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Morphological and Physiological Study of the Effect of Histamine on the Isolated Perfused Rabbit Lung

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Summary

Histamine causes interstitial pulmonary edema, but whether this is the result of an increased permeability of the pulmonary circulation or only of the bronchial circulation remains to be determined. In order to selectively study the effect of histamine on the pulmonary circulation, we used an isolated perfused rabbit lung preparation because in this species, the bronchial circulation is poorly developed. Edema formation was assessed by continuously monitoring the weight of the lung perfused at constant pressure. These studies were supplemented by electron microscopic observations using hemoglobin as an ultrastructural tracer for microvascular permeability. We found that histamine (8.0 μg base/100 ml of perfusate) did not cause lung weight to increase. Ultrastructural studies showed that histamine, at this dosage, did not cause a greater leakage of hemoglobin than occurred in the control lungs. Thus, we have concluded that histamine does not increase the permeability of the pulmonary microcirculation in the isolated perfused rabbit lung.

Introduction

The ability of histamine to increase vascular permeability to macromolecules in the systemic circulation, and thereby cause edema, has been demonstrated morphologically (1–3) and physiologically (4–8). In the lung, morphological data from this laboratory (9) indicate that histamine increases the permeability of the bronchial venules but not of the pulmonary microvasculature. Our findings suggested a difference between the responses of the two microvascular beds of the lung to histamine.

On the other hand, *Brigham* and *Owen* (10) have reported that histamine increases the flow of lymph and the quantity of lymph protein from the lungs of unanesthetized sheep and concluded that histamine increases the permeability of the pulmonary microcirculation. It is difficult to reconcile their observations with ours particularly since the contribution of the bronchial circulation to the formation of lung lymph is not known.

In order to more precisely identify the site of action of histamine within the lung, we resorted to examining the effect of this drug in an isolated perfused rabbit lung preparation. Since in the rabbit the bronchial circulation is limited to the main and lobar bronchi (11) development of permeability edema in the absence of intrapulmonary bronchial microvessels would be unequivocal evidence that histamine increases the permeability of the pulmonary microcirculation.

The isolated perfused rabbit lung preparation has two advantages: 1) it allows monitoring of edema development by continuously weighing the lung and 2) it avoids complicating effects of the systemic reactions to histamine. Physiological indices of edema development were supplemented with electron microscopic studies using hemoglobin (68,000 daltons) as an ultrastructural tracer for changes in microvascular permeability (12).

Methods

Constant Pressure Perfusion of the Isolated Lung

Lungs were isolated from adult, New Zealand White rabbits weighing from 2.0 to 4.0 kg under sodium pentobarbital anesthesia. After instituting artificial ventilation, the chest was opened by removing the sternum with a portion of the sternal ribs and the rabbit was then heparinized (10 mg/kg, iv). A large bore

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cannula (5.0 mm i.d.) was placed in the left atrium via the atrial appendage and exsanguination begun. During this procedure, the pulmonary artery was cannulated via the outflow tract of the right ventricle. The lungs were removed from the thorax and a heavy ligature placed around the atrio-ventricular groove. The lungs were wrapped in plastic wrap to minimize drying and placed in a plexiglass chamber containing a 60 liter water bath at the bottom. The chamber temperature was maintained at 36 to 38 °C. Perfusion was started using a Krebs-Ringer bicarbonate buffer containing 5.0 grams bovine serum albumin and 100 mg dextrose per 100 ml. Some red cells remained in the perfusate, but the hematocrit was less than 2.0%.

The perfusion pressure was kept constant and controlled by adjusting the height of the pressure regulators connected to the pulmonary artery and vein cannulas. Vascular pressures were selected so that the entire lung would be in zone III conditions at the end of expiration. The pulmonary artery and vein pressures were monitored with Statham P23Db transducers via catheters placed in the respective cannulas located as close (2.0 cm) as possible to the lung. All pressures were measured relative to the base of the lung. Flow of the perfusate was monitored with a Biotronex electromagnetic flowmeter (cannulating type, 0.25 inch i.d.). Lung weight was monitored with a Grass force transducer in series with a double pan balance. This system allowed us to detect changes in lung weight as small as 12.5 mg although the noise in the system was usually about 60 mg. All data were recorded on an Electronics for Medicine DR-8 Photorecorder. The data were also stored on magnetic tape (Hewlett Packard FM Instrumentation Tape Recorder) to facilitate computer assisted analysis of the analog data.

In order to maintain normal P_{CO_2} and pH, the perfusate was equilibrated with a 6% CO_2 in air gas mixture and the lung was ventilated with this mixture also. Periodic samples of the perfusate were taken for gas and pH analysis with a Radiometer blood gas analyzer.

Assessment of Edema Formation

Edema formation was assessed from the changes in lung weight and perfusate flow. A continuously increasing lung weight in the presence of a constant or decreasing flow of perfusate was considered as evidence of edema formation. Changes in perfusate flow at a constant perfusion pressure were interpreted as changes in resistance due to changes in vessel diameter.

Data Analysis

The data stored on magnetic tape were digitized with a PDP-12 LINC at 12.5 Hz and time weighted averages were taken over two respiratory cycles (f = 24 breaths per minute) once every 15 seconds. The parameters analyzed were: pulmonary artery and vein pressures, perfusate flow, and lung weight.

Protocol

A typical protocol consisted of allowing the lung to stabilize and then recording all parameters for 30 minutes. Sufficient histamine diphosphate was then added to the main reservoir to provide a concentration of histamine base equal to 8.0 μg per 100 ml and all parameters were then recorded for another 30 minutes. Preliminary experiments to determine the dose-response curve for histamine showed that all doses from 2.0 to 18.0 μg of histamine base per 100 ml produced an immediate increase in vascular resistance and a decrease in lung weight attributed to the decrease in vessel diameter. A dose of 8.0 μg/100 ml was finally chosen because it was the lowest dose which appeared to cause a continuous increase in lung weight after vascular resistance had stabilized.

Morphological Studies

The effect of histamine on permeability was studied morphologically in three preparations using hemoglobin as a tracer. After the lung preparation was stable for 30 minutes, sufficient hemoglobin was added to the main reservoir to yield a final concentration between 2.5 and 4.0 g per 100 ml. (The hemoglobin was dissolved in Krebs-Ringer bicarbo

nate containing sufficient bovine serum albumin so that final solution had a colloid oncotic pressure equal to the standard perfusate.)

The hemoglobin was visualized in the tissue by a cytochemical reaction for peroxidase activity (12). This method allows morphological detection of about 1.0 g percent of hemoglobin in plasma or tissue. As controls, we used two additional preparations perfused with the hemoglobin-albumin mixture and to which saline was added instead of histamine. Histamine or saline was added to the perfusate 5 minutes after the hemoglobin solution and the lungs were fixed 10–15 minutes later by intratracheal administration of 2% paraformaldehyde : 2.5% glutaraldehyde (F:G) in 0.1 M Na cacodylate buffer (pH 7.2 to 7.4).

For each lung, samples for electron microscopy were taken from three regions in the periphery of the left lower lobe (upper, middle, lower).

Semiquantitative Assessment of Vascular Leakage

From each of the three regions of the lung sampled as described above, three blocks were selected at random for sectioning. Thus, for each lung nine blocks were sectioned. Thin sections were mounted on coded grids and examined in an electron microscope. From each grid, 10 micrographs were taken at random hence, 90 electron micrographs were taken from each rabbit lung. The electron micrographs were photographically enlarged to a final magnification of 30,000 times.

Each electron micrograph was evaluated by two independent observers without knowing the origin of the material. Interstitial edema was assessed by measuring the relative area of the collagenous portion of the pulmonary interstitium which was electron lucent according to equation 1:

$$1.\ \text{Swelling} = \frac{\text{Electron lucent area}}{\text{Total area of interstitial space}} \times 100$$

Severity of extravascular leakage of tracer was determined according to equation 2.

$$2.\ \text{Leakage} = \frac{\text{Intensity of histochemical reaction x surface area occupied by reaction product}}{\text{Total area of interstitial space}} \times 100$$

The intensity of the histochemical reaction was determined by visual evaluation of the electron micrographs on a scale of 0 to 3. A score of 0 corresponding to no visible reaction product; a score of 3 corresponding to the same concentration of hemoglobin in the extravascular space as in the intravascular space.

Differences in the mean index of swelling (equation 1) and leakage (equation 2) between the experimental and control groups as well as between animals of the same group and between different areas of the same lung were subjected to a student's t-test to determine statistical significance. Since the purpose of this experiment was to determine whether histamine caused a greater leakage of hemoglobin than occurred in the control lungs, we performed a student's t-test for positive "t" values using a probability of less than 0.05 to declare a statistically significant difference; that is, a one sided or sign considered t-test was performed.

Results

Physiological Responses of the Isolated Lung to Histamine

In five pairs of isolated lungs, histamine was added to the perfusion system following a 30–45 minute control period during which the lung had stabilized. The response from one experiment is shown in Figure 1 and the data are summarized in Table 1A. In each preparation, histamine caused an immediate increase in vascular resistance (indicated by a decrease in flow at constant perfusion pressure) and a reduction in lung weight. In animal No. 4, these parameters remained stable for the duration of the experiment (see Figure 1) and in animal No. 3, they were stable for only the last ten minutes of the run.

In animal No. 2, vascular resistance increased and lung weight decreased throughout the run. In animals No. 1 and 5, vascular resistance fell and lung weight increased after the

Tab. 1 Circulatory Responses of Isolated Perfused Rabbit Lungs

An. No.	control Ppa	Pla	Q̇	PVR	Lung Wt.	5 minute post-histamine Ppa	Pla	Q̇	PVR	Lung Wt.	30 minute post-histamine Ppa	Pla	Q̇	PVR	Lung Wt.
						A.									
1	15.8	7.8	167	0.048	23.5	16.7	7.5	80	0.114	20.0	16.0	8.0	141	0.057	21.2
2	15.7	5.4	315	0.033	13.5	15.9	4.9	300	0.037	13.0	16.0	4.7	280	0.040	12.0
3	13.9	6.3	255	0.030	21.8	14.4	6.3	136	0.060	18.4	15.6	6.7	49	0.182	16.5
4	15.8	6.2	336	0.029	20.2	15.6	5.7	186	0.053	17.5	15.4	5.8	183	0.052	17.5
5	15.3	7.0	180	0.047	24.3	15.3	7.0	112	0.074	22.0	15.8	7.8	138	0.058	22.8
						B.									
	control					5 minutes post-saline					30 minutes post-saline				
6	14.1	7.3	367	0.019	21.4	14.0	7.3	369	0.018	21.5	14.3	6.9	353	0.021	20.8
7	13.5	8.0	510	0.011	24.0	13.5	8.0	500	0.011	24.0	13.8	8.4	478	0.011	23.0
						C.									
	control					5 minutes post-hemoglobin					5 minutes post-histamine				
8	9.6	7.4	126	0.017	32.6	9.6	7.4	115	0.019	32.0	10.4	7.4	96	0.031	30.2
9	8.8	6.6	128	0.017	24.4	9.5	6.5	75	0.040	21.9	10.8	5.7	39	0.131	17.4
10	14.5	6.1	251	0.033	23.0	14.9	5.8	165	0.055	21.6	14.5	5.9	25	0.344	17.8
						D.									
	control					5 minutes post-hemoglobin					5 minutes post-saline				
11	12.8	7.6	126	0.041	–	10.7	6.7	22	0.182	–	17.1	6.7	20	0.520	–2.8*
12	15.1	9.7	275	0.020	28.3	15.3	9.4	252	0.023	25.8	15.7	9.1	229	0.029	25.0

Ppa: pulmonary artery pressure (mm Hg); Pla: left atrial pressure (mm Hg); Q̇: perfusate flow (ml/min); PVR: pulmonary vascular resistance (mm Hg)/(ml/min); Lung Wt.: lung weight (grams).

* Due to technical problems total lung weight was not known, and the decrease in lung weight exceeded the range of the recorder, but the decrease was greater than 2.8 grams.

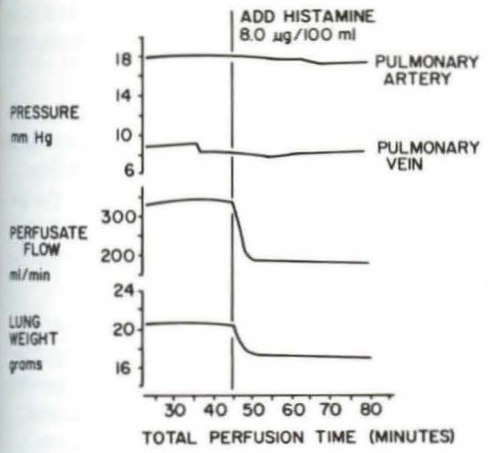

Fig. 1 Steady-state responses of pulmonary hemodynamics and lung weight to a single dose of histamine. After the initial response, lung weight remained constant indicating that edema liquid was not accumulating in the lung

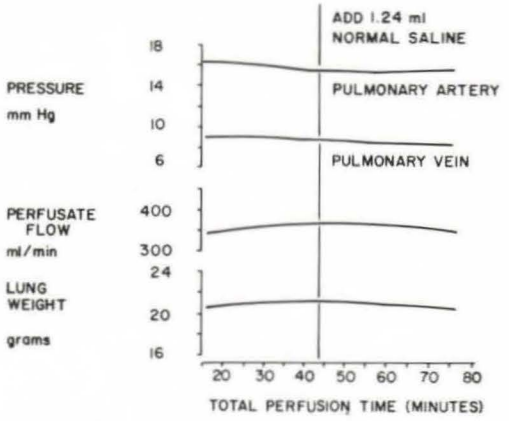

Fig. 2 The effect of the addition of saline (control) to the perfusate on pulmonary hemodynamics and lung weight. These data indicate that our preparation was stable for the duration of our experiments

initial changes caused by histamine. In these three preparations, lung weight changed in the same direction as one would predict from the responses of vascular resistance, therefore it was not possible to determine from the changes in lung weight whether or not edema liquid was accumulating in the lung. However, in two of the five preparations, lung weight did not increase and vascular resistance remained constant, indicating that histamine did not cause edema.

The data in Table 1B and in Figure 2, show the effect of adding saline vehicle to the perfusate in place of histamine. From these data, it can be seen that the preparation was stable during the time that we made our observations.

The physiological responses of the hemoglobin perfused lungs to histamine is shown in Figure 3 and Table 1C. Table 1D shows the response of the hemoglobin perfused lung when saline was added in place of histamine. In both preparations, the addition of the hemoglobin was associated with a decrease in perfusate flow and lung weight. The decrease in weight is interpreted as a decrease in vascular volume corresponding to the increase in vascular resistance indicated by the reduction in perfusate flow. The reason for this

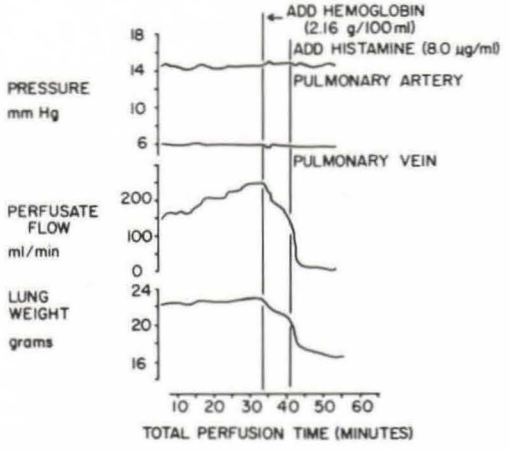

Fig. 3 Effect of hemoglobin and histamine on the hemodynamics and lung weight. Although the hemoglobin reduced perfusate flow and lung weight, the response to histamine was not different from that seen in Figure 1

response to hemoglobin is unclear, but the control data indicate that the hemoglobin perfusate did not cause edema. Furthermore, hemoglobin did not interfere with the response to histamine. That is, when histamine was added to the perfusate, lung weight and perfusate flow showed a further decrease just as occurred in the previous series of experiments.

Tab. 2 Index of Hemoglobin Leakage in the Interstitial Space of the Left Lower Lobe

	Control	Histamine
Corner Vessels**		
Upper	38.69 ± 8.47 (42)	58.85 ± 7.18*,[1] (52)
Middle	44.86 ± 12.79 (36)	28.02 ± 6.97 (53)
Lower	46.31 ± 9.07*,[2] (38)	23.42 ± 6.10*,[2] (57)
Alveolar Septa		
Upper	3.05 ± 2.32*,[1] (18)	33.29 ± 7.90*,[1] (38)
Middle	20.00 ± 6.82 (24)	26.84 ± 14.14 (37)
Lower	3.86 ± 2.47 (22)	6.97 ± 3.85 (33)

Values are means ± S.E.

* The difference between these two values is statistically significant ($p < 0.05$)

The numbers in parentheses represent the numbers of electron micrographs.

** Corner vessels are those located at the junction of three or more alveolar septa.

[1] Sign considered t-test.
[2] Sign ignored t-test.

Anatomical Correlates

Vascular Leakage

The results of the semiquantitative assessment of hemoglobin leakage are summarized in Table 2. Figures 4 through 6 are representative electron micrographs of the histamine treated and control lungs. In histamine treated lungs there was a significantly greater leakage of hemoglobin out of the corner and septal vessels in the upper regions of the left lower lobe of the lung. Histamine did not cause a significantly greater leakage of hemoglobin than occurred in the control lungs in the middle and lower portions of the left lower lobe for both the corner vessels and the septal vessels. However, if the data for all regions of the lobe and for both corner and septal vessels are pooled, histamine did not cause a significantly greater leakage than occurred in the control lungs. (Mean index was 31.93 for the controls and was 29.30 for the histamine treated lungs.)

Overall the morphological results confirm the impressions from the physiological experiments that histamine did not cause permeability edema in the isolated lung. However, the regional difference in the permeability changes indicated that such a conclusion may not be completely warranted.

It should also be noted that for the corner vessels, the index of leakage of hemoglobin in the lower portion of the lobe was greater in the control lungs than in the histamine treated lungs. An unpaired t-test was performed on these data and showed that the absolute value of this difference is significantly greater than zero at the 0.05 probability level. The reason for this observation is unclear, but suggests that the permeability of the control lungs may have been quite high to begin with.

The data in Table 3 summarize the relative degree of interstitial edema. In general, both the control and the histamine treated lungs

Tab. 3 Index of Swelling of the Interstitial Space of Left Lower Lobe

	Control	Histamine
Corner Vessels		
Upper	66.19 ± 8.27 (42)	66.83 ± 7.69 (52)
Middle	51.80 ± 10.09 (36)	52.17 ± 6.32 (53)
Lower	53.95 ± 9.66 (38)	62.03 ± 7.43 (57)
Alveolar Septa		
Upper	16.66 ± 5.60*,[1] (18)	37.89 ± 7.26*,[1] (38)
Middle	25.83 ± 9.76 (24)	38.78 ± 7.71 (37)
Lower	19.77 ± 8.69 (22)	33.18 ± 7.41 (33)

Values are means ± S.E.

* The amount of edema in the histamine treated lung is significantly greater than in the control lung ($p < 0.05$).
[1] Sign considered t-test

Figs. 4–6 Comparison between isolated rabbit lungs perfused with hemoglobin (control) and hemoglobin with histamine (experimental). All sections were examined unstained. Hemoglobin (Hb) was detected indirectly by the generation of the osmiophilic reaction product of polymerized diaminobenzidine (12)

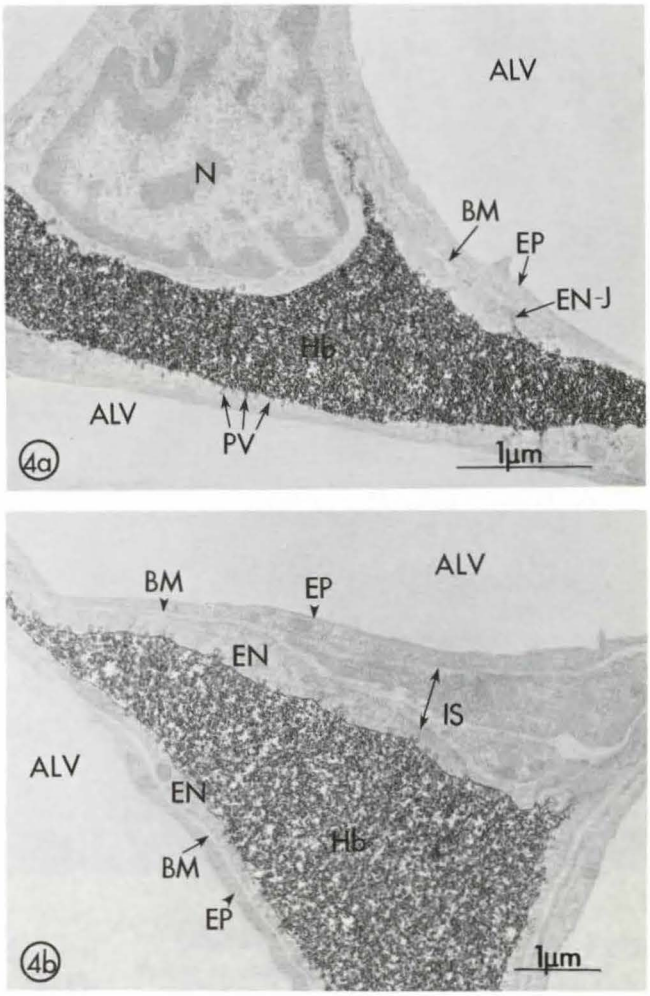

Fig. 4
Control (a) and experimental (b) lungs. The reaction product is confined to the intravascular compartment, to pinocytotic vesicles (PV) open onto the capillary lumen and the luminal portion of the endothelial cleft (EN-J). The interstitial space is of normal width and electrondensity. Swelling and leakage indices = 0. IS-interstitium; BM-basement membrane; ALV-alveolus; EP-squamous epithelial cell; EN-endothelium; N-nucleus

showed evidence of interstitial edema to equal degrees. The only exception occurred in the interstitium surrounding the septal blood vessels in the upper part of the lobe.

The fact that the control lungs had interstitial edema may reflect the generally held belief that the isolated lung is a gradually deteriorating one. Indeed, the numerical values that would have been obtained if one examined lungs of normal animals fixed *in situ* immediately after sacrifice would be zero. The values in Table 3 indicate the percent increase in the width of the interstitial space.

Another interesting finding is that the amount of interstitial edema was not greater at the base of the lung than at the upper parts of the lobe. Perhaps this is because the left lower lobe of the rabbit had a vertical height of only 5.0 to 6.0 cm.

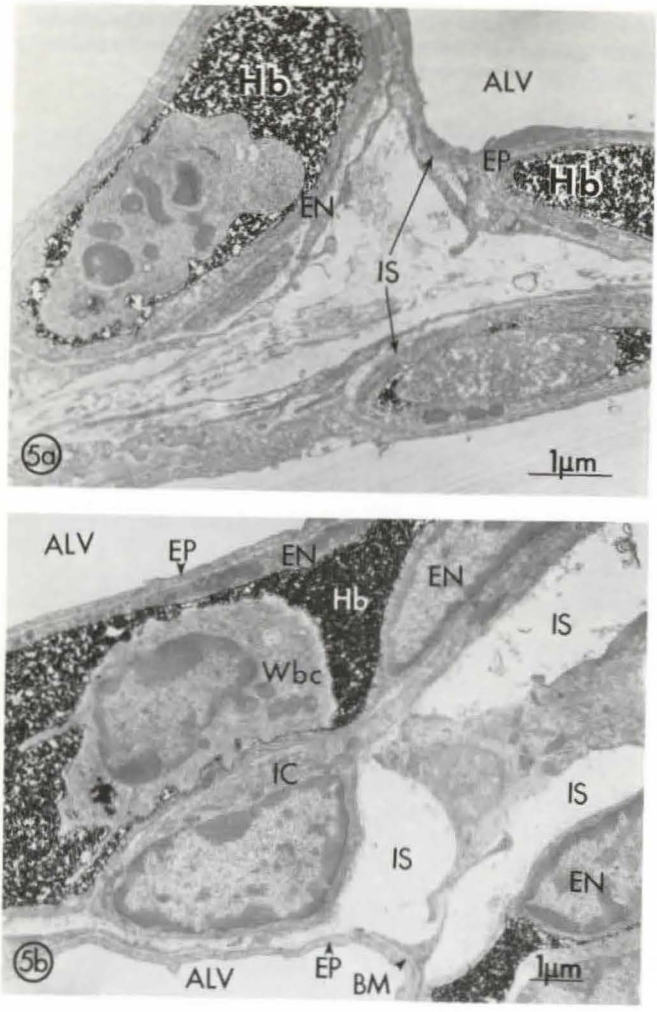

Fig. 5
The electromicrographs illustrate swelling and electronlucency of the collagenous portions of the interstitium, presumably reflecting edema fluid accumulation. The thin portion of the alveolar-capillary barrier is anatomically unchanged. Control (a) and experimental (b) lungs. WBC-white blood cells; IC-interstitial cell

Discussion

We have presented physiological data indicating that histamine did not cause permeability edema in the isolated rabbit lungs. We have also presented morphological data indicating that histamine did not consistently cause a greater leakage of stroma-free hemoglobin than occurred in the control lungs.

Physiological Data

Our data showed that following the administration of histamine the lungs did not gain in weight continuously. From this pattern of response we have concluded that histamine did not increase the permeability of the pulmonary microvessels. This finding is in contrast to what would have been predicted by the experiment of *Brigham* and *Owen* (10) in the unanesthetized sheep. It may be argued that their technique was more sensitive to changes in vascular permeability. However, using their data we estimated that, if edema was developing, the weight of the rabbit lung should have increased by 0.561 grams during the first 30 minutes following the administration of histamine. This estimate assumes that lymph did not leave the isolated lung and the

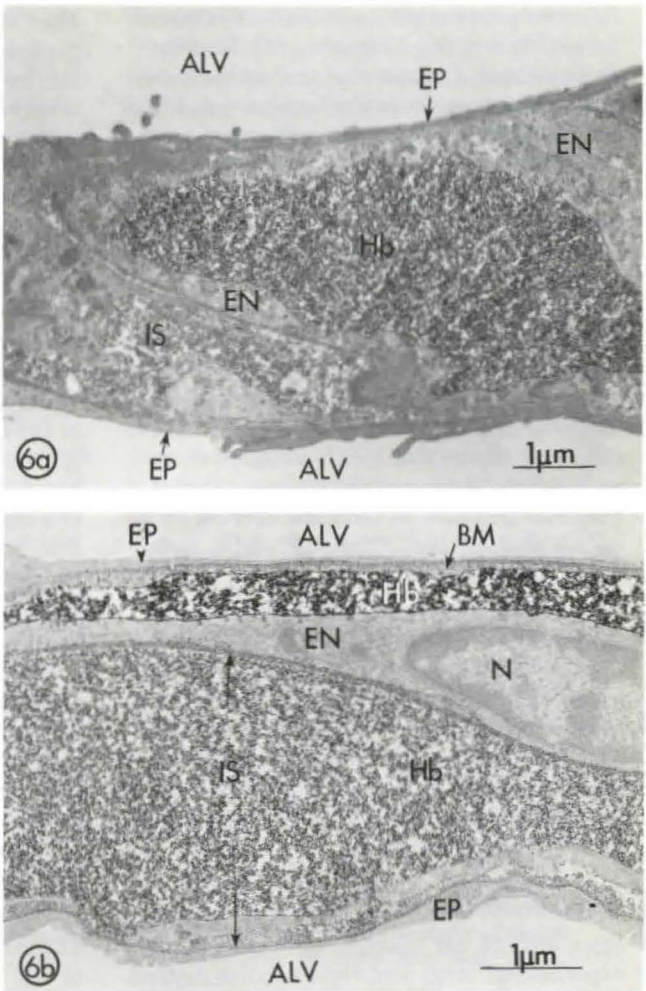

Fig. 6
Marked widening of the collagenous portion of the interstitium and extravascular accumulation of hemoglobin in control (a) and experimental (b) lungs. The epithelial cells prevent the escape of tracer into the alveoli

increase in lung water reported by *Brigham* and *Owen* (10) occurred at a uniform rate. Since the sensitivity of our recording system was 25 mg/mm deflection, a 0.5 gram increase in lung weight was well within the limits of our technique. It should also be noted that this technique provides a reliable means of assessing histamine induced edema in systemic vascular beds (4).

Although we interpreted constant perfusate flow and lung weight as convincing evidence that the perfused lung was not becoming edematous, it might be argued that an increase in microvascular permeability, caused by histamine, was counterbalanced by a decrease in capillary pressure due to increased pre-capillary resistance. However, it seems unlikely that these changes in permeability and hydrostatic pressure could have been so well balanced in two out of five preparations. Moreover, *Brody* and *Stemmler* (13) and *Glazier* et al. (14) have presented evidence indicating that histamine in the lung causes venoconstriction rather than arteriolar constriction as would be necessary for the preceding argument to be valid.

The effect of histamine on the permeability of an isolated lung is controversial. Histamine

failed to produce edema in isolated rabbit lungs (15) and dog lungs (18, 19). However, *Dawson* et al. (16) concluded that histamine increased the extravascular compartment of isolated cat lungs. These investigators observed the same decrease in lung weight as we did following histamine administration, but used an indicator dilution technique to measure vascular volume changes. With these techniques, they concluded that vascular compartments decreased more than total weight. Since *Lehr* et al. (17) from this laboratory have shown that histamine markedly alters the distribution of blood flow in the rabbit lung it is likely that *Dawson* et al. (16) underestimated the intravascular volume of their preparation because of the uneven distribution of blood flow in histamine treated lungs.

The physiological technique that we used to determine whether edema liquid was accumulating in the lung is not adequate by itself to determine if permeability changes had occured following the administration of histamine. To assess changes in permeability we used hemoglobin as a protein tracer for morphological studies. The advantage of this approach is that it enabled us not only to detect permeability changes but also to visually identify the site of leakage.

Morphological Data

Since leakage was found in both control and experimental lungs a semiquantitative method for the analysis of the anatomic data was developed. The nature of the histochemical reaction precluded the application of more rigorous morphometric methods (20).

The results of these studies show that histamine did not consistently cause a greater leakage of hemoglobin (m.w. 68,000) than occurred in the control lungs. Although histamine caused a greater leakage of hemoglobin from the corner and septal vessels in the upper portion of the left lower lobe, such effect was absent in other portions of the lobe. Furthermore, the leakage of hemoglobin from the corner vessels of the lower portion of the lobe was greater in the control lungs than in the histamine treated ones.

No differences in degrees of interstitial swelling (probably due to edema fluid accumulation) were found between control and histamine treated lungs, except in the case of the septal vessels of the upper portion of the lungs. Taken together, the morphological observations generally support the physiological data that histamine did not cause a greater amount of edema than was found in the control lungs. The findings that there was significantly greater leakage of hemoglobin from the corner and septal vessels in the upper portions of the lobe is an interesting one, but difficult to explain. One is tempted to conclude that as the experiment progressed, the lower regions of the lungs were not well perfused and hence did not receive any histamine. However, this argument is not supported by the fact that hemoglobin was found intravascularly in the base of the lung and by the fact that the amount of edema found in the lower portion of the lung was not greater than that seen in the upper regions (see Table 2).

That we have found evidence of interstitial edema and protein leakage in the control lungs as well as the histamine treated lungs suggests that our isolated lungs may have deteriorated so much that the addition of histamine could not further increase permeability. We have no real means of assessing this possibility, but wish to note the following:

The control lungs for the physiological part of this study did not progressively gain weight during the experiment. The experimental lungs showed a vigorous vasoconstrictor response when histamine was added to the perfusate suggesting that they had not seriously deteriorated.

No anatomic damage of the microvascular endothelium was detected inspite of the mild interstitial edema. Finally, the appearance of hemoglobin reaction product in the interstitium may have been the result of normal transcapillary protein exchange. *Brigham* and *Owen* (10) reported lymph/plasma ratios of albumin of 0.85 in their unanesthetized sheep. Since hemoglobin has a molecular weight similar to that of albumin, one would expect that in steady-state, the amount of hemoglobin

in the interstitium would approach that seen in the capillaries. Moreover, the tetrameric molecule of hemoglobin is in equilibrium with dimers and monomers which may more easily cross the endothelium (12). Although we fixed our lungs within 15 minutes after the addition of the hemoglobin, wash-in of molecules the size of albumin proceeds rapidly at first and then decays exponentially with time. For example, *Staub* (21) has presented data indicating that 30 minutes after an intravenous injection of I-125 labelled albumin, the specific activity of pulmonary lymph is already 20% that of simultaneously sampled plasma. The specific activity of the interstitium must rise faster than that in the lymph and so it may not be unexpected to find hemoglobin in the interstitium of our isolated perfused lungs within 15 minutes.

Michel et al. (22) have presented evidence indicating that the morphological techniques similar to those used by us were not sensitive enough to detect the normal transcapillary movement of protein. In their experiments, they used horseradish peroxidase as a protein tracer. The difference between their results and ours may be related to the fact that we were able to use relatively higher concentrations of tracer (hemoglobin) than they were able to use in their dog experiments and that lymphatic drainage of the interstitium is not operating in an isolated perfused lung preparation, which would promote the accumulation of tracer in the interstitium.

General Discussion

The role of histamine on the permeability of pulmonary microvessels is controversial. Data from this lab (9) has shown that injections of histamine in the dog result in an increased permeability of only the bronchial circulation. *Brigham* and *Owen* (10) infused histamine intravenously into awake sheep and measured movement of endogenous proteins from pulmonary vessels to lymphatics. They showed a dose-related increase in lung lymph flow and protein clearance. This effect was less marked when histamine was injected into the left atrium, and they concluded that histamine increased the permeability of pulmonary microvessels rather than that of bronchial vessels. *Propst* et al. (23) using saline-filled dog lungs found that histamine increases the permeability of the alveolar-capillary membrane to low molecular weight solutes but not to albumin. *Goetzman* and *Visscher* (19) using an *in vitro* isolated saline-filled dog lung lobe preparation also found that histamine does not increase the permeability of the alveolar-capillary membrane to albumin. In the present study we used an animal with poorly developed bronchial circulation and we found no evidence that histamine increases the transvascular movement of proteins in the pulmonary circulation. The difficulty in interpreting the data from the studies of *Brigham* and *Owen* (10) is that the contribution of the bronchial circulation to the formation of the lymph is uncertain. While it is likely that the bulk of the lymph is formed by the pulmonary circulation under normal conditions, the close proximity of the bronchial vessels to the peribronchial lymphatics and their sensitivity to the action of certain mediators of permeability suggest that the "systemic" vessels of the lung may influence the composition of the pulmonary lymph in pathologic states.

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