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The ultrastructure of rat lung following acute primary blast injury

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Summary. While a number of workers have described the effects of blast waves upon the lung at both the macroscopic and light microscopic level, studies involving the use of the electron microscope have not been reported. In the experiments reported here the ultrastructural changes seen in lungs from rats exposed to a blast wave impacting on the right side of the chest are described. Considerable damage to the right lower lobe was observed which took the form of tearing of the inter-alveolar septa with capillary rupture and intra-alveolar haemorrhage. Changes to the alveolar epithelium and type II pneumocytes were also noted. Lesions were also identified in the left lung; these included intra-alveolar oedema with a minimal amount of interstitial oedema together with increased pinocytosis and isolated rupture of the alveolar epithelium. 'Ballooning' of the endothelium into the lumen of the capillary was also observed. There was an indication that lesions noted in the left lung at the electron microscopic level may be progressive in the first 24 hours following injury.

Keywords: blast, pulmonary contusion, pulmonary oedema, electron microscopy, histopathology, rat

Pulmonary contusion was first described in 1761 by Morgagni in a victim of a carriage crash (Morgagni 1761, cited by Fallon 1940). Vehicular accidents still account for the majority of traumatic chest injuries and pulmonary contusions in civilian medical practice (Wilson *et al.*, 1977).

In a military environment, pulmonary contusion may be produced by non-penetrating impact of projectiles upon body armour, the penetration of lung parenchyma by fragments and bullets, and by exposure to blast overpressure. Detonation in air of high explosive produces a region of instantaneous rise in pressure, generally termed a blast wave, that travels radially from the explosive device at a velocity initially greater than the velocity of sound in air. Impact of the blast wave upon the thorax gives rise to two phenomena:

(i) the propagation of a stress (pressure) wave in the thorax.
(ii) gross displacement of the thoracic wall leading to compression of intra-thoracic structures.

The role of these responses in the aetiology of pulmonary (and bowel) contusions following exposure to blast is not clear; present evidence suggests that for 'short' duration blast waves, stress coupling is the principal injury mechanism (Cooper *et al.*, 1990) but with 'long' duration waves thoracic compression dominates (Jonsson 1979).

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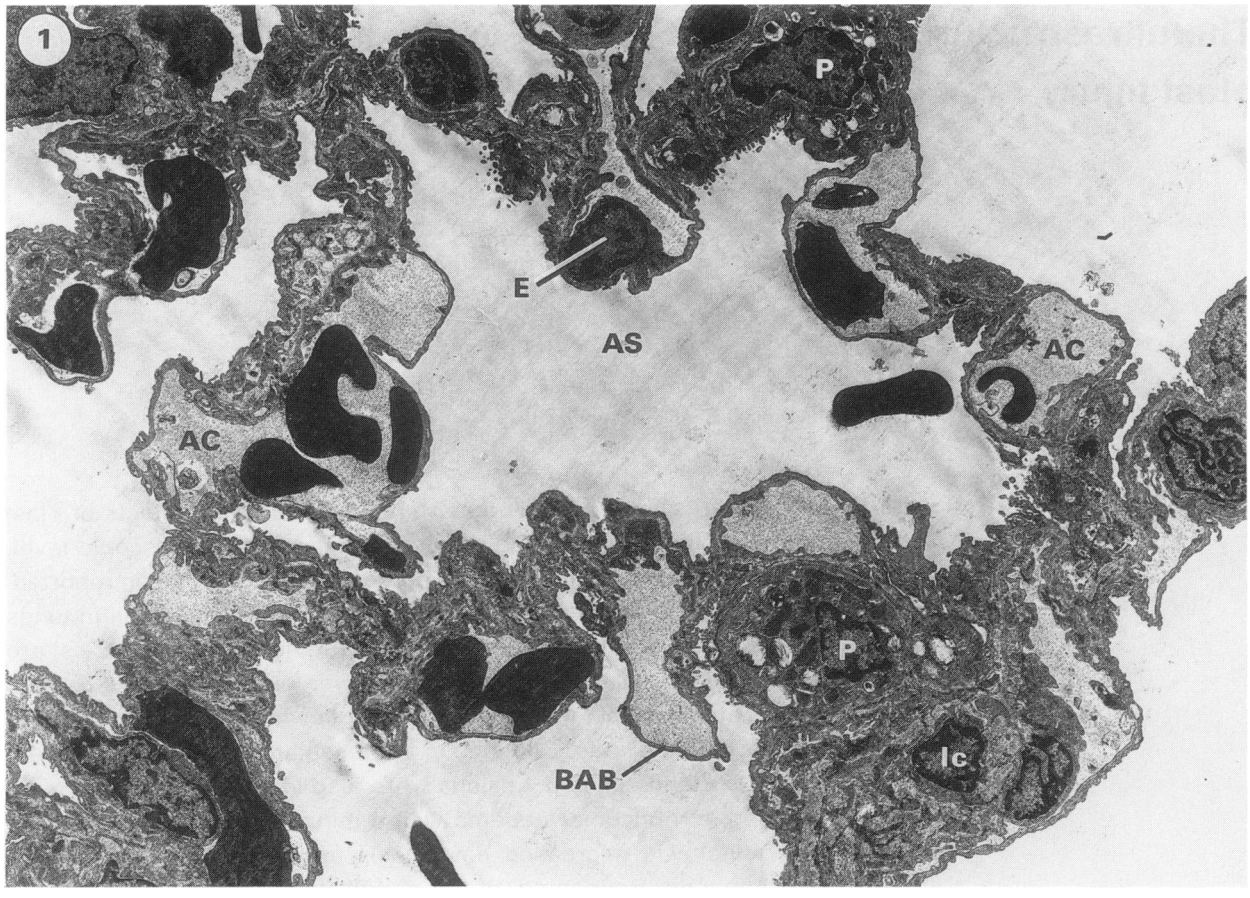

Figure 1. Low power electron micrograph of control rat lung. × 2080. AC, Alveolar capillary; AS, alveolar space; BAB, blood–air barrier; P, Type II pneumocyte; E, capillary endothelial cell; Ic, interstitial cell.

The pulmonary contusions produced in this context are generally termed 'blast lung' and are plainly evident as areas of haemorrhage on gross inspection of the lungs immediately after exposure.

Clinically, these contusions may be difficult to detect in the absence of overt symptoms such as breathlessness, haemoptysis or a decreased Pa,O_2 and the lung may continue to accumulate extravascular haemorrhagic or oedema fluid that may lead to deterioration in lung function and in the condition of the patient. The implication of these clinical sequelae is that haemorrhage may continue within the acutely injured lung and that areas of the lung initially apparently undamaged may later bleed and show other signs of damage.

There are a number of questions therefore concerning the aetiology of blast contusions and the subsequent clinical progress that may in part be answered by assessment of such lesions using electron microscopy:

(1) Are overtly normal non-haemorrhagic regions of the lung damaged at the sub-cellular level in blast injury?
(2) Does the area of haemorrhage strictly define the actual area of parenchymal injury?
(3) From which part of the vascular tree does the blood evident in blast-induced pulmonary contusion actually originate?

A few researchers have described 'torn' capillaries and breaks in alveolar septa in blast damaged lungs at the light microscope level (Zuckerman 1940) but in our experience it is difficult to distinguish, with certainty, infrequent traumatic breaks in alveolar walls from the effects of random sectioning in alveolar fields. Damage to capillaries in otherwise normal septa cannot be identified with confidence at the light microscopic level. Such identification is critical to the identification of minimal injury.

The resolution of these questions would provide insight into the relative roles of stress wave coupling and

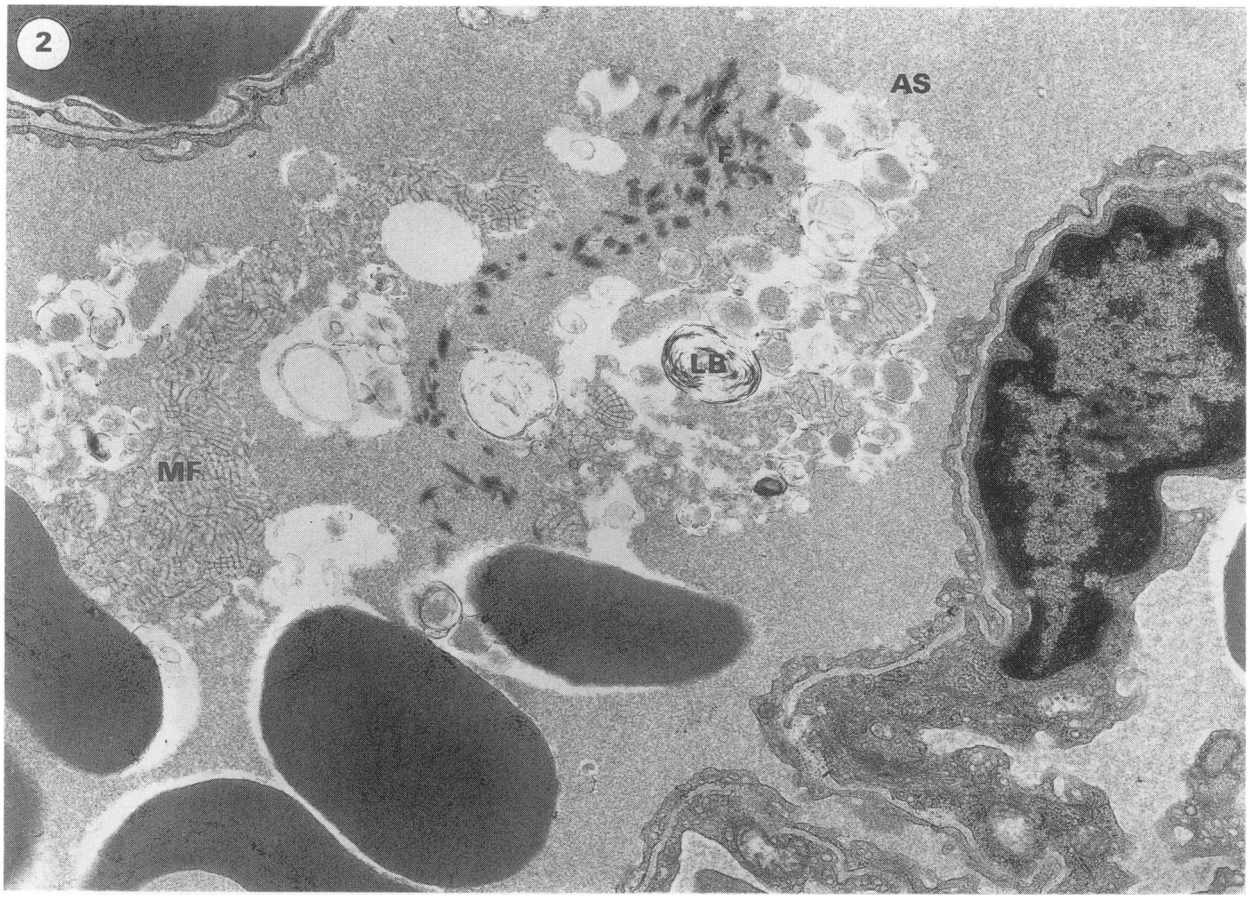

Figure 2. Electron micrograph of right lower lobe of rat killed immediately after blast injury to the right lateral thorax showing intra-alveolar haemorrhage with intense osmiophillic material and the beginning of fibrin clots formation. No apparent damage to the cells of the blood–air barrier. × 10700. F, Fibrin clot; AS, alveolar space; MF, myelin figures; LB, lamellated whorls of surfactant.

gross thoracic compression. The former would lead to injury at capillary level due to loss of energy as the wave interacts with the tissue/air interspace; the latter would also cause damage to larger blood vessels due to shear arising from gross distortions of the parenchyma.

The purpose of this preliminary study is to describe in general terms the electron microscopical appearance of normal and blast injured rat lungs at 30 minutes and 24 hours after exposure to a blast wave.

Materials and methods

Materials

The components of Spurr's low viscosity epoxy resin were supplied by TAAB Laboratories Equipment Ltd, Reading, Berkshire, UK, and all other chemicals were obtained from British Drug Houses PLC, Poole, Dorset, UK.

Animals

Six female Porton Strain rats wt 200–250 g were obtained from the Animal Breeding Unit at Porton and kept at an ambient temperature of 20°C and humidity 40–60%. They were anaesthetized with 40 mg/kg sodium pentobarbitone (Sagatal) injected intraperitoneally.

Production of pulmonary blast injury

The anaesthetized rats were placed on their left sides on a plane metal surface; the right lateral surface was then exposed to a blast-wave from a specially constructed

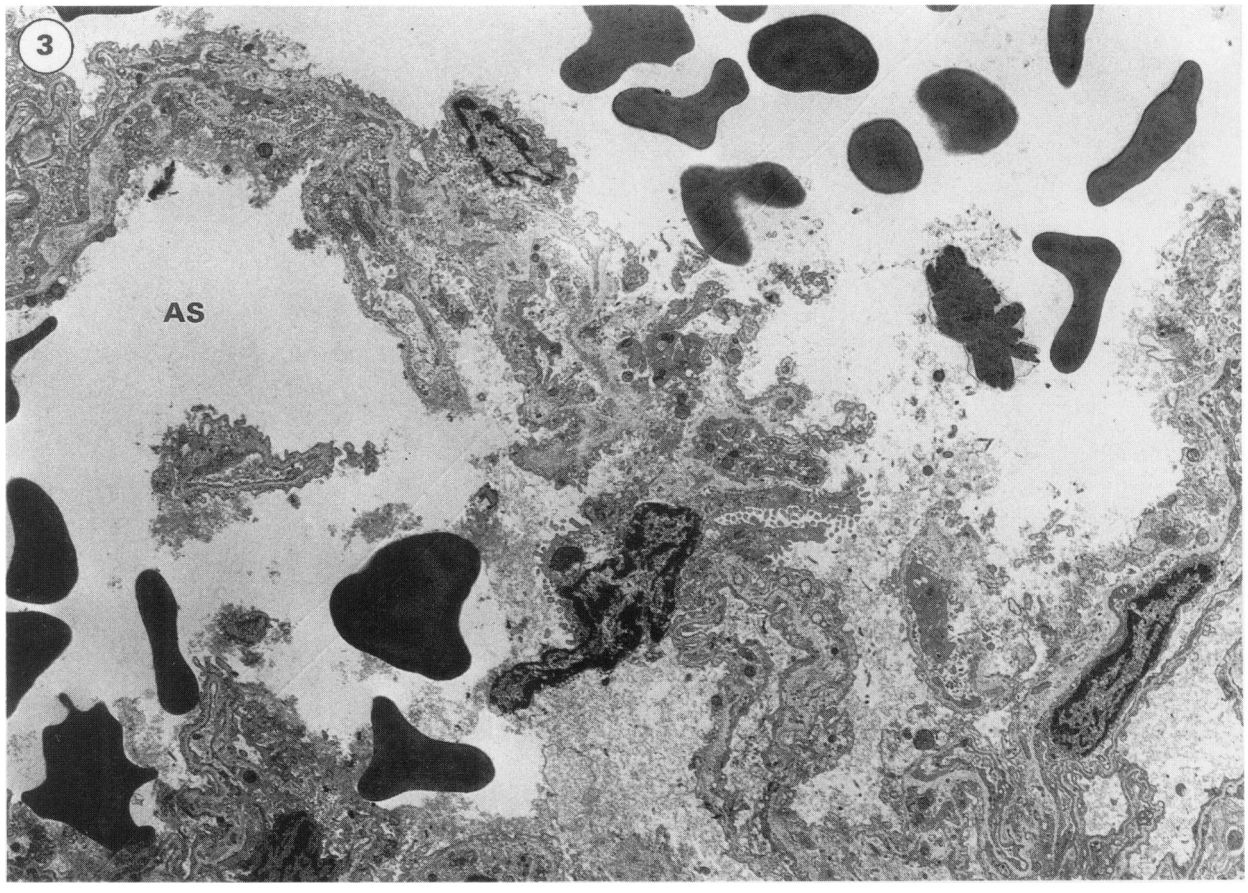

Figure 3. Electron micrograph of the right lower lobe of rat killed immediately after blast injury to the right lateral thorax showing an area of interstitial disruption with associated intra-alveolar haemorrhage. × 2330. AS, Alveolar space.

blast-wave generator. In simple terms, compressed air at a pressure of 10 MPa was suddenly applied to an aluminium diaphragm of thickness 0.55 mm. The diaphragm disrupted and a blast wave discharged from the diaphragm housing. The housing was positioned 5 cm from the right lateral thorax of the anaesthetized rat. The peak reflected overpressure at this distance from the housing was approximately 480 kPa with a positive phase duration of about 1.3 ms. Exposure of a rat under these circumstances results in a 'moderate' pulmonary contusion, generally confined to the right lung and accessory lobe.

The animals were divided into three groups of two and treated as follows:

Group A Animals anaesthetized and *not* exposed to blast wave.

Group B Animals exposed to a blast wave and killed within 30 minutes of injury.

Group C Animals exposed to a blast wave and killed 24 hours after injury.

Autopsy and tissue selection

The animals were killed by intraperitoneal injection of Sagatal (sodium pentobarbitone 60 mg/ml). The thorax was opened and the lungs rapidly removed. Paramedian slices, approximately 3 mm in thickness, were taken from the left lung and right lower lobe and placed in 3% glutaraldehyde buffered with 0.1 M cacodylate buffer, pH 7.4, containing 3 mM calcium chloride and maintained at 40°C. The remainder of the left lung and right lower lobe was immersed in 10% neutral phosphate buffered formalin.

Light microscopy

After fixation, tissue was dehydrated and embedded in

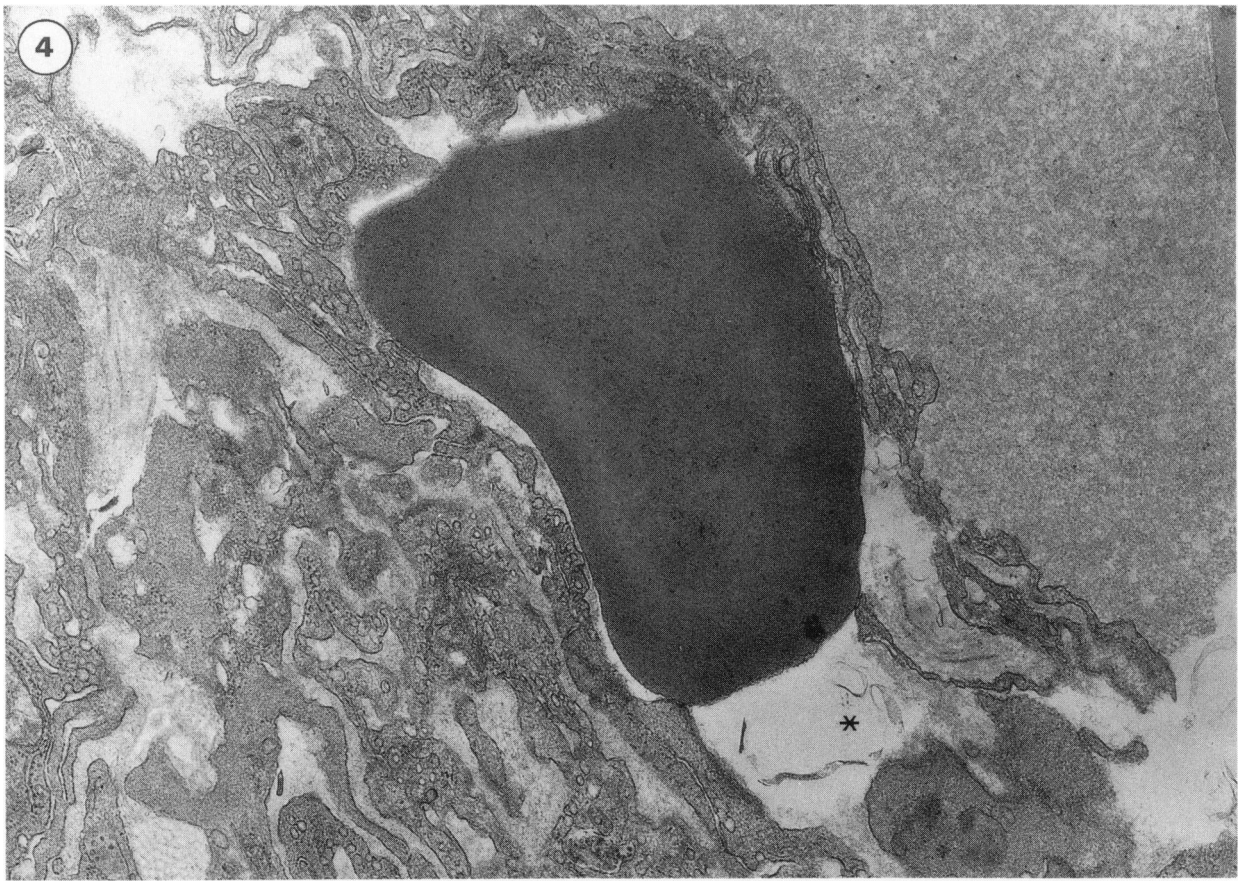

Figure 4. Electron micrograph of the right lower lobe of rat killed immediately after blast injury to the right lateral thorax showing *alveolar capillary rupture. × 16220.

paraffin wax. Sections 5±2 μm thick were cut and stained with haematoxylin and eosin.

Electron microscopy

After fixation for 30 minutes the 3-mm slices were diced into approximately 1 mm cubes and further fixed in glutaraldehyde for at least 8 hours. Subsequent dehydration and filtration in Spurr's low viscosity resin (Spurr 1969) was carried out using standard techniques utilizing a Lynx processor (Leica UK Ltd, Cambridge, UK). Polymerization was carried out at 70°C for at least 12 hours. At least five blocks from each lobe were cut on an Ultracut E (Leica UK Ltd, Cambridge, UK) ultramicrotome using both glass and diamond knives. Sections 1 μm thick were cut for light microscopy and stained for 20 minutes at 60°C with 1% toluidine blue in 1% sodium tetraborate. Sections were then cut for electron microscopy, 60–90 nm thick, picked up on copper grids and stained with alkaline lead citrate using an Ultrostainer (Leica UK Ltd, Cambridge, UK). They were then examined and micrographs taken using a Phillips EM300 electron microscope operated at 80 kV.

Results

Macroscopic appearances of the lung

Macroscopic examination of the lungs 30 minutes following the injury showed extensive haemorrhages of the right lung. The haemorrhagic areas were surrounded by regions of congestion with areas of pale discolouration. The left lung appeared only slightly congested.

By 24 hours after the injury haemorrhagic areas were present in both the right and left lung together with varying degrees of congestion and pale discolouration. Small amounts of froth were observed to exude from the cut surfaces.

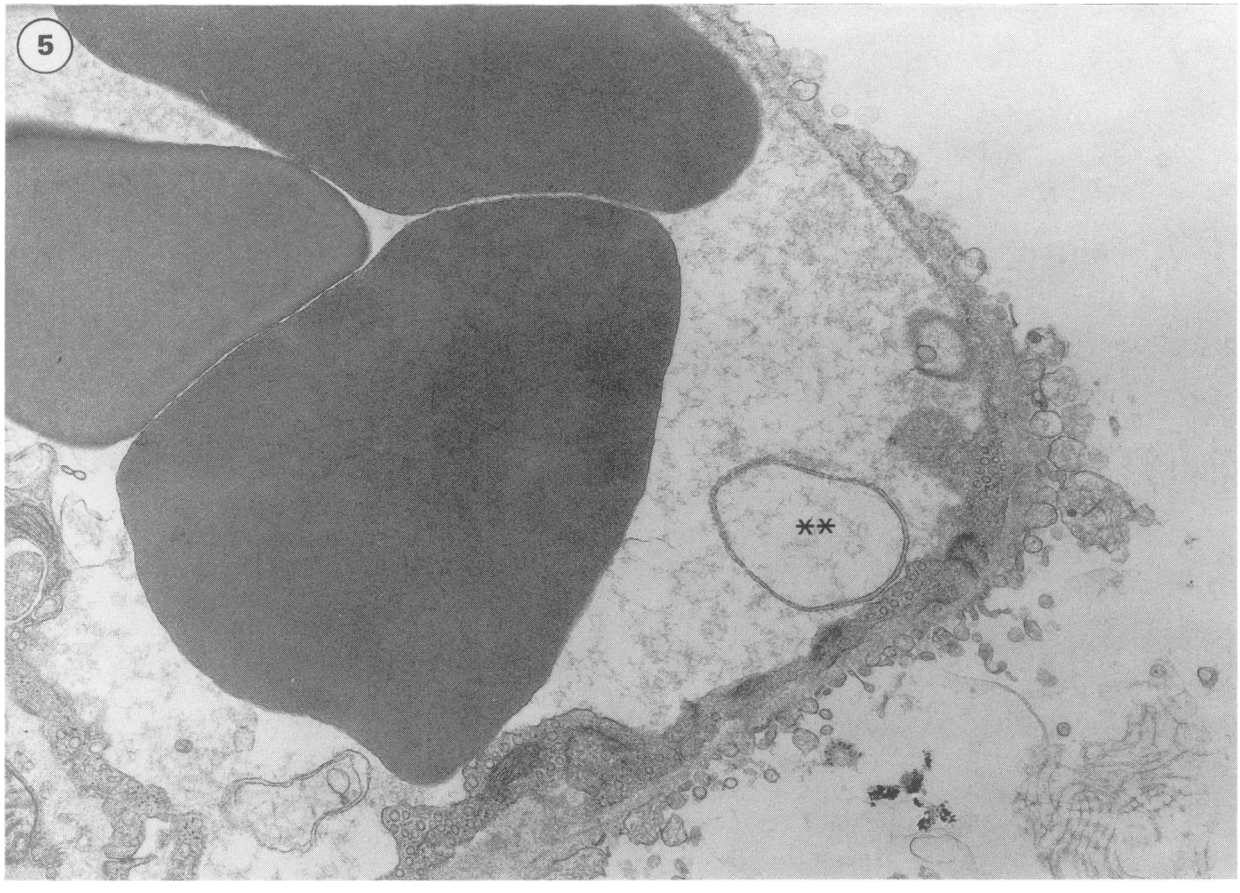

Figure 5. Electron micrograph of the right lower lobe of rat killed immediately after blast injury to the right lateral thorax. Type 1 epithelial cell necrosis and ** ballooning of the endothelium. × 7180.

The lungs from control animals, which were anaesthetized only, showed no abnormalities.

Light microscopy

Group A: Control (anaesthetic only) killed 30 minutes after induction of anaesthesia. The structure of the normal Porton rat has been described previously (Colgrave *et al.* 1979). One of the animals showed an area of slight congestion and a few free erythrocytes were present in the alveolar spaces. No other abnormalities were observed.

Group B: Animals killed within 30 minutes of injury by blast wave. In animals from this group damage was most evident in the right lower lobe. This took the form of haemorrhage which seemed to be of two types.

Type A. This was characterized by the presence of blood filled alveoli together with associated damage to inter-alveolar septa.

Type B. The alveoli were again blood filled but there appeared to be no damage to inter-alveolar septa.

Blood was also present in bronchioles and in perivascular interstitial spaces. The endothelium of arterioles and venules appeared normal.

The left lungs of animals from this group showed areas of severe congestion with one animal also showing an area of Type B haemorrhage. No erythrocytes were present in bronchioles or in the interstitium surrounding both bronchioles and blood vessels.

Group C: Animals killed 24 hours after injury by blast wave. The damage observed in the lungs of animals from this group was similar to that found in the lungs of animals killed within 30 minutes of injury. Both Type A and Type B haemorrhages were observed in all the tissue studied, being most severe in the right lower lobe. Damage to the inter-alveolar septa was more severe than that found previously in the lungs of animals which

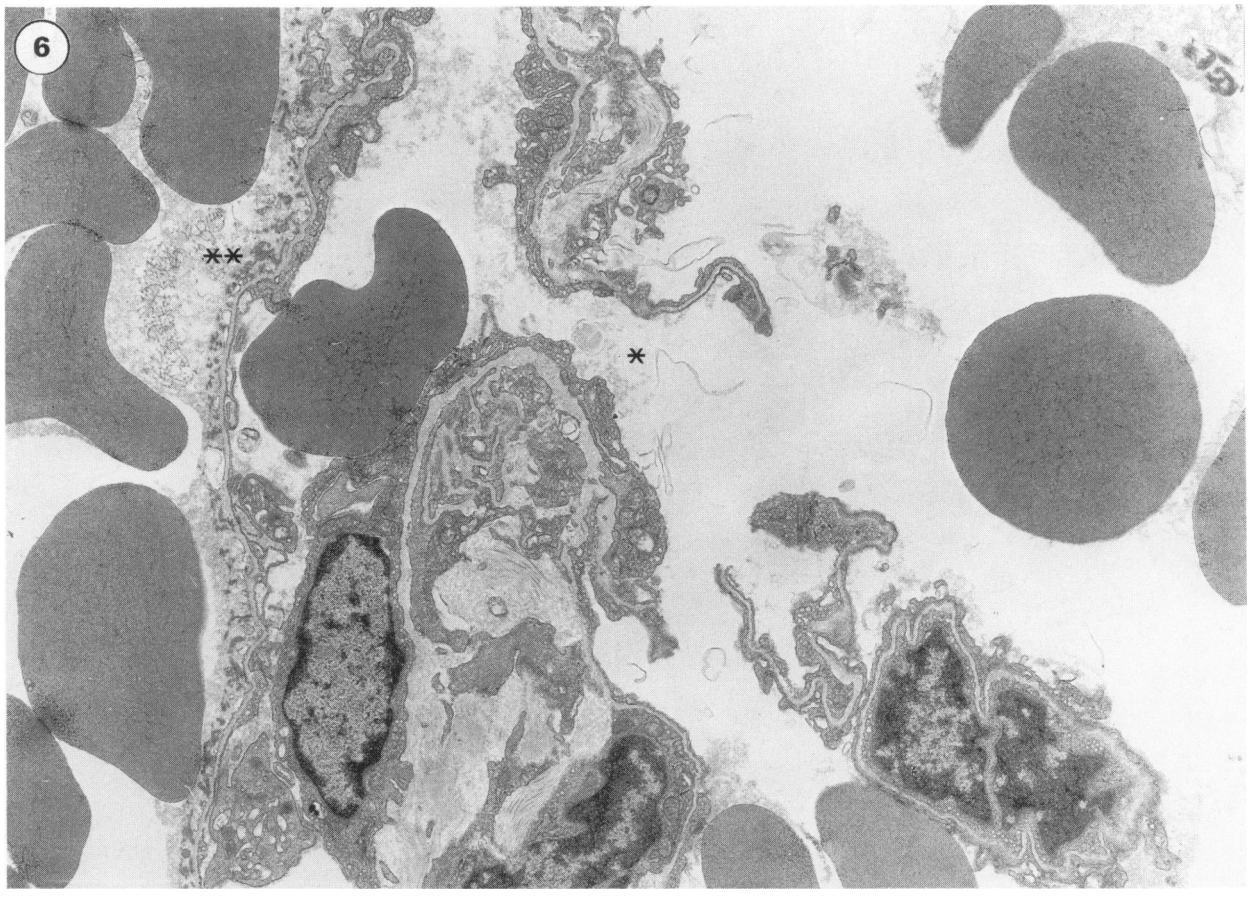

Figure 6. Electron micrograph of the left lung of rat killed 24 hours after blast injury to the right lateral thorax showing **an area of Type 1 epithelial cell necrosis and *capillary rupture. × 12760.

had been killed within 30 minutes of injury. In the left lung, areas of severe congestion interdispersed with Type B haemorrhage were observed, the haemorrhage having a subpleural distribution. A fibrinous intra-alveolar oedema was present in some alveoli situated adjacent to the haemorrhagic areas. This seemed to be more evident in the right lower lobe rather than in the left lung.

Bronchiolar lumina from both lungs were filled with blood and in the right lower lobe erythrocytes were present in both peribronchiolar and perivascular interstitial spaces.

Electron microscopy

The ultrastructure of the normal Porton rat lung has been described previously (Colgrave *et al.* 1979). In the lungs of control animals (Figure 1) the only differences in appearance from that commonly described were very isolated alveolar capillary changes which took the form of 'ballooning' (localized expansion of the cytoplasm leading to the plasma membrane expanding and taking on the appearance of a small balloon) (Figure 1).

Group B: Animals killed within 30 minutes of injury by blast wave. Left lung. Minor changes were observed in the type 1 epithelial and endothelial cells in the region of the blood–air barrier. Damage to the epithelium took the form of increased pinocytosis with an isolated area of 'blebbing' (a change similar to ballooning but only very small dilatations are seen) with associated rupture of the cell. In the endothelium, areas of increased pinocytosis and 'ballooning' into the lumen of the capillary were observed; these were more frequent than in the lung of control animals.

The structure of the vasculature, bronchiolar epithelial cells and associated interstitium was unchanged.

Type II pneumocytes were essentially normal in structure with isolated incidences of loss of integral

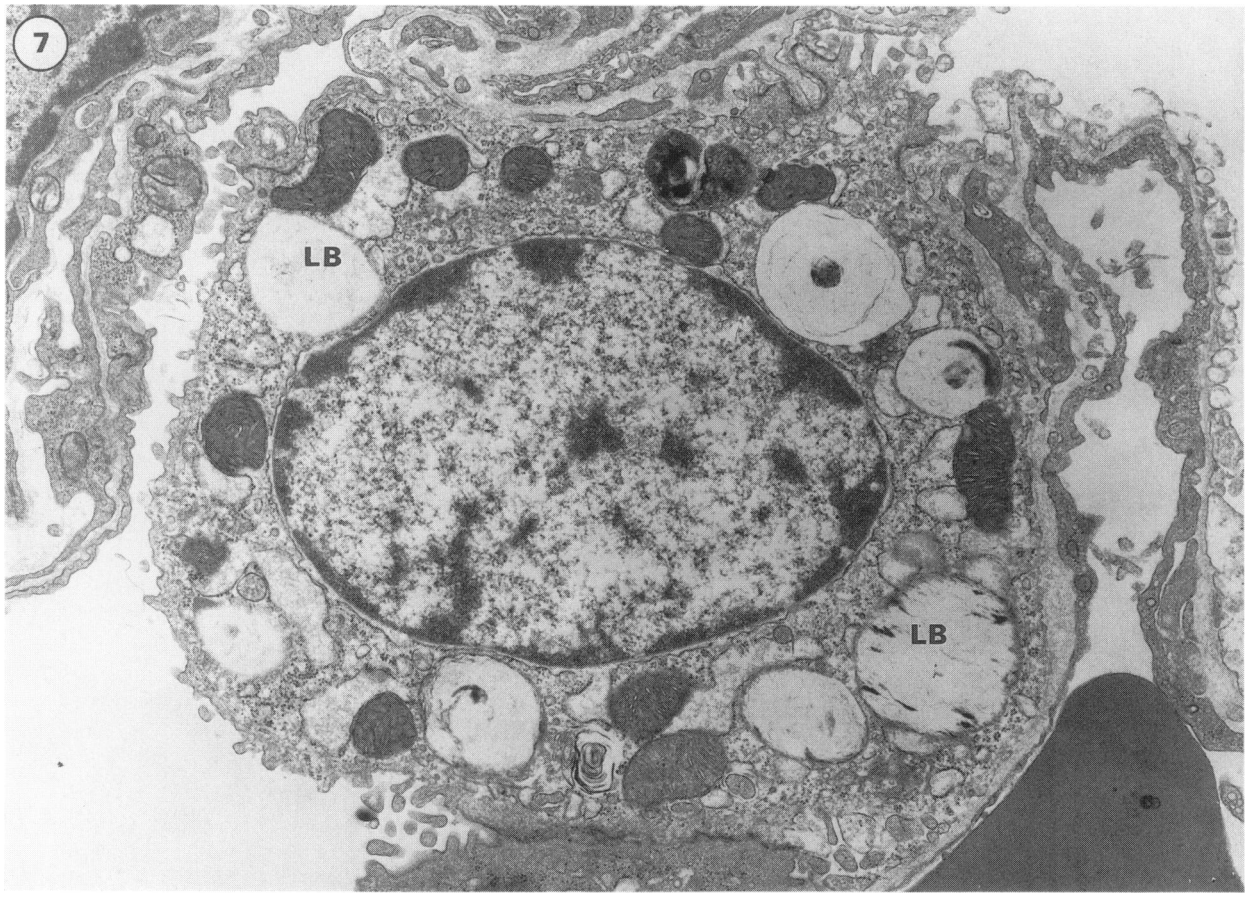

Figure 7. Electron micrograph of the left lung of rat killed 24 hours after blast injury to the right lateral thorax showing loss of lamellar body structure from a type II pneumocyte. × 10700. LB, Lamellar body.

structure or enlargement of lamellated bodies being observed.

Right lower lobe. Observation of the ultrastructure of the right lower lobes showed widespread areas of haemorrhage. Two types of lesion could be distinguished.

In the first type of lesion (Figure 2) the alveolar spaces were filled with erythrocytes interdispersed in amorphous osmiophilic material. However, little fibrin clot formation could be distinguished. The interstitium of the alveolar walls showed no changes in ultrastructure with all the capillary membranes intact. There was extensive 'ballooning' of the endothelium with little change to type 1 epithelial cell structure except for increased pinocytosis with isolated areas of thickening. Type II pneumocytes appeared normal in ultrastructure.

In the second type of lesion (Figures 3–5) the alveolar spaces were filled with erythrocytes dispersed in amorphous osmiophilic material in which the beginning of fibrin clot formation could be distinguished. A few of the erythrocytes were misshapen. The alveolar walls showed areas of complete interstitial disruption with associated capillary rupture. Any intact capillaries showed evidence of damage; extensive endothelial 'ballooning' (see Figure 5) and increased pinocytosis with 'blebbing' and loss of structure (necrosis) of epithelium. Type II pneumocytes again showed isolated instances of loss of structure or enlargement of lamellated bodies.

An isolated area of peribronchiolar interstitial haemorrhage was present in the right lobe of the lungs of one animal.

Group C: Animals killed 24 hours after the blast injury. In the lungs of animals from this group the changes in ultrastructure appeared to be more severe and extensive than those found in the lungs of animals killed 30 minutes after injury.

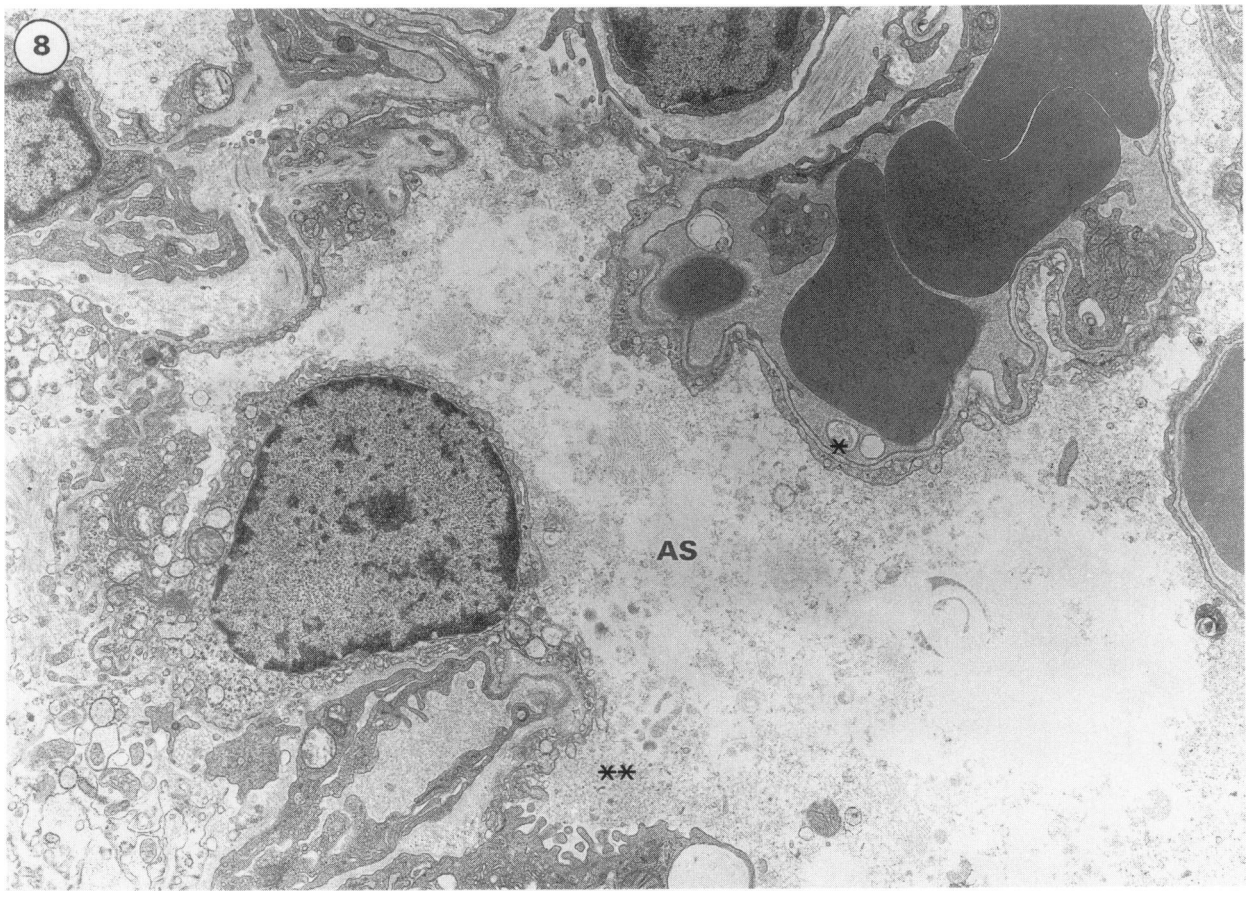

Figure 8. Electron micrograph of the right lower lobe of rat killed 24 hours after blast injury to the right lateral thorax showing an area of intra-alveolar oedema with associated alveolar cell changes. ×7180, AS, Alveolar space. *Capillary endothelial cell ballooning; **Type 1 epithelial cell blebbing.

Left lung (Figures 6–7). The main findings in the left lung were areas of both interstitial and intra-alveolar oedema together with isolated areas of haemorrhage. The damage appeared similar to that described as the first type of lesion described in the right lower lobe of the lung of animals which were killed within 30 minutes of injury except for more severe damage to the components of the alveolar blood–air barrier.

Microemboli with characteristic accumulations of both leucocytes and platelets were found in the lumen of the larger arterioles ane venules. Slight accumulations of amorphous fluid and a few macrophages were observed in the lumens of bronchioles. The epithelium was unchanged.

Right lower lobe (Figures 8–10). Changes in the lobes of the right lower lobes were similar to those described above though damage was generally more severe and some cellular proliferation was observed; however, no microemboli were seen.

Discussion

Pulmonary contusions have been described as progressive lesions (Taylor *et al.* 1982, Nichols *et al.* 1968, Fulton & Peter 1970, Fulton 1974). On the other hand, Trinkle (Trinkle *et al.* 1973, Trinkle 1977) has taken the view that *untreated* pulmonary contusions are not progressive lesions and has suggested that the apparent progressive nature of pulmonary contusions might be, at least in part, dependent upon the treatment given to patients with such lesions. Many patients with pulmonary contusions also have extrathoracic injuries. Fulton (1974) stated that 80% of patients with pulmonary contusions have extrathoracic injuries and need intravenous infusions. The investigation of the incidence of pulmonary contusions in such

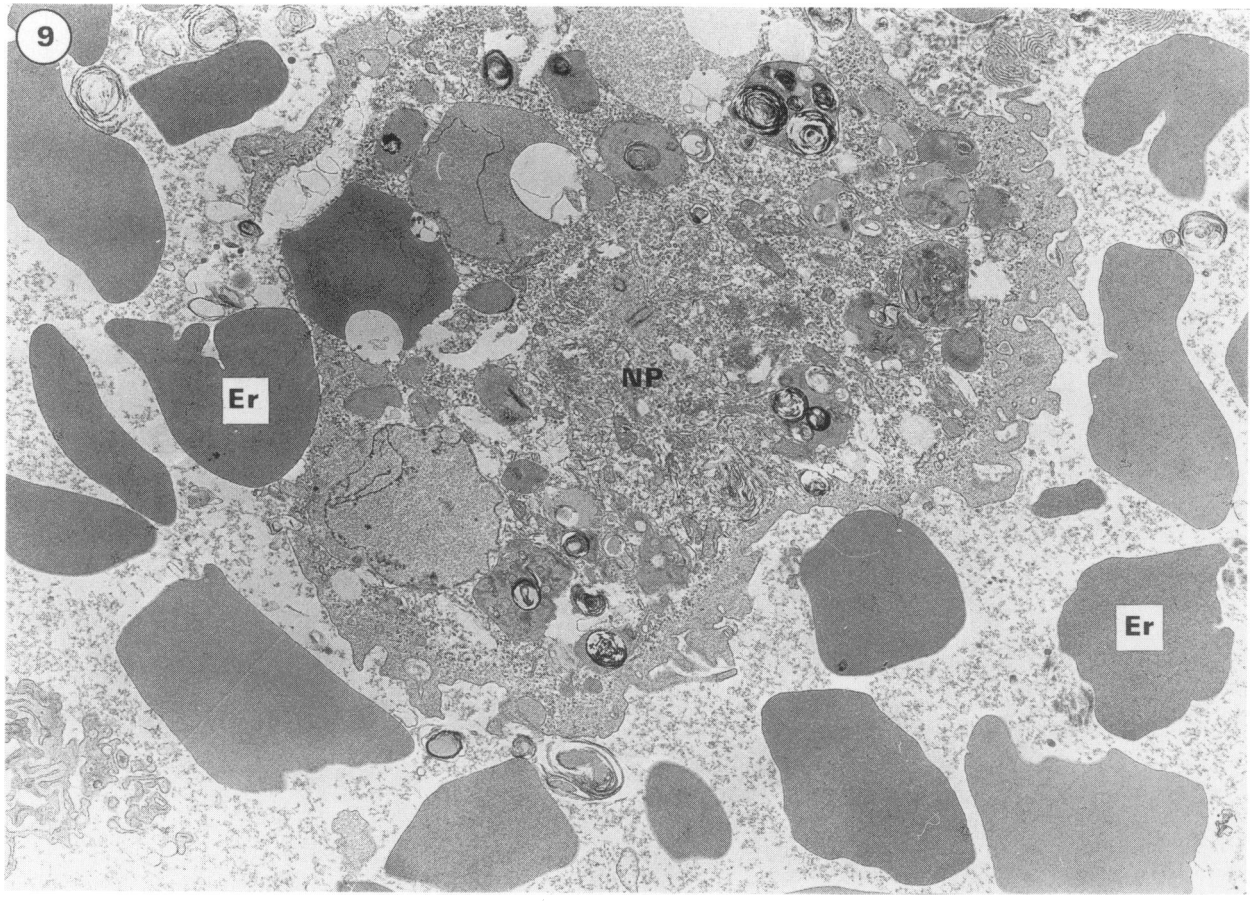

Figure 9. Electron micrograph of the right lobe of rat lung killed 24 hours after blast injury to the right lateral thorax showing an area of intra-alveolar haemorrhage with a necrotic Type II pneumocyte. ×6110. NP, Necrotic Type II pneumocyte; Er, misshapen erythrocyte.

patients is greatly complicated by the presence of the extrathoracic injuries. Such patients are at risk of developing the post traumatic pulmonary insufficiency syndrome (Adult Respiratory Distress Syndrome (ARDS)) which with its characteristics of a widespread high protein containing pulmonary oedema may be indistinguishable from the effects of an extending pulmonary contusion. Indeed, unwise fluid transfusion in patients with pulmonary contusion may lead to an extension of that contusion.

In an experimental series, of which those preliminary experiments described in this paper form a part, an attempt has been made to study pulmonary contusions without the complicating factors of other injuries or the infusion of intravenous fluids. In the present experiments changes have been demonstrated using electron microscopy which extend these and other light microscopic observations (Brigham 1982). These histopathological alterations occurred not only in the areas of damage which were obvious at the light microscopic level but also in the apparently normal areas of both the right and left lobes of the lung. In these apparently normal areas the changes were not accompanied by bleeding but took the form of loss of structure of lamellated bodies of type II pneumocytes, blebbing and focal necrosis of type 1 epithelial cells. Intra-alveolar oedema and a minimal amount of interstitial oedema was also observed. These ultrastructural modifications are similar to those reported by Bachofen and Weibel (1982) in cases of ARDS. The cause of these changes is at present obscure but a number of hypotheses, some of which may be tested experimentally, are suggested:

(1) Damage to one area of lung may release factors into the circulation which promote further damage in distant parts of the organ: this theory is similar to that

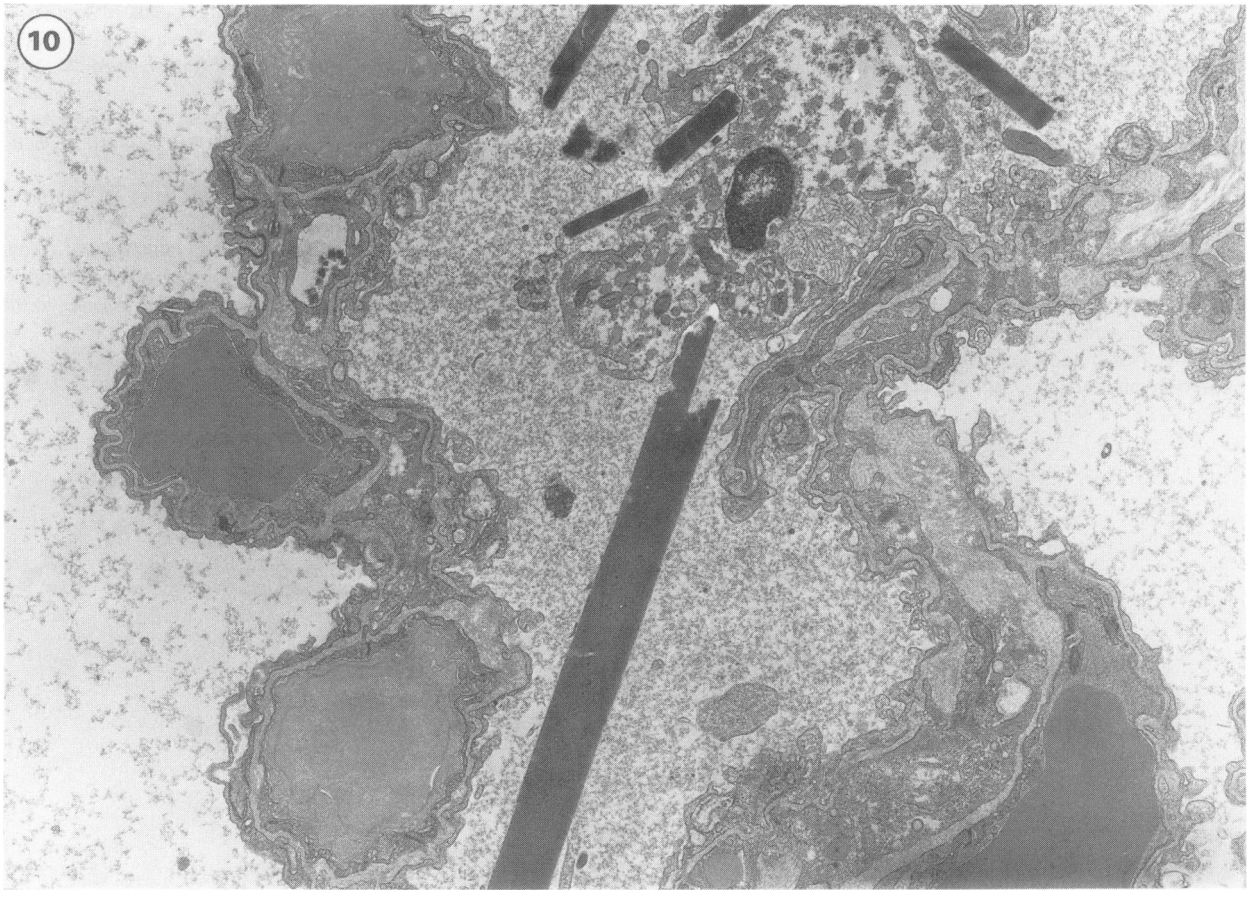

Figure 10. Electron micrograph of the right lobe of rat lung killed 24 hours after blast injury to the right lateral thorax showing an area of intra-alveolar oedema with associated alveolar cell damage together with dense osmiophilic rod-shaped particles which contain a significant amount of iron. ×7180.

discussed by Brigham (1982) in considering possible aetiologies of ARDS.

(2) Blood released in areas of obvious damage may be distributed widely in the whole lung by reflux to undamaged areas. The effect of this blood on inter-alveolar septa at the electron microscopic level is unknown.

(3) Damage to areas of lung may trigger reflex changes in other parts of the lung. The importance of the reflex production of vasoconstriction and increases in pulmonary capillary pressure as a result of pulmonary contusions is unknown.

There is a suggeston in these results that the electron microscopic changes seen in pulmonary contusions become more marked in the first 24 hours following injury. This would lend weight to the contention that pulmonary contusions are progressive lesions, at least at the cellular level. Only a few animals were used in this part of the study and considerable variability in the extent of lesions produced in rat lungs by the blast producing device used should be noted. Further experiments will be needed to confirm the potentially significant observations reported in this preliminary paper.

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