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Three-dimensional Analysis of Permeability Pathways across Capillary Endothelia

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Continuous capillaries have very high hydraulic conductivity and provide a partially selective diffusion barrier between the blood and the interstitium. Electron-dense tracers, which are used intravitally to test the conductivity of capillary permeability pathways, are found in the interstitial compartments. It is uncertain, however, whether the tracers have crossed the capillary wall through interendothelial junctions or via the vesicular membranous compartments from two-dimensional electron micrographs. Previous studies (Chen and Wagner 1990, Wagner and Chen 1991) indicate that electron-dense patches of terbium precipitate were often found in regions bounded by pericytes and the abluminal capillary surfaces in the rete mirabile. To answer this question, i.e., whether vesicular pathways are the source of interstitial tracer deposits, three-dimensional information is needed. A reconstruction image from continuous series of thin sections provides a three dimensional map of the areas of dense tracer deposits and any associated pathways via interendothelial junctions or the vesicular membranous compartments across the endothelium.

The rete capillaries were administrated with tracer, fixatives and embedded in British araldite resin as previous reports (Chen and Wagner 1990, Wagner and Chen 1990, Wagner and Chen, 1991). Ribbons of serial thin sections were picked up on slotted grids coated with Formvar film and observed with a Zeiss CEM 902 transmission electron microscope. Sections in the mid-range of a series were examined first. If a patch or dense terbium deposit between a pericyte and endothelial cell was detected, the adjacent sections were examined to determine the distribution of the tracer toward opposite ends of the series. High magnification micrographs (X 30,000) of these regions in all adjacent sections of the series were taken. The negatives of series were sequentially image-registered by Image-I analysis software. Then the registered and digitalized images were exported to the Silicon graphics IRIS workstation. The three dimensional volume visualization system, voxelview, was used to render exterior surfaces and interior structures in sections. The three-dimensional projections of the vesicular system or interend othelial junctions may reveal the permeability pathways across the capillary wall.

When the interstitial plaques were traced through serial sections, the amount of terbium deposits and the plaque areas within mid-series decreased continually to sparse deposits at both ends of the series. In two series, fused vesicular profiles filled with terbium seem to form a continuous channel from the lumen to the interstitium. The three-dimensional reconstruction of membranous compartments showed the continuity of totally electron-dense terbium within a trans-endothelial channel of fused vesicles with the dense interstitial patch deposits. The results indicate that the endothelial vesicular system has provided this transcellular pathway. None of four inter-endothelial clefts formed a paracellular pathway for terbium (Figure 1). Previously, analyses of ultrathin serial sections had not revealed any communications of bound compartments across the endothelial cell (Bundgaard et al. 1979, Frokjaer-Jensen, 1980, Frokjaer-Jensen et al. 1988). The data indicates that communications of membrane compartments might occur by formation of transendothelial channels of fused vesicles or by connection of two close racemose structures on opposite membranes of endothelium.

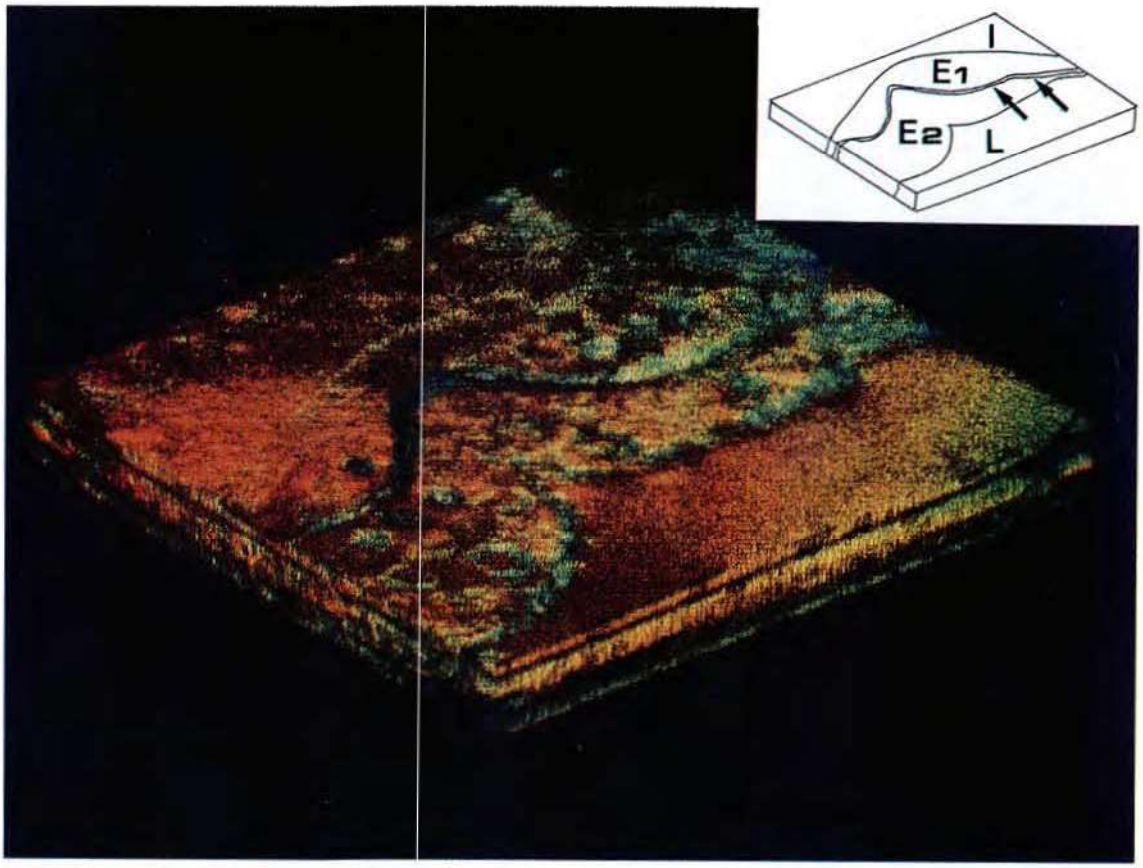

Fig. 1. The three-dimensional projections of the interendothelial junction after reconstruction of eleven sections. The in-terendothelial clefts and adjacent vesicular profiles are filled dense depositions of terbium indicated by blue color. E1 and E2: endothelium, I; interstitium, L: capillary lumen. Arrow: interendothelial junctions between endothelia.

REFERENCES

Bundgaard M, J. Frokjaer-Jensen, C Crone. 1979. Endothelial plasmalemmal vesicles as elements in a system of branching invaginations from the cell surface. Proc. Natl. Acad. Sci. USA. **76:** 6439-6442.

Chen, S-C., RC Wagner. 1990. Improved detectability of capillary permeability tracers by electron spectroscopic imaging. Microvasc. Res. **40:** 439-442.

Frokjaer-Jensen J. 1983. The plasmalemmal vesicular system in capillary endothelium. Conventional electron microscopic (EM) thin sections compared with the picture arising from ultrathin (≈ 140 Å) serial sectioning. Prog. Appl. Microcirc. **1:** 17-34.

Frokjaer-Jensen J, RC Wagner, SB Andrews, P Hagman, TS Reese. 1988. Three-dimensional organization of the plasmalemmal vesicular system in directly frozen capillaries of the rete mirabile in the swim bladder of the eel. Cell Tissue Res. **254:** 17-24.

Wagner, R.C., S-C Chen. 1990. Ultrastructural distribution of terbium across capillary endothelium: detection by electron spectroscopic imaging and electron energy loss spectroscopy. J. Histochem. Cytochem. **38:** 273-282.

Wagner, R.C., S-C Chen. 1991. Transcapillary transport of solute by the endothelial vesicular system: evidence from thin serial section analysis. Microvasc. Res. **42:**139-150.