Electron microscopic pathological patterns of alveolar septum in acute dextran-induced and alloxan-induced pulmonary edema in dogs

Koji Nakahara[1], Michio Arakawa[1], Kenjiro Kambara[1], Hiroshi Oda[1], Senri Hirakawa [1], Shizuko Shoumura [2] and Hideo Isono[2]

[1]Second Department of Internal Medicine and [2]First Department of Anatomy, Gifu University School of Medicine, Gifu 500, Japan

Summary. We studied the incidence of electron microscopic pathological patterns of the alveolar septum observed 30 min after induction of pulmonary edema by dextran-70 infusion (6 dogs, dextran group) and by alloxan injection (6 dogs, alloxan group).

For comparable amounts of extravascular lung water in both dextran and alloxan groups, which were twice as much as control group (6 dogs), we characterized the pathological changes.

The incidence of the electron microscopic pathological patterns that appeared in dextran group compared with that in control group was significantly high in terms of the widening of the interstitial space, dispersion and disarray of collagen fibrils, and erythrocytes in the interstitial space. The incidence in alloxan group compared with that in control group was significantly high in terms of the swelling of epithelial cells and endothelial cells as well as the widening of the interstitial space, and dispersion and disarray of collagen fibrils.

We conclude that dextran causes interstitial changes exclusively and alloxan causes cellular changes primarily coupled with secondary interstitial changes in acute pulmonary edema.

Key words: Pulmonary edema, Dextran-induced edema, Alloxan-induced edema, Electron microscopic study, Extravascular lung water

Introduction

In pulmonary edema of diverse etiologies, various electron microscopic pathological patterns of the alveolar septum have been reported (Cottrell et al., 1967; Teplitz, 1968; Cunningham and Hurley, 1972;

Noble et al., 1974; Smith and Heath, 1974; Dearden et al., 1977; Defouw and Berendsen, 1978). The pathological patterns frequently observed are: widening of the interstitial space; dispersion and disarray of collagen fibrils; focal disruption of epithelial cellular continuity; and swelling of epithelial and endothelial cells.

However, to our knowledge, their exact incidence has not yet been well documented and true pathological characteristics remain unanswered, the main reason being simply the lack of the quantitative trial due to the laborious and time-consuming work involved. In addition, unavoidable artifacts similar to pathological patterns occur during the process of fixation of the specimen (Wangensteen et al., 1981) and introduce other difficulties in analyzing the electron microscopic changes. For fair assessment of the characteristic changes of the alveolar septum in pulmonary edema from different etiologies, we have to estimate the incidence of electron microscopic pathological changes as objectively as possible.

Dearden et al. (1977) finely graded the extent of the electron microscopic changes of the alveolar septum in ethchlorvynol-induced pulmonary edema. However they did not refer to the incidence of the electron microscopic changes. There is an exceedingly excellent study done by Montaner et al. (1986) who quantified extensively the incidence of pathological changes confined to the alveolar epithelial damage in high pressure and oleic acid-induced low pressure pulmonary edema. Our primary interest was to obtain a whole picture of pathological changes occurring in the alveolar septum. In this connection, Kitamura et al. (1985) in our laboratory electron microscopically quantified the incidence of the artifacts resembling pathological patterns in pulmonary edema in intact dog lung and concluded for the first time that the incidence of the artifacts is less than 10% at most.

There are two experimental types of pulmonary edema which have been studied as representative models

of increased-pressure and increased-permeability edema. Dextran has been used to produce increased-pressure edema, while alloxan has been used to produce increased-permeability edema. In dextran-induced pulmonary edema, Noble et al. (1974) observed striking morphological changes in the alveolar interstitium including widening of the interstitial space, dispersion and disarray of collagen fibrils, and focal swelling of the endothelial cells. In alloxan-induced pulmonary edema, Cottrell et al. (1967) found the degeneration of both endothelium and epithelium, and the appearance of fibrin within the alveoli. Unfortunately, neither of them quantified the incidence of these changes and pathological characteristics are not clearly delineated.

Accordingly, we aimed to objectively demonstrate the pathological characteristics for comparable amounts of extravascular lung water in acute dextran-induced and alloxan-induced pulmonary edema in dogs.

We found that the incidence was significantly high in the changes in the interstitial space in acute dextraninduced pulmonary edema, while cellular changes associated with the changes in the interstitial space were significantly high in acute alloxan-induced pulmonary edema.

Materials and methods

Animal preparation

We anesthetized 18 dogs, weighing 12.5±2.5 (mean ± SD) kg, with pentobarbital sodium (25mg/kg). The dogs breathed spontaneously through an intratracheal tube. The methods of obtaining the extravascular lung thermal volume by the thermal-dye indicator dilution technique were essentially the same as described elsewhere (Yasuda et al., 1984; Kambara et al., 1985; Arakawa et al., 1985; Iinuma et al., 1986). Briefly, using two thermistor-mounted Swan-Ganz catheters, (93A-431H-7.5F, Edwards Laboratories Inc.), we placed the tip of one catheter in the right atrium for injecting ice-cold indocyanine green solution, and placed the tip of the other catheter in the aortic root for recording the thermodilution curve. We also placed the tip of Sones catheter in the aortic root through which we withdrew blood constantly and recorded the dye-dilution curve.

Experimental protocol

Production of pulmonary edema

We produced two types of pulmonary edema, one by infusion of dextran-70 (Macrodex, Pharmacia, AB) at a rate of 100 ml/kg over 30 minutes in 6 dogs (dextran-induced pulmonary edema, dextran group) and the other by a bolus injection of alloxan (alloxan monohydrate, Kishida chemical Co.) of 100 mg/kg in 6 dogs (alloxan-induced pulmonary edema, alloxan group). Six dogs without intervention served as control (control group). The interventions were done for 30 minutes and their samples for electron microscopy were excised.

Measurements of extravascular lung water

Using the thermal-dye indicator dilution technique, we measured the extravascular lung thermal volume during the experiment. The extravascular lung thermal volume was calculated as a product of cardiac output and the difference in mean transit time between the thermodilution curve and dye-dilution curve both being recorded in the aortic root (Yasuda et al., 1984; Arakawa et al., 1985; Iinum et al., 1986; Kambara et al., 1986). This extravascular lung thermal volume included the volume of the solid tissue in the lung and the volume equilibrated with cardiovascular wall (Yasuda et al., 1984; Kambara et al., 1985).

To doubly confirm the severity of pulmonary edema, the postmortem extravascular lung water was measured gravimetrically (Pearce et al., 1965; Selinger et al., 1975) at the end of the experiment in 14 dogs.

Electron microscopy

We excised several tissues from the different portions of the surface of the right lower lobe. Tissues were collected randomly in the control group, while tissues were collected from the portions where pulmonary edema appeared distinct at macroscopic level in both dextran and alloxan groups.

Tissues were immediately minced into 1 mm cubes, and fixed in 2.5% glutaraldehyde solution 0.1 M cacodylate buffer at pH 7.4 for 2 hours. They were then postfixed in 1% osmium tetroxide in the same buffer for one hour, dehydrated through graded concentrations in ethanol, and embedded in Epon 812. Thin sections were cut with a LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi-HS-8 electron microscope.

In each dog from control group (6 dogs), dextran group (6 dogs) and alloxan group (6 dogs), 20 electron micrographs were randomly taken, without prior knowledge to which group they belonged, from different regions of lung tissues at a primary magnification of 2,400-5,000 times, and photographically enlarged resulting in a final magnification of 4,700-10,000 times. The total number of electron micrographs was 360. To attain complete isolation from prior knowledge, we further numbered all electron micrographs serially, and mixed them randomly by shuffling. We examined electron micrographs for 13 electron microscopic pathological patterns (Table 1) which were well documented in pulmonary edema. Observers were assigned to give a plus if patterns were clearly present and to give a minus if patterns were only questionably present or absent. According to the generally accepted definition, items NO.3 through NO.8 are cellular changes representing typical patterns encountered in increased-permeability edema (Albertine, 1985). Among others, swelling of alveolar epithelial cells and capillary endothelial cells is a very important pathological entity and reflects early cellular injury (Teplitz, 1979).

To evaluate the inter-observer variation as to the

judgement on 13 pathological patterns, two observers independently examined electron micrographs. To evaluate the intra-observer variation, one observer examined electron micrographs twice on separate occasions.

Statistical analysis

Values were expressed as mean ± one standard deviation. For the judgement of intra-observer variation and inter-observer variation, and for the comparison with baseline as to EVLTV, Student's t test was used for paired and unpaired data. Comparisons of the incidence of pathological patterns among control group, alloxan group and dextran group were made with one-way analysis of variance and subsequently with the extended Tukey's test and p<0.05 was accepted as indicating significant differences.

Results

Extravascular lung water

The values for extravascular lung thermal volume (EVLTV) and postmortem extravascular lung water (EVLW) are shown in Table 2. Both values were in good agreement at the end of the interventions except for the expected difference due to thermally equilibrated volume. (EVLTV=1.28 × EVLW − 0.26, r = 0.91) (Fig. 1).

The extravascular lung water remained within normal values during baseline period in three groups, and almost doubled at similar amounts on the average in both dextran group and alloxan group at the end of the interventions.

Electron microscopic findings

Reproducibilities of the observation judgement

As to the incidence of each electron microscopic pathological pattern listed in Table 1, the reproducibilities of the observation judgement were excellent in one observer for 3 groups (Fig. 2). The reproducibilities were also excellent in two observers for control and alloxan groups (Fig. 3). However, small disagreement occurred as to the item of swelling of epithelial cells in dextran group (p<0.05) (Fig. 3).

Control group

The incidence of 13 pathological patterns is shown in Table 3. A representative electron micrograph is shown in Fig. 4, showing normal appearance of cellular and interstitial components. The incidence of each of the 13 electron microscopic pathological patterns was less than 10% which was consistent with our previous study (Kitamura et al., 1985).

Dextran group

The incidence of 13 pathological patterns is shown in Table 3. Electron micrographs are shown in Figs. 5-7. The incidence was significantly high compared with that of control group in the changes in the interstitial space, such as widening of the interstitial space (75.8 ± 4.9%), dispersion and disarray of collagen fibrils (73.3 ± 5.2%), and erythrocytes in the interstitial space (12.5 ± 8.8%). The incidence of the erythrocytes in the intersitial space was significantly high compared with that of alloxan group, for comparable amounts of extravascular lung water. The incidence of the cellular changes (items NO.3 − 8) was less than 11%, indicating an equal incidence to the control group.

Alloxan group

The incidence of 13 pathological patterns is shown in Table 3. Electron micrographs are shown in Figs. 8-11. The incidence was significantly high compared with the control group in the cellular changes, such as swelling of epithelial and endothelial cells (30.8 ± 12.4%, and 29.2 ± 10.7%) as well as the changes in the interstitial space, such as widening of interstitial space (60.0 ± 8.9%) and dispersion and disarray of collagen fibrils (61.7 ± 7.5%). The incidence of the swelling of epithelial cells and endothelial cells was significantly high compared with that of dextran group, for comparable amounts of extravascular lung water.

Discussion

We presently studied the incidence of electron microscopic pathological changes of the alveolar septum in acute pulmonary edema produced by dextran-70 and alloxan. From our findings, we first conclude that dextran causes interstitial changes exclusively and alloxan primarily causes cellular changes coupled with secondary interstitial changes.

Dextran-induced pulmonary edema

Noble et al. (1974) infused 10% dextran-40 into dogs and found focal swelling of the endothelial cells, widening of the interstitial space, and dispersion and disarray of collagen fibrils in the early stage of edema. On the other hand, Defouw and Berendsen (1978) produced increased-pressure edema in isolated dog lung perfused with the perfusate mainly consisting of 6% dextran. They reported that the thickness of both endothelial and epithelial cells was not significantly increased, while wide swelling of the interstitium was clearly present. We currently found that the incidence of swelling of epithelial and endothelial cells in dextran-induced pulmonary edema was not significantly high compared with that in the control state. In addition, dextran did not alter the speed of equilibration of ^{125}I-albumin across the pulmonary microvascular wall in unaesthetized sheep (Arakawa et al., 1988). Therefore we would conclude that dextran does not cause cellular changes.

Table 1. Electron microscopic pathological patterns in the alveolar septum.

1. Erythrocytes within the alveoli.
2. Fibrin within the alveoli.
3. Swelling of epithelial cells.
4. Increase of epithelial cellular vesiculation.
5. Epithelial cellular vacuolization.
6. Swelling of endothelial cells.
7. Increase of endothelial cellular vesiculation.
8. Endothelial cellular vacuolization.
9. Widening of the interstitial space.
10. Dispersion and disarray of collagen fibrils.
11. Erythrocytes in the interstitial space.
12. Focal disruption of epithelial cellular continuity.
13. Subendothelial blister.

Table 2. Extravascular lung thermal volume and extravascular lung water in control group and two experimental groups.

	BW (kg)	EVLTV (ml/kg)		EVLW (g/kg)
		baseline	final	
control group	13.2 ± 2.0	6.3 ± 0.6		4.9 ± 0.5
dextran group	12.7 ± 3.0	7.1 ± 1.0	$15.9 \pm 4.5+$	$11.8 \pm 2.3^*$
alloxan group	11.6 ± 2.7	6.2 ± 1.6	$12.4 \pm 2.0+$	$11.5 \pm 2.9^*$

Values are means $\pm$ SD.
EVLTV: extravascular lung thermal volume.
EVLW: postmortem extravascular lung water.
* p $<$ 0.05 comparisons were made among the three groups by one-way analysis of variance.
$+$ p $<$ 0.05 comparisons were made with baseline by Student' s t test.

Table 3. Incidence of 13 electron microscopic pathological patterns.

pattern NO.	control group	dextran group	alloxan group
1	2.5 ± 4.8	15.8 ± 13.6	9.2 ± 17.7
2	4.2 ± 6.6	13.3 ± 9.8	11.7 ± 10.3
3	6.7 ± 7.5	10.8 ± 6.6	$30.8 \pm 12.4^*$◆
4	4.2 ± 6.6	3.3 ± 4.1	7.5 ± 7.6
5	3.3 ± 4.1	5.8 ± 2.0	16.7 ± 11.7
6	5.8 ± 4.9	5.8 ± 9.2	$29.2 \pm 10.7^*$◆
7	5.8 ± 7.4	2.5 ± 4.2	9.2 ± 4.9
8	3.3 ± 4.1	5.8 ± 2.0	12.5 ± 11.7
9	8.3 ± 8.2	$75.8 \pm 4.9^*$	$60.0 \pm 8.9^*$
10	5.0 ± 4.4	$73.3 \pm 5.2^*$	$61.7 \pm 7.5^*$
11	0.8 ± 2.0	$12.5 \pm 8.8^*$◆	0.8 ± 2.0
12	6.7 ± 7.5	5.0 ± 4.5	15.8 ± 15.6
13	0.8 ± 2.0	5.0 ± 6.3	1.7 ± 2.6

Values are means $\pm$ SD
* p $<$ 0.05 Comparisons were made with control group by one-way analysis of variance.
◆ p $<$ 0.05 Comparisons were made within dextran group and alloxan group by one-way analysis of variance.
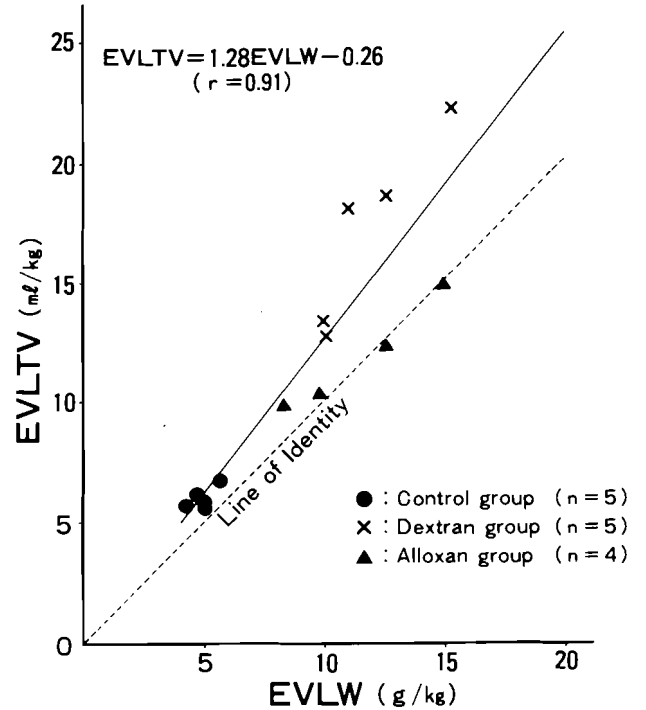

Fig. 1. Agreement of extravascular lung thermal volume (EVLTV) and postmortem extravascular lung water (EVLW). The EVLTV was determined by thermal-dye double indicator dilution method and EVLW by gravimetric method. Since EVLTV includes solid tissue besides water volume, EVLTV exceeds EVLW as theoretically predicted. The gravimetric method was not done in one dog in control group, one dog in dextran group and two dogs in alloxan group.
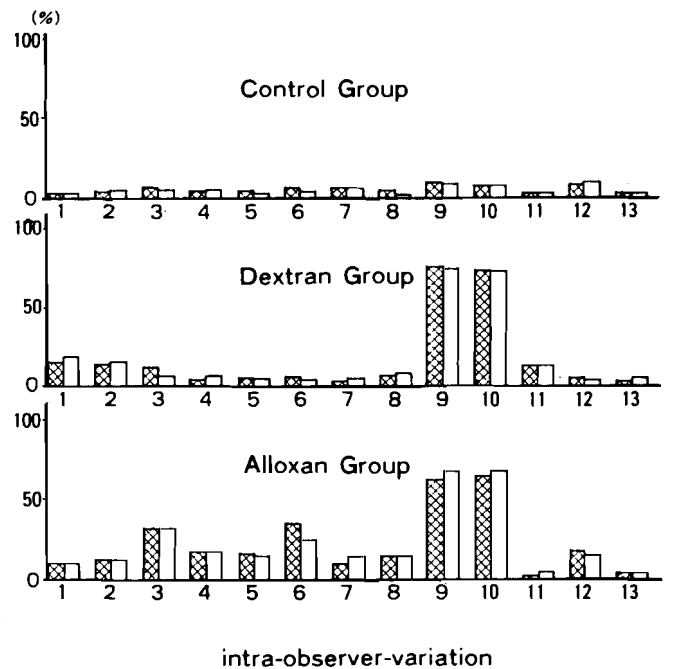

Fig. 2. Incidence and reproducibility on interpretation of electron microscopic pathological patterns in one observer. The agreement in one observer was quite satisfactory.
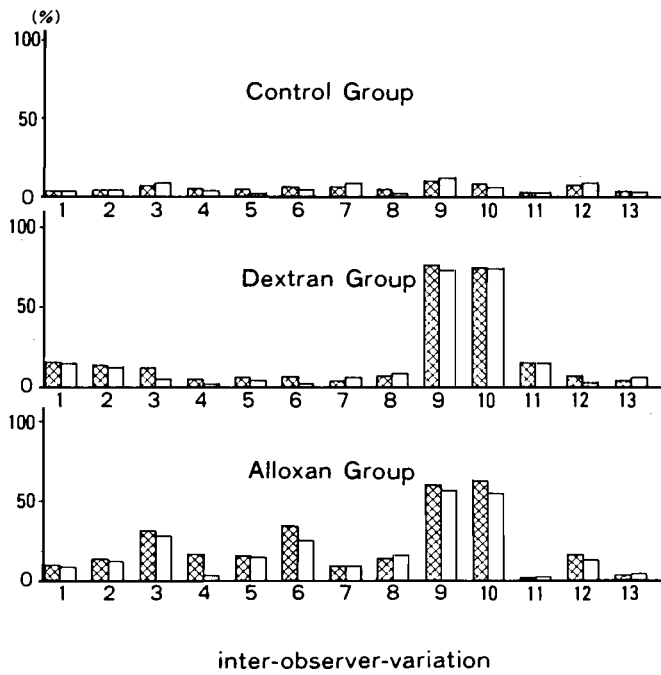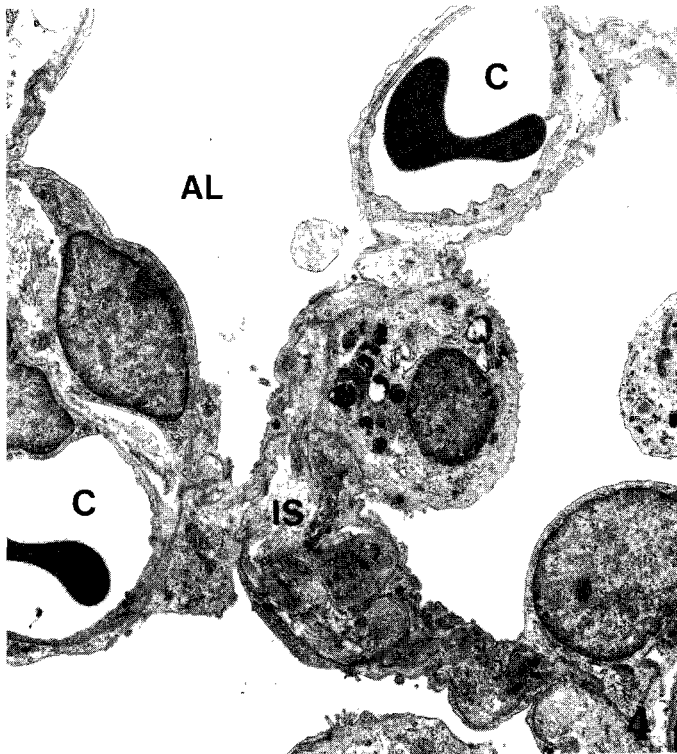
Fig. 3. Incidence and reproducibility on interpretation of electron microscopic pathological patterns in two observers. One disagreement occurred as to item NO. 3 in dextran group. Otherwise reproducibility was excellent in two observers.

Abbreviations
alveoli (AL), epithelial cells (EP), endothelial cells (EN), interstitial space (IS), capillary (C), erythrocyte (E), leukocyte (L), subendothelial blister (SB).

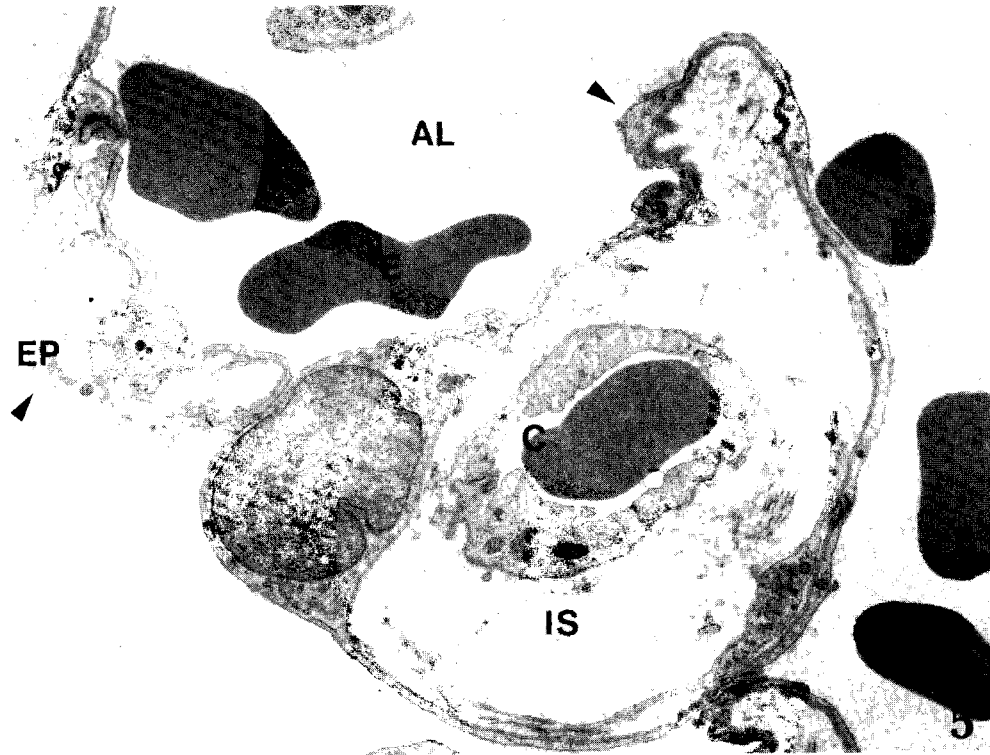**Fig. 4.** Fine structure of alveolar septum from a control dog. × 4,700

Electron microscopic patterns in pulmonary edema

Fig. 5. Electron micrograph from a dog with dextran-induced pulmonary edema. Erythrocytes (E) within the alveoli (AL), widening of the interstitial space (IS), dispersion and disarray of collagen fibrils, and swelling (arrow head) of epithelial cells (EP) are shown. × 6,000
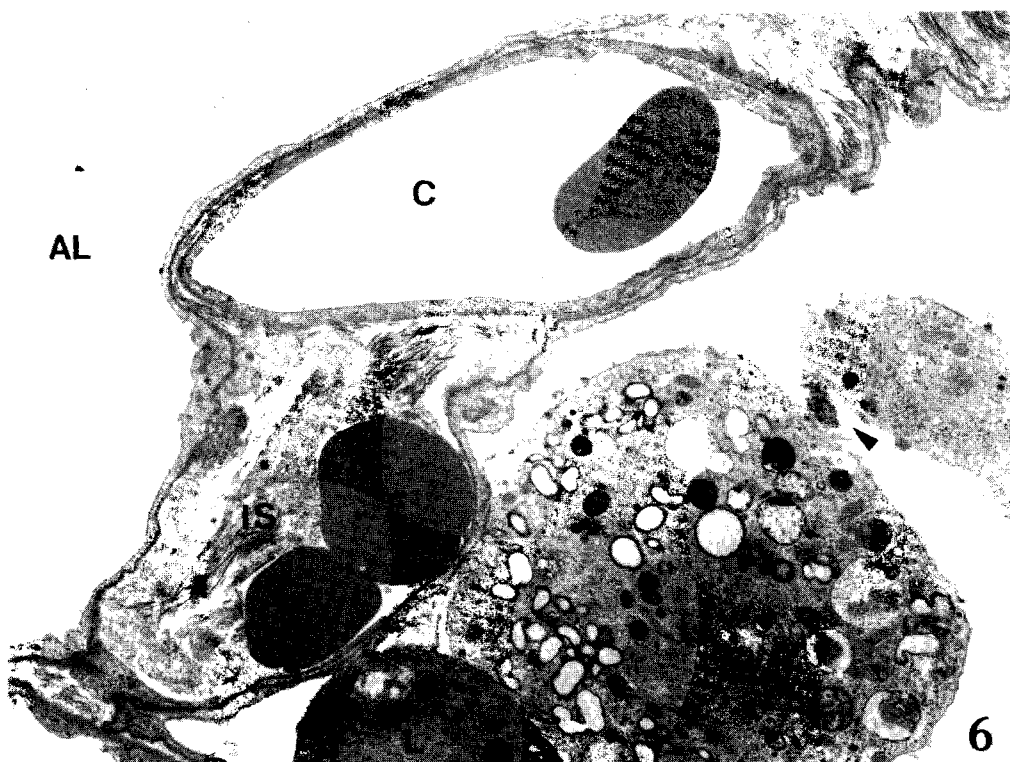

Fig. 6. Electron micrograph from a dog with dextran-induced pulmonary edema. Erythrocytes (E) within the interstitial space (IS), fibrin (arrow head) within the alveoli, and widening of the interstitial space (IS) are shown. × 6,000
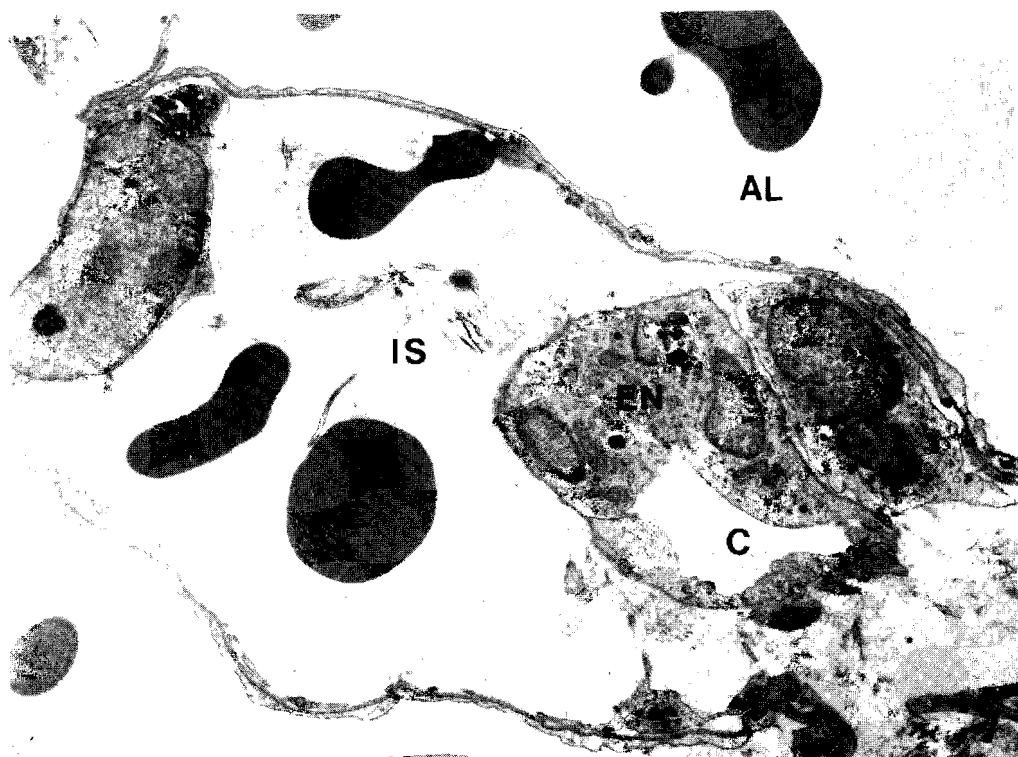
Fig. 7. Electron micrograph from a dog with dextran-induced pulmonary edema. Erythrocytes (E) within the alveoli (AL) and interstitial space (IS), widening of the interstitial space (IS), dispersion and disarray of collagen fibrils, and swelling of endothelial cell (EN) are shown. × 6,000
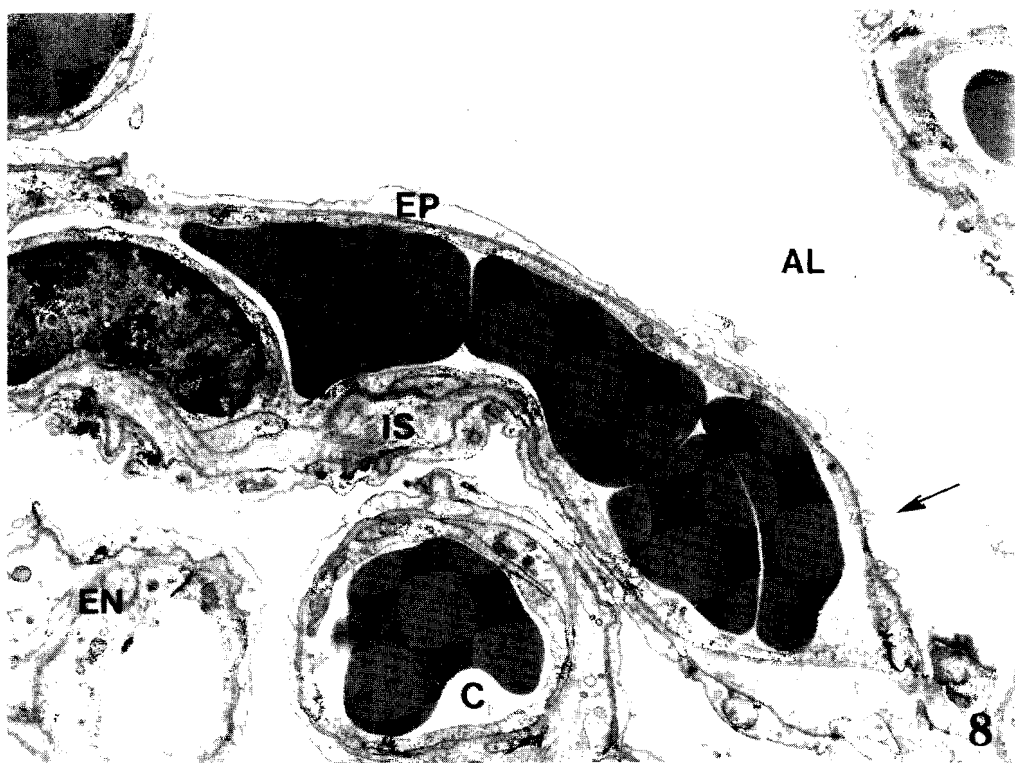

Fig. 8. Electron micrograph from a dog with alloxan-induced pulmonary edema. Swelling of epithelial cells (EP) and endothelial cells (EN) and focal disruption (arrow) of epithelial cellular continuity are shown. × 6,000

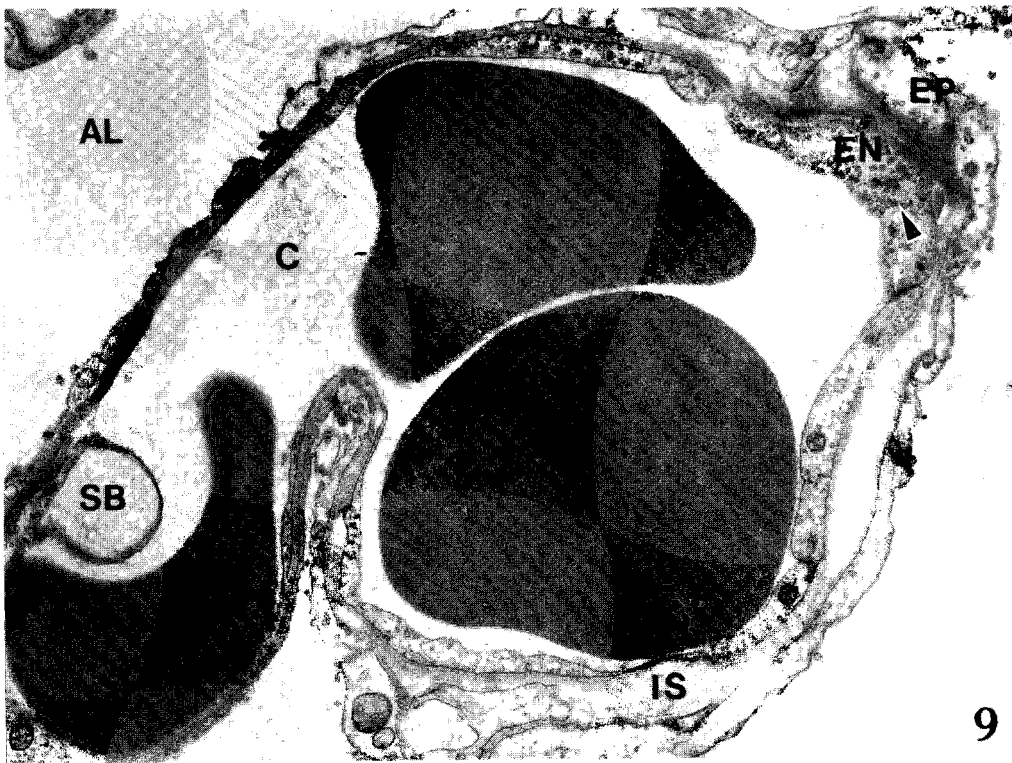*Electron microscopic patterns in pulmonary edema*

Fig. 9. Electron micrograph from a dog with alloxan-induced pulmonary edema. Swelling of epithelial cells (EP) and endothelial cells (EN), increase of endothelial cellular vesiculation (arrow head), and subendothelial blister (SB) are shown. × 10,000
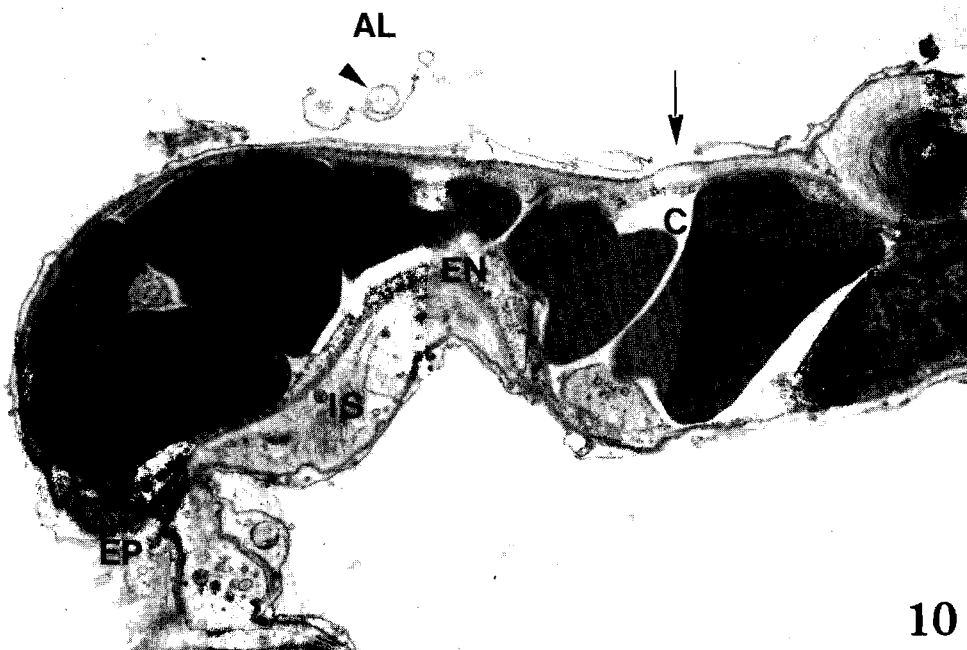

Fig. 10. Electron micrograph from a dog with alloxan-induced pulmonary edema. Swelling of epithelial cells (EP) and endothelial cells (EN), focal disruption (arrow) of epithelial cellular continuity, vacuole in the desquamated epithelium (arrow head) and widening of the interstitial space (IS) are shown. × 6,000
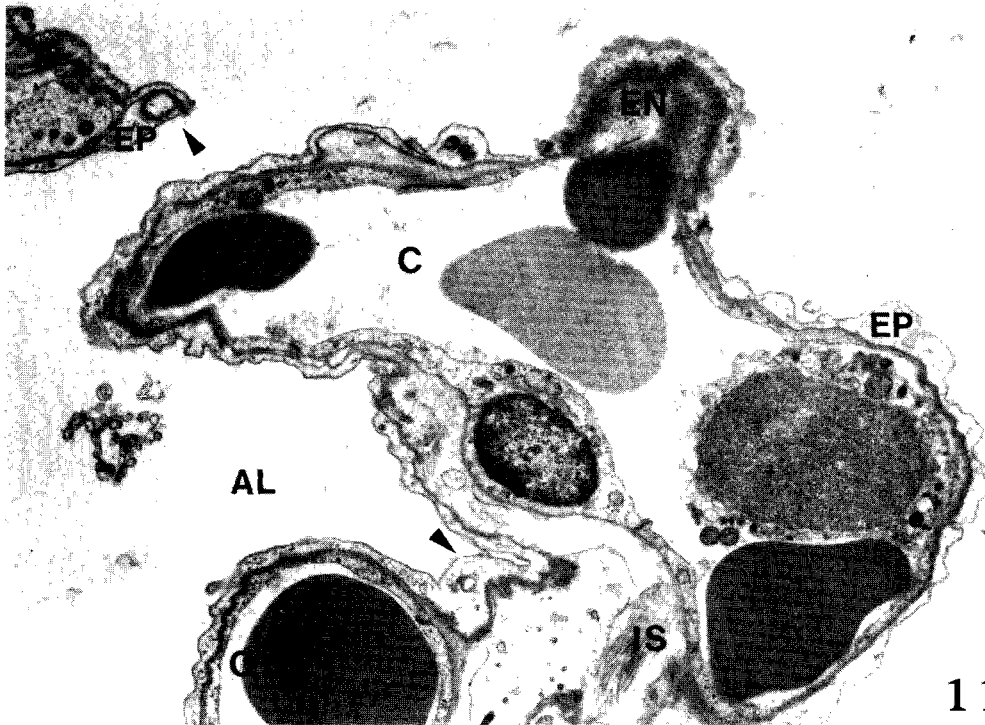
Fig. 11. Electron micrograph from a dog with alloxan-induced pulmonary edema. Swelling of epithelial cells (EP) and epithelial cellular vacuolization (arrow head) are shown. × 6,000

Defouw and Berendsen (1978) reported the increase of the epithelial and endothelial cellular vesiculation which was disclosed by a sophisticated method using stereological techniques. Furthermore, their magnification for photography was high (81,500 times). The magnification in our study (4,700-10,000 times) is too low to precisely identify and quantify vesicles. For these reasons, even though we found no significant increase of vesiculation, we cannot interpret the incidence of vesicles or discuss the role of vesicles.

Unfortunately, as we used standard fixation procedures, we failed to stain dextran particles. Simionescu et al. (1972) stained dextran particles with a minor but important modification of routine fixation-staining process and observed the distribution of dextran particles electron microscopically. The visualization of dextran particles, functioning as a tracer, will provide additional confirmative evidence as to the specific histological changes in the alveolar septum (Michel, 1985).

Alloxan-induced pulmonary edema

From our present study, the following features were concluded to be characteristic in alloxan-induced pulmonary edema: (1) a significantly high incidence of cellular changes which, is however low relative to the incidence of interstitial changes, (2) poor correlation of cellular changes with the grade of pulmonary edema, and (3) focal involvement in cellular changes in the epithelium and endothelium. The latter focal involvement may

prevent the observer from judging the electron microscopic changes perfectly and account for the low ratio of cellular changes to interstitial changes in alloxan-induced pulmonary edema. For these reasons, we must be aware that cellular changes alone may not be sensitive indicators to predict the severity of pulmonary edema in alloxan-induced pulmonary edema.

In alloxan-induced pulmonary edema, cellular changes were reported to occur in both alveolar epithelium and capillary endothelium (Cottrell et al., 1967), but the involvement preponderance has not been evaluated. We questioned which side was more damaged between epithelium and endothelium and our study showed the same incidence of cellular changes between alveolar epithelium and capillary endothelium, indicating that alloxan causes cellular changes equally in both epithelium and endothelium.

Although vacuoles were observed in other varieties of increased-permeability edema (Finegold, 1967; Coalson et al., 1970; Dearden et al., 1977), vacuoles have never been described in alloxan-induced pulmonary edema. In alloxan-induced pulmonary edema, vacuoles were observed with a variety of incidence in the swollen segment of alveolar epithelium and capillary endothelium. Incidentally, in dextran-induced pulmonary edema, vacuoles were observed only as frequently as in the control group. Therefore, vacuoles might be a sign of cellular changes in alloxan-induced pulmonary edema.

Although one report (Cottrell et al., 1967) concludes that the appearance of fibrin within the alveoli is an

outstanding feature of alloxan-induced pulmonary edema, both Noble et al. (1974) and we confirm that the fibrin is present within the alveoli even in dextran-induced pulmonary edema. In reality, fibrin deposit within the alveoli was even present in intact lung (control group) probably as an artifact, and also present in alloxan-induced and dextran-induced pulmonary edema at a similar incidence and at higher incidence than control group. However, the incidence in any condition was not statistically significant. Therefore care should be taken when considering fibrin as an indicator. However it might be concluded from our results that fibrin may appear in the alveoli depending on the grade of edema irrespective of type of edema, either increased-pressure edema or increased-permeability edema.

Finally, we have to bear in mind that morphological changes are invariably influenced by the localization of tissue sampling site, severity of pulmonary edema, and the length of exposure to intervention and so on. These conditions may limit, to some extent, the fair assessment of morphological changes in this kind of study.

References

Albertine K.H. (1985). Ultrastructural abnormalities in increased-permeability pulmonary edema. In: Clin Chest Med. Matthay M.A. (ed). 6. Saunders. C. Philadelphia. pp. 345-369.

Arakawa M., Yasuda Y., Kambara K., Iinuma J., Miyazaki H., Yamaguchi M., Takaya T., Nagano T.,Goto M., Suzuki T., Tanaka T., Miyamoto H. and Hirakawa S. (1985). Pulmonary blood volume and pulmonary extravascular water volume in men. Jpn. Circ. J. 49, 475-486.

Arakawa M., Jerome E.H., Enzan K., Grady M. and Staub N.C. (1988). Effect of dextran-70 on lung liquid and protein exchange in awake sheep. FASEB. J.2, 6 A 1703.

Coalson J.J., Hinshaw L.B. and Guenter C.A. (1970). The pulmonary ultrastructure in septic shock. Exp. Mol. Pathol. 12, 84-103.

Cottrell T.S., Levine O.R., Senior R.M., Wiener J., Spiro D. and Fishman A.P. (1967). Electron microscopic alterations at the alveolar level in pulmonary edema. Circ. Res. 21, 783-797.

Cunningham A.L. and Hurley J.V. (1972). Alpha-naphthyl-thiourea-induced pulmonary oedema in the rat: A topographical and electron-microscope study. J. Pathol. 106, 25-35.

Dearden L.C., Glauser F.L. and Smeltzer D. (1977). The effect of ethchlorvynol on pulmonary ultrastructure in dogs. Am. J. Pathol. 87, 525-536.

Defouw D.O. and Berendsen P.B. (1978). Morphological changes in isolated perfused dog lungs after acute hydrostatic edema. Circ. Res. 43, 72-82.

Finegold M.J. (1967). Interstitial pulmonary edema-an electron microscopic study of the pathology of staphylococcal enteroxemia in Rhesus monkeys. Lab. Invest. 16, 912-924.

Iinuma J., Arakawa M., Yasuda Y., Kambara K., Miyazaki H., Segawa T. and Hirakawa S. (1986). Fluid volume balance between pulmonary intravascular space and extravascular space in dogs. Jpn. Circ. J. 50, 818-828.

Kambara K., Yasuda Y., Iinuma J., Arakawa M. and Hirakawa S. (1985). A problem intrinsic to the measurements of the pulmonary extravascular water volume by the thermal-dye technic with the sampling site in the bifurcation of the aorta-Thermal equilibrium with the aortic wall-. Jpn. Circ. J. 49, 301-310.

Kitamura Y., Shomura S., Isono H., Nakahara K., Oda H., Yasuda Y. and Hirakawa S. (1985). Basic study on the incidence of the ultrastructural changes in the experimental pulmonary edema, with special reference to the ultrastructural change in non-treated dogs. Acta. Sch. Med. Univ. Gifu. 33, 1005-1020 (In Japanese).

Michel R.P. (1985). Lung microvascular permeability to dextran in α-naphthylthiourea-induced edema: site of filtration, patterns of accumulation, and effects of fixation. Am. J. Pathol. 119, 474-484.

Montaner J.S.G., Tsary J., Evans K.G., Mullen J.B., Burns A.R., Walker D.C., Wiggs B. and Hogg J.C. (1986). Alveolar epithelial damage: A critical difference between high pressure and oleic acid-induced low pressure pulmonary edema. J. Clin. Invest. 77, 1786-1796.

Noble W.H., Kovacs K. and Kay J.C. (1974). Fine structural changes in haemodynamic pulmonary oedema. Can. Anaesth. Soc. 21, 783-797.

Pearce M.L., Yamashita J. and Beazell J. (1965). Measurement of pulmonary edema. Circ. Res. 16, 482-488.

Selinger S.L., Bland R.D., Demling R.H. and Staub N.C. (1975). Distribution volumes of [^{131}I] albumin, [^{14}C] sucrose, and ^{36}Cl in sheep lung. J. Appl. Physiol. 39, 773-779.

Simionescu N., Simionescu M. and Palade G. (1972). Permeability of intestinal capillaries pathway followed by dextrans and glycogens. J. Cell. Biol. 53, 365-372.

Smith P. and Heath D. (1974). The ultrastructure and time sequence of the early stage of paraquat lung in rats. J. Pathol. 114, 177-184.

Teplitz C. (1968). The ultrastructural basis for pulmonary pathophysiology following trauma. J. Trauma. 8, 700-714.

Teplitz C. (1979). Pulmonary cellular and interstitial edema. In: Pulmonary edema. Fishman A.P. and Renkin E.M. (eds). Am. Phys. Soc. Maryland. pp. 97-111.

Wangensteen D., Bachofen H. and Weibel E.R. (1981). Effects of glutaraldehyde or osmium tetroxide fixation on the osmotic properties of lung cells. J. Microsc. 124, 189-196.

Yasuda Y., Hirakawa S., Arakawa M., Kambara K. and Iinuma J. (1984). A problem in the measurements of pulmonary extravascular water volume by double indicator dilution method, using heat and dye. -Thermal diffusion into the left ventricular wall-. Jpn. Circ. J. 48, 580-590.