DIFFERENTIAL ACTIVITY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN RHESUS SKELETAL MUSCLE : ENZYME-HISTOCHEMICAL INVESTIGATION OF THE OPERATION OF A SECONDARY GLYCOLYTIC PATHWAY IN SLOW MUSCLE FIBRES

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ABSTRACT

The rhesus extraocular muscle and diaphragm were tested histochemically for the presence and localization of glucose-6-phosphate dehydrogenase (G-6-PD) activity. The histochemical profile of the *in situ* activity of the enzyme varied considerably in relation to the muscle fibre types. Qualitative variations in G-6-PD activity were reflected by a differential staining of the muscle fibres. Localization of G-6-PD activity mainly at mitochondrial locations in the slow fibres, and its apparent absence in the fast fibres, suggests possible operation of pentose cycle therein, attributable probably to the constant functioning of these specially active muscles. The possible contribution of G-6-PD activity to the overall muscle metabolism is discussed.

INTRODUCTION

THE finding[1] that the activities of G-6-PD and 6-phosphogluconate dehydrogenase in skeletal muscle are very low has led to the general belief that skeletal muscle uses, almost exclusively, the Embden-Meyerhof glycolytic pathway for glucose metabolism, and the contribution of pentose cycle to this purpose is insignificantly low. Pentose cycle operation, involving G-6-PD activity, has been recorded in muscle under anoxia[2,3], foetal muscle[4], and normal and atrophic/denervated skeletal muscle[3,6]. Though the activity of G-6-PD, a triphosphopyridine nucleotide(TPN)-linked enzyme, has often been investigated *in vitro*—using radioactive labelled compounds and quantitative assay methods—hardly any attempt seems to have been made to demonstrate *in situ* G-6-PD activity in skeletal muscles having a heterogeneous fibre-architecture, like the extraocular muscles and the diaphragm. A preliminary report[7] from this laboratory described the successful histochemical demonstration of G-6-PD activity in avian skeletal muscle fibres, using the reactions of the cellular electron transfer system. We have not yet come across any other report describing the histochemical characterization of G-6-PD activity in skeletal muscle, especially that of mammals.

The present report, thus, embodies the results of the histochemical investigation for the demonstration of G-6-PD activity in the various component fibres of the diaphragm and extraocular muscle of the rhesus monkey (*Macaca mulatta*). An attempt has been made to elaborate the precise metabolic topography of G–6–PD activity in these muscles, in relation to its involvement in the muscle metabolism.

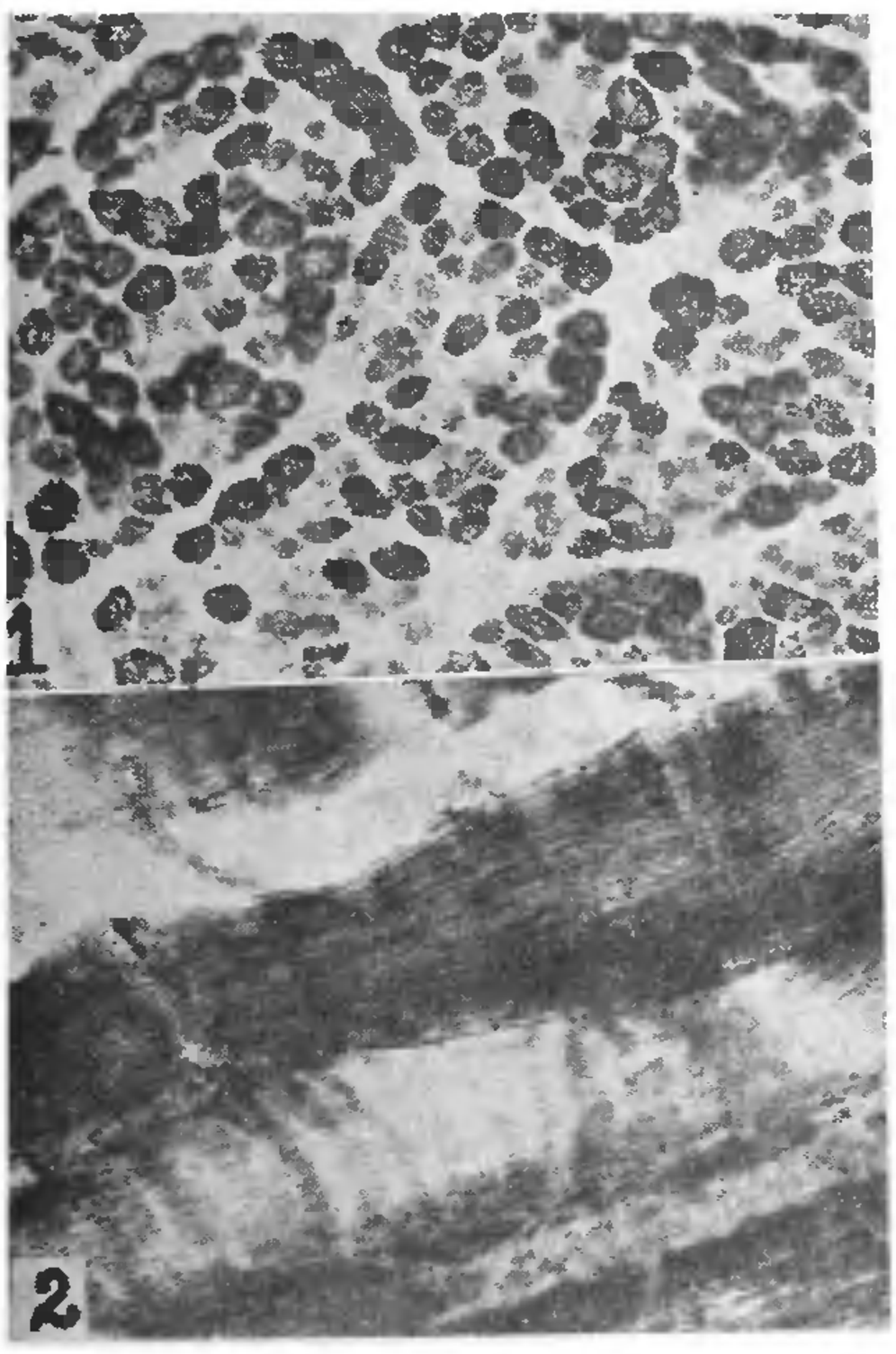
MATERIALS AND METHODS

Fresh muscle pieces obtained from autopsy were frozen quickly at — 20° C in the cryostat using the quick-freeze timer, and 10 micron sections were cut. After initial treatment[7] the sections were incubated in a medium[8] containing nitro-blue tetrazolium for half an hour. The control sections were incubated in a medium lacking either TPN or the substrate, and were processed simultaneously. After brief rinsing in distilled water the sections were post-fixed in 10% neutral buffered formalin and mounted in glycerol jelly.

RESULTS

Microscopic examination of the incubated sections revealed a distinct differential staining of the muscle fibres (Figs. 1, 2). A positive result, in the form of heavy deposition of formazan (reduced tetrazolium salt), was obtained in the slow fibres. Such fibres appeared dark, granular, and mitochodria-laden. The fast fibres, that appeared light in contrast, exhibited no significant formazan deposition. The pattern of staining for G–6–PD activity was uniform throughout ; formazan deposition being superior in slow fibres, which seemed to predominate the total fibre population in both diaphragm and extraocular muscle. It was of interest to observe that whereas the slow fibres were, as usual, smaller than the fast ones in the diaphragm, majority of these were bigger than the fast variety in the extraocular muscle (Fig. 1). The G-6-PD activity appeared to be confined to granular loca-

tions, probably mitochondrial, in the slow fibres (Figs. 1, 2)—the fast fibres (lacking in mitochondrial substance), thus, did not show a positive histochemical reaction for the enzyme activity. Control sections showed a very faint and undifferentiated colouration—corresponding probably to unspecific and non-enzymic reduction of the tetrazolium salt.



FIGS. 1–2. Fig. 1. Histochemical demonstration of glucose-6-phosphate dehydrogenase in rhesus extraocular muscle. Only the slow fibres (darkly stained) show a positive reaction. The fast fibres (light, unstained, and small) appear to lack demonstrable enzyme activity. × 125. Fig 2. Same as in Fig. 1, in rhesus diaphragm, a longitudinal section, × 500.

TABLE I

Histochemical characterization of glucose-6-phosphate dehydrogenase activity in the rhesus skeletal muscle fibres

Muscle	Enzyme activity*	
	Slow fibres	Fast fibres
Extraocular muscle	+++	±
Diaphragm	+++	+

* ++++, strong; +, traces; ±, not detectable.

DISCUSSION

The significance of the demonstration of G-6-PD activity in muscle tissue has partly been emphasised in an earlier report[7]. Pigeon *Pectoralis major*, as also that of green parakeet (both predominated by slow, mitochondria-abundant fibres), was shown to possess histochemically detectable G-6-PD activity. About the same time Green and Landau[9] demonstrated positive G–6–PD activity, and pentose cycle operation, in red striated muscle (slow)—the myocardium of foetal and adult mice. Our present report of apparently mitochondrial localization of G–6–PD activity in the slow muscle fibres is in agreement with the histochemical study of this enzyme in other tissues, but is at variance with the information derived from homogenization studies[8], where G–6–PD activity appears largely in the soluble or non-particulate centrifugal fractions[10]. Coupling our present finding with the fact that G–6–PD activity increases with increasing lipid content[10], and that pentose cycle provides reduced TPN for fatty acids' synthesis[10], we are in a position to correlate the possible involvement of G–6–PD activity in lipid synthesis in slow fibres with the high level of available lipids usually observed in such fibres[11].

It has been known that the slow fibres, unlike the fast ones, are relatively poor in glycolytic enzymes—thus, having very low metabolic capacity for glucose utilization for energy production. Under such 'metabolic lag' the alternative significance of G–6–PD activity in partly enhancing the feeble rate of glucose utilization in the slow fibres can possibly be appreciated. Besides this, the demonstration of preferential G–6–PD activity in slow fibres also seems to suggest their capacity for intracellular synthesis of fatty substance, the fuel for their sustained contraction. Though the present histochemical demonstration of G–6–PD activity does not help make a quantitative assessment of the overall contribution of pentose cycle to the glucose metabolism in skeletal muscle, it does, in fact, substantiate the existence of pentose cycle activity in the diaphragm and extraocular muscle of the rhesus monkey. Such a possibility of the operation of pentose cycle in constantly active muscle has been suggested earlier[3].

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ON THE GENUS *ZORNIA* GMEL. (FABACEAE) IN INDIA AND CEYLON

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THERE has been much confusion in the identity and nomenclature of species of the genus *Zornia* Gmel. in India. Till recently the genus was believed to be represented in the country by one species, namely, *Z. diphylla* Pers. (Baker)[1]. Later work has, on the one hand, shown that, the plant occurring widely all over the country is not *Z. diphylla* Pers. but an entirely different species, and on the other, that the genus is represented in India by at least three species; a fourth species occurs in the adjacent region of Ceylon. The existing regional floras treat the material under one species and the characters distinguishing these four species in India and Ceylon are not properly understood.

The purpose of the present note is to give a brief account of this taxonomic riddle and to give a dichotomous key of these four taxa.

Baker[1] mentioned only one species of the genus *Zornia* Gmel. from the Indian subcontinent, namely *Z. diphylla* Pers. He mentioned two varieties, namely :

(*i*) var. *zeylonensis* Baker (in western parts of peninsular India and Ceylon) ;

(*ii*) var. *walkeri* (Arn.) Baker (in Ceylon).

Mohlenbrock[2] studied the genus in detail and reported two species from India. He stated that the plant found in larger part of India is *Zornia gibbosa* Span.

Mohlenbrock[2] further stated that the true *Z. diphylla* (L.) Pers. is based on *Hedysarum diphylla* L., and that this species is confined only to southern parts of peninsular India and Ceylon. He mentioned *Z. diphylla* var. *zeylonensis* as its synonym. A specimen of this variety quoted by Mohlenbrock (*Thwaites* 3600) has been examined, and it perfectly matches with *Z. diphylla* (L.) Pers. Thwaites[3] mentioned this taxon under the name *Z. conjugata* Sm. This was followed also by Santapau[4] in his *Flora of Khandala*. This plant was treated by Gamble[5] under the name *Z. zeylonensis* Pers.

Wagh[6] wrote a note on the genus *Zornia* in India, wherein he gave the distinguishing characters of the two species found in India and also their distribution.

Recently Ravi[7] has described another species of the genus, namely, *Zornia quilonensis* from Kerala. It differs from *Z. diphylla* in its articles being reticulate and having scabrous prickles, and bracts and leaflets being punctate.

The variety treated by Baker[1] as *Zornia diphylla* var. *walkeri* is treated by Mohlenbrock[2] as *Zornia walkeri*. One collection quoted by Baker, namely, *Thwaites* 3599, is also cited by Mohlenbrock under *Z. walkeri*, but strangely Mohlenbrock did not mention *Z. diphylla* var. *walkeri* in the synonymy of *Z. walkeri*. The sheet *Thwaites* 3599 is available in Calcutta Herbarium and has been examined. It