

## **Pathogenesis of high-altitude pulmonary oedema: direct evidence of stress failure of pulmonary capillaries**

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*Pathogenesis of high-altitude pulmonary oedema: direct evidence of stress failure of pulmonary capillaries. J.B. West, G.L. Colice, Y-J. Lee, Y. Namba, S.S. Kurdak, Z. Fu, L-C. Ou, O. Mathieu-Costello. ©ERS Journals Ltd 1995.*

**ABSTRACT:** The pathogenesis of high-altitude pulmonary oedema (HAPE) is disputed. Recent reports show a strong correlation between the occurrence of HAPE and pulmonary artery pressure, and it is known that the oedema is of the high-permeability type. We have, therefore, proposed that HAPE is caused by ultrastructural damage to pulmonary capillaries as a result of stress failure of their walls. However, no satisfactory electron microscopy studies are available in patients with HAPE, and animal models are difficult to find.

Madison strain Sprague-Dawley rats show a brisk pulmonary pressure response to acute hypoxia and are susceptible to HAPE. We exposed 13 Madison rats to a pressure of 294 torr for up to 12.5 h, or 4 rats to 236 torr for up to 8 h. Pulmonary arterial or right ventricular systolic pressures measured with a catheter increased from  $30.5 \pm 0.5$  (SEM) in controls (n=4) to  $48 \pm 2$  torr (n=11). The lungs were fixed for electron microscopy with intravascular glutaraldehyde.

Frothy bloodstained fluid was seen in the trachea of three animals. Ultrastructural examination showed evidence of stress failure of pulmonary capillaries, including disruption of the capillary endothelial layer, or all layers of the wall, swelling of the alveolar epithelial layer, red blood cells (RBCs) and oedematous fluid in the alveolar wall interstitium, proteinaceous fluid and RBCs in the alveolar spaces, and fluid-filled protrusions of the endothelium into the capillary lumen.

These appearances are consistent with the ultrastructural changes we have previously described in rabbit lung when the capillaries are exposed to high transmural pressures, and we conclude that the pathogenesis of HAPE is stress failure of pulmonary capillaries.

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The pathogenesis of high altitude pulmonary oedema (HAPE) remains obscure in spite of a great deal of research. Current hypotheses include: increased pulmonary capillary permeability caused by hypoxia or oxygen radicals [1]; leakage of fluid through small pulmonary arteries as a result of severe pulmonary hypertension [2]; overperfusion of some lung regions causing capillary damage through increased shear stress [3]; centrogenic origin due to cerebral oedema, multiple pulmonary emboli, antidiuresis with consequent fluid retention, and fluid shift from the periphery of the body to the lungs [4]. However, none of these hypotheses fits all the facts.

We have recently proposed that HAPE is due to damage to the walls of pulmonary capillaries as a result of very high wall stresses caused by increased capillary transmural pressures [5, 6]. These high capillary pressures are the result of uneven hypoxic pulmonary vasoconstriction as originally proposed by HULTGREN [3]. Extensive studies in our laboratory have shown that raising the capillary transmural pressure causes ultra-

structural damage to the capillary walls, including disruptions of the capillary endothelial layer, alveolar epithelial layer, and sometimes all layers of the wall [5, 7–10]. The result is a high-permeability form of oedema [11].

Madison strain Sprague-Dawley rats have been shown to develop high pulmonary artery pressures when exposed to hypoxia, and some of the animals subsequently develop pulmonary oedema. We have, therefore, used these animals as a model for HAPE.

In this paper, we report studies on rats exposed to simulated high altitude, in which ultrastructural examination of the lung showed the typical changes of stress failure of pulmonary capillaries that we have seen in rabbit lung with high capillary transmural pressures. We propose that this mechanism explains the strong correlation with pulmonary artery pressure, the occurrence of high-permeability pulmonary oedema, and the appearance of inflammatory markers in the alveolar fluid which have been described in HAPE.

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## Methods

### Animals

Twenty nine Madison strain Sprague-Dawley rats, body weight (BW) 290–327 g were used. Thirteen rats were exposed to a pressure of 294 torr in a low-pressure chamber for 3–12.5 h. Four rats were exposed to a pressure of 236 torr for up to 8 h. The progressive decompression of the chamber, and its recompression at the end of the exposure period, took about 20–30 min each. Another four animals breathed 8.5% oxygen in a plexiglass chamber for up to 12 h. Eight animals were used as controls, and were not exposed to low pressures or hypoxic gas. The protocols were approved by the Animal Subjects Committees of The University of California, San Diego (UCSD) and Dartmouth Medical School.

Measurements of pulmonary artery and right ventricular pressures were carried out *via* implanted catheters inserted 48 h prior to the experiment. The procedure used for catheterization under deep anaesthesia with combined intramuscular injection of ketamine (60 mg·kg<sup>-1</sup> BW) and intraperitoneal injection of pentobarbital (20 mg·kg<sup>-1</sup> BW), has been described in detail elsewhere [12]. Briefly, the right external jugular vein was isolated and a small incision was made. A 12.5 cm Silastic catheter (0.012 in internal diameter (ID), 0.025 in outside diameter (OD)), filled with heparinized saline and connected *via* a Statham transducer to a Grass polygraph (Model 7B), was inserted through the incision into the right ventricle and the pulmonary artery with the aid of a 7 cm introducer (PE 90), whilst guided by the typical pressure tracings. When the catheter was in the pulmonary artery (~7 mm from the pulmonary valve), the introducer was slipped out over the catheter and removed. The catheter was then secured in the jugular vein by basket-weave sutures, exteriorized at the back of the animal's neck, flushed with heparinized saline and sealed with a stainless steel metal plug.

Pressure measurements were performed immediately after taking the rats out of the low-pressure chamber, and transferring them to a plexiglass box, where they breathed 8.5% oxygen if they had been exposed to a pressure of 294 torr, 6.6% oxygen if they had been exposed to 236 torr, or ambient room air (20.9% O<sub>2</sub>) if they were controls. Pulmonary artery or right ventricular pressures were obtained in eight animals exposed to a pressure of 294 torr, three animals that were exposed to a pressure of 236 torr, all animals exposed to 8.5% oxygen gas, and four controls.

### Preparation of lungs for electron microscopy

The animals were anaesthetized using a combination of ketamine (60 mg·kg<sup>-1</sup> BW *i.m.*) and pentobarbital (20 mg·kg<sup>-1</sup> BW *i.p.*). They were placed supine and a cannula inserted into the trachea. The chest was rapidly opened and the lungs were inflated to a pressure of 20 cmH<sub>2</sub>O before being held at 5 cmH<sub>2</sub>O. After clamping

the right atrium and cutting the left atrium for outflow, the lungs were perfused with saline (NaCl·l<sup>-1</sup> 11.06 g, osmolarity 350 mOsm, heparin·l<sup>-1</sup> 20,000 U) *via* a cannula inserted directly into the right ventricle. When the outflow appeared clear of blood cells (approx. 1–3 min), fixative (phosphate-buffered 2.5% glutaraldehyde, total osmolarity 500 mOsm, pH adjusted to 7.4) was perfused for 10 min. Both saline and fixative perfusions were carried out at an inflow pressure of 25–30 cmH<sub>2</sub>O, generated by the height of the perfusion reservoirs and measured *via* a glass manometer connected to the circuit by a T-tube inserted close to the inflow cannula.

After fixation, the trachea was ligated, the lungs were excised and stored in glutaraldehyde in a refrigerator at 4°C for 5–30 days. One slab ~2 mm thick, was taken perpendicular to the anteroposterior axis at about half the distance from the bottom of the left lobe as well as from macroscopically abnormal dark-stained areas in each animal. The slabs were systematically cut into thin vertical slices and tissue blocks by use of the lung vertical section sampling method described by MICHEL and CRUZ-ORIVE [13]. Additional samples were systematically taken from macroscopically dark areas in other regions of either right or left lungs. The specimens were rinsed overnight in 0.1 M phosphate buffer adjusted to 350 mOsm with NaCl (pH 7.4), and postfixed for 2 h in a solution of osmium tetroxide in 0.125 M sodium cacodylate buffer adjusted to 350 mOsm with NaCl (total osmolarity 400 mOsm, pH 7.4). They were then dehydrated in increasing concentrations (70–100%) of ethanol, rinsed in propylene oxide, and embedded in Araldite. Two blocks were selected randomly from each sample. Sections (1 µm) were cut from each block with an LKB Ultratome III. They were stained with 0.1% toluidine blue aqueous solution. Ultrathin sections (50–70 nm) were contrasted with uranyl acetate and bismuth oxynitrate [14], and examined with Zeiss 10 or Philips EM300 electron microscopes.

### Statistical analysis

Pressure measurements are expressed as group mean ±SEM. Group means were compared by Student's *t*-test and differences taken as significant for value of *p* less than 0.05.

## Results

### Tolerance of the hypoxia

Ten of the 13 rats exposed to a barometric pressure of 294 torr survived. The period of exposure was 8.5–12.5 h. Three rats died, two after 3 h, and one after 4 h. Of the four rats exposed to a barometric pressure of 236 torr, three survived but one died during the measurement of pulmonary artery pressure. All the rats exposed to 8.5% oxygen survived.

### *Pulmonary artery or right ventricular pressures*

In all rats exposed to low barometric pressure or low oxygen concentration, increases in pulmonary artery or right ventricular pressure were seen. The mean pulmonary arterial systolic pressure in the control animals breathing air was  $30.5 \pm 0.5$  (SEM) torr ( $n=4$ ). During exposure to 294 torr, 236 torr barometric pressure and 8.5% oxygen gas, the mean pulmonary arterial or right ventricular systolic pressure was  $49 \pm 2$  torr ( $n=15$ ). There were no significant differences between the pressures measured in the animals that had been exposed to 294 ( $49 \pm 2$  torr;  $n=8$ ), as compared with 236 torr ( $45 \pm 3$  torr;  $n=3$ ) or 8.5% oxygen gas ( $51 \pm 3$  torr;  $n=4$ ). As indicated in Methods, the pressures were measured after the hypobaric exposure while the animals were breathing 8.5 or 6.6% oxygen, respectively.

### *Macroscopic appearance of lungs and light microscopy*

Bloodstained frothy fluid was seen in the trachea of two animals exposed to a pressure of 294 torr, and in one animal exposed to a pressure of 236 torr. The lungs of animals exposed to severe hypoxia (294 or 236 torr barometric pressure or 8.5% oxygen gas) showed various degrees of macroscopic abnormalities in over half of the 21 animals. These ranged from irregular sparse dark areas to very large haemorrhagic regions. Substantial alveolar oedema fluid and the presence of red blood cells in the alveoli in an animal who died in the chamber at 294 torr barometric pressure is shown in figure 1.

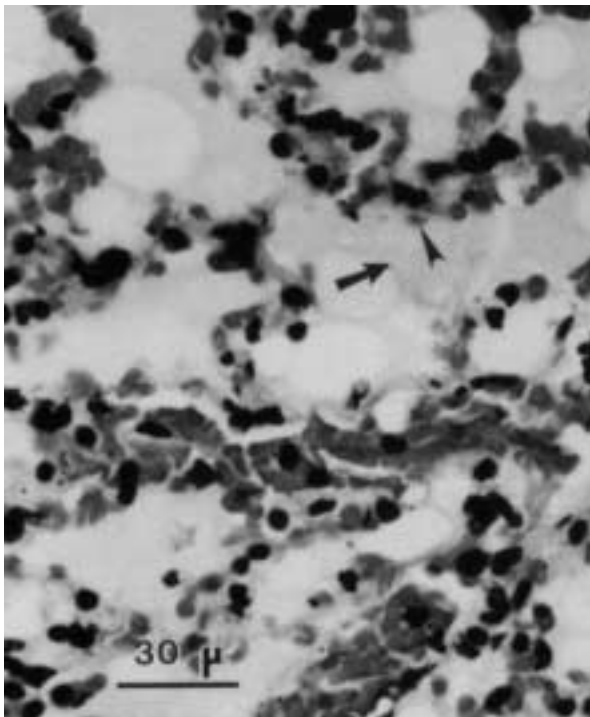


Fig. 1. – Light micrograph of haematoxylin and eosin stained paraffin-embedded section, showing alveolar oedema (arrow) and red blood cells (arrowhead) in alveoli of a Madison rat exposed to 294 torr barometric pressure. (Internal scale = 30  $\mu$ m).

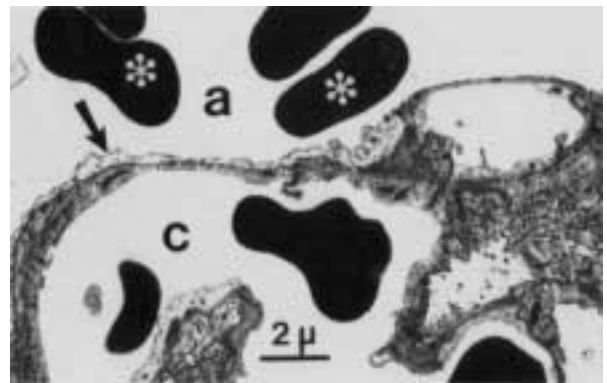


Fig. 2. – Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat exposed to 236 torr barometric pressure for 6.5 h. Note red blood cells (\*) in alveolar space (a) and swelling of epithelial lining (arrow). c: capillary. (Internal scale = 2  $\mu$ m).

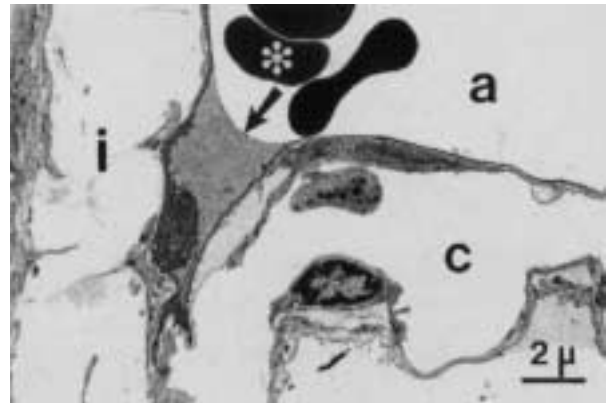


Fig. 3. – Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure. Red blood cells (\*) and granular material (arrow) in alveolar space (a). Note interstitial (i) oedema. c: capillary. (Internal scale = 2  $\mu$ m).

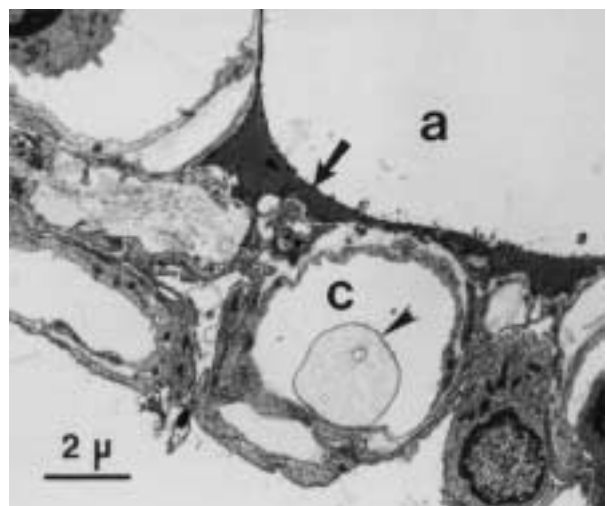


Fig. 4. – Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure (same animal as in figs 3, 5 and 6). Note fluid-filled protrusion (arrowhead) of the endothelium into the capillary (c) lumen and granular material (arrow) in the alveolar space (a). (Internal scale = 2  $\mu$ m).

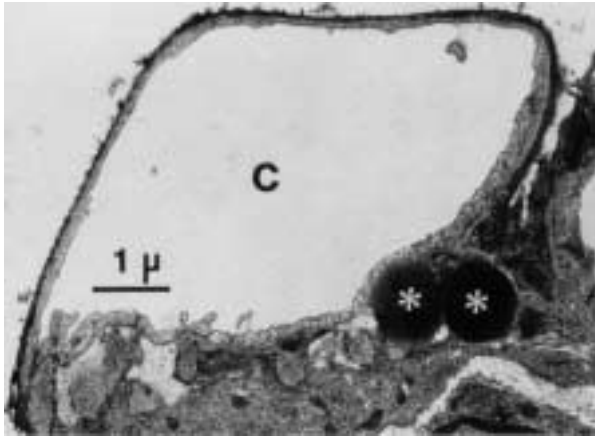


Fig. 5. — Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure (same animal as in figs 3, 4 and 6). Note red blood cells (\*) in interstitium. c: capillary. (Internal scale = 1 μm).

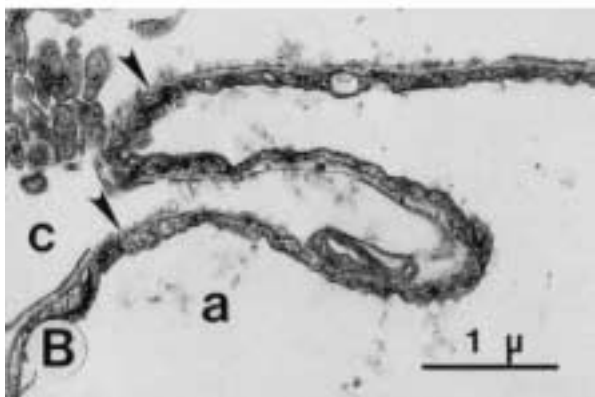
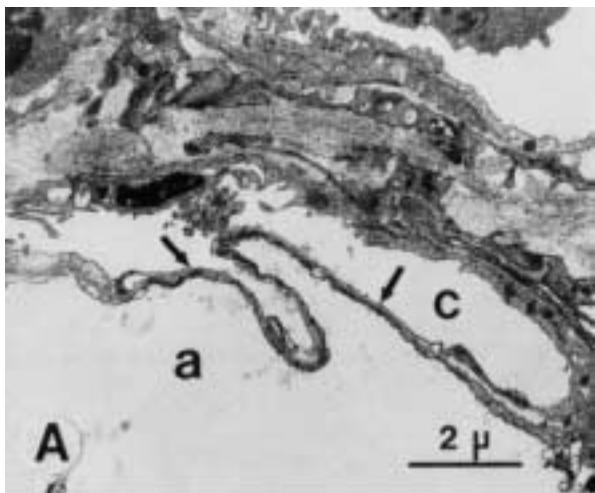


Fig. 6 — Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure (same animal as in figs 3–5). A) Disruption of capillary wall (arrows). (Internal scale = 2 μm). B) Enlargement of portion of endothelial disruption from (A) showing exposed basement membrane (arrowhead). a: alveolar space; c: capillary. (Internal scale = 1 μm).

#### *Ultrastructural appearances of lung parenchyma*

Normal electron microscopic appearances were seen in the control lungs, but morphological evidence of

haemorrhagic pulmonary oedema was seen in several animals exposed to hypoxia. Red blood cells were found in the alveolar spaces (fig. 2) as well as electron-dense granular material which represents oedema fluid with a high protein concentration (fig. 3). There was also oedema of the interstitium of the alveolar wall (figs. 3 and 4), fluid-filled protrusions of the endothelium into the capillary lumen (fig. 4), and obvious swelling of alveolar epithelial cells (fig. 2).

There was clear evidence of disruptions of the capillary endothelial and alveolar epithelial layers consistent with stress failure of pulmonary capillaries [7]. Figure 5 shows red blood cells in the interstitium of the alveolar wall, indicating disruption of the capillary endothelial layer in the proximity. An example of a break in the endothelium is seen in figure 6. Figure 7 shows complete rupture of the blood-gas barrier, with a red blood cell passing through from the capillary lumen into the alveolar space. Figures 3–9 are from animals with the most severe lesions who died in the low-pressure

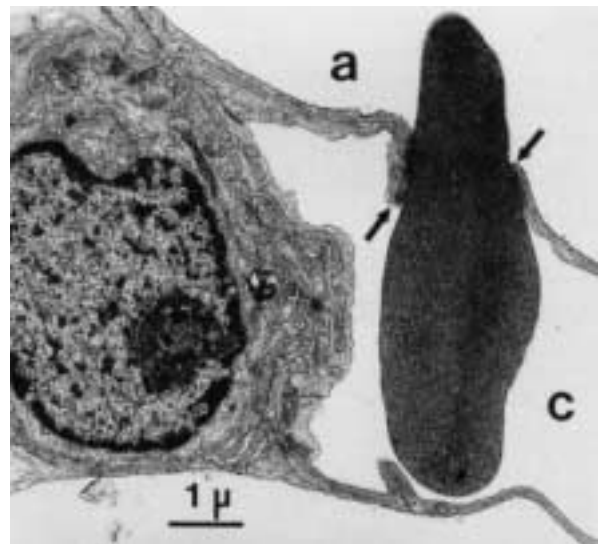


Fig. 7. — Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat exposed to 294 torr barometric pressure for 4 h. Complete rupture of the blood-gas barrier (arrows), with red blood cell passing into the alveolar space (a). c: capillary. (Internal scale = 1 μm).

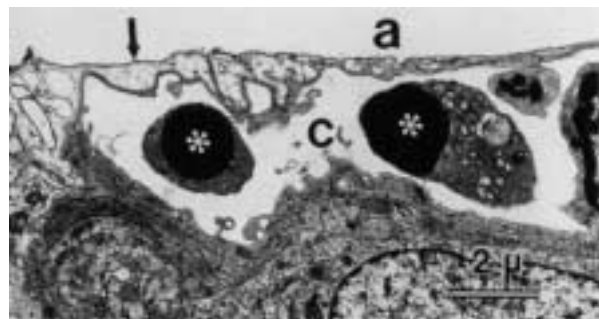


Fig. 8. — Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure. Note red cell material (\*) in capillary (c) macrophage and swelling of epithelial lining (arrow). a: alveolar space; c: capillary. (Internal scale = 2 μm).

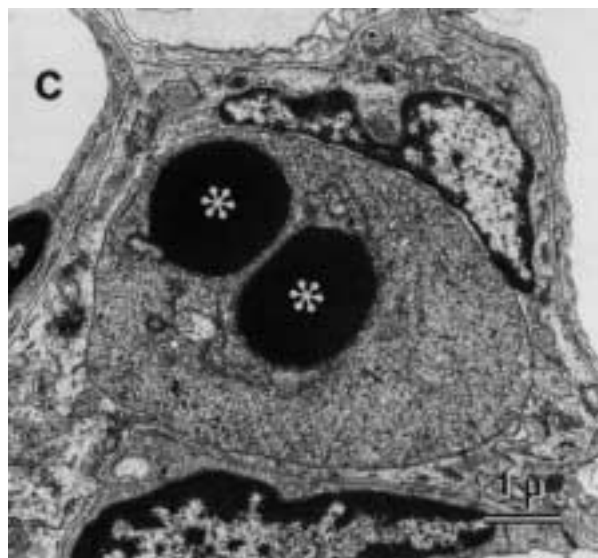


Fig. 9. — Electron micrograph showing ultrastructure of lung parenchyma in a Madison rat after 3 h exposure to 294 torr barometric pressure (same animal as in fig. 8). Note red cell material (\*) in macrophage in the interstitium. c: capillary. (Internal scale = 1  $\mu$ m).

chamber. In these rats, macrophages showed large dark intracellular phagosomes suggesting lysis of red blood cells. These were seen in the intravascular compartment (fig. 8), interstitial space (fig. 9) and in alveolar spaces.

## Discussion

### *Correlation between HAPE and pulmonary hypertension*

There is now very good evidence that the occurrence of HAPE is strongly correlated with the height of the pulmonary arterial pressure. Direct pressure measurements in patients with HAPE show high values [15]. Patients who develop HAPE tend to have an unusually high degree of hypoxic pulmonary vasoconstriction [16]. Exercise at high altitude, which increases pulmonary arterial pressure, is a known risk factor [17]. There is an association with a restricted pulmonary vascular bed, for example in patients with unilateral absence of a pulmonary artery [18]. Finally, reducing the pulmonary arterial pressure, for example by giving the calcium channel blocker, nifedipine, usually causes rapid disappearance of the oedema [19], and nifedipine is also effective in preventing HAPE in a high risk group [20]. All these facts suggest that HAPE is caused in some way by high vascular pressures in the pulmonary circulation.

### *The oedema fluid of HAPE is of the high-permeability type*

SCHOENE *et al.* [21] and HACKETT *et al.* [22] obtained samples of alveolar fluid by bronchoalveolar lavage (BAL) in patients with HAPE. They reported that the fluid was of the high-permeability type with a large

concentration of high molecular weight proteins and many cells. In one study [21], the protein concentration of the fluid ( $616 \pm 3$  mg·dl<sup>-1</sup>) exceeded that seen in most cases of the adult respiratory distress syndrome, one of the best examples of a disease producing a high-permeability type of oedema. In addition, there were numerous red blood cells and white blood cells in the alveolar fluid. Increased concentrations of leukotriene B<sub>4</sub> and complement fragment C5a were also found (see below). These observations strongly suggest that HAPE is associated with damage to the walls of the pulmonary capillaries. The problem, therefore, is to reconcile a hydrostatic pressure basis for the disease with the development of abnormalities in the capillary walls.

### *Stress failure of pulmonary capillaries as a mechanism for HAPE*

Several years ago, we studied the effect of raising the pressure in capillaries in rabbit lung and showed that this caused ultrastructural changes in the capillary walls [5, 7]. The changes consisted of disruption of the capillary endothelial layer, alveolar epithelial layer, and sometimes all layers of the capillary wall. At the time, we suggested that this might be the pathogenic basis of HAPE [5], but there were no electron micrograph studies of the lung in that disease, and it is difficult to find animal models. The present study presents direct evidence of ultrastructural changes typical of stress failure of pulmonary capillaries in an animal model of HAPE. We chose Madison strain Sprague-Dawley rats because these have previously been shown to be particularly susceptible to HAPE [23].

The morphological changes that we report here in the rats exposed to low barometric pressures include: disruptions of the capillary endothelial layer (fig. 6); or disruptions of all layers of the capillary wall (fig. 7); swelling of the alveolar epithelial layer (figures 2, 3 and 8); red blood cells and proteinaceous fluid in the alveolar spaces (figs 2–4); red blood cells and oedematous fluid in the alveolar wall interstitium (figs 3 and 5); and fluid-filled protrusions of the endothelium into the capillary lumen (fig. 4). All these morphological changes are typical of stress failure of pulmonary capillaries and have been described in our rabbit preparation [7, 9, 10].

The mechanism of the pulmonary hypertension during acute exposure to high altitude is known to be hypoxic pulmonary vasoconstriction. Since this chiefly occurs in small pulmonary arteries [24], it is not immediately clear why some pulmonary capillaries would be exposed to the high pressure. The explanation is presumably that given by HULTGREN [3], who suggested that the vasoconstriction is uneven, with the result that some capillaries are not protected from the increased pressure in the pulmonary arteries. Direct evidence for uneven vasoconstriction in alveolar hypoxia has not yet been obtained, although the very patchy nature of the oedema in HAPE is consistent with it [15, 25]. Also DAWSON *et al.* [26] showed that alveolar hypoxia nearly doubled the dispersion of transit times through the pulmonary

circulation of a lobe of dog lung, which strongly suggested that hypoxic vasoconstriction was uneven.

A striking feature of stress failure of pulmonary capillaries is that some of the breaks are rapidly reversible when the pressure is reduced. In one study carried out in rabbit lung, the autologous blood perfusion was performed at a high pressure, and the pressure was then reduced for 3 or 6 min before intravascular fixation [10]. It was found that about 70% both of the epithelial and endothelial breaks closed, and these were chiefly the breaks that were small, and those associated with an intact basement membrane. This behaviour probably explains why it is relatively difficult to find actual breaks in the endothelial and epithelial layers, although the consequences of the breaks, such as red blood cells in the interstitium of the alveolar wall and the alveolar spaces are obvious. The rapid reversibility of most of the disruptions caused by stress failure may also explain why patients with HAPE often improve rapidly when they descend to a lower altitude. The rapid reversibility also explains why it is relatively difficult to see breaks in the pulmonary capillaries of galloping racehorses that bleed into their lungs, although the same mechanism of stress failure is responsible [27].

#### *Possible role of inflammation in the aetiology of HAPE*

Bronchoalveolar lavage studies in patients with HAPE show the presence of inflammatory markers, including leukotriene B<sub>4</sub>, other lipoxygenase products of arachidonic acid metabolism, and C5a complement fragment in the lavage fluid [21]. At first sight, these findings seem to argue for some other mechanism than stress failure of pulmonary capillaries. However, an important feature of the ultrastructural changes in stress failure is that the basement membrane of capillary endothelial cells is frequently exposed [7]. An example from the present study is seen in figure 6. The exposed basement membrane is electrically charged and highly reactive, and can be expected to activate leucocytes and platelets. In BAL studies of the rabbit preparation, leukotriene B<sub>4</sub> is seen in the lavage fluid [11]. Platelet activation will result in the formation of small thrombi, which are a feature of the pathology of HAPE [28].

However BÄRTSCH and co-workers [29, 30] showed that patients with early HAPE do not have increased blood levels of platelet factor IV or beta-thromboglobulin, which are indicators of platelet activation. They also showed no increase in factor XII, the first step of contact activation in the coagulation cascade. Possibly, these negative results can be explained by the dilution in circulating blood of substances produced in small, scattered sites throughout the lungs.

#### *Previous related work*

There have been no previous studies in which the morphological features of stress failure of pulmonary capillaries have been sought in a model of HAPE.

However, MOOI *et al.* [31] studied the ultrastructural changes that occurred in rat lungs when the animals were exposed to acute decompression in a hypobaric chamber. These studies were carried out in the context of aircraft pilots who may need to bale out at extreme altitudes. The pressure was reduced to 265 torr over a period of 1 h, and the exposure was up to 5 h. The electron microscopy appearances included: swelling and disruption of type 1 alveolar epithelial cells; disruption of capillary endothelial cells; and red blood cells in the alveolar wall interstitium and alveolar spaces. These findings are similar to those described here, and may have been due to the pulmonary hypertension as a result of the acute alveolar hypoxia.

In an early study by VISWANATHAN *et al.* [32] a variety of animals, including dogs, monkeys, guinea-pigs, rats and mice, was exposed to a simulated altitude of 30,000 feet (9144 m) whilst being housed in a revolving drum which kept them constantly in motion. Some animals developed pulmonary oedema, with so-called degenerative changes in the alveolar epithelial and capillary endothelial cells. The authors attributed the changes to "hypoxic stress".

#### *Implications for therapy*

If HAPE is caused by stress failure of pulmonary capillaries, the main objective should be to reduce the pulmonary arterial pressure. The pressure is high because of hypoxic pulmonary vasoconstriction, and the best way to reduce it is by rapid descent to a lower altitude which increases the alveolar oxygen partial pressure A (PaO<sub>2</sub>). In addition, oxygen should be given if this is available. Calcium channel blockers, such as nifedipine, are also effective because they reduce the pulmonary artery pressure [19].

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