Hypertrophy-induced Increase of Intermediate Filaments in Vascular Smooth Muscle

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ABSTRACT The distribution of filaments was studied in hypertrophied rabbit vascular smooth muscle. Hypertrophy was induced by partial ligation of the portal-anterior mesenteric vein. 14 d after ligation, there was an approximately threefold increase in the number of intermediate filaments per cross-sectional area, as compared to control values. The actin:intermediate:myosin filament ratio was 15:1.1:1 in control and 15:3.5:0.5 in hypertrophied portal-anterior mesentric vein vascular smooth muscle. Comparison of the filament ratios with the increase in volume density of the hypertrophied cells suggests that the number of myosin filaments per cell profile remained approximately the same as in controls, whereas the number of actin filaments increased in proportion to the increase in cell volume.

Intermediate (10 nm diameter) filaments are ubiquitous components of most, if not all, eukaryotic cells (see reference 39 for review). In nonneural tissue, they were first described by Ishikawa et al. (35) in cultured cells and distinguished from actin filaments through the difference in size and the failure of the 10-nm filaments to bind the heavy meromyosin subfragment of myosin. Recent studies have shown that the major component of the intermediate filaments in muscle is a protein of ~50,000–55,000 mol wt (15). In nonmuscle cells, other than nerve, a related protein of slightly higher mass (55,000–58,000 daltons) forms the 10-nm filaments, although the two proteins may also coexist in the same cell (5, 20, 24). A marked increase in the number of intermediate filaments, frequently in the form of cables, can be induced in muscle and in other cells by drugs such as cytochalasin B and Colcemid (CIBA Pharmaceutical Co., Summit, N. J.) (4, 29, 33, 34, 38, 55, 56).

In normal adult mammalian smooth muscle, intermediate filaments generally surround dense bodies on which actin filaments insert (2). Intermediate filaments are very numerous in cultured vascular (45–47) and other smooth muscles (11–13, 60). In previous studies of normal adult vascular smooth muscle, occasional cells were found containing massive quantities of intermediate filaments that appeared to displace the normal actin and myosin filament lattice (50, 52). Although it was recognized that such proliferation of intermediate filaments in adult smooth muscle represented some form of cellular pathology, its specific cause was not known. We now show that a large increase in the number of intermediate filaments in vascular smooth muscle occurs during hypertrophy induced by increasing vascular distending pressure. In addition, we also describe changes found in the number and distribution of actin and myosin filaments of hypertrophied vascular smooth muscle. These studies are being pursued to enable us to eventually distinguish the secondary effects of increased pressure on vascular smooth muscle from possible primary pathology of cell organelles that may cause hypertension.

MATERIALS AND METHODS

New Zealand white male rabbits (1.8–2.7 kg) were anesthetized with Innovar-vet (Pitman-Moore, Inc., Washington Crossing, N. J.) and their abdominal cavity was opened via midline abdominal incision. The portal-anterior mesenteric vein (PAMV) was isolated and a 000 silk ligature was placed around the vein just before the vein divided into the two major hepatic branches. The ligature was tied down until the vein was seen to bulge and the abdomen was closed with 000 silk.

Rabbits were placed in two categories, sham-operated and experimental. The sham-operation involved the placement of a loose 000 silk ligature around the PAMV at the same site as in the experimental group.

On the fourteenth postoperative day, the rabbits were killed by cervical dislocation and a 4–5 cm section of the PAMV on the high pressure side of (caudal to) the ligature was removed. Longitudinal strips of the PAMV were stretched to ~1.75 times their excised length and incubated in a modified Krebs solution (51) aerated with 95% O_2–5% CO_2 for 30 min at 37°C.

After incubation, the tissues were fixed for 2 h in 2% glutaraldehyde in 0.07 M cacodylate buffer (pH 7.4) containing 4.5% sucrose. All tissues were stored in cacodylate buffer in the refrigerator overnight and postfixed for 90 min in 2% osmium tetroxide in 0.1 M cacodylate buffer.

All tissues were rinsed with 0.1 M cacodylate buffer and block stained with saturated aqueous uranyl acetate for 90 min. They were then dehydrated in graded alcohols and embedded in Spurr's resin (54).

Thin sections, cut with a Porter-Blum MT2B ultramicrotome (DuPont Instruments, Wilmington, Del.) with diamond knives, were stained with alkaline lead citrate and examined with a Hitachi HU-11E microscope operated at 75 kV.

To facilitate the counting of filaments within the cells, electron micrographs

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of the media were taken at a magnification of × 31,000. Whole cell profiles were randomly selected and enlarged six times. Each cell profile was divided into a series of grids 5 cm × 5 cm. The number of actin (5–8 nm), myosin (15–18 nm), and intermediate (10 nm) filaments (50–52) were counted per grid area, excluding areas containing mitochondria and nuclei, and then expressed per micrometer.

Each cell profile was divided into peripheral and central portions. The peripheral area included those grids following the periphery of the cell profile that extended from the plasma membrane a distance of 5 cm towards the core of the profile at the 31,000 × 6 magnification (or an ~0.27 μm annulus).

Stereology

For stereological analysis, electron micrographs of the portal vein media were taken at a magnification of × 4,800 and enlarged three times. Each micrograph was covered with a sheet of tracing paper containing a square grid pattern and whole cell profiles were randomly selected and traced. The centers of 6 × 6-mm squares formed by the intersections of horizontal and vertical grid lines were used to determine areal densities of the profiles (point counting). The intersections of parallel vertical lines 12 mm apart with membranes of the cell profiles were used to determine surface densities (intersection counting).

The following stereological terminology used is that of Weibel (62): A_a, areal density, i.e., the area of the component related to the containing area; V_v, volume density, i.e., the volume of the component related to the containing volume; and S_v, surface density, i.e., the surface area of the component per unit containing volume.

The Delesse principle (17) states that the A_a of a cell profile is an unbiased estimate of its V_v, i.e., $A_a = V_v$. For that reason, A_a and V_v are used interchangeably.

The A_a of the cell profiles was obtained from the equation: $A_a = P_a \cdot (d/m)^2 / n = V_v$, where P_a is the total number of points falling on all the smooth muscle cell profiles per micrograph, d is the distance between the grid lines, m is the magnification of the print, and n is the number of cell profiles per micrograph.

The test line length (L_S) of the system was obtained from the equation: $L_s = (0.5)(P_a)(d)/m$.

The S_v of the cell profiles was obtained from the equation: $S_v = \pi^2 \cdot I_a / 4\ L_s$, where I_a is the total number of intersections of the horizontal lines with the membranes of the cell profiles.

The circular smooth muscle layer of the PAMV media in transverse section appears as an oriented or anisotropic group of smooth muscle cells. The formulas derived above assume that these cells possess a geometrical configuration of a right circular cylinder (19).

Estimation of Cell Length

The procedure of Cooke and Fay (16) was utilized in obtaining isolated cells and measuring cell length. Before fixation and treatment with potassium hydroxide, the longitudinal strips of the PAMV were stretched to ~1.75 times their excised length.

RESULTS

Ultrastructural studies were confined to the media of the PAMV. The distended regions of the PAMV examined were divided into two segments, the first 1–1.5 cm of the PAMV on the high pressure side of and immediately adjacent to the ligature, and the second, 2–3 cm long segment caudal to the first.

2 wk after the placement of the ligature, the wall thickness on the high pressure side of the constricted PAMV was visibly increased, confirming the observations of Johansson (36) on rat portal vein. A significant increase in the number of intermediate filaments was the most striking ultrastructural alteration observed in the smooth muscle cells of the PAMV distal to the coarctation. This change was consistent in both segments on the high pressure side of the coarctation (Figs. 1 and 2). In

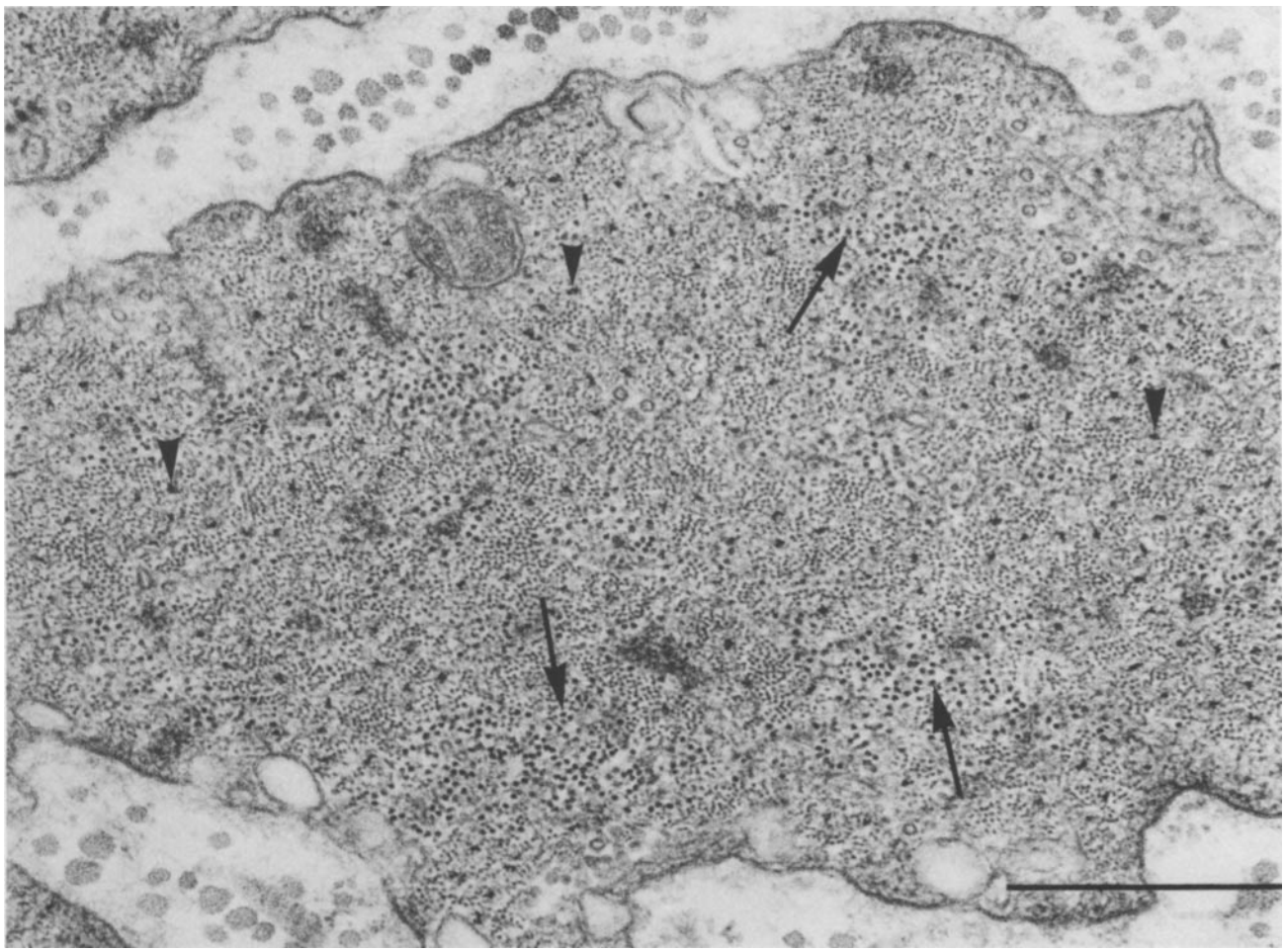

FIGURE 1 Transverse section of a smooth muscle cell from hypertrophied PAMV illustrating the large increase and nonuniform distribution of intermediate filaments (some shown by arrows). Arrowheads indicate the myosin filaments. Bar, 0.5 μm. × 74,400.

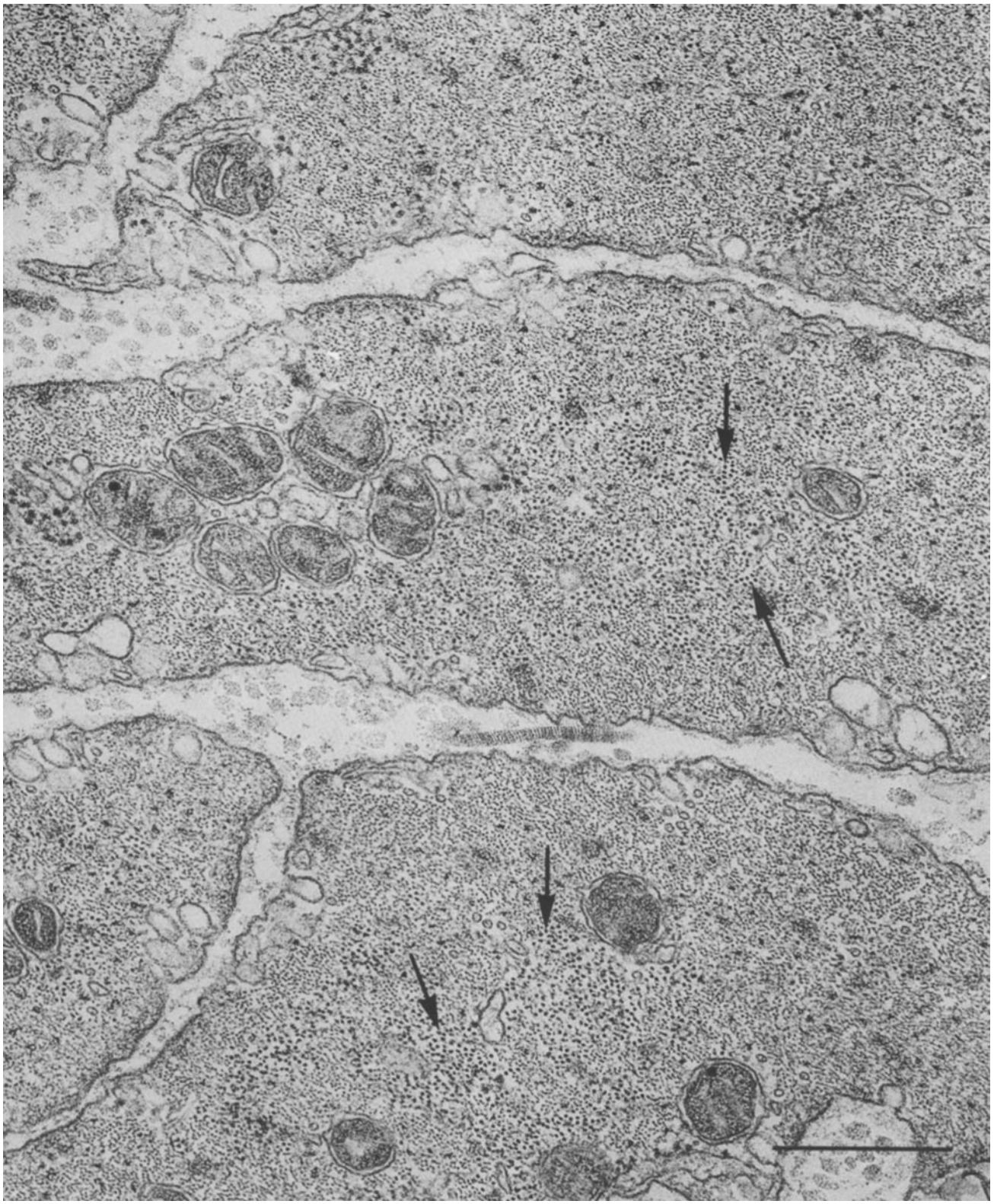

FIGURE 2 Transverse section of a bundle of smooth muscle fibres from hypertrophied PAMV showing the predominantly central location of the intermediate filaments (arrows). Bar, 0.5 μm. × 61,200.

the majority of transverse sections, the intermediate filaments were localized in the central portion of the cell profile and were nonuniformly distributed.

In general, the intermediate filaments were increased 256% per cell cross section in the hypertrophic PAMV. Peripherally, the 10-nm filaments were increased 150%, whereas centrally, they showed a massive increase of 345% per cell cross section (Table I). The actin:intermediate (A/I) filament ratio decreased from a control value of 13.7 to 4.6 in the hypertrophied PAMV. A/I and actin:myosin filament (A/M) ratios were converted

TABLE I

Actin, Myosin, and Intermediate Filament Counts and Ratios from Sham-operated and Hypertrophied PAMV

		Total cell profile*			Peripheral			Central		
		A	M	I	A	M	I	A	M	I
		Mean ± SE			Mean ± SE			Mean ± SE		
Total filament counts‡	Sham	1,733 ± 12	112 ± 3	112 ± 11	1,797 ± 22	116 ± 4	85 ± 4	1,692 ± 22	111 ± 5	140 ± 18
	Hypertrophied	2,091 ± 34	67 ± 1	449 ± 31	2,558 ± 37	73 ± 1	221 ± 22	1,607 ± 51	63 ± 1	622 ± 52
A:M ratio	Sham		15.4 (2.7)			15.4 (2.7)			15.2 (2.7)	
	Hypertrophied		31.1 (5.5)			35.2 (6.3)			25.5 (4.5)	
A:I ratio	Sham		13.8 (6.8)			21.2 (10.4)			12.1 (5.9)	
	Hypertrophied		4.6 (2.3)			11.6 (5.7)			2.6 (1.3)	

Values in parenthesis represent mass ratios calculated from filament counts and the following filament diameters: A, 7 nm; M, 16.5 nm; I, 10 nm.
A, actin filaments; M, myosin filaments; I, intermediate filaments.

* Data from eight animals; 80 sham grids counted and 155 experimental grids counted; approximately five cell profiles per animal were counted; SE was calculated from the number of grids counted.

‡ Total filament counts for actin, myosin, and intermediate filaments represent the number of filaments per square micrometer.

into their equivalent mass ratios by conventional stereological method (17, 59, 62). The calculated A/I mass ratio in the hypertrophied PAMV exhibited a threefold decrease as compared with the control A/I mass ratio (Table I). This decrease is consistent with the large increase in the intermediate filaments.

There was also a change in the number and distribution of the thick (myosin) and thin (actin) filaments. In the control vessels, actin and myosin were uniformly distributed throughout the cells and the intermediate filaments were associated with the dense bodies (Fig. 3), in agreement with previous studies (2, 44). In contrast, filament distribution was rather nonuniform in the smooth muscle cells of the hypertrophied vessels. Peripherally, actin filaments increased by 42%, whereas myosin decreased by 40%. Centrally, the number of actin and myosin filaments per unit area were decreased by 5 and 43%, respectively (Table I). Throughout the whole cell, the number of actin filaments per unit area were increased by 21%, whereas myosin filaments decreased by 40%.

The A/M filament ratio approximately doubled in hypertrophied vascular smooth muscle (Table I) from the control value of 15:1 (Table I and reference 18). The calculated A/M mass ratio in hypertrophied vascular smooth muscle increased by approximately twofold (Table I) compared with a control value of 2.7. The latter control value is comparable to the A/M ratio of 2.3 obtained by SDS gel electrophoresis in the porcine hepatic portal vein (14).

Stereological analysis of the cell profiles revealed an ~60% increase in V_v in hypertrophied cells (Table II). In addition, the S_v of hypertrophied cells was decreased ~14% as compared with the sham cells (Table II). Of those cell profiles that contained nuclei, the V_v of the nuclei from hypertrophied cells showed a small but significant increase. However, the S_v of the nuclei from hypertrophied cells was decreased by ~16% (Table III).

Isolated smooth muscle cells from hypertrophied PAMV showed a small but significant increase in cell length over that of cells from sham-operated animals (Table II).

DISCUSSION

The development of distention-induced hypertrophy of smooth muscle has been observed in the coarcted aorta (2, 8, 9, 43, 61), portal vein (36), and obstructed small intestine (26, 28). Manifestations of hypertrophy include evidence of mitosis and an increase in wall thickness and the number and size of smooth muscle cells in the distended segment (1, 6, 8, 9, 21, 25, 30, 32, 63). These changes are absent on the "low-pressure" side of the coarctation.

The ultrastructure of hypertrophied vascular smooth muscle, to our knowledge, has not been previously described. In our study, a massive increase in the number of intermediate filaments was the most notable ultrastructural change in hypertrophied vascular smooth muscle. While this manuscript was in preparation, Gabella (28) also reported an increase in the number of intermediate filaments in hypertrophied intestinal smooth muscle. Intermediate filaments are also increased in hypertrophied cardiac muscle (22), but not to the extent as described in this or in Gabella's (1979) study. In the hypertrophied PAMV, the intermediate filaments were irregularly oriented and frequently concentrated in the central portion of the fibers. Preliminary SDS gel electrophoresis suggested that polymerization of preexisting intermediate filament subunits may have contributed to the increase in intermediate filaments (Berner, Holtzer, Sourlyo, and Sourlyo, unpublished observations).

The stereological principle, which states that the relative areas of cell profiles are directly proportional to the V_v of that cell (17, 62), was utilized in the present study to illustrate a 60% increase in the V_v of hypertrophied cells, less than the reported three to fourfold increase in the cell volume of hypertrophied intestinal smooth muscle (27). Assuming that the geometrical configuration of the cells is that of a right circular cylinder, the surface/volume ratio of the cylinder is equivalent to $2/r$, where r is the radius. Any increase in the radius would produce a decrease in the surface/volume ratio. This study shows a 14% decrease in S_v of the hypertrophied cells and it may be concluded that the radius has also increased. Although the length of the hypertrophied cell shows a significant 5% increase, the size of the population is too small to warrant any definitive conclusion regarding an increase in cell length. It may be stated that the hypertrophied are at least as long as the cells from sham-operated animals. Therefore, the increase in V_v and cell length, in conjunction with the decrease in S_v imply that the increase in the average cross-sectional area reflects an overall increase in cell volume of the hypertrophied cells, rather than shortening.

The nuclei from hypertrophied cells exhibited an 11% increase in V_v and a corresponding decrease in S_v. The ratio of the A_a of the nucleus to the A_a of the whole cell profile (A_a/A_{ac}) was decreased by 29% in hypertrophied cells (Table III). These findings indicate a small but significant increase in the nuclear volume of hypertrophied cells which corresponds with the findings in hypertrophied intestinal smooth muscle (27).

Throughout the whole cell, the total number of myosin filaments per unit area decreased by 40%. Stereological analysis of cell V_v reveals a 60% increase. This would indicate that there was no net change in the number of myosin filaments per cell cross section and that the increased volume of the cell was occupied by actin and intermediate filaments.

The maximal active force per unit cross-sectional area or per medial cross-sectional area is decreased in hypertrophied smooth muscle (28, 31, 36). The decrease in the number and distribution of myosin filaments and the massive increase in intermediate filaments reported in this study may account for the decrease in force generation during hypertrophy. Gabella (28) made a similar conclusion regarding the increase in intermediate filaments in hypertrophied intestinal smooth muscle. This explanation may be oversimplified, however, in view of the numerous geometrical and biochemical factors which may influence the contractile apparatus of and tension development by smooth muscle (2, 37, 41, 42). It is of interest that the majority (3, 10, 23, 48, 53), though not all (40), of the studies of systemic hypertensive vascular smooth muscle also show a decrease in the maximal active tension generated in response to a variety of contractile agonists. It will be of interest to determine whether comparable changes in actin to myosin filament ratios and in the number and distribution of intermediate filaments also occur in more slowly progressing systemic hypertension as they did in response to the relatively rapid increase in distending pressure used in this and in Gabella's (28) study. It is apparent that just as an increase in the volume of the endoplasmic reticulum represents a relatively nonspecific response to a variety of forms of injury (1, 25, 49, 57, 58), including hypertension and hypertrophy, an absolute and relative increase in the intermediate filaments of smooth muscle can also occur in response to a variety of interventions, such as cell culture (11–13, 45–47) and pressure induced hypertrophy (present study and reference 28). Therefore, continued assessment of ultrastructural changes that are secondary to

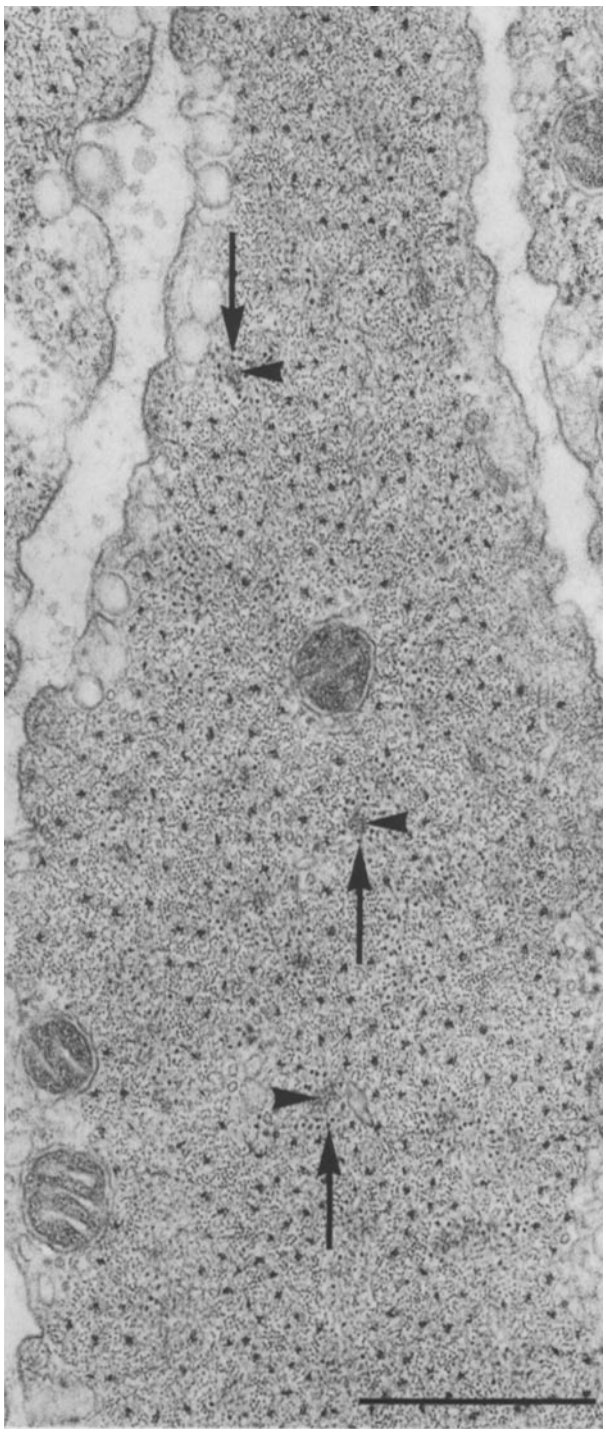

FIGURE 3 Transverse section of a bundle of smooth muscle fibres from control PAMV illustrating the uniform distribution of thick filaments and actin filaments. The intermediate filaments (arrows) are seen associated with dense bodies (arrowheads). Bar, 0.5 μm. × 61,200.

TABLE II

Areal Density, Surface Density, and Cell Length Values from Sham-operated and Hypertrophied PAMV

	Sham (n = 12)*	Hypertrophied (n = 19)	P‡
A_{ac}§	6.6 ± 0.5 μm²	10.6 ± 1 μm²	$P < 0.001$
S_{vc}	2.9 ± 0.3 μm²/μm³	2.5 ± 0.1 μm²/μm³	$P < 0.001$
Cell length	258 ± 10 μm	273 ± 19 μm	$P < 0.005$

* n, total number of micrographs; 155 sham cell profiles and 152 experimental cell profiles were used. Values represent the mean ± SEM. SEM was calculated from the number of micrographs analyzed. SEM for cell lengths was calculated from n = 29 sham isolated cells and n = 16 hypertrophied isolated cells.

‡ P values < 0.05 were considered significant.

§ A_{ac}, areal density of whole cell profiles; S_{vc}, surface density of whole cell profile.

TABLE III

Areal and Surface Densities of Nuclei from Sham-operated and Hypertrophied PAMV

	Sham (n = 10)*	Hypertrophied (n = 14)	P‡
A_{an}§	4.4 ± 0.4 μm²	4.9 ± 0.3 μm²	$P < 0.005$
S_{vn}	4.3 ± 0.3 μm²/μm³	3.6 ± 0.2 μm²/μm³	$P < 0.001$
A_{an}/A_{ac}	0.7 ± 0.1	0.5 ± 0.05	$P < 0.001$

* n, total number of micrographs; 21 sham nuclear profiles and 25 hypertrophied nuclear profiles were used. Values represent the mean ± SEM. SEM was calculated from the number of micrographs analyzed.

‡ P values < 0.05 were considered significant.

§ A_{an}, areal density of the nucleus; S_{vn}, surface density of the nucleus; A_{an}/A_{ac}, ratio of nuclear areal density to whole cell profile areal density.

hypertension, rather than part of its causative mechanism, is warranted.

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