AD705068

UNIVERSITY OF OKLAHOMA MEDICAL CENTER

EFFECTS OF ENDOTOXIN ON PULMONARY CAPILLARY PERMEABILITY, ULTRASTRUCTURE AND SURFACTANT

L. H. Harrison, J. J. Beller, L. B. Hinshaw,

J. J. Coalson, and L. J. Greenfield

Technical Report No. 16
University of Oklahoma Medical Center THEMIS Contract

This document has been approved for public release and sale; its distribution is unlimited.

Reproduction in whole or in part is permitted for any purpose of the United States Government

Reproduced by the
CLEARINGHOUSE
for Federal Scientific & Technical
Information Springfield Va. 22151

DDC
RECEIVED
MAY 11 1970
C

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.
800 Northeast Thirteenth Street
Oklahoma City, Oklahoma 73104

34

EFFECTS OF ENDOTOXIN ON PULMONARY CAPILLARY PERMEABILITY, ULTRASTRUCTURE AND SURFACTANT

L. H. Harrison, J. J. Beller, L. B. Hinshaw,

J. J. Coalson, and L. J. Greenfield

Technical Report No. 16
University of Oklahoma Medical Center THEMIS Contract

March 23, 1970

Research sponsored by the Office of Naval Research
Contract N00014-68-A-0496
Project NR 105-516

Reproduction in whole or in part is permitted for
any purpose of the United States Government

This document has been approved for public release
and sale; its distribution is unlimited.

MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE
OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.

TABLE OF CONTENTS

Page

INTRODUCTION..... 1

METHODS..... 1

RESULTS..... 4

PATHOLOGY..... 5

DISCUSSION..... 7

SUMMARY.....10

REFERENCES.....12

ACKNOWLEDGEMENTS

The authors acknowledge the technical assistance of Mrs. Dennice Noel, Mr. Hubert Jennings and Mr. David Gordon.

INTRODUCTION

The respiratory insufficiency which usually accompanies clinical gram-negative bacteremic shock remains a poorly understood but often lethal complication (11). In animals subjected to endotoxin shock, the pulmonary changes reported include increased pulmonary vascular resistance and abnormal ventilation/perfusion ratios (5). Microscopically in dogs or primates, pulmonary edema, hemorrhage, platelet microemboli, vascular endothelial damage, sequestration of leukocytes, and alveolar cell necrosis have been observed (19). Selective studies of the vascular bed of the limb after exposure to endotoxin show increased capillary permeability (7, 17). Since this abnormality may play a role in the pathogenesis of lung damage by permitting losses of fluids, electrolytes, and proteins into the interstitium, this study was undertaken to determine changes in pulmonary capillary permeability using a modified technic of isogravimetric perfusion of the lung.

METHODS

In 1948, Pappenheimer and Soto-Rivera (13) reported a technic for determination of capillary permeability by isogravimetric perfusion of the isolated forelimb. Recently, this technic has been extended to the isolated lung in our laboratory and by Gaar (3) although we have included in addition the functional component of bronchial ventilation.

Thoracotomy was performed on 24 healthy adult mongrel dogs of either sex which had been screened for Dirofilaria immitis. The animals were anesthetized with sodium pentobarbital (30 mg/kg in normal animals; 10 mg/kg in shocked animals) and a cuffed endotracheal tube inserted. One femoral artery was cannulated and the dog heparinized (200 units/kg), after which 800 - 1000 cc

of blood was collected and filtered through a fine Teflon mesh. At the same time, the lower lobe of the left lung was excised and weighed, and polyethylene cannuli inserted into the pulmonary artery, vein and bronchus. The lobe was wrapped in a small plastic bag to prevent drying and then was placed on a supporting tray so that the pulmonary vein was uppermost in order to prevent torsion (Figure 1). The supporting tray was attached to a strain-gauge balance (7) for continuous measurement of lobe weight. The polyethylene cannuli inserted in the arterial and venous lines were advanced to the orifices of their respective vessels. The reference point for these pressures was taken at the level of the pulmonary vein. All pressures were recorded on an oscillograph by means of a single Statham P23Db pressure transducer.

Venous resistance to flow was regulated by a screw clamp and venous return to a 250 ml. graduated burette was circulated through a disc deoxygenator primed with autologous blood from the donor dog. Temperature of the perfusate was maintained at 36-37° C by heat exchanger and monitored continuously by thermister probe. A mixture of 95% nitrogen and 5% carbon dioxide was used in the deoxygenator at a flow rate of 3 l/min. Blood was returned from the deoxygenator to the pulmonary artery by means of a calibrated occlusive roller pump.

The lobe was ventilated by means of a Harvard respirator using ambient air at a constant volume of 4 cc/gram lobe weight and a constant rate of ten cycles/min. Intrabronchial pressures were monitored by means of a saline-filled, graduated U-tube. Blood gas determinations and pH were obtained in some preparations on samples from the arterial and venous cannulae.

After excision of the lobe, blood flow was restored within 5-7 minutes at low levels (100 cc/min.) and the preparation allowed to stabilize. Venous

pressure was increased by the screw clamp in increments of 1-2 mm. Hg. while increases in lobe weight were observed carefully. This process was continued until the highest venous pressure had been achieved which did not produce a continuous gain in weight. This was recorded as a first in a series of "isogravimetric" points. Subsequent points were taken by altering venous resistance and blood flow inversely in such a manner that the lobe maintained a steady weight equal to that of the first isogravimetric point. After several such points had been established, and while the preparation was at an isogravimetric point, the arterial and venous lines were occluded simultaneously by clamps. Within two seconds the arterial and venous pressures had equilibrated and thus were equal to the pressure within the pulmonary capillaries. This was designated as the isogravimetric capillary pressure (Pc_i). Several such pressures at zero flow were recorded during the course of a two hour perfusion (Figure 2). Immediately following perfusion the lobes were weighed and biopsy specimens obtained for light and electron microscopy. Adjacent lung tissue was used for pulmonary surfactant assay determined by the surfactometer and quantitated on the basis of hysteresis loop area as reported previously (4). Values less than 1.5 inches (2) were assumed to be abnormal on the basis of probability of normality .025.

Three groups of dogs were utilized in the study. Group I consisted of lungs from six normal dogs for which normal isogravimetric curves were established after which endotoxin was added to the perfusate. The endotoxin dosage was calculated on the basis of perfusate volume equivalent to an LD_{80} dose in the intact animal. After one hour of circulation a second isogravimetric curve was obtained. Group I consisted of lungs from eight dogs which had survived an intravenous LD_{80} dose of purified E. Coli endotoxin (Difco)

administered 8-16 hours prior to surgery. Group III consisted of lungs from 10 normal animals and served as the control group undergoing perfusion for the same length of time as in Groups I and II.

RESULTS

The mean isogravimetric capillary pressures for the normal control (Group III) and the endotoxin shock survivors (Group II) were 5.9 $\pm$ 0.2 mm. Hg. (SEM) and 7.4 $\pm$ 0.7 mm. Hg., respectively (Figure 3). The small but significant difference between these values ($P<0.05$) was not observed when the normal control was compared to Group I in which endotoxin was added to the perfusate of otherwise normal lung (Table I).

Decreased pulmonary surfactant was observed in two of the ten normal lobes perfused in Group III but only one was clearly abnormal. The lungs of endotoxin survivors in Group II showed minimally decreased surfactant in 3 of 8 dogs. In Group I, 5 of 6 dogs showed marked reductions in pulmonary surfactant after endotoxin was added to the perfusate. Inflation pressures were slightly higher in the shocked lungs and both groups showed a gradual rise of 3-5 cm. saline over the course of a two hour perfusion with the same volume of ventilation.

Perfusion blood gas determinations averaged 31 mm. Hg. PCO_2 and 37 mm. Hg. PO_2 with pH of 7.32 from blood leaving the deoxygenator. The pulmonary venous blood determinations ranged from PO_2 values of 92 - 128 mm. Hg. and PCO_2 13 - 17 mm. Hg. with no significant differences between the groups, although the number of determinations was small.

Calculation of pulmonary vascular resistance showed that total resistance to flow in the lungs of endotoxin-shocked dogs was increased significantly

above normal at low flow rates. However it approached and became indistinquishable from normal controls at higher flows (Figure 4). No significant increase in pulmonary vascular resistance was observed when endotoxin was added to the perfusate of a normal lung (Group I). Selective pre- and postcapillary resistance changes were determined utilizing pressure differences between observed isogravimetric capillary pressures and arterial or venous pressures. There was no significant difference when endotoxin was added to the perfusate (Group I), but in Group II, changes in precapillary resistance accounted for the greater portion of the total increase in pulmonary vascular resistance in these endotoxin-shocked dogs (Figure 5). This represents an exaggeration of the relationship between pre- and postcapillary resistances observed in the normal dog lung (Figure 6). However at low flows pulmonary venous resistance in Group II was increased significantly above that of the control group at similar flow rates (Figure 7).

PATHOLOGY

In the control lungs perfused for 2-3 hours, only focal areas of atelectasis were seen. Some pulmonary artery branches accompanying the respiratory bronchioles showed an accumulation of red blood cells in the adventitial coat. Sections stained with PAS showed occasional stained cells along the septal wall which were normal in distribution.

In the dogs receiving endotoxin during perfusion, (Group I) atelectatic areas were noted which seemed to radiate around alveolar ducts. There was an increase in thickness of the alveolar capillary walls and pre-arteriolar vessels were filled with red blood cells. Similarly postcapillary venules were dilated. Sections stained with PAS demonstrated positive material

within cells in the alveolar walls, usually neutrophils, in approximately half of the dogs in this group.

In Group II both atelectasis and focal areas of pulmonary edema were observed. Postcapillary venules were more congested and dilated in this group. In one of the animals, thrombi consisting of fibrin, leukocytes and red blood cells were demonstrated, but this finding was not observed in any of the other animals. PAS positive material in polymorphonuclear leukocytes was found abundantly in all animals in this group (Figure 8).

On electron microscopy, the control perfused lungs in Group III showed some capillary ectasia. The only pathologic finding of the endothelium was an occasional bleb due to cytoplasmic edema. There was no congestion of polymorphonuclear leukocytes or platelets within the capillary spaces. The alveolar type I and type II cells were within normal limits.

In Group I after endotoxin, both platelets and polymorphonuclear leukocytes were seen within capillaries and the endothelial lining was quite attenuated without rupture (Figure 9). There was marked edema of the perivascular space with separation of reticular and collagen fibers. Marked widening of the alveolar capillary wall was evident. Type I alveolar cells showed no significant change but type II cells showed degenerative fatty vacuoles within the cytoplasm.

In Group II the capillaries contained abundant polymorphonuclear leukocytes, scattered strands of fibrin, and platelets. Within the cytoplasm of the leukocytes, a marked decrease of lysosome granules was noted. Osmiophilic granules resembling glycogen which were diastase-susceptible at the light microscopic level, were greatly increased within the cytoplasm (Figure 10).

Leukocytes were seen in intravascular, perivascular, and alveolar spaces. The capillary endothelium was attenuated but intact, and edematous changes were noted in the perivascular spaces. The alveolar Type I epithelium showed severe edema with occasional loss of continuity of this lining layer. Within the type II cells, the cytoplasm was markedly vacuolated, and the microvilli were blunted.

DISCUSSION

Since isogravimetric capillary pressure varies inversely with capillary permeability, it can be used as an index of the integrity of the capillary membrane. The observed decrease in pulmonary capillary permeability in canine endotoxin survivors is in marked contrast to observations made in peripheral beds where endotoxin increased capillary permeability (7, 17). It is possible that the combination of low pressure and high flow made the lung vasculature "privileged" in terms of susceptibility to endotoxin damage. The hydrostatic pressure exerted on the capillaries of the dog extremity is more than twice as great (17 mm. Hg.) as that found in the normal lung, suggesting that transmural pressure may play an important role in the etiology of capillary damage. The fact that significant differences in pulmonary vascular resistance and Pc_i were observed only in the lungs of animals receiving endotoxin before pulmonary perfusion seems to implicate intermediate pathologic processes occuring in vivo. Although the mechanism is unknown, the endothelial cell thinning observed on electron microscopy may have played a role in the reduced permeability of the capillaries in endotoxin survivors.

The presence of large numbers of leukocytes filling the pulmonary capillaries has been observed in monkeys (12) and in rats (2). McKay et al (12)

attributed the neutropenia seen after endotoxin shock to sequestration of the neutrophils in the pulmonary capillaries and their intravascular destruction. Endothelial rupture, described by De Palma et al (2) in rat tissues, was not observed in endotoxin studies in monkeys (12) nor was it found in this study.

Phagocytosis has been shown to cause lysis of specific granules in leukocytes (9) however, endotoxin alone has not been felt to cause degranulation of polymorphonuclear leukocytes. Several investigators have shown that endotoxin exerts a labilizing effect upon lysosomes (10, 20) and in this study a loss of lysosomal granules was observed in leukocytes sequestered in alveolar capillary vessels. The finding of granular material ultrastructurally resembling glycogen has not been previously described, although the release of glycogen from hepatic cells after endotoxemia has been documented (2). It was originally assumed that the neutrophils had phagocytized glycogen released from the liver, but in Group I in which the lungs were isolated from hepatic circulation, this same accumulation was seen frequently. Rubenstein et al (16) using an immunoflourescence technique, demonstrated endotoxin within the cytoplasm of neutrophils sequestered in pulmonary capillaries. They described circumscribed patches of immunoflourescent particles (15-20 mu) associated with distorted or rupturing leukocytes. Since endotoxin is a known lipopolysaccharide (14) and should be PAS-positive on staining, a preparation of dried pure endotoxin was made on two groups of slides and fixed with Bouin's, Carnoy's, or formalin fume fixative. One group of slides was digested with diastase and then both groups were treated with PAS. Both groups of endotoxin slides showed intensely positive PAS staining demonstrating resistance to diastase digestion. Although a final definition of the material seen in the

neutrophils in this study is not possible, the granules would appear to be glycogen. The source of particulate glycogen in the isolated perfused lungs is unknown. After phagocytosis of the material into polymorphonuclear neutrophils, the displacement of lysosomes within these cells may reduce their ability to phagocytize bacteria.

Preservation of pulmonary surfactant at normal levels in almost all of the control lungs demonstrates the stability of the ex vivo perfusion system. The marked reduction in surfactant in Group I after endotoxin was added to the perfusate suggests a direct effect on the lining membrane. On electron microscopy, the appearance of abundant free osmiophilic material in air spaces confirmed this disruption of the lining layer. Although a direct cellular effect of endotoxin has been well documented (15), cellular damage alone would not alter surfactant levels until there was failure of replenishment after the turnover time of 18 hours. Reduced compliance with a higher inflation pressure at the same tidal volume was also observed in this group although the differences between groups were not significant. In Group II, only minimal decreases in surfactant were observed in 3 of 8 dogs who survived endotoxin shock. The reasons for this preservation of surfactant are not known, but may be related to factors which permitted the animal to survive an LD_{80} dose of endotoxin.

As noted by other investigators, it is likely that a significant part of the circulatory difficulties experienced by a patient in shock with reduced pulmonary flow can be attributed to increased pulmonary vascular resistance. Since increased blood flow was associated with reduction of pulmonary vascular resistance to normal control levels, there would be ample reason to restore cardiac output and blood volume to optimal levels in the clinical situation. The beneificial effects of isoproterenol on pulmonary

perfusion in endotoxin shock in sheep has been well demonstrated (5). However the increase in the venous component of resistance at low pulmonary flows could be responsible for the clinical "wet lung" and respiratory distress syndrome simply by ultra filtration, particularly if fluid therapy is too aggresive. Additional caution would be indicated in the use of positive-pressure ventilation, with or without the addition of supplemental oxygen, since both measures can produce increases in pulmonary vascular resistance.

SUMMARY

A method is described for measuring pulmonary capillary permeability by isogravimetric perfusion of excised dog lungs. The isogravimetric capillary pressures were determined in (1) normal canine lungs perfused with blood to which endotoxin had been added; (2) excised lungs of endotoxin-shocked dogs; (3) perfused lungs of normal control dogs. No increase in pulmonary capillary permeability was noted but a statistically significant decrease in permeability was found in lungs of survivors of endotoxin shock. Pulmonary surfactant was observed to decrease only when endotoxin was added to the perfusate of normal lungs. Surfactant was preserved in survivors of endotoxin shock. Light and electron microscopy revealed granular inclusions within polymorphonuclear neutrophils in the lungs of dogs after end oxin shock or lungs perfused ex vivo with endotoxin and was compatible with phagocytized glycogen. The endothelial cell thinning observed in endotoxin survivors may have been responsible for the decrease in pulmonary capillary permeability. Both pre- and postcapillary vascular resistances were increased at low blood flows but returned to control levels at normal blood flows. The relative increase in postcapillary pulmonary vascular resistance at low blood

flows may play a role in the clinical "wet lung" type of respiratory insufficiency seen frequently in bacteremic shock, although no increase in capillary permeability was observed.

REFERENCES

1. Cohn, Z. A. and Morse, S. I.: Functional and metabolic properties of polymorphonuclear leukocytes. II. The influence of a lipopolysaccharide endotoxin. J. Experimental Med. 111: 689, 1960.

2. De Palma, R. G., Coil, J., Davis, J. H. and Holden, W. D.: Cellular and ultrastructural changes in endotoxemia: A light and electron microscopic study. Surgery. 62: 505, 1967.

3. Gaar, K. A., Jr., Taylor, A. E., Owens, L. J. and Guyton, A. C.: Pulmonary capillary pressure and filtration coefficient in the isolated perfused lung. Amer. J. Physiol. 213(4): 910, 1967.

4. Greenfield, L. J. and Duncan R. C.: Quantitative dynamic pulmonary surfactant determinations using the pneumatic surfactometer. Surgery. 64: 351, 1968.

5. Halmagyi, D. F. J., Starecki, B. and Harver, J.: Mechanism and pharmacology of endotoxin shock in sheep. J. Appl. Physiol. 18: 544, 1963.

6. Hinshaw, L. B., Kuida, H., Gilbert, R. P., and Visscher, M. G.: Influence of perfusate characteristics on pulmonary vascular response to endotoxin. Amer. J. Physiol. 191: 293, 1957.

7. Hinshaw, L. B., Reins, D. A. and Hill, R. J.: Response of isolated liver to endotoxin. Canadian J. Physiol. and Pharmacol. 44: 529, 1966.

8. Hinshaw, L. B., Solomon, L. A., Holmes, D. D., and Greenfield, L. J.: Comparison of canine responses to E. coli organisms and endotoxin. Surg. Gynecol. and Obstet. 127: 981, 1968.

9. Hirsch, J. C. and Cohn, Z. A.: Degranulation of polymorphonuclear leukocytes following phagocytosis of microorganisms. J. Exptl. Med., 112: 1005, 1960.

10. Janoff, A. and Kaley, S.: Studies in lysosomes in tolerance, shock, and local injury induced by endotoxin. In Bacterial Endotoxin, pp. 631, Rutgers University Press, eds. M. Landy and W. Braun, 1964.

11. MacLean, L. D., Mulligan, W. G., McLean, A. P. H., and Duff, J. H.: Patterns of septic shock in man - a detailed study of 56 patients. Annls. of Surg. 166(4): 543, 1967.

12. McKay, D. G., Margaretten, W. and Csavossy, I.: An electron microscope study of endotoxin shock in Rhesus monkeys. Surg. Gynecol. and Obstet. 125: 825, 1967.

13. Pappenheimer, J. R. and Soto-Rivera, A.: Effective osmotic pressure of the plasma proteins and other quantities associated with the capillary circulation in the hindlimbs of cats and dogs. Amer. J. Physiol. 153: 471, 1948.

14. Ribi, E.: Preparation, purification, and structure of endotoxin. J. Okla. State Med. Assoc., 59: 413, 1966.

15. Rosenberg, J. C. and Rush, B. F.: Blood lactic acid levels in irreversible hemorrhagic and lethal endotoxin shock. Surg. Gynecol. and Obstet. 126: 1247, 1968.

16. Rubenstein, H. S.: Discussion, Part III, In Bacterial Endotoxin, p. 250, Rutgers University Press, eds. M. Landy and W. Braun, 1964.

17. Solomon, L. A. and Hinshaw, L. B.: Effect of endotoxin on isogravimetric capillary pressure in the forelimb. Amer. J. Physiol. 214(3): 443, 1968.

18. Stish, R. J., McLean, L. D. and Visscher, M. D.: Device for continuous recording of weight change suitable for physiological measurements. J. Appl. Physiol. 9: 297, 1956.

19. Vaughn, D. L., Gunter, C. A. and Stookey, J. L.: Endotoxin shock in primates. Surg. Gynecol. and Obstet. 126: 1309, 1968.

20. Weissmann, G. and Thomas, L.: On a mechanism of tissue damage by bacterial endotoxins. In Bacterial Endotoxin, pp. 602, Rutgers University Press, eds. M. Landy and W. Braun, 1964.

LEGEND FOR FIGURES

Figure 1. Diagrammatic representation of the isogravimetric pulmonary perfusion apparatus. The lower lobe of the canine lung is supported on a tray which records slight changes in total weight during perfusion and ventilation. Venous pressure is altered by means of the constricting device to permit the determination of isogravimetric pulmonary capillary pressure points.

Figure 2. Dog No. 2191, normal. Mean isogravimetric pressures determined by varying venous pressures at different blood flows for a normal canine lung ventilated with air. At all flow rates above zero, the arterial pressure is greater than venous pressure with no net transfer of fluid, and the mean capillary pressure is presumably the same at all blood flows. The measured zero flow isogravimetric capillary pressure is equal to all pressures opposing filtration.

Figure 3. Comparison of mean isogravimetric capillary pressures obtained from normal controls and from dogs surviving endotoxin shock. The slight but significant increase in isogravimetric capillary pressure observed in the endotoxin survivors was not observed when endotoxin was added to the perfusate of a normal lung. PC_i, isogravimetric capillary pressure.

Figure 4. Determination of pulmonary vascular resistance in normal dogs, group 3, and survivors of endotoxin shock, group 2. Resistance is calculated on the basis of millimeters of mercury of pressure differential over flow and expressed as millimeters of mercury per cubic centimeter per minute per 100 grams of lung. Significant

increases in resistance are observed at all but the highest blood flow rates.

Figure 5. Pulmonary arterial versus pulmonary venous resistance 24 hours after the administration of endotoxin. Comparison of precapillary and postcapillary pulmonary vascular resistances, mean ± standard error of the mean, at different blood flows calculated from the pressure differences between observed capillary pressures and pulmonary arterial or venous pressures and expressed as millimeters of mercury per cubic centimeter per minute per 100 grams of lung. Pulmonary arterial or precapillary resistance shows a significant increase at flows of 500 to 600 cubic centimeters per 100 grams of lung which is an exaggeration of the normal pattern as seen in Figure 6. Q, blood flow.

Figure 6. Pulmonary arterial versus pulmonary venous resistance in the normal dog. Comparison of precapillary and postcapillary pulmonary vascular resistances, mean ± standard error of the mean, observed in isogravimetric perfusion of the normal canine lung. Resistance is expressed as millimeters of mercury per cubic centimeter per minute per 100 grams of lung. Blood flow, Q, shows as cubic centimeters per 100 grams of lung.

Figure 7. Comparison of postcapillary pulmonary vascular resistances between normal dogs and those in a state of endotoxin shock, group 2. At blood flows less than 400 cubic centimeters per 100 grams of lung, there is a significant increase in postcapillary resistance above control levels. Resistance is expressed as millimeters of mercury per cubic centimeter per

minute per 100 grams of lung. Q, blood flow.

Figure 8. a) A low power micrograph of the lung from a dog in a state of endotoxin shock, group 2, shows strongly periodic-acid Schiff positive material predominantly within the leukocytes within the septal wall. The alveolar spaces are filled with transudate and some alveolar macrophages. b) An adjacent section was treated by diastase digestion. The cells now show the disappearance of periodic-acid Schiff positive material. Periodic-acid Schiff with a control diastase digestion, counterstained with hematoxylin and eosin, X165.

Figure 9. The effects of the addition of endotoxin to the perfusate in group 1 are shown in this electron micrograph. The capillaries, C, are filled with neutrophils, N, showing an increase of glycogen particles, arrows, and a few strains of fibrin, F. Edematous changes within the perivascular space, P, has separated the reticular and collagen fibers. The endothelial lining shows evidence of early cytoplasmic blebbing, B. The type I epithelial lining is intact. The alveolar spaces, A, show no evidence of pulmonary edema. Uranyl acetate and lead citrate, X5846.

Figure 10. Electron micrograph of lung from a dog in a state of endotoxin shock in group 2. The capillaries, C, contain abundant neutrophils, some of which are sparsely granulated. In the neutrophil, N, which is markedly degranulated, the cytoplasm contains many glycogen particles. The endothelium in this particular field is not edematous; however, endothelial blebbing was noted focally in dogs in a state of systemic endotoxin shock. Edema of the perivascular space, P, can be demonstrated. The alveolar type I

epithelium, ATi, is likewise edematous and is actually disrupted in focal areas, arrow. The type II epithelial cell, ATii, shows marked cytoplasmic vacuolization and severe blebbing of microvilli. Uranyl acetate and lead citrate, X2825.

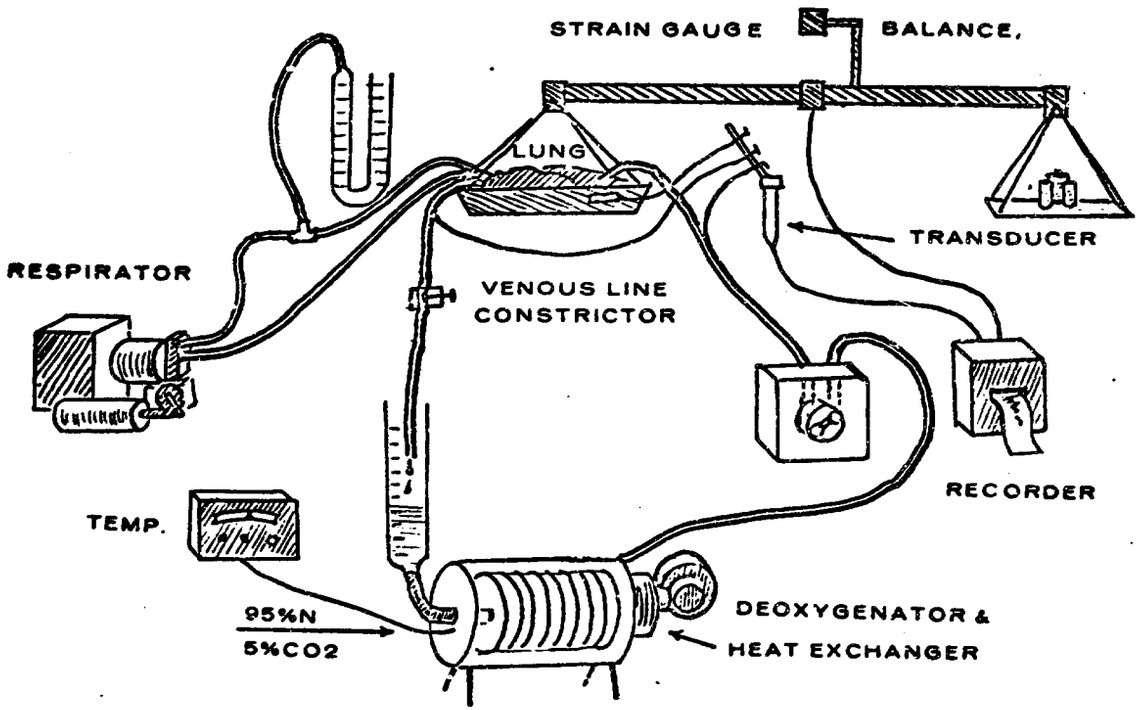

STRAIN GAUGE
BALANCE,
LUNG
TRANSDUCER
RESPIRATOR
VENOUS LINE
CONSTRICTOR
RECORDER
TEMP.
95%N
5%CO2
DEOXYGENATOR &
HEAT EXCHANGER

Figure 1

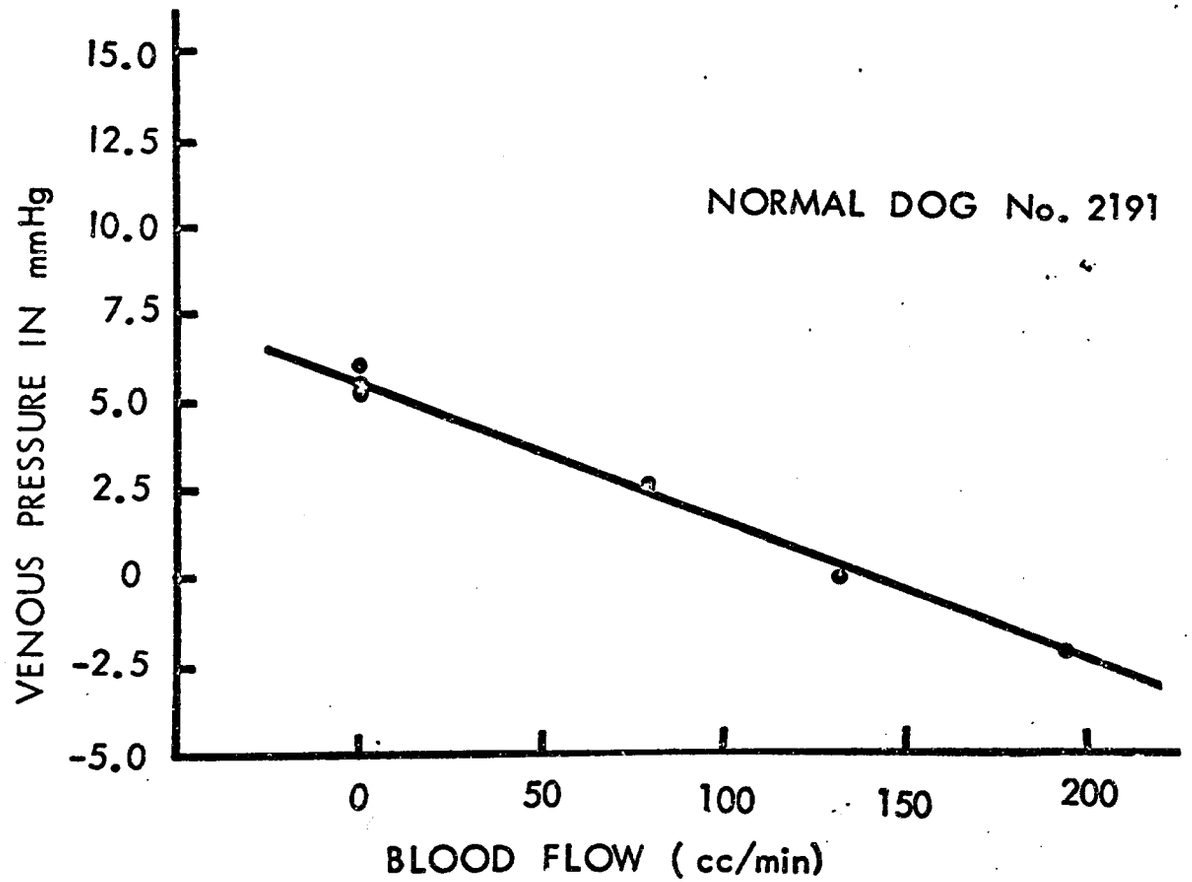

NORMAL DOG No. 2191
VENOUS PRESSURE IN mmHg
15.0
12.5
10.0
7.5
5.0
2.5
0
-2.5
-5.0
0
50
100
150
200
BLOOD FLOW (cc/min)

Figure 2

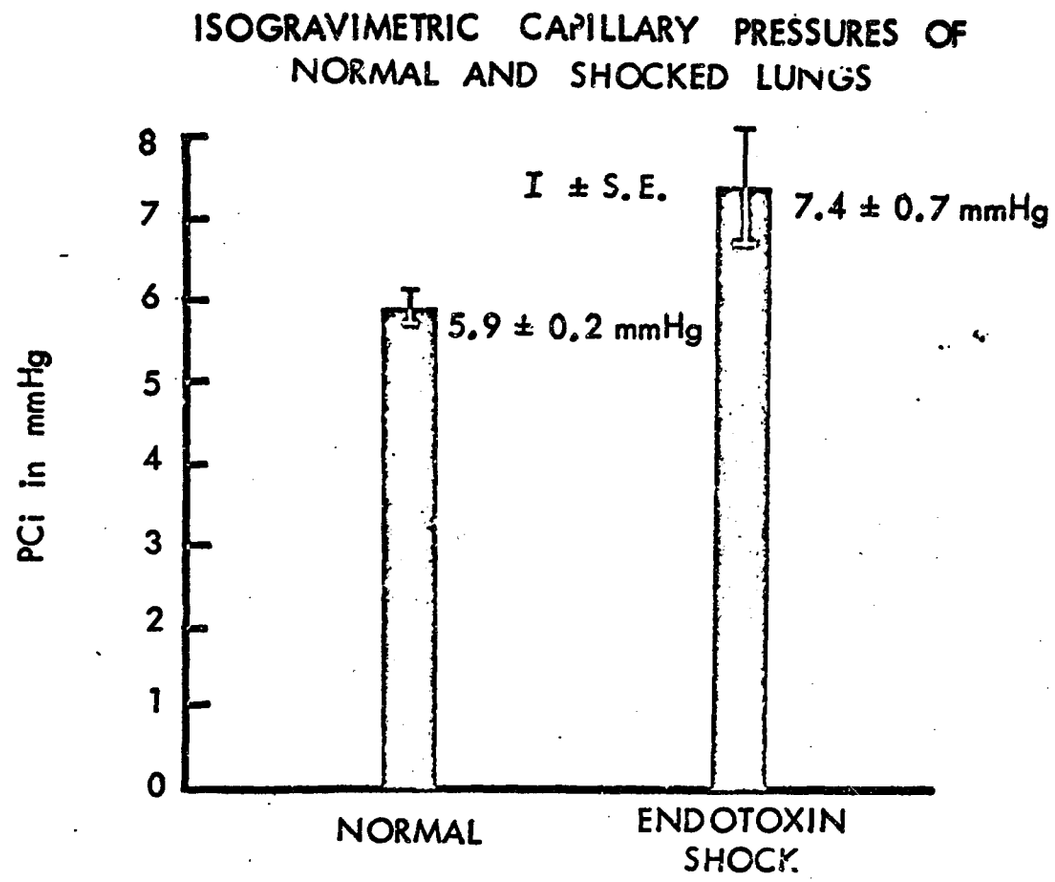

ISOGRAVIMETRIC CAPILLARY PRESSURES OF
NORMAL AND SHOCKED LUNGS
PCi in mmHg
8
7
6
5
4
3
2
1
0
I ± S.E.
5.9 ± 0.2 mmHg
7.4 ± 0.7 mmHg
NORMAL
ENDOTOXIN
SHOCK

Figure 3

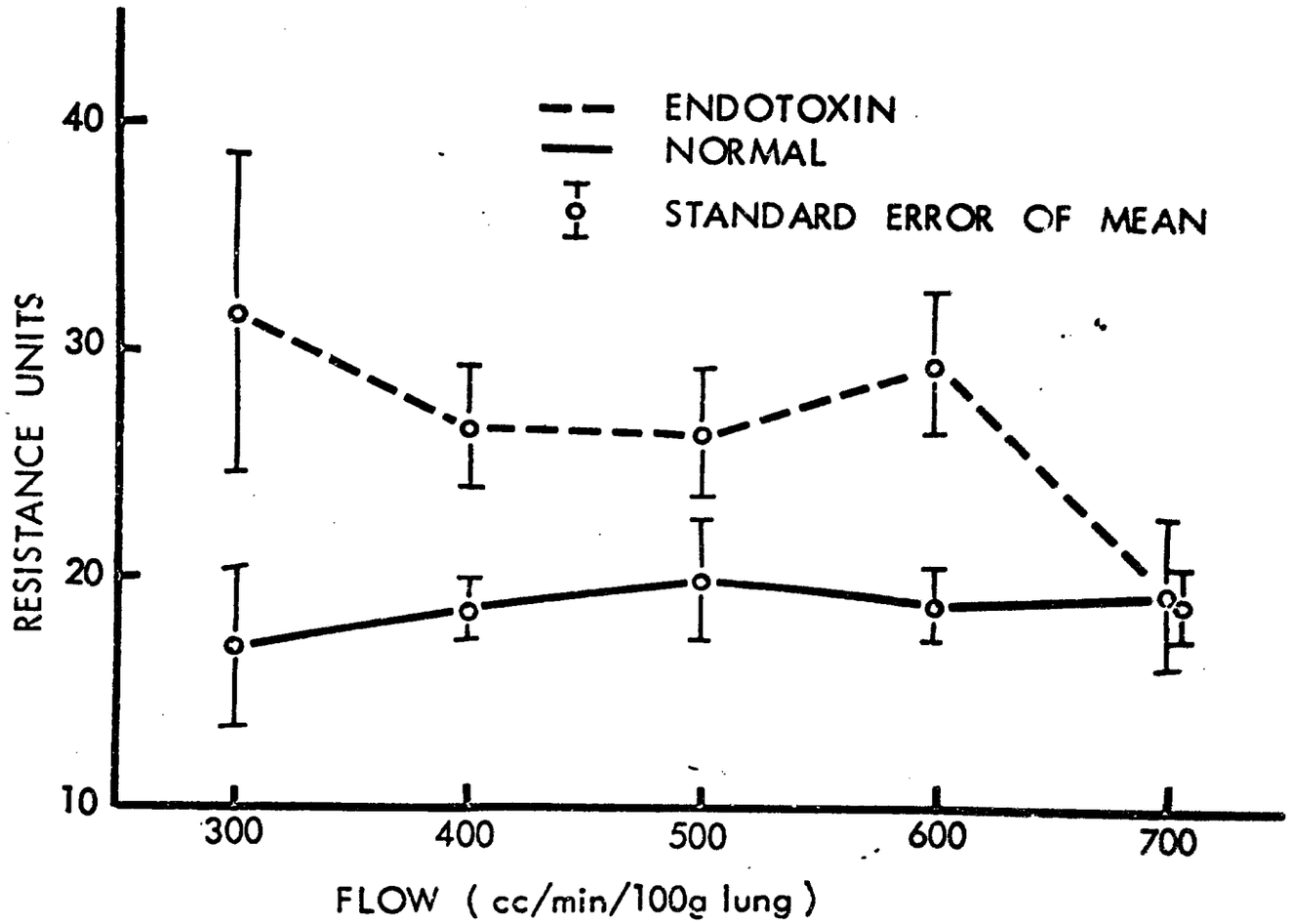

ENDOTOXIN
NORMAL
STANDARD ERROR OF MEAN
RESISTANCE UNITS
40
30
20
10
300
400
500
600
700
FLOW (cc/min/100g lung)

Figure 4

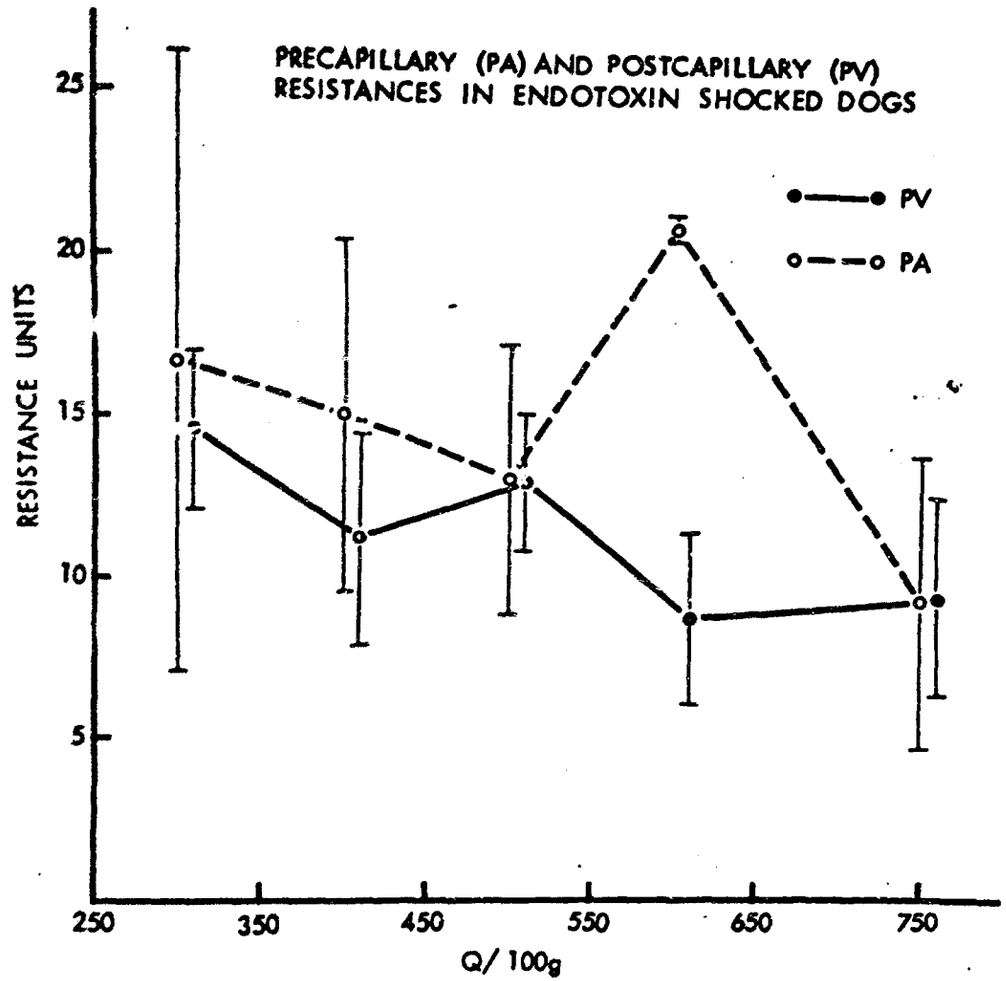

PRECAPILLARY (PA) AND POSTCAPILLARY (PV)
RESISTANCES IN ENDOTOXIN SHOCKED DOGS
PV
PA
RESISTANCE UNITS
25
20
15
10
5
250
350
450
550
650
750
Q/ 100g

Figure 5

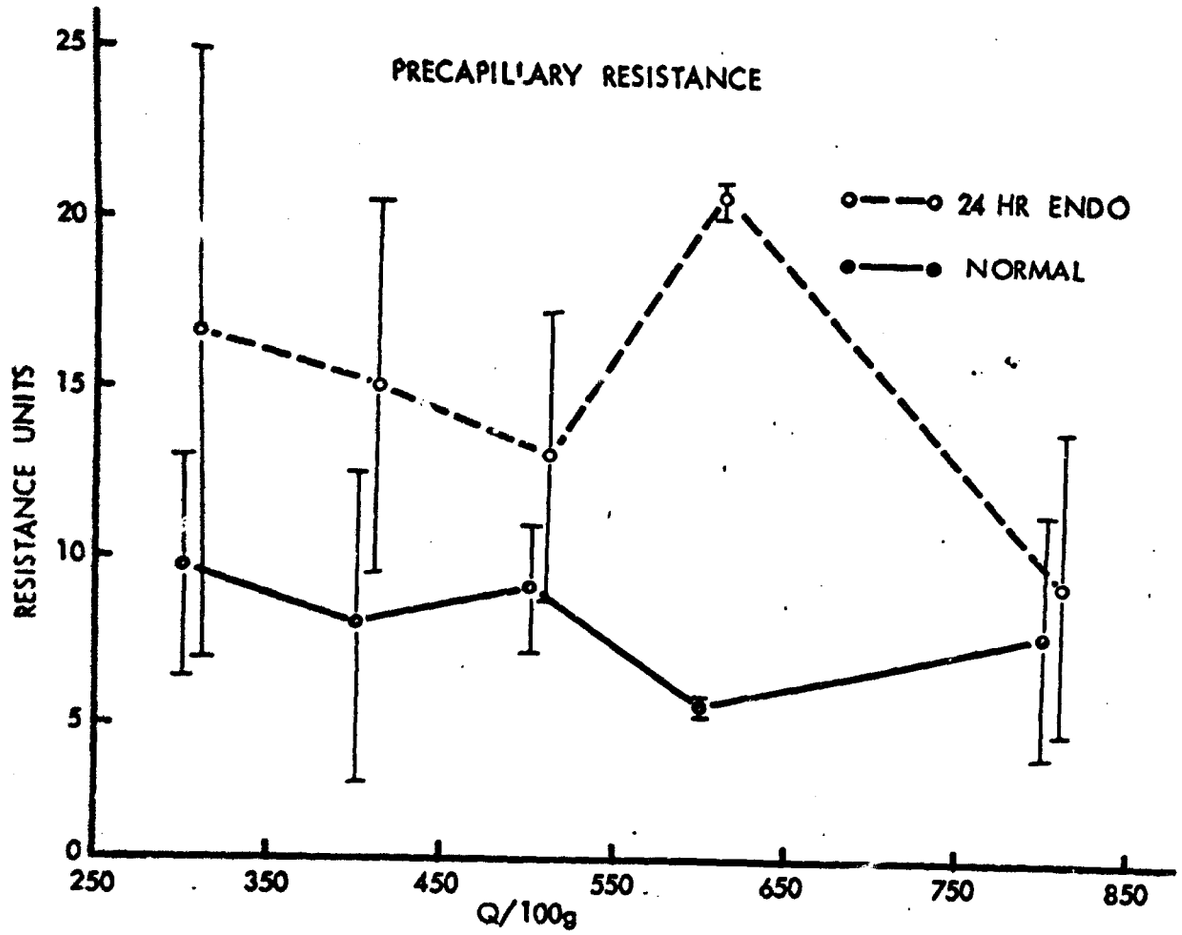

PRECAPILLARY RESISTANCE
24 HR ENDO
NORMAL
RESISTANCE UNITS
0
5
10
15
20
25
250
350
450
550
650
750
850
Q/100g

Figure 6

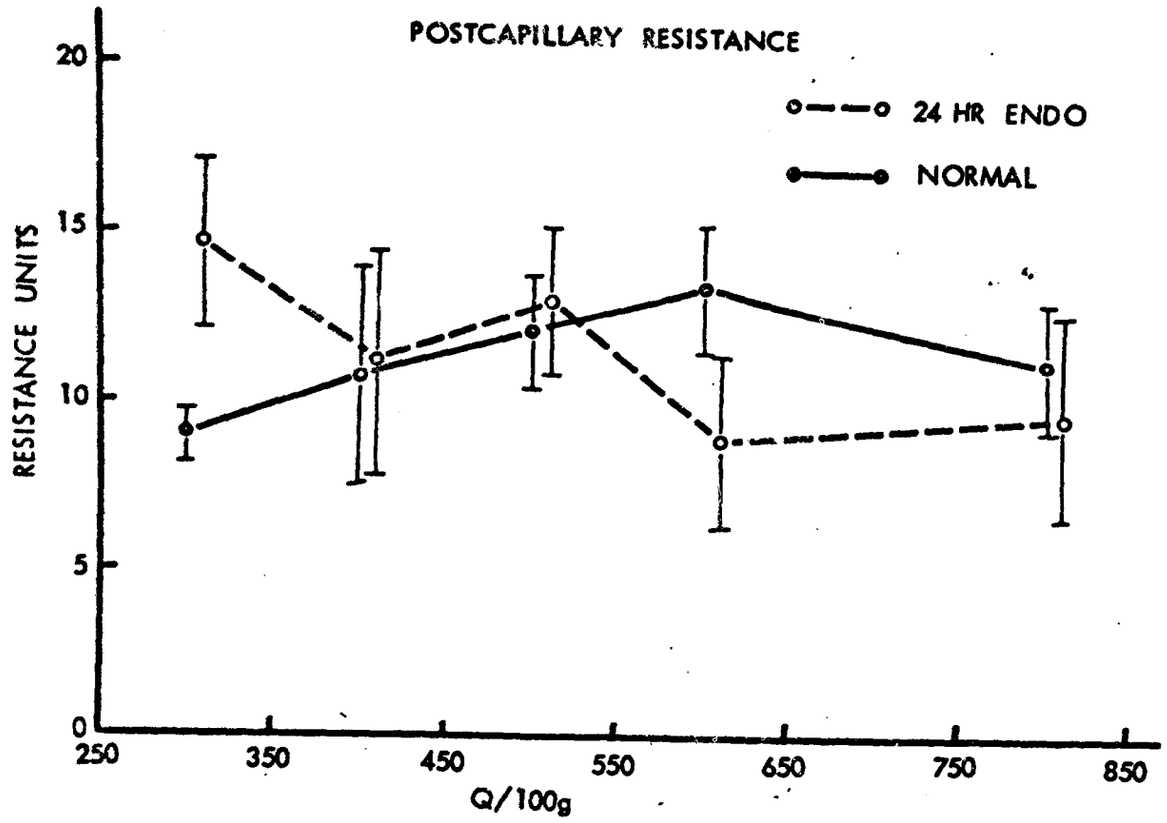
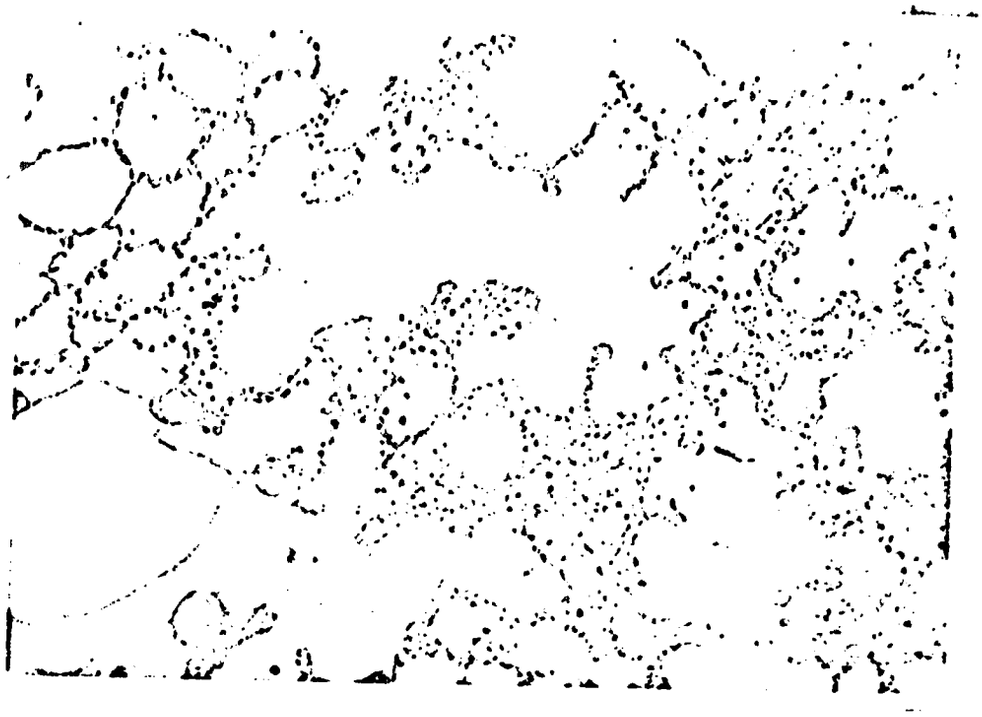

POSTCAPILLARY RESISTANCE
24 HR ENDO
NORMAL
RESISTANCE UNITS
20
15
10
5
0
250
350
450
550
650
750
850
Q/100g

Figure 7

NOT REPRODUCIBLE

a



b

Figure 8

NOT REPRODUCIBLE

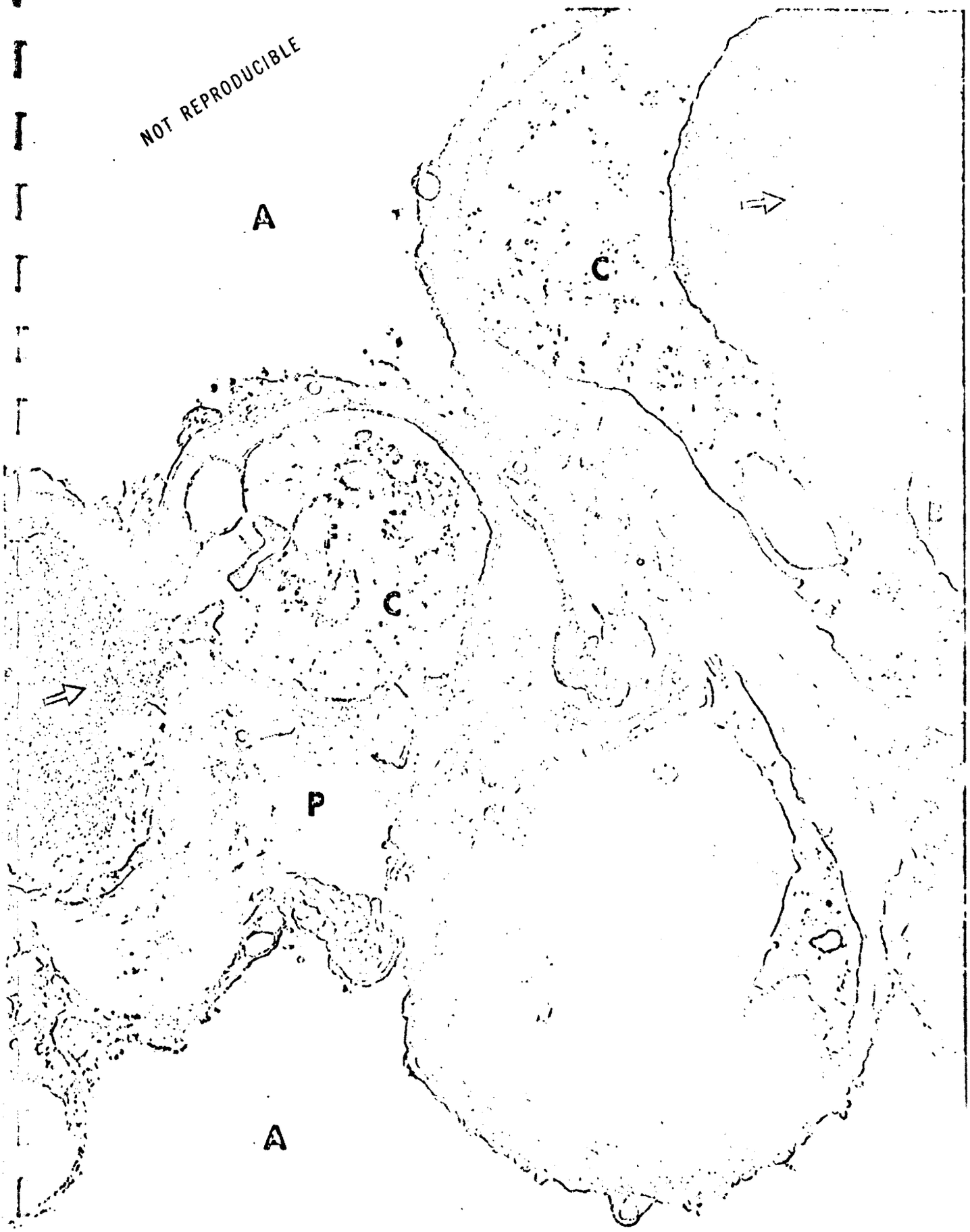


Figure 9

NOT REPRODUCIBLE

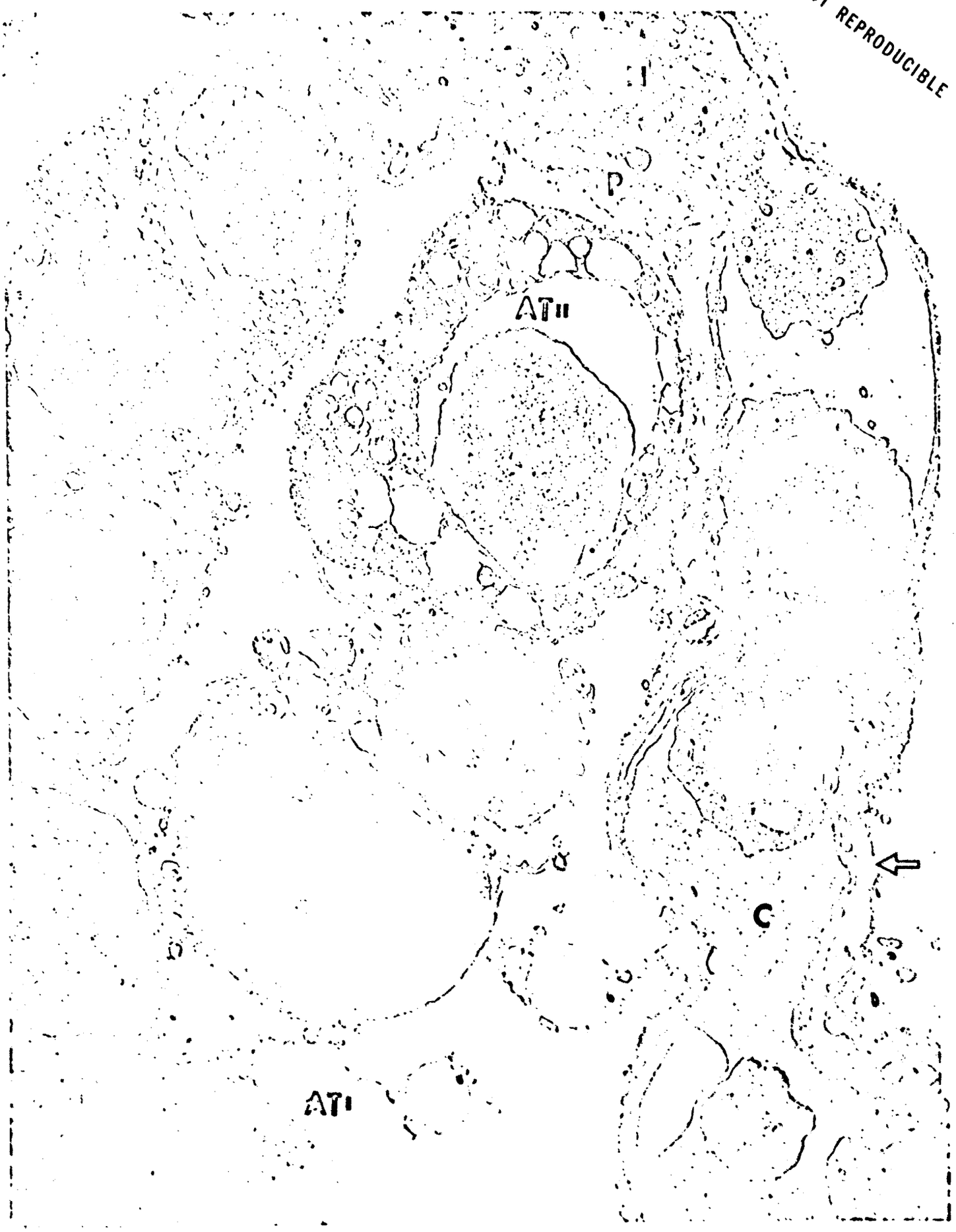


Figure 10

TABLE I

ALTERATIONS IN PULMONARY SURFACTANT AND PULMONARY CAPILLARY PERMEABILITY AFTER EXPOSURE TO ENDOTOXIN

DOG NO.	DOG WEIGHT	SURFACE TENSION AREA Sq. In.	CONTROL PC_i* mm.Hg.	ENDOTOXIN PC_i mm.Hg.	PERFUSION TIME HOURS
		GROUP I Endotoxin added to the perfusate of normal lung			
3351	19.0	.99	8.0	7.0	2.5
531	25.0	1.00	-	-	2.5
931	13.0	.94	5.5	6.0	2.5
1036	20.8	-	6.0	6.5	2.5
1070	24.5	.99	4.0	5.75	2.5
1101	18.0	1.27	6.0	6.25	2.5
Mean ± S. E. M.		1.04 ± .06	5.9 ± 0.6	6.3 ± 0.1	
		GROUP II Survivors of Endotoxin Shock			
1111	17.0	2.84		5.0	2.0
358	15.5	1.72		6.0	2.0
1232	16.5	1.75		10.5	2.0
1358	19.8	1.00		8.9	2.0
1057	13.0	2.22		6.0	2.0
3311	13.8	-		8.75	2.0
3343	15,0	1.40		7.5	2.0
3924	18.8	2.24		6.3	2.5
Mean ± S. E. M.		1.88 ± .23		7.4 ± 0.7	
		GROUP III Control Perfusions			
1212	20.5	2.77	4.9		2.0
539	22.7	2.15	5.9		2.0
1036	20.8	1.89	6.0		2.5
1101	18.0	2.20	6.0		2.0
1268	24,0	1.21	6.5		2.0
1601	25.0	.46	5.0		2.0
3734	17.4	2.30	7.25		2.0
3303	19.4	2.00	6.1		2.5
2191	25,0	1.60	5.6		2.0
1722	22.0	1.60	5.9		2.0
Mean ± S. E. M.		1.84 ± .20	5.9 ± 0.2		

*Pulmonary capillary pressure (isogravimetric)

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) MEDICAL CENTER RESEARCH AND DEVELOPMENT OFFICE OF THE UNIVERSITY OF OKLAHOMA FOUNDATION, INC.	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP Unclassified

3. REPORT TITLE

EFFECTS OF ENDOTOXIN ON PULMONARY CAPILLARY PERMEABILITY, ULTRASTRUCTURE AND SURFACTANT

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Technical Report

5. AUTHOR(S) (First name, middle initial, last name)

L. H. Harrison, J. J. Beller, L. B. Hinshaw, J. J. Coalson, and L. J. Greenfield

6. REPORT DATE March 23, 1970	7a. TOTAL NO. OF PAGES 29	7b. NO. OF REFS 20
8a. CONTRACT OR GRANT NO. N00014-68-A-0496 b. PROJECT NO. NR 105-516 c. d.	9a. ORIGINATOR'S REPORT NUMBER(S) 16 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10. DISTRIBUTION STATEMENT

This document has been approved for public release and sale; its distribution is unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY Office of Naval Research

13. ABSTRACT

A method is described for measuring pulmonary capillary permeability by isogravimetric perfusion of excised dog lungs. The isogravimetric capillary pressures were determined in 1) normal canine lungs perfused with blood to which endotoxin has been added; 2) excised lungs of endotoxin-shocked dogs; 3) perfused lungs of normal control dogs. No increase in pulmonary capillary permeability was noted but a statistically significant decrease in permeability was found in lungs of survivors of endotoxin shock. Pulmonary surfactant was observed to decrease only when endotoxin was added to the perfusate of normal lungs. Surfactant was preserved in survivors of endotoxin shock. Light and electron microscopy revealed granular inclusions with polymorphonuclear neutrophils in the lungs of dogs after endotoxin shock or lungs perfused ex vivo with endotoxin and was compatible with phagocytized glycogen. The endothelial cell thinning observed in endotoxin survivors may have been responsible for the decrease in pulmonary capillary permeability. Both pre- and postcapillary vascular resistances were increased at low blood flows but returned to control levels at normal blood flows. The relative increase in postcapillary pulmonary vascular resistance at low blood flows may play a role in the clinical "wet lung" type of respiratory insufficiency seen frequently in bacteremic shock, although no increase in capillary permeability was observed.

DD FORM 1473 (PAGE 1)

S/N 0101-807-6811

UNCLASSIFIED