

## CROSS-STRIATED ARRAYS OF FILAMENTS IN ENDOTHELIUM

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Cytoplasmic filaments measuring approximately 60–70 Å in diameter have frequently been observed in endothelial cells (2, 4, 7, 8, 16, 22–24, 27). These filaments are either dispersed throughout the cytoplasm or aggregated in bundles. A contractile function has been ascribed to these filaments (4, 7, 8, 22, 23, 27), and there is some evidence that endothelial cells can contract (12, 15).

The present report describes a type of cross-striated array of filaments in the cytoplasm of endothelial cells of cerebral cortical small arteries and arterioles from hypertensive rats. It is suggested that these structures represent contractile elements. The relationship of endothelial contractility to the increased permeability of the endothelial cell junctions of these small arterial vessels is discussed.

### MATERIALS AND METHODS

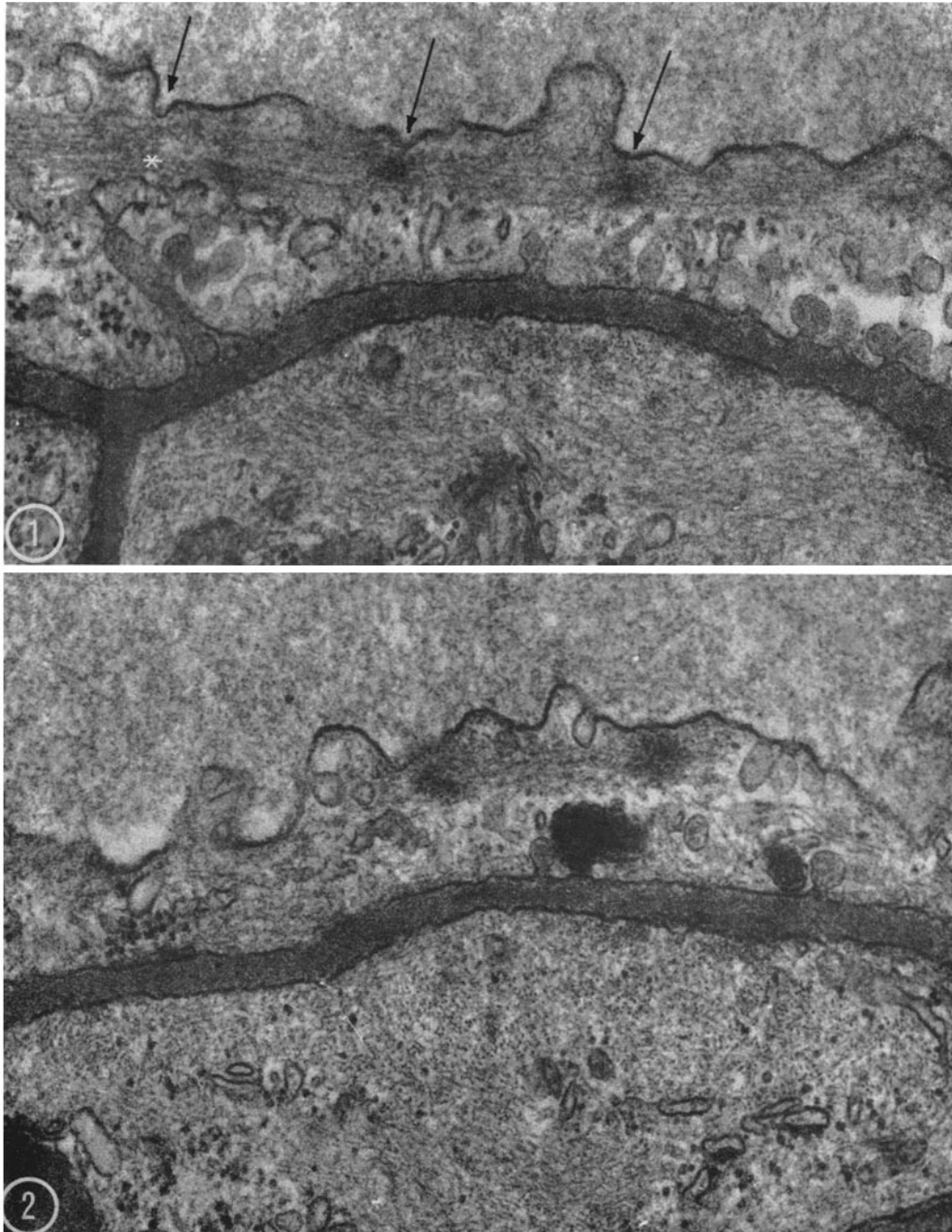
Male Columbia Sherman rats with experimental renal hypertension (26) of varying duration and control animals were utilized in these experiments. Some of the hypertensive and control animals were injected intravenously with colloidal carbon (Pelikan C11/1431a, Gunther Wagner Co., Hanover, Germany) (14) and/or horseradish peroxidase Type II (Sigma Chemical Co., St. Louis, Missouri) (20) for assessment of vascular permeability. The animals were sacrificed, and portions of the cerebral cortex were fixed at room temperature in formaldehyde-glutaraldehyde (6). Tissue from animals injected with horseradish peroxidase was incubated for 15 min in a Tris buffer (pH 7.6) containing 3-3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide (20) to yield a final product detectable by electron microscopy. The tissues from all animals were dehydrated with acetone and embedded in Araldite. Thin sections were then cut, stained with lead citrate (21) or uranyl acetate and lead citrate, and examined in a Philips EM 200 electron microscope.

### OBSERVATIONS AND DISCUSSION

Cross-striated bundles of filaments are found within the cytoplasm of endothelial cells of small cerebral cortical arteries and arterioles from hypertensive animals (Figs. 1–4). Seven endothelial cells, comprising approximately 5% of the endothelial cells examined, contain these bundles of

filaments. Such structures are not found in a comparable number of endothelial cells from control animals. The filaments are arrayed parallel to the long axis of the vessels and are always located at the luminal borders of the endothelial cells. The individual filaments measure approximately 60–70 Å in diameter and are of an indeterminate length. Electron-opaque bands of material measuring up to 1,000 Å in width are distributed along the long axis of the filament bundles. The dense bands frequently appear contiguous with the inner surfaces of the endothelial cell membranes (Figs. 1–3). They vary from one to seven in number and have a regular spacing of approximately 0.5  $\mu$ .

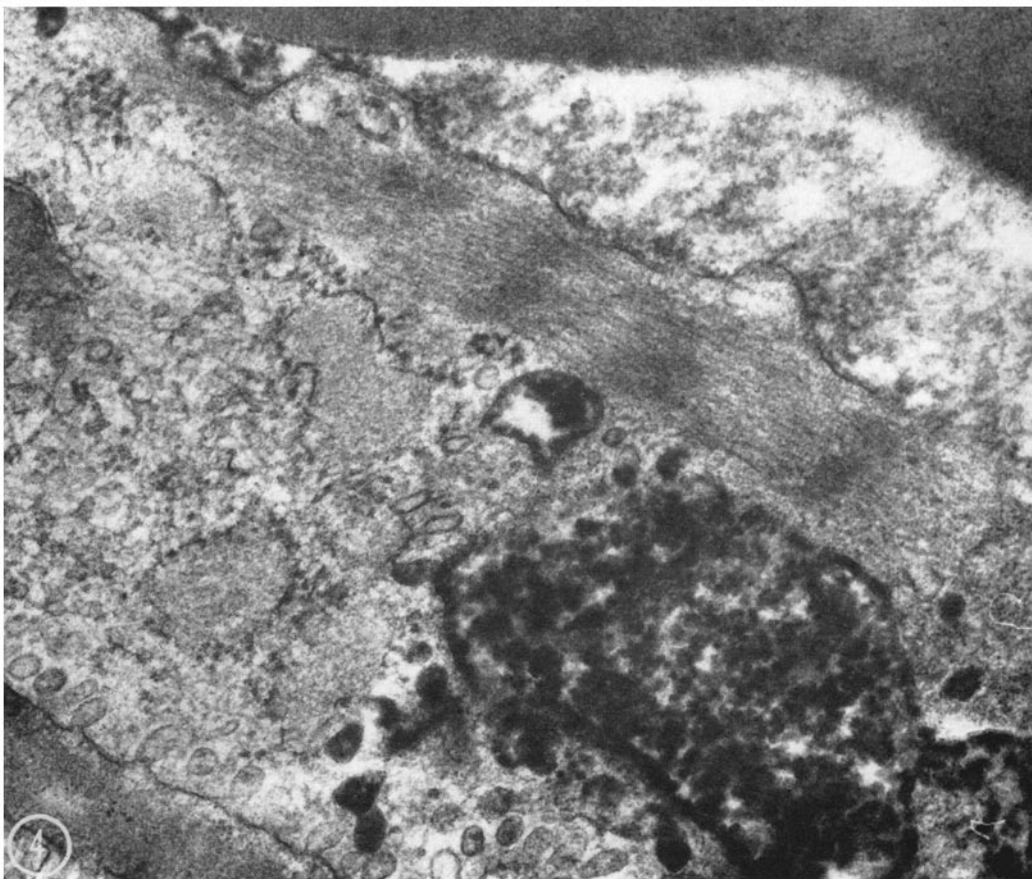
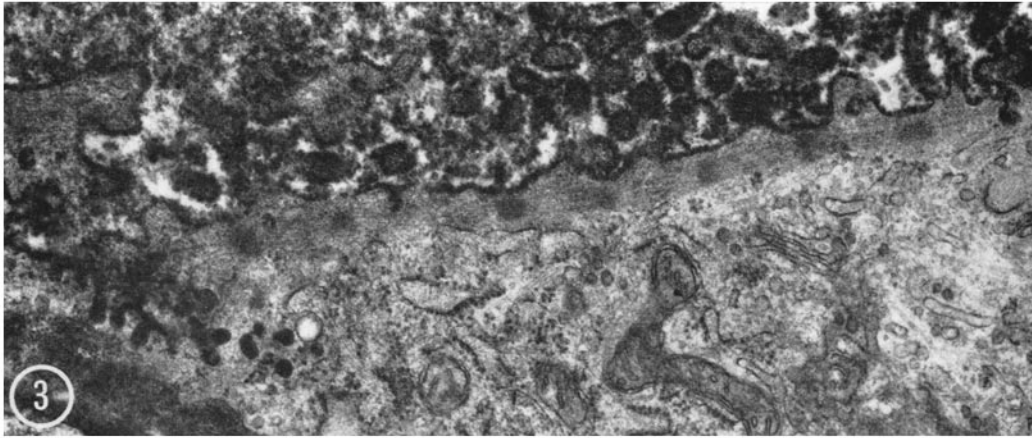
The cross-striated arrays of filaments are reminiscent of skeletal and cardiac muscle. The electron-opaque bands resemble the Z lines of striated muscle, the dense bodies of smooth muscle, and the dense zones of the contractile fibrils of myoepithelial cells (3). The resemblance to muscle is further supported by the fact that the diameter of the filaments (60–70 Å) is similar to that of actin (9). The absence of thicker filaments in endothelium could be related to the preparative techniques utilized. It is possible that myosin filaments would be revealed by the use of special methods that have been applied to smooth muscle (10, 19) and renal (18) cells, as well as by transverse sections. Another explanation for the presence of only thin filaments could reside in the functional state of the endothelium at time of fixation, since it has recently been shown that relaxed smooth muscle contains solely actin filaments (11). The possibility of actin-myosin interaction is not precluded, however, by the absence of myosin-like filaments, for there is some evidence that myosin in living smooth muscle may exist in a relatively disaggregated form that is still able to promote the sliding of interdigitated actin filaments (17). In this regard, actomyosin that is antigenically similar to that of uterine smooth muscle has recently been demonstrated in vascular endothelium by the fluorescent antibody technique (1). The absence of A and I bands could be due to the contraction of the endothelium. However, sarcomere lengths less than 1.2  $\mu$  are never seen in contracted striated muscle.



FIGURES 1-4 are electron micrographs of arterial vessels from the cerebral cortex of hypertensive animals. The arterioles in Figs. 3 and 4 are from animals injected intravenously with horseradish peroxidase.

FIGURE 1 Bundles of filaments are present at the luminal border of this endothelial cell. These filaments are oriented parallel to the longitudinal axis of the cell. Three electron-opaque bands of material (arrows), one of which is partially out of the plane of the section (\*), are distributed along the long axis of the filament bundles at a regular spacing of about  $0.5 \mu$ . The diameter of the filaments (60-70 A) is comparable with that of the filaments in the subjacent smooth muscle cell. Stained with uranyl acetate and lead citrate.  $\times 59,000$ .

FIGURE 2 A few filaments at the luminal border of this endothelial cell are delimited by electron-opaque bands of material. Stained with uranyl acetate and lead citrate.  $\times 59,000$ .



**FIGURE 3** Note the myofibrillar appearance of the cross-striated filament aggregates in the luminal portion of this endothelial cell. Seven electron-opaque bands are readily discernible. Several of the dense bands appear to be contiguous with the inner surface of the endothelial cell plasma membrane, and the extreme left band is closely associated with the intercellular junction. Peroxidase is seen within the lumen and apposed to the luminal aspect of the endothelial cell surface membrane. Stained with lead citrate.  $\times 29,000$ .

**FIGURE 4** Sarcomere-like arrays of filaments are present in this obliquely sectioned endothelial cell. The filament bundles are wider than those depicted previously. The peroxidase lies within an infolding of the endothelium as well as in the lumen of the vessel. Stained with lead citrate.  $\times 56,000$ .

The sarcomere-like arrays of filaments, which could represent the structural basis for endothelial contraction, are seen solely in segments of small arteries and arterioles in which endothelial cell junctions exhibit increased permeability, as evidenced by the penetration of exogenous peroxidase (5). The ultrastructure of endothelial cell junctions is particularly significant in pathological situations characterized by an increase in vascular permeability (13, 25). If the observed filaments have contractile properties, they may function to increase the tension across endothelial cell junctions. Their longitudinal orientation at the luminal borders of the cells at approximately the same level as the zonulae occludentes is consistent with such a function. The forces of an increased hydrostatic pressure within the lumina of these vessels and a longitudinal contraction of the endothelial cells would both tend to separate the membranes of these tight junctions, perhaps, as the evidence suggests (5), to an extent that permits the passage of small protein molecules (exogenous peroxidase).

Endothelial contractility has previously been proposed as the mechanism underlying the increase in vascular permeability and the separation of endothelial cell junctions that follow the administration of histamine and serotonin (12, 15). This suggestion was based upon the observations that the endothelial cells lining venules frequently bulged into the lumen, had numerous nuclear indentations or "pinches," and rarely exhibited subnuclear arrays of filaments with electron-opaque patches. These patches had a spacing that was approximately similar to that described in the present report. Cross-striated arrays of filaments have recently been described in the endothelial cells of arterioles in rat myometrium (23). In contrast to the present report, however, these filaments were found in the basal portions of the endothelial cells where their contraction might reasonably produce distortion of endothelial cells, causing them to bulge into the vascular lumina and to narrow these channels. No protrusion of endothelial cells into the lumina of arteries and arterioles from hypertensive animals was observed.

Since contractile properties are presently being ascribed to cells other than muscle cells, the suggestion that the cross-striated arrays of filaments in endothelium represent contractile elements seems reasonable. If this is indeed the case, the designation of endothelial cells as myoendothelial cells would appear appropriate.

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#### REFERENCES

1. BECKER, C. G., and G. E. MURPHY. 1969. Demonstration of contractile protein in endothelium and cells of the heart valves, endocardium, intima, arteriosclerotic plaques, and Aschoff bodies of rheumatic heart disease. *Amer. J. Pathol.* 55:1.
2. BENSCH, K. G., G. B. GORDON, and L. MILLER. 1964. Fibrillar structures resembling leiomyofibrils in endothelial cells of mammalian pulmonary blood vessels. *Z. Zellforsch. Mikrosk. Anat.* 63:759.
3. ELLIS, R. A. 1965. Fine structure of the myoepithelium of the eccrine sweat glands of man. *J. Cell Biol.* 27:551.
4. FAWCETT, D. W. 1959. The fine structure of capillaries, arterioles and small arteries. In *The Microcirculation*. S. R. M. Reynolds and B. Zweifach, editors. University of Illinois Press, Urbana. 1.
5. GIANCOMELLI, F., J. WIENER, and D. SPIRO. 1969. Ultrastructure and permeability of cerebral vessels in experimental hypertension. *Amer. J. Pathol.* 55:33a. (Abstr.)
6. GRAHAM, R. C., and M. J. KARNOVSKY. 1966. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *J. Histochem. Cytochem.* 14:291.
7. HAMA, K. 1961. On the existence of filamentous structures in endothelial cells of the amphibian capillary. *Anat. Rec.* 139:437.
8. HAN, S. S., and J. K. AVERY. 1963. The ultrastructure of capillaries and arterioles of the hamster dental pulp. *Anat. Rec.* 145:549.
9. HANSON, J., and J. LOWY. 1963. The structure of F-actin and of actin filaments isolated from muscle. *J. Mol. Biol.* 6:46.
10. KELLY, R. E., and R. V. RICE. 1968. Localization of myosin filaments in smooth muscle. *J. Cell Biol.* 37:105.
11. KELLY, R. E., and R. V. RICE. 1969. Ultrastructural studies on the contractile mechanism of smooth muscle. *J. Cell Biol.* 42:633.
12. MAJNO, G., and M. LEVENTHAL. 1967. Pathogenesis of histamine-type vascular leakage. *Lancet.* 2:99.
13. MAJNO, G., and G. E. PALADE. 1961. Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an elec-

- tron microscopic study. *J. Biophys. Biochem. Cytol.* 11:571.
14. MAJNO, G., G. E. PALADE, and G. I. SCHOEFL. 1961. Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: A topographic study. *J. Biophys. Biochem. Cytol.* 11:607.
  15. MAJNO, G., S. M. SHEA, and M. LEVENTHAL. 1969. Endothelial contraction induced by histamine-type mediators. An electron microscope study. *J. Cell Biol.* 42:647.
  16. PALADE, G. E. 1953. Fine structure of blood capillaries. *J. Appl. Phys.* 24:1424.
  17. PANNER, B. J., and C. R. HONIG. 1967. Filament ultrastructure and organization in vertebrate smooth muscle. Contraction hypothesis based on localization of actin and myosin. *J. Cell Biol.* 35:303.
  18. PEASE, D. 1968. Myoid features of renal corpuscles and tubules. *J. Ultrastruct. Res.* 23:304.
  19. PEASE, D. 1968. Structural features of unfixed mammalian smooth and striated muscle prepared by glycol dehydration. *J. Ultrastruct. Res.* 23:280.
  20. REESE, T. S., and M. J. KARNOVSKY. 1967. Fine structural localization of a blood-brain barrier to exogenous peroxidase. *J. Cell Biol.* 34:207.
  21. REYNOLDS, E. S. 1963. The use of lead citrate as an electron-opaque stain in electron microscopy. *J. Cell Biol.* 17:208.
  22. RHODIN, J. A. G. 1962. Fine structure of vascular walls in mammals. *Physiol. Rev.* 42 (Suppl. 5):48.
  23. RÖHLICH, P., and I. OLÁH. 1967. Cross-striated fibrils in the endothelium of the rat myometrial arterioles. *J. Ultrastruct. Res.* 18:667.
  24. WEISS, L. 1962. The structure of fine splenic arterial vessels in relation to hemoconcentration and red cell destruction. *Amer. J. Anat.* 111:131.
  25. WIENER, J., R. G. LATTES, B. G. MELTZER, and D. SPIRO. 1969. The cellular pathology of experimental hypertension. IV. Evidence for increased vascular permeability. *Amer. J. Pathol.* 54:187.
  26. WILSON, C., and F. B. BYROM. 1939. Renal changes in malignant hypertension; experimental evidence. *Lancet.* 1:136.
  27. ZWILLENBERG, L. O., and H. H. L. ZWILLENBERG. 1963. Zur struktur und funktion der hülsencapillaren in der milz. *Z. Zellforsch.* 59:908.