DEHYDROGENASE ACTIVITY AND ITS DIURNAL VARIATIONS IN DIFFERENT MUSCLES OF THE SCORPION, HETEROMETRUS FULVIPES

BY POKALA VENKATESWARA RAO AND SEPUR GOVINDAPPA

(Department of Zoology, Sri Venkateswara University, Tirupati)

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ABSTRACT

Histologically different types of muscle fibres have been observed. The dehydrogenase activity in the muscles of pedipalp, leg and heart has been investigated. The heart and pedipalpal muscle have higher and lower dehydrogenase activity respectively than the leg muscle. The pattern of carbohydrate breakdown seems to be different in the leg muscle due to the interference of lipid metabolism, perhaps because of the differences in the muscle fibre composition of the muscle. As the scorpion happens to be the nocturnal animal, there are conspicuous differences in the dehydrogenase activity during day and night times. During night time there seems to be stress on the anaerobic phase of metabolism. In general, increase in dehydrogenase activity during night has been correlated with increased activity of the organism. Hence this shows the existence of diurnal variations in the dehydrogenase activity.

INTRODUCTION

Information about carbohydrate metabolism in the invertebrates is less extensive in comparison with that in vertebrates. A sizable amount of this evidence comes from arthropods, in particular the insects (Watanabe and Williams, 1951; Weis-Fogh, 1952; Zebe, 1954; Zebe and Mc Shan, 1957; George and Bhaktan, 1963). The only report available on the dehydrogenase activity in arachnids deals with hepatopancreas (Vijayalakshmi, 1964).

The leg muscles have been reported to differ in their dehydrogenase activity from the flight muscles in insects (Zebe and Mc Shan, 1954; George and Bhaktan, 1963). Moreover it has been suggested that the differences in muscle fibre composition may underlie these differences (Bhaktan and...
George, 1963). The degree of activity has been found to vary for the same tissue under different physiological conditions. Differences in dehydrogenase activity have been reported between active and blocked cells (Bodine et al., 1954), between tissues of normal and castrated rats (Ekstein and Abraham, 1959), between tissues of active and aestivating snails (Ekstein et al., 1958), between tissues of different sexes (Mc Shan et al., 1954) and between tissues of animals of different ages (Mc Shan et al., 1954). Effect of starvation (Allen, 1962) and temperature (Fukudar and Kurogi, 1958) have also been studied. Changes due to acclimation and acclimatization are reported (Vijayalakshmi, 1964).

The present investigation attempts at investigating the possible differences and diurnal variations in dehydrogenase activity of different muscles in the scorpion.

**MATERIAL AND METHODS**

Scorpions of the species, *Heterometrus fulvipes* have been used. The histology of the muscle fibres has been studied through sectioning and staining. Mallory's triple connective tissue stain has been used.

For enzyme assay spectrophotometric ferricyanide reduction method (Colowick and Kaplan, 1955) has been adopted. The animals were always dissected alive either at 8 a.m. or at 8 p.m. at 18° C. in a cold room. Female scorpions in the size range of 8 to 10 cm. alone were used. Muscles were collected from the pedipalpal chela and the patella of the leg before the heart was collected. The tissues were chilled to 0° C. and homogenized in 0.25 M sucrose solution. The homogenates were centrifuged at 2500 rpm. at 18° C. and the supernatants were used.

Optimum pH for the dehydrogenase activity was determined.

**RESULTS**

The results of the present investigation can be detailed as follows:

*Morphological and histological features of the muscles*

The muscle collected from the pedipalp is the flexor of the movable finger. It is translucent white in colour. All the muscle fibres are of one and the same type. The cortex of each muscle fibre as seen in cross-section (*Pl. XIII* Fig. 1) consists of radiating lamellar fibrils or sarcostyles and the central medulla of ordinary non-fibrillar sarcoplams. They are all striped.
Dehydrogenase Activity in Different Muscles of *H. fulvipes*

The muscle collected from the leg is the extensor tibia. It comprises of two histologically different types of muscle fibres (Pl. XIII, Fig. 2). Nearly two-thirds of the muscle fibres are of the same type as those of pedipalpal muscle. These occupy more or less the interior forming the core of the muscle. More peripherally located are the second type of the muscle fibres with an outstanding cortex with extensively developed radiating sarcostyles almost obliterating the medulla which contains the nuclei. All the fibres are striped. The muscle is light muddy brown in colour and very compact.

The heart is a long tubular elastic structure. The muscle fibres are annular and striated (Pl. XIII, Fig. 3).

**Enzyme Activity**

At pH 6.0 the dehydrogenase activity was found to be maximum and hence this was taken as the optimum.

Dehydrogenase activities are maximum in the heart and least in the pedipalpal muscle. The same trend in the enzyme activities has been observed both at 8 a.m. as well as at 8 p.m. (Table I).

**Table I**

*Dehydrogenase activities in the muscles of scorpion during day and night times—expressed in μg. ferrocyanide/mg./min.*

(Mean ± standard deviation)

<table>
<thead>
<tr>
<th>Time</th>
<th>Pedipalpal muscle</th>
<th>Leg muscle</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SDH</td>
<td>MDH</td>
<td>LDH</td>
</tr>
<tr>
<td>8 a.m.</td>
<td>60·03</td>
<td>180·65</td>
<td>160·72</td>
</tr>
<tr>
<td></td>
<td>±13·58</td>
<td>±50·47</td>
<td>±24·32</td>
</tr>
<tr>
<td>8 p.m.</td>
<td>364·31</td>
<td>81·85</td>
<td>319·30</td>
</tr>
<tr>
<td></td>
<td>±4·23</td>
<td>±5·20</td>
<td>±75·69</td>
</tr>
</tbody>
</table>

In all the muscles studied there is a general increase in the activity of succinate and lactate dehydrogenases (SDH and LDH) at 8 p.m. over that of 8 a.m. (Table II). In both pedipalpal muscle and heart there is a general drop in the malate dehydrogenase (MDH) activity at 8 p.m., whereas in the leg muscle alone there is a rise (Table II).
TABLE II

Percentage rise or drop in dehydrogenase activity at 8 p.m. over that at 8 a.m.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Muscle</th>
<th>SDH</th>
<th>MDH</th>
<th>LDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pedipalpal muscle</td>
<td>+506.60</td>
<td>-101.70</td>
<td>+98.65</td>
</tr>
<tr>
<td>2.</td>
<td>Leg muscle</td>
<td>+71.12</td>
<td>+111.50</td>
<td>+132.00</td>
</tr>
<tr>
<td>3.</td>
<td>Heart</td>
<td>+77.98</td>
<td>-11.12</td>
<td>+133.40</td>
</tr>
</tbody>
</table>

Note: + and − signs indicate rise and drop respectively.

DISCUSSION

The dehydrogenase activity in the scorpion muscle is found to be as high as that in the insect muscle and is therefore higher than in the vertebrate muscle. The situation is similar to that in the insect muscles where the dehydrogenase activity is 5 times that of frog (Roeider, 1961), considerably higher than that in the vertebrate muscle (Watanabe et al., 1951) and twice the highest value obtained for the bird muscle (George and Talesera, 1961).

It is clearly noticed that the dehydrogenase activity is relatively low in the pedipalpal muscle (Table I). This pedipalpal muscle is metabolically least active and the heart is the most active. The leg muscle is nearly as active as the heart. This is to be expected as the heart is persistently active, day-in and day-out. The leg muscle is consistently active, all the time the animal indulges in locomotor activity, as it operates the distal joints of the double knee of the leg. On the other hand the pedipalpal muscle operating the movable finger of the chela indulges in activity rather inconsistently only at times when the animal uses its chela, for prehensile purposes. The higher dehydrogenase activity in the leg muscle and the heart is consistent with the established fact that there exists a relationship between activity and concentration of oxidative enzymes (Proser and Brown, 1961; Govindappa and Swami, 1965). It has been shown even among insects that the wing muscles indulging in long sustained activity have higher dehydrogenase activity than the leg muscle (Zebe and Mc Shan, 1957; George and Bhaktan, 1963; Bhaktan and George, 1963).

Within the given 8 a.m. muscle, the differences in the activities of the three dehydrogenases are slight (Table I) and not so very significant when compared to the activities of 8 p.m. muscles. This observation clearly
indicates that both aerobic and anaerobic phases of metabolism prevail equally during daytime. All the muscles seem to breakdown the carbohydrate substrate anaerobically as efficiently as they can do aerobically.

In 8 p.m. muscles there is a general increase in succinate (SDH) and lactate dehydrogenase (LDH) activities in all the muscles over the 8 a.m. muscles (Table II). This rise in activity of the dehydrogenases perfectly fits in with the reported rise in locomotor activity and oxygen consumption in these animals at 8 p.m. (Gopalakrishnareddy, 1966). This also finds a correlation with the rise in spontaneous nervous activity of the nerve cord at this hour (Pampapathi Rao, 1963). As the locomotor and neural activities are energy dependent, the energy requirement might have been met by the animal during night times from both increased aerobic and anaerobic phases of the metabolism, and this is evidenced by the increased succinate (SDH) and lactate dehydrogenase (LDH) activities respectively (Table II). The relative increase in lactate dehydrogenase (LDH) activity between daytime and night time in the leg muscle (130%) and heart (133·4%) is greater than the relative increase in succinate dehydrogenase (SDH) activity (71·12% and 77·99% respectively). This observation clearly indicates that the anaerobic metabolism has been stepped up more than the aerobic metabolism in these muscles. The situation in pedipalpal muscle is different, in that, there is a greater rise in aerobic metabolism as evidenced by succinate dehydrogenase (SDH) activity (506·6%) during night time than the anaerobic phase (98·65%). From this evidence it appears that the leg muscle differs from the pedipalpal muscle and leans more towards cardiac muscle. Although there is general rise of lactate dehydrogenase (LDH) activity in both leg muscle and heart, the causal factors behind this rise seems to be different. In the heart there is a marked drop in malate dehydrogenase (MDH) activity by 11·2% during night time (Table II), suggesting that the aerobic phase of metabolism has been stepped up only up to the production of malic acid and thereafter it has taken a different pathway. The same situation is prevailing in the case of pedipalpal muscle, where there is a drop in malate dehydrogenase (MDH) activity by 101·7% during night time (Table II). This observation suggests the possibility of diversion of aerobic metabolism at the level of malic acid and leading on to the production of pyruvic acid, possibly by the catalytic activity of malic enzyme (Fruton and Simmonds, 1960). Probably this pyruvic acid might have been responsible for the increased lactate dehydrogenase (LDH) activity of both pedipalpal muscle and heart.

The pattern of metabolism in the case of leg muscle is evidently different in that there is an increase even in malate dehydrogenase (MDH) activity
during night time (Tables I and II). In this case there is simultaneous increase both in aerobic and anaerobic phases of metabolism. This fact clearly suggests that these two phases of metabolism are independent in this muscle. This might be possible in this muscle because of the probable participation of two different sets of muscle fibres with different phases of metabolism. From the histological studies it has been shown that this muscle consists of two different muscle fibres (Pl XIII, Fig. 2). From the present histological and biochemical evidences, it can be said that one of the two types of muscle fibres may be involved in glycogen breakdown, while the other may be concerned with the lipid breakdown. It is suggested that the lipid breakdown in one set of muscle fibres might account for the increased succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) activities, while the glycogen breakdown in the other set of muscle fibres may account for increased lactate dehydrogenase (LDH) activity in the leg muscle of the scorpion.

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**EXPLANATION OF PLATE XIII**

**Fig. 1.** Pedipalpal muscle in cross-section (1 × 160).

**Fig. 2.** Leg muscle in cross-section showing the distribution of two types of muscle fibres, (1 × 100), stained in Mallory's triple connective tissue stain.

**Fig. 3.** Heart in longitudinal section showing the striped muscle fibres (1 × 400).