ACUTE LOCAL ANAPHYLACTIC INFLAMMATION OF THE LUNGS*

PAUL R. CANNON, M.D., THEODORE E. WALSH, M.D., AND CHARLES E. MARSHALL, B.S.

(From the Departments of Pathology and Surgery (Division of Otolaryngology), University of Chicago, Chicago, Ill.)

The phenomenon of acute local anaphylactic inflammation is not clearly understood, either as to its mechanism of development or its relationship to immunity. Its specificity is generally agreed upon, but the type and source of antibodies are still uncertain. Although there is evidence that the local anaphylactic reaction results from the union of antigen with precipitins in the blood and tissues, some investigators attribute it to a specifically changed reaction-capacity of tissues and suggest that this may be entirely independent of demonstrable antibodies, either in the blood or tissues. Some also regard the entire process as harmful, to be eliminated whenever possible, whereas others consider it as ordinarily beneficent, although occasionally injurious.

A part of the uncertainty relating to the entire problem has come from the use of complex antigens, particularly bacterial cultures, vaccines, or animal proteins containing several antigenic components. Furthermore, these materials have usually been injected into solid tissues, such as the skin, joints, kidneys, heart and aorta—organs in which interstitial, parenchymal and vascular lesions cannot be readily differentiated. Inasmuch as there is considerable evidence that the primary reaction of local anaphylaxis is vascular, it is desirable, in order that the primary lesions may not be obscured by secondary effects, to study its development in a vascular organ containing a minimal quantity of interstitial and parenchymal tissue. The lungs of rabbits, because of their vascularity, seem well suited for such a study, especially since they play such an important part in the development of the anaphylactic reaction in this species. They afford an excellent opportunity, furthermore, for the nontraumatic introduction of the antigen into the air passages where the reaction can develop as a surface phenomenon on a "blood-tissue"

* Received for publication March 26, 1941.

barrier; *viz.*, the membrane which lines the alveolus and separates it from the lumen of the intra-alveolar capillary.

Only a few investigators have made histological studies of anaphylactic inflammation in the lungs. Earlier workers [1,2] observed the development of pneumonitis in guinea pigs sensitized to a foreign serum following inhalation of a spray of the serum; Schlecht and Schwenker [3] described pulmonary hemorrhages and foci of acute alveolitis appearing as early as 6 hours after inhalation. Opie [4] injected 0.2 cc. of horse serum through the thoracic wall into the lung of an immunized rabbit and found that it caused "localized consolidation with leucocytes and edema surrounding a central focus of necrosis," whereas, "the same antigen injected into a normal rabbit was absorbed from the lung with no noteworthy change." Fried [5] injected horse serum directly into the trachea of normal and of sensitized rabbits and noted in the latter an intensified inflammation of the lungs, characterized by edema, hemorrhage, leukocytic infiltration, deposition of fibrin, consolidation and necrosis.

The following experiments were performed with the object of studying histologically the pulmonary lesions which might develop in normal and protein-sensitized rabbits following the entrance of a solution of protein into the lungs.

Materials and Methods

Rabbits from a source carefully guarded against snuffles were made hypersensitive by several subcutaneous injections, at intervals of from 5 to 7 days, of a solution of dried egg white or of crystalline egg albumin (crystallized three times). The latter substance is a relatively homogeneous purified antigen which is but slightly irritative to normal tissues. For some of the tests a solution of partially purified egg albumin, representing the albumin fraction after the first precipitation with ammonium sulfate, was used. This is referred to as albumin precipitate. The rabbits, when adequately sensitized, as shown by an intradermal injection of 0.1 cc. of a 2 per cent solution of the protein, were paired with normal rabbits and a solution of either crystalline egg albumin or of the albumin precipitate was instilled simultaneously into their nostrils. (It is well known that light fluids, when introduced into the nostrils of unanesthetized rabbits, flow

readily down the trachea into the lungs. This obviates the necessity for intratracheal injection or for direct injection through the thoracic wall with the complicating elements of surgical trauma, hemorrhage, shock or interference by anesthesia.) At varying intervals the animals were killed by air embolism and the lungs fixed *in situ* with a Zenker-formaldehyde solution. Celloidin sections were prepared and stained with hematoxylin and eosin. In some instances rabbits were sensitized passively by the intravenous or intraperitoneal injection of serum from rabbits hypersensitive to crystalline egg albumin and the effects following instillation of the solution of protein into their lungs observed. In some of the animals, also, the precipitative titers, as determined by the collodion particle agglutination method,[6] were determined at the time of instillation of the protein solution.

Results

Findings in Normal Rabbits

Sixteen normal rabbits were used as controls and their lungs examined from 4 to 48 hours after intranasal instillation of the protein solutions. Eleven of the animals were killed 24 hours after the instillation. The effects of the entrance of the egg albumin into their lungs were for the most part slight. Despite the fact that several blocks of tissue were taken from each lung, and that an effort was made to select the most abnormal looking areas, most of the sections showed no significant change. An occasional slight area of acute alveolitis was seen in a few instances, with an associated minimal edema. Acute hemorrhage was never seen and the perivascular lymphatics usually appeared normal. There was no phlebitis or arteritis and no thrombosis. Almost all sections were described as normal and inflammation, when present, was minimal. Crystalline egg albumin or a solution of dried egg white, therefore, may be regarded as practically nontoxic in the amounts and concentrations that entered the lungs of these normal rabbits.

Findings in Rabbits Sensitized Against Egg Albumin

Nineteen hypersensitive rabbits were treated simultaneously with the controls. The effect of the entrance of the protein solutions into their lungs, however, was strikingly different, being

characterized by acute edema, alveolitis, bronchitis and pneumonic consolidation (Figs. 1 and 2). The severity of the reaction depended essentially upon the degree of sensitivity of the animals, the amount of material which apparently entered the lungs and the length of time after its instillation. The lymph flow was markedly increased (Fig. 3) and at times hemorrhage occurred, both into lymphatic spaces and into alveoli (Fig. 4). Acute arteritis and phlebitis were present in some animals, with, in some instances, mural thrombosis in small arteries and veins (Fig. 6). Infarction also occasionally developed. The effect was as if the nontoxic solution of protein had become toxic, at times extremely so. Thus, the primary and outstanding effect was upon blood vessels and was made evident particularly by increased capillary permeability. Only when the effect was more intense did arteritis, phlebitis, infarction and marked pneumonic consolidation occur.

Findings in Rabbits Passively Sensitized

An attempt was made to ascertain whether the pulmonary lesions which appeared so strikingly in actively sensitized animals could be demonstrated also in those passively sensitized. Sera from several rabbits which were strongly hypersensitive to crystalline egg albumin were pooled and 50 cc. were injected intravenously into two normal rabbits (P_3 and P_4). At the end of 24 hours the animals were found to be definitely hypersensitive to egg albumin and samples of their sera were taken. A solution of precipitated egg albumin was then instilled intranasally and, at the end of 24 hours, a second sample of blood was taken, the animals were sacrificed and their lungs prepared for histological study. The two samples of sera, with serum from a normal animal as a control, were tested against collodion particles to which crystalline egg albumin had been adsorbed. The precipitative titers of both hypersensitive rabbits showed a decline from 1:1920 to 1:120 as a consequence of the intranasal instillation of the albumin and its combination with antibody in the lungs. Sections of the latter (Figs. 7 and 8) showed acute edema, deposition of fibrin within alveoli, early acute alveolitis and hemorrhage, with erythrocytes present in dilated perivascular lymphatic spaces and alveoli.

Two additional rabbits, with two controls, were similarly treated (P6 and P7) and sacrificed at the end of 48 hours. Sections from the lungs of the normal rabbits showed nothing unusual in one and only a few tiny areas of acute alveolitis in the other. In the sensitized animals, however, the pulmonary inflammation was intense, being characterized by marked acute focal alveolitis, dilation of the perivascular lymphatic spaces and accumulations of mononuclear cells and masses of fibrin in the alveolar spaces.

Inasmuch as passive sensitization in the preceding experiments was accomplished by way of the blood stream, an attempt was made to produce it directly within the alveolar spaces. Fox [7] has shown that immune sera, when introduced into the lungs by way of the bronchi, are absorbed but slowly into the blood stream. This is due, presumably, to the slow diffusibility of the larger globulin molecules. Ten cc. of serum from a rabbit hypersensitive to crystalline egg albumin were instilled into the nostrils of a normal rabbit. Twenty minutes later 2 cc. of a 4.4 per cent solution of albumin precipitate were then instilled intranasally. The animal was sacrificed 30 hours later. Sections showed a more marked acute edema and focal alveolitis than were seen in rabbits P3 and P4, with many polymorphonuclear leukocytes centered around eosinophilic masses (precipitate?). This finding would suggest an antigen-antibody reaction occurring largely on or within the alveolar walls, with the increased capillary permeability and acute inflammation ensuing.

It is obvious, therefore, that the local anaphylactic reaction is essentially the same, whether it occurs in rabbits actively or passively sensitized. This must mean that the determining element in the reaction is humoral, presumably the anaphylactic antibody and not primarily a changed reaction-capacity of the tissues.

Discussion

These findings corroborate those of others that, whether in general or local anaphylaxis, much of the reaction is vascular and manifests itself as an increased capillary permeability as the result of specific injury to endothelium. Moon [8] has recently called attention to the striking similarity between the circulatory

disturbances seen in anaphylactic shock and in experimental or clinical traumatic shock and has emphasized the point that in anaphylaxis "the capillary endothelium is a chief point of injury." The injury occurs, presumably as a result of the union of antigen and antibody within the sensitized endothelium. Manwaring, Chilcote and Hosepian,[9] and Petersen and Levinson [10] have shown that in anaphylactic shock the endothelium may be damaged to the point of allowing erythrocytes to pass through. Zander [11] has shown recently, by means of the application of a partial vacuum to the skin of rabbits, that allergic inflammation leads to an increased tendency to capillary hemorrhage or, as he calls it, an increased capillary fragility. Direct evidence of the effect of an antigen-antibody reaction upon vascular permeability is furnished in the experiments of Abell and Schenck.[12] These observers studied the action of horse serum introduced into the moat of an ear chamber in rabbits sensitized to horse serum. They observed contraction of arterioles, with stoppage of circulation, an increased tendency of leukocytes to adhere to the endothelium and passage of leukocytes in large numbers through the walls of capillaries and venules. Leukocytes, at times, also formed clumps large enough to cause embolic blockage in capillaries and venules. With repeated introduction of horse serum into the moat, even extravasation of erythrocytes and endothelial destruction occurred. Rich and Follis [13] more recently have concluded that, as a result of studies of the Arthus phenomenon in the corneas of sensitized rabbits, "the sensitivity that determines necrosis appears to be limited to the blood vessels, and especially to the endothelium" whereas "the cells of the tissues at large are not themselves sensitized."

The exact cause of the increased capillary permeability is uncertain. Lowered oxygen tension with resulting asphyxia of the vessel wall is usually considered of great importance as a cause of such a change. It is possible that this may occur also when antigen and precipitin combine on or within endothelial cells. The interference with cellular respiration might be manifested quickly by the increased permeability which is so conspicuous a feature of the anaphylactic reaction. A final answer cannot be given, however, until such intracellular precipitation can be demonstrated in living endothelium.

SUMMARY

Rabbits made hypersensitive (actively or passively) to purified egg albumin, and normal controls, were given simultaneous intranasal instillations of a solution of purified egg albumin. They were sacrificed at varying intervals, the lungs were fixed *in situ* in a Zenker-formaldehyde solution and sections were stained with hematoxylin and eosin.

In the normal animals pulmonary inflammation was minimal and frequently indiscernible. In the hypersensitive rabbits, however, the entrance of egg albumin into the lungs engendered the development of acute pneumonitis, characterized by edema, alveolitis, bronchitis and pneumonic consolidation. The perivascular lymphatics were dilated and contained many erythrocytes. Acute arteritis and phlebitis occurred at times and mural thrombosis was occasionally seen. The findings were essentially identical, whether the rabbits were actively or passively sensitized.

The experiments indicate, therefore, that the primary effect of the antigen-antibody reaction in the lungs was an increased capillary permeability, followed later by severer vascular injury. They show, furthermore, that the effect is due to a humoral element, presumably the anaphylactic antibody, rather than to a changed reaction-capacity of the tissues. The intensity of the reaction depends, apparently, upon the varying intensities of the antigen-antibody union.

REFERENCES

1. Friedberger, E. Die Anaphylaxie mit besonderer Berücksichtigung ihrer Bedeutung für Infektion und Immunität. *Deutsche med. Wchnschr.*, 1911, **37**, 481–487.

2. Ishioka, S. Zur Histologie der anaphylaktischen Pneumonie. *Deutsches Arch. f. klin. Med.*, 1912, **107**, 500–507.

3. Schlecht, H., and Schwenker, G. Über die Beziehungen der Eosinophilie zur Anaphylaxie. *Deutsches Arch. f. klin. Med.*, 1912, **108**, 405–428.

4. Opie, E. L. Inflammatory reaction of the immune animal to antigen (Arthus phenomenon) and its relation to antibodies. *J. Immunol.*, 1924, **9**, 231–245.

5. Fried, B. M. Allergic lobar pneumonia. *J. Exper. Med.*, 1933, **57**, 111–119.

6. Cannon, P. R., and Marshall, C. E. An improved serologic method for the determination of the precipitative titers of antisera. *J. Immunol.*, 1940, **38**, 365–376.

7. Fox, J. P. The permeability of the lungs to antibodies. *J. Immunol.*, 1936, **31**, 7–23.

8. Moon, V. H. The pathology and mechanism of anaphylaxis. *Ann. Int. Med.*, 1938, **12**, 205–216.

9. Manwaring, W. H.; Chilcote, R. C., and Hosepian, V. M. Hepatic reactions in anaphylaxis. VIII. Anaphylactic reactions in isolated canine organs. *J. Immunol.*, 1923, **8**, 233–238.

10. Petersen, W. F., and Levinson, S. A. Studies in endothelial permeability. II. The rôle of the endothelium in canine anaphylactic shock. *J. Immunol.*, 1923, **8**, 349–359.

11. Zander, Ernst. Changes in blood vessels (capillary fragility) with inflammation. *J. Exper. Med.*, 1937, **66**, 637–651.

12. Abell, R. G., and Schenck, H. P. Microscopic observations on the behavior of living blood vessels of the rabbit during the reaction of anaphylaxis. *J. Immunol.*, 1938, **34**, 195–213.

13. Rich, A. R., and Follis, R. H., Jr. Studies on the site of sensitivity in the Arthus phenomenon. *Bull. Johns Hopkins Hosp.*, 1940, **66**, 106–122.

DESCRIPTION OF PLATES

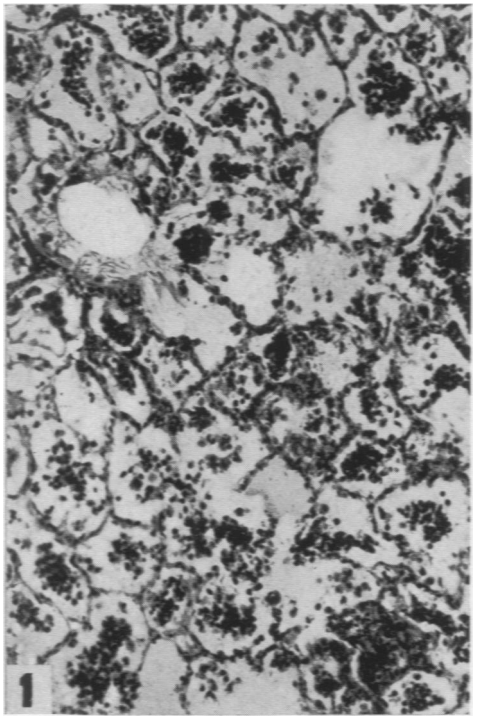
PLATE 122

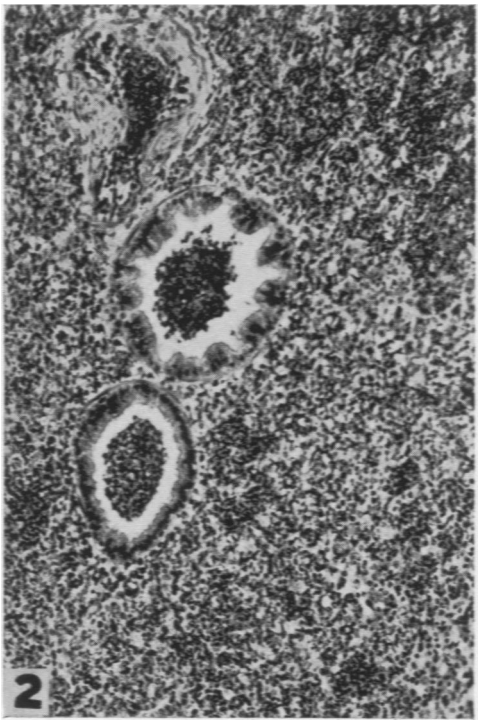
FIG. 1. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 10 per cent crystalline egg albumin. Acute edema and alveolitis are present. × 125.

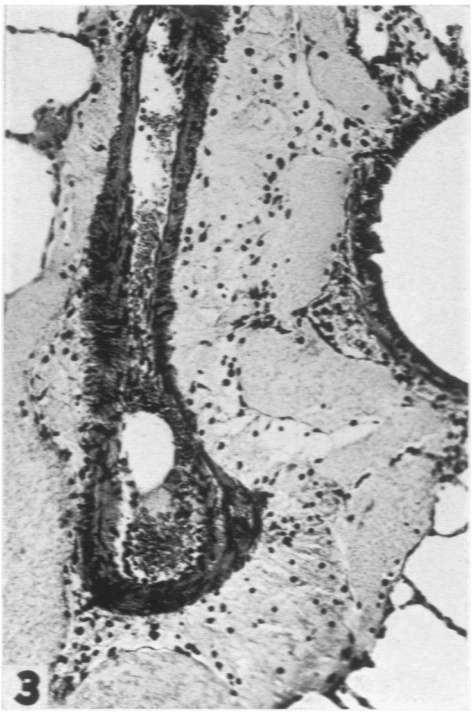
FIG. 2. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of 5 per cent crystalline egg albumin. Taken from an area showing acute bronchitis and bronchopneumonia. × 125.

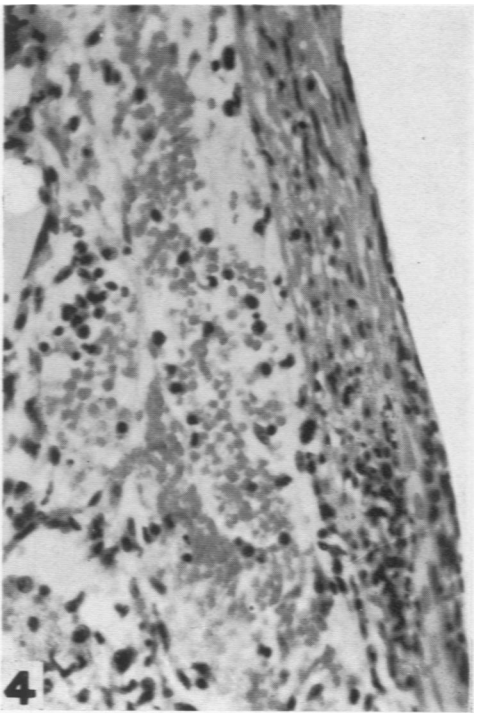
FIG. 3. From the lung of a rabbit actively sensitized against crystalline egg albumin 8 hours after intranasal instillation of crystalline egg albumin, showing marked dilation of a perivascular lymphatic space. × 130.

FIG. 4. From the lung of a rabbit actively sensitized against crystalline egg albumin 24 hours after intranasal instillation of crystalline egg albumin. The increased vascular permeability is shown by the presence of erythrocytes in the perivascular lymphatic space. × 290.









Cannon, Walsh and Marshall

Anaphylactic Inflammation of the Lungs

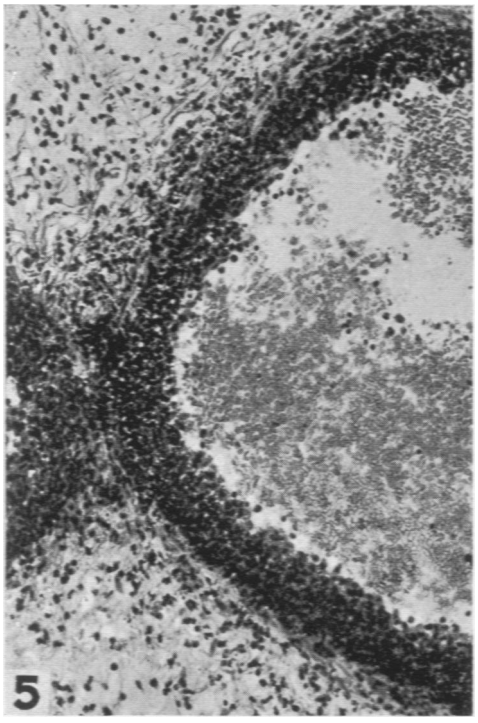
PLATE 123

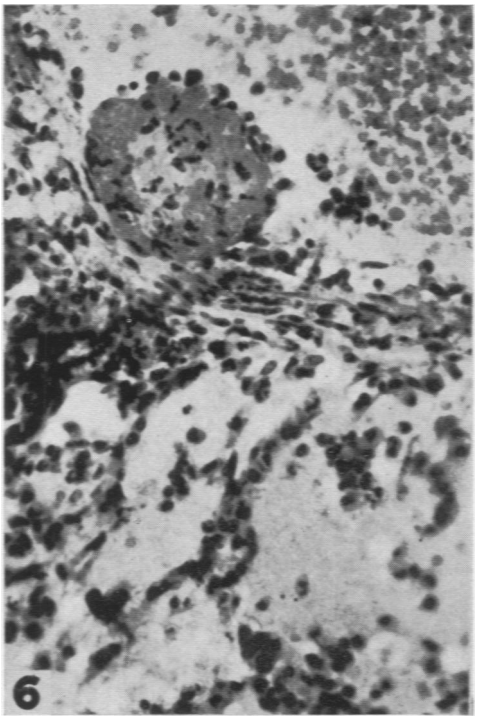
FIG. 5. From the lung of a rabbit actively sensitized against dried egg white scales 24 hours after intranasal instillation of a solution of dried egg white. Acute phlebitis and acute lymphangitis are seen. × 160.

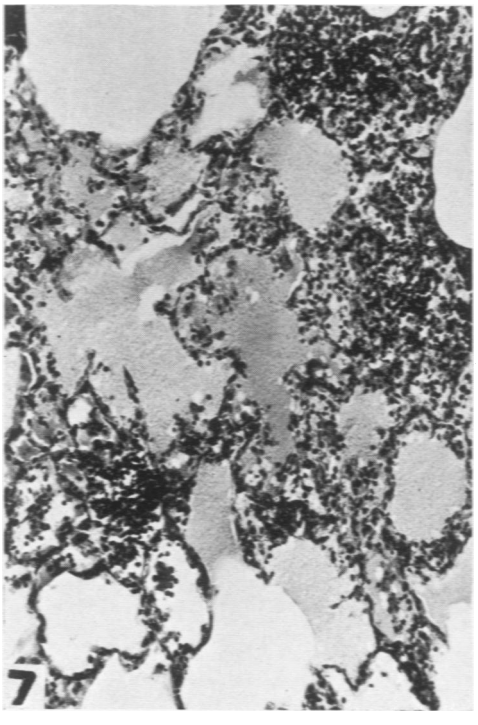
FIG. 6. Photomicrograph from the lung of the same rabbit used for Figure 1, demonstrating an early mural thrombus in a pulmonary vein. × 275.

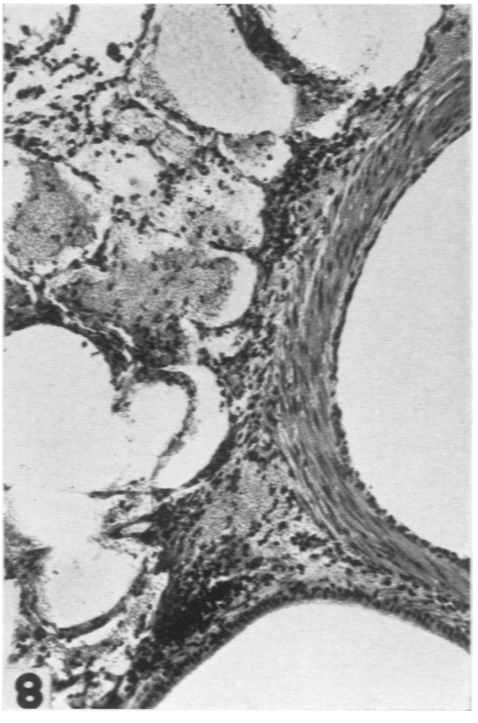
FIG. 7. Photomicrograph of the lung of a rabbit passively sensitized to crystalline egg albumin 24 hours after intranasal instillation of albumin precipitate. Acute pulmonary edema and acute alveolitis are seen. × 120.

FIG. 8. Photomicrograph of the lung of the same rabbit shown in Figure 7 to illustrate acute edema and acute hemorrhage into the alveolar and perivascular lymphatic spaces. × 130.









Cannon, Walsh and Marshall

Anaphylactic Inflammation of the Lungs