Histamine and Interstitial Pulmonary Edema in the Dog

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ABSTRACT

Histamine, administered to dogs by various routes, produced peribronchial interstitial edema. Using colloidal carbon as an electron-dense tracer, it was shown that bronchial venules had become leaky at the same time that the minute blood vessels of the pulmonary circulation remained unaffected by the histamine. The effect of histamine was rapid and brief in duration: wide interendothelial gaps appeared in the walls of bronchial venules through which blood and tracer escaped into the interstitium. This action of histamine seemed to be related to the presence in the bronchial venular endothelium of abundant cytoplasmic fibrils that presumably have contractile capacity. Water content of the lungs was consistent with morphologic evidence that histamine promoted the accumulation of fluid in the lungs. The same pattern of response was observed after bradykinin or compound 48/80, a mast cell degranulator. In contrast to these three agents, serotonin did not affect bronchial venular permeability to colloidal carbon. Consideration of these observations in the light of the large extent of the bronchial venular plexus, raises the possibility that the bronchial venular system may play an important reabsorptive role under normal circumstances and that it may also be involved in the genesis of certain types of interstitial pulmonary edema.

KEY WORDS serotonin bradykinin 48/80 permeability bronchial circulation endothelial contraction electron microscopy colloidal carbon

In the systemic circulation, histamine, serotonin, and other vasoactive substances selectively affect the venular end of the microcirculatory bed (1-4). Whether the consecutive segments of the microcirculation of the lung react differently to such agents, is unknown. Also unknown is whether the minute blood vessels of the bronchial and pulmonary circulations differ with respect to ultrastructure and permeability. These uncertainties merit clarification since the lung in many species is rich in histamine and other humoral substances that are capable of altering vascular permeability (5). Attempts by others to determine the effects of a variety of humoral agents on the permeability of small pulmonary blood vessels (6-8) have been inconclusive because the agents were administered in such quantities, and by such routes, that they exerted systemic as well as local effects.
We undertook to determine the direct effects of histamine, serotonin, and bradykinin on the permeability of the minute blood vessels of the pulmonary and bronchial circulations, using colloidal carbon as an electron-dense tracer for pathologic capillary permeability (1, 2). Where relevant, we compared the effects produced by local application of the agent to those produced by more remote routes of administration, i.e., intravenously or by airways.

**Methods**

**General.**—The experiments were performed on dogs. The agents used—histamine, serotonin, and bradykinin—have been identified as normal constituents in the lungs of many animal species (5). Three types of experiments were done to distinguish clearly between local and systemic effects and to clarify local mechanisms of action: in the first group (9 dogs), histamine, bradykinin, serotonin, and 48/80 (a mast-cell degranulator), were injected subpleurally; in the second group (17 dogs), histamine was administered intravenously; in the third group (3 dogs), histamine was nebulized into the airways of one lobe so that the rest of the lungs served as control. Details of dosage and technique are presented at appropriate places below. Hemodynamic indexes were monitored during the intravenous infusions or the aerosolization of histamine. Special care was exerted to exclude from study animals infested with Dirofilaria immitis since it has been shown that infestation of dogs with this parasite is associated with extensive growth of bronchial vessels (9).

**Subpleural Injections.**—Nine healthy mongrel dogs weighing 11–20 kg were used for these experiments. They were anesthetized using a solution of 5% chloralose in propylene-glycol (100 mg/kg body weight) injected intravenously supplemented by succinylcholine (0.5 mg/kg); additional injections of succinylcholine were made every 30 minutes thereafter as necessary. Each dog was ventilated using a piston pump (Harvard Apparatus Co.) connected to the trachea by a cuffed tube. The expiratory line was submerged under a column of water 5 cm high. Minute ventilation was adjusted according to body weight using the Kleinman-Radford nomogram (10). Ventilation was held constant throughout except for periodic (every 5–10 minutes) hyperinflations to avoid atelectasis, using transpulmonary pressures of about 30 cm H₂O. In three of the nine dogs, the pulmonary artery pressure was monitored as described in the subsequent sections.

The left caudal lobe of the lung was exposed by thoracotomy through the fourth intercostal space, and the agent under study was injected subpleurally with a tuberculin syringe and a 30 gauge needle. The volume of the injection varied between 50 and 100 μlitters. Two or three subpleural injections were usually made in each animal over widely separated areas of the ventrolateral aspect of the lobe. The agents used for subpleural injection were:

1. Histamine (histamine phosphate, Eli Lilly Co.) in doses of 0.1–100 μg of histamine base.
2. Serotonin (5-hydroxytryptamine creatinine sulfate, Sigma Chemical Co.) in doses of 0.1–10 mg.
3. Bradykinin (bradykinin tri-acetate, Sigma Chemical Co.) in doses of 0.1–10 μg.
4. The mast-cell degranulator 48/80 (a condensation product of p-methoxyphenethylmethylamine with formaldehyde, Burroughs Wellcome Co.) in doses of 0.3–0.7 μg.
5. Isotonic saline, 100 μlitters.

**Carbon Labeling.**—Colloidal carbon (Pelikan biological ink, Guenther Wagner Pelikan Co., batch C 11/1431a) was administered intravenously in doses of 0.5–1.0 ml/kg body weight immediately after the subpleural administration of each agent.

**Intravenous Infusion of Histamine.**—Experiments were performed on 17 healthy mongrel dogs weighing 19–25 kg and anesthetized and ventilated as described in the preceding section. The first seven were used to determine the dosage of histamine that would consistently cause sufficient leakage of carbon to be detected with the dissecting microscope. Colloidal carbon was injected intravenously immediately before starting the intravenous infusion of histamine. Histamine base (3–30 μg/kg min⁻¹) was infused for 30–90 minutes.

The remaining ten dogs were divided into two groups, test and control. The test group of five dogs received an intravenous infusion of histamine phosphate, 7 μg/kg min⁻¹ of histamine base for 90 minutes; the five control dogs were infused for 90 minutes with isotonic saline. Dogs of both groups were injected intravenously with colloidal carbon just before the infusions were started. In each of these ten dogs, the left hemithorax was entered through the fourth intercostal space and the left cranial lobe was removed to facilitate access to the heart. Pulmonary artery pressure was measured with a radiopaque polyethylene catheter (Kifa, U. S. Catheter Co.) 15 cm long, inserted directly in the main pulmonary artery and attached to a Statham P23Db strain gauge. Left atrial pressure was measured using a polyethylene catheter inserted into the atrial
appendage. Mean pressures were obtained electronically. Pulmonary blood flow was measured with an electromagnetic flow meter (Biotronex) and transducers 14–16 mm in diameter, placed about the main pulmonary artery. Calibration was done in vitro by mounting the transducers on segments of pulmonary artery that were perfused with steady levels of blood flow. Stroke volume was obtained by electronic integration of each flow pulse. Instantaneous heart rate was measured with a tachometer (Electronics for Medicine). Cardiac output was determined by multiplying the average stroke volume and the average heart rate during two respiratory cycles. Photographic records of the hemodynamic studies were obtained with a multichannel oscillographic recorder (Electronics for Medicine).

Inflation pressure was monitored by connecting a lateral pressure tap of the endotracheal tube to a Statham P23Db strain gauge. Static lung compliance was determined by recording the pressure-volume curve of the lungs: with a calibrated syringe successive increments of 100 ml in volume were introduced, pausing after each increment until stress-relaxation was completed (3–5 seconds). Before determining each pressure-volume curve, the lungs were inflated up to 30 cm H$_2$O for two respiratory cycles to ensure a uniform volume-history of the lungs. Aortic blood pressure was monitored after cannulating either the carotid or femoral artery with polyethylene tubing. Aortic blood samples were drawn anaerobically at regular time intervals and analyzed immediately after sampling for pH, oxygen, and carbon dioxide tensions with conventional electrolytic techniques (Radiometer). Each experiment was ended by an overdose of pentobarbital administered intravenously, and a lung biopsy sample was taken. The water content of the lungs was then determined by a method described previously from this laboratory (11). The statistical significance of the difference between the mean water content of the control and histamine infused lungs was examined by the unpaired t-test.

Aerosol of Histamine.—Three dogs weighing 20–25 kg were prepared in a manner similar to that described in the preceding section. In addition, the airways to the left caudal lobe and to the right lung were cannulated separately by means of a tracheal divider and ventilated independently by using two piston pumps. The adequacy of separation was tested by inflating each lung separately. Histamine, in a concentration of 2.75 mg/ml was administered continuously by aerosol to the left caudal lobe for periods of 90 minutes using a DeVilbiss nebulizer that was located in series with the ventilatory pump; the injection of colloidal carbon was made at the start of aerosolization. The total dose of histamine base administered varied between 5 and 7 mg. Hemodynamic variables, compliance, blood gases, and water content of lungs were obtained as described in the preceding section.

Anatomic Studies.—Biopsy specimens of the lung were taken at regular intervals after subpleural injection and at the close of the experiments involving either intravenous or intrabronchial administration of agents. In either instance, the tissue was fixed promptly by immersion in a cold solution of diluted (1:5) formaldehyde-glutaraldehyde fixative (12) in 0.2M cacodylate buffer, pH 7.4. After fixation, the sites of carbon leakage (Fig. 1a) were identified using a dissecting microscope (20x). These areas, as well as the surrounding unaltered tissue, were sampled for: (a) clearance in glycerin (2) prior to examination by means of the dissecting microscope (20–40x), (b) embedding in paraffin in preparation for light microscopy and (c) postfixation in buffered OsO$_4$ and embedding in Epon 812 for electron microscopy.

Since the anatomical details of the connections between the bronchial and pulmonary circulations are not entirely clear (13–15), we attempted to demonstrate these connections for ourselves. In four dogs, simultaneous injections of silicone polymer material (Microfil) were made into the aorta after clamping it at the diaphragm and into the pulmonary artery. Different colors were used for the two injections; different injection pressures were used for the two circuits: approximately 100 mm Hg for the aorta and 15 mm Hg for the pulmonary artery. The silicone polymer was contained in two polyethylene bags, each raised to the proper hydrostatic level to obtain the desired infusion pressure. At the end of the infusion with Microfil, the lungs were fixed by introducing a solution of 10% formalin into the airways at a pressure of 20 cm H$_2$O. After fixation for 24 hours, the bronchi were dissected with fine scissors, and slices 3–5 mm in thickness were cleared in glycerin for examination with a dissecting microscope.

Results

Subpleural Injections of Histamine and Bradykinin

The effects of histamine and bradykinin on the permeability of the minute blood vessels of the lungs were similar and will be described together. The total number of injections was 18 for histamine and 7 for bradykinin.

After a single injection of colloidal carbon the lungs became uniformly black within a few seconds and resumed their original pink
color within 10–15 minutes as the carbon was cleared from the circulation. The same sequence occurred if a subpleural injection of histamine or bradykinin immediately preceded the intravenous injection of carbon except at the site where the agent had been injected subpleurally. There a characteristic series of changes ensued: within 10-20 seconds after the subpleural injection, the area surrounding the site of injection became very congested and gradually assumed a dark red color. The dark red area persisted thereafter, becoming more

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and more sharply demarcated as the remainder of the lung again became pink; by the end of the experiment, the sites of injection were clearly identified as dark red areas on the surface of the lungs, 0.5-1.0 cm in diameter. These dark red areas did not inflate as well as the surrounding lung, producing a characteristic "dimple" on the surface of the inflated lung. In five dogs in which saline was substituted for histamine or bradykinin, the site of subpleural injection merged imperceptibly with the rest of the lung except for slight hemorrhage and carbon deposition along the needle tract; this tract was used to identify the site of injection for the biopsies that followed. Moreover, saline injections did not result in the formation of a "dimple" during inflation.

Microscopic examination of the lung tissue surrounding the injection site showed that the subpleural injection of histamine or bradykinin had consistently caused carbon to leak from bronchial vessels (Fig. 1a, c, d). The leaky blood vessels were located in the walls of terminal bronchioles and small bronchi several millimeters removed from the end of the needle tract. Although hemorrhage and leakage of carbon identified the needle tract, no carbon or fluid was observed in adjacent alveolar septa or pulmonary venules. Between the end of the needle tract and the leaky bronchial or bronchial venules, the lung tissue seemed normal in both test and control animals (Fig. 1b), and the intervening minute vessels of the pulmonary circulation showed no increase in permeability. In essence, these observations on the bronchial venules corresponded to the histamine type of leakage described by Majno et al. (1, 2) for the systemic venules in the cremaster muscle of the rat.

**Early Changes**—Leakage of carbon from bronchial venules (10-50μ in diameter) was detected in biopsy samples taken as early as 1.5 minutes after injection. The number of venules affected varied with the dose and with the agent. Histamine, 0.1-1.0 μg, caused

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**Figure 2**

Portion of a leaky bronchiolar venule, ten minutes after subpleural injection of histamine. The arrow points to carbon escaping from the vascular lumen through a gap in the endothelium (END). L = lumen; P = pericyte. Uranyl acetate and lead citrate.
leakage of only a few venules; 10 μg caused extensive carbon leakage in the venules of terminal bronchioles and of the short segments of small bronchi; larger quantities, i.e., up to 100 μg, caused marked leakage of carbon from venules in the larger segmental bronchi, several centimeters away from the injection site. Equivalent quantities of bradykinin caused much more carbon leakage than did histamine. After either agent, biopsy samples taken 1.5-10 minutes after subpleural injection showed that carbon had passed.
through gaps between endothelial cells (Fig. 2). The escaped carbon was tightly packed against the basement membrane or against the pericyte. Along with the carbon particles in the walls of the venules and in the perivascular connective tissue, were leukocytes, red blood cells, and plasma. In comparison with normal controls, the perivascular connective tissue around the leaky vessels was edematous, i.e., the collagen fibers were separated by an amorphous, slightly electron-dense material. Similar material distended the adjacent lymph vessels.

**Late Changes.**—In biopsy samples taken 30-60 minutes after the injection, interstitial edema was no longer present and the vessels appeared normal. The only indication of increased permeability was the extravascular deposits of carbon. Some of the carbon particles were still trapped in the basement membrane; others were scattered in the perivascular connective tissue or ingested by macrophages (Fig. 3).

**SUBPLEURAL INJECTIONS OF 48/80**

Eleven injections were made in six dogs. This agent, which causes mast cells to degranulate and, presumably, to discharge their contents (16), produced changes in the lung identical to those produced by histamine and bradykinin, i.e., carbon leakage from bronchial venules. In addition, degranulated mast cells occurred in the vicinity of the leaky bronchial and bronchiolar vessels. Although degranulated mast cells were also present near pulmonary or pleural venules and near alveolar capillaries, these pulmonary vessels did not leak carbon.

**SUBPLEURAL INJECTIONS OF SEROTONIN**

Nine injections were made in five dogs. Within a few seconds after the regular sequence—subpleural injection of serotonin followed by the intravenous injection of carbon—the area of injection became very hyperemic and dark red; this discoloration persisted for the rest of the experiment (60 minutes). Microscopically, the histamine type of leakage of carbon from bronchial venules was not observed, even after huge doses, e.g., up to 10 mg. In addition to the usual hemorrhage and leakage of the tracer along the tract, serotonin produced marked congestion of alveolar capillaries, pulmonary veins, and bronchial venules. However, no tracer particles were seen in the interstitium of the lung or bronchi, nor was separation of the intercellular junctions observed.

**SUBPLEURAL INJECTION OF SALINE**

Five injections were made in five dogs. By both light and electron microscopy, the area of hemorrhage and tracer leakage around the needle tract was much smaller than in the preceding experiments, and the surrounding lung tissue was not congested. The pulmonary and bronchial vessels adjacent to the site of injection were normal. In only one instance did electron microscopy reveal a few carbon particles in the wall of a single peribronchial venule. Majno et al. (1) observed similar changes in cremasteric venules of rats injected subcutaneously with saline. They attributed such minimal leakage to the local release by trauma of substances that enhance permeability.

**INTRAVENOUS INFUSIONS OF HISTAMINE**

These experiments were intended to provide an alternate route for introduction of histamine, one more comparable to the routes most often used by others. To standardize our preparation and to enable comparison with the results of others, observations of pulmonary and circulatory performance were also made.

The results of infusing 7 μg/kg min⁻¹ of histamine in five dogs are illustrated in Figure 4. Each experiment lasted for 90 minutes. The cardiac output and aortic blood pressure fell promptly, reaching a steady level 10-20 minutes after the start of the infusion. On the average, pulmonary artery pressure increased but the results showed no consistent pattern in individual instances: it increased in two dogs and decreased in two; records were not obtained in a fifth dog because of technical difficulties. Left atrial pressure either remained unchanged or decreased slightly (2-3 mm Hg). Static lung compliance decreased in

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FIGURE 4
Hemodynamic and pulmonary effects of intravenous infusion of histamine. Each point represents the average value for at least three dogs. Circles = histamine; triangles = saline.

each dog immediately after the start of the infusion and, except for one dog, remained low throughout the infusion period; in this exception, compliance returned to control values after 35 minutes of infusion. In each dog, arterial blood Po\textsubscript{2} decreased progressively and Pco\textsubscript{2} increased despite unaltered minute ventilation. In each dog metabolic acidosis also developed during the infusion, manifested by a progressive decrease in arterial pH, out of proportion to the increments in arterial Pco\textsubscript{2}.

Control experiments involving the injection of a suspension of colloidal carbon in saline (without histamine) are also shown in Figure 4. Immediately after injection, cardiac output and aortic blood pressure decreased. But, by 30 minutes, these parameters had returned to control levels. Static compliance remained within control limits throughout the experiment. Except for one dog that had a considerable drop in arterial Po\textsubscript{2} at the end of the experiments, arterial oxygen tensions did not decrease from control values by more than 4 mm Hg.

The water content of control lungs (saline infusion only) (Fig. 5) averaged 4.0 ml/g dry weight, with a range of 3.8 to 4.1. This average value for open-chest, artificially ventilated dogs, differed slightly but significantly (P < 0.001) from previous control values reported by Levine et al. (11) from our laboratory in closed-chest, spontaneously breathing dogs. After infusing 7 µg/kg min\textsuperscript{-1} of histamine, the water content (per gram of dry lung) increased, averaging 4.4 ml with a range of 4.2 to 4.6 (Fig. 5). Virtually identical results (4.4 ml with a range of 4.1 to 4.8) were obtained in other dogs that had received doses ranging from 3 to 30 µg/kg min\textsuperscript{-1}. These average values for water content of the lungs after the infusion of histamine significantly exceeded (P < 0.001) those obtained after
infusing the suspension of colloidal carbon in saline (without histamine).

**Morphologic Observations.**—After the infusion of histamine, the lungs appeared diffusely congested and focally atelectatic. The bronchial mucosa was congested and edematous. Spotty leakage of carbon from venules had occurred along the entire length of the bronchial tree. Leakage was most pronounced in large and segmental bronchi, particularly at bifurcations; it was unrelated to atelectasis.

By light and electron microscopy, the changes in the bronchial venules after intravenous infusion were identical with those observed after subpleural injection except that many more leaky venules were found in large bronchi than in the peripheral ones. The alveolar capillaries were engorged with red blood cells. Many capillaries contained clumps of carbon, platelets, and fibrin. These microthrombi were associated with swelling of endothelial and alveolar cells, and focal accumulation in the adjacent alveolar spaces of amorphous electron-opaque material, myelin figures, red blood cells, and alveolar macrophages. The nonspecific nature of these changes was indicated by the normal appearance of the lung in areas where the microthrombi were absent. In no instance, however, had carbon leaked from the alveolar capillaries or pulmonary venules; nor had endothelial junctions widened.

**INTRAVENOUS INFUSIONS OF SALINE**

At the end of saline infusion, the lungs appeared uniformly normal in color and consistency. Random pieces of lungs were biopsied and the tissue was fixed for electron microscopy as in the preceding experiments. Examination of the bronchial tree with the aid of a dissecting microscope failed to reveal any carbon leakage. The mucosal vessels were normal. Light and electron microscopy revealed only occasional microthrombi containing carbon in the alveolar capillaries associated with swelling of endothelial and epithelial cells as in the dogs that received histamine by infusion. The presence of microthrombi in both the histamine infusion and saline infusions indicated that these changes were not related to the effect of histamine per se.

**AEROSOL OF HISTAMINE**

**Physiological Observations.**—In three dogs, histamine administered by aerosol to the left caudal lobe caused a slight decrease in cardiac output and aortic blood pressure, but never to the extent induced by the infusion of histamine (Fig. 4). Mean pulmonary artery pressure increased but never exceeded control levels by more than 8 mm Hg. Left atrial pressure tended to decrease toward the end of the aerosol period. Static compliance of the lobe that received the aerosol of histamine decreased to approximately the same extent and in the same pattern as during histamine infusion. As in the case of histamine infusion, arterial oxygen tension during histamine aerosolization decreased in all dogs. At autopsy, the water content of the left caudal lobe (per gram of dry weight) was increased, ranging from 4.8 to 7.9 ml (Fig. 5). In each instance, the water content of the left caudal lobe exceeded that of the right lung, which ranged from 3.8 to 4.3 ml even though the degree of congestion was identical in both lungs.

**Morphologic Studies.**—Even though the aerosol had been confined to one lobe, both lungs appeared congested and focally hemorrhagic. The bronchial mucosa of the left caudal lobe, which had been exposed to the aerosol of histamine, was markedly congested and edematous. Leakage of carbon was extensive in the proximal segment of the lobar bronchus; it was patchy in the other bronchi, concentrating, as in the case of the histamine infusion, at bronchial bifurcations. The bronchi of the opposite lung, which had not been exposed to the aerosol, were normal—there was no edema, congestion or leakage of the tracer from bronchial venules. Light and electron microscopy of both the left caudal lobe and right lung revealed marked congestion of the pulmonary blood vessels, with focal areas of alveolar edema and hemorrhage. In no instance, however, were tracer particles found in the interstitium of the lungs in the vicinity of alveolar capillaries or pulmonary venules.
FIGURE 6
Cast of the pulmonary and bronchial microcirculations. The cross section of a segmental bronchus reveals the rich submucosal and peribronchial venular plexuses (large arrows) connected by venular radicles (fine arrows) perforating the muscular layer. BrA = bronchial artery; PA = pulmonary artery.

Discussion
The present study demonstrated that in the lung of the dog the subpleural administration of histamine, bradykinin, or 48/80 resulted in submucosal and peribronchial accumulation of fluid; the fluid originated from leaky bronchial venules. In contrast, the minute vessels of the pulmonary circulation were unaffected. The effect of histamine was independent of the route of administration, producing the same picture by subpleural, intravenous, and intratracheal routes: selective increase in the permeability of bronchial venules demonstrated by light and electron microscopic evidence of peribronchial edema and leakage. In addition, the water content of the lung was increased.

Coincidental with the peribronchial accumulation of fluid, the interendothelial junctions of bronchial venules developed gaps approximately 1000–5000 Å wide. Through these gaps, carbon particles, about 250–500 Å in diameter, left the vascular space and accumulated either in the interstitial space or the venular wall (Fig. 2).

The subpleural administration of serotonin, over a wide range of concentrations, failed to elicit histaminelike leakage either in bronchial venules or in any other vessels of the dog lung. This lack of effect of serotonin is strikingly different from the marked leakage of colloidal carbon produced by serotonin in the cremaster muscle of the rat (1). We have no adequate explanation for the failure of subpleural serotonin to increase the permeability of either the bronchial or pulmonary vessels.

Bronchial Circulation.—By an extensive series of anatomical studies, including light and electron microscopy as well as injection techniques, we sought to establish the reason for preferential leakage of fluid from bronchial and bronchiolar vessels after histamine and bradykinin. In nearly all aspects, we verified the observations of Miller (13) and of McLaughlin et al. (15) on the anatomy of the bronchial microvascular bed. Particularly relevant to the present study is the disposition of the bronchial venous circulation: a submucosal venous plexus lies parallel to the epithelium lining the bronchial lumen and to a second
venular plexus located in the peribronchial connective tissue; short venular radicles traverse bronchial muscle to bridge the two plexuses (Fig. 6). In the small bronchi, the two plexuses approximate each other closely because the bronchial wall is thin; in the terminal bronchioles, the two plexuses merge and only a single plexus can be identified. The physiological significance of this anatomical arrangement is conjectural. Among the possible roles is regulation of heat exchange in the airways as a consequence of the distribution of blood between deep and superficial layers.

Ultrastructurally, the capillaries and venules of the bronchial circulation differ from the corresponding minute blood vessels of the pulmonary circulation in three major respects: the bronchial venules have a thicker endothelium, a greater number of pericytes and a plentiful supply of bundles of fine cytoplasmic filaments in the endothelial cells (Fig. 7).

**Leaky Bronchial Venules.**—By injection studies, as well as by conventional microscopic studies, the leaky vessels corresponded to bronchial venules. The ultrastructural criteria that we adopted for the electron microscopic identification of the leaky vessels were based on diameter and wall composition. Since the

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**FIGURE 7**

Detail of the endothelial cytoplasm of a bronchiolar venule. The black arrow indicates the numerous fibrils. C = carbon; F = cytofibrils; L = lumen; M = mitochondria; RBC = erythrocyte. Uranyl acetate and lead citrate.
bronchial circulation is part of the systemic circulation, we followed the classification of Rhodin (17), who defined systemic capillaries as endothelial tubes less than 8μ in diameter encircled by a basement membrane and a discontinuous layer of pericytes; larger tubes (8-50μ) of similar structure were designated as venules. Accordingly, the blood vessels which leaked carbon following histamine or histaminelike agents were almost exclusively bronchial venules; only occasionally did bronchial capillaries become leaky.

Histamine Type of Leakage.—Majno et al. (1, 2), working with the cremaster muscle in the rat, introduced the concept that the structural basis of increased permeability produced by histamine and other mediators of inflammation is the widening of interendothelial junctions of venules. Subsequently, Buckley and Ryan (4), using the rat mesentery, came to similar conclusions. Rowley (3) postulated, on the basis of experiments on rat skin, that the mechanism by which interendothelial junctions became wider after histamine was downstream venoconstriction, which increased the intraluminal pressure of venules and consequently the tangential stretching forces within their walls. The sparing of capillaries was attributed by Rowley to their smaller radius, which led to less wall tension and to less tangential stretching for any given transmural pressure (Laplace's law). However, hemodynamic evidence for a constrictive action of histamine on systemic veins is inconclusive. Indeed, Diana and Kaiser (18), using the isogravimetric technique of Pappenheimer and Soto-Rivera (19), were unable to demonstrate that histamine produced an increase in postcapillary resistance in the dog hind limb. Moreover, Majno et al. (20), as well as Buckley and Ryan (4), using direct in vivo microscopic visualization of veins in the rat cremaster muscle and mesentery, failed to detect venoconstriction on topical application of histamine. Nor could they induce interendothelial gaps in venules by passive increments in venous pressure which they produced by partial occlusion of veins in the extremity (20).

Majno et al. (21) proposed an alternative explanation to venoconstriction, the active contraction of endothelial cells, to account for the opening of interendothelial junctions of venules after histamine. As indirect evidence of endothelial contraction, they described the frequent occurrence of characteristic deformities of endothelial nuclei following the application of histaminelike agents. And, as a possible morphological basis for this contractility, they proposed the abundant cytofilaments which are present in some endothelial cells. In support of this hypothesis is the demonstration by Becker and Murphy (22), using fluorescein-labeled antibodies, that endothelial cells contain actomyosin.

The results of the present study are consistent with the hypothesis of Majno et al. (21). Thus, comparison of venules of similar diameter and wall structure in the pulmonary and bronchial circulations disclosed that the endothelium of the bronchial venules is very rich in cytofibrils (Fig. 7), whereas the endothelial cells of pulmonary veins of the same caliber are either devoid of, or have very few, fibrils. Although it was not possible for us to apply to the lung the type of fixation used for the cremaster muscle, the striking difference between the cytofibril content of bronchial and pulmonary endothelium suggests that bronchial venular endothelium has a much greater potential for contraction than does pulmonary venular endothelium.

Physiological Implications.—Although our physiological observations were simply intended to standardize our preparation and to relate our observations to those of others, they not only support some earlier observations but also raise questions about others. For example, our determinations of pulmonary compliance and blood gas composition support the conclusion of Colebatch et al. (23, 24) that intense bronchoconstriction of terminal airways occurs after histamine and 48/80. Moreover, the hemodynamic changes that we observed correspond to those noted by Feeley et al. after histamine (25) and by Brashear et al. (26) after 48/80. On the other hand, our observations do suggest some caution in the
interpretation of experiments which have attempted to define the site of action of histamine on the lungs in terms of active changes in pulmonary vascular caliber (7, 8, 24) without considering passive changes in vascular dimensions and pulmonary compliance that may be produced by perivascular accumulation of fluid (27).

With respect to the accumulation of fluid in the lungs, conventional teaching deals with two general categories: (1) a primary hydrodynamic imbalance in the normal equilibrium of forces across the alveolocapillary membrane resulting in excess filtration over reabsorption, or (2) damage of the membrane by toxic agents with secondary fluid accumulation despite normal intravascular pressures. Accordingly, Staub et al. (28) studied two prototypes of experimental pulmonary edema, increased pulmonary capillary pressure and intravenous administration of alloxan. Using light microscopy, they noted that, irrespective of the mechanism used to produce the pulmonary edema, the first visible sites of fluid accumulation were the perivascular, peribronchial connective tissue sheets. They were inclined to favor the idea that fluid had formed at the alveolar level and had been directed by mechanical force (29) to the vicinity of the smaller bronchi instead of remaining at the site of formation. However, subsequent studies by Whayne and Severinghaus (30), using hypoxia, raised the possibility that the fluid in the same perivascular, peribronchial space had simply traversed the adjacent pulmonary arteriolar walls. Our studies of hemodynamic pulmonary edema (31), using hemoglobin as a molecular tracer, support the idea that the fluid had originated from alveolar vessels. Whether, to what extent, and under what conditions precapillary vessels contribute appreciably to the genesis of pulmonary edema, remains to be settled.

Our experiments point to the bronchial circulation as a possible source of interstitial pulmonary edema. For example, they raise the possibility that inflammatory processes of the bronchi may contribute to peribronchial edema by increasing the permeability of the adjacent network of bronchial venules. Moreover, in clinical states involving the discharge of endogenous chemical mediators, such as endotoxemia (32), anaphylactoid reaction and severe hypoxia (33–35), the peculiar susceptibility of bronchial venules to these agents may cause them to leak even though pulmonary venules retain their normal permeabilities.

Conversely, the localization of edema fluid in the vicinity of bronchial venules not only raises the possibility that these veins may be involved in the genesis of peribronchial and perivascular interstitial edema, but may also play a reabsorptive function for fluid brought to the peribronchial vicinity from elsewhere, e.g., the alveolar level. Favoring this notion is the marked disproportion shown in our silicone casts between the sparse arterial supply to the bronchi and the abundant venous plexus. However, little is known about the rate at which water that enters the interstitial space is brought to these peribronchial areas or about the dimensions of the reabsorptive surface provided by the bronchial venules. Consequently, the reabsorptive role that we are proposing for bronchial venules remains speculative.

Finally, our ultrastructural observations are consistent with a variety of physiological observations that have been difficult to explain. For example, Haddy (36) observed that bradykinin did not induce the formation of pulmonary edema in an isolated perfused lung preparation. Similarly, Goetzman and Visscher (37), also using an isolated perfused lung preparation, were unable to demonstrate an increase in the permeability of the alveolocapillary membrane to albumin following the administration of massive doses of histamine intravascularly. Our observations, which identify the bronchial circulation as the site of action for the effects of these two agents on permeability, suggest that the lack of effect of histamine and bradykinin in the experiments of Haddy and of Goetzman and Visscher was due to the absence of a bronchial circulation in their preparations.
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