Pulmonary Injury Induced by C3a and C5a Anaphylatoxins

Norma P. Stimler, PhD, Tony E. Hugli, PhD, and Colin M. Bloor, MD

Homogeneous anaphylatoxins C3a (human or porcine), C5a (porcine), and the porcine classic anaphylatoxin, a mixture of C5a and C5a des Arg, isolated from complement-activated serum, were shown to induce acute pulmonary injury in the guinea pig following intrabronchial instillation. The gross physiologic response to these factors is characterized by respiratory distress with rapid, shallow breathing. Administration of 8–17 μg/kg of porcine classic anaphylatoxin proved lethal in 50% of the animals treated. The acute response (less than 20 minutes after instillation) of pulmonary tissue to insult by the anaphylatoxins is characterized by constriction of the smooth muscle walls in both bronchioles and pulmonary arteries and by focal atelectasis. Aggregates of platelets and leukocytes in pulmonary vessels and in other organs such as the chambers of the heart were commonly observed after intrabronchial administration of the anaphylatoxins. Although C3a was never lethal in guinea pigs even when doses as high as 500 μg/kg were administered by the intrabronchial route, this anaphylatoxin did induce the same pattern of acute pulmonary injury as C5a. *In vitro* experiments employing guinea pig platelets indicated that these cells aggregate in the presence of 10^{-10} M porcine C5a but are not affected by C3a (human or porcine) even at levels up to 10^{-6} M. Hence, platelet aggregation as observed *in vivo* may be directly affected by C5a, but in the case of C3a, secondary mediators must be involved. Anaphylatoxin preparations were also shown to induce contraction of guinea pig lung strips *in vitro:* this effect was not inhibited by antihistamines at concentrations that blocked contraction to exogenous histamine. The *in vivo* response to anaphylatoxin could be blocked with high doses of the antihistamine chlorpheniramine but not by corresponding doses of diphenhydramine. (Am J Pathol 1980, 100:327-348)

THE LUNGS constitute a common portal of entry for many inflammatory agents, and a significant proportion of the pulmonary lesions that result may be mediated by immunopathologic mechanisms involving complement activation.[1-6] Several model systems have been developed to investigate the role of complement in pulmonary disease. Studies by Vasquez [7] and Hagadorn et al [8] in which rats were given intravenous injections of rabbit antiserum to rat lung demonstrated severe pneumotoxic alveolitis with marked cyanosis and tachypnea, resulting in death within 2–30 minutes.

To distinguish between humoral and cellular origins of the immune reaction involved in antigen-induced pulmonary disease, Richerson [9] immu-

From the Department of Pathology, University of California at San Diego, La Jolla, California, and the Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California.

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Address reprint requests to Dr. Norma P. Stimler, Department of Pathology, UCSD School of Medicine, M-012, La Jolla, CA 92093.

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nized guinea pigs with various antigens, some favoring antibody production and others the development of cell-mediated hypersensitivity. These animals were subsequently challenged intratracheally with an aerosol of the antigen that had been injected. Histologic examination indicated that complement-fixing antibody mediated a severe hemorrhagic pneumonia with evidence of polymorphonuclear leukocyte infiltration within 4–6 hours. Recently, Schertzer and Ward [10,11] presented evidence that the extent of pulmonary injury observed in rats infused intratracheally with antigen–antibody complexes of varying composition is directly related to their complement fixing activity *in vitro*. Thus it is apparent that at least some aspect of complement activation is involved in the immunopathologic mechanisms of pulmonary injury.

Complement activated by either the classic or alternative pathway results in the production of biologically active peptides C3a and C5a from the third and fifth components of complement, respectively.[12,13] The complete chemical structures of human,[14] porcine,[15] and rat [16] C3a and of the C5a polypeptide chains from human [17] and porcine [18] blood have recently been determined. Both peptides possess the well-known spasmogenic properties of an anaphylatoxin,[19,20] and C5a is also a potent mediator of human polymorphonuclear leukocyte chemotaxis.[21] The spasmogenic activities of these materials are controlled *in vivo* by a serum carboxypeptidase of the B type.[22] Exopeptidase action results in complete abrogation of the spasmogenic properties of human and porcine C3a. Porcine C5a, in contrast, escapes total inactivation by the serum carboxypeptidase, retaining 20–40% of the initial spasmogenic activity.[23]

The work presented in this study was undertaken to assess the potential role that the anaphylatoxic peptides C3a and C5a may play in complement-mediated pulmonary injury. Our model system was designed to study responses in guinea pig lung to infusion of chemically pure C3a or C5a. This is the first investigation of the acute response of pulmonary tissue in guinea pig induced by these biologically active peptides in a pure form.

Materials and Methods

Purification of the Anaphylatoxins

Porcine C5a and C5a des Arg (ie, the classic anaphylatoxin*), were purified from porcine serum activated for complement in the presence or absence of ε-aminocaproic acid,

* Complement components are named in accordance with the World Health Organization Committee on Complement Nomenclature (1968). Porcine classic anaphylatoxin is defined as C5a des Arg obtained by activation of complement in the absence of the serum carboxypeptidase inhibitor, ε-aminocaproic acid. In activated porcine serum the spasmogenic activity of C5a des Arg is equivalent to 20–40% of intact C5a activity.

respectively, according to the procedure described by Gerard and Hugli.[23] Porcine C3a and C3a des Arg were isolated in the course of C5a preparation following SP-Sephadex chromatography and were further purified by chromatography on a column of QAE-Sephadex under the same conditions as used for the final purification step of the C5a and classic anaphylatoxins. Human C3a was isolated by the same procedure as used for porcine C3a. All peptide materials were homogeneous by the criteria of polyacrylamide gel electrophoresis, cellulose acetate electrophoresis, and amino acid composition. Anaphylatoxin activities were assessed with the use of terminal strips of guinea pig ileum as described by Cochrane and Müller-Eberhard.[20] Chemotactic activities were determined by the use of a modified chemotaxis under agarose technique.[24]

Treatment of Guinea Pigs

Albino guinea pigs of either sex (Hartley), weighing 250–700 g, were anesthetized with an intramuscular injection of a mixture of Rompun (xylazine, 20 mg/ml, Haver–Lockhart) and Ketaset (ketamine hydrochloride, 100 mg/ml, Bristol Laboratories, (1:1 vol/vol, 0.2–0.5 ml/animal). Lidocaine (lidocaine hydrochloride, 20 mg/ml, Med-Tech) was injected subcutaneously (0.5 to 1 ml) at the sites of incisions. The animals were entubated either by tracheostomy or by direct laryngoscopy. Some animals were artifically respirated via a tracheal cannula with a small animal respirator (Harvard Apparatus) adjusted to a tidal volume of 5–6 ml and a rate of 60 respirations/minute.

Peptide materials that were instilled into the lungs were dissolved in normal saline to give a total volume of 0.3 ml. Methyl green (0.1%) was included as a tracer in order to localize the actual site of administration. Solutions were administered to the lungs via a small catheter inserted approximately 1 cm past the carina into the right or left mainstem bronchus. Control guinea pigs were treated in the identical manner but received 0.3 ml saline containing 0.1% methyl green. In experiments where guinea pigs were pretreated with antihistamines, the animals were given an intraperitoneal injection of diphenhydramine hydrocholoride (Benadryl, 10 mg/ml, Parke-Davis, 20 mg/kg body wt) 30 minutes to 1 hour before the administration of the anaphylatoxins.

Electrocardiograms were monitored in some animals at 1–3-minute intervals throughout the experiment. Guinea pigs were maintained for 20–30 minutes after the intrabronchial administration of peptide solutions. The lungs were then excised, with the heart left attached, inflated under positive pressure, and fixed in formalin (10% formalin in 0.1 M sodium phosphate, pH 7.6). In some cases the liver, kidney, spleen, and brain were also excised and fixed.

Histologic Preparation

After fixation the lungs were examined visually to determine which lobe received the bulk of the anaphylatoxin–methyl green solution. The affected lobe, an adjacent lobe, and one lobe from the opposite lung were bisected, postfixed in Bouin's fixative, and processed for light microscopy. Five-micron sections were stained with hematoxylin and eosin (H&E). Periodic acid–Schiff (PAS), reticulum, and phosphotungstic acid–hematoxylin (PTAH) stains were used in selected cases. Sections of the other organs (heart, liver, kidney, spleen, and brain) were processed in a similar manner.

Slides were examined under the light microscope, comparing sections from anaphylatoxin-treated guinea pigs with sections from control animals. Representative fields are shown in Figures 1–4.

Vascular Permeability Measurements

Changes in vascular permeability were assessed according to the method described by Scherzer and Ward.[10] ^{125}I-labeled bovine serum albumin (BSA) (prepared by chloramine-T

labeling of commercial BSA [25]) was mixed with unlabeled BSA to give a specific radioactivity of 2×10^7 cpm/mg. A sample of 100 μg of ^{125}I-BSA in 0.5 ml normal saline was injected intravenously in an external jugular vein immediately before the anaphylatoxin was instilled intratracheally. The animals were killed after 30 minutes, the lungs were perfused through the aorta with Tyrode buffer (a volume equal to 10% of the animal's body weight), and the ^{125}I-BSA remaining in the lungs was determined by scintillation counting. An index of vascular permeability was computed from the ratio of ^{125}I cpm in the lungs to the ^{125}I cpm in 1 ml of blood collected at the time of sacrifice. These values were determined for 5 animals treated with 10 μg/kg of porcine classic anaphylatoxin (only those animals that survived the treatment were included), 5 animals treated with 200 μg/kg C3a, and 7 control animals that received only saline.

In Vitro Experiments

The platelet-aggregating activity of the porcine anaphylatoxins was tested with the use of both human and guinea pig platelets. Human and guinea pig platelet-rich plasma (PRP) was prepared from blood containing 0.4% sodium citrate. PRP (1 ml) was stirred in the photometer cuvette of a platelet aggregometer (Bausch and Lomb), anaphylatoxin solutions were added after the baseline was established, and changes in light scattering were recorded at 550 nm.

The ability of anaphylatoxins to contract guinea pig lung strips *in vitro* was assessed as described by Lulich et al [26] for cat lung strips. Guinea pig lungs were perfused with Tyrode buffer, and strips ($2 \times 3 \times 10$ mm) containing terminal airways and small blood vessels were suspended in Tyrode buffer in a muscle bath apparatus under a tension of 0.3 g. Anaphylatoxins were added to the bath, and the magnitude of the contraction produced was compared with that produced by a known concentration of histamine. To determine whether the contractions observed were the result of histamine release by the anaphylatoxins, antihistamines (chlorpheniramine or diphenhydramine at 10^{-6} M) were added to the bath 2 minutes before exposure to either anaphylatoxin or histamine. The tissues were washed and allowed to relax between subsequent tests. Contractions were recorded by means of an isotonic smooth muscle transducer (Harvard Apparatus, #387). Changes in length were amplified 40-fold and recorded at a chart speed of .01 cm/sec (Harvard Apparatus Recorder #350, chart mover #480).

Results

The native C3a and C5a anaphylatoxins, which were purified to homogeneity from zymosan-activated human or porcine serum, gave dramatic physiologic responses following intrabronchial infusion into guinea pigs (Table 1). The quantity of each peptide administered was adjusted to correspond to that quantity present in 2–5 ml of activated serum.[15,23] All peptides that display spasmogenic activity on the guinea pig ileum resulted in respiratory distress when infused into guinea pig lungs. These included porcine C5a, porcine classic anaphylatoxin, human and porcine C3a, and partially purified anaphylatoxin fractions from guinea pig serum activated either in the presence or absence of a serum carboxypeptidase inhibitor. The onset of rapid, shallow breathing (tachypnea) began 30 seconds after infusion. In animals surviving this treatment, tachypnea continued for approximately 20 minutes, and then respiration gradually returned to nor-

Table 1—Physiologic Response of Guinea Pigs to Intrabronchial Infusion of Anaphylatoxins

	No. of Animals	Dose (μg/kg)	Tachypnea*	Lethal
Porcine C5a	10	8–10	+	0
Porcine C5a des Arg	10	8–17	+	5
Porcine C3a	14	200–500	+	0
Human C3a	7	200–500	+	0
Guinea pig C5a des Arg†	3	0.2	+	1
Guinea pig ZAS†	3	3–4 μg C5a + 50–100 μg C3a	+	1
Porcine C3a des Arg	6	200–500	–	0
Saline (0.3 ml)	10		–	0

* Onset 30 seconds after infusion with 20-minute duration.

† Guinea pig serum was activated with zymosan in the presence or absence of IM EACA to give ZAS or "classic" anaphylatoxin, respectively. The material was partially purified and concentrated as indicated in Materials and Methods. The quantity administered to each guinea pig was the equivalent of 1 ml serum.

mal. No change was observed in the animal's electrocardiograms following treatment with the anaphylatoxins.

As indicated in Table 1, the porcine C5a des Arg at 8–17 μg/kg proved lethal in 50% of the animals tested. The partially purified anaphylatoxin preparations from guinea pig serum, containing either a mixture of C5a and C3a or C5a des Arg and C3a des Arg (See Table 1), were also lethal for one of every three animals tested at doses equivalent to 1 ml of the animal's serum. Artificial respiration did not alter the outcome of these experiments. In contrast, as indicated in Table 1, porcine C5a, at doses similar to the porcine C5a des Arg, never resulted in death. Human or porcine C3a (or C3a des Arg) at intrapulmonary doses as high as 500 μg/kg were never lethal.

Whole lungs excised 20 to 30 minutes after infusion of any of the spasmogenically active peptide preparations usually showed numerous small hemorrhagic foci. When the anaphylatoxin was delivered to a single lobe, as indicated by the localization of methyl green, that lobe was often pale and significantly smaller than an adjacent or untreated lobe.

The characteristic histologic appearance of pulmonary tissue treated with C5a is shown in Figure 1. This particular guinea pig was treated intrabronchially with 15.5 μg/kg of porcine C5a des Arg and died within 5 minutes as a result of the treatment. The anaphylatoxin solution was concentrated in the left lower lobe, as indicated by the relative intensity of tracer dye (Figure 1A). In all animals treated with this material, the bronchioles showed severe smooth muscle constriction, the epithelial layer was thrown into heavy folds obstructing the lumen, the smooth muscle walls of the arteries were thickened in contraction, and focal atelectasis was ap-

parent (Figure 1B). Occasionally pronounced vascular congestion was also observed. Focal aggregates of neutrophils and platelets, enmeshed in what appeared to be a fibrin network, were commonly found in the large pulmonary vessels of the treated lungs and often appeared to occlude smaller vessels. These cellular aggregates were also observed in areas of the lung that did not receive the anaphylatoxins directly, as indicated by the absence of tracing dye (Figure 1C). Although evidence of either airway or vascular smooth muscle contraction was not apparent, small thrombi were clearly present. In addition, thrombi were often observed in the right ventricle of the heart of treated animals. Lungs from animals infused with saline containing methyl green showed none of the above effects (Figure 1D).

Intrapulmonary infusions of porcine C3a at doses of 200–500 μg/kg in guinea pigs, although never lethal, resulted in a histologic appearance similar to that obtained with the porcine C5a des Arg (Figure 2). Furthermore, the platelet and leukocyte aggregates observed in pulmonary vessels and in the heart following administration of C3a (Figure 2 B–D) were larger and more abundant than were ever seen following infusions of either C5a or C5a des Arg.

In order to quantitate the vascular changes that result from treatment with these peptides, the ^{125}I-BSA entrapped in the lungs after a 30-minute intrapleural exposure to porcine C5a des Arg, to C3a, or to saline was determined. These data are presented in Table 2. These results indicate a significant ($P < .01$) increase in the plasma components trapped in the lungs of guinea pigs treated with anaphylatoxins over that of control animals.

Since the spasmogenic action of anaphylatoxins is believed to be mediated by vasoamines in the guinea pig ileum,[20] it was of interest to determine whether this mechanism also occurs in pulmonary tissue *in vivo*. Guinea pigs were pretreated with diphenhydramine (20 mg/kg) to block histamine uptake at the H_1 receptor sites. Animals were subsequently treated with porcine C3a or C5a des Arg as described above. The spasmo-

Table 2—Changes in Vascular Permeability Observed 20 Minutes After Intrabronchial Administration of Anaphylatoxins in Guinea Pigs

	Number of animals	Vascular permeability*	Control
Saline	7	0.28 ± .097	100%
Porcine C5a des Arg	5	0.49 ± .076	175%
C3a (200 μg/kg)	5	0.45 ± .087	161%

* Vascular permeability index calculated as ^{125}I in lungs/^{125}I in 1 ml blood ± SEM.

genic response was not altered by this treatment (Figure 3). No thrombi were observed; however, a known side effect of diphenhydramine is inhibition of platelet aggregation.[27] Control experiments indicated that selected antihistamine levels afforded protection from intrabronchial instillation of histamine.

When animals were treated in the same manner with chlorpheniramine (10 mg/kg) instead of diphenhydramine, the spasmogenic response to intratracheal anaphylatoxins was blocked and the lungs had the same appearance as the saline or C3a des Arg treated controls (Figure 3).

In order to ensure that subtle species differences in the anaphylatoxins did not account for the effects observed, partially purified anaphylatoxin fractions from guinea pig serum, activated in the presence (C3a + C5a) or the absence (C5a des Arg) of ϵ-aminocaproic acid, were infused under the same conditions as were the porcine and human anaphylatoxins. The histologic sections taken from animals treated with guinea pig factors were indistinguishable from those treated with human porcine anaphylatoxins (Figure 4).

In Vitro Experiments

In order to determine the source of the cellular aggregates observed, the platelet aggregating abilities of the several anaphylatoxins were assessed with the use of human or guinea pig citrate-treated platelet-rich plasma. The results of this experiment are presented in Table 3. Porcine C5a was effective in causing aggregation of guinea pig platelets at 10^{-10} M. Human C5a induced comparable aggregation at 10^{-9} M. The porcine C5a des Arg aggregated guinea pig platelets at 10^{-7} M. Human or guinea pig C3a was not effective in aggregating guinea pig platelets at concentrations as high as 10^{-6} M, and none of the peptides were capable of aggregating human platelets at any of the concentrations listed above.

In order to confirm the observation of bronchospasm *in vivo*, parenchymal strips of guinea pig lungs were suspended in a muscle bath apparatus and tested for smooth muscle contraction by anaphylatoxins. The results are shown in Text-figure 1. Moderate contractions were observed with 10^{-6} M C3a (porcine or human) and with 4×10^{-8} M porcine C5a des Arg. The response to 50 ng/ml of histamine (4.5×10^{-7} M) is shown for comparison. As observed with the guinea pig ileum assay,[20] the lung tissue was rapidly desensitized, and a fresh strip was required for each determination. The contractions to histamine (50 ng/ml) could be blocked effectively by either of the H_1 blocking drugs, diphenhydramine (10^{-6} M) or chlorpheniramine (10^{-6} M). When the chlorpheniramine concentration was increased to 10^{-3} M, the anaphylatoxin-induced contractions were

Table 3—Platelet Aggregation by the Serum Anaphylatoxins

Anaphylatoxin	% Aggregation*	
	Guinea pig PRP	Human PRP
Porcine C5a		
1.2×10^{-10} M	50	ND†
1.2×10^{-8} M	61	0
1.2×10^{-7} M	100	0
Porcine C5a des Arg		
1.2×10^{-8} M	7	ND
1.2×10^{-7} M	114	ND
4.8×10^{-7} M	ND	0
Human C5a		
4.2×10^{-10} M	9	ND
8.3×10^{-9} M	83	0
3.3×10^{-7} M	ND	0
Porcine C3a		
1.1×10^{-8} M	0	0
1.1×10^{-6} M	0	0
Human C3a		
1.1×10^{-7} M	0	0
1.6×10^{-6} M	0	0

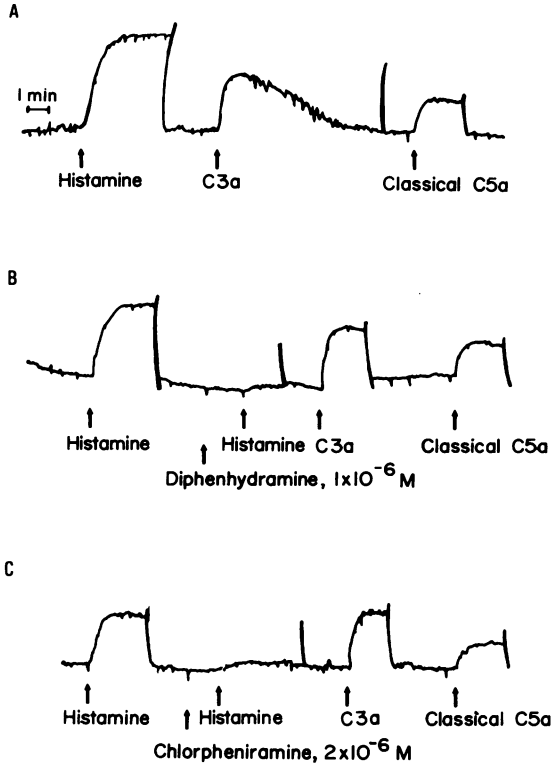
* % aggregation, compared with that produced by 2 μM ADP.
† Not determined.

completely inhibited. Since the dose of chlorpheniramine used in the whole animal experiments was 10 mg/kg, the effect of the higher concentration *in vitro* may be more representative of the effect observed *in vivo*.

Discussion

The results of this study show that locally applied preparations of anaphylatoxins (ie, human or porcine C3a, porcine C5a, and the porcine C5a des Arg), produce severe pulmonary injury in the guinea pig. During the acute phase of the response (ie, within 30 minutes following intrabronchial infusion) the characteristic symptoms were bronchoconstriction and tachypnea. On gross examination the affected lobes were relatively pale and much smaller than normal. Pathologic changes included contraction of bronchiolar and pulmonary vascular smooth muscle, focal atelectasis, and formation of leukocyte- and platelet- containing aggregates in vessels of the lungs and in the right ventricle of the heart. Changes were seen in parts of the lung not directly reached by the anaphylatoxins. The porcine C5a des Arg, but none of the other preparations, was lethal for approximately 50% of the animals following intrabronchial instillation.

Previous reports have indicated that certain of the isolated tissue and whole animal responses to anaphylatoxin challenges are mediated by the



TEXT-FIGURE 1—Contraction of guinea pig lung strips *in vitro* in response to the anaphylatoxins. A, Anaphylatoxins alone: Histamine, 50 ng/ml, C3a, 10 μg/ml, porcine C5a des Arg (classic C5a), 400 ng/ml. B, Anaphylatoxins in the presence of 10^{-6} M diphenydramine, same concentrations of all materials as in A. C, Anaphylatoxins in the presence of 2×10^{-6} M chlorpheniramine, same concentrations of all materials as in A.

release of vasoamines, particularly histamine. Contraction of the guinea pig ileum,[20] the cutaneous wheal and flare following intradermal injection,[28] and the vasculitis observed in experimental immune complex disease [29] can all be inhibited by antihistamines. Furthermore, direct application of C5a to mast cells, leukocytes, or platelets from certain animal species results in release of intracellular histamine.[30,31] It was important, therefore, to determine whether the pulmonary response that we observed following intrabronchial instillation of C3a or C5a resulted from release of histamine as well. Our data indicate that the bronchoconstrictive response of guinea pigs to spasmogenically active complement peptides was not blocked *in vivo* by pretreating animals with 20 mg/kg of the H_1 blocking agent, diphenhydramine, although they were protected from histamine. However, chlorpheniramine, which also blocks histamine uptake at H_1 receptors, abrogated the anaphylatoxin effect when administered at the relatively high dose of 10 mg/kg. Comparable experiments *in vitro* using isolated guinea pig lung strips showed that neither of the anti-

histamines, at 1–2 $\times 10^{-6}$M, concentrations that were effective in blocking contractions to exogenous histamine, was capable of blocking anaphylatoxin-induced contractions. Nonetheless, when the chlorpheniramine concentration was increased to 10^{-3}M, both C3a- and C5a-induced contractions were blocked. Since chlorpheniramine at elevated levels is known to act nonspecifically in inhibiting responses to mediators other than histamine,[32] it was assumed that the inhibitory effects observed only at high concentrations of antihistamine were not related to blockade of the histamine response.

Such results appear to be in conflict with early reports on experimental immune complex disease, indicating that vasoamines, particularly histamine, were responsible for the majority of the effects observed.[29] Rabbits, prepared for serum sickness by injection with BSA and treated with an antihistamine, were significantly protected from the increased vascular permeability changes usually observed in immune complex-treated animals that did not receive the drug. These investigators also showed that in the rabbit the source of the released hisamine was circulating platelets. It is well established that the immune complexes in experimental serum sickness trigger pulmonary inflammatory reactions by the generation of the anaphylatoxic peptides C3a and C5a via complement activation. Furthermore, C5a has been shown to trigger platelet activation *in vitro*. One must therefore expect that when anaphylatoxin is introduced into the bloodstream of a guinea pig, either endogenously or exogenously, the primary reaction is the release of platelet vasoamines. Howver, anaphylatoxins that are introduced intrabronchially may interact with a variety of other cell types (such as pulmonary macrophages, mast cells, and smooth muscle cells) before reaching the circulation. It may well be that the bronchoconstriction that is observed after instillation via the airways is the result of functions of these peptides other than histamine release.

Bodammer [33] and Bodammer and Vogt [34] have presented physiologic data indicating that guinea pigs infused intravenously with porcine classic anaphylatoxin show an impairment of air efflux from the lungs, resulting in hyperinflation. In contrast, we observed that when single lobes were treated with either C3a or C5a by intrabronchial instillation, those lobes were collapsed and frequently atelectatic. This apparent discrepancy can be explained by differences in the method of administering the materials. Intravenous administration of anaphylatoxins uniformly exposes all areas of the lungs from the vascular side, including the large airways supplied by the bronchial artery. However, when the same materials are instilled intrabronchially in small volumes, the solutions are unevenly distributed within the lung, usually involving only one lobe. Contraction of smooth

muscle is predicted to be localized, resulting in lobar atelectasis, while the remaining untreated lobes inflate (and deflate) in a relatively normal manner.

The generation in guinea pigs of cellular aggregates in pulmonary vessels and in chambers of the heart may be either a direct or indirect effect of the C5a. Porcine C5a, 10^{-10}M, and human C5a, 10^{-9}M, did cause aggregation of guinea pig platelets in plasma. On the other hand, neither porcine nor human C3a induced aggregation at concentrations as high as 10^{-6}M (human platelets were aggregated by neither C3a nor C5a, in agreement with previous results [35]). Grossklaus et al [35] found aggregation of guinea pig platelets, prepared in a similar manner, on exposure to either porcine C5a at 10^{-9}M or C3a at 10^{-6}M. We believe that these conflicting data for C3a could be explained if the preparation was contaminated with trace quantities of C5a: as little as 0.1% C5a in the C3a preparation would result in the aggregation observed. In our study C5a was eliminated by recycling the C3a preparations over a QAE Sephadex column.[23] In addition, human platelets could not be aggregated by either human or porcine C3a or C5a, in agreement with previous observations.[35]

Since C3a is incapable of aggregating guinea pig platelets *in vitro,* formation of the cellular aggregates observed *in vivo* must involve a secondary mediator. The conclusion that C3a has no direct effect on blood components that could result in the formation of cellular aggregates is supported by the observation of Mulfelder et al,[36] who reported that C5a but not C3a can induce leukocytes to produce procoagulant activity. Furthermore, it is likely that the cellular aggregates induced by C5a result, at least in part, from secondary mediator effects.

It is well known that both anaphylatoxins cause rapid increases in vascular permeability [28]; consequently the extent of extravasation of intravenous ^{125}I-BSA in the lungs following intrabronchial instillation of C3a or C5a was determined. The extent of extravasation that we observed is similar to that reported by Desai et al [37] following 20-minute intratracheal exposure to 1000 ED_{50} units of C5fr (the C5-derived chemotactic fragment) or a 60-minute exposure to 1000 ED_{50} units of the synthetic chemotaxin, f-Met-Leu-Phe. These data, together with other physiologic and pathologic findings, indicate that C3a, as well as C5a, is capable of inducing pulmonary injury in guinea pigs.

Our finding that porcine C5a des Arg was lethal in guinea pigs, and C5a was not, was surprising, considering that C5a is more active than the classic anaphylatoxin in contracting the guinea pig ileum, enhancing vascular permeability, and inducing leukocyte chemotaxis. Intravenous administration of porcine C5a des Arg in guinea pigs was also reported to be lethal,[38]

although these studies did not contain data on the effect of homogeneous C5a. Along these lines of investigation, Henson et al [39] have found that human C5a des Arg, which can be isolated in quantity, is more active than C5a in causing neutrophil accumulation in the lungs of rabbits after intratracheal instillation. Due to the presence of a circulating carboxypeptidase, the C5a des Arg molecule is presumably the physiologic form of this mediator. The biologic potency of this material *in vivo* suggests that it is the most important of all the forms of anaphylatoxin.

We have shown that the initial products of complement activation, C3a and C5a, are capable of producing acute pulmonary injury in the guinea pig. The secondary metabolite, C5a des Arg, has a much more dramatic effect *in vivo* than its precursor C5a, suggesting a prominent role for this complement-activation peptide as a mediator in pulmonary disease processes.

Note added in proof: A recent investigation by Gerard and Mugli [40] has demonstrated that the classic anaphylatoxin isolated in the absence of serum carboxypeptidase inhibitors is C5a des Arg. The des Arg form of the C5a molecule obtained from both human and porcine serum possesses intrinsic anaphylatoxin activity.

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[*Illustrations follow*]

Figure 1—Sections from the lungs of a guinea pig injected intrabronchially with 15.5 μg/kg porcine C5a des Arg. This guinea pig died within 5 minutes after treatment. **A**—Section from the left lower lobe, showing constriction of the smooth muscle layer in a bronchus and an artery. (H&E, ×60) **B**—Section from the same lobe, showing focal atelectasis around a constricted bronchus. (H&E, ×60) **C**—Section from a contralateral lobe, showing an arteriole occluded by leukocytes. (H&E, ×200) **D**—Section from saline-treated control lung. (H&E, ×60) (With a photographic reduction of 5%)

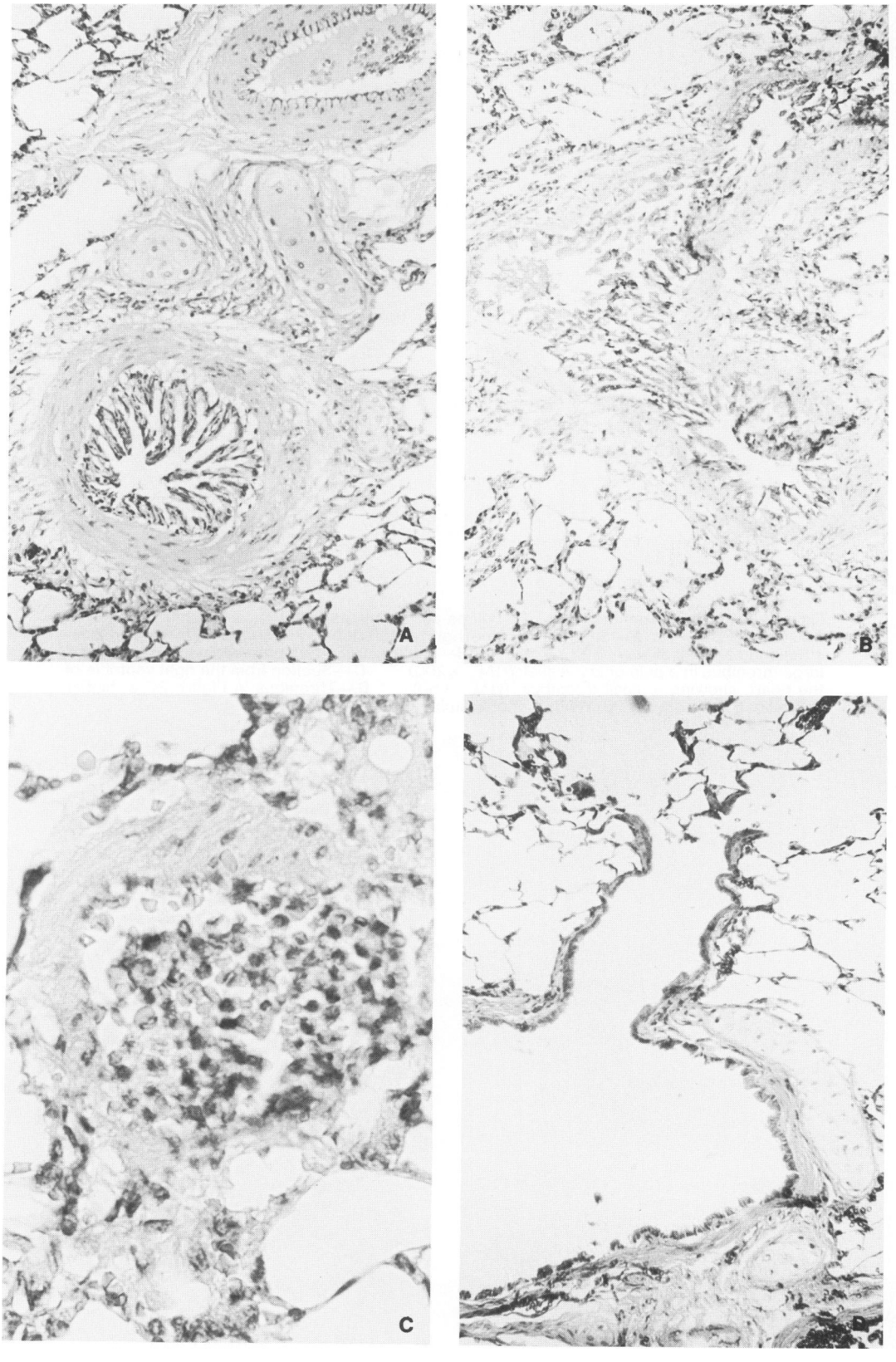
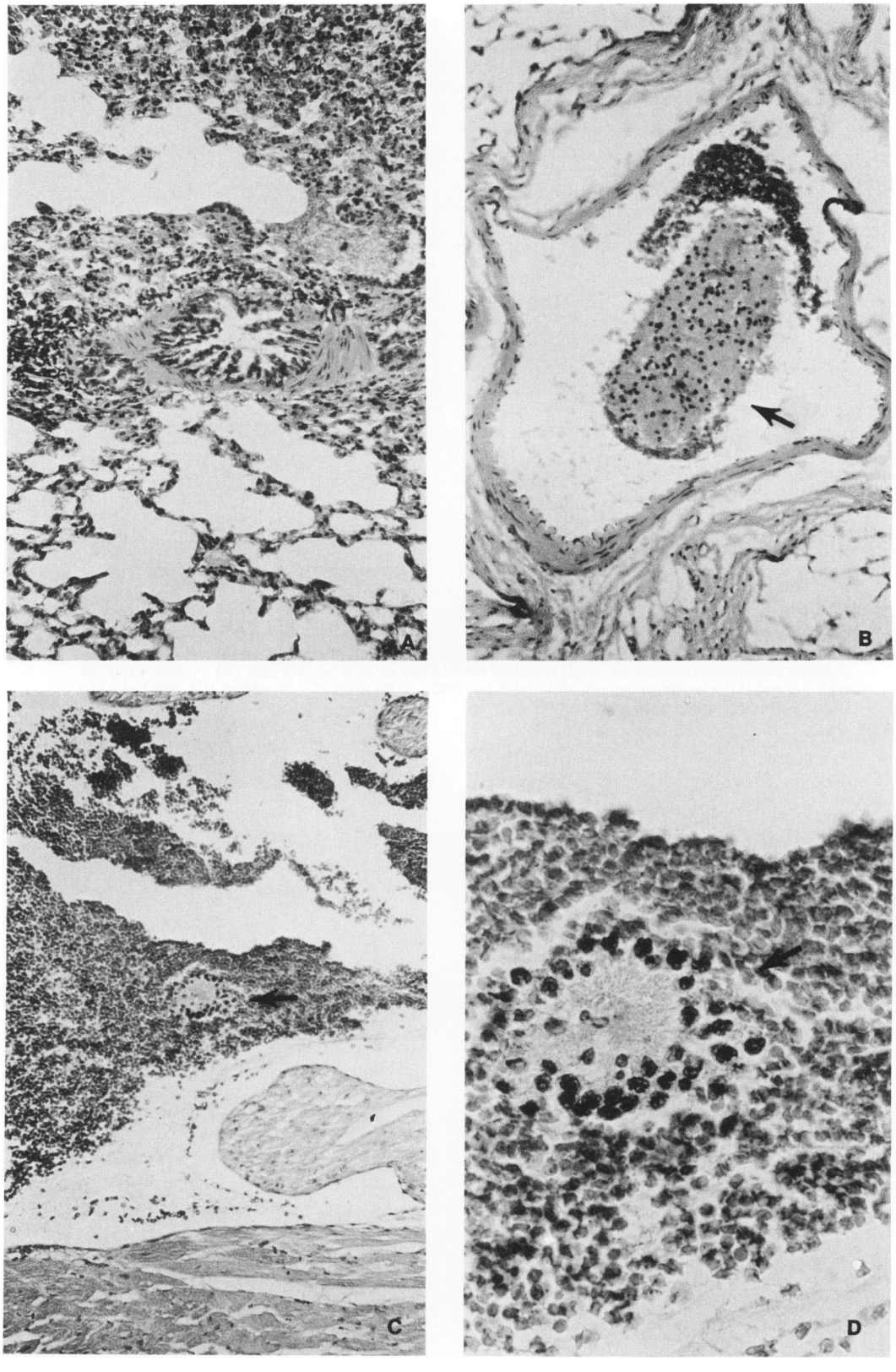
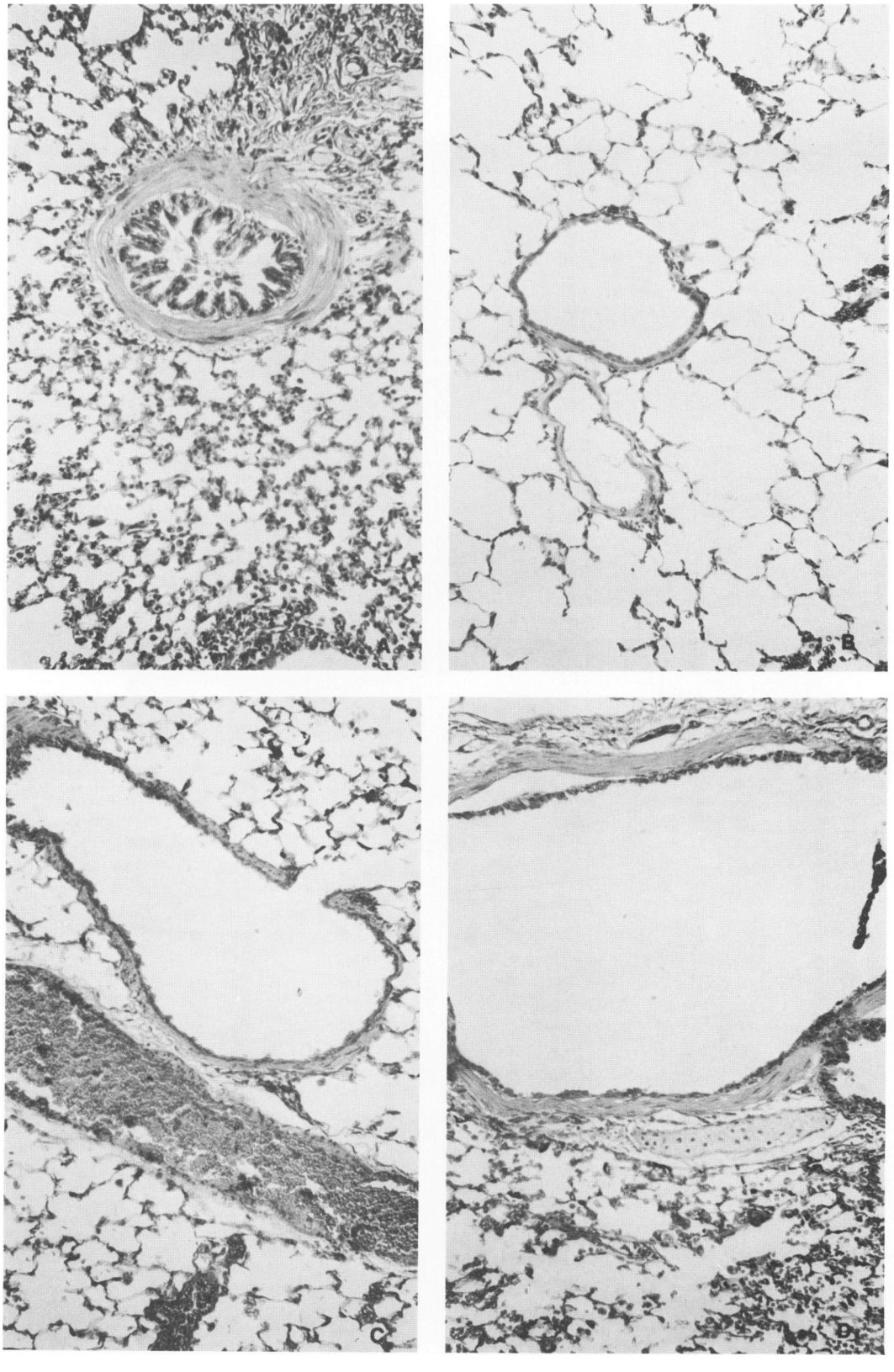

A
B
C

Figure 2—Sections from the lungs of a guinea pig 30 minutes after intrabronchial injection of 200 μg/kg C3a. **A**—Section from the right middle lobe, showing smooth muscle constriction in a small airway. (H&E, ×60) **B**—Section from the right middle lobe, showing a large thrombus in a pulmonary vessel. (H&E, ×200) **C**—Section from the right ventricle of the heart, showing a small thrombus. (H&E, ×60) **D**—Thrombus in Figure 2C at higher magnification. (H&E, ×200) (With a photographic reduction of 5%)



2

Figure 3—Sections from the lungs of guinea pigs pretreated intraperatoneally with 20 MG/kg diphenhydramine followed by intrabronchial instillation of 200 μg/kg C3a **(A)** or saline **(B)**, or with 10 mg/kg chlorpheniramine followed by 200 μg/kg C3a **(C)** or saline **(D)**. (H&E, ×60) (With a photographic reduction of 5%)



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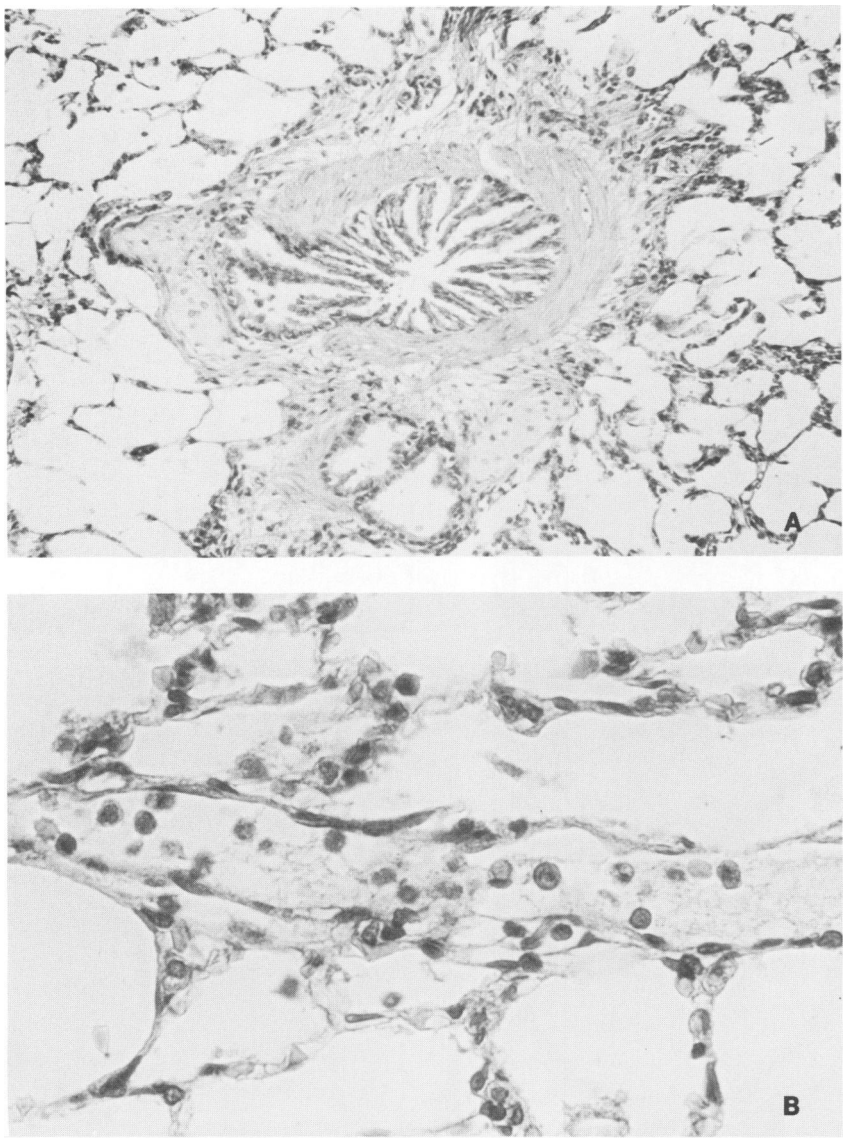

Figure 4—Sections from the lungs of a guinea pig given intrabronchial injections of the equivalent of 1 ml guinea pig serum activated with zymosan in the presence of EACA and partially purified. This guinea pig died as a result of the treatment within 10 minutes. **A**—Small constricted bronchus with mild atelectasis. H&E, ×60) **B**—Small vessel containing numerous leukocytes and proteinaceous material. (H&E, ×200)