The Effect of Temperature on the Macroscopic and Microscopic Appearance of Crustacean Muscle

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The copepod *Cyclops agilis*, when grown at different temperatures, is known to grow to different adult sizes. The effect of growth on the size and structure of the thoracic body muscles was compared in *Cyclops* grown at 10°, 15° and 25°C, by using the light and electron microscopes.

The minimum diameters of muscle fibres measured in transverse light-microscope sections of whole *Cyclops* were found to be significantly larger in those grown at 15°C than in those grown at 25° and 10°C. The mean minimum fibre diameter of those grown at 15°C was $14.1 \pm 0.58 \mu\text{m}$ compared with $9.6 \pm 0.25 \mu\text{m}$ for those grown at 25°C and $10.6 \pm 0.33 \mu\text{m}$ for those grown at 10°C. Fibre cross-sectional area was calculated from the maximum and minimum diameters of the fibre, the cross-section of the fibre being treated as an ellipse. A significant increase in mean fibre area was found for *Cyclops* grown at 15°C ($263 \pm 21.8 \mu\text{m}^2$) compared with that for those grown at 25°C ($142 \pm 7 \mu\text{m}^2$) and at 10°C ($156 \pm 14 \mu\text{m}^2$). No difference was recorded in the number of fibres seen in leg muscles in the *Cyclops* grown at the three temperatures. From an estimation of the number of myofibrils seen in a fibre it was concluded that increased fibre size is reflected in an increase in the number of myofibrils/fibre and hence in the amount of contractile material. The constancy of the interfibrillar spacing measurements found for *Cyclops* grown at the three temperatures supports this conclusion.

No significant difference in either sarcomere length or A-band length was detected in *Cyclops* muscles at the two different temperatures examined (15° and 25°C). The mean sarcomere length for those grown at 15°C was $2.09 \pm 0.04 \mu\text{m}$ compared with $1.99 \pm 0.04 \mu\text{m}$ for those grown at 25°C. The A-band length was also found to vary considerably from fibre to fibre in each *Cyclops* examined. The mean A-band length for those grown at 15°C was $1.71 \pm 0.03 \mu\text{m}$ compared with $1.64 \pm 0.3 \mu\text{m}$ for those grown at 25°C. These measurements include those of a very small number of fibres with large sarcomere length seen in *Cyclops* from both temperature groups. These fibres differed in appearance from most of the fibres in that they had a much denser Z-line and a larger mean sarcomere length of $3.8 \pm 0.14 \mu\text{m}$ and A-band length of $2.8 \pm 0.12 \mu\text{m}$. These may be similar to the slow fibres described in *Macrocyclus albidus* (Fahrenbach, 1967).

The results reported here confirm the finding in mammals (Macallum, 1898; Goldspink, 1962) that the number of fibres in a muscle does not normally change once differentiation of the tissue is complete, whereas the size of the fibres is determined by the physiological conditions to which the muscle is subjected, in this case temperature.

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Histochemical Characteristics of Muscle Fibres in the Diaphragm

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This study attempts to define and explain histochemical variations in the muscle fibres of the diaphragm in mammals of various body sizes.

Fresh frozen transverse sections of muscle fibres were treated to demonstrate aerobic metabolism, anaerobic metabolism and lipid distribution. These properties were demonstrated by histochemical methods for succinate dehydrogenase activity, phosphorylase activity and the Sudan Black B method for lipids respectively. Incubation of sections with ATP and Ca^{2+} at pH 9.4 and subsequent treatment with Co^{2+} and ammonium sulphide demonstrated myosin adenosine triphosphatase activity. It may distinguish between fibres with a fast and a slow intrinsic speed of contraction due to the presence or absence of a deposit in the myofibrils.

By using serial frozen sections and back projection, histochemical profiles of about 400 individual muscle fibres were made on each sample taken from the diaphragms of shrews, mice, rats, rabbits, cats, dogs, sheep, humans, pigs, oxen and horses. From these profiles the following types of muscle fibres were found: fast-contracting, aerobic; fast-contracting, combined; fast-contracting, anaerobic; slow-contracting, aerobic; slow-contracting, combined.

Studies on myosin adenosine triphosphatase showed that the percentage of fast-contracting fibres increased with decreasing body size; this phenomenon can be related to the faster speed of contraction of the diaphragm in smaller mammals.

Biochemical studies have shown that the ability for aerobic metabolism is greater in the diaphragms of smaller mammals. Histochemically this trend was seen, but differences in succinate dehydro-

genase activity between fibres were not as distinct as the differences in adenosine triphosphatase activity. The proportion of aerobic and anaerobic fibres varied between species, but was not influenced by body size. Phosphorylase activity tended to show an opposite trend to that of adenosine triphosphatase. The horse had nearly 100% of its fibres high in phosphorylase activity, but in the shrew diaphragm no fibres with phosphorylase activity were seen.

Fast-contracting muscle fibres were also high in phosphorylase activity, showing that faster-contracting fibres used mainly an anaerobic metabolism. In smaller animals the proportion of fibres with high adenosine triphosphatase activity exceeded that of fibres with high phosphorylase activity, so aerobic metabolism may be used by fast-contracting fibres.

Some Properties of a Protease from *Bacillus polymyxa*

By P. J. GRIFFIN and W. M. FOGARTY. (*Department of Industrial Microbiology, University College, Dublin 4, Irish Republic*)

Preliminary investigations dealing with the elaboration of proteolytic and starch-degrading enzyme systems by *Bacillus polymyxa* N.C.I.B. 8158 and the influence of an acid-catalysed extract of peat on these processes have been described (Griffin & Fogarty, 1971a). The protease alone was secreted in high yield when arabinose was used as carbon source. The effects of different factors influencing the proteolytic and milk-clotting activity of the enzyme have been recorded (Griffin & Fogarty, 1971b).

The protein in the cell-free supernatant from a peat extract-arabinose medium was fractionated by precipitation with ammonium sulphate, dialysed and concentrated with acetone. Further purification was effected by gel filtration on Sephadex G-100. An overall 70-fold increase in specific activity was thus achieved.

Dialysis of the protease against 20 mM-phosphate buffer, pH 7.0, resulted in irreversible loss in activity. Addition of 1 mM-calcium chloride to the phosphate buffer reversed this effect. Dialysis against 20 mM-tris-HCl buffer, pH 7.0, resulted in only a slight loss in activity. The ionic composition of the buffer used had a significant effect on enzyme activity, e.g. Britton and Robinson's buffer, phosphate buffer maleate buffer, and citrate-phosphate buffer gave activities of 83, 85, 67.7 and 53% respectively, of that observed with tris-HCl buffer, pH 7.0.

The enzyme showed highest activity at pH 7.0 for casein and hide powder, 83% and 82% of the respective substrates being rendered soluble in 5% (w/v) trichloroacetic acid in 2 h. The protease caused considerable breakdown of a variety of proteins.

There was complete inhibition of activity by 1 mM-EDTA and by 1 mM-*o*-phenanthroline, 66% inhibition by 2 mM- α '-bipyridyl, 7% inhibition by 5 mM-sodium oxalate and 34% inhibition by 5 mM-sodium citrate. The effects of a variety of other inhibitors on activity of the enzyme were also examined.

Dialysis of the enzyme preparation against 1 mM-EDTA, pH 7.0, for 3 h and then against 20 mM-tris-HCl buffer, pH 7.0, for 12 h, followed by addition of Mn^{2+} (final concentration 1 mM), gave an increase of 56% in activity over the original non-dialysed material. Dialysis against 1 mM-EDTA for 12 h resulted in no activity on the addition of Mn^{2+} .

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Extracellular Enzymes of a *Bacillus* sp. Associated with Increased Permeability in Sitka Spruce (*Picea sitchensis*)

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Destruction of tori and bordered pit membranes in the sapwood of Sitka spruce (*Picea sitchensis*) during water storage has been shown to result in increase in permeability of the material to treatment with preservative (Dunleavy & Fogarty, 1971). Evidence indicates that destruction of the pit membranes may be caused by the action of bacterial extracellular enzymes. The importance of pectinolytic activity in the degradation of the pits has been suggested (Ward & Fogarty, 1971). Certain enzymes, e.g. cellulases, are known to have highly detrimental effects on the structural properties of wood. We now report the results of a study of the nutritional factors influencing the elaboration of extracellular enzymes by an organism associated with permeability increase in water-stored spruce.

The bacterium, a *Bacillus* sp., isolated from water-stored Sitka spruce (W. M. Fogarty, unpublished work), was grown at 27°C in liquid media