Specific Binding Sites for Albumin Restricted to Plasmalemmal Vesicles of Continuous Capillary Endothelium: Receptor-mediated Transcytosis

Lucian Ghitescu, Anton Fixman, Maya Simionescu, and Nicolae Simionescu

Institute of Cellular Biology and Pathology, Bucharest-79691, Romania

Abstract. The interaction of homologous and heterologous albumin-gold complex (Alb-Au) with capillary endothelium was investigated in the mouse lung, heart, and diaphragm. Perfusion of the tracer in situ for from 3 to 35 min was followed by washing with phosphate-buffered saline, fixation by perfusion, and processing for electron microscopy. From the earliest time examined, one and sometimes two rows of densely packed particles bound to some restricted plasma membrane microdomains that appeared as uncoated pits, and to plasmalemmal vesicles open on the luminal front. Morphometric analysis, using various albumin-gold concentrations, showed that the binding is saturable at a very low concentration of the ligand and short exposure. After 5 min, tracer-carrying vesicles appeared on the abluminal front, discharging their content into the subendothelial space. As a function of tracer concentration 1–10% of plasmalemmal vesicles contained Alb-Au particles in fluid phase; from 5 min on, multivesicular bodies were labeled by the tracer. Plasma membrane, coated pits, and coated vesicles were not significantly marked at any time interval. Heparin or high ionic strength did not displace the bound Alb-Au from vesicle membrane. No binding was obtained when Alb-Au was competed in situ with albumin or was injected in vivo. Gold complexes with fibrinogen, fibronectin, glucose oxidase, or polyethyleneglycol did not give a labeling comparable to that of albumin. These results suggest that on the capillary endothelia examined, the Alb-Au is adsorbed on specific binding sites restricted to uncoated pits and plasmalemmal vesicles. The tracer is transported in transcytotic vesicles across endothelium by receptor-mediated transcytosis, and to a lesser extent is taken up by pinocytotic vesicles. The existence of albumin receptors on these continuous capillary endothelia may provide a specific mechanism for the transport of albumin and other molecules carried by this protein.

ALBUMIN is largely responsible for the oncotic pressure of plasma and interstitial fluid and serves as a carrier for important molecules such as fatty acids, testosterone, estradiol, and thyroid hormones (4, 7, 12, 16, 36). In heart myocytes (12) and hepatocytes (16), it has been shown that the uptake of fatty acids is mediated by an albumin receptor.

Although the endothelial transport of serum albumin was extensively investigated, the cellular mechanism of this phenomenon is unknown. Physiological data obtained have been interpreted by some investigators to be indicative of a prevalent convective process (15, 21, 31), whereas others consider that the estimation of albumin transport by the so-called "albumin escape rate" lacks any physiological significance (17, 18). Estimates of lymph/plasma ratio revealed that the amount of albumin passing from plasma into the interstitial fluid varies greatly from one organ to another (6, 19, 20, 23, 33, 38–40). Considering the type of endothelium (continuous, fenestrated, discontinuous) encountered in these various organs, no firm correlation can be established between the endothelial structure and the rate of albumin transport. Thus, other properties of endothelial cell may account for the remarkable difference in the rate, speed, and kinetics of albumin uptake in various tissues.

We report evidence that in the capillary endothelium of the mouse lung, heart, and diaphragm, the albumin transport occurs by a process that implies specific adsorption on binding sites restrictively located to plasmalemmal vesicles. Part of these results were previously presented in abstract form (11, 26).

Materials and Methods

Animals

Reagents

polyethyleneglycol, M_r 20,000 (PEG)[1] from J. T. Baker Products, Phillipsburg, NY; and native ferritin 6× crystallized, cadmium free, pI 3.9-4.2, from Miles Laboratories, Inc., Elkhart, IN. Human plasma fibronectin was prepared according to the method of Vuento and Vaheri (35).

Preparation of Colloidal Gold

Colloidal gold particles with an average diameter of 5 nm were prepared by reducing HAuCl₄ with white phosphorus (28). To the monodisperse solution of colloidal gold, one of the following macromolecules was added in the concentration (per milliliter Au solution) indicated: BSA, pI 4.75 (100 μg), (b) mouse albumin, pI 4.7 (100 μg); as other plasma proteins we used (c) bovine fibrinogen, pI 4.3-4.55 (100 μg) and (d) human fibronectin, pI 6.0 (30 μg); as a nonplasmatic protein we used (e) glucose oxidase, pI 3.8-5.2 (100 μg); and as an uncharged molecule we used (f) PEG, M_r 20,000 (1 mg). The pI values were determined by chromatofocusing in a fast protein liquid chromatography system using a Mono P column equilibrated with 20 mM bis Tris-HCl buffer, pH 6.4, and eluted with Polybuffer 74 pH 4.0 (Pharmacia Biotechnology, Uppsala, Sweden). All mixtures were centrifuged at 170,000 g for 1 h; the pellets were resuspended in phosphate-buffered saline (PBS). The complexes obtained were mouse albumin-gold, bovine albumin-gold, collectively termed Alb-Au, fibrinogen-gold, fibronectin-gold, glucose oxidase-gold, and PEG-Au. The pI of one complex, as estimated by isoelectrofocusing in 1% Agarose IEF, Pharmalyte pH 3.5-10.0 (Pharmacia Biotechnology), was not much different from that of the protein used, e.g., Alb-Au, pI 5.2 (vs. albumin pI 4.75). As determined by ¹²⁵I-BSA adsorbtion, 1 ml gold colloid at a concentration having $A^{0.1cm}_{515nm} = 0.1$ was found to retain 18-40 μg BSA (different tracer preparations). Before use, the solutions were centrifuged for 5 min at ~12,000 g; aliquots examined by electron microscopy showed that the complexes were in monomeric dispersion.

Experiments with Albumin-Gold

In Situ. The inquiry was addressed to three capillary networks provided with continuous endothelium: lung, heart, and diaphragm. For heart and diaphragm experiments, after ether anesthesia and laparotomy, a catheter introduced into the abdominal aorta was used as inlet, and another catheter was placed into vena cava abdominalis as outlet. The vasculature was washed free of blood by perfusing PBS at 37°C for 5 min at a flow rate of 3 ml/min. For experiments on lung, after thoracotomy, a catheter was placed into the pulmonary artery, and the right atrium was cut open. In most experiments we used BSA-gold complex as probe. Alb-Au at 37°C and at concentrations corresponding to $A^{0.1cm}_{515nm} = 0.1$ and 0.4 was perfused continuously at a flow rate of 3 ml/min for 3, 5, or 10 min. In lung experiments the tracer at concentration of 0.4 was administered intermittently in 0.1-ml aliquots for 3, 5, 10, 15, and 35 min, to a total of 1.0 ml. The unbound tracer was washed out by perfusing PBS, at 37°C, 3 ml/min, for 3 min. Fixation was carried out by perfusing either the triple fixative (27) or 2.5% formaldehyde, 1.5% glutaraldehyde, and 2.5 mM CaCl₂ in 0.1 M HCl-Na cacodylate buffer, pH 7.4; immersing tissue specimens for 60 min in the triple fixative; then staining en bloc with 0.5% uranyl acetate. Specimens were embedded in Epon-812 by the standard procedure. Thin sections cut on an American Optical Ultracut Microtome (American Optical Scientific Instruments, Buffalo, NY) or a Reichert Ultramicrotome (Reichert Scientific Instruments, Buffalo, NY) were stained with uranyl acetate and lead citrate, and examined with a Philips 400-HM electron microscope.

In Vivo. After anesthesia and laparotomy, the portal vein and hepatic artery were ligated to minimize the tracer uptake by the liver. 200 μl Alb-Au at concentration of $A^{0.1cm}_{515nm} = 8.0$ was injected into the iliac vein. After 3 min, small fragments of heart and diaphragm were fixed by immersion in the same aldehyde mixture as above, then postfixed in buffered OsO₄ and further processed for electron microscopy.

Control Experiments

Control experiments were of the same general design as the Alb-Au experiments in situ, but with certain distinctive steps.

Competition with Albumin. The lung vasculature was washed free of blood with PBS containing 0, 0.1, 0.25, 1.0, 2.5, and 30 mg/ml BSA. The tracer (Alb-Au at $A^{0.1cm}_{515nm} = 0.1$), perfused for 3 min, and the post-tracer PBS contained the same concentration of competing albumin. In diaphragm and heart, this competition was done with 3% BSA only.

Effect of High Ionic Strength. After perfusion of 1 ml Alb-Au ($A^{0.1cm}_{515nm} = 0.1$) and 20 mg native ferritin pI 3.9-4.2 in PBS, the vessels were washed with 10

ml PBS containing 0.45 M NaCl.

Competition with Heparin. To elucidate if charge interactions play any significant role in the binding of the Alb-Au to the endothelial cell surface, we paralleled the high ionic strength effect with competition experiments using as a competitor a strongly anionic macromolecule, heparin. Perfusion was performed for 3 min with the above mentioned mixture of tracers and then with PBS, both containing 4 mg/ml of heparin.

Experiments with Fibrinogen-Gold. The tracer perfused was 6 ml fibrinogen-gold given over 3 min.

Experiments with Fibronectin-Gold. Instead of Alb-Au, 6 ml of fibronectin-gold were perfused for 3 min.

Experiments with Glucose Oxidase-Gold. The vasculature was perfused with 6 ml of the gold complex with this nonplasmatic protein, at the same flow rate as above.

Experiments with PEG-Gold. Performed both in situ and in vivo, these procedures consisted of perfusing, instead of Alb-Au, similar amounts of PEG-Au for from 3 to 10 min (in situ) or 3 min (in vivo).

Specimens collected from all these control experiments were prepared for electron microscope examination as described above.

Morphometric Analysis. To assess the saturability of Alb-Au binding, 6 ml tracer at concentrations of $A^{0.1cm}_{515nm} = 0.01, 0.025, 0.05, 0.1, 0.25, 0.53$, and 0.95 were continuously perfused through the lung at 2 ml/min under the general experimental conditions described above under In Situ. The effect of competing BSA on Alb-Au binding was quantitated by including in this morphometric analysis the set of experiments described under Competition with Albumin. For each experiment six blocks were selected from different regions of the lung and 30 micrographs were taken randomly at the same magnification. On each print at a final magnification of 97,500 the integrated lengths of the plasma membrane proper profile and of the uncoated pits and plasmalemmal vesicles open to the capillary lumen were measured with a planimeter (Keuffel and Esser Co., Switzerland). Concomitantly, the gold particles within the three topographical locations were recorded.

Results

General

The interaction of Alb-Au with endothelium was similar in the three capillary beds examined but the transendothelial passage of this complex was more rapid and more pronounced in the lung than in the heart and diaphragm microvessels. As a general pattern, the Alb-Au binding was restricted to some microdomains of the plasma membrane appearing as small (~100-150 nm in diameter) uncoated pits, and to the membrane of plasmalemmal vesicles. In these two locations most particles were characteristically adsorbed in one or sometimes two rows. Although the endothelial transit is known to be slow and relatively reduced in situ, Alb-Au was markedly transported by vesicles through the endothelial cell, and to a lesser extent was also internalized into multivesicular bodies. The results obtained with homologous (mouse) and heterologous (bovine) albumin were similar.

Binding of Alb-Au

From the earliest time points examined (3 min), Alb-Au particles were characteristically bound primarily to two restricted microdomains of the luminal endothelial surface: small uncoated pits (Fig. 1), and the great majority (~90%) of open plasmalemmal vesicles (Figs. 2 and 3). Transitional forms between labeled uncoated pits and labeled vesicles were frequently detected. Vesicle diaphragms were not particularly conspicuous, though a fuzz could be observed in some cases (Fig. 3). The plasma membrane and coated pits were essentially unlabeled. As seen in cross-section, particles were usually adsorbed on vesicle membrane in a single or two rows at an average density of ~10-15 particles per vesicle profile cut at its largest diameter (~80 nm) (Figs. 2 and 3). In ~10% of plasmalemmal vesicles, Alb-Au particles appeared randomly
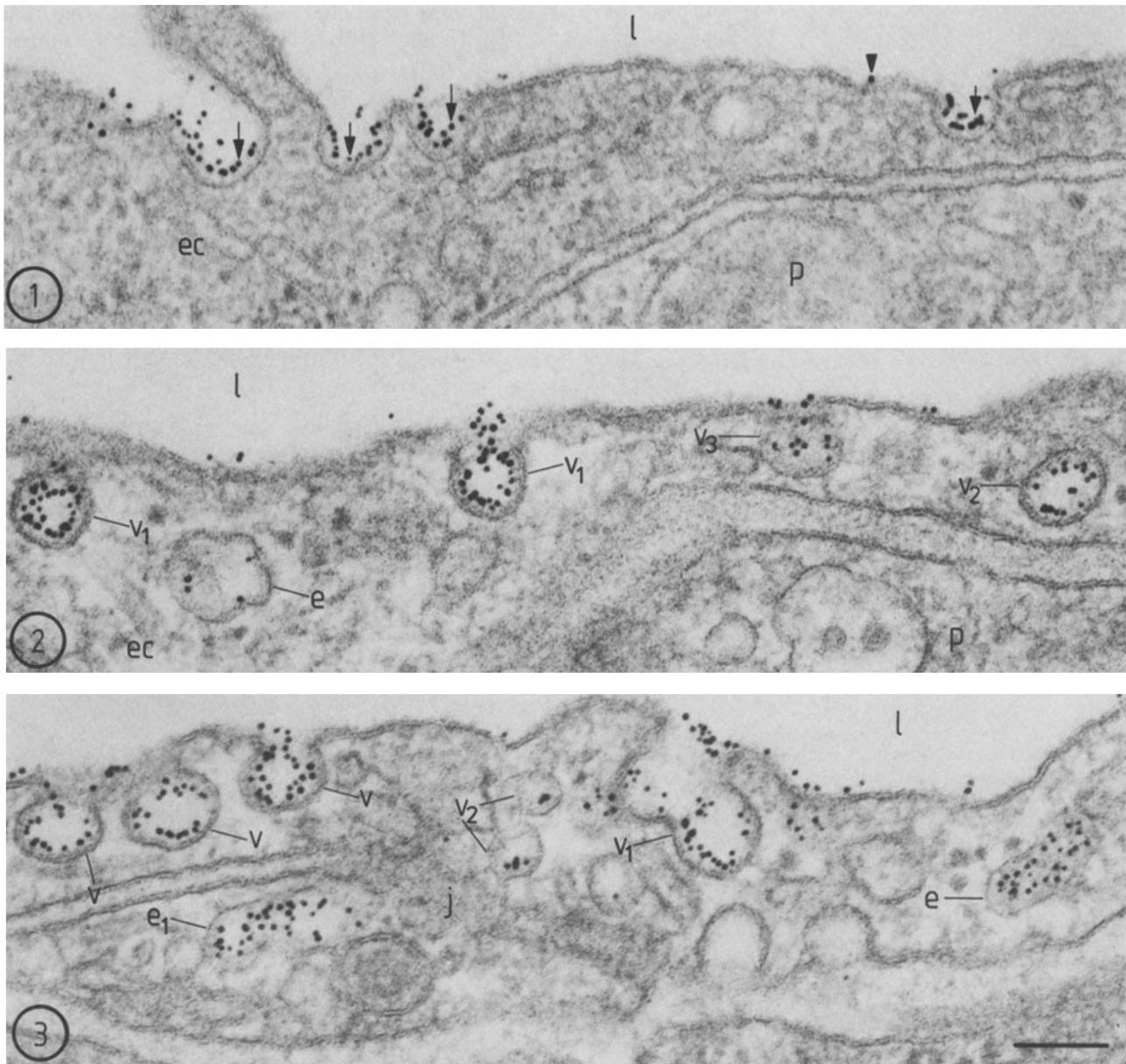
Figures 1–3. These and all other figures illustrate the interaction of bovine serum Alb-Au with alveolar capillary endothelium at different time intervals of tracer perfusion in the mouse lung. Abbreviations used in Figs. 1–10: *ec*, endothelial cell; *e*, endosome; *v*, plasmalemmal vesicle; *l*, lumen; *p*, pericyte; *pm*, plasma membrane. Fig. 1: At 3 min, one or two rows of Alb-Au particles were adsorbed on uncoated pits (arrows) of the luminal aspect of the endothelial cell (*ec*). Extremely rare particles could be detected on plasma membrne (arrowhead). Fig. 2: At 3 min, most plasmalemmal vesicles open on the luminal front (v_1) or apparently internalized (v_2) were marked by one or two rows of densely packed particles adsorbed on vesicle membrane. In v_3, the section is probably tangential through a labeled vesicle. Polymorphic, larger vesicles contained an amorphous osmophilic material (in contrast to the relatively clear content of plasmalemmal vesicles [washed by PBS]), tentatively identified as endosomes (*e*) were marked by various number of particles. Fig. 3: At 5 min, while separate (*v*) or fused plasmalemmal vesicles (v_1) were marked by adsorbed Alb-Au particles, most of these were accumulated within tubulovacuolar structures tentatively identified as endosomes (*e*). The structure in e_1 probably belongs to another endothelial cell (tangential section through junction, *j*). v_2, tangentially cut vesicles. Bar, 1 μm for all figures.

distributed, suggesting a fluid-phase uptake (Fig. 6). The morphometric analysis of the Alb-Au binding at different tracer concentrations showed that the plasmalemmal vesicle membrane had an affinity for Alb-Au five to six times higher than the plasma membrane proper (Fig. 11). The shape of the curve depicting the number of Alb-Au particles bound per micrometer real length of open plasmalemmal vesicle membrane as a function of tracer concentration suggested, within a rather large dispersion of values, that the degree of vesicle labeling increased with ligand concentration before reaching a saturation level of ~60 gold granules/μm of vesicle surface profile.

Transcytosis of Alb-Au

At 3 to 5 min, some labeled vesicles were apparently internalized while maintaining the one or two rows of adsorbed

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particles. Starting at 5 min and increasing with time and concentration, a number of vesicles were detected discharging their content on the abluminal endothelial front: particles appeared shortly retarded against the basal lamina (Figs. 4–7). At 10 min, Alb-Au particles were seen in the subendothelial interstitia (Fig. 7). Occasionally, tracer particles were detected along transendothelial channels and beyond their abluminal openings (Fig. 5). Alb-Au particles were not observed in intercellular junctions at any time interval. The appearance of Alb-Au particles in the subendothelium was usually more rapid in the lung than in heart and diaphragm capillaries.
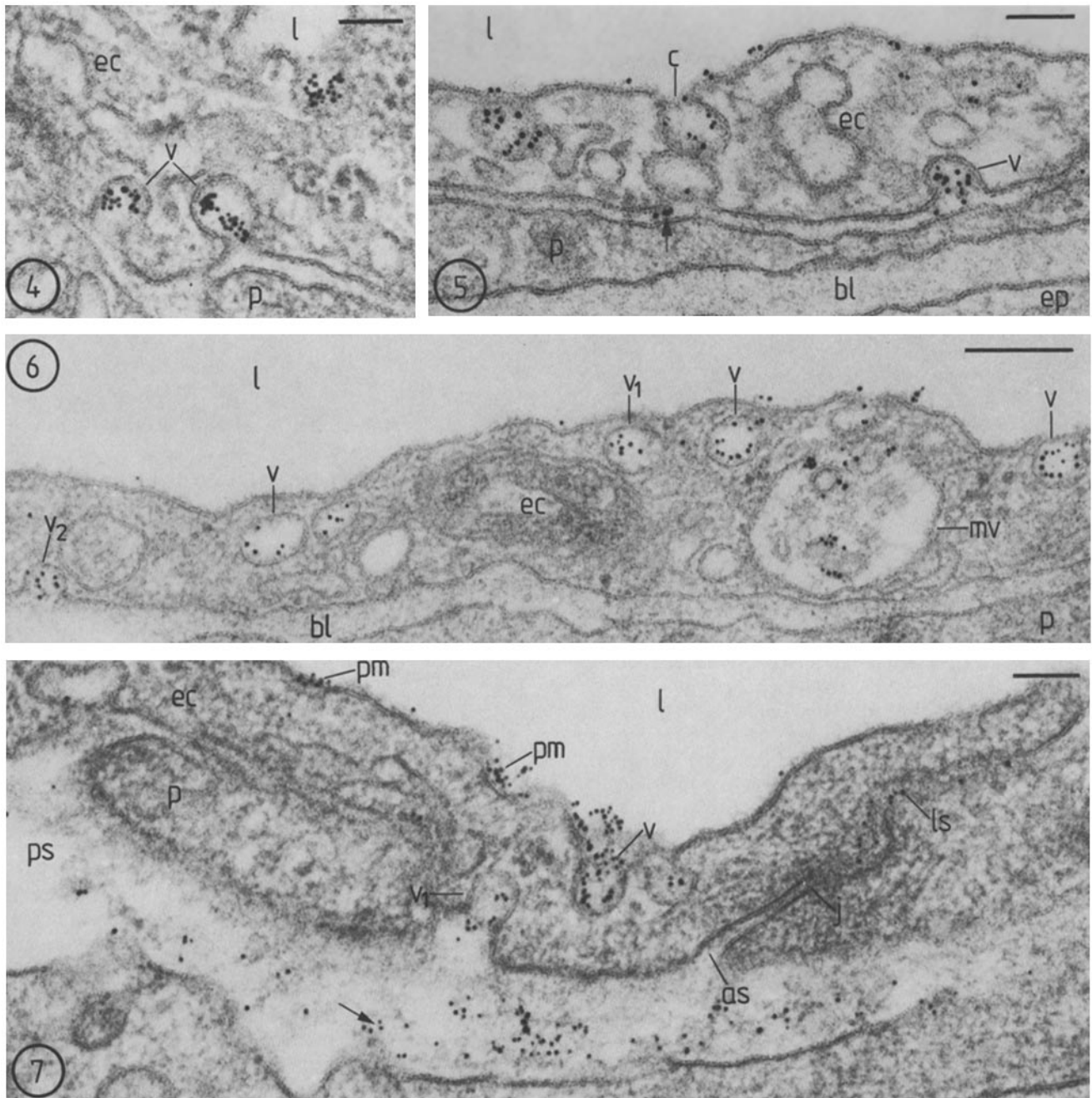
Figures 4–7. Fig. 4: At 10 min, transcytotic vesicles (*v*) opened on the abluminal front of endothelium (*ec*). Bar, 0.1 μm. Fig. 5: At 5 min, occasional transendothelial channels (*c*) were marked by Alb-Au particles, some of which occurred at their abluminal opening (arrow). *V*, a transcytotic vesicle discharging its particles into the subendothelial interstitium. *bl*, basal lamina; *ep*, alveolar epithelial cell type II. Bar, 0.1 μm. Fig. 6: Characteristic picture at 10 min and longer intervals: Alb-Au particles were adsorbed on the membrane of most plasmalemmal vesicles in different locations (*v*) including those open on the abluminal front (v_2), mark vesicles in which the uptake appeared to be in fluid phase (v_1), and decorated multivesicular bodies (*mv*). Bar, 0.2 μm. Fig. 7: At 35 min (arrow) numerous Alb-Au particles appeared in the pericapillary space (*ps*), apparently discharged by vesicles open on the abluminal front (v_1). Some particles occurred in the luminal part of the intercellular space (*is*) but were absent from the abluminal part of the latter (*as*) beyond the junction (*j*). Bar, 0.1 μm.
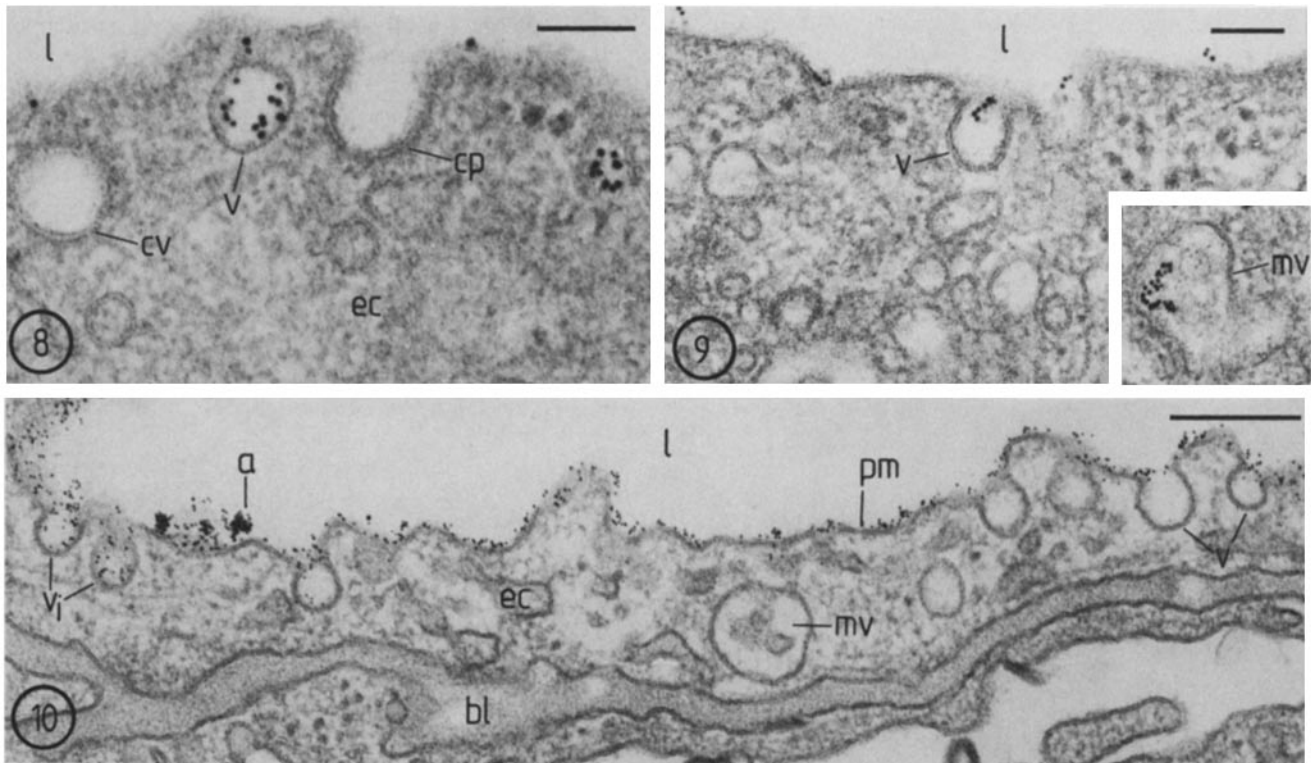
Figures 8–10. Fig. 8: While plasmalemmal vesicles were consistently labeled by Alb-Au particles, neither coated pits (*cp*) nor coated vesicles (*cv*) bound significantly this tracer. Bar, 0.1 μm. Fig. 9: Competition with 3% albumin inhibited the characteristic Alb-Au labeling of plasmalemmal vesicles which only rarely contained few particles, whereas a number of multivesicular bodies (*mv*) were marked by the tracer (inset). Bars, 1 μm for Fig. 9 and inset. Fig. 10: Alveolar capillary, 3 min after perfusion with glucose oxidase-Au complex: particles labeled almost uniformly the plasma membrane, some vesicle diaphragms (*v*), and plasmalemmal vesicles (*V*₁). At this time interval, multivesicular bodies (*mv*) were not marked by the tracer. Small aggregates (*a*) were seldom encountered. *bl*, basal lamina. Bar, 0.2 μm.

Fluid-Phase Endocytosis of Alb-Au

Although coated pits and coated vesicles were barely marked by Alb-Au (Fig. 8), ~10% of total plasmalemmal vesicles contained such particles taken up in fluid phase (Fig. 6). At 5 min, many multivesicular bodies were labeled by the particles located within their matrix (Fig. 6). Concomitantly, various quantities of tracer were observed in polymorphic tubulo-vacuolar features (~150–250 nm in diameter), which displayed a slightly osmiophilic material. These structures were tentatively identified as components of the endosomal or prelysosomal compartment (Figs. 2 and 3). At later time points (15 to 35 min) tracer particles could be seen in vacuoles, presumably lysosomes, containing autophagic material. Although endosomes and lysosomes cannot be identified with certainty solely on morphologic grounds, the accumulation of Alb-Au in easily distinguishable multivesicular bodies is evidence that the tracer is delivered to the lysosome compartment. In the lung, the extent of labeling of the latter was slightly increased from ~20–30% at 10 min, to ~60% at 35 min after Alb-Au perfusion. Higher concentration of tracer apparently did not result in a parallel increase in the number of marked multivesicular bodies.

Controls

In Vivo Experiments. Labeling of plasmalemmal vesicles by Alb-Au particles was markedly reduced as compared with in situ experiments, both in terms of percentage of marked vesicles (<10%) and in number of particles per vesicle (about two) that usually occupied the center of vesicle. Plasmalemma and coated pits were not decorated, but some multivesicular bodies were labeled by few particles. The overall aspect of Alb-Au interaction with endothelium was quite similar to that observed after competition with albumin.

Competition with Albumin. Competition with albumin gave results largely similar to those emerged from the in vivo experiments (see above): while transcytosis of Alb-Au was practically abolished, a certain degree of endocytosis still occurred, as demonstrated by the decoration of some multivesicular bodies (Fig. 9). Fig. 12 shows the effect of different concentrations of competing BSA on Alb-Au binding to the membrane of uncoated pits and luminally open plasmalemmal vesicles, as estimated by morphometry. Colloid particles with $A^{0.1cm}_{515nm} = 0.1$, had 18–40 μg BSA adsorbed per milliliter gold sol and were 50% less bound to the vesicle membrane when competed with ~100 μg/ml albumin.

High Ionic Strength. High ionic strength resulted in the displacement of native ferritin but not of Alb-Au particles from the interior of plasmalemmal vesicles.

Competition with Heparin. Competition with heparin did not impair the binding of Alb-Au but prevented the adsorption of native ferritin.

Experiments with Fibrinogen-gold, Fibronectin-gold, and PEG-gold. In our experimental conditions, none of these tracers was significantly bound to plasmalemma proper or coated pits. Very rare vesicles or their diaphragms contained
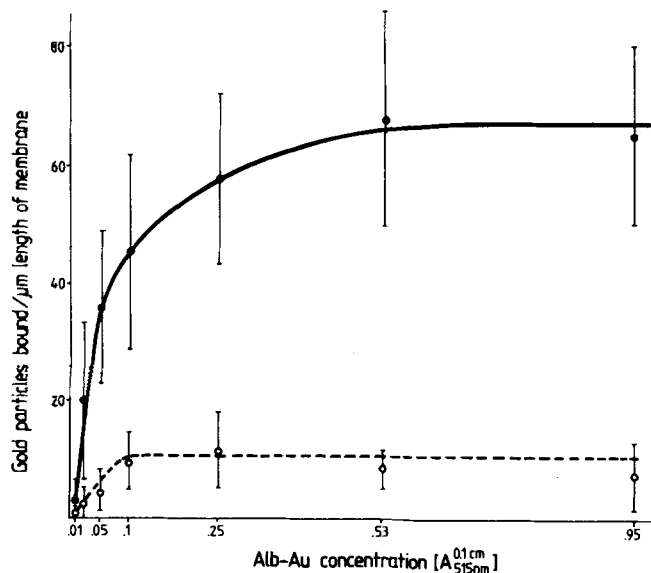
Figure 11. The number of Alb-Au particles bound per micrometer length of membrane, as a function of tracer concentration. Dotted line: particle density on the plasma membrane proper. Continuous line: density of the tracer on the plasmalemmal vesicle membrane surface. The length of the bars represents twice the standard deviation. For each experimental condition a 50–80-μm length of endothelium was analyzed.
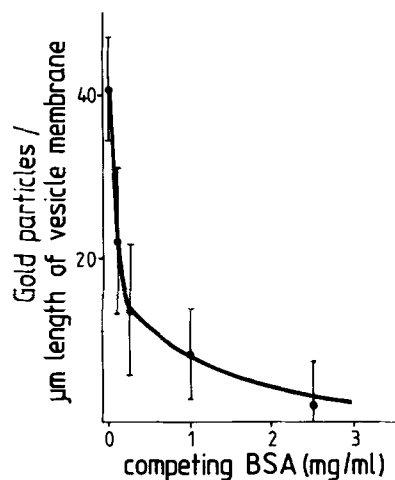

Figure 12. Alb-Au binding to the plasmalemmal vesicle membrane as a function of concentration of competing albumin. 60–80 μm of vesicle membrane profile length was analyzed for each experiment.

one or two particles. Occasionally, multivesicular bodies were marked by few particles. Glucose oxidase-gold complex, exposing a nonplasmatic protein of pI 3.8–5.2 comparable to albumin (pI 4.75), decorated rather uniformly plasma membrane, coated pits, and plasmalemmal vesicles (Fig. 10).

Discussion

These studies reveal: (a) the specific adsorptive binding of Alb-Au complex to plasmalemmal vesicles, and (b) provide evidence for the transport of Alb-Au complex across capillary endothelium of mouse lung, heart, and diaphragm. In the endothelia investigated in these experiments, both homolo-

gous and heterologous albumin gave similar binding patterns: the data presented refer to heterologous albumin.

Alb-Au binds specifically and saturably to uncoated pits of plasma membrane and to most (~90%) plasmalemmal vesicles. The binding is not electrostatic in nature since high ionic strength or competition with a negatively charged macromolecule such as heparin does not displace the adsorbtive binding of Alb-Au (pI 5.1) but prevents the vesicle uptake of the even more anionic native ferritin (pI 3.8–4.2). The binding of Alb-Au is, however, abolished by the endogenous albumin (experiments in vivo) and by exogenous albumin (in situ). Morphometric analysis of the competition experiments with albumin indicates that Alb-Au and monomeric albumin compete with similar affinities for a limited number of the same binding sites located on the plasmalemmal vesicle membrane. Colloidal gold complexes with other plasma proteins such as fibrinogen and fibronectin as well as PEG-Au do not bind in a significant manner to vesicles. The uniform, undiscriminating binding of glucose oxidase-gold (pI 3.8–5.2) appears to be a nonspecific electrostatic interaction comparable to that reported for other nonphysiologic membrane markers of similar pI such as hemeundecapeptide (4.3) and anionic hemeundecapeptide (3.85) (reference 9). The scarcity of Alb-Au binding to plasma membrane does not support the postulate that in vivo, albumin is electrostatically attached to a fiber matrix covering the endothelium (5). The number of densely packed Alb-Au particles adsorbed to vesicle membrane is much greater than expected from a Poisson distribution (for comparison with the ferritin labeling of similar vesicle, see reference 2). This indicates that the vesicle loading with Alb-Au does not depend solely on the diffusion from the lumen but on strong attractive forces on the vesicle membrane. All of these results suggest that Alb-Au complex binding to endothelial uncoated pits and plasmalemmal vesicles is mediated by specific binding sites or receptors. Recently, Sage et al. have reported that a unique albumin-binding glycoprotein is constitutively synthesized by bovine, porcine, and human endothelial cells in culture. Immunofluorescence staining of endothelial cells showed a granular distribution for this 43K protein (22).

Endothelial transport of Alb-Au complex takes place both by vectorial transcytosis (vesicles), and by convective transcytosis (channels) (24–26). Such observations together with those reporting vesicular transport of other plasma constituents such as low density lipoproteins (32, 34), glycosylated albumin (38, 39), albumin and fibrinogen (40), and insulin (14, 30) strongly contradict the hypothesis on the nonfunctionality of endothelial vesicles in transport coined by Bundgaard et al. (3) and Frøkjaer-Jensen (8). Since binding of Alb-Au complex is not affected by high ionic strength and strongly anionic heparin but is abolished by competition with low concentrations of free albumin, it appears that in lung, heart, and diaphragm capillaries the endothelial passage of Alb-Au takes place at least in part by receptor-mediated transcytosis.

There is a suggestion that some of the Alb-Au-carrying vesicles are formed upon binding of ligand to its receptor followed by their clustering in uncoated pits. This presumption has to be checked by experiments with monovalent tracer. A similar ligand binding to uncoated pits was formerly observed during interaction of low density lipoprotein with arterial endothelium in situ (34).

Since Alb-Au complex does not bind to coated pits and vesicles, and multivesicular bodies are labeled by this tracer, the carriers to this compartment are probably a fraction of plasmalemmal vesicles that contain the ligand in fluid phase. This is supported by the observation that Alb-Au endocytosis is not abolished by albumin competition; it is presumed that this process is nonspecific, as seems to be the case with the pinocytosis of the control tracers used in our experiments. Accordingly, it can be assumed that plasmalemmal vesicles, although uniform in structure, actually represent at least two populations of functionally different vesicles: transcytotic vesicles (the vast majority) and pinocytic vesicles (a small fraction). Though most endothelial vesicles appear to be constitutive (24), at least some may be formed by substrate-induced vesiculation, as suggested by transitional forms (from pit to vesicle) detected in these experiments. The routing to multivesicular bodies may either express a normal alternative pathway for albumin or be the consequence of the fact that Alb-Au is a multivalent ligand.

Taking into account the broad differences in the lymph/plasma concentration of albumin in various organs (33), as well as endothelial structural and functional characteristics in each capillary network, modulation in the density and affinity of endothelial albumin binding sites may occur from one vascular bed to another.

The absence of Alb-Au labeling of coated pits in the endothelia examined is at variance with the strong and exclusive Alb-Au binding to coated pits in the endothelium of liver sinusoids (10). In vivo, specific binding sites for albumin may secure the uptake of the minimum amount of this protein or its associated molecules needed by a given tissue. Upon receptor saturation, more albumin can be transported in fluid phase.

The case of albumin transport is not unique; as reported, other plasma molecules cross the endothelium by adsorptive or receptor-mediated transcytosis: low density lipoprotein (32, 34), transferrin (13, 29), glycosylated albumin (38, 39), and insulin (14, 30).

Albumin binding sites constitute a relatively large family present on hepatitis B viruses (1), prokaryotes (streptococcus [37]), and eukaryotes (hepatocytes [16], heart myocytes [12]).

It is known that in addition to its role in maintaining the colloid osmotic pressure of plasma and interstitial fluid, albumin is also an important carrier for various molecules (fatty acids, testosterone, estradiol, thyroid hormones), which are presented by the ligand at the surface of the target cell (4, 12, 16). Accordingly, the receptor-mediated transcytosis of albumin in endothelium represents not only a transport mechanism for albumin per se but also the albumin-mediated delivery of other important molecules required by the metabolic needs of various tissues.

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