MYOFILAMENTS IN SMOOTH MUSCLE OF GUINEA PIG'S TAENIA COLI

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The fine structure of myofilaments in vertebrate smooth muscle is still obscure because of the difficulty in demonstrating intracellular structure in the electron microscope. The present interest of this field is focused on the problem of how myosin filaments exist in the natural state. Although several reports have indicated the presence of two types of filaments, in some studies (1–3) the reported diameter of the purported thick filaments, less than 60–70 A, seemed to be too small, or in other studies the thick filaments were observed only under a specific condition, in the presence of collagenase (4) or in acid pH (5). At the present time, vertebrate smooth muscle is generally considered to consist of one type of filament containing both actin and myosin filament in the natural condition, from the evidence provided by electron micrographs (6–9) and X-ray diffraction diagrams (10, 11). The present studies were intended to clarify the fine structure of mammalian smooth muscle, paying special attention to the question of whether or not there exist two types of filaments under nearly physiological conditions.

MATERIALS AND METHODS

Sectioning: Several pieces of taenia coli were removed from a male guinea pig immediately after slaughter. Tissues were fixed in 4% glutaraldehyde buffered with 0.1 M cacodylate at pH 7.2 for 4 hr, washed with the same buffer, and postfixed in a 1% OsO_4-phosphate at pH 6.8 for 1 hr. After being washed with distilled water several times, tissues were stained with a 2% uranyl acetate for 1.5 hr before dehydration (the block staining method, 12, 13). Tissues were then dehydrated in graded ethyl alcohols and were embedded in Epon 812 as usual. Sections were stained with lead citrate (14).

Negative staining: The essential techniques used were according to Huxley (15). Several pieces of fresh taenia coli were homogenized at an appropriate speed in a Politoron homogenizer (Politoron Company, Switzerland) for 15 sec in the modified relaxing medium, i.e. 0.15 M KCl, 1 mM $NaHCO_3$ (pH 7.8), 4 mM ATP, and 5 mM $MnCl_2$. Manganese ion was used for inhibiting the excitation and the contraction of smooth muscle in concentrated potassium solutions (16). Homogenate was dropped on the collodion-filmed grid coated with carbon and was

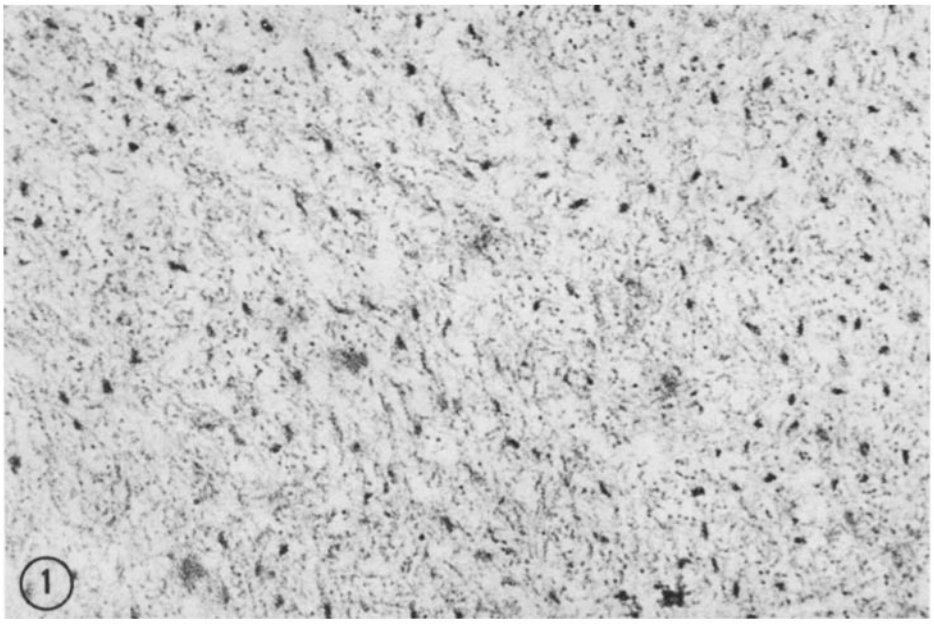

FIGURE 1 Cross section of smooth muscle. Two types of filament are clearly observed. Notice the random distribution of filaments. Dense bodies are poorly developed. × 67,500.

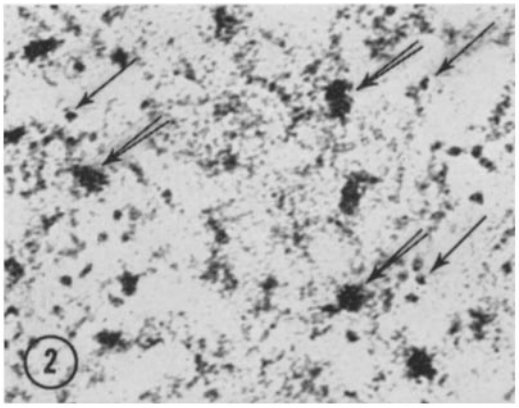

FIGURE 2 Larger magnification of cross section. Thick filaments (demonstrated by double arrow) and thin filaments (single arrow) are randomly distributed. × 165,000

stained directly with 2–3% uranyl acetate without previous washing with solvent.

RESULTS AND COMMENTS

In the cross section two types of filament were clearly observed as shown in Fig. 1. However, the filaments were distributed in a very disordered manner and no definite relation could be demonstrated between thick and thin filaments. In a larger magnification it was noticed that the thick filament was composed of several subunits (demonstrated by double arrows) among the randomly distributed thin filaments (single arrow) (Fig. 2). The minimum diameters of 1087 filaments were plotted in Fig. 3. Two peaks of population were obtained, as expected from the "two types of filament" principle of muscle. One peak is distributed in a rather narrow range of 40–70 A, and the other broadly 120–170 A. These sizes are in good accord with those from striated muscle. The ratio of the number of thick filaments to the number of thin filaments was nearly $\frac{1}{5}$, from the above population diagram. This value, of course, was not conclusive since the number of the thin filaments might be miscounted becasue of their small size.

Although the evidence in the longitudinal section was not so clear as that in the cross section, the thick filament could be distinguished from the thin filament as shown in Fig. 4. Filaments were arranged parallel to the longitudinal axis of each cell.

Fig. 5 shows the presence of two types of myofilaments negatively stained. The structure of the

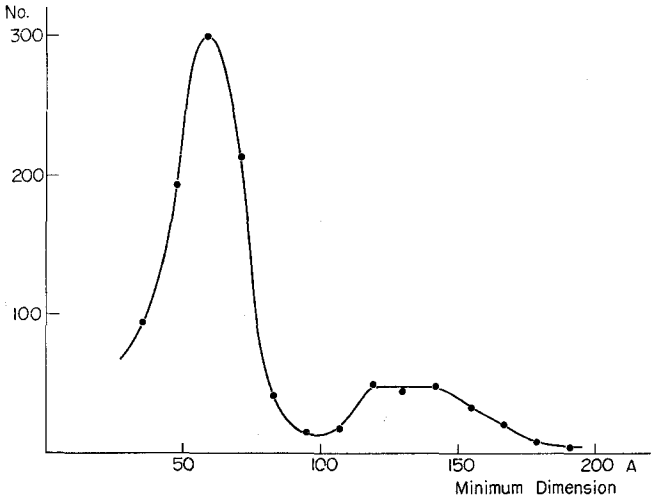

FIGURE 3 Frequency distribution of minimum diameters of myofilaments in cross section. 1087 filaments were measured. Two populations of diameters are noted from the diagram.

FIGURE 4 Longitudinal section of smooth muscle. Two types of filaments are distinguished. × 60,000.

thin filaments was essentially the same as that in the previous reports (4, 6). The thick filaments showed the central core, the lateral projections, and the tapering ends like those from skeletal muscle (15). Compared with the separated filaments of the skeletal muscle, the width of the thick filament measured at the central core area, 130–180 A, was larger, and the length was shorter, 0.7–1.0 μ, even in the case which had the tapered form at both ends. In some places a thin filament was lying alongside a thick filament, and the projections of the thick filament were touching the thin filament (indicated by arrows in Fig. 5); this appears to be evidence of interaction of the thick and thin filaments.

It may be concluded from the above results that there exist two types of myofilaments in smooth muscle of taenia coli under natural conditions. Generally speaking, it is difficult to demonstrate clearly myofilaments of vertebrate smooth muscle in sections, due to the difficulty of fixing or staining the intracellular structure. The block staining method seems to be a good one to overcome such a difficulty. The interaction of thick and thin fila-

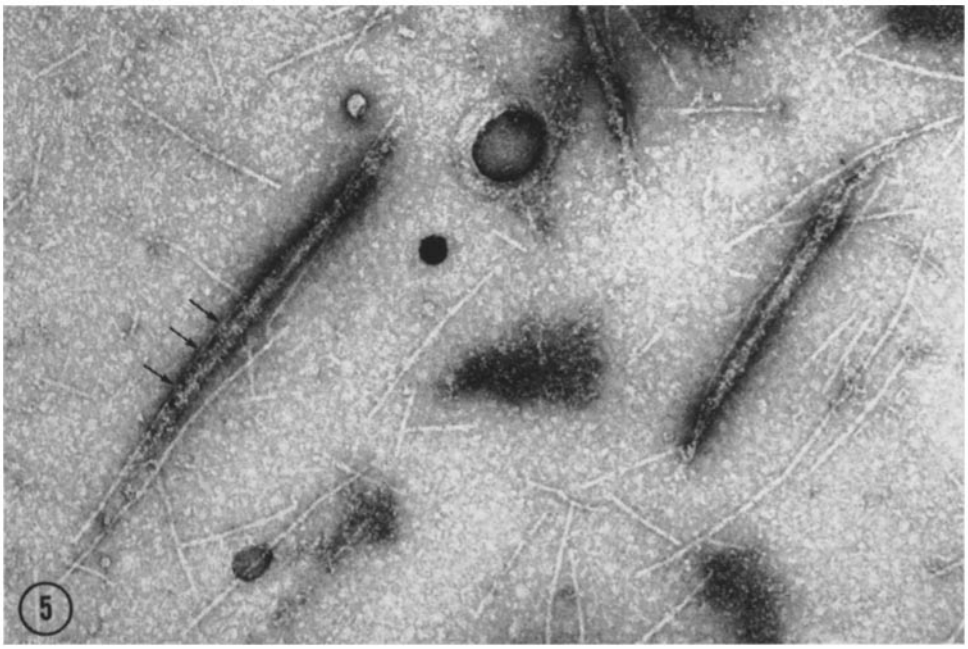

FIGURE 5 Separated filaments from taenia coli homogenized in the modified relaxing medium. Negatively stained with 2% uranyl acetate. Two types of filaments are seen. Arrows indicate the interaction of thick and thin filaments. × 90,000.

ments in vertebrate smooth muscle occurs easily in the process of ATP exhaustion. Negative staining experiments, then, should be conducted as soon as possible after homogenization of materials. Under such a caution, thick filaments were clearly observable together with thin filaments. These observations, especially on shapes and sizes of filaments, are in good agreement with the recent results obtained by Kelly and Rice (5) and by Rice et al. (17), though the material and the conditions of preparation were quite different. X-ray diffraction analysis did not show a typical myosin pattern (10, 11); this may be explained by the relatively small number of thick filaments compared with the number of thin filaments, or by the inconsistency of the filament axis between different cells even though there is a parallel arrangement of filaments in each cell.

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