CYTOPLASMIC FILAMENTS IN DEVELOPING AND ADULT VERTEBRATE SMOOTH MUSCLE

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ABSTRACT

An extensive study of adult and developing smooth muscle has revealed the widespread occurrence of a distinct filament with an average diameter of about 100 A (termed the 100 A filament). Unlike that of myofilaments, their appearance in longitudinal section is uniform, but in transverse section they have a round profile, occasionally exhibiting a less electron-opaque core. The 100 A filaments are almost invariably preserved under a variety of fixation procedures, whereas myofilaments, particularly the thicker filaments, are preserved inconsistently. The 100 A filaments appear to be randomly oriented throughout the cytoplasm, either singly or in small groups, although they are sometimes concentrated in the juxtanuclear region of the smooth muscle cells. The intimate association of 100 A filaments with dark bodies, in both developing and adult smooth muscle cells, may indicate that these filaments either play a role in dark body formation or, at least, constitute a part of the dark body.

The 100 A filaments are conspicuous in developing smooth muscle cells and occasionally form networks or clusters; they appear to decrease in relative number as maturation proceeds, but considerable numbers are still present in adult tissue.

INTRODUCTION

The well documented interdigitating sliding filament mechanism of contraction, originally proposed for striated muscle (14, 15), has led to the expectation of two myofilament types in vertebrate smooth muscle. However, unlike the myofilaments of striated muscle, smooth muscle myofilaments appear to be difficult to preserve consistently, a factor responsible not only for the current controversy as to the actual presence of thick myosin filaments (8, 9, 26, 28, 29, 35), but also for the wide variation in reported diameters of both thick and thin myofilaments (3). This variation, together with the anticipation of the presence of only two filament types, could be sufficient to mask the presence of other filaments, whether contractile in nature or not.

A specific class of filaments with an average diameter of 100 A was noted in smooth muscle cells grown in culture (4). These filaments were tentatively termed "100 A filaments" because of their similarity to those reported by Ishikawa et al. (17) in maturing striated muscle in culture. Although it was suggested that the 100 A filaments play a role in dark body formation or at least form a part of the substructure of the dark body (4), their nature and significance remain obscure. These 100 A filaments have now been found to occur in a variety of embryonic and adult smooth muscle cells. Because they form a considerable proportion of the total filament component of smooth muscle cells, they may have inadvertently been included within the myofilament category in past analytical studies.

We have, therefore, applied several methods of preservation in order to define the 100 A filament and to distinguish it from myofilaments.

MATERIALS AND METHODS

I. Developing Smooth Muscle Cells

Smooth muscle cells from the gizzard of chick embryos maturing both in vivo and in vitro were used.

(*a*) Cultures of 10–16-day old embryonic gizzards grown for 5 days on collagen-coated Falcon cooper dishes (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.) were fixed in collidine-buffered 2.5% glutaraldehyde (pH 7.4) followed by postfixation in unbuffered 2% OsO_4. They were then block-stained with 1–2% aqueous uranyl acetate solution for 1 hr. A more detailed description of the preparation procedure has been reported previously (4).

(*b*) The gizzards of 8–10-day old embryos were fixed by dropping fixative on the *in situ* organs for 5 min. The tissue was then dissected and further fixed for 1 hr by the same procedure described above.

II. Adult Smooth Muscle Cells

Smooth muscle from a variety of vertebrate tissues, including the adult chick gizzard, the guinea pig and zebra finch (*Taeniopygia castanotis*) ureter, the rat vas deferens and the guinea pig taenia coli, were examined. The tissues were fixed by dropping the following fixatives on the *in situ* organs for 5 min. The tissue was then dissected, cut into small blocks 2 mm x 2 mm, and further fixed in:

OsO_4-FIXATION: Tissues were fixed in 2%, phosphate-buffered OsO_4 (pH 7.4) for 2 hr.

OsO_4-GLUTARALDEHYDE–OsO_4 FIXATION: Tissues were fixed initially with 1%, phosphate-buffered OsO_4 (pH 7.4) for 1 hr, further fixed with 4% glutaraldehyde for 1 hr, and finally fixed with OsO_4 for 1 hr (T. Kanaseki, M. Imaizumi, and Y. Uehara, in preparation).

GLUTARALDEHYDE FIXATION: Tissue was fixed in 2–4% phosphate-buffered glutaraldehyde (pH 7.4) for 1 hr, washed in buffer for 1–3 hr, and postfixed in OsO_4 for 1 hr (33).

MODIFIED KARNOVSKY'S FIXATION: Tissue was fixed with a phosphate-buffered mixture of 2% paraformaldehyde and 2% glutaraldehyde (pH 7.4) for 1 hr, washed in buffer overnight, and then post-fixed in OsO_4 for 1 hr (20).

Most of the fixed tissue blocks were block-stained in a 2% aqueous uranyl acetate solution for 1 hr. After brief dehydration through a graded series of alcohol, the tissue blocks were embedded in Araldite (24). Thin sections were cut on a Huxley-Cambridge or LKB ultramicrotome with glass knives. Sections were examined with a Hitachi Hu 11B electron microscope after single staining with lead citrate (37), or double staining with lead citrate and uranyl acetate.

OBSERVATIONS

I. 100-A Filaments in Cultured Chick Gizzard

Free ribosomes, rough surfaced endoplasmic reticulum, and Golgi complexes are conspicuous in cultured chick gizzard cells (4). In an early stage of maturation (10 day embryo, cultured 5 days), smooth muscle cells contained two different filament types that were scattered throughout the cytoplasm (Fig. 1). One type tended to form fairly well organized small bundles, often associated with dark bodies. These filaments had a poorly defined profile and an uneven electron opacity throughout their length. They ranged from 30 to 80 A in diameter, with an average of about 50 A, and they were similar to the thin myofilaments that are generally considered to consist of actin (21, 23, 26, 28).

The other type of filament was thicker, and each filament maintained a fairly uniform diameter, ranging from 80 to 110 A (these filaments are described as "100 A filaments"). They were scattered randomly, with respect to the fiber axis, throughout the cytoplasm and occasionally penetrated myofilament bundles without showing any particular orientation to them. They had a distinct boundary and a uniform electron opacity along their length. The 100 A filaments often exceeded 3 μ in length in a single section.

With further maturation (16 day embryo, cultured 5 days), myofilaments increased in number. The 100 A filaments were well-defined, and were both within myofilament bundles and at their periphery (Fig. 2).

The 100 A filaments occasionally formed large clusters or masses (Fig. 3, see also reference 4). There were few organelles within these clusters, except for particles with a diameter of 200–250 A, similar to ribosomes, some of which appeared to be closely associated with the 100 A filaments. The 100 A filaments sometimes emerged from the clusters and mingled with adjacent myofilaments (Fig. 3). Diffuse rodlike structures, 200–500 A in diameter and up to 2000 A in length, similar to dark bodies and associated with developing and mature myofilament bundles, were common within the clusters of 100 A filaments. Some of the 100 A filaments appeared to penetrate the dark body-like structures (Fig. 4). Longitudinal striations seen in the matrix of these structures were possibly 100 A filaments. The texture of the dark

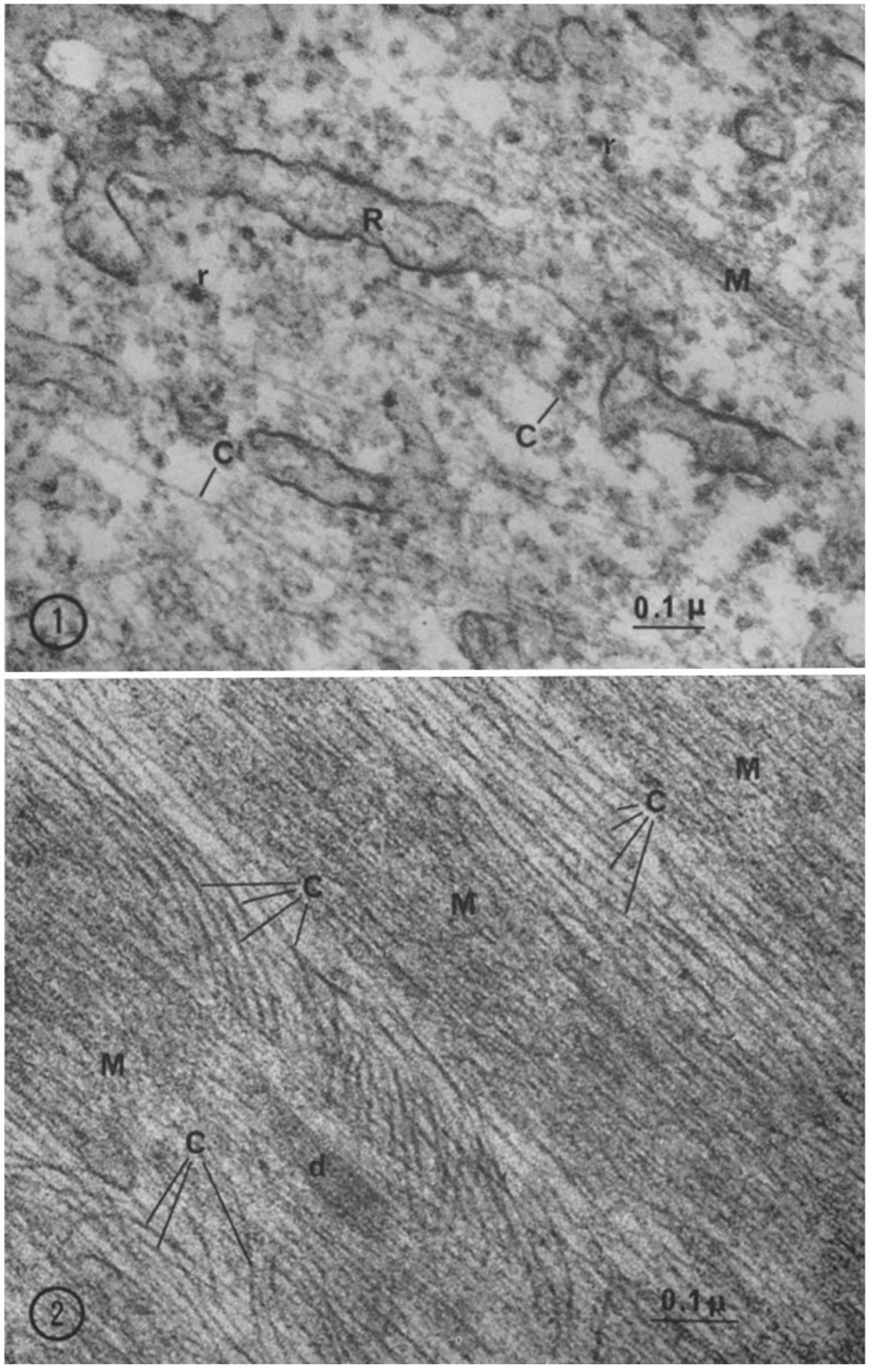


FIGURE 1 Cultured smooth muscle, early stage of maturation, longitudinal section (chick gizzard, 10-day old embryo, cultured 5 days). Note: filaments 80–100 A in diameter (100 A filaments) (*C*) and thin filaments (*M*) 30–80 A in diameter in a small bundle (myofilaments). Free ribosomes (*r*); rough surfaced endoplasmic reticulum (*R*). Glutaraldehyde fixation. × 110,000.

FIGURE 2 Cultured smooth muscle, more advanced stage of maturation, longitudinal section (chick gizzard from 16-day old embryo, cultured 5 days). Myofilament bundles (*M*) contain thin filaments 30–80 A in diameter and have associated dark bodies (*d*). 100 A filaments (*C*) are between these bundles or intermingled with them, in an irregular manner. Glutaraldehyde fixation .× 130,000.

bodies suggests that they may be largely composed of 100 A filaments.

II. 100-A Filaments in Embryonic Chicken Gizzard

Developing smooth muscle cells taken directly from the gizzards of 8–10-day old chick embryos were similar to the cultured cells (Fig. 5). The general structure of embryonic smooth muscle cells of chick gizzard has been described by Bennett and Cobb (1).

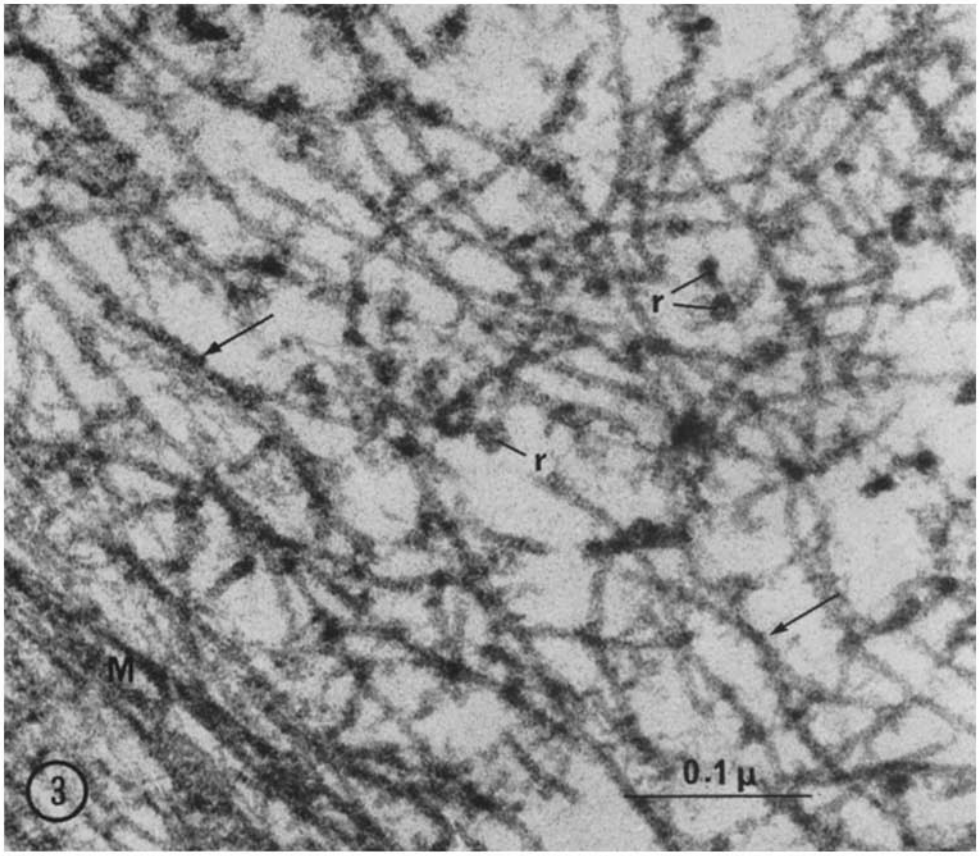
At an early stage, the myofilaments appeared to consist of a single type, measuring from 30 to 80 A in diameter. They tended to form bundles, often aligned with the long axis of the muscle cell. In addition, 100 A filaments were again identified, with a distribution and relationship similar to those described for cultured smooth muscle cells (Fig. 5). Occasionally, the 100 A filaments were in the form of networks or clusters which were also quite similar in appearance to those described above in cultured smooth muscle cells (Fig. 6). Within these networks, vesicular or vacuolar membranous systems and lipid droplets were sometimes seen. In more advanced smooth muscle cells, both in cultured and in embryonic tissues, myofilaments gradually increased in number and became arranged in a much more organized fashion. Sometimes, thicker filaments with a diameter of 150–200 A were encountered in these bundles; these filaments resembled the thicker myofilaments described by several authors in adult smooth muscle cells (6, 21, 27).

Microtubules, about 250 A in diameter, were present in the smooth muscle cells. No spatial relationship between these microtubules and 100 A filaments was seen.

III. The 100-A Filaments in Adult Smooth Muscle Cells

Smooth muscle cells of adult tissue contained a few cytoplasmic organelles and abundant myofilaments which occupied nearly the whole area of the cytoplasm. Compared to the myofilaments, the 100 A filaments were few, but still they could be identified in a variety of smooth muscle cells, including those or chick gizzard, guinea pig and finch ureter, rat vas deferens, and guinea pig taenia coli. In order to compare the effects of fixatives on 100 A filaments and myofilaments, tissues were preserved by several different fixation procedures. In some tissues which were fixed by a modified Karnovsky's method or with glutaraldehyde, most of the filaments preserved were thin, 40–80 A myofilaments; very few thick myofilaments (150–200 A) were seen (Fig. 7). As indicated in this figure, the 100 A filaments were identified among the thin filaments by their larger size and more well-defined rounded profiles, features which are more apparent at higher resolution (Fig. 8). However, in some tissues, fixed with glutaraldehyde and OsO_4, there were thicker filaments with a diameter of from 150 to 200 A in addition to the thin myofilaments, and they closely resembled the thicker myofilaments which have been suggested to represent myosin in filamentous form (6, 21, 27). The 100 A filaments were also preserved, being nearly intermediate in size between the thin and thick myofilaments (Fig. 9), and their smoother round profiles in transverse section were conspicuous against the irregular, thicker myofilaments. The 100 A filaments were better defined in tissue prepared by the triple fixation procedure, but under these conditions most of the myofilaments that were preserved were thicker ones, and only a few thin filaments were seen (Fig. 10). Furthermore, 100 A filaments were particularly conspicuous in some smooth muscle cells of tissue fixed with osmium tetroxide without block staining. These cells were frequently encountered in the central region of large tissue blocks, where myofilaments were poorly preserved and the cytoplasm was occupied by fine granular material which may have resulted from the disintegration of myofilaments (Fig. 11). There, the 100 A filaments could be traced for up to 5 μ along their rather wavy course, and again they appeared to have a close association with dark bodies, sometimes passing from one dark body to another. Some of the 100 A filaments appeared to branch, but this may be due to superimposition of filaments.

As in developing tissue, 100 A filaments in adult tissue appeared to be in particular association with dark bodies, and were sometimes observed to be completely embedded within the matrix of dark bodies and to have an electron-transparent halo around them (Fig. 12). The 100 A filaments were usually distributed rather randomly throughout the cytoplasm, sometimes singly and sometimes in small bundles, although they were occasionally observed to be aggregated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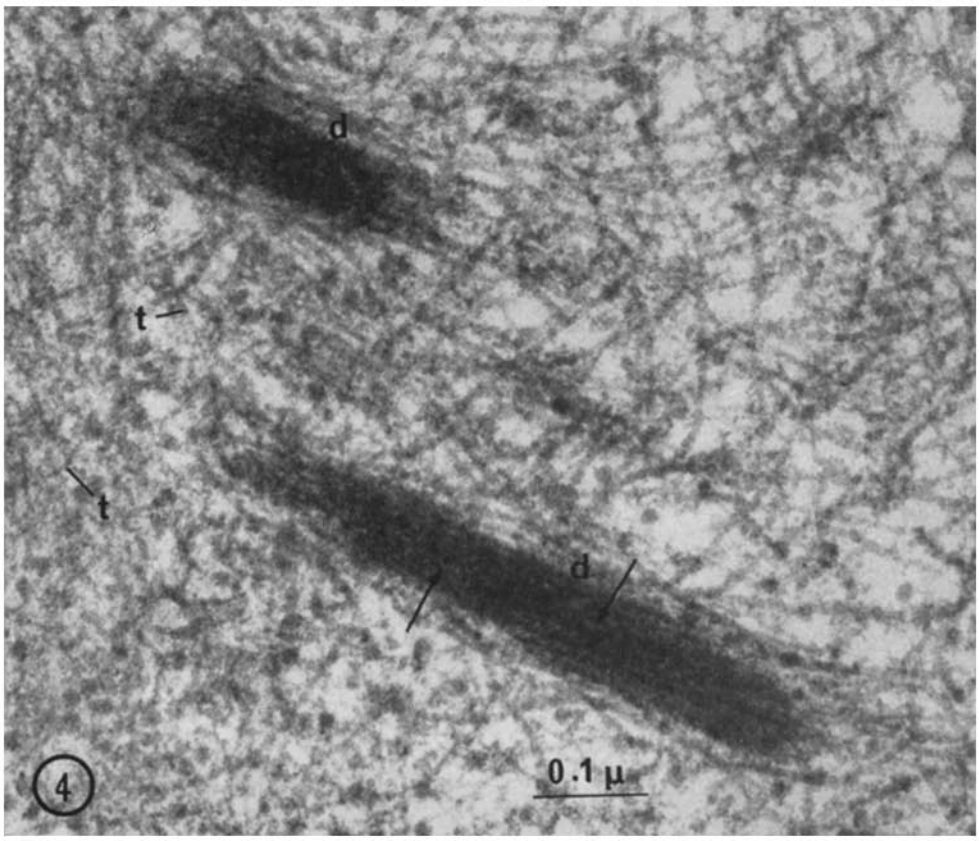
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in the juxtanuclear region of the cell, where a number of mitochondria and other cytoplasmic organelles were present (Fig. 13). At higher magnification, some of the 100 A filaments appeared to have a less electron opaque core, giving the impression that they were hollow, cylindrical structures (Inset of Fig. 13). However, the 100 A filaments were much smaller than microtubules, which are usually 200–250 A in diameter. This is consistent with results obtained for intermediate-sized filaments in striated muscle cells maturing in culture (17).

The number of 100 A filaments in smooth muscle cells appeared to vary not only between species but also between different organs of the same species, the largest number being found in smooth muscle cells of the finch ureter. However, this estimate is based on superficial observation, and statistical studies are needed.

In the endothelial cells of capillaries within smooth muscle tissue, similar filaments were also encountered. Their size and appearance were quite consistent with the 100 A filaments reported above, and they tended to be in the form of small bundles (Fig. 14).

DISCUSSION

The present study has indicated the widespread occurrence of a class of filaments with an average diameter of 100 A (described as "100 A filaments") in a variety of developing and adult vertebrate smooth muscle cells. These filaments are different in size and appearance from either thin filaments, which have generally been considered to consist of actin, or thicker filaments, which have been suggested to represent myosin in filamentous form (6, 21, 27).

It has been shown that myofilaments, particularly the thicker filaments, are highly labile and, therefore, inconsistent in appearance, depending on the preservation procedure applied (3, 21) and/or on the state of stretch or contraction of the muscle cells (21, 23). In contrast to the thick myofilaments, the 100 A filaments reported here were preserved consistently and retained their nearly constant size and appearance throughout a variety of fixation procedures. They were preserved even in tissue fixed with a single osmium tetroxide fixation, where in some cells almost all myofilaments appeared to disintegrate into a fine granular material. Their involvement in a sliding filament mechanism of contraction is highly unlikely, because of their random orientation and scattered distribution. The widespread occurrence of similar filaments in other cell types, such as rat liver epithelial cells (2), cells of blood forming organs (36), mononuclear phagocytes (7), and endothelial cells (Fig. 14), may also suggest that they are not in the myofilament category.

In a recent paper on the organization of myofilaments in smooth muscle, Lowy and Small (23) described the presence of round filaments which appear to be equivalent to the 100 A filaments reported here. However, they suggested that these were artificial products derived from the disaggregation of myosin ribbons. This seems unlikely because, with a variety of fixation methods, the number of 100 A filaments was not found to be in inverse proportion to that of the thick filaments; there appeared to be no particular spatial relationship of the 100 A filaments to thicker filaments; 100 A filaments were occasionally accumulated in the juxtanuclear region of the cell, a region containing no myofilaments; and there seldom occurred any linear arrangement of 100 A filaments in transverse section. Also, little evidence of any approximation or fusion of adjacent 100 A filaments has been noted.

Several authors have indicated that myofila-

FIGURE 3 Portion of network of 100 A filaments in cultured smooth muscle (chick gizzard from 10-day old embryo, cultured for 5 days). Some of the 100 A filaments (*arrows*) are intermingled with a myofilament bundle (*M*). Electron-opaque particles (*r*), presumably ribosomes, sometimes appear to be in in close association with 100 A filaments. Glutaraldehyde fixation. × 250,000.

FIGURE 4 Longitudinal section of dark-body-like structures (*d*) occasionally seen in cultured smooth muscle (from 10-day old chick gizzard, 5 days in culture). 100 A filaments appear to penetrate these structures, giving them the appearance of having longitudinal striations within their matrices (*arrows*). A few thin filaments (*t*) can be seen in the vicinity of these structures, but appear to have no association with them. Glutaraldehyde fixation. × 150,000.

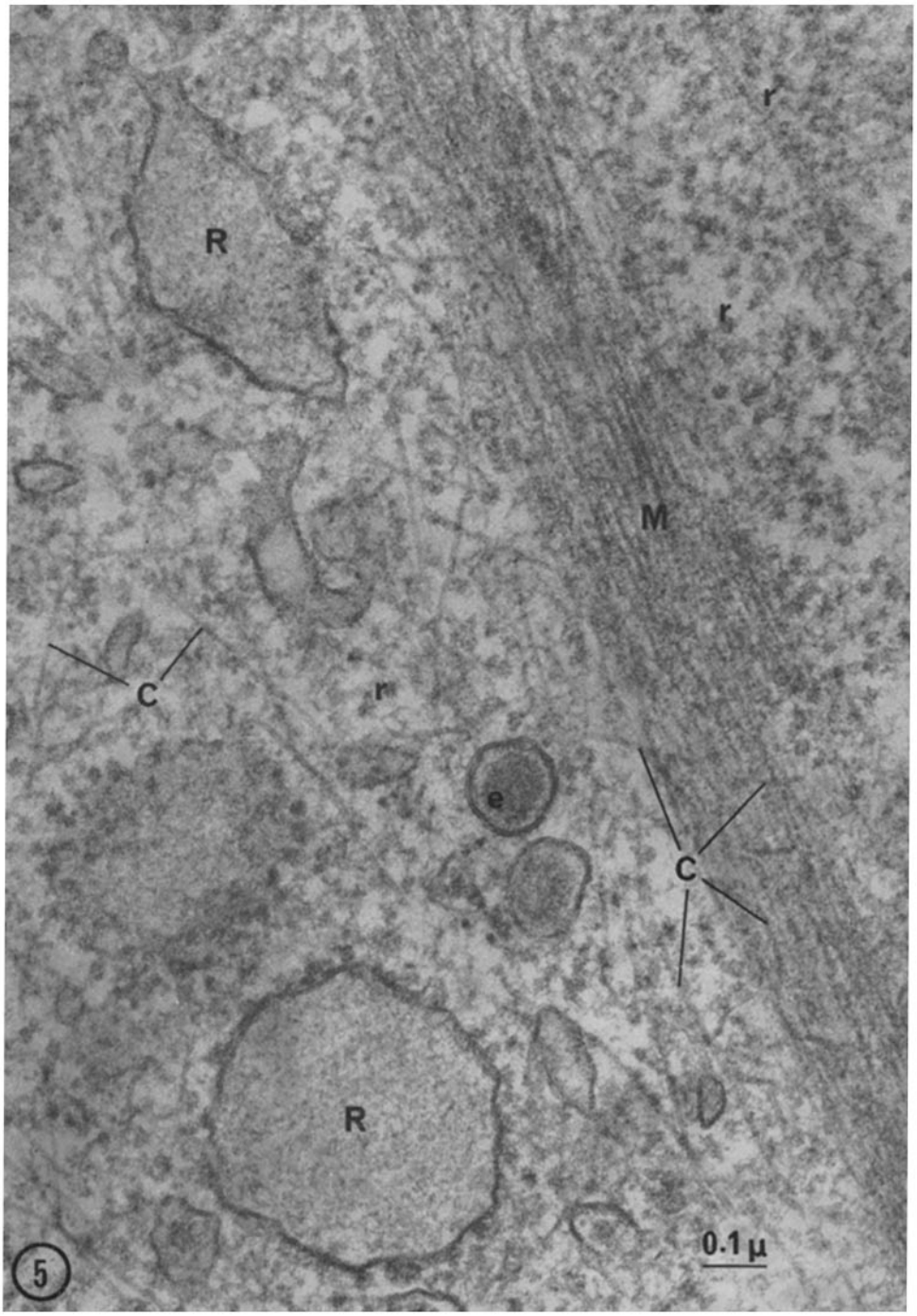


FIGURE 5 Longitudinal section of an embryonic smooth muscle cell of chick gizzard (10-day old embryo). 100 A filaments (*C*) are randomly orientated throughout the cytoplasm, some of them passing into bundles of myofilaments (*M*), 40–30 A in diameter. Free ribosomes (*r*); dilated rough surfaced endoplasmic reticulum (*R*) containing flocculent material; membrane-bounded electron-opaque bodies (*e*). Glutaraldehyde fixation. × 88,000.

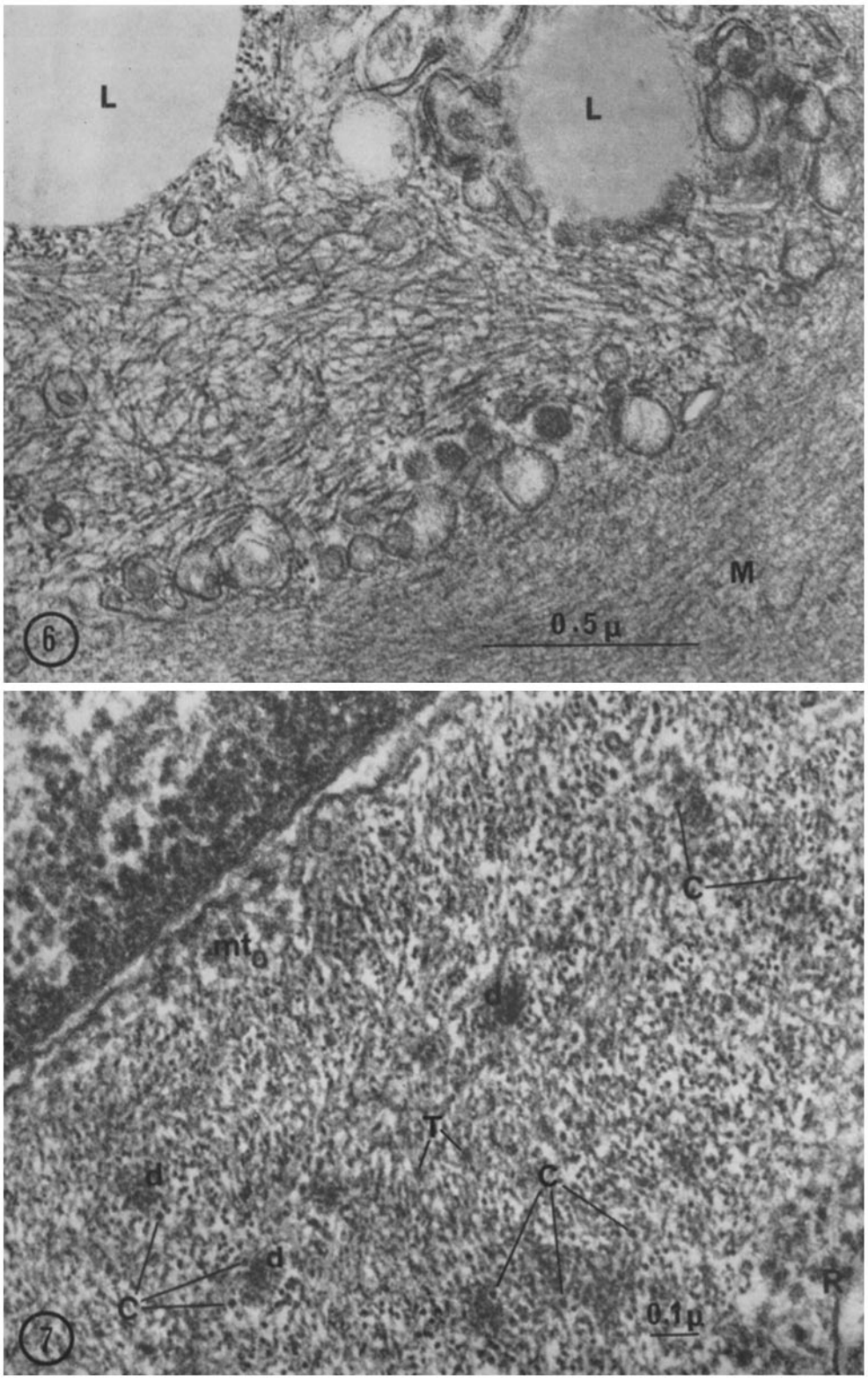


FIGURE 6 Transverse section of developing smooth muscle cell (gizzard, 10-day old chick embryo), showing aggregation of 100 A filaments adjacent to a myofilament bundle (*M*). Some of the 100 A filaments appear closely applied to the surface of a lipid droplet (*L*) (upper left corner). Glutaraldehyde fixation. × 65,000.

FIGURE 7 Transverse section of a smooth muscle cell from guinea pig taenia coli fixed with a modified Karnovsky's method, containing a number of thin myofilaments and few thick myofilaments (*T*). 100 A filaments (*C*) can be clearly distinguished from myofilaments by their size and appearance. These 100 A filaments appear to be in close association with dark bodies (*d*). *R*, rough surfaced endoplasmic reticulum; *mt*, microtubules. × 75,000.

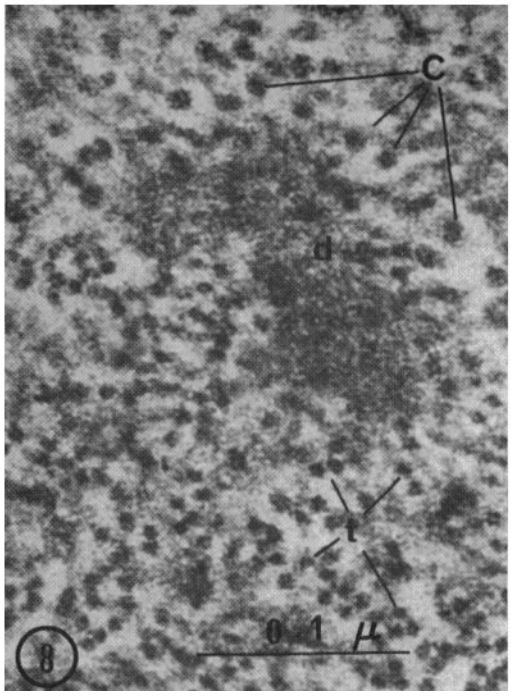
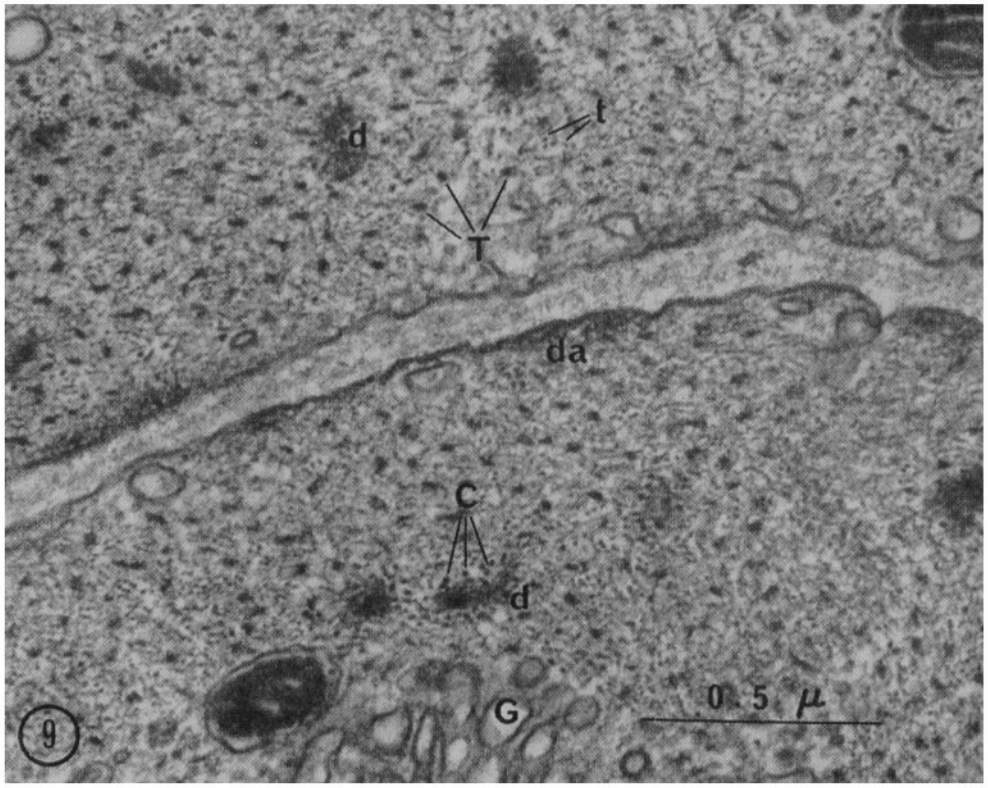


FIGURE 8 Transverse section of smooth muscle cell from guinea pig taenia coli. 100 A filaments (*C*) can be easily distinguished from thin myofilaments (*t*) by their different diameters. Fixation: modified Karnovsky's method. *d*, dark bodies. × 280,000.

FIGURE 9 Transverse section of smooth muscle cells, adult finch gizzard; fixation 4% glutaraldehyde. In addition to both thin (*t*) and thick (*T*) myofilaments, 100 A filaments (*C*) can be identified. These are intermediate in size between the two myofilament types. Note apparent close association of 100 A filaments with dark bodies (*d*). *da*, dense area. *G*, Golgi complex. × 65,000.



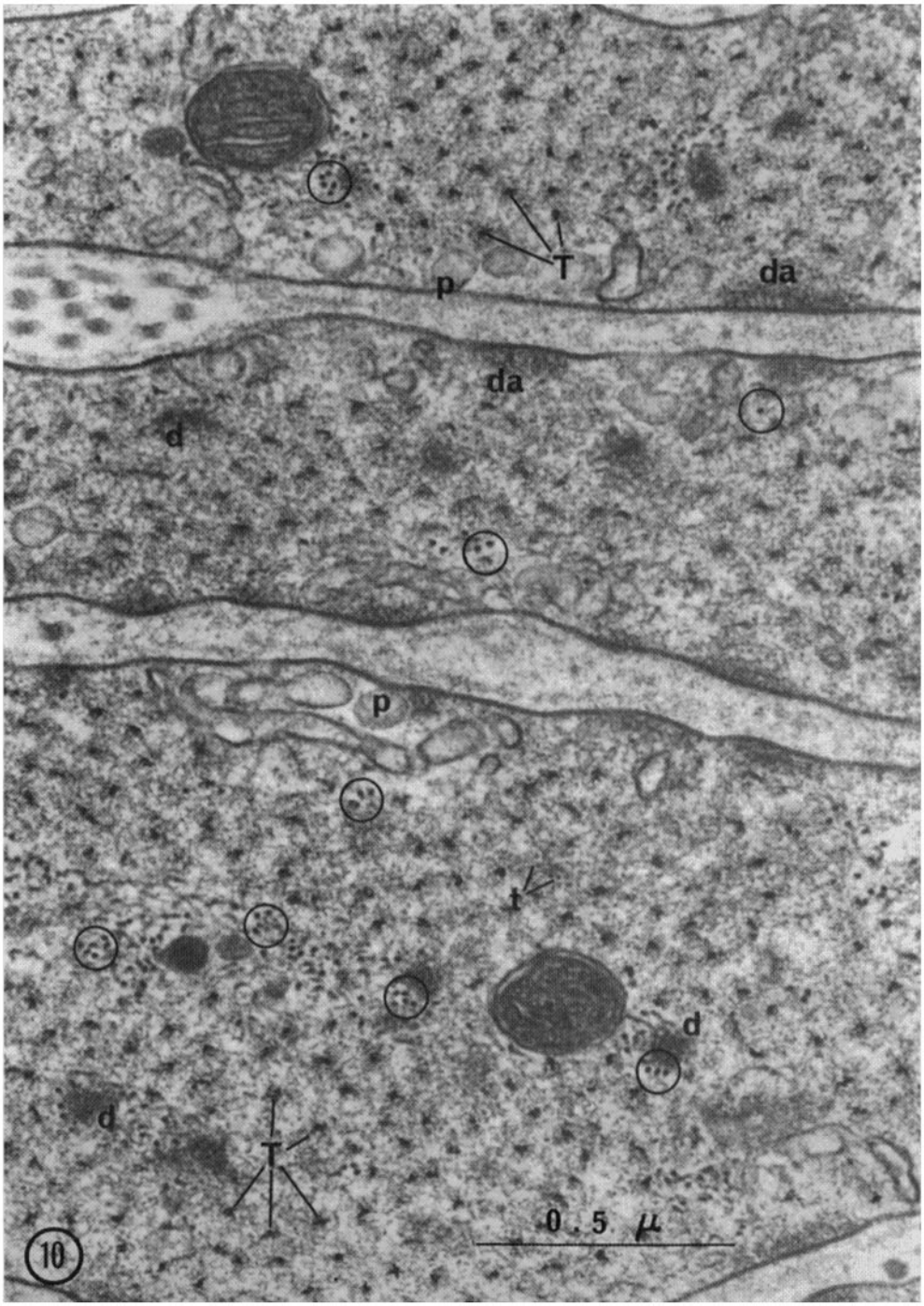
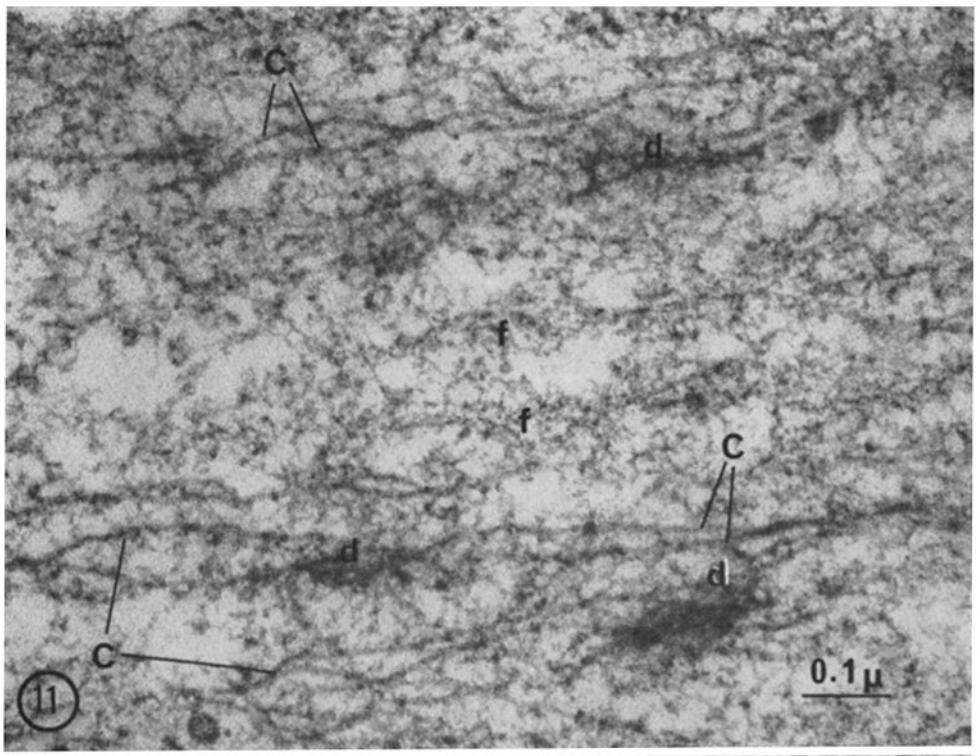


FIGURE 10. Transverse section of smooth muscle cells, adult finch ureter; fixation 1% OsO_4, followed by 4% glutaraldehyde, followed by 1% OsO_4. Thick myofilaments (*T*) exhibiting an irregular profile are in greater numbers than thin myofilaments (*t*). 100-A filaments (*encircled*) can be identified among these myofilaments, and are either in small groups or scattered singly. Again, some of the 100 A filaments are closely associated with dark bodies (*d*). *da*, dense area; *p*, pinocytotic vesicles. × 72,000.



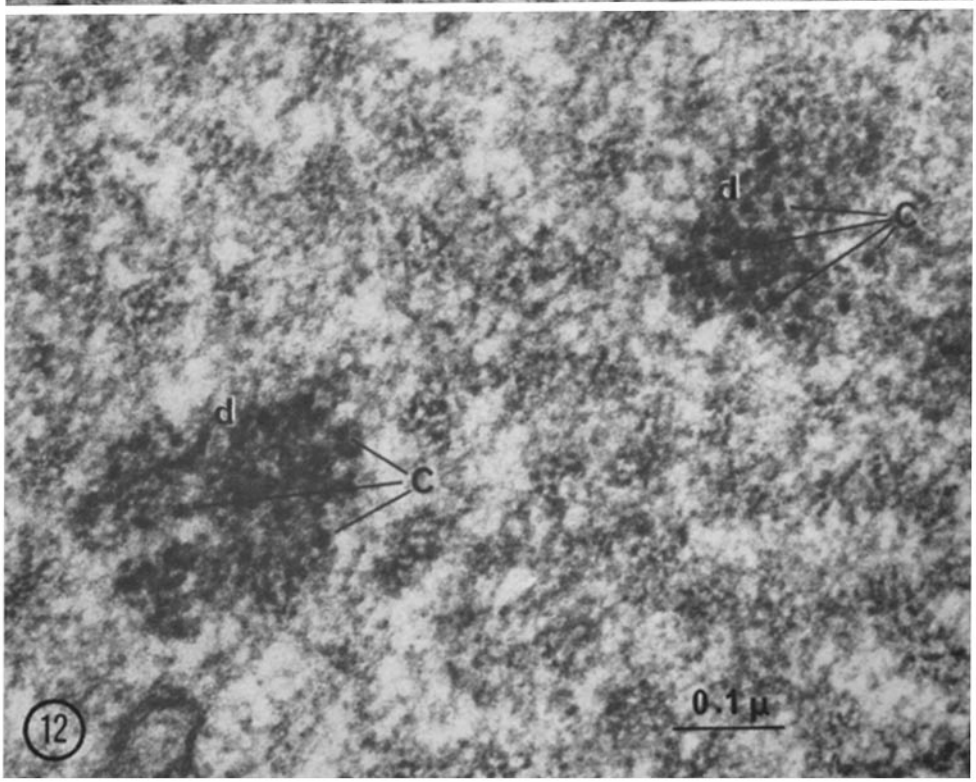
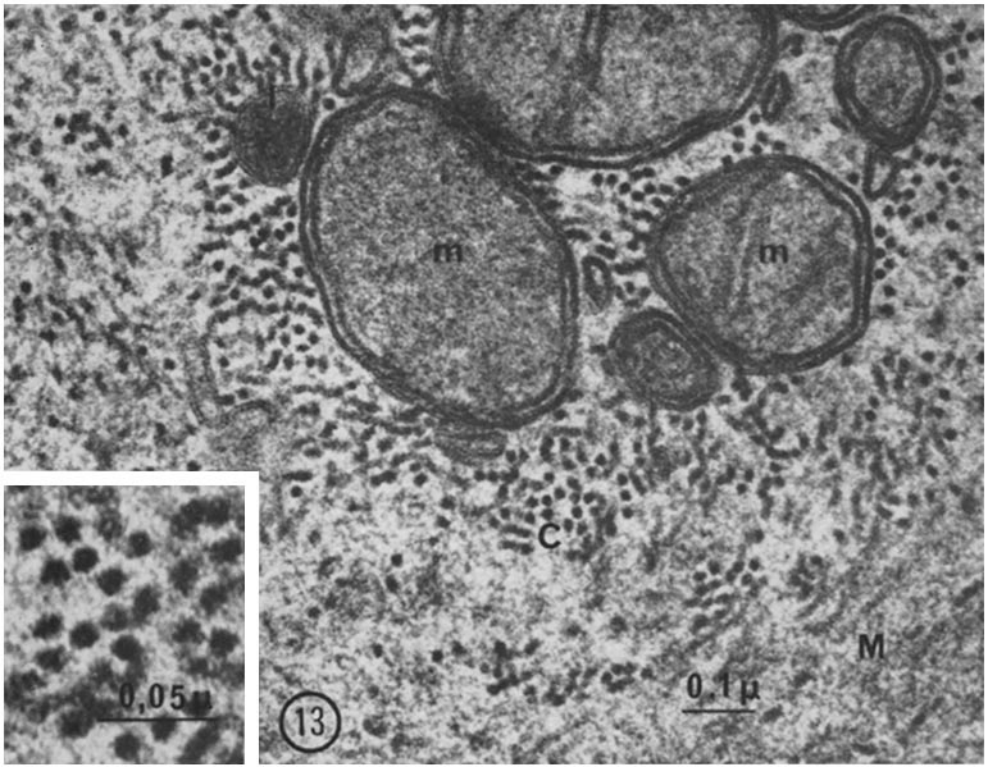


FIGURE 11 Longitudinal section of a smooth muscle cell, adult guinea pig taenia coli. (OsO_4 fixation, without block staining in 2% uranyl acetate). In the central region of large tissue blocks, myofilaments are often poorly preserved, the only indication of their presence being a fine flocculent material (*f*). 100 A filaments (*C*), however, are particularly apparent under these circumstances. Here, the 100 A filaments are closely associated with dark bodies (*d*) and appear to pass from one dark body to another. × 120,000.

FIGURE 12 Transverse section of dark bodies (*d*) in guinea pig vas deferens. 100 A filaments (*C*) appear well preserved; some of them are surrounded by a halo, enabling easy identification in the dark body matrix. Thin myofilaments are not well preserved. × 155,000.



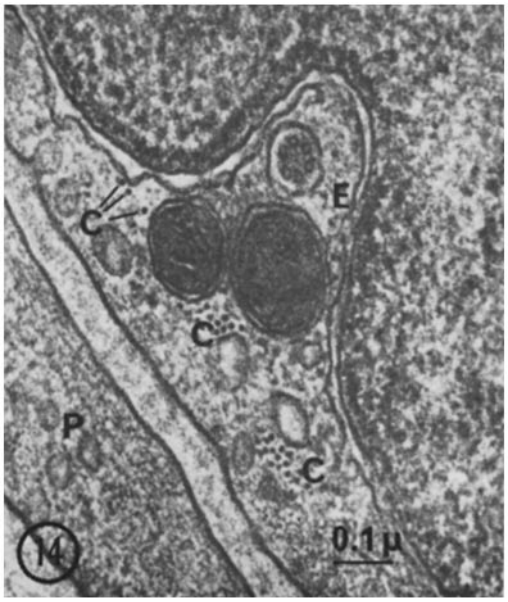


FIGURE 13 Transverse section of a smooth muscle cell, adult guinea pig ureter, showing accumulation of 100 A filaments (*C*) in juxtanuclear region, with cytoplasmic organelles, such as mitochondria (*m*), electron-opaque bodies, possibly lysosomes (1). *M*, myofilament bundles, cut tangentially. OsO_4-glutaraldehyde-OsO_4 fixation. × 100,000. Inset: High resolution micrograph of an area of Fig. 13, showing an electron-transparent central core apparent in some 100 A filaments. × 320,000.

FIGURE 14. Part of an endothelial cell of a capillary (*E*), demonstrating the presence of 100 A filaments (*C*). *P*, pericyte. OsO_4-glutaraldehyde–OsO_4 fixation. × 75,000.

ments converge upon and enter dark bodies (22, 25, 32), suggesting that dark bodies are attachment devices which anchor the myofilaments (30). The present study has demonstrated the intimate association of 100 A filaments with dark bodies in both developing and adult smooth muscle. This may suggest that 100 A filaments are either involved in dark body formation or at least constitute an important part of dark bodies in adult smooth muscle cells. From the published micrographs of dark bodies (10, 11), it is also apparent that some of the filaments which are in close association with them are larger than thin filaments, and probably represent 100 A filaments.

It has been shown that the 100 A filaments in other cell types, such as fibroblasts, cultured striated muscle cells, or chondrogenic cells, do not show detectable binding with heavy meromyosin, suggesting that they may consist of some protein other than actin (18). Tropomyosin is a possibility, since it has been shown to be present in a much higher proportion than actomyosin in vertebrate smooth muscle (13, 19, 34). Several authors have indicated that tropomyosin constitutes a part of the Z-band material of striated muscle (5, 12, 16). Assuming that dark bodies are homologous to Z-bands, though some doubt surrounds this point (31), 100 A filaments may contain tropomyosin, since they constitute an important part of dark bodies as indicated above. To determine the nature of 100 A filaments, further studies, including extraction experiments for structural protein, are necessary.

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