STRUCTURAL CHANGES IN MUSCLE DURING CONTRACTION

Interference Microscopy of Living Muscle Fibres

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IN spite of the numerous investigations which have been made into the changes of the striations of muscle when it contracts, there is little agreement at the present day on either the nature or the significance of these changes. Several factors contribute to this unsatisfactory position. The only contractions that could be studied in living muscle by the earlier workers[1] were the slow waves that occur in freshly isolated insect fibres; the broad striations and small diameter of these fibres are favourable for the interpretation of the microscope image, but the local nature of the contractions and the difficulty of applying passive stretch make it impossible to say whether the changes seen in the striations are accompaniments of 'activation', of tension development or of shortening. Observations on fixed material, whether with visible light or with the electron microscope, are also subject to this limitation as well as to the uncertainties in the effect of the fixative. Isolated fibres from frog muscle, however, give satisfactory propagated twitches and tetani, in which activation is complete very early after the first stimulus[2], while tension develops more slowly even if the contraction is isometric, and changes of length, both during stimulation and in the resting muscle, can be controlled by holding the tendon ends. This preparation therefore provides a basis for correlating visible changes with the sequence of events which take place during a contraction, and Buchthal and his colleagues[3] have endeavoured to exploit this possibility. Their conclusions are, however, open to the objection that they used the ordinary light microscope, which cannot be expected to provide a reliable image of unstained striations (alternate bands of high and low refractive index) the repeat distance of which is 2–3 μ, in a fibre of 50–100 μ diameter.

The phase-contrast microscope is equally unsuitable for a specimen of these dimensions; but an interference microscope in which the reference beam does not traverse the specimen would be expected to give a satisfactory 'optical section' of the fibre. An instrument of this kind was therefore built, the optical components being made to our specification by Messrs. R. and J. Beck. The basic principle was first described by Smith[4] (see also Huxley[5]), but further developments were incorporated to allow a water-immersion objective of n.a. 0·9 to be used. Gross refraction effects due to the cylindrical shape of the fibre were abolished by adding serum albumin to the Ringer's solution to bring its refractive index close to the average value for the fibre contents. A solid cone of illumination, n.a. 0·5–0·6, was always employed. Under these conditions, the fibre was completely invisible with ordinary light, but with the interference arrangement an excellent image of the striations (and also of sarcoplasm, nuclei and granules) was obtained. The contrast between *A*- (higher refractive index) and *I*-bands could be controlled or reversed by changing the background path-difference between the two beams; the measured widths of the bands were independent of this adjustment (Fig. 1). The fibre was photographed on moving film by a series of ten flashes from a discharge tube at intervals of about 20 msec., and could be stimulated by pulses of current synchronized with these flashes.

Passive stretch. The sarcomere length *s* could be changed by passive stretch or release from about 2·0 to 4·2 μ, the value at the extended length in the body being 2·5 μ[6]. Almost the whole of this change of length took place in the *I*-bands (Fig. 2). The measured width of each *A*-band remained constant at 1·4–1·5 μ except for a fall to about 1·3 μ as *s* was reduced in the range 2·5–2·0 μ; but this fall may well not be real, as its amount is less than the resolving power of the optical system. When a fibre was stretched rapidly (20–30 per cent in 5 msec.) the new ratio of *A*- to *I*-band widths appeared to be established without delay (less than 2 msec.).

Isometric twitches. Fibres were stimulated at a wide range of lengths with the tendon ends held stationary, and the twitch tensions, measured simultaneously with the *RCA* 5734 transducer, were normal. No change in the widths of the bands could be detected, except that when slight shortening of the region of the fibre in the field of view took place the changes were similar to those in isotonic shortening of the same extent.

Isotonic contractions (Fig. 3). Fibres were photographed during twitches and short tetani under isotonic conditions, with various initial lengths up to 3·2 μ per sarcomere. As in passive shortening, it was the *I*-bands that became narrower, the band-width

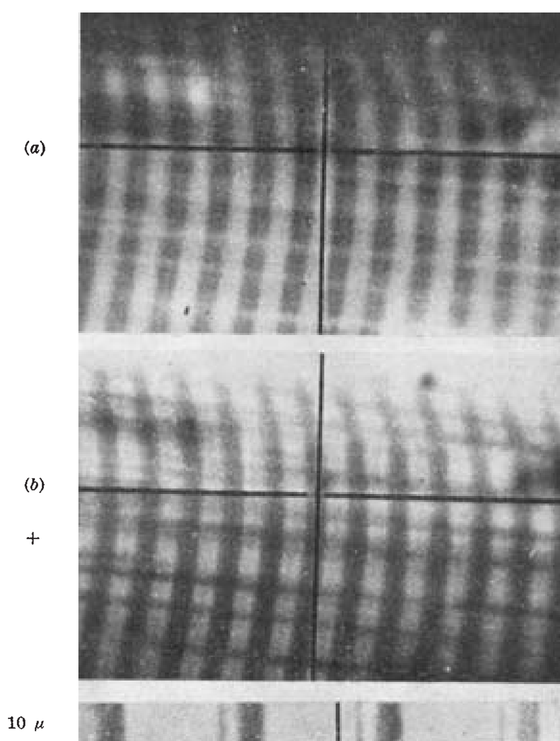

Fig. 1. Muscle fibre in negative (*a*) and positive (*b*) contrast. *A*-bands (higher refractive index) light in (*a*), dark in (*b*). Note that the threads of sarcoplasm have a refractive index slightly higher than even the *A*-bands. Sarcomere length, 3·0 μ

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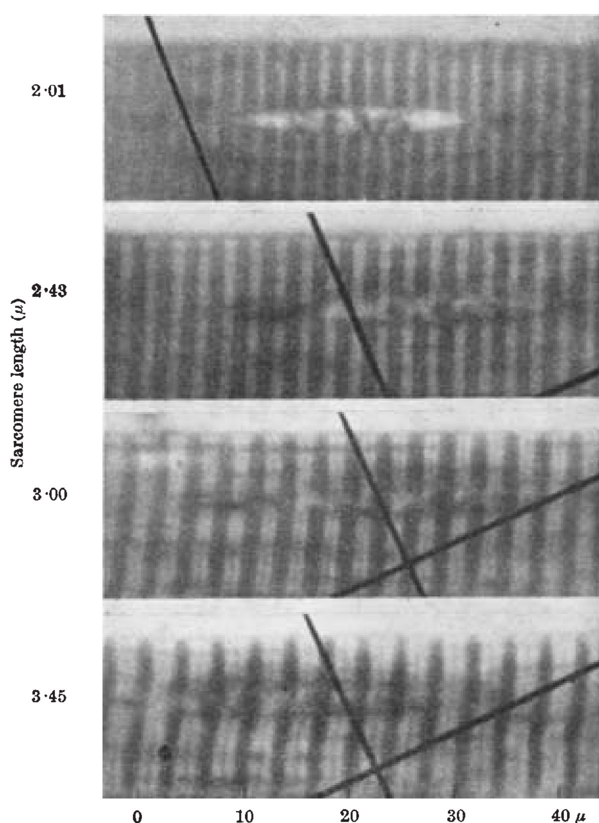

Fig. 2. Passive stretch of a muscle fibre. Positive contrast (*A*-bands dark). Sarcomere lengths indicated beside the photographs. Almost all the change of length is in the *I*-bands (light)

of *A* being constant down to a sarcomere length of $s = 2{\cdot}5\ \mu$ and falling only slightly down to $s = 2{\cdot}0\ \mu$. On further shortening, studied largely by ciné photography of slow contractions induced by constant-current stimulation, *A*-band width decreased definitely in all cases; but there were additional phenomena which were not the same in every experiment. The following sets of changes were observed on several occasions:

(*a*) Striations became extremely faint on shortening beyond a sarcomere length of about $1{\cdot}8\ \mu$ (cf. Speidel[7]).

(*b*) The dense band narrowed to about half the sarcomere length, after which both bands narrowed in proportion.

(*c*) At $s = 1{\cdot}8\ \mu$, a very narrow dense band was visible at the centre of the former *A*-band, and on shortening to $s = 1{\cdot}7\ \mu$ additional dense lines appeared midway between these (cf. Jordan's observations on stained preparations[8]).

The similarity of the changes during passive shortening and during isotonic contraction, and the absence of change during isometric twitches, show that the changes in the ratio of widths of the *A*- and *I*-band depend simply on the length of the fibre, and are unaffected by 'activation' or by tension development as such. The approximate constancy of *A*-band width under a wide range of conditions (including shortening within the physiological range) agrees with the observations of Krause and of Engelmann[1], and also with those of H. E. Huxley and J. Hanson on separated myofibrils reported in the accompanying communication, though it is in conflict with the results of Buchthal *et al.*[3]. The natural conclusion, that the material which gives the *A*-bands their high refractive index and also their birefringence is in the form of submicroscopic rods of definite length, was put forward by Krause, and receives strong support from the observations reported here. The identification of this material as myosin[9], and the existence of filaments (presumably actin) extending through the *I*-bands and into the adjacent *A*-bands, as shown in many electron microscope studies, makes very attractive the hypothesis that during contraction the actin filaments are drawn into the *A*-bands, between the rodlets of myosin. (This point of view was reached independently by ourselves and by H. E. Huxley and Jean Hanson in the summer of 1953. It has already been mentioned by one of those authors[10] and is further discussed by them in the accompanying article.)

If a relative force between actin and myosin is generated at each of a series of points in the region of overlap in each sarcomere, then the tension per filament should be proportional to the number of these points, and therefore to the width of this zone of overlap. If the myosin rods are $1{\cdot}5\ \mu$ long and the actin filaments $2{\cdot}0\ \mu$, the isometric tetanus tension should fall linearly as the fibre is stretched over the

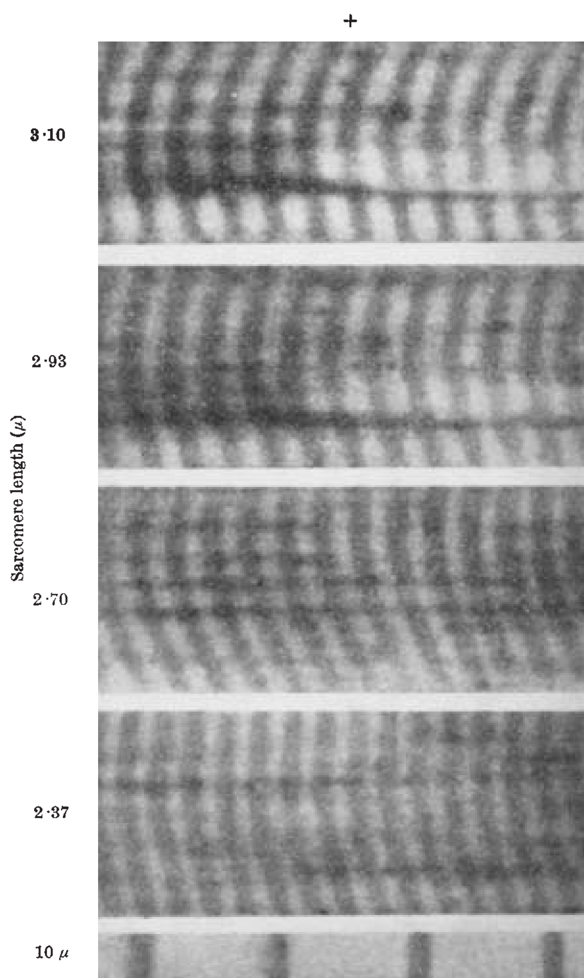

Fig. 3. Muscle fibre during a short isotonic tetanus. Positive contrast (*A*-bands dark). As in passive stretch (Fig. 2), the *A*-bands remain of almost constant width

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range of sarcomere lengths from 2·0 to 3·5 μ; this is in fair agreement with observation[11]. In arthropod striated muscle, there is a wide range of sarcomere lengths *in situ*, and narrowness of striation appears to be correlated with high speed of contraction[12]. This would be expected if the relative velocity between actin filaments and myosin rods in any one zone of overlap were the same for muscles of different sarcomere lengths, since the number of sarcomeres shortening in series per unit length is inversely proportional to sarcomere length. On this basis it would also be expected that the muscle with longer sarcomeres would be capable of producing a greater tension, but we are not aware of any experimental results on this point.

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Changes in the Cross-Striations of Muscle during Contraction and Stretch and their Structural Interpretation

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In recent papers[1-3], we have described evidence concerning the location and arrangement of the two principal structural proteins, actin and myosin, in striated muscle at rest length. This evidence indicates that myosin is located in the anisotropic or A-bands, in the form of longitudinal filaments about 110 A. in diameter, spaced out in a hexagonal array 440 A. apart; these filaments are continuous from end to end of the A-band, and appear to be responsible for its high density and birefringence. Actin, on the other hand, is present in both the A-bands and the relatively isotropic or I-bands, in the form of filaments about 40 A. in diameter; these extend from the Z-lines, through the I-bands, and into the A-bands, where they lie between the myosin filaments and terminate on either side of the H-zone; the myosin filaments seem to have a somewhat greater thickness in this zone. Hasselbach[4] has reached similar conclusions about the location of actin and myosin, though his concept of the details of their arrangement is different from ours. We shall now describe evidence that during stretch, and during contraction down to about 65 per cent of rest length, the length of the A-bands remains constant within the limits of accuracy of our measurements (5–10 per cent), the changes in length of the muscle being taken up by changes in the length of the I-bands alone; further shortening beyond the point where the I-bands vanish (about 65 per cent of the rest length) is accompanied by the formation of contraction bands where the A-bands have come into contact with the Z-lines. These changes appear to take place by a process in which actin filaments slide out of or into the parallel array of myosin filaments in the A-bands; as shortening proceeds, the actin filaments fold up in the A-band, and this folding continues after the I-bands have been fully retracted. The myosin filaments remain at constant length until forced to shorten by excessive contraction of the sarcomeres.

Previous work on the changes in cross-striation accompanying stretch or contraction (reviewed by Jordan[5] and Buchthal, Knappeis and Lindhard[6]) has given results which in general we consider to be unreliable, for the following reasons. Observations made on intact muscle fibres in conventional light microscopes are liable to be misleading because of optical artefacts due to the thickness of the fibres. (This difficulty has been surmounted by the technique described by A. F. Huxley and R. Niedergerke in the accompanying paper.) Furthermore, normal contraction is so rapid that the changes taking place *during* the process are difficult to see and record. If fixed and sectioned material is used, it is possible to avoid optical artefacts and the necessity for rapid observation, but other kinds of artefacts are introduced. In spite of all these considerations, a number of workers, notably Speidel[7], have given accounts of changes of band pattern during stretch and contraction which we recognize as generally correct; but they do not establish the details of the changes with the precision necessary for satisfactory interpretation.

In order to avoid optical artefacts, we have used isolated myofibrils about 2 μ in diameter prepared by blending glycerol-extracted rabbit psoas muscle[8]. They are admirable objects for high-resolution microscopy in phase-contrast illumination or polarized light, and will contract when treated with adenosine triphosphate[9]. This contraction is a much slower process than contraction *in vivo*, and therefore provides favourable circumstances for detailed observation of the band changes taking place. The evidence that the mechanism of contraction in glycerol-extracted muscle treated with adenosine triphosphate is similar to that of normal contraction in living muscle has already been adequately discussed by Szent-Györgyi[8] and Weber and Portzehl[10]. We have also devised a simple technique for stretching isolated fibrils during observation. A suspension of fibrils, mounted as a very thin layer on a slide under a coverslip, is examined in the microscope until a fibril is found with one end embedded in a fibre fragment adhering to the coverslip, and its other end in a fragment attached to the slide. Movement of the coverslip in the appropriate direction will then produce the desired stretch or will permit the fibril to shorten if adenosine triphosphate is present. Very small movements can be produced with great ease by gentle pressure on the edge of the coverslip, for the thin layer of liquid provides smooth and highly viscous lubrication.

Photographs used for measurement were taken on microfile film at a magnification of × 370 or × 550, and an apochromatic phase-contrast objective of n.a. 1·15 was employed.

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