Striation Patterns of Ox Muscle in Rigor Mortis

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PLATES 208 AND 209

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ABSTRACT

Ox muscle in rigor mortis offers a selection of myofibrils fixed at varying degrees of contraction from sarcomere lengths of 3.7 to 0.7 μ. A study of this material by phase contrast and electron microscopy has revealed four distinct successive patterns of contraction, including besides the familiar relaxed and contracture patterns, two intermediate types (2.4 to 1.9 μ, 1.8 to 1.5 μ) not previously well described.

In the course of a study on beef (1) it was found that various muscles of the ox enter rigor mortis in differing states of contraction which are undoubtedly related to the strains present in the muscles of the hung carcass. While some muscles such as the psoas and latissimus dorsi pass into rigor in a fully relaxed condition, others such as the longissimus dorsi, semimembranosus, and tensor fasciae latae show a range of contraction in the myofibrils of the one muscle, from relaxation down to extreme contraction. These muscles offer for study a complete sequence of striation patterns. While most of these patterns have been previously well recorded for other species, some interesting intermediate forms have here been observed in the isolated myofibrils by phase contrast and electron microscopy. The relation between the patterns and sarcomere length has been established for shortening from 0 to 80 per cent of maximum length.

Experimental

Early experiments were carried out on longissimus dorsi muscles excised immediately after death, stored in the cold until rigor was complete, and fixed in 10 per cent formaldehyde. Later experiments were carried out on various muscles excised in the boning room, 1 day after slaughter. Fixed samples were blended for 5 minutes in water in a chilled blendor. Unfixed muscle was blended for 2 minutes in cold 0.08 M KCl. Fixed or unfixed samples for the electron microscope were washed twice with water or 0.08 M KCl respectively. Phase contrast photomicrographs were taken with a Zeiss stand W. microscope and electron micrographs with a Phillips model EM-100B instrument.

RESULTS

Phase Contrast Microscopy:

No difference could be observed in the patterns, their distribution, or length ranges, between fixed muscle and unfixed rigor muscle. The patterns fell into the same length ranges, irrespective of the muscle examined. The patterns could be divided into four distinct types of definite length range.

Type I (3.7 to 2.4 μ). This is the familiar relaxed pattern frequently described (Figs. 1 *a* and *b*). A dark A band is split by a wide light H band. The A band is cylindrical and thicker than the I band. The N lines, generally not visible under phase contrast, appear clearly in this muscle at the centre of the I bands. The I band shortens with the sarcomere. This usual type is designated type IA to distinguish it from a less common species falling into the same length range, type IB. This latter type is illustrated in Figs. 1 *c* and *d* and is characterized by a concentration of the A band material at the outer edges of the A band, lengthening it slightly and leaving a wide light centre in which the M line can be seen. The N lines also can be seen in Fig. 1 *c*. As the sarcomere length of type IA approaches 2.5 μ, the H band disappears and a dark line appears in its place. At 2.4 μ the pattern passes over to

Type II (2.4 to 1.9 μ). This new form is marked by a broad dense band with a lighter centre lying across the A band (Figs. 1 *e* and *f*). The sarcomere has a convex profile. The residue of the I band persists through the length range and the whole sarcomere appears to shorten proportionately. At *ac.* 1.9 μ there is a sudden transition to Type III.

Type III (1.8 to 1.5 μ). This type (Figs. 1 *g* and *h*) is of much lower contrast than the previous form. The Z bands are less dense. A grey band with a central dark line occupies the middle of the sarcomere. The edges of the grey band are dense, giving in some cases the appearance of a triple striation (Figs. 1 *g*, *m*, and *n*). The fibril has no marked protuberances on its profile. At about 1.5 μ the contrast becomes very low, in fact in some fibrils even Z bands can barely be distinguished. Below 1.5 μ the well known contraction pattern emerges. The transition in a single fibril is seen in Figs. 1 *m* and *n*.

Type IV (1.5 to 0.7 μ). Here marked thickening of the Z bands occurs, while the detail in the centre of the sarcomere is reduced to a double striation (Figs. 1 *i* and *j*) which becomes a single faint striation (Fig. 1 *k*) and finally disappears as shortening proceeds. The fibril in Fig. 1 *l* is the most extreme shortening observed but the length of a sarcomere is seldom less than 1.1 μ.

Electron Microscopy:

Type IA. The shadowed myofibrils in Figs. 2 *a* and *b* have the same appearance as those under phase contrast. In addition, the M line and two fine lines between the Z band and the prominent N line are visible.

Type II. The well defined dark central band of the phase contrast micrographs appears in the shadowed fibril of Fig. 2 *c* only as a dark shadow overlaying the M band, and is barely visible in the stained fibril (Fig. 2 *d*). A dark line, presumably the N line, is visible in the very short I band of the shadowed fibril, but in the stained fibril appears almost as part of a composite Z band.

Type III. The broad dark central band in Figs. 2 *e* and *f* does not appear to have well defined edges. The M and Z lines are the only other well defined features.

Type IV. Both stained and shadowed (Fig. 2 *g*) fibrils show heavy Z bands, with no detail other than M lines. There is a suggestion of a central broad band showing in slight relief.

DISCUSSION

The pattern-length relationship appears to be the same for the sarcomeres of all the muscles examined. Indeed, toad (2, 3) and rabbit (4) muscle appear to fall into the same length ranges as ox muscle, while blowfly (5) and crab muscle (6) have longer sarcomeres. The "rest length" of the sarcomere is not easily decided in the present case as muscles may be under strain in the hung carcass and a whole range of sarcomere types may be present in one muscle.

The type IB myofibril has already been recorded by Draper and Hodge (2, Fig. 9) and Hanson and Huxley (7, Fig. 31). Philpott and Szent-Gyorgyi (8) demonstrated the "series elastic component" of muscle as a broad light area appearing in the centre of the A band either on stretching or isometric contraction. Their patterns appear to be identical with those of type IB. As the type IB fibrils fall into the lower half of the type I length range they undoubtedly represent isometric contractions rather than stretching. Huxley and Niedergerke (3), however, record no change of pattern during isometric contraction of living fibres, while Huxley and Hanson (4) found an increase in density at the centre of the A band in fibrils held at the ends.

The shortening of the I band down to the emergence of type II agrees with current contraction theories (7) of the absorption of the I band into the A band, but in the type II fibril the A band is definitely shorter. The N line is separated from the A band by a small gap, the same size as the residual I band in type II. Fig. 2 *d* gives the impression that the N line has come hard against the Z band, perhaps functioning as a "back-stop." The residual I band then persists on shortening down to the transition point at 1.9 μ.

The dark central band of type II fibrils and its swollen profile indicate a concentration of some part of the A substance at the center. This is in contrast to the concentration of A substance at the outer edges of the A band in isometric contraction. Huxley and Niedergerke (3) record the appearance at 1.8 μ and Huxley and Hanson (4) at 2.1 μ of a dark band in the center of the A band. As the former work was carried out on living fibres it would appear that type II fibrils belong to the physiological contraction range. The fibril of period 2.0 μ illustrated by Hanson and Huxley (7, Fig. 26 *a*) appears to be identical with the Type II fibrils here recorded.

At 1.8 to 1.9 μ some profound rearrangement of the sarcomere occurs as shown by the sudden drop in contrast and change of pattern. A resemblance to A and I bands is seen but this may not bear any simple relation to the original bands.

After a further low contrast transition at 1.5 μ it is obvious that accumulation of material in the

Z bands to form "contraction bands" is occurring. The electron micrographs show the M line as the only feature of unvarying appearance in all four types, and agree with the change of Z bands to contraction bands. The identity of location of the "Z bands" of type III and the contraction bands of type IV can be seen in the transition along the fibrils of Figs. 1 *m* and *n*.

The above results suggest that the mechanism of contraction with the onset of rigor mortis is in no way different from that of living fibres (as far as they go) or from that induced in isolated myofibrils by ATP. While the absorption of the I band into the A band is a satisfactory explanation of the upper physiological range of contraction (2, 3, 7), it is clear that a much more complicated theory will be necessary to accommodate the full range of contraction possible in muscle.

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Explanation of Plates

Plate 208

Fig. 1. Phase contrast micrographs. × 2,800. Sarcomere lengths given in microns. Figures in brackets are widths of A bands or, (in *g* and *h*), of the central grey band.

a. Fibril from psoas muscle (unfixed) type IA.

b. Semimembranosus (unfixed) type IA.

c. Longissimus dorsi (unfixed) type IB.

d. Latissimus dorsi (unfixed) type IB.

e. and *f*. Longissimus dorsi (fixed) type II.

g and *h*. Longissimus dorsi (fixed) type III.

i to *l*. Longissimus dorsi (fixed) type IV.

m and *n*. Longissimus dorsi (fixed) showing transition from type III to type IV.

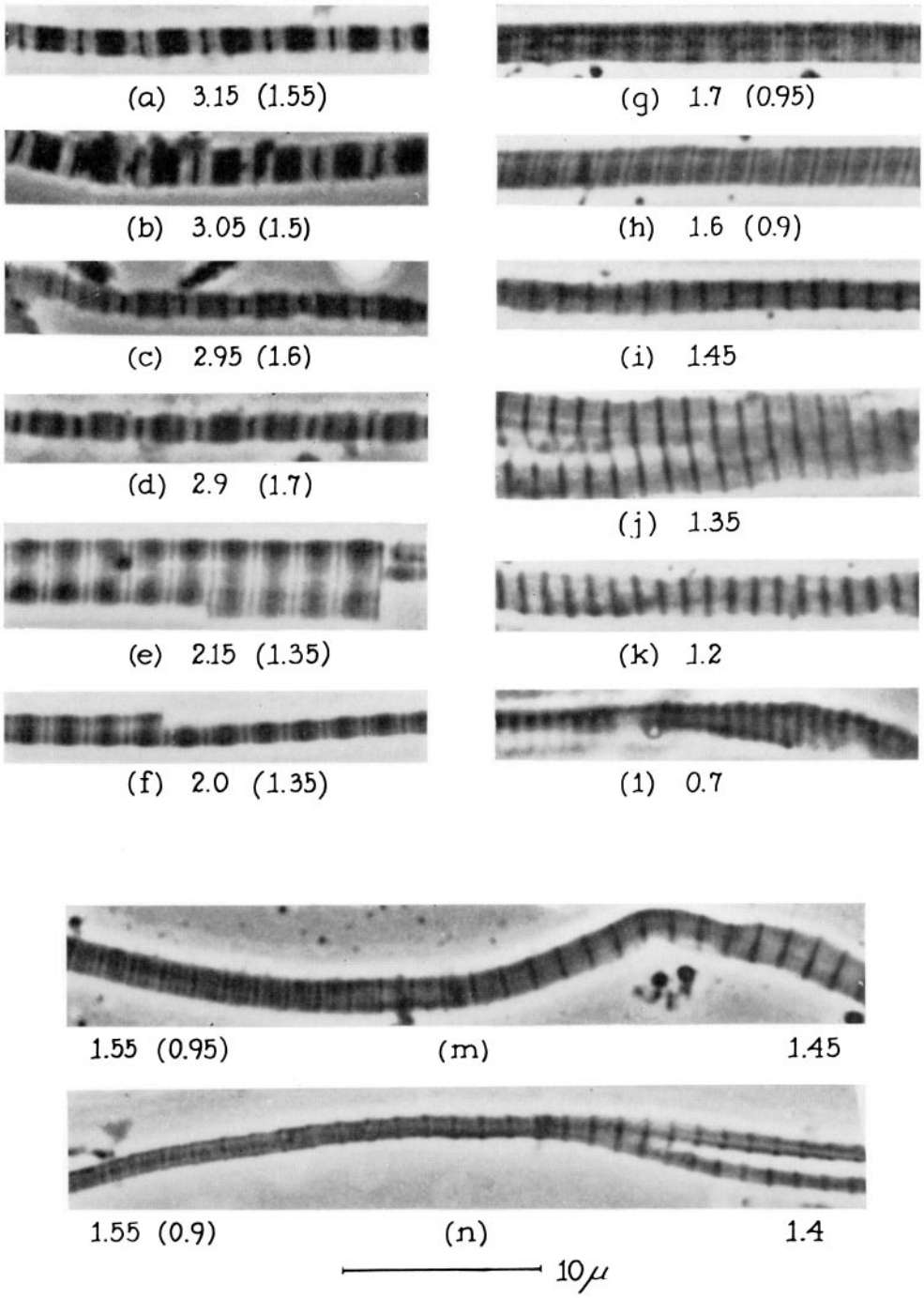

FIG. 1

(Locker: Striation patterns)

PLATE 209

FIG. 2. Electron micrographs. × 15,000. Sarcomere lengths given in microns. Figures in brackets are widths of A bands.

a and *b*. Fibrils from latissimus dorsi muscle (unfixed) shadowed with uranium, type IA.

c. Longissimus dorsi (fixed) shadowed with uranium, type II.

d. Longissimus dorsi (fixed) stained briefly with 0.1 per cent phosphotungstic acid, type II.

e. Longissimus dorsi (fixed) stained briefly with 0.1 per cent phosphotungstic acid, type III.

f. Longissimus dorsi (unfixed) shadowed with uranium, type III.

g. Longissimus dorse (unfixed) shadowed with uranium, type IV.

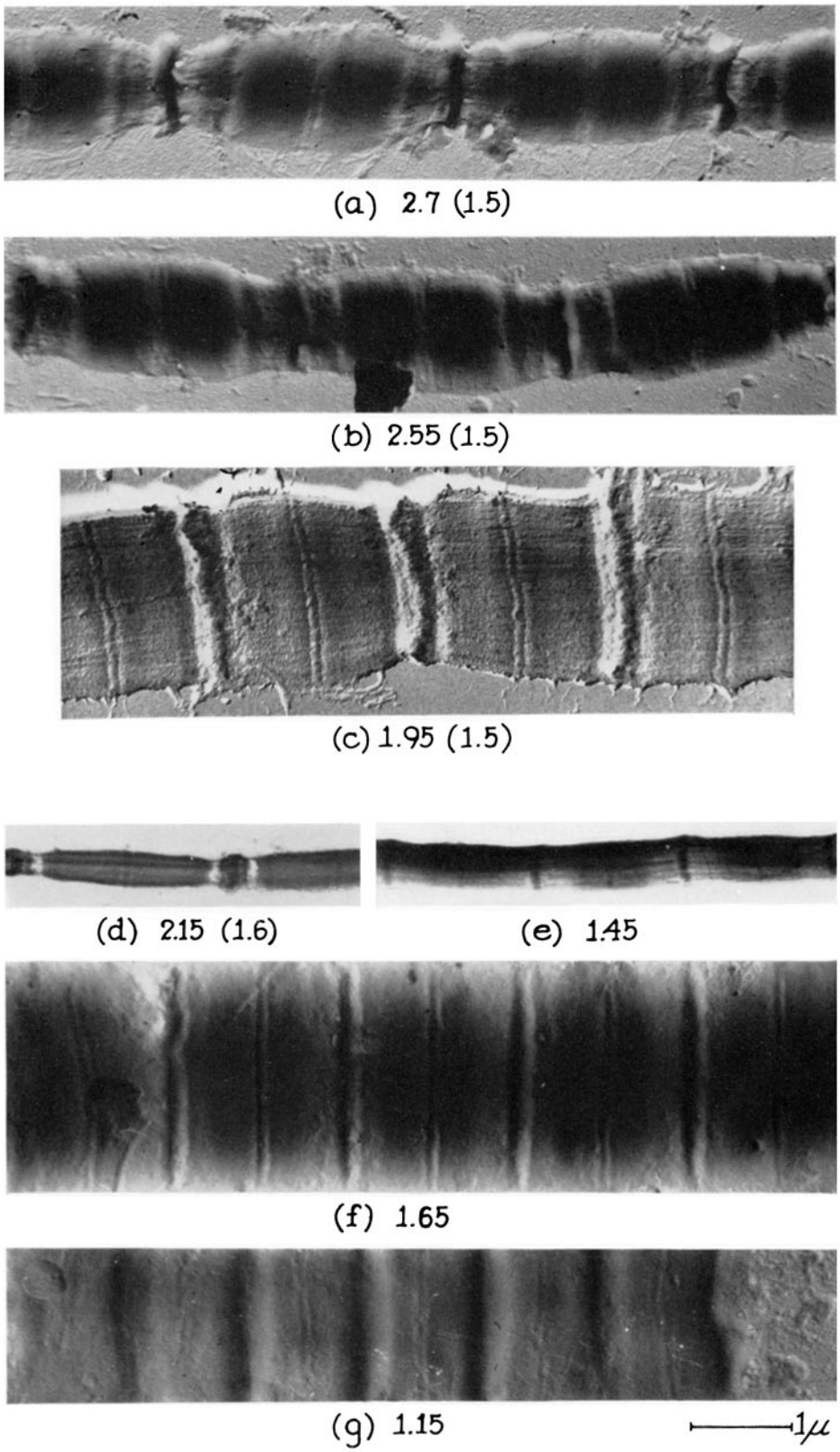

FIG. 2

(Locker: Striation patterns)