SARCOPLASMIC RETICULUM AND EXCITATION-CONTRACTION COUPLING IN MAMMALIAN SMOOTH MUSCLES

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ABSTRACT

The sarcoplasmic reticulum (SR) was studied in the smooth muscles of rabbit main pulmonary artery, mesenteric vein, aorta, mesenteric artery, taenia coli, guinea pig mesenteric artery, and human uterus, and correlated with contractions of the smooth muscles in Ca-free media. SR volumes were determined in main pulmonary artery (5.1%), aorta (5%), portal-anterior mesenteric vein (2.2%), taenia coli (2%), and mesenteric artery (1.8%): because of tangentially sectioned membranes these estimates are subject to a correction factor of up to +50% of the values measured. Smooth muscles that contained a relatively large volume of SR maintained significant contractile responses to drugs in the virtual absence of extracellular calcium at room temperatures, while smooth muscles that had less SR did not. The unequal maximal contractions of main pulmonary artery elicited by different drugs were also observed in Ca-free, high potassium-depolarizing solution, indicating that they were secondary to some mechanism independent of changes in membrane potential or calcium influx. Longitudinal tubules of SR run between and are fenestrated about groups of surface vesicles separated from each other by intervening dense bodies. Extracellular markers (ferritin and lanthanum) entered the surface vesicles, but not the SR. The peripheral SR formed couplings with the surface membrane: the two membranes were separated by gaps of approximately 10 nm traversed by electron-opaque connections suggestive of a periodicity of approximately 20–25 nm. These couplings are considered to be the probable sites of electromechanical coupling in twitch smooth muscles. Close contacts between the SR and the surface vesicles may have a similar function, or represent sites of calcium extrusion. The presence of both thick and thin myofilaments and of rough SR in smooth muscles supports the dual, contractile and morphogenetic, function of smooth muscle.

INTRODUCTION

It is generally accepted that activation of smooth muscle contraction is mediated by a rise and inactivation (relaxation) is mediated by a fall in cytoplasmic-free calcium concentration (for reviews see Somlyo and Somlyo, 1968 *a*; 1970 *b*; Hurwitz and Suria, 1971). The relative contribution to activation by intracellularly released, as compared to inward moving extracellular, calcium, has not been established. In a recent study on reptilian smooth muscle (Somlyo et al., 1971 *c*) a semi-

quantitative estimate of the sarcoplasmic reticulum was obtained and it was concluded that these structures were present in sufficient abundance to serve as possible intracellular sources and sinks of activator calcium.

The purpose of the present study was to provide a quantitative estimate of the sarcoplasmic reticulum (SR) in some mammalian smooth muscles and to correlate the volume and organization of the SR with contractile properties. We were particularly interested in whether there is a correlation between the distribution of the SR and the ability of different mammalian smooth muscles to contract in the absence of extracellular calcium. We have selected for the correlation of ultrastructure with function the smooth muscles of the portal-anterior mesenteric vein and the main pulmonary artery of the rabbit, because these two blood vessels contain functionally different types of smooth muscle: the former generates action potentials and develops relatively phasic K-contractures, while the latter responds to excitatory drugs with graded depolarization and develops tonic K-contractures (Somlyo and Somlyo, 1968 *b*; 1969; Somlyo et al., 1969).

The investigation of the SR was complemented with the use of extracellular markers to ascertain that the structures examined represented a true SR that does not communicate with the extracellular space, and with examination of tilted specimens to evaluate the effects of tangentially sectioned membranes (Peachey, 1965 *a*; Loud, 1967; 1968) on the visualization of SR. We examined the disposition of the SR in a number of mammalian vascular smooth muscles, taenia coli, and human uterus, with particular attention to the question whether the SR formed sufficiently close relationships with the surface membrane to function as a source of calcium released by the action potential, as in striated twitch muscle fibers (for reviews see Bianchi, 1968; Peachey, 1968; Sandow, 1970).

The results indicate some correlation between the contractile properties and the SR volume, and show a close relationship of the SR to the surface membrane and to surface vesicles. The most significant physiological observation made, in our opinion, is the finding that different drugs produce unequal maximal contractions in *depolarized* pulmonary artery smooth muscle bathed in Ca-free solutions. Preliminary reports of some of these observations have been published (Devine and Somlyo, 1970; Somlyo and Somlyo, 1970 *b*; Somlyo et al., 1971 *b*).

METHODS

Main pulmonary arteries, portal-anterior mesenteric veins, small mesenteric arteries, thoracic aortas, and taenia coli were obtained from New Zealand White male rabbits (1.8–2.7 kg) killed by cervical dislocation; small (approximately 500 μm in diameter) mesenteric arteries for lanthanum tracer experiments were obtained from guinea pigs (0.30–0.35 kg) killed by a blow on the head; human uterine smooth muscle was obtained at caesarian section. The excised tissues were placed in oxygenated Krebs' bicarbonate solution at 37°C (for composition see Somlyo et al., 1971 *c*) and processed as follows:

(*a*) Rabbit main pulmonary artery (MPA), portal-anterior mesenteric vein (PAMV), thoracic aorta (AO), taenia coli (TC), and human uterus smooth muscle were stretched to approximately 1.5 times their excised length, incubated for 30 min in Krebs' bicarbonate solution, and fixed for 2 hr in 2% glutaraldehyde (prepared from 8% glutaraldehyde, Polysciences, Inc., Rydal, Penn.) containing 4.5% (0.13 M) sucrose in 0.075 M cacodylate buffer (pH 7.4).

(*b*) Small mesenteric arteries (MA), approximately 500 μm in diameter, were incubated for 30 min in Krebs' bicarbonate solution and fixed unstretched for 2 hr in 2% glutaraldehyde containing 4.5% sucrose in 0.075 M cacodylate buffer (pH 7.4) or in 2% glutaraldehyde in Krebs' bicarbonate solution (pH 7.4).

All tissues were rinsed in buffer with or without 6% sucrose (0.15 M) for 5–30 min and postfixed for 2 hr in 2% osmium tetroxide in 0.05 M cacodylate buffer, or Krebs' bicarbonate buffer, and block stained with saturated aqueous uranyl acetate for 30 min, dehydrated in ethanol, and embedded in Spurr's resin (Spurr, 1969).

Extracellular Markers

Strips of rabbit PAMV and MPA were stretched to approximately 1.5 times their excised length and incubated in ferritin prepared as previously described (Somlyo et al., 1971 *c*). After 90 min, the tissues were fixed for 2 hr in 2% glutaraldehyde in 0.075 M cacodylate buffer, pH 7.4, containing 4.5% sucrose, rinsed in buffer with or without sucrose, and postfixed in 2% osmium tetroxide in 0.05 M cacodylate buffer with or without 3% sucrose for 2 hr and blockstained with saturated aqueous uranyl acetate for 30 min, dehydrated, and embedded in Spurr's resin.

Small mesenteric arteries from guinea pigs and portal-anterior mesenteric veins from rabbits were fixed in 0.1 M cacodylate-buffered 2% glutaraldehyde–2% formaldehyde fixative, pH 7.4, for 2 hr and postfixed in 2% osmium tetroxide containing 2% lanthanum nitrate at pH 7.7 (Revel and Karnovsky, 1967). The tissues were rapidly dehydrated and embedded in Epon 812.

Thin sections (approximately 40–90 nm), cut with a Porter-Blum MT2B ultramicrotome with diamond knives, were stained with alkaline lead citrate, and examined with an AEI 6B or a Hitachi HU11E electron microscope.

Sarcoplasmic Reticulum Relationships Viewed with Specimen Tilting

The Hitachi HU11E electron microscope was fitted with a HK3A wide-range tilting stage ± 30° with full 360° azimuth control and a large bore polepiece.

The tilt stage and large bore polepiece, primarily designed for metallurgy, do not permit as high a resolution as that available in the normal mode of operation. In addition, frequent alignment of the microscope is necessary at varying degrees of tilt, and this tends to build up contamination of the specimen and limits the usefulness of this type of tilt stage for biological specimens.

Volume of Sarcoplasmic Reticulum

Micrographs of groups of transversely sectioned smooth muscle cells were obtained at a primary magnification of 8000, enlarged photographically to a final magnification of 48,000, and montages were constructed. The inclusion of a number of slightly obliquely-sectioned cell profiles was unavoidable. The volume of the SR was estimated by integration by weight of portions of the SR cut from micrographs of mitochondrion- and nucleus-free profiles of smooth muscle cells and expressed as a per cent value (Somlyo et al., 1971 *c*). These determinations were made on identically fixed tissues.

The extent to which tangential sectioning of membranes influences the SR volumes observed (Peachey, 1965 *a*; Loud, 1967; 1968) was determined in selected tilt series and the SR volume "synthesized" from the tilt series to include the total SR visible at −30°, +30°, and 0° tilt.

Physiological Studies

Circumferential strips of main pulmonary artery (MPA), helical strips of thoracic aorta (AO), longitudinal strips of portal-anterior mesenteric vein (PAMV), and strips of taenia coli (TC) were obtained from rabbits. The composition of the Krebs' and Ca-free (with 4 mM EGTA) Krebs' solutions as well as the methods of recording tension have been previously published (Somlyo et al., 1971 *c*). The composition of the depolarizing solution was as follows in mM: K_2SO_4, 76.0; KCl, 10.0; $KHCO_3$, 16.0; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgCl_2$, 1.2; dextrose, 5.6. Calcium was omitted from the Ca-free depolarizing solution and 4 mM EGTA (ethylene glycol bis [β-aminoethyl ether]) was added. The EGTA containing, Ca-free Krebs' and depolarizing solutions were adjusted to a pH of 7.4 with NaOH and KOH, respectively. For experiments with Ca-free Krebs' solution the preparations were incubated in this solution for half an hour before stimulation with drugs. For experiments with Ca-free depolarizing solution the preparations were incubated for 15 min in Ca-free Krebs' solution and for an additional 15 min in Ca-free depolarizing solution before addition of the drugs. Under the latter conditions the Ca-free depolarizing solution itself elicited no contraction, probably minimizing the possibility of release of intracellular calcium by depolarization.

RESULTS

Contractile Responses of Pulmonary Artery, Portal-Anterior Mesenteric Vein, Thoracic Aorta, and Taenia Coli in Normal, Ca-Free Krebs' and Ca-Free Depolarizing Solutions

The contractile responses of two types of smooth muscle (MPA and PAMV) are illustrated in Fig. 1. In normal Krebs' solution the maximal response elicited by acetylcholine is significantly less than the maximal contraction stimulated by norepinephrine, as shown by the additional contraction produced by the catecholamine when added in the presence of a maximal acetylcholine-induced contraction. The inequality of the maximal contractile responses of a smooth muscle to different stimulating agents is characteristic, different from the behavior of fast-striated muscles, and has been discussed elsewhere (Somlyo and Somlyo, 1968 *a*, *b*; 1970 *a*, *b*; Somlyo et al., 1969). In the Ca-free solution at 24°C the PAMV did not contract when stimulated with acetylcholine, and a barely detectable contraction was produced by norepinephrine. In contrast, MPA smooth muscle maintained significant contractile responses to both acetylcholine and norepinephrine in Ca-free solutions. The unequal maximal contractile responses of MPA smooth muscle to the two drugs were also observed in Ca-free, depolarizing solution.

The results of experiments similar to that in Fig. 1 are tabulated in Table I, where the maximal contractile response indicated, in each case, is the tension developed in response to the supramaximal concentration of norepinephrine (vascular smooth muscle) or acetylcholine (TC). Taenia coli smooth muscles were stimulated with supramaximal concentrations of serotonin (10 μg/ml) followed by acetylcholine. The response to the latter was always greater than the maximal response to serotonin. The average values of the contractile

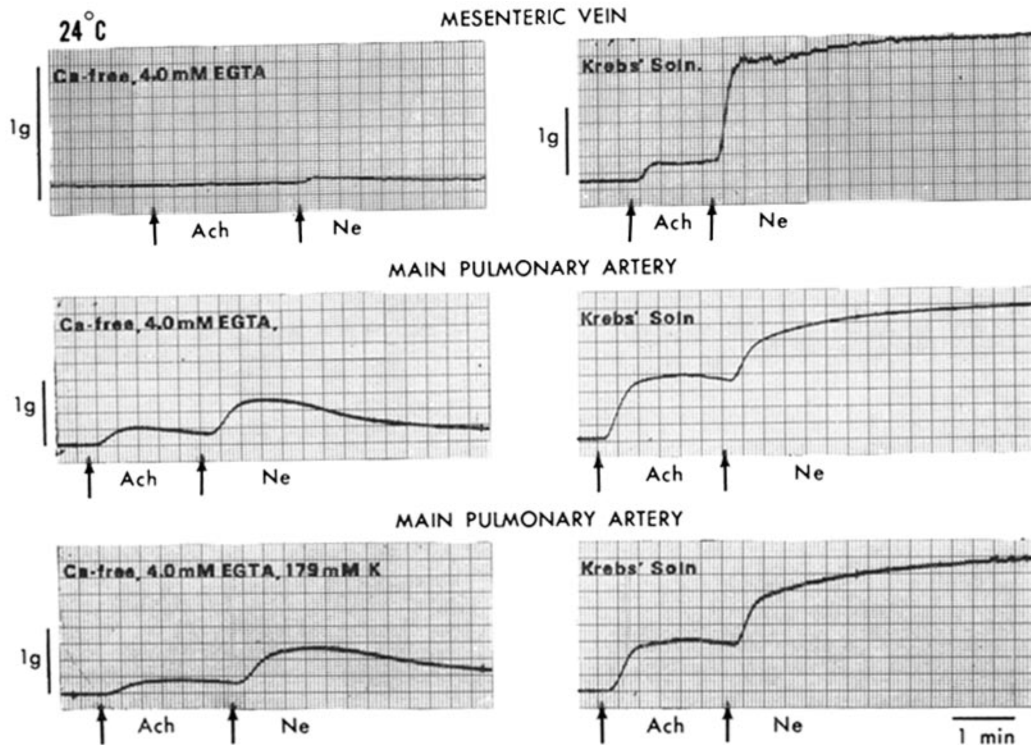


FIGURE 1 Contractile responses of two types of vascular smooth muscle in normal and in Ca-free solutions. The contractions in the right-hand column illustrate the responses in normal (Ca-containing) solution to supramaximal concentrations of acetylcholine (Ach, 5 μg/ml) and of norepinephrine (Ne, 10 μg/ml). The left-hand column shows the effect of the same drugs on the same preparations placed in Ca-free Krebs' (two upper records) or Ca-free depolarizing (left lower record) solution. The amplifier gain was increased in the upper left-hand record (see vertical calibration). Note that the contractile response of the portal-anterior mesenteric vein (mesenteric vein) is almost completely abolished in the Ca-free solution, while the main pulmonary artery smooth muscle still develops sizeable contractions in this medium. The unequal maximal contractions produced by the two drugs still persist in the *depolarized* main pulmonary artery smooth muscle in Ca-free, high K medium (left bottom panel). For further explanation see text.

responses shown in Table I, normalized to the maximal contractile response of a given preparation in Krebs' solution at room temperature as 100%, are summarized in Table II.

The most clearly evident result shown in Tables I and II is the very significant difference in behavior between MPA and AO smooth muscle on the one hand, and the smooth muscle of the TC and PAMV on the other. The smooth muscle of the large arteries exhibits sizeable (approximately 50% of the control in Krebs' solution) contractile responses after half an hour in calcium-free solutions at room temperature, while the TC and PAMV smooth muscles contract minimally (5% of control) or not at all under similar conditions. The second observation is that, when compared to the contractile response in Krebs' solution at the same temperature, the contractions in Ca-free solutions are reduced when the muscles are incubated at 37°C as compared to room temperature. Similar observations on turtle oviduct smooth muscle, suggesting that the intracellular calcium is more readily lost into Ca-free solutions at higher temperatures (Somlyo et al., 1971 *c*), prompted us to study the behavior of mammalian smooth muscles in Ca-free solutions at room temperature.

Although inspection of Table I seems to suggest that the reduction of contractility at the lower temperatures in Krebs' solution varies among the different smooth muscles, we do not feel that the

TABLE I

Maximal Tension (mg)

	Animal No.	37°C Krebs' solution	37°C Ca-free 4.0 mM EGTA solution	23.5° ± 1.5°C Krebs' solution	23.5° ± 1.5°C Ca-free 4.0 mM EGTA solution	23.5° ± 1.5°C Ca-free 4.0 mM EGTA 179 mM K solution
Main pulmonary artery	1	2845	750	1436	931	
	2			1693	1193	
	3	1574		1002		525
	3	1280		1120		420
	4			1910	650	
	4			1950		650
	5			305	173	22
	6	2305		835	341	122
	7	1772	417	1793	921	909
	8			2436	1266	1039
	9	1877	563	1801	521	980
Thoracic aorta	1	2960	924	769	717	
	4			1193		555
	5			450	111	139
	6	2396		1585	613	717
	7	2058	412	1274	902	529
	8			1339	648	865
	9	2444	443	1034	558	943
Taenia coli	1	6432	0	3869	117	
	5			4040	0	0
	6	3700		3000	67	9
	7	4882	0	3302	116	116
	8			2742	23	499
	9	5658	0	3631	155	111
Mesenteric vein	1	3705	20	2275	50	
	2			3578	0	
	5			1700	0	15
	6	1979		1886	19	9
	7	2073	0	1515	9	9
	8			2698	10	10
	9	1802	0	1915	4	29

TABLE II

Mean Maximal Tension (%) + SD

	37°C Krebs' solution	37°C Ca-free 4.0 mM EGTA solution	23.5 ± 1.5°C Krebs' solution	23.5 ± 1.5°C Ca-free 4.0 mM EGTA solution	23.5 ± 1.5°C Ca-free 4.0 mM EGTA 179 mM K
Main pulmonary artery	158 ± 69.1	35 ± 15.0	100	50 ± 14.4	37 ± 17.5
Thoracic aorta	233 ± 108.0	65 ± 47.9	100	55 ± 24.1	53 ± 21.8
Taenia coli	148 ± 18.4	0 ± 0	100	2 ± 1.5	5 ± 7.2
Mesenteric vein	125 ± 31.4	0.3 ± 0.5	100	3 ± 3.9	5 ± 5.9

Summary of data in Table I, normalized to the tension developed (= 100%), by each strip, in Krebs' solution at room temperature.

present data are sufficient for evaluating the significance of these results. Major variations in the sensitivity of different smooth muscles to reduction in temperature have been summarized elsewhere (Somlyo and Somlyo, 1970 *b*).

ULTRASTRUCTURE

Rabbit Portal-Anterior Mesenteric Vein

The filament organization of the longitudinal smooth muscle of rabbit PAMV consisted of the recently described (Devine and Somlyo, 1971; Rice et al., 1971; Somlyo et al., 1971 *a, d*) quasi-rectangular array of thick filaments with intervening rows of thin filaments (Fig. 2). In transverse section, the most common localization of the SR was at the periphery of the muscle fibers, adjacent to the surface vesicles and to the nonspecialized surface membrane. In regions occupied by dense bodies, both surface vesicles and SR were absent. The centrally located SR was rather limited in this type of smooth muscle, with the exception of the area of the nuclear poles where occasionally a prominent collection of SR mixed with Golgi apparatus was present (Fig. 4). In transverse sections the tubular elements of SR were seen either in a radial orientation, or running parallel to the surface membrane or as transversely sectioned profiles, suggesting an organization into a three-dimensional network.

In longitudinal sections, the longitudinally oriented SR tubules were running adjacent to the surface membrane: an exceptionally long SR-membrane contact is shown in Fig. 3. The comparison of longitudinally sectioned material (Figs. 3–6) and transversely sectioned material (Fig. 2) suggested that the most common disposition of the SR is one of longitudinally oriented tubules. A characteristic arrangement of the SR in tangential sections was that of longitudinal tubules running between surface vesicles and forming fenestrated loops around them (Figs. 5, 6, 8), with the groups of surface vesicles being separated from each other by adjacent dense bodies. This arrangement is similar to that found in the turtle oviduct (Somlyo et al., 1971 *c*) and guinea pig ileum (Gabella, 1971), and the fenestration of longitudinally running SR tubules around the surface vesicles was characteristic of every type of smooth muscle examined by us (Figs. 6, 8, 13, 26, 29, 30). The separation of longitudinal groupings of surface vesicles by intervening dense bodies has been previously suggested on the basis of freeze-etch studies of mesenteric artery smooth muscle and vas deferens (Devine et al., 1971).

Characterization of Surface Vesicles and Sarcoplasmic Reticulum with Extracellular Markers (Lanthanum and Ferritin)

Regardless of whether the extracellular marker was applied before (ferritin) or after fixation (lanthanum), the marker was seen in surface vesicles that had an obvious connection with the extracellular space (Figs. 7, 7 A) as well as in the surface vesicles that had no *apparent* communication with the extracellular space (Figs. 7, 8). The appearance of such "free-floating" vesicles could be accounted for by the fact that the vesicle neck, particularly if unusually long (Fig. 7 A), may not have been included in the plane of section; this would obviously be the case when the vesicles were tangentially sectioned (Fig. 8). Tilt stage examinations (see below and Fig. 13) indicated another reason for the appearance of free-floating vesicles in electron micrographs: when the neck of the vesicle is tangential to the incident electron beam the resultant electron micrograph does not show the membrane of the neck region, as would also be suggested from theoretical considerations (Peachey, 1965 *a*; Loud, 1967; 1968). Extracellular markers, when present in sufficient concentrations in the regions examined, entered what appeared to be free-floating vesicles, and the absence of ferritin from an occasional vesicle was uncommon enough to be due to chance. The density of ferritin was usually lower even in the surface vesicles connected to the extracellular space by a visible neck, than in the adjacent extracellular space. It was our general impression that vesicles communicate with the extracellular space rather than being truly pinocytotic, and we therefore refer to them as surface vesicles.

The tubular elements identified by us as the SR in smooth muscle were not penetrated by the extracellular markers (Figs. 7, 8, 19) and represent a *true SR*, not a system of longitudinal tubules communicating with the extracellular space described in cardiac muscle (Forssmann and Girardier, 1970; Rubio and Sperelakis, 1971), nor one similar to the T system of developing muscle (Ishikawa, 1968).

Coupling between the SR and Surface Membrane

The elements of SR made close contact (approximately 10 nm) with the surface membrane (Figs. 10–12) and the surface vesicles (Figs. 9, 13). The gap between the two membranes was traversed by electron-opaque connections that, in appropriate sections, had a periodicity of approximately 20–25 nm. The SR often contained some electron-opaque material in its lumen, and the SR–surface membrane coupling most closely resembled the couplings described in cardiac muscles (Sommer and Johnson, 1968; Fawcett and McNutt, 1969; Walker et al., 1971) and in developing skeletal muscle (Edge, 1970; Kelly, 1971). The relatively consistent spacing of the electron-opaque bridges, in our material, suggests that they are structural components of couplings, rather than chance deposits. This conclusion must remain tentative, pending a more extensive study of sections optimally oriented to reveal the periodicity of the electron-opaque connections and their relationships to the two membranes.

Tilt Stage Examination of the SR in Smooth Muscle Cells

Tilting the specimen through a range of +30° to −30° confirmed the usefulness of this method (Peachey, 1965 *a*; Loud, 1967; 1968) in visualizing membranes that appeared blurred or were even invisible due to the tangential incidence of the electron beam. Elements of tubular SR often became blurred at one angle of tilt, whereas other elements, previously obscure, now formed a sharp image (Figs. 13 A-C). In the tilt series illustrated (Figs. 13 A-C), the SR volumes at the three different angles of tilt were 6.7%, 7.5%, and 6.7%, respectively. The SR volume synthesized (by tracing onto a single micrograph the SR membranes seen at all three tilt angles) to include all the membranes visible from all three tilt angles was 10.1%, although we should emphasize that this portion of the smooth muscle cell was selected as an illustrative example because it contained a fairly large amount of SR, and the SR volume was greater than the average values obtained in random sections (Table III).

It has been suggested (Loud, 1968) that a correction factor of +50% should be added to stereologically determined SR volumes, in order to correct for the underestimate due to nonvisualization of membranes tangential to the incident electron beam. In some of our material, as also indicated by our tilt stage examination (see above), a similar correction factor appears to be applicable, although it may be argued that in examining transverse sections of longitudinally running tubules the "tangential underestimate" would be less. Since, at least in AO and MPA smooth muscle, radially oriented tubules (subject to tangential error in transverse sections of smooth muscle) were common and since we probably tended to err in our measurements by excluding rather than including doubtful profiles, we suspect that a valid correction factor applicable to our material in Table III is close to the maximum value of +50% of the measured estimates.

Except where stated otherwise, the tissues were fixed in 2% glutaraldehyde in cacodylate buffer containing 4.5% sucrose, postfixed in 2% osmium tetroxide, and block stained in aqueous uranyl acetate; the pretreatment schedules are given in Methods. Rabbits were used except where stated otherwise. All sections were stained with alkaline lead citrate. All calibration lines are 0.1 μm unless stated otherwise.

Abbreviations

AO, aorta
COL, collagen
DB, dense body
EL, elastic tissue
G, Golgi apparatus
M, mitochondria
MA, mesenteric artery
MPA, main pulmonary artery
MT, microtubule
MYO, myofilaments
PAMV, portal-anterior mesenteric vein
R, ribosomes
SM, smooth muscle
SR, sarcoplasmic reticulum
SV, surface vesicle
TC, taenia coli

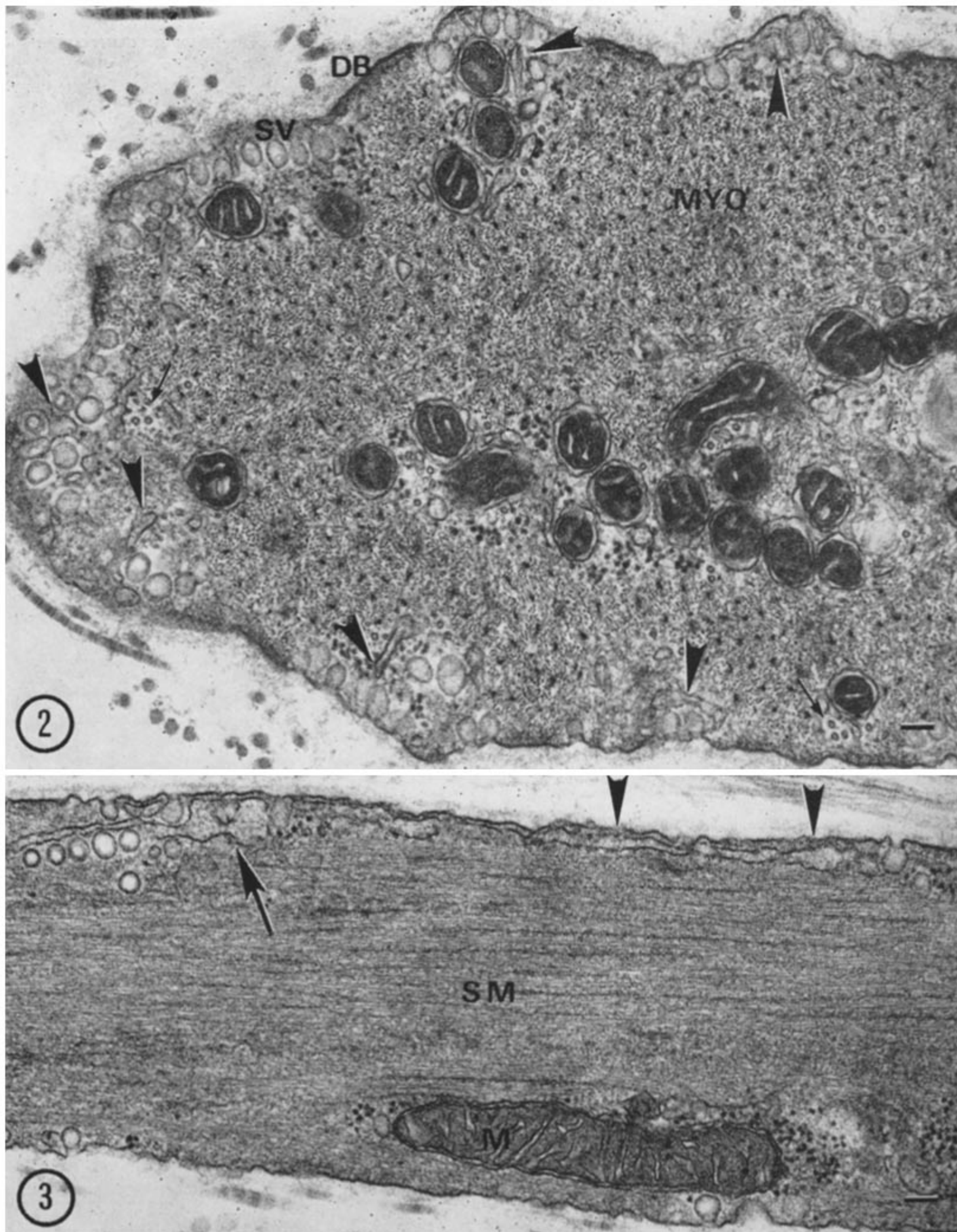
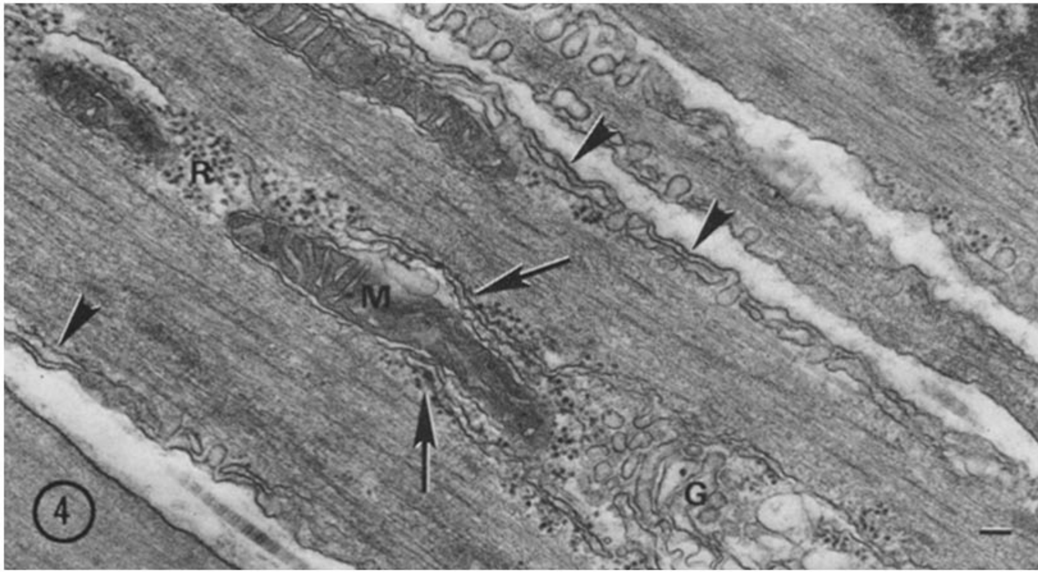


FIGURE 2 Transverse section of a portion of a smooth muscle cell of PAMV showing the SR approaching the surface vesicles and cell membrane (arrowheads). The surface vesicles are absent at the membrane areas occupied by dense bodies, and the SR does not approach the cell membrane at these areas. Microtubules (small arrows) are present close to the SR and mitochondria. Some mitochondria are in close apposition to surface vesicles. Thick and thin filaments are present, with a regular spacing of approximately 70 nm between the thick filaments. PAMV: × 47,000.

FIGURE 3 Longitudinal section through a vascular smooth muscle cell showing an unusually long SR tubule–cell membrane relationship (arrowheads) and a dilated portion of the SR beside some surface vesicles (large arrow). Thick and thin filaments are present in the cytoplasm, and a mitochondrion is in close association with a surface vesicle. PAMV: × 47,000.



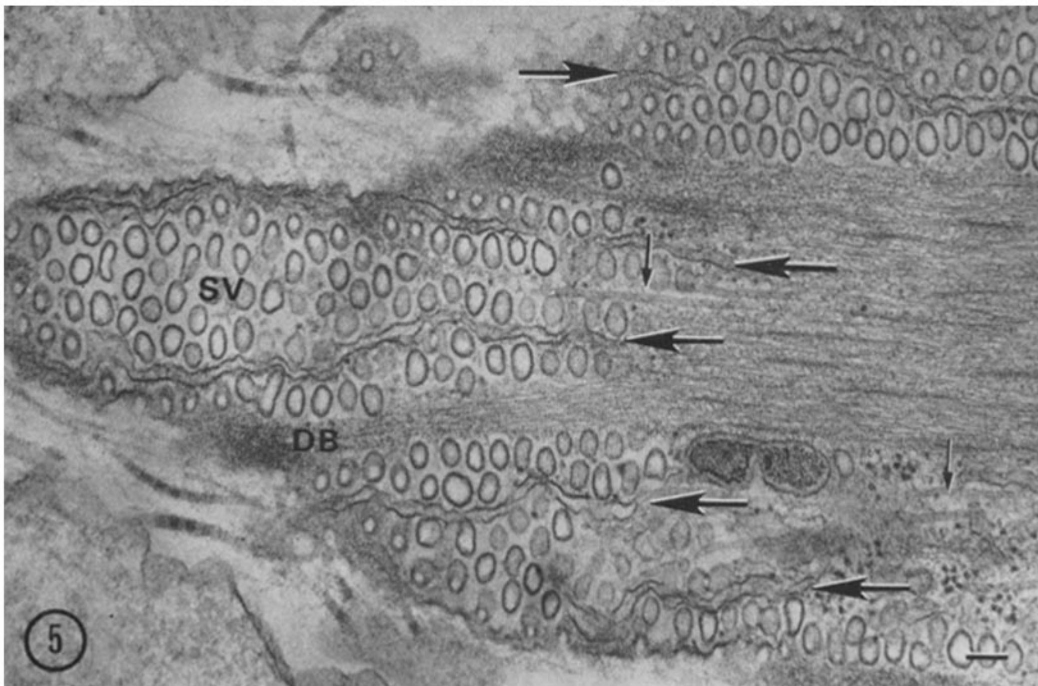


FIGURE 4 Longitudinal section through a vascular smooth muscle cell showing peripheral and central SR. The peripheral SR is close to the cell membrane and surface vesicles (arrowheads); the central SR (more extensive than that usually found) is continuous with the Golgi apparatus and forms a series of longitudinal tubules (large arrows). Ribosomes are more numerous adjacent to the central than to the peripheral SR. Some longitudinally sectioned thick and thin myofilaments are present. PAMV: × 42,400.

FIGURE 5 A tangential section showing finger-like elements of SR (large arrows) running longitudinally through groups of surface vesicles separated from each other by dense bodies. Some microtubules (small arrow) are also present near the surface vesicles. PAMV: × 53,800.

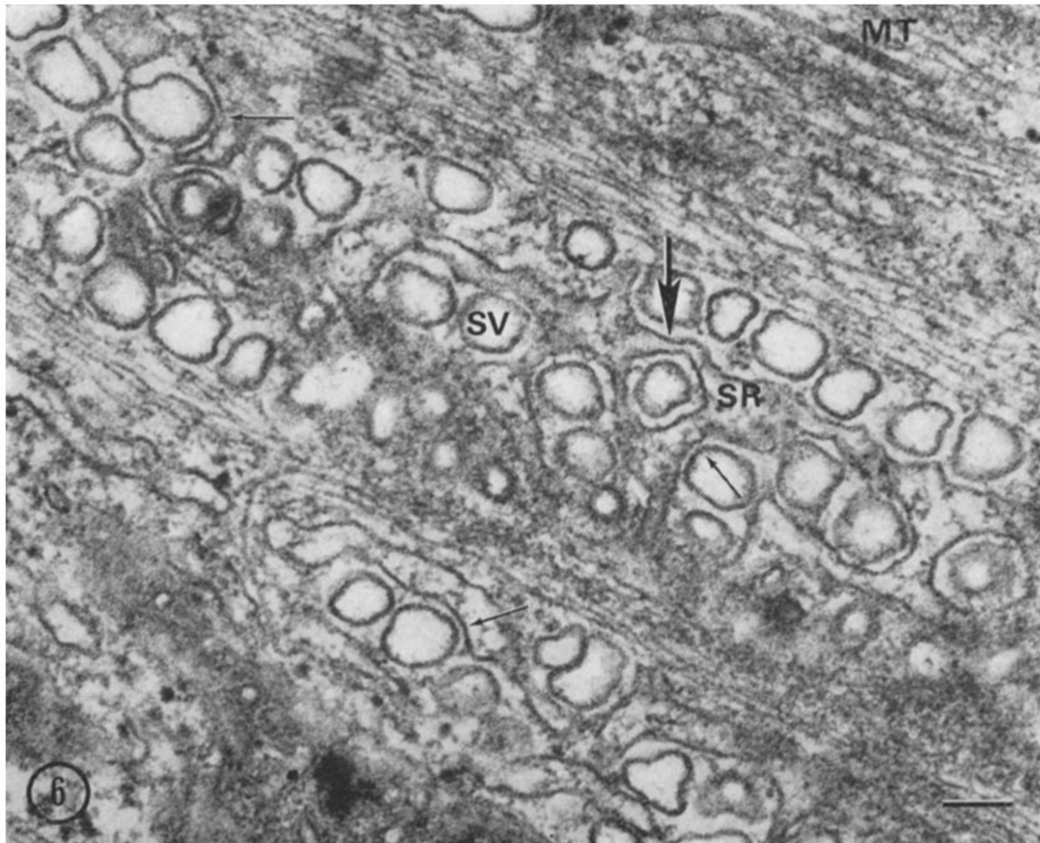


FIGURE 6 An area of longitudinally sectioned vascular smooth muscle cell showing, in tangentional view, a lacelike network of SR associated with and encircling some surface vesicles (large arrow). Occasional close relationships of the SR and the surface vesicles are present with a gap of approximately 10 nm between the two apposing membranes (small arrows). Stained with permanganate in addition to alkaline lead citrate. PAMV: × 95,000.

Main Pulmonary Artery, General Observations, and Distribution of SR

There have been a few previous studies of main pulmonary artery ultrastructure (Reale and Ruska, 1965; Verity and Bevan, 1968; Silva and Ikeda, 1971), but it seems worthwhile to note some general observations facilitated by the more recent improvements in preservation of smooth muscle for electron microscopy.

Transverse sections of the media of the MPA revealed layers of smooth muscle cells (with multiple processes) separated from each other by intervening lamellae of connective tissue elements (Figs. 14, 16). This lamellar arrangement appears to be characteristic of both large and small arteries, although the extent of elastic tissue differs greatly in different arteries. In small arteries, the lamellar arrangement is more evident when the vessels are not allowed to collapse before fixation (for review see Somlyo and Somlyo, 1968 *a*). Smooth muscle cells within a single layer made contact with each other by processes and sometimes by relatively broad areas of cell-to-cell contact. At higher magnification (Fig. 15) some of the contacts were seen to be gap junctions (Revel and Karnovsky, 1967; Uehara and Burnstock, 1970). Occasional close contacts between smooth muscle cells of rabbit main pulmonary artery have been previously reported (Verity and Bevan, 1968). It is possible that the gap junctions that we have seen after glutaraldehyde–osmium tetroxide fixation and block staining with uranyl acetate (Brightman and Reese, 1969) would appear as

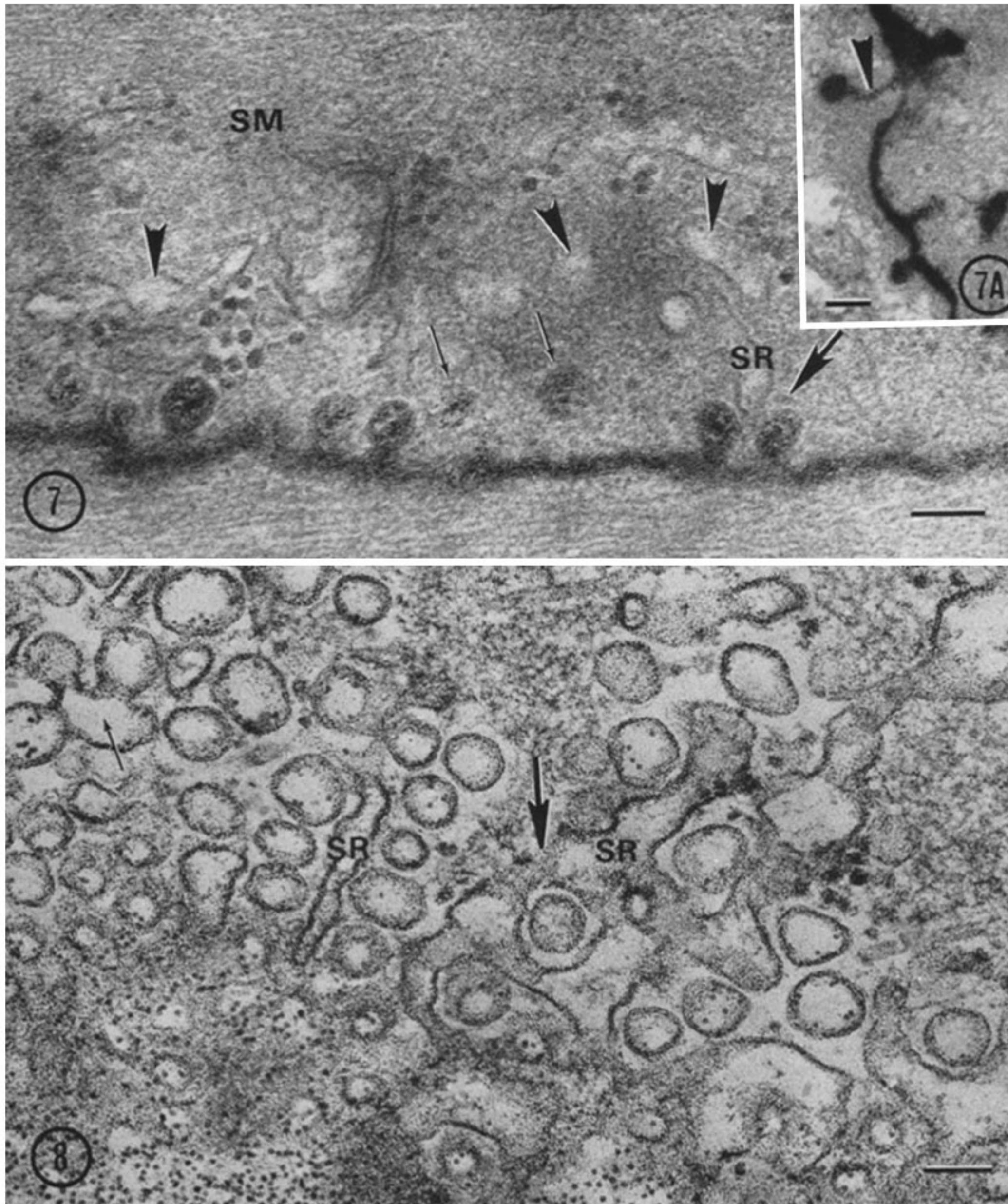


FIGURE 7 There are deposits of lanthanum in the surface vesicles (large arrow) and in the extracellular space between portions of two vascular smooth muscle cells. Some lanthanum deposits are in "free-floating" vesicles not visibly connected to the cell membrane (small arrows), the connection presumably being out of the plane of this section. The SR (arrowheads) approaches the surface vesicles (large arrow).

FIGURE 7 A A surface vesicle connected by an unusually long neck (containing lanthanum) (arrowhead) to the extracellular space between two vascular smooth muscle cells. Guinea pig mesenteric artery, lanthanum treatment, not block stained. Fig. 7, × 100,000; Fig. 7 A, × 60,000.

FIGURE 8 A tangential section through a peripheral portion of a PAMV smooth muscle cell showing ferritin in the extracellular space, attached to the basement membrane and in the surface vesicles. No ferritin is present in the SR which lies between, or forms a fenestrated lacelike network around, the vesicles (large arrow). Some surface vesicles have a common opening (small arrow). PAMV, incubated in Krebs' solution containing ferritin for 90 min. × 93,000.

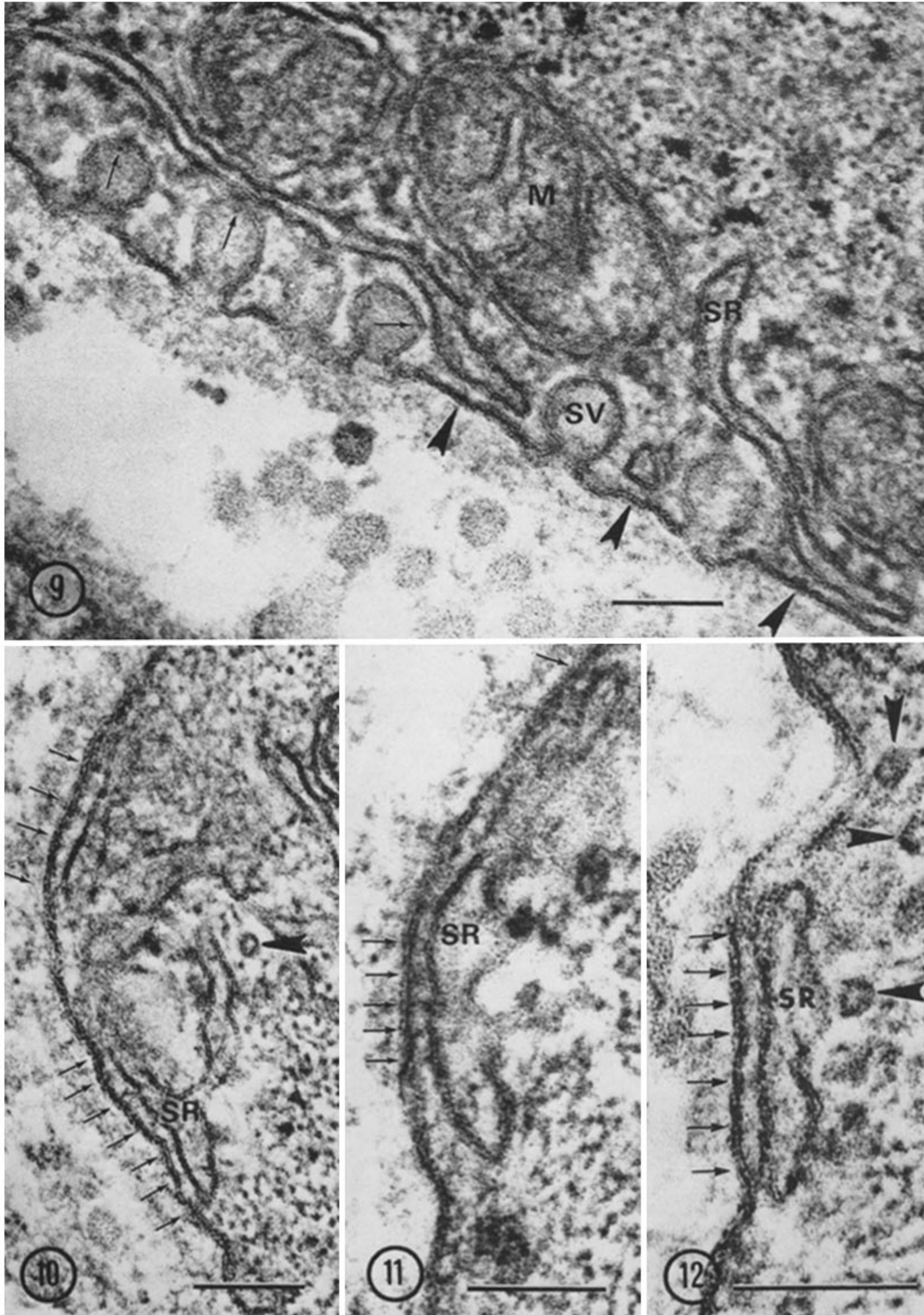


FIGURE 9 A tubular element of SR in close relationship with surface vesicles (small arrows) and the cell membrane (arrowheads). PAMV: × 165,000.

FIGURES 10, 11, and 12 Examples of the SR-membrane relationship (coupling) in which the outer leaflets of the SR and the cell membranes are separated by an approximately 10 nm gap. Electron-opaque material present between the two apposing membranes has a "periodicity" of approximately 20–25 nm (small arrows). Some electron-opaque material is also present in the SR lumen. Microtubules are adjacent to the SR (arrowheads). PAMV; Fig. 10, × 165,000; Fig. 11, × 207,000; Fig. 12, × 276,000.

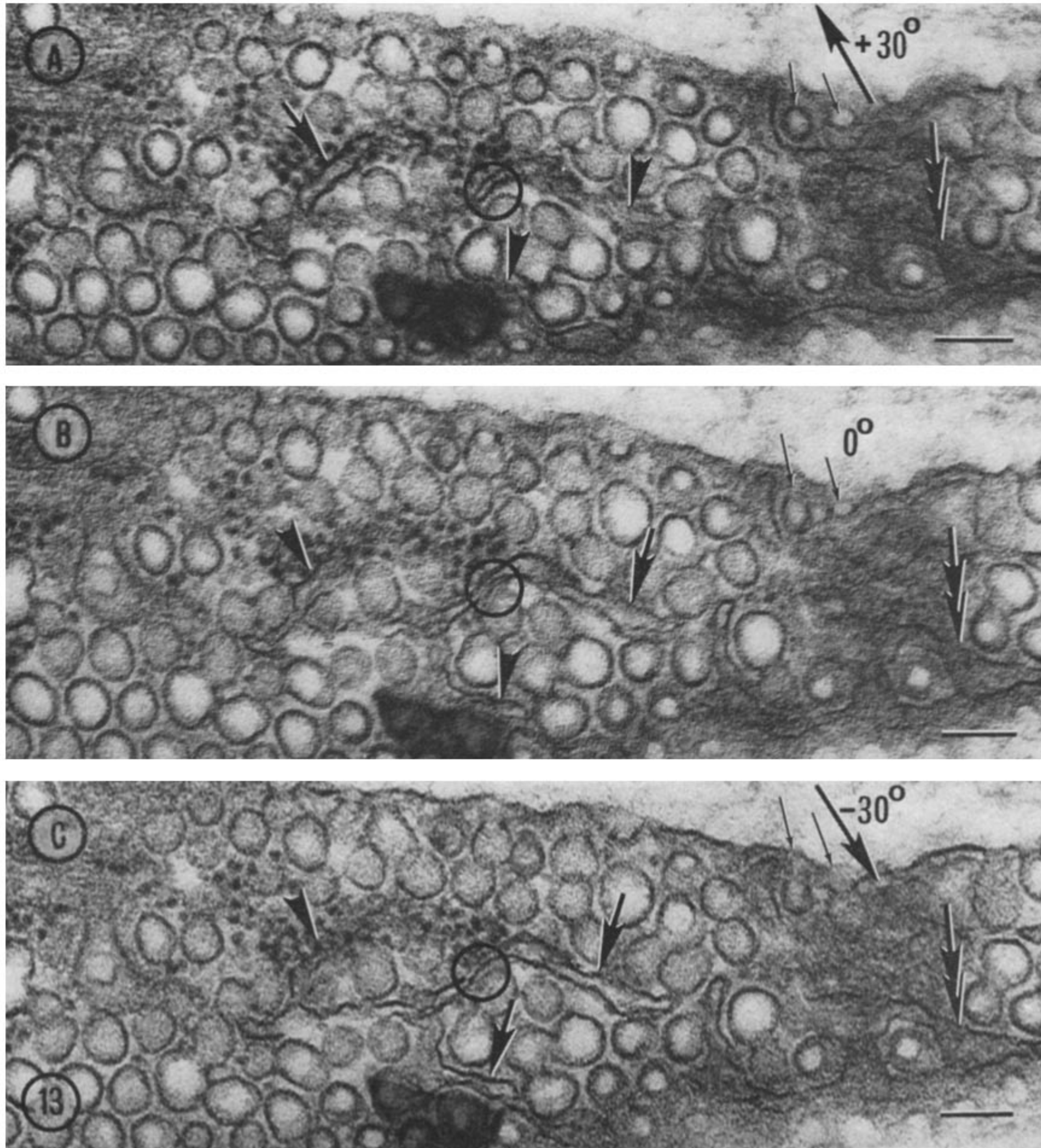


FIGURE 13 A, B, C A tilt series from (A) +30° through (B) 0° to (C) −30° through the tip of a vascular smooth muscle cell sectioned tangentially through surface vesicles, sarcoplasmic reticulum, and basement membrane. The direction of tilt is indicated by the long arrows. The appearance of the vesicles alters slightly and, in some instances, the opening to the extracellular space (small arrows, Fig. 13 C) changes in appearance and even disappears (small arrows Fig. 13 A) with the tilt angle. The sarcoplasmic reticulum in Fig. 13 B, at 0° tilt, is only partially visualized, as revealed by comparison with the micrographs at + 30° and − 30° that show membranes previously blurred (at 0° tilt) due to tangential incidence of the electron beam. The complete large short arrows show well-defined SR, and the arrowheads show ill-defined SR which is visualized better by varying the tilt. The circled region shows how the relationship between the SR and vesicle membranes changes with the tilt angle. A loop of SR encircles one of the surface vesicles (double arrow). This series illustrates how the volume of the SR may be underestimated from a single micrograph. PAMV: × 100,000.

TABLE III

Percentage Volumes of Sarcoplasmic Reticulum

Animal no.	PAMV	MPA	AO	MA	TC
1	2.7 (55)	—	—	—	—
2	2.2 (25)	4.2 (12)	3.5 (9)	—	1.5 (29)
3	2.6 (33)	5.3 (7)	6.9 (15)	—	1.7 (16)
4	1.5 (11)	6.0 (13)	4.8 (21)	1.8 (28)	1.4 (15)
5	—	—	—	1.8 (16)	—
6	—	—	—	—	3.6* (20)
Mean	2.2 (124)	5.1 (32)	5.0 (45)	1.8 (44)	2.0 (80)

The percentage of cell volume (excluding nucleus and mitochondria) occupied by the sarcoplasmic reticulum. Portal-anterior mesenteric vein (PAMV), main pulmonary artery (MPA), aorta (AO), mesenteric artery (MA), and taenia coli (TC). The number of cells measured is given in parentheses after the percentage value.
* In this taenia coli, discussed in text, there was a more than usual amount of collagen.

nexuses (Dewey and Barr, 1962; Cobb and Bennett, 1969) with permanganate fixation, but we did not explore this point. The presence of cell contacts with gap junctions in MPA smooth muscle suggests that the rhythmic contractions of similar preparations in Na-free solutions (Somlyo and Somlyo, 1971 *a*) can be ascribed to conducted activity. The smooth muscle cells of the subendothelial region appeared less regularly packed (within a greater extracellular space), suggesting that in this region conducted activity over extensive distances is less likely to occur than in the deeper and more regularly organized media. The total amount of connective tissue components was much larger in the MPA (and AO) than in the PAMV and TC, although the collagen content of the latter was variable. The shape of the smooth muscle cells of the MPA (and AO) was irregular with several processes, these cells resembling in this respect the smooth muscle cells of the turtle aorta (Somlyo et al., 1971 *c*), and did not lend itself to a reliable estimate of the cell diameter.

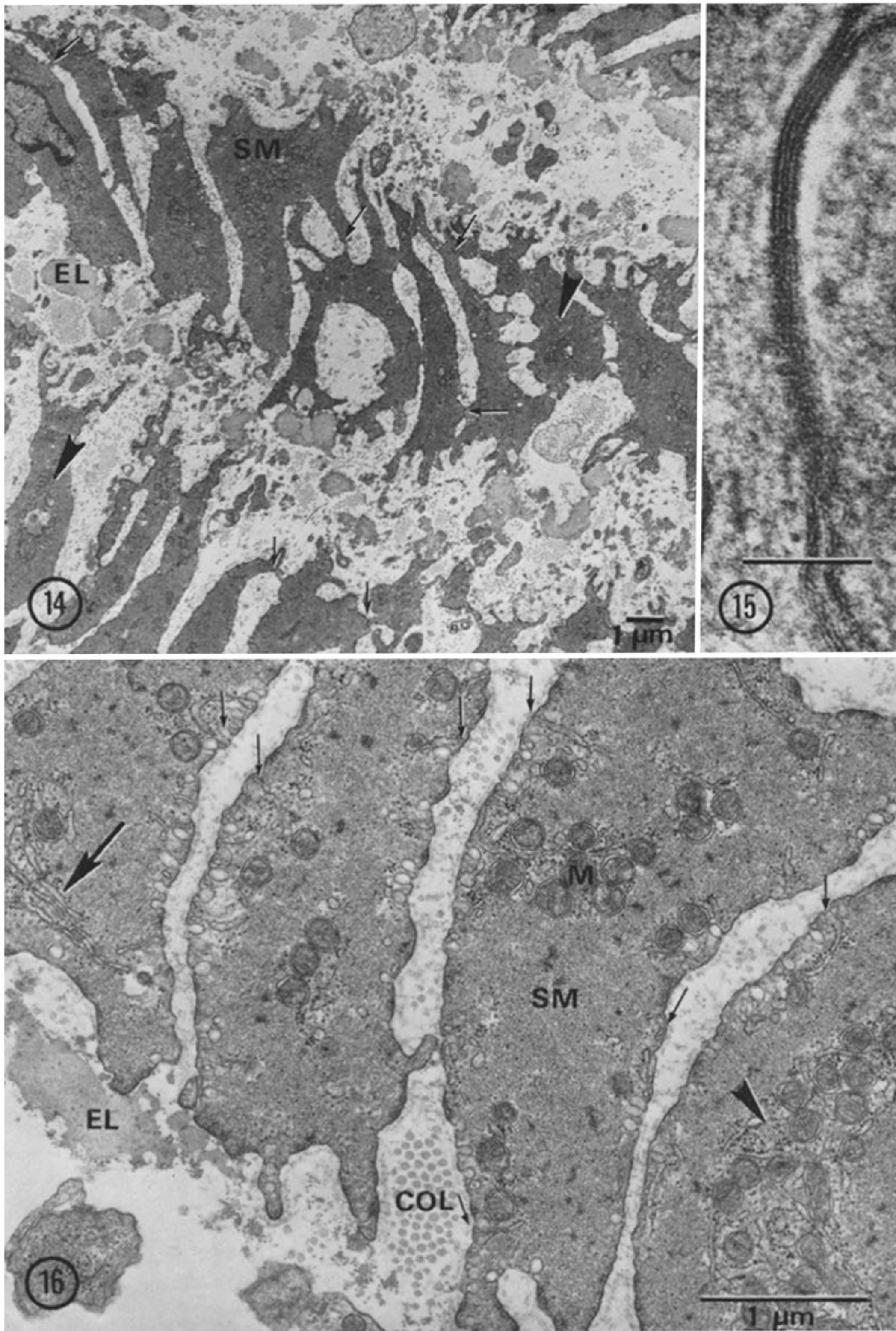
In transverse sections, there was a voluminous system of SR tubules (Figs. 16, 17, 19, 20) and, in particular, the centrally located SR appeared much more prominent than in the average PAMV fiber. The SR included both segments covered with ribosomes (rough SR) and smooth SR, and in many places the two types of tubules were continuous. The centrally located SR tubules often formed radial extensions to the periphery (Figs. 19, 20, 23). Regions of relatively regular-stacked SR membranes were a characteristic feature of MPA (Figs. 16, 17, 19, 20) and AO (see below) (Figs. 24, 25) smooth muscle. In tangential section the stacked regions of SR tubules had a fenestrated appearance (Fig. 23), but the presence of ribosomes on some of the tubular membranes and the failure of extracellular markers to penetrate these tubules (Fig. 19) indicated that these tubules were part of the sarcoplasmic reticulum, and bore only superficial resemblance to the tubular systems of developing muscle (Ishikawa, 1968) that communicate with the extracellular space.

In longitudinal sections, groups of SR and mitochondria separated longitudinally running fibrillar elements into bundles including dense bodies (Fig. 18). Conglomerations of SR tubules and mitochondria were found at the nuclear poles in MPA (and other smooth muscles), a localization mentioned in previous studies of smooth muscle (Shoenberg, 1958; Reale and Ruska, 1965). Microtubules, previously noted in smooth muscle by others (Sachs and Daems, 1966; Sandborn et al., 1967), were commonly seen running longitudinally and associated with mitochondria and SR. Fenestrations of a lacelike network of SR overlying surface vesicles, like those illustrated in the other smooth muscles (Figs. 6, 8, 26, 29, 30), were also seen in longitudinally oriented, tangential sections of MPA.

Close (approximately 10 nm) contacts between the SR and the surface membranes were also observed in MPA smooth muscle (Figs. 20, 22, 23).

Myofilaments and Intermediate Filaments in MPA Smooth Muscle

The two types (thick and thin) of myofilaments previously described in PAMV (Devine and


SM
EL
14
1 µm
15
M
SM
EL
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16
1 µm

Somlyo, 1971; Rice et al., 1971; Somlyo et al., 1971 *a*; 1971 *d*) were also observed in rabbit MPA smooth muscle (Figs. 16, 21). The thin-to-thick filament ratio was high: this seems characteristic of vertebrate smooth muscles thus far examined (Rice et al., 1970; 1971; Devine and Somlyo, 1970). Rosettes formed by two or three rows of thin filaments surrounding a thick filament were common (Fig. 21), and this lattice configuration allowed space for a central thick filament but not for a large (e.g., ribbon-like) structure. The variation in the diameters of the thick filaments occupying the central position in such rosettes was sufficiently great (Fig. 21) to be compatible with tapered filaments sectioned at different levels of their taper within a single cross-section. Intermediate-size (10–12 nm) filaments described previously in other smooth muscles (Rice et al., 1970; Somlyo et al., 1971 *a*; Uehara et al., 1971) were also observed in MPA smooth muscle as well as in the other smooth muscles examined.

Thick filaments were reproducibly demonstrated in several embeddings, and their apparent absence from some micrographs taken at low magnifications was due to technical factors such as oblique sectioning (Somlyo et al., 1971 *d*). At higher magnifications, thick filaments were readily distinguished by their position within the rosette and by their diameter which is smaller than that of the ribosomes: the presence of thick filaments was also verified in longitudinal sections. The best ordered rosettes were usually seen towards the periphery and at the tips of the MPA smooth muscle fibers.

Aorta

The general structure of the rabbit aorta has been described previously (Bierring and Kobayasi, 1963; Seifert, 1963; Stein et al., 1969) and we shall summarize only those observations pertinent to the distribution of SR.

In all major aspects, the distribution of SR in AO smooth muscle resembled its distribution in the MPA. In transverse section, stacked tubules of rough and smooth SR were present (Figs. 24, 25) and were connected to the periphery through radially oriented tubules. In longitudinal section the oblique segmentation into fibrillar and organelle-rich regions observed in MPA, and previously noted in rat aorta (Cliff, 1967), was observed.

Small Mesenteric Artery, Taenia Coli, and Human Uterus

In MA smooth muscle the extent of SR was much more limited than in the large arteries (see Table III), and its distribution (although not examined in as great detail) seemed similar to that in PAMV. Both the characteristic fenestration around and contacts with surface vesicles (Fig. 26) and the SR surface membrane couplings (Fig. 27) were present.

The distribution of the SR in the TC resembles that seen in the PAMV. The diameter of the TC muscle fibers (2.5–7.5 μm through the nuclear region) may have been somewhat larger than that of the PAMV muscle fibers (2–6 μm), but

FIGURE 14 Low magnification view of transversely sectioned MPA showing portions of three layers of smooth muscle, with each layer being separated from the adjoining one by elastic tissue and collagen. The cells within the same layer come close to each other in the form of long processes or plaquelike areas, and in some cases these form presumptive or possible gap junctions (small arrows) but the membranes cannot be resolved at this magnification (for an example at a higher magnification, see Fig. 15). Collections of SR and mitochondria are indicated by arrowheads. MPA: × 5,760.

FIGURE 15 A gap junction between two MPA smooth muscle cells. The distance between the two outer leaflets of the cell membrane is approximately 2.5 nm. MPA: × 204,000.

FIGURE 16 Low magnification view of a group of transversely sectioned MPA smooth muscle cells illustrating the variations in the distribution of SR. In one fiber there is a large central grouping of SR with mitochondria (arrowhead), while in other fibers there is more peripheral SR (small arrows), or the layered SR is present (large arrow). Some of the variations in SR distribution may reflect the level of sectioning, since longitudinal sections show a very prominent mass of central SR at the nuclear poles. Elastic tissue and collagen are present. MPA: × 25,500.

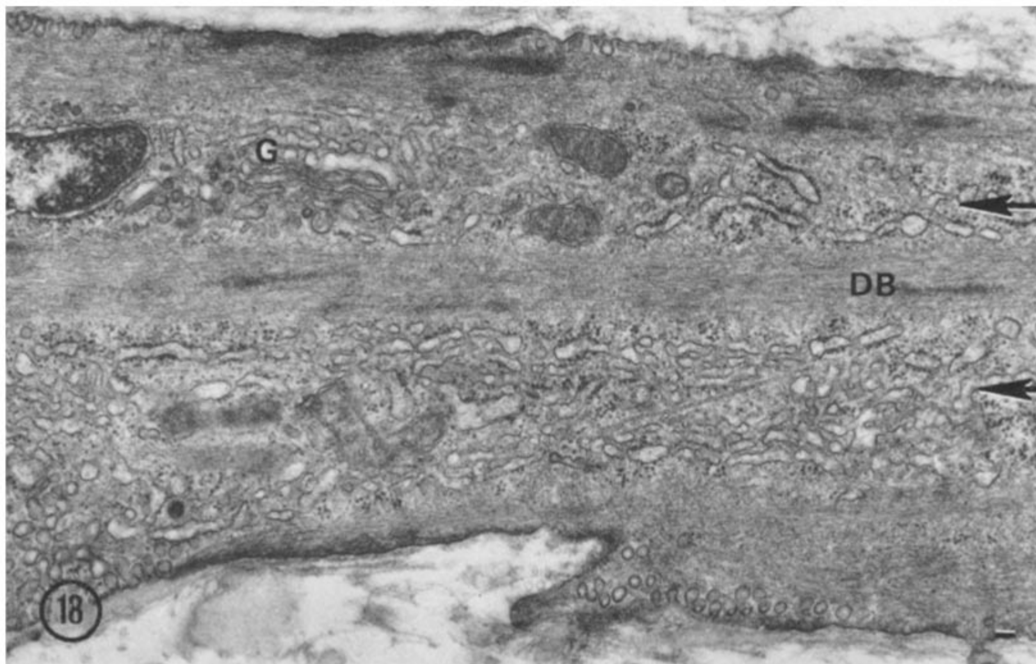
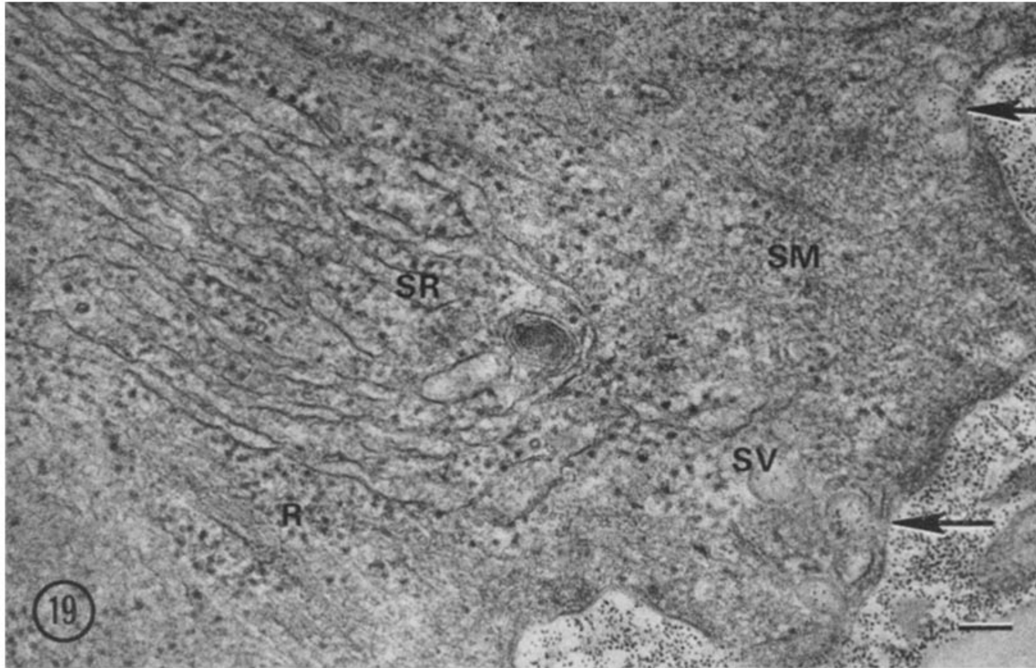


FIGURE 17 Transverse section through MPA smooth muscle cell showing two groups of central "layered" SR (arrows). Ribosomes are present on some SR membranes. Microtubules (commonly seen in association with SR and mitochondria) are present. MPA: × 42,400.

FIGURE 18 Longitudinal section through MPA smooth muscle. The longitudinally arranged region of SR and mitochondria (large arrows) presumably corresponds to the regions of layered SR seen in transverse sections but possibly not as closely packed (Figs. 17, 18, 20). The bundles of SR and mitochondria are separated from each other by fibrillar elements containing dense bodies. One longitudinal layer of SR extends from the Golgi apparatus near the nucleus, but the other is not associated with the nucleus and approaches the cell membrane at some regions. MPA: × 25,500.



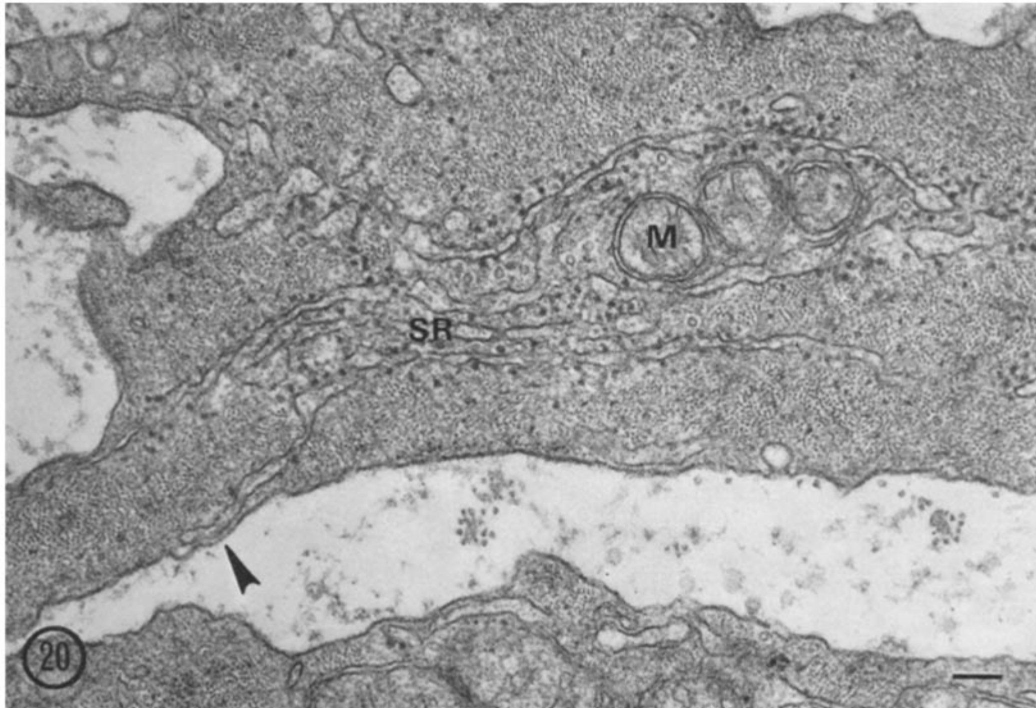


FIGURE 19 A section through an MPA smooth muscle cell showing ferritin in the extracellular space, and in surface vesicles (large arrows), but not in the layered SR, part of which has ribosomes attached to the membranes. MPA, incubated in Krebs' solution containing ferritin for 90 min. × 69,000.

FIGURE 20 Transversely sectioned MPA smooth muscle cell with an element of central layered SR connecting with the peripheral SR in close apposition to the cell membrane (arrowhead). The thick and thin myofilament arrangement can be seen in this region of the cell. MPA: × 63,000.

since this measurement depends on the degree of stretch of the individual muscle fiber, and since all cells do not have a circular profile, we do not know whether the difference in diameter reflects a true discrepancy of the two cell sizes. The question is of some functional interest, because (if most of the SR is near the cell membrane) a more extensively developed peripheral SR in a larger fiber would yield quantitative estimates of SR that would be similar to (or conceivably less than) those of a more scantily developed peripheral SR in a smaller fiber. The central SR of the TC, like that of the other smooth muscles, was directly connected with the peripheral SR forming the couplings (Fig. 28). The characteristic grouping of SR tubules threading their way and forming fenestrated networks around groups of surface vesicles, each grouping being separated from the adjacent one by dense bodies, was also seen in longitudinal sections of TC (Figs. 29, 30).

The well-developed SR of human uterine smooth muscle at term and the connections between centrally located and peripheral SR previously described in OsO_4-permanganate–fixed tissues (Laguens and Lagrutta, 1964) were also observed in our material (Fig. 30). Thick and thin myofilaments and intermediate filaments (Fig. 31) were also present, as noted previously (Somlyo et al., 1971 *a*). Higher magnification views showed that surface couplings were formed between the plasma membrane and the junctional SR (Fig. 32).

Estimates of SR Volumes

The SR volumes of different smooth muscle preparations measured from montage preparations (see Methods) are shown in Table III. The greater volume of SR in the MPA and AO smooth muscles as compared to the other smooth muscles studied is of a sufficient magnitude to be clearly detectable by inspection of representative electron micrographs. We consider this difference to be sufficiently great to be very significant, even with the admittedly limited accuracy of volume measurements. The variations in the SR volumes among the other smooth muscles are far too small to be significant without a much more extensive series of determinations, and these differences could have been due to vagaries of sampling within a block or to differences among different animals. We had the distinct impression that the volume of SR in a given smooth muscle in different animals, or even within different regions of the same smooth muscle strip of one animal, can be quite variable. There appeared to be some association, in smooth muscles that normally have a less extensive SR (PAMV, TC), between the appearance of a more than usually prominent SR and the presence of a more extensive network of collagen. This association, for example, was found in the TC having the highest SR volume in Table III. Perhaps this was due to the presence of a larger amount of rough SR in these smooth muscles (see Discussion), but we did not attempt to separate the rough and smooth SR volumes since the two systems were continuous. The previous impression, based on a review of published material (Somlyo and Somlyo, 1968 *a*), that there is only a "small amount of SR" in AO smooth muscle must be considered erroneous in the light of the present comparative and more quantitative study.

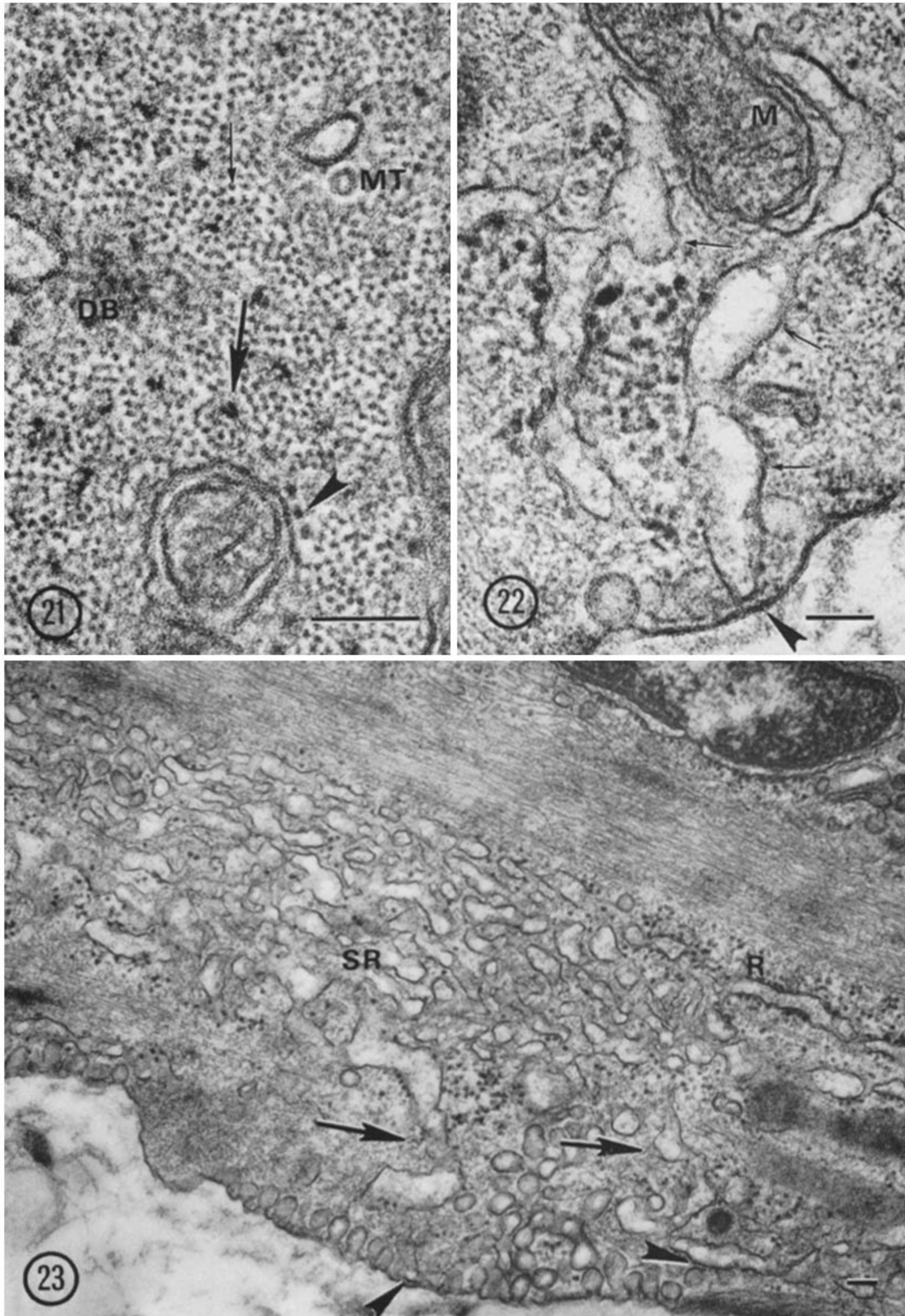
DISCUSSION

Our major conclusion is that there is in mammalian smooth muscles a sarcoplasmic reticulum that, while being variable in different smooth muscles, is sufficiently well developed and or-

FIGURE 21 A high magnification of rosettes formed by thick and thin filaments in MPA. There is an amorphous material surrounding the thick filaments (large arrow) and beyond this region rows of thin filaments are present (small arrow). The thick filament spacing is approximately 60–70 nm; some intermediate-size (10–12 nm) filaments (arrowhead) are present close to a mitochondrion. MPA: × 165,000.

FIGURE 22 Transverse section of MPA smooth muscle showing a portion of SR approaching the cell membrane. The tubular SR has some small varicosities (small arrows). The distance between the SR and the cell membranes is approximately 10 nm (arrowhead). MPA: × 103,500.

FIGURE 23 A branching network of SR, a variation of layered SR, lying longitudinally in an MPA smooth muscle cell; the branches (arrows) form contact with the surface vesicles (arrowheads). Some ribosomes are attached to portions of the SR. MPA: × 42,400.


MT
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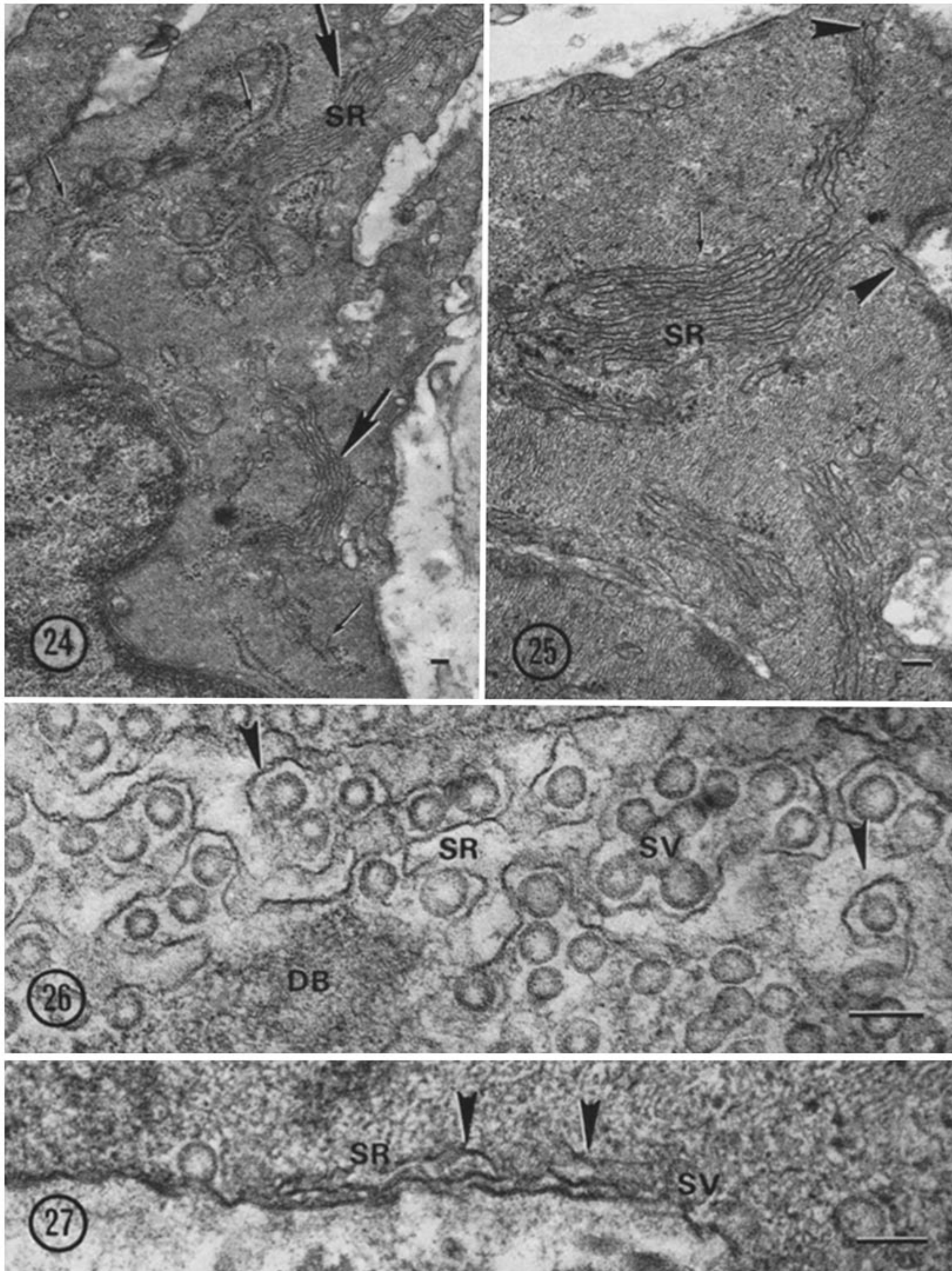

FIGURE 24 Transverse section of aortic smooth muscle cell with two regions of layered SR (large arrows) and portions of a rough SR (small arrows). Only part of the large number of cell processes can be seen in this micrograph. The SR arrangement is essentially the same as that found in the MPA (cf. Figs. 14–23). AO: × 25,500.

FIGURE 25 A portion of layered SR (small arrow) connected to peripheral SR in close relationship with the cell membrane (arrowheads). AO: × 42,400.

FIGURE 26 A portion of a mesenteric artery smooth muscle cell showing surface vesicles threaded by a network of SR, some of which encircles the vesicles (arrowheads). MA, unstretched. The fixative was buffered with Krebs' bicarbonate solution. × 107,500.

FIGURE 27 An element of SR (arrowheads) lying beside the cell membrane of a mesenteric artery smooth muscle cell; a distance of approximately 10 nm separates the two membranes. MA, unstretched. × 103,500.

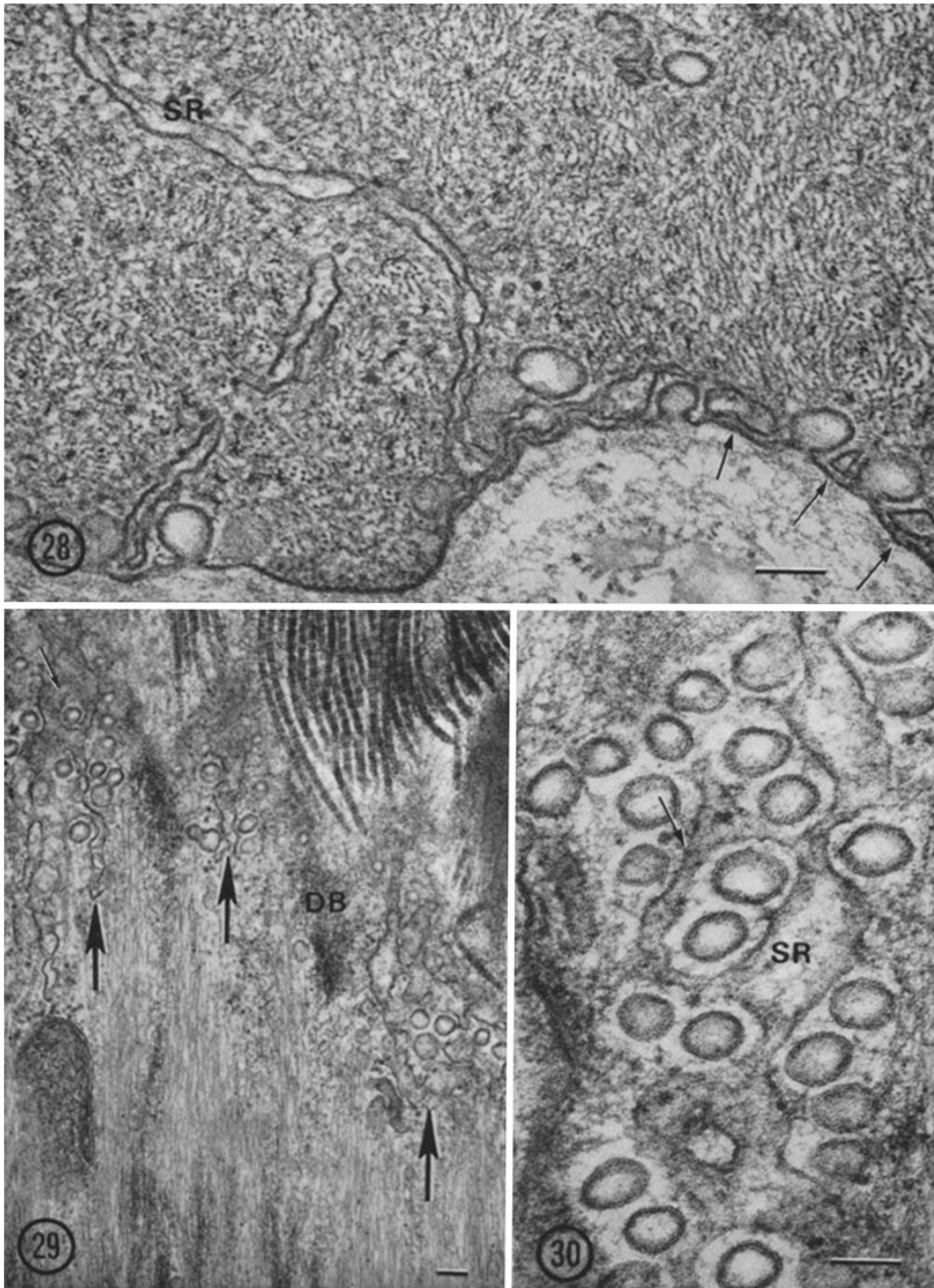

FIGURE 28 Section through a taenia coli smooth muscle cell showing an element of SR approaching the cell membrane where it forms a close contact. Small elements of the SR between the surface vesicles (small arrows) possibly represent transversely sectioned parts of the tubular SR encircling the vesicles (see Figs. 29 and 30). TC: × 103,500.

FIGURES 29 and 30 Low and high magnification views of longitudinally sectioned TC smooth muscle showing the longitudinally arranged SR (large arrows, Fig. 29) running between the surface vesicles (Fig. 29) and encircling some of them (small arrow, Fig. 30). TC: Fig. 29, × 42,400; Fig. 30, × 103,500.

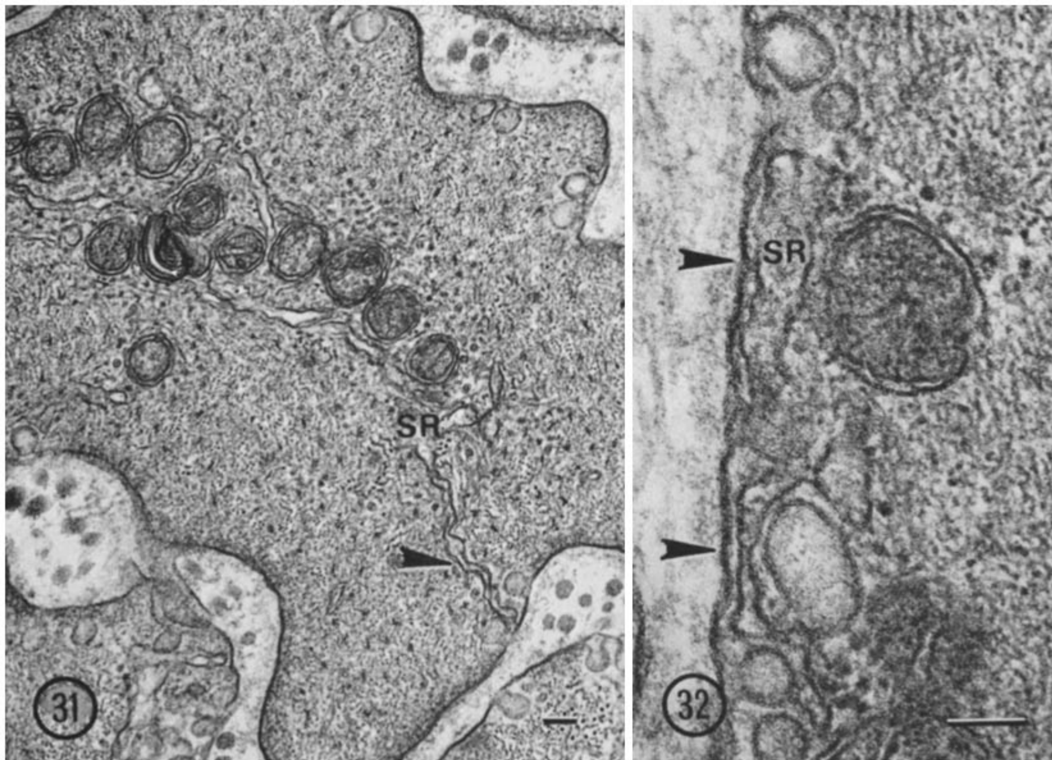

FIGURE 31 Transversely sectioned human uterine smooth muscle cell showing an element of central SR continuing peripherally (arrowhead). Thick and thin myofilaments are also present. Human uterine smooth muscle, 6% sucrose in buffer wash only. × 42,400.

FIGURE 32 High magnification of a region of human uterine smooth muscle showing SR in close relationship (approximately 10 nm) with the cell membrane (arrowheads). Some electron-opaque substance is in the SR. Electron-opaque densities are also present in the gap between the SR and cell membrane. Human uterine smooth muscle, 6% sucrose in buffer wash only. × 103,500.

ganized in its relationship to the surface membrane to function as a Ca store in the processes of excitation-contraction and inhibition-relaxation coupling. Although a number of authors have made mention of elements of SR in mammalian smooth muscle (Caesar et al., 1957; for reviews see Somlyo and Somlyo, 1968 *a*; Burnstock, 1970), the present studies are the first to demonstrate that the smooth-surfaced tubules observed are part of a closed SR into which extracellular markers (ferritin, lanthanum) do not penetrate. This extends our previous observations on the SR of reptilian smooth muscles (Somlyo et al., 1971 *c*) to several mammalian smooth muscles including the human uterus. Our initial reports of these findings (Devine and Somlyo, 1970; Somlyo and Somlyo, 1970 *b*) were followed by observations on the presence of SR in intestinal smooth muscle by Gabella (1971) although the latter author appears to be unaware of the earlier reports.

It was surprising to find that the tonic smooth muscle of the pulmonary artery (Somlyo and Somlyo, 1968 *b*; 1969; Somlyo et al., 1969) contained a larger volume of SR than the "twitch-muscle" of the portal-anterior mesenteric vein, because the volume of the SR in twitch-striated muscles is generally greater than that in tonic-striated muscles. We also had the distinct impression that a relatively large proportion of the SR present in the PAMV was peripheral, whereas in the MPA smooth muscle the centrally located SR was more voluminous. The possibility that the SR of main pulmonary artery smooth muscle that we estimated included a proportion of rough

SR that does not necessarily contribute to calcium storage (but see below) cannot be ruled out. We have included the entire SR in our estimates because there was no sharp distinction between the SR to which ribosomes were attached and the adjoining and continuous smooth-surfaced SR, and because deposits of strontium accumulated by SR continuous with the rough SR have been observed (Fig. 4; Somlyo and Somlyo, 1971 *b*). The latter observations are not yet sufficiently quantitative to conclusively resolve the question of Ca storage by rough SR.

The ability of main pulmonary artery smooth muscle to contract in the virtual absence of extracellular calcium, and the absence of significant contractions in PAMV under similar conditions, are in apparently good correlation with volume and distribution of SR in these two muscles. The larger volume of more centrally located SR of the main pulmonary artery and aorta may be less readily depleted of its calcium stores than the more scanty and peripherally located SR of the portal-anterior mesenteric vein and taenia coli. The possibility that the correlation is fortuitous cannot be ruled out. Both central and peripheral elements of the SR can accumulate divalent cations (Somlyo and Somlyo, 1971 *b*).

It has been estimated (Somlyo et al., 1971 *c*) that, *assuming the release of 20% of the calcium stored by the SR*, the SR would have to be about 4% of the cell volume for a threshold and about 7% for a maximal contraction. The assumptions utilized for these estimates are too numerous and the quantitation of the SR in smooth muscle is probably too inaccurate to permit us to attach great significance to the fact that some of the smooth muscles sampled in the present study had an SR volume of less than 4%. We suspect, as the most probable source of discrepancy, the assumption that only 20% of calcium stored by the SR can be released during excitation-contraction coupling. The rabbit PAMV develops a twitch in response to a *single* action potential (Somlyo and Somlyo, 1968 *b*; 1970 *a*), and the calcium released by the action potential from the SR appears to suffice for activating contraction, since the possible calcium current that could be moving inward during a single smooth muscle action potential (Goodford, 1967) is nearly two orders of magnitude below that required for activating a contraction (Somlyo et al., 1971 *c*). Analysis of the magnitude of electrically stimulated twitches of rabbit TC as a function of extracellular Ca concentration also suggests an intracellular source of activator Ca (M. J. Siegman, personal communication). It is also possible that twitch contractions triggered by single action potentials in smooth muscles containing relatively small amounts of SR (PAMV, TC) involve only the peripheral portion of the smooth muscle fiber, where the SR-sarcolemma couplings are located. The (uncorrected) SR of the rat ventricle has been estimated (Page et al., 1971) to occupy 3.5% of cell volume (corresponding to an approximately 5.2% volume corrected for tangentially sectioned membranes), and it is generally accepted that mammalian cardiac muscle is activated by internal stores of calcium. Thus, while the frog sartorius contains a total SR volume of approximately 13% and a terminal cisternal volume of 5% (Peachey, 1965 *b*), considerably smaller volumes of SR consisting of primarily tubular (rather than cisternal) structures are sufficient to activate twitch contractions in other vertebrate muscles.

The different amounts of SR in the various smooth muscles appear to correlate with the participation of smooth muscle in protein synthesis (see below) and, perhaps, with the extent of glycogen synthesis (Knoth et al., 1969; Somlyo et al., 1971 *c*). Significant changes in the SR volume of aortic smooth muscle occur with maturation (Karrer, 1961) and with aging, although observations regarding the effect of the latter are conflicting (Stein et al., 1969; Cliff, 1970). There is a marked proliferation of the SR in the estrogen-treated and the pregnant uterus (Shoenberg, 1958; Ross and Klebanoff, 1967; 1971), and an increase in the volume of SR is also one of the characteristic responses of vascular smooth muscle to various types of injury (for reviews see Somlyo and Somlyo, 1968 *a*; Ross and Klebanoff, 1971). Technically, all of these factors and the vagaries of fixation for electron microscopy might be expected to introduce a rather wide variability of SR volumes in smooth muscle. If the rough SR, which is generally the most enlarged under the conditions listed above, contributes to the storage of calcium for excitation-contractiong coupling, then major variations in the intracellular calcium stores of smooth muscles may be anticipated depending on the functional state of a given preparation.

The relationship of the peripheral SR tubules

to the surface membrane involved an approximately 10 nm gap that, occasionally though by no means invariably, was traversed by densities suggestive of 20–25 nm periodicity (Figs. 10–12). These couplings, shown to be sites of cation accumulation (Somlyo and Somlyo, 1971 *b*), would seem to be the most probable sources from which calcium can be released by the action potential: a twitch mechanism proposed on the basis of electrophysiological studies of rabbit portal-anterior mesenteric vein (Somlyo and Somlyo, 1968 *a*; *b*; 1970 *b*; Devine and Somlyo, 1970) and subsequently extended to reptilian smooth muscles (Bozler, 1969; Somlyo et al., 1971 *c*). The mechanism of signal transmission between the two (SR and surface) membranes is subject to the same uncertainties voiced about direct electrical transmission across the (10 nm) triadic junctions of striated muscles (Forssmann and Girardier, 1970; Franzini-Armstrong, 1970; 1971), because membrane-to-membrane distances at the smooth muscle SR couplings are also significantly greater than at the gap junctions (compare Figs. 10–12 with Fig. 15). A feasible alternative to direct electrical coupling (in striated twitch muscles) has been suggested by Bianchi (1961; Bianchi and Bolton, 1966), who proposed that the influx of "trigger" calcium during the action potential or during K-depolarization may release activator Ca from the SR.

The close contacts between the SR and the surface vesicles (Figs. 9, 13) were infrequently traversed by electron-opaque material, and periodicity of "bridges" could not be expected to occur over the short distances of contact. We cannot be certain whether the small electron opacities between surface vesicles and SR were fortuitous. Perhaps, both the surface couplings and the contacts between the SR and surface vesicles participate in electromechanical coupling, but the possibility that the fenestrated regions around surface vesicles are more concerned with uptake and outward transport (rather than release) of calcium cannot be excluded. It might be noted that in rabbit portal-anterior mesenteric vein the mitochondria often make extensive contacts with surface vesicles, and the distances (4.4 nm) between the mitochondrial and surface vesicle membranes are less (Somlyo et al., in press) than the intermembranous distance at the SR–surface membrane couplings. The possibility that the mitochondrial–surface vesicle contacts are sites of ion transport has been suggested (Somlyo et al., in press), and a close relationship of mitochondria to surface vesicles has also been observed in freeze-etch preparations of guinea pig taenia coli (P. Goodford, personal communication).

The observation that intracellular calcium stores are less readily depleted in calcium-free media at lower than at higher temperatures (Somlyo et al., 1971 *c*; present study) has also been independently established by Keatinge (1971) in studies of sheep carotid artery; his calculations indicate that the half-hour incubation in Ca-free media with ethylenediamine tetraacetate (EDTA) is sufficient to remove the extracellular, unbound calcium and that the temperature-sensitivity of the loss of contractility in such solutions has a high activation energy compatible with an effect on active Ca-transport (W. R. Keatinge, personal communication). The residual contractile response of smooth muscle in Ca-free solutions at low temperatures appears to be a useful method for studying that portion of drug-induced activation that is dependent on intracellular stores of calcium. In general, the present observations confirm the impression that internal calcium stores contribute to activation at least in aortic (Altura and Altura, 1969[1]; Goodman and Weiss, 1971), main pulmonary artery (present study), and sheep carotid artery (Keatinge, 1971) smooth muscle, but leave open the question whether maximal drug-induced contraction in some smooth muscles is activated, at least in part, by influx of extracellular calcium (Somlyo and Somlyo, 1970 *b*). Electrophysiological studies suggest that norepinephrine does increase the calcium permeability of vascular smooth muscle membranes (Somlyo and Somlyo, 1971 *a*), although this has been contested on the basis of certain ^{45}Ca flux studies (van Breemen and Lesser, 1971). Acetylcholine-induced contractions have been related to changes in membrane permeability (Durbin and Jenkinson, 1961), and acetylcholine also increases calcium influx into smooth muscle (Potter et al., 1970). The present studies should not be construed to indicate that the SR is the *sole* source of activator Ca in *all* mammalian smooth muscles, but are consistent with earlier

[1]In view of the low concentration of EDTA (0.08 mM) used, in the presence of Mg, by these authors, it is uncertain whether extracellular (contaminant) Ca also contributed to activation of the contractions in their "Ca-depleted" preparations.

suggestions from this laboratory that the extent and contribution of the SR to excitation-contraction coupling may vary in different smooth muscles.

The unequal maximal contractions elicited by drugs in main pulmonary artery smooth muscle in Ca-free media cannot be explained by the simple hypothesis (Somlyo and Somlyo, 1968 *a*; 1970 *b*; Somlyo et al., 1969) that different drugs produce only different degrees of inward movement of extracellular calcium, although this probably does occur in Ca-containing solutions (Somlyo and Somlyo, 1971 *a*). The unequal maximal contractions of *depolarized* (with 179 mM K) smooth muscles in Ca-free solutions clearly indicate a mechanism of control that is not directly dependent on the membrane potential, relating them to the early observations of Evans, Schild, and Thesleff (1958) on the action of drugs on depolarized smooth muscles in solutions containing calcium. Not only amines (present study) but angiotensin, a polypeptide, can also stimulate contraction of rabbit aortic strips in high (179 mM) K, Ca-free solutions (Somlyo and Somlyo: unpublished observation). In Ca-free depolarizing solution (in view of the small fraction of the total SR in direct contact with the surface membrane), it is probable that both the central and the peripheral elements of the SR would have to contribute in order to release sufficient calcium to activate contraction. Two possible models may be suggested to account for the excitatory action of drugs in the absence of extracellular calcium. The first possibility, suggested by previous workers (Edman and Schild, 1962; Keatinge, 1968 *a*; Bozler, 1969), is that drugs, presumably after penetrating the surface membrane, have a direct action on intracellular calcium stores (now primarily identified with the SR) and release calcium by a direct action on the SR membrane. Recent studies (Takagi and Uchida, 1970; Meyer et al., in press; Baudouin et al., in press) suggest that excitatory drugs (acetylcholine and angiotensin) can release Ca from microsomal preparations of smooth muscle, but it is not known whether such preparations include complete couplings of surface membrane with drug receptors and attached SR, or whether the drug effect is due to a direct action on the SR membrane. The second possible mechanism is that drugs increase the permeability of the surface membrane that forms couplings with the peripheral SR, and that this triggers the release of calcium from the junctional SR; the calcium concentration of the coupled SR may be replenished by diffusion down a concentration gradient from the more central portions (? uptake sites) of SR. This model is essentially analogous to the one suggested by Winegrad (1970) for striated muscle, where the longitudinal tubules are uptake sites where calcium is transferred to the terminal cisternae. Alternatively, the ion permeability change produced at the couplings may initiate a similar change in the permeability of the junctional SR to Ca, and this permeability change may be propagated as a regenerative calcium current flowing between adjacent active and inactive regions of the SR membrane (Somlyo et al., 1972) in a manner that, except for the absence of its all or none character, is similar to the propagation of an action potential along surface membranes. The action of trigger calcium (Bianchi, 1961; Bianchi and Bolton, 1966) manifested also by the regenerative release of calcium from the striated muscle SR (Ford and Podolsky, 1970) and by the oscillatory responses of skinned-muscle fibers to caffeine (Endo et al., 1970) could be due to the same underlying mechanism. It should be noted that, because of the very rapid efflux of sodium from smooth muscles (Keatinge, 1968 *b*; Jones and Karreman, 1969; for review see Somlyo and Somlyo, 1968 *b*), ions other than Ca are less likely to account for the propagation of a signal, is such occurs, along the SR of *depolarized* smooth muscle in Ca-free solutions (present study).

The extent to which mitochondrial uptake of calcium, in addition to uptake by the SR, contributes to relaxation of smooth muscles, and whether under certain conditions mitochondrial calcium can also be released by drugs to activate contraction, remains to be determined. This possibility is suggested by the observations that barium is accumulated by the mitochondria of rabbit portal-anterior mesenteric vein after barium-induced contractures in Ca-free solutions (Somlyo et al., 1972) and that strontium is accumulated by both the mitochondria and the SR in vascular smooth muscles (Somlyo and Somlyo, 1971 *b*). Peachey (1964) had noted some years ago that strontium and barium are accumulated by mitochondria of the smooth muscle in the toad bladder.

The organization of myosin in filamentous form, often surrounded by orbits of thin filaments

forming a rosette, in main pulmonary artery smooth muscle supports recent observations on the thick filaments of mammalian smooth muscles (Rice et al., 1970; 1971; Devine and Somlyo, 1971; Somlyo et al., 1971 *a*; *d*), while the presence of the two sets of myofilaments together with an abundant rough SR in many fibers is consistent with the dual, contractile and morphogenetic (Ross and Klebanoff, 1971; Ross, 1971; for review see Somlyo and Somlyo, 1968 *a*), roles of smooth muscle.

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