التفريق الهستوكيميائى لألياف عضلة الصدر الكبرى فى نوعين من الحمام (كولومبيا ليفيا)

د. ى. شاهين ، د .س. الموجى

ملخص

لقد وجد ثلاث أنواع من الألياف العضلية فىعضلة الصدر الكبرى فى الحمام المستأنس وهىألياف حمراء والياف بيضاء والياف وسط بين الاثنين .بينما كانت الألياف العضلية فى الحمام البرى تتكون من نوعين الاحمر والأبيض .

وقد نسبت هذه الصـفة الكلية للقـدرة علىالطيران .

Dept. of Anatomy and Histology, Cairo Univ. and Dept. of Physiology, Zagazig Unive.

Head of Dept. : **Prof. Dr. A.N. Georgi**

HISTOCHEMICAL DIFFERENTIATION OF THE PECTORALIS MAJOR MUSCLE FIBRES OF TWO ALLIED FORMS OF COLUMBA LIVIA

(with one table and two figures)

By

Y.M. Shahien and S.A. El Mougy

(Received at 12/8/1974

SUMMARY

The pectoralis major muscle of the domestic form of Columba livia revealed the presence of three types of muscle fibres, red, white and intermediate, while that of the wild form was made up of 2 distinct components of fibres, red and white. This morphological characteristic seems to be correlated with flight ability.

INTRODUCTION

Recently, there has been a considerable interest in the metabolism of red muscle as compared with white voluntary skeletal muscle of vertebrate. Histochemical differences have been demonstrated between these two basic types of muscle fibres. The red muscle (type I) is rich in succinic dehydrogenase, fat and lipase. The white muscle (type II) is rich in glycogen, poor in fat and its related enzymes (OGATA, 1958 ; DUBOWITZ and PEARSE, 1960, 1961 : JINNAI, 1960 ; BECKETT, 1962 : ENGEL, 1962, STEIN and PADYKULA 1962 ; HENNEMAN and OLSON, 1965 ; NISHIYAMA, 1965 ; BEATTY, BASINGER, DULLY and BOCEK, 1966 ; EDGERTON, 1968 and EDGERTON and SIMPSON, 1969). According to the available literature, the breast muscle of the pigeon is histochemically classified into the two basic types of muscle fibres by oxidative enzyme reactions GEORGE and NAIK, 1958 b & c, and 1959 ; GEORGE and SCARIA, 1958 a and b ; GEORGE and TALESARA, 1960 and 1961 ; CHINOY, 1963 and 1970 ; DUBOWITZ, 1963 & 1966 ; NENE and GEORGE, 1965 and GEORGE and BERGER, 1966. GEORGE and BERGER (1966), attributed the difference in muscle fibres to their functional adaptation at the molecular level. GUTH and SAMAHA (1969) and BARNARD, EDGERTON, FURUKAWA and PASER (1971) suggested that both white and red fibres are fast contracting while the intermediate fibre has a slow contracting time.

The histochemical studies on the pectoralis muscle of two allied forms of columba livia revealed an interesting difference with a possible physiological significance and the results of that study are reported in this short communication.

MATERIALS AND METHODS

Well-fed, healthy, adult pigeons (Columba livia) of the domestic (220-250 g) and wild forms (145-160 g), were decapitated and allowed to bleed. Pieces of the pectoralis major muscle were quickly excised and frozen for sectioning at 15-25 u. The sections were then directly transferred too freshly prepared incubation medium at 40°C for 15 minutes for the demonstration of succinic dehydrogenase (SDH) activity, adopting the method prescribed by NACHLAS, TSOU, DE COUZA, CHENG and SELIGMAN (1957).

Additional small blocks of pectoralis muscle fixed directly in cold neutral formalin, calcium formol and carnoy were used to demonstrate lipase, fat and glycogen using the tween 80, sudan black and PAS reactions (PEARSE, 1968).

Sections were mounted in 50% glycerine and the number of broad fibres per square counted according to the method described by GEORGE and NAIK (1959).

In addition, some necessary measurements were included : weight of the bird, wing area, wing span, wing shape and weight of the wing muscles.

RESULTS AND DISCUSSION

The observed results indicated variability in the SDH activity in the two allied forms of C. livia. The domestic form revealed the presence of a small band of narrow red muscle fibres (type I) towards the centre of the muscle, surrounded by intermediate and broad white muscle fibers (type II). Some narrow fibers were scattered at random towards the periphery (Fig. 1). The diameter of the narrow fibers ranged from 25-29 microns, that of the broad fibres ranged from 180-130 microns and 37-40 microns in the intermediate fibres, in the proportion of 1 : 4 : 2 respectively. The presence of the intermediate fibres in the domestic form agreed with the findings of GERMINS, D'ALORA and WASHRMANN (1965) in chicks. On the other hand, the wild form demonstrated in the present investigation sharply differentiated two distinct types without any intermediate forms (Fig 2). The diameter ranged from 30-35 microns in the narrow fibres and 65-110 microns in the broad fibres, in the proportion of 1 : 25. The distribution of narrow and broad fibres demonstrated, in the wild form agreed well with GEORGE and NAIKE (1959) on C. livia although differing in the proportion of narrow : broad. It was noted that there was a predominance of type II fibres in the periphery of the M. pectoralis major of the pigeon. The absence of type I fibres from the periphery of the fasiculi of the muscles examined refelcted the funtional differences between the fibre types. Type I fibres were surrounded by greater numbers of capillaries than the relative by anaerobic type II fibres (ROMANUL, 1964). The aerobic fibres may be distributed in the central regions of the fasiculi in order to prevent compression of their capillaries by rigid perimysium and consequent interruption of their oxygen supply. Type I fibres in contact with the relative by avascular perimysium may also be at a slight disadvantage because they have smaller surface areas through which oxygen may diffuse. Thus the association of the oxidative fibres with the more muscular central regions of the fasiculi is well marked in the pigeon due to the greater overall metabolic rates and greater oxygen demands of their muscle fibres in birds than in other vertebrates.

Failure of demonstrating the intermediate form of muscle fibre by using the histochemical reactions, lead us to differentiate between the two allied forms of C. livia. This was due to difference in stainability among the different types of fibres which could not be so clearly demonstrated as in case of the use of SDH (OGATA and MORI, 1964). GEORGE and TALESARA (1961) demonstrated that even homologous muscles of birds may show great variations in microscopic structure and enzyme activity, recording greater SDH activity in the good flyers than in the poor flyers. Also, the findings of REVEL (1962), LASIEWSKIN, GALEY and VASQUEZ (1965) ; GAUTHIER and PADYKULA (1966) ; HALL — CRAGGS (1968) and EDGERTON, CERCHMAN and CARROW (1969), suggested that oxidative was directly related to metabolic activity and frequency or total amount of chronic contractile activity.

In the light of the present findings, the difference in enzyme activity may be accounted to the difference in flying habits. The occurrence of small, large and physiologically slow intermediate fibres was explained through the fact that the reciprocal enzyme activities may represent different states of contraction. Therefore, the higher the proportion of intermediate fibres-relative to red and white fibres — the slower the contraction time for that muscle (GUTH and SAMAHA, 1969 and BARNARD, EDGERTON, FURUKAWA and PASER, 1971). The marked variation in diameter between the fibres of different enzyme activity supported by the fact that these fibres were of different typts, as did the consistency of both reaction and size in the red muscle of the fish (DUBOWITY and PEARSE, 1960).

In view of the present observation and the biophysical calculations in table I, it becomes valid to relate the obtained results with a possible difference in the flight ability of the two allied forms of C. livia. This hypothetical assumption may put farward when bearing in mind previous findings in other birds, for example, the parakeet (PRITTAVULA KRAMERI) and the bee-eater (MEROPS ONENTALIS). The M. pectoralis major consists of only the narrow variety of fibres, the broad ones being absent altogether. There are other species, for example, the domestic fowl (GALLUS DOMESTICUS) and the kite (MILVUS MIGRANS) in which there is the broad variety of muscle fibers. Thus further studies should be engaged with flight energy and estimate flight ranges of the two allied forms of C. Livia. activities may represent different states of contraction. Therefore, the higher the proportion of intermediate fibres relative to red and white fibres the slower the contraction time for that muscle (GUTH and SAMAHA 1969 and BARNARD EDGERTON FURUKAWA and PASER, 1971). The marked variation in diameter between the fibres of different enzyme activity supported by the fact that these fibres were of different types, as did the consistency of both reaction and size in the red muscle of the fish DUBOWIT and PEARSE, 1960).

In view of the present observation and the biophysical calculations in table I, it becomes valid to relate the obtained results with a possible difference in the flight ability of the two allied forms of C. livia. This hypothetical assumption may be put forward, when bearing in mind previous findings in other birds, for example, the parakeet (Prittavula krameri) and the bee-eater (Merops onentalis). The M. pectoralis major consists of only the narrow variety of fibres, the broad ones being absent altogether. There are other species, for example, the domestic fowl (Gallus domesticus) and the kite

(Milvus migrans) in which there is the broad variety of muscle fibers. **Thus** further studies should be engaged with flight energy and estimate flight **ranges** of the two allied forms of C. livia.

TABLE 1. **Biophysical calculations of the two allied forms of columba livia**

	Wild	Domestic
Wing loading	0.06 kg/meter²	0.08 Kg/meter²
Aspect ratio	0.07	0.09
Power — wight ratio	10.7%	20%
Gliding angle	2.8 Kg/meter	4.17 Kg/meter
Power loading	7.4 Kg/Hp	13.2 Kg/HP

REFERENCES

Barnard, R.J., Edgerton, V.R.; Furukawa, T. and Paser, J.B. (1971). Histochemical, biochemical and contractile properties of red, white and intermediate fibres. Am. J. Physiol. 220 : 410-414.

Beatty, C.H., Basinger, G.M., Dully, C.C. and Bocek, R.M. (1966). Comparison of red and white voluntary skeletal muscles of several species of primates. J. Histochem. Cytochem. 14 : 590.

Beckett, E.B., (1962). Some applications of histochemistry to the study of skeletal muscle Rev. Canad. Biol. 21 : 391.

Chiney, N.J. (1963). Histochemical localisation of myoglobin in the pigeon breast muscle J. Anim. Norphol. Physiol. 10 : 74-76.

Chiney, N.J. (1970). Histochemical localisation in animal tissues of a special peroxidase which generates the free radical of ascorbic acid. Stain technology 45 : 99-103.

Dubowitz, V. and Pearse, A.G.E. (1960). Reciprocal relationship of phosphorylase and oxidative enzymes in skeletal muscle. 180, 701-702.

Dubowitz, V. and Pearse, A.G.E. (1960). A comparative histochemical study of oxidative enzyme and phosphorylase activity in skeletal muscle. Histochemie. 2 : 105.

Dubowitz, V. and Pearse, A.G.E., (1961). Enzymatic activity of normal and dystrophic human muscle ; A histochemical study. J. Path. Bact. 81.

Dubowitz, V. 1963. Enzymatic maturation of skeletal muscle. Nature London. 197 : 1215.

Dubowitz, V. 1966. Enzyme histochemistry of developing human muscle. Nature, London 211 : 884.

Edgerton, V.R. 1968. Histochemical changes in rat skeletal musc e after exercise. Ph. D. Thesis East Lansing, Mich. Michigan State Univ.

Edgerton, V· R., Gerchman, L. and Carrow, R.E. 1969. Histochemical changes in rat skeletal muscle after exercise. Exp. Neurol. 24 : 110.

Edgerton, V.R., and Simpson, B.R. 1969. The intermediate fibre of rats and guinea pigs. J. Histochem. Cytochem. 17 : 828-838.

Engel, W.K. 1962. The essentiality of histo-and cytochemical studies of skeletal muscle in the investigation of neuromuscular disease. Neurology. 12 : 778.

Gauthier, F. G. and Padykula, H., 1966. Cytological studies of fibre types in skeletal muscle J. Cell. Biol. 28 : 333.

George, J.C. and Naik, R. M., 1958b. Relative distribution of the mitochondria int the two types of fibres in the pectoralis major muscle of the pigeon. Nature (Longon),, 181, 782-783.

George, J.C. and Naik, R.M., 1958c. The relative distribution and the chemical nature of the full store of the two types of fibres in the pectoralis major muscle of the pigeon. Nature (London), 181, 709-710.

George, J.C. and Naik, R.M., 1959. Studies on the structure and physiology of the flight mnscles of birds. 4 Observations on the fiber architecture of the pigeon. Biol. Bull., 116, 239-247.

George, J.C. and Scaira, K.S., 1958a. Histochemical demonstration of lipase activity in the pectoralis major muscle of the pigeon. Nature (London), 181, 783.

George, J.C. and Scaria, K.S., 1958b. A histochemical study of the dehydrogenase activity in the pectoralis major muscle of the pigeon and certain other vertebrate skeletal muscles Quart. J. Micro. Sci, 99, 469-473.

George, J.C. and Talesara, C.L., 1960. Studies on the structure and physiology of the flight muscles of birds. 9. A quantitative study of the distribution pattern of succinic dehydrogenase in the pectoralis major muscle of the pigeon. Biol. Bull, 118, 262-268.

George, J.C., and Talesara, C.L., 1961. Histochemical observations on the succinic dehydrogenase and cytochrome oxidase activity in pigeon breast muscle. Quant. J. Micro. Sci., 102, 131-141.

George, J.C., and Talesara, C.L., 1961. A quantitative study of the distribution pattern of certain oxidizing enzymes and a lipase in the red and white fibres of the pigeon breast muscle, J. Coll. Comp. Physiol. 58 : 253.

George, J.C., and Berger, A.J., 1966. Avian Myology London. Academic Press.

Germino, N.I., D'Albora, H. and Washrmann, J.P., 1965. Succinic dehydrogenase in the development of the skeletal muscles of chicks . Acta Anat. 62, 434-444.

Guth, L. and Samaha, F.J. 1969.Qualitative differences betwcen actomyesin ATPase of slow and fast mammalian mo!cle. Exptl. Neurol. 25 : 138-152.

Hall-Craggs, E.G.B. 1968. The contraction times and enzyme activity of two rabbit laryngeal muscles. J. Anat. 102 : 241.

Henneman, E. and Olson, C.B.,1965.Relations between structure and function in the design of skeletal muscle. J. Neurophysiol. 28 : 581-585.

Jinnai, D. 1960.Functional differentiation of skeletal muscles.Acta Med. Okayama. 14: 159.

Lasiewski, R.C., Caley F.R., and Vasquez, C. 1965. Morphology and physiology of the pectoral muscles of humming-birds. Nature, London 206 : 404.

Nachlas, M.N., Tsou, C., De Souza, E., Cheng, C.S. and Seligman, A.M., 1957. Cytochemicol demonstration of succinic dehydrogenase by the useof a new p-nitrophenil substituted ditetrazol. J. Histoche. Cytochem. 5, 420-436.

Nene, R.V., and George, J.C., 1965. Histophysiological study of some muscles of the avian pectoral appendage. Pavo. 3 : 35-46.

Nishiyama, A. 1965.Histochemical studies on the red, white and intermediate muscle fibres of some skeletal muscles. I. Succinic Dehydrogenase activity and physiological function of intercostal muscle fibres. Acta· Med ckayama 19 : 177.

Ogata, T., 1958. A histochemical study of red and white muscle fibres. Part I. Activity of the succinoxidase system in muscle fibres. Acta Med. Okayama. 12 : 216-227.

Ogata, T. and Mori, M. 1964. Cited by Ommer, P.A. 1971 Histochemical differentiation of skeletal muscle fibres in the Bovine fetus. Experienta. 27 : 173.

Pearse, A.G.E., 1968. Histochemistry Theoretical and Applied. J. and A. Churchill Ltd. London.

Revel, J.P. 1962. The sarcoplasmic reticulum of the bat cricothyroid muscle. J. Cell. Biol. 12 : 571.

Rommanul, F.C.A. 1964. Histochemical studies of enzymes in individual muscle fibres. Arch. Neurol. 11 : 355.

Stein, J.M., and Padykula, H. A., 1962. Histochemical classification of individual skeletal muscle fibres of the rat. Am. J. Anat. 110 : 103.

Author's adress. **Dr. Y.M. Shahien.** Depart. of histology Fac. of ved. Med. Cairo University.

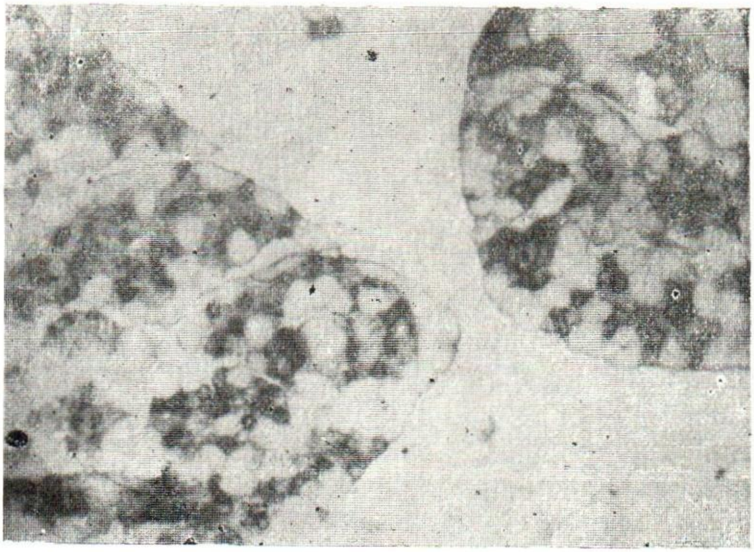

Fig. 1. Transverse secttion of M. pectoralis major stained for SDH activity showing junction of several fasiculi. Note, red white and intermediate fibres. White fibres predominate in preiphery of fasiculi, X 60

Fig. 2. Section of M. pectoralis major stained for SDH. Characteristic distribution of centrally situated red muscle fibres. X 950