The Lung in Hemorrhagic Shock

I. In Vivo Observations of Pulmonary Microcirculation In Cats

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HEMORRHAGIC HYPOTENSION produces morphologic changes in the lungs of experimental animals and humans.[1-4] Many of these structural alterations are of a nonspecific nature and can often be related to factors other than shock, such as manipulation of the lung, retransfusion, or fixation techniques. Sequential studies of events during life, which lead to these morphologic changes, are poorly documented. Such sequential observations of the lung, carried out with in vivo microscopy of the pulmonary microcirculation in cats during hemorrhagic shock are described in this paper. These observations are correlated with the light microscopic appearance of the same lungs studied by serial sections.

Methods

Twenty healthy mongrel cats (*Felis domesticus*) weighing 2.2–4.1 kg were anesthetized with intraperitoneal pentobarbital sodium, 15 mg/kg. Cannulations of the femoral artery and femoral vein were carried out using catheters primed with saline. Systemic arterial pressure was monitored through the femoral artery catheter. Ventilation with a mixture of 95% oxygen and 5% carbon dioxide was maintained through a closed tracheostomy incision. Preliminary experiments proved that cats in shock could not be maintained on room air alone. The lungs were intermittently insufflated during periods of direct observation. The process of insufflation consisted of maintaining the lungs in the inflated state for periods of 2–3 min, without exceeding the capacity of the thoracic cavity. Gas runoff was maintained through a leakvalve arrangement which permitted the O_2–CO_2 mixture to flow through the lung at a rate of 2 liters/min. Intratracheal pressure was measured with a strain guage pressure transducer and was never allowed to exceed 4 cm of water. Periodically, the cats were ventilated with room air for 5-min intervals. This was delivered through the tracheal cannula by a volume respirator pump (Harvard apparatus). Observations of the microcirculation were made through an open

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thoracotomy in the right sixth intercostal space, allowing the right middle lobe to be viewed with a Leitz dissecting microscope using a modified quartz rod transillumination technique.[5-7] A 16-mm Bolex movie camera, mounted on one eyepiece of the dissecting microscope, was used to photograph circulatory changes at various periods during the entire procedure, at magnifications of ×50–150.

Heparin, 200 USP units, was instilled in the cannulas to prevent coagulation, but precautions were taken to avoid passage of any of this heparin into the animals. Thus, the cats were not anticoagulated. Fifteen cats were bled in a stepwise sequence from the femoral artery and the blood delivered into a closed plastic reservoir at a rate adjusted so that the mean arterial blood pressure fell slowly and consistently from the mean control level of 150 mm Hg to a shock level of between 60 and 70 mm Hg. It was kept at this level for 2 hr. The blood in the reservoir was anticoagulated for storage but was not returned to the animals until the end of the 2-hr shock period. Blood pressure was maintained using saline replacement through a venous cannula. No more than 25 cc of saline was given to any animal in these experiments. Following the 2-hr shock period, all of the shed blood was reinfused slowly. At various intervals during the procedure 35-mm color positive transparencies were taken in order to record minute-to-minute changes in vascular wall-to-lumen ratios. These can best be documented and compared by using consecutive transparencies taken of the same area, at matched magnifications.

An additional 5 animals were studies in exactly the same manner as the 15 previously described, except that they were not bled. All of the animals were sacrificed after return of the shed blood by perfusing the lungs in vivo, with 4% cacodylate buffered gluteraldehyde instilled in the endotracheal cannula. Two animals were perfused in addition, through the pulmonary artery, with this same fixative. One animal was perfused (via the mainstem bronchus containing a cannula leading into the right middle lobe) with osmium tetroxide to determine the comparable effects of fixation methods. Intratracheal perfusion pressure never exceeded 10 cm of water. This operation was carried out with the lungs in situ, in order that overinflation might be avoided.

Serial light microscopic sections of paraffin-embedded tissues were placed on 70-mm milar film and stained with the Masson trichrome stain.[8] These were serial sections of the right middle and right lower lobes and frequently numbered as many as 4000–5000. These were subjected to routine screening. The significance of using this technique is threefold: (1) Vascular and alveolar comparisons can be made from one area to another; (2) small vascular alterations, which are not normally detected by routine sections, may be documented; and (3) tissue alterations which may be missed because of routine lung sampling errors, can be detected.

Results

In vivo microscopic observations

Direct observations of the pulmonary microvascular bed before, during, and after a period of hemorrhagic shock reveal the following sequence of events.

Before bleeding (pre-shock period). During the pre-shock period, capillaries contain single files of red blood cells which do not exhibit the rouleaux effect.[9] The cells do not stick together, seem to repel each other, and are periodically interrupted by white cells or plasma intervals containing no cells. This state of the circulation continues essen-

tially unchanged throughout the entire period of observation in the 5 control cats that were not bled (Fig 1). No microaggregates (platelet agglutinations, white cell clumps, or combinations of these) are visible. None of the formed elements of the blood stick to the endothelium of the vessel walls, and perfusion is relatively uniform throughout the observed regions, except for the periodic selective perfusion previously described in normal cat lungs.[10]

During and after bleeding (2-hr shock period). As systemic blood pressure is reduced, constriction of the pulmonary arterioles can be observed. This constriction is documented by an increased wall/lumen ratio and can best be described as a decrease in the width of the flowing cell column within the arteriole (Fig 2). As previously reported [11] this constriction occurs in the distal precapillary segment of these vessels. As a result of this precapillary arteriolar constriction, alveolar capillaries immediately become bloodless and are difficult to visualize. The alveolar rings (seen on the pleural surface as a red ring of blood when compared to the alveolar wall seen in a single layer) representing the septa, contain numerous capillaries. These soon lose their red color, which is due to circulating erythrocytes, and a blanching effect develops in most areas of the lung being viewed (Fig 3). The number of involved capillaries which become bloodless in any given field of observation is related to the degree, severity, and duration of shock. However, this is not in linear relation to the drop in pressure.

Corresponding with flow cessation followed by the probable occurrence of increased permeability of vessels (mainly capillaries),[12] there is a visible widening of the interstitial compartments which is the manifestation of pulmonary edema.[13] This widening may be clearly identified about 30 min after the induction of bleeding.

As pulmonary blood flow decreases, the animals develop a compensatory tachycardia of 150–170 beats/min. Subsequently, a few arteriolar-to-capillary pathways which had been closed may reopen, usually with a narrowed lumen and jerky, pulsatile flow. At the same time many areas remain partially perfused in spite of a decrease in the number of capillaries maintaining flow. Such phenomena as granular flow, followed by to-and-fro ("rocking chair") flow, may be observed during the first half hr of shock. As time passes, blood flowing through these arterioles begins to agglutinate into "sludge", which soon results in a stagnant situation in many regions of the lung. Leukocytes specifically become spherical and cling to the endothelium of arterioles and capillaries.

Numerous small vessels (both venules and veins) become congested

with blood cell agglutinations. Settling of these cell masses to the lower side of lower vessels is frequent when flow ceases.[14] The known consequences of such settling have been well described.[15–17] At no time during this series of experiments were pulmonary venules observed actively to constrict. Rather, width of the venules depended upon the mass of cellular material passing through the lumen.

Confluence of the capillaries to form large venules is common in the normal lung. The flow in these large venules is normally nonpulsatile and rapid. However, in the shock animal stagnation of the blood takes place in these vessels within 30 min of the onset of bleeding, and they bulge and sacculate progressively as time passes. Subsequent to the onset of stagnation in these venules, the alveoli become partially collapsed and opaque while being viewed. Segmentation of the vessels, both arterioles and venules, with sluggish, pulsatile, alternating to-and-fro flow may be observed.

On the occasions during the experiment when the lungs are mechanically ventilated rather than insufflated, the pulsatility of flow increases. As the lung expands, a rapid forward surge of blood in the arterioles occurs, which produces a subsequent runoff into the capillaries at full inspiration. Venule blood moves slowly forward in a regular, segmental, jerky manner which is characterized by a rapid forward component followed by a slow backward motion. This venous pulsatility is probably associated with changes in pressure, while the arteriolar motion is associated with respiration.

Reversals in direction of flow in capillaries and arterioles, bypass of the alveolar capillary beds, no visible color change in erythrocytes passing from arterial to venous blood, and occasional increases in the rate of flow in large venous streams, are all indicative of alterations in the pattern and pathways of pulmonary blood flow. Such "functional shunting" occurs during the first hr of shock. The morphology of these shunts is undetermined.

In regions of reduced or impeded flow during shock, smooth, spherical gas pockets enveloped by films of liquid (optically refractile qualities characteristic of a liquid-gas interface) are noted. They appear after long periods (approximately 1 hr in most animals) in the perivascular and peritubular connective tissues in the interstitium of the lung. These measure 4–8 μ in diameter when first visualized, and form a dark interface with surrounding tissues. At the onset, these gas pockets, or "bubbles," are small and difficult to visualize. They become increasingly more distinct during the remainder of the second hr of shock (Fig 4). The bubbles are observed to disappear as they are filled by fluid in some

animals. This is accompanied by an increase in the interstitial edema already present. In some cases, the bubbles coalesce to produce larger bubbles. Three of the animals were seen to develop focal hemorrhages in these areas of bubble formation. This hemorrhage was subpleural, perivascular, peritubular, and came from the terminal portion of the pulmonary arteriole in the precapillary sphincteric area where constriction can be shown on serial sections, and from the capillaries just distal to that. Bleeding was rapid initially, but ceased as the blood formed an arteriolar tamponade. The hemorrhage into these areas of bubble formation is shown in Fig 5B and 6. The bubbles are not observed in areas where flow is maintained, where the capillary beds remain open, or in any of the control animals.

During and after return of blood (post-shock period). During and after return of the shed blood, the entire vascular bed becames massively engorged with "sludging" and agglutination of erythrocytes. This was noted in all of the animals during the reinfusion period. Observations were made in 3 of the cats for up to 1 hr following return of the shed blood. Some of the red cell masses move slowly while others become stagnant without resumption of flow. Of particular interest is the vein and venule network. As transfusion progresses, a few rapid streams are seen in veins and venules, but frequently there is minimal flow. Venous congestion is striking as a specific feature of the circulation at this time. Even with improvement in the hemodynamic status manifested by increased blood volume and pressure, and with assisted respiration the irregularity and atelectasis of the alveoli continues to increase in various areas. Resumption of circulatory function is not uniform and perfusion remains poor (Fig 5A).

The areas of interstitial bubble formation which appear during the second hr of shock, are either completely or partially replaced by edema fluid and are no longer visible, or are obliterated by interstitial hemorrhage after the entire shed blood volume is returned. All animals show further exacerbation of the profound pulmonary edema and new areas of hemorrhage may be noted as the blood volume is restored. These hemorrhages are subpleural, interstitial, and focal (Fig 5B and 6).

Serial light microscopic sections

After perfusion with gluteraldehyde (or osmium in one animal) the serial lung sections were examined on 70-mm milar film. The type of fixation had no apparent effect on lung morphology. All of the animals that were examined had been transfused. Light microscopic changes

observed in the postshock lung are highly variable within the same animal from one area to the next, and from animal to animal. These alterations in morphology range from minimal pulmonary congestion to peritubular and periarterial hemorrhage, intralveolar hemorrhage, focal atelectasis, and severe interstitial edema (Fig 6). The most consistent findings are capillary engorgement and interstitial edema beneath the alveolar capillary membrane.

Increased thickness of the alveolar lining is observed in various areas in all animals, with frequent desquamation of the alveolar epithelium, manifested as cells lying free in the alveolar spaces. The lymphatics are difficult to recognize at the light microscopic level in these lungs, but they appear as large, dilated spaces adjacent to the bronchioles, arteries, and veins. The subpleural lymphatics form a network, which is observed in vivo to be engorged with lymphatic fluid. Deep lymphatics are not observed in vivo. In the lung sections, they are identified as large empty spaces. These dilated lymphatics are not to be confused with the interstitial space occupied by the bubbles. None of these changes are seen in the lungs of control cats, whose morphology appears normal. The bubbles were conspicously absent in *all* control animals.

Discussion

A sequence of microvascular events has been observed in the lungs of shocked cats, which may help to explain the abnormal anatomic changes found in these animals at autopsy. The consistent occurrence early in the course of shock of precapillary arteriolar constriction, visualized as an increased wall-to-lumen ratio, suggests an alteration in the permeability of these vessels or a compensatory adjustment to a markedly reduced central blood volume, as the initial event in the development of pulmonary damage secondary to hemorrhagic shock. The stimulus for this constriction could be neural, one or more of the many vasoactive substances which appear in the circulation in shock,[18] or a combination of neural and humoral stimuli. It may also be simply a matter of reaching a critical closing pressure. The lung itself contains many vasoactive substances which could be released or activated by currently unknown mechanisms in shock. Other investigators have demonstrated that by occluding the main stem bronchus and all of its surrounding vessels at the hilum, the lung is at least partially protected in shock.[19] This suggests that the initial insult is not hypoxic but rather is secondary to neural and/or humoral stimuli.

The edema, hemorrhage, and morphologic damage to the alveolar

walls can be expected to lead to a diffusion problem across the alveoli. Together with the sluggish and irregularly inadequate perfusion of alveolar vessels, this may account for the problem of decreased arterial saturation that has been well documented in hemorrhagic shock.[20,21]

In normal animals, arterio-venous pathways apparently allow very little flow. These shunts are small but become large following hemorrhage, as observed in this study. This could, of course, play an important role in the causation of the arterial oxygen unsaturation that is seen in shocked animals. The development of a diffusion block superimposed on anteriolar constriction and bloodless alveolar rings may then lead to subsequent hypoxic and ischemic injury to the lung itself.

Some investigators have attempted to explain the phenomena of the shock lung on the basis of intravascular coagulation in the microcirculation.[22,23] This has been related to either microembolic or thrombotic occlusion of multiple small blood vessels. In this regard, we were unable to document the appearance of such microemboli early in the course of shock, but rather noted a decrease in pulmonary vascular diameter and stagnation of flow prior to the development of a sludged appearance of the blood. After infusion of the shed blood, and despite the resultant rise in systemic arterial pressure, the capillary bed did not reopen to any significant extent during periods of observation for up to 1 hr. This continued perfusion defect may be a major factor in irreversibility of the shock state. Regarding this matter, such questions as critical re-opening pressure of vessels, pulmonary vasospasm, pulmonary vascular reactivity, and the degree of vascular blood pooling are yet to be solved.

A striking observation that was made in these studies was the appearance of interstitial bubbles in areas of ischemic lobules. These occurred only after about 1 hr of shock, and were not in any of the control animals. Because of their refractile characteristics, they were considered to represent interstitial accumulations of gas. It is reasonable to hypothesize that these bubbles represent gas that has dissected into the alveolar septums through small defects in the alveolar epithelium covering the septal walls. The importance of these bubbles is further seen in the fact that many of them are subsequently observed to be the focal point of interstitial hemorrhage and replacement by pulmonary edema. Their presence is an indication of tissue weakening and separation within alveolar septums. This eventually permits blood to enter the alveolar compartments if rupture of the wall occurs after vascular rupture and interstitial bleeding.

A final item of probable importance is the observation of dilated superficial pulmonary lymphatics in shock. Since pulmonary edema is a consistent finding in the lungs of these shock animals, it is imperative that the mechanism and dynamics of this system be studied.

Summary

Sequential in vivo observations of the vascular events occurring in the lungs of cats during hemorrhagic shock have been made and correlated with postmortem light microscopic appearances. Precapillary arteriolar constriction occurs early in shock and is followed by slowing and often reversals of blood flow; white blood cells adhere to artery and capillary endothelium; sludging of blood occurs, capillaries and venules close and frequently remain empty during the shock period; collateral pathways appear which are not seen during the control period; bubbles are seen in the interstitium of the ischemic alveolar walls after 1 hr of shock, and are thought to represent air that has dissected into the alveolar septums through defects in the alveolar epithelium; vascular engorgement and poor perfusion continue after return of the shed blood; the alveoli continue to be misshapen, and an edematous, hemorrhagic lung is produced.

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[Illustrations follow]

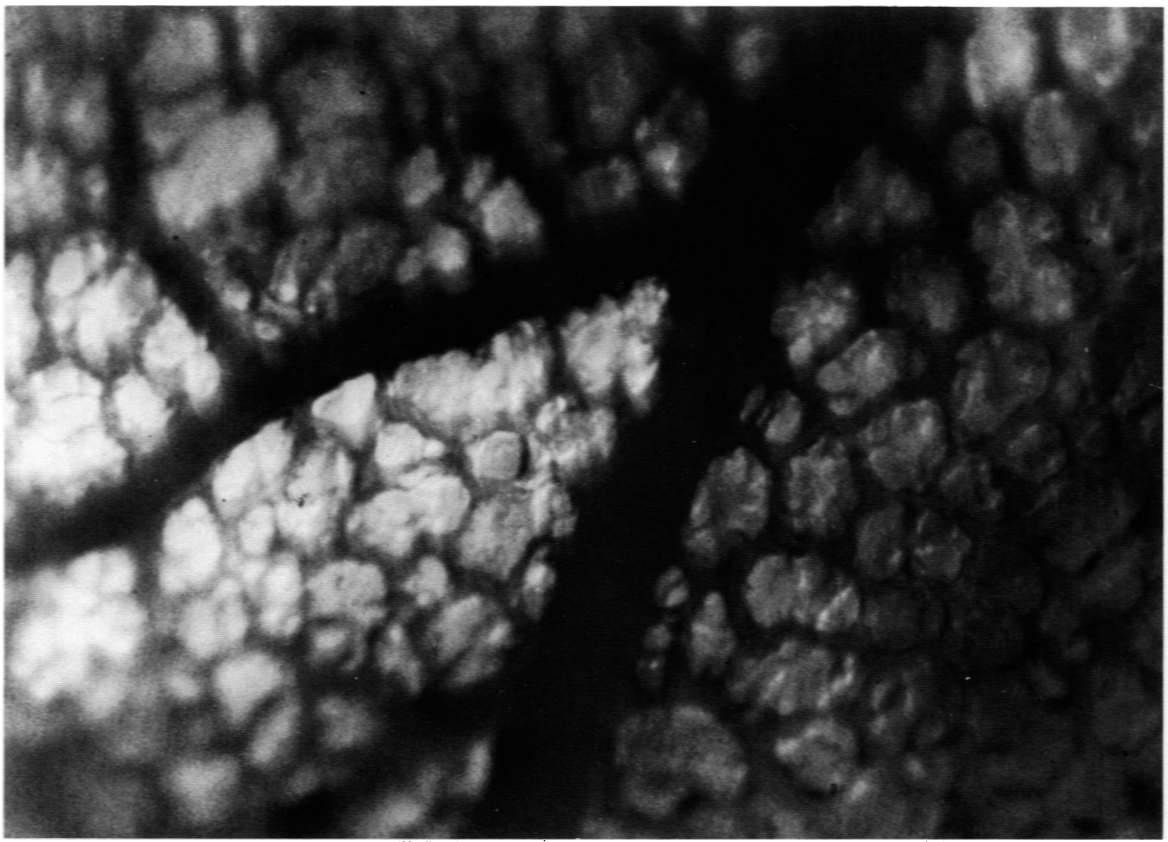
Legends for Figures

All illustrations (except Fig 6) were transferred from 16-mm color motion picture film.

Fig 1. Photomicrograph of branching pulmonary arteriole (50 μ diameter) in control animal. All septal vessels are full of blood and appear dark in this and subsequent black and white photographs. Flow in this vessel and other vessels of this size is so rapid that it is difficult to observe individual cells during control periods or in control animals. Capillaries running across alveolar walls are not seen at this magnification. × 75.

Fig 2. Pulmonary arteriole beginning to undergo constriction, as shown by increasing wall/lumen ratio. Vessel is comparable to arteriole shown in Fig 1, but is not in same lung. Width of flowing cell column is narrowing and less blood is present in septal branches, which are lighter than those in Fig 1. Taken just at initiation of bleeding, and capillaries are receiving less blood as constriction takes place. × 75.

Fig 3. Septal rings seen on surface have become relatively free of blood in this photomicrograph made 15 min after animal had been bled to a shock level. Rings appear as dark lines rather than widened structures seen in Fig 2. Capillaries in alveoli are bloodless and arteriole has undergone constriction which is irregular when compared with arterioles in Fig 1 and 2. × 75.



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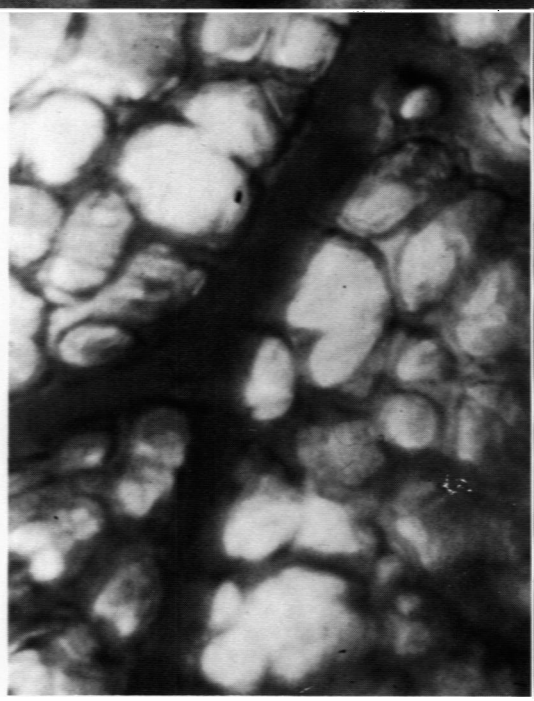

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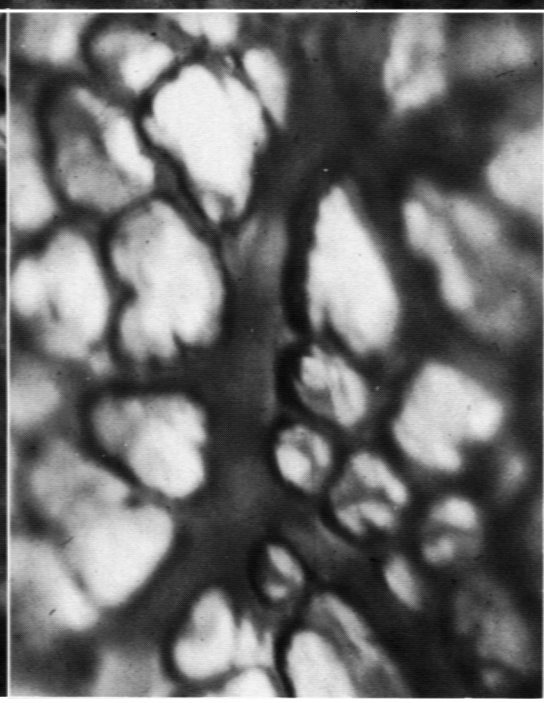

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Fig 4. A. This pulmonary arteriole, supplying adjacent alveoli, is undergoing slowing of flow. Flow is granular-appearing, irregular, and was observed to reverse directions several times. "Bubbles" which developed in interstitial areas appeared at 1 hr and 5 min. This photograph was made 1 hr 45 min after induction of shock. **B.** Sequential photograph of the same area shown in Fig 4A demonstrates formation of additional "bubbles" and further accentuation of decreased flow. Large masses of sludge are present in this vessel which was photographed 1 hr 50 min after shock induction. Compare this with one made 5 min previously and note change in distinctness of alveolar walls. × 150.

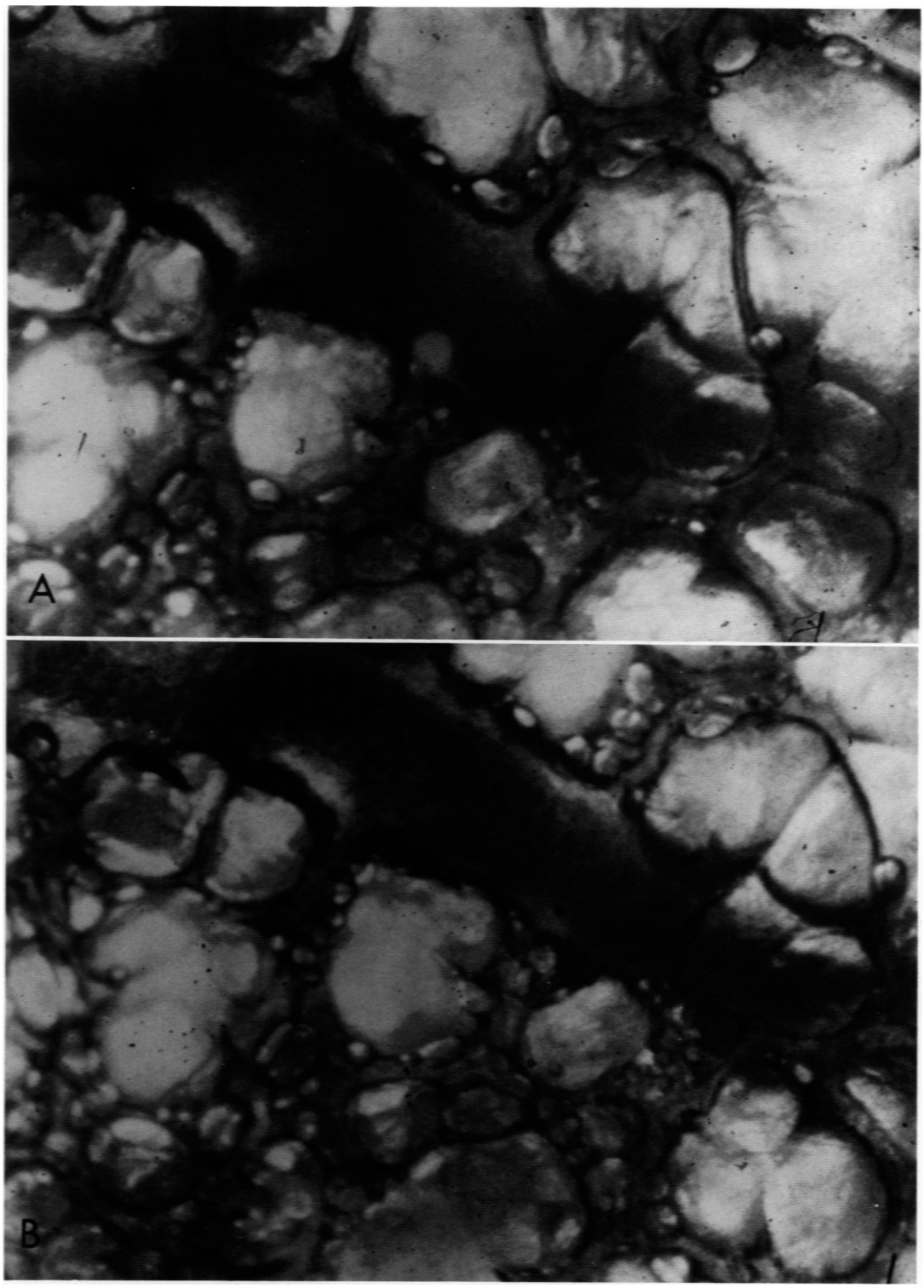
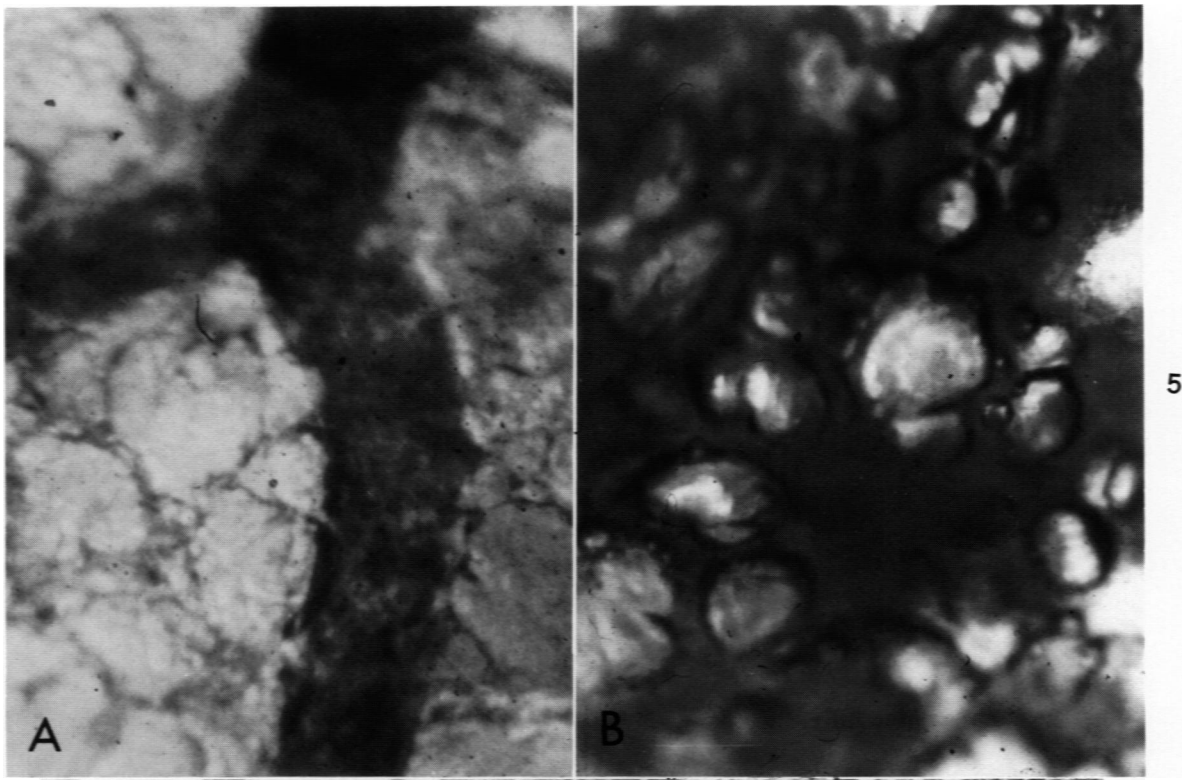

A
B

Fig 5. A. Pulmonary arteriole contains large aggregates of red cells which are hardly moving. Alveolar walls are indistinct and difficult to visualize. No blood is present in alveoli at this time (immediately following return of the shed blood) and vessels remain closed with very few areas demonstrating resumption of circulatory function. **B.** Areas of interstitial bubble formation were completely obliterated in some instances when capillaries ruptured and blood was set free in interstitial space. As shown in this photomicrograph of such an area, most of the "bubbles" have been replaced by blood. × 150.

Fig 6. Such morphological alterations as the ones shown in this lung section consisting of peritubular and periarterial hemorrhage, intralveolar hemorrhage, and interstitial edema were seen in these shock animals. H & E. × 200.



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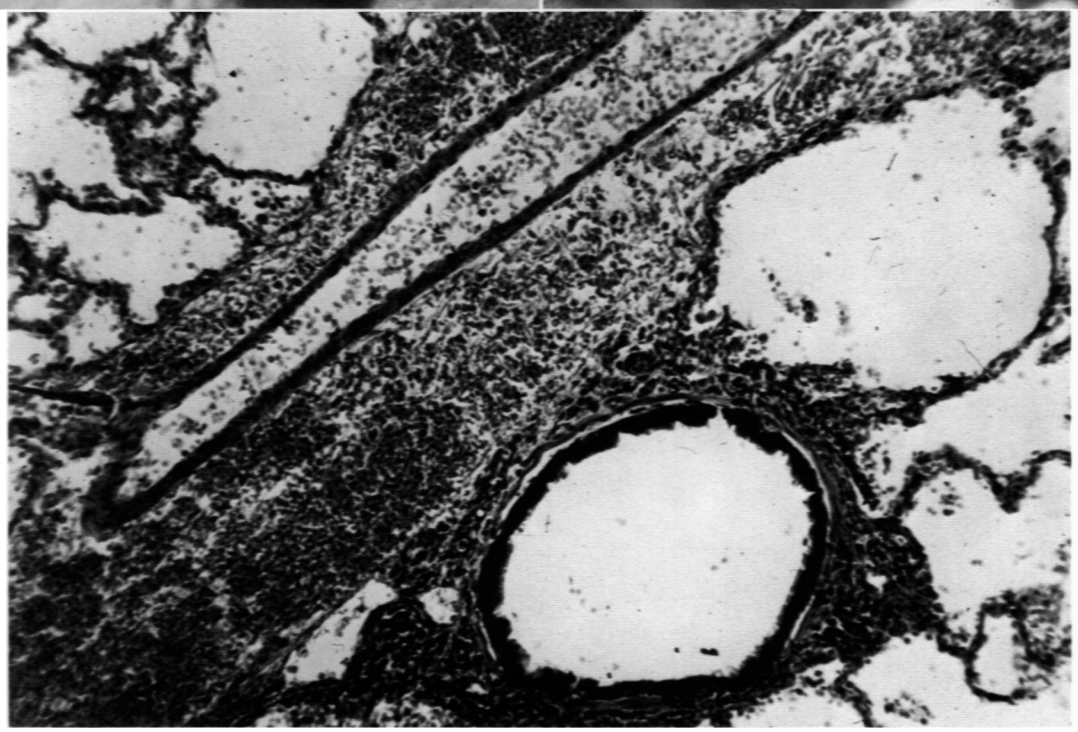

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