

Immunocytochemical Studies of Endothelial Cells In Vivo.

I. The Presence of Desmin Only, or of Desmin Plus Vimentin, or Vimentin Only, in the Endothelial Cells of Different Capillaries of the Adult Chicken

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Abstract. It is currently believed that the intermediate filaments of endothelial cells contain vimentin subunits exclusively. This inference, however, is derived from studies of only a few types of endothelial cells. By double indirect immunofluorescence and immunoelectron microscopy, we have now examined the endothelial cells of the micro- and macrovasculature of a variety of tissues and organs of adult chicken in vivo for their content of desmin and vimentin. Endothelial cells of the peritubular capillary in the renal cortex, the hepatic sinusoid, and the splenic sinusoid were found to contain only desmin; those of the exocrine

pancreas capillary contained both desmin and vimentin; and the endothelial cells of the macrovasculatures and of all the other microvasculatures examined, including the vasa recta of the renal medulla, contained only vimentin. Such heterogeneity suggests that different types of adult chicken endothelial cells may have different embryological origins. To the extent that desmin and vimentin intermediate filaments may be functionally distinct, these results also suggest that different capillary endothelial cells may have different functional properties.

THE endothelial cells that form the innermost layer of blood vessels and lymphatics are of great physiological importance. They regulate the traffic of molecules and cells between the tissues and the circulation, control vascular tone by synthesizing vasoactive agents, and maintain a nonthrombogenic surface throughout the vasculature (27). Despite this importance, endothelial cells have until recently been among the least well investigated cell types. Although readily accessible endothelial cells, such as those of the large blood vessels like the aorta, have been studied in vivo and have been placed in culture in vitro, less accessible endothelial cells, such as those of the smaller vessels and capillaries, have not as yet had their molecular ultrastructure extensively investigated. This is because capillary endothelial cells are not only difficult to isolate and culture (38), but even in vivo they are often so thin that they are difficult to study by conventional immunofluorescence microscopy. However, there is evidence that endothelial cells are not all alike, and are differentiated to meet specific demands from their tissue environment (20, 30, 34); therefore, results obtained with aorta endothelium in vivo or in culture do not necessarily apply to all (e.g., capillary) endothelial cells.

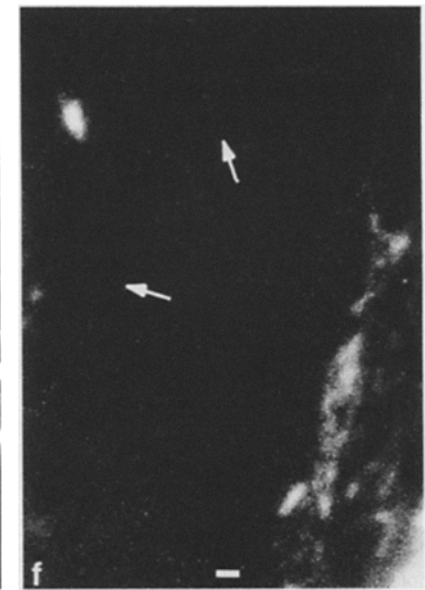
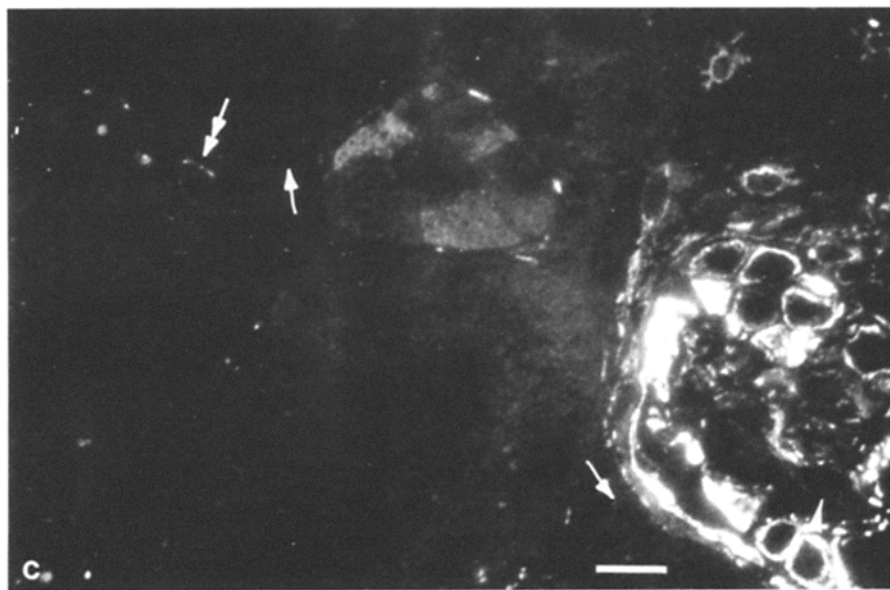
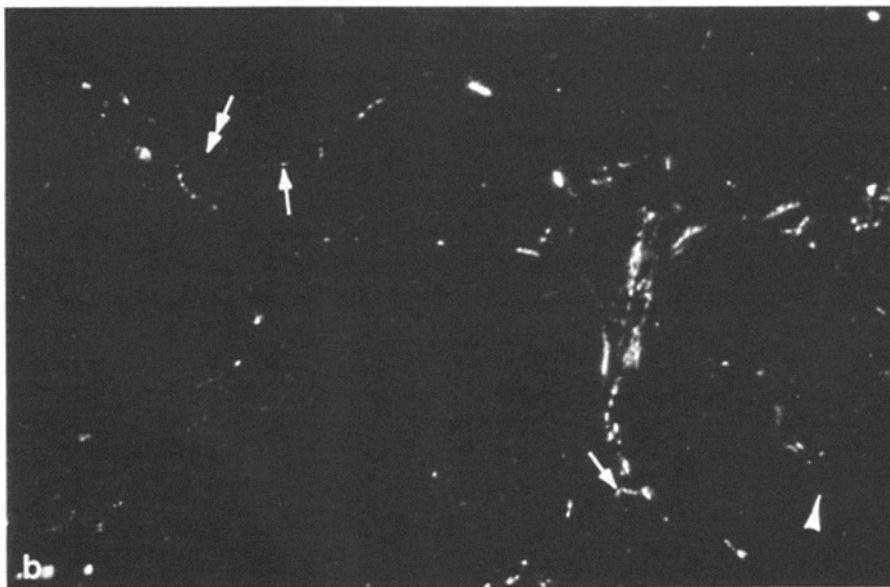
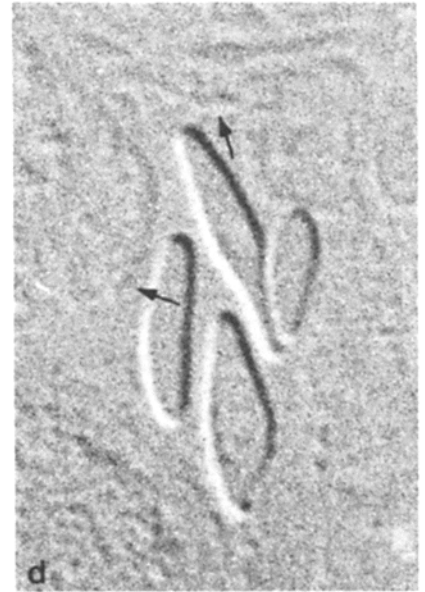
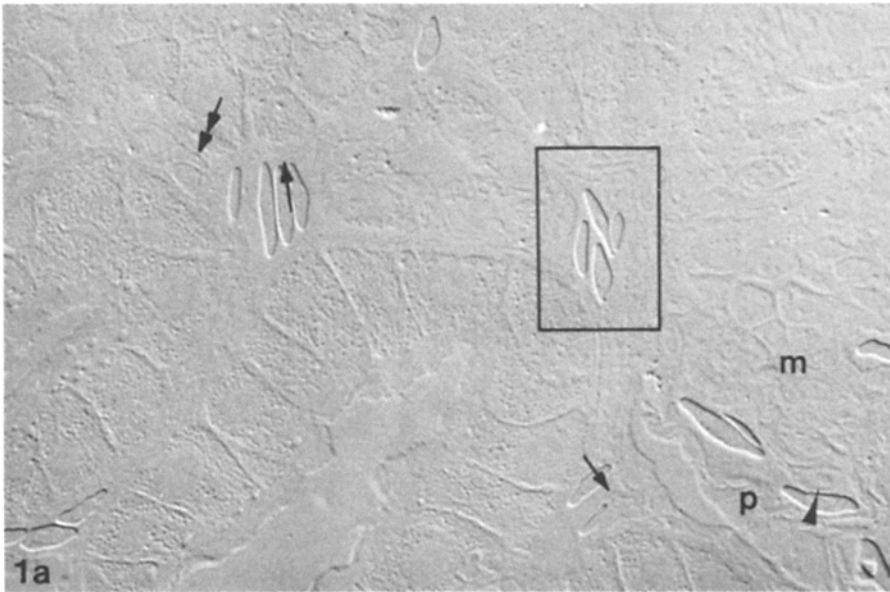
Under these circumstances, a general and powerful approach to the study of the molecular ultrastructure of endothelial cells is immunoelectron microscopy, using techniques of frozen sectioning and immunolabeling developed

in this laboratory (31, 32). In this paper, we begin a systematic immunocytochemical study of the structural proteins of endothelial cells of different tissues and organs, with an investigation of the intermediate filament proteins desmin and vimentin. From studies of endothelial cells of large vessels in vivo (4) or in culture (4, 12, 17, 25) that were found to contain only vimentin and no desmin, it has been assumed that vimentin is generally the only intermediate filament protein in endothelial cells. We show, however, that depending on the chicken organ, endothelial cells of different capillaries contain either desmin only, vimentin only, or desmin and vimentin. These results indicate that endothelial cells of different vasculatures are differentiated in their cytoskeletons. This suggests that they may have different embryological origins, as well as different functional characteristics.

Materials and Methods

Immunochemical Reagents

Guinea pig antibodies against chicken erythrocyte vimentin and rabbit antibodies against chicken gizzard desmin were affinity-purified and cross-absorbed with the heterologous antigen as described (33). It is important to emphasize that cross-reactivity between these resultant antibodies is absent (33). For the secondary antibodies used in immunofluorescence, affinity-purified and cross-absorbed goat antibodies to guinea pig IgG and to rabbit IgG were conjugated to rhodamine and fluorescein (5). For double



immunolabeling, either a combination of rhodamine goat anti-rabbit IgG and fluorescein goat anti-guinea pig IgG, or a combination of fluorescein goat anti-rabbit IgG and rhodamine goat anti-guinea pig IgG was used. For double immunoelectron microscopy, a combination of ferritin goat anti-rabbit IgG and Imposil (iron-dextran) goat anti-guinea pig IgG was employed (11, 19). These goat antibodies were also affinity-purified and cross-absorbed.

Preparation of Tissues

Adult White Leghorn hens were anesthetized with sodium pentobarbital, and sample tissues were removed and promptly immersed in fixative in small pieces. In some animals, after thoracotomy, a cannula was inserted into the left ventricle of heart and fixative was perfused at a pressure of 100–150 mmHg. Perfusion-fixed tissues were also cut into pieces and further fixed by immersion. Fixatives used were 3% formaldehyde (prepared freshly from paraformaldehyde) or a mixture of 3% formaldehyde and 0.1–0.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, at room temperature. Total fixation time was 60 min. After fixation they were kept in 0.5% formaldehyde in 0.1 M sodium phosphate buffer, pH 7.4, at 4°C for up to a week. For cryosectioning, tissue pieces were infused with 2.3 M sucrose in 0.01 M sodium phosphate buffer, pH 7.4, for 15–30 min, and rapidly frozen in liquid nitrogen. Semi-thin sections (0.5–1.0 µm in thickness) and ultra-thin sections (0.05–0.10 µm in thickness) were cut by an ultramicrotome (model MT-2B; Dupont Co., Sorvall Instruments Div., Newtown, CT) with cryoattachment (31, 32).

Immunofluorescence Microscopy

Semi-thin frozen sections mounted on glass slides were immunolabeled according to Tokuyasu et al. (33). When tissues were fixed with a fixative containing glutaraldehyde, sections were treated with 0.5 mg/ml sodium borohydride for 5 min to quench the fluorescence caused by the fixative (22). After pretreatment with 2% gelatin and rinsing, sections were sequentially incubated with primary antibodies for 20–30 min, rinsed for 10 min, incubated with secondary antibodies for 20–30 min, rinsed for 10 min, and mounted with 90% glycerol in Tris-HCl buffer, pH 8.5. Sections were observed with a Zeiss photomicroscope III equipped with epifluorescent illumination and a 63× Nomarski objective lens. The specimens were photographed on Kodak Tri-X film.

Immunoelectron Microscopy

Ultra-thin sections were immunolabeled using ferritin- and Imposil-conjugated secondary antibodies as described (32). For pretreatment of the thawed frozen sections, a mixture of 1% normal goat serum and 2% dextran (mol wt 10,000) was used. Incubations with primary and secondary antibodies were performed for 30 min each. Sections were adsorption-stained and embedded in methylcellulose as described (32), and were observed with a Philips EM-300 electron microscope operated at 60 or 80 kV.

Results

Renal Cortex

The avian kidney is a structural hybrid of the reptilian and mammalian kidneys (7, 28). In the renal cortex, there are solely reptilian-type nephrons without a loop of Henle, while in the renal medulla, there are only mammalian-type nephrons with a loop of Henle. The peritubular capillaries in the renal cortex, as seen among reptilian-type nephrons, have lumen diameters up to ~30 µm. When semi-thin frozen sec-

tions were prepared and doubly labeled for desmin and vimentin, both antigens were found to coexist in many places along the capillary wall (Fig. 1). At the innermost portion of the capillary wall, however, desmin labeling that was recognized to be in an endothelium (Fig. 1, *a* and *b*, single arrows; and Fig. 1, *d* and *e*, single arrows) was not accompanied by vimentin labeling (corresponding arrows in Fig. 1, *c* and *f*, respectively). On the other hand, some cells around the peritubular capillary were stained only for vimentin and were probably fibroblasts (double-headed arrows in Fig. 1, *a–c*). The absence of vimentin staining in erythrocytes was probably caused by the close packing of the fixed hemoglobin molecules, as erythrocyte ghosts have been positively stained under similar conditions (33). The above findings suggested that endothelia, and other cells in and around the peritubular capillary, have either desmin, or vimentin, or both, but because the endothelium and the pericytes are both very thin and closely juxtaposed, and also because the fluorescent labels were sporadic, immunoelectron microscopy was necessary to confirm the results. Desmin and vimentin were doubly labeled by ferritin and Imposil, respectively. It was clearly demonstrated (Fig. 2) that the endothelium of the peritubular capillary is labeled for desmin only, whereas the adjacent pericyte contained both desmin and vimentin. Fibroblasts in the connective tissue around the capillary were found to contain only vimentin (not shown). It is therefore concluded that endothelial cells of the peritubular capillaries in the renal cortex contain desmin and little or no vimentin.

On the other hand, endothelial cells of the glomerular capillaries were not definitively labeled for desmin (arrowhead, Fig. 1, *a* and *b*) or vimentin (corresponding arrowhead in Fig. 1 *c*). Endothelial cells of arterioles were strongly labeled for vimentin (arrow, Fig. 3, *a* and *c*) but did not stain for desmin (corresponding arrow, Fig. 3 *b*). Immunoelectron microscopic labeling (not shown) confirmed these findings. By contrast, endothelial cells of intrarenal portal veins, as in the case of the peritubular capillary endothelium, appeared to contain only desmin (corresponding arrows in Fig. 3, *d–f*). Other cells in the same field (arrowheads, Fig. 3, *d–f*), presumably smooth muscle cells, contained both desmin and vimentin.

Renal Medulla

Capillaries of the renal medulla (vasa recta) also have a large lumen and a thin wall. When labeled for vimentin and desmin, however, they gave different results from the renal cortical peritubular capillaries. The innermost portion of capillaries was strongly and continuously labeled for vimentin alone (corresponding arrows, Fig. 4, *a–c*). Desmin labeling was observed in cells that were more exterior, and was always accompanied by vimentin labeling (arrowheads in Fig.

Figure 1. A semi-thin frozen section of renal cortex. Nomarski (*a* and *d*) and double immunofluorescence micrographs for desmin (*b* and *e*) and vimentin (*c* and *f*). (*a–c*) Although desmin and vimentin coexist in many areas, only desmin is seen in the innermost portion of the peritubular capillary wall (arrows). The endothelium of the glomerular capillary (arrowhead) is not definitively stained for vimentin or for desmin. Mesangial cells (*m*) and podocytes (*p*) in the glomerulus as well as fibroblasts (double arrows) are stained for vimentin only. Bar, 10 µm. (*d–f*) Higher magnification of an area of a rectangle in *a*. The cytoplasm of endothelial cells is stained for desmin, but not for vimentin (arrows). Bar, 1.0 µm.

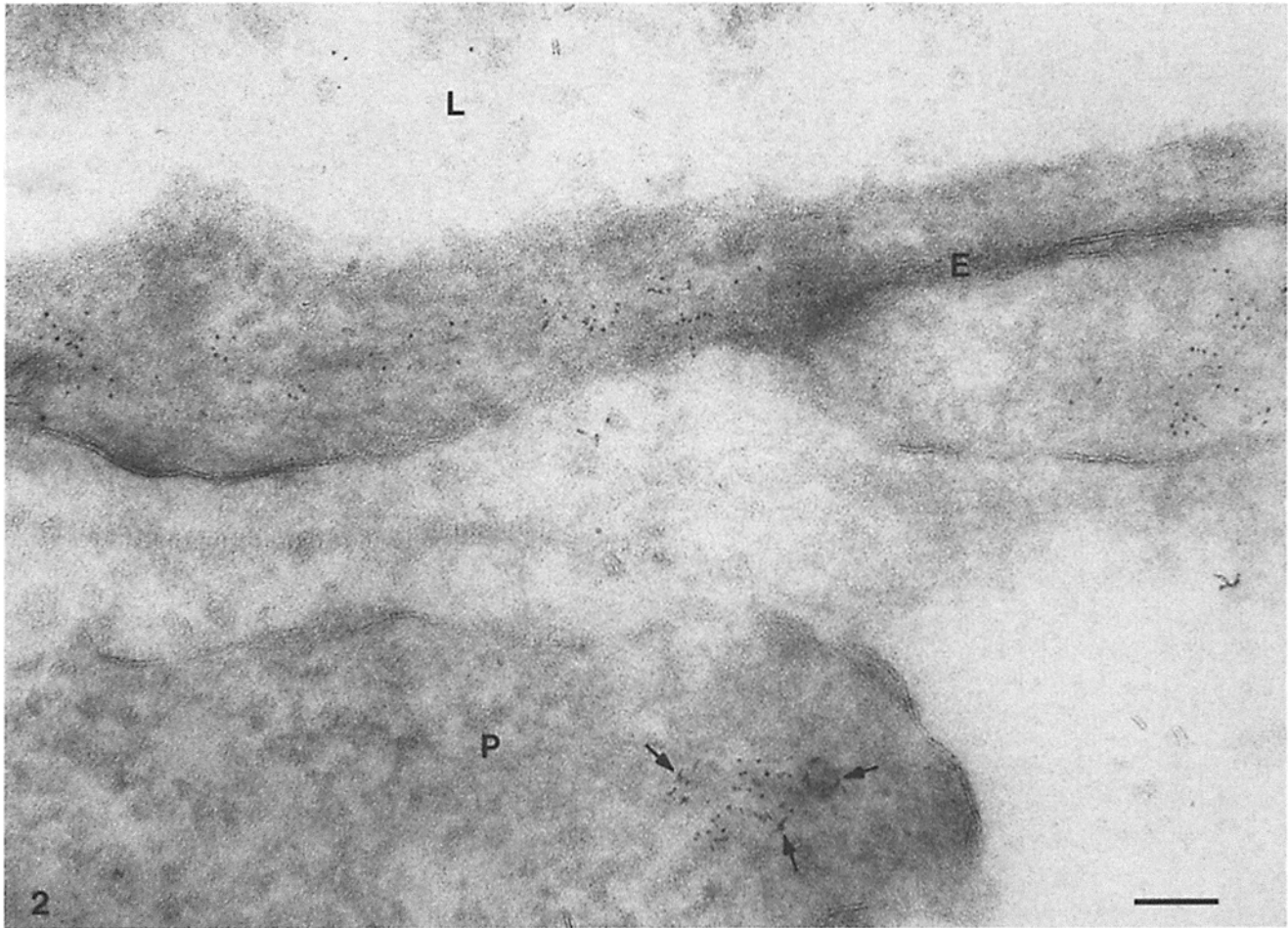


Figure 2. An ultra-thin frozen section of the peritubular capillary in the renal cortex. Double immunolabeling for desmin and vimentin, marked with ferritin and Imposil, respectively. (This combination is the same for all double immunoelectron micrographs.) The endothelium (*E*) is virtually labeled with ferritin alone, while the pericyte (*P*) is labeled with both ferritin and Imposil. Imposil particles are marked with arrows. *L*, lumen of capillary. Bar, 0.1 μ m.

4). This same pattern was observed in the renal papilla (not shown). The conclusion is that the capillary endothelial cells of the renal medulla contain vimentin only.

Liver

The sinusoid in chicken liver is lined with endothelial cells and Kupffer cells as in mammalian liver (6, 23). The endothelial cells are thin and narrow, and adhere closely to the sinusoidal wall, while the Kupffer cells have an irregular shape and often protrude into the lumen. In doubly labeled immunofluorescence micrographs, the results obtained appeared very similar to those of the renal cortex; desmin and vimentin were seen mostly to coexist along the sinusoidal wall, but some desmin labeling was observed (arrows, Fig. 5, *a* and *b*) in the innermost layer of the sinusoid without accompanying vimentin labeling (corresponding arrows in Fig. 5 *c*). As Kupffer cells were found to be diffusely labeled for vimentin only (corresponding arrowheads in Fig. 5, *a-c*), those cells that were labeled solely for desmin were likely to be endothelial cells. Endothelial cells of the interlobular portal vein in a portal area were also labeled for desmin (arrows, Fig. 5, *d* and *e*) but not for vimentin (corresponding arrows in Fig. 5 *f*) whereas those of the interlobular hepatic

artery were stained for vimentin but not for desmin (corresponding arrowheads in Fig. 5, *d-f*).

The above immunofluorescence results for the sinusoid were confirmed by immunoelectron microscopy (Fig. 6). Since cells in and around the hepatic sinusoid have enormous numbers of endogenous ferritin particles in the cytoplasm, ferritin conjugates could not be used for immunolabeling. Therefore, single labeling for either desmin or vimentin was carried out with Imposil conjugates. Endothelial cells, as well as cells beneath them in the space of Disse, were labeled for desmin (Fig. 6 *a*). On the other hand, the endothelial cells did not label for vimentin (Fig. 6 *b*). It was concluded from these results that liver endothelial cells contain desmin only, whereas Kupffer cells contain vimentin only.

Spleen

The splenic sinusoid that is lined by the flat littoral endothelium was observed by double immunofluorescence microscopy (Fig. 7, *a-c*). Labeling for desmin was seen along the inner surface of the sinus wall (arrows in Fig. 7, *a* and *b*). In some places, however, desmin labeling was seen as two parallel lines, only the exterior one of which was also labeled for vimentin (corresponding arrowheads in Fig. 7, *a-c*). The

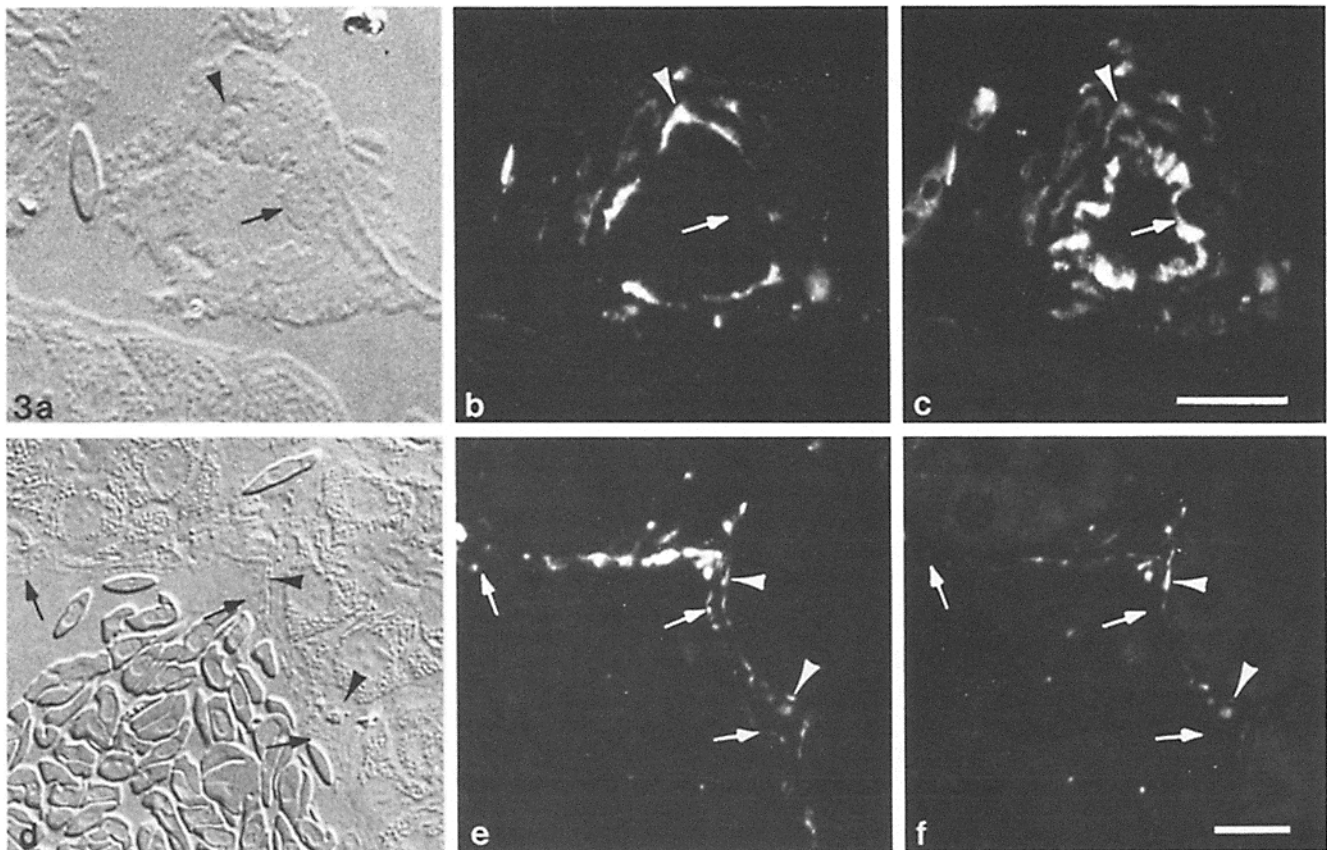


Figure 3. Nomarski (*a* and *d*) and double immunofluorescence pictures of the renal cortex for desmin (*b* and *e*) and vimentin (*c* and *f*). (*a-c*) An arteriole. The arteriolar endothelium (*arrow*) is solely stained for vimentin. The smooth muscle cells (*arrowheads*) are stained for both desmin and vimentin. Bar, 10 μ m. (*d-f*) An intrarenal portal vein. The endothelium is labeled (*arrow*) only for desmin. Cells exterior to the endothelium, possibly smooth muscle cells, are stained for both desmin and vimentin (*arrowheads*). Bar, 10 μ m.

continuous desmin staining along the interior surface of sinusoid was apparently from the endothelium and the structures labeled for both proteins were likely perisinusoidal cells, which are on the outer surface of the sinusoid.

Pancreas

Capillaries in the exocrine portion of pancreas were examined (Fig. 7, *d-f*). Although there were a few focal areas that stained only for vimentin, the capillary wall was generally labeled for both desmin and vimentin, including the innermost portion of the endothelial cytoplasm (corresponding arrows in Fig. 7, *d-f*). Two parallel lines of labeling for both desmin and vimentin were observed along some capillary walls (corresponding arrowheads in Fig. 7, *d-f*), which probably correspond to endothelium and pericyte.

Double immunoelectron microscopy confirmed the above results (Fig. 8). The endothelial cells were labeled with both ferritin and Imposil, indicating the presence of desmin and vimentin, respectively.

Cardiac Muscle

Transverse sections of cardiac muscle were immunofluorescently labeled for desmin and vimentin (Fig. 9). As reported earlier for these antibodies (33), desmin but not vimentin was seen in cardiac muscle cells. Capillary endothelial cells were stained solely for vimentin (corresponding arrows in

Fig. 9, *a-c*). This result was confirmed by immunoelectron microscopy (Fig. 10); endothelial cells were labeled only with antivimentin, but pericytes were labeled with both antibodies.

The endothelium of capillaries in skeletal muscle (anterior latissimus dorsi) and smooth muscle (gizzard) were labeled similarly to those in cardiac muscle (not shown), for vimentin and not for desmin.

Brain

Since endothelial cells of brain capillaries are likely to play a major role in the blood-brain barrier, and therefore, some differentiation was possible, capillaries of the cerebral cortex and the midbrain were examined. Endothelial cells as well as astrocytes around the capillary were labeled for vimentin, but not for desmin (not shown) by immunofluorescence microscopy. The results show that brain capillaries are similar to those of muscle type in the composition of intermediate filaments.

Large Vessels

The aorta, the femoral artery, the mesenteric artery and vein, and the cranial renal portal vein (a branch from the external iliac vein) were examined by immunofluorescence. Endothelial cells of these vessels were all strongly labeled for vimentin and not for desmin (the result with femoral artery is

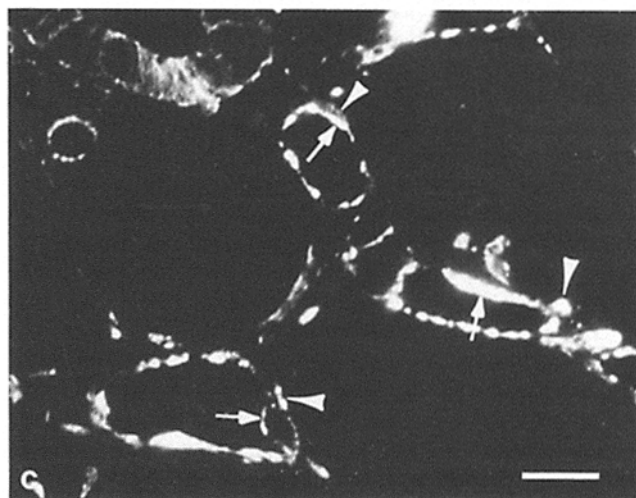
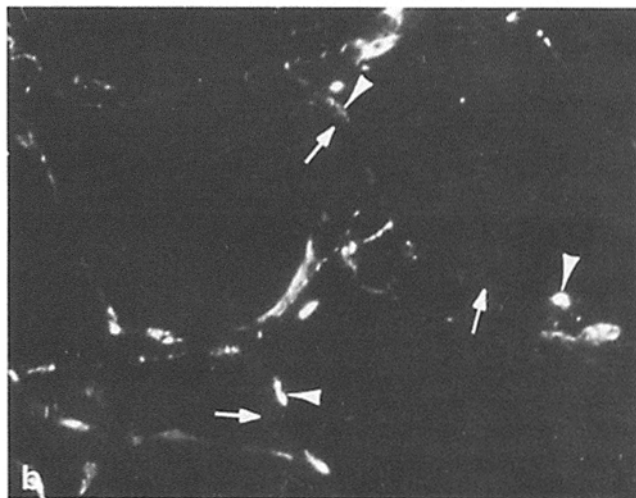
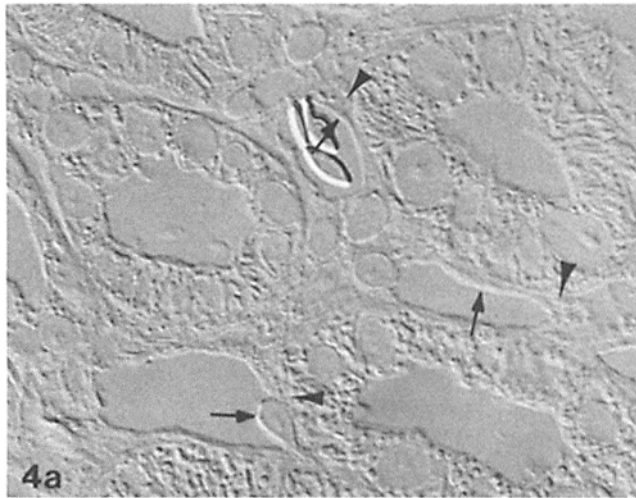


Figure 4. The renal medulla. Nomarski (a) and double immunofluorescence micrographs for desmin (b) and vimentin (c). The endothelium of vasa recta (arrows) is stained brightly for vimentin, but not for desmin, while the pericyte is stained for both proteins (arrowheads). Bar, 10 μ m.

Table I. Summary of Results of Immunolabeling of Endothelium

	Desmin	Vimentin
Renal cortex		
Peritubular capillary	+	-
Glomerular capillary	-	\pm
Arteriole	-	+
Intrarenal portal vein	+	-
Renal medulla		
Vasa recta	-	+
Liver		
Sinusoid	+	-
Interlobular artery	-	+
Interlobular portal vein	+	-
Spleen		
Sinusoid	+	-
Pancreas		
Capillary in the exocrine portion	+	+
Muscle		
Cardiac muscle	-	+
Skeletal muscle	-	+
Smooth muscle	-	+
Brain		
Cerebral cortex	-	+
Midbrain	-	+
Large vessels		
Aorta	-	+
Femoral artery	-	+
Cranial renal portal vein	-	+
Mesenteric artery and vein	-	+

shown in Fig. 9, *d-f*; others not shown). Smooth muscle cells of tunica media were stained relatively strongly for both desmin and vimentin in the aortic arch, but in smooth muscle cells of other vessels desmin labeling was much stronger than vimentin.

Discussion

By means of immunocytochemistry, particularly immunoelectron microscopy of ultra-thin frozen sections of intact tissues, we have examined the endothelial cells of a wide range of vasculatures *in vivo* for the presence of desmin and vimentin in their intermediate filaments. The results of this study are summarized in Table I. Several conclusions can be derived from these results. (a) The intermediate filaments of different endothelia can contain either desmin only, vimentin only, or both together. (b) The endothelium containing desmin occurs in capillaries and small portal veins, while all the endothelial cells of large vessels contain vimentin. (c) The endothelium containing only desmin is present only in large-diameter capillaries, often termed sinusoids. (d) The endothelia that are classified morphologically as the continuous type may have different intermediate filament proteins; e.g., vimentin only in muscle and brain capillaries, and both desmin and vimentin in pancreas capillaries.

There are several reasons why the variation in the subunit composition of intermediate filament proteins in endothelial cells has not heretofore been detected. In the first place, biochemical studies have been carried out only with cultured endothelial cells derived from large vessels (4, 12). Secondly, since prior immunofluorescence studies of tissues used

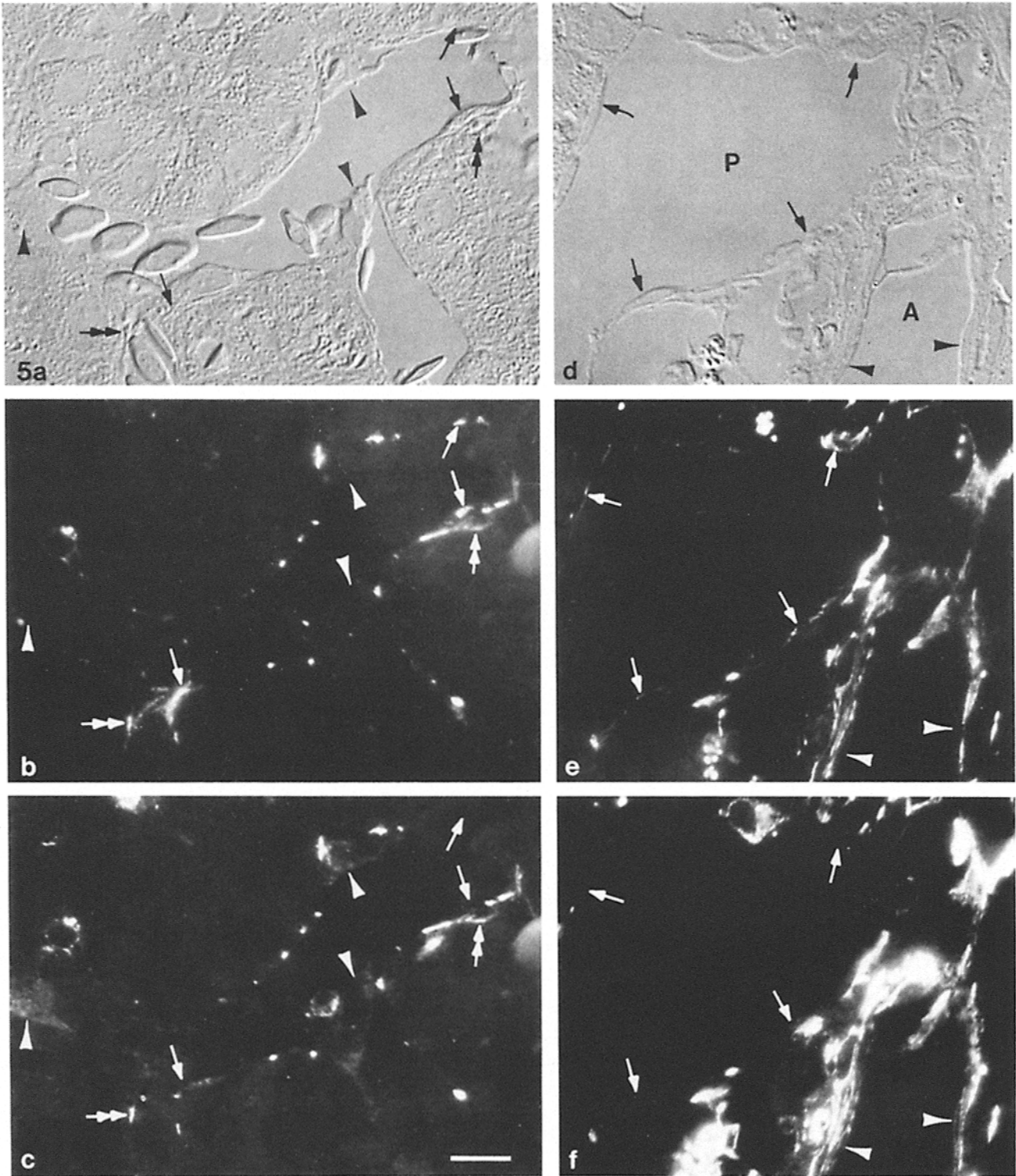


Figure 5. Liver. Nomarski (*a* and *d*) and double immunofluorescence micrographs for desmin (*b* and *e*) and vimentin (*c* and *f*). (*a-c*) Endothelial cells, recognized by the flat cytoplasm, are stained only for desmin (*arrows*), whereas Kupffer cells with the large cytoplasm protruding into the lumen are diffusely stained only for vimentin (*arrowheads*). Many spots that are stained for both desmin and vimentin along the sinusoidal wall are supposed to be in the processes of fat-storing cells (*double arrows*). (*d-f*) A portal area. The endothelium of interlobular hepatic artery (*A*) is stained only for vimentin (*arrowheads*). In contrast, the endothelium of intralobular portal vein (*P*) is stained only for desmin (*arrows*). Bar, 10 μ m.

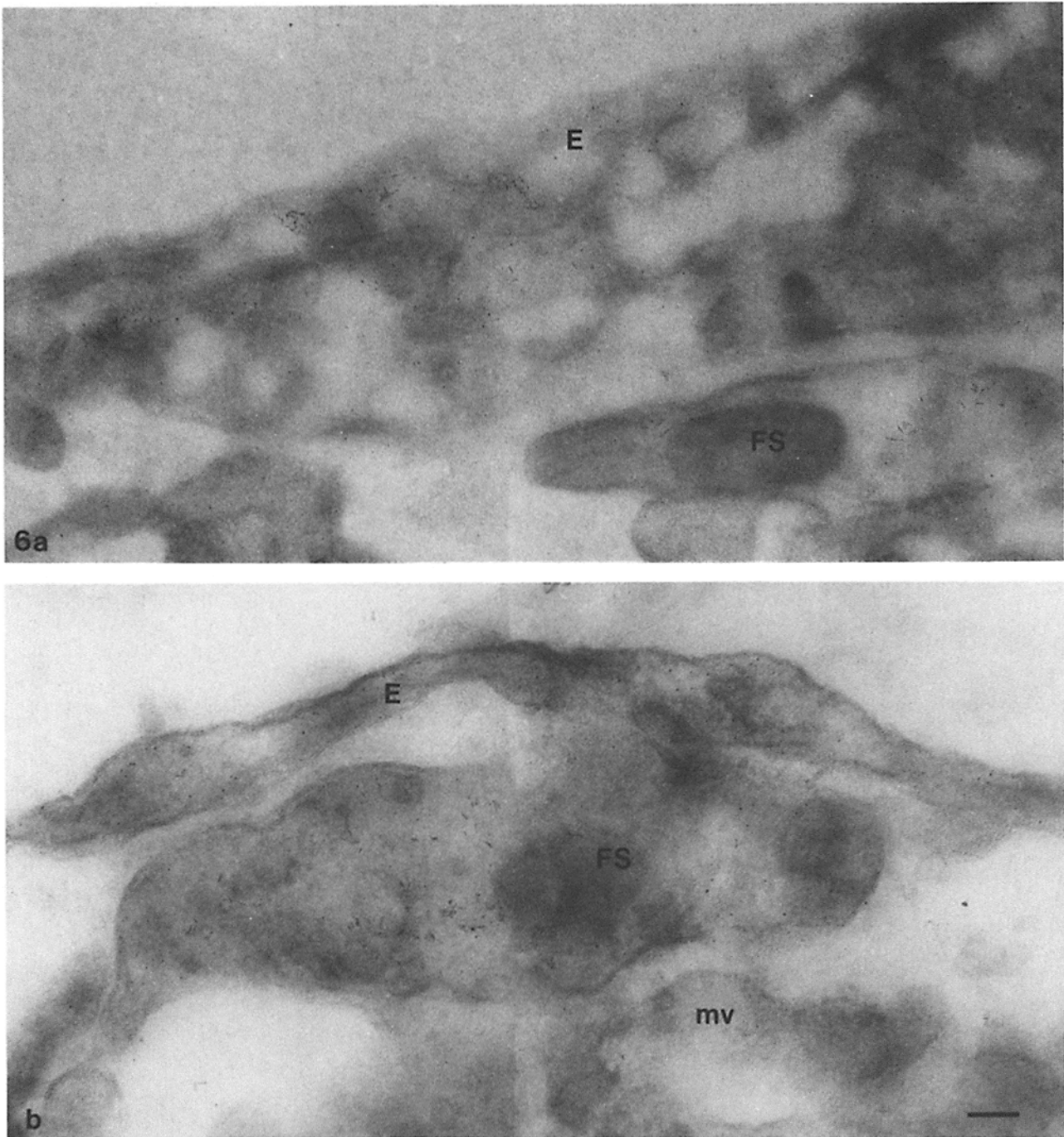


Figure 6. Immunoelectron microscopy of the hepatic sinusoid. Because of the presence of the endogenous ferritin, Imposil is used as a marker for desmin (*a*) and vimentin (*b*). (*a*) Desmin exists both in the endothelium and in the fat-storing cell (*FS*). (*b*) Vimentin is observed in the processes of fat-storing cells (*FS*) in the space of Disse, but not in the endothelium (*E*). *mv*, microvilli of liver parenchymal cells. Bar, 0.1 μm .

cryostat sections of 2 μm or thicker, it was difficult to make a clear distinction between endothelial cells and cells juxtaposed to them (e.g., in liver [26]). Thirdly, the immunofluorescent labeling of pericapillary cells could easily be confused with that in endothelial cells, as the latter labeling was seen only sporadically, especially in capillaries of kidney and liver (Figs. 1 and 5). By using a combination of immunofluorescence microscopy of semi-thin frozen sections (0.5–1.0 μm), and immunoelectron microscopy of ultra-thin frozen

sections (0.05–0.10 μm), we could distinguish with certainty the contributions of desmin and vimentin to the intermediate filaments of endothelial cells. It was important as well that the antidesmin and antivimentin primary antibodies used in this study had been carefully absorbed with the heterologous protein to eliminate any possible cross-reactivity (33).

In the course of these immunocytochemical studies of endothelial cells, the desmin and vimentin contents of a variety of pericapillary cells were also observed. These results will

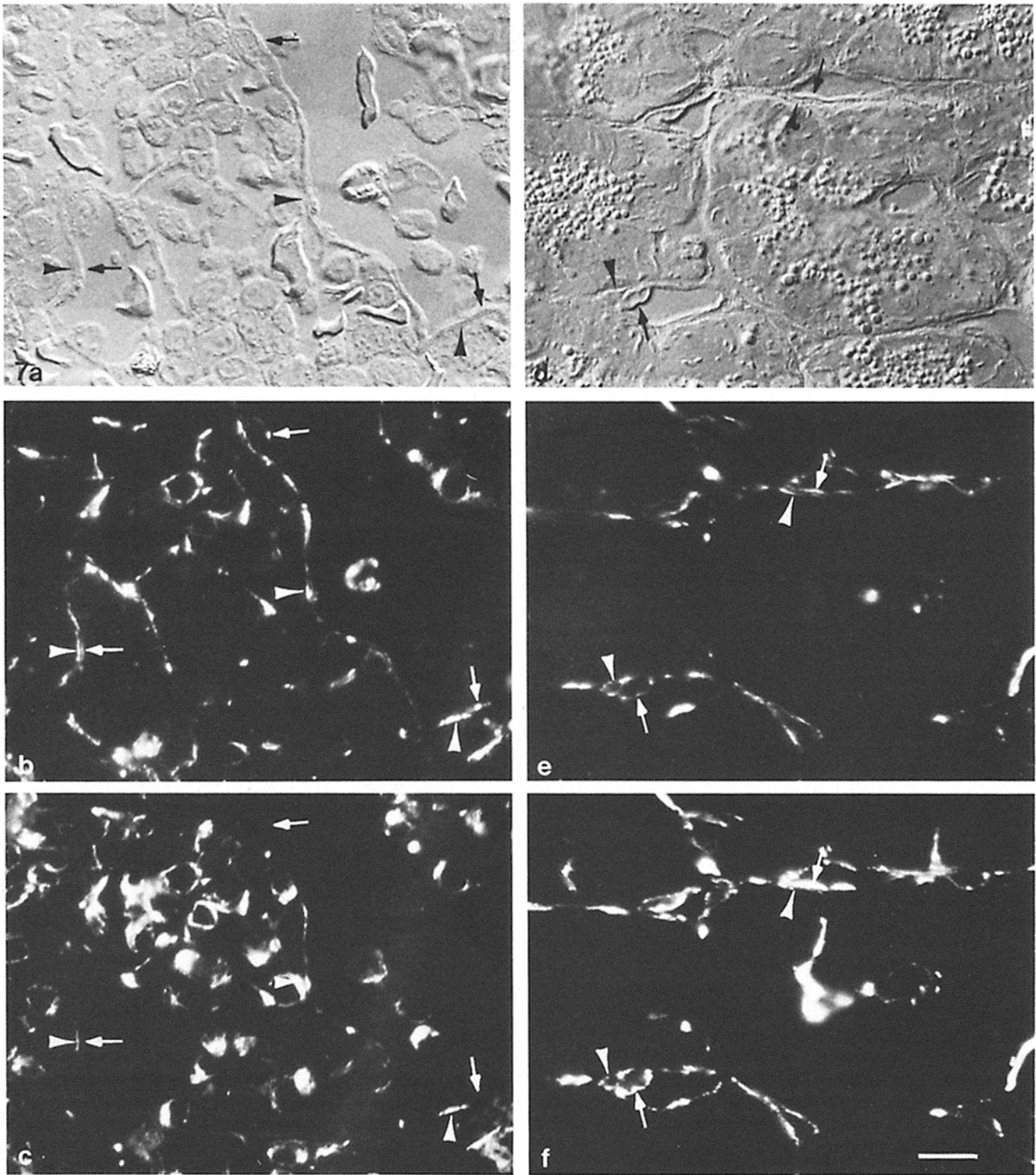


Figure 7. Nomarski (*a* and *d*) and double immunoelectron micrographs for desmin (*b* and *e*) and vimentin (*c* and *f*). (*a-c*) The red pulp of spleen. The littoral cells of splenic sinusoid are labeled only for desmin (*arrows*), but perisinusoidal cells exterior to them in the sinusoidal wall are stained for both desmin and vimentin (*arrowheads*). (*d-f*) Exocrine portion of pancreas. Endothelial cells (*arrows*) as well as pericytes (*arrowheads*) are labeled for both desmin and vimentin. Bar, 10 μ m.

be reported separately (Fujimoto, T., and S. J. Singer, manuscript in preparation).

What is the significance of the fact that some endothelial cells have desmin instead of, or along with, vimentin as a major component of intermediate filaments? Expression of

intermediate filament proteins has been thought to be a cell- and tissue-specific phenomenon (13, 21). In general, the expression of desmin has been considered restricted to mature muscle cells, and endothelial cells have not been regarded as related to muscle. However, our results suggest that some en-

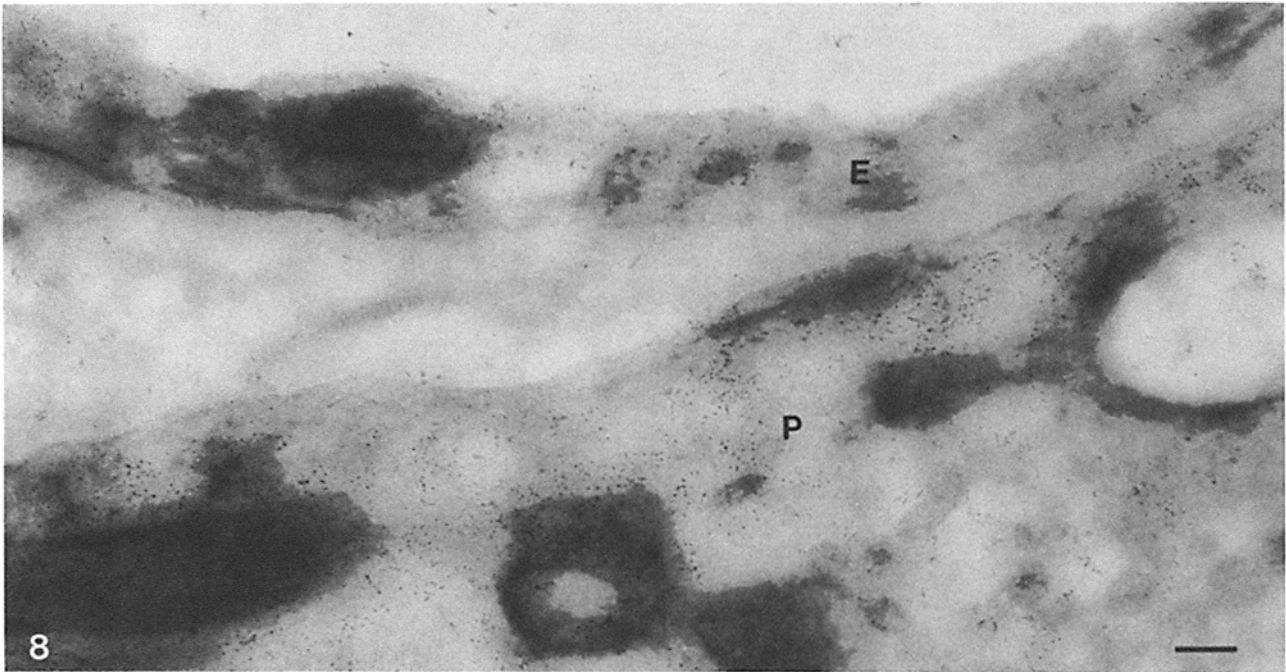


Figure 8. Immunoelectron microscopy of the exocrine pancreas. Desmin (ferritin) and vimentin (Imposil) are doubly labeled. Both the endothelium (*E*) and the pericyte (*P*) have both proteins. Bar, 0.1 μ m.

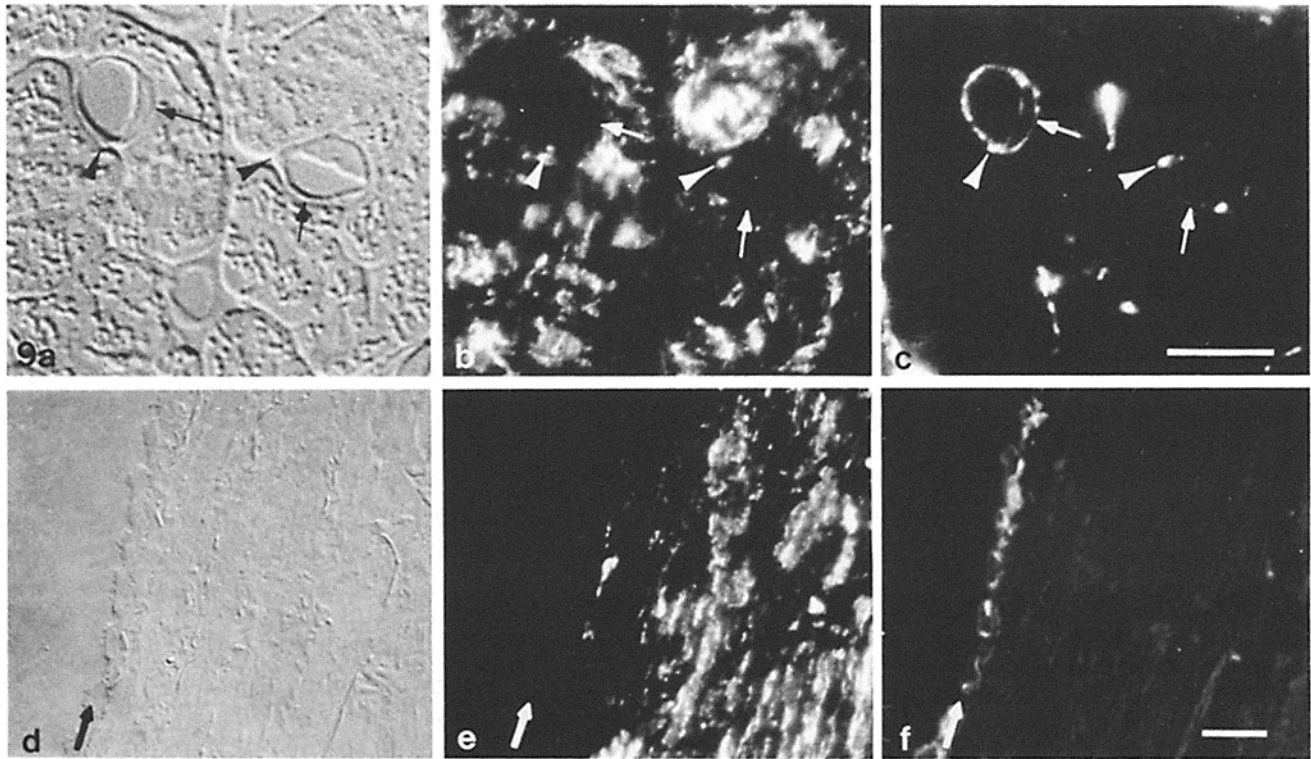


Figure 9. Nomarski (*a* and *d*) and double immunofluorescence micrographs for desmin (*b* and *e*) and vimentin (*c* and *f*). (*a-c*) A transverse section of cardiac muscle. The endothelial cells of capillaries (*arrows*) are stained only for vimentin. Some spots (*arrowheads*) exterior to the endothelium are doubly stained for both desmin and vimentin, and probably belong to the pericyte. Note that the cardiac muscle cells are only positive for desmin and not for vimentin. Bar, 10 μ m. (*d-f*) Femoral artery. The endothelium (*arrow*) is labeled only for vimentin. The smooth muscle cell in tunica media are stained brightly for desmin and only weakly but definitely for vimentin. Bar, 10 μ m.

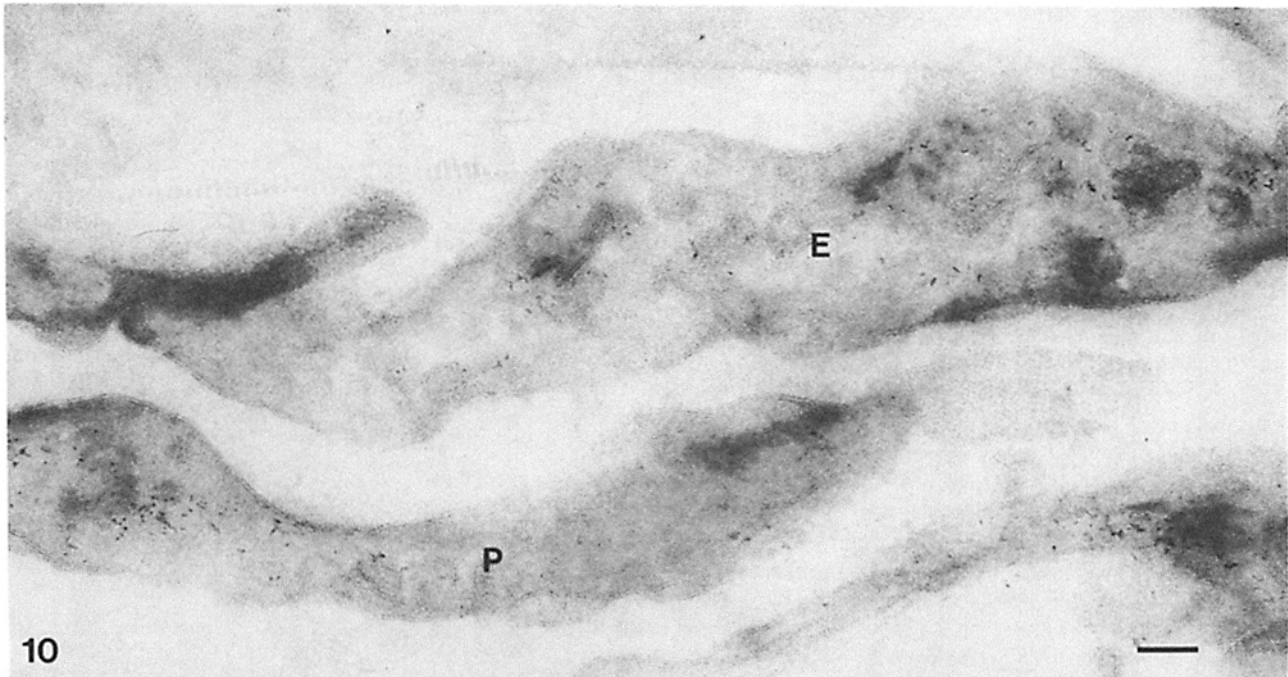


Figure 10. Double immunoelectron microscopy of a capillary in the cardiac muscle. The endothelium (*E*) is labeled only with Imposil (vimentin), but the pericyte (*P*) is labeled with both ferritin (desmin) and Imposil. Bar, 0.1 μm .

endothelial cells have something in common with muscle cells, either in their embryological origin or in their function, or else that the association of desmin with muscle cells is not as specific as had been thought.

There are two different theories about the embryological origin of endothelial cells (3, 34). One is the angioblast theory; it assumes that all endothelial cells arise by the growth and extension of the pre-existing endothelium in the yolk sac. The other theory assumes that endothelial cells can arise by differentiation of local mesenchymal cells. The latter theory is generally favored at present (3, 34, but see 36). As evidence in its favor, for example, the capillary endothelial cells of some organs are reported to express organ-specific antigens on their cell surface (2, 3). This phenomenon is thought to reflect the diverse embryological origin of endothelial cells in different organs. The variety of intermediate filament proteins expressed in the endothelium might then also reflect a different embryological origin of some endothelial cells as compared with others.

The findings with renal capillaries are intriguing in regard to this latter suggestion. As remarked briefly in Results, the avian kidney has reptilian-type nephrons in the cortex and mammalian-type nephrons in the medulla (7, 28). It is peculiar that while the vasa recta interposed among mammalian-type nephrons are supplied with blood only from efferent glomerular arterioles, peritubular capillaries around reptilian-type nephrons receive blood not only from efferent glomerular arterioles but from a renal portal system (1, 29) that does not exist in the adult mammalian kidney. Our present results showed that the endothelium of cortical peritubular capillaries and that of medullary vasa recta have different intermediate filament proteins, desmin and vimentin, respectively. It therefore seems likely that the occurrence of desmin in the kidney endothelium is closely related to the development of the portal system.

This idea is further supported by finding desmin in the hepatic sinusoidal endothelium, which receives blood from its own portal system. In fact, endothelial cells of the hepatic sinusoid are known to have several peculiar characteristics (e.g., absence of the following components associated with large vessel endothelia: Factor VIII (16, 18), Ulex europaeus I receptor (16), basement membrane collagen type V, and the Weibel-Palade body (18) in rat and/or human liver), and hence are suspected to be of different origin from other endothelial cells (16). Also true of both the renal and hepatic portal systems is that desmin is limited to the endothelium of the capillaries and small portal veins within the organ and is not found in veins of the portal system exterior to the organ (i.e., the cranial renal portal vein of kidney and the mesenteric vein of liver). These observations suggest that the endothelium of the capillaries and the small veins of the portal system develop differently from the rest of the vasculature.

We may consider whether desmin intermediate filaments have any special function as compared with vimentin intermediate filaments in the endothelium. At this time, functional differences between desmin and vimentin intermediate filaments have not been established. However, all the cells that have previously been reported to express desmin are cells specialized for contractility. It is conceivable that desmin is required for the efficient and coordinated function of the actomyosin system in specialized contractile cells. The presence of desmin in littoral cells of the splenic sinusoid is interesting in this respect, because bundles of thin filaments running parallel to the cell's longitudinal axis and containing dense bodies (8) are assumed to be in a sarcomeric arrangement (10, 35). Isolated littoral cells were shown to contract *in vitro* in response to Mg-ATP (35). These results would seem to be consistent with the idea that desmin is expressed in those cells in which the actomyosin system is structured for a contractile function. However, there are similar bundles

of thin filaments in endothelial cells of arteries and arterioles (14, 24, 37) that contain vimentin and not desmin, and in vitro contractility has been demonstrated with such cells (9). At this time, therefore, we cannot say with certainty which of the two consequences of our findings is correct; that (a) endothelial cells containing desmin may have special contractile activity; or (b) the presence of desmin in the intermediate filaments is not of itself an indicator of contractile function for a cell.

It is not known whether mammalian endothelial cells show a diversity in intermediate filament proteins similar to what we have found in chicken. Regarding the splenic sinusoidal endothelium, however, it should be noted that labeling with antivimentin has been reported in human spleen (10, 15), although the overall structure of the endothelial cell looks similar to that of the chicken. Because the possible presence of desmin was not examined, it is not ruled out that the splenic endothelium in mammals contains desmin, and therefore exhibits some differences from other mammalian endothelial cells. The expression of desmin and vimentin in the endothelial cells of mammalian tissues should be investigated.

It is axiomatic that every capillary is uniquely constructed to meet the demands of organs and tissues (20, 30, 34). Our present study shows that even the composition of such a basic structure as intermediate filaments is variable among endothelial cells. It is possible that other cytoskeletal proteins, as well as membrane proteins associated with the cytoskeleton, are expressed and organized differently in different endothelial cells. In subsequent papers of this series we will examine these questions.

We wish to thank Mrs. Margie Adams and Mr. Michael McCaffery for their excellent technical assistance. We also thank Dr. Kiyoteru Tokuyasu and Dr. Sidney S. Sobin for their invaluable advice and critical reading of the manuscript.

These studies were supported by a grant from the Weingart Foundation, and by U.S. Public Health Service grant GM-15971 to S. J. Singer, who is an American Cancer Society Research Professor.

Received for publication 21 May 1986, and in revised form 3 July 1986.

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