GLUTAMIC, OXALOACETIC TRANSAMINASE (GOT) ACTIVITY IN SKELETAL MUSCLES OF SOME FRESHWATER FISHES

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ABSTRACT

Quantitative estimation of Glutamic, Oxaloacetic Transaminase (GOT) activity in the skeletal muscles of two freshwater fishes, Barbus tor, Day (Physostomi-Cyprinidae) and Heteropneustes fossilis, Bloch. (Physostomi-Siluridae) has been done, colourimetrically. It is observed that the GOT enzyme activity is closely associated with the red fibres and is directly proportional to the concentration of red fibres in the muscles.

In B. tor, the activity is more in the hypaxial area than in the epaxial and in H. fossilis, it is more in the epaxial area than in the hypaxial area. The activity is maximum in the lateral line area of the skeletal muscles of both fishes, wherein the concentration of red fibres is maximum.

INTRODUCTION

When a labelled amino-acid is administered to an animal a number of amino-acids of liver proteins are found to contain the labelled nitrogen (Schoenheimer, 1942; Stetten and Schoenheimer, 1944; Shemin, 1950; Wilson, King and Burris, 1954; Meister, 1957). When dietary requirements are satisfied, the excess nitrogen of the metabolites is utilised by the animals for building of new amino-acids, either by deamination or by transamination. All known natural amino-acids, perhaps with the exception of threonine and lysine, participate in the enzyme transamination (Meister, 1957).

In animals there is no physiological storage of proteins or amino-acids. In caloric deprivation as a result of starvation, excessive work or muscular exhaustion, the animal survives by oxidation of carbon chains of the amino-acids of body proteins. Most of the amino nitrogen is excreted as urea or uric acid. Glutamate appears to be an important compound as it transaminates with oxaloacetate to form aspartic acid, which aids in urea synthesis.
In some freshwater fishes examined, it was observed that in certain regions of skeletal musculature, a number of free amino-acids were released after intense activity, while in other regions, most of the free amino-acids almost vanished (Narawane, unpublished). It was, therefore, felt that an inquiry into the enzyme systems catalysing the breakdown of proteins and amino-acids in the muscles, may help in the understanding of the problem of energy production in the different areas of skeletal muscles of fishes. The present paper reports on the GOT enzyme activity.

**Material and Methods**

Two fishes, of diverse habits and habitats, *Barbus tor*, Day (Physostomi-Cyprinidae) and *Heteropneustes fossilis*, Bloch. (Physostomi-Siluridae) were selected for this work. The former is an active, fast swimming animal with 'carangiform' locomotion, while the latter is a small, comparatively less active and an 'air breathing' animal, with an 'anguilliform' locomotion and living in shallow waters. For the purpose of this work the skeletal muscles of both fishes were divided into three regions, lengthwise, as epaxial, hypaxial and lateral line areas. The specimens were collected locally. Ten specimens of *B. tor* (about 4 months old, average length 15 cm.) and twelve specimens of *H. fossilis* (about 4 months old, average length 10 cm.) were used in these experiments.

Muscles in each area were pooled by taking thin slices along the length and depth for homogenation in distilled water at 0°C. Clear homogenates, after centrifuging, were stored at 4°C. and were used within 30 minutes of the killing of the animals. For quantitative estimations of the enzyme activity, Dubach (1958) method—a modification of Cabaud et al. procedure (1956) was used. The method is based on the principle that amino group of aspartic acid is reversibly transferred by GOT to ketoglutaric acid and glutamic and oxaloacetic acids are formed in the process. The oxaloacetic acid is decarboxylated to pyruvic acid by addition of aniline citrate. The pyruvic acid is then condensed with 2·4 dinitrophenylhydrazine to form hydrozones which, when extracted with toluene, form a red coloured complex with ethanolic KOH. The intensity of the colour is proportional to the pyruvic acid, which is itself proportional to the GOT activity. The colour density was measured with Engel Colourimeter (Kipp and Zonen), with filter No. 50 (range 450–500 m\(\mu\)). Reference curve was prepared with sodium pyruvate. In the blanks, the GOT activity was blocked by addition of a few drops of 1 gm./ml. solution of trichloroacetic acid. As the enzyme and all the end products were soluble in water, the GOT activity was determined in terms of...
micrograms of pyruvic acid formed, per mg. of dry weight of the tissue homogenate, in 30 minutes, at 25-27°C. 30-minute period was taken, as the velocity of the pyruvic acid formation was maximum up to about 30 to 35 minutes, at 25-27°C. The substrate used uniformly, contained a mixture of Aspartic acid, α-Ketoglutaric acid and K₂HPO₄, at pH 7.4.

RESULTS AND DISCUSSION

The results are shown in Table I.

**TABLE I**

GOT activity in skeletal muscles of *B. tor* and *H. fossilis*

<table>
<thead>
<tr>
<th>Area of muscle</th>
<th><em>B. tor</em></th>
<th><em>H. fossilis</em></th>
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<tbody>
<tr>
<td>Epaxial</td>
<td>046·25 ± 3·5</td>
<td>080·15 ± 6·7</td>
</tr>
<tr>
<td>Hypaxial</td>
<td>130·20 ± 8·6</td>
<td>054·35 ± 5·8</td>
</tr>
<tr>
<td>Lateral line</td>
<td>710·11 ±12·5</td>
<td>302·77 ±8·6</td>
</tr>
</tbody>
</table>

Unit: μg. Pyruvic acid released by tissue homogenate, equivalent to 1 mg. of its dry weight, at the end of incubation for 30 minutes, at 25-27°C.

In both fishes it was observed that the muscles in the epaxial, hypaxial and lateral line areas differed markedly in their morphology, in terms of their fibre lengths and diameters of the fibre bundles. Biochemically, they differed, in terms of their protein, carbohydrate and fat contents. The comparatively narrow red fibres contain more minerals, more free amino-acids, more mitochondria and more respiratory pigments, than the white fibres, which have more fat and glycogen contents. The enzyme activities, such as, ATPase, nonspecific acid and alkaline phosphatases, succinic dehydrogenase (Narawane, 1964), etc., are not uniform in the three areas of muscles. It was also observed that in *B. tor*, hypaxial area contains more red fibres, while in *H. fossilis*, epaxial area contains more red fibres, than the hypaxial one. Lateral line areas of both fishes contain maximum number of red fibres. The differential concentration of red and white fibres in the three areas of musculature, in the two fishes, is significant with reference to their locomotory patterns.

*B. tor* swims with jerks, while travelling over long distances, across, with or against the current. The movements are marked with comparatively
quicker contractions of the hypaxial area, particularly in the trunk region, suggesting that the hypaxial muscles contract and relax faster than the epaxial muscles. *H. fossilis* swims over shorter distances and the movements are mostly vertical with quick side twists like an anguilla or a water snake. The movements are marked with quicker contractions of epaxial area and lashing of the caudal peduncle in vertical plane or in screw-like movements.

The results of the present experiments indicate, that, (1) In *B. tor* GOT activity is more in the hypaxial than in the apaxial area, while in *H. fossilis*, it is more in epaxial area than in the hypaxial area. (2) GOT activity is closely associated with the red fibres, like the SDH activity (Narawane, 1964). Therefore, the differential concentration of red and white fibres in different regions of the skeletal musculature is the most significant single factor, responsible for the variations in the utilisation of metabolic fuels, in the epaxial, hypaxial and lateral line areas of the skeletal musculature of fishes, and that, the patterns of locomotory movements fit in the scheme of energy utilisation by the muscles.

**REFERENCES**


