

## Myofilaments from Smooth Muscle.\* (19158)

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Myofilaments with a 400 Ångstrom period have been described in skeletal muscle(1-3) and in cardiac muscle(4). This paper is a preliminary report of the finding in smooth muscle of filaments resembling those of cardiac and skeletal muscle with respect to dimensions and periodic structure.

**Method.** Five millimeter wide rings were cut from the small intestine of freshly killed turtles and chickens, inverted, and stretched to their approximate maximum physiologic length. The mucosa was stripped off and the muscle rings were fixed in 10% neutral formalin or frozen rapidly in isopentane chilled in liquid nitrogen. The frozen specimens were then dehydrated for two weeks in a vacuum cryostat at  $-65^{\circ}\text{C}$ . The formalin fixed material was washed 24 hours in running tap water. Small portions of muscle treated by either method were suspended in water and fragmented with a micro-blender. The resulting suspension was examined with a phase microscope and found to consist of fragments and groups of smooth muscle cells mixed with a small amount of connective tissue fibers and unrecognizable material. Irrelevant material was removed by differential centrifugation and drops of the final suspension were placed on Formvar or collodion films on standard electron microscope grids. The preparations were air dried and shadowed, usually with nickel, and examined with an electron microscope (RCA EMU-2C).

**Observations.** Fragments of smooth muscle

fibers could readily be identified at low magnification upon visual inspection of the fluorescent screen. Figure 1 is an electron micrograph of such a fragment. It reveals the presence of fine filaments extending parallel to the long axis of the muscle cell (B). They range from 150 Å to 230 Å in diameter and appear to be embedded in extrafilamentary muscular substance which appears homogeneous at low magnifications.

Electron micrographs taken at a higher magnification show a definite periodicity that can be readily made out in many of these. The periods range from 322 Å to 743 Å. However, about 90% of all the measurements range from 322 Å to 550 Å. The average period length is 448 Å with a median of 432 Å.

In Fig. 1 there may be seen a number of coarser fibers of a different nature, many of which run crossways to the primary axis of the muscle fragment (A). These are identified as collagen and distinguished from myofilaments by their relatively greater thickness (diameter 400 Å to 550 Å) and their consistently greater longitudinal periodicity (average 660 Å). In many of them the subbanding and polarized nature typical of collagen(5) can be recognized. However, it is not always easy to distinguish myofilaments from collagen filaments, especially in the range where the observed measurements overlap. Similar filaments (Fig. 2 and 3) may be seen in fragmented formalin fixed chicken gizzard.

No clear cut myofibrils, such as readily break out on fragmentation of skeletal or cardiac muscle, have been noted in the present smooth muscle material. Fibrils resembling the "paramyosin" of clam muscle(1,6) have not been observed.

**Summary.** Myofilaments have been found in the smooth muscle of the gut of the turtle

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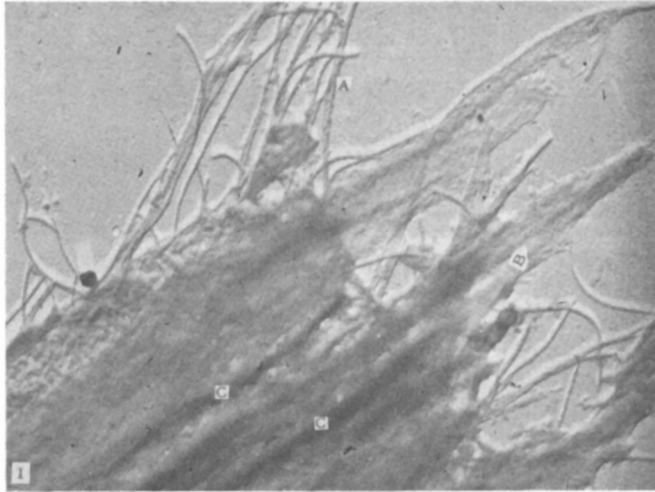


FIG. 1. Electron micrograph of a portion of a smooth muscle fiber from the gut of a turtle, freeze dried, and shadowed with nickel at an angle of 4:1. The greater number of fibers extending to the right and left of the central portion at 'A' are collagen, whereas groups of fibers at 'B' are myofilaments. Darkened longitudinal ridges at 'C' may constitute bundles of filaments embedded in extrafilamentary material and they are visible in fixed material with phase and ordinary bright-field microscopes and would be referred to as smooth muscle myofibrils. Collodion film. 10800  $\times$ .

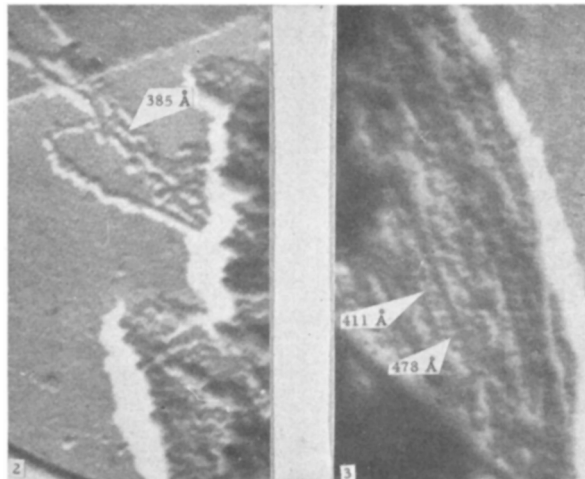


FIG. 2. Electron micrograph of the broken end of a smooth muscle fiber from a chicken gizzard. Formalin fixation, shadowed with nickel at an angle of 6:1. Collodion film. 24200  $\times$ .

FIG. 3. Electron micrograph of a thin sheet of fibers from smooth muscle cell in chicken gizzard. Formalin fixation, shadowed with nickel at an angle of 4:1. Collodion film. 24200  $\times$ .

and chicken. They resemble closely the corresponding filaments of skeletal and cardiac muscle, revealing a repeating period of about 400 Å. and ranging in diameter from 150 Å to 230 Å. Interfilamentary material is also

observed in smooth muscle; but is fairly evenly distributed, and not disposed in repeating bands, as in striated muscle.

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