





Search for

Within ☒ This book ☐ All books ☐ PubMed

NCBI » Bookshelf » Madame Curie Bioscience Database » Channels and Transporters » Channels across Endothelial Cells

Channels across Endothelial Cells

Radu V Stan*

* Corresponding Author: Dartmouth Medical School, Angiogenesis Research Center, Department of Pathology, HB 7600, Borwell 502W, 1 Medical Center Drive, Hanover, NH 92093-0651, Email: Radu.V.Stan@Dartmouth.edu

The evolution of multicellular organisms entailed the formation of biological compartments separated by epithelial cellular barriers. As a consequence, strategies have evolved to move/ exchange materials between these compartments for nutrition, wastes removal and information exchange, while still maintaining their identity. These exchanges occur either across barrier cells (transcellular) or in between adjacent cells (paracellular). Among the strategies employed for transcellular exchange are the formation of physical patent channels/pores that cut through the cells and the formation of “functional channels” by shuttling vesicles during transcytosis. In this chapter, I will summarize the knowledge of the transcellular exchange systems using the vascular endothelium as a barrier model. We will focus on the components, structure and function of the endothelial organelles such as fenestrae, vesiculo-vacuolar organelles and transendothelial channels as well as the vesicular carriers involved in transcytosis (i.e., plasmalemmal vesicles or caveolae), which form either physical or functional channels across endothelial cells.

Table of Contents

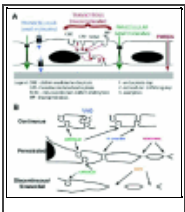
In this Page

- [Introduction](#)
- [Vascular Permeability—Pathways of Transendothelial Exchange](#)
- [Pores across Endothelium](#)
- [Conclusions](#)
- [References](#)

Introduction

A critical step in the evolution of multicellular organisms (metazoans and higher) was the establishment of different biological compartments to carry out specific functions. This entailed the development of epithelial cellular barriers between different environments as well as the development of strategies to selectively move molecules between compartments (i.e., for delivery of nutrients, elimination of wastes as well as ensuring molecular communication between distant cells and tissues) while maintaining their distinct composition.

Madame Curie Bioscience Database
2000-



situated either across (i.e., transcellular) or in between (i.e., paracellular) the cells forming the barrier. While the **paracellular transport** occurs via the intercellular junctions, there are at least three **transcellular** routes described so far: (a) water and small molecules are moved via **membrane transporters** that have a different distribution/orientation on opposite fronts of a barrier cell; (b) **transcytosis**, defined as

Figure 1

A) Schematic of the pathways taken by molecules across (more...)

the transport of macromolecular cargo from one front of a polarized cell to the other within membrane-bounded carrier(s), and (c) **pores or channels** which are patent openings through the barrier cells with or without selective permeability for different molecules. Together, these processes ([Fig. 1A](#))

contribute to the success of multicellular organisms (reviewed in refs. [1, 2](#)).

The transcellular exchange systems have achieved a high level of complexity and sophistication in mammals. Among different types of epithelia, the vascular endothelium has the most diversified repertoire of specific structures that might carry out these exchanges. During decades of research on the topic, several endothelial subcellular structures that might participate in this function have been identified, such as caveolae, transendothelial channels (TEC), vesiculo-vacuolar organelles (VVO), fenestrae and intercellular junctions. We will visit the endothelial structures involved in mediating the exchanges of water and solutes between the blood plasma and the interstitial fluid/tissues across endothelial cells.

Vascular Permeability—Pathways of Transendothelial Exchange

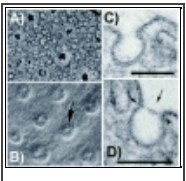


Figure 3

Transmission electron micrographs of endothelial caveolae (more...)

Vascular endothelium is a highly differentiated cellular monolayer with the organization of a simple squamous epithelium that lines the entire cardiovascular system. It constitutes a quasi-ubiquitous presence in organs and tissues throughout the body forming one of the largest vertebrate “organs” ([3](#)). Endothelia have been classically defined into three main structural types, depending on their content of specific structures: the continuous, fenestrated and discontinuous endothelium ([Fig. 1B](#), reviewed in ref. [1](#)). The **continuous** endothelium forms an uninterrupted barrier between the blood and tissues. It features a large population of caveolae or plasmalemmal vesicles,^{[4,5](#)} extremely few TEC^{[6](#)} and virtually no fenestrae. In addition to relatively much fewer caveolae ([Fig. 3](#)), the **fenestrated** endothelium features pores such as fenestrae ([Fig. 4](#)), transendothelial channels ([Fig. 4](#)) and endothelial pockets.^{[3,7-11](#)} **Discontinuous or sinusoidal** endothelium lines the sinusoids in the liver and bone marrow. It has extremely few caveolae and sinusoidal gaps and is characterized by the presence of pores called fenestrae or gaps, which are much larger and heterogeneous in size than the fenestrae of the fenestrated endothelium (reviewed in ref. [12](#)).

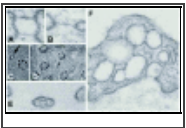


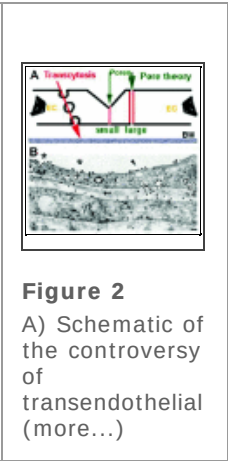
Figure 4

Pores across endothelial cells. Electron micrographs (more...)

Endothelium performs important functions in the physiology of vertebrates.^{[1,13-16](#)} Its primary function is the control of transendothelial exchanges of water and solutes (both small and large molecules) between the blood plasma and the underlying tissues, also known as microvascular permeability. By this endothelium has a critical role in the delivery of nutrients to tissues and clearing of wastes, as well as in the functional integration at organ and organism level allowing the information exchange between tissues situated at distance one from another (i.e., hormones,

etc.). The dominant character of transendothelial transport is clearly expressed in the average thickness of endothelium of less than 0.3 μm as well as at the expression of specific structures that mediate this function.

Although endothelium is a highly dynamic tissue, it is generally recognized that it mediates a **“basal” microvascular permeability**, defined as the permeability of microvessels to fluid and hydrophilic solutes that occurs in normal tissues and an **increased microvascular permeability**, which is the large increase in permeability to fluid and plasma proteins that occurs in acutely or chronically inflamed tissues or in tumors. The basal permeability is a continuous process that occurs with small physiological variations by mechanisms that are largely unknown. It is also not known whether the increased permeability is an exaggeration in rate of the basal mechanisms or it occurs via completely different mechanisms.



Endothelium differs from other epithelia on its much higher (2-3 orders of magnitude) permeability to water and solutes (both small and especially large molecules). This higher permeability was found by physiologists to have both a diffusive and convective component. The best fitting model (known as the “pore theory”) (Figs. 1A, 2A) was that the exchange takes place through two types of patent (open), water-filled, cylindrical or slit-like channels: small pores with diameter of $<11\text{ nm}$ and $\sim 15.1\text{ units}/\mu\text{m}^2$ and large pores with diameter of $\sim 50\text{ nm}$ and $\sim 0.2\text{--}0.02\text{ units}/\mu\text{m}^2$.¹⁷⁻¹⁹

The structural aspects of the fluid and solute exchanges across endothelium have been approached by cell biologists mainly by electron microscopy alone or combined with in vivo tracer studies with a variety of probes of variable chemical and physical properties (Fig. 2B, reviewed in refs. 20, 21). The above-mentioned tracer studies are in agreement with the pore theory in the case of the small pores being represented by the paracellular pathway. Also there is agreement in the case of fenestrated and discontinuous endothelia where structural equivalents of large pores have been found such as TEC and fenestrae. No porelike structural equivalents were found for the large pores by electron microscopy in continuous endothelia (where most of the data supporting the “pore theory” had been obtained) with the exception of VVOs in the venular endothelium.²² This established a long-standing controversy (schematized in Fig. 2A) regarding the structural basis of capillary permeability for macromolecules between the capillary physiologists and the cell biologists/morphologists interested in the topic. In the case of the continuous endothelium, the tracer studies have instead documented the alternative concept/process of transcytosis the equivalent of a “functional pore” across epithelia (see below).²³⁻²⁵

Functional Pores across Endothelium—Transcytosis

At its simplest, transcytosis is the transport of macromolecular cargo from one side of a polarized cell to the other (e.g., apical to basolateral) within membrane-bounded carriers (i.e., vesicles). It is a landmark function of all epithelia where it is performed in several distinct steps (Fig. 1A) such as: (1) **endocytosis**, or uptake of cargo at one front of the cells forming barrier, (2) **intracellular trafficking** of cargo in membrane bound vesicles, and, (3) **exocytosis** or fusion of these vesicles with the opposite front of the barrier cells and discharge of the cargo. The endocytotic step was shown

to occur by either caveolae-mediated endocytosis (CAV-ME), clathrin-mediated endocytosis (CME), macropinocytosis (MP) or noncaveolae, nonclathrin pathways (NCNC).^{2,20,26}

All these pathways have been shown to operate in transcytosis in the endothelium as well. Clathrin-mediated endocytosis was shown to be important in endothelium of the testis for receptor-mediated transport of hormones such as LH/CG and FSH, as a mechanism for specific delivery to target organs.²⁷⁻³⁰ Macropinocytosis-like pathways operate via the megalin (gp330)/ LDL-receptor binding protein (LRP) system involved in transport across the brain endothelium of beta-amyloid binding Apolipoprotein J,³¹⁻³⁶ Receptor Associated Protein (RAP) as well as transport of IgG and nutrients from mother to fetus in mammalian placenta.³⁷⁻⁴¹ It has been shown that the syncytiotrophoblast (STB) layer has drastically reduced levels of caveolin 1 and caveolae^{39,42} and that the transport of IgG across the STB layer occurs via the Fcγ Receptor 1b2 in by a NCNC pathway.⁴³ The same NCNC pathway seems to operate in the fetal endothelium where the IgG and FcγRIIb2.^{41,43,44} This system might also play a role in the lung⁴⁵ and brain⁴⁶ where Fc Receptors were demonstrated on the endothelium. A very interesting finding was that one of the IgG receptors FcRn binds albumin at an acidic pH, that the lifespan of albumin is shortened in FcRn-deficient mice, and that the plasma albumin concentration of FcRn-deficient mice (i.e., either the α-chain or β2-microglobulin of the FcRn heterodimer) is less than half that of wild-type mice.⁴⁰ The FcRn receptor functions in an acidic prelysosomal internal compartment where molecules destined to transcytosis might be sorted. Its function is to protect albumin and IgG from degradation in lysosomes diverting them to the opposite front of the cells. However, a direct demonstration of the trafficking of albumin and other relevant plasma proteins in endothelium is still lacking.

The above transcytotic pathways exist for regulated, receptor-mediated transport across cells in all polarized epithelia,^{2,47,48} a broader tissue category to which the vascular endothelium belongs.⁴⁹ However, this does not explain the higher (2-3 orders of magnitude) permeability of endothelium to plasma proteins that occurs in an adsorptive manner (low affinity) and does not seem to make use of receptor mediated systems, unless these pathways could be modulated as to account for such an increase. Caveolae-mediated transcytosis has been proposed to fulfill this gap and to be major players in basal vascular permeability. This put them at the center of the controversy outlined above and thereby the subject of intense study. Data pro or against caveolar were obtained in attempts to answer whether; (1) vesicular transport (transcytosis), although admittedly occurring, has a meaningful contribution to vascular permeability, and (2) if yes, whether caveolae are implicated in this transport (for more detailed reviews, see refs. [1](#), [17](#), [19-21](#), [26](#)).

Caveolae (or plasmalemmal vesicles) ([Fig. 3](#)) were first described in the endothelium of continuous type by Palade⁴ but they occur in all types of endothelia as well as most mammalian cell types (for reviews see refs. [1](#), [20](#), [26](#)). They are morphologically defined as spherical invaginations of plasma membrane of regular shape and size (~70 nm outer diameter),^{5,50,51} which can occur single or in grape-like clusters attached to either front of the endothelium. Only in select endothelia of continuous type (i.e., in lung, tongue, kidney vasa recta) and in all fenestrated and sinusoidal endothelia, caveolae are provided with a Stomatal Diaphragm (SD).^{52,53} The SD is a thin (~5-7 nm) protein barrier of variable diameter (<40 nm), which occurs in their stomata (hence the name) or in between vesicles that are part of a cluster. The function of the

SD is not known (for reviews see refs. 1, 20, 26).

Among the most important arguments for caveolae as transcytotic carriers are:

- a. Endothelial caveolae are numerous, commensurate with the massive exchanges of water and solutes that occur continuously. By EM, rarely seen transendothelial pores in the continuous endothelium are at a much lower surface density than postulated by the pore theory.^{3,54-60} Morphologically defined caveolae were shown to mediate the transport of different tracers across endothelium in situ.^{50,51,61-74} Increased numbers of caveolae correlate with increased transcytosis of tracers in experimental diabetes.⁷⁵ Most importantly, caveolae can be targeted for transport of cargo in tissue specific manner; a monoclonal antibody against a lung endothelial caveolar antigen was taken up and transported across the endothelial barrier via caveolae only in the lung thus establishing proof of principle for tissue targeted delivery of drugs via transcytosis.^{72,76}
- b. The transport of tracers is inhibited by N-ethyl maleimide (NEM), known to interfere with vesicular docking and fusion to target membranes^{65,67} and filipin, a cholesterol-binding drug known to disrupt caveolae.⁶⁷ Caveolae from lung vasculature contain molecules involved in docking and fusion such as VAMP-2,^{72,77,78} NSF and SNAP 25.^{74,79}
- c. Caveolae contain molecules involved in vesicle fission such as dynamin⁸⁰⁻⁸³ or intersectin.⁸⁴ Importantly, endothelial caveolae pinch off from plasma membranes in cell-free assays and their detachment requires dynamin2 and GTP hydrolysis in Ecs.^{85,86}
- d. Caveolae are capable of endocytosis in a dynamin2, glycolipid, tyrosine kinase and actin-dependent manner. Once internalized caveolae can either: fuse with an intracellular compartment called a "caveosome" or fuse with Rab5 containing early endosomes as shown in other cell types⁸⁷⁻⁹⁸ or in endothelium.⁹⁹⁻¹⁰⁷ The finding that the internalization of vesicles that participate in transcytosis can be modulated obviates an advantage over a system of rigid, permanent pores insensitive to such modulations.
- e. Evidence of a transcellular route across endothelium was obtained by live microscopy in situ in the rat cremaster¹⁰⁸ who showed different kinetics for the transport of a small pore probe as compared with a vesicular transport probe (a stearyl dye that fluoresces only in membrane bound vesicles).

Taken together, these data show that the transcellular transport via uncoated vesicles is a fact, that the integrity of lipid rafts is necessary for the transport of proteins across endothelium, that caveolae contain the molecules necessary for either membrane fission or membrane docking and fusion and that they are internalized in an inducible, regulated manner. All of these lines of evidence are consistent with the participation of caveolae in transcytosis.

There are arguments against the importance of transcytosis in vascular permeability as well as against caveolae-mediated transcytosis.

- a. Three-dimensional reconstructions by serial sectioning of fixed tissues showed that most endothelial vesicles would either be fused to the plasma membrane or form branching structures whose lumen communicate with one cell front or

another but not with both at the same time. Few free vesicles were encountered, which was taken as evidence against vesicular transport. However, only occasional TEC were recorded as well, as already pointed out. A counterargument used by the proponents of transcytosis was that the chemical fixation is a slow process and it involves mainly proteins. During its progress, many morphological changes could occur that could lead to result the formation of the racemose structures as a manifestation of the "lowest free energy" in the system.²³ The volume density of caveolae in chemically fixed samples seems to be significantly higher (~70%) as compared with rapidly frozen specimens,^{109,110} which was used by the opponents of vesicular transport as an argument against its importance.^{52,109,110} Also, in continuous endothelium the frequency of caveolae varies widely depending on the organ. And this variation does not correlate with permeability, suggesting that caveolae have also other functions. Indeed caveolae have been also involved in eNOS signaling,¹¹¹ lipoprotein metabolism¹¹²⁻¹¹⁴ and mechanotransduction.^{115,116}

- b. Capillary permeability increases with the increase in the vascular blood pressure, which has been explained by the existence of patent channels. An alternative explanation could be that transcytosis is pressure sensitive. There is some evidence that indicates that the number of endothelial caveolae and their loading of tracers are increased and the thickness of endothelium is decreased in high blood pressure.¹¹⁷⁻¹²³ The transport rate is insensitive to temperature downshifts and to chemical fixation by aldehydes suggesting that it is not active and it does not depend on proteins, respectively.^{18,124-126} No morphological evidence as to the integrity of the endothelium was presented. Recent papers have suggested that NEM and filipin have actually an increasing effect on permeability to macromolecules in an ex-vivo lung preparation as well as in a peritoneal dialysis model.^{17,127,128} The perfusion times used were rather lengthy and no morphological documentation of the capillary endothelium is presented to assess its integrity in such preparations (discussed in refs. [21](#), [66](#)). Some indication for this comes from the measurements of albumin permeability in isolated rat hindquarters,¹²⁴ which were several fold increased as compared with measurements in intact animals.¹²⁹
- c. Cav1 is essential for the formation of caveolae yet its overexpression seems to have an inhibitory effect on the caveolar internalization and cargo uptake into other cell types than ECs.^{130,131} In the same vein treatment of mice with Cav1 scaffolding domain fused to the transducing domain of antennapedia lowered the increased permeability in inflammation as well as that in tumors. VEGF increased permeability is also inhibited by the Cav1 scaffolding domain.^{132,133} It is not clear however, whether the basal and the increased permeability of the endothelium occur through the same mechanisms.
- d. A serious challenge to the importance of transcytosis via caveolae in basal permeability comes from data obtained in caveolin1 -/- mice. These mice lack morphologically distinct caveolae in all nonmuscle cells, endothelium included¹³⁴⁻¹³⁶ thereby showing that caveolin and caveolae are not essential either for the survival of these mice or for the normal development of their vasculature. In absence of caveolae the albumin concentration in the cerebrospinal fluid is the same as in wild type. The albumin uptake into cells (i.e., fibroblasts) or tissues (i.e., aortic rings) was found decreased in these mice.¹³⁷ The baseline permeability to albumin of large vessels (i.e., aorta) was

found as decreased whereas the permeability of the capillaries was increased.¹³⁸ In keeping with this, albumin concentration in serum was found slightly but significantly decreased. The raise in basal capillary permeability of CAV1-/- mice was reduced by L-nitro arginine methyl ester (L-NAME), a nonspecific inhibitor of endothelial nitric oxide synthase (eNOS).¹³⁸ This study, tied in with data showing that the negative regulation of eNOS by caveolin 1 decreases the increased permeability in tumors and inflammation.^{132,133,139-142} This suggests that in absence of caveolae, the mechanisms of increased permeability are turned on, which could compensate the loss of caveolae and to mediate an increase in basal permeability. Lastly, the failure of L-NAME to decrease the permeability in wild-type mice suggests that NO-independent mechanisms concur to the basal, physiological permeability. In other words, the increased permeability in inflammation or tumors occurs by a different mechanism and is not just a modulation (i.e., increase) of the mechanism of basal permeability.

Taken together, the consensus is that, depending on their physical properties molecules cross the continuous endothelium as follows: water and small molecules (e.g., ions, glucose) use a paracellular pathway as well as a transcellular pathway via transporters, whereas the pathway employed by macromolecules is transcellular. A role for membrane-bound vesicles in the transcellular pathway is not disputed. It is still debated whether the exchanges occur through transient TECs formed by vesicular fusion or by shuttling vesicles during transcytosis.¹⁹ The role of caveolae in transendothelial exchanges is still unresolved but in the light of data from CAV1-/- mice it is unclear whether the control of permeability occurs via caveolae participating in transcytosis, via caveolin regulation of eNOS or both. In summary, the data imply several possibilities with respect to caveolar involvement in basal permeability: (i) Caveolae play the major role in transcytosis and in CAV1-/- mice compensatory mechanisms are switched on in their absence. (ii) Exchange of macromolecules across endothelium occurs via multiple partially redundant vesicular pathways, caveolae being one of them. (iii) Transcytosis of macromolecules occurs via a pathway other than caveolae that is sensitive to lipid rafts disassembly (i.e., macropinocytosis or related novel NCNC pathway).

Pores across Endothelium

As mentioned above, physical pores across endothelium have been found. These are the fenestrae, gaps, TEC and VVOs. Due to space constraints, I will discuss only the morphology and functional data, referring the reader to more comprehensive reviews.

Endothelial Fenestrae

Endothelial fenestrae (Fig. 4B-E), the landmarks of the endothelia of fenestrated type, are circular windows resembling “boat portholes” that cut through the cell body. They are arranged in ordered linear arrays within large planar clusters called “sieve plates”. Individual fenestral pores have a remarkably constant diameter (~62-68 nm).^{3,8,9} Their circumference is delineated by the fenestral rim that occurs where the luminal aspect of plasma membrane continues with the abluminal one under a sharp angle. As

in the case of the SDs, electron dense material has been demonstrated in the cytoplasm within the sharp angle formed by the plasma membrane. The circumference of most fenestral pores is apparently round, but as demonstrated by Maul, 20-30% of them have an octagonal symmetry, which can be photographically enhanced by rotating the image several times around its center.¹⁴³ The octagonal symmetry has been clearly established by deep-etch of rapidly frozen specimens⁵³ and high resolution scanning EM.¹⁴⁴

The fenestrae are provided with a Fenestral Diaphragm (FD)⁷ in all cases (i.e., in kidney peritubular capillaries, all endocrine and exocrine glands, intestinal villi) except in capillaries of kidney glomerulus (Fig. 4E).¹⁴⁵⁻¹⁴⁸ Although of a slightly larger diameter, the FDs are morphologically similar to the SDs by TEM. They appear as thin protein barriers anchored in the fenestral rim and provided with a central knob or density, as demonstrated in parallel and perpendicular sections. Moreover, in en face views obtained in oblique or grazing sections, the central density seems to be connected to the fenestral rim by thin fibrils.¹⁴⁹ Bearer and Orci have exquisitely demonstrated the intimate organization of the FD.⁵³ In rapidly frozen deep-etch specimens, they have shown the FD to consist of radial fibrils, starting at the rim and interweaving in a central mesh, the equivalent of the central density/knob seen by TEM. Unfortunately, the SDs of caveolae and TEC could not be resolved in the same detail due to their smaller size and experimental conditions (i.e., angle of shadowing).⁵³ It was readily apparent that the caveolar diaphragms resembled those of the transendothelial channels both featuring a central particle.⁵³ Close examination also reveals hints to the same radial fibrils pattern as for FDs. PV1 protein was shown to be a key component of the FDs, as in the case of the SDs.¹⁵⁰⁻¹⁵³

Finally, perfusion fixation of vascular beds provided with fenestrated endothelia where the fixatives have been dissolved in fluorocarbons (oxygen carriers used as blood plasma expanders) combined with tannic acid staining, revealed the presence of large (up to 400 nm) tufts or fascinae fenestrae on the luminal side of FDs and their absence from the luminal side of fenestrae.¹⁵⁴ These tufts were absent from the caveolar SDs. The fibers forming these tufts have been interpreted as the morphologic equivalent of the heparan sulfate proteoglycans (HSPGs) shown to reside on the luminal surface of fenestrae by other means (see below).

Fenestrated capillaries occur in organs that are involved in reabsorption of water and small solutes (e.g., kidney and intestine) or hormones (e.g., endocrine glands) from the interstitium into the blood. Most of the data in the literature argues that the fenestrated capillaries are highly permeable to water and small hydrophilic solutes but their permeability to macromolecules is basically the same as that of the continuous endothelium.¹⁵⁵ Although, the relative contributions to permeability of caveolae and their SDs, TEC, fenestrae and VVOs is not clear, these permeability characteristics have been attributed to fenestrae, which are considered the equivalents of the functional pores of the pore theory of capillary permeability in the fenestrated endothelium (reviewed in ref. 19). TEC were considered just double diaphragmed fenestrae and VVOs did not exist as a concept yet. The low albumin penetrance was explained by the contribution of the proteoglycans tufts as well as the basement membranes to the overall permeability. It has been calculated that the permeability to water and small solutes could be explained with just a fraction of the fenestrae being open, therefore the function of the proteoglycans would be that of a permeability-reducing plug.¹⁵⁶ Tracer experiments show that all fenestrae are permeable to

horseradish peroxidase ($\varnothing$ 4 nm)⁸ whereas a few permit the exit of ferritin ($\varnothing$ 11 nm),⁸ dextrans ($\varnothing$ 12.5-22.5 nm) and glycogen ($\varnothing$ 22 and 30 nm).¹⁵⁷ As shown by Bearer and Orci,⁵³ the pores in between the fibrils of fenestrae have dimensions of 5-6 nm, therefore the passage of the larger tracers must be accommodated by fenestrae with larger pores. As in the case of the fenestrae, all these tracers gained access to few caveolae past their SDs.^{157,158} An interpretation of these data would be that the diaphragms are dynamic structures and the degree of their permeability could be modulated by yet unknown factors. Another possibility that cannot be discarded at present would be the artifact creation in perfusion experiments or during processing in experiments where the tracers were injected in circulation.

In a mouse model the fenestrae and TEC were shown to disappear from the pancreatic islet vessels upon targeted genetic ablation of VEGF-A secretion from the pancreatic islet cells. The endothelium of islet capillaries became continuous with increased number of vesicles resembling caveolae. Functional assays of absorption of hormones secreted by the islet cells (e.g., insulin) showed that the effect of lack of fenestrae was a delayed absorption of these hormones. This constitutes strong evidence in favor of fenestrae as involved in the rapid exchange of water and small solutes such as peptide hormones.¹⁵⁹

The functions of VVOs have been extensively reviewed recently.²² They have been involved in increased permeability related to inflammation and tumors, permeability to macromolecules as well as particulates. Interestingly, their SDs seem to play a restrictive role in the permeability of particulates.¹⁶⁰ The results obtained in cells in culture¹⁶¹ bolster the claims for VVOs as an organelle involved in the permeability induced by cytokines. However, it is not clear how their restricted localization to the venular endothelium would contribute to the overall permeability in a particular vascular bed, accepting the hypothesis that the bulk of transendothelial exchanges would occur at the level of the capillaries.

Transendothelial Channels

Transendothelial channels (TEC) (Figs. 4A,C) are patent pores spanning the endothelial cell (EC) body from one front to the other. TEC are rarely found in continuous endothelia where they seem to be formed by the fusion of either one caveola/plasmalemmal vesicle with both luminal and abluminal aspects of the plasmalemma or by chains of usually two to four caveolae.^{5,6,162} In the fenestrated endothelia, TEC occur in the attenuated part of the endothelial cell and are provided with two SD (one luminal and one abluminal).^{3,9,162,163} As in the case of caveolar SDs, the fine structure of the SDs of TEC could not be clearly resolved by the deep-etch procedure of Bearer and Orci due to technical limitations.⁵³ However, close inspections of the micrographs reveal the same pattern of radial fibrils and a central density. PV1 protein is also present in these diaphragms at both fronts of the endothelial cell.

Vesiculo-Vacuolar Organelles (VVO)

Vesiculo-vacuolar organelles (VVOs) are morphologically defined as chains of interconnected vesicles of variable size that form intricate transendothelial channels

spanning the cytoplasm of the endothelial cells from one front of EC to the other.¹⁶⁴ They are usually provided with SDs at the connection points between vesicles and vacuoles as well as at the level of their stoma or communication with the extracellular space.¹⁶⁰ The SDs of the VVOs closely-resemble those of caveolae by TEM. A recent review discusses the biology of VVOs in great detail.²²

VVOs have been first described in the tumor vasculature¹⁶⁵ and subsequently in the normal endothelium of the post-capillary venules.¹⁶⁴⁻¹⁶⁶ The definition of VVOs is purely morphological therefore there is a difficulty in discerning VVOs from clusters of caveolae or other vesicles that occur in most endothelia^{3,5} in absence of specific biochemical markers. This raised doubts as to their status as a bona fide, novel organelle,¹⁶³ but this status might be bolstered by recent EM data showing that in caveolin 1 -/- mice (lacking caveolae in all nonmuscle cell types, endothelia included), the venules were still provided with structures resembling VVOs (Fig. 4E).^{26,134,135} However, the relationship between these structures and VVOs remains to be clarified following biochemical evaluation.

Endothelial Pockets

These are infrequent structures that by EM resemble a pocket or a large vacuole formed by cellular processes that contain fenestrae with the usual structure¹⁰ (reviewed in ref. 1). The information on these is scarce and, so far, they seem to occur in very low numbers only in the fenestrated endothelia. No functional data is available on these structures.

Conclusions

Undoubtedly our understanding of the mechanisms of the exchanges across cellular barriers has greatly advanced in the last years. What is clear is that there are many pathways by which material can be moved from one side of the barrier to the other. Which pathways are important for each individual molecule is a matter of further inquiry. As for the mechanisms of transendothelial exchange, the controversy is still there, as we still do not have clear information on the molecular players that allow its increased and continuous protein leakage.

Acknowledgements

I would like to thank G.E. Palade, C. Carriere, B. Jacobson and L. Ghitescu for advice and long and fruitful collaborations. I would like to apologize for relying on reviews and not being able to cite all the primary sources of the data due to constraints of space.

References

1. Stan RV. Endothelial diaphragms: Nanogates of vascular permeability *Anat Rec* 2005 In press.
2. Tuma PL, Hubbard AL. Transcytosis: Crossing cellular barriers. *Physiol Rev.* 2003; 83: 871–932. [[PubMed](#)]
3. Simionescu M, Simionescu N, Palade GE. Morphometric data on the endothelium of blood capillaries. *J Cell Biol.* 1974; 60: 128–152. [[PubMed](#)] [[Free Full text in PMC](#)]
4. Palade GE. Fine structure of blood capillaries. *J Applied Physics.* 1953; 24: 1424.
5. Palade GE, Bruns RR. Structural modulations of plasmalemmal vesicles. *J Cell Biol.* 1968; 37: 633–649. [[PubMed](#)] [[Free Full text in PMC](#)]
6. Simionescu N, Simionescu M, Palade GE. Permeability of muscle capillaries to small heme-peptides. Evidence for the existence of patent transendothelial channels. *J Cell Biol.* 1975; 64: 586–607. [[PubMed](#)] [[Free Full text in PMC](#)]
7. Gautier A, Bernhard W, Oberling C. Sur l'existence d'un appareil lacunaire pericapillaire du glomerule de Malpighi, revele par la microscopie electronique. *Comptes rendus de la seances de la Societe de Biologie.* 1950. pp. 1605–1607.
8. Clementi F, Palade GE. Intestinal capillaries. I. Permeability to peroxidase and ferritin. *J Cell Biol.* 1969; 41: 33–58. [[PubMed](#)] [[Free Full text in PMC](#)]
9. Clementi F, Palade GE. Intestinal capillaries. II. Structural effects of EDTA and histamine. *J Cell Biol.* 1969; 42: 706–714. [[PubMed](#)] [[Free Full text in PMC](#)]
10. Milici AJ, Peters KR, Palade GE. The endothelial pocket. A new structure in fenestrated endothelia. *Cell Tissue Res.* 1986; 244: 493–499. [[PubMed](#)]
11. Milici AJ, Furie MB, Carley WW. The formation of fenestrations and channels by capillary endothelium in vitro. *Proc Natl Acad Sci USA.* 1985; 82: 6181–6185. [[PubMed](#)] [[Free Full text in PMC](#)]
12. Braet F, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: A review. *Comp Hepatol.* 2002; 1: 1. [[PubMed](#)] [[Free Full text in PMC](#)]
13. Aird WC. Spatial and temporal dynamics of the endothelium. *J Thromb Haemost.* 2005; 3: 1392–1406. [[PubMed](#)]
14. Stevens T, Rosenberg R, Aird W. et al. NHLBI workshop report: Endothelial cell phenotypes in heart, lung, and blood diseases. *Am J Physiol Cell Physiol.* 2001; 281: C1422–1433. [[PubMed](#)]
15. Ghitescu L, Robert M. Diversity in unity: The biochemical composition of the endothelial cell surface varies between the vascular beds. *Microsc Res Tech.* 2002; 57: 381–389. [[PubMed](#)]
16. Cines DB, Pollak ES, Buck CA. et al. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood.* 1998; 91: 3527–3561. [[PubMed](#)]
17. Rippe B, Rosengren BI, Carlsson O. et al. Transendothelial transport: The vesicle controversy. *J Vasc Res.* 2002; 39: 375–390. [[PubMed](#)]
18. Rippe B, Haraldsson B. Transport of macromolecules across microvascular walls: The two-pore theory. *Physiol Rev.* 1994; 74: 163–219. [[PubMed](#)]
19. Michel CC, Curry FE. Microvascular permeability. *Physiol Rev.* 1999; 79: 703–761. [[PubMed](#)]
20. Stan RV. Structure and function of endothelial caveolae. *Microsc Res Tech.* 2002; 57: 350–364. [[PubMed](#)]
21. Predescu D, Vogel SM, Malik AB. Functional and morphological studies of protein transcytosis in continuous endothelia. *Am J Physiol Lung Cell Mol Physiol.* 2004; 287: L895–901. [[PubMed](#)]
22. Feng D, Nagy JA, Dvorak HF. et al. Ultrastructural studies define soluble macromolecular, particulate, and cellular transendothelial cell pathways in

- venules, lymphatic vessels, and tumor-associated microvessels in man and animals. *Microsc Res Tech.* 2002; 57: 289–326. [[PubMed](#)]
23. Palade GE. Role of plasmalemmal vesicles.
In: Crystal RG, West JB, eds. The Lung: Scientific Foundations. New York: RavenPress Ltd. 1991: 359–367.
24. In: Simionescu N ed. Transcytosis and traffic of membranes in endothelial cells.
Berlin: Springer-Verlag. 1981
25. Simionescu N. Cellular aspects of transcapillary exchange.
Physiol Rev. 1983; 63: 1536–1579. [[PubMed](#)]
26. Stan RV. Structure of caveolae. *Biochim Biophys Acta.* 2005
27. Misrahi M, Beau I, Ghinea N. et al. The LH/CG and FSH receptors: Different molecular forms and intracellular traffic.
Mol Cell Endocrinol. 1996; 125: 161–167. [[PubMed](#)]
28. Ghinea N, Milgrom E. A new function for the LH/CG receptor: Transcytosis of hormone across the endothelial barrier in target organs.
Semin Reprod Med. 2001; 19: 97–101. [[PubMed](#)]
29. Vu Hai MT, Lescop P, Loosfelt H. et al. Receptor-mediated transcytosis of follicle-stimulating hormone through the rat testicular microvasculature.
Biol Cell. 2004; 96: 133–144. [[PubMed](#)]
30. Ghinea N, Mai TV, Groyer-Picard MT. et al. How protein hormones reach their target cells. Receptor-mediated transcytosis of hCG through endothelial cells.
J Cell Biol. 1994; 125: 87–97. [[PubMed](#)] [[Free Full text in PMC](#)]
31. Zlokovic BV, Martel CL, Mackic JB. et al. Brain uptake of circulating apolipoproteins J and E complexed to Alzheimer's amyloid beta.
Biochem Biophys Res Commun. 1994; 205: 1431–1437. [[PubMed](#)]
32. Zlokovic BV. Cerebrovascular transport of Alzheimer's amyloid beta and apolipoproteins J and E: Possible anti-amyloidogenic role of the blood-brain barrier. *Life Sci.* 1996; 59: 1483–1497. [[PubMed](#)]
33. Zlokovic BV, Martel CL, Matsubara E. et al. Glycoprotein 330/megalin: Probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. *Proc Natl Acad Sci USA.* 1996; 93: 4229–4234. [[PubMed](#)] [[Free Full text in PMC](#)]
34. Chun JT, Wang L, Pasinetti GM. et al. Glycoprotein 330/megalin (LRP-2) has low prevalence as mRNA and protein in brain microvessels and choroid plexus.
Exp Neurol. 1999; 157: 194–201. [[PubMed](#)]
35. Calero M, Rostagno A, Matsubara E. et al. Apolipoprotein J (clusterin) and Alzheimer's disease. *Microsc Res Tech.* 2000; 50: 305–315. [[PubMed](#)]
36. Shayo M, McLay RN, Kastin AJ. et al. The putative blood-brain barrier transporter for the beta-amyloid binding protein apolipoprotein j is saturated at physiological concentrations. *Life Sci.* 1997; 60: PL115–118. [[PubMed](#)]
37. Fuchs R, Ellinger I. Endocytic and transcytotic processes in villous syncytiotrophoblast: Role in nutrient transport to the human fetus.
Traffic. 2004; 5: 725–738. [[PubMed](#)]
38. Van de Perre P. Transfer of antibody via mother's milk.
Vaccine. 2003; 21: 3374–3376. [[PubMed](#)]
39. Linton EA, Rodriguez-Linares B, Rashid-Doubell F. et al. Caveolae and caveolin-1 in human term villous trophoblast. *Placenta.* 2003; 24: 745–757. [[PubMed](#)]
40. Chaudhury C, Mehnaz S, Robinson JM. et al. The major histocompatibility complex-related Fc receptor for IgG (FcRn) binds albumin and prolongs its lifespan. *J Exp Med.* 2003; 197: 315–322. [[PubMed](#)] [[Free Full text in PMC](#)]
41. Takizawa T, Anderson CL, Robinson JM. A novel Fc{gamma}R-defined, IgG-containing organelle in placental endothelium.
J Immunol. 2005; 175: 2331–2339. [[PubMed](#)]

42. Lyden TW, Anderson CL, Robinson JM. The endothelium but not the syncytiotrophoblast of human placenta expresses caveolae. *Placenta*. 2002; 23: 640–652. [[PubMed](#)]
43. Lyden TW, Robinson JM, Tridandapani S. et al. The Fc receptor for IgG expressed in the villus endothelium of human placenta is Fc gamma RIIb2. *J Immunol*. 2001; 166: 3882–3889. [[PubMed](#)]
44. Gafencu A, Heltianu C, Burlacu A. et al. Investigation of IgG receptors expressed on the surface of human placental endothelial cells. *Placenta*. 2003; 24: 664–676. [[PubMed](#)]
45. Kim KJ, Malik AB. Protein transport across the lung epithelial barrier. *Am J Physiol Lung Cell Mol Physiol*. 2003; 284: L247–259. [[PubMed](#)]
46. Schlachetzki F, Zhu C, Pardridge WM. Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. *J Neurochem*. 2002; 81: 203–206. [[PubMed](#)]
47. Simons K, Wandinger-Ness A. Polarized sorting in epithelia. *Cell*. 1990; 62: 207–210. [[PubMed](#)]
48. Caplan MJ. Membrane polarity in epithelial cells: Protein sorting and establishment of polarized domains. *Am J Physiol*. 1997; 272: F425–429. [[PubMed](#)]
49. Muller WA, Gimbrone Jr MA. Plasmalemmal proteins of cultured vascular endothelial cells exhibit apical-basal polarity: Analysis by surface-selective iodination. *J Cell Biol*. 1986; 103: 2389–2402. [[PubMed](#)] [[Free Full text in PMC](#)]
50. Bruns RR, Palade GE. Studies on blood capillaries. I. General organization of blood capillaries in muscle. *J Cell Biol*. 1968; 37: 244–276. [[PubMed](#)] [[Free Full text in PMC](#)]
51. Bruns RR, Palade GE. Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. *J Cell Biol*. 1968; 37: 277–299. [[PubMed](#)] [[Free Full text in PMC](#)]
52. Noguchi Y, Shibata Y, Yamamoto T. Endothelial vesicular system in rapid-frozen muscle capillaries revealed by serial sectioning and deep etching. *Anat Rec*. 1987; 217: 355–360. [[PubMed](#)]
53. Bearer EL, Orci L. Endothelial fenestral diaphragms: A quick-freeze, deep-etch study. *J Cell Biol*. 1985; 100: 418–428. [[PubMed](#)] [[Free Full text in PMC](#)]
54. Frokjaer-Jensen J. Three-dimensional organization of plasmalemmal vesicles in endothelial cells. An analysis by serial sectioning of frog mesenteric capillaries. *J Ultrastruct Res*. 1980; 73: 9–20. [[PubMed](#)]
55. Frokjaer-Jensen J. The plasmalemmal vesicular system in striated muscle capillaries and in pericytes. *Tissue Cell*. 1984; 16: 31–42. [[PubMed](#)]
56. Frokjaer-Jensen J. The endothelial vesicle system in cryofixed frog mesenteric capillaries analysed by ultrathin serial sectioning. *J Electron Microsc Tech*. 1991; 19: 291–304. [[PubMed](#)]
57. Frokjaer-Jensen J, Wagner RC, Andrews SB. et al. 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*. 1988; 254: 17–24. [[PubMed](#)]
58. Bundgaard M, Frokjaer-Jensen J, Crone C. Endothelial plasmalemmal vesicles as elements in a system of branching invaginations from the cell surface. *Proc Natl Acad Sci USA*. 1979; 76: 6439–6442. [[PubMed](#)] [[Free Full text in PMC](#)]
59. Bundgaard M. Vesicular transport in capillary endothelium: Does it occur? *Fed Proc* 1983; 42:2425-2430.
60. Bundgaard M. The three-dimensional organization of smooth endoplasmic reticulum in capillary endothelia: Its possible role in regulation of free cytosolic calcium. *J Struct Biol*. 1991; 107: 76–85. [[PubMed](#)]
61. Milici AJ, Watrous NE, Stukenbrok H. et al. Transcytosis of albumin in capillary endothelium. *J Cell Biol*. 1987; 105: 2603–2612. [[PubMed](#)] [[Free Full text in](#)

[PMC\]](#)

62. Ghitescu L, Galis Z, Simionescu M. et al. Differentiated uptake and transcytosis of albumin in successive vascular segments. *J Submicrosc Cytol Pathol.* 1988; 20: 657–669. [[PubMed](#)]
63. Ghinea N, Simionescu N. Anionized and cationized hemeundecapeptides as probes for cell surface charge and permeability studies: Differentiated labeling of endothelial plasmalemmal vesicles. *J Cell Biol.* 1985; 100: 606–612. [[PubMed](#)] [[Free Full text in PMC](#)]
64. Predescu D, Palade GE. Plasmalemmal vesicles represent the large pore system of continuous microvascular endothelium. *Am J Physiol.* 1993; 265: H725–733. [[PubMed](#)]
65. Predescu D, Horvat R, Predescu S. et al. Transcytosis in the continuous endothelium of the myocardial microvasculature is inhibited by N-ethylmaleimide. *Proc Natl Acad Sci USA.* 1994; 91: 3014–3018. [[PubMed](#)] [[Free Full text in PMC](#)]
66. Predescu SA, Predescu DN, Palade GE. Plasmalemmal vesicles function as transcytotic carriers for small proteins in the continuous endothelium. *Am J Physiol.* 1997; 272: H937–949. [[PubMed](#)]
67. Schnitzer JE, Allard J, Oh P. NEM inhibits transcytosis, endocytosis, and capillary permeability: Implication of caveolae fusion in endothelia. *Am J Physiol.* 1995; 268: H48–55. [[PubMed](#)]
68. Palade GE. Transport in quanta across endothelium in blood capillaries. *Anat Rec.* 1960; 136: 254.
69. Palade GE. Blood capillaries of the heart and other organs. *Circulation.* 1961; 24: 368–388. [[PubMed](#)]
70. Wagner RC, Chen SC. Transcapillary transport of solute by the endothelial vesicular system: Evidence from thin serial section analysis. *Microvasc Res.* 1991; 42: 139–150. [[PubMed](#)]
71. Ghitescu L, Bendayan M. Transendothelial transport of serum albumin: A quantitative immunocytochemical study. *J Cell Biol.* 1992; 117: 745–755. [[PubMed](#)] [[Free Full text in PMC](#)]
72. McIntosh DP, Tan XY, Oh P. et al. Targeting endothelium and its dynamic caveolae for tissue-specific transcytosis in vivo: A pathway to overcome cell barriers to drug and gene delivery. *Proc Natl Acad Sci USA.* 2002; 99: 1996–2001. [[PubMed](#)] [[Free Full text in PMC](#)]
73. Predescu D, Predescu S, McQuistan T. et al. Transcytosis of alpha1-acidic glycoprotein in the continuous microvascular endothelium. *Proc Natl Acad Sci USA.* 1998; 95: 6175–6180. [[PubMed](#)] [[Free Full text in PMC](#)]
74. Predescu SA, Predescu DN, Palade GE. Endothelial transcytotic machinery involves supramolecular protein-lipid complexes. *Mol Biol Cell.* 2001; 12: 1019–1033. [[PubMed](#)] [[Free Full text in PMC](#)]
75. Pascariu M, Bendayan M, Ghitescu L. Correlated endothelial caveolin overexpression and increased transcytosis in experimental diabetes. *J Histochem Cytochem.* 2004; 52: 65–76. [[PubMed](#)]
76. Carver LA, Schnitzer JE, Anderson RG. et al. Role of caveolae and lipid rafts in cancer: Workshop summary and future needs. *Cancer Res.* 2003; 63: 6571–6574. [[PubMed](#)]
77. McIntosh DP, Schnitzer JE. Caveolae require intact VAMP for targeted transport in vascular endothelium. *Am J Physiol.* 1999; 277: H2222–2232. [[PubMed](#)]
78. Feng D, Flaumenhaft R, Bandeira-Melo C. et al. Ultrastructural localization of vesicle-associated membrane protein(s) to specialized membrane structures in human pericytes, vascular smooth muscle cells, endothelial cells, neutrophils, and eosinophils. *J Histochem Cytochem.* 2001; 49: 293–304. [[PubMed](#)]
79. Schnitzer JE, Liu J, Oh P. Endothelial caveolae have the molecular transport machinery for vesicle budding, docking, and fusion including VAMP, NSF, SNAP, annexins, and GTPases. *J Biol Chem.* 1995; 270: 14399–14404. [[PubMed](#)]

80. Henley JR, Krueger EW, Oswald BJ. et al. Dynamin-mediated internalization of caveolae. *J Cell Biol.* 1998; 141: 85–99. [[PubMed](#)] [[Free Full text in PMC](#)]
81. Oh P, McIntosh DP, Schnitzer JE. Dynamin at the neck of caveolae mediates their budding to form transport vesicles by GTP-driven fission from the plasma membrane of endothelium. *J Cell Biol.* 1998; 141: 101–114. [[PubMed](#)] [[Free Full text in PMC](#)]
82. Henley JR, Cao H, McNiven MA. Participation of dynamin in the biogenesis of cytoplasmic vesicles. *Faseb J.* 1999; 13(Suppl 2): S243–247. [[PubMed](#)]
83. Yao Q, Chen J, Cao H. et al. Caveolin-1 interacts directly with dynamin-2. *J Mol Biol.* 2005; 348: 491–501. [[PubMed](#)]
84. Predescu SA, Predescu DN, Timblin BK. et al. Intersectin regulates fission and internalization of caveolae in endothelial cells. *Mol Biol Cell.* 2003; 14: 4997–5010. [[PubMed](#)] [[Free Full text in PMC](#)]
85. Schnitzer JE, Oh P, McIntosh DP. Role of GTP hydrolysis in fission of caveolae directly from plasma membranes. *Science.* 1996; 274: 239–242. [[PubMed](#)]
86. Schnitzer JE, Oh P, McIntosh DP. Role of GTP hydrolysis in fission of caveolae directly from plasma membranes [published erratum appears in *Science* 1996; 274:1069]. *Science.* 1996; 274: 239–242. [[PubMed](#)]
87. Parton RG, Joggerst B, Simons K. Regulated internalization of caveolae. *J Cell Biol.* 1994; 127: 1199–1215. [[PubMed](#)] [[Free Full text in PMC](#)]
88. Smart EJ, Estes K, Anderson RG. Inhibitors that block both the internalization of caveolae and the return of plasmalemmal vesicles. *Cold Spring Harb Symp Quant Biol.* 1995; 60: 243–248. [[PubMed](#)]
89. Choudhury A, Dominguez M, Puri V. et al. Rab proteins mediate Golgi transport of caveola-internalized glycosphingolipids and correct lipid trafficking in Niemann-Pick C cells. *J Clin Invest.* 2002; 109: 1541–1550. [[PubMed](#)] [[Free Full text in PMC](#)]
90. Mundy DI, Machleidt T, Ying YS. et al. Dual control of caveolar membrane traffic by microtubules and the actin cytoskeleton. *J Cell Sci.* 2002; 115: 4327–4339. [[PubMed](#)]
91. Pelkmans L, Kartenbeck J, Helenius A. Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. *Nat Cell Biol.* 2001; 3: 473–483. [[PubMed](#)]
92. Pelkmans L, Puntener D, Helenius A. Local actin polymerization and dynamin recruitment in SV40-induced internalization of caveolae. *Science.* 2002; 296: 535–539. [[PubMed](#)]
93. Pelkmans L, Burli T, Zerial M. et al. Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. *Cell.* 2004; 118: 767–780. [[PubMed](#)]
94. Sharma P, Sabharanjak S, Mayor S. Endocytosis of lipid rafts: An identity crisis. *Semin Cell Dev Biol.* 2002; 13: 205–214. [[PubMed](#)]
95. Sharma DK, Brown JC, Choudhury A. et al. Selective stimulation of caveolar endocytosis by glycosphingolipids and cholesterol. *Mol Biol Cell.* 2004; 15: 3114–3122. [[PubMed](#)] [[Free Full text in PMC](#)]
96. Sharma DK, Choudhury A, Singh RD. et al. Glycosphingolipids internalized via caveolar-related endocytosis rapidly merge with the clathrin pathway in early endosomes and form microdomains for recycling. *J Biol Chem.* 2003; 278: 7564–7572. [[PubMed](#)]
97. Kirkham M, Fujita A, Chadda R. et al. Ultrastructural identification of uncoated caveolin-independent early endocytic vehicles. *J Cell Biol.* 2005; 168: 465–476. [[PubMed](#)] [[Free Full text in PMC](#)]
98. Damm EM, Pelkmans L, Kartenbeck J. et al. Clathrin- and caveolin-1-independent endocytosis: Entry of simian virus 40 into cells devoid of caveolae. *J Cell Biol.* 2005; 168: 477–488. [[PubMed](#)] [[Free Full text in PMC](#)]

99. Aoki T, Nomura R, Fujimoto T. Tyrosine phosphorylation of caveolin-1 in the endothelium. *Exp Cell Res.* 1999; 253: 629–636. [[PubMed](#)]
100. Shajahan AN, Tiruppathi C, Smrcka AV. et al. Gbetagamma activation of Src induces caveolae-mediated endocytosis in endothelial cells. *J Biol Chem.* 2004; 279: 48055–48062. [[PubMed](#)]
101. Shajahan AN, Timblin BK, Sandoval R. et al. Role of Src-induced dynamin-2 phosphorylation in caveolae-mediated endocytosis in endothelial cells. *J Biol Chem.* 2004; 279: 20392–20400. [[PubMed](#)]
102. Vogel SM, Minshall RD, Pilipovic M. et al. Albumin uptake and transcytosis in endothelial cells in vivo induced by albumin-binding protein. *Am J Physiol Lung Cell Mol Physiol.* 2001; 281: L1512–1522. [[PubMed](#)]
103. Minshall RD, Tiruppathi C, Vogel SM. et al. Endothelial cell-surface gp60 activates vesicle formation and trafficking via G(i)-coupled Src kinase signaling pathway. *J Cell Biol.* 2000; 150: 1057–1070. [[PubMed](#)] [[Free Full text in PMC](#)]
104. John TA, Vogel SM, Minshall RD. et al. Evidence for the role of alveolar epithelial gp60 in active transalveolar albumin transport in the rat lung. *J Physiol.* 2001; 533: 547–559. [[PubMed](#)] [[Free Full text in PMC](#)]
105. Tiruppathi C, Song W, Bergenfeldt M. et al. Gp60 activation mediates albumin transcytosis in endothelial cells by tyrosine kinase-dependent pathway. *J Biol Chem.* 1997; 272: 25968–25975. [[PubMed](#)]
106. Yamaguchi T, Murata Y, Fujiyoshi Y. et al. Regulated interaction of endothelin B receptor with caveolin-1. *Eur J Biochem.* 2003; 270: 1816–1827. [[PubMed](#)]
107. Foster LJ, De Hoog CL, Mann M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc Natl Acad Sci USA.* 2003; 100: 5813–5818. [[PubMed](#)] [[Free Full text in PMC](#)]
108. Vogel SM, Easington CR, Minshall RD. et al. Evidence of transcellular permeability pathway in microvessels. *Microvasc Res.* 2001; 61: 87–101. [[PubMed](#)]
109. Wood MR, Wagner RC, Andrews SB. et al. Rapidly-frozen, cultured, human endothelial cells: An ultrastructural and morphometric comparison between freshly-frozen and glutaraldehyde prefixed cells. *Microcirc Endothel Lymph.* 1986; 3: 323–358.
110. McGuire PG, Twietmeyer TA. Morphology of rapidly frozen aortic endothelial cells. Glutaraldehyde fixation increases the number of caveolae. *Circ Res.* 1983; 53: 424–429. [[PubMed](#)]
111. Gratton JP, Bernatchez P, Sessa WC. Caveolae and caveolins in the cardiovascular system. *Circ Res.* 2004; 94: 1408–1417. [[PubMed](#)]
112. Razani B, Combs TP, Wang XB. et al. Caveolin-1-deficient mice are lean, resistant to diet-induced obesity, and show hypertriglyceridemia with adipocyte abnormalities. *J Biol Chem.* 2002; 277: 8635–8647. [[PubMed](#)]
113. Frank PG, Lee H, Park DS. et al. Genetic ablation of caveolin-1 confers protection against atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2004; 24: 98–105. [[PubMed](#)]
114. Frank PG, Lisanti MP. Caveolin-1 and caveolae in atherosclerosis: Differential roles in fatty streak formation and neointimal hyperplasia. *Curr Opin Lipidol.* 2004; 15: 523–529. [[PubMed](#)]
115. Rizzo V, McIntosh DP, Oh P. et al. In situ flow activates endothelial nitric oxide synthase in luminal caveolae of endothelium with rapid caveolin dissociation and calmodulin association. *J Biol Chem.* 1998; 273: 34724–34729. [[PubMed](#)]
116. Rizzo V, Sung A, Oh P. et al. Rapid mechanotransduction in situ at the luminal cell surface of vascular endothelium and its caveolae. *J Biol Chem.* 1998; 273: 26323–26329. [[PubMed](#)]
117. Arnal JF, Dinh-Xuan AT, Pueyo M. et al. Endothelium-derived nitric oxide and vascular physiology and pathology. *Cell Mol Life Sci.* 1999; 55: 1078–1087.

[[PubMed](#)]

118. Hillman N, Cox S, Noble AR. et al. Increased numbers of caveolae in retinal endothelium and pericytes in hypertensive diabetic rats. *Eye*. 2001; 15: 319–325. [[PubMed](#)]
119. Kurozumi T, Imamura T, Tanaka K. et al. Effects of hypertension and hypercholesterolemia on the permeability of fibrinogen and low density lipoprotein in the coronary artery of rabbits. Immunoelectron-microscopic study. *Atherosclerosis*. 1983; 49: 267–276. [[PubMed](#)]
120. Kurozumi T, Imamura T, Tanaka K. et al. Permeation and deposition of fibrinogen and low-density lipoprotein in the aorta and cerebral artery of rabbits-immuno-electron microscopic study. *Br J Exp Pathol*. 1984; 65: 355–364. [[PubMed](#)]
121. Sakata N, Ida T, Joshita T. et al. Scanning and transmission electron microscopic study on the cerebral arterial endothelium of experimentally hypertensive rats fed an atherogenic diet. *Acta Pathol Jpn*. 1983; 33: 1105–1113. [[PubMed](#)]
122. Traub O, Berk BC. Laminar shear stress: Mechanisms by which endothelial cells transduce an atheroprotective force. *Arterioscler Thromb Vasc Biol*. 1998; 18: 677–685. [[PubMed](#)]
123. Wiener J, Loud AV, Giacomelli F. et al. Morphometric analysis of hypertension-induced hypertrophy of rat thoracic aorta. *Am J Pathol*. 1977; 88: 619–633. [[PubMed](#)] [[Free Full text in PMC](#)]
124. Rippe B, Kamiya A, Folkow B. Transcapillary passage of albumin, effects of tissue cooling and of increases in filtration and plasma colloid osmotic pressure. *Acta Physiol Scand*. 1979; 105: 171–187. [[PubMed](#)]
125. Haraldsson B, Johansson BR. Changes in transcapillary exchange induced by perfusion fixation with glutaraldehyde, followed by measurements of capillary filtration coefficient, diffusion capacity and albumin clearance. *Acta Physiol Scand*. 1985; 124: 99–106. [[PubMed](#)]
126. Rosengren BI, Carlsson O, Venturoli D. et al. Transvascular passage of macromolecules into the peritoneal cavity of normo- and hypothermic rats in vivo: Active or passive transport? *J Vasc Res*. 2004; 41: 123–130. [[PubMed](#)]
127. Carlsson O, Rosengren BI, Rippe B. Transcytosis inhibitor N-ethylmaleimide increases microvascular permeability in rat muscle. *Am J Physiol Heart Circ Physiol*. 2001; 281: H1728–1733. [[PubMed](#)]
128. Rosengren BI, Al Rayyes O, Rippe B. Transendothelial transport of low-density lipoprotein and albumin across the rat peritoneum in vivo: Effects of the transcytosis inhibitors NEM and filipin. *J Vasc Res*. 2002; 39: 230–237. [[PubMed](#)]
129. Renkin EM, Gustafson-Sgro M, Sibley L. Coupling of albumin flux to volume flow in skin and muscles of anesthetized rats. *Am J Physiol*. 1988; 255: H458–466. [[PubMed](#)]
130. Thomsen P, Roepstorff K, Stahlhut M. et al. Caveolae are highly immobile plasma membrane microdomains, which are not involved in constitutive endocytic trafficking. *Mol Biol Cell*. 2002; 13: 238–250. [[PubMed](#)] [[Free Full text in PMC](#)]
131. Le PU, Guay G, Altschuler Y. et al. Caveolin-1 is a negative regulator of caveolae-mediated endocytosis to the endoplasmic reticulum. *J Biol Chem*. 2002; 277: 3371–3379. [[PubMed](#)]
132. Gratton JP, Lin MI, Yu J. et al. Selective inhibition of tumor microvascular permeability by cavtratin blocks tumor progression in mice. *Cancer Cell*. 2003; 4: 31–39. [[PubMed](#)]
133. Bucci M, Gratton JP, Rudic RD. et al. In vivo delivery of the caveolin-1 scaffolding domain inhibits nitric oxide synthesis and reduces inflammation. *Nat Med*. 2000; 6: 1362–1367. [[PubMed](#)]
134. Zhao YY, Liu Y, Stan RV. et al. Defects in caveolin-1 cause dilated cardiomyopathy and pulmonary hypertension in knockout mice. *Proc Natl Acad Sci USA*. 2002; 99: 11375–11380. [[PubMed](#)] [[Free Full text in](#)

PMC]

135. Drab M, Verkade P, Elger M. et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. *Science*. 2001; 293: 2449–2452. [[PubMed](#)]
136. Razani B, Engelman JA, Wang XB. et al. Caveolin-1 null mice are viable but show evidence of hyperproliferative and vascular abnormalities. *J Biol Chem*. 2001; 276: 38121–38138. [[PubMed](#)]
137. Schubert W, Frank PG, Razani B. et al. Caveolae-deficient endothelial cells show defects in the uptake and transport of albumin in vivo. *J Biol Chem*. 2001; 276: 48619–48622. [[PubMed](#)]
138. Schubert W, Frank PG, Woodman SE. et al. Microvascular hyperpermeability in caveolin-1 (-/-) knock-out mice. Treatment with a specific nitric-oxide synthase inhibitor, L-name, restores normal microvascular permeability in Cav-1 null mice. *J Biol Chem*. 2002; 277: 40091–40098. [[PubMed](#)]
139. Gratton JP, Yu J, Griffith JW. et al. Erratum: Cell-permeable peptides improve cellular uptake and therapeutic gene delivery of replication-deficient viruses in cells and in vivo. *Nat Med*. 2003; 9: 1221. [[PubMed](#)]
140. Gratton JP, Yu J, Griffith JW. et al. Cell-permeable peptides improve cellular uptake and therapeutic gene delivery of replication-deficient viruses in cells and in vivo. *Nat Med*. 2003; 9: 357–362. [[PubMed](#)]
141. Bernatchez PN, Bauer PM, Yu J. et al. Dissecting the molecular control of endothelial NO synthase by caveolin-1 using cell-permeable peptides. *Proc Natl Acad Sci USA*. 2005; 102: 761–766. [[PubMed](#)] [[Free Full text in PMC](#)]
142. Bauer PM, Yu J, Chen Y. et al. Endothelial-specific expression of caveolin-1 impairs microvascular permeability and angiogenesis. *Proc Natl Acad Sci USA*. 2005; 102: 204–209. [[PubMed](#)] [[Free Full text in PMC](#)]
143. Maul GG. Structure and formation of pores in fenestrated capillaries. *J Ultrastruct Res*. 1971; 36: 768–782. [[PubMed](#)]
144. Apkarian RP. The fine structure of fenestrated adrenocortical capillaries revealed by in-lens field-emission scanning electron microscopy and scanning transmission electron microscopy. *Scanning*. 1997; 19: 361–367. [[PubMed](#)]
145. Oberling C, Gautier A, Bernhardt W. La structure des capillaires glomerulairevue au microscope electronique. *Presse medicale Parisienne*. 1951; 59: 938–940.
146. Pease DC. Electron microscopy of the vascular bed of the kidney cortex. *Anat Rec*. 1955; 121: 701–712. [[PubMed](#)]
147. Reeves WH, Kanwar YS, Farquhar MG. Assembly of the glomerular filtration surface. Differentiation of anionic sites in glomerular capillaries of newborn rat kidney. *J Cell Biol*. 1980; 85: 735–753. [[PubMed](#)] [[Free Full text in PMC](#)]
148. Yamada E. The fine structure of the mouse kidney glomerulus. *J Biophys Biochem Cytol*. 1955; 1: 551–566. [[PubMed](#)]
149. Friederici HH. On the diaphragm across fenestrae of capillary endothelium. *J Ultrastruct Res*. 1969; 27: 373–375. [[PubMed](#)]
150. Stan RV, Tkachenko E, Niesman IR. PV1 is a key structural component for the formation of the stomatal and fenestral diaphragms. *Mol Biol Cell*. 2004; 15: 3615–3630. [[PubMed](#)] [[Free Full text in PMC](#)]
151. Stan RV. Multiple PV1 dimers reside in the same stomatal or fenestral diaphragm. *Am J Physiol Heart Circ Physiol*. 2004; 286: H1347–1353. [[PubMed](#)]
152. Stan RV, Kubitza M, Palade GE. PV-1 is a component of the fenestral and stomatal diaphragms in fenestrated endothelia. *Proc Natl Acad Sci USA*. 1999; 96: 13203–13207. [[PubMed](#)] [[Free Full text in PMC](#)]
153. Stan RV, Ghitescu L, Jacobson BS. et al. Isolation, cloning, and localization of rat PV-1, a novel endothelial caveolar protein. *J Cell Biol*. 1999; 145: 1189–1198. [[PubMed](#)] [[Free Full text in PMC](#)]

154. Rostgaard J, Qvortrup K. Electron microscopic demonstrations of filamentous molecular sieve plugs in capillary fenestrae. *Microvasc Res.* 1997; 53: 1–13. [\[PubMed\]](#)
155. Granger DN, Granger JP, Brace RA. et al. Analysis of the permeability characteristics of cat intestinal capillaries. *Circ Res.* 1979; 44: 335–344. [\[PubMed\]](#)
156. Levick JR, Smaje LH. An analysis of the permeability of a fenestra. *Microvasc Res.* 1987; 33: 233–256. [\[PubMed\]](#)
157. Simionescu N, Simionescu M, Palade GE. Permeability of intestinal capillaries. Pathway followed by dextrans and glycogens. *J Cell Biol.* 1972; 53: 365–392. [\[PubMed\]](#) [\[Free Full text in PMC\]](#)
158. Milici AJ, Bankston PW. Fetal and neonatal rat intestinal capillaries: Permeability to carbon, ferritin, hemoglobin, and myoglobin. *Am J Anat.* 1982; 165: 165–186. [\[PubMed\]](#)
159. Lammert E, Gu G, McLaughlin M. et al. Role of VEGF-A in vascularization of pancreatic islets. *Curr Biol.* 2003; 13: 1070–1074. [\[PubMed\]](#)
160. Feng D, Nagy JA, Pyne K. et al. Pathways of macromolecular extravasation across microvascular endothelium in response to VPF/VEGF and other vasoactive mediators. *Microcirculation.* 1999; 6: 23–44. [\[PubMed\]](#)
161. Chen J, Braet F, Brodsky S. et al. VEGF-induced mobilization of caveolae and increase in permeability of endothelial cells. *Am J Physiol Cell Physiol.* 2002; 282: C1053–1063. [\[PubMed\]](#)
162. Milici AJ, L'Hernault N, Palade GE. Surface densities of diaphragmed fenestrae and transendothelial channels in different murine capillary beds. *Circ Res.* 1985; 56: 709–717. [\[PubMed\]](#)
163. Roberts WG, Palade GE. Endothelial fenestrae and fenestral diaphragms. In: Rissau W, Rubanyi GM, eds. *Morphogenesis of Endothelium.* Amsterdam: Hardwood Academic Publishers. 2000: 23–41.
164. Dvorak AM, Kohn S, Morgan ES. et al. The vesiculo-vacuolar organelle (VVO): A distinct endothelial cell structure that provides a transcellular pathway for macromolecular extravasation. *J Leukoc Biol.* 1996; 59: 100–115. [\[PubMed\]](#)
165. Kohn S, Nagy JA, Dvorak HF. et al. Pathways of macromolecular tracer transport across venules and small veins. Structural basis for the hyperpermeability of tumor blood vessels. *Lab Invest.* 1992; 67: 596–607. [\[PubMed\]](#)
166. Vasile E, Dvorak AM, Stan RV. et al. Isolation and characterization of caveolae and vesiculo-vacuolar organelles from endothelial cells cultured with VPF/VEGF and from human lung. *Mol Biol Cell.* 2000; 11: 121.