CONTRACTION AND CROSS-STRIATION OF MUSCLE

by

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INTRODUCTION

The relation of the cross-striation of the muscle to contraction has been most extensively studied by BUCHTHAL[1-3] who came to the conclusion that only the A band contracts, while the I band stretches. His finding is in agreement with WEBER's statement that only the A bands contain myosin[4,7,10]. It is difficult to imagine, however, how the muscle could contract more than 50% if half of it does not participate in the contraction. The filaments observed in the electron microscope seem to run continuously through A and I bands and there is reason to believe that these filaments are actin threads[5,6,8]. They could thus be expected to be accompanied by myosin and be contractile. On the other hand, if myosin is localized in the A bands only, one has to assume part of the muscle's actin to be free of myosin.

It appears to be simple to decide by direct microscopic observation how far the two bands, A and I, participate in contraction. The difficulty is with the material. It is very difficult, if not impossible, to maintain a uniform lasting contraction in living muscle under the microscope. This difficulty was solved by SZENT-GYÖRGYI[7,8] with the introduction of the glycerinated psoas muscle. If the muscle is extracted with glycerol, its ATP is removed while the contractile microstructure remains undamaged. If the glycerol is washed out, the muscle suspended in saline and ATP added, contraction of any desired extent can be produced, depending on the length to which the muscle is allowed to shorten. If the ATP is then washed out, the muscle remains indefinitely in this contracted state and can be studied at ease under the microscope. If the psoas muscle is used for this preparation, one has the additional advantage of parallel running fibers, which, owing to the lack of connective material, can easily be disintegrated into thin fiber bundles or even single fibers.

EXPERIMENTAL

Thin fiber bundles of the psoas muscle of the rabbit were used in all experiments. Immediately after the death of the animal narrow strips of the psoas (10–12 cm long and 3–4 mm wide) were attached *in situ* by ligaments to applicator sticks, cut free of the rest of the psoas and immersed, together with the sticks, into 50% watery glycerol solution of 0° C, and kept at this temperature for 48 hours. Beyond this time they were stored at −20° C in 50% glycerol.

If fibers stretched beyond the rest-length were required, the psoas bundle was stretched immediately after the death of the animal and attached to the applicator stick in a stretched condition. If fibers of rest-length were desired, the muscle was fixed at its natural length *in situ*. If muscle of equilibrium length was needed, then one end of the muscle only was tied to the stick, while the other

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end was cut free and fixed to the stick after it had shortened spontaneously to its equilibrium length, which was mostly 10% shorter than the rest-length. Before observation these muscles were separated with the help of tweezers into fiber bundles or even single fibers.

If contracted muscle was desired, the starting material was muscle of rest-length. With the help of the tweezers thin fiber bundles were isolated containing 3–6 fibers. The bundle was laid over a hook, then both ends were fixed with a clamp. The muscle was straightened by a gentle pull on the clamp, and immersed in this condition for 5 minutes into a solution csntaining 0.08 *M* KCl, 0.0005 *M* $MgCl_2$ and 2 mg per ml ATP, then transferred for 10 minutes into distilled water to wash out the ATP. The temperature of both the ATP solution and the distilled water was 0° C (isometric contraction). A number of bundles were allowed to shorten slowly in ATP while pulling against a weight. When the desired final length was reached, the bundles were transferred to distilled water, as above (30–50% contraction). Psoas fibers will not contract at 0° C by more than 50% of their rest-length. To make stronger contraction possible the experiment has been performed at 10° C, but otherwise as described above.

In taking the *microphotograms* the following procedure was employed: A larger number of fibers of each type was observed under the microscope using high power magnification and oil immersion. Fibers treated identically all looked alike. The best fields were photographed, 10 or more pictures being taken of 3 or more fibers of each preparation. The negatives were taken on Kodak Micro-file M 402 film with a Leica camera and its Micro-Ipso attachment. All pictures were taken with the same magnification and an identical enlargement factor was used in making the prints.

RESULTS

The uniformity among fibers which were treated similarly was already apparent on simple microscopic inspection. This was further borne out by the obvious similarity in the enlarged prints of the same type of material.

On the prints the cumulative height of 10 adjacent compartments was measured and expressed in percentage of the same value for rest-length fibers. This served as the measure of contraction, and was checked against the degree of contraction (or stretch) measured on the whole fiber. This procedure served several purposes. It proved that no striations disappear (or appear) even under conditions of extreme contraction (or stretching). It also helped to exclude errors such as observing a torn fiber while the whole bundle appeared to be intact. Finally it showed that no disfiguration occurred during the preparation of the single fibers. The agreement between the degree of contraction measured on the whole muscle and on the prints of single fibers was always satisfactory (within 5%) except for the maximally (78%) contracted fibers. Here the measurement of the microphotograms revealed that the sarcomers contracted by 60% of their rest-length only, and further shortening was due to curving and wrinkling of the fibers.

For measurements of the relative width of the bands only those parts of the prints were used in which the bands lay straight over one, or preferably several centimeters, in which the widths of the bands were uniform throughout this length, and the demarcation of the A and I bands was sharp. Since absolute values were not needed a millimeter scale was put obliquely across adjacent A and I bands, thus allowing a more exact determination of their relative widths. Focusing experiments showed that the width of the bands did not depend on the level at which the picture was taken. This was done by taking a picture first at the highest level at which any structure could be discerned, then taking subsequent pictures moving the objective down by 0.010 mm each time, until the lowest limit of visibility was reached. Such pictures look identical. This result is in agreement with similar data of BUCHTHAL[1].

Measurement of the microphotograms showed that the relative width of the A and I bands is always 1:1 independently of the degree of contraction or stretch. It will be seen from the pictures on Fig. 1. that over the whole range studied both the A and I

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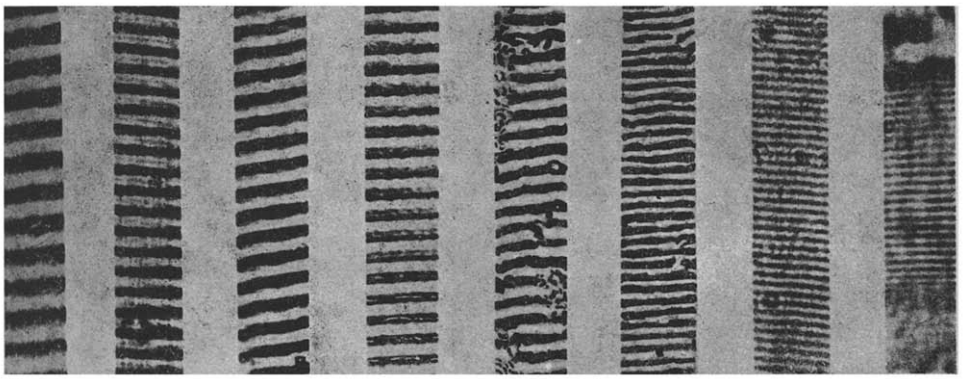
bands occupy 50–50% of the height of the compartment, that is both A and I bands contract and stretch equally, at least under the conditions of the experiments descibed. This would indicate that both bands contain contractile material and that the actin filaments are accompanied by myosin in the I bands as well as in the A bands.



a b c d e f g h

Fig. 1. Microscopic pictures of single fibers of the glycerinated psoas muscle of the rabbit. (Unstained). a: 140% stretched, b: rest-length, c: isometric contraction, d: equilibrium length, e — h contracted: e: by 22.5%, f: by 40%, g: by 50%, h: by 78%

SUMMARY

Microphotograms were taken of glycerinated psoas fibers of the rabbit in different stages of contraction and stretch. A and I bands retain their relative width of 1:1 over the whole range. It is concluded that both bands are equally capable of contraction and both contain contractile material.

RÉSUMÉ

L'auteur présente des microphotogrammes de fibres de psoas glycérinées du lapin dans différents stades de contraction et d'extension. Les bandes A et I conservent leur largeur relative de 1:1 dans tout le domaine étudié. L'auteur conclue que les deux espèces de bandes ont la même capacité de contraction et que toutes deux contiennent du matériel contractile.

ZUSAMMENFASSUNG

Microphotogramme von glycerinierten Psoasfasern vom Kaninchen in verschiedenen Stadien der Kontraktion und Dehnung wurden aufgenommen. Die A und I Banden behalten in dem ganzen Bereich ihre relative Breite von 1:1. Es wird geschlossen, dass beide Banden die gleiche Kontraktionsfähigkeit haben und dass beide kontraktiles Material enthalten.

REFERENCES

[1] F. BUCHTHAL, G. G. KNAPPEIS, AND J. LINDHARD, *Skand. Arch. Physiol.*, 73 (1936) 163.
[2] F. BUCHTHAL AND G. G. KNAPPEIS, *Acta Physiol. Scand.*, 5 (1943) 256.
[3] F. BUCHTHAL AND G. G. KNAPPEIS, *Acta Physiol. Scand.*, 6 (1943) 123.
[4] D. NOLL AND H. H. WEBER, *Arch. ges. Physiol. Pflugers*, 235 (1934) 234.
[5] G. ROZSA, A. SZENT-GYÖRGYI, AND R. W. WYCKOFF, *Biochim. Biophys. Acta*, 3 (1949) 561.
[6] G. ROZSA, A. SZENT-GYÖRGYI, AND R. W. WYCKOFF, *Exptl Cell Res.*, 1 (1950) 194.
[7] A. SZENT-GYÖRGYI, *Biol. Bull.*, 96 (1949) 140.
[8] A. SZENT-GYÖRGYI, *Chemistry of Muscular Contraction*, 2nd ed., Acad. Press, New York, 1951, pp. 83–86.
[9] H. H. WEBER, *Ergeb. Physiol., exptl Pharmakol.*, 36 (1934) 109.
[10] H. H. WEBER, *Naturwissenschaften*, 27 (1939) 33.

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