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Contraction of Isolated Smooth-Muscle Cells—Structural Changes

(cell volume/cell membrane/myofilaments/contractile mechanism)

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ABSTRACT The contraction of isolated smooth-muscle cells has been correlated with evagination of the cell membrane, a marked change in myofilament orientation, and a decrease in cellular volume. Both localized and full contractions have been elicited in the same cell by varying the intensity of electrical stimulation. These results may be explained by a model of the smooth-muscle cell in which the contractile apparatus extends between attachment sites on the cell membrane that are relatively closely spaced.

The relation of structure to function of smooth-muscle cells within multicellular preparations is exceedingly difficult to analyze owing to variable interactions, electrical and mechanical, between neighboring cells and the extracellular matrix. It is particularly difficult to determine the degree of contraction of each individual fiber within the intact tissue. The technique, recently described by Bagby *et al.* (1), for isolating in viable form individual smooth-muscle cells from the stomach muscularis of *Bufo marinus* makes possible an analysis of the contractile process in single isolated fibers. We, therefore, investigated structural changes associated with contraction of these isolated cells in response to variable electrical stimulation. The results indicate that a remarkable evagination of the surface membrane is associated with those parts of the cell undergoing contraction. Simultaneous reorientation of myofilaments and a decrease in cell volume were also noted. These changes provide insight into the arrangement of the contractile apparatus within the smooth-muscle cell.

MATERIALS AND METHODS

Cell Preparation. A suspension of isolated smooth-muscle cells from the stomach of *Bufo marinus* was prepared by a modification of the method of Bagby *et al.* (1). Slices of the stomach muscularis, 0.5-mm thick, were obtained by use of a Stadie–Riggs tissue slicer. They were incubated at 30.5° for 45 min in 0.1% trypsin (Sigma, type III) and 0.1% collagenase (Sigma, type I) in amphibian Ringer solution (Na^+, 95.26 mM; K^+, 3.0 mM; Ca^{+2}, 1.8 mM; Mg^{+2}, 1.0 mM; Cl^-, 81.6 mM; HCO_3^-, 20 mM; SO_4^-, 1.0 mM; HPO_4^-, 0.56 mM; $H_2PO_4^-$, 0.14 mM; glucose, 11.1 mM) and then reincubated for 30 min in 0.05% collagenase (Sigma, type I) in amphibian Ringer solution at 30.5°. Slices were then washed in amphibian Ringer solution, and the tissue was dispersed into single cells by being passed rapidly into and out of a medicine dropper. The resulting cell suspension was studied during the ensuing 8 hr. Even after this period, numerous cells capable of contractile responses were still present.

Electrical Stimulation, Video Recording, and Photomicrography. A few drops of cell suspension were placed on a microscope slide on the stage of a Zeiss Nomarski microscope, and the cells were allowed to settle. Contraction of the cells was initiated by passage of current between a glass micropipette filled with 3 M NaCl, the cathode, and a silver–silver chloride anode. The micropipette was advanced near the cell, while the anode was positioned at the periphery of the fluid pool. Stimulation was with square waves at 10 pps and 2 msec duration; the stimulus amplitude was varied between 1 and 100 V, and current intensity was monitored. The contractile responses of the individual fibers were recorded by a 16-mm Bolex motion picture camera and/or a Sony Video Recording System.

Analysis of Cell Volume. Film records were analyzed to determine if volume changes accompany the contractile responses of the smooth-muscle cells. In order to obtain information regarding volume from the two-dimensional images, the cell was assumed to be representable as an ellipsoid whose cross-sectional profile maintained a constant eccentricity in all functional states. It then follows from geometrical considerations* that the ratio of volumes (V) of a given cell at two different contractile states is given by:

$$V_1/V_2 = (A_1)^2/(A_2)^2 \times L_2/L_1,$$

where A is the area of the two-dimensional projection of the cell and L is its length. The area contained within a tracing of the outline of the cell was determined with a planimeter, and its length was determined with a plan measurer. Area and length measurements were made at rest, at maximal contraction, and at intermediate stages. Measurements were taken so that each cell was represented no more than once within intervals corresponding to shortening to 99.9–80.0, 79.9–60.0, 59.9–40.0, and 39.9–20.0% of resting length. The validity of the method of volume measurement was tested by applying it also to cells exposed to solutions of varying osmotic pressure.

* The volume (V) of an ellipsoid is given by $V = 1/6\pi\, abL$, where L is the longest axis and a and b are the remaining two axes. The area (A) of the two-dimensional projection of the ellipsoid is given by $A = \pi/4\, aL$. If one assumes that a/b is identical in two ellipsoids, which is equivalent to assuming identical eccentricity of their cross-sectional profiles, then the ratio of volumes of the two ellipsoids is given by:

$$V_1/V_2 = (A_1)^2/(A_2)^2 \times L_2/L_1.$$

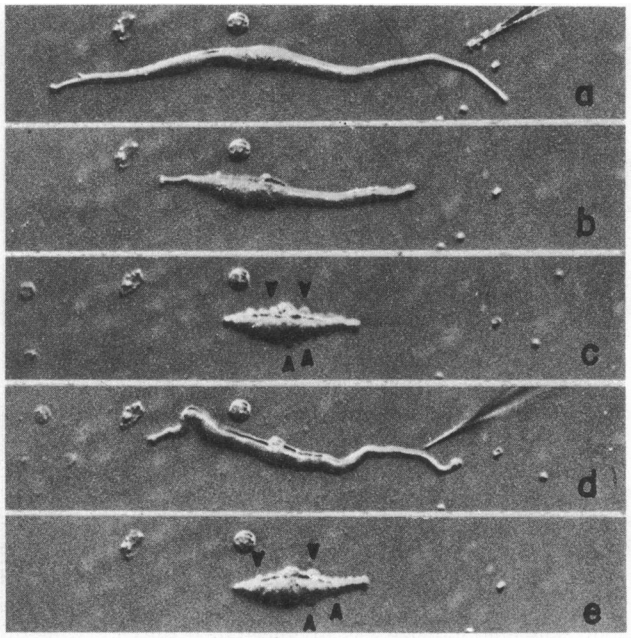

FIG. 1. Five frames from a film record showing synchronous contraction of an isolated smooth-muscle cell in response to electrical stimulation. Time intervals: (*a*) just before stimulation; (*b*) 5.0 sec after initiation of contraction; (*c*) 12.0 sec after initiation of contraction—cell maximally contracted; (*d*) about 10 min after initiation of contraction—cell maximally relaxed; (*e*) 8.8 sec after initiation of second contraction—cell maximally contracted. Note, especially along the cell margin, the irregularity of the surface (*darts*) in the fully contracted cell (*c,e*) compared with the relaxed cell (*a,d*). ×310.

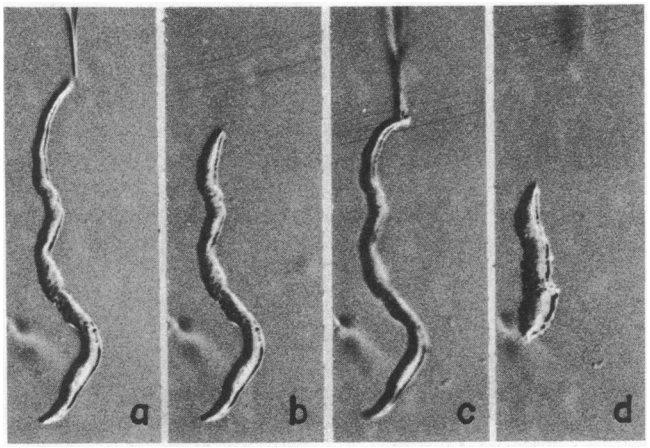

FIG. 2. Four frames from a film record showing both localized and full synchronous contraction of an isolated smooth-muscle cell in response to electrical stimulation. Time intervals: (*a*) just before stimulation; (*b*) 6.3 sec after initiation of contraction—localized contraction of the cell was maximal; (*c*) about 5 min after initiation of localized contraction—cell maximally relaxed and microelectrode repositioned for stimulation; (*d*) 12.5 sec after initiation of second contraction—cell maximally contracted. Note that localized contraction in *b* results in no change in length or shape of the cell except in that part nearest to the stimulating electrode. The first, localized contraction was initiated with a lower stimulus strength and a greater separation between cell and electrode than the second, synchronous contraction. ×300.

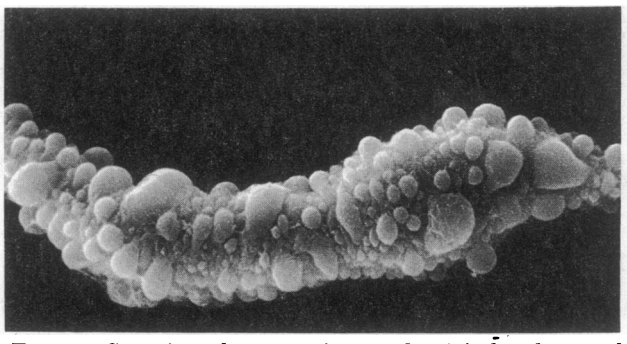

FIG. 3. Scanning electron micrograph of isolated smooth-muscle cell fixed after shortening in response to electrical stimulation to 30% of its resting length. ×1800.

Electron Microscopy. Structural changes associated with the contraction of the isolated cells were studied with the scanning and transmission electron microscope. For this purpose, a cell suspension was placed on a glass slide coated with 4% gelatin in amphibian Ringer solution. Contraction of a cell lying on the gelatin surface was initiated as described above, and the cell was fixed by the injection of 2.5% glutaraldehyde (Tabb) in amphibian Ringer solution from a micropipette (30-μm tip) positioned near the cell. The cell was continuously observed and its activity was recorded on videotape. Fixation resulted in adhesion of the cells to the gelatin surface. Although fixation of multicellular smooth muscle preparations with glutaraldehyde is usually accompanied by contraction (2, 3), no change in length or shape was noted when the isolated cells were fixed in the manner described. After excess gelatin was trimmed from the glass slide, it was immersed in 2.5% glutaraldehyde at 4° for several hours, post-fixed in osmium tetroxide, stained *en bloc* with uranyl acetate, and dehydrated. For scanning electron microscopy, slides containing a small piece of gelatin with the attached cell were dried by the critical-point method (4) and coated with a 100–200 Å layer of Au–Pd (70:30). The slides were observed with a JSM-U3 scanning electron microscope (J.E.O.L. Ltd.) and the cell, whose contractile activity had been monitored, was identified from the videotape records. For transmission electron microscopy, slides were infiltrated with epon after dehydration. The gelatin slab was then removed from the glass surface and embedded in epon. Thin sections of the cell, whose contractile activity had been monitored, were stained with uranyl acetate and lead citrate. Transmission electron-microscopic observations were made with an RCA EMU-4 electron microscope.

RESULTS

The pattern of response of isolated smooth-muscle cells to electrical stimulation may be divided into three general classes. In response to stimulation with relatively high current intensity (0.3 mA, average) most of the fibers appeared to be activated synchronously over their entire extent, and shortening occurred along all parts of the cell. Selected frames from a cinematographic record of such a cell are shown in Fig. 1. The transition from the relaxed to the contracted state is accompanied by an apparent change in the texture of the cell surface from a smooth contour (Fig. 1*a*) to one that is more highly irregular (Fig. 1*c*). Similar changes of the cell surface were also associated with contractions initiated by acetylcholine (100 μg/ml) or amphibian Ringer solution in

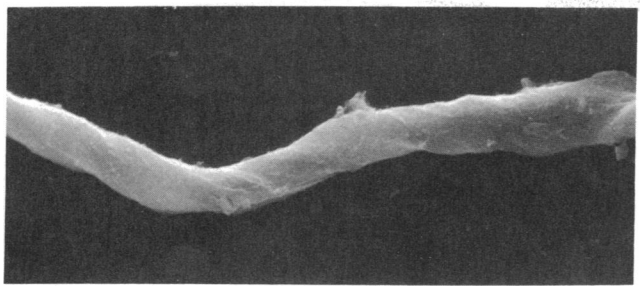

FIG. 4. Scanning electron micrograph of isolated smooth-muscle cell fixed in related state. ×2000.

which the Na^+ salts were substituted by K^+ on a molar basis. Upon cessation of electrical stimulation, the fiber typically appeared to relax and gradually approached its resting length. Reextension of the cell is accompanied by the apparent return of a smooth contour to the fiber surface (Fig. 1*d*). As shown in Fig. 1*e*, the cell may be restimulated to contract. The second pattern of response, seen in about 10% of the cells studied, was characterized by a contraction that spread as a wave from the point of stimulation. The portions of the cell that shortened had a more irregular surface than those portions of the cell that had not contracted. In some instances, waves of contraction began at several points along the cell. In the third pattern of response, elicited by stimulation with low current intensities (0.03 mA, average), contraction was limited to the region near the electrode as shown in Fig. 2. Force does not appear to be transferred to more distal regions of the cell, as judged by the absence of any detectable change in length or shape. By application of a higher stimulus strength, the entire cell may be activated and a synchronous contraction is initiated (Fig. 2*d*).

Electron-microscopic observations were undertaken in order to assess ultrastructural changes associated with contraction. Fig. 3 shows a typical scanning electron micrograph of a cell fixed at the point of maximal contraction. The cell surface is overwhelmed by bulbous and mound-like evaginations. This image is to be compared with that of a relaxed smooth muscle cell (Fig. 4) in which the cellular surface is generally smooth. The smooth appearance was characteristic of cells fixed in a relaxed state, irrespective of whether they had been made to contract previously.

Typical transmission electron micrographs of isolated smooth-muscle cells fixed in the relaxed and fully contracted states are shown in Figs. 5 and 6, respectively. The relaxed cell (Fig. 5) is characterized by the absence of bulbous projections and a generally longitudinal orientation of myofilaments. The bulbous projections of the contracted cell (Fig. 6) are relatively free of myofilaments whose dense packing and apparently random orientation characterizes the inner nonevaginated regions of the cell. Furthermore, the plasma membrane surrounding the evaginations is relatively free of plasma-membrane dense bodies, which are abundant along those sections of the membrane not involved in these outpocketings.

Cinematographic records of the contractile responses of over 50 cells were analyzed for changes in cell volume. Fig. 7 indicates that contraction of the smooth-muscle cell is associated with a decrease in volume; at 30 ± 1% of resting length, volume decreased to 79 ± 3% of the volume of the relaxed cell. This volume decrease accompanying contraction

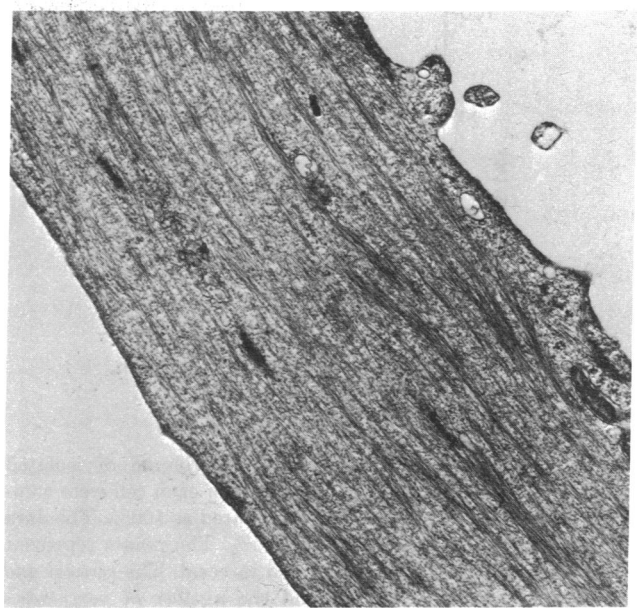

FIG. 5. Transmission electron micrograph of a longitudinal section through an isolated smooth-muscle cell fixed in the relaxed state. Note the absence of evaginations of the plasma membrane and the generally longitudinal orientation of the myofilaments. ×18,000.

is highly significant ($P < 0.001$). In order to check the validity of these measurements of volume change, the method was applied to individual cells immersed in solutions of various osmotic pressures. Volume of the smooth muscle cells so determined varied inversely with osmotic pressure in a man-

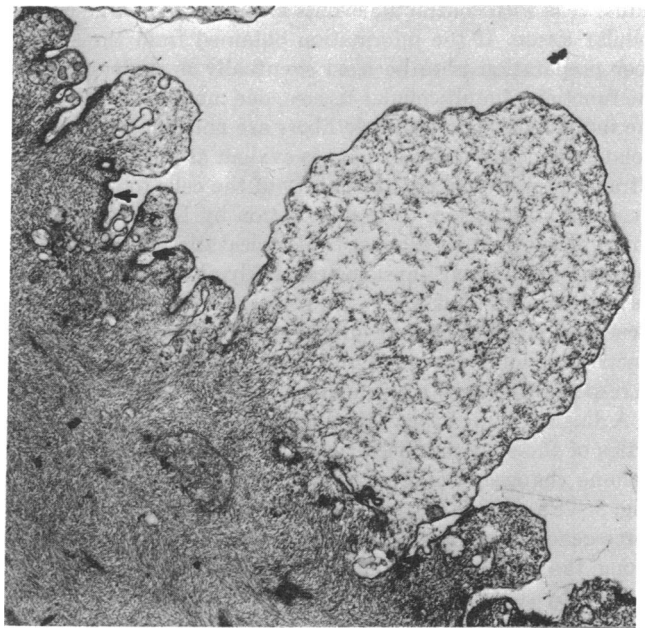

FIG. 6. Transmission electron micrograph of a longitudinal section through an isolated smooth-muscle cell fixed after shortening to 32% of its resting length. The evaginations are relatively free of myofilaments and contain few, if any, plasma-membrane dense bodies (*arrows*) which, however, abound along the membrane surrounding the nonevaginated regions. The inner, withheld regions are characterized by densely packed, randomly oriented myofilaments. ×18,000.

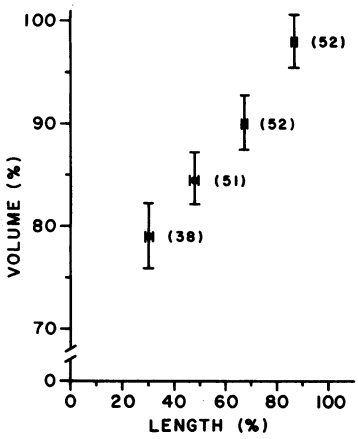

FIG. 7. The effect of contraction on volume of isolated smooth-muscle cells. Volumes and lengths of each cell were compared to those at rest, which were designated as 100%. The data are grouped into length intervals of 20%. The points represent the mean length and volume for each interval. The *vertical and horizontal bars* indicate the SE and the *number in parentheses* indicates the number of observations.

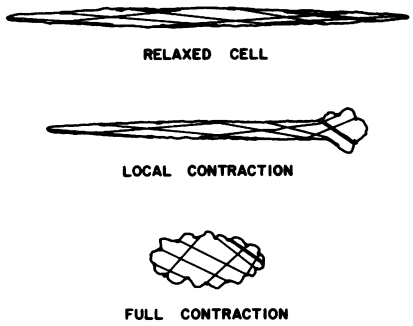

FIG. 8. Schematic representation of a smooth-muscle cell showing how the contractile apparatus is attached to the cell surface. The *densities* along the cell membrane represent plasma-membrane dense bodies and the *lines* connecting them represent the contractile apparatus. One of these lines has been widened to facilitate identification in the three different contractile states.

ner similar to that observed in isolated striated-muscle fibers (5). The method of analyzing the cinematographic records appears, therefore, to yield reasonable estimates of volume change.

DISCUSSION

The present study represents the first attempt to study structure and function of a single isolated smooth-muscle cell. Studies of this nature may eliminate the uncertainties inherent in attempting to correlate structural changes in individual cells with contractile events measured in a large multicellular tissue. If the information obtained from the single-fiber preparation is to be used eventually in understanding the function of multicellular tissues, one must be certain that the individual smooth-muscle fibers are not damaged by the isolation procedure. No damage is evident as judged from the ultrastructural data and the ability of the cells to respond to electrical stimulation, or to stimulation by K^+ or acetylcholine. The contractile response to electrical stimulation appears to occur via normal physiological mechanisms, as judged by its reversibility and Ca^{+2} requirement (6). The observed structural changes are not unique to contraction initiated by electrical stimulation but were also observed after contraction in response to K^+ or acetylcholine.

A diagrammatic explanation of the special contractile properties of the smooth-muscle cell, and the ultrastructural and volume changes correlated with its contraction is shown in Fig. 8. The essential feature of this model is the attachment of force-generating units to relatively closely spaced dense bodies along the plasma membrane, a feature that has been described by several investigators (7–9). One additional feature of the model is that the angle between the contractile apparatus and cell membrane increases upon shortening of the muscle cell. The observed ability of the smooth-muscle cell to contract in three distinctly different patterns follows directly from the proposed model. Because the contractile units are attached between closely spaced points at the cell membrane, activation along a short region of the cell would produce only localized shortening. Uniform activation of the entire cell or activation spreading in a wavelike manner would result in a uniform or wavelike contraction, respectively. The wavelike spread of contraction appears to be similar to nodes of contraction observed in cultured chick amnion (10).

Ultrastructural analysis of cells fixed at various stages of contraction and relaxation indicate that contraction is correlated with: (*a*) the formation of evaginations of the cell membrane that are characterized by the absence of myofilaments within and a paucity of plasma-membrane dense bodies, and (*b*) a reorientation of myofilaments from a predominantly longitudinal orientation in relaxed cells to a more random orientation in contracted cells. Folds of the cell membrane somewhat similar to blebs have been reported (7, 11), but the extent of these changes was not as pronounced as in the present study due probably to restrictions imposed upon changes in cellular dimensions in the intact tissue. As can be seen in Fig. 8 the behavior of the proposed model is consistent with our observations. The formation of cellular evaginations may be understood as being due to the application of an inwardly directed force at plasma-membrane dense bodies coupled with a general need to increase cellular diameter to accommodate the volume displaced by shortening of the cell. Thus, those regions of the sarcolemma without dense bodies are forced outward to form blebs. One other possible explanation for the appearance of blebs is that it represents a mechanism for maintaining constant surface area in going from a cylindrical towards a spheroidal shape. Such geometrical considerations do not appear, however, to be the major factor underlying bleb formation since highly shortened cells that are in the initial stages of relaxation are relatively free of blebs. Likewise, blebs are absent in cells that have been shrunk to 50% of their volume by hyperosmotic solutions. The absence of blebs in cells swollen to 200% of their volume in hyposmotic solution would argue against bleb formation being the result of differences in deformability of the membrane in different regions of the cell. Thus, bleb formation appears to be a correlate solely of the active process of contraction. The observed change in myofilament orientation is also consistent with the model; moreover, it may provide an explanation for the decrease in form birefringence characteristic of contraction (12) in smooth muscle.

The decrease in volume upon contraction may also be understood from the model in Fig. 8. Because the need to

increase fiber diameter upon contraction is resisted by an inwardly directed component of the force at the cell membrane, an increase in intracellular pressure upon contraction might be expected. Net loss of volume from the cell would therefore result, as was observed. Because of considerable scatter in the data relating length and volume, it is not possible to determine the precise manner in which they are related. The scatter most probably results from errors due to changes in the orientation of the cells during contraction; such errors would be expected to distribute normally. On the other hand, a consistent error leading to an apparent decrease in volume would result if the fibers were flattened by the effects of gravity in the relaxed state and upon contraction become more rounded in cross-section. However, this does not appear to be so since cross-sections as well as stereoscopic observations with the scanning electron microscope reveal no flattening of relaxed muscle cells attributable to gravity. The volume decrease appears, therefore, to be attributable only to forces associated with contraction. A decrease in volume associated with contraction of smooth muscle has been suggested (13, 14) as a result of differences in cytoplasmic density and intercellular distance between contracted and relaxed smooth muscle. In striated muscle a volume decrease of similar magnitude to that in the present study has been noted during isotonic contraction (15). It is also undoubtedly due to an increase in intracellular pressure (16), although the resistance to an increase in fiber diameter in striated muscle probably results from structures different than in smooth muscle.

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1. Bagby, R. M., Young, A. M., Fisher, B. A. & McKinnon, K. (1971) *Nature* **234**, 351–352.
2. Cooke, P. H. & Fay, F. S. (1972) *Exp. Cell Res.* **71**, 265–272.
3. Small, J. V. & Squire, J. M. (1972) *J. Mol. Biol.* **67**, 117–149.
4. Anderson, T. F. (1951) *Ann. N.Y. Acad. Sci.* **13**, 130–133.
5. Blinks, J. R. (1965) *J. Physiol.* **177**, 42–57.
6. Fay, F. S. & Delise, C. M. (1972) *Physiologist* **15**, 132.
7. Lane, B. P. (1962) *J. Cell Biol.* **27**, 199–213.
8. Rosenbluth, J. (1965) *Science* **148**, 1337–1339.
9. Pease, D. C. & Molinari, S. (1960) *J. Ultrastruct. Res.* **3**, 447–468.
10. Lewis, M. R. & Lewis, W. H. (1917) *Amer. J. Physiol.* **44**, 67–74.
11. Kelly, R. E. & Rice, R. V. (1969) *J. Cell Biol.* **42**, 683–694.
12. Fisher, E. (1944) *J. Cell. Comp. Physiol.* **23**, 113–130.
13. Nemetschek-Gansler, H. (1967) *The Cellular Biology of the Uterus*, ed. Wynn, R. M. (Appleton-Century Crofts, New York), p. 353.
14. Meigs, E. B. (1911) *Amer. J. Physiol.* **29**, 317–329.
15. Sato, T. G. (1954) *Annot. Zool. Jap.* **27**, 165–171.
16. Gordon, A. M., Huxley, A. F. & Julian, F. J. (1966) *J. Physiol.* **184**, 170–192.