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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Goldspink, G. (1962). *Proc. R. Ir. Acad. B*, 62, 135.

Macallum, J. B. (1898). *Bull. John Hopkins Hosp.* 6, 208.

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-