A Regular Actin Filament Lattice in a Vertebrate Smooth Muscle

Previous studies on the localization of contractile proteins in vertebrate smooth muscles led to contradictory results. A number of investigators who studied tissue sections by electron microscopy observed only randomly distributed thin filaments that were interpreted as consisting of actin[1–6]. Recently, some authors were able to demonstrate 2 types of filaments (comparable to that in cross-striated muscles) of which the thick filaments are believed to represent the myosin component[7–9]. ELLIOTT[10], using X-ray diffraction methods, found evidence only for actin filaments but not for thick myosin filaments. Further studies of ELLIOTT and LOWY[11] revealed an equatorial reflexion which was assigned to a regular lattice of the actin filaments. In electron microscopy, however, a regular packing of actin filaments was never detected in any vertebrate smooth muscle. This report demonstrates that under appropriate preparative techniques regions with a three dimensional order of the actin filaments can be observed in the intestinal muscle of the mouse. The previously proposed models of the contraction mechanism of vertebrate smooth muscles are discussed in the light of these findings.

Material and methods. The tissue studied was the circular muscle of the mouse large intestine. To obtain relaxed muscles, pieces of the intestine were placed in a modified Krebs solution[12] containing $1:10^4$ Suprarenin (Hoechst). The tissue was fixed for 30 min in 5% glutaraldehyde in cacodylate buffer, postfixed with OsO_4, dehydrated in graded alcohols and embedded in Epon 812. Sections were stained with 5% uranyl acetate in methanol and lead citrate and examined with a Siemens Elmiskop IA microscope.

Results and discussion. Figure 1 shows a cross-sectioned fiber of the circular muscle of the mouse's large intestine. The prominent structures in the fiber are points of 70 Å diameter, which represent cross sections through thin filaments. The filaments are not randomly distributed about the fiber, but clearly ordered into bundles of different sizes leaving areas free of filaments. Because the groups of filaments are often in contact with one another, the resulting impression is of a netlike filament pattern, which characterizes the cross-sectioned muscle fiber. In the filament-free areas different cell structures are seen: mitochondria, cross sections through cisternae of the endoplasmic reticulum and microtubules, granules with an average diameter of 120 Å (which probably represent glycogen reserves), and diffuse, sometimes filamentous appearing material of which the nature is obscure. Thick filaments, corresponding to myosin filaments of cross-striated muscle were not detected.

When analyzing the filament fields in detail, it will be obvious that the filaments are often arranged in a hexagonal lattice (Figure 2). The filaments are closely packed in these regions, and neighbouring filaments (diameter 70 Å) are separated by small spaces of 40–50 Å. Therefore, the centre to centre distance is 110–120 Å. Occasionally lateral interactions between filaments were observed, but lacking any regularity. It is supposed that they are due to the preparation. Besides the latticelike arrangements described above, other areas can be detected, especially

[1] J. S. T. MARK, Anat. Rec. *125*, 473 (1956).
[2] R. CAESAR, G. A. EDWARDS and H. RUSKA, J. biophys. biochem. Cytol. *3*, 867 (1957).
[3] H. Z. GANSLER, Zellforsch. mikrosk. Anat. *52*, 60 (1960).
[4] J. A. G. RHODIN, Physiol. Rev. *42* (Suppl. 5), 48 (1962).
[5] B. J. PANNER and C. R. HONIG, J. Cell Biol. *35*, 303 (1967).
[6] B. J. PANNER and C. R. HONIG, J. Cell Biol. *44*, 52 (1970).
[7] Y. NONOMURA, J. Cell Biol. *39*, 741 (1968).
[8] H.-G. HEUMANN, Zool. Anz., Suppl. *33* (1969).
[9] R. E. KELLY and R. V. RICE, J. Cell Biol. *42*, 683 (1969).
[10] G. F. ELLIOTT, Proc. R. Soc., Lond., Ser. B *160*, 467 (1964).
[11] G. F. ELLIOTT and J. LOWY, Nature, Lond. *219*, 156 (1968).
[12] B. BÜLBRING and K. GOLENHOFEN, J. Physiol. *193*, 213 (1967).

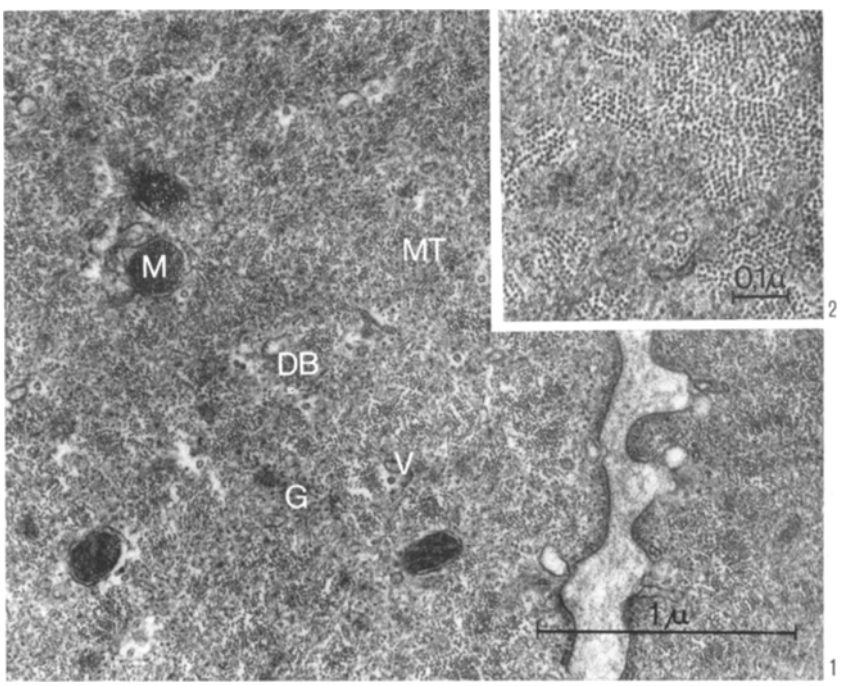

Fig. 1. Transverse section of a smooth muscle cell of the mouse large intestine. Thin filaments were distributed in a typical pattern. Structures such as mitochondria (M), vesicles of ER (V), microtubules (MT), glycogen granules (G) are easily identified. DB, dense body.

Fig. 2. Detail of cross-sectioned filaments demonstrating their arrangement in a regular lattice.

at the border of the filament groups, where the filaments are more irregularly distributed and more widely separated.

Randomly dispersed in the cytoplasme dark-stained areas are found in which filaments can with difficulty be identified. Longitudinal sections through the muscle cell show (Figure 3) that these structures are spindle-shaped bodies (often referred to as 'dense bodies') arranged parallel to the long axis of the cell. The filaments appear to converge upon and enter these bodies. The striking resemblance of the dense bodies to Z-discs of cross-striated muscles tempts one to assume that the function of both structures is the same.

The resulting distribution of actin filaments into regularly packed bundles corresponds to results of ELLIOTT and LOWY[11] obtained by X-ray diffraction methods. In the living unstimulated taenia coli muscle of the guinea-pig, the authors obtained an equatorial reflexion with a spacing of about 115 Å, which was, according to ELLIOTT and LOWY, the consequence of an orderly side by side packing of actin filaments. The striking agreement in the dimensions of the regular lattice, identified in electron microscope and with X-ray diffraction methods led one to conclude that identical structures were involved. A diffraction diagram similar to the taenia coli muscle of the guinea-pig could so far only be detected in an invertebrate smooth muscle, the anterior byssus retractor of *Mytilus* (ABRM)[13]. In cross-striated muscles, however, where orderly arranged filaments were only found neighbouring the Z-disc[14], the actin reflexion pattern was accordingly sparsely developed[15].

The new information about the organization of actin filaments in the smooth muscle of the mouse intestine yields some arguments for and against the hitherto proposed models of vertebrate smooth muscle contraction. According to PANNER and HONIG[5,6], all longitudinal filaments of gizzard smooth muscle are composed of actin; myosin exists in relatively unaggregated form (dimers or tetramers) between actin filaments. The authors suggested that contraction was imparted by numerous myosin dimers which formed linkages between actin filaments. Arguments against this hypothesis are: 1. The small myosin units were never seen in tissue sections; 2. The interactin distance found in the present work seems too small for functional myosin aggregates. KELLY and RICE[9] found in the relaxed taenia coli of the guinea-pig only thin filaments, randomly distributed within the fiber. In contrast, intermingled thick and thin filaments were found in contracted muscles. These results could be partly confirmed, since besides the type of fiber described above, others were found in which thick and thin filaments were present. Because these fibers are especially numerous in muscles contracted before fixation, it is probable that they represent fibers in state of contraction. KELLY and RICE conclude from their results that the myosin filaments may aggregate in contraction. Yet, the supposition of alternating aggregation and disaggregation of myosin molecules during contraction and relaxation seems to be contradictory to the speed of contraction[6].

To understand these discrepancies, the very fragile nature of the thick filaments has to be considered, too. If they are stabilized only in contracted fibers, where they form linkages with adjacent thin filaments, then they may survive electronmicroscopical preparation procedures. The present report provides some indications for this hypothesis. When comparing the distribution of filaments found in the intestine muscle of the mouse with that of an invertebrate smooth muscle, for instance the ABRM of *Mytilus*[16], one can recognize certain similarities. In both types of muscles one finds sets of thin filaments arranged in a regular lattice and attached to Z-elements (= I-band). In the areas between the thin filaments, thick filaments (A-band) are found in the ABRM in intestine muscle, however, diffuse material which perhaps consists of disintegrated myosin filaments. In the state of contraction, both types of muscle have a similar appearance. When suitable preparative procedures have been developed, the correctness of the proposed hypothesis might be proved[17].

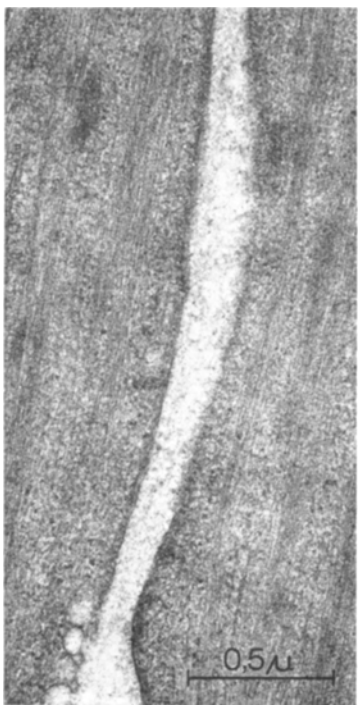

Fig. 3. Longitudinal sectioned muscle cells. Only thin filaments are present. 2 dense elongated bodies, which are traversed by filaments, are seen in upper left-hand corner.

Zusammenfassung. In den glatten Muskelfasern aus dem Dickdarm der Maus wurde eine charakteristische Verteilung der Aktinfilamente gefunden. Die Filamente sind zu Bündeln zusammengelagert, die parallel zur Faserlängsachse ausgerichtet sind. Innerhalb der Bündel liegen die Filamente in dichter hexagonaler Packung. Die Dimensionen des gefundenen Filamentmusters stimmen sehr gut mit den durch Röntgenbeugungsanalysen ermittelten überein.

H.-G. HEUMANN

Lehrstuhl für Zellenlehre der Universität, Berliner Strasse 15, D-69 Heidelberg (Germany),
16 April 1970.

[13] J. LOWY and P. J. VIBERT, Nature, Lond. *215*, 1254 (1967).
[14] G. G. KNAPPEIS and F. CARLSEN, J. Cell Biol. *13*, 323 (1965).
[15] H. E. HUXLEY and W. BROWN, J. molec. Biol. *30*, 383 (1967).
[16] H.-G. HEUMANN and E. ZEBE, Z. Zellforsch. mikrosk. Anat. *85*, 534 (1968).
[17] I am indebted to Prof. E. SCHNEPF for much valuable criticism and discussion, to Mrs. I. VOCKE for technical assistance.