Carbon Deposition in Bronchial and Pulmonary Vessels in Response to Vasoactive Compounds (36589)

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The effect of compounds that increase vascular permeability can be conveniently studied at the morphologic level by marking the leaking vessels with circulating particulate matter. Escaping particles become caught against the basement membrane and cause blackening of the affected vessels (1, 2). With this technique it has been possible to detect gaps between endothelial cells of small vessels (almost exclusively venules) after treatment with histamine-type mediators of inflammation (1), probably due to endothelial contraction (3); however, the small vessels of certain organs (*e.g.*, brain, testis, Gasserian ganglion) fail to leak when challenged with these mediators (4).

In the present work, we have extended our previous studies on the topography of vascular labeling with carbon black (4) by examining the effects of mediators on the bronchial and pulmonary circulations in the rat. In addition to histamine, 5-hydroxytryptamine (5-HT) and bradykinin, we have tested epinephrine because it is known to induce pulmonary edema (5).

Materials and Methods. One hundred seventy male Wistar rats from the Ivanovas Farm (Kissleg-Allgäu, Germany) weighing 100–150 g were divided into 17 equal groups for two series of experiments.

First series (60 rats, intravenous injection of carbon plus mediators, 24 hr study). Ten rats were kept as untreated controls; the remaining rats were injected in groups of 10 according to the following scheme: under light anesthesia (intraperitoneal Nembutal, Abbott, S.A., Zoug, Switzerland, 4 mg/100 g body wt), the animals received first in the left jugular vein 0.5 ml of a 10% dilution of colloidal carbon (Pelikan Biological Ink, batch No. C11/1431a, Günther Wagner, Hannover, Germany) in NaCl, 0.9%. Immediately thereafter they received one of the following intravenous injections: 1 ml of NaCl (0.9%) either alone or containing histamine phosphate (Laboratoires Vifor, Geneva, Switzerland, 100 μg/100 g body wt), 5-HT (serotonin creatinine sulfate, Merck Co., Darmstadt, Germany, 15 μg/100 g body wt), bradykinin (bradykinin acetate, Sigma Chemical Co., St. Louis, MO, 75 g/100 g body wt), or epinephrine chlorhydrate (Adrenalin, Laboratories Vifor, 1 μg/100 g body wt). This last compound was administered very slowly, the time of injection varying between 6 and 12 min. All animals were killed 24 hr later.

Second series (110 rats, study of the chronology of vascular labeling after epinephrine). Ten rats were kept as untreated controls; the remaining rats were anesthetized with Nembutal and injected with colloidal carbon (as above). Immediately thereafter they received intravenously 1 ml of NaCl (0.9%) either alone or with epinephrine (as above). Animals were killed in groups of 10 at the following times: 2 and 10 min, 1, 5 and 24 hr.

All rats were killed during Nembutal anesthesia by injecting the trachea with 3% glutaraldehyde in cacodylate buffer at pH 7.2–7.4 under a pressure of 20 cm of water. After 4 to 6 min the lungs were removed (together with the heart, thymus, mediastinum, esophagus and trachea) and placed in cacodylate-buffered glutaraldehyde for 2 more hr. Tissue blocks of 1 mm^3 were then cut from the middle lobe of the right lung for electron microscopy, and the remaining parts of the organ were kept for histologic examination. For histology, fixation was completed in alcohol–formol (4 parts of absolute

TABLE I. Carbon Labeling of Lung Vessels After iv Injection of Vasoactive Compounds.

Group	Treatment	Degree of carbon labeling	
		Pulmonary vessels	Bronchial vessels
1	NaCl	±	0
2	Histamine	±	+++
3	Bradykinin	±	++
4	5-HT	±	++
5	Epinephrine	+++	±

alcohol and 1 part of 10% neutral formalin); paraffin secretions were stained with hematoxylin and eosin, or very lightly with neutral red for better detection of carbon deposits. The intensity of vascular carbon labeling was graded in blind studies by three observers using an arbitrary scale from 0 to ++ (Table I). For electron microscopy, the blocks were kept for 3 more hr in glutaraldehyde (giving a total time of 5 hr), then left in cacodylate buffer overnight postfixed for 2 hr in 2% OsO_4 in *s*-collidine buffer at pH 7.4, dehydrated and embedded in Epon 812. Thick sections were cut on a Porter Blum MT-2 ultramicrotome using glass knives and stained with toluidine blue or Paragon triple stain (6); thin sections from selected areas were cut with diamond knives mounted on uncovered grids stained with uranyl acetate and lead citrate, coated with a thin layer of carbon and examined with a Philips 300 or a Zeiss EM 9A electron microscope.

Results. Gross evaluation of lung color was not possible because of the fixation technique. Histologically, at 24 hr, control animals treated with NaCl showed only occasional tiny grains of carbon in the walls of small pulmonary vessels. The same was observed after treatment with histamine, 5-HT or bradykinin; however, such animals also showed a striking selective labeling of peribronchial venules (Fig. 1) similar to that described in muscle, skin or subcutaneous tissue after intra-arterial injection of these mediators (4). A quite different pattern of carbon distribution was seen with epinephrine: there was virtually no peribronchial venular labeling, but the deposition of carbon in small pulmonary vessels was much heavier and more widespread than that seen in the controls or in those treated with the other agents (Table I).

Electron microscopy at 24 hr showed that most of the carbon in animals given epinephrine was in phagocytic vacuoles inside endothelial cells of capillaries and small venules; the particles formed clusters, usually surrounded by a distinct membrane. In some cases these phagocytic bodies were only partially filled with carbon; they also contained small vesicles similar to those present in the cytoplasm, and thus resembled multivesicular bodies. Carbon was also present in the extracellular space of the septa, in phagocytic vacuoles of septal macrophages and more rarely in type I epithelial cells. Carbon was also observed free in alveoli, in alveolar macrophages or in circulating granulocytes and monocytes. The last finding was much more evident in epinephrine-treated animals than in controls. The few spots of carbon seen in the pulmonary vessels of the control group were found in endothelial cells as in the epinephrine-treated group, but the carbon deposits in the controls were much less frequent and very small. Generally, the phagocytic endothelial cells showed no signs of injury except when the intracellular carbon masses were very large, in which case the cell seemed to have fewer organelles than normal and the cell membrane facing the lumen

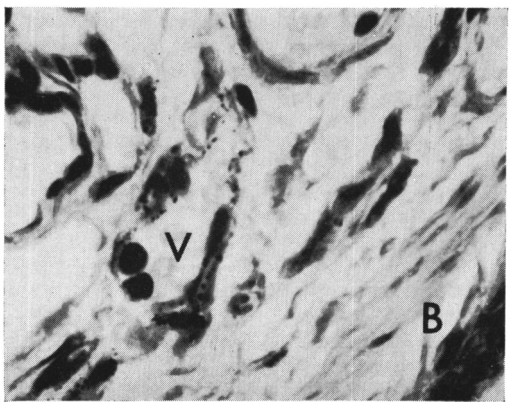

FIG. 1. Carbon labeling of peribronchial vessels 24 hr after iv histamine. Carbon is deposited in the wall of a peribronchial venule. (B) bronchial epithelium (×400).

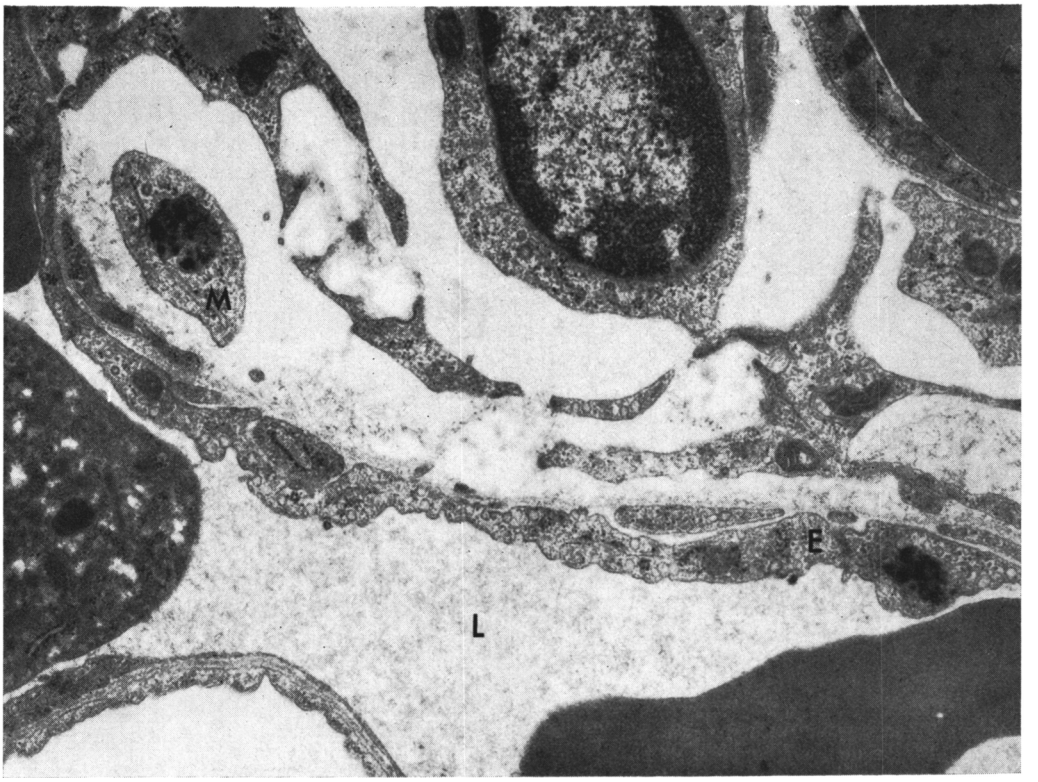

FIG. 2. Carbon uptake in lungs 5 hr after iv epinephrine. Carbon is in the cytoplasm of an endothelial cell facing the lumen (L) of a small vessel, and in a septal macrophage (M). Note moderate interstitial edema (×18,500).

showed some discontinuities; these changes were only seen following epinephrine treatment. There was no erythrocyte extravasation or leukocytic infiltration in the septa.

In examining the chronology of epinephrine-induced carbon uptake by the endothelium, we eliminated animals that developed overt pulmonary edema with respiratory difficulty and nasal frothing (as sometimes happened when epinephrine was injected too quickly). In the groups killed at 2 and 10 min after either NaCl or epinephrine, the only feature was the presence of circulating carbon particles in the plasma; no changes were found in endothelial cells—in particular there were no interendothelial gaps, such as appear in venules responding to histamine-type mediators (1). Neither were gaps found at any later stage in the present experiments with epinephrine.

At 1 hr, a minor degree of septal edema was visible in animals treated with epinephrine (our method of fixation precluded the evaluation of alveolar edema). Carbon was still circulating, either free or in phagocytic vacuoles of granulocytes and monocytes. A few carbon particles were now seen in cytoplasmic vesicles of endothelial cells: such particles were usually single, and most of the vesicles were close to the luminal surface. At 5 hr, the epinephrine-treated animals had a more severe interstitial edema than at 1 hour (Fig. 2). Carbon particles had accumulated in endothelial cells of small pulmonary vessels (Fig. 2) and were often surrounded by a membrane (Fig. 3). By now, there was no circulating carbon except in leukocytes. Particles were also seen in the interstitial tissue of septa, in phagocytic vacuoles of septal macrophages (Fig. 2) and occasionally in epithelial cells.

The changes at 24 hr have been described above. When compared with animals killed at 5 hr, those killed at 24 hr showed more

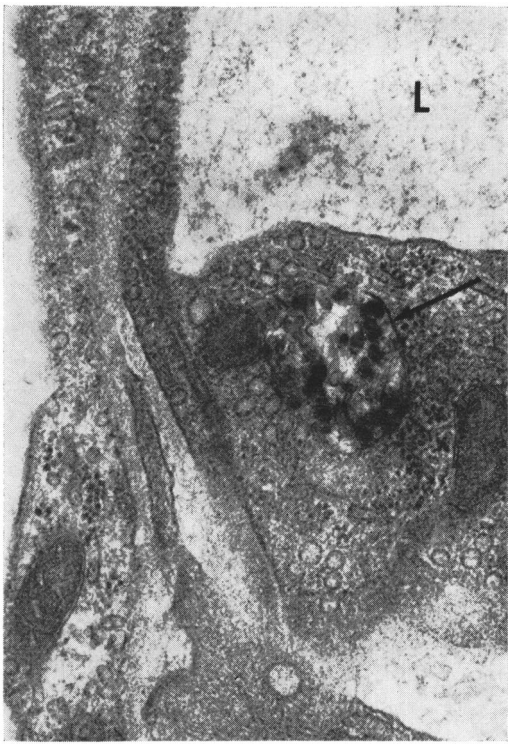

FIG. 3. A detail of carbon deposition in pulmonary endothelium 5 hr after iv epinephrine. A phagocytic vacuole surrounded by a membrane (arrow) is filled with particulate matter; the remaining cytoplasm is normal (×38,000).

carbon in the septal macrophages.

Discussion. Our results show that bronchial and pulmonary vessels differ in their reaction to various vasoactive compounds. The carbon labeling of *peribronchial venules* with histamine-type mediators is similar to the response observed in other territories of the systemic circulation (*e.g.*, subcutaneous tissue, skin, muscle) after local or intra-arterial challenge (2, 4, 7). That these venules are part of the bronchial circulation we have verified in additional rats by carbon perfusion from the left ventricle. Such an activity of histamine, 5-HT and bradykinin on bronchial vessels is particularly significant since these substances, after intravenous injection, come immediately in contact with pulmonary vessels and only later do they reach the bronchial circulation. This indicates that these agents maintain at least part of their activity after passing through the pulmonary circulation. On the other hand, epinephrine (injected by the same route as the other agents tested) does not produce any labeling of bronchial venules. It could be supposed that the amine is detoxified by pulmonary vessels, but this is unlikely since it retains its ability to increase systemic pressure (5). In additional experiments (not reported here) we have not noted carbon deposition in small vessels of the systemic circulation such as in the masseter muscle, skin or subcutaneous tissue after intracarotid injection of epinephrine (in amounts similar to those used here); neither are there reports in the literature showing that epinephrine acts in the same way as histamine-type mediators (7).

The behavior of *pulmonary vessels* is strikingly different from that of the bronchial circulation. Firstly, they show no labeling in response to histamine-type mediators; this might be ascribed to hemodynamic conditions of the pulmonary circulation, although certain systemically supplied vascular territories (such as brain, testis and Gasserian ganglion) show a similar lack of response (4). Secondly, epinephrine (which does not act on bronchial venules) stimulates the uptake of colloidal carbon by pulmonary vessels. To check that this phenomenon does not take place exclusively with carbon, we have performed experiments similar to those reported above using saccharated iron oxide (Ferrum Haussman, Haussman Laboratories A. G., St. Gallen, Switzerland); the results were virtually the same as with carbon.

It is known that epinephrine induces lung edema (5); under our conditions this loss of fluid takes place without the formation of gaps between endothelial cells of pulmonary small vessels (or at least of gaps large enough to allow the passage of carbon or saccharated iron oxide). This further supports the suggestion that epinephrine produces edema by a mechanism that differs from that of histamine-type mediators. Endothelial uptake takes place when edema has already started; with our techniques it is impossible to establish whether this is due to a direct effect of epinephrine, or is mediated indirectly through its hypertensive or edema-producing action.

It is well established that particulate mat-

ter given intravenously is taken up by the organs of the reticuloendothelial system. Accumulation of carbon particles in the endothelium of small vessels in several organs, including lungs, has been reported in rats or mice overloaded with this substance (8, 9). In the present experiments, although the dose of carbon was much lower than that used in the overloading studies, pulmonary endothelial cells of rats receiving carbon and NaCl sometimes showed phagocytic vacuoles. This suggests that endothelial phagocytosis in the lung may play a physiological role in the sequestration of circulating particulate material.

It is known that sudden and heavy overload with carbon causes massive accumulation of particulate matter in the lungs and eventually pulmonary thrombosis (10, 12). We did not see such lesions in our animals, probably because of the small amount of carbon administered. However, it could well be that with larger doses, the quantity of phagocytosed particles is so great that endothelial damage occurs, initiating local thrombosis.

Summary and Conclusions. After intravenous injection of low doses of collodial carbon into rats, a small number of particles are normally found inside phagocytic vacuoles in endothelial cells of small pulmonary vessels. Histamine-type mediators of inflammation injected intravenously induce carbon labeling of bronchial venules while leaving pulmonary vessels virtually unaffected. Under the same conditions, epinephrine does not affect bronchial vessels but greatly enhances the phagocytosis of circulating particles by the endothelium of pulmonary vessels.

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