Microvascular Pericytes Contain Muscle and Nonmuscle Actins

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ABSTRACT We have affinity-fractionated rabbit antiactin immunoglobulins (IgG) into classes that bind preferentially to either muscle or nonmuscle actins. The pools of muscle- and nonmuscle-specific actin antibodies were used in conjunction with fluorescence microscopy to characterize the actin in vascular pericytes, endothelial cells (EC), and smooth muscle cells (SMC) in vitro and in situ. Nonmuscle-specific antiactin IgG stained the stress fibers of cultured EC and pericytes but did not stain the stress fibers of cultured SMC, although the cortical cytoplasm associated with the plasma membrane of SMC did react with nonmuscle-specific antiactin. Whereas the muscle-specific antiactin IgG failed to stain EC stress fibers and only faintly stained their cortical cytoplasm, these antibodies reacted strongly with the fiber bundles of cultured SMC and pericytes. Similar results were obtained in situ. The muscle-specific antiactin reacted strongly with the vascular SMC of arteries and arterioles as well as with the perivascular cells (pericytes) associated with capillaries and post-capillary venules. The nonmuscle-specific antiactin stained the endothelium and the pericytes but did not react with SMC. These findings indicate that pericytes in culture and in situ possess both muscle and nonmuscle isoactins and support the hypothesis that the pericyte may represent the capillary and venular correlate of the SMC.

Of particular interest to vascular cell biologists is the relationship among endothelial cells (EC),[1] smooth muscle cells (SMC), and pericytes, especially with regard to the nature of their interactions in vascular injury, wound healing, and new blood vessel growth (7, 23, 31). Although a great deal is known about the origin and function of EC and SMC, very little is known about the origin and role of the pericyte. In fact, the pericyte is currently defined solely by its location surrounding the EC within the microvascular basement membrane (31). Studies on the cell biology of the pericyte have been hindered because no biochemical or microscopic markers are available for conclusive identification of these cells either in vitro or in situ.

Antibodies to actin, while difficult to obtain, have been invaluable probes for studying the form, distribution, and function of actins in muscle and nonmuscle cells (2, 4–6, 11, 12, 14, 17–20, 22, 26, 27, 37). Antibodies with differential binding affinities for the various isoactins that occur in muscle and nonmuscle cells have also been reported (2, 6, 20).

In this series of experiments, we have taken advantage of the fact that polyclonal rabbit antiactin immunoglobulins (IgG) bind to isoactins in both muscle and nonmuscle cells. Using affinity chromatography, muscle and nonmuscle-specific actin antibodies were purified and used to stain vascular cells in vitro and in situ. These isoactin-specific ligands were used to determine the composition of the pericyte actin pool and to compare it with that of vascular SMC and EC. Our results indicate that the pericyte, in vitro as well as in situ, possesses both muscle and nonmuscle isoactins.

MATERIALS AND METHODS

Fractionation of Antiactin IgG

Rabbit antibodies against chicken gizzard smooth muscle actin were prepared according to the method of Herman and Pollard (19). Specificity of antiactin antisera has been previously described (18, 19). Immune IgG were precipitated twice from serum by ammonium sulfate fractionation, then dissolved in and dialyzed against phosphate-buffered saline (PBS; 0.015 M sodium phosphate, pH 7.5, 0.15 M NaCl, 0.02% NaN_3). 5 ml of immune IgG (10–15 mg/ml) was mixed with 1 ml of Sepharose 4B-nonmuscle (platelet) actin (0.5 mg actin/ml Sepharose 4B) (30) by rotation for 6 h at 25°C and was poured

[1] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle's medium; EC, endothelial cell(s); IgG, immunoglobulins; SMC, smooth muscle cell(s).

into a 0.75 × 10-cm column. The nonadherent IgG were eluted with PBS in 2-ml fractions. Glycine (0.2 M, pH 2.8) was used to release 1-ml fractions of the column-bound immune IgG into prechilled tubes containing four drops of 0.5 M sodium phosphate, pH 7.5. These neutralized fractions were immediately dialyzed against several hundred volumes of PBS. The nonadherent IgG were mixed with 1 ml of Sepharose 4B-smooth muscle (chicken gizzard) actin (0.45 mg actin/ml Sepharose 4B) under the same experimental conditions as described above. Glycine (0.2 M, pH 2.8) was used to release the anti-muscle actin IgG. These IgG populations with known differential affinities to muscle and nonmuscle actins were then used to stain cultured cells and frozen tissue sections in the studies described below. To test the level of cross-reactivity of the isoactin antibodies, the order of the affinity chromatography steps was reversed. This reversal failed to alter the fluorescence staining patterns.

Tissue Culture

PERICYTES: Pericytes were cultured from capillary fragments isolated from bovine retinas as previously described (16). Briefly, capillary fragments were isolated by collagenase digestion of minced retinas followed by sieving. The capillary fragments were plated into tissue culture flasks in Dulbecco's modified Eagle's medium (DME) supplemented with 10% calf serum. The pericytes were identified and distinguished from EC by their larger size and irregular morphology, by their noncontact-inhibited growth patterns, and by their lack of staining with antisera to bovine Factor VIII, criteria used by others to identify retinal capillary pericytes (1, 3, 8, 13). The pericytes were distinguished from SMC in culture by slower growth rate, their irregular morphology, the absence of dense bodies, and the lack of "hill and valley" growth pattern characteristic of SMC at confluence (6, 16).

SMOOTH MUSCLE CELLS: Vascular SMC were cultured from the medial layer of bovine aortas by the explant procedure of Ross (32). At confluence, the cells formed a "hill and valley" pattern, characteristic of SMC in culture (6).

ENDOTHELIAL CELLS: EC were isolated from bovine aortas by collagenase treatment using the procedure of Jaffe et al. (21). The cells were grown in DME supplemented with 10% calf serum, maintained a nonoverlapping, contact-inhibited morphology at confluence, and stained positively for Factor VIII antigen (21) using antisera kindly providly by Dr. Edward Kirby (Temple University Medical School, Philadelphia, PA).

In Vitro Staining Studies

EC, SMC, and pericytes were grown on glass microscope coverslides in DME supplemented with 10% calf serum as described above. For antibody staining, the cells were rinsed in 100 ml of DME warmed to 37°C and equilibrated to 5% CO_2 before fixation for 5 min in 4% formaldehyde in DME buffered with 10 mM HEPES, pH 7.3. The cells were washed and permeabilized for 30 s with absolute acetone cooled with dry ice. The cells were then equilibrated in several hundred milliliters of PBS before incubation with the antiactin IgG at the following concentrations: (*a*) antiactin IgG, unfractionated (80–100 μg/ml); (*b*) antiactin IgG, muscle-specific (5–50 μg/ml); (*c*) antiactin IgG, nonmuscle-specific (5–50 μg/ml); (*d*) rhodamine-labeled goat anti-rabbit IgG (50 μg/ml, Cappel Laboratories, Cochranville, PA).

Staining was accomplished by incubation in the primary antisera for 60 min at room temperature followed by washing and incubation with the fluorescently labeled goat anti-rabbit IgG (*d* above) for 60 min at room temperature. The following combination of antibodies (letters in parentheses refer to list above), and cells were examined: (*i*) Pure populations of EC, SMC, and pericytes with each of the primary antiactin IgG (*a–c*); (*ii*) EC and pericytes with muscle-specific antiactin IgG (*b*); (*iii*) EC and pericytes with nonmuscle-specific antiactin IgG (*c*); (*iv*) EC and SMC with muscle-specific antiactin IgG (*b*); (*v*) EC and SMC with nonmuscle-specific antiactin IgG (*c*); (*vi*) pericytes and SMC with nonmuscle-specific antiactin IgG (*c*); (*vii*) pericytes and SMC with muscle-specific antiactin IgG (*b*); (*vii*) pure and mixed cultures of EC, SMC, and pericytes with either nonimmune IgG or immune IgG (80 μg/ml) depleted of antiactins by affinity chromatography.

In Situ Staining Studies: Preparation of Tissues for In Situ Localization of Isoactins

CONVENTIONAL FROZEN SECTIONS: C57 Black mice (25–35 g) were purchased from the Jackson Laboratory (Bar Harbor, ME) and Sprague-Dawley rats (125–145 g) were purchased from Charles River Breeding Labs Inc. (Wilmington, MA). Rodents were anesthetized with Metofane vapors before the pericardial cavity was exposed. 30 ml of warmed (37°C) physiological saline was introduced into the left ventricle, with the right atria opened as an outflow point. Fixation was accomplished with 50 ml of 3% formaldehyde in physiological saline at a perfusion rate of 2.5 ml/min and 100-mm Hg of pressure followed by 100 ml of saline. The excised organs were frozen in *n*-methylbutane cooled with dry ice. 5-μm sections were prepared on a cryostat (Model CTD, International Equipment Co., Needham Heights, MA) at −25°C.

ULTRATHIN FROZEN SECTIONS: Sprague-Dawley rats were anesthetized with Metofane vapors before infusion of PBS (37°C) via the left ventricle. Fixation was accomplished with 50 ml of 3% formaldehyde in PBS, pH 7.4 as described above. Tissues of interest were cut into small pieces (~1 mm^3) and were placed in 3% formaldehyde for 1 h at room temperature, washed twice in PBS, and stored at 4°C in 0.1% formaldehyde in 0.05 M NaH_2PO_4, pH 7.4, 0.02% NaN_3. Before cutting, the tissues were washed once in 0.1 M NaH_2PO_4, pH 7.4 and equilibrated in 2.3 M sucrose in the same buffer for 30 min at room temperature. The tissue was removed with fine forceps and placed on a copper block before freezing with liquid nitrogen. The frozen tissue blocks were transferred to an ultracryomicrotome (Model FC-4, Reichert-Jung, Buffalo, NY), and 0.5-μm sections were cut on dry glass knives at −70°C. The tissue sections were picked up in a drop of the 2.3 M sucrose–phosphate buffer solution suspended across a 3-mm diameter platinum loop, before placement onto a chromalum-coated glass slide. The ultrathin sections were stored at −20°C or stained with antibodies directly using 5–10 μl of the affinity-purified antiactins.

MICROSCOPY AND PHOTOMICROGRAPHY: Fluorescently labeled cells and tissue sections were observed with phase contrast and fluorescence optics using a Zeiss Diavert inverted light microscope (Carl Zeiss, Inc., Thornwood, NY) equipped for rhodamine and fluorescein fluorescence. Results from the experiments and the controls were recorded through a Zeiss 1.4 numerical aperture planapochromat objective lens (63×) on Tri-X negative film (ASA 1,000) and processed in Acufine developer as previously described (18, 19).

RESULTS

Affinity-Fractionation of Antiactin IgG

Quantitation of the polyclonal antiactin IgG fractionation indicated that 0.7% of the immune IgG could be recovered from the platelet actin–Sepharose 4B column, whereas 0.9% of the immune IgG pool could be released from the muscle actin–Sepharose 4B. When the chromatographic steps were reversed and the whole immune IgG pool was fractionated over muscle actin–Sepharose 4B before nonmuscle actin–Sepharose 4B, pool recoveries were nearly identical (0.86 and 0.75% for the muscle and nonmuscle antiactins, respectively). The resulting staining patterns using the IgG fractions obtained from the two separation methods were indistinguishable, indicating that the specificities of the two pools were not dependent on the order of the fractionation, but on the specificity of antibody to the muscle or nonmuscle actin.

In Vitro Staining Studies

EC CULTURES: When aortic EC were stained with the unfractionated antiactin IgG, bright specific fluorescence was observed (Fig. 1 *a*). Numerous actin-positive stress fibers were seen traversing the cytoplasm. Bright fluorescence associated with membrane ruffles was also noted, whereas the nuclei were seen as negative images in the stained cytoplasms. Visualization of EC stained with 10 μg/ml muscle-specific antiactin IgG revealed virtually no cytoplasmic fluorescence (Fig. 1 *b*). If a higher concentration (40 μg/ml) of this muscle-specific antiactin was used, slight EC staining was apparent indicating weak cross-reaction between the muscle-specific actin antibodies and the nonmuscle actin. In contrast, the nonmuscle-specific antiactins IgG reacted strongly with the EC isoactins (Fig. 1 *c*). The fluorescent images of cells stained with 20 times less of the affinity-purified actin IgG (5 μg/ml for nonmuscle type vs 100 μg/ml for the unfractionated IgG) were virtually indistinguishable from the images obtained after staining with unfractionated antiactin IgG (Fig. 1, *a* and *c*).

VASCULAR SMC CULTURES: When the unfractionated

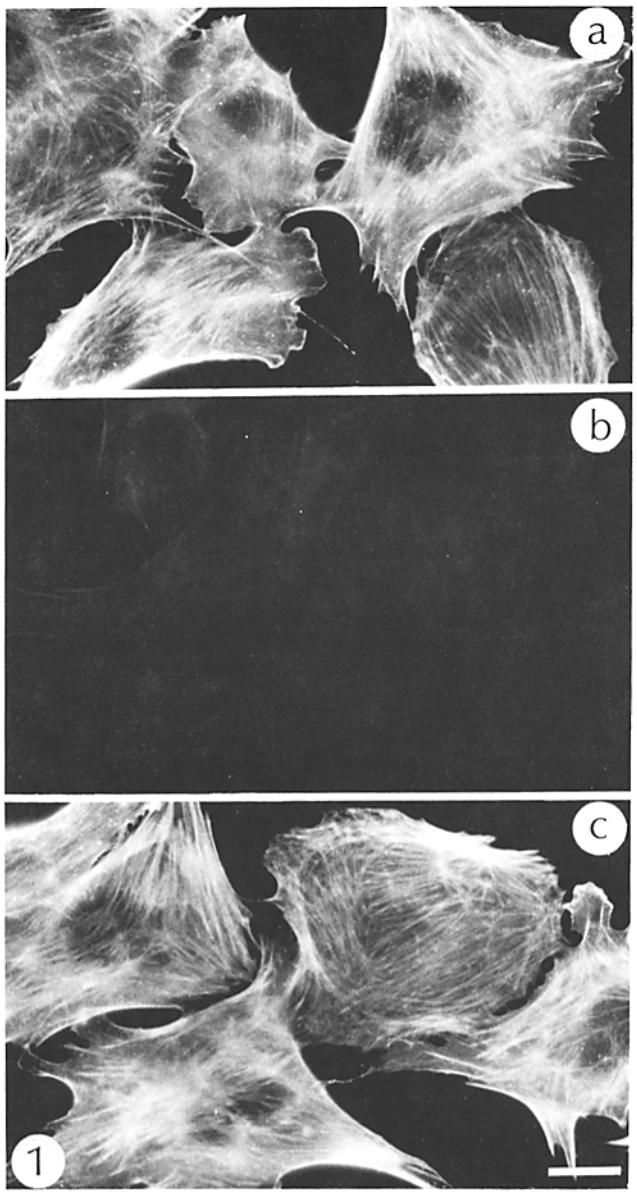

FIGURE 1 Staining of bovine aortic EC with antiactin IgG. The unfractionated immune IgG (80 μg/ml) stained numerous actin-containing cables (a). No fibers were visible when the cells were stained with affinity-purified muscle-specific antiactin IgG (10 μg/ml) (*b*). The staining pattern of EC stained with affinity purified nonmuscle specific anti-actin IgG (10 μg/ml) (c) is indistinguishable from the pattern obtained with the unfractionated antisera (a). Bar, 10 μm. × 1,000.

antiactin was used to localize actin in SMC, numerous fluorescent cables were apparent (Fig. 2, *a* and *b*). When the cultured SMC were stained with nonmuscle-specific antiactin IgG, the cytoplasmic fibrils were barely visible in fluorescence (Fig. 2, *c* and *d*). Very fine fibers were evident (Fig. 2*c*), but these were not reminiscent of the broad, brightly stained cables seen with the unfractionated antiactin or the muscle-specific antiactins. Interestingly, the cortical or membrane-associated actins in the cultured SMC always reacted strongly with the nonmuscle-specific antiactin IgG (Fig. 2, *c* and *d*). The muscle-specific antiactin IgG reacted strongly with the SMC fibrils; and, these fluorescence images (stained with 50 times less IgG) were indistinguishable from the unfractionated antiactin staining patterns (compare Fig. 2, *a* and *b* with Fig. 2*e*).

PERICYTE CULTURES: When pericytes were stained with unfractionated antiactin IgG, a fantastic assortment of fibrillar and diffuse cytoplasmic fluorescence was apparent (Fig. 3). Broad (1.0–5.0 μm) and fine fibers (0.2–1.0 μm) were stretched across the cytoplasm along the substrate attachment plane, terminating at distinct sites along the basal plasma membrane. In regions of motile cytoplasm (documented by time-lapse videomicrographic analysis; Herman, I., unpublished observations), a uniform, granular meshwork of fluorescence was evident (Fig. 3*a*, arrows). Additionally, a bright rim of fluorescence was obvious at the extreme margins of motile lamellae, filopodia, membrane spikes, and ruffles. When the pericytes were stained with either the muscle-specific or nonmuscle-specific antiactin IgG, fibrous and diffuse fluorescence was revealed (Figs. 3, *b* and *c*). These findings contrasted with those obtained with the EC and SMC where the differential affinities of the fractionated antiactin IgG were apparent in the resulting staining patterns.

MIXED VASCULAR CELL CULTURES: If mixed cultures of pericytes and EC were stained with nonmuscle-specific antiactin IgG, all of the cells stained (Fig. 4) and the pericytes could not be distinguished from EC by fluorescence (4, *a* and *b*). Similarly, if pericytes and SMC were stained with the muscle-specific antiactin IgG, all of the cells in the culture were brightly fluorescent (Fig. 4, *c* and *d*). However, if mixed populations of pericytes and EC were stained with the muscle-specific antiactin IgG, only the pericytes were reactive (Fig. 4, *e–h*). The pericytes were obvious among the weakly positive images of the EC which were readily visible by phase contrast microscopy. SMC could be distinguished from EC or pericytes if the appropriate antiactin IgG was utilized (Fig. 4, *i–l*). In mixed cultures of SMC and EC stained with the muscle-specific antiactin IgG, only the SMC cells displayed bright, fibrous fluorescence (Fig. 4, *i* and *j*). Endothelial stress fibers were not stained, though intact EC could be observed in phase contrast images. On the other hand, in mixed cultures of SMC and pericytes stained with the nonmuscle-specific antiactins, the SMC appeared as negative outlines in the field of brightly stained pericytes (Fig. 4, *k* and *l*).

Tissue Section Studies

ARTERIOLES AND ARTERIES: Whereas the results of the experiments on cultured cells suggested that the isotype-specific antiactins could discriminate pericytes and SMC from EC in vitro, it was important to determine if similar results could be obtained in complex tissue samples. Cryosections were prepared from a variety of perfused-fixed tissues. Results revealed that the fractionated IgG could discriminate among the various vascular cells present in the tissue sections (Figs. 5 and 6).

The selective staining with the anti-muscle actin antibodies was demonstrated in the cerebral vascular system (Fig. 5, *a* and *b*), in the renal vascular system (Fig. 5, *c* and *d*), and in the great elastic arteries emanating from the heart (Fig. 5, *e–g*). In relatively thick sections (30 μm) of brain, the smooth muscle in the medial layers of brain arteries (not shown) and arterioles were positively identified. In Fig. 5*a*, the longitudinal aspect of a cerebral arteriole is seen with its media, consisting of a single layer of fluorescent SMC, after staining with muscle-specific antiactin. Since the muscle cells are

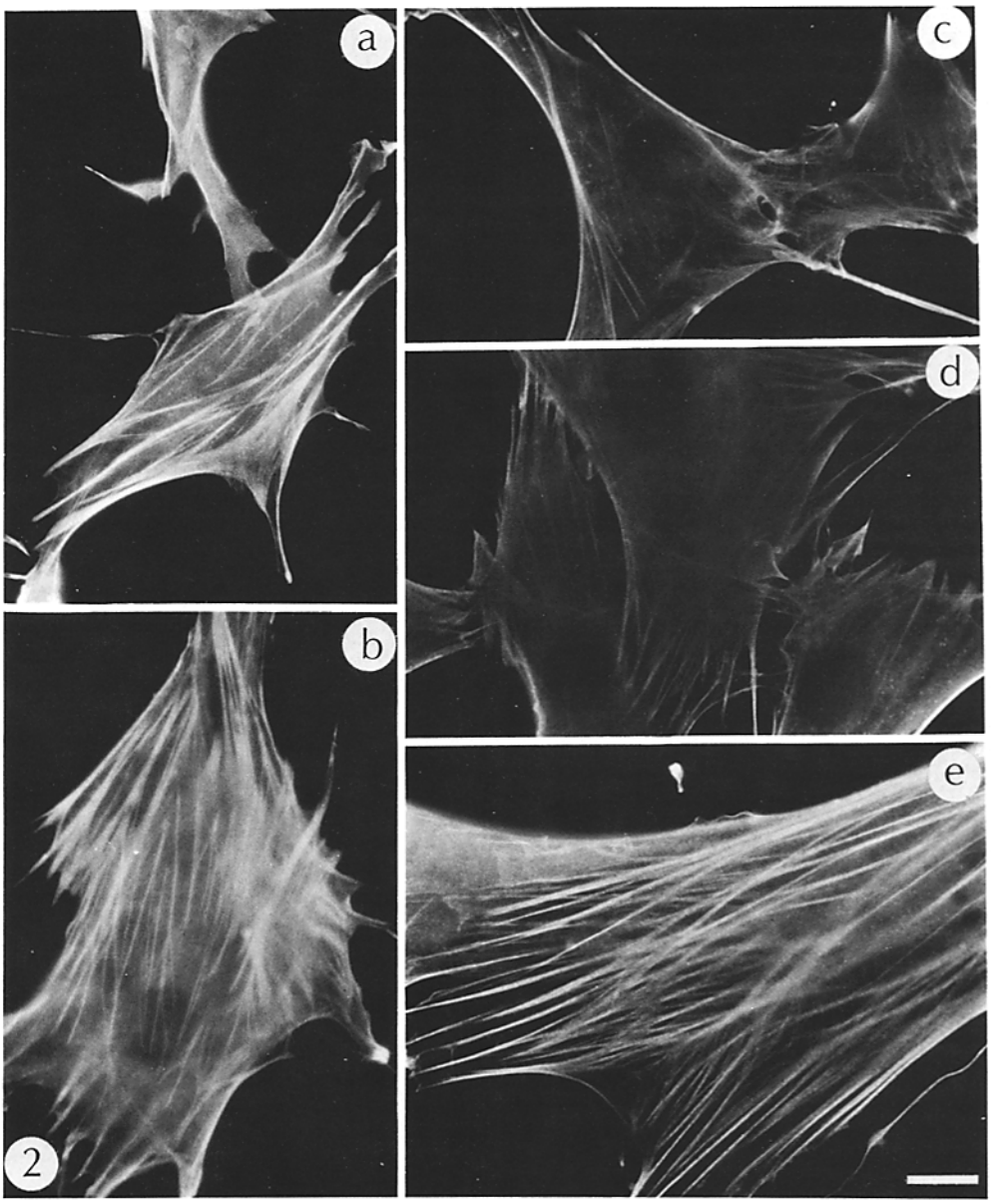

FIGURE 2 Staining of aortic SMC with antiactin IgG. The unfractionated immune IgG staining (*a* and *b*) revealed numerous actin-positive fibers. In c and *d*, the nonmuscle-specific antiactin IgG bound to the cell membrane–associated actin present in spikes, retraction fibers, and membrane ruffles. At this antibody concentration (10 μg/ml), some very fine fibers were weakly stained. In *e*, which was stained with 10 μg/ml muscle-specific antiactin IgG, bright fibers are seen traversing the cytoplasm. Bar, 10 μm. × 1,000.

circularly arranged with respect to the long axis of the vessel, the SMC appear in cross-section with their nonreactive nuclei as dark circular profiles. The lower arrows in Fig. 5*b* mark the endothelium of the intimal layer in phase contrast, which is nonreactive with the muscle-specific antiactin.

The staining of a small, muscular artery and an elastic artery with the muscle-specific antiactin IgG is shown in Figure 5, *c–g*. The toluidine blue–stained parallel section through the longitudinally oriented muscular artery (Fig. 5*d*) clearly illustrates the presence of EC lining the lumen of this blood vessel (black arrows). After staining with the antimuscle actin IgG, only the medial SMC are fluorescent (Fig. 5*c*). In this orientation, the SMC appear in cross-section so that the unstained nuclei appear as black holes amidst the brightly stained cytoplasms. The specificity of smooth muscle staining is apparent in the elastic arteries where the elastic laminae (which are 2.0 μm in diameter and are spaced at 8-μm intervals in the medial layer) do not stain (Fig. 5*f*). The elastic fibers (which are autofluorescent in the green range of the spectrum) can be clearly identified (Fig. 5*g*) using the appropriate barrier and excitation filters in the fluorescent microscope. The toluidine blue–stained parallel section reveals the presence of the endothelial layer (Fig. 5*e*), which is nonreactive with the anti-muscle actin IgG (Fig. 5*f*).

VENULES AND CAPILLARIES: At the microvascular level, the muscle-specific antiactins recognized perivascular cells adjacent to the endothelial abluminal surface (Fig. 6). As in the fluorescent antibody staining experiments on the vascular cells in vitro, the nonmuscle-specific antiactins stained the perivascular cells (pericytes) and EC (Fig. 6, *a* and *b*). In longitudinally oriented, ultrathin nonmuscle antiactin-stained sections of post-capillary venules, pericytes possessed brightly stained cytoplasms; but, in contrast to the cells in vitro, no stress fibers were evident.

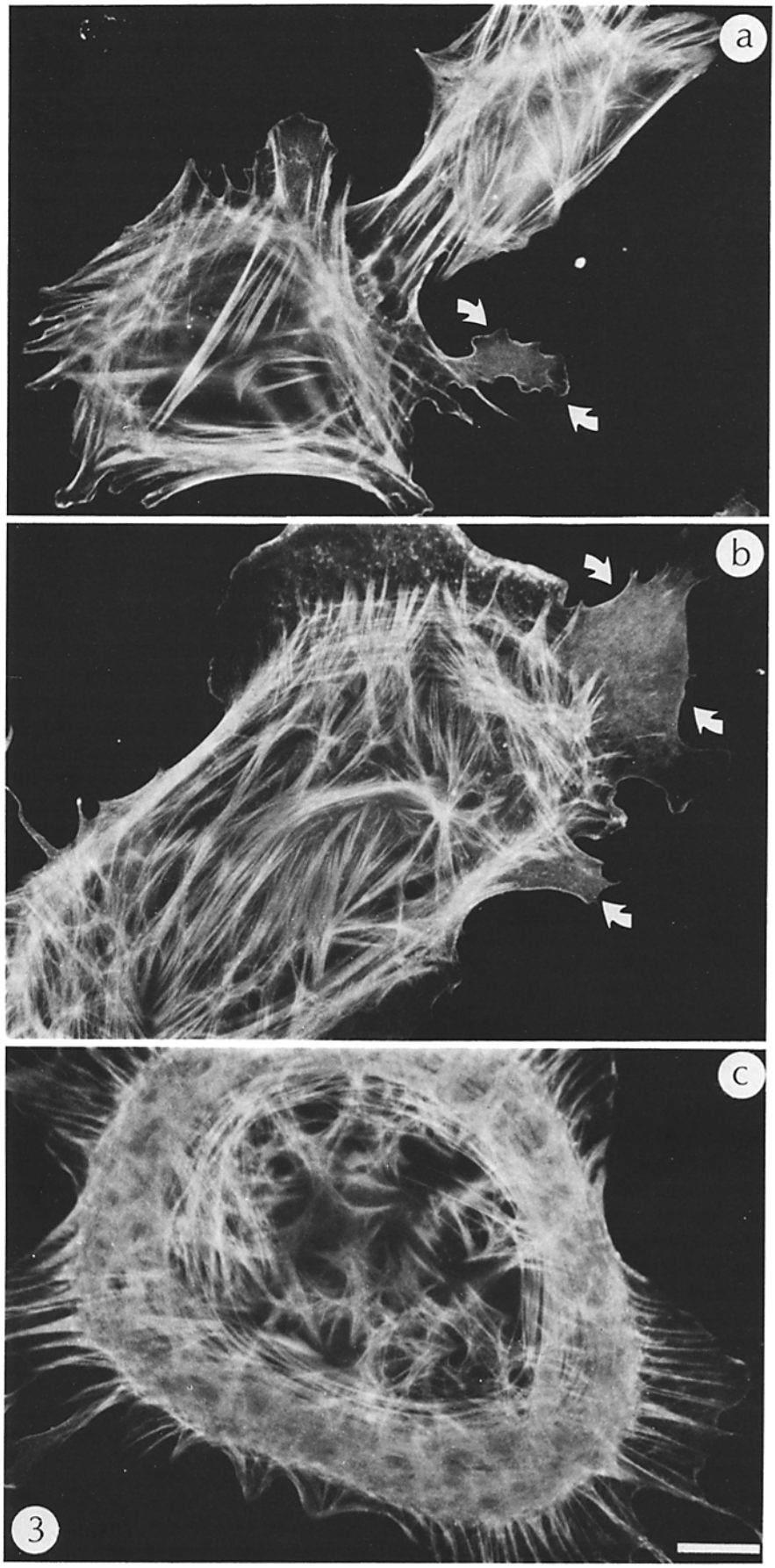

FIGURE 3 Staining of retinal pericytes with antiactin IgG. Fluorescence images of retinal capillary pericytes after staining with the unfractionated antiactins (*a*) revealed massive fiber bundles that were actin-positive and regions of motile cytoplasm where there was a granular meshwork of diffuse actin staining (lower cell in *a*, arrows). In *b* and *c*, retinal pericytes were stained with either the muscle-specific (*b*) or nonmuscle-specific (*c*) antiactin IgG (10 μg/ml). The fibrous and diffuse staining in *b* and *c* were comparable, suggesting equivalent cross-reaction of the muscle and nonmuscle-specific antibodies with the pericyte actin pools. Bar, 10 μm. × 1,300.

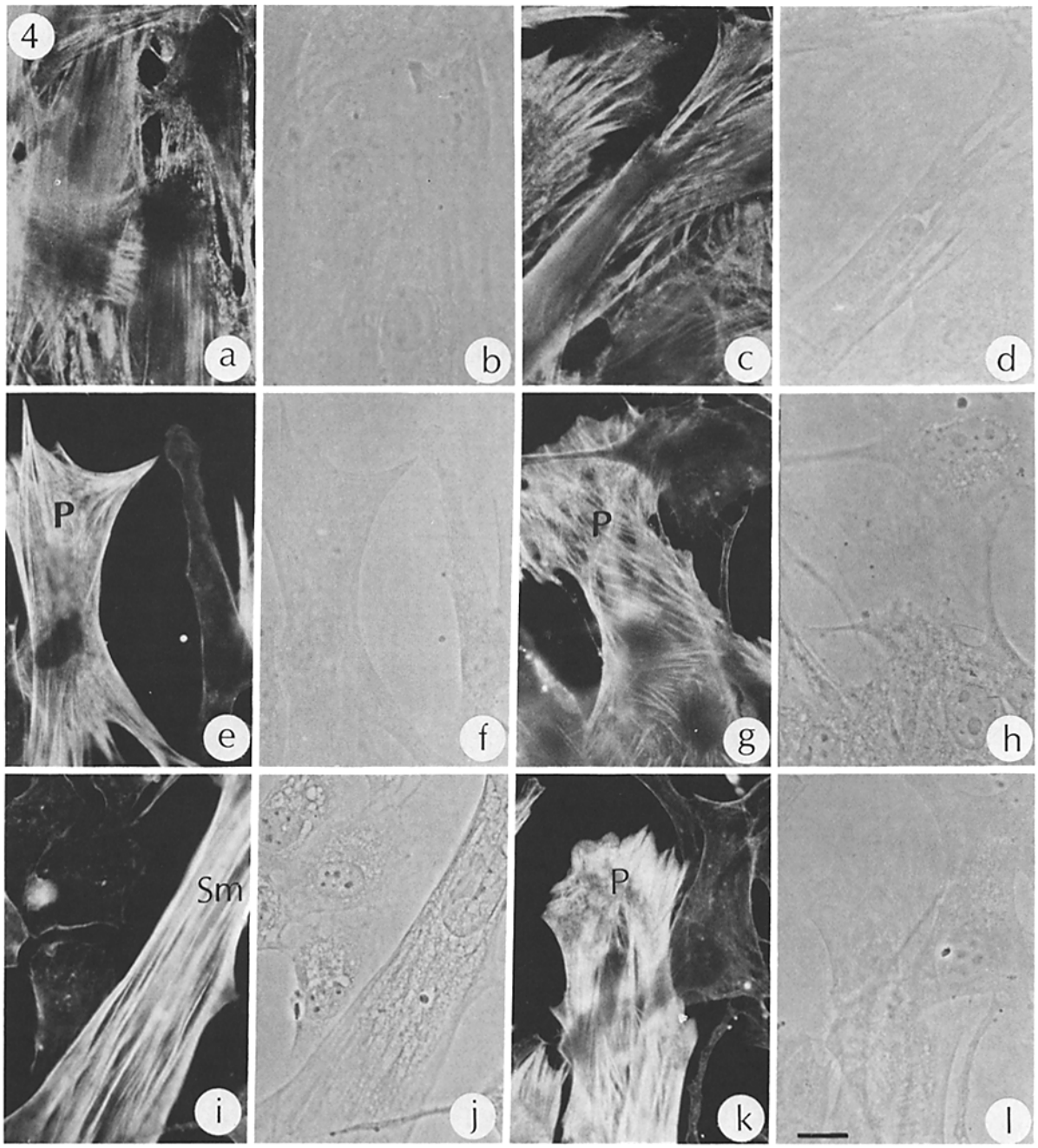

FIGURE 4 Staining of mixed cultures of pericytes, SMC, and EC with affinity-fractionated antiactin IgG. Fluorescence and phase contrast micrographs of EC and pericytes, SMC and EC, and pericytes and SMC. In *a* and *b*, the EC–pericyte cultures were stained with 5 μg/ml nonmuscle-specific antiactin IgG, and all of the cells are positive. In *c* and *d*, the SMC and pericyte cultures were stained with 5 μg/ml muscle-specific antiactin IgG, and all of the cells are positive. In *e–h*, pericyte–EC cultures were stained with 5 μg/ml muscle-specific antiactin IgG. Brightly stained pericytes (*P*) are evident among negatively stained EC, which are apparent in the phase contrast images (*f* and *h*). The dark shadow across the fluorescent pericyte is caused by a nonfluorescent (negative) EC process. In the SMC–EC culture (*i* and *j*) stained with 5 μg/ml muscle-specific antiactin IgG, a brightly stained SMC (*Sm*) is apparent. Four EC seen in phase contrast (*j*) are very weakly stained. In *k* and *l*, pericytes and SMC were stained with 15 μg/ml nonmuscle-specific antiactin IgG, and negative images of the SMC can be seen crossing the fluorescent pericytes (*P*). The SMC cortical cytoplasm is weakly stained, indicating some cross-reaction with the nonmuscle antiactin. Bar, 10 μm. × 800.

The number of perivascular cells associated with capillaries varies considerably and this variation in pericyte number was readily observed in cardiac muscle and in the other tissues examined (not shown), utilizing either the nonmuscle or muscle-specific antiactin IgG (Fig. 6, *c–h*). When cardiac muscle capillaries were transversely sectioned and stained with the muscle-specific antiactin IgG, pericytes were seen in association with some, but not all of the capillary profiles (Fig. 6, *g* and *h*). As in longitudinally oriented microvessels stained with the muscle-specific antibodies, the endothelial cytoplasms were not stained. In the cardiac muscle, the EC of pericyte-poor capillaries were seen only after staining with the nonmuscle actin antibodies. Here, the sarcomeric actin present in the I-bands of the cardiac muscle was nonreactive (Fig.

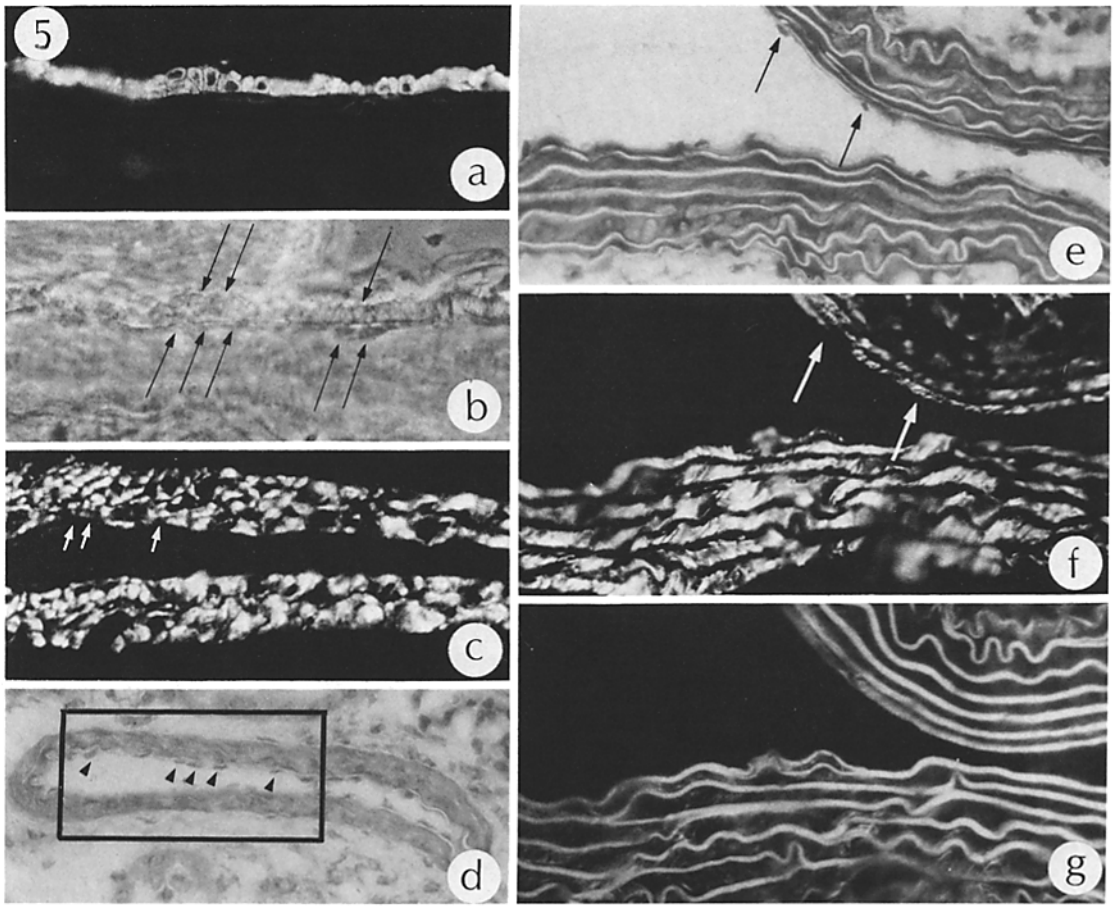

FIGURE 5 Identification of vascular smooth muscle in cryosectioned arterioles and arteries using muscle-specific antiactin IgG. In *a*, the fluorescence image of a longitudinally oriented brain arteriole is seen after staining with anti-muscle actin antibodies. The corresponding phase contrast image (*b*) reveals that the EC (lower black arrows) are not stained under these conditions. In *c*, the fluorescently stained media of a kidney cortical muscular artery sectioned in longitudinal profile is seen after reaction with 5 μg/ml anti-muscle actin antibodies. The corresponding toluidine blue-stained bright field image (*d*) reveals the specificity of the probe for the medial SMC since neither the EC (black arrows, *d*; white arrows, *c*) nor the other kidney epithelial cells are stained. In *e–g*, the specificity of the muscle actin probe is demonstrated after staining of an elastic artery. The EC (*e*, black arrows) are nonreactive (*f*, white arrows) with the muscle-specific actin probe. Elastic laminae (*g*) interdigitate between the layers of smooth muscle apparent in *f*. (*a* and *b*) × 200; (*c*) × 150; (*d*) × 540; (*e–g*) × 200.

6 *d*) with the nonmuscle antiactin. In contrast, pericyte-rich capillaries were detected after staining the ultrathin frozen sections of cardiac muscle with the muscle-specific (or the nonmuscle specific) antibodies (Fig. 6, *e–h*). The pericyte processes associated with the abluminal endothelial surface were brightly stained after incubation with the muscle-specific antiactin. In the longitudinal profile of the cardiac muscle capillary shown in Fig. 6 *e*, the endothelial cytoplasm is not stained, but is evident in the phase contrast photograph (Fig. 6 *e*). Moreover, the sarcomeric actin within the cardiac muscle I-bands was stained with the muscle-specific antiactin IgG, indicating that the vascular SMC, pericytes, and the cardiac muscle cells all possess actins recognizable by the muscle-specific antiactin IgG.

DISCUSSION

Polyclonal vs Monoclonal Antibodies

We have demonstrated that fractionated antiactin IgG, when used in conjunction with fluorescence microscopy, can be utilized as a specific vascular cell marker, allowing the discrimination among EC, SMC, and pericytes. Since the actin antibodies utilized in this study react across species and tissue boundaries, they could, in principle, bind both muscle and nonmuscle actins (19). Although smooth muscle and non-muscle γ isoactins possess the same isoelectric pH, sequencing studies have revealed that the chicken gizzard smooth muscle γ isoactin is more similar in sequence to striated muscle actin than to γ isoactin present in nonmuscle cells (9, 15, 25, 28, 33–35, 39). We have capitalized on this by performing sequential, differential absorption on solid-phase matrices using actins purified from nonmuscle and muscle sources. Because both muscle-specific (smooth or skeletal) and nonmuscle-specific antiactins can be obtained from this polyclonal sera, this avenue of antiactin purification may be more efficient and economical than utilizing monoclonal technology (2) or cloning the specific isoactin genes (33, 38, 39) to sort out amino acid sequence heterogeneities. Recently, Pardo and his co-workers (29) performed a similar affinity-fractionation of polyclonal actin antibodies to demonstrate the association of γ-actin with skeletal muscle mitochondria.

The Vascular Pericyte

Much speculation surrounds the embryonic origin of peri-

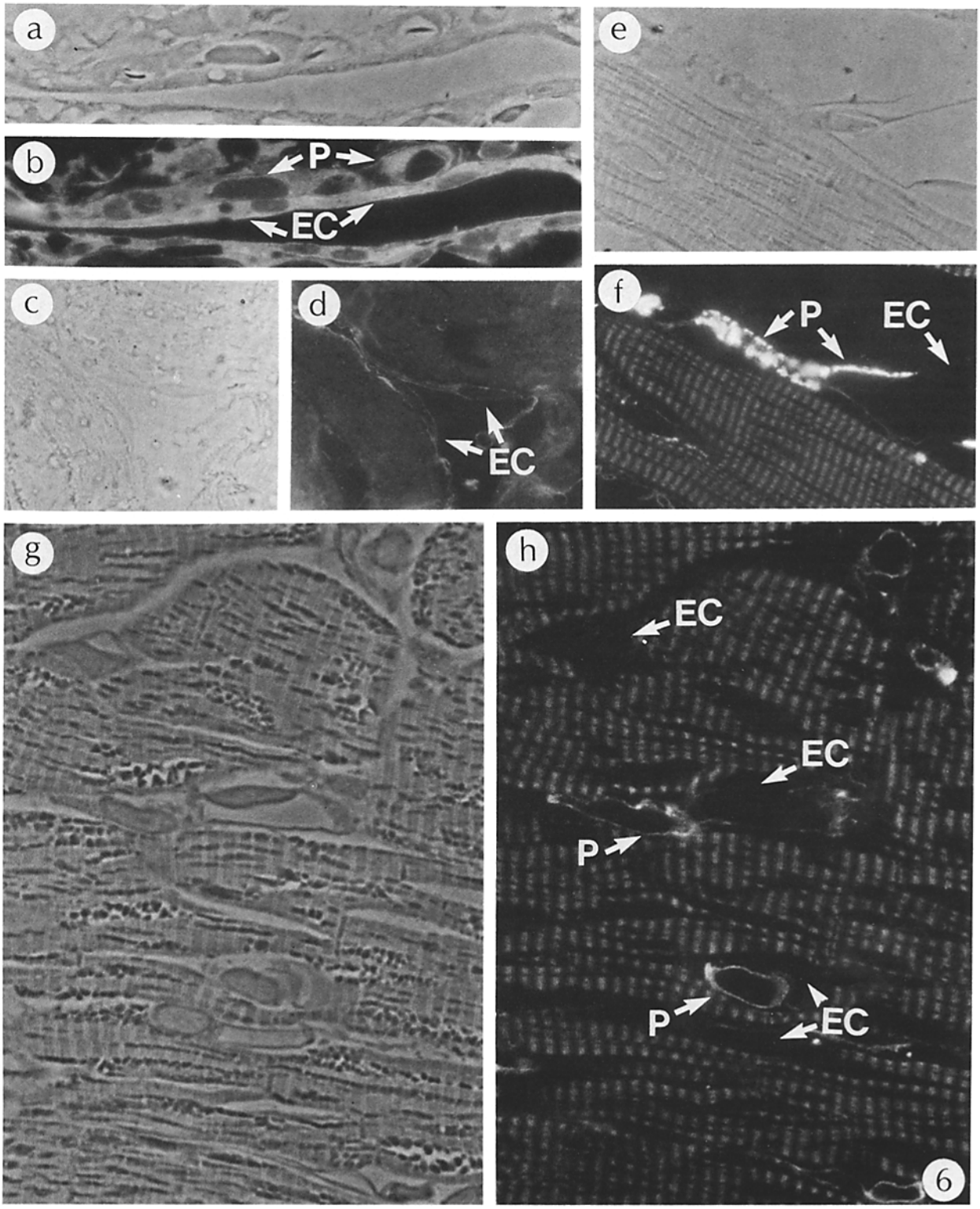

FIGURE 6 Localization of microvascular pericytes in ultrathin cryosectioned cardiac muscle. Phase contrast (*a*, *c*, *e*, and *g*) and fluorescence (*b*, *d*, *f*, and *h*) micrographs of 0.5 μm cardiac muscle tissue sections stained with anti-nonmuscle (*b* and *d*) and muscle actin (*f* and *h*) antibodies. In *b*, a longitudinally oriented postcapillary venule is seen after reaction with the nonmuscle actin antibodies. As in tissue culture staining experiments (Figs. 3 and 4), the pericytes (*P*) react strongly with this probe, in contrast to the SMC, which are not stained under these conditions. The intimate association of pericytes with endothelium (*EC*) is clearly revealed in the longitudinal profile of this microvessel. Pericyte absence (*c* and *d*) and presence (*e–h*) within the capillary bed is demonstrated using either the nonmuscle specific (*d*) or muscle-specific antiactins. Endothelial cytoplasms are stained with the nonmuscle reactive (*d*), but not the muscle-reactive (*f* and *h*) antiactins. The intermittent, patchy fluorescence is due to the staining of the finger-like pericyte processes that wrap around the endothelium (*f* and *h*). Note that the actin in the pericyte processes and the cardiac muscle I-bands are stained, indicating immunologic identity among the vascular smooth muscle cell, pericyte, and cardiac muscle cell α-actins. (*a* and *b*) × 1,300; (*c–f*) × 650; (*g* and *h*) × 1,300.

cytes and the functional relationship between the endothelium and pericytes at the microvascular level (7, 31). A variety of functions have been postulated for the pericyte including the regulation of capillary contraction (23, 36, 40) and control of capillary proliferation (24). However, the lack of appropriate markers for pericytes has prevented a thorough examination of the problem. Although the presence of Factor VIII antigen has been demonstrated to be a definitive marker for the endothelium, the lack of cross-reactivity across species boundaries has limited this marker to use in bovine and human tissue. The vascular SMC is readily identified by its location within the vessel wall and by the types of actin and myosin present within its cytoplasm. The use of the fractionated antiactins provides a means for conclusive differentiation of pericytes from EC and SMC in situ.

Since pericytes react with the muscle and nonmuscle-specific antiactins, it is very likely that these cells contain both smooth and nonmuscle isoactins. Recent experiments utilizing two-dimensional gel electrophoresis to analyze column-purified pericyte actin indicate that pericytes possess α, β, and γ isoactins in a molar ratio of 1:2.3:2.7 (Herman, I. M., unpublished data). Whether particular pericyte isoactins serve certain cellular functions is a question currently under investigation.

Staining pericytes in vitro with either antiactin IgG reveals continuous fluorescence of stress fibers (Fig. 3, *b* and *c*), indicating that muscle and nonmuscle isoactins may be present along the same fibers. At low antibody concentrations (2–5 μg/ml), a spotted appearance of the fibrous staining is sometimes apparent, suggesting there may be domains of muscle and nonmuscle isoactins along pericyte fiber bundles or individual actin filaments; or, that a given domain is undersaturated at the concentration of antibody used.

The presence of both muscle and nonmuscle actin staining in the pericytes may reflect a pluripotent disposition of the cells. Pericyte pluripotentiality has been previously suggested (10, 31). Diverse stimuli such as microvasculature injury or capillary sprouting during embryonic development and tumor growth might dictate the expression of actin isotypes needed by pericytes for their participation in these diverse biological events. Interestingly, co-cultures of pericytes with EC or SMC did not induce alterations in the staining properties of any of these cells in that EC remained positive for Factor VIII antigen and nonmuscle actin, whereas pericytes remained negative for Factor VIII antigen and positive for both the muscle and nonmuscle-specific actins. The smooth muscle–like morphology of the pericytes in culture, the similarity of their location adjacent to the endothelium in situ, and the demonstrated presence of SMC actin indicate that pericytes may be similar in character to SMC. Thus, at the microvascular level, the pericyte may be functional in regulating venular and capillary permeability, contraction, and blood flow. Clearly, more work is needed to better define the role of the pericyte in the microvasculature. The specific pools of antiactin have already proven to be useful tools for the identification of pericytes in situ and will permit investigation of how these cells are modulated by external factors during hemostatic and disease processes.

We thank Dr. Judah Folkman for his helpful comments concerning this work, Drs. Donald Ingber, Stanley Jacobson, and Michelle Marks for help in preparing the cryosectioned materials, Dr. Bruce Batten for the use of his fluorescent microscope, Liz Sproul for her help with the cell cultures, and Bonnie Troped and Yvonne Waters for manuscript preparation. We also appreciate the positive criticisms and suggestions provided by the Journal of Cell Biology reviewers.

This work was supported in part by grants from the National Society to Prevent Blindness to Drs. Herman and D'Amore, an American Heart Association Grant-In-Aid to Dr. Herman, and National Institutes of Health grant EY05318. Dr. Herman is a recipient of an Established Investigatorship Award from the American Heart Association.

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