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HISTOCHEMICAL CHARACTERIZATION OF THE PHYSIOLOGICAL HETEROGENEITY OF FIBRE TYPES IN THE RHESUS EXTRAOCULAR MUSCLE

C. L. TALESARA AND P. KUMAR

Muscle Physiology and Histochemistry Unit, Department of Zoology, University of Delhi, Dilhi-7

ABSTRACT

To study the fibre-architecture, and allied energy metabolism, the rhesus extraocular muscle was put to a battery of histochemical and biochemical tests. In the present preliminary report, based on the histochemical measure of the *in situ* activity of succinic dehydrogenase and the lipid content, the muscle was found to possess at least six types of fibres varying perceptibly in their histophysiological characteristics. The histochemical profile of the extraocular muscle suggests that it follows a predominantly aerobic type of cellular metabolism, and varies considerably from the relatively simple architecture of the usual skeletal muscles —revealing a unique histophysiological specialization.

Key words: Extraocular muscle, fibre-types, succinic dehydrogenase, slow and fast fibres, oxidative metabolism, light and dark fibres.

INTRODUCTION

THOUGH most mammalian skeletal muscles follow a closely identical histophysiological pattern, being mixtures in varying proportions of bigger white (twitch/fast) and smaller red (tonic/slow) fibres, the validity of this classical description does not hold good in the case of some specialized muscles, *viz.*, the extraocular muscles[1-2]. In erstwhile studies the mammalian extraocular muscles have been characterized as having slow and fast fibres, largely on the basis of cholinesterase staining[3-4], specific innervation[5-6] certain physiological attributes[7-11], and ultrastructure[3,12-17]. Most of these reports provide a rather generalized picture of an otherwise uniquely specialized muscle. In the present study we report, demonstrating histochemically, the actual plan of fibre-pattern—which in itself is unique and probably of significant metabolic implication—in the extraocular muscle of the rhesus monkey (*Macaca mulatta*).

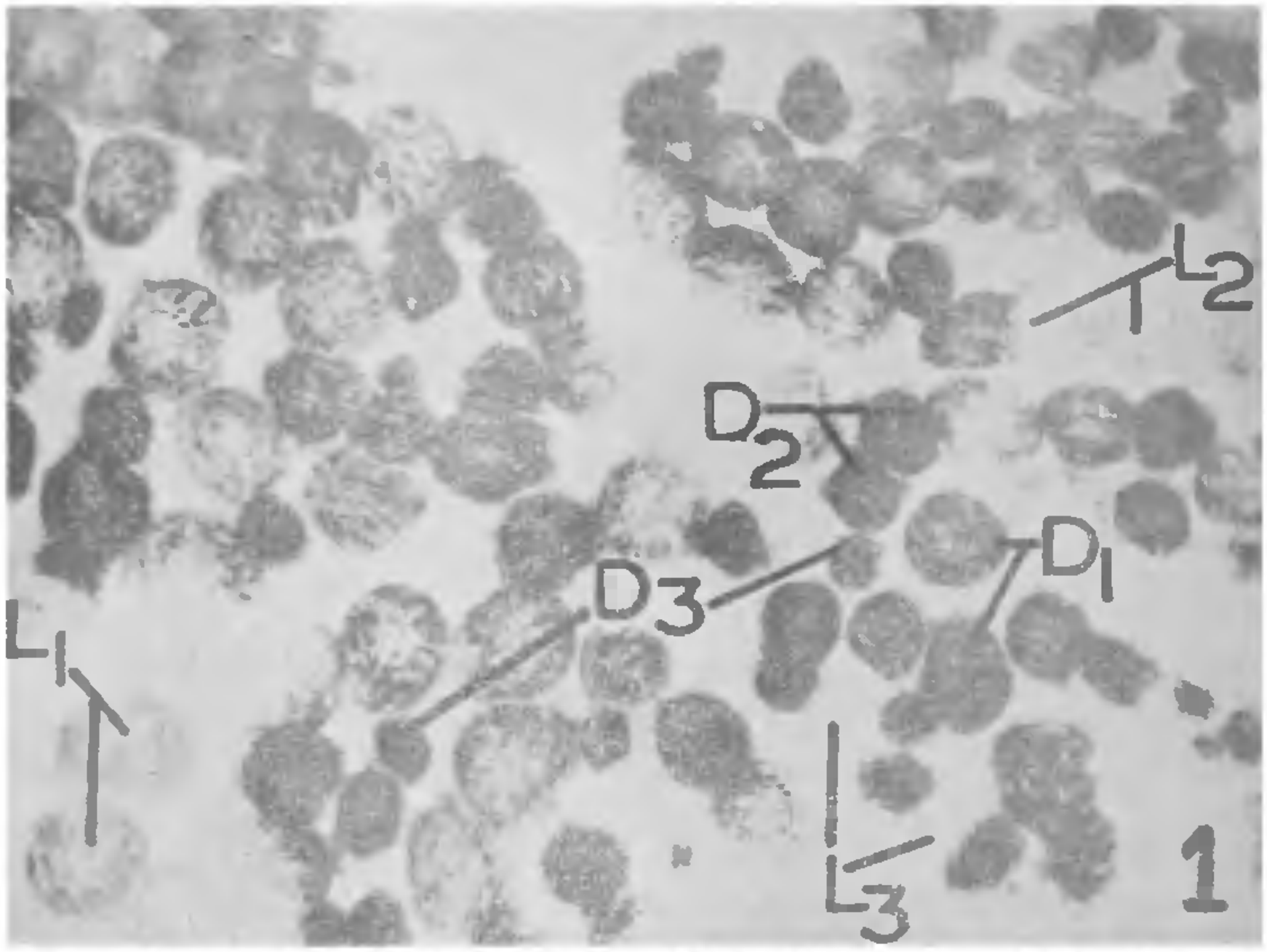
MATERIALS AND METHODS

The muscle pieces excised during autopsy were sectioned fresh-frozen at 10 microns in a cryostat, and proceeded for the routine staining reaction for succinic dehydrogenase (SDH) activity. The reaction medium[18] contained nitro-blue tetrazolium as an electron acceptor. Fresh-frozen sections of the muscle were also utilized for the histochemical localization of sudanophilic lipids with a view to verify and evaluate the picture obtained for SDH activity, since both the lipid content and SDH activity are indicative of the capacity for oxidative metabolism of the muscle fibres. Lipids were stained by the usual Sudan Black B solution, made to saturation in 70% alcohol. Subsequently, the sections were rinsed briefly in cold 70% alcohol to remove excess stain and then fixed in cold calcium-formol. To ascertain the structural uniformity of the various extraocular muscles of each eye, sections were cut from a single composite block of all the extraocular muscles.

RESULTS

Microscopic examination of the sections treated for the localization of SDH activity (Fig. 1) and lipids (Fig. 2) revealed an almost identical differential pattern of staining of the various component fibres of the extraocular muscle. A distinct segregation of the fibres into two major types, *viz.*, dark (D) and light (L) was found, corresponding to two different fibre populations. These two main types were further shown to be composed of big, medium, and small fibres differing in their overall histochemical profile. Thus, in all six sub-types of the fibres were found to be histochemically distinguishable. The three types of dark fibres (Fig. 1—D_1, D_2, D_3) showed a marked lack of sarcoplasm and apparently mitochondrial localization of SDH activity, as revealed by heavy formazan (reduced tetrazolium salt) deposition. No significant SDH activity could be recorded in the light type of fibres (Fig. 1—L_1, L_2, L_3). The muscle as a whole had an evident predominance of dark fibres (granulated, mitochondria-laden) of all the three subtypes (Table I). Most of the smaller light fibres (Figs. 1 and 2, L_3) had an essentially clear sarcoplasm and exhibited no apparent SDH activity or lipid content. The six fibre-types of the extraocular muscle followed discernible local variations in their overall distribution. Some areas of the muscle, particularly the peripheral locations, had a somewhat superior representation of the smaller fibres having low SDH activity and lipid content. The differences in the diameter of the fibres were obvious (Table I); the three types of light fibres were bigger in dimension than their respective counterparts of the dark variety. The localization of lipids, unlike that of SDH activity, was both

mitochondrial and extramitochondrial. The intensity of staining for lipids and SDH activity, which imparted a darker appearance to the heavily granulated fibres, was comparable for each fibre (Table I).



FIGS. 1–2. Fig. 1. Differential activity of succinic dehydrogenase in the various component fibres of the rhesus extraocular muscle. Light fibres : big (L_1), medium (L_2), small (L_3), Dark fibres : big (D_1), medium (D_2), small (D_3), × 200. Fig. 2. Same as Fig. 1, histochemical demonstration of lipids, × 200.

TABLE I

Characterization of the fibre-types of the rhesus extraocular muscle on the basis of histochemical attributes and fibre counts

	Light fibres (L)			Dark fibres (D)		
	Big (L_1)	Medium (L_2)	Small (L_3)	Big (D_1)	Medium (D_2)	Small (D_3)
SDH activity* ..	++	+	±	+++++	++++	++++
Lipid content ..	+++	++	+	+++++	+++++	+++++
% population/unit area ..	12	15	13	23	22	15
Total % population/unit area (L : D)			40 : 60			
Fibre diameter (μ) ..	62	43	32	48	38	23

* +++++, very strong; ++++, strong; +++, appreciable; ++, slight; +, insignificant; ±, doubtful.

DISCUSSION

The morphological definition of various fibres of extraocular muscle in terms of their structural and physiological characteristics has varied in previous investigations. There is complete agreement, however, that the dark (slow) fibres—in contrast to fast twitch fibres—have multiple motor nerve ending, and both twitch and tonic fibres are of constant occurrence in the mammalian extraocular muscles. A casual mention[16] of the possible presence of some other fibre-types which may differ in fine structure from typical slow fibres has been made on the basis of the type of nerve ending. The present report makes obvious that there are indeed several other types of fibres besides the typical fast and slow ones in the rhesus extraocular muscle. Though these fibres may have closely approaching physiological and mechanical attributes, they do appear to possess quite distinct histochemical individuality—an expression of their varying inherent metabolism. Coupling the present report with our other qualitative and quantitative histochemical investigations[19] we are in a position to conclude that the rhesus extraocular muscle is uniquely different in its fibre-architecture and allied energy metabolism from most other skeletal muscles of the body. It follows a largely oxidative pattern of cellular metabolism, as indicated by a distinct predominance of mitochondria-rich and lipid-abundant fibres having high SDH activity. The dependence of extraocular muscle on a largely oxidative type of metabolism, utilizing fats as the chief substrate, indicates the slow and sustained character of its mechanical activity. The presence of a few fast contracting white fibres suggests the capacity of the extraocular muscle for differential phasic contraction, besides the usual tonic one. The histochemical heterogeneity of the extraocular muscle fibres reflects their metabolic differences in relation to their varying physiological grade of contractility. The histochemical characterization of this muscle also reflects the extensive heterogeneity of the skeletal muscle in response to local physiological demand, manifested equally in both structure and function.

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