A Histochemical Study of Dehydrogenase Activity in the Pectoralis Major Muscle of the Pigeon and certain other Vertebrate Skeletal Muscles

By J. C. GEORGE *and* K. S. SCARIA

(From the Laboratories of Animal Physiology and Histochemistry, Department of Zoology, M.S. University of Baroda, Baroda, India)

SUMMARY

Certain dehydrogenases in the breast muscle of the pigeon and fowl and the leg muscle of the fowl and frog were studied histochemically by the use of 2:3:5:triphenyl tetrazolium chloride. The dehydrogenase activity was found to have a relationship with the colour and the mitochondrial content of the individual muscle fibres. In the pigeon breast muscle, however, the broad white fibres did not show the presence of any of the enzymes studied. It is therefore concluded that these fibres in the pigeon breast muscle are a unique system in which none of the oxidative processes concerned takes place; they cannot be considered as analogous to the white fibres of the other muscles studied.

INTRODUCTION

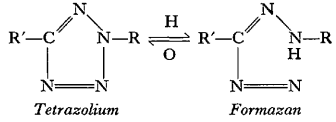
SINCE George and Jyoti (1957) showed that fat is the chief fuel during sustained activity of the breast muscle of flying birds and George and Naik (1958*a*) that the white and red fibres in the pectoralis major muscle of the pigeon are respectively loaded with glycogen and fat, this muscle has become 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 (1958*b*) 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. George and Scaria (1956) showed 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 (1958). Recently George, Nair, and Scaria (1958) studied the alkaline phosphatase activity in this muscle by histochemical methods and found that the enzyme is located mostly in the narrow fibres and only at the border in the broad fibres. They suggested that most of the transphorylation reactions in the muscle takes place in the narrow fibres and the presence of alkaline phosphatase at the periphery of the broad fibres may be for the transport of glycogen from the broad fibres to the narrow ones. Other workers have recently studied the dehydrogenase activity in the pigeon breast muscle by chemical methods (Chappel and Perry, 1953). In this paper we report 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 gastrocnemius and the pectoralis major muscles of the fowl (*Gallus domestica*) and the former muscle of the frog (*Rana*

[Quarterly Journal of Microscopical Science, Vol. 99, part 4, pp. 469–473, Dec. 1958.]

tigrina) for comparison. The following dehydrogenases were studied: succinic, malic, lactic, D-glucose, and glycerophosphate dehydrogenase.

Method

Several authors have used the TTC (2:3:5:triphenyl tetrazolium chloride) method for the study of succinic dehydrogenase in animal tissues (Straus and others, 1948; Black and Kleiner, 1949; Seligman and others, 1949; Black and others, 1950). We followed the method of Straus and others (1948) 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 dehydrogenase (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 intervention 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 our 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 cuvette. DPN was omitted from the mixture for succinic dehydrogenase, since this enzyme does not require the cofactor (Baldwin, 1953). Sections about 50 to 80 μ thick, prepared according to the method of George and Scaria (1958), 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. We 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 sunlight was recommended (Pearse, 1954) for hastening the reaction and reducing the period of incubation. We have noticed 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.

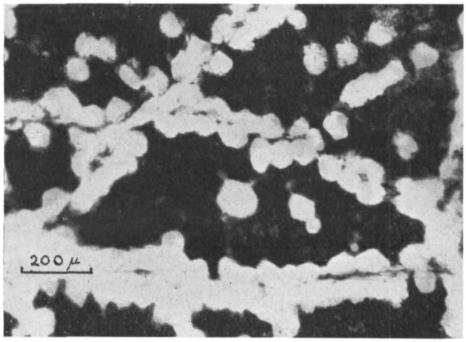


FIG. 1. Photomicrograph of a transverse section of the pectoralis major muscle of the pigeon, showing localization of succinic dehydrogenase in the narrow fibres. The broad fibres stand out as clear, colourless areas.

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 (fig. 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 we could not detect. This conclusion 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.

From our observations on other muscles also, a correlation could be drawn between the dehydrogenase activity, the colour of the muscle, and their 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	++++	+
Fowl	pectoralis major	white	very few	+++	++
Fowl	gastrocnemius	pale red	more than in pectoralis major of fowl	++	+++
Pigeon	pectoralis major	red	more than in any of the other muscles	+	++++

The abundance of mitochondria was determined by microscopic examination of the stained sections.

+, minimum; ++++, 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. We are 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 of 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. Further investigations which are in progress will, we hope, throw more light on the physiology of these unique fibres in the pigeon breast muscle and perhaps on the general problem of muscular contraction as well.

We are grateful to the Sigma Chemical Company, U.S.A., for their generous gift of the TTC and DPN used in this study.

REFERENCES

BALDWIN, E., 1953. *Dynamic aspects of biochemistry*. Cambridge (University Press).
BLACK, M. M., and KLEINER, I. S., 1949. Science, **110**, 660.

BLACK, OPLER, S. R., and SPEER, F. D., 1950. Am. J. Path., **26**, 1097.
CHAPPEL, J. B., and PERRY, S. V., 1953. Biochem. J., **55**, 586.
DEFENDI, V., and PEARSON, B., 1955. J. Histochem. Cytochem., **3**, 61.
GEORGE, J. C., and JYOTI, D., 1957. J. Anim. Morph. Physiol., **4**, 119.
—— and NAIK, R. M., 1958*a*. Nature, **181**, 709.
—— —— 1958*b*. Ibid., **181**, 782.
—— and SCARIA, K. S., 1956. J. Anim. Morph. Physiol., **3**, 91.
—— —— 1958. Nature, **181**, 783.
—— NAIR, S. M., and SCARIA, K. S., 1958. Curr. Sci., **27**, 172.
PAUL, M. H., and SPERLING, E., 1952. Proc. Soc. Expl. Biol. Med., **79**, 352.
PEARSE, E. A. G., 1954. *Histochemistry, theoretical and applied.* London (Churchill).
—— and SCARPELLI, D. G., 1958. Nature, **181**, 702.
SELIGMAN, A. M., GOFSTEIN, R., and RUTENBURG, A. M., 1949. Cancer Res., **9**, 366.
STRAUS, F. H., CHERONIS, N. D., and STRAUS, F., 1948. Science, **108**, 113.