The results presented here provide evidence that permeability of intercellular gap junctions is qualitatively different from that of nonjunctional membranes and that material can pass between cell cytoplasms by way of channels not connected to extracellular space. The data also support the hypothesis that gap junctions are a site of electrotonic coupling between cells.

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12. In contrast to the crayfish axon, Procion Yellow does enter some other cells. It entered the intrafusal muscle fibers of frog in the region of the sensory endings, although it did not enter undamaged extrafusal muscle fibers. In desheathed frog sciatic nerve, myelin and Schwann cell cytoplasm were stained although no axoplasmic staining was observed. In both tissues, results consistent with a reversible local anesthetic action of the dye were found. Some of the staining results observed in these and other tissues also suggest that Procion Yellow binds to cell membranes.
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17. We thank S. B. Waxman for assistance with some of these experiments and Dr. P. G. Model for helpful discussion. Supported in part by PHS grant 5 P01 NB 07512 and NSF grant GB-6880.

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Hemoglobin as a Tracer in Hemodynamic Pulmonary Edema

Abstract. *Stroma-free hemoglobin is an electron-opaque molecule useful as a tracer for the ultrastructural study of pulmonary capillary permeability. After this tracer was infused into the isolated pulmonary lobe of the dog, the endothelial junctions of the capillaries, as revealed by electron microscopy, act like distensible pores, thus allowing the tracer to escape when the pulmonary artery pressure was raised above 50 millimeters of mercury.*

Shortly after it is injected intravenously into the mouse, horseradish peroxidase (molecular weight, 40,000) passes through the clefts between endothelial cells of lung capillaries, traverses potential sites of obstructions within these clefts (cell junctions) (*1*), and enters the pericapillary interstitial space (*2*). It has been proposed that the junctions within the clefts between the capillary endothelial cells correspond to the "small pore system" and the plasmalemmal vesicles to the "large pore system" referred to by physiologists (*2, 3*).

However, there is no consensus concerning the proposed role for the junctions. Indeed, it has been claimed that most of the junctions are sealed by fusion of their walls and that only few form open channels, approximately 50 Å in diameter (*4*). Accordingly, plasmalemmal vesicles have been proposed (*4*) as the anatomic counterpart of both the small (*5, 6*) and large (*7, 8*) pore systems of the physiologists.

Since it is known that, in pulmonary edema, proteins of greater molecular weight than that of horseradish peroxidase do pass from the vascular lumen to the interstitial space (*9*), we undertook to examine the ultrasonic basis for this increased permeability of the alveolo-capillary membrane in experimental pulmonary edema. Using stroma-free hemoglobin (molecular weight, 64,500) as a tracer molecule, we found that high luminal pressures seem to widen the calibers of the interendothelial junctions in pulmonary capillary walls and allow the passage of hemoglobin from the capillary lumen into the interstitial tissue.

Ten mongrel dogs, weighing 12 to 15 kg, were anesthetized with pentobarbital

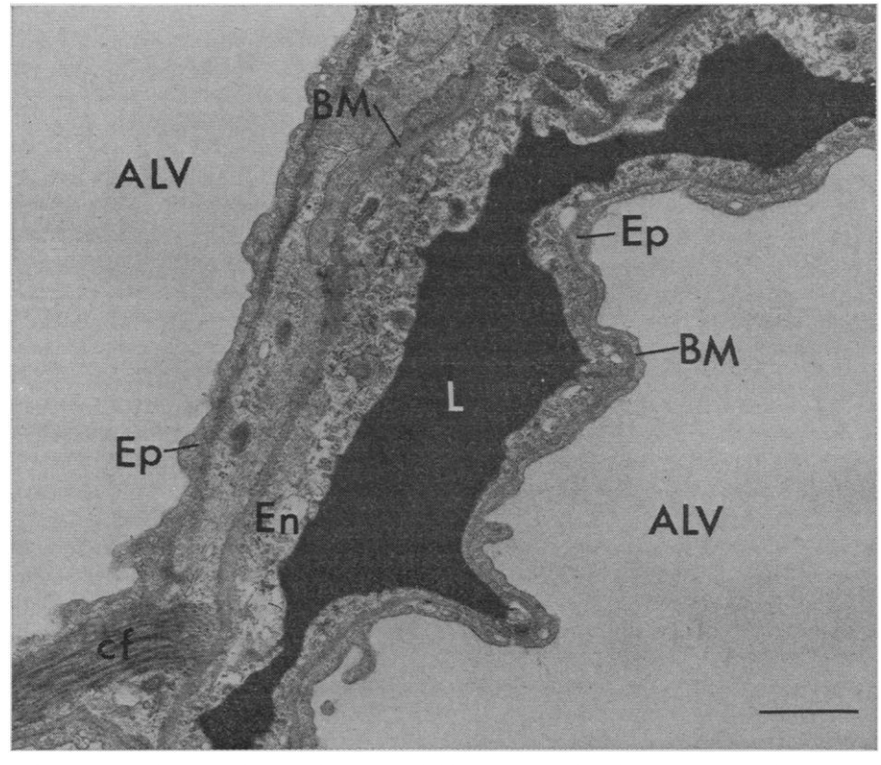

Fig. 1. Dog lung perfused with hemoglobin at normal pulmonary arterial pressure for 20 minutes. In the peroxidase-reacted sections, hemoglobin appears as an electron-opaque reaction product which uniformly fills the vascular lumen (*L*); *En*, endothelium; *Ep*, epithelium; *BM*, basement membrane; *ALV*, alveolus; *cf*, collagen fibers. Section reacted with peroxidase, stained with uranyl acetate and lead citrate. The bar at the lower right corner indicates 1 μm.

(intravenously; 30 mg/kg); they were ventilated by a piston pump (Harvard Apparatus) connected to a cuffed endotracheal tube. After thoracotomy, the left lobar pulmonary artery was cannulated with polyethylene tubing (inside diameter, 6 mm). A solution of stroma-free hemoglobin, 6 to 7 g per 100 ml of fluid (*10*), warmed to 36° to 38°C, was allowed to flow from a plastic bag into the cannulated artery. About 1000 ml of the hemoglobin solution was used for each experiment. Pulmonary artery pressure was monitored through the side tap of the cannula with a strain gauge (Statham P 23 Db) connected to a recording oscilloscope (Electronics for Medicine, White Plains, New York). Perfusion pressures were accurately controlled by varying the height of the plastic bag. Left atrial pressure was held constant during perfusion by periodic withdrawal of blood from the carotid artery.

After an initial perfusion for 5 to 10 minutes with hemoglobin solution to wash out the blood from the lobe, the pulmonary vein from the left lower lobe was ligated, and the capillary bed was exposed to a known hydrostatic pressure of hemoglobin solution; the experiment was continued for another 10 to 15 minutes. The oncotic pressure of each hemoglobin solution was measured with a modified Hansen manometer (*11*); solutions of bovine albumin were used to construct a reference curve. The oncotic pressures of the solutions in the bag ranged from 18 to 22 mm-Hg, a value comparable to that of normal plasma oncotic pressures.

Biopsies of the lobe were obtained by clamping peripheral pieces of lung tissue, about 2 to 3 cm in greatest diameter, while the lung was inflated at approximately its normal end-expiratory position. The biopsies were obtained from 2 to 20 minutes after the beginning of the perfusion and at pulmonary pressures which ranged from normal values (10 to 15 mm-Hg) to 70 mm-Hg. At the conclusion of each experiment, the rest of the lobe was dried and its water content was estimated by subtraction (*12*).

The tissue was fixed for 4 hours in cold (4°C), 2.5 percent glutaraldehyde or in diluted glutaraldehyde-formaldehyde (*2*). After being washed in cold, 0.1*M* cacodylate buffer, *p*H 7.4, slices (0.5 mm thick) were incubated in the Graham-Karnovsky medium (*13*) and then fixed in a solution of 1.3 percent osmium tetroxide in *s*-collidine buffer, *p*H 7.2. The tissue was dehydrated and embedded in Epon 812. Thin sections, unstained or stained with uranyl acetate and lead citrate (*14*), were examined in a RCA EMU 3H electron microscope.

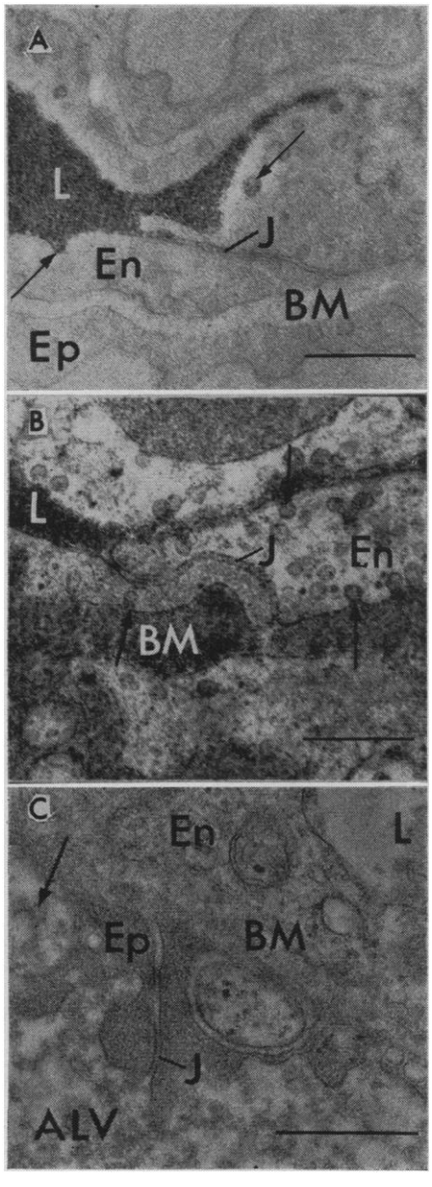

Fig. 2. (A) Dog lung perfused for 20 minutes with hemoglobin at normal pulmonary arterial pressures. Hemoglobin fills the lumen (*L*) of the capillary and extends partly into the intercellular cleft (*J*) from the luminal side. Hemoglobin-filled pinocytotic vesicles (arrow) are limited to the luminal side of the endothelium. Unstained section reacted with peroxidase. The bar indicates 0.5 μm. (B) Dog lung perfused for 20 minutes with hemoglobin at 50 mm-Hg. Hemoglobin is present in the capillary lumen (*L*) and extends through the intercellular cleft (*J*) into the basement membrane and pericapillary space. Hemoglobin is present in pinocytotic vesicles (arrows) both on the luminal and basement aspects of the endothelium. Section reacted with peroxidase, stained with uranyl acetate and lead citrate. The bar indicates 0.5 μm. (C) Dog lung perfused for 3 minutes at 70 mm-Hg. Hemoglobin is present in the capillary lumen (*L*) in the basement membrane and has lifted part of a squamous alveolar cell away from the basement membrane (arrow). The hemoglobin extends through a cleft between squamous alveolar cells (*J*) into the alveolar space (*ALV*). Section stained with uranyl acetate and lead citrate. The bar indicates 0.5 μm.

Hemoglobin was identified by light microscopy from the brown product of its interaction with benzidine and osmium tetroxide (*15*). After 5 to 20 minutes of perfusion with the stroma-free hemoglobin solution at normal pulmonary arterial pressures (10 to 15 mm-Hg), the lung appeared normal. The electron-opaque hemoglobin reaction product was uniformly distributed within the lumens of the pulmonary vessels. None of the reaction product reached the interstitial space (Figs. 1 and 2A), even though it did extend from the lumen into the intercellular space, stopping at the junctions between the endothelial cells (Fig. 2A). Pinocytotic vesicles containing hemoglobin were consistently found on the luminal-side of the endothelial cell, but rarely were they found on the tissue side of the endothelium, that is, in the vicinity of the basement membrane (Fig. 2A). In keeping with the lack of morphologic evidence for the accumulation of excess fluid outside of the vessels, the water content of these lobes at autopsy was within normal limits (*12*), that is, 3.8 g per gram of dry weight. The same results were obtained when the experiments were repeated at a higher pulmonary arterial pressure (25 mm-Hg).

On the other hand, when the pulmonary arterial pressure was increased to 50 mm-Hg and the lung was again perfused with a hemoglobin solution for 5 to 20 minutes, pulmonary edema occurred. Edema was subsequently verified at autopsy by the high water content of the lobes, which averaged 6.5 g per gram of dry tissue. Light microscopy revealed widespread accumulation of the brown reaction product (hemoglobin) not only in the interstitial space but also in some of the alveoli. Electron microscopy showed that the hemoglobin extended, without interruption, from the capillary lumen through many clefts between endothelial cells, passing the endothelial junctions, into the interstitial space (Figs. 2B and 3). In the interstitium, hemoglobin permeated both the endothelial and epithelial basement membranes, accumulated in the widened

collagenous portion of the alveolar septum, and filled the spaces between the collagenous fibers (Fig. 3). In addition, in some areas, hemoglobin-filled clefts between the squamous alveolar cells were found connecting the interstitium with the hemoglobin-filled alveoli (Fig. 2C). However, hemoglobin in the alveoli was associated with degenerative changes of the squamous alveolar cells, such as increased electron opacity or swelling.

Within the endothelium, the number and pattern of distribution of the pinocytotic vesicles seemed to be the same as in the lungs, described above, of dogs with more normal pulmonary arterial pressures. However, one distinct difference in the high-pressure group (50 mm-Hg) was the presence of hemoglobin in the vesicles near the basement membrane even though the vesicles in the middle of the endothelium were usually free of hemoglobin. Rarely did we observe a chain of hemoglobin-containing vesicles extending from the luminal to the basement membrane aspect of the cell. Moreover, whenever the vesicles near the basement membrane contained hemoglobin, the basement membrane also contained hemoglobin.

When the perfusion pressure was raised to 70 mm-Hg, hemoglobin solution filled the airways; light and electron microscopy revealed that hemoglobin had accumulated in large amounts in the pericapillary interstitial space and in the air spaces. The water content of the lobe after only 3 minutes of perfusion was greatly increased, averaging 8 g per gram of dry tissue.

Thus, a concentrated solution of stroma-free hemoglobin (7 g per 100 ml) can be used as a molecular marker in ultrastructural studies of capillary permeability. As a marker, hemoglobin has the advantage of being a naturally occurring animal protein which, unlike extraneous markers, such as horseradish peroxidase, is devoid of undesirable pharmacological activity. Ultrastructurally, hemoglobin can be identified through its reaction product with benzidine and osmium (*13*). Since the amount of haptoglobin in blood is small, most of the injected hemoglobin circulates in an unbound form.

We found no evidence that pinocytosis was involved in the transcapillary transport of hemoglobin into the interstitial space at normal or high pulmonary capillary pressures. This conclusion is based on the following evidence. (i) At normal pressures (10 to 15 mm-Hg), vesicles which opened at the basement membrane did not contain hemoglobin. (ii) At high pressures, many vesicles within the endothelium were free of hemoglobin even though there was hemoglobin both in the interstitial space and in the vesicles opening at the basement membrane. (iii) When vesicles near the basement membrane did contain hemoglobin, the basement membrane also contained hemoglobin, which suggests that the source of the hemoglobin in the vesicles was the basement membrane rather than the luminal aspect of the cell. Even though hemoglobin was occasionally found in plasmalemmal vesicles, which were apparently lying free in the endothelium, these hemoglobin-filled vesicles could not be construed as evidence of transcapillary transport because of the possibility that such vesicles may communicate with the capillary lumen at other planes of section (*2*, *3*).

As long as the pulmonary capillary pressure remained less than 50 mm-Hg, the capillary endothelial junctions were impermeable to hemoglobin, either because such junctions are ordinarily sealed by fusion of adjacent cell membranes (zonulae occludentes) (*4*) or because they form exceedingly narrow slits, which have been estimated to be approximately 50 Å wide (*2*, *3*), that is, too narrow to allow the passage of hemoglobin which is about 60 Å in diameter (*6*). When intravascular pressures were greater than 50 mm-Hg, hemoglobin promptly traversed the endothelial junctions.

Although hemoglobin extended from the lumen to the interstitial space at the high capillary pressures, the anatomical reason for this passage through the endothelial junctions could not be established. However, several lines of evidence suggest that the junctions had widened: (i) There was no anatomical evidence of cell damage or of transport by auxiliary mechanisms, such as pinocytosis. (ii) Small molecules, such as horseradish peroxidase (molecular weight, 40,000) did not escape from capillaries to the interstitial space at normal pulmonary arterial pressures (*16*), whereas larger molecules (hemoglobin; molecular weight, 64,500) did escape at the higher pressures (*17*). Unfortunately, technical limitations, as well as artifacts due to processing of

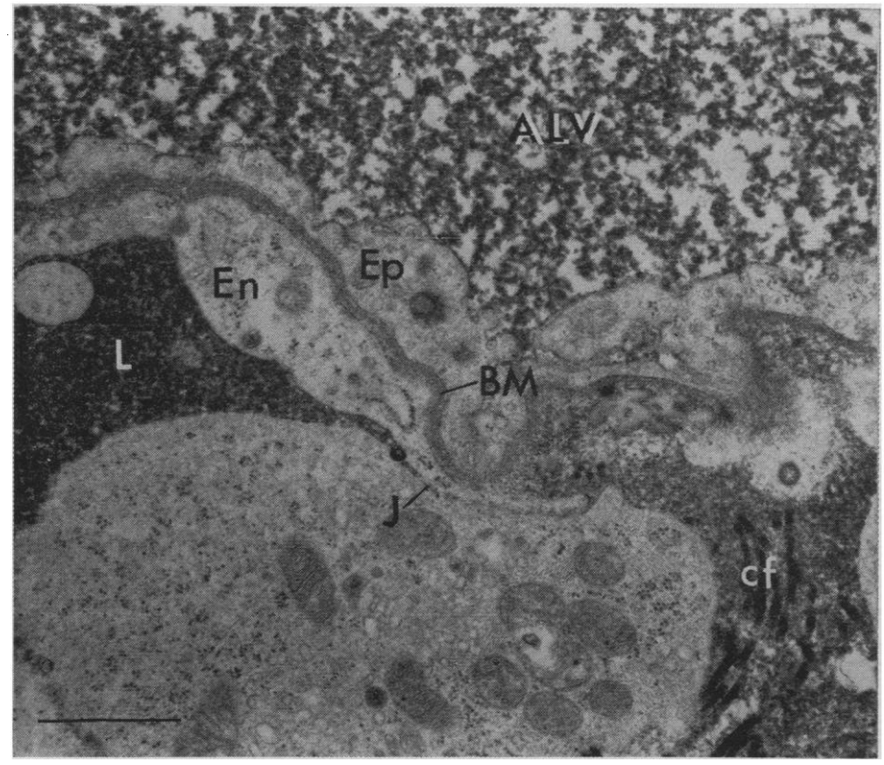

Fig. 3. Dog lung perfused for 5 minutes at high pulmonary arterial pressure (50 mm-Hg). Hemoglobin extends from the capillary lumen (*L*) into the interstitial space through an endothelial cleft (*J*). Hemoglobin has collected around the collagen fibers (*cf*) of the interstitial space, permeated the basement membrane (*BM*) and is found in form of a coarsely granular electron-opaque product in the alveolar space (*ALV*). Section reacted with peroxidase, stained with uranyl acetate and lead citrate. The bar indicates 1 μm.

tissues, discouraged us from attempts to demonstrate such widening. On the other hand, the notion of labile "pore" size is consistent with the increase in the concentrations of proteins which occurs in the interstitial fluids of the limb as venous pressure is increased (*18*) as well as with the sudden increase in the concentrations of albumin and dextran in the right thoracic lymphatic duct, which occurs in the dog after rapid expansion of the plasma volume by intravenous infusions (*19*).

In addition to its theoretical importance, change in the endothelial pore size due to hemodynamic factors is of practical interest because of its role in predisposing to hemodynamic edema by enhancing the passage of proteins into the interstitial space, thereby influencing the balance of forces that is involved in Starling's law of capillary exchange. Moreover, the presence of "tight junctions" between squamous alveolar cells (*2*) which favors the accumulation of fluid in the interstitial space, plus the existence of "collagen" sumps in the interstitial space where fluid may accumulate, is consistent with the clinical observation that, early in the course of pulmonary edema, interstitial edema (stiff lung) may exist without evidence of alveolar edema (rales).

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Rhapidosomes: Absence of a Highly 2′-O-Methylated RNA Component

Abstract. *Rhapidosomes of* Saprospira grandis *do not contain RNA that is highly methylated at the 2′-oxygen of the sugar residues. Neither is such an RNA associated with the particles as a contaminant. A cursory examination of the rhapidosomes of two other marine organisms gave similar results.*

Rhapidosomes, rod-shaped particles produced by various marine flexibacteria (*1*), have been reported by Correll and Lewin (*2*, *3*) to be composed of 70 percent protein and 30 percent RNA of which 40 to 90 percent of the sugar residues are methylated at the 2′–oxygen position. However, we found only a small amount of nucleic acid [less than 2 percent relative to protein by weight (*4*)] in purified preparations of the particles. Furthermore, the nucleic acid is more than 95 percent labile to standard alkaline hydrolysis, thus ruling out a high degree of methylation on the 2′–oxygen (*5*). In addition, crude rhapidosome preparations isolated according to Correll and Lewin (*2*) contained no RNA that was unusual with respect to the amount of *O*–methylation (*6*). The results of three groups of experiments, in which rhapidosomes of an increasing degree of purity were examined, support these conclusions.

In two separate experiments, RNA was extracted with phenol from rhapidosomes of *Saprospira grandis* DAW-2 by the procedure of Correll and Lewin (*2*) and chromatographed on diethylaminoethyl(DEAE)-cellulose (Cl^-) (*3*). In each case, that fraction, which, like the presumed rhapidosomal RNA (*2*, *3*) and transfer RNA (tRNA), was eluted between 0.3 and 0.7*M* NaCl, was hydrolyzed in alkali (0.3*N* NaOH, 18 hours, 37°C). The neutralized hydrolyzates were absorbed on DEAE-cellulose (CO_3^{-2}). With both preparations, essentially all of the material was eluted between 0.03 and 0.15*M* $(NH_4)_2CO_3$ (Fig. 1) in three partially resolved peaks, the components of which were identified by their spectral and electrophoretic properties as mononucleotides. Insignificant quantities of di- or oligonucleotides stable to alkali were detected, in marked contrast to the report (*3*) which stated that 93 percent of the nucleic acid isolated from the rhapidosomes remained as oligonucleotide fragments after alkaline hydrolysis.

The rhapidosome fractions were also isolated (*2*) from *S. grandis* WH, *Microscilla tractuosa* EG-13, and *Cytophaga diffluens* GOL-12 (*3*). A small amount of nucleic acid was obtained from the *S. grandis* WH preparation via phenol extraction (*2*), but it was almost completely (greater than 95 percent) labile to alkali. No nucleic acid could be isolated from the rhapidosomes of *M. tractuosa* or *C. diffluens*, although material absorbing in the ultraviolet, but exhibiting only end absorption (that is, increasing absorption from 320 to 220 nm with no minimum between), was obtained in all cases. We are thus unable to support the claim (*3*) that the rhapidosomes of these organisms contain RNA which is 75 to 97 percent resistant to alkali.

Rhapidosomes from *S. grandis* DAW-2 of a higher degree of purity were isolated as follows. Cells (116 g, wet weight) were harvested in late log phase from 40 liters of medium, suspended in distilled water, and allowed to lyse (*7*). After the bulk of the cell debris was removed by centrifugation at 5000*g*, the supernatant liquid was centrifuged and the fraction sedimenting