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The Search for Fc Receptors on Human Tissues and Human Endothelial Cells in Culture (41140)

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Abstract. Human endothelial cells in culture and in tissue sections were examined for Fc receptors by several different methods, including the binding of FITC- and ferritin-labeled antibodies to IgG, rosette formation with sensitized sheep erythrocytes, and binding of opsonized latex particles. Although human polymorphonuclear leukocytes reacted positively in all of these tests, endothelial cells freshly isolated or cultured from umbilical cord veins were consistently negative. A small number of cells cultured from umbilical arteries formed rosettes with sheep erythrocytes, but cells from pulmonary arteries did not. Fc receptors could not be found in vessels of the umbilical cord or in lung tissue sections, but vessels in the placenta contained prominent Fc receptors. Cultured endothelial cells that were suspended by trypsin took up latex beads and immune complexes by endocytosis. Undisturbed monolayers of cells were much less active. This finding suggests that the endothelium may be stimulated by proteolytic enzymes to ingest large particles or complexes. and that even without Fc receptors, the endothelium can remove potentially harmful materials from circulation by phagocytosis.

One of the major functions of vascular endothelium is the transport of materials between blood and tissues. This involves membrane transport for some amines (1, 2) and prostaglandins (3), but macromolecules require other mechanisms, such as uptake and transport through cytoplasmic vesicles (4–6). Large particles may be taken up by micropinocytosis (7–9) or phagocytosis (10–15). Since the endothelium is continually exposed to circulating antibodies, antigens, and immune complexes, it may regulate the passage of these agents into the tissues and thereby affect the development of immune reactions.

Several types of cell bind aggregated IgG and immune complexes by interaction between the Fc fragment of the antibody molecule and specific Fc receptors on the cell membrane (16). Lymphocytes (17, 18), macrophages (19–21), and polymorphonuclear leukocytes (21–23) have Fc receptors that can be demonstrated by the binding of antibodies and antibody-bearing cells. Fc receptors have been identified on placental vessels (24–28), trophoblastic tissues (29–31), and on human renal glomeruli (32). Endothelial cells cultured from human umbilical veins are reported to have Fc receptors (33), but cells cultured from bovine pulmonary arteries lack them (34).

The culture of endothelial cells from various human vessels permits the examination of endothelial functions associated with specific vessels (35–39). Endothelial cells cultured from human umbilical veins, umbilical and pulmonary arteries were examined for Fc receptors. Tissue from lung, umbilical cord, and placenta was used to compare the reactions of endothelium *in situ* with those of cultured cells.

Materials and Methods. *Cells and tissues.* Endothelial cells were isolated from veins and arteries of human umbilical cords by brief digestion with trypsin or collagenase as previously described (37, 38). Human pulmonary arterial endothelial cells were isolated from human lungs obtained at autopsy within 3–5 hr postmortem (38). The detached endothelial cells were concentrated by centrifugation, washed once, and plated in culture plates or flasks in Medium 199 (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 20% fetal calf serum and 10% human serum and antibiotics. All cultures were maintained at 37° in an atmosphere of 5% CO_2. The umbilical cord endothelial cells were used

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without transfer, and the pulmonary artery cells were transferred once before examination.

All endothelial cells used in this study were identified by three criteria: their appearance in phase contrast microscopy was that of an epithelioid cell monolayer, they stained positively for factor VIII antigen by indirect immunofluorescence (38, 39), and they had angiotensin I converting enzyme activity, as measured by the hydrolysis of [^{3}H]hippuryl diglycine (37, 38). Since smooth muscle cells and fibroblasts, the only possible contaminating cell types, have different morphologic characteristics from endothelial cells and do not have the endothelial markers, the cells used for this study were considered to be homogeneous cultures of endothelial cells.

Human polymorphonuclear leukocytes were isolated by centrifugation of blood on Ficoll–Hypaque gradients (40) followed by dextran sedimentation. The cells were recovered by centrifugation, washed three times with Hanks' balanced salt solution, and adjusted to a final concentration of 4×10^6 cells/ml before use.

Human placentas and umbilical cords were obtained from normal deliveries or full-term Caesarean sections. Cross-sections of 2–4 mm were cut from the cords and from the vascular portion of the central placental cotyledon. Lung tissue was obtained from autopsy material within 3 hr postmortem. All tissues were washed in phosphate-buffered saline (pH 7.4) to remove blood, and then cut into small pieces and frozen in liquid nitrogen. Cryostat sections (4 μm thick) were cut and air dried on slides without fixation.

Immune reagents. Human Cohn fraction II (Sigma Chemical Co., St. Louis, Mo.) was diluted to 1% in normal saline, heated to 63° for 20 min, and then centrifuged (400*g*, 15 min) to remove large aggregates. Aggregated human IgG conjugated to fluorescein-isothiocyanate (FITC-IgG, Cappel Laboratories, Inc., Downingtown, Pa.) was prepared the same way.

Antiovalbumin antibodies were produced in rabbits in response to injection of 500 μg of ovalbumin in complete Freund's adjuvant. The antiserum was purified on a column of ovalbumin coupled to Sepharose 4B. The equivalence point was estimated from a precipitin curve, and soluble complexes were prepared by addition of four- to ninefold excess antigen to the antiserum.

Sheep erythrocytes (Cordis Laboratories, Miami, Fla.) were washed with saline and suspended at 2% (v/v) in Hanks' balanced salt solution. Equal volumes of cell suspension and rabbit IgG and IgM antibodies to sheep erythrocytes (Cordis Laboratories, Miami, Fla.) were mixed and incubated at 37° for 30 min with gentle shaking. The sensitized cells (EAs) were washed once in Hanks' solution before use.

Methods for Fc receptors. Cultured endothelial cells and cryostat tissue sections were examined for Fc receptors by immunofluorescence, immunoelectron microscopy, rosette formation, and a latex bead binding assay.

i. Immunofluorescence. Endothelial cells were detached from confluent monolayers by brief exposure to trypsin, collagenase, or by scraping with a rubber spatula. After washing with medium 199, the suspension was adjusted to approximately 4×10^6 cells/ml and incubated with aggregated human IgG (1 mg/ml) at 4° for 30 min. The cells were centrifuged, washed, and resuspended to 1 ml with Medium 199. FITC-labeled anti-human IgG (0.2 ml) was added, and the cells were allowed to stand for 20 min at 4°. The cells were then washed three times and finally resuspended to 0.25 ml in Medium 199. A drop of cell suspension was examined by light and fluorescence microscopy. Cell suspensions that were incubated only with FITC-labeled antibody were controls. Suspensions of polymorphonuclear leukocytes were treated in a similar manner.

Monolayers of cultured endothelial cells or air-dried tissue sections were also examined for Fc receptors by immunofluorescence. The cultured cells were washed with Medium 199, covered with a solution of aggregated human IgG, and incubated for 20 min at 4°. The protein solution was removed, the monolayers were washed, and FITC-labeled anti-human IgG was added.

Control cultures were incubated only with antibody after exposure to Medium 199 alone. After a second incubation for 20 min at 4°, the cells were washed, covered with 90% glycerine in phosphate-buffered saline, and examined by light and fluorescence microscopy. Tissue sections were treated with aggregated human FITC-labeled IgG in a moist chamber for 30 min and then washed and mounted as described. Some sections were treated with unlabeled aggregated IgG prior to addition of the FITC-labeled material to establish that FITC binding was specific for aggregated IgG.

ii. Electron microscopy. Suspended endothelial cells were incubated with aggregated IgG and washed as described above. Controls were incubated without IgG. The cells were centrifuged to a pellet and fixed with 2.5% glutaraldehyde. After washing overnight in phosphate-buffered saline, the cells were incubated with ferritin-conjugated anti-human IgG (Cappel Laboratories, Inc., Downingtown, Pa.) diluted 1:10 for 20 min at room temperature. The samples were washed, centrifuged to concentrate the cells, and postfixed with 2% osmium tetroxide before embedding in Epon. Small pieces of umbilical and placental tissues were incubated with aggregated IgG and ferritin–antibody in a similar manner.

iii. Rosette formation. Untreated (E) and antibody-bearing (EA) erythrocytes were mixed with suspended cells or layered onto cell monolayers or tissue sections. The samples were incubated for 30 min in a moist chamber at room temperature, then washed gently with balanced salt solution, and examined by phase contrast or light microscopy for adherence of erythrocytes. Some tissue sections were fixed with 4% formaldehyde and stained with hematoxylin and eosin prior to inspection. A positive rosetting test was recorded if more than five erythrocytes adhered to a single endothelial cell at the cell membrane.

iv. Latex particle binding. Polystyrene latex beads coated with aggregated human IgG (RA test, Hyland Laboratories, Costa Mesa, Calif.) were washed three times in Hanks' solution and resuspended at a concentration of 2.5 mg/ml of protein. One milliliter of endothelial or neutrophil cell suspension was mixed with an equal volume of beads and incubated for 30 min at 4°. The cells were washed to remove unbound beads and fixed for electron microscopy.

Phagocytosis was stimulated in endothelial cells and leukocytes by adding human aggregated IgG, latex particles, or immune complexes. Particles within cells were then identified by immunofluorescence or electron microscopy. Cell suspensions or endothelial monolayers were incubated with IgG, latex, or immune complexes for 1 hr at 37°. The preparations were washed, and cytocentrifuge slides were made from the cell suspensions. The slides or cell monolayers in flasks were fixed, and processed for microscopy as described above.

Results. *Identification of Fc receptors.* Although several different methods were used to detect Fc receptors, they could not be found on either freshly isolated or cultured endothelial cells from umbilical cord veins. A small number of cells cultured from umbilical arteries and suspended with collagenase before testing formed rosettes with sheep EAs, but pulmonary arterial endothelial cells were consistently negative when tested with this method. In contrast, polymorphonuclear leukocytes were strongly positive for Fc receptors with any of the methods applied. Table I summarizes the results of immunofluorescence, immunoelectron microscopy (ferritin-labeled anti-IgG), IgG-EA rosette formation, and binding of opsonized latex beads. Table II compares the reaction of endothelial cells with EAs and the reaction of neutrophils with EAs in the rosette forming assay.

Among the tissues tested for IgG binding, both lung and umbilical cord were negative, but placental tissue displayed Fc receptors by all three of the methods used (Table I). In addition, when the rosette forming test was applied to cryostat sections of placenta, sheep erythrocytes (EAs) clustered around the vessels in the tissue. Figure 1 contrasts the binding of EAs to placental tissue and umbilical cord.

Phagocytosis of immune complexes and latex beads. Polymorphonuclear leuko-

TABLE I. DETECTION OF Fc RECEPTORS IN CULTURED ENDOTHELIAL CELLS AND TISSUES

Sample	FITC-anti-IgG	Ferritin-anti-IgG	IgG-EA rosettes	Latex bead binding
Endothelial cells				
Umbilical Vein				
Freshly isolated T	–	–	–	–
Freshly isolated C	–	N.D.	–	N.D.
Suspended T	–	–	–	–
Suspended S	–	–	–	–
Monolayer	–	–	–	–
Umbilical artery				
Suspended C	–	N.D.	±	N.D.
Monolayer	–	N.D.	–	N.D.
Pulmonary artery				
Suspended	–	N.D.	–	N.D.
Monolayer	N.D.	N.D.	–	N.D.
Polymorphonuclear Leukocytes	+	+	+	+
Tissue sections				
Lung	–	N.D.	–	N.D.
Umbilical cord	–	N.D.	–	N.D.
Placenta	+	+	+	+

Note. N.D., not done; T, trypsin; C, collagenase; S, scraped. Endothelial cells were isolated by either trypsin or collagenase treatment of vessels, and cultured cells were suspended by trypsin or collagenase treatment of the monolayers. Some cultures of umbilical venous cells were detached by scraping.

cytes readily took up opsonin-coated latex beads in preference to uncoated beads, and the particles could be seen in the cytoplasm with transmission electron microscopy. Endothelial cells took up opsonized and unopsonized beads equally well. Figure 2 shows several beads in the cytoplasm of an endothelial cell, and others can be seen entering the cell by endocytosis. Monolayers of endothelial cells did not take up latex particles, and only occasionally took up insoluble immune complexes.

Discussion. We examined cultured endothelial cells for the presence of Fc re-

TABLE II. ROSETTE FORMATION WITH SENSITIZED SHEEP ERYTHROCYTES (SRBC) AND ENDOTHELIAL CELLS OR POLYMORPHONUCLEAR LEUKOCYTES

Cell type	Treatment	Percentage rosettes formed	
		Sensitized SRBC	Unsensitized SRBC
Umbilical vein	Freshly isolated	4 (0–6)	3 (2–4)
	Cultured (trypsin)	2 (0–4)	4 (1–6)
	Cultured (collagenase)	0	0
	Cultured (scraped)	3 (2–4)	2 (1–4)
Umbilical Artery	Cultured (collagenase)	14 (11–20)	0
Pulmonary Artery	Cultured (collagenase)	0	0
Polymorphonuclear neutrophils	Suspended	83 (80–85)	2 (0–4)

Note. Endothelial cells were freshly isolated from umbilical cord veins by either trypsin or collagenase digestion. the cultured cells were detached from monolayer cultures by treatment with trypsin, collagenase, or by scraping with a rubber spatula. Data are percentage rosettes formed, and numbers in parentheses are the range of values in two to four separate determinations.

ceptors for IgG by several different methods, including the binding of labeled antibodies to IgG, rosette formation with sensitized sheep erythrocytes, and the binding of opsonized latex particles. Human polymorphonuclear leukocytes reacted positively in each of these tests, but endothelial cells were almost entirely negative. Cells cultured from umbilical arteries were weakly positive in a rosetting assay, but cells from umbilical veins or pulmonary arteries were consistently negative.

Although enzymes used to suspend cultured cells might affect the protein components of the cell membrane, there were no demonstrable Fc receptors on intact monolayers or on cells suspended by scraping. Thus, it is unlikely that enzymatic dispersion of the cells destroys Fc receptors.

Dedifferentiation of cells in culture might account for loss of surface receptors. However, sections of both umbilical cord and lung that contained vessels with intact endothelium were negative by several different methods. In addition, primary cultures of umbilical venous endothelial cells contain a large number of cells from the original vessel, and it is unlikely that Fc receptors would be lost in the brief (approximately one week) period before testing.

Other investigators failed to detect Fc receptors on bovine pulmonary arterial cells by rosette formation with sheep erythrocytes (34). However, Shadforth and coworkers reported that Fc receptors on endothelial cells from human umbilical veins can be demonstrated by direct binding of FITC-aggregated human γ-globulin (33). We used an indirect immunofluorescence method which involves reaction with unlabeled human aggregated IgG followed by FITC-labeled anti-human IgG. This method clearly demonstrates Fc receptors on polymorphonuclear neutrophils, and it is more sensitive than direct immunofluorescence (41). A recent report by Lyss *et al.* (42) suggests that viral infection of endothelium can stimulate the expression of IgG and C3 receptors. These authors found that neither IgG- nor IgM-sensitized sheep erythrocytes adhered to normal endothelial cells, even in the presence of purified complement components. However, cells that were infected with herpes simplex virus, type I, bound the sensitized erythrocytes in rosette formation (42).

As others have reported (24) we found that Fc receptors were present on vessels in sections of human placenta. Placenta also has receptors for IgG on trophoblastic tissue (24–28) that may participate in active transfer of maternal IgG to the fetus (29, 31). The placental Fc receptors may protect the fetus from immune complexes that form within the placental circulation following transfer of maternal IgG antibodies against paternally derived fetal alloantigens.

The lack of Fc receptors on umbilical veins may indicate that these vessels are simply conduits that are not involved in the removal of immune complexes. The lack of Fc receptors in adult lung tissue and pulmonary endothelial cells is more surprising, since the lung is a target organ for immune complex disease (43). Possibly, as suggested by studies in virally infected cells (42), the expression of Fc receptors is controlled by external factors.

The phagocytic function of endothelial cells may be more important than receptor binding of immunoglobulins for transport and disposal of immune reactants. Although binding and subsequent phagocytosis of immune complexes and foreign particles by neutrophils involves Fc receptors (44, 45), phagocytosis by endothelial cells appears to depend upon other mechanisms. The situation is complicated by the fact that some forms of endothelial transport involve endocytosis and vesicle formation distinct from phagocytosis (4–9). Since the cultured endothelial cells in our experiments took up both opsonized and unopsonized latex particles, as well as immune complexes, the reaction is probably nonspecific. However, these findings suggest that endothelial cells can remove particles or potentially damaging agents from circulation if a cell membrane stimulus is provided. Trypsin used to suspend the cells could be such a stimulus. Suspended endothelial cells readily took up latex particles and insoluble immune complexes, but cell monolayers did not. Trypsin can affect

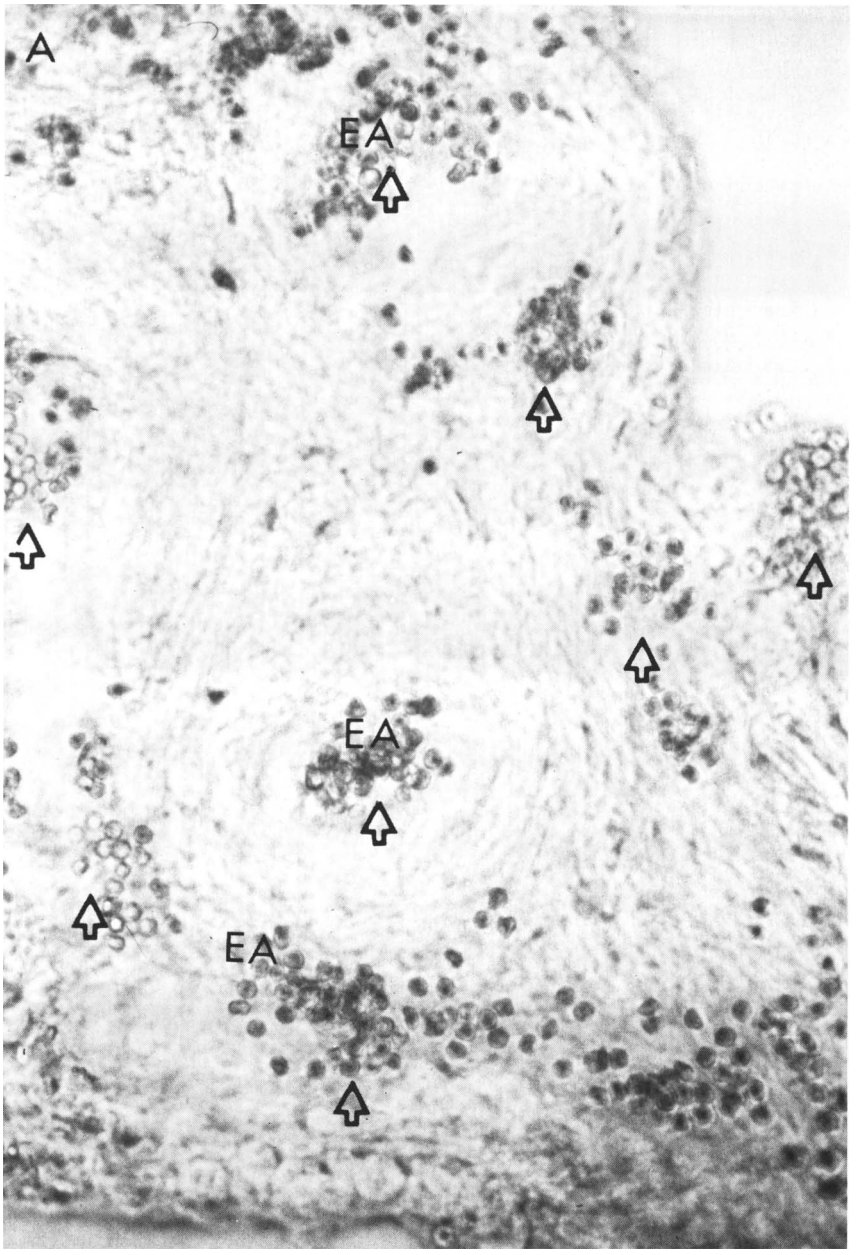

FIG. 1. Binding of sensitized sheep erythrocytes (IgGEA) to tissues. (Magnif = 200×). (A) Erythrocytes are bound to placental vessels in clusters (EA) indicated by the arrows.

the endothelial cell membrane, as indicated by its ability to stimulate formation of prostacyclin (46).

Several studies indicate that endothelium has a phagocytic function in vivo (10–15), and it has been suggested that vasoactive agents, such as histamine, serotonin, or angiotensin (13) stimulate this process. However, phagocytosis is also influenced by the size of the material to be ingested (47).

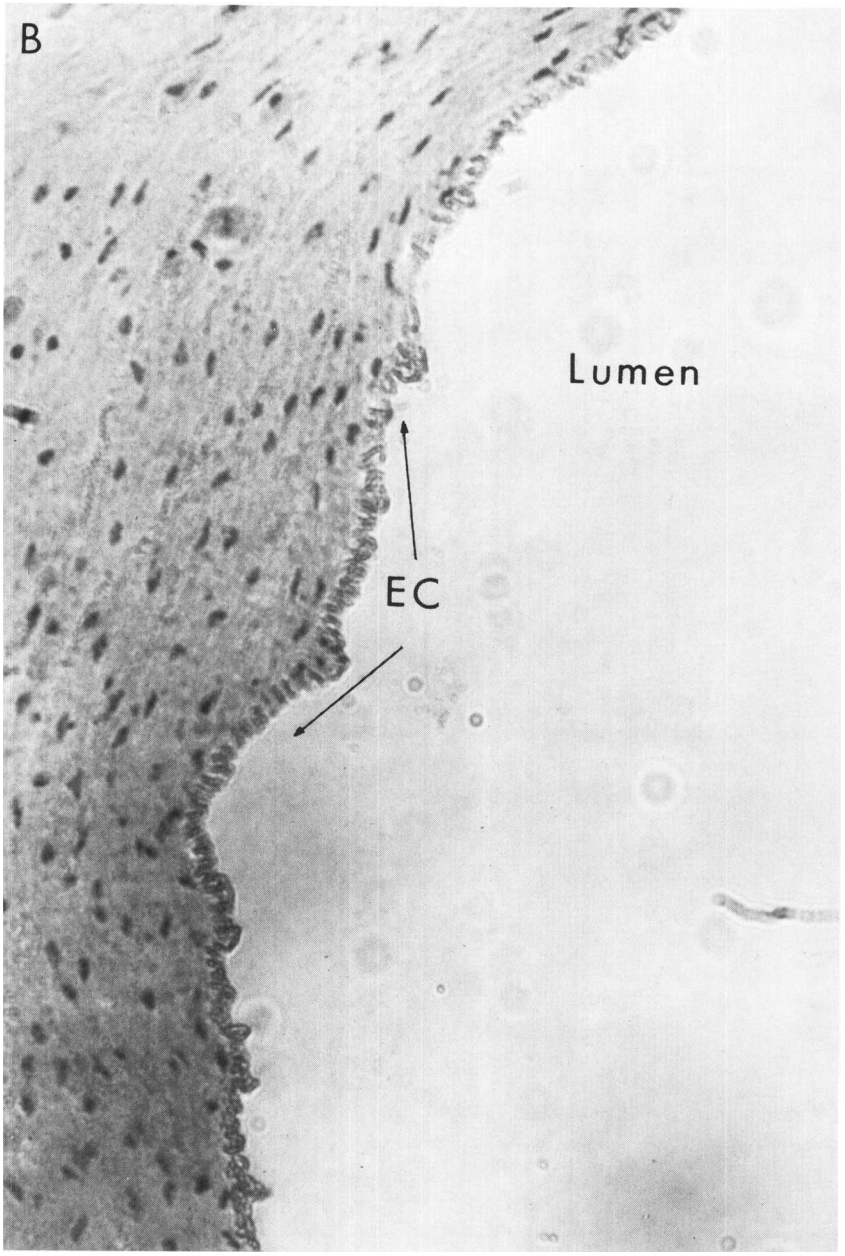

FIG. 1. (B) No bound erythrocytes are seen in section through umbilical cord. The vessel lumen is on the right and the endothelium (EC) is indicated by arrows.

Even though the reticuloendothelial system plays a major role in removal of immune complexes from circulation (10, 48), complexes and carbon markers can be found within the walls of small vessels (48) and glomeruli (49) following the injection of preformed complexes into experimental animals. Phagocytosis by endothelium is most evident when the reticuloendothelium is overloaded with carbon (10). Our experiments with cultured endothelial cells support a phagocytic function for endothelium, and suggest that this function may be stimulated by proteases or other

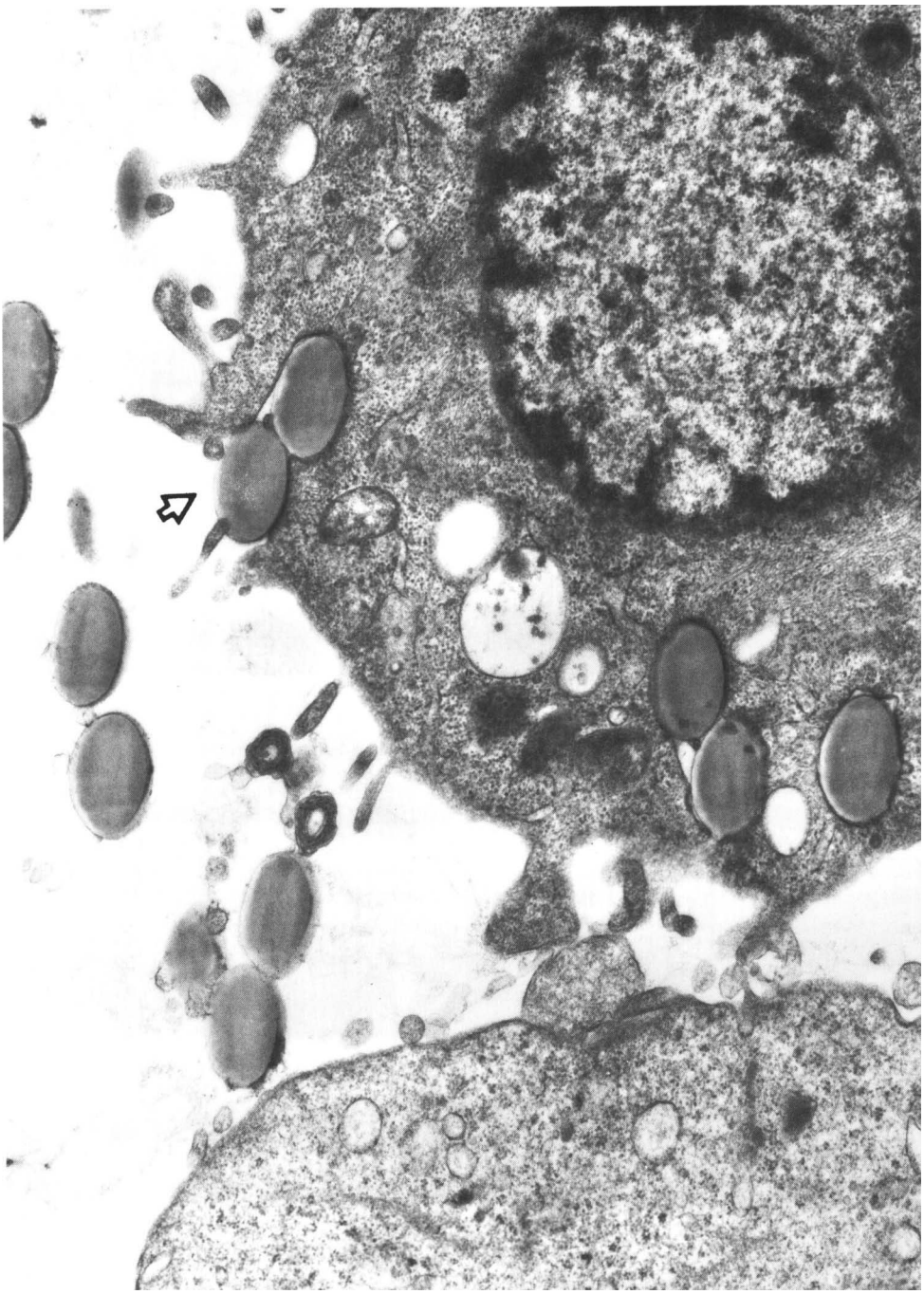

FIG. 2. Phagocytosis of latex particles by cultured endothelial cell. Endothelial cells cultured from human umbilical veins were suspended with trypsin (0.25%) and incubated with latex beads. Note beads in vesicles within the cytoplasm and incoming beads at arrow. Magnif = 20,000×.

materials that affect the cell membrane. Such a mechanism could contribute to the development of immune complex vasculitis by facilitating transport of complexes into the vessel wall.

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