Br. J. exp. Path. (1972) **53**, 347.

PULMONARY OEDEMA INDUCED BY ANTU, OR BY HIGH OR LOW OXYGEN CONCENTRATIONS IN RAT—AN ELECTRON MICROSCOPIC STUDY

BARBARA MEYRICK, J. MILLER* AND LYNNE REID

From the Department of Experimental Pathology, Institute of Diseases of the Chest, Brompton Hospital, London S.W.3

Received for publication February 1972

Summary.—Pulmonary oedema has been experimentally induced in rats using either an intraperitoneal injection of alpha-naphthyl thiourea (ANTU) or by variation of atmospheric oxygen content. For the first time an electron microscopic study of the development of ANTU-induced lung oedema has been made. At 2 hours blebbing and scalloping of endothelial cells and interstitial oedema were observed, each showing increasing severity with increase of dose—3, 10 and 50 mg/kg doses. By 6 hours, in the 50 mg/kg treated animals, epithelial damage was also apparent. No alteration in mean alveolar size or in the distribution of alveolar lining cells was found. The breathing rate of the rats was slowed for the first 3 hours and then increased well above normal levels.

Hyperoxic conditions (99–100% O_2 at 1 atm/pressure) produced interstitial oedema by 6 hours, followed at 24 hours by both endothelial and epithelial damage. Hypoxic conditions (7–10% O_2 at 1 atm/pressure) did not produce these effects. Both hyperoxic and hypoxic conditions were associated with a significant increase in the mean alveolar size, an apparent decrease in number of Type III pneumonocytes and in the macrophages when related to alveolar cell number, while the counts of Types I and II pneumonocytes remained within the normal range.

HUMAN pulmonary oedema has many causes, acting in a variety of ways. Although it is not always possible to correlate them closely with clinical manifestations, several different "models" can be studied experimentally, one of the most widely investigated being that produced by alpha-naphthylthiourea (ANTU) (Latta, 1947; Hesse and Loosli, 1949; Richter, 1952).

Recently, with the light microscope, Bohm (1966) compared the effect of a range of agents causing pulmonary oedema and showed that ANTU gave rise to a relatively less severe oedema than the other agents, in that the treated animals survived for more than 8 hours. Using intravenous colloidal carbon as a marker, he considered that in ANTU-induced oedema the fluid escape was from the venules; that either after intraperitoneal injection of ammonium sulphate or intracisternal injection of thrombin and fibrinogen in saline or India ink, the oedema fluid came from the capillaries; and that after adrenaline administration leakage occurred from both venules and capillaries. Teplitz (1968) used the electron microscope to trace with ferritin and saccharated iron oxide (administered intravenously) the fluid leakage after ANTU administration: in contradiction to

* Present address Royal Naval Physiological Laboratory, Alverstoke, Hants.

Bohm's report (1966) of a venular leak, he considered that the protein leaked from the capillaries.

Other substances capable of producing oedema experimentally include alloxan (Cottrell *et al.*, 1967), anthrax toxin (Dalldorf *et al.*, 1969), acid solutions (Alexander, 1968) and ammonium sulphate (Hayes and Shiga, 1970).

Using the electron microscope, hyperoxic conditions have also been reported to cause pulmonary interstitial oedema (Kistler, Caldwell and Weibel, 1967; Kapanci *et al.*, 1969; Adamson, *et al*,. 1970). Kistler, Caldwell and Weibel (1967) reported that 98·5% oxygen for 48 hours was necessary before any structural alteration was detected in rat lung; Kapanci *et al.* (1969) repeated these experiments with monkey lung and also reported no detectable change before 48 hours' exposure. However, recently in the dog lung, Coalson, Beller and Greenfield (1971) reported ultrastructural changes by one hour.

The purpose of this study was to examine with the electron microscope the development of lung changes occurring after ANTU administration and high (99–100%) and low (7–10%) concentrations of oxygen at one atmosphere, paying particular attention to the interstitial space, endothelium and epithelium. Three doses of ANTU (3, 10 and 50 mg/kg) were given to see if there was a dose-related effect. The morphological changes, mean alveolar size, type of alveolar cells present and their relative proportions in all three experiments were compared. In the ANTU-treated rats the breathing rates were also noted; this was not done for the oxygen groups since, when in the compression chambers, the animals could not be clearly seen.

MATERIAL AND METHODS

ANTU

The effect of increasing the dose of ANTU was investigated using three levels—3, 10 or 50 mg/kg: this was suspended in olive oil and the concentration adjusted so that each animal received between 1·0 and 1·2 ml, injected intraperitoneally, using a size 18 needle (Richter, 1952). Control animals were treated with either olive oil or saline.

The animals were male, 70-day-old, S.P.F. rats (Carworth Europe) and weighed between 350 and 500 g. Three experiments with ANTU were carried out, each on separate occasions. In the first experiment, 8 rats were used, 2 for each of the three different doses of ANTU—3, 10 and 50 mg/kg. They were killed 1–12 hours after injection. From these results it was decided that a dosage of 50 mg/kg most consistently gave pronounced oedema and so this dose was given to 8 of the 12 rats in each of the two subsequent experiments, these animals being killed between 2 and 9 hours later, the remaining rats serving as controls. On a separate occasion 9 additional animals were used to compare the effect on the breathing rate of olive oil and saline. The results from these experiments were combined, giving a total of 41 animals for analysis (Table I).

The animals were anaesthetized by intraperitoneal injection of 2 ml of Veterinary " Nembutal " (pentobarbitone sodium). The chest was opened, the trachea, lungs and heart removed and their weights recorded. The lungs were distended with 2% glutaraldehyde in cacodylate buffer pH 7·2 at a pressure of 23 cm H_2O and the volume of fluid used was recorded; the volume of each lung was then measured separately by fluid displacement.

For electron microscopy the left lung was used in Experiments I and II, and the right lower lobe in III; the right lower lobe was used for light microscopy and processed as described below.

Variation in atmospheric oxygen pressure (hyperoxia and hypoxia)

Four experiments were carried out at the Royal Naval Physiological Laboratories, Gosport, two under conditions of low oxygen concentration (7 and 10%) and two at increased levels (90–96% and 99–100%), each concentration for 6 and 24 hours. The percentage of carbon dioxide in the chambers was monitored at intervals and was always less than 0·1%.

TABLE I.—*ANTU Dose and Animal Survival Time—all Four Experiments Combined*

Number of animals	Dose ANTU (mg/kg)	Survival time (h)
1	3	1
1	3	2
1	10	6
1	10	9½*
2	50	2
2	50	3
2	50	4
5	50	6
1	50	6½*
2	50	7½*
1	50	8
2	50	9*
1	50	12
22		

+ 8 controls received olive oil alone
+ 3 controls received saline alone
+ 8 controls received no treatment

41 animals in all

* These animals died; the remainder were killed by intraperitoneal injection of " Nembutal ".

TABLE II.—*O_2 and CO_2 Concentrations and Duration of Exposure*

Experiment	No. of rats used	% O_2	% CO_2	Duration of exposure (h)
		HYPOXIA		
I	3	10	$<0\cdot2$	6
II	3	7	$<0\cdot1$	24
		HYPEROXIA		
III	3	90–96	$<0\cdot2$	6
IV	3	99–100	$<0\cdot1$	24

The 18 rats used were from the same source as above, weighing between 215 and 275 g (45–50 days old).

Experiments were carried out on two separate occasions, I with III and II with IV (Table II); on each occasion 3 control animals were used. In these experiments the left lung was used for electron microscopy, the right for light microscopy. Lung and heart weight and volume were not obtained.

Preparation of tissue

Lungs were fixed for microscopy as above. Tissue for electron microscopy was taken from the mid-region of the left lung just below the hilum. Blocks for light microscopy were processed through graded alcohols, chloroform and embedded in wax. Sections were cut at 4 μm and stained with haematoxylin and eosin.

Blocks for electron microscopy, each 1 mm^3, were cut from the lung after fixation at room temperature for one hour. These blocks were then fixed for a further hour, transferred to cacodylate buffer at 4° overnight, post-fixed in 1% osmium tetroxide in cacodylate buffer at room temperature for one hour, washed, dehydrated and embedded in Araldite. Using glass knives and an LKB Ultratome III, pale gold sections were cut; these were stained with either Karnovsky's lead hydroxide (1961) or uranyl acetate followed by Reynold's lead citrate (1963) and examined with an AEI EM6B.

Quantification of results

Using the light microscope ($\times$ 25 objective and $\times$ 8 eyepiece) the alveolar number and size were assessed, by estimating from 10 fields the mean number of alveoli per microscopic field, and then the student's *t*-test was applied.

With the electron microscope the alveolar size was assessed by counting the number of alveolar walls transected by the grid bars demarcating the same two sides of each of 20 squares. Twenty squares were counted from each of three different sections from each animal. In these same squares macrophages and all of the three types of cell whose nucleus contributed to the alveolar lining were identified and counted, the mean estimated and the *t*-test applied.

RESULTS

ANTU

Within a few hours of any injection, the animals huddled in the corner of the cage. The animals that had received only 3 mg/kg of ANTU did not show any other ill effects, those receiving 10 mg/kg had difficulty in breathing after 2–3 hours and those which had 50 mg/kg after only 30–40 minutes. In the last group the

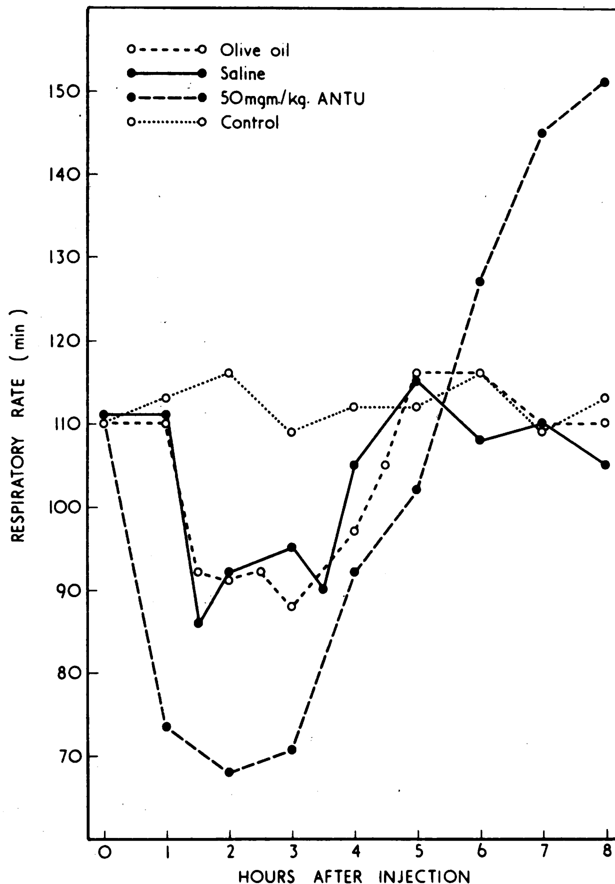


FIG. 1.—Breathing rate of animals shows an initial fall during the first hour in the ANTU-treated animals followed by a rise between 3–7 hours after injection; after olive oil or saline injection a milder fall was seen.

feet and mucous membranes became blue, the coat ruffled and, rather later, weakness of the limbs was apparent.

Breathing rates

The control rats breathed approximately 110 times/min when awake and active and at a slightly slower rate, 95–100 times/min, when still or asleep. For the first hour after administration of 50 mg/kg of ANTU the average breathing rate of the animals fell to 73/min (Fig. 1); this persisted for the next 2 hours, the rate then rising steadily over the next 4 hours to reach 140–145/min. The breathing rate of the rats that received olive oil remained in the normal range for only one hour and then fell to 86/min for 2 hours before returning to normal. A similar fall and recovery to the normal level was seen in the animals treated with saline. The olive oil and saline control animals huddled in the corner of the cage for at least the first hour after injection, as did the ANTU-treated animals during the whole experiment. It seemed that the injection contributed to the initial fall in respiratory rate but in the ANTU-treated animals this fall was more pronounced and occurred sooner.

One opening the chest, it was only those animals that had received 50 mg/kg of ANTU that showed mottling of the lungs and this only after 3 hours: by 4 hours, the lungs appeared blue and pleural effusions were present. An increase in weight of the trachea and lungs on removal from the body was looked for only in the 50 mg/kg of ANTU animals; the control lungs weighed 2·5 g and the lungs from animals 2 hours after injection weighed 4 g.

Light microscopy

Lungs from the animals injected with only olive oil appeared similar to the normal. The lungs of the animals injected with 3 mg/kg of ANTU showed no abnormality after one hour, but by 2 hours there was a little eosinophilic oedema fluid around blood vessels and occasionally in alveoli. After 10 mg/kg of ANTU the lungs were similar but with an increased amount of eosinophilic material in the alveoli, including those along the pleural edge. Two hours after 50 mg/kg of ANTU large amounts of oedema fluid were found around the blood vessels but little eosinophilic material appeared in alveoli. By 6 hours there were white blood cells and more eosinophilic material in the alveolar spaces and the pleura was swollen. No significant alteration in mean alveolar size was found, nor did the electron microscope studies reveal any increase in mean alveolar size or any significant variation in number and type of cell contributing to the alveolar lining.

Ultrastructure

The ultrastructure of the alveolar wall in the olive oil injected rats was similar to the normal (Low, 1953; Kistler *et al.*, 1967; Meyrick and Reid, 1968, 1970).

3 *mg/kg*.—By 2 hours there were the first oedematous changes, with blebbing of the endothelium in many of the capillaries, and an increase of pinocytic activity was seen in the remaining areas of normal endothelium. A little oedema fluid was found outside the basement membrane within the interstitium. The alveolar lining cells and macrophages appeared normal.

10 *mg/kg*.—The interstitial oedema was increased and there was material in the alveoli. The endothelial blebbing was more marked and appeared to be one

of two types depending on the overall thickness of the endothelium. When the endothelium was thin, localized blebbing was seen (Fig. 2); when it was thicker the appearance was of scallops; both types were seen in each animal (Fig. 3). If a pericyte was associated with a blood vessel, this appeared to remain in contact with the basement membrane; the oedema fluid separated the endothelium but not the pericyte from the basement membrane (Fig. 3). The alveolar lining cells appeared normal save that there were a few swollen Type I pneumonocytes (Fig. 3) but this appearance has also been noted in occasional control animals. The majority of macrophages contained foamy vacuoles.

50 *mg/kg*.—By 2 hours the interstitial oedema and endothelial blebbing and scalloping were very marked. The higher the dose of ANTU administered, the more pronounced the effect. The interstitial fibroblasts showed an increase in rough endoplasmic reticulum and platelets within capillaries were rounder than in the normal lung.

At 6 hours the lungs were similar but most of the endothelial damage was of the thicker, scalloped type. Some of the capillaries were collapsed due to (i) swelling and lifting of endothelium, (ii) migration of white cells into the interstitial space associated with compression of capillaries or (iii) platelet aggregations within vessels. The alveolar oedema fluid increased in amount and included fibrin. Ingestion of this material by macrophages could be seen (Fig. 4). Granulocytes could be seen escaping from lung capillaries (Fig. 5).

While some Type I pneumonocytes appeared normal, others were electron

---

EXPLANATION OF PLATES

FIG. 2.—10 mg/kg ANTU. Capillary in an alveolar wall showing blebbing (*) of endothelium (end). The cytoplasmic extensions of the Type I pneumonocyte (I) appear pale and swollen. There is oedema fluid in the interstitial space (int). Type III pneumonocyte (III). Pericyte (p). Alveolus (alv). Glut + OsO_4. × 7,500.

FIG. 3.—10 mg/kg ANTU. Capillary showing both blebbing (*) and scalloping (←) of endothelium. Fluid is seen between endothelium and basement membrane (b.m.). The pericyte (p) remains in contact with the basement membrane. Fluid in interstitial space (int). Alveolus (alv). Glut + OsO_4. × 5600.

FIG. 4.—50 mg/kg ANTU. Ingestion of intra-alveolar fibrin (f) by an alveolar macrophage (mac). Alveolus (alv). Glut + OsO_4. × 5600.

FIG. 5.—50 mg/kg ANTU. A granulocyte (gr) escaping from a capillary (cap), situated between endothelium (end) and the broken basement membrane (bm) of the capillary. A process from a pericyte is seen. Type I pneumonocyte (I). Glut + OsO_4. × 8,450.

FIG. 6.—50 mg/kg ANTU. An electron dense Type I pneumonocyte (I). The Type I pneumonocyte on the other side of the alveolar wall is normal in appearance. Alveolus (alv). Glut + OsO_4. × 8,450.

FIG. 7.—50 mg/kg ANTU. A Type III pneumonocyte showing disorganized microvilli (mv) (cf. Fig. 2), and numerous fibrils (fi), many in cross-section. Oedema fluid is seen in the interstitial space (int). Alveolus (alv). Glut + OsO_4. × 8,450.

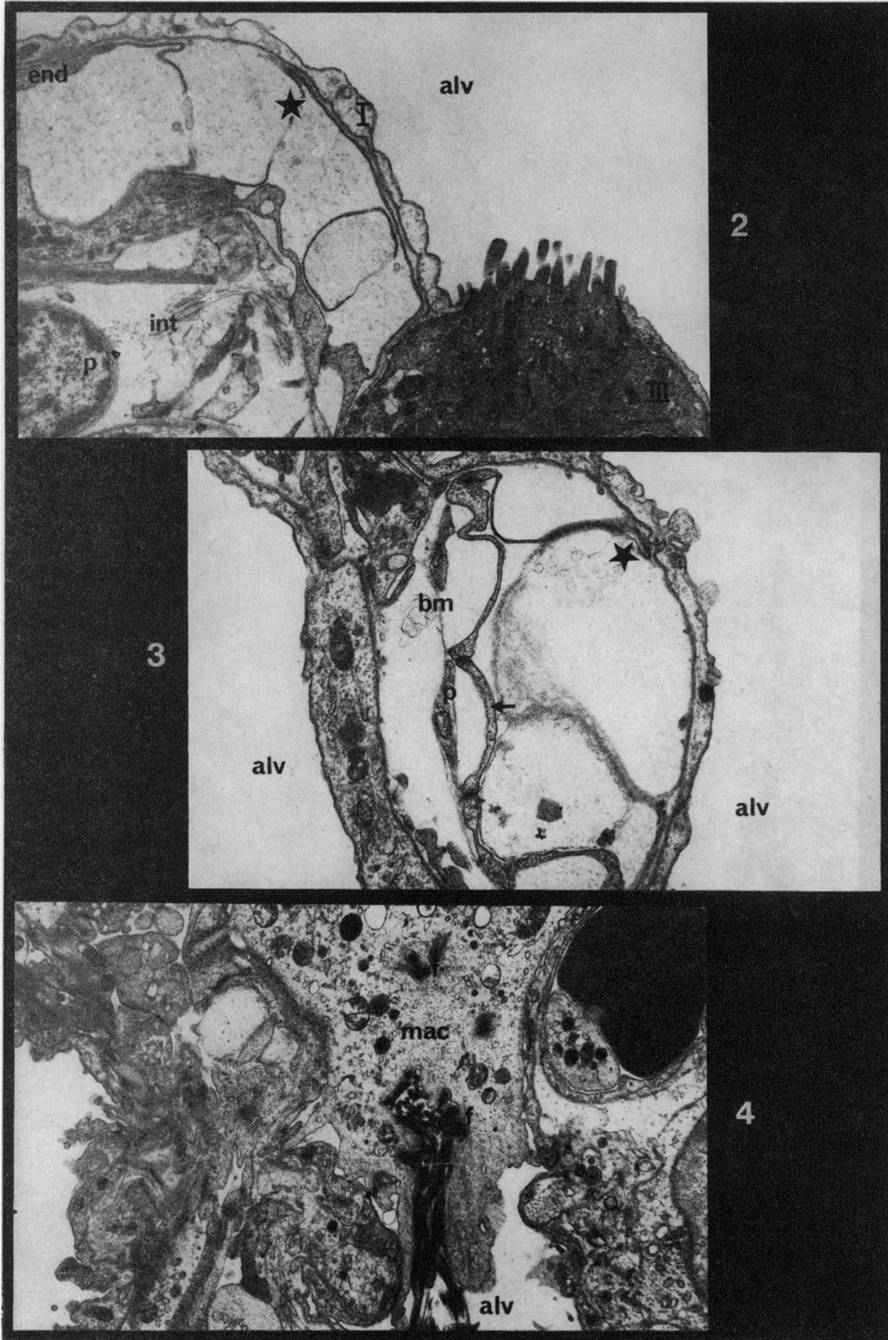
FIG. 8.—50 mg/kg ANTU. Alveolar wall showing irregularly shaped platelets (pl) within a capillary. The endothelium (end) appears very thin. There is fibrin (f) in the alveoli (alv). Type II pneumonocyte (II). Glut + OsO_4. × 5600.

FIG. 9.—50 mg/kg ANTU. Deposition of fibrin (f) on base area of alveolar wall where the epithelial lining has disappeared. Alveolus (alv). Capillary (cap). Glut + OsO_4. × 8,450.

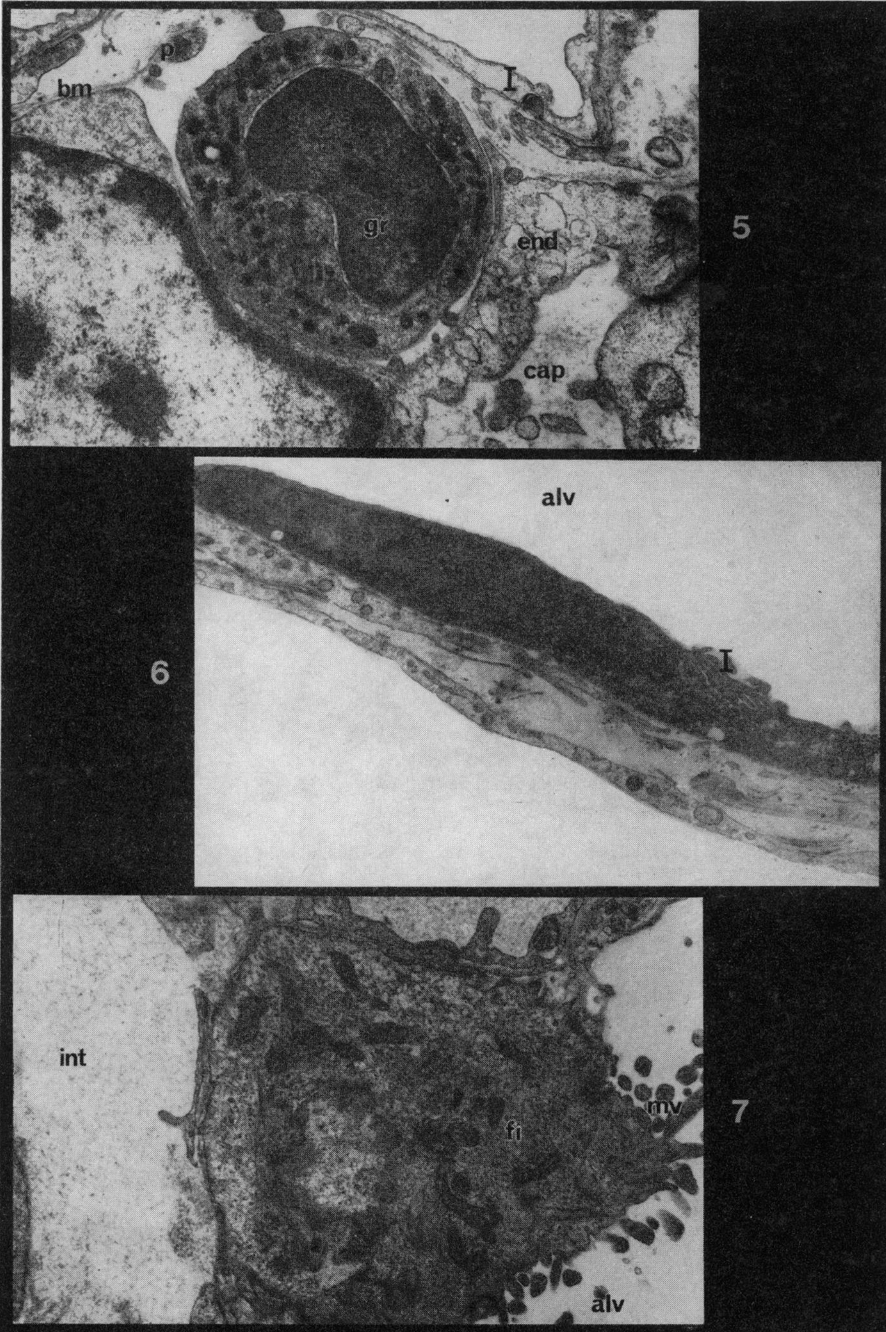
FIG. 10.—Hyperoxia. The endothelium (end) remains in contact with the capillary basement membrane (bm). There is fluid in the interstitial space (int). Alveolus (alv). Glut + OsO_4. × 9,000.

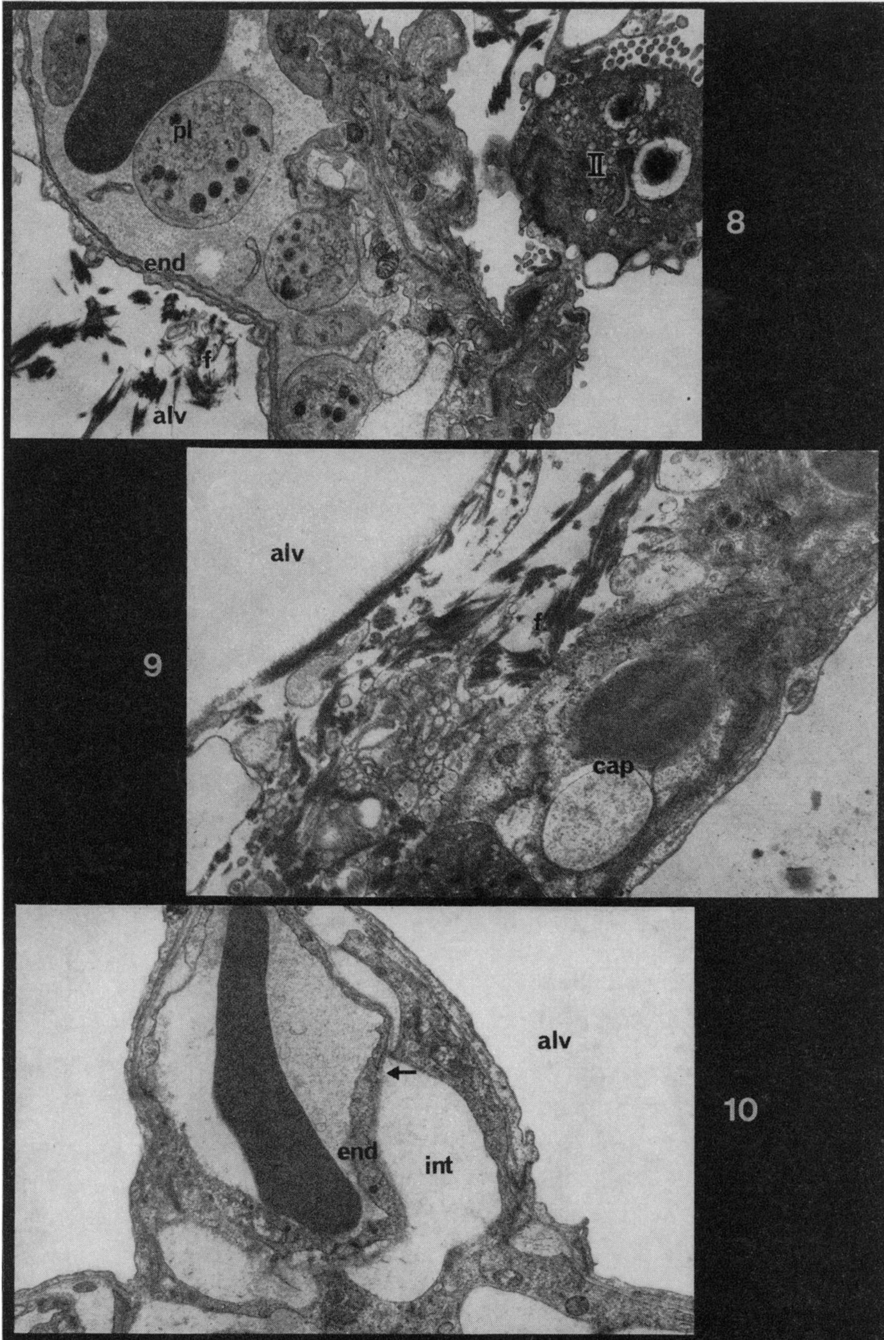
FIG. 11.—Hyperoxia. A collapsed capillary (cap) with electron dense endothelium. Alveolus (alv). Fibroblast (fib). Interstitial oedema (int). Collagen (co). Glut + OsO_4. × 3700.

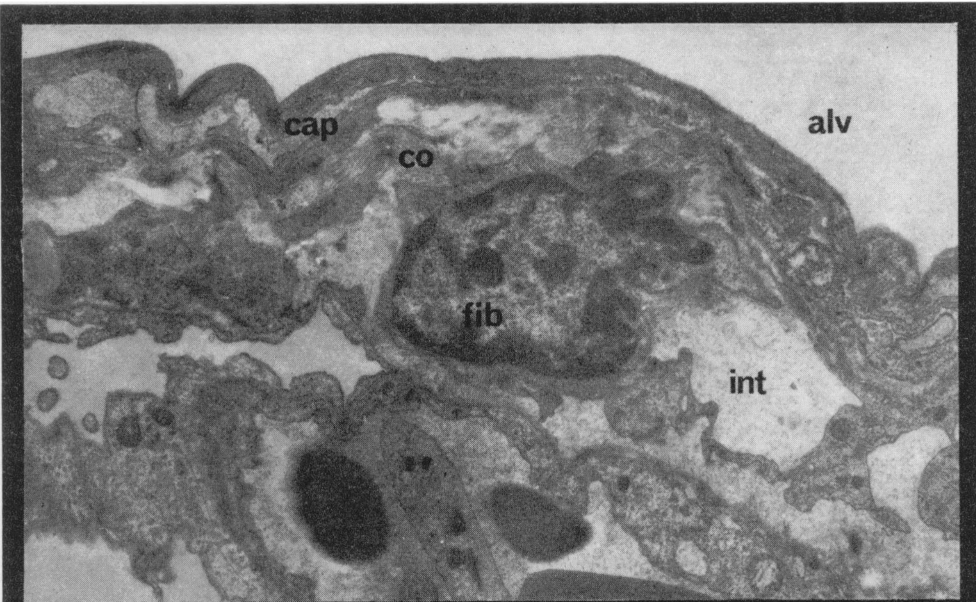
FIG. 12.—Hypoxia. Type III pneumonocyte illustrating reduction in its alveolar surface and few microvilli (mv) restricted by Type I pneumonocyte (I). Alveolus (alv). Glut + OsO_4. × 5400.

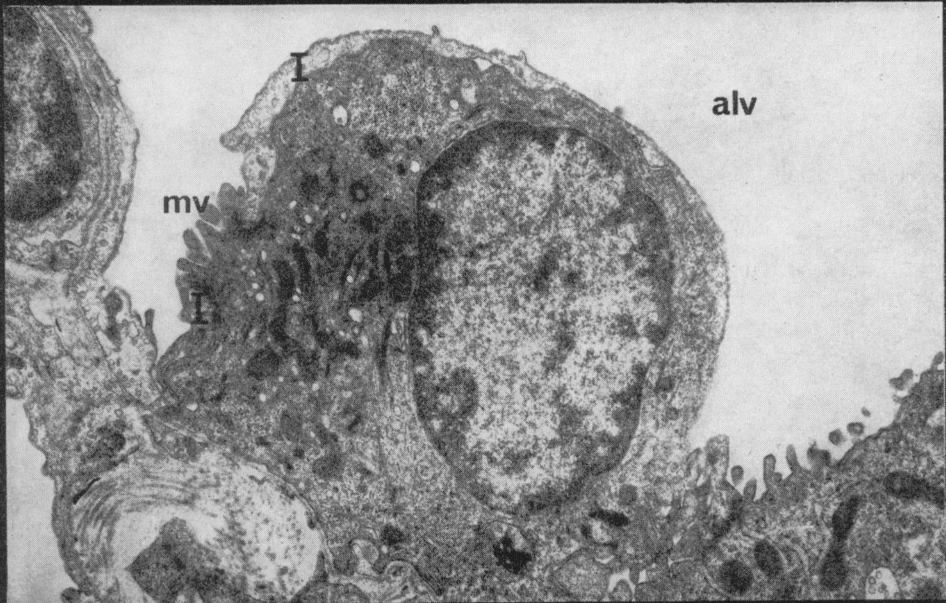


Meyrick, Miller and Reid


p
bm
I
gr
end
cap
5
alv
I
6
int
mv
fi
alv
7


pl
II
8
end
f
alv
alv
9
f
cap
alv
10
end
int


cap
co
alv
fib
int
11


I
alv
mv
I
12

dense and looked necrotic (Fig. 6). The Type II pneumonocytes contained fewer osmiophilic bodies than usual: the Type III pneumonocytes (Meyrick and Reid, 1968) had disorganized microvilli and contained vesicles in the supranuclear region where many fibrils were congregated (Fig. 7).

By 9 hours, areas of endothelium appeared electron dense and the lumen of many capillaries contained mainly irregularly shaped platelets (Fig. 8). Occasionally the alveolar surface was devoid of epithelial lining cells and fibrin had deposited over bare areas (Fig. 9); the remaining Type I pneumonocytes were pale and swollen. The Type II pneumonocytes contained dilated rough endoplasmic reticulum and the Type III pneumonocytes were still disorganized. Macrophages, lymphocytes and polymorphonuclear leucocytes were all found within the alveolar space. In summary, ANTU-induced oedema produces at 2 hours both endothelial damage and interstitial oedema, and at 6 hours epithelial damage. There was no alteration in alveolar size.

Variation in atmospheric oxygen content (hyperoxia and hypoxia)

All the animals in the experiments survived save 2, one from Experiment II and the other from Experiment IV; the former died after 4 hours in hypoxic conditions, the latter when the chamber returned to normal atmospheric air; these 2 animals were not used for electron microscopy. Microscopically the animals under hypoxic conditions retained a normal colour and on opening the chest the lungs appeared normal save for haemorrhagic foci 0·5–1·0 mm in diameter in the lung of the one that died. The animals under hyperoxic conditions appeared slightly blue and those in Experiment III had limb weakness. On opening the chest the lungs were blue.

Light microscopy

After 6 hours under hyperoxic conditions, the lungs appeared normal but after 24 hours the lungs appeared similar to those of the ANTU-treated rats in that there was eosinophilic oedema fluid around the large blood vessels and in alveoli. In one animal eosinophilic material was found in subpleural alveoli. In the hypoxic group even after 24 hours the lungs appeared normal. In both the hyperoxic and hypoxic animal lungs a significant increase in mean alveolar size was found with the light microscope (Table III, $P = < 0{\cdot}01$). With the electron microscope the alveoli were also shown to be larger, but only in the hyperoxic animals did the result achieve significance at the 5% level (Table III, $P = < 0{\cdot}05$).

TABLE III.—*Comparison Between Effects of Hypoxia and Hyperoxia (Mean Values)*

	Control	Hypoxia	Hyperoxia
Light microscope			
Alveoli/field	107·9	93·9 ($P = < 0{\cdot}01$)	91·9 ($P = < 0{\cdot}01$)
Electron microscope			
Distance/alveolar intersection	65·4	70·5 ($P = < 0{\cdot}9$)	85·3 ($P = < 0{\cdot}05$)
% Type III pneumonocytes	5·0	1·5 ($P = < 0{\cdot}8$)	1·2 ($P = < 0{\cdot}05$)
% ratio macrophages	15·2	10·3 ($P = < 0{\cdot}05$)	9·9 ($P = < 0{\cdot}05$)

Ultrastructure

The lungs after 6 hours of high oxygen concentration showed no endothelial lifting but interstitial oedema seemed to cause the endothelial cells to encroach on the capillary lumen (Fig. 10). Types I, II and III pneumonocytes and macrophages all appeared normal. After 24 hours some capillaries were empty, the damaged endothelial cells were in apposition, although the interstitial swelling was, if anything, less perhaps because the fluid had now escaped into the alveolar space. Some Type II pneumonocytes appeared more electron dense than normal (Fig. 11). The Type III pneumonocytes were normal in appearance save that the microvilli seemed fewer, most of the cell being now covered by cytoplasm from an adjacent Type I pneumonocyte and therefore with a smaller free alveolar surface area (Fig. 12). It is possible that the whole cell could be covered by cytoplasm from Type I pneumonocytes leading to the reduced number of Type III pneumonocytes counted. Type II pneumonocytes showed no recognizable changes. Proteinaceous material was seen in alveoli.

In the hypoxic group abnormality was seen only after 24 hours, this being in the Type III pneumonocytes, similar to that in the hyperoxic group. One animal lung also had a few pale swollen Type I pneumonocytes.

Quantification of alveolar cells.—The mean number of type I and type II pneumonocytes was within the normal range in both the hypoxic and hyperoxic rats but after 24 hours a decrease in the number of Type III pneumonocytes was found in both groups, significant at the 5% level only in the hyperoxic group (Table III). In both groups a significant reduction was found in the number of alveolar macrophages relating to alveolar cell number at 24 hours (Table III, $P = < 0{\cdot}05$).

It is therefore apparent that hyperoxia is capable of causing pulmonary oedema at 6 hours before the morphological changes in the endothelium or epithelium seen by 24 hours are apparent. Hypoxia, however, did not cause these changes. Both hypoxia and hyperoxia of 24-hour duration led to enlarged alveoli and reduction of Type III pneumonocytes, perhaps only because their identification was difficult due to loss of their microvilli; the number of macrophages was also reduced.

DISCUSSION

By 2 hours, pulmonary oedema had been produced with the three doses of ANTU investigated (3, 10 and 50 mg/kg) and a dose-related effect on severity was seen. With 3 mg/kg, the only dose studied at one hour, the lung looked normal; therefore at least with the sublethal dose oedema was not immediately apparent. With the light microscope, at the end of 2 hours and after each dose, oedema was seen first around blood vessels with connective tissue sheaths; only later did fluid accumulate in the alveolar space. Examination with the electron microscope, however, revealed that by 2 hours alveolar wall, or interstitial, oedema was already apparent as well as endothelial lifting and blebbing. Epithelial damage was apparent at the end of 6 hours only in the animals given 50 mg/kg. Thus it seems that in ANTU-induced oedema endothelial damage and interstitial oedema are already present when only perivascular oedema is seen in the light microscope.

Staub, Nagano and Pearce (1967) have reported that in pulmonary oedema fluid first accumulated in perivascular spaces. The findings here suggest that

oedema develops by leakage through damaged endothelial cells of the intra-alveolar blood vessels with the excess fluid draining to the perivascular spaces and, with the light microscope, being first apparent there. It is only when leakage through the endothelium is so great that this drainage route is overwhelmed, that fluid accumulates within the aolveolar wall and increases its thickness. Oedema of the alveolar space was apparent 2 hours before epithelial damage was seen in the animals given 50 mg/kg; this had become apparent between 4 and 6 hours. Again leakage through the cell, this time epithelial, was apparent before there was any morphological change in the cell. Whether the epithelial damage arises from the accumulation of oedema or from a delayed effect of the ANTU is not clear, but the fact of the delay seems to favour the former. In this study it has not been possible to distinguish between venules and capillaries, although Bohm (1966) using the light microscope claimed that with a colloidal suspension of carbon black he detected a venular leak, and Teplitz (1968) using the electron microscope and ferritin and saccharated iron oxide as a tracer, reported a capillary one.

High oxygen concentrations (99–100%) are important because of their clinical use. They have been shown to cause pulmonary oedema earlier than has been reported in recent experiments. With the light microscope the lung appeared normal after 6 hours, but electron microscopy showed intra-alveolar wall oedema with no detectable damage of endothelial or epithelial cells. Increased passage of fluid through the alveolar capillary wall had evidently occurred before damage was apparent morphologically and was not yet sufficient to cause a detectable increase in the size of the perivascular space, a change disclosed with the light microscope after 24 hours. At this time both endothelial and epithelial damage was seen with the electron microscope and there was also leakage into the alveolar space.

Other studies have not reported a change until 48 hours. Kistler *et al.* (1967) examined the lungs of 44–50 day old rats at 6, 24, 48 and 72 hours' exposure to 98·5% O_2 at one atmosphere and found no morphological changes before 48 hours, when interstitial oedema and endothelial damage was noted. Later Kapanci *et al.* (1969) reported endothelial damage at 4 days in monkeys' lungs exposed to similar conditions. Bowden, Adamson and Wyatt (1968) exposed mice to 90% O_2 at one atmosphere for 1, 2, 10 and 14 days and also found no morphological change before 48 hours. In a later study (Adamson *et al.*, 1970), details of the time of appearance of endothelial and epithelial damage were not given. In 1971, Coalson *et al.* reported both endothelial and epithelial damage in dogs after only one hour of exposure to 100% oxygen administered by a ventilator. From our studies it seems that with the oedema induced by ANTU as well as by high levels of oxygen, the endothelial cell is the susceptible one, being the site of the earliest damage.

Although hypoxia is known clinically to produce pulmonary oedema in animals, no evidence of interstitial oedema or endothelial or epithelial damage was found with light or electron microscopy in these experiments, perhaps because the hypoxic conditions (7 or 10% O_2 at one atmosphere) was not severe enough or the duration too short. The animals appeared distressed initially, but adjusted to the conditions in a short time. Schulz (1959) reported endothelial blebbing by 90 min at 5000 m (this content of oxygen is approximately equal to 10–11% O_2 at one atmosphere), but these results could be attributed to the reduction in atmospheric pressure. Reidbord and Spitz (1966) showed endothelial vacuolization after 25 min in an enclosed chamber, but since no control of CO_2 was made, its increase

could be responsible, since Schaefer, Avery and Bensch (1964) reported alveolar oedema and an increase in lung weight in guinea-pigs exposed to 15% CO_2 for one hour at 1 atm. The more extreme damage reported by Kisch (1965) was not encountered.

A significant increase in alveolar size was found in both oxygen groups. Oedema itself does not cause this change since the animals given ANTU were similar to the normal. Since oedema reduces surfactant activity and causes a rise in surface tension (Adamson *et al.*, 1970), reduced alveolar size would be expected. It seems likely, therefore, that these animals were breathing at increased lung volumes during the experiment so that some change in the tissue of the alveolar wall had occurred. If bronchoconstriction were part of the response to abnormal oxygen levels (Milic-Emili and Ruff, 1971), this could explain these changes. In man, bronchial spasm has been reported (Greene, 1965) and increase in resistance to airflow (Sharp *et al.*, 1958) but there seems to be a species difference since in dogs (Cook *et al.*, 1959) and guinea-pigs (Rech and Borison, 1962) it was not evident. Such a mechanism in the rat would explain our findings. It is also of interest that in 1934, Prinzmetal produced " emphysema " in rats by giving them low oxygen levels to breathe.

The effect reported here was in adult animals and is different from the effect on the growing lung. Burri and Weibel (1971) reported that when rats are exposed to hypoxic and hyperoxic conditions from the 23rd to the 44th day of life, the lung volumes increase and decrease respectively.

The change in alveolar size seems to be not necessarily part of the changes seen in oedema, nor is the decrease in number of macrophages and Type III pneumonocytes, since none of these was seen in the ANTU-induced oedema. The Type III pneumonocytes in the animals given ANTU did, however, show an increase in fibrils and had irregular microvilli at their apical edge; an increase in vesicles was also seen in some cells. Since in some cases the fibrils, especially within the microvilli, appear as microtubules, it may be that these cells are concerned with the fluid balance within the alveoli. An increase in fibrils was also seen in brush cells in airways. A significant decrease in number of Type III pneumonocytes was found only in both oxygen groups. Electron microscopic examination suggests that the decrease in number of Type III cells may be apparent rather than real and may be caused by disappearance of microvilli and extension of the cytoplasmic covering from an adjacent Type I pneumonocyte over the apical alveolar surface of the Type III cell. This suggests that normal oxygen levels are necessary for the normal functioning of this cell.

The reason for the decrease in macrophages is not clear, It may only reflect the increase in alveolar size, a normal number of macrophages being distributed through an increased volume.

The pattern of breathing, bradypnoea followed by tachypnoea, needs further analysis. The slow breathing was not expected, but may be caused by a reflex action in the airways or an accentuation of shock. From the clinical picture of oedema, the rapid breathing would be expected and may arise from stimulation of Type J receptors in the alveolar wall (Paintal, 1970; Meyrick and Reid, 1971); the reason for its delay is not clear.

We would like to thank the Royal Naval Physiological Laboratory, Alverstoke, Hants. (Director: Dr V. Hempleman), for allowing us to use their facilities for the

hyperoxic and hypoxic experiments. We are also indebted to Professor B. Benjamin, Director of Statistical Studies at the Civil Service College, for his help with the statistical analysis.

REFERENCES

ADAMSON, I. Y. R., BOWDEN, D. H. & WYATT, J. P. (1970) Oxygen Poisoning in Mice. Ultrastructural and Surfactant Studies during Exposure and Recovery. *Archs Path.*, **90**, 463.

ALEXANDER, I. G. S. (1968) The Ultrastructure of the Pulmonary Alveolar Vessels in Mendelson's (Acid Pulmonary Aspiration) Syndrome. *Br. J. Anaesth.*, **40**, 408.

BOHM, G. M. (1966) Vascular Permeability Changes During Experimentally Produced Pulmonary Oedema in Rats. *J. Path. Bact.*, **92**, 151.

BOWDEN, D. H., ADAMSON, I. Y. R. & WYATT, J. P. (1968) Reaction of the Lung Cells to a High Concentration of Oxygen. *Archs Path.*, **86**, 671.

BURRI, P. H. & WEIBEL, E. R. (1971) Morphometric Estimation of Pulmonary Diffusion Capacity. II. Effect of PO_2 on the Growing Lung. *Resp. Physiol.*, **11**, 247.

COALSON, J. J., BELLER, J. J. & GREENFIELD, L. J. (1971) Effects of 100 per cent Oxygen Ventilation on Pulmonary Ultrastructure and Mechanics. *J. Path. Bact.*, **104**, 267.

COOK, C. D., MEAD, J., SCHREINER, G. L., FRANK, N. R. & CRAIG, J. M. (1959) Pulmonary Mechanics During Induced Oedema in Anaesthetized Dogs. *J. appl. Physiol.*, **14**, 177.

COTTRELL, T. S., LEVINE, O. R., SENIOR, R. M., WEINER, J., SPIRO, D. & FISHMAN, A. P. (1967) Electron Microscopic Alterations at the Alveolar Level in Pulmonary Odeema. *Circulation Res.*, **21**, 783.

DALLDORF, F. G., BEALL, F. A., KRIGMAN, M. R., GOYER, B. A. & LIVINGSTON, H. L. (1969) Transcellular Permeability and Thrombosis of Capillaries in Anthrax Toxaemia. An Electron Microscopic and Biochemical Study. *Lab. Invest.*, **21**, 42.

GREENE, G. D. (1965) In *Respiration*, vol. II, Ed. W. O. Fenn and H. Rahn. Washington, D.C.: Am. Physiol. Soc. p. 1585.

HAYES, J. A. & SHIGA, A. (1970) Ultrastructural Changes in Pulmonary Oedema Produced Experimentally with Ammonium Sulphate. *J. Path. Bact.*, **100**, 281.

HESSE, F. E. & LOOSLI, C. G. (1949) The Lining of the Alveoli in Mice, Rats, Dogs and Frogs Following Acute Pulmonary Oedema Produced by ANTU Poisoning. *Anat. Rec.*, **105**, 299.

KAPANCI, Y., WEIBEL, E. R., KAPLAN, H. P. & ROBINSON, F. R. (1969) Pathogenesis and Reversibility of the Pulmonary Lesions of Oxygen Toxicity in Monkeys. *Lab. Invest.*, **20**, 101.

KARNOVSKY, M. J. (1961) Simple Methods for "Staining with Lead" at High pH in Electron Microscopy. *J. biophys. biochem. Cytol.*, **11**, 729.

KISCH, B. (1965) Electron Microscopy of Capillary Haemorrhage in the Lungs. *Expl Med. Surg.*, **23**, 117.

KISTLER, G. S., CALDWELL, P. R. B. & WEIBEL, E. F. (1967) Development of Fine Structural Damage to Alveolar and Capillary Lining Cells in Oxygen-poisoned Rat Lungs. *J. Cell Biol.*, **32**, 605.

LATTA, H. (1947) Pulmonary Edema and Pleural Effusions Produced by Acute Poisoning in Rats and Dogs. *Bull. Johns Hopkins Hosp.*, **80**, 181.

LOW, F. N. (1953) Pulmonary Alveolar Epithelium of Laboratory Mammals and Man. *Anat. Rec.*, **117**, 241.

MEYRICK, B. & REID, L. (1968) The Alveolar Brush Cell in Rat Lung—a Third Pneumonocyte. *J. Ultrastruct. Res.*, **23**, 71.

MEYRICK, B. & REID, L. (1970) The Alveolar Wall. *Br. J. Dis. Chest*, **64**, 121.

MEYRICK, B. & REID, L. (1971) Nerves in Rat Intra-acinar Alveoli: an Electron Microscopic Study. *Resp. Physiol.*, **11**, 247.

MILIC-EMILI, J. & RUFF, F. (1971) Effects of Pulmonary Congestion and Edema of the Small Airways. *Bull. Physio-path. Resp.*, **7**, 1181.

PAINTAL, A. S. (1970) In *Breathing.* (Hering-Breuer Centenary Symposium, Ciba Foundation, London). London: Churchill. p. 59.

PRINZMETAL, M. (1934) Relation of Inspiratory Distension of Lungs to Emphysema. *J. Allergy*, **5**, 493.

RECH, R. H. & BORISON, H. L. (1962) Vagotomy-induced Pulmonary Edema in the Guinea-pig. *Am. J. Physiol.*, **202**, 499.

REIDBORD, H. E. & SPITZ, W. U. (1966) Ultrastructural Alterations in Rat Lungs. *Archs Path.*, **82**, 80.

REYNOLDS, E. S. (1963) The Use of Lead Citrate at High pH as an Electron-opaque Stain in Electronmicroscopy. *J. Cell Biol.*, **17**, 208.

RICHTER, C. P. (1952) The Physiology and Cytology of Pulmonary Edema and Pleural Effusion Produced in Rats by Alpha-naphthylthiourea (ANTU). *J. thorac. Surg.*, **23**, 66.

SCHAEFFER, K. E., AVERY, M. E. & BENSCH, K. (1964) Time Course of Changes in Surface Tension and Morphology of Alveolar Epithelial Cells in CO_2-induced Hyaline Membrane Disease. *J. clin. Invest.*, **43**, 2080.

SCHULZ, H. (1959) In *The Submicroscopic Anatomy and Pathology of the Lung.* Berlin: Springer.

SHARP, J. T., GRIFFITHS, G. T., BUNNELL, I. L. & GREENE, D. G. (1958) Ventilatory Mechanics in Pulmonary Oedema in Man. *J. clin. Invest.*, **37**, 111.

STAUB, N. C., NAGANO, H. & PEARCE, M. L. (1967) Pulmonary Edema in Dogs, especially the Sequence of Fluid Accumulation in Lungs. *J. appl. Physiol.*, **22**, 227.

TEPLITZ, C. (1968) The Ultrastructural Basis for Pulmonary Pathophysiology Following Trauma. *J. Trauma*, **8**, 700.