EXPERIMENTAL PULMONARY EMBOLISM WITH SERUM-INDUCED THROMBI

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Since Virchow demonstrated the relation between pulmonary artery obstruction and infarction of the lung, numerous techniques have been employed to duplicate experimentally the pathologic and physiologic events that follow embolization of the lesser circulation. The introduction into the venous blood of a variety of substances has not succeeded in reproducing fully the dynamic factors involved in the production of thrombi, their release, their passage to the lung, and the mechanisms by which they may be removed from the circulation.

To investigate some of these factors in a more physiologic and controlled setting than has heretofore been available, use was made of the observation that the systemic infusion of thrombin-free serum induced massive thrombosis in vascular segments containing stagnant blood far removed from the site of infusion.[1] This method of thrombus formation is simple and reproducible; the thrombi formed are initially nonadherent and of uniform composition, can be of predetermined size, and can be produced without significant intimal injury or systemic disturbance in the veins and arteries of a variety of animals.[2] The present report describes some of the observations resulting from an adaptation of this technique to the study of pulmonary embolism.

METHODS AND MATERIAL

The basic technique of thrombus formation has been described previously.[1] Forty mongrel dogs, 15 to 20 kg. in weight, were anesthetized with sodium pentobarbital, and a segment of each external jugular vein was freed from its surrounding structures, and its tributaries ligated. Thirty ml. of heterologous, thrombin-free, canine serum eluate,* independent of the weight of the animal, was then infused into an antecubital vein in 30 seconds. Within 60 seconds after completion of the infusion,

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* This eluate was prepared by treating pooled canine serum with barium sulfate, centrifugating and eluting the adsorbate with citrate, as previously reported in detail.[1] The eluate represented a 4-fold concentration by volume of the thrombosis-accelerating activity of the parent serum and was used in place of serum in these and subsequent experiments to reduce the quantity of fluid administered.

2 serrefine clamps were placed on each jugular vein, isolating segments ranging from 1 to 14 cm. and averaging 4 cm. in length. The presence of a thrombus forming a cast of the isolated segment was determined by removing one of the vein segments 10 minutes after isolation and examining its contents. Thrombi also invariably formed behind the distal clamp where stasis was partial.

Following confirmation of thrombus formation in one jugular vein, the length and diameter of the contralateral segment was measured and the proximal clamp on this vein removed, leaving the distal clamp *in situ*. The thrombus formed in the measured segment was eased proximally by gently raising the distal end of the vein; prompt movement of the thrombus toward the heart was readily visible through the vein wall. In 20 of these animals, tidal volume, minute volume, and rate of respiration were recorded on a kymograph attached to a Tissot spirometer before, during, and for 10 minutes after release of the thrombus. Simultaneous electrocardiographic tracings were obtained on a multichannel, direct writing recorder.

In a second group of 28 animals, a large volume of freshly formed thrombus was released into the venous circulation. Single clamps were placed on both isolated jugular veins, and in some instances on both femoral veins as well, within 60 seconds after eluate infusion. Extensive thrombosis occurred in the venous system behind these clamps. Ten minutes later the clamps were removed, and as much thrombus as possible was moved toward the heart by gentle massage of the neck and legs distal to the sites of clamping. In many of these animals serum eluate infusion followed by clamping of a vein and subsequent release of thrombi was repeated at 10-minute intervals as many as 8 times.

Three groups of control animals were also studied. One such group of 27 animals was treated in every respect like the experimental series except that the serrefine clamps were replaced by silk ligatures and the thrombi formed thus confined to their veins of origin. A second control group consisting of 14 dogs was subjected to eluate injection without vein isolation, while a third group of 13 dogs was subjected to vein isolation without eluate infusion.

All experimental and control animals were sacrificed by the intravenous injection of sodium pentobarbital at intervals from minutes to weeks after completion of the appropriate procedure. Each animal received 1 mg. of heparin per kg. of body weight 5 minutes before death to prevent postmortem clotting. In dogs sacrificed 12 or more hours after embolization, surgical asepsis was used. No antibiotic agents were administered.

At necropsy the heart and lungs of each animal were removed *en bloc* from the junctions of the venae cavae to the ascending aorta without loss of blood. The venous pathways from the areas of jugular vein isolation to the heart were dissected *in situ*. Blood was drained by gravity from the heart through a 40 μ sieve to retain small, loose thrombi. The right and left cardiac chambers were examined for macroscopically visible thrombi and the lungs for evidence of infarction. The pulmonary arteries were minutely dissected, and the size, location and gross appearance of all thrombi found were recorded. Each lobe was then sectioned transversely from apex to base at 5 mm. intervals and the cut surfaces examined grossly for evidence of pulmonary artery thrombosis. Recovered thrombi were fixed in 10 per cent formalin in isotonic saline for histologic examination. When no macroscopic thrombi were found, the distal portions of one or more lobes were similarly fixed and examined histologically for minute emboli in small peripheral pulmonary arteries. In several instances, large paraffin sections of entire lobar segments were also examined.

RESULTS

Control Studies

Seventeen of the 27 dogs in which both jugular veins were isolated after serum eluate infusion, but in which the isolating clamps were

not removed, were sacrificed 15 to 120 minutes after eluate injection. Thrombi forming complete casts of the isolated segments were present in both jugular veins of all these animals. Examination of the venous pathways from these veins to the heart, the cardiac chambers and the pulmonary arteries revealed, in 2 animals, minute threadlike thrombi measuring 3 by 1.5 mm. or less within a small pulmonary artery branch. In the 10 remaining dogs, permitted to survive for 1 to 12 days following eluate injection, similar small pulmonary artery thrombi ranging from 1 to 25 mm. in length, and 0.5 to 2 mm. in diameter were found in 5 animals.

Among the 14 nonanesthetized dogs in which no surgical manipulation was performed but which were allowed to survive up to 3 days following an injection of serum eluate, filiform thrombi similar to those seen in the previous series were observed in 8.

In contrast with these findings, no trace of thrombus was found in the hearts or lungs of 12 of 13 animals that did not receive serum eluate but were subjected to anesthesia, venoclysis, and the systemic infusion of 30 ml. of isotonic saline followed by vein clamping. None of the 13 dogs showed evidence of thrombosis in any of the 13 isolated vein segments. In only one dog, sacrificed after 24 hours, was a single thrombus measuring 5 by 2 mm. found in a pulmonary artery. However, in 6 of the 13 animals in this group, including that in which the pulmonary thrombus was found, small thrombi were observed at necropsy in the foreleg veins that had served as the sites of venoclysis.

Embolization Studies With Single Measured Thrombi

Size of the Embolus Released. A series of 25 vein segments were measured with calipers; the contained thrombi were then removed and similarly measured. The average thrombus was 4 mm. shorter and 2 mm. narrower than its corresponding vein, and in no instance was the discrepancy in diameter greater than 3 mm. Of particular importance was the fact that no thrombus in this group was less than 4 mm. in diameter. Furthermore, such thrombi when measured ***in vitro*** at repeated intervals revealed no reduction in length or diameter for at least 4 hours after removal from the vein.

Results Obtained Within 4 Hours of Embolization. Twenty of the 40 dogs in which a single embolus was released were sacrificed within 4 hours of embolization (Table I). A red, nonadherent thrombus consistent with all, or part, of that originally released was found in the pulmonary arteries or in the right heart in every instance. In 10 of these animals, a single embolus consistent with that released was recovered intact. In the remaining 10, the embolus had fractured, as evidenced by

the uncovering of 2 to 4 pieces in 7 of these dogs, or by the finding, in 3, of a segment that could only have been a portion of the original embolus. Of those 7 animals in which more than one embolus was found, fragments were uncovered in opposite lungs in 3, in different lobes on the same side in 2, and in the right ventricle and a pulmonary artery in the remaining 2.

TABLE I

RECOVERY OF PULMONARY EMBOLI FOLLOWING RELEASE OF A SINGLE THROMBUS

Time of sacrifice after embolization	No. of dogs	No. of dogs in which emboli were recovered			No. with infarcts
		Total	In right heart	In pulmonary arteries	
3 min. to 4 hr.	20	20	8*	16	0
1 day to 28 days	20	10	1	10	0
1 to 4 days	6	6	1	6	
5 to 13 days	9	4	0	4	
15 to 28 days	5	0	0	0	
Total	40	30	9	26	0

* Four dogs revealed embolic fragments in a pulmonary artery as well.

All or part of the embolus was found in the right ventricle in 8 of the 20 animals. In 2 instances it was wedged securely between the ventricular wall and the chordae tendineae of the tricuspid valve (Fig. 1), and the chordae had left a distinct impression on the surface of the embolus. In one additional dog, an embolus was found in the pulmonary artery behind a cusp of the pulmonic valve.

In 6 of the 20 dogs, additional threadlike thrombi, similar to those observed in the control animals, and measuring less than 2 mm. in diameter, were also found in the pulmonary arteries or ventricular chambers. These thrombi, however, were readily distinguishable by their size and appearance from those released from the jugular vein.

No animal in this group showed any change in tidal volume, minute volume, rate of respiration, cardiac rate or rhythm, or electrocardiographic configuration during, or for 10 minutes after, release of the embolus.

Results Obtained 24 Hours or Longer After Embolization. Twenty additional dogs in which a single embolus was released were sacrificed 1 to 28 days after embolization (Table I). Although one or more small thrombi were found in each of the 6 animals sacrificed between 1 and 4 days, many of these measured less than 5 mm. in length and 1 mm. in

diameter, and could not be distinguished from similar minute thrombi noted in the control animals. In 4 of these 6 animals, however, additional larger thrombi measuring 2 to 3 mm. in diameter were also found in the pulmonary arteries, and, in one instance, in the right ventricle as well. These latter thrombi were believed to represent all or part of the emboli originally released and to have undergone a variable degree of reduction in size in the intervening period. All of these thrombi were red and nonadherent.

Of the 9 dogs sacrificed from 5 to 13 days after embolization, residual emboli were found in only 4. Of these 4 animals, adherent emboli up to 6 mm. in length and 1 mm. in width were present in 3, and the remaining nonadherent thrombus appeared to have been dislodged at the time of dissection. Microscopic examination of 2 of the larger of these emboli showed early organization at the periphery, with fibrin essentially free of red cells making up the bulk of the remainder (Fig. 2). In the 5 dogs sacrificed after 15 days or longer, no residual evidence of embolization could be found, nor was there any trace of the threadlike thrombi observed earlier. In those instances in which the lung tissue peripheral to the site at which emboli were found was examined histologically, no abnormalities were noted, nor were evidences of infarction, either gross or microscopic, observed.

With the exception of one thrombus found in the right ventricle 24 hours after release, no intracardiac emboli were noted in any animal of this series sacrificed after the first day.

Embolization Studies With Multiple Thrombi

Results Obtained Within 4 Hours of Embolization. Ten dogs were sacrificed within 4 hours of embolization (Table II). No emboli were

TABLE II

RECOVERY OF PULMONARY EMBOLI FOLLOWING RELEASE OF MULTIPLE THROMBI

Time of sacrifice after embolization	No. of dogs	No. of dogs in which emboli were recovered			No. with infarcts
		Total	In right heart	In pulmonary arteries	
3 min. to 4 hr.	10	10	5	10	0
1 day to 6 mo.	18	16	3	16	2
1 day	4	4	2	4	0
4 to 7 days	4	4	1	4	1
13 to 16 days	4	4	0	4	0
43 days to 6 mo.	6	4*	0	4*	1
Total	28	26	8	26	2

* One embolus found on microscopic examination only.

found in the venous pathways between the sites of vein isolation and the heart. The pulmonary arteries of all animals contained large amounts of dark red, nonadherent emboli, usually filling all the major pulmonary arteries (Fig. 3). Measurements of the recovered mass of thrombus were unreliable because of twisting, cohesion and friability, but estimates in several animals ranged from 40 to 100 cm. in total length. Nor was there any way of determining, during the course of an experiment, how much of a peripheral vein was actually emptied of thrombus at each release and massage, and, consequently, how much fresh thrombus was likely to form at the next clamping. The variability in the amount of emboli released was evident from the fact that, in several dogs, release of the contents of 2 femoral veins produced as great an apparent volume of pulmonary emboli as did 4 or more releases from 4 peripheral veins. The possibility also existed that pulmonary artery narrowing caused by previously released emboli may have produced sufficient stasis to induce local thrombosis upon injection of fresh serum eluate.

Emboli were also found in the chamber of the right ventricle in 5 of the 10 dogs in this group. In each instance the intracardiac emboli were enmeshed, totally or in part, in the chordae tendineae of the tricuspid valve.

Several animals in this group showed an increase in minute volume and rate of respiration. There were no significant changes in tidal volume, and alterations in cardiac rate and rhythm were minor. These and additional measurements of cardiopulmonary function will be reported in detail elsewhere.

Results Obtained 24 Hours or Longer After Embolization. Observations in the 18 dogs sacrificed 1 day to 6 months after embolization with multiple thrombi are summarized in Table II. No emboli were recovered in the venous pathways between the sites of vein isolation and the heart. Of the 12 animals sacrificed 1 to 16 days after embolization, emboli were found in the pulmonary arteries in every instance in contradistinction to the much smaller incidence of persistence in the animals subjected to a single embolus. Although there was some suggestion that the amount of thrombus in the pulmonary arteries was decreasing in volume during the first week, this could not be evaluated accurately in view of the unreliability of the measurements. After this first week, however, the decrease in the amount of residual emboli was striking, and in the 4 animals sacrificed between 13 and 16 days, scattered organizing thrombi not exceeding 5 cm. in total length were all that remained.

Traces of organized emboli were still to be found in 4 of the 6 animals sacrificed between 43 days and 6 months after embolization. In 3 of these—in one instance after 5 months—the original volume of thrombus released had been reduced to a few adherent nubbins which proved on

microscopic examination to be old, organizing emboli. In 2 specimens, the pattern of organization had produced strands resembling the "bridging" frequently observed in human pulmonary arteries at necropsy (Fig. 4), and changes suggesting an earlier phase of this phenomenon were noted in other organizing emboli (Fig. 2). In one additional animal, a small organized embolus, overlooked grossly, was found on microscopic examination. In the remaining 2 dogs, examined after 67 days and 6 months respectively, all traces of the original mass of thrombus had disappeared.

Pulmonary infarcts, small in proportion to the total amount of thrombus reaching the lungs, were noted in only 2 animals in this group. In one of these, sacrificed 7 days after embolization, 3 infarcts were found. These measured up to 2 by 1 cm., had a firm red appearance on gross examination, and presented the classical features of recent hemorrhagic infarction on microscopic examination. In the other animal, sacrificed 67 days after embolization, 2 small firm areas proved, on microscopic examination, to be old, organized infarcts even though no traces of the original emboli could be found.

One-half the animals sacrificed within the first 24 hours showed variable amounts of thrombi in the right ventricle as well as in the pulmonary arteries (Table II). In two of these the thrombi were entwined about the chordae tendineae of the tricuspid valve. A similar thrombus which had become adherent and showed microscopic evidence of early organization was also found in one animal sacrificed on the seventh day following massive embolization.

No spontaneous deaths occurred in any of the 28 animals subjected to massive embolization.

Discussion

The modifications of the technique of serum-induced thrombosis described in this communication provide a unique opportunity for study of the mechanism whereby autologous pulmonary emboli are handled by the animal. It is evident from the control data, as well as from previous studies,[2] that the method is highly reproducible and that interfering factors are minimal. Postmortem clots which might be confused with the experimental emboli are completely preventable by heparin, and the occasional filiform thrombi found in the hearts and pulmonary arteries of both control and experimental animals differ radically in size and appearance from those intentionally released. The relative roles of lysis and organization, as well as mechanical factors, in the behavior and eventual disposition of the experimental emboli can therefore be determined with a high degree of reliability.

When single measured emboli were released, thrombi consistent with

those sent off were invariably recovered in dogs examined within 4 hours after embolization. In view of the observed discrepancy between the vein measurement and the actual size of the thrombus released, no definite reduction in volume could be demonstrated within this period. After 24 hours, however, reduction in size became clearly evident with increasing time. Five days after embolization only a few residual emboli could be found, and after 2 weeks no traces were uncovered. Allowing for the possibility that a few of the residual emboli may have been lost or unrecognized in the course of dissection, it is apparent that a significant reduction in volume occurred, presumably by lysis.

Further evidence in support of the remarkable efficiency of thrombolysis was provided by those animals subjected to multiple emboli. From a volume of thrombus filling virtually every major radicle of the pulmonary arterial tree, a dramatic reduction to less than 5 cm. in total length was noted within 2 weeks. After 6 weeks, small organized nubbins were all that remained, and in some instances there was nothing left to indicate that a massive embolization to the lung had occurred.

No evidence of adherence between the embolus and the wall of the pulmonary artery was observed prior to the fifth day. All emboli persisting beyond this time, however, showed varying amounts of adherence and organization. When the volume of embolic material was small, as in the animals subjected to single emboli, about 30 per cent of the dogs sacrificed after the fifth day showed evidences of residual emboli. Although no trace of an embolus was found in this group after 2 weeks, the microscopic appearance of some of the organizing emboli observed earlier (Fig. 2) suggested that an occasional persistent fragment might have been observed beyond this time if the series had been larger, or that a nubbin partially incorporated into the wall of the vessel might have been overlooked. On the other hand, significant though markedly reduced amounts of thrombi were noted in every animal subjected to massive embolization and sacrificed within 16 days, and traces of organized embolic material were still present in one such animal after 5 months. It is apparent that persistence of emboli bears a direct, though obviously nonlinear, relation to the volume of thrombus reaching the lung. Furthermore, the effectiveness of the lytic mechanism, particularly in the first few days following embolization, appears to play a critical role in determining the amount of thrombotic material persisting long enough to undergo organization. Such local factors as the extent of contact of the embolus with the living tissue of the vessel wall undoubtedly also contribute to the rate and extent of the organization process.

The influence of certain mechanical factors on the behavior and fate

of pulmonary emboli is also evident from these experiments. In the group of animals subjected to a single embolus, fracture of the embolus occurred in one-half the dogs examined within the first 4 hours following release. The distribution of these fragments in different lungs or lobes, or in both heart and lung suggests that fracture occurred, in almost all instances, prior to entry of the embolus into the smaller pulmonary arterial branches and probably in the right heart itself. Although the possibility of fragmentation at the point of release was not entirely excluded, some additional observations make this unlikely. In several experiments, for example, thrombi released to narrowed portions of the veins proximal to the isolated segments were invariably intact in contrast with those permitted to reach the heart.

An additional mechanical factor was suggested by the presence of emboli in the chambers of the right heart. This delay in passage to the lung, though usually brief, was occasionally as long as 24 hours in the group subjected to single emboli. In those dogs subjected to massive embolization, emboli were found in the right heart after 7 days, and in one such animal not in this experimental series, there was evidence of an organized embolus in the right heart after 36 days.[3]

The infrequency and small size of the pulmonary infarcts found in these animals, and their occurrence only in dogs subjected to multiple emboli, is consistent with the general experience that infarcts are not readily produced in the presence of a normal bronchopulmonary circulation even when obstruction is extensive. Still more striking was the absence of spontaneous deaths, or even severe respiratory distress, either immediate or delayed, in any of the animals of this series. It is possible that transient right to left shunts, recently demonstrated in dogs subjected to massive embolization with serum-induced thrombi [4] may serve as a protective mechanism in ameliorating the hemodynamic load on the right heart. Survival of these animals may also be due in some measure to delay of emboli in the right heart, together with the remarkable efficiency of the lytic mechanism.

The source of the filiform thrombi found in the heart and pulmonary arteries of both control and experimental animals was frequently uncertain. Some of these thrombi occurring in the experimental group may have represented a portion of the embolus originally released from the jugular vein. Minute thrombi formed in the small ligated venous tributaries were occasionally included in the isolated venous segment and could be found attached to the control thrombus or to the recovered embolus itself. These small twigs could conceivably have been broken from the main body of an embolus in its passage to the lungs.

Such a mechanism, while possible in the experimental animals, cannot

explain the occurrence of these thrombi in the control groups. A source of such thrombo-emboli, common to both experimental and control groups, was the venoclysis site at which thrombi could be demonstrated in some instances even in the absence of eluate infusion. Of greater significance, however, seems to be the high degree of correlation between the occurrence of filiform thrombi and the infusion of serum eluate regardless of what else may have been done. Following such infusion, it is possible that thrombi of this type may form in areas of retarded blood flow in peripheral veins or pulmonary arteries. Supporting this concept is the fact that the incomplete thrombi which invariably form behind the second clamps occasionally resemble the filiform thrombi observed in the heart and lungs. Similar thrombi have also been produced in earlier experiments by simple external digital pressure on a peripheral vein following eluate infusion.[1]

Summary

The fate of serum-induced, autologous thrombi, released from peripheral veins as single or multiple emboli, was determined in a series of dogs by sacrificing the animals at intervals from minutes to months after embolization.

Marked reduction in the volume of the emboli was noted within the first 2 weeks, and no residua were observed after this time in dogs subjected to single emboli. Following massive embolization, traces of emboli persisted for longer periods; the effectiveness of thrombolysis, however, was such that a volume of thrombus filling virtually the entire pulmonary arterial tree was reduced after 6 weeks to a few small organized nubbins, or had entirely disappeared.

Adherence and organization of emboli were not noted prior to the fifth day, but were observed in every embolus encountered beyond this time.

Delay in passage of emboli through the right heart occurred in some instances, and such emboli occasionally persisted as endocardial thrombi. Fracture of emboli prior to entry into the pulmonary arteries was also observed in several animals.

No spontaneous deaths occurred. Rare pulmonary infarcts were observed only in those animals subjected to massive embolization, and, when present, were small in relation to the amount of emboli reaching the lung.

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[*Illustrations follow*]

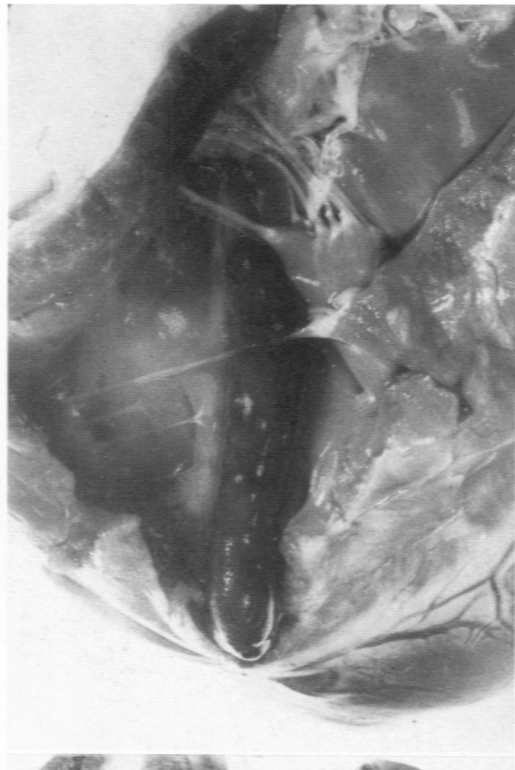
LEGENDS FOR FIGURES

FIG. 1. An embolus recovered from the right ventricle 12 minutes after release. Note the well defined shape of the thrombus and compression by one of the chordae tendineae of the tricuspid valve.

FIG. 2. Early organization of a single embolus recovered from a pulmonary artery 15 days after release. Note organization into strands, suggesting an early "bridging" effect. Hematoxylin and eosin stain. × 58.

FIG. 3. Massive embolism to the lung. Dog sacrificed immediately following the release of multiple thrombi. Note that all pulmonary radicles are filled with thrombi.

FIG. 4. Strands of organized embolus resembling "bridging" in a pulmonary artery 95 days after massive embolization. Hematoxylin and eosin stain. × 50.



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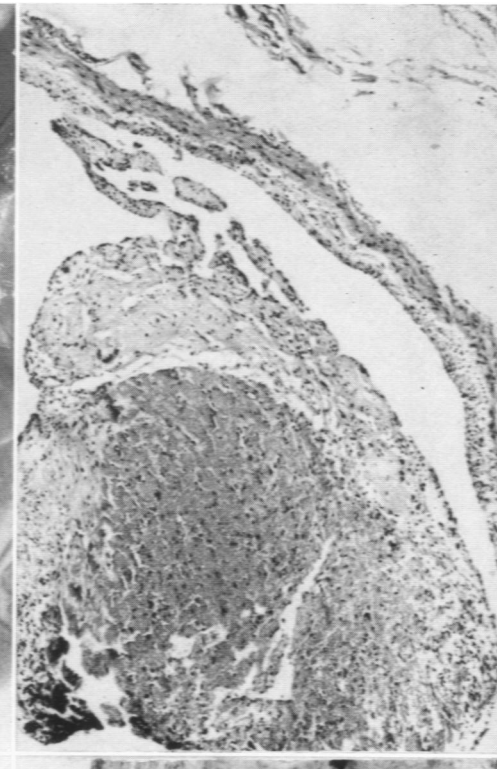

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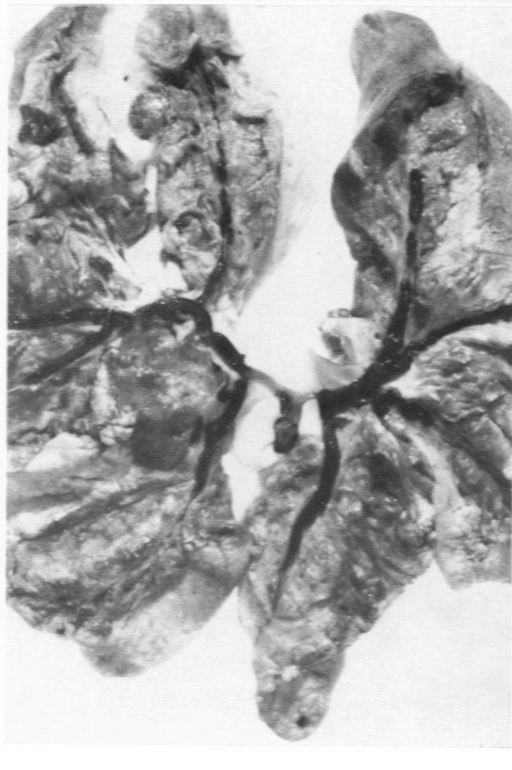

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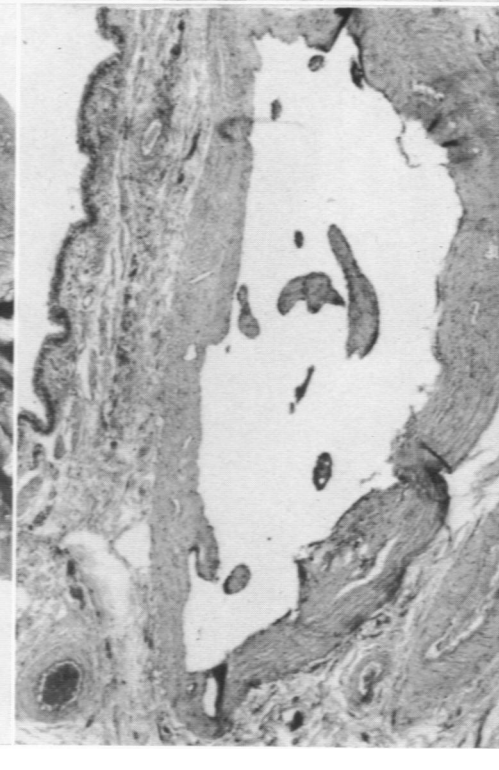

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