The role of pinocytic vesicles in the transport of materials across the walls of small blood vessels

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The purpose of this presentation is to focus attention on the pinocytic vesicle system in endothelial and mesothelial cells and to present some results of studies on the structure, function, and biochemical properties of vesicles which might be relevant to the problem of transport across these cells and their membranes.

Small blood vessels can be characterized by the anatomy of their endothelial lining. In one type of vessel the endothelium forms a continuous cell barrier without any apparent discontinuities or channels across the cytoplasm. Vessels of this type are found in cardiac and skeletal muscle, lung, connective tissue, brain, and retina. In this paper this type of vessel will be referred to as “muscle-type” capillaries. A second type of vessel is found in liver, kidney, endocrine glands, intestine, choroid and ciliary body, and other sites. The endothelium which lines capillaries of this type may have openings or holes across the cytoplasm of the endothelial cells (such as are found in liver and glomerular capillaries); or the cytoplasm of the endothelial cells may be extremely thin in places which results in an apparent blood-tissue barrier of the thickness of a single membrane (examples of this type are found in the intestine, endocrine glands, and choroid).

The endothelial cells lining the vessels of the first type (muscle-type) frequently possess a system of microvesicles—called pinocytic vesicles—which line the plasma membranes of these cells. Capillaries in some tissues such as heart and lung have large numbers of these vesicles, while those in the retina and brain have relatively few. When these vesicles were first described it was hypothesized that they might serve as a transport system for the movement of salts, water, and large molecules across the cytoplasm of the endothelial cells. Since this suggestion was proposed many studies have been reported which show that this vesicle system is associated with the movement of tracer particles under certain experimental conditions.

A vesicle system with similar properties is found in mesothelial cells which line the serosal surfaces of the rat mesentry. These mesothelial cells are more accessible to experimental manipulation than are the endothelial cells of capillaries, and they have been used as a model system for combined morphologic and biochemical studies.
Fig. 1. A segment of capillary endothelium from an isolated rat heart perfused with iron oxide. The dense iron particles are seen within pinocytic vesicles. Tissue fixed in OsO₄. (From Jennings, M. A., Marchesi, V. T., and Florey, H. W.: Proc. Roy. Soc., Ser. B. 156: 14, 1962.) (×100,000.)

Fig. 2. Part of a mesothelial cell lining the serosal surface of the rat mesentery. The surface membranes form typical pinocytic vesicles similar to those found in capillary endothelium. Tissue fixed in glutaraldehyde followed by OsO₄. (×60,000.)
Observations

Pinocytic vesicles of vascular endothelium. The evidence that pinocytic vesicles of muscle-type capillaries serve to transport materials across the endothelial barrier is based on studies of electron-dense tracer particles with the electron microscope. Tracer particles such as ferritin or iron oxide were introduced into the circulation of intact animals and, at various times afterward, tissue was removed and processed for microscopic study. The electron-dense tracers could be identified in the bloodstream, and, in time, some particles were also found in the extravascular tissue spaces. Particles were seen within pinocytic vesicles of the endothelial cells but they were not seen in the interspaces between the endothelial cells. Therefore it was concluded that the particles which passed across the endothelial barrier were transported by way of the vesicle system. Further experimental evidence was provided by similar studies using the isolated, perfused heart preparation. A high concentration of tracer particles was introduced directly into the arterial circulation of the hearts, and the transport of particles by the pinocytic vesicles was readily demonstrated (Fig. 1). In this experimental system there was a much greater movement of tracer particles across the endothelium than could be achieved in the intact animal. Even in this case the particles traversed the endothelium only by way of the pinocytic vesicles.

Fig. 3. Part of a mesothelial cell lining the serosal surface of the mesentery. This mesentery was immersed in cold distilled water for two minutes, then fixed immediately in glutaraldehyde followed by OsO₄. The arrows point to distorted remnants of pinocytic vesicles. Most of the vesicles have been pulled out to form linear membranes. (x40,000.)
Pinocytic vesicles of mesothelium. The mesothelial cells which line the serosal surfaces of the mesentery have vesicles which are the same size (700 to 900 Å), and shape, and they occur in the same distribution as those found in heart capillaries (Fig. 2). These vesicles are formed by "unit membranes" which are directly continuous with the linear cell membranes. Apart from the vesicular configuration the membranes which make up the vesicles are indistinguishable from other parts of the cell membrane when viewed by conventional thin section electron microscopy.

To test the stability and reversibility of the vesicles and their membranes, strips of mesentery were incubated in hypotonic media for varying periods of time. Electron micrographs of mesothelial cells at various stages of osmotic lysis show that the pinocytic vesicles pull out as the cells swell, and the vesicle membranes eventually revert to the linear form (Fig. 3).

Transport function of mesothelial vesicles. The same experimental approaches described earlier for the demonstration of transport activity by endothelial cells have been used to show transport activity in mesothelium. Tracer particles introduced into the peritoneal cavities of animals pass across the mesothelium within pinocytic vesicles. However, the mesothelium has an additional virtue as an experimental system in that the transport of tracer materials can be studied easily under controlled in vitro conditions. When strips of mesentery are incubated in vitro in a balanced salt medium containing a tracer particle such as ferritin the particles can be identified.

Fig. 4. Part of a mesothelial cell lining the serosal surface of the mesentery. This mesentery was incubated for five minutes in a balanced salt medium containing 0.1 per cent ferritin, then fixed in glutaraldehyde followed by OsO₄. Ferritin molecules have been taken up from the medium by the mesothelial cell, transported across the cytoplasm, then released into the extracellular space. Some ferritin is still within pinocytic vesicles. (×75,000.)
free in the connective tissue spaces of the mesentery within minutes after initial exposure (Fig. 4). By studying preparations fixed at different intervals after exposure to the ferritin it was clear that the ferritin molecules passed across the mesothelial lining cells by way of the pinocytic vesicles. Ferritin was not seen free in the cytoplasm of intact mesothelial cells nor was any detected in the narrow (100 to 150 Å) spaces between adjacent mesothelial cells.

Properties of an adenosinetriphosphatase associated with pinocytic vesicles. Many observers have reported that blood capillaries of all types have an ATPase activity localized presumably in their endothelial linings.8-12 These observations were based on histochemical techniques using the light microscope. Recently the Wachstein-Meisel lead salt method for the demonstration of ATPase activity13 has been modified for use with the electron microscope, and this technique has been applied to studies of small blood vessels in different tissues.14-16 One of the most interesting findings was the localization of ATPase activity in the pinocytic vesicles in capillaries of the muscle type. The success of this technique was based on the use of glutaraldehyde as a fixative17 since this aldehyde fixative provided preservation of the structure of the tissue which was adequate for electron microscopic study while still retaining some of the enzymatic activity.

Fig. 5 shows the localization of ATPase activity on the membranes of pinocytic vesicles of capillary endothelium. The reaction product is localized only on parts
of the cell membrane which form vesicles; the linear membrane appears unreactive. Since this observation was made on tissue fixed in glutaraldehyde it was not clear whether the linear membrane was truly nonreactive or whether the activity on that portion of the membrane was inhibited by the fixative.

This problem was resolved by incubating fresh, unfixed mesentery in a modified histochemical medium for the demonstration of ATPase activity. Figs. 6 and 7 show clearly that even when the incubation is performed with unfixed tissue the reaction product is confined solely to the inner surfaces of the parts of the cell membrane which form vesicles. This activity was demonstrated in the vesicles in both freshly isolated mesentery and in mesentery which was incubated in vitro for one hour before the reaction was run. This latter observation suggested that the enzyme or enzymes responsible for the reaction were bound to the vesicle membranes. This point was substantiated when histochemical reactions were carried out on mesothelium following lysis by osmotic shock. Fig. 7 shows that even when most of the vesicles were pulled out to form linear membranes they were still reactive, and the reactive substances were not dissolved or destroyed by numerous washes in cold distilled water. Fig. 7 also shows that the enzymatic activity is on the membrane itself and is not dependent upon the vesicular configuration of the membrane.

![Image](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932955/)
Electron microscopic histochemical studies of glutaraldehyde-fixed tissue were also carried out on different vascular beds, and the presence of ATPase activity within the endothelium correlated with the vesicle populations of the different vessels. Thus the endothelial cells lining the capillaries of heart and lung which have large numbers of vesicles were highly reactive,\textsuperscript{14, 15} while the endothelial cells of capillaries of brain and retina with few vesicles were essentially unreactive.\textsuperscript{10-15}

An ATPase activity has been associated with the movements of sodium and potassium across membranes of other cell types such as brain, kidney, and red cells.\textsuperscript{10-23} This activity is stimulated by magnesium, sodium, and potassium and specifically inhibited by the cardiac glycoside ouabain. Thus the demonstration of ATPase activity in the pinocytic vesicles was significant since it raised the possibility that the vesicle membrane might somehow be involved in the transport of small electrolytes such as sodium and potassium as well as large molecules.

In order to determine whether the ATPase associated with the pinocytic vesicles was also activated by Mg, Na, and K and inhibited by ouabain, quantitative assays of ATP hydrolysis were carried out on strips of osmotically lysed mesentery.\textsuperscript{7} Since the ATPase activity of the vesicles remained firmly bound to the mesothelial membranes after lysis—and they seemed to be the only parts of the cell showing activity—it was assumed that the breakdown of ATP reflected the activity of these structures.

The results of these experiments showed that the ATPase of the mesothelial membranes was activated by magnesium alone, and was specific for nucleotide substrates.

**Fig. 7.** Part of a mesothelial cell from a strip of mesentery which was immersed in cold distilled water for two minutes, then incubated unfixed in an ATPase reaction medium for two minutes. Most of the pinocytic vesicles have pulled out to form linear membranes (as shown in Fig. 3). Reaction product is now localized on these linear membranes. (×60,000.)
with a pH optimum in the neutral range. However, there was no indication that the ATPase activity was stimulated by sodium and potassium nor was it sensitive to ouabain. These findings suggested that the ATPase on the vesicles was not similar to the Na-K ATPase of other membranes nor was it due to a nonspecific acid or alkaline phosphatase.

In order to explore further some of the chemical properties of this membrane-bound ATPase and to verify the finding made on the lysed mesentery strips, methods were devised for the isolation of mesothelial cells free from the connective tissue, blood vessels, adipose cells, and assorted cell types of the whole mesentery. Mesenteries were removed from rats following perfusion with saline and incubated in a buffered medium with collagenase (Worthington) which separated the mesothelial cells from the other cell types. The mesothelial cells were preferentially harvested on a discontinuous sucrose gradient. Fig. 8 shows the appearance of isolated mesothelial cells following this treatment. These cells were then disrupted by ultrasonication and a "membrane" fraction was prepared by differential centrifugation (Fig. 9).

Experiments with aliquots of washed and dialyzed membrane fractions gave ATP hydrolysis rates of 20 to 40 micro-

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<tr>
<th>Table I. Properties of membrane bound adenosinetriphosphatase</th>
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<tr>
<td>Activated by Mg$^{2+}$ or Ca$^{2+}$ ions</td>
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<tr>
<td>Not stimulated by Na$^{+}$ or K$^{+}$, or inhibited by ouabain</td>
</tr>
<tr>
<td>pH optimum in 6-8 range</td>
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<tr>
<td>Active toward nucleotide substrates (ATP, ADP, AMP)</td>
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<td>Not active toward glycerophosphate, hexosediphosphate, or glucose-6-phosphate</td>
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Fig. 8. Phase contrast photomicrograph of a preparation of isolated mesothelial cells. These cells were freed from the connective tissue and other cellular elements of the mesentery by the separation of a collagenase-treated preparation on a discontinuous sucrose gradient. The mesothelial cells remain attached to one another in sheets, and this provides the basis for their separation from the other cells.
moles of inorganic phosphate released per milligram of protein per hour. This activity was stimulated by magnesium and calcium independently, and the activity was unaffected by sodium, potassium, or ouabain. These results are summarized in Table I.

**Discussion**

The results presented here show that the pinocytic vesicles of endothelial and mesothelial cells are specific adaptations of the cell membrane which can function in the transport of large molecules. The portions of the cell membrane which form the vesicles appear to have an enzyme system (ATPase) not present on the linear membrane. This ATPase is not a nonspecific acid or alkaline phosphatase nor is it similar to the sodium-potassium activated ATPase found on other cell membranes. Rather, some of the properties of this ATPase are similar to those of myosin ATPase of muscle (activation by magnesium and calcium, insensitivity to ouabain).

The system of pinocytic vesicles as a possible transport mechanism applies to other epithelial barriers as well as the vascular endothelium and mesothelium described above. One stage in the transport of tracer particles across the endothelium

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*Fig. 9. Membrane fraction prepared from isolated mesothelial cells. Mesothelial cells were disrupted by ultrasonication and a membrane fraction prepared by differential centrifugation. This fraction is made up largely of "unit membrane" structures. (x50,000.)*
of the cornea has been shown to involve vesicles,25,26 and recent observations on the movement of tracer particles across the endothelial walls of the canal of Schlemm27 also implicate a vesicle mechanism.

Several important questions and problems relating the pinocytic vesicle system to transport across epithelial barriers remain to be resolved. There is as yet no clear indication as to the physiological significance of the vesicle system in vivo nor is there any simple way to investigate this point. Even if the vesicle system does operate in vivo, there would be no reason to assume that it is the only route—or even the principal route—for the trans-epithelial passage of materials. Thus the demonstration of a transport function by vesicles does not rule out the possibilities of movement via intercellular or other channels.

To assess the possible contribution of the vesicle system to transport across endothelial and mesothelial barriers it might be profitable to find out whether vesicle formation or function can be modified experimentally and what effect this would have on vascular or mesothelial permeability. As one approach to this problem one might explore the hypothesis that the ATPase associated with vesicle membranes plays a role in the formation of the vesicle configuration perhaps in the formation and movement of membrane or its energy utilization analogous in some way to the ATPase involved in muscle contraction.

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