this enzyme does not require the co - factor (Baldwin,1953). Sections about 50μ to 80μ thick, pre - pared according to the method described in chapter 3, were immersed in the respective incubation media, covered with a lid, and incubated for 5 to 30 min. at 37°C. They were then washed in buffer, fixed in 10% neutral formalin, washed in water and mounted in glycerine jelly without counterstaining.

Shelton and Schneider (Pearse, 1954) claimed that freezing destroys endogenous activity of succinic dehydrogenase. I have observed that frozen sections prepared as cited above do show endogenous activity when directly transferred to TTC solution; thus freezing alone does not destroy endogenous activity. But the endogenous activity was found to be lost

when the sections, were kept for 10 to 15 min. in cold 0.1 M phosphate buffer at pH 7.2 before transferring them to the TTC solution. So in this study all the sections were placed in phosphate buffer for 10 to 15 min. to ensure complete loss of endogenous activity, and then transferred to the respective incubation media. Exposure of the incubation medium to bright sun light was recommended (Pearse, 1945) for hastening the reaction and reducing the period of incubation. It must be mentioned here that this procedure might produce erroneous results since colour development under such conditions is not due to enzymic activity alone, because the solution itself turns red on exposure to sunlight.

Results and Discussion

It was found that the pigeon breast muscle contains all the dehydrogenases except D-glucose dehydrogenase. Colour development in the sections was rapid enough to be visible within 5 to 10 min. of incubation. Microscopic examination of the sections revealed that the enzymic activity is confined only to the narrow fibres as was indicated by the deep-red colour developed in them. The colour development was so intense as to obscure the boundary of the individual narrow fibres (Figure 1). Short periods of incubation clearly showed the mitochondria as deep-red spots in the narrow fibres. The broad fibres were completely blank and stood out from the rest of the fasciculus as clear, colourless

areas. It could, therefore, be concluded that the broad fibres in the pigeon breast muscle do not contain any dehydrogenase; or, if they contain any, only extremely minute traces, which could not be detected. This conclusion

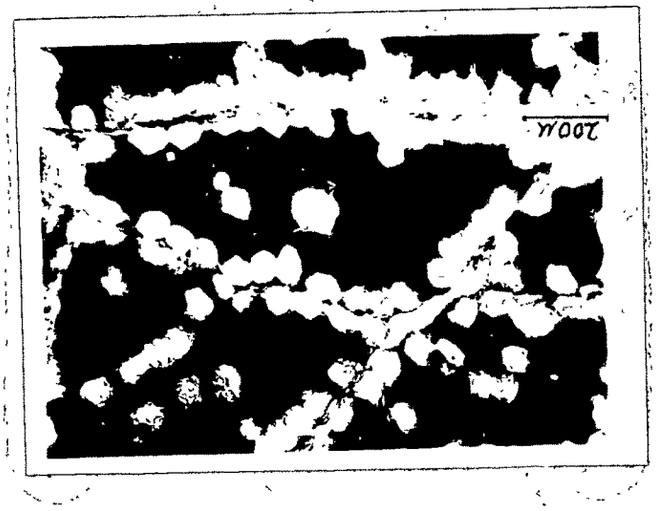


Figure 1. Photomicrograph of a transverse section of the pectoralis major muscle of the pigeon, showing localization of succinic dehydrogenase in the narrow fibres. The broad fibres stand out as clear, colourless areas.

is further substantiated by the fact that when sublethal doses of TTC were administered to the pigeon intravenously or intramuscularly and the bird was killed after a day or two, formazan could be detected at the centres of highest metabolic activity such as liver, kidney, adipose tissue and the heart and breast muscles. In breast muscle, the colour due to formazan could be noticed only in the narrow fibres. The breast muscle of the dove very closely resembled that of the pigeon with respect to succinic dehydrogenase activity with the difference that when thick sections were incubated for a very long time, a faint pink colour could

be detected in the broad fibres.

From observations on other muscles also, a correlation could be drawn between the dehydrogenase activity, the colour of the muscle and the mitochondrial content (Table 1). A similar relationship was shown by Paul and Sperling (1952), who used other methods.

Table 1

Dehydrogenase activity in various muscles

Animal	Muscle	Colour	Abundance of mitochondria	Time taken for maximum colour development	Intensity of the colour
Frog	gastrocnemius	white	none	x x x x	x
Fowl	pectoralis major	white	very few	x x x	x x
Fowl	gastrocnemius	pale red	more than in pectoralis major of fowl	x x	x x x
Pigeon	pectoralis major	red	more than in any of the above muscles	x	x x x x
Dove	do	do	same as in pigeon	x	x x x x
Bat	do	do	do	x	x x x x

The abundance of mitochondria was determined by microscopic examination of the stained sections.
x , minimum; x x x x , maximum.

In the leg muscle of the fowl, where different types of fibres varying from red to white with all intermediate forms occur, dehydrogenase activity was detected in all the fibres. But the mitochondrial content and the dehydrogenase activity was found to vary, the maximum and the minimum being in the red and the white fibres respectively. In the

pigeon, on the other hand, the white fibres in the breast muscle did not show any indication of the presence of any of the enzymes for which tests were made. I am therefore led to believe that none of the oxidative processes concerned takes place in the broad fibres of the pigeon breast muscle. If this conclusion is correct, the broad white fibres in the pectoralis major muscle of the pigeon should be considered as unique and not as analogous to the white fibres of any of the other muscles studied.

It was possible to distinguish more than two types of fibres in the breast muscle of the bat as judged from the degree of enzyme activity and the number of mitochondria as revealed by the formazan. The narrow red fibres showed the maximum activity of succinic dehydrogenase and the maximum number of mitochondria and some of the broad white fibres the minimum. Fibres which were intermediate in diameter between the broad and narrow ones showed varying degrees of activity depending on the diameter of the fibres. The mitochondrial density of the fibres also varied according to the diameter. Thus, just as in the case of the leg muscle of the fowl there was found to be a direct and clear cut correlation between the diameter and mitochondrial density of the fibres and their enzyme activity. But the breast muscle of the bat differs from the leg muscle of the fowl in that the bulk of the former is made up mostly of narrow fibres, a feature naturally associated with greater activity and the broader fibres are much less in number and occupy

the superficial region of the muscle. Further evidence that the enzyme activity in muscle fibres is related to the size of the fibres is seen in the recent work of Nachmias and Padykula (1958). It should be pointed out that the fibres in the breast muscle of the bat which did not show the presence of lipase (Chapter 3) are really the broad fibres which show the minimum activity of succinic dehydrogenase. The conclusion that can be drawn from this study is 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 the pigeon is an extreme case of specialization the significance of which will be discussed later.