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PULMONARY OEDEMA: ITS DEVELOPMENT AND EFFECT ON THE
DISTRIBUTION OF VENTILATION AND BLOOD FLOW

by

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ABSTRACT

This thesis describes a series of experiments undertaken to investigate some aspects of the pathogenesis of pulmonary oedema and the functional changes associated with its development. Studies were made in anaesthetised dogs, isolated dog lungs and in man. The isolated lung series afforded the opportunity of investigating the effect of a range of vascular and alveolar pressures on the rate of fluid accumulation during normoxia and hypoxia, and to correlate the results with histological evidence. The results showed that direct fluid leakage from larger vessels (arteries up to 200 μ and veins up to 750 μ in diameter) made a significant contribution to oedema development. Hypoxia caused an increase in the arterial contribution and was invariably accompanied by peri-arterial haemorrhage. It is suggested that random sites of micro-rupture in arteriolar walls may be the main source of fluid in hypoxic pulmonary oedema.

In a further series it was shown that inducing small known amounts of oedema in vertical isolated lungs led to diversion of ventilation and blood flow away from the dependent regions. These effects on basal ventilation and blood flow were exaggerated at low lung volumes and partially reversed at higher lung volumes. It is indicated that the changes were brought about by interstitial oedema fluid surrounding conducting airways and vessels, causing closure of small airways and narrowing of extra-alveolar vessels. Similar changes were found in the distribution of ventilation and blood flow in a group of patients with liver cirrhosis, thought to have interstitial oedema. It is suggested that studies of regional pulmonary ventilation and blood flow, together with measurements of overall gas exchange, may provide a means of diagnosing the early stages of oedema in patients.

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CHAPTER I

INTRODUCTION

Pulmonary oedema is a pathological state in which there is extra-vascular accumulation of fluid in the lung. In its acute stage it results in gross impairment of ventilation and diffusion and can be a direct or contributory cause of death. Two situations where lung oedema can itself be the cause of death are hypoxia of high altitude and inhalation of irritant gases. More often, however, it is a complication of other diseases. Table I (Cameron, 1948) illustrates the diversity of diseases which were identified at autopsy in one hundred cases of pulmonary oedema. Table II (also from Cameron) shows the frequency at which lung oedema occurs in certain selected diseases. Since pulmonary oedema is associated with so many conditions, it is important to investigate its pathogenesis so that its treatment or prevention can be more effective.

Fluid Exchange in the Pulmonary Circulation

General considerations : (See Addendum at back of thesis)

Starling's Law (1896) governs the bulk movement of water between capillaries and the surrounding tissues. It states that water passes out of the capillaries (filtration) as a result of capillary hydrostatic pressure, and passes into the capillaries (absorption) as a result of the osmotic pressure of the plasma proteins. A modified version of Starling's Law is given below. It takes not only the capillary parameters into account, but also the hydrostatic and osmotic pressures of the liquid surrounding the capillary. The forces favouring filtration have a plus (+) sign, and those favouring absorption a minus (-) sign.

TABLE I

Main necropsy findings in 100 cases of pulmonary edema*

<u>Pathology</u>	<u>No. of cases</u>
Severe coronary disease	34
Congestive cardiac failure	32
Carcinoma of various sites, including lung	27
Wasting, moderate	25
Wasting, severe	19
Bronchopneumonia	23
Hypertensive disease	18
Chronic bronchitis alone	14
Carcinoma of liver	11
Obstruction of pulmonary veins by carcinoma	11
Massive pulmonary embolism	10
Cerebral hemorrhage	9
Cerebral tumor	7
Tuberculosis	6
Cirrhosis of liver	6
Fractured skull	3
Multiple fractures (excluding skull)	2

*Cameron, G.R., 1948.

TABLE II

Frequency of pulmonary edema in 500 necropsies of cardiovascular disease, cerebral injury, and multiple fractures*

<u>Pathology</u>	<u>Total no. of cases</u>	<u>No. showing pulmonary edema</u>
Hypertensive disease (excluding chronic nephritis)	94	81 (86%)
Chronic nephritis	50	37 (74%)
Complete coronary obstruction	66	45 (68%)
Cerebral hemorrhage	66	44 (67%)
Mitral stenosis	84	55 (65%)
Fractured skull	38	24 (63%)
Multiple fractures (excluding skull)	28	17 (61%)
Pulmonary embolism	<u>74</u>	<u>23 (31%)</u>
Total	500	326 (65%)

*Cameron, G.R., 1948.

When these forces are balanced, then there is no nett fluid movement.

$$\text{Fluid movement} = k (P_c - \pi_{pla} - P_{il} + \pi_{il})$$

where k = filtration coefficient (ml/sec/cm²/mm Hg), P_c = capillary hydrostatic pressure (mm Hg), π_{pla} = osmotic pressure of plasma (mm Hg), P_{il} = pericapillary or interstitial liquid hydrostatic pressure (mm Hg), and π_{il} = osmotic pressure of the interstitial liquid (mm Hg).

Work by Guyton and Lindsay (1959) indicates that Starling's Law of transcapillary fluid exchange can also be applied to the pulmonary circulation. They found that the rate of fluid accumulation in the extravascular lung tissue was directly related to the intravascular forces ($P_c - \pi_{pla}$) and that pericapillary forces had a negligible effect. In contrast, Levine et al (1965 and 1967) demonstrated in dogs that the pericapillary forces, particularly the interstitial liquid pressure, significantly influenced fluid accumulation. Later experiments from the same laboratory (Mellins et al, 1969) showed a relationship between oedema fluid formation and the forces in the Starling equation when pleural pressure was included as an index of interstitial liquid pressure. Therefore, Starling's Law may well be applicable to the lung circulation, in that there is in the normal situation a balance of forces between blood inside the vessels and the fluid surrounding them, so that net movement of fluid in or out of the vessels is minimal.

Work published to date, however, does not clearly define the application of Starling's Law to the lung because, though capillary pressure and the plasma osmotic pressure are to some extent known (7 and 25 mm Hg respectively), the magnitude and influence of the extravascular forces are not yet established. For example, interstitial liquid pressure has only been estimated indirectly. In dog experiments in

which fluid accumulation was studied while varying the parameters in the Starling equation, Levine et al (1957) estimated P_{il} to be of the order of -9mm Hg. Meyer et al (1968) implanted perforated hollow capsules in the lungs of dogs, and some weeks later measured a mean pressure of around -6mm Hg within them. They considered this pressure to be representative of interstitial liquid pressure because there was free fluid interchange between the capsule and the surrounding lung tissue, but again, this was only an indirect measurement of interstitial liquid pressure. During oedema development the pressure within the capsules became positive. The other unknown extravascular force is the osmotic pressure of the interstitial liquid (π_{il}). This has been assumed to be the same as pulmonary lymph (5mm Hg) because no direct sampling of interstitial liquid has been possible.

Lymphatics and lymph flow

Miller (1947) has suggested that the lung's accessibility to the environment, and therefore its susceptibility to invasion by foreign bodies, may be the teleological reason for its otherwise excessive lymph system. Lymph vessels enter the lung at the hilum and form branching channels in the interstitial tissue surrounding pulmonary bronchi, arteries and veins. There is also an extensive lymph network in the subpleural connective tissue which, in man, is linked with the deeper lymph vessels by channels running in the interlobular septa (von Hayek, 1960). Those lymph vessels associated with the airways lie external to the muscularis and between it and the accompanying arteriole. A second lymph network lies between the fibrous and muscular layers, amongst the bronchial veins. The peribronchial lymph vessels extend peripherally as far as the alveolar ducts, but are absent in the alveolar septa (Drinker, 1945; von Hayek, 1960;

Cottrell, 1967). Also associated with the airways are the lymph nodes, which are predominantly along the tracheal tree and at the branching angles. Arterial lymphatics lie between the arteries and adjoining lung parenchyma and are considered to extend peripherally to the alveolar septa junctions. The venous lymphatics are not distinct in man but in dogs they form discreet channels and are in communication with the peri-bronchiolar lymphatics (Miller, 1911).

Since the alveolar septa lack lymph vessels, fluid from the capillaries was thought by Drinker (1945) to enter the lymph vessels only when the septa had become engorged. Simple valves in the lymph channels direct the fluid towards the hilum, where it drains via the right lymphatic duct into the right subclavian vein. The mechanism of lymph flow is not clearly understood, but it is possible that negative pressure in the subclavian vein during inspiration, together with the pumping action of the respiratory excursions, provide the means of propulsion. Drinker (1945) and Uhley et al (1961), collecting lymph from the right lymphatic duct in dogs, showed that normally pulmonary lymph flow is minimal. It was increased by ventilation at high tidal volumes, and during pulmonary oedema development. Possibly the latter was due to an increased driving pressure associated with a positive interstitial liquid and therefore lymph pressure in the lung.

Pathogenesis of pulmonary oedema

As the Starling equation and experimental evidence suggest, there is normally a balance of forces such that nett fluid transfer into or out of the pulmonary vessels is slight and well within the capacity of the lymphatic drainage system. It is also evident from the discussion of transcapillary fluid exchange in the lung that any event which brings about a prolonged increase in the rate of capillary filtration is a potential cause of pulmonary oedema. I shall consider only the three

main factors which result in increased capillary filtration, and emphasise the first because it is considered to be the cause of "haemodynamic oedema" which is the main subject of this thesis.

1. A rise in capillary hydrostatic pressure.
2. A decrease in the osmotic pressure of the plasma proteins.
3. An increased filtration coefficient.

These factors, particularly the first and third, interact. For example, a rise in hydrostatic pressure may cause an increase in the number of capillaries open (Hall, 1953) or capillary distension (Glazier et al, 1969), both of which would result in an increased vessel wall area for filtration. Also increasing filtration would be a fall in the osmotic pressure of the plasma proteins. One situation where an altered filtration coefficient by itself might be considered the cause of pulmonary oedema is during lung tissue damage (and therefore altered capillary permeability characteristics) resulting from inhalation of toxic gases.

Taking the first factor separately now; capillary hypertension can be brought about in a number of ways, of which left ventricular failure is perhaps the most common. Left atrial pressure (usually around 4 mm Hg) rises and the increased pressure is reflected back via the pulmonary veins to the capillaries. There will be a corresponding rise in pulmonary arterial pressure and if this generalised pulmonary hypertension is prolonged, besides causing pulmonary oedema, it can lead to right heart failure. The second factor, a lowered plasma osmotic pressure, is often caused by an upset in the water or ion balance of the body and is usually associated with an increased blood volume, which in turn may contribute to systemic and pulmonary

hypertension. In this way the first and second factors may interact.

Pulmonary capillaries have been the accepted site of fluid exchange and therefore pathogenesis of lung oedema, but evidence is accumulating that larger vessels may also be involved. They are, after all, subjected to all the intra and extravascular forces that capillaries are, though naturally the intravascular hydrostatic pressures will be different in arterioles and venules from capillaries. Also, in larger vessels the wall thickness may be the main factor limiting fluid exchange.

In the systemic circulation, Landis (1954, 1963) and Courtice and Simmonds (1949) have shown there is a gradient of capillary hydrostatic pressure from the arterial to the venous side. One might expect, therefore, tissue fluid to be produced at the arterial end and reabsorbed at the venous end of the capillary, assuming uniform permeability along its length. However, Rous et al (1950) showed that, despite the hydrostatic gradient, water soluble dyes pass with greater ease through the low pressure venous end than the arterial end of the capillary, indicating a gradient of permeability along its length. A similar gradient of permeability was shown by Böhm (1966) in the pulmonary circulation. Venules were found to be more permeable than capillaries when lung oedema was induced experimentally by alpha-naphthyl thiourea (A.N.T.U.) administration in rats. Since A.N.T.U. is thought to act specifically in increasing capillary permeability, the interpretation of these results may not be clear cut. There is, however, a possibility that venules as well as capillaries may be involved in fluid exchange in the lung, and therefore contribute towards oedema fluid formation. What about pulmonary arterioles which in the lung are particularly thin walled?

An in vivo situation in which the arterioles may be the principal site of fluid transudation rather than capillaries is oedema of high altitude. The syndrome of altitude oedema was first described by Hurtado in 1937 and since then hundreds of cases have been recorded. It seems to occur most commonly in young people 12 to 36 hours after rapid ascent to altitudes over 10,000 feet. Residents of altitude, returning after a short stay at sea level, are particularly susceptible. Cases which have undergone cardiac catheterisation have shown that although the pulmonary arterial pressure was grossly elevated, left atrial pressure remains low and pulmonary venous wedge pressures indicate a normal hydrostatic pressure in the capillaries (Fred et al, 1962; Hultgren et al, 1964). Drinker (1945) and others have suggested that hypoxic oedema is brought about by a direct toxic effect on the capillary walls, making them abnormally permeable. Other studies (Henry et al, 1947; Goodale et al, 1970) do not consider this to be the case. Only when anoxia was extreme (15-20 mm Hg) was the permeability of the capillaries to albumin increased. Oedema of altitude therefore arises from a situation of arterial hypertension without capillary hypertension, and apparently little direct effect of hypoxia on capillary permeability. Could the hypertension (which is generally thought to be caused by arteriolar constriction) give rise to fluid transudation upstream of the constriction site; namely from the arterioles and small arteries? A preliminary report by Whayne and Severinghaus (1968) suggests that pulmonary arterial hypertension could well lead to oedema formation. They blocked the terminal bed of isolated rat lungs with polystyrene microspheres and then raised the arterial pressure to 100 mm Hg. Fluid accumulation in the interstitial space was demonstrated after ten minutes.

Sequence of fluid accumulation in the lungs

The sequence of fluid accumulation in the lung has conventionally been considered to be firstly in the alveolar septa, followed by filling of the interstitial space surrounding progressively larger and larger vessels and airways (Drinker, 1945). Since the fluid was thought to originate from the alveolar capillaries, then it was expected that alveolar oedema would occur early in the sequence. More recently, Staub et al (1967) have shown that alveolar oedema is a feature of the later stages of oedema development. In histological studies of lungs rapidly frozen at a variety of stages of oedema development, Staub et al (1967) showed that oedema fluid accumulation was first detectable in the interstitial connective tissue surrounding extra-alveolar vessels and airways. This occurred before there was any evidence of alveolar oedema, or even thickening of the alveolar walls. These authors did not suggest that the extra-alveolar vessels might be the site of fluid origin, but indicated that the preferential fluid flow into their surrounding interstitial spaces may have resulted from the negative pressure there described by Meyer et al (1960). Lymph vessels, present in the perivascular/^{and}peribronchial interstitial tissue, transport the fluid to the hilum and complete the drainage of excess fluid from the lung. Staub et al (1967) emphasised that, only in the event of lymphatic drainage being insufficient to keep pace with oedema fluid formation, would fluid begin to fill alveoli.

Investigations described in Chapters II and III

The studies described in Chapters II and III are concerned with the investigation of many of the subjects covered in this section entitled "Fluid Exchange in the Pulmonary Circulation". In Chapter II the experiments were designed to determine whether leakage from arterioles might be the primary site of fluid origin in pulmonary oedema of high

altitude. Dogs were studied under anaesthesia whilst altitude was simulated by the administration of a hypoxic gas mixture (10% oxygen in nitrogen). Preceding the hypoxic period, the capillaries of the left lung were functionally separated from their arteries upstream by micro-sphere embolisation on that side. In response to hypoxia, pulmonary arterial hypertension developed and was maintained for two hours. At the conclusion of the experiments, biopsies from the lungs were studied histologically to determine the degree of oedema development. Perivascular oedema fluid cuffing in the right, patent lung was compared with that in the left lung, where capillary flow and possible hypertension were absent. Thus the presence of oedema in the left lung would indicate that its origin was upstream from the embolisation site; namely from arterioles or small arteries.

In Chapter III the development of oedema has been studied in isolated dog lungs, both during normoxia and hypoxia. By controlling the relationships between alveolar and vascular pressures (such that the former exceeded the latter), the capillaries were functionally separated both from the arterial and venous compartments. Continuous weighing of the lung during experiments allowed the rate of fluid transudation from each vascular compartment to be estimated independently. Thus the relative contributions were determined of arteries, capillaries and veins to oedema formation in the lung under given static vascular and alveolar pressures. The sizes of the vessels involved in fluid exchange were investigated by mixing a water soluble dye (Evans Blue) with the blood so that the passage of water out of vessels could be detected histologically. Vascular and alveolar pressures were varied from experiment to experiment to determine how changes in either the intra or the extravascular hydrostatic pressure parameters in the Starling equation would affect the rate of fluid transudation across the walls of

extra-alveolar vessels. Lung biopsies, taken at intervals, enabled correlations to be made between oedema development (as assessed by weight gain) and the histological appearance of the tissue. Thus the sequence of fluid accumulation in the lung was studied under static and flow conditions and during normoxia and hypoxia.

Functional defects arising from the presence of pulmonary oedema

The most important effect of pulmonary oedema is impairment of gas exchange. A number of workers studying gas exchange during the induction of oedema in dogs have shown either by measuring the diffusing capacity of carbon monoxide (Said et al, 1964; Staub et al, 1967), or by the breathing of low oxygen mixtures (Williams, 1955) that diffusion impairment is not the main cause of arterial hypoxaemia. The histological studies of Staub et al (1967) support this finding in that they showed minimal thickening of the blood/gas barrier by oedema fluid. On the basis of measurements of venous admixture during oxygen breathing, both Williams and Said et al attributed the arterial hypoxaemia to the presence of large pure shunts. The causes of the shunt, however, were different. Williams suggested it was due to oedema fluid filling of alveoli and airways. Said et al, on the other hand, must have been studying a much less acute stage of oedema development because they found that the shunt could be reversed by ventilation at high tidal volumes. They interpreted this to indicate that blood was flowing through atelectatic regions of the lungs which could be reopened by hyperinflations. Atelectasis can be considered to be an extreme case of ventilation/perfusion inequality which appears to accompany the early stages of oedema development. At the acute stage, gross and irreversible impairment of gas exchange is essentially due to the absence of ventilation. Another cause of ventilation/perfusion inequality, and hence arterial hypoxaemia, could be a change in the regional distribution of blood flow.

West et al (1965) showed reduced blood flow in the dependent regions of vertical isolated lungs in the presence of interstitial oedema.

Interrelated with impairment of gas exchange, and possibly causing a reduction in ventilation, are changes in the mechanical properties of lungs. In the presence of oedema in dogs and isolated lungs, Cook et al (1959) showed a fall in dynamic pulmonary compliance which was accompanied by a reduction in static compliance during inspiration in the tidal breathing range. When oedematous lungs were inflated with fluid (thus minimising the surface forces), Cook et al found that the pressure/volume characteristics of these lungs were not significantly different from normal fluid filled lungs. They therefore suggested that the difference in elastic properties between air filled normal and oedematous lungs was a function of surface tension forces being altered by the presence of alveolar oedema, such that lung units became unstable at higher volumes than normal. Airways resistance was found to increase only in the presence of oedema fluid in the large airways (Sharp et al, 1958; Levine et al, 1965). Hughes et al (1970) showed that interstitial oedema caused an increase in gas trapping in isolated lungs. They attributed this to peribronchiolar oedema fluid interfering with the normal distending effect on small airways of the expanded lung parenchyma, so that airway closure occurred at higher lung volumes than in the absence of oedema. Such closure of lung units may in fact be the same phenomenon observed by Said et al (1964) in the early stages of oedema formation.

Detection and quantitation of pulmonary oedema

In the clinical situation it is difficult to obtain meaningful measurements to quantitate the severity of pulmonary oedema. Current methods include auscultation of the chest, chest X-rays, and,

more recently, multiple indicator dilution techniques (Levine et al, 1965; Chinard, 1969) and chest impedance measurements (Severinghaus, 1971) to determine lung water. But, of these methods, auscultation and chest X-rays are only qualitative, and only then in the presence of gross oedema. Multiple indicator dilution techniques are to some degree quantitative in that they assess the relative amounts of fluid in the intra and extravascular compartments. The errors, however, are so great that in fact only gross oedema can reliably be detected. The same applies to chest impedance measurements, which assess the total fluid in the chest. There is therefore at present no reliable clinical method of detecting pulmonary oedema in its early stages, when the amounts of excess extravascular fluid are small.

Animal studies allow some degree of oedema quantitation and these include wet to dry lung weight ratios (Uhley et al, 1961), microscopic observation of autopsy material (Drinker, 1945) and continuous weighing of excised lungs (Jagner et al, 1961) or open chest preparations (Sarnoff et al, 1955). Oedema has also been evaluated experimentally by the accompanying fall in lung compliance (Cook et al, 1959) and impaired gas exchange (Williams, 1955; Said et al, 1961). In intact dogs, Drinker (1945) developed a technique, later modified by Uhley et al (1961) for the collection of lymph from the right thoracic duct whose main source of lymph is the lung. This might be considered the most meaningful of all the methods of quantitation.

Investigations described in Chapters IV and V

The studies described in Chapters IV and V are concerned

with the physiological effects of early pulmonary oedema; in particular its effect in changing the distribution of ventilation and blood flow and lung mechanics. Could such changes be used to diagnose early pulmonary oedema?

In Chapter IV experiments are described in which Xenon-133 was used to measure the distribution of ventilation and blood flow in isolated dog lungs in the vertical position. Measurements were made both before and after inducing controlled amounts of interstitial oedema by raising pulmonary venous pressure. The effect of changing lung volume on the distribution of ventilation and blood flow in the presence of oedema was also investigated. Inspiratory and expiratory pressure/volume curves, made before and after oedema induction, gave information on changes in lung mechanics. Further information on the effect of oedema on lung mechanics was obtained by inflating the lung at slow and fast rates during the measurements of ventilation distribution. End expiratory lung gas volumes, measured both by spirometry and the concentration of Xenon in the expired gas, indicated the degree of gas trapping in the lung associated with changes in mechanics.

In Chapter V studies are described in which Xenon-133 was used to measure the distribution of ventilation and blood flow in patients with liver cirrhosis, who were thought to have early pulmonary oedema. All the patients were suffering to some extent from arterial hypoxaemia and it was therefore of interest

to determine whether gross mismatching of ventilation and blood flow might be the main cause of their impaired gas exchange. The patients' total lung capacities were estimated by gas dilution and therefore it was possible to demonstrate by spirometry the amount of gas remaining in the lung at functional residual capacity and residual volume. These volumes indicated the degree of gas trapping, which was then correlated with the distribution of inhaled gas. The possibility of abnormal distributions of ventilation and blood flow being used for the diagnosis of early oedema is discussed.

CHAPTER II

PULMONARY ARTERIOLES AND THE DEVELOPMENT OF HYPOXIC OEDEMA IN ANAESTHETISED DOGS

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Whayne and Severinghaus (1968) showed that arterial hypertension in isolated rat lungs caused the development of interstitial pulmonary oedema. Since the entire microcirculation had been blocked by polystyrene microspheres previous to the hypertension, they concluded that the oedema fluid had originated from arterioles and small arteries upstream of the embolisation site. They also found perivascular fluid cuffs containing some red cells in the lungs of rats which had been swimming while breathing a gas mixture of 8-10% oxygen in nitrogen, showing that hypoxic oedema had developed. The experiments, however, lacked measurements of pulmonary arterial and left atrial pressures, which would have helped in determining the main site of origin of the oedema.

In the series described here, the dog was chosen as the experimental animal because its size afforded the possibility of inserting catheters to monitor pulmonary arterial and left atrial pressures during hypoxia. The same technique of polystyrene microsphere embolisation was used to separate the arterial from the capillary bed, but embolisation was limited to the left lung. Thus the capillaries of the left lung were protected against subsequent hypoxic hypertension and any fluid transudation into the interstitial space will have originated upstream from arterioles or arteries. The left atrial catheter was used to confirm a normal pulmonary venous pressure during hypoxia and therefore prohibit any venous contribution towards oedema formation. Experiments described in this chapter were designed to determine whether perivascular oedema fluid accumulation could occur in vivo, in the absence of capillary flow or hypertension.

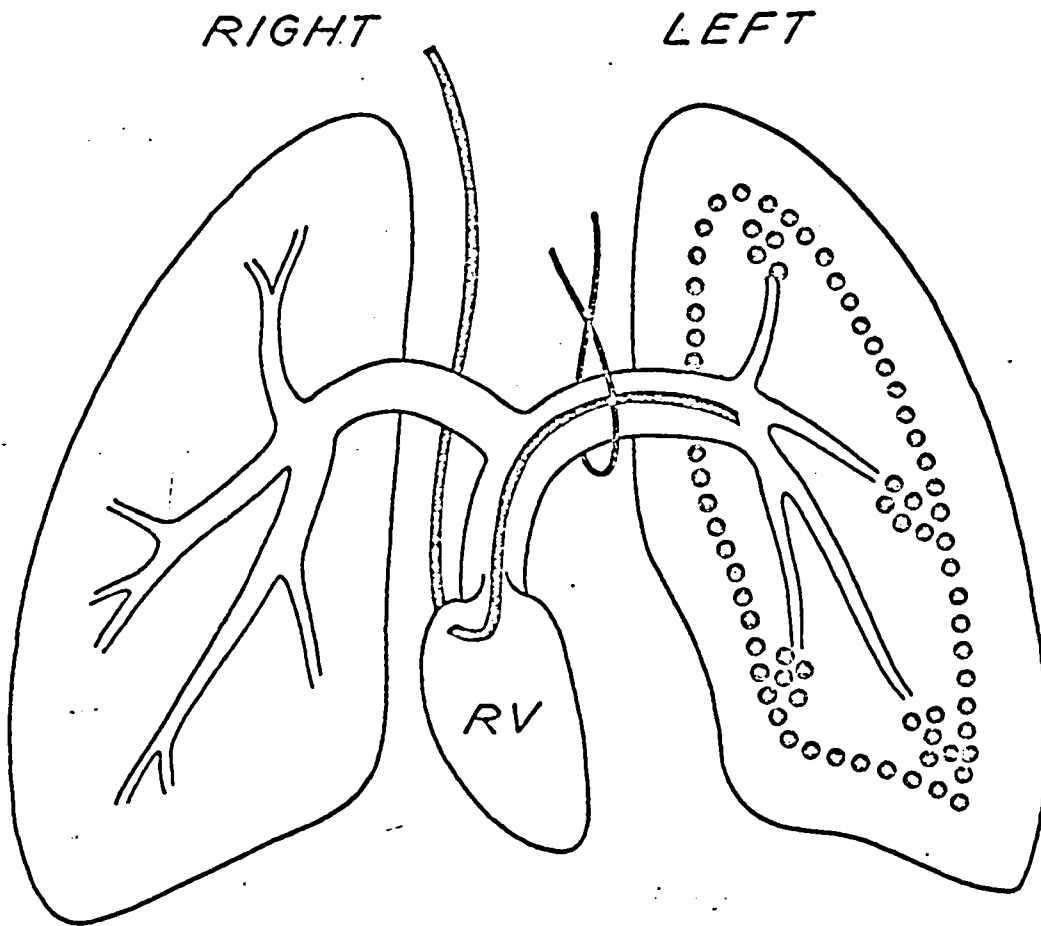


Fig.1 The embolisation procedure

METHODS

Young mongrel dogs weighing between 10-15 Kg were studied. Anaesthesia was induced with pentobarbital (30 mg/kg) injected intravenously, and muscle relaxation obtained with gallamine (20 mg/30 min) to permit artificial ventilation via a tracheotomy. A Bird respirator powered an enclosed bellows (Air Shields Ventimeter) coupled to a conventional anaesthetic circuit containing a carbon dioxide (CO_2) absorber. End tidal oxygen tension was continuously recorded by an electrode coupled into the breathing circuit just beyond the expiratory valve in such a way that the inspiratory positive pressure in the circuit forced end tidal gas from the previous breath through the electrode. The inflow rates of nitrogen (N_2) and oxygen (O_2) were adjusted manually with needle valves and flow meters to obtain the desired end tidal O_2 tension. Tracheal CO_2 tension was recorded by an infra-red analyser and ventilation was adjusted to hold CO_2 tension between 30-40 mm Hg. Catheters were placed in the left carotid artery and in the left pulmonary artery via the jugular vein.

The left atrium and main pulmonary artery were exposed through the left fourth intercostal space and a snare of nylon fishing line was passed around the left pulmonary artery within the pericardium. A polythene catheter was placed in the left atrial appendage using a purse-string suture. Pressures in the carotid artery, left or main pulmonary artery and left atrium were recorded with pressure transducers. Through a midline abdominal incision, a 25 cm long vinyl tube with internal diameter 3.5 mm was inserted, one end in the aorta and the other in the inferior vena cava. A screw clamp on this tube made it possible to vary this aortocaval shunt. With the animal supine, polystyrene microspheres 12-35 μ in diameter, diluted 20 fold with heparinised blood, were infused from a height of 15 cm above the mid chest level into the left pulmonary artery after tightening the snare around the catheter (see Figure 1). Blockage of the terminal arterial bed was considered complete when the pressure in the infusion catheter (monitored from a side

arm), fell less than $0.5 \text{ cm H}_2\text{O/sec}$ when the tubing from the infusion flask was occluded. The snare was then released, and mean pulmonary arterial pressure (P_a) was recorded.

During the three hour hypoxic period which followed embolisation, both the end tidal O_2 tension and aorto-caval shunt flow were adjusted to obtain maximum elevation of pulmonary arterial pressure. Arterial O_2 and CO_2 tensions and pH were obtained with appropriate electrodes (Radiometer types 5044, 5036 and 5021 respectively) at 15-30 minute intervals. Metabolic acidosis was controlled by intravenous administration of sufficient $1.0M \text{ NaHCO}_3$ to keep arterial pH above 7.5. After three hours of hypoxia, the animals were sacrificed with 50-50 mg/kg pentobarbital injected intravenously, followed immediately by 5 ml of India ink to indicate which areas of the lung were still perfused. With the airway pressure held at $20 \text{ cm H}_2\text{O}$, clamps were placed on the lung apex and anterior and posterior segments of lower lobes of both lungs so that biopsies of about 3 cm in diameter could be formalin fixed in the inflated state. 12 μ sections were cut and stained with haematoxylin and eosin.

Estimation of oedema

Since wet to dry lung weight ratios and lung density were not significantly different in test and control lungs, a histological assessment of oedema was therefore made. The 12 μ sections of lung were scanned, and for each vessel (arteries and veins) whose shortest diameter (external to the wall) exceeded 150 μ the vessel area and perivascular cuff area were calculated, assuming both to be circular with diameter equal to the minimum observed diameter. Vessels cut so obliquely that the ratio of long to short diameter exceeded three were excluded, as were vessels in atelectatic areas. The vessel and cuff areas were totalled for each section and the total cuff area was expressed as a percentage of the vessel area.

The experiments consisted of one test group and two control groups. In Group I, the experimental group, embolisation of the left

lung was followed by three hours of hypoxic hypertension. Control Group II was also unilaterally embolised, but was ventilated with oxygen and was normotensive for three hours. Control Group III was made hypoxic and hypertensive but without embolisation. The left lung was protected against pulmonary hypertension by partial or total occlusion of its pulmonary artery by means of the nylon snare. Those experiments where India ink demonstrated either incomplete embolisation of the left lung, or incomplete perfusion of the right (due to presence of excess emboli refluxed from the left pulmonary artery) were discarded. Only the successful experiments are reported here.

RESULTS

Group I

In the six embolised, hypertensive dogs, microsphere obstruction of the terminal left pulmonary arterial bed elevated mean pulmonary arterial pressure 3-5 mm Hg. When hypoxia was induced, pulmonary arterial pressure rose 5-17 mm Hg within five minutes. The aorto-caval shunt was then opened and adjusted together with the inspired O_2 tension to obtain maximum elevation of the pulmonary arterial pressure. This maximum level tended to fall gradually with time, so that during the three hour hypoxic period mean pulmonary arterial pressure was 30-60 mm Hg. Mean maximal left atrial pressure remained between 6-8 mm Hg (6.4 mm Hg was the average). Systemic arterial pressure fell with high shunt flow but was always above a systolic pressure of 80 mm Hg. An arterial oxygen tension of about 30 mm Hg was found to be most effective in elevating pulmonary arterial pressure.

In the animals reported here, pulmonary oedema fluid was found neither in the airways nor in the alveoli when the lung tissue was examined histologically. In contrast, those discarded experiments in which emboli had refluxed from the left lung into the right showed profuse airway and alveolar oedema. Perivascular oedema cuffs were found both in the right (perfused) lungs and left (non-perfused) lungs. Figure 2 illustrates an artery filled with India ink in a section from the right lung. A small perivascular fluid cuff has formed round this artery. In the embolised left lung the area of the perivascular cuffs was greater than in the control right lung, ($P < 0.02$ student's paired t test), in spite of absence of capillary flow. Non-perfusion of capillaries in the left lung thus failed to 'protect' from oedema formation. Figures 3 and 4 are photomicrographs of an artery and vein from the left lung, the artery showing emboli in its lumen and both vessels showing large oedema cuffs. Some of the microspheres appear to have been scattered through the lung by the microtome. Average cuff areas are given in Table III for the six experiments in Group I. There was no consistent difference between apical and lower lobes except that lower lobes had more atelectasis. One of the high altitude native dogs brought from Leadville (experiment 27) showed the largest cuffs. These dogs were studied about two weeks after their arrival at sea level.

Group II

In four dogs the effect of polystyrene microspheres per se was determined, in the absence of hypoxia or pulmonary arterial hypertension. Oxygen tension was held above 200 mm Hg during the three

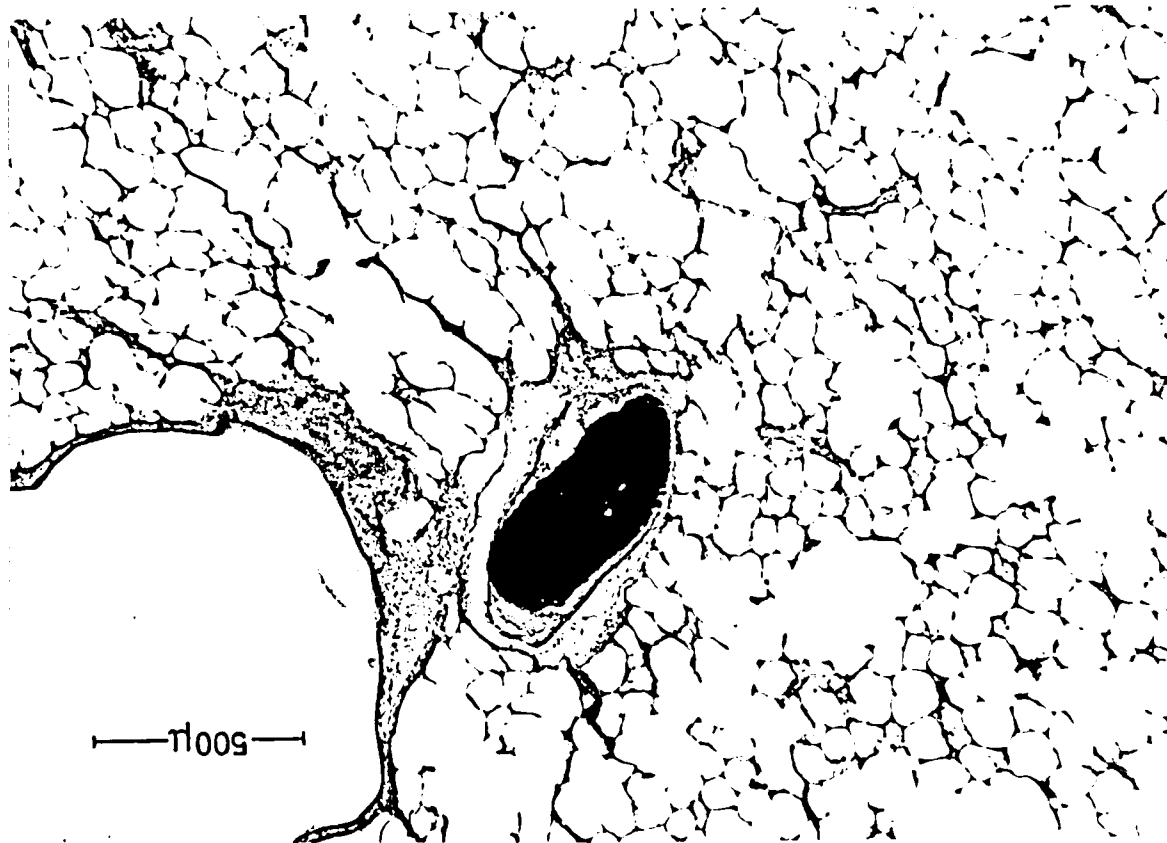


Fig.2 12 μ section of the right lung showing an artery with oedema fluid cuffing. The India ink in the vessel lumen demonstrated that blood was flowing through the lung.

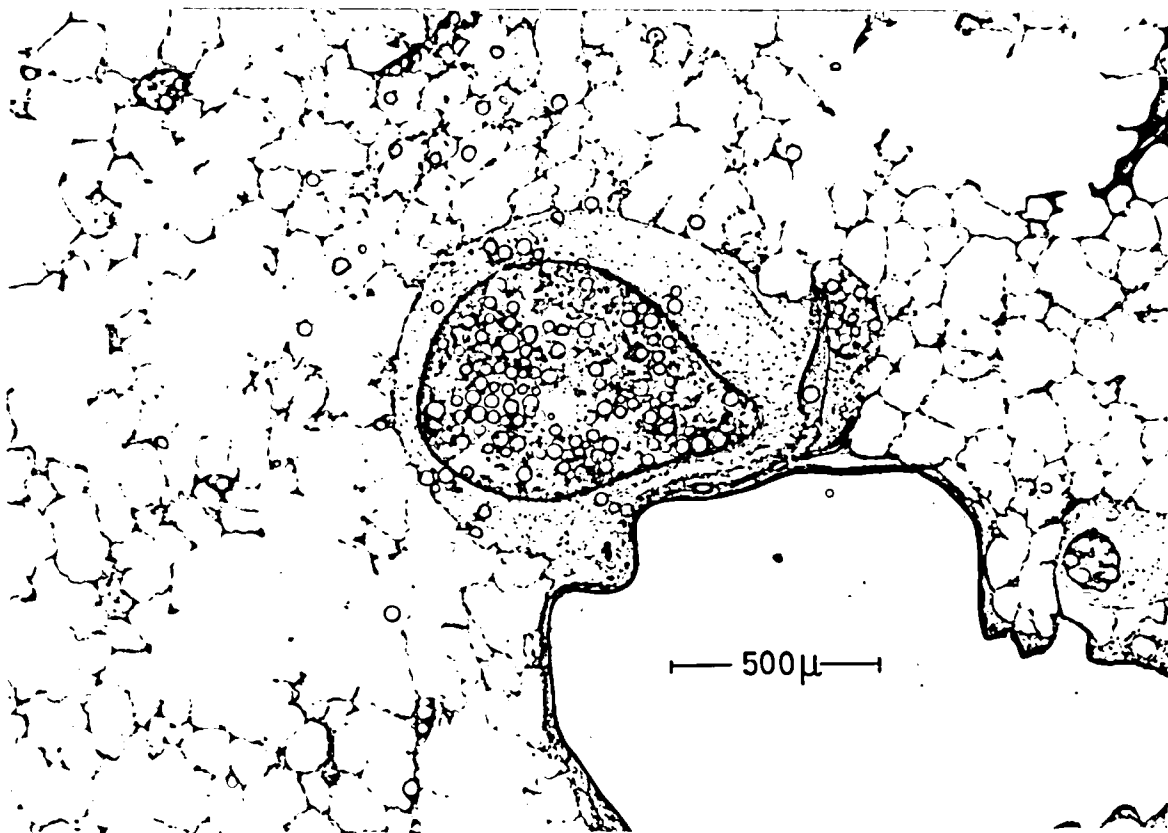


Fig.3 12 μ section showing an artery in the left lung, (Group I), microspheres in the lumen, and a perivascular cuff of oedema fluid.

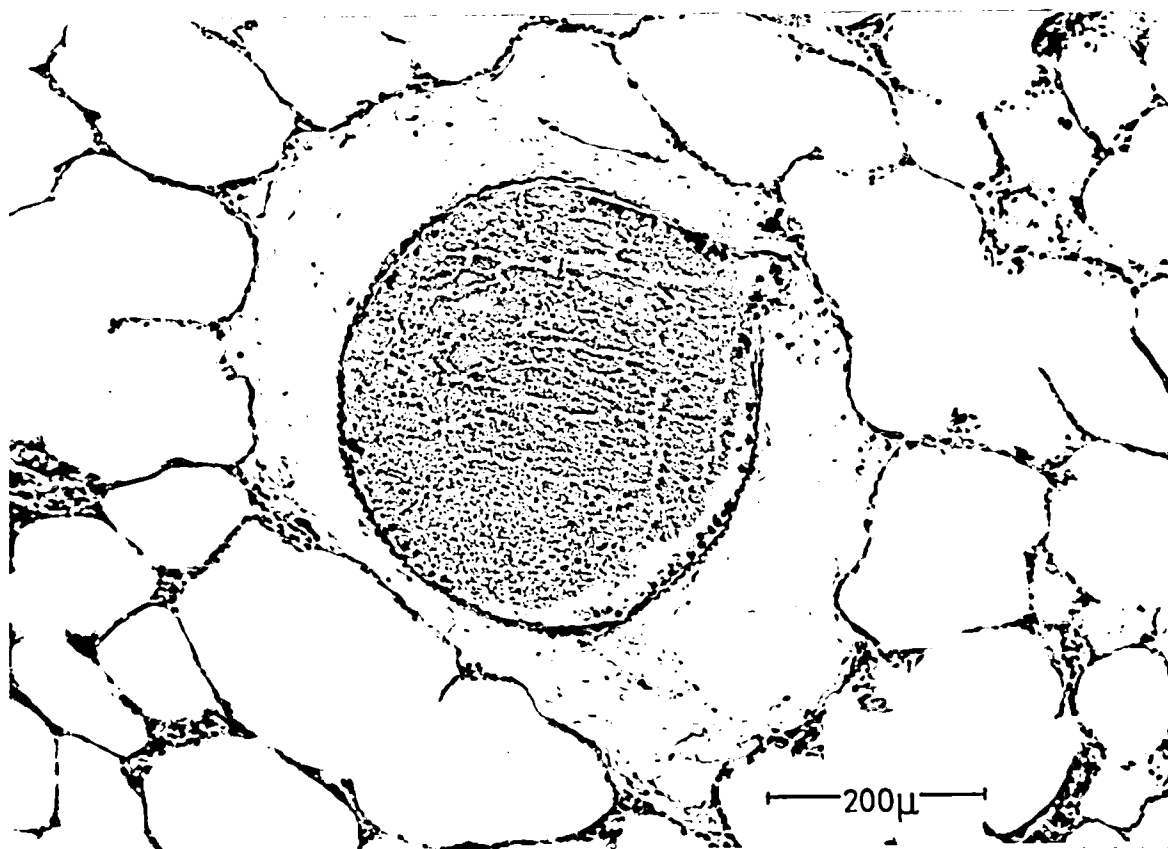


Fig.4 12 μ section showing oedema fluid cuffing round a vein in the left lung (Group I).

TABLE III

CUFF AREA, % OF VESSEL AREAGroup 1 Hypoxic hypertensive

<u>LEFT LUNG</u>						<u>RIGHT LUNG</u>				
Embolized						Non-embolized				
Expt.no.	Apex	L.Ant.	L.Post.	MEAN	n	Apex	L.Ant.	L.Post.	MEAN	n
10	74	48	Atel.	65	22	-	40	32	39	11
12	-	59	80	72	62	-	38	26	30	45
14	48	20	41	33	53	34	19	72	48	34
22	77	44	68	64	48	22	32	Atel.	30	14
26*	73	56	27	52	23	55	43	Atel.	48	16
27*	112	159	78	98	36	68	49	53	55	23
64 ⁺ 22sd						42 ⁺ 10sd				

Group I experiments. Mean perivascular cuff area expressed as a percentage of vessel area, in experiments where there was unilateral embolisation, three hours hypoxia with pulmonary hypertension. *Dogs native to altitude (10,500 ft.).

hour period, and no aorto-caval shunt was used. In one experiment (No.25, Table IV) embolisation was associated with significant perivascular oedema. In this early experiment the blood/microsphere mixture was introduced by syringe, with intermittent monitoring of back pressure, while in subsequent experiments the mixture was infused at a constant pressure. It may be that the pulmonary artery pressure was transiently elevated sufficient to give rise to the perivascular oedema. Some degree of cuffing was found in all the non-embolised lungs, but this may be the normal situation. Figure 5 illustrates the histological appearance of an artery with minimal cuffing. Both the perfused and embolised lungs of Group I had significantly more oedema than the comparable lungs of the normotensive controls of Group II ($P < .001$ and $< .002$ respectively by unpaired t test).

Group III

In six dogs hypoxia was introduced in the absence of unilateral embolisation. Instead, an attempt was made to maintain the left pulmonary arterial pressure low by tightening the snare around the left artery while hypertension was induced by hypoxia and arterio-venous shunting. In three of the experiments the snare was tightened such that the artery was occluded and in the other three, the left pulmonary arterial pressure was held in the normal range by partial occlusion, though the procedure was never completely dependable. In all six experiments some perivascular cuffing was observed (Table V). However, cuffing was greater by 22% in the right lung, continuously exposed to hypertension ($P < 0.15$ with paired t test on the two lungs in each animal, and < 0.005 by unpaired t test). The mean cuff area

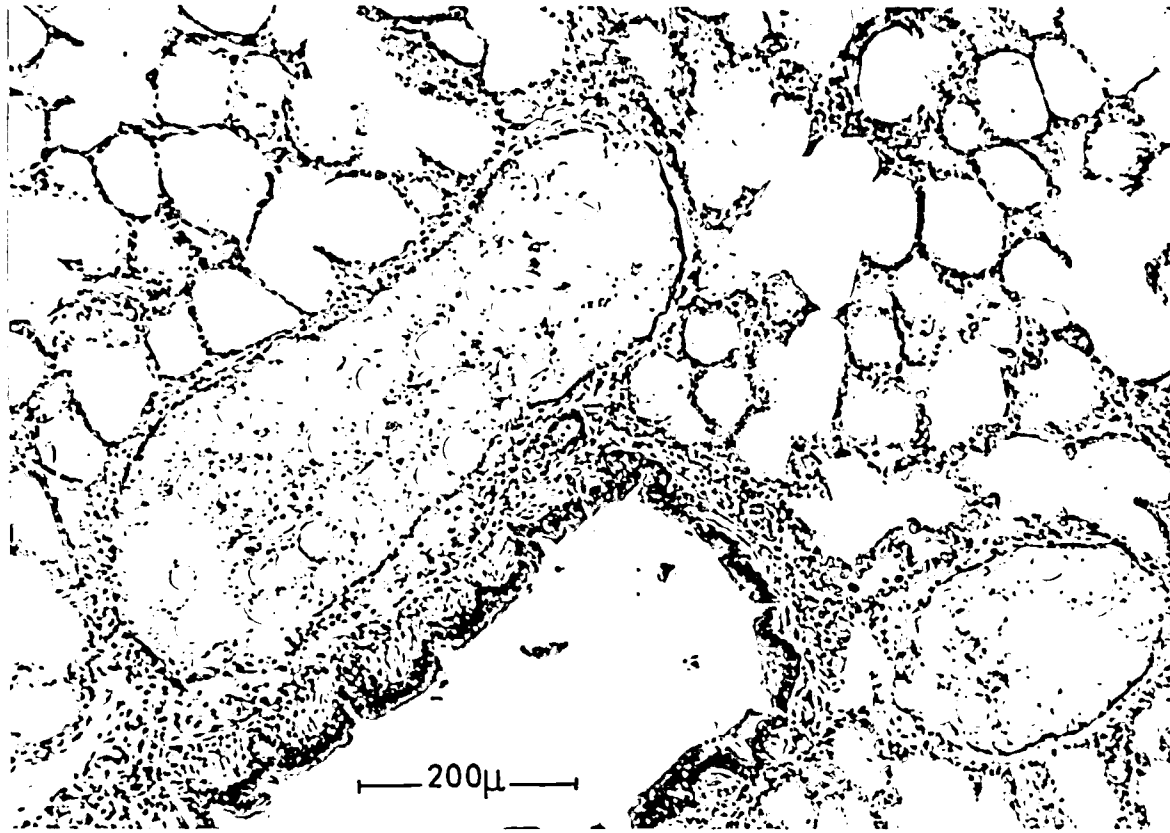


Fig. 5 12 μ section showing arteries which were embolised but not subjected to hypertension or hypoxia in the left lung (Group II). Note the minimal perivascular cuffing under these control conditions.

TABLE IV
CUFF AREA, % OF VESSEL AREA

Group II Normoxic normotensive

<u>LEFT LUNG</u>						<u>RIGHT LUNG</u>					
Embolized						Non-embolized					
Expt.no.	Apex	L.Ant.	L.Post.	MEAN	n	Apex	L.Ant.	L.Post.	MEAN	n	
23	-	-	10	10	34	17	9	34	15	73	
24	-	-	8	8	31	10	30	5	18	58	
25	-	-	60	60	18	15	14	16	19	71	
28	23	22	42	29	21	18	-		18	4	
				26 ⁺ -24 ^{sd}					17 ⁺ -2 ^{sd}		
31	No Emboli								23	70	

Group II, control experiments. Unilateral embolisation followed by three hours of hyperoxia and normal pulmonary arterial pressure.

TABLE V
CUFF AREA, % OF VESSEL AREA

Group III - Hypoxic Non-Embolized

Normotensive						Hypertensive				
Expt.no.	Apex	L.Ant.	L.Post.	MEAN	n	Apex	L.Ant.	L.Post.	MEAN	n
29	29.1	37.2	11.4	25.9	24	63.9	44.2	33.1	46.7	30
33	60.0	52.2	26.6	46.3	37	43.2	68.2	61.2	66.8	25
36	23.9	55.2	32.1	37.3	25	31.1	58.1	38.8	42.7	25
37	19.5	25.0	38.5	27.7	21	46.6	97.1	29.1	57.7	27
38	23.3	13.2	48.4	28.3	24	106.1	69.7	66.2	80.7	39
39	35.3	18.9	26.5	26.9	30	52.3	30.9	13.5	32.3	31
32 ⁺ 8sd						54 ⁺ 17sd				

Group III experiments. The left pulmonary artery was snared to maintain its pressure at normal level. Right pulmonary arterial pressure was more than 30 mm Hg for the 3 hours of hypoxia. There was no embolisation.

of 32% in the 'normotensive' side was significantly greater than in the 17% of the Group II controls ($P < .002$).

DISCUSSION

Limitations of the experimental model

The pulmonary pressor response to hypoxia of dogs has been noted by Viswanathan et al (1969) to be rather weak. One of the high altitude dogs used in this series was one of the most responsive to the stimulus of hypoxia but no such elevation of Pa pressure comparable to Maltgren et al's mean pressure of over 100 mm Hg was achieved. The polystyrene microspheres may have injured the endothelium at their impaction sites and thus induced some fluid leakage, particularly when upstream pressure was elevated. Although lung biopsies were fixed in the inflated state, they undoubtedly underwent shrinkage in the formalin fixation process. In taking cuff to vessel area ratios it was assumed that the shrinkage in the tissue would be uniform so that the two areas, though underestimated, could be compared. One of a number of questions arising from the study was the magnitude of the normal cuff to vessel area ratio. This point will be discussed in the light of a subsequent series of isolated lung experiments described in the next chapter.

Origin of perivascular cuffs. The presence of fluid in periarterial spaces constitutes no evidence for an arterial origin because of the rapid free movement of fluid in the interstitial space. Therefore, perivascular fluid cuffing can only be a sign of early pulmonary oedema. However, in lungs having no capillary flow, but exposed to elevated pulmonary arterial pressure, the resulting perivascular oedema may have

been caused by transarterial leakage. Supporting this suggestion is the finding in Group I that, after three hours of hypoxic hypertension, perivascular cuffs were larger on the non-perfused side than on the perfused side. The most likely explanation being that in the embolised lungs the elevated pulmonary arterial pressure was transmitted down to the embolisation site, undiminished by flow resistance. The mechanism of transarterial leakage that might be postulated from the results in this study is that pulmonary hypertension distends the medium and large arteries sufficiently to cause discrete and randomly distributed leakage sites. The transudation may vary from almost protein-free water to whole blood, depending upon the size of the disruption.

Distensibility of pulmonary arteries. The proposed hypothesis is that pulmonary hypotension distends the medium and large arteries sufficiently to cause them to leak. That these vessels are highly distensible has been recently established by Maloney & Castle (1970) in frog lungs where arteries 300-400 μ diameter were shown to enlarge radially about 2% per cm H_2O transmural pressure rise, over the range of 1-27 cm H_2O . There is evidence supporting a paradoxical distension of pulmonary arteries in hypoxic vasoconstriction (Alzamora-Castro et al, 1961; Arias Stella and H. Kruger, 1963; Fred et al, 1962; Hultgren et al, 1962; Roy et al, 1967) although Sackner & Dubois (1965) place the hypoxia induced volume increase in either capillaries or veins.

Hypothesis linking arterial leakage to hyaline membrane formation.

Arias Stella and Kruger (1963) showed hyaline membranes in autopsied

cases of pulmonary oedema of altitude. Respiratory distress syndrome of the newborn is associated with pulmonary hypertension, arteriolar obstruction and normal venous morphology (Lauweryns, 1968). The possibility should be considered that the protein seen in the alveoli leaked through arterial walls, filled the interstitial space and then flowed to the alveoli, where exposure to air denatured and precipitated the protein, and where the normal capillary pressure permitted the water to be reabsorbed.

Conclusion

Pulmonary perivascular oedema cuffs occur in lungs which have no capillary blood flow and normal capillary pressure, when the pulmonary arterial pressure is elevated following embolisation of the capillary bed with polystyrene microspheres. Similar oedema occurs in the lungs with no embolisation but pulmonary hypertension. The results are compatible with the hypothesis that a site of vascular leakage, in hypoxic oedema is the arteriolar wall. This study is not conclusive however, because it is possible that the transarterial leakage on the embolised side may have been partly due to damage by the microspheres. However, the minimal oedema seen when lungs were embolised in the absence of hypoxia and pulmonary hypertension diminishes the direct effect of the microspheres and supports the transarterial leakage hypothesis.

The next chapter describes a more detailed study, using an isolated dog lung, of the possible role of arterioles and venules in

the development of oedema both during hypoxia and normoxia. In the greater proportion of the experiments, the larger vessels are again functionally separated from the capillaries, but by a method less hazardous than embolisation of the microcirculation. The experiments which are described will be seen to lend support to the hypothesis that arterioles make a significant contribution to the development of hypoxic pulmonary oedema.

CHAPTER III

EXTRA-ALVEOLAR VESSELS AND OEDEMA DEVELOPMENT IN ISOLATED DOG LUNGS BOTH DURING NORMOXIA AND HYPOXIA

This chapter describes a wider investigation of pulmonary vascular permeability. In addition to a more detailed study of the role of arterioles in oedema formation, the experiments have been designed to make a comparison between the arterial, capillary and venous compartments and their separate contributions to the development of pulmonary oedema in normoxia and hypoxia. Besides studying the sites of fluid transudation into the interstitial space, I have determined the effects of varying vascular and alveolar pressure independently on the rate of oedema development. Histology and continuous weighing of the lung afforded information on the origin of the oedema fluid, its rate of formation and its sequence of accumulation. The greater proportion of the experiments are in a static "no flow" situation in which the capillaries (intra-alveolar vessels) can be functionally separated from the rest of the pulmonary vasculature (extra-alveolar vessels).

The presence of two types of pulmonary vessels: intra-alveolar and extra-alveolar, was first shown by Macklin in 1946, using burettes filled with a Latex suspension to separate large from small vessels. Similarly, Howell and his colleagues (1961) used kerosene instead of latex to study changes in vascular volume of the larger

vessels with lung inflation. At high transpulmonary pressures the volume of the compartment made up of larger vessels expanded, while that of small vessels collapsed. This concept of two types of vessels is therefore functional rather than morphological and the junction between the two has not been experimentally defined. However, Riley (1959) proposed that the expanded compartment (extra-alveolar vessels) includes all arteries and veins, down to vessels in the alveolar septal junctions, whereas the compressed compartment (intra-alveolar vessels) consists of vessels in the alveolar septa. How the functional separation of intra and extra-alveolar vessels can be brought about in an isolated lung is described below.

The isolated lung preparation allows the various pressures affecting the pulmonary circulation to be varied independently and over a large range. West et al (1964) have described how the distribution of blood flow in the vertical isolated lung is affected by arterial, venous and alveolar pressure. A summary is shown in the diagram in Figure 6. Zone I is the situation mentioned in the previous paragraph whereby intra-alveolar vessels can be functionally separated from the extra-alveolar vessels. When alveolar pressure is greater than arterial pressure then the intra-alveolar vessels, which are directly exposed

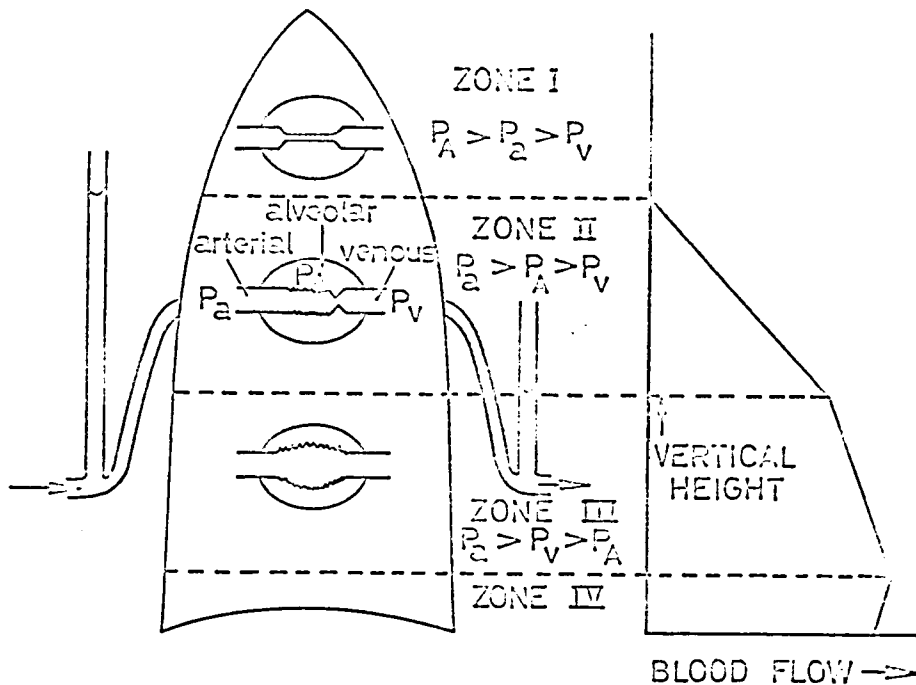


Fig.6 Diagram to show how the distribution of blood flow in the vertical isolated lung is affected by arterial, venous and alveolar pressure. In Zone I alveolar pressure exceeds arterial and venous pressures and there is no flow because the intra-alveolar vessels are collapsed. In Zone II arterial pressure exceeds alveolar pressure which in turn exceeds venous pressure. The vessels behave like Starling resistors and flow is determined by the arterial-alveolar pressure difference. In Zone III venous pressure exceeds alveolar pressure and flow is determined by the arterial-venous pressure difference. At low lung volumes there is a Zone IV in which interstitial pressure may exceed the venous pressure, such that flow is determined by the arterial-interstitial pressure difference.

to alveolar pressure, are collapsed and there is therefore no flow. The extra-alveolar vessels, which are not directly exposed to alveolar pressure, remain held open by the expanded lung parenchyma. Zone I experiments described in this chapter were designed to study the contribution of extra-alveolar vessels to interstitial oedema fluid accumulation in the lung.

In Zone II arterial pressure exceeds alveolar, but alveolar pressure exceeds venous pressure. Flow is determined by the difference between arterial and alveolar pressure. In Zone III venous pressure is greater than alveolar pressure and flow is determined by the arterial-venous pressure gradient. More recently a fourth zone of reduced blood flow in the most dependent region of the lung has been demonstrated by Hughes et al (1968) at medium and low lung volumes. Hughes suggested that flow in this region might be determined by the difference between arterial and interstitial pressure surrounding the extra-alveolar vessels rather than the arterial-venous difference.

Measurements of the distribution of blood flow in the lung using radioactive CO_2 (West & Dollery, 1960) and xenon 133 (West et al, 1964) confirm that blood flow falls off rapidly towards the top of the lung and that the apex may be underperfused during resting conditions. Also, at low lung volumes or situations of raised interstitial pressure as in interstitial oedema, there is a basal region of reduced blood flow (Hughes et al, 1968). When pulmonary arterial pressure is raised above the top of the lung, as in exercise and horizontal posture, the lungs become completely perfused. This would also be the case in pulmonary hypertension whether caused by hypoxia or left heart failure,

but these two conditions also result in pulmonary oedema and hence an increased area of reduced basal blood flow or Zone IV. In summary the proportion of the lung in a particular Zone can be altered by the vascular and transpulmonary pressures and this is further complicated by the pulsatile nature of the pulmonary blood flow.

In this series of experiments the conditions have been simplified by using a lung in the horizontal position where vertical height was minimal so that hydrostatic effects were unimportant and the lung could thus approximate to a single horizontal unit. Interstitial oedema development was studied in lungs under conditions of Zones I, II and III, both during normoxia and hypoxia.

METHODS

Isolated greyhound lungs were used in the horizontal position. By varying the relationships between alveolar, arterial and venous pressures, the lungs were studied in Zone I, Zone II and Zone III conditions, as defined in the introduction to this chapter.

Greyhound dogs weighing between 20-32 kg were anaesthetised by intravenous injection of sodium thiopental (20 mg/kg body weight) and heparinised (2,000 I.U./kg). The left lung was rapidly removed after death by exsanguination and glass cannulas tied into the artery, bronchus and left atrium. Sixty lungs were studied whose weights on excision varied between 83-159 g. After the initial weighing, the lung was supported horizontally in a perspex box and placed on a balance (Gallenkamp Model 7), as shown in Figure 7, so that it could be weighed throughout the experiment to assess oedema development. All

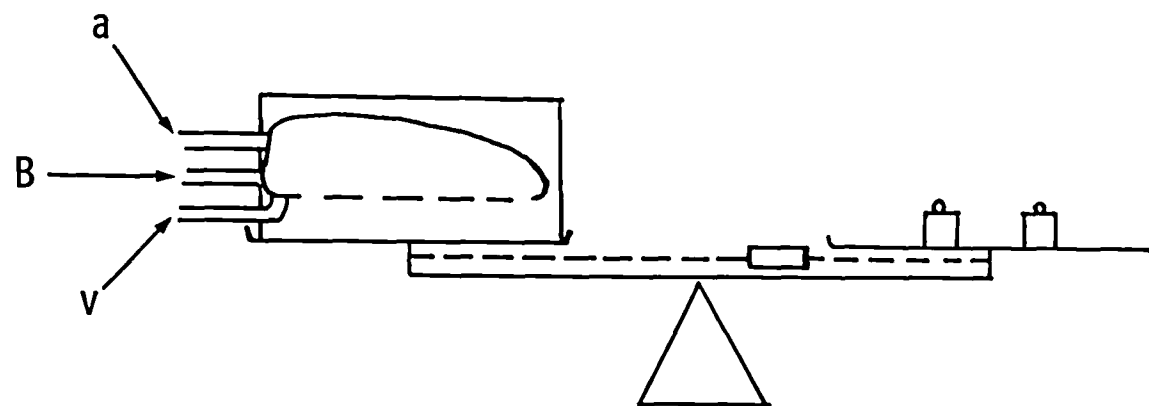


Fig.7 Diagram to show the lung placed horizontally in a perspex box with arterial (a), bronchial (B) and venous (v) cannulas protruding from the front of the box.

connections to the box were of soft Penrose drain tubing, so that maximum flexibility allowed the balance to swing freely during weighing. Expansion of the lung was by positive pressure inflation with either air or nitrogen. A water manometer was used to measure the inflation pressure relative to pleural pressure which was atmospheric.

In the flow experiments the lung was perfused with autologous blood at 37°C by means of a roller pump, which also varied the flow rate and in flow pressure. The pump pulsations were damped out and the steady blood flow passed through a filter and heating column before entering the pulmonary arterial cannula. Blood leaving the lung flowed into a venous reservoir and, by adjusting its height, the venous pressure could be varied. Saline manometers were used to measure arterial and venous pressures relative to the dependent part of the lung. Blood flow was measured by collecting timed samples in a measuring cylinder. In the hypoxia experiments pulmonary venous oxygen tension was measured using an oxygen electrode (Radiometer type 5044).

In the Zone I experiments in which the permeability of the extra-alveolar vessels was studied, Evans blue dye mixed with blood was used to mark the passage of fluid from the intra-vascular to the extra-vascular compartment. The dye concentration (0.6%) exceeded that which would all be bound to serum albumen (Courtice & Simmonds, 1949) so that free dye would pass with water into the interstitial space. That the dye is inert per se has been shown by Courtice et al (1949).

Each experiment lasted at least two hours during which serial lung weighings and biopsies were taken. The biopsies were carefully clamped to prevent deflation on excision and frozen, thus preserving

the state of interstitial oedema in the lung by arresting any further exchange of fluid between the vascular and interstitial compartments. The method of freezing was to plunge the biopsy into liquid nitrogen (-195.8°C), using small amounts of tissue (2-3 g) so that freezing was very rapid and therefore shrinkage minimal. In three experiments, in which tissue close to the surface was required, Freon 12 cooled to -155°C with liquid nitrogen was used. This freezing agent, though not so cold, 'wets' the lung surface better than liquid nitrogen and will therefore freeze more rapidly. Staub & Storey (1962) have shown that the outer 4 mm of tissue freezes without shrinkage. From each biopsy a block of tissue was taken for freeze-drying and another for cryotomy. These blocks (approximately 5 mm thick x 8 mm x 5 mm) were cut from the biopsy using a bandsaw. The tissue was never kept out of liquid nitrogen long enough for it to thaw.

Freeze-drying, sectioning and staining

Three blocks of tissue were freeze-dried together (Pearse-Speedivac freeze-dryer, Edwards High Vacuum Ltd.) for twenty-four hours at a temperature of -20°C and pressure of 0.001 mm Hg. The blocks were then double-embedded in 2% celloidin and Paraffin wax (Balwax 1, R.A. Lamb Co.). Sections 6 μ thick were cut on a Cambridge rocking microtome. Where thin sections (2 μ) were required, a specially sharpened razor blade was used instead of the standard microtome knife and a technique described by Wigglesworth (1959). The 6 μ sections were stained with haematoxylin and eosin and the 2 μ sections with P.A.S. and Orngne G.

Cryotomy

The lung tissue was placed in a drop of water on an aluminium mounting block and frozen to it by gently lowering it into liquid nitrogen. Since lung tissue is too friable to obtain even relatively thick sections on the cryostat, the surface of the block left after each 20 μ cut was observed instead. For this a Nikon binocular microscope held in a metal frame was mounted onto the front of the cryostat. Cellophane paper secured across the frame kept the cryostat closed, maintaining an internal temperature of less than -10°C , but at the same time allowing illumination of the block of tissue with photo flood lighting. A zoom lens in the microscope gave magnifications up to forty times and exact dimensions of blood vessels and airways were measured using a precalibrated graticule in one eyepiece. A camera (Nikon, Microflex P.F.M.) mounted on another eyepiece allowed photographs to be taken to record fields or individual vessels as the tissue was sectioned. Kodachrome II colour film (KPA 135-36P) was used.

Estimation of oedema and site of fluid transudation into the interstitial space

Continuous weighing of the lung was the method of estimating overall fluid accumulation in the interstitial space. Since the biopsies were serial, the time of the first appearance of fluid cuffing could be ascertained for each experiment and the corresponding lung weight gain. Both the sections from freeze-dried tissue and the cryostat tissue were used to calculate vessel and fluid cuff areas from measurements of vessel diameters and their corresponding perivascular cuffs. As described in the previous chapter, the cuff areas were expressed as

a percentage of the vessel areas. Further, these ratios were compared for vessels of diameters ranging from 50 μ to over 300 μ . In experiments where dye was mixed with the blood to estimate the size range of permeable vessels, measurements of diameters were made in vessels in which dye was present simultaneously in the lumen, wall and perivascular fluid cuff.

EXPERIMENTAL CONDITIONS

Zone I experiments (36 lungs)

In all experiments alveolar pressure exceeded arterial and venous pressures and intra-alveolar vessels were therefore collapsed. Surface tension forces which tend to hold these vessels open were taken into account when the alveolar and vascular pressures were chosen. Pain and West (1966) have shown that in the deflation state, pulmonary blood flow occurred up to 4.0 cm above the point at which arterial pressure equals alveolar pressure. It rose higher in the inflation state and the difference was attributed to the perivascular pressure of intra-alveolar vessels being altered by changes in the surface tension of the alveolar lining layer. On the basis of these findings, blood flow under Zone I conditions was avoided by studying the lungs in the deflation state and with the alveolar pressure always exceeding the vascular pressures by at least 5 cm H₂O. The extra-alveolar vessels were held open by the expanded lung parenchyma and were therefore filled with blood.

Three groups of experiments were carried out to investigate the contribution of the extra-alveolar vessels alone to oedema formation

both in normoxia and hypoxia. In Group I (24 lungs) the arterial and venous pressures were equal and, by combining different transpulmonary pressures with a variety of vascular pressures, their roles in the formation of oedema were studied. In Group II (6 lungs) the separate contribution of extra-alveolar arteries was studied by raising arterial pressure to 20 cm H₂O while venous pressure remained below the level of the lung. Similarly in Group III (6 lungs) the extra-alveolar venous contribution was assessed by raising venous pressure to 20 cm H₂O while arterial pressure remained low. Evans blue dye was not necessary to determine whether extra-alveolar vessels were permeable, and therefore contributing to interstitial fluid accumulation, because the presence of perivascular oedema when intralveolar vessels were collapsed would in itself show that the fluid had originated from extra-alveolar vessels. However, dye was mixed with the blood in all Zone I experiments to investigate the size range of permeable extra-alveolar vessels, and it was also useful in determining the extent of extra-alveolar vessels and checking that the intra-alveolar vessels were indeed collapsed (by absence of dye). This was also checked by histologically counting the number of red cells present per 100 μ of alveolar septum in the 2 μ thick lung sections, as described by Glazier et al (1969), who showed a correlation between the number of red cells and the capillary pressure at different levels in the lung.

Zone II experiments (10 lungs)

There were two groups of experiments under Zone II conditions: one with forward flow through the lung (artery to vein, six lungs) and the other with retrograde flow (veins to artery, four lungs). In both

groups the inflow pressure ^{was} 20 cm H₂O and the outflow pressure was below the lung. In the five normoxic experiments initial flow varied between 80 to 150 ml/min with no significant difference between forward and retrograde flow. In the five hypoxia experiments for the same inflow pressure (20 cm H₂O) forward flows were 210-305 ml/min initially and the two initial retrograde flows were 156 and 172 ml/min respectively.

Zone III experiments (14 lungs)

In these experiments arterial pressure was set around 20 cm H₂O above the lung and then venous pressure was raised until a minimum arterial-venous difference was obtained. The pressures were then set to give a mean of 20 cm H₂O, which was maintained throughout the experiments by small adjustments of flow when necessary. Flow varied between 60-90 ml/min in the five normoxia experiments and 62-84 ml/min in the three hypoxia experiments. Three normoxic and three hypoxic experiments were also carried out under static conditions, with the whole vascular bed filled and arterial and venous pressures both at 20 cm H₂O. They will be referred to as "Static Zone III experiments". Alveolar pressure in all fourteen experiments was 10 cm H₂O.

RESULTS

Zone I experiments

Two hours was found to be a convenient experimental period because interstitial oedema developed, but at the same time the interstitial compartment was not completely filled because alveolar oedema

was found to be absent. For this reason, the total weight gain after two hours was chosen for comparisons between lungs. In the sixty experiments, three normoxic Zone II lungs (flow artery to veins) did not develop interstitial oedema in two hours and three hypoxic "Static Zone III" lungs developed alveolar oedema by two hours. Results from this study and unpublished data from other isolated lung experiments in this laboratory have shown that a gain of more than 35% of the original weight on excision is required before alveolar oedema is evident. Isolated lungs were weighed on excision and again at the end of the experiment, in both instances with the vascular compartment drained, so that the weight gain represents extra-vascular fluid. I have expressed weight gain as a percentage of the original weight on excision in order to take differences in lung size into account. For example in identical experimental conditions, one would expect a large lung to gain more weight in a given time than a small one and this can be seen in Tables VI and VII.

Group I: equal vascular pressures

In a variety of vascular and alveolar pressure combinations, the separate effects of vascular and alveolar pressure on the development of oedema were studied. Figure 8 illustrates how the rate of weight gain varied with the level of equal arterial and venous pressures. Each line represents one experiment and initial lung weights were similar (101-105 g). Figure 9 shows data from twenty experiments to indicate the vascular effect. Equal arterial and venous pressures are plotted against the total weight gain over two hours, expressed as a percentage of the original lung weight on excision. Alveolar pressures

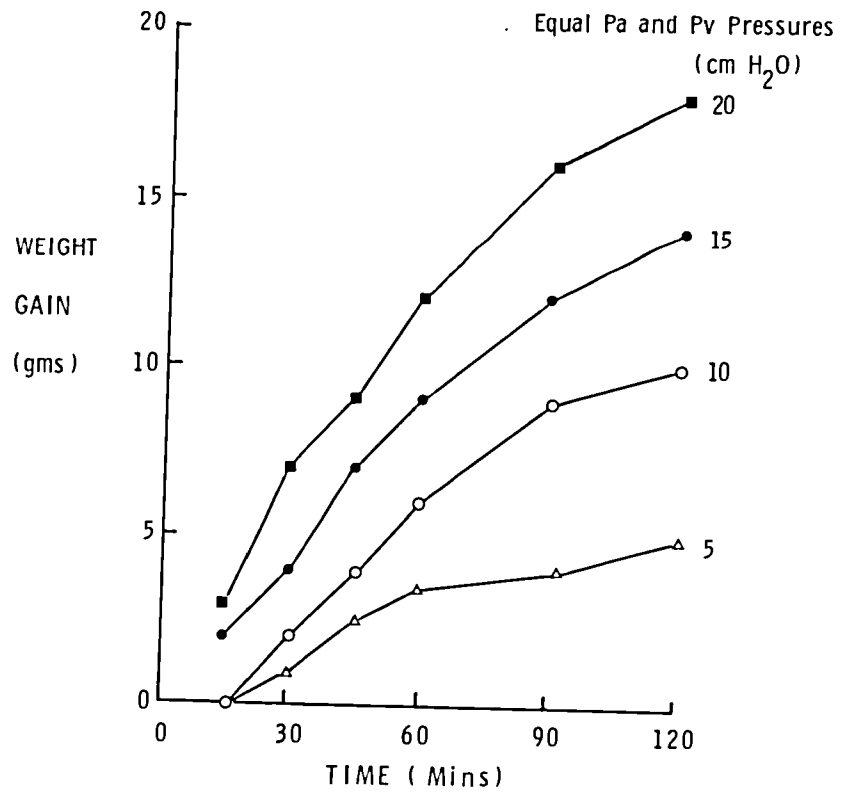


Fig.8 The weight gain is plotted at intervals during the two hour experimental period. Each line represents one experiment at the equal arterial and venous pressures labelled. Alveolar pressures varied between 17-30 cm H₂O.

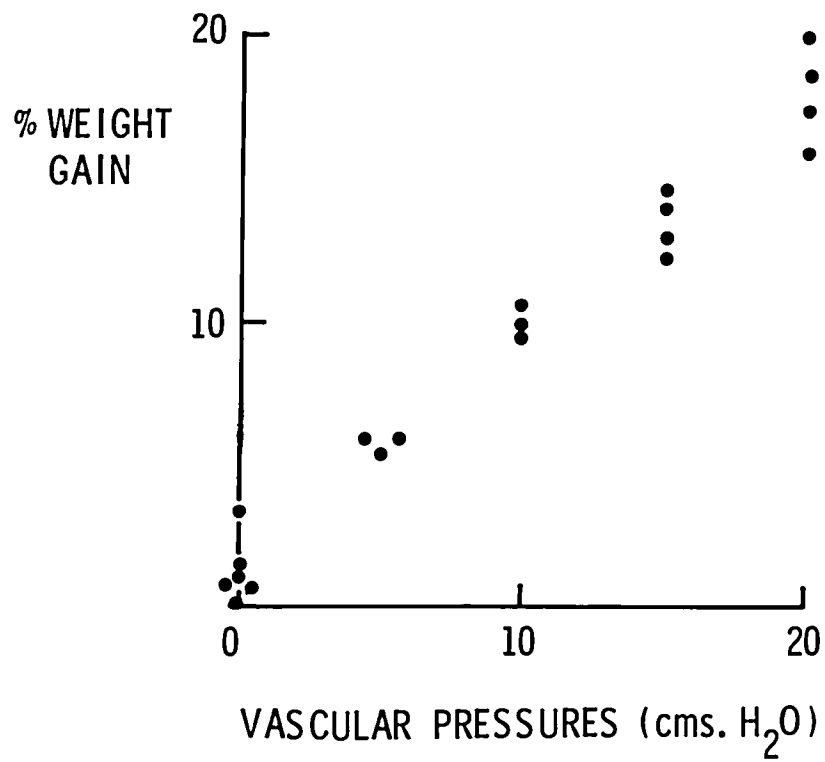


Fig.9 Vascular pressures plotted against the total weight gain over two hours, expressed as a percentage of the original lung weight. Alveolar pressures varied between 5-30 cm H₂O.

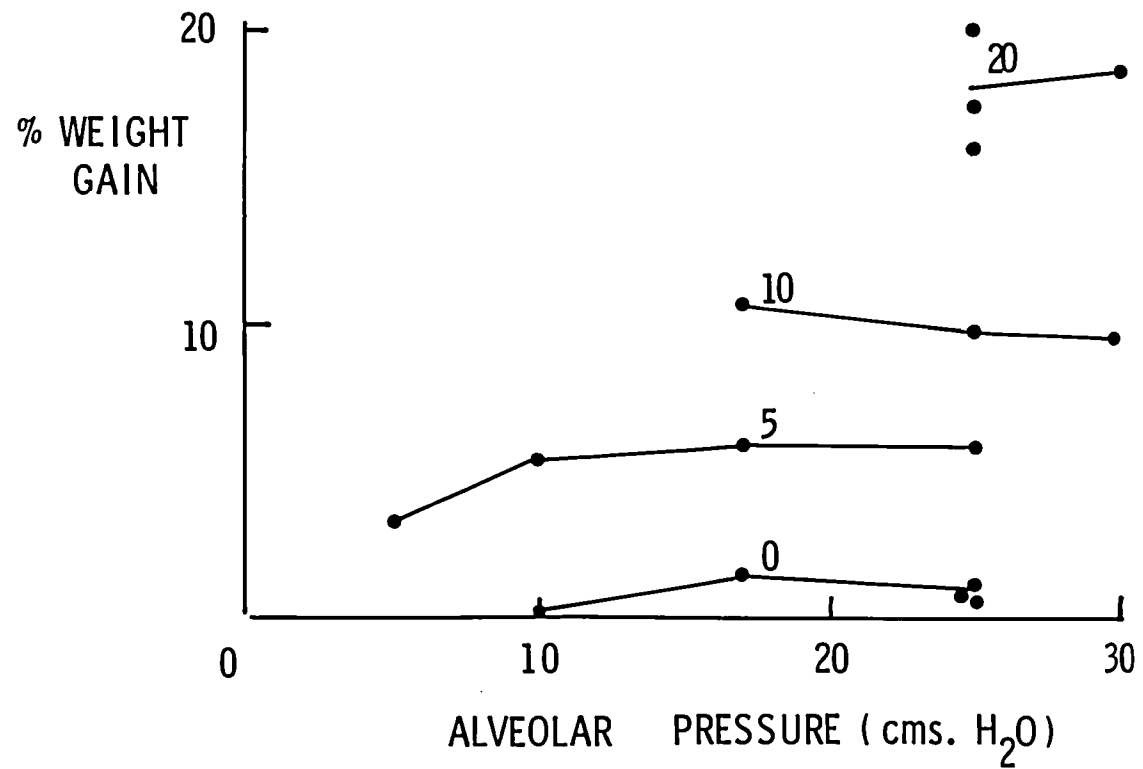


Fig.10 Alveolar pressures plotted against percent weight gain. Vascular pressures varied between 0-20 cm H₂O. Horizontal lines show the levels of vascular pressures.

varied between 5-30 cm H₂O. There was a linear relationship between vascular pressure and the rate of oedema formation. Figure 10 is a graph of percentage weight gain from the same experiments but this time plotted against alveolar pressure. Vascular pressures varied between 0-20 cm H₂O as shown by the horizontal lines. For the same vascular pressure, the alveolar pressure does not seem to have an effect on the rate of oedema formation.

Group II ($P_a = 20$ cm H₂O) and Group III ($P_v = 20$ cm H₂O)

In Figure 11 the rates of weight gain are shown for normoxia experiments where only either arterial pressure or venous pressure was raised to 20 cm H₂O above the lung. Also plotted for comparison is an experiment where both arterial and venous pressures were 20 cm H₂O, and finally, the top curve is an example of a "Static Zone III" experiment (where the whole vascular bed was filled, including capillaries). Initial lung weights varied between 102-109 g. Tables VI and VII show the serial weight gains in the Zone I and Static Zone III experiments respectively. Figure 12 and Tables VIII and IX show the corresponding data from the hypoxia experiments. Note how the rate of weight gain has been increased by hypoxia in all the experimental conditions except that of a raised venous pressure only. Within the two hour experimental period plateauing of the weight gain was not evident in the majority of experiments, indicating that leakage from one segment of the vasculature did not to any extent seem to influence the rate of leakage from other segments. On this basis, therefore, the last column in Tables VI, VII, VIII and IX represents the possible relative contribution of each of the three compartments to weight gain, the Static Zone

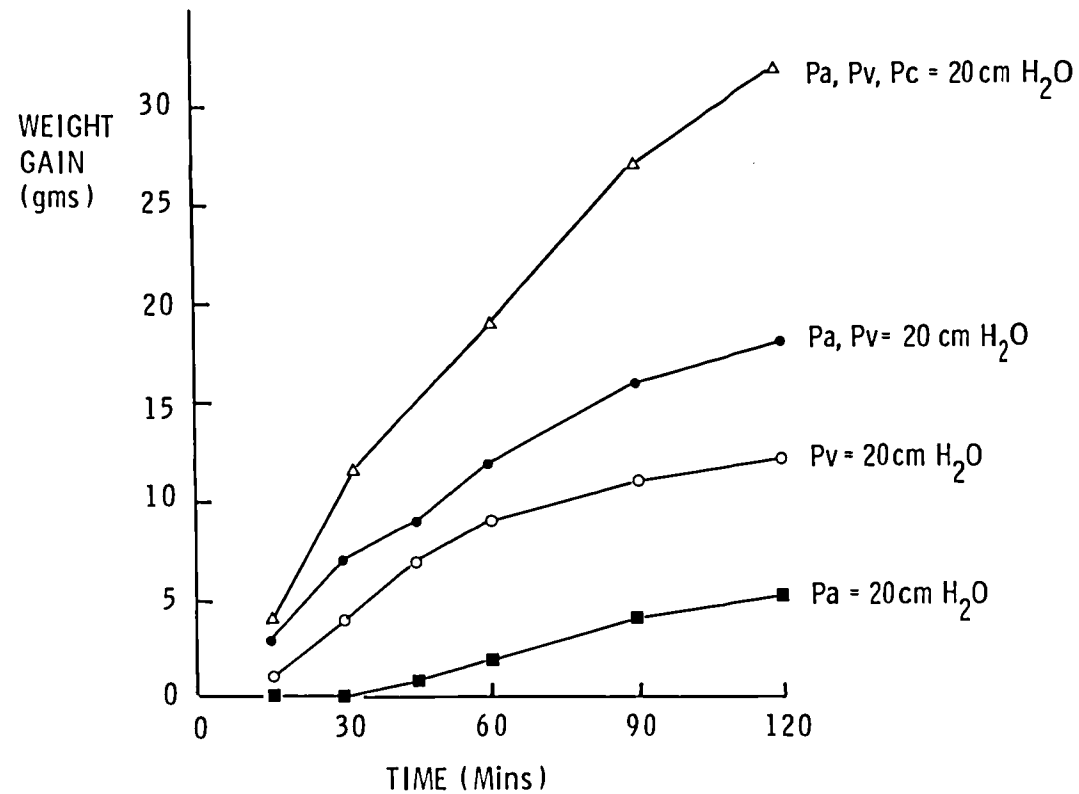


Fig.11 The weight gain is plotted at intervals during the 2 hour experimental period. Each line represents one experiment at the vascular pressures indicated. The top curve is a "Static Zone III" experiment, where capillaries as well as extra-alveolar vessels were filled, and alveolar pressure was 10 cm H₂O. The other three experiments were under Zone I conditions, with alveolar pressure at 25 cm H₂O.

TABLE VI

ZONE I EXPERIMENTSN O R M O X I A

Vascular pressures (cm H ₂ O)	P alveolar (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean wt. gain	Relative %
		Initially	+½hr.	+1hr.	+1½hr.	+2hr.			
	25	123	127	134	141	146	18.7		
P arterial = 20	25	131	136	140	146	152	16.0		
P venous = 20	25	115	121	125	131	138	20.0	18.0	63.0
	25	*104	111	116	120	122	17.3		
	25	156	156	158	160	162	3.8		
P arterial = 20	25	*102	102	104	106	107	4.9	4.6	16.1
P venous = -20	25	98	98	99	101	103	5.1		
	25	*103	107	112	114	115	11.6		
P arterial = -20	25	138	144	149	153	158	14.5	13.1	46.0
P venous = 20	25	113	116	120	123	128	13.3		

*Plotted in fig. 11 . The last column shows the relative contribution of all extra-alveolar vessels, and separate contributions of extra-alveolar arteries and veins to interstitial fluid accumulation.

TABLE VII
STATIC ZONE III EXPERIMENTS

N O R M O X I A

Vascular pressures (cm H ₂ O)	P alveolar (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean wt. gain	Relative %
		Initially	+½hr.	+1hr.	+1½hr.	+2hr.			
	10	*109	121	128	136	141	29.4		
P _a , P _v , P _c = 20	10	135	148	154	161	169	25.2	28.5	100.0
	10	93	100	106	110	116	24.8		

Results from three "Static Zone III" experiments. The final column shows 100% because the whole vascular bed was filled, thus allowing both extra-alveolar and intra-alveolar vessels to contribute to oedema formation.

*Plotted in fig. 11 .

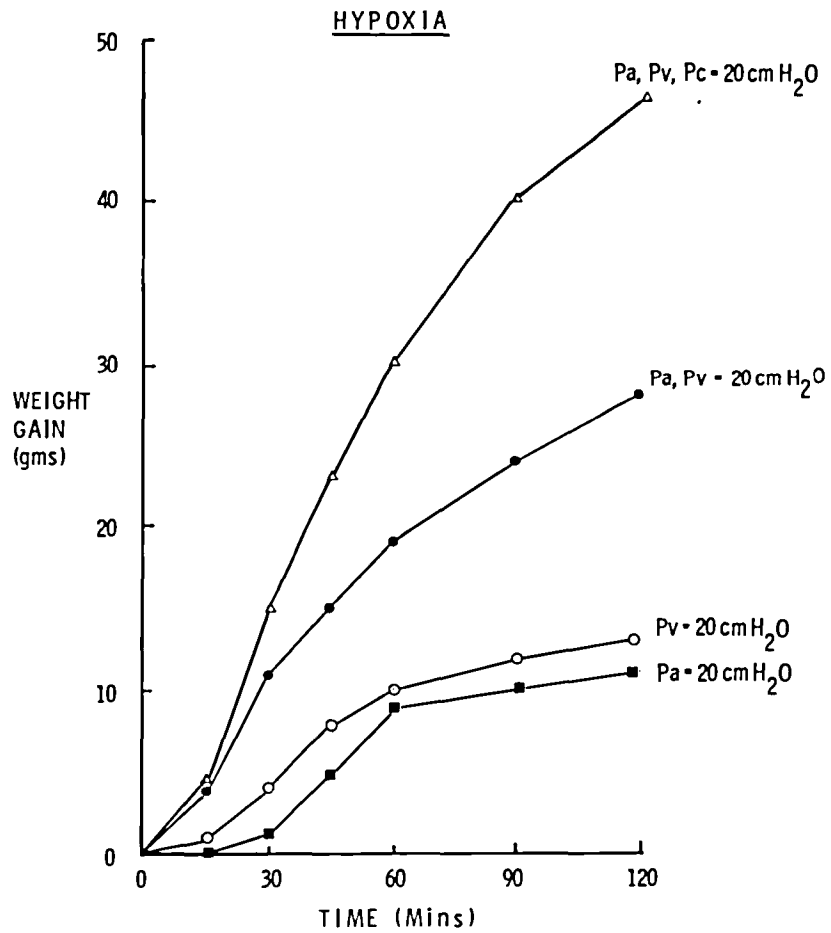


Fig.12 The weight gain is plotted at intervals, during the two hour experimental period of hypoxia. Each line represents one experiment at the vascular pressures indicated. The top curve is a "Static Zone III" experiment, where capillaries as well as extra-alveolar vessels were filled, and alveolar pressure was 10 cm H₂O. The other three experiments were under Zone²I conditions, with alveolar pressure at 25 cm H₂O.

TABLE VIII

ZONE I EXPERIMENTSHYPOXIA

Vascular pressures (cm H ₂ O)	P alveolar (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean wt. gain	Relative %
		Initially	+½hr.	+1hr.	+1½hr.	+2hr.			
P _a , P _v = 20	25	*107	118	125	131	135	26.2		
	25	94	100	104	108	113	20.2	24.8	63.6
	25	122	130	139	149	156	27.9		
P arterial = 20 P venous = -20	25	*111	112	120	121	122	9.8		
	25	126	130	133	138	141	11.9	10.4	26.7
	25	116	119	122	124	127	9.5		
P arterial = -20 P venous = 20	25	*102	106	112	113	114	12.8		
	25	92	94	97	102	106	15.2	13.8	35.4
	25	134	139	143	147	152	13.4		

*Plotted in fig. 12 .

The final column shows the relative contributions of all extra-alveolar vessels, extra-alveolar arteries and extra-alveolar veins to interstitial fluid accumulation.

TABLE IX
STATIC ZONE III EXPERIMENTS

H Y P O X I A

Vascular pressures (cm H ₂ O)	P alveolar (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean wt. gain	Relative %
		Initially	+½hr.	+1hr.	+1½hr.	+2hr.			
	10	*109	124	139	149	156	43.1		
P _a , P _c , P _v = 20	10	120	132	141	152	165	37.4	39.0	100.0
	10	96	105	113	122	132	37.5		

*Plotted in fig. 12 .

Results from three "Static Zone III" experiments. All vessels were filled so that there was a maximum contribution of 100% to oedema formation.

III experiments being 100%. Under these static, Zone I conditions, during normoxia extra-alveolar arteries accounted for 16% of the oedema fluid, extra-alveolar veins 46% and the alveolar septal capillaries 38%. Similarly, during hypoxia arteries contributed 27%, veins 35% and capillaries 38%.

Zone II and Zone III experiments

Figure 13 illustrates the rates of weight gain during Zone II and Zone III normoxic experiments and Table X shows serial and per cent weight gains. The Zone III per cent weight gain during flow is less than in the static situation and that may be because the veins (and possibly some capillaries) were at pressures less than 20 cm H₂O during the flow due to the likely pressure drop down the vessels. Also, one would expect the contribution of arteries plus capillaries (Zone II) to be greater than for arteries alone (Zone I). Similarly, the weight gain should be greater for veins plus capillaries (Zone II) than for veins alone (Zone I). In the Zone III experiments, the arterial pressure was higher than 20 cm H₂O and venous pressure lower. The a-v difference varied from lung to lung (5.5-9.0 cm H₂O) and when it was large, the venous pressure was 16.0 cm H₂O instead of 20 cm H₂O. From the Zone I study one would expect proportionally less fluid to pass out from veins, (during flow this might also involve the pressure in the capillaries) and therefore total weight gain to be less. Under Zone II conditions it is possible that some of the fluid which had left the vessels with an elevated pressure then entered the permeable vessels on the low pressure side.

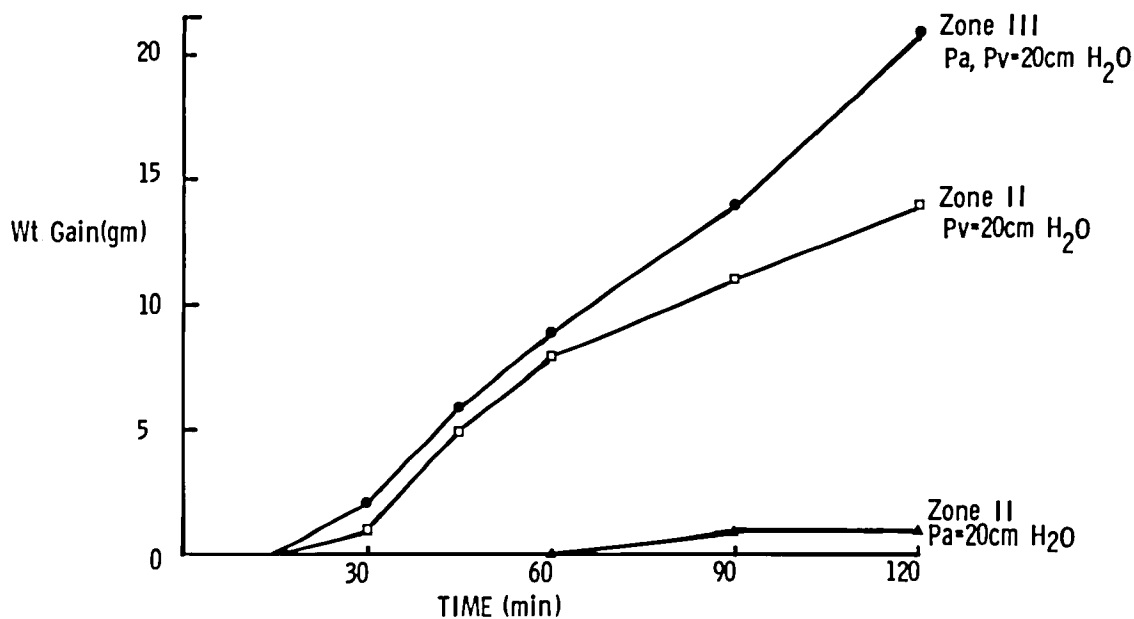


Fig. 13 The weight gain is plotted at intervals during the 2 hour experimental period. The Zone II experiment under conditions of raised venous pressure only was done by retrograde perfusions. Alveolar pressures were 10 cm H₂O in all the experiments.

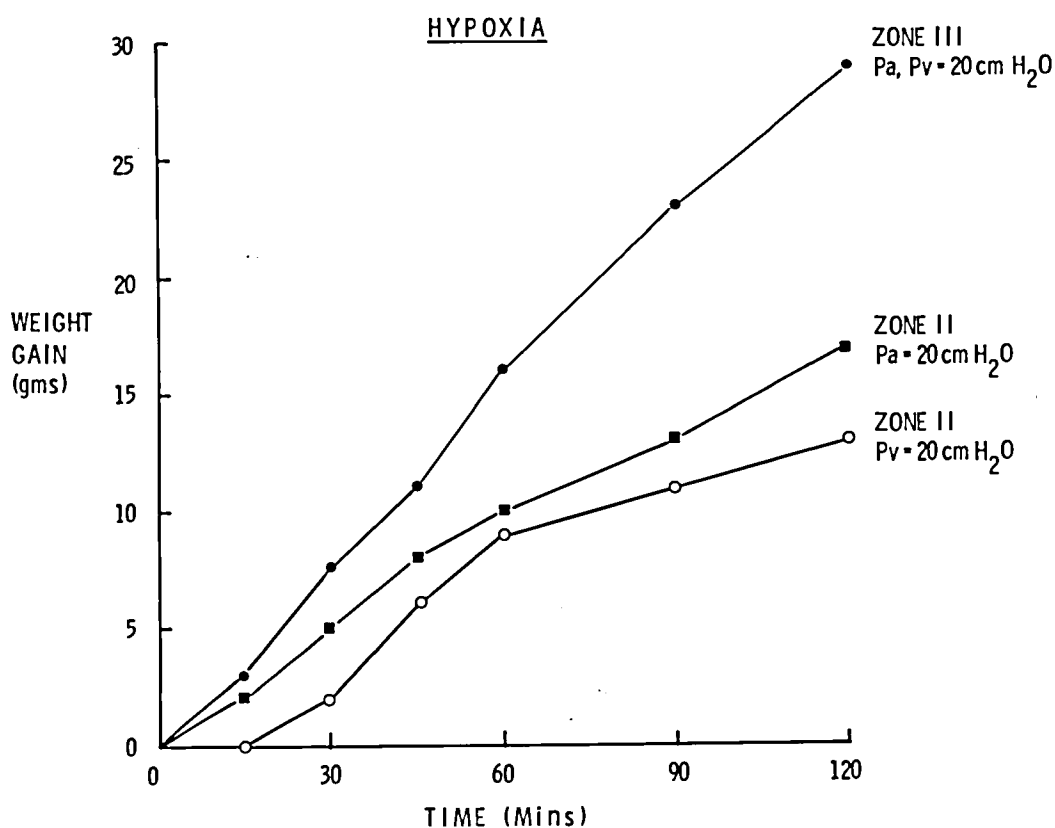


Fig. 14 The weight gain is plotted at intervals during the 2 hour experimental period of hypoxia. The Zone II experiment under conditions of raised perfusion. Alveolar pressures were 10 cm H₂O in all the experiments.

TABLE X

FLOW EXPERIMENTSN O R M O X I A

Vascular pressures (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean % wt. gain
	Initially	+½hr.	+1hr.	+1½hr.	+2hr.		
P arterial = 20	149	149	149	150	151	1.3	1.3
P venous = -2	*116	116	116	117	117	0.9	
	120	120	121	122	122	1.7	
P arterial = -2	*115	116	123	126	129	12.2	13.2
P venous = 20	84	85	89	93	96	14.3	
P _a = 21.9, P _v = 17.9	89	91	97	105	111	24.7	20.1
P _a = 23.0, P _v = 18.5	135	136	146	152	159	17.8	
P _a = 25.0, P _v = 16.0	*119	121	128	133	140	17.6	
P _a = 20.5, P _v = 17.5	115	117	127	133	141	22.6	
P _a = 23.2, P _v = 16.8	122	123	132	138	144	18.0	

*Plotted in fig. 13 .

TABLE XI

FLOW EXPERIMENTSH Y P O X I A

Vascular pressures (cm H ₂ O)	Lung weight (gm)					Total wt. gain (% of initial)	Mean %-wt. gain
	Initially	+½hr.	+1hr.	+1½hr.	+2hr.		
P arterial = 20	*121	126	131	134	138	14.0	11.6
P venous = -2	108	111	113	117	119	10.2	
	94	96	99	102	104	10.6	
P arterial = -2	*113	115	122	124	126	11.5	12.9
P venous = 20	98	100	103	107	112	14.3	
P _a = 23.1, P _v = 18.0	*119	127	135	142	148	24.4	29.0
P _a = 22.8, P _v = 16.4	129	138	147	160	169	31.0	
P _a = 24.0, P _v = 16.1	101	112	120	127	133	31.6	

*Plotted in fig. 14 .

Experimental evidence supporting this hypothesis was obtained from an experiment in which, under Zone I conditions, both arterial and venous pressures were raised to 20 cm H₂O above the lung for two hours and then lowered to 20 cm H₂O below. The lung weighed 125 gms initially and gained 25 gms in the first two hours. Only the venous blood had dye mixed with it and during this time dye-stained fluid in the perivenous spaces migrated to peribronchial and periarterial spaces, confirming the presence of connections between the perivenous and peribronchial spaces described by Miller (1911) in dogs. When the pressures had been lowered for two hours, the lung had lost 15 gms in weight and dye-stained fluid entered small permeable arteries (previously containing no dye). Fluid also re-entered the veins, but this was not detectable by presence of dye since they had previously been filled with dye mixed with blood. However, both levels of the arterial and venous reservoirs were seen to rise, confirming re-entry of fluid from the interstitial space into the vascular compartment.

The rates of weight gain during hypoxia experiments are shown in Figure 14 and Table XI. With an elevated arterial pressure only (the model for altitude) there was a striking increase in weight gain over the two hour experimental period which, if anything, has been underestimated because of the venous reabsorption of oedema fluid described in the two previous paragraphs. This increased arterial contribution to oedema fluid contrasts with the venous contribution during retrograde flow which was apparently unchanged by hypoxia. Its increase may have been masked by arterial reabsorption, but under Zone I conditions too the weight gain due to fluid leakage from extra-alveolar veins was

not affected by hypoxia. During the flow experiments with hypoxia, venous blood leaving the lung had oxygen tensions varying between 35-41 mm Hg.

Pulmonary vascular resistance

Throughout the flow experiments inflow and outflow pressures were kept constant. Changes in pulmonary vascular resistance were therefore reflected by changes in flow. Development of interstitial oedema was generally accompanied by a fall in flow rate and therefore a rise in overall pulmonary vascular resistance. The fall in flow rate did show some correlation with the amount of weight gained. During hypoxia initial flows were higher than those during normoxia which was a surprising finding since it indicates that overall pulmonary vascular resistance was lower. Further, vascular resistance seemed to be higher during retrograde flow than forward flow, but the number of experiments are not sufficient for this observation to be significant. With the development of hypoxic oedema there were quite marked falls in flow in the three elevated arterial pressure experiments (210 to 196 ml/min, 234 to 256 ml/min and 305 to 184 ml/min). The two retrograde flow experiments showed smaller falls (156 to 143 ml/min and 172 to 166 ml/min).

HISTOLOGY

Zone I experiments

Tissue just under the pleural surface of the three lungs frozen with Freon 12 was used to test whether the intra-alveolar vessels were indeed collapsed during Zone I conditions. Sections 2 μ thick

were examined at x 700 magnification and the number of red cells present per 100 μ alveolar septum was recorded in 15 fields and 40 alveolar septa. There was a mean value of 2.8 ± 0.04 , which is within the range reported by Glazier et al (1969) for Zone I in vertical isolated lungs. The sparsity of red cells confirms that the lungs were indeed in Zone I conditions. Figure 15 shows the histological appearance of a Zone I lung at x 350 magnification to illustrate this sparsity of red cells in the alveolar septal capillaries due to compression of the septa by alveolar pressure (25 cm H₂O) exceeding vascular pressure (10 cm H₂O). Both Staub (1962) and Glazier et al (1969) found that, under Zone I conditions, some of the corner vessels at the junction of alveolar septa contained red cells. In the present study, besides the presence of red cells in corner vessels as illustrated in Figure 15, dye was seen in some corner vessels showing they were open. They were therefore uninfluenced by the alveolar pressure and could for this reason be classified as extra-alveolar vessels; a finding consistent with the Riley model (1959). Figure 16 shows the size distribution of 269 corner vessels containing dye under Zone I conditions, measured at x 700 magnification. Measurements were limited to vessels less than 20 μ diameter. The mean diameter of corner vessels open is 12.5 μ . Intra-alveolar vessels contained no dye.

Having shown that intra-alveolar vessels were indeed collapsed in Zone I, we have confidence that the intra and extra-alveolar vessels were functionally separated and therefore that the fluid contributing to oedema development in Zone I lungs will have originated from extra-alveolar vessels. This finding infers that vessels other than the

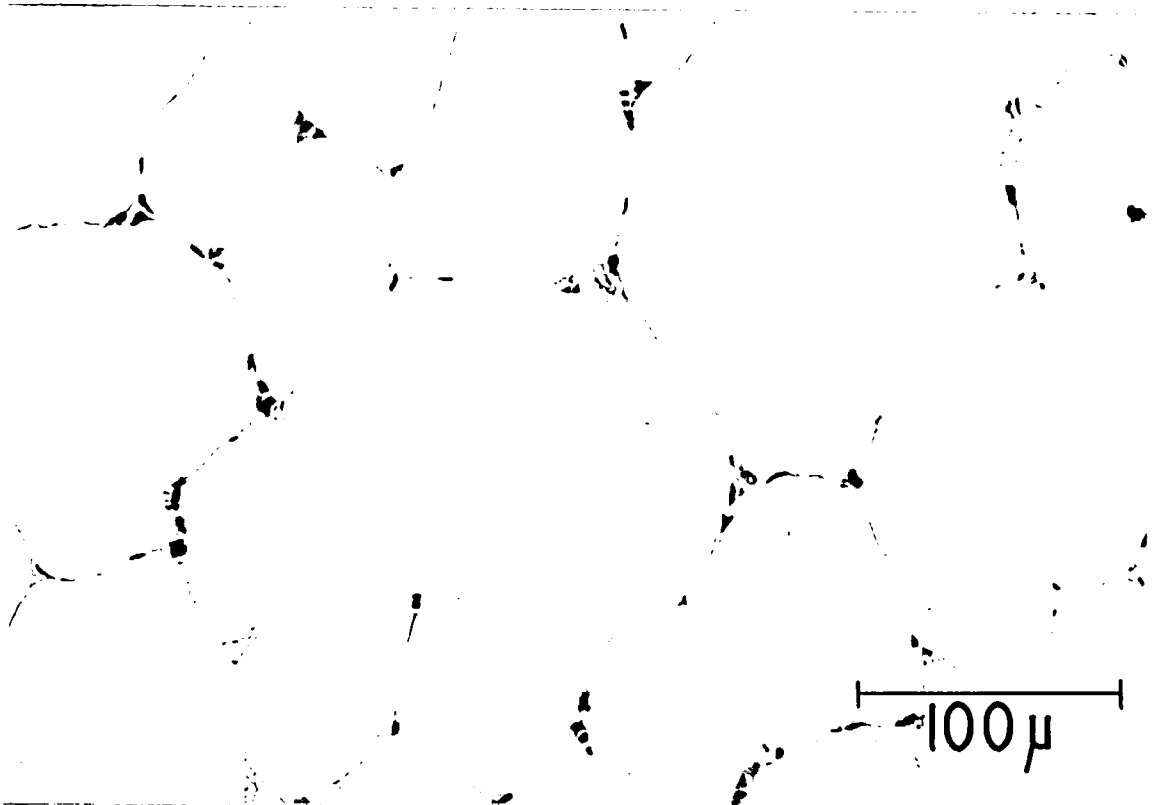


Fig.15 2μ section of lung tissue showing the sparsity of red cells in intra-alveolar capillaries during Zone I conditions.

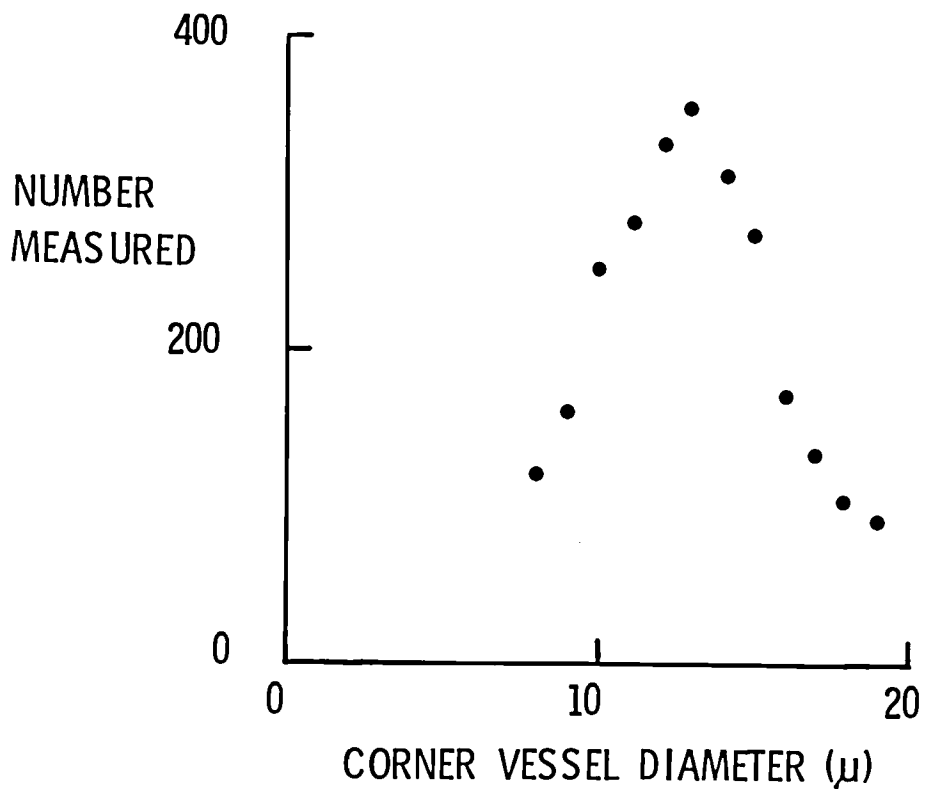


Fig.16 Vessel diameter in microns is plotted against the number of vessels measured from 2μ sections of lung under Zone I conditions.

expected intra-alveolar capillaries are permeable and this being so, they may have a significant role in oedema development in vivo. Before the specific origin of the fluid in Zone I conditions is discussed, some general points can be made. Fluid cuffing round the majority of vessels was first histologically evident when lungs had gained between 4-6% of their original weight on excision. As one might expect, the time course of the appearance of perivascular cuffing was related to the vascular pressures so that high vascular pressures gave rise to interstitial fluid accumulation more rapidly than low pressures. Figure 9 showing the time course of weight gain at different vascular pressures illustrates this point.

Normal histological appearance

Sections of lung biopsies taken immediately after setting up the preparation showed that in the normal situation there is an interstitial space of finite size containing, amongst other structures, lymph vessels, protein elements and small amounts of fluid. Figures 17 and 18 illustrate the normal appearance of an artery and vein respectively, before the lung has gained any weight from interstitial fluid accumulation.

Cuff area to lumen area ratios

When, as shown in Figure 19, vessel size is compared with the ratio of perivascular cuff area to lumen area in normal or 'control' lungs, the ratio is inversely proportional to vessel size. That is, larger vessels have proportionally smaller perivascular cuffs than small vessels. The proportion of vessels in control lungs with measurable cuffs is shown, in Figure 20, to increase with vessel size. In

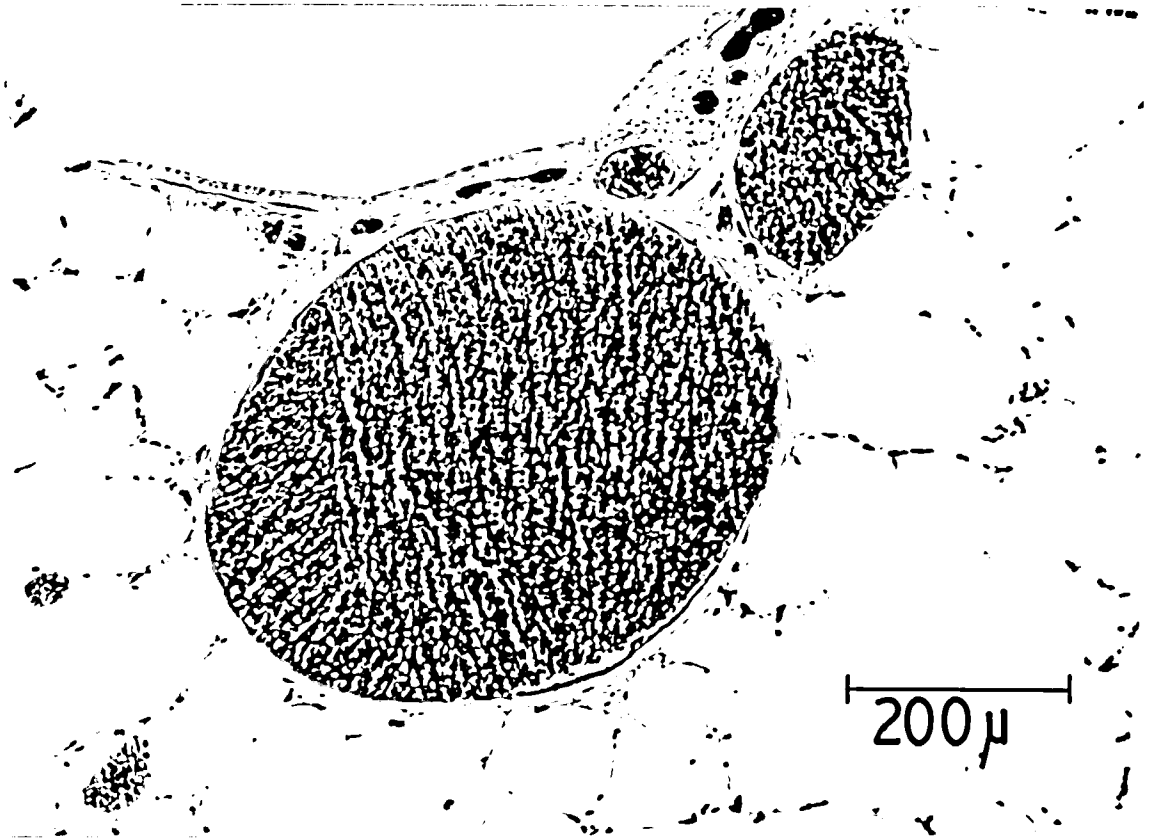


Fig.17 6 μ section showing arteries containing blood mixed with dye. Note the minimal perivascular cuffing in this tissue which was taken immediately the preparation had been set up.

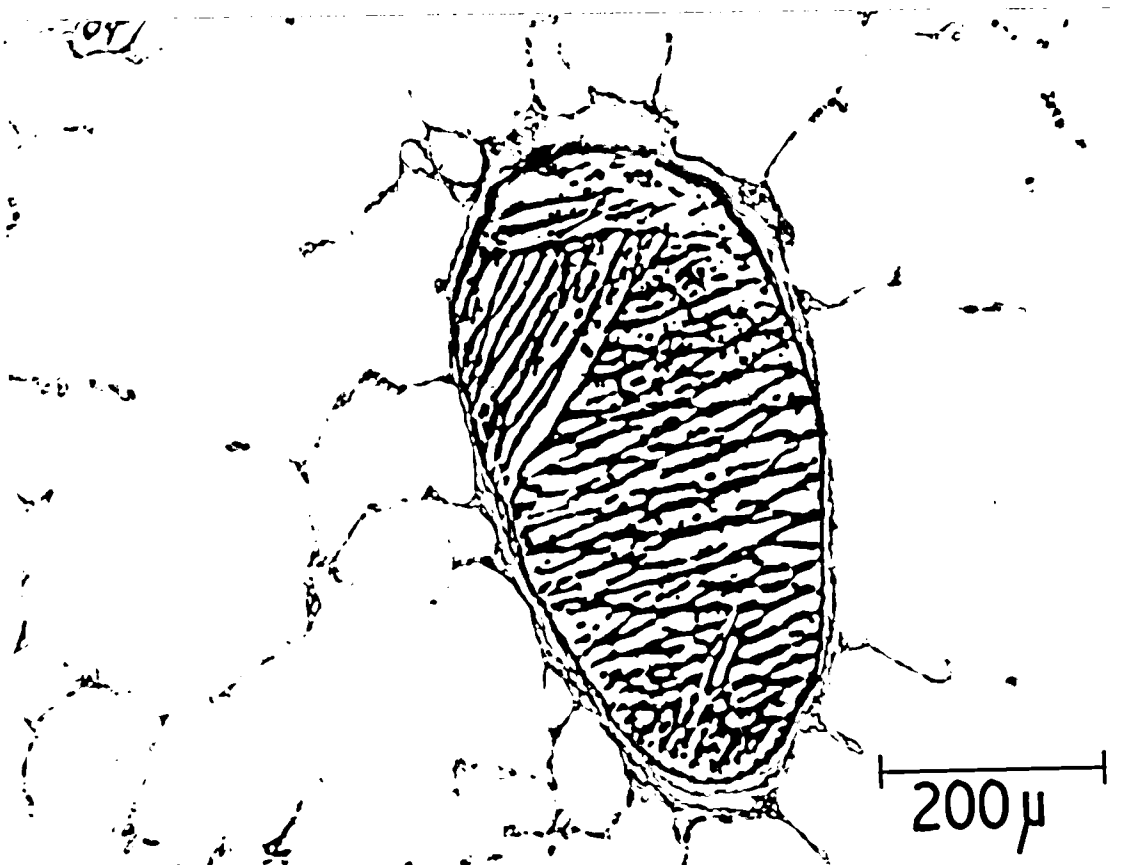


Fig.18 6 μ section showing a vein containing blood mixed with dye. Again this biopsy was taken as soon as the preparation was set up. Notice the minimal perivenous cuffing.

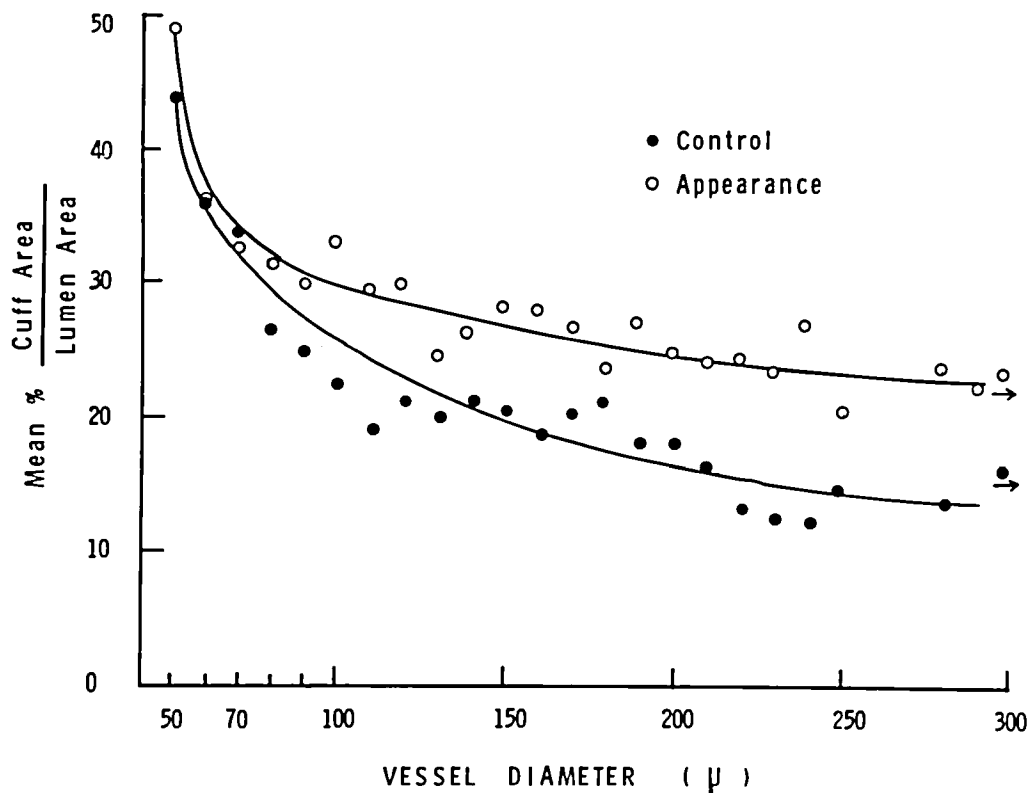


Fig.19 Vessel diameter in microns is plotted against the mean % cuff area/lumen area ratio. • represents measurements made on tissue taken immediately the preparation was set up. ○ represents tissue when the lungs had gained between 4-6% of their initial weight.

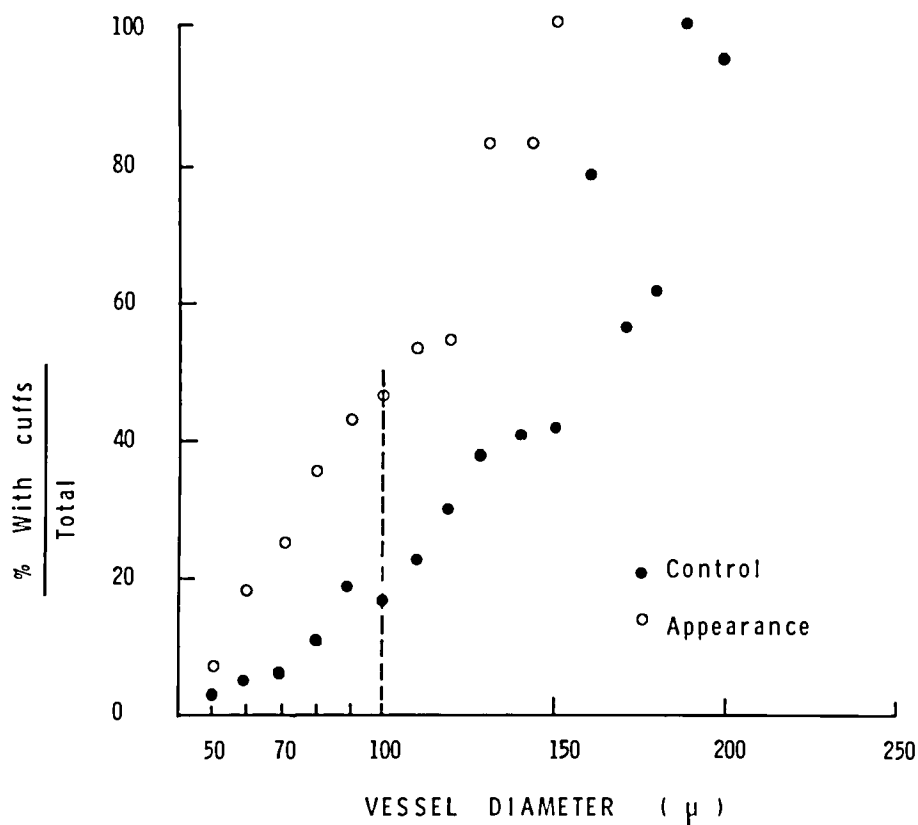


Fig.20 Vessel diameter in microns is plotted against the percentage of vessels with cuffs over the total number of vessels measured. • represents measurements made on the initial biopsy. ○ represents measurements made on tissue taken when there was a 4-6% increase in lung weight.

the presence of a 4-6% weight gain (which arbitrarily marks the 'appearance' of perivascular oedema) the proportion of vessels with cuffs increased considerably. For example, more than twice the number of vessels 100 μ in diameter now have measurable fluid cuffs. Similarly, in Figure 19 it can be seen that the cuff to lumen area ratio has increased with a 4-6% weight gain (appearance). Each point represents the mean ratio calculated from measurements on 21 or more vessels in the range greater than 300 μ and up to 208 vessels in the 50-60 μ range. A paired t test on the control and appearance ratios at each size range showed the change with weight gain to be a significant one ($P < 0.01 - 0.001$). The information gained from cuff to lumen area ratios does not, however, indicate anything specific about the site of origin of the fluid except that a greater proportion of small vessels have cuffs and that their cuff area is proportionately larger, both of which are consistent with small (50 μ) extra-alveolar vessels being more permeable than the large ones. In fact when comparing cuff to lumen area ratios of arteries and veins separately, the results are misleading. With a 4-6% weight gain the mean cuff to lumen area of 100 μ arteries is 36%, while that for veins is only 27% (the values being significantly different, $P < 0.001$), giving the impression that arteries may be more permeable than veins. In the previous section on weight gains, we showed the reverse to be true; namely veins were more permeable than arteries since their contribution to weight gain in two hours was considerably greater. The explanation for the larger area ratios in arteries may be that the parenchymal border of the peri-arterial space is apparently more loosely attached to the outer

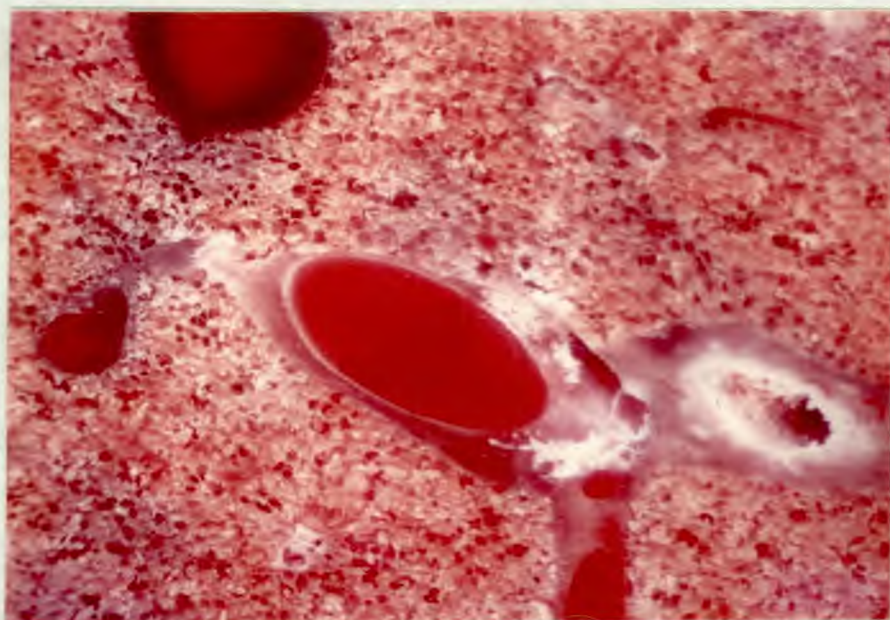


Fig.21 The cut surface of a frozen lung biopsy showing two veins in the top left quadrant, an artery in the middle and an airway in the right lower quadrant. Dye mixed with venous blood has migrated into the perivenous space which can just be seen to join with the periarterial space. X 30.

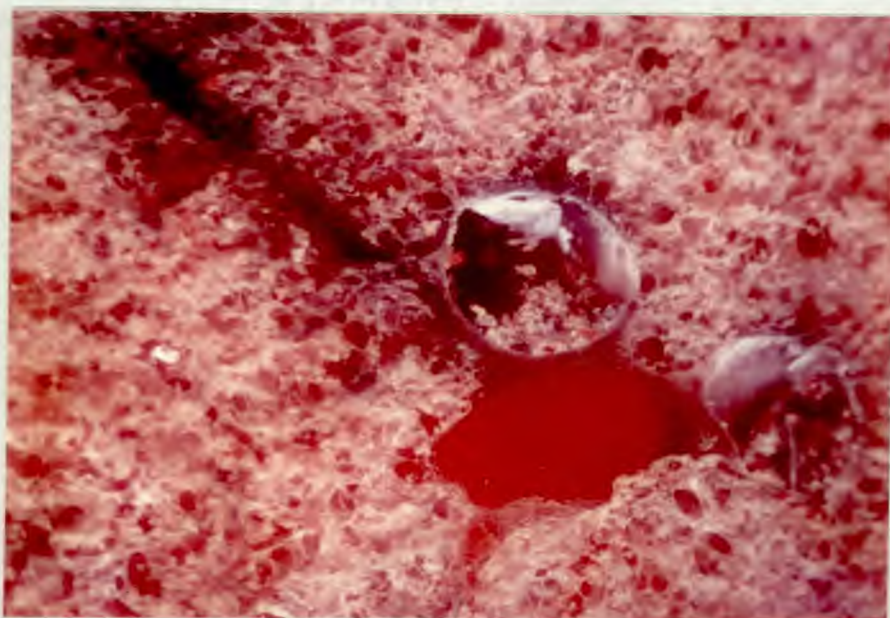


Fig.22 The cut surface of a frozen lung biopsy showing two airways and an artery. Dye, from a vein out of the picture in the top left corner, is seen migrating to the peribronchial and periarterial spaces. X 55.

wall of the vessel than is the case in veins (von Hayek, 1960) and hence interstitial fluid tends to accumulate initially more round arteries than veins. This slightly preferential accumulation round arteries indicated by the area ratios in no way reflects the original site of origin of the oedema fluid since the peri-arterial and perivenous spaces are continuous (Miller, 1911). This observation has been confirmed in the present study and is illustrated by Figures 21 and 22. In both cases dye was mixed with venous blood only. In Figure 21 dye has passed with fluid from the veins into the perivenous space, which can just be seen to join with the peri-arterial space. Figure 22 shows another biopsy at a later stage where the dye-stained perivenous fluid has migrated to the peribronchial and arteriolar spaces. These connections may be extremely important in vivo because fluid passed out of the high pressure arterial side (as in exercise or at altitude) can be readily reabsorbed by the low pressure venous side. Such reabsorption of fluid into the vascular space has been discussed earlier in the Results section.

Histological appearance in the presence of interstitial oedema

The artery in Figure 23 and vein in Figure 24 show obvious perivascular oedema. Note in both the figures the thin alveolar septa, emptied of red cells, confirming collapse of intra-alveolar vessels in Zone I conditions. Surrounding both artery and veins are cuffs of oedema fluid which can only have originated from extra-alveolar vessels. Both the artery and veins shown in the Figures 23 and 24 are permeable extra-alveolar vessels as determined by the dye studies discussed in the next paragraph. Note also the absence of alveolar oedema - a finding



Fig.25 6μ section showing an artery with perivascular fluid cuffing. Note the sparsity of red cells in intra-alveolar vessels characteristic of Zone I conditions.

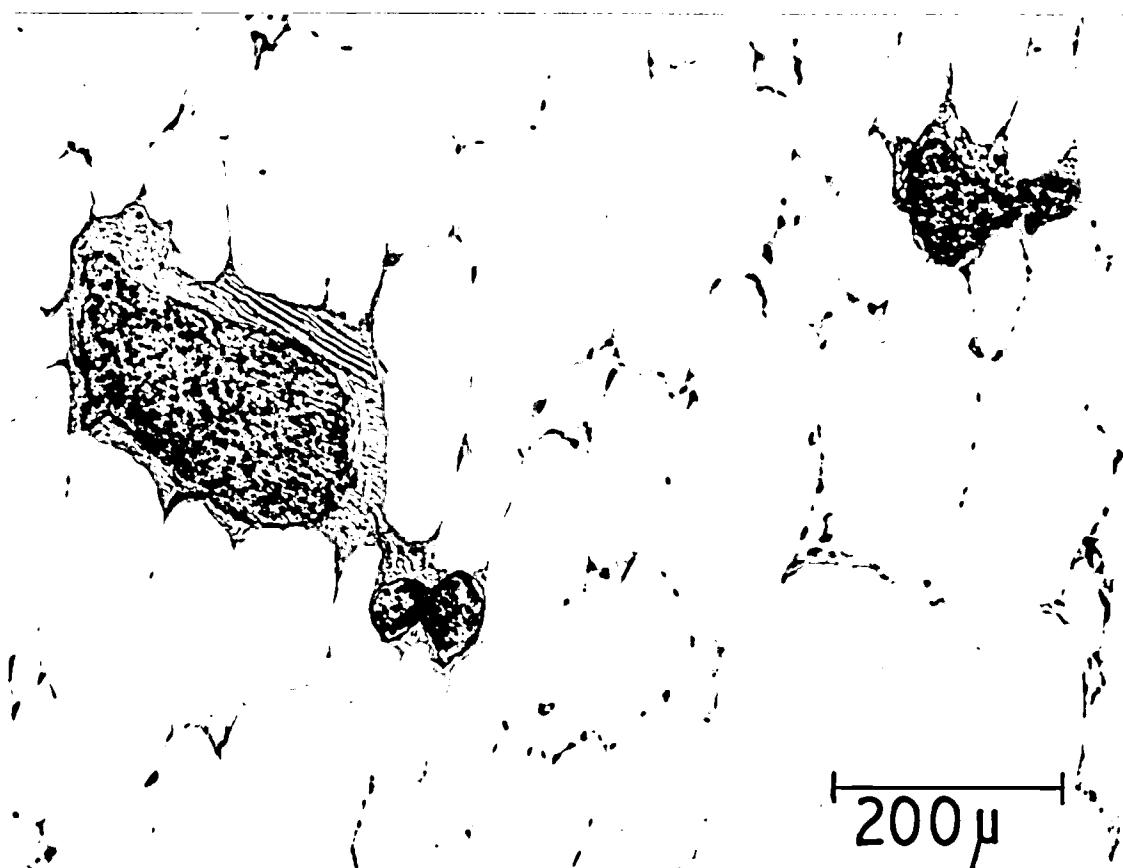


Fig.24 6μ section showing a number of veins with perivascular cuffs. Note the sparsity of red cells in the intra-alveolar vessels characteristic of Zone I conditions.

common to all experiments except the three hypoxia "Static Zone III".

Dye indication of permeable extra-alveolar vessels

From the weight gain and histological evidence of interstitial fluid accumulation during Zone I conditions, we can say that some extra-alveolar vessels must be permeable. To determine the size range of permeable extra-alveolar arteries and veins, dye was mixed in with the blood to mark the passage of water and proteins into the interstitial space. The criterion for permeability was presence of dye in the lumen, wall and perivascular space concomitantly, and by this criterion arteries up to 200 μ in diameter and veins up to 750 μ diameter were found to be permeable. Whereas this was not conclusive evidence of permeability, it might be a good indication because all observed vessels larger in diameter than the limits mentioned had unstained walls. In such vessels the dye-stained fluid in the perivascular space would have originated from smaller vessels, and subsequently migrated towards the hilum. Figures 25 and 26 are photomicrographs of the surfaces of frozen biopsies in the cryostat showing a permeable artery of 150 μ diameter and permeable vein of 615 μ diameter. In the latter the dye has almost all migrated into the interstitial space. Both vessels show dye-stained walls. It is of interest that both vessels have small branches (arterial, 78 μ diameter, and venous, 162 μ diameter) whose contribution to the oedema fluid cuff one would expect to be highly significant. Indeed the arterial fluid cuff seems to be largest in the vicinity of the branch. Where large impermeable vessels have small permeable branches, it is likely that the fluid from them will flow directly into the interstitial space surrounding

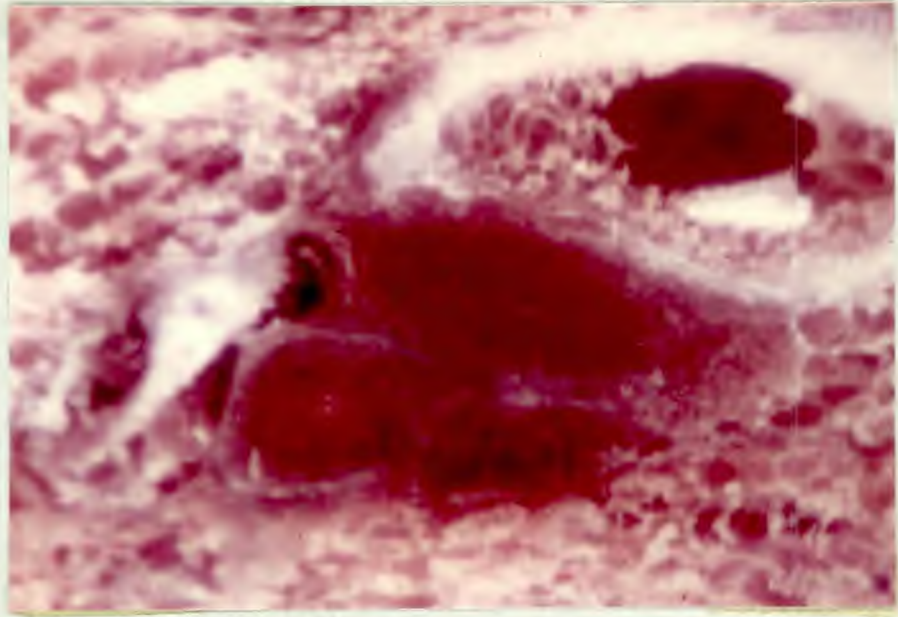


Fig.25 The cut surface of a frozen lung biopsy showing an artery and its branch adjacent to an airway. The lumen, wall and periarterial space are all dye-stained indicating the artery to be permeable. X 150.

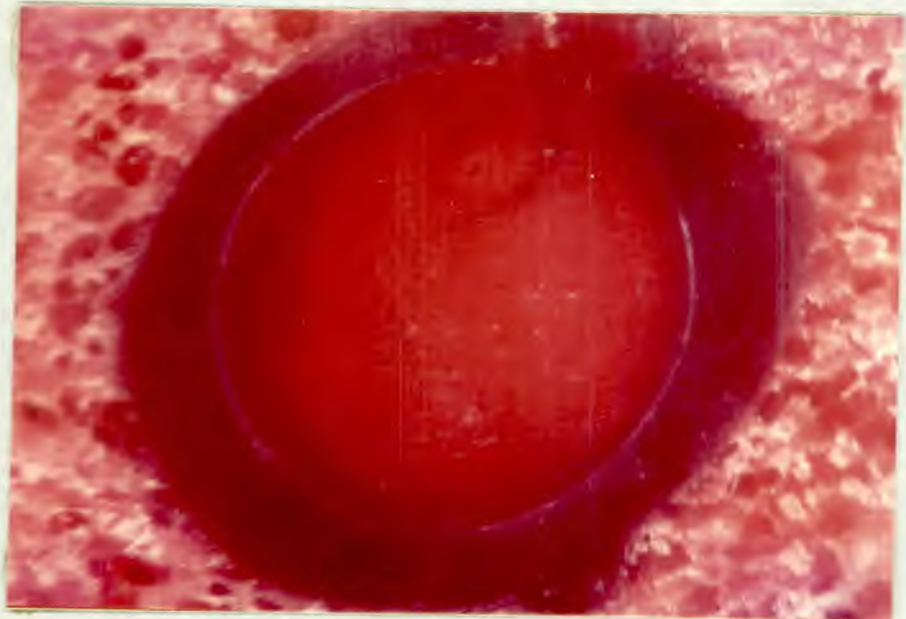


Fig.26 The cut surface of a frozen lung biopsy showing a permeable vein. Dye mixed with the venous blood has migrated into the perivenous space. Notice concomittant staining of lumen, wall and surrounding space. X110.

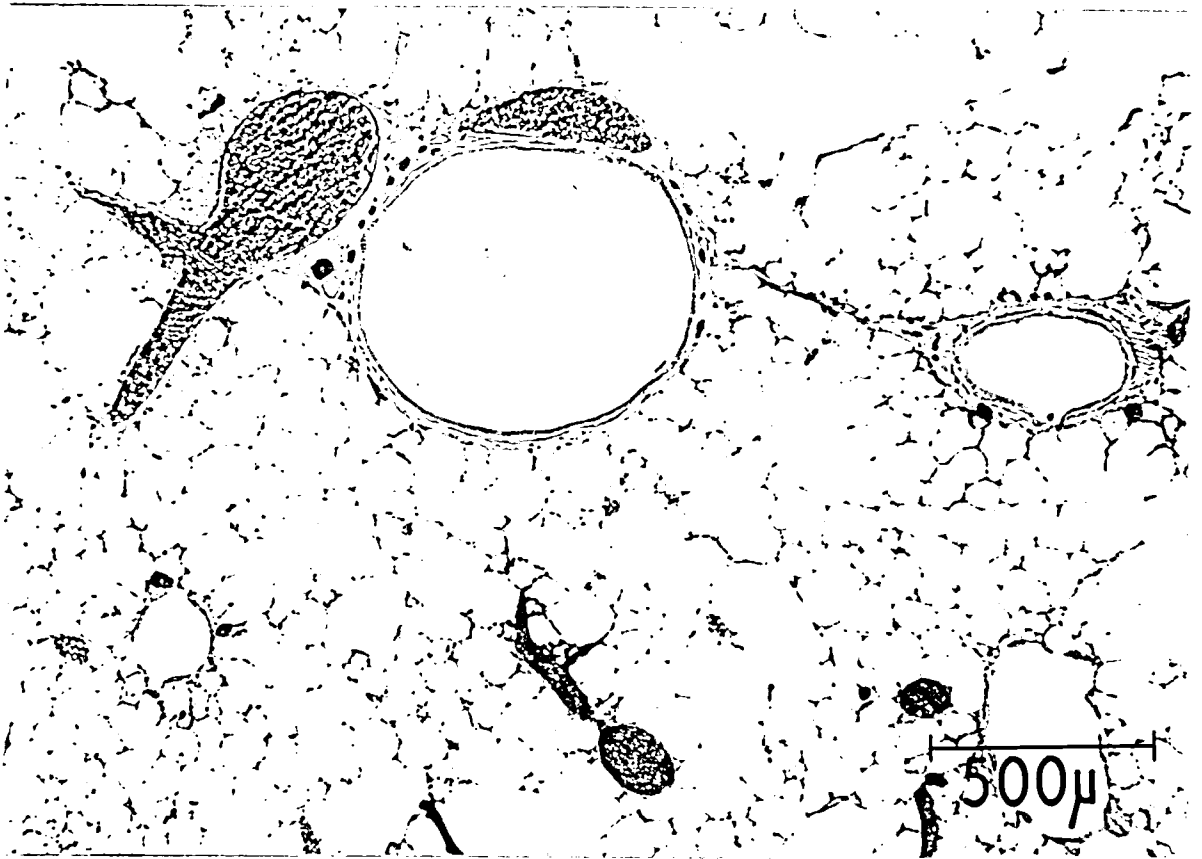


Fig.27 6 μ section of Zone I lung showing an artery adjacent to an airway. The main artery is not permeable, but its two branches are.

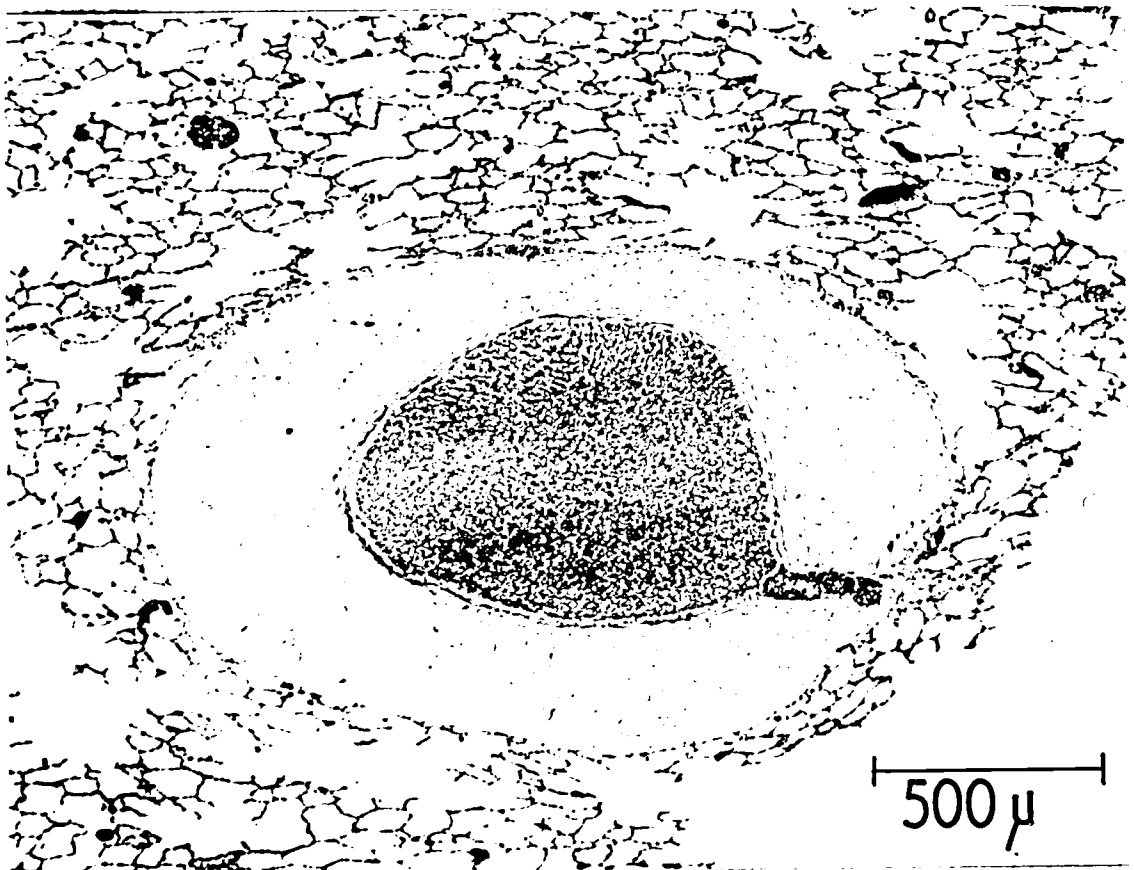


Fig.28 6 μ section of Zone III lung showing an impermeable vein with its permeable branch.

the large vessel. Figures 27 and 28 show an impermeable artery and vein respectively, each having small permeable branches. Figure 27 is a section from a Zone I lung, whereas Figure 28 is from a Zone III flow experiment in which the venous pressure was elevated. Note the sparsity of red cells in the alveolar septa in Figure 27 and contrast it with the numerous red cells in the capillaries in Figure 28.

Hypoxia

The histological appearance during Zone I hypoxic experiments was very similar to all the photomicrographs already introduced. One major difference however was the incidence of arteriolar haemorrhage into the perivascular space which was not once seen in the normoxic series. The haemorrhage was never alveolar, but confined to the interstitial space, indicating microrupture of arterioles. Figure 29 is the surface of a frozen biopsy showing the peri-arteriolar and peribronchial spaces filled with blood. It is of interest too that the size range of permeable arteries, as judged by the criterion previously mentioned, was increased by hypoxia and the largest permeable artery seen was 620 μ in diameter.

Flow experiments - Zone II

Figure 30 illustrates the histological appearance of the lung at the end of Zone II (forward flow) experiments. An immediate difference between this section and those in Zone I is the increased number of alveolar septal red cells. Interstitial fluid accumulation is minimal and confined to the peri-arterial space; that round veins and airways being negligible. The appearance contrasts with Figure 31, in which there was retrograde flow. Notice the perivenous fluid

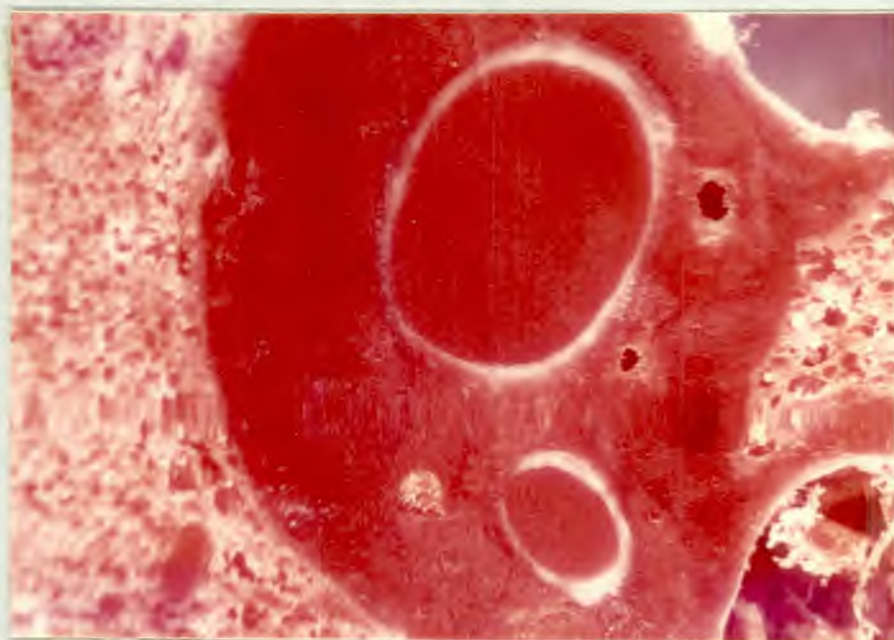


Fig.29 The cut surface of a frozen lung biopsy showing two arteries with their surrounding interstitial spaces filled with blood. X 30.

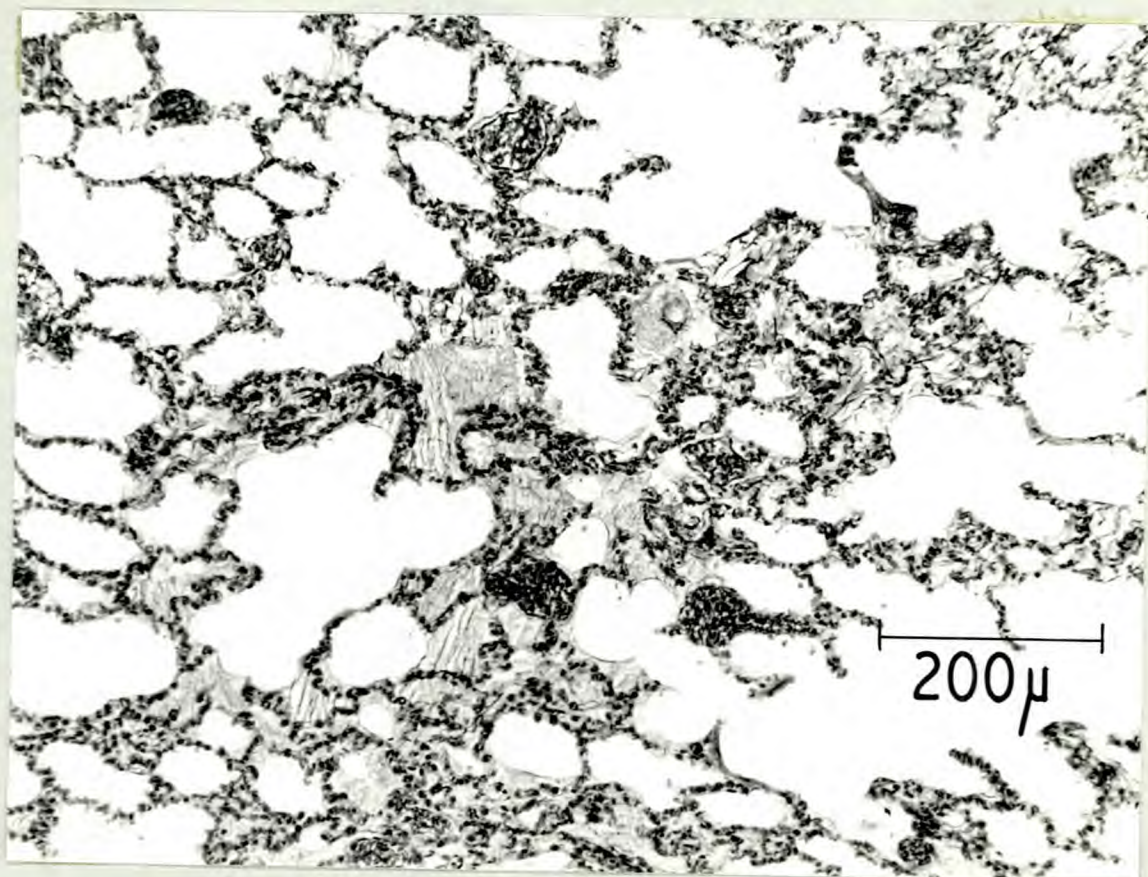


Fig.32 6 μ section showing the appearance of alveolar oedema.

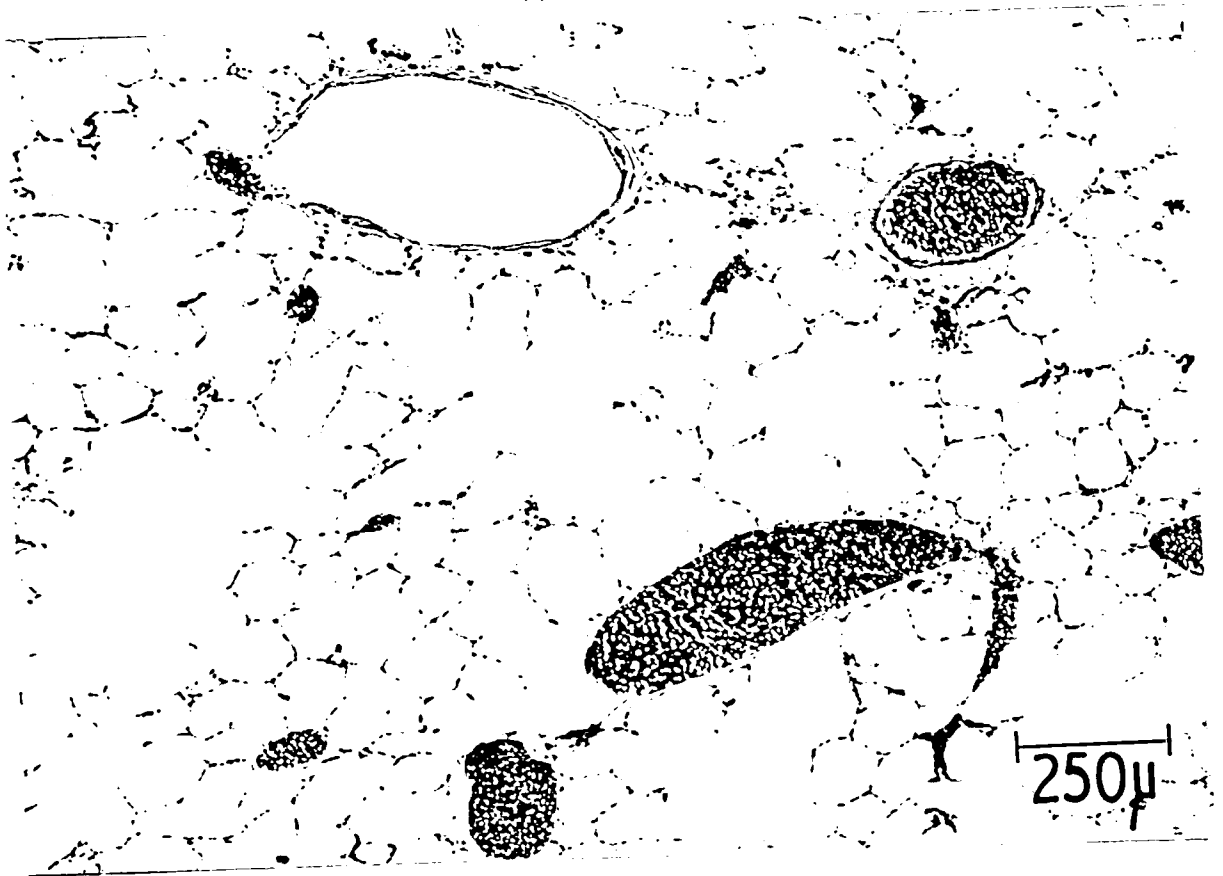


Fig.30 6 μ section of Zone II lung (forward flow). Note some peri-arterial fluid but minimal perivascular cuffing.

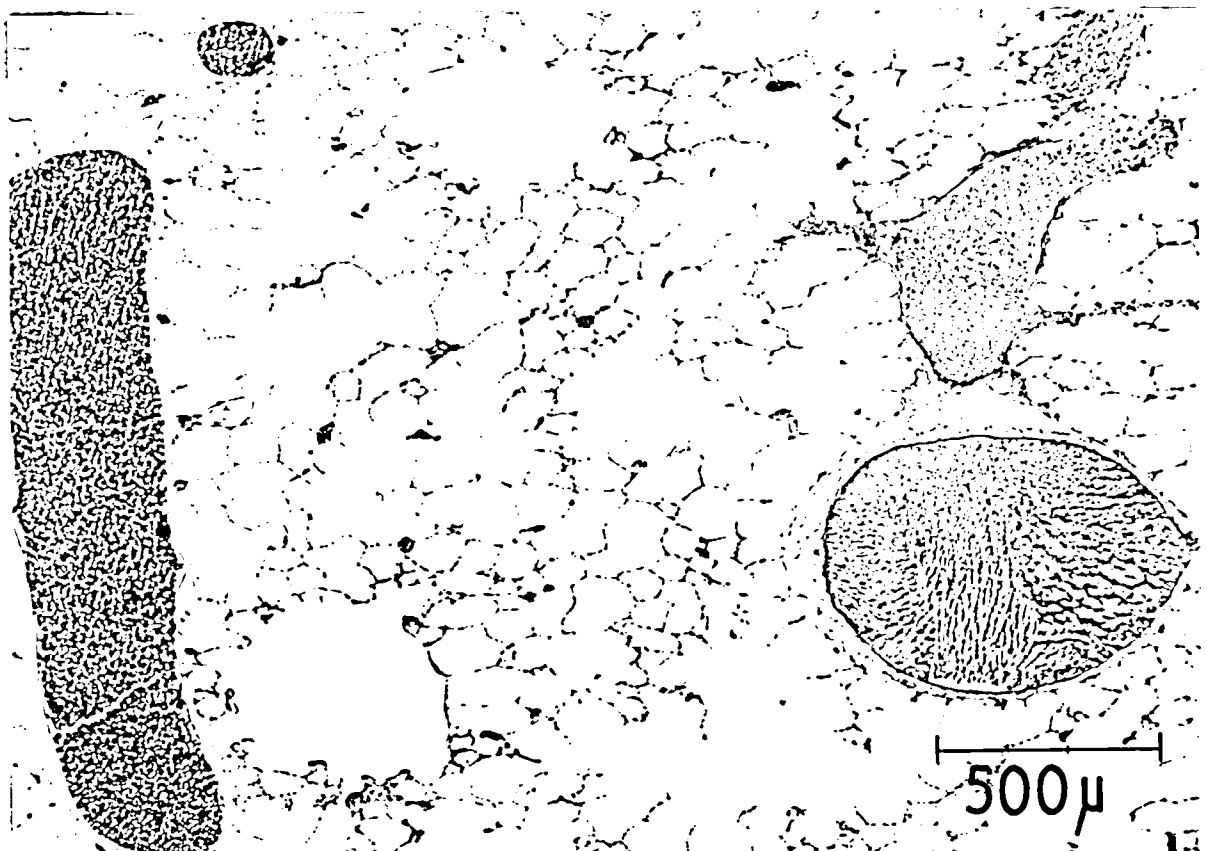


Fig.31 6 μ section of Zone II lung (retrograde flow). Note perivascular fluid but minimal perivascular cuffing.

cuffing and absence of peri-arterial cuffing. In both cases there is absence of alveolar or airway oedema.

The hypoxia experiments in which there was forward flow showed peri-arteriolar haemorrhage very similar to that shown in Figure 29; the venous appearance being normal. In addition, there was alveolar oedema whose appearance is illustrated in Figure 32. Retrograde flow with hypoxia did not give rise to any haemorrhage, whether alveolar or perivascular.

Zone III

Figures 33 and 28 are sections of lung frozen in Zone III conditions. The elevated venous pressure clearly shows a congesting effect on the alveolar septal capillaries. Venous fluid cuffing was very marked (Figure 28) and peri-arterial cuffing evident. There was, however, absence of alveolar oedema in the normoxic series. Hypoxic lungs in Zone III showed both peri-arterial and alveolar haemorrhage.

Pulmonary vascular resistance

In accordance with previous studies of the distribution of blood flow in the lung (West et al, 1965; Hughes et al, 1968) there was an overall rise in vascular resistance associated with interstitial oedema. The increased resistance was attributed to narrowing of arterioles caused by the perivascular oedema fluid. Figure 34 from the present series illustrates such an artery, distorted by the surrounding fluid cuff. The irregular shape of its cross section suggests a significant fall in internal diameter, giving rise to increased resistance.

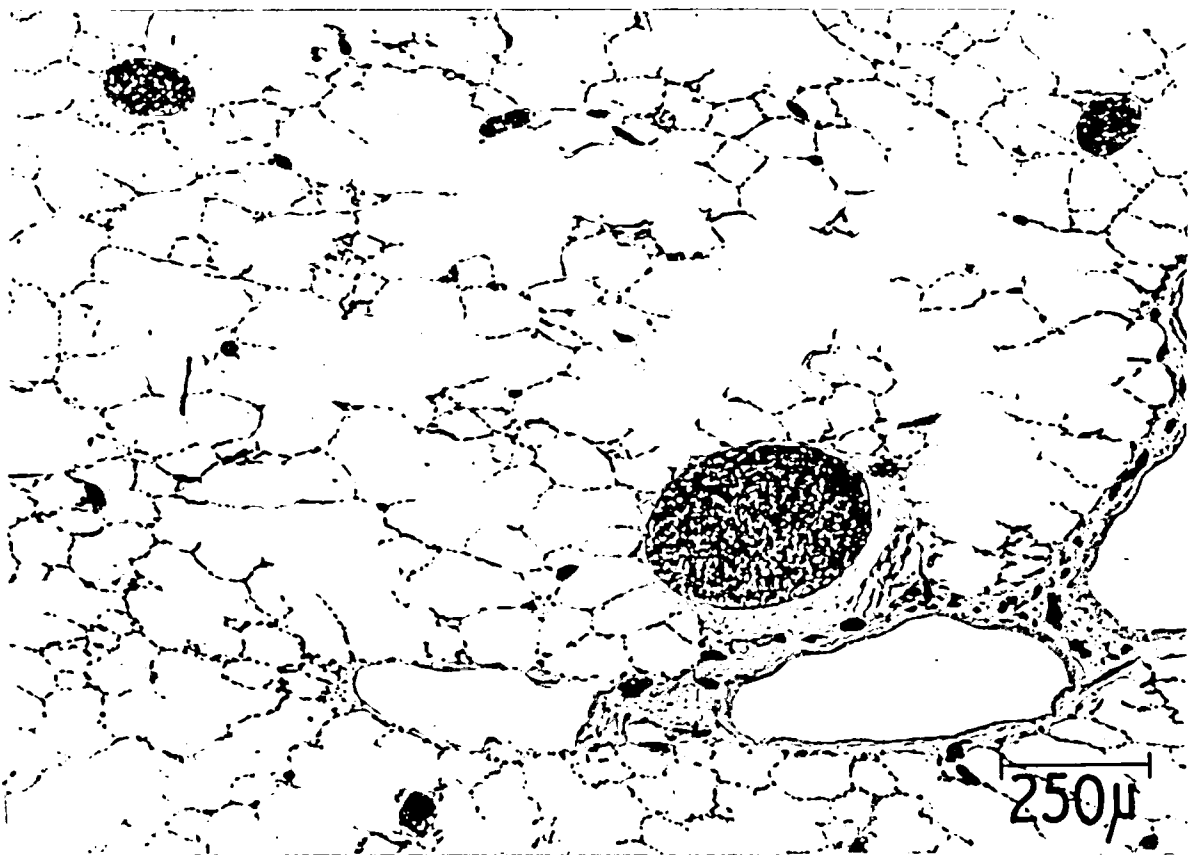


Fig.33 6 μ section of Zone III lung. Note the congested appearance of the intra-alveolar vessels and the periarterial fluid cuffing. Perivenous cuffing under Zone III conditions is illustrated in Fig.28.

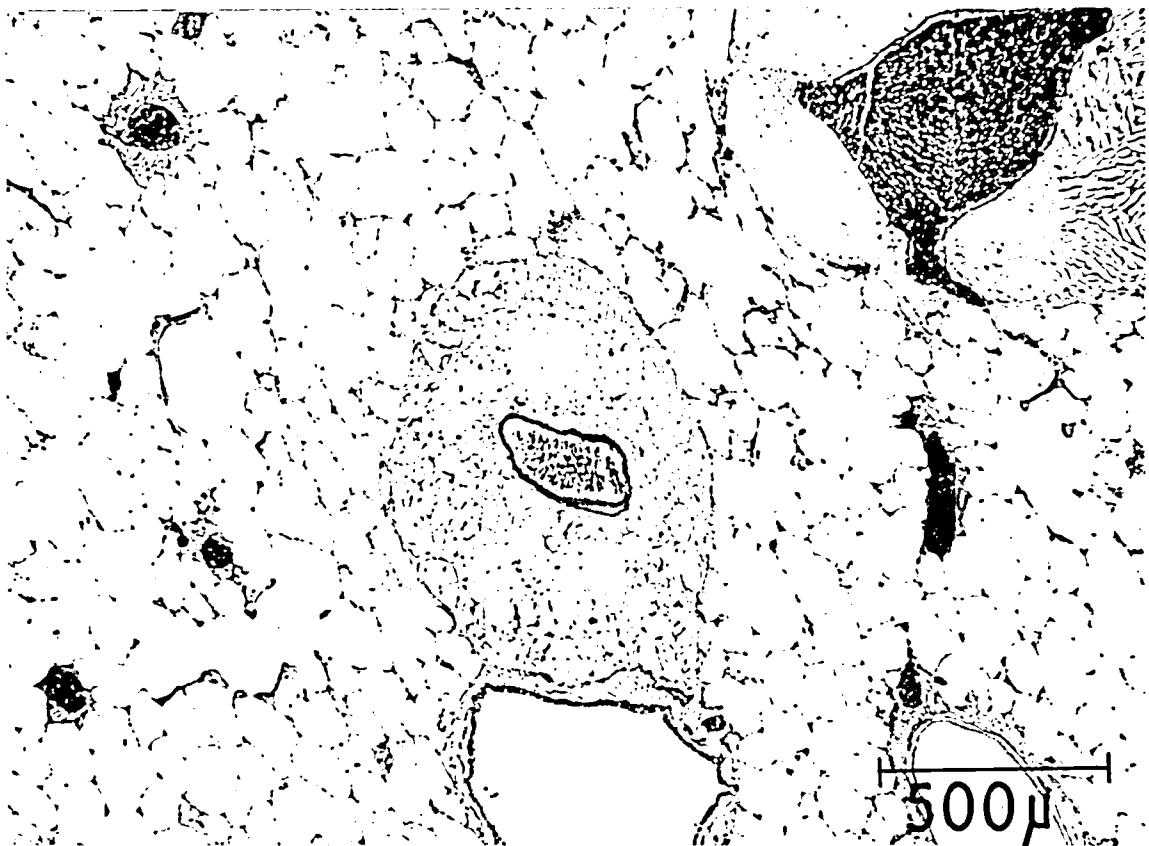


Fig.34 6 μ section of Zone I lung showing an artery and vein both considerably distorted by their surrounding cuffs of oedema fluid.

DISCUSSION

Advantages of the isolated lung preparation over the whole animal in studying the site of fluid leakage in pulmonary oedema.

An in vivo study has all the advantages of normal physiology but all the difficulties associated with the controlling, or attempting to control, the numerous parameters which influence the specific area of investigation. An isolated lung on the other hand allows complete control of vascular and alveolar pressures, state of lung inflation and blood flow rate. Many features of this preparation have shown in the past that its function, for some hours, is relatively normal. Gas exchange and pulmonary compliance are normal and the lungs respond to constrictory and dilatory stimuli such as drugs and the breathing of low oxygen gas mixtures. It is true, however, that lymphatic drainage from the lung has been stopped so that the rate of oedema accumulation is exaggerated, but this does not prohibit the investigation of the origin of oedema fluid under a variety of experimental conditions, nor does it prevent the study of some parameters influencing oedema formation.

Indices of pulmonary oedema

In the previous study it was only possible to take lung biopsies at the end of an experiment, and from the tissue histological

measurements and calculations of cuff area was the most consistent method of oedema assessment. In the isolated lung the primary index of oedema was gain in lung weight which was monitored serially and correlated with the histological appearance of biopsies taken at the same time. During each experiment the vascular and alveolar pressures were held constant so that the weighings, separated in time, were made under identical experimental conditions. It does mean, however, that to infer that all the weight gain was due to extra-vascular fluid, the vascular volume has to remain constant. With time it is quite possible that vessel tone would change, but, by confining the experiments to two hours, gross changes were avoided. Weighing the lungs with the vasculature drained on excision and again at the end of the experiment served to check the total weight gain during the experiment and it was found that the two totals corresponded well; the greatest disparity being 3 gm. This would indicate that the vascular volume changes were indeed minimal. The cuff to lumen area ratio does increase with relatively little fluid accumulation (4-6% increase in lung weight), so that it seems to be a fairly sensitive index of early oedema development. It does not, however, indicate either the sites of origin of oedema fluid, nor the rates of accumulation in different segments of the pulmonary vasculature.

Vascular permeability

Some extra-alveolar vessels have been shown to be permeable and fluid from those vessels has given rise to interstitial oedema. Although veins up to 750 μ and arteries up to 200 μ diameter were included, it is probable that the major contribution to oedema would

be from the smaller extra-alveolar vessels which have the thinnest walls and greatest surface area. Nevertheless, the extra-alveolar venous contribution to fluid accumulation was in excess of that of the intra-alveolar capillaries. Wiederhielm (1966) showed an eleven-fold difference in the permeability of highly diffusible dyes between venous and arterial capillaries. The difference between extra-alveolar venous and capillary permeability would have to be considerably greater than 11-fold when wall thickness and surface area are taken into account. Evidence for the existence of two distinct populations of pores, differing in size, has been reported by Landis (1964) using the passage of two dyes, one highly diffusible and the other bound to albumen. He attributed the gradient of permeability described by Rous (1930) and others to the increased number of larger pores in venous capillaries and venules. These relatively large gaps (over 1000 Å) between the endothelial cells of venules had earlier been demonstrated by Majno and Palade (1961) in electromicrographs of muscle. One might speculate that in the pulmonary venules and veins the number of such leaks might be greater than in the venous systemic circulation, but there is no proven explanation at this time for the seemingly excessive permeability of extra-alveolar veins.

The contribution of extra-alveolar arteries to oedema development was about a third of that from extra-alveolar veins and this finding may be related to a wider size range of permeable veins resulting from the overall thinner wall thickness and larger surface area than arteries of comparable sizes (Wiener et al, 1966). In the static situation, 63% of the interstitial oedema fluid originated from extra-

alveolar arteries and veins and 57% from intra-alveolar capillaries. One parameter which would favour fluid transudation via permeable extra-alveolar vessels in vivo might be their greater transmural hydrostatic pressure gradient. The pressure surrounding capillaries is considered to be approximately alveolar, or slightly less because of alveolar surface forces. Interstitial pressure surrounding extra-alveolar vessels would be related to the lung distending forces and therefore might approximate pleural pressure or be even more negative. Even taking the slightly lower hydrostatic pressure in the veins as compared with capillaries into account, the transmural pressure gradient would be greater for extra-alveolar vessels than capillaries.

Summarising:-

$$\frac{P_{\text{transmural cap}}}{P_{\text{transmural ex}}} = \frac{P_{\text{cap}} - P_{\text{alv}}}{P_{\text{ex}} - P_{\text{pl}}} = < 1$$

Effect of alveolar and pleural pressures on oedema formation

In accordance with the findings of Wagner et al (1961) and Mellins et al (1969) the results reported here show in Figure 10 that changes in alveolar pressure did not alter the rate of oedema formation. Therefore in these experiments the transmural pressure (constant vascular pressure on the one side and interstitial pressure on the other), was not apparently affected by variations in alveolar pressure, indicating that interstitial pressure did not change appreciably with alveolar pressure. Lending further support to this inference, there was a linear relationship between vascular pressures and oedema formation (Figure 9) despite the alveolar pressures varying between 5-30 cm H₂O and therefore the state of lung inflation varying considerably.

This result is unexpected, particularly as changes in pleural pressure (and therefore lung inflation) were shown by Mellins et al (1969) to affect the rate of oedema formation. In addition, Howell et al (1961), when they observed that increasing lung volume increased the vascular volume of larger vessels, concluded that interstitial pressure becomes more negative with lung inflation. Whether positive or negative pressure inflation was used, they found that the pressure surrounding the large vessels was not alveolar pressure. Since then it has generally been assumed that interstitial pressure approximates pleural pressure, or may be more negative than it.

A study which might help to resolve the disparity between the effects of alveolar and pleural pressure on the extra-alveolar vascular contribution to oedema formation is one by Pratt (1968). He measured the pressure in distal arteries during static inflation with negative and positive inflation with results, summarised below, which suggest that the interstitial pressure is higher with positive pressure because the parenchymal traction on the vessel is counteracted by the alveolar pressure.

<u>Inflation</u>	<u>Alveolar pressure</u> (cm H ₂ O)	<u>Pleural pressure</u> (cm H ₂ O)	<u>Arteriolar pressure</u> (cm H ₂ O)
-ve	0	-14	-17
+ve	14	0	-3

Note that in negative pressure inflation the arteriolar pressure is lower than pleural pressure suggesting interstitial pressure may too be lower. If indeed interstitial pressure is raised by positive pressure inflation, this might partly explain why intermittent positive pressure

ventilation is often effective in the clinical treatment of oedema.

Interstitial liquid pressure

As I mentioned in Chapter I, Starling's Law may govern the movement of fluid from the extra-alveolar vascular compartment into the interstitial space or vice versa.

$$F = k(P_{va} - \pi_{pla} - P_{il} + \pi_{il})$$

where F = fluid flux (ml/sec/cm²), k = filtration coefficient, P_{va} = extra-alveolar vascular hydrostatic pressure, π_{pla} = osmotic pressure of blood plasma (cm H₂O), P_{il} = interstitial liquid pressure (cm H₂O) and π_{il} = osmotic pressure of interstitial fluid (cm H₂O). Two of the pressures are reasonably well established: (1) the colloid osmotic pressure of plasma is about 15 cm H₂O in the dog and is written with a negative sign since it causes fluid movement into the vascular space. (2) The colloid osmotic pressure of interstitial liquid is considered to be similar to that of pulmonary lymph, about 5 cm H₂O. At equilibrium the flux is zero and, by extrapolating backwards in Figure 8 to zero weight gain, we find the extra-alveolar vascular pressures would have to be around -1 cm H₂O. As shown below, the interstitial pressure calculated from these values is -11 cm H₂O, which is of the right order to be comparable with Meyer et al's in vivo experimental findings when recording the pressure inside perforated hollow capsules implanted in the lungs of dogs (1968).

$$F_{ex} = k_{a,v} (-1 - 15 - (-11) + 5) = 0$$

where F_{ex} = flux from extra-alveolar vessels and $k_{a,v}$ = filtration coefficient for extra-alveolar arteries (a) and veins (v).

Hypoxia and pulmonary vascular permeability

Brinker (1945), Henry (1967) and others reported increased pulmonary lymph formation in response to severe hypoxia (P_{aO_2} 15-20 mm Hg), but more recent evidence suggests that moderate hypoxia does not seem to affect capillary permeability (Mullgren, 1967; Goodale, 1970). As will be discussed below, the present results indicate that only arterial permeability seems to be affected by hypoxia.

Static experiments

The Zone I experiments with arterial pressure only raised and Static Zone III experiments show a large increase in the rate of fluid accumulation associated with hypoxia. The pulmonary arterial pressure was the same as in the normoxic experiments (20 cm H_2O) so that the difference in the rate of fluid accumulation cannot be completely explained on the basis of an elevated arterial pressure. In fact the results seem to suggest that the combination of hypoxia and a moderately elevated pulmonary arterial pressure affects arteriolar permeability in particular. Clements (1961) has shown that stretching of capillaries and oxygen toxicity both cause increased capillary permeability. Casten (1954) suggested that stretching of the capillary pores might be a cause of oedema development. Whereas, in the previous chapter hypoxic oedema was largely attributed to the elevated arterial pressure per se, causing random sites of micro-rupture to develop, these experiments would seem to add that hypoxia may also have a weakening effect on the endothelial cell junctions so that, for the same arterial pressure, they are more susceptible to mechanical failure. Certainly, in Zone I experiments the sites of micro-rupture must have been located in extra-alveolar arterioles and were large enough to allow red cells to enter the peri-arterial space. When capillaries were

subjected to an elevated pressure in combination with hypoxia (Static Zone III experiments), there was alveolar haemorrhage originating from the intra-alveolar capillaries.

Flow experiments

Zone II (forward flow) and Zone III experiments both showed increased rates of oedema fluid accumulation. The peri-arteriolar haemorrhage in the Zone II experiments one might expect to have originated from arterioles sufficiently upstream to still be subjected to a pressure not much less than 20 cm H₂O. Certainly, their origin was not from intra-alveolar capillaries because alveolar haemorrhage was absent. Only when venous pressure also was elevated (Zone III) was alveolar haemorrhage found. A curious finding was that in retrograde Zone II flow conditions the rate of fluid accumulation not only was unaffected by hypoxia, but was of the same magnitude as in the normoxic Zone I experiments with venous pressure only elevated. Such a result leads one to speculate whether there was venular vasoconstriction in response to hypoxia. This effect was apparently abolished in Zone III when the mean vascular pressure in the pulmonary circulation was 20 cm H₂O.

Site of pulmonary vascular response to hypoxia

The majority of evidence published indicates that the dominant pulmonary vascular response to hypoxia is arteriolar constriction (Aviado, 1960; Bain, 1963; Staub, 1963; Bergofsky, 1963; Sackner, 1966). Pathological and radiological evidence supports paradoxical distension of the arteries concomitantly (Duke, 1961; Fred, 1962; Hultgren, 1962; Arias Stella, 1963; Roy, 1967). There are, however,

some studies suggesting venous constriction is the dominant hypoxic response (Duke, 1963; Katz, 1962; Fred, 1962; Morgan, 1968), and others would predict that both arterial and venous constriction occur simultaneously (Fishman, 1961). In the experiments described, for the same vascular pressures, retrograde flow rate was less than that during forward perfusion, indicating overall vascular resistance to be greater during retrograde flow. It would be tempting to infer that this would suggest predominantly venular constriction, but the only tiny shred of evidence I have to support this is that the rate of fluid accumulation during retrograde flow was similar to that during Zone I conditions with venous pressure only raised.

Conclusion

The origin of fluid in pulmonary oedema, whether caused by hypoxia at altitude or left heart failure, has until recently been assumed to originate exclusively from capillaries. This isolated lung series has demonstrated that in normoxia pulmonary vessels other than capillaries may be permeable and therefore take an active part in fluid exchange in vivo. Evidence has been presented for the existence of a gradient of vascular permeability in the lung, similar to that in the systemic circulation. During hypoxia the results show that fluid transudation is particularly enhanced from arterioles. A combination of hypoxia and an elevated pulmonary arterial pressure may be the cause of mechanical failure in endothelial cells, allowing passage of fluid and red cells into the interstitial space.

The next chapter introduces the effects of interstitial oedema on the lung; in particular its effect on the distribution of ventilation and blood flow and lung mechanics.

CHAPTER IV

EFFECT OF INTERSTITIAL OEDEMA ON VENTILATION AND PERFUSION

DISTRIBUTION IN ISOLATED DOG LUNGS

The experiments described in this chapter were designed to investigate the effect of controlled amounts of oedema on lung physiology. The series was intended to answer such questions as - "are there changes in pulmonary mechanics associated with early oedema, and if so, how do they affect the distribution of ventilation and blood flow in the lung? If there is regional mismatching of ventilation and blood flow, could this lead to impaired gas exchange? Finally, could changes in the distribution of ventilation and blood flow provide a method of diagnosing early oedema in patients?"

The regional distribution of pulmonary ventilation has been extensively studied in humans (Fowler, 1952; West and Dollery, 1960; Milic-Emili et al, 1966; Robertson et al, 1969) and in isolated lungs (Zarardini and West, 1966; Faridy et al, 1967) but there is no published work on its distribution in the presence of pulmonary oedema. This chapter describes measurements of ventilation distribution made in vertical isolated lungs both before and after the induction of interstitial oedema.

The previous measurements of blood flow distribution associated with perivascular oedema have shown an inverted pattern only under specialised conditions of a raised venous pressure, low flow and narrow arterial-venous pressure gradient (West et al, 1965⁷; Hughes et al, 1968; Ritchie et al, 1969). In order to investigate why oedema

should apparently only affect the pattern of blood flow under such limited conditions, measurements were made of the distribution of blood flow under conditions of low venous pressure, both before and after the induction of interstitial oedema.

Methods

Isolated greyhound lungs were used in the vertical position after the methods of West et al (1964). Greyhound dogs weighing between 20 and 35 Kg were anaesthetised with intra-venous injection of barbiturate and heparinised. The left lung was rapidly removed after death by exsanguination. Glass cannulas were tied into the artery, bronchus and left atrium. The lung was suspended vertically in a Perspex box placed on a balance (Gallenkamp model 7) and weighed continuously during the experiment to assess oedema development. All connections to the box were of soft Penrose drain tubing so that maximum flexibility allowed the balance to swing freely during weighing. Measurements of lung weight were always done under identical conditions. After flow had been stopped, pulmonary arterial (P_a) and pulmonary venous (P_v) pressures were set at a fixed level, 10 cm below the bottom of the lung. Transpulmonary pressure (TPP) was 5 cm H_2O . The lung could be expanded either by lowering the pressure in the box, or by connecting a syringe to the bronchial cannula and applying positive pressure. The latter was used during ventilation measurements.

The lung perfusion circuit is shown in Figure 35. Venous blood from an anaesthetised mongrel dog was removed via two catheters

placed in the jugular veins and passed successively through a roller pump, an air reservoir to damp out pulsations, a filter and a heat exchanger so that it entered the lung at about 37°C . The temperature of the entering blood was measured by a thermometer in a T connector just before arterial cannula. Blood leaving the lung was collected in a venous reservoir with a float-actuated mercury switch controlling a second roller pump which returned the blood to the dog via a femoral vein. Except during oedema induction, the venous reservoir was set below the lung (Zone II conditions, West et al, 1964). Interstitial oedema was induced by raising the venous reservoir 2-5 cm above the top of the lung, until the lung had gained 20 or 30% of its original weight on excision. Arterial pressure (P_a) and venous pressure (P_v) were measured with saline manometers relative to the hilum. A differential pressure transducer (Sanborn 268 B) measured transpulmonary pressure (TPP); box or pleural pressure on the one side and airway pressure on the other. Blood flow was measured by collecting timed samples in a measuring cylinder. Before every measurement of lung weight, ventilation and blood flow a standard volume history was used. The lung was inflated with negative box pressure to TPP $2\frac{1}{2}$ cm H_2O and held for one minute. During this time the lung was scanned 30 cm vertically from base to apex by a pair of scintillation counters to obtain background count rate. TPP was then lowered to 10 cm H_2O and the lung ventilated tidally twice between 6-10 cm H_2O . After setting TPP to 5 cm H_2O measurements of ventilation and blood flow distribution were made as described below.

Regional distribution of ventilation

At TPP 5 cm H_2O a 5cc bolus of xenon-133 gas (containing about 2 mc) was injected into the bronchial cannula and the lung was rapidly or slowly inflated with a syringe containing a predetermined volume of air. This volume measured beforehand was the inspired volume when the lung was inflated with negative pressure from TPP 5-24 cm H_2O . With the lung held inflated, a further scan was performed. Blood flow was stopped during all scans. Before the next measurement, xenon was washed out of the lung and the volume history manoeuvre and background scan were repeated. The mean rate of inhalation before each measurement was determined from the volume trace by the slope of the first 50 cc inspired. Although there was sometimes difficulty in accurately determining the mean flow rate for the first 50 cc in a fast breath, the absolute rates were not as important as the difference (approximately 100-fold) between the fast and slow breaths. The measurement of the distribution of a slow inhalation in the presence of interstitial oedema was repeated in four lungs whose TPP at the time of bolus injection was zero, and 10 cm H_2O .

Expired xenon concentration in lungs with interstitial oedema

At the end of a ventilation distribution measurement, during which the lung was scanned at TPP 24 cm H_2O , the lung was slowly deflated to zero TPP into a spirometer. Expired xenon concentration (in counts per second) was monitored by a lead-shielded counter placed close to the tubing connecting the bronchial cannula to the spirometer. Pressure, volume and xenon concentration were displayed against time on an ultra-violet recorder.

Distribution of blood flow

Each measurement was preceded by the fixed volume history described. When the lung was held at TPP 5 cm H₂O, an injection of 2-5 mc xenon-133 dissolved in saline was made into the tubing close to the arterial cannula. When count rates over the dependent part of the lung were steady, blood flow was stopped and the lung scanned following inflation to TPP 24 cm H₂O. After the induction of interstitial oedema blood flow distribution measurements were repeated and additional measurements made under conditions of raised flow and increased lung volume.

Lung volumes and pressure/volume relationships

The lung volume (TPP 24 cm H₂O) was determined by a gas dilution technique. Xenon was mixed with a known volume of air (400-600 cc) in a bag and the concentration measured. The bag was then connected to the lung at TPP 5 and mixing of gas in lung and bag brought about a new steady xenon concentration. Initial and final xenon concentrations were recorded by means of a lead shielded counter in the rebreathing circuit. Knowing the two concentrations and the initial gas volume, the lung volume was calculated. Regional lung volumes were determined by scanning the lung at TPP 24 cm H₂O after the equilibration procedure described above. Measurements of both total and regional lung volumes were repeated after oedema induction. During slow inflations from TPP 5-24 cm H₂O, electrical outputs from the differential pressure transducer and syringe were displayed on an X/Y recorder to give pressure/volume curves. The deflation curves from TPP 24-0 cm H₂O were similarly displayed, except that volume was

recorded from a spirometer. Thus the pressure/volume relationships on inflation and deflation were studied both initially and after oedema induction. Using the total lung volume and the pressure/volume curves, lung volumes at TPP 24, 5 and zero could be calculated. Volume and pressure signals were also displayed against time on an ultra-violet recorder.

Counting methods and calculations

The specification and geometry of the scintillation counters have been described elsewhere (West et al, 1964). A time constant of 0.2 sec and scanning speed of 0.5 cm/sec were used and data from the counters collected in two ways. First, the scanned counts were passed to rate meters to be displayed as count rates on an ultra-violet recorder with a marker signal for each cm of vertical distance travelled. Secondly, a scaler-timer chain accumulated the counts over each cm of vertical distance covered by the counters. The information was fed sequentially into a data logger with punch tape output. The data was processed by computer as described by Kingaby et al (1968). The blood flow index ($\dot{Q}$) and ventilation index ($\dot{V}$) were calculated according to the methods of Ball et al (1962). A summary of the calculations is given below:-

$$\dot{V} = \frac{Cr}{Cr_{eq}} \times F_{I^{eq}} \times \frac{F_b}{TLC}$$

$$\dot{Q} = \frac{Cr}{Cr_{eq}} \times F_{I^{eq}} \times \frac{F_i}{TLC}$$

where Cr = regional count rate during a measurement, Cr_{eq} = regional count rate at equilibration, $F_{I^{eq}}$ = inspired concentration of Xenon at

equilibration, F_b = Xenon concentration of bolus, F_i = Xenon concentration in saline injection. Since the alveoli in the isolated lung are uniformly expanded at TPP $24 \text{ cm H}_2\text{O}$, we have expressed our blood flow and ventilation ratios as 'per alveolus'. The ventilation ratios were normalised to give a mean ratio of unity. We feel justified in doing this because our method of scanning the lung meant that ventilation at all levels was truly represented. In addition to the computer calculation of ventilation or blood flow per alveolus, the distribution of fast and slow breaths could be directly compared.

Histology

Five minutes after the end of each experiment small pieces of lung tissue were taken from the apical, middle and lower lobes and put into formol saline for fixation. After wax embedding, 6μ sections were cut, mounted and stained with haematoxylin and eosin.

RESULTS

Twelve lungs weighing between 91-156 g were studied both immediately the preparation was set up (30-50 min) and after interstitial oedema had been induced. The early measurements took about an hour to complete, during which time weight gain was minimal (0-4 g). Lung weight was closely followed during the period of oedema induction so that increases of either 20% or 30% the original weight on excision could be achieved. Figure 36 shows a peripheral airway partially surrounded by a small cuff of oedema fluid. The amount of oedema surrounding small airways varied considerably from a barely detectable widening of the peribronchial space to a cuff of fluid completely

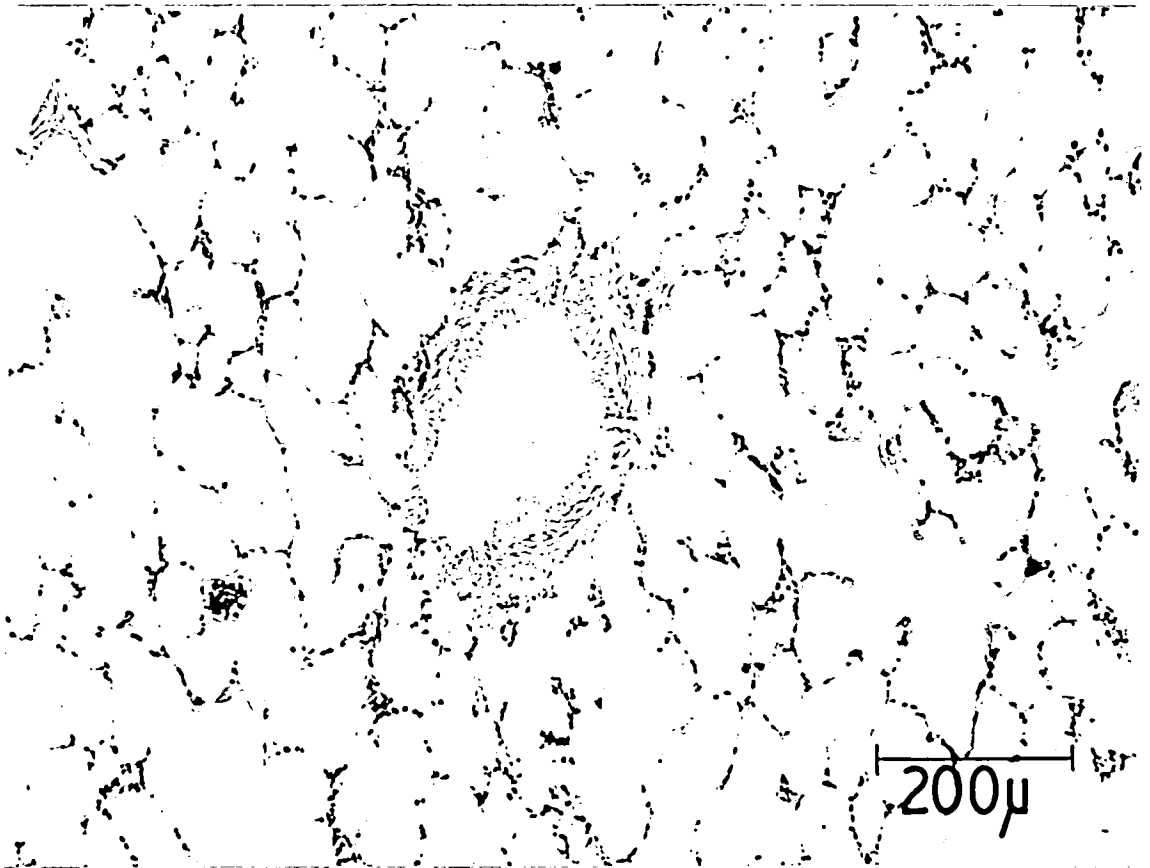


Fig.36 6 μ section of a biopsy taken from the lower lobe at the end of an experiment. Note some oedema fluid accumulation round the small airway.

surrounding the bronchus. In no experiment was oedema fluid histologically visible in the alveoli. Perivascular and peribronchial oedema, present in all lungs, was invariably more marked in the lower lobes than the apical lobe, though no systematic measurements of widths of fluid cuffs were made. Preferential accumulation of perivascular oedema fluid in the basal lobe of isolated lungs has already been described by Hughes et al (1968).

Regional distribution of fast and slow inspirations

Figure 37 shows the mean initial distributions with standard errors of fast and slow breaths. The bottom graph shows a comparison of the distributions. Notice that the standard errors are greater for the distribution of fast breaths than for slow breaths. Figure 38 shows the mean distributions with 20% weight gain and Figure 39 with 30% weight gain. Because the initial pattern of ventilation varied considerably from lung to lung, the subsequent changes in distribution of ventilation with oedema were more evident when compared directly with their initial distribution. If they were identical initially and after oedema induction, then the points would run along unity. Figures 40 and 41 show graphs of such distribution ratios for six lungs which gained 20% of their original weight and six lungs which had a 30% weight gain. The standard errors show that the distribution of the slow breath changes with oedema, whereas that of the fast breath was not so significantly altered. Also, these two figures show that there was no significant difference in the distribution ratio with 20% weight gain as compared with a gain of 30%.

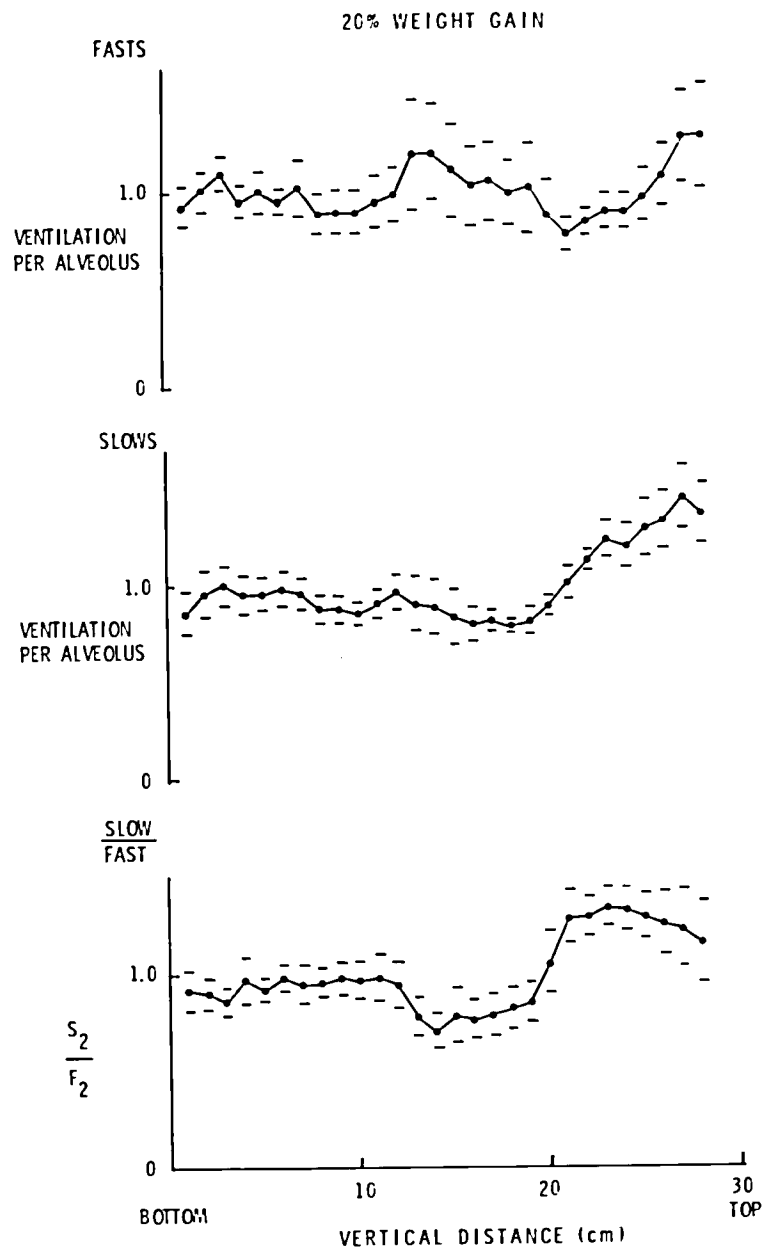


Fig. 58 Vertical distance up the lung is plotted against the ventilation per alveolus. Mean distributions of fast and slow breaths are shown with standard errors for the six lungs which gained 20% of their initial weight. The bottom graph compares the two distributions.

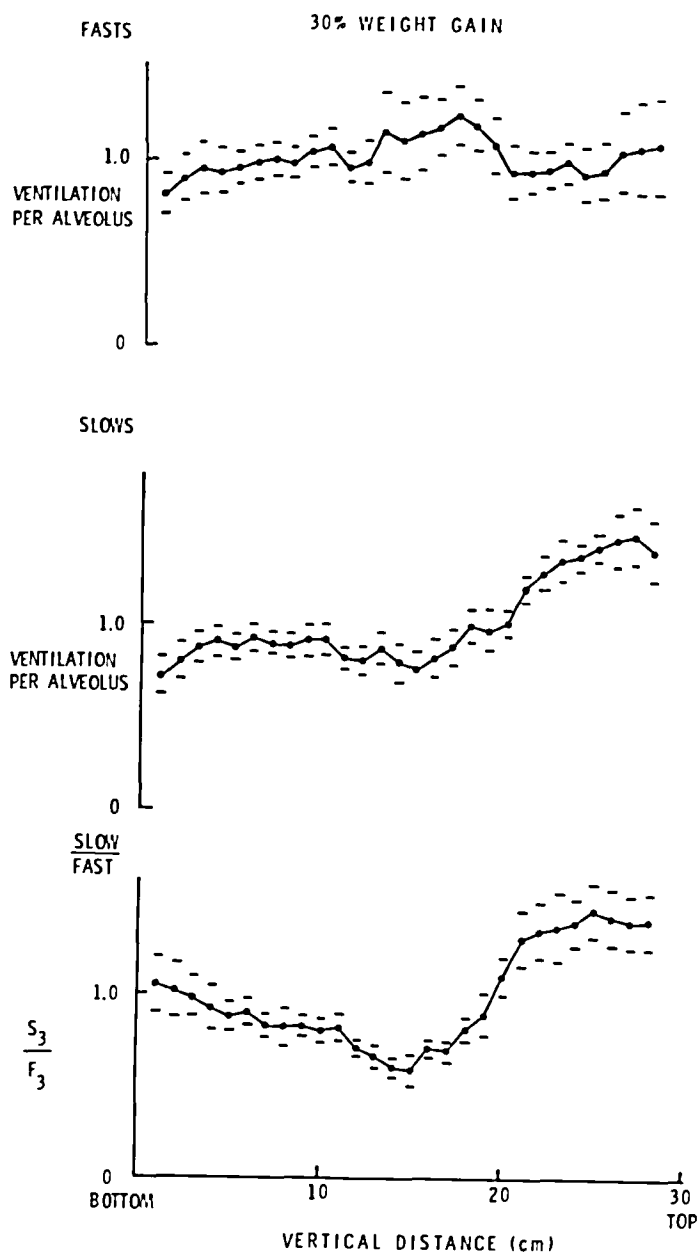


Fig. 59 Vertical distance up the lung is plotted against the ventilation per alveolus. Mean distributions of fast and slow breaths are shown with standard errors for the six lungs which gained 30% of their initial weight. The bottom graph compares the two distributions.

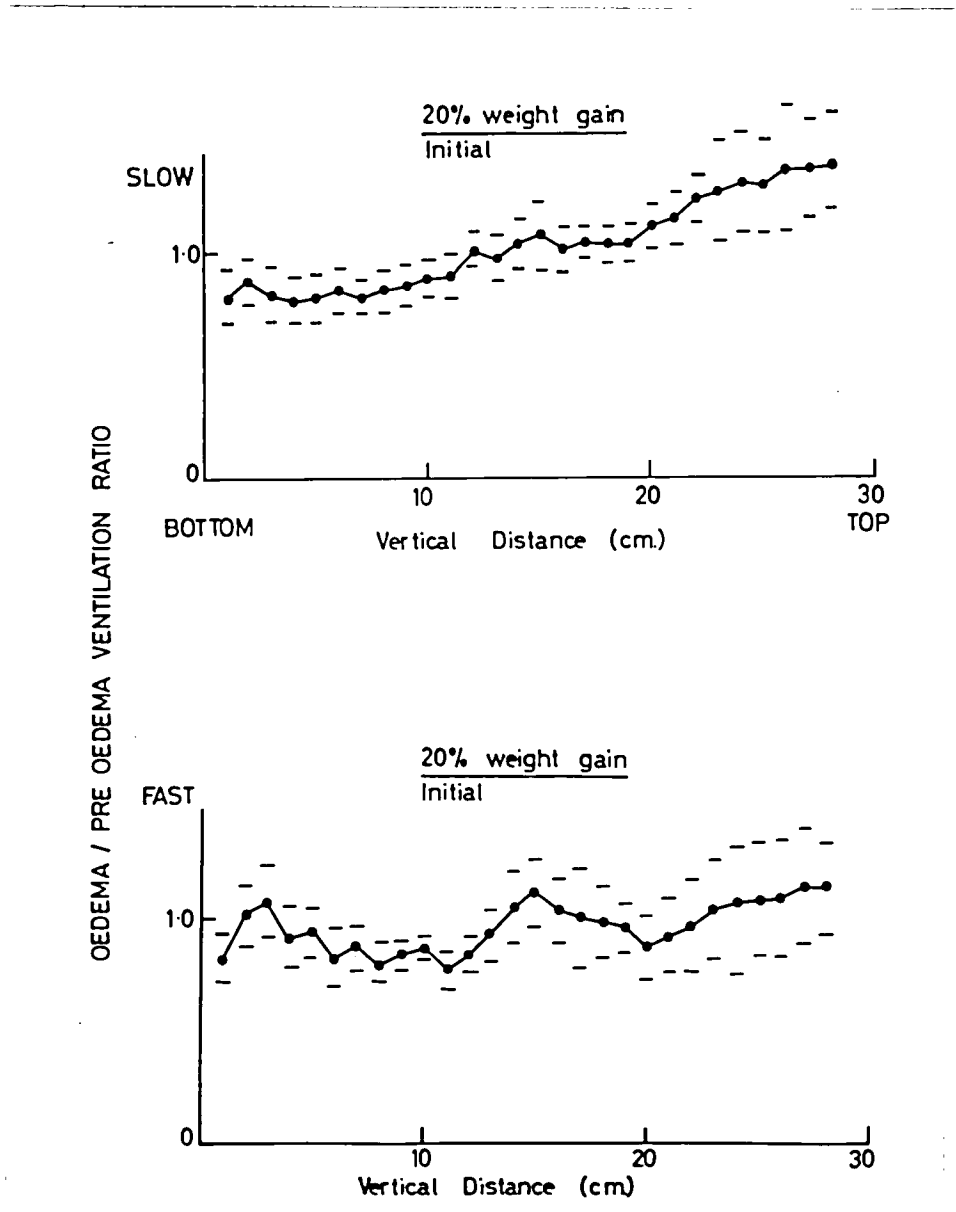


Fig.40 Vertical distance up the lung is plotted against the oedema/pre-oedema ventilation ratio. Mean distributions of this ratio are shown with standard errors for slow and fast breaths.

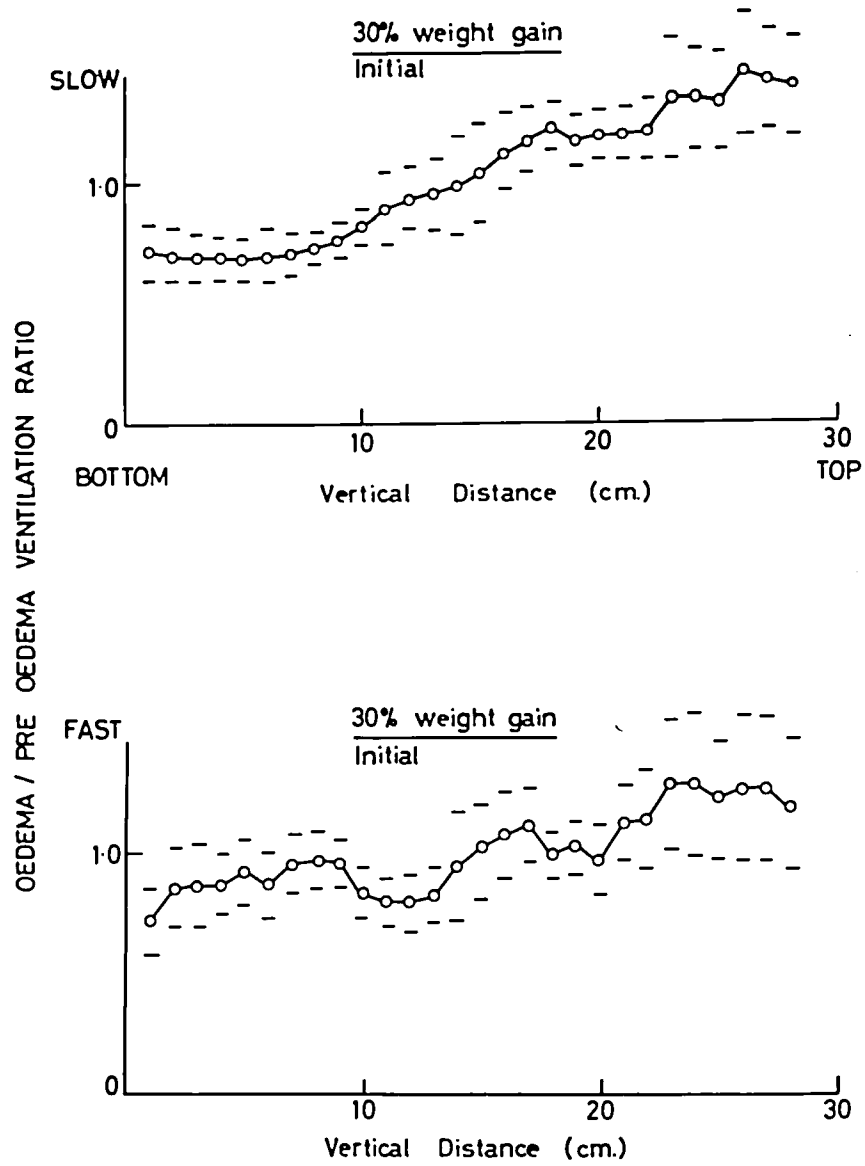


Fig.41 Vertical distance up the lung is plotted against the oedema/pre-oedema ventilation ratio. Mean distributions of this ratio are shown with standard errors for slow and fast breaths.

The rates of inhalation were 20-40 cc/sec for the slow breaths and 2.7-4.8 lit/sec for fast breaths. The effect of positive versus negative pressure inhalation on the distribution of fast and slow breaths was tested. Distributions of slow breaths were identical regardless of the type of inhalation; fast breaths showed slight variations but these may have been due to the slower rate of inhalation with negative pressure inflation (0.8-1.7 lit/sec). Boluses of 20 cc compared with 5 cc showed that bolus size did not seem to affect the distribution patterns.

The effect of time, in the absence of interstitial oedema, on the distribution of a slow inspiration

Isolated perfused lung preparations tend to develop oedema spontaneously with time. To test whether the changes seen in the distribution of a slow breath after interstitial oedema induction were indeed a consequence of oedema and not an aging lung preparation, we made serial measurements of ventilation distribution in one preparation where there was no weight gain (and therefore no oedema) with time. This was achieved by maintaining vascular pressures 20 cm below the lung, except during ventilation measurements when there was flow. Figure 42 shows results from this experiment to illustrate that the effect of time per se on the distribution of a slow breath was negligible.

Expired xenon concentration in lungs with interstitial oedema

Figure 43 shows the changes in xenon concentration with volume of gas slowly expired from a lung with interstitial oedema. Lung counts have been suppressed. Following the xenon free dead space

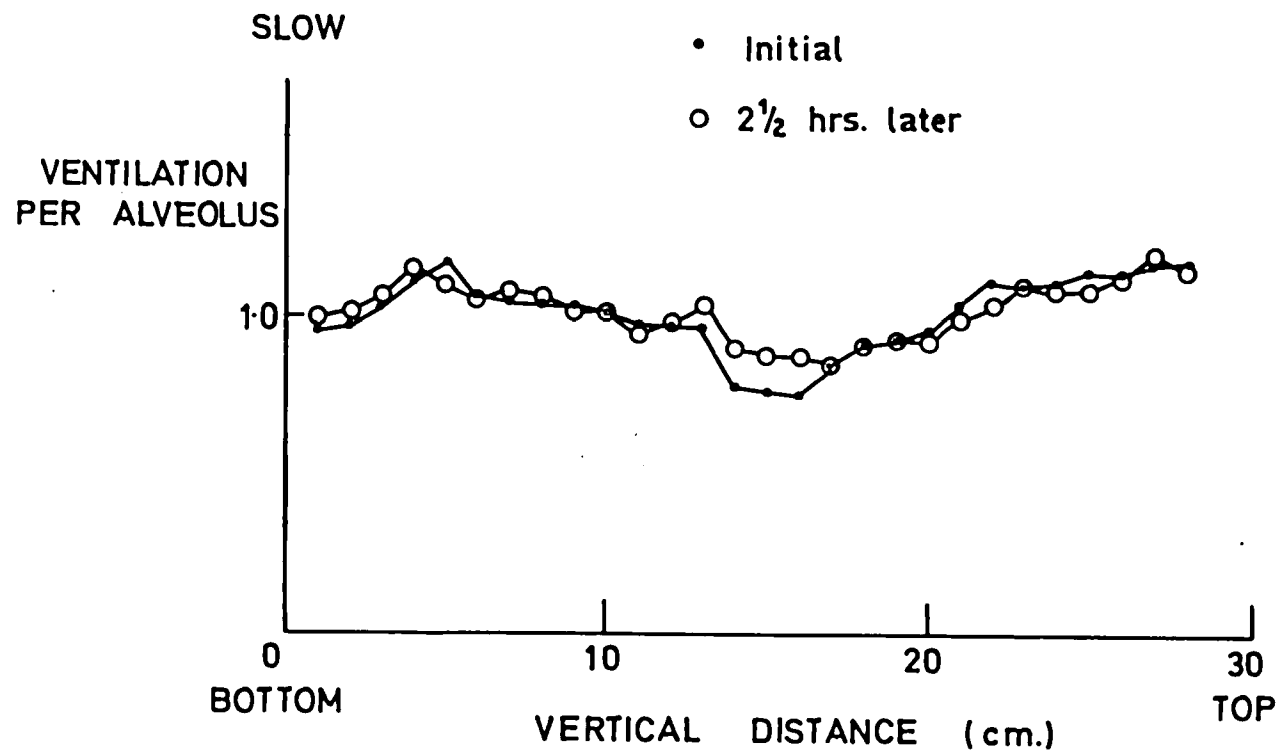


Fig. 42 Vertical distance up the lung is plotted against the ventilation per alveolus. • represents the initial distribution of a slow breath and ○ its distribution 2½ hours later.

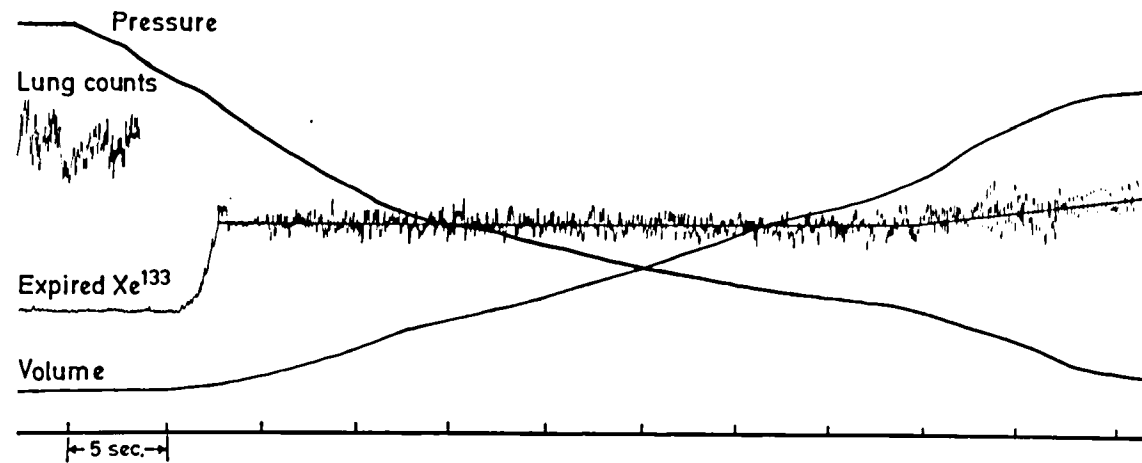


Fig. 43 Copy of an ultraviolet recording of TPP, expired gas volume and expired xenon concentration displayed against time. Notice the slight increase in xenon concentration towards end expiration.

volume the concentration rose sharply and plateaued. Towards end expiration the slope of the xenon concentration slightly steepened. Three other lungs showed similar results.

Lung volumes and pressure/volume relationships

Lung volume measurements (TPP 24) made initially and after oedema induction did not differ by more than 10%. Regional lung volume at TPP 24 did not change with interstitial oedema either. Table XII shows how the volumes of gas remaining in the lung at TPP 5 and zero both increased in the presence of interstitial oedema. They have been expressed here as a percentage of the total lung volume TPP 24 cm H₂O. The mean initial volume ratio at TPP 5 cm H₂O was 44.3%, which rose to 59.0% with oedema. Similarly, the mean initial volume ratio at TPP zero was 21.3% and this rose to 42.4% in the presence of interstitial oedema. These increases are highly significant ($P < .001$ in both cases with the t test). Figure 44 shows results from one experiment illustrating the pressure/volume relationship on deflation from TPP 24 to zero, both initially and after oedema induction. Note how the upper part of the curve is relatively unchanged by interstitial oedema, but the pressure/volume curve has been significantly altered at lower lung volumes, giving rise to a marked increase in the volume of gas remaining in the lung at zero TPP. Figure 45 shows inspiratory pressure/volume curves from experiment 6 in Table XII. It illustrates the point made above that the inspiratory volume (for TPP change 5-24 cm H₂O) was diminished (i.e. lung volume at TPP 5 was increased). Further, Figure 45 demonstrates how the initial slope of the pressure/volume curve has become flatter with

TABLE XII

<u>$V_5/V_{24}\%$</u>		<u>$V_0/V_{24}\%$</u>	
Initially	Oedema	Initially	Oedema
44	56	11	40
43	62	25	48
38	60	18	43
53	67	21	50
47	52	19	37
42	54	16	35
42	71	29	46
26	44	22	38
53	66	21	47
55	55	39	39
47	55	19	47
42	61	16	39

where V_0 , V_5 and V_{24} are the lung volumes at TPP 0,5 and 24 cm H_2O respectively.

The volumes of gas remaining in the lung at TPP 5 and zero cm H_2O expressed as a percentage of the lung volume at TPP 24 cm H_2O .

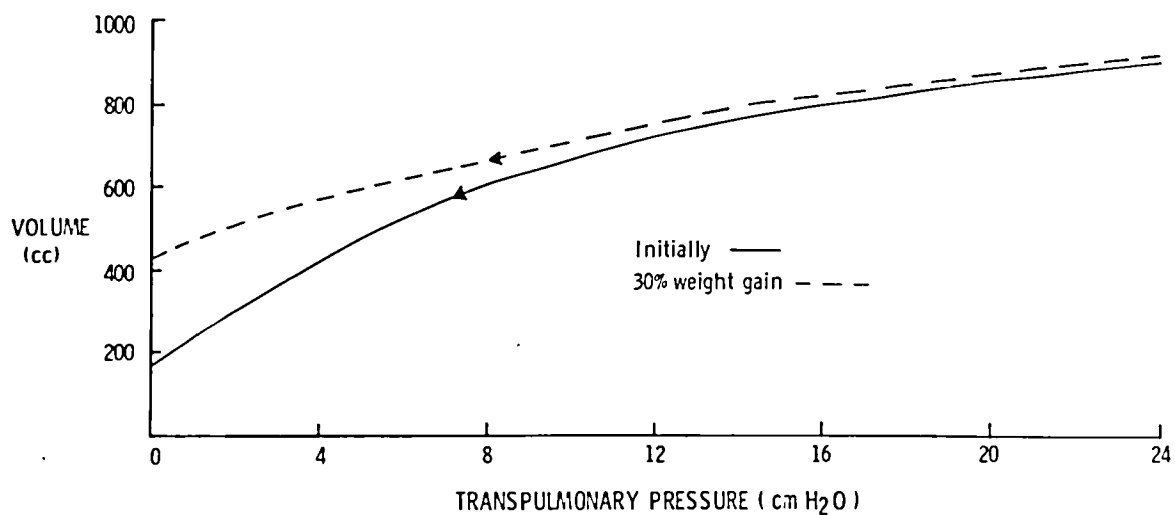


Fig. 44 Transpulmonary pressure is plotted against the volume of gas exhaled from the lung as it was deflated from TPP 24 to zero.

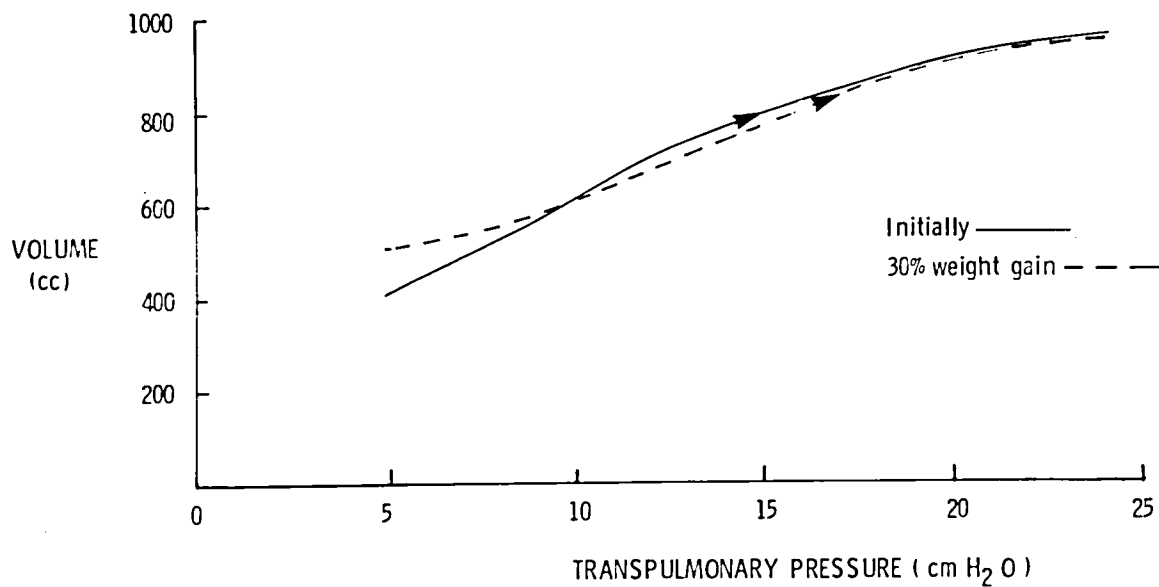


Fig. 45 Transpulmonary pressure is plotted against the volume of gas inhaled by the lung as TPP was increased from 5 to 24 cm H₂O.

presence of interstitial oedema. The change in volume per cm pressure change from TPP 5-6 cm H₂O varied initially from lung to lung between 50-71 cc (mean 60 cc). After oedema induction the volume change was 30-42 cc (mean 37 cc) for the same pressure change. Similar results were found in all six other lungs in which inspiratory and expiratory pressure/volume curves were recorded. Initial and final pressure/volume curves were identical in the 'control' lung which had no weight gain.

Effect of preinspiratory lung volume on the distribution of a slow breath in the presence of oedema

Figure 46 shows the results from an experiment in which regional ventilation distribution measurements of a slow breath were made at three different lung volumes corresponding to TPP 0, 5 and 10 cm H₂O. Notice that the usual measurement (TPP 5 cm H₂O) shows the inverted pattern of ventilation distribution found in the presence of interstitial oedema. The effect of lowering the TPP to zero is to markedly diminish further the amount of gas going to the base of the lung. Increasing the TPP to 10 cm H₂O has the opposite effect: namely of improving basal ventilation and diminishing apical ventilation, or making the overall pattern more even. In summary, lowering the lung volume enhances the effect of interstitial oedema on the distribution of ventilation, while increasing lung volume diminishes the effect. Similar results were seen in three other preparations.

Blood flow distribution

In six experiments the blood flow distribution was measured within one hour of the preparation being set up and again after inter-

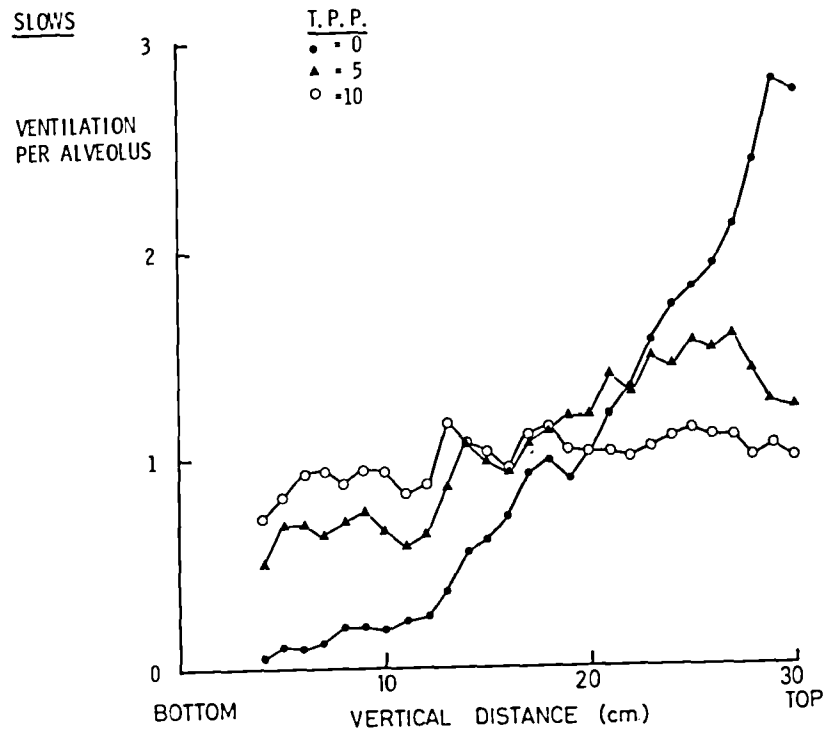


Fig.46 Vertical distance up the lung is plotted against the ventilation per alveolus. The ventilation distributions are shown of slow breaths inhaled from TPP zero, 5 and 10 cm H_2O .

stitial oedema had been induced, as indicated by a 30% gain in lung weight. For the initial measurement, P_a pressure was set to 2 cm above the hilum by adjusting the flow (124-136 cc/min). Venous pressure was always below the lung (15 cm below the hilum). After induction of interstitial oedema, the measurement was repeated with identical flow and venous pressure. P_a pressure rose between 1.7-13.7 cm (mean 8.1) as a result of oedema, indicating an increase in overall pulmonary vascular resistance. Figure 47 shows the results in the six experiments. Initially all but one lung had no region of reduced blood flow to the base, but after inducing interstitial oedema, there was a marked reduction of blood flow to the base in all lungs.

Effects of increasing blood flow and TPP on the distribution of blood flow in the presence of interstitial oedema

The same distribution of blood flow after 30% weight gain shown in the first graph in Figure 47 is again plotted in Figure 48. When the flow was raised from 132-275 cc/min, P_a rose from 7.5 cm above the hilum to just above the top of the lung (14 cm above the hilum). The measurement was repeated and, as can be seen in Figure 48, blood flow to the base was increased. Returning the flow to its original rate, P_a fell to 6.8 cm above the hilum. Raising the TPP to 20 cm H_2O instead of 5 cm H_2O further lowered P_a to 2.7 cm above the hilum and in this lung removed the basal region of decreased blood flow. Two other lungs showed less marked increases in basal blood flow, with flow and TPP such that, though basal regions were better perfused, there still remained a small area of reduced blood flow.

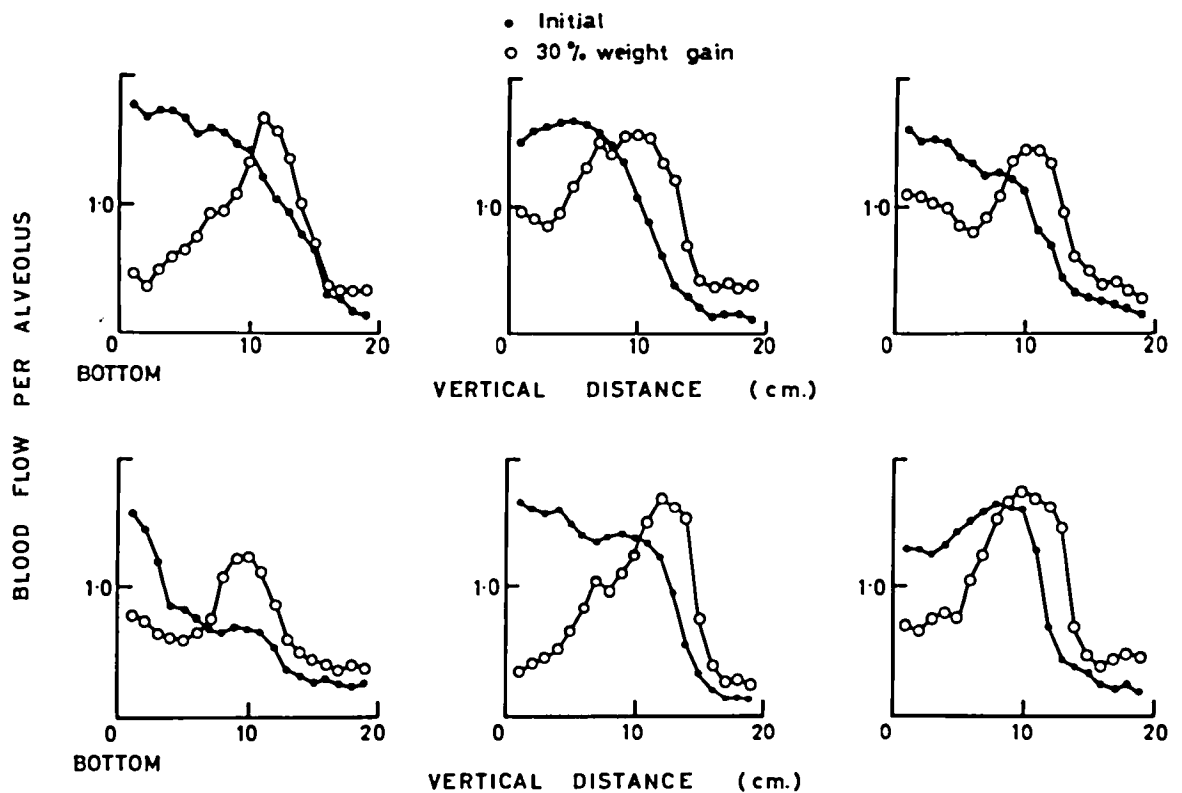


Fig.47 Vertical distance up the lung is plotted against blood flow per alveolus. The pre and post oedema distributions of blood flow are shown for the six lungs. TPP was 5 cm H₂O.

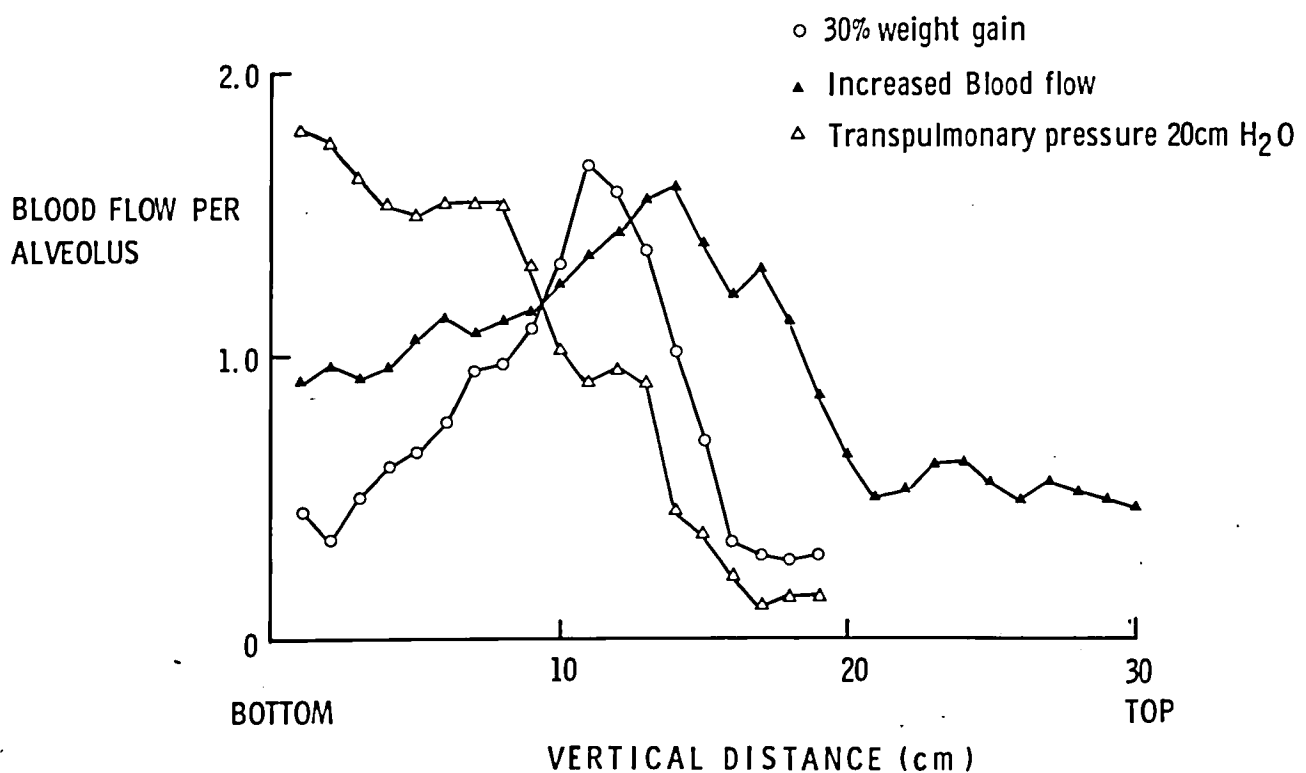


Fig.48 Vertical distance up the lung is plotted against blood flow per alveolus. ○ represents the distribution after 30% weight gain shown in the first graph in Fig.47. ▲ represents the distribution after blood flow was increased and △ the distribution after the TPP was increased to 20 cm H₂O.

Effect of interstitial oedema on the distribution of ventilation/
perfusion ratio

Figure 49 shows how the distribution of ventilation/perfusion ratio ($\dot{V}/\dot{Q}$) was altered by the presence of interstitial oedema. The initial distribution is very similar to that described by West in his nine slice model of the upright lung; the $\dot{V}/\dot{Q}$ ratio being slightly less than unity over a considerable portion of the lung and then rising sharply towards the level of no flow in the upper lobe. In the lower part of the lung perfusion was slightly in excess of ventilation whereas higher up, where the flow fell off rapidly, there was an excess of ventilation. In the presence of interstitial oedema the reduction in basal blood flow was considerably greater than the reduction in basal ventilation during a slow breath, and hence the raised $\dot{V}/\dot{Q}$ in that region. Since blood gases were not studied in this series, we are unable to say whether the basal mismatching of blood flow and ventilation associated with oedema was sufficient to impair gas exchange.

DISCUSSION

Ventilation distribution

The ventilation distribution measurements were designed to investigate the effect of interstitial oedema on regional compliance and regional resistance. Otis et al (1956) pointed out that the distribution of a tidal volume between two units in parallel depends upon their time constants (compliance x resistance). When the distance between the site of bolus injection and the lung units is small (less than 20 cc in our case), then the distribution of a slow breath will

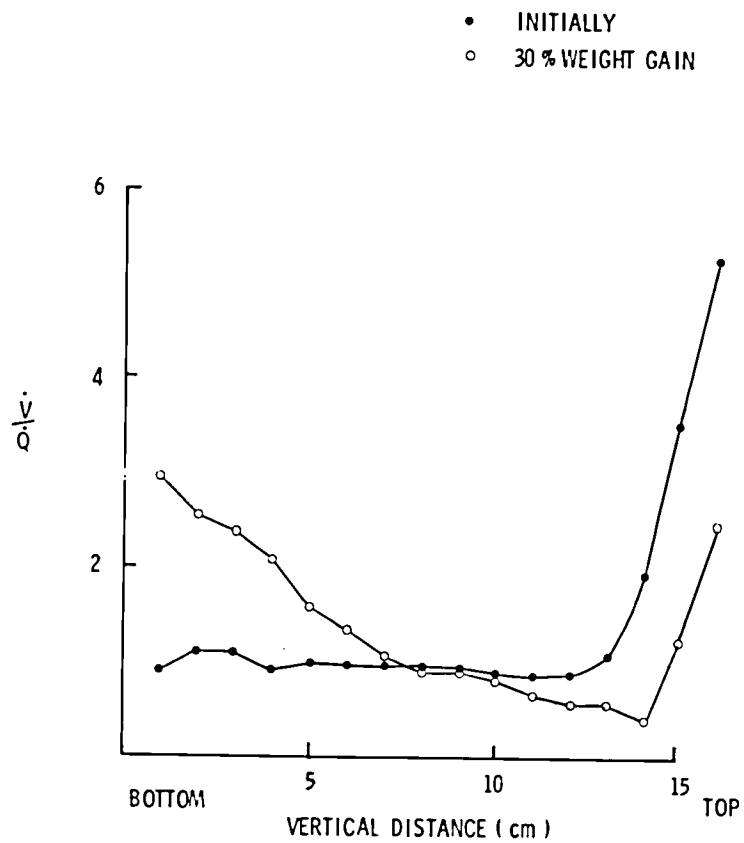


Fig. 49 Vertical distance up the lung is plotted against the ventilation/perfusion ratio. • represents the pre-oedema distribution and ○ the distribution after the lung had gained 30% of its initial weight.

be compliance dominated and that of a fast breath resistance dominated. Hence by using slow and fast inflation rates we were able to distinguish between changes in compliance and changes in resistance associated with the presence of interstitial oedema.

Just for interest, Figure 50 is a plot of the regional time constants measured for the twelve experiments, both initially and after oedema induction. It can be seen that initially the lungs showed a slightly longer basal time constant when compared with that of the apex; 1.5 and 1.1 respectively. In the presence of interstitial oedema, lung resistance was little changed but basal compliance decreased, therefore a longer time constant was shown by the apical regions (base 1.0, apex 1.45). These values are not absolute; they only serve to compare one region with another.

Effect of time

Faridy et al (1966) and others have shown that the pressure/volume characteristics of isolated lungs only altered irreversibly after a considerable period of continuous ventilation and that the changes were due to depletion of surfactant. Short term changes in pulmonary compliance were shown by Collier & Mead (1964) to be reversed by forced inflations. In the present series the lungs were not ventilated continuously and the volume history preceding each measurement included inflation to and holding of the lung at a TPP of 24 cm H₂O for one minute. Figure 44 shows that the distribution of a slow breath (and therefore the compliance) was unaffected by time when oedema, as judged by weight gain was avoided. However, it is of interest that two lungs, not included in this series because the

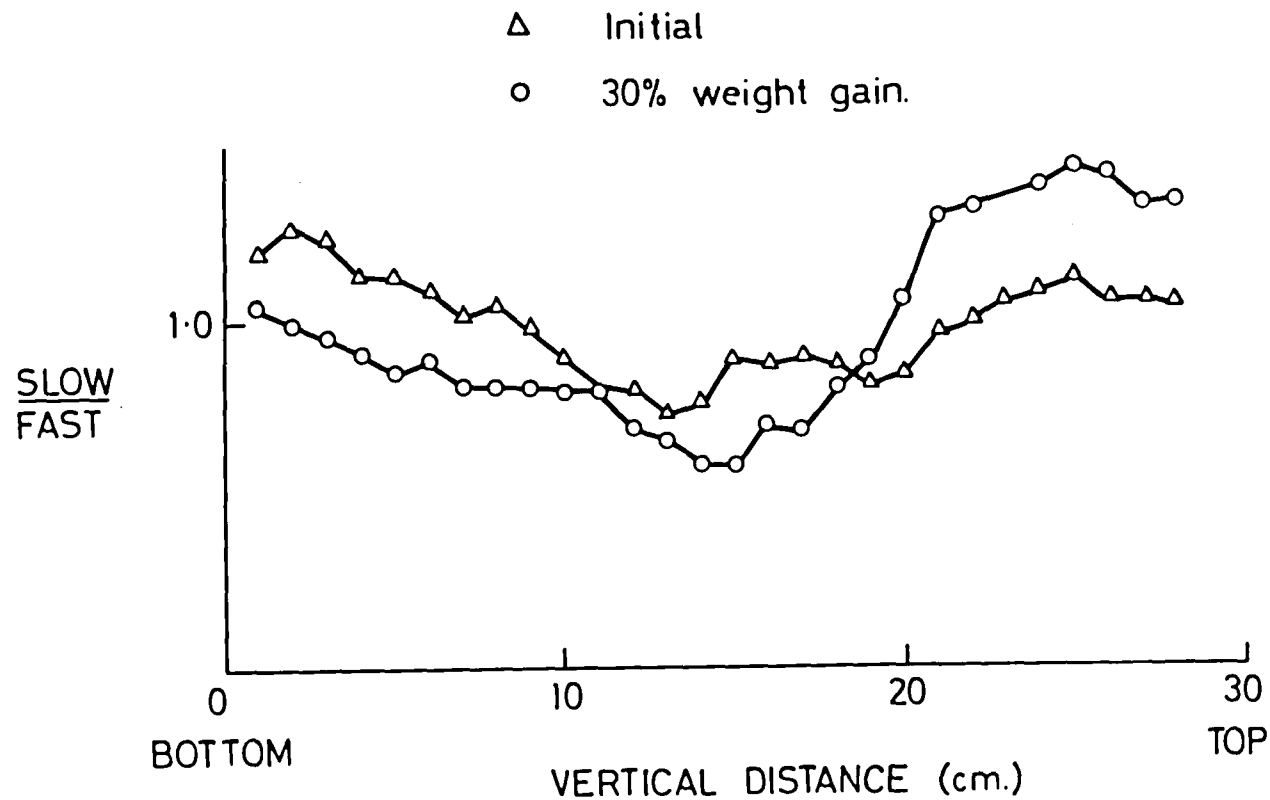


Fig.50 Vertical distance up the lung is plotted against the slow/fast ratio. Mean distributions of the ratio are shown both pre-oedema and after a 30% gain in lung weight.

vasculature was not filled with blood, did show an irreversible fall in compliance with time, but this was associated with the presence of interstitial air. Ventilation distribution was changed and lung volumes at TFP 0 and 5 cm H₂O were increased in a way very similar to the changes associated with presence of interstitial fluid.

Effect of interstitial oedema

Interstitial oedema seemed to have little effect on lung resistance, but basal compliance was reduced. This change in compliance was associated with preferential accumulation of oedema fluid in the dependent region. The ventilatory movements of the lower lobe were noticeably reduced and, as a consequence, the washout of xenon from this region took longer than initially. Three main factors might account for the change in lung compliance associated with early oedema; atelectasis, alveolar stiffness and closure of small peripheral airways.

Atelectasis and alveolar stiffness

Presence of atelectasis would have diminished the lung volume, whereas the results show an increase in volume at TFP 0 and 5 cm H₂O. Both this and the absence histologically of atelectasis suggest that it was not a contributing factor. Alveolar stiffness might be caused by either fluid in the septa or in the lumen as well as the alveolar walls. An electromicrographic study by Cottrell et al (1967) describes how in "haemodynamic oedema" fluid in the alveolar septa caused dispersion of the collagen fibrils from their normal orientation in bundles. Such a change in the geometry of the elastic elements might be a feasible explanation for a fall in septal compliance except that our results and others (Cook et al, 1959) have shown that oedema only

affected lung compliance at low lung volumes. At higher lung volumes the pressure/volume relationship was little changed. It is therefore a little surprising that the disorientation of septal collagen does not affect compliance throughout the range of lung volumes. Alveolar oedema or fluid in the alveolar spaces as well as septa cannot be completely ruled out as a possible cause of decreased lung compliance despite its absence histologically. I have confirmed the sequence of oedema formation in the lung described by Staub et al (1967) showing alveolar fluid to be the final stage before alveolar haemorrhage and foam in the airways. From experiments described in the previous chapter it was shown that a lung weight gain in excess of 35% of the original weight on excision was necessary before the alveolar stage of oedema was reached. Hence, by carefully controlling the lung weight in this study, the possibility of alveolar oedema was minimised. Another indication that oedema fluid was predominately confined to the interstitium is that total and regional lung volumes were not decreased in the presence of oedema.

Closure of small airways

Figure 43, showing an increase in xenon concentration at end expiration, is suggestive of basal airway closure for the following reasons. Dolfuss et al (1967), using a similar xenon bolus technique, showed that on slow inspiration from residual volume (RV), gas first goes to the upper lung regions. They therefore receive a greater proportion of the xenon bolus than the basal regions. Further, the upper regions tend to empty last and hence the increase in xenon concentration towards end expiration. This phenomenon Dolfuss et al attributed to

basal airway closure occurring at RV, which prevented gas entering the lower regions, until lung volume had increased sufficiently to reopen basal lung units. Airway closure was therefore considered to be the cause of the reversed distribution of ventilation at RV and it may account for the similar distribution of slow inspirations in lungs with interstitial oedema.

The fall in basal compliance associated with interstitial oedema may also have been caused by airway closure because there was an increase in lung volumes at TPP 0 and 5 cm H₂O (Table XII). For reasons already given in the previous section, it is unlikely that small peripheral airways were fluid filled. It is possible, however, that formation of menisci from very small amounts of bronchiolar fluid might lead to their effective closure. Similarly, the surfactant lining layer, which has been shown by Macklem et al (1970) to have a stabilising function on peripheral airways, might be altered by oedema fluid. This however would not necessarily make bronchioles unstable because oedema fluid itself seems to have surface lowering properties (Pattle, 1958). Secondly, an increase in smooth muscle tone in bronchioles is an unlikely cause of airway closure in an isolated lung preparation ventilated with air. However, peribronchiolar oedema fluid might well cause airway closure by its compressive effect on small airways as well as the presence of interstitial fluid interfering with normal airway distention by the expanded lung parenchyma (Hughes & Rosenzweig, 1970). Further, the inverse relationship between TPP and basal compliance (Figure 46) is consistent with peribronchial oedema causing a rise in airway closing pressures which could be overcome by raising TPP. That

peri-airway oedema was mainly peripheral in its distribution is confirmed both by the histology and the fact that regional lung resistance was little changed. Macklem & Mead (1967) demonstrated that the peripheral airways contribute only approximately 10% of the total airways resistance so that a peripheral increase would have little effect on the total resistance. This would account for the distribution of fast breaths, which are mainly resistance dependent, being relatively unaltered by the presence of interstitial oedema. Cook et al similarly found that after inducing pulmonary oedema in anaesthetised dogs lung resistance was little changed. In contrast, Sharp et al (1958) reported marked rises in airways resistance in patients with acute pulmonary oedema, but this was in the presence of foam in the airways.

It is concluded that early oedema may have caused closure of peripheral airways which decreased lung compliance in the oedematous regions, but did not significantly alter the resistance. Increasing lung volume improved basal lung compliance, possibly by reopening some previously closed airways.

BLOOD FLOW DISTRIBUTION

This study has shown that there is a reduction in basal blood flow in the presence of interstitial oedema when venous pressure was below the lung. Previous investigations (West et al, 1965; Hughes et al, 1958 and Ritchie et al, 1969) have only shown reduced basal blood flow associated with oedema when venous pressure was elevated (Zone III) and there was a small arterial-venous pressure difference. Under vascular conditions similar to ours (Zone II),

Ritchie et al (1969) found no basal blood flow reduction and Hughes et al found reductions in only four out of twenty-two lungs with oedema. Both these studies, however, were carried out under relatively high transpulmonary pressures, 12-15 cm H_2O in the former and 10 cm H_2O in the latter case, contrasting with our 5 cm H_2O . We believe that their relatively high states of lung inflation might account for the different results because, when we raised TPP to 20 cm H_2O the reduction in basal blood flow was eliminated (Figure 48). The importance of lung inflation on pulmonary blood flow distribution in the absence of oedema has been reported previously by Hughes et al in humans (1969) and isolated lungs (1968). It may be that the effects of oedema on pulmonary vascular resistance are only apparent below a certain level of TPP and that in previous studies a high TPP may have obscured the changes associated with oedema.

Site of increased vascular resistance

The fact that increasing TPP does affect the distribution of blood flow suggests that the site of increased vascular resistance is in the extra-alveolar vessels of the lung. It has been known for some time that the pulmonary vessels can be functionally separated into intra and extra-alveolar vessels (Macklin, 1946; Howell et al, 1961). As the lung is inflated to high lung volumes intra-alveolar vessels are compressed whilst extra-alveolar vessels are expanded by the pull of the inflating lung parenchyma. West et al (1965) attributed the increased basal vascular resistance to oedema fluid interfering with this tethering effect of the lung parenchyma on extra-alveolar vessels, causing them to close. Hughes et al (1968) proposed that

perivascular oedema might bring about a Starling resistor effect in the extra-alveolar vessels. Ritchie et al (1969) rapidly froze the surface of isolated perfused lungs under conditions of low total blood flow, narrow arterial-venous pressure difference and ^{showed an} inversion of the distribution of blood flow. Finding no anatomical evidence of vessel closure, they proposed instead that some rheological phenomenon might be an alternative explanation for the increased vascular resistance in the dependent region. I consider that even a slight narrowing would be sufficient to cause the reduced basal blood flow, without evoking closure.

Possible mechanism to account for increased resistance being preferentially basal

The reasons why interstitial oedema should cause a preferential reduction in blood flow in the dependent regions is not altogether clear. West et al (1965) suggested that the interstitium of the lung forms a continuous fluid column and therefore interstitial pressure would be higher at the base, but in fact one would expect the hydrostatic gradient of interstitial liquid pressure to be matched by a similar gradient of intra-vascular pressure, assuming blood and interstitial fluid to have similar densities. Hence the transmural pressure for extra-alveolar vessels would be similar at all levels in the lung. It may be, however, that initial drainage of interstitial fluid to the base causes accumulation and eventual fluid cuffing preferentially in that region, before developing higher up the lung.

Recent studies on the mechanics of hydrothoraces (Agostoni et al, 1969) suggest that accumulation of fluid in the dependent part

of the pleural space compresses the lung in that region, but that the expansion of the upper zones is unaffected. Normally there are points of contact between the two pleural surfaces (Agostoni et al, 1964) which allow free movement of pleural liquid, but bring about gradients of surface and liquid pressures. Both pressures are slightly sub-atmospheric at the lung base and become more negative with vertical height (1 cm H₂O/cm vertical height for liquid pressure and a smaller change for surface pressure). When, as in a hydrothorax the pleural membranes become separated by sufficient fluid to break the points of contact, the surface pressure becomes one and the same as the liquid pressure and the lung is compressed in this region.

The perivascular space may well behave as an extension of the pleural space (von Hayek, 1961) with points of contact between its limiting membrane and the outside of the vessel wall, which would account for the expanding effect of lung parenchyma on extra-alveolar vessels. The normal surface pressure for extra-alveolar vessels is not known but there is evidence that it is less than alveolar pressure (Macklin, 1946) and may be less than pleural pressure (Howell et al, 1961). As fluid accumulates contact between the limiting membrane and vessel wall would be lost so that perivascular surface pressure becomes equal to the liquid pressure. Meyer et al (1968), using perforated capsules implanted in the lung, showed that interstitial liquid pressure changes from negative to positive values with the accumulation of oedema fluid. A rising interstitial liquid pressure would tend to diminish vascular transmural pressure and hence vessel diameter, causing increased vascular resistance. Because of the effect of

gravity and the continuity of the interstitial space, oedema fluid will tend to preferentially accumulate in the dependent parts of the lungs (Hughes et al, 1968). Therefore an increase in vascular resistance would first be evident in the lung bases. Higher up the lung, where interstitial fluid is insufficient to break the points of contact, transmural pressure would be greater and therefore vascular resistance less. Perhaps the reason why the increased basal resistance was partially reversed at higher TPPs was that, in addition to a fall in interstitial liquid pressure, expansion of the surface limiting membrane may reduce the thickness of the perivascular fluid cuff sufficiently to restore the points of contact between the limiting membrane and vessel wall.

CONCLUSIONS

The results indicate that in the early stage of pulmonary oedema, interstitial fluid accumulation affects the calibre of extra-alveolar vessels and the patency of small airways. Since fluid tends to accumulate in the dependent regions initially, its effect in increasing vascular resistance and reducing lung compliance are predominately basal. It is possible that in vivo interstitial oedema might bring about sufficient mismatching of ventilation and blood flow in the dependent lung regions to cause arterial hypoxaemia. This early stage of pulmonary oedema may

therefore account for the hitherto unexplained hypoxaemia which occurs in patients during apparently asymptomatic periods between episodes of overt pulmonary oedema. The next chapter describes measurements of regional ventilation and blood flow in such patients to test the above hypothesis.

CHAPTER V

REGIONAL DISTRIBUTION OF VENTILATION AND BLOOD FLOW IN PATIENTS
WITH EARLY OEDEMA ASSOCIATED WITH LIVER CIRRHOSIS

In patients with liver cirrhosis there is progressive scarring of the liver tissue, resulting in some degree of cellular failure and portal hypertension. Clinical signs of the disease include ascites and pulmonary oedema. There is frequently a high circulating level of aldosterone (which is normally metabolised in the liver) resulting in sodium retention and, associated with it, an increased blood volume. Other contributing factors to the formation of pulmonary oedema in liver cirrhosis may be a reduction in plasma protein concentration, increased capillary permeability, the presence of portocaval pulmonary shunts and impaired lymphatic drainage of the lungs. It is likely therefore that patients with liver cirrhosis have interstitial oedema present chronically which, at this early stage, cannot be detected clinically.

METHODS

The age, sex and lung volumes of the five patients are given in Table XIII. None of the patients had clinically diagnosable pulmonary oedema at the time of the study, but all showed arterial hypoxaemia (as can be seen in the last column of Table XIII) and had histories of one or more episodes of pulmonary oedema. Blood flow and ventilation distributions were measured after the methods of Ball et al (1962) with modifications described by Hughes et al (1968) and Milic-Emili (1966)/

TABLE XIII

<u>Patient</u>	<u>Sex</u>	<u>Age</u>	<u>Total lung capacity</u> (T.L.C. litres)	<u>Functional residual capacity</u> (F.R.C. % T.L.C.) normally about 50%	<u>Residual volume</u> (R.V. % T.L.C.) normally about 25%	<u>P_aO₂</u> (mm Hg)
M	F	29	6.45	62	34	65
P	F	37	5.11	50	31	54
T	F	12	2.90	56	28	46
G	M	18	4.85	47	24	75
D	M	32	6.06	43	23	75

respectively. The patients were studied whilst sitting erect and care was taken to keep their position relative to the counters as fixed as possible during a complete study of ventilation and blood flow.

Blood flow distribution

A 40 cm polythene catheter was introduced under local anaesthesia into an ante-cubital vein so that its tip was beyond the shoulder. While the patient paused at the end of a normal expiration (FRC), 1-2 mc of xenon dissolved in saline were injected and the catheter flushed with saline. After five seconds (allowing time for the bolus to reach and xenon gas to be evolved in the lungs), the patient inhaled slowly to total lung capacity (TLC) and held his breath while the lungs were scanned 30 cm vertically by paired scintillation counters. The time taken for the counters to scan the lungs was kept constant for any one patient and varied between patients from 10-25 seconds. After washing out the activity, a background scan was completed before the next measurement. In the patient D the blood flow distribution was measured at TLC as well as FRC.

Ventilation distribution (FRC and RV)

After tidally breathing room air for a few minutes the patient was asked to pause at the end of a normal expiration (FRC) during which time a tap was turned connecting the patient with a spirometer containing oxygen and xenon in a known concentration (about 1 mc/lit). After a slow (< 0.5 lit/sec) inhalation of labelled gas to TLC, two pairs of scintillation counters scanned the chest vertically while the patient held his breath. After washing out the radiation by hyperventilation, a background scan was completed before the next manoeuvre.

The distribution of gas at residual volume (RV) was similarly determined, but the single breath was taken from maximum expiration (RV) to TLC. To take the volume of lung at each vertical level into account, regional lung volume was determined by scanning after the patient had equilibrated with xenon in the spirometer, whose concentration was monitored throughout by a counter incorporated in the rebreathing circuit. During this manoeuvre, the closed spirometer circuit was primed with oxygen and contained a fan to circulate the gas, and soda lime absorbers to remove CO_2 produced during the 5-5 minutes rebreathing period. As the lungs were scanned during a measurement, the count rates from each pair of scintillation counters were displayed on an ultra-violet recorder, with a marker signal for each cm of vertical distance travelled.

Counting methods and calculations

The specification and geometry of the paired scintillation counters have been described elsewhere (Hughes et al, 1968). A time constant of 0.2 sec and a scanning speed between 0.5 - 3 cm/sec (depending on the ability of the patient to hold his breath) were used and data from the counters fed into a data logger. The data was processed by computer as described by Kingaby et al (1968). The blood flow index ($\dot{Q}$) was calculated according to the methods of Ball et al (1962); a summary of which is given below:-

$$\dot{Q} = \frac{Cr}{Cr_{eq}} \times F_{Ieq} \times \frac{F_i}{TLC}$$

where Cr = regional count rate during a measurement, Cr_{eq} = regional count rate at equilibration, F_{Ieq} = inspired concentration of xenon at

equilibration, F_i = xenon concentration in the saline injection.

A detailed description of the principles involved in the calculation of regional FRC and RV can be found in the paper by Milic-Emili et al (1966). Therefore only a summary will be given here. The nett regional count rate during a measurement (Cr) is divided by the nett regional count rate at equilibrium (Cr_{eq}) and multiplied by the xenon concentration of the gas inspired at equilibrium (F_{Ieq}) to obtain the regional xenon concentration (Fr).

$$Fr = \frac{Cr}{Cr_{eq}} \times F_{Ieq} \quad (1)$$

The inspiratory capacity (IC) to total lung capacity (TLC) ratio is the regional xenon concentration in ml/lit (Fr_{IC}) divided by the concentration of xenon inspired by the IC measurement (F_{IIC}) in ml/lit.

$$\frac{IC_r}{TLC_r} = \frac{Fr_{IC}}{F_{IIC}} \quad (2)$$

If TLC is taken to be one, then FRC is one minus IC. Therefore, to calculate the FRC to TLC ratio,

$$\frac{FRC}{TLC} = 1 - \frac{Fr_{IC}}{F_{IIC}} \quad (3)$$

Similarly the RV to TLC ratio can be calculated using (1) and (2) in the form below, where the single breath is a vital capacity (VC).

$$\frac{VC_r}{TLC_r} = \frac{Fr_{VC}}{F_{IVC}}$$

Hence

$$\frac{RV}{TLC} = \frac{1 - Fr_{VC}}{F_{IVC}}$$

We therefore obtain the regional lung volumes at FRC and RV as a percentage of TLC. Since all the alveoli are the same volume at TLC

(Milic-Emili, 1966), the ventilation to each region will depend upon its starting volume. This was deduced by Milic-Emili et al (1966) from the shape of the pressure volume curve of the lung and from measurements of pleural pressure at TLC. For example, alveoli with a small starting volume (at the lung base) will receive a greater proportion of the inspired gas than alveoli with a higher starting volume (at the lung apex). Hence the regional distribution of ventilation on inspiration from FRC has an inverse relationship to the regional distribution of volume at FRC.

RESULTS

Regional distribution of lung volume (RV and FRC)

Figure 51 shows the results for each patient. Regional lung volumes at RV and FRC are expressed as a percentage of TLC. It can be seen that regional RV increased down the lungs, contrasting with the normal distribution (Sutherland et al, 1966) shown in Figure 52. In the same figure standard errors of the mean results in the patients demonstrate a significant difference between their distribution of RV and that of normals. In the dependent regions the RV in the patients exceeded 50% of the volume of these regions at full inspiration (TLC), indicating increased trapping of gas in the basal regions. These results are compatible with those described in the previous chapter, where there was an increased volume of gas remaining in the isolated lung associated with early oedema. Figures 51 and 52 also show the regional distribution of FRC in patients and normals. Even at FRC there seems to be some basal gas trapping present in the liver cirrhotic patients.

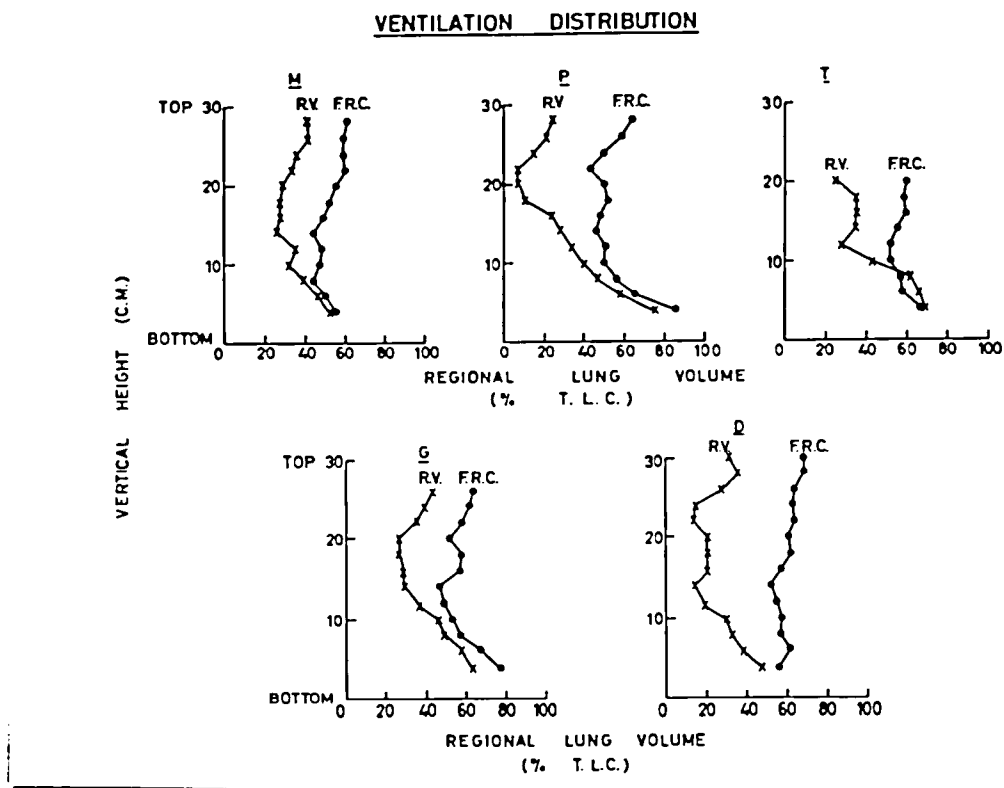


Fig.51 Vertical distance up the lung is plotted against the regional lung volume as a percentage of TLC. The distributions of regional lung volume at RV and FRC are shown for each patient.

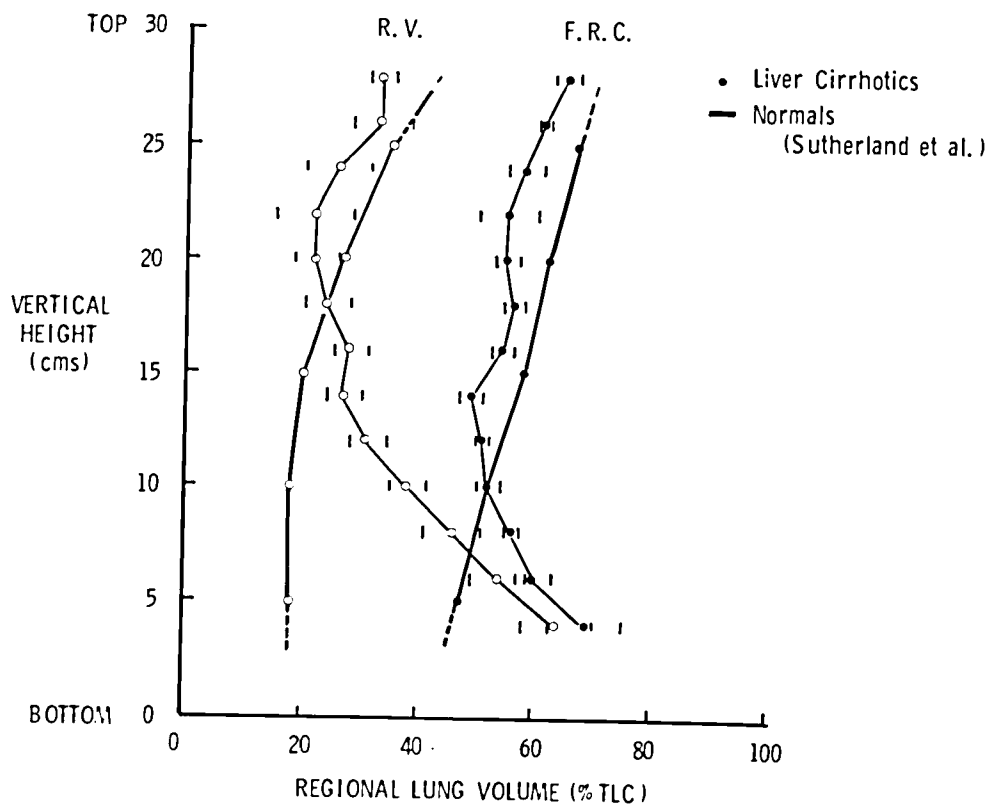


Fig.52 Vertical distance up the lung is plotted against the regional lung volume as a percentage of TLC. The mean distributions of regional lung volume in the five patients and Sutherland's normal subjects are shown at RV and FRC. Standard errors on the patient data are also included.

Regional distribution of blood flow

Figures 53 and 54 show the results for each patient and mean results with standard errors respectively. The blood flow distribution at FRC in normal subjects (Hughes et al, 1968) is also plotted in Figure 54 for comparison. As can be seen, there was considerable variation in the pattern of distribution, with only two patients (G & D) showing abnormally reduced basal blood flow. Taking the group as a whole, however, the results indicate that the pattern of perfusion in the liver cirrhotics was not appreciably different from that found in normal subjects. One interesting result was the marked improvement in basal blood flow in the patient D when a measurement was made at TLC. Such an improved basal blood flow was seen in the previous chapter when the measurement was made at TPP 20 cm H₂O instead of 5 cm H₂O.

DISCUSSION

Regional distribution of ventilation

The results of the regional distribution of FRC and RV in liver cirrhotics have shown that gas volumes in the dependent regions of the lungs were markedly increased in comparison with normal subjects. This means that, on inspiration to TLC, there was a reduction in basal ventilation, particularly when inspiring from RV. To a lesser degree there was also basal reduction in ventilation when the patients inspired from FRC, indicating that the ventilation distribution during resting tidal breathing may well be abnormal. Isolated lungs were shown in the previous chapter to have reduced basal ventilation associated with interstitial oedema, which was exaggerated at low lung volumes and

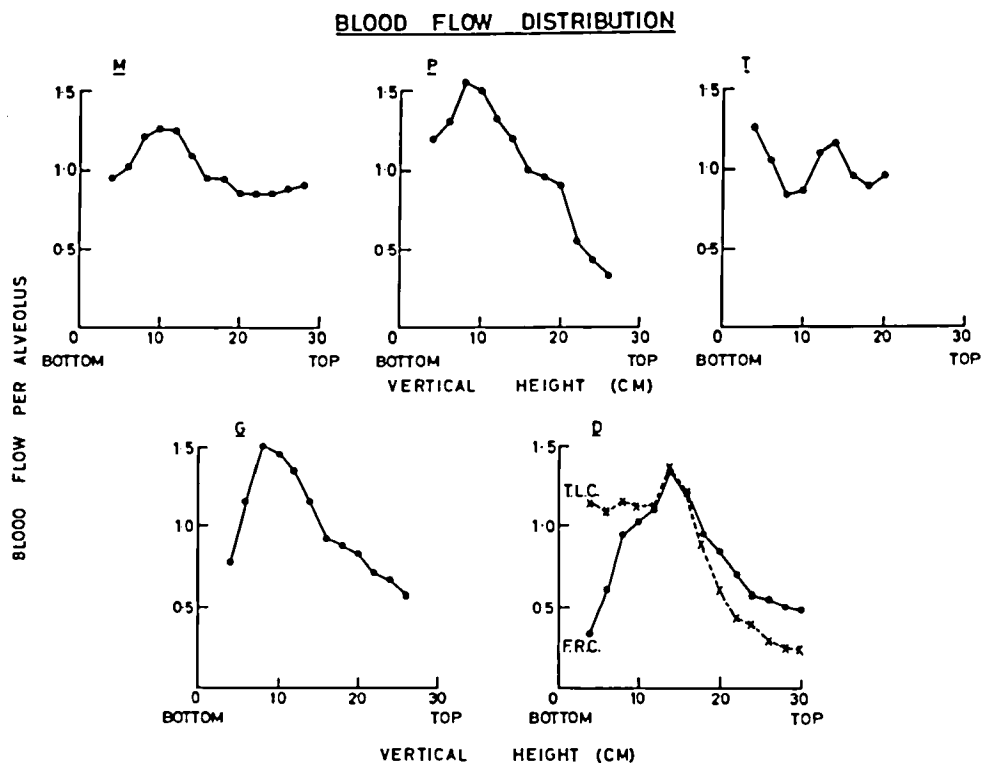


Fig.53 Vertical distance up the lung is plotted against blood flow per alveolus. The regional distribution of blood flow at FRC is shown for each patient. Blood flow distribution at TLC is shown for the patient D.

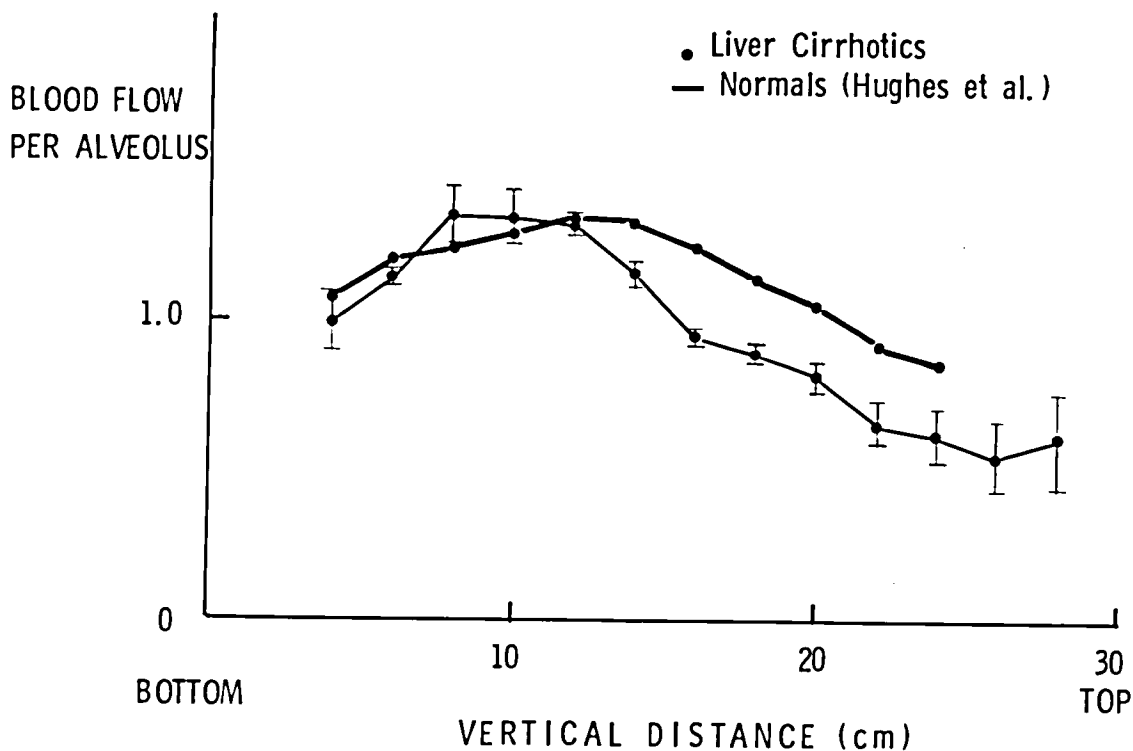


Fig.54 Vertical distance up the lung is plotted against blood flow per alveolus. The mean distributions of regional blood flow in the five patients and Hughes' normal subjects are shown. Standard errors on the patient data are also included.

reduced at higher lung volumes. The similarity between the results of the two studies leads one to speculate whether early oedema leading to gas trapping at higher lung volumes than normal, may be the cause of the abnormal distribution of ventilation in patients with liver cirrhosis.

Regional distribution of blood flow

Three patients showed relatively normal distributions of blood flow. However, it is of interest that the two patients showing the greatest reductions in dependent blood flow (G and D) also had the lowest FRC to TLC ratios. Also, when D raised his lung volume to TLC during the measurement, the basal region of reduced blood flow almost disappeared. There is insufficient data here, but perhaps in vivo an increased lung volume might improve basal blood flow in a way similar to that described in isolated lungs in the previous chapter.

Ventilation/perfusion ratio

The reduction in lower zone ventilation in four out of five liver cirrhotic patients was more marked than the reduction in perfusion so that one might expect a decreased ventilation/perfusion ratio in the basal regions. Figure 55 shows the regional distribution of $\dot{V}/\dot{Q}$ for each patient. The overall $\dot{V}/\dot{Q}$ ratio was measured separately on each subject and was taken into account in calculating the regional distributions. It can be seen that the ventilation/perfusion ratio in the lower third of the lungs was less than 0.6 in four of the five patients. Such low ventilation/perfusion areas may have contributed significantly to the arterial hypoxaemia which was present in all the patients to varying degrees ($\text{PaO}_2 = 46-75 \text{ mm Hg}$).

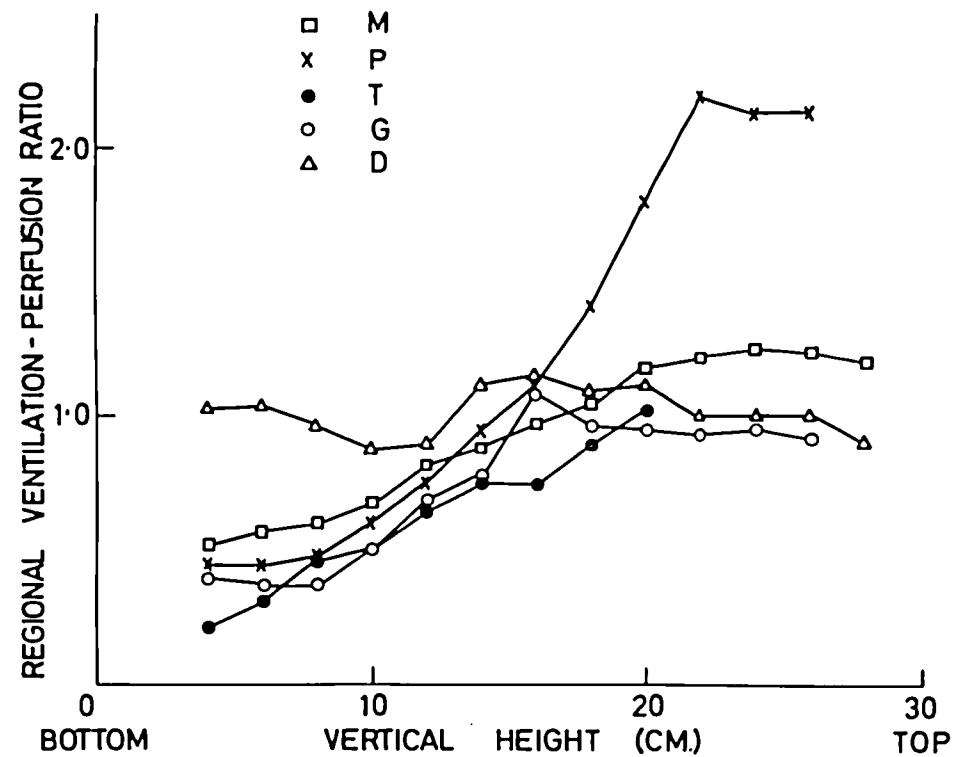


Fig. 55 Vertical distance up the lung is plotted against the ventilation/perfusion ratio. The regional ventilation/perfusion ratio is shown for each patient.

CONCLUSIONS

In cirrhosis the distribution of pulmonary ventilation was more abnormal than that of blood flow. It is suggested that the reduction in basal ventilation was caused by the presence of interstitial oedema fluid giving rise to closure of small airways and therefore gas trapping at lung volumes higher than in normal subjects. Exaggerated mismatching of ventilation and blood flow in the dependent regions may be an important cause of impaired gas exchange, resulting in arterial hypoxaemia in patients with early pulmonary oedema. Measurements of regional ventilation and blood flow, together with blood gases, may provide a suitable means of diagnosing the interstitial stage of pulmonary oedema.

CHAPTER VI

CONCLUSIONS

The purpose of the studies described in this thesis was to investigate the pathogenesis of pulmonary oedema, its sequence of development, and the effect of the early, interstitial stage on the distribution of ventilation and blood flow in the lung.

Experiments described in Chapter II were intended to clarify the puzzling situation of hypoxic oedema development at altitude, where capillary hypertension is absent. Drinker (1945) and other workers had observed that lymphatic flow from the lung was increased during hypoxia and assumed that the hypoxic gas mixture directly affected the permeability characteristics of the capillaries, causing an increased filtration rate and hence oedema fluid accumulation. Later Goodale et al (1970) demonstrated that capillary permeability was only changed when hypoxia was severe; more severe than the levels of hypoxia which have caused altitude oedema in man. The results in Chapter II would suggest that random sites of microrupture in the arterioles make them the primary site of pathogenesis in altitude oedema. Though the experiments did not rule out the direct effect of hypoxia on capillaries, they did provide evidence that the arterial hypertension associated with hypoxia was the major cause of oedema and not the toxic effect of hypoxia per se on the pulmonary vessels. In Chapter III the results confirmed that arterioles make a significant contribution to oedema development during hypoxia. At the same time, venous and capillary contributions were not increased by hypoxia,

showing that it seemed to primarily affect arterial permeability. Whereas in normoxia the arterial contribution was slight, the rate of interstitial oedema accumulation from arterioles was increased considerably during hypoxia, and the maximum size of contributing vessel was raised from a diameter of 200 μ to 620 μ . The presence of red cells in the interstitial space, which invariably accompanied hypoxic oedema development, supports the hypothesis of micro-rupture rather than an increase in permeability. Since the arterial pressure was at the same level during hypoxia as normoxia (when the pressure seemed to have no direct effect on the walls), these experiments would indicate that hypoxia may have a weakening effect on the endothelial cell junctions of arterioles, making them more susceptible to mechanical failure when subjected to an abnormally elevated pressure.

The experiments described in Chapter III were mainly concerned with the site and sequence of oedema development. Histological changes were correlated with weight gain, as an index of oedema development. The preparation afforded the opportunity of separating alveolar capillaries from the larger extra-alveolar vessels, so that oedema development from arterial, capillary and venous compartments could be studied independently under static conditions. During normoxia the arterial contribution to oedema development was small (16%); nevertheless the dye experiments showed that arterial vessels much larger than capillaries were involved in fluid exchange. The venous contribution to oedema formation was considerable (46%) and even exceeded that of capillaries (37%) in the static situation. This surprising finding was supported by the relatively large size of permeable veins (up to

750 μ in diameter). One must however bear in mind that this venous contribution to oedema formation has been over-estimated when one considers the in vivo situation where venous pressure will always be lower than capillary pressure.

These results are compatible with Böhm's["] observation (1966) that pulmonary venules are more permeable than capillaries. In addition the results demonstrate the involvement of larger veins as well as venules in oedema formation when venous pressure was elevated.

The fact that vessels other than capillaries may be involved in fluid exchange in the lung leads one to speculate whether their role in oedema development in vivo may have been underestimated. Certainly, results from Chapters II and III provide evidence of the considerable role of arterioles and small arteries in hypoxic oedema formation. Another situation in which the larger vessels (in particular venules and veins) may make a substantial contribution is during left ventricular failure. Here, when pulmonary venous pressure is elevated, there is hypertension throughout the lung vasculature (corresponding to Zone III in the isolated lung) and the venous contribution to oedema formation may nearly equal that of capillaries. It is therefore not surprising that the onset of acute pulmonary oedema can be so rapid in left ventricular failure.

Histological findings reported in Chapter III confirm the sequence of oedema fluid accumulation in the lung described by Staub et al (1967). Oedema fluid was first apparent surrounding arteries, airways and veins and only at a later stage (if at all), was alveolar oedema evident. In the Zone I experiments, all the oedema fluid originated from extra-alveolar vessels, but during flow experiments

it will have also originated from capillaries. It is of interest that Staub's sequence still applied during the Zone III flow situation of raised venous pressure (and therefore elevated capillary pressure), when one might expect capillary transudation to be considerable. It may therefore be concluded from the present results that initially fluid from the capillaries drained into the interstitial space, where, perhaps drawn by the negative interstitial pressure round conducting vessels and airways, it tracked retrograde towards the hilum to accumulate in the perivascular and peribronchial spaces. Simultaneously there may have been some direct leakage of fluid from arteries and veins into the perivascular space, so that fluid of capillary origin would accumulate together with fluid from extra-alveolar vessels. Assuming oedema accumulation to be in excess of its lymphatic drainage, the interstitial space would become filled, and only then would one expect fluid filling of alveoli and airways.

Since oedema fluid is histologically detectable in the lung with only a relatively small gain in lung weight (4-6%), it would be of interest to know whether the presence of small quantities of interstitial fluid affects lung physiology. If some change in lung function could be detected at this early stage of pulmonary oedema in patients, then appropriate treatment might prevent the development of an acute situation. In many clinical centres radioactive gas techniques have become almost routine as a method of assessing the ventilation and blood flow aspects of lung function. Using these techniques, the normal distributions of ventilation and blood flow have been

established in patients (Milic-Emili et al, 1966; West et al, 1960) and vertical isolated lungs (Zardini et al, 1966; West et al, 1964). It is also known how changing certain parameters such as lung volume will affect the patterns of ventilation and blood flow (Sutherland et al, 1968; Hughes et al, 1968). In Chapter IV I have investigated the effect of controlled amounts of interstitial oedema on the distribution of ventilation in a vertical isolated lung preparation. Oedema was induced by raising pulmonary venous pressure. Distributions before oedema induction were compared with the measurements made after a 20 or 30% gain in lung weight. The changes in ventilation distribution were less marked than those shown by the blood flow, but both were to some extent diverted away from oedematous areas in the dependent regions of the lung. Basal mismatching of ventilation and blood flow was evident in the presence of interstitial oedema and was exaggerated at low lung volumes, presumably because of more widespread airway closure. The reversible arterial hypoxaemia associated with early oedema described by Said et al (1964) may well have been caused by airway closure. Hyperinflations returned the A-a gradient to normal by reopening previously closed small airways. Distribution measurements made at a higher lung volume improved basal ventilation for the same reason.

The distension of the lung was varied to gain some insight as to how the oedema fluid was affecting the traction of the lung tissue on airways and extra-alveolar vessels, thus affecting their calibre. At low lung volumes (TPP 5 cm H₂O) basal interstitial oedema greatly affected vessels and airways, causing a marked reduction in both ventilation and blood flow in that region. As lung volume was increased

(TPP 20 cm H₂O), the effects of the oedema fluid were partially or totally reversed, so that ventilation and blood flow were both improved to that region. Measurements made after fast or slow inhalations showed that the regional airways resistance was only slightly increased, but that lung compliance was more markedly affected. The results indicate that the fall in lung compliance associated with interstitial oedema was due to closure of small peripheral airways, which had become unstable at higher lung volumes than normal because of the oedema fluid surrounding them. It would seem therefore that interstitial oedema interferes with the traction of the lung tissue on extra-alveolar vessels and small airways. These changes in the mechanical properties of the lung accompanying early oedema can be partially or totally reversed by increasing the lung volume; thereby reopening previously closed lung units and increasing the calibre of the extra-alveolar vessels. It is however of interest that changing the lung volume did not affect the rate of fluid transudation from extra-alveolar vessels (Chapter III). One might have expected the interstitial pressure to be lowered by increased inflation, thereby causing an increased driving pressure across the vascular wall. Whether the explanation is, as Pratt's study (1968) would suggest, in the fact that positive pressure inflation was used is debatable, but intermittent positive pressure ventilation does seem to meet with some success in the clinical treatment of pulmonary oedema.

Having shown measureable changes in the distribution of ventilation and blood flow associated with early oedema in isolated lungs, it was desirable to investigate whether similar techniques

could be applied to patients. Chapter V describes studies of the regional distribution of ventilation and blood flow in five patients with liver cirrhosis in whom the presence of early oedema was likely. All were suffering to some degree from arterial hypoxaemia of unknown cause. The results show that their ventilation distributions were more abnormal than their blood flow distributions, but the nett result was gross mismatching of ventilation and blood flow in the lower third of the lung. Such an area of grossly unequal ventilation and blood flow might to a large extent account for their arterial hypoxaemia. Their patterns of ventilation are similar to those shown in the isolated lung series in the presence of interstitial oedema. The blood flow distributions showed much less marked basal reduction than the isolated lung series and the reason for this is not clear. One patient (D) showed a reversal in the reduction in basal blood flow when he increased his lung volume from FRC to TLC. It might seem from the studies reported in Chapters IV and V and from Said's observations, that in the presence of early oedema, breathing in the upper range of the lung volume would improve both the ventilation and blood flow distributions; making the overall $\dot{V}/\dot{Q}$ ratio of the lung closer to unity and hence improving gas exchange.

Abnormalities in the distributions of ventilation and blood flow were shown in patients with liver cirrhosis but though the presence of interstitial oedema was likely, there was no definite proof of it. The ideal group of patients in whom to examine the distributions of ventilation and blood flow would have been those recovering from left heart failure and severe pulmonary oedema. A study at a time shortly after the episode of heart failure and later after complete recovery would be a very suitable test for the radioactive gas technique of diagnosing interstitial oedema. Further investigations along these lines would be of value towards the development of a method of diagnosing oedema in its early stages so that ultimately it would be possible to avoid the acute stage. Finally, since early oedema is associated with arterial hypoxaemia due to gross regional ventilation/perfusion inequality, it might be beneficial to correlate the overall gas exchange in a patient with his regional abnormalities of ventilation and blood flow.

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ADDENDUM TO GENERAL CONSIDERATIONS OF FLUID EXCHANGE

The forces governing the bulk movement of water between capillaries and the surrounding tissues can be summarised by Starling's Law.

$$\text{Fluid movement} = k(P_c - \pi_{pla} - P_{il} + \pi_{il})$$

where k = filtration coefficient, $\text{ml}/(\text{sec.cm}^2.\text{mm Hg})$, P_c = capillary hydrostatic pressure (mm Hg), π_{pla} = osmotic pressure of plasma (mm Hg), P_{il} = interstitial liquid hydrostatic pressure (mm Hg) and π_{il} = osmotic pressure of the interstitial liquid (mm Hg).

Fluid exchange between the intravascular and interstitial fluids depends on filtration and diffusion which are opposed by the colloid osmotic pressure of the blood proteins. The hydrostatic and osmotic forces in the Starling equation will now be considered.

Capillary hydrostatic pressure (P_c)

Direct measurements of the hydrostatic pressure in frog mesentary capillaries by Landis in 1926 (Am. J. Physiol. 75 : 548-570) resulted in pressures of 14.5 cm H_2O and 10 cm H_2O at the arterial and venous ends of the capillary respectively. He used glass micropipettes of 5μ diameter and obtained values of an accuracy to within a few mm Hg. His measurements showed that, although 70 - 80% of the arterial to venous pressure drop occurred in the arterioles, 20 - 30% of the total could be accounted for by the capillaries. He also observed that P_c (which tended to cause passage of water out of the capillaries), was slightly less than the osmotic pressure of the plasma proteins (tending to draw water into the capillaries), so that there was a net balance of forces across the capillary wall. The differences in P_c which he observed at the arterial and venous ends of the capillary were compatible with Starling's view that these pressures favour filtration at the arteriolar end and a balancing absorption at the venular end.

There are/...

There are no direct measurements of P_c in the lung, but pressures measured by fine catheters wedged in venules suggest P_c in the lungs to be between 5-15 mm Hg (Fishman, 1963). Since the π_{pla} is about 25 mm Hg, one would expect capillary absorption of fluid to be favoured. Such net absorption would minimise the amount of interstitial fluid in the lung, thus protecting the blood/gas barrier from any widening and hence ensuring maximum efficiency in gas exchange.

Two reasons for P_c being so much lower in the pulmonary circulation are that the pulmonary arterial tree is more distensible and less elastic than the systemic circulation. For these reasons and because vascular resistance is less in the lungs, the systolic pulmonary arterial pressure approximates 25 mm Hg instead of the 120 mm Hg existing in systemic arteries. In man this results in mean P_c in the lung and systemic circulation of about 12 and 28 mm Hg respectively. These values apply to capillaries at the level of the heart, and, due to the gravitational hydrostatic gradient, they will become greater below the heart and less above the heart. P_c naturally also depends on the downstream or venous pressure (P_v) and is rapidly elevated with raised P_v . Alterations in the calibre of arteries in particular will raise P_c with vasodilation and lower P_c with vasoconstriction. It will follow that, providing the other parameters in the Starling equation remain constant, an increase in P_c will favour filtration and a decrease will favour absorption.

Interstitial liquid hydrostatic pressure (P_{il})

It is now recognised that total interstitial pressure is made up of the sum of the liquid pressure and the solid pressure (Guyton et al, 1971). In connection with Starling's law of capillary exchange, we are primarily concerned with the liquid pressure component (P_{il}).

Early measurements of tissue liquid pressures made by McMaster (J. Expt. Med. 84 : 473-494) in 1946 using a needle inserted into the subcutaneous tissue of mice and man gave values between 0.4-4.9 mm Hg. More recent measurements made by Wiederhielm (The Pulmonary Circulation and Interstitial Space, Univ. Chicago Press, pp. 29-40, 1969) in the subcutaneous tissue of bat wings gave an average P_{il} of 1.25 mm Hg.

These/...

These positive values of P_{il} contrast with subsequent measurements using different techniques, in which P_{il} was invariably negative under normal conditions. Negative values of -6.4 mm Hg and -9.5 mm Hg were obtained by Guyton (Circ. Res. 16 : 452-460, 1965) and Meyer (1968) in perforated capsules chronically implanted into subcutaneous and lung tissue respectively. Other workers (Scholander et al, 1968; Ladegaard-Pederson (Circ. Res. 26 : 765-770, 1970) and Snashall et al (Clin. Sci. 41 : 35-53, 1971), using a cotton wick placed into subcutaneous tissue of a large variety of species, have found P_{il} to be between -2 and -6 mm Hg. The conflicting positive and negative results can be resolved if the positive pressure effects of the solid tissue elements are taken into account (Hughes, Bull. Physio-path. resp. 7 : 1095-1123, 1971). It is likely that needle measurements represent a measurement of total interstitial pressure, including the solid and liquid components, whereas the capsule and wick techniques measure only the liquid component. Therefore P_{il} is almost certainly negative and possibly more negative in the lung than in other tissues. A negative pericapillary pressure will tend to favour filtration of fluid out of the capillaries, but in the lung, even the combined values of P_c and P_{il} may not exceed the balancing absorption effect of the osmotic pressure of the plasma proteins, so that net fluid movement under normal circumstances would be minimal. P_{il} in the lung has been shown by Meyer et al (1968) to be more negative at high lung inflation and to become positive in the presence of pulmonary oedema.

Osmotic pressure (π)

When two aqueous solutions of the same solute, but of different concentrations, are separated by a semipermeable membrane, a force called osmotic pressure operates to drive solvent through the membrane from the less concentrated solution into the more concentrated solution, so that the solutions tend to become equal in concentration. The osmotic pressure of a given solution is directly proportional to the solute concentration in moles/litre and the absolute temperature. This^{is} called Van't Hoff's Law (Z.physik.Chem. 1 : 481-508, 1887) and is generally written:-

$$\pi = C R T$$

where/...

where C = concentration of solute in moles/lit., R = gas constant (0.082) and T = absolute temperature. Since equimolar dilute solutions of different solutes at the same temperature have the same osmotic pressure, osmotic pressure is determined by the number of molecules of solute present in unit volume of solute. From Van't Hoff's equation, blood plasma would have an osmotic pressure of 7.9 atmospheres, assuming that capillary walls were perfect semipermeable membranes (i.e. permeable to water only). Because biological membranes are permeable to most solutes as well as to water, they do not behave as a physical chemist's definition of a semipermeable membrane. This means that the total osmotic pressure of the body fluids are not manifested as actual pressures. An expression "osmotic reflection coefficient" σ was introduced by Staverman (Rec. trav. chim. 70 : 344-352, 1951) for the modification of Van't Hoff's equation to include leaky membranes. The value of σ ranges from unity in the perfect semipermeable membrane to less than zero in one where the mobility of the solute exceeds that of the solvent. Van't Hoff's equation therefore becomes

$$\pi = C R T \sigma$$

when biological membranes are being considered.

Only the plasma proteins, which are not freely diffusible through the capillary membranes, contribute a measurable osmotic pressure in blood plasma and this is of the order of 25 mm Hg (Landis and Pappenheimer, 1963).

π_{pla} is often referred to as the "colloid osmotic pressure" or "oncotic pressure". Because of their osmotic pressure, the plasma proteins play an important part in the regulation of the distribution of water in the body. Albumin, with a molecular weight of 69,000, is the chief osmotically active protein in normal mammalian plasma and contributes about 65% of the total

π_{pla} . The globulins, with molecular weights ranging from 45,000 to 1,000,000 contribute a further 15% to π_{pla} and the rest of the total is due to unidentified components.

Plasma/...

Plasma does not, however, behave as an ideal fluid with regard to its osmotic pressure, and hence it became necessary to include further modifications to Van't Hoff's law to account for special phenomena exhibited by the plasma proteins. The deviations of plasma from an ideal fluid are taken into account by the osmotic coefficient. This is the ratio of the rate of change of the osmotic pressure to that of the concentration $\left(\frac{d\pi}{dC}\right)$. In the plasma there is a disproportionate increase in the colloid osmotic pressure for a given increase in concentration (and hence the deviation from Van't Hoff's law), so that the magnitude of π_{pla} is equivalent to a 12% concentration of an ideal solute, instead of the 7% actual concentration of proteins. The osmotic coefficient is therefore high in plasma. It follows that the amplification of osmotic forces in plasma has a distinct advantage in maintaining a constant blood volume. In tissue fluid we have the reverse situation in that the osmotic coefficient is low and the forces drawing fluid into the tissues are therefore small. As fluid passes from the vascular compartment into the tissue, the plasma proteins are concentrated to give a force restoring fluid to the blood. Again, the restoring force (π_{pla}) as the proteins become more concentrated is disproportionate to that which would occur if plasma proteins behaved as ideal solutes. This disproportionate increase in the π_{pla} as a function of protein concentration can be partly explained by two phenomena. First there is the Donnan Effect (Chem. Rev. 1: 73-90, 1924) which states that if the membrane is impermeable to one of the solutes, the concentration of the diffusible solutes will be less in the solution which contains the non diffusible solute. Applied to the capillary, the non diffusible solutes refer to the plasma proteins and the diffusible solutes to all the freely diffusible components in plasma, which will be discussed in a section on diffusion. The second cause for the deviation of plasma proteins from the Van't Hoff's equation is the existence of electrostatic interactions between protein molecules and between protein and salts.

Almost/...

Almost all the remarks concerning osmotic pressure have so far been directed at the plasma proteins, but the interstitial liquid osmotic pressure (π_{il}) may also have a role to play in capillary fluid exchange. Its protein content is unknown, though indirect estimates made from lymph samples from a variety of sites have indicated interstitial liquid can have a concentration from 0.1-5%. Miseroocchi and Agostoni (J. Appl. Physiol. 30 : 208-213, 1971) have found protein concentrations in the pleural fluid of dogs and rabbits of 1.06 and 1.33% respectively. Pappenheimer and Soto Rivera (Am. J. Physiol. 1952 : 471-491, 1948) and Levine et al (1967) have estimated π_{il} to be about 5 mm Hg in both dog limb and lung tissue. Lunde and Waaler (J. Physiol. 205 : 1-18, 1969) have postulated a much higher value for π_{il} in the lung (up to 20 mm Hg) which would strongly influence pulmonary fluid balance.

Filtration coefficient (k)

The constant k in Starling's equation determines the rate at which fluid passes from the blood into the interstitial space or vice versa. Measurements of k have been made in single capillaries in a number of species including frogs, rats, rabbits, cats and dogs. Micromanipulation techniques were used by Landis in 1928 (Am. J. Physiol. 82 : 217-238) to test the validity of Starling's hypothesis. He showed that when P_c exceeded 12 cm H₂O, water left the capillary and when P_c was less than 10 cm H₂O, then fluid was reabsorbed from the interstitial space. He concluded that between these two levels of P_c , there was no fluid movement and therefore a balance between π_{pla} and P_c . Landis took this result as evidence that capillary walls are relatively impermeable to plasma proteins. The values of k which he computed from the slope of the straight line resulting from plots of fluid movement against $P_c - \pi_{pla}$ was 0.0056 μ^3 of fluid per sec. per μ^2 of capillary wall per cm H₂O difference between P_c and π_{pla} . Gaar et al (1967) found a higher value for k in isolated dog lungs (0.070), but a similar

straight/...

straight line relationship between fluid movement and $P_c - \pi_{pla}$ once P_c had been raised above 25 mm Hg (the value for π_{pla}). In isolated rabbit lungs Lunde and Wanler (1969) found that a left atrial pressure in excess of 10 mm Hg resulted in extravasation of fluid.

The capillary filtration coefficient can be altered in a number of ways. For example, increasing P_c will increase the filtration area as well as the driving pressure and hence increase filtration. A fall in blood temperature will decrease k since it is a function of the plasma viscosity. Capillary injury will cause vasodilation as well as altering the membrane permeability characteristics, both resulting in increased filtration. Another consequence of injury may be an overall increase in the porosity of the capillary wall, either by widening of existing pores or by the creation of new pores from gaps appearing between damaged endothelial cells.

Diffusion

It has already been mentioned that, of the many constituents of blood, the plasma proteins make the only significant contribution to the osmotic pressure. This is because all the other components can exchange with the pericapillary fluid by diffusion. These diffusible components fall into two groups: the lipid soluble molecules like O_2 and CO_2 , and the lipid insoluble molecules like water, sodium chloride, glucose and urea. It has been suggested by Pappenheimer (Physiol. Rev. 33 : 387-423, 1953) and others that the entire surface area of the capillary wall is available for diffusion of lipid soluble molecules, whereas the area for diffusion of lipid insoluble molecules represents only 0.1% of the total area of the capillary wall. There is good evidence that this 0.1% area is in fact composed of aqueous channels between endothelial cells and these pores, as they are usually called, were found by electron microscopy (Fawcett, D.W. Urbana, Ill. Univ. Illinois Press, 1959) to be about 90 Å wide and 0.5 - 1 μ in length.

Before/...

Before considering the differences between diffusion of lipid soluble and lipid insoluble molecules a few general comments can be made which apply to both. The rate at which solute molecules diffuse is directly proportional to their concentration (moles per litre) in the solvent and the temperature. Diffusion therefore takes place to mix solute and solvent, so that when the concentration of a substance in a solution varies from one region to another, there will be net movement (flux) of particles from regions of high concentration to regions of low concentration. For a given concentration gradient, the flux will depend on the ease with which the molecules move through the solvent. That is, the less frictional resistance, the greater the flux. Frictional resistance to a molecule will depend on the size of the molecule and the viscosity of the diffusion medium. Large molecules will have higher frictional resistance than small molecules and hence more difficulty in diffusing. The measure of the ease of motion is called the diffusion coefficient (D). These principles may be summarised in Fick's Law:

$$\frac{dn}{dt} = D A \frac{dC}{dx}$$

where $\frac{dn}{dt}$ is the flux in unit time, A is the cross-sectional area and $\frac{dC}{dx}$ the concentration gradient.

The small, lipid soluble molecules diffuse via the endothelial cell membranes and their diffusion is so rapid as not to be a limiting factor in exchange rates under normal circumstances. Only during heavy exercise (when the blood transit time is greatly decreased) or at altitude (when the diffusion gradient for O₂ is reduced) may the blood/gas exchange rates become limiting.

Diffusion of lipid insoluble molecules is a function of molecular size relative to pore size. For a molecule as small as water,

diffusion/...

diffusion through pores of 90 Å diameter will be completely unrestricted and therefore very rapid, but for larger molecules the diffusion rate will increase rapidly as pore radius increases. In order to estimate the mean pore dimensions, the following formula is used:

$$\bar{r} = 2 \sqrt{\frac{8\eta k}{A_v \Delta x}}$$

where $\bar{r}$ is the mean pore radius, η the viscosity, k the filtration coefficient, A_v the apparent pore area as determined by diffusion experiments using labelled water, and Δx the path length through the pore. The upper limit of pore size may be estimated by the size of the largest molecule which just fails to pass through the membrane. This process of restricted diffusion of lipid insoluble molecules through pores has been called "molecular sieving". Karnovsky (J. Gen. Physiol. 52 : 64-95s, 1968) has shown that molecules up to a molecular weight of 10,000 are only moderately restricted. Larger molecules like albumin ($M = 69,000$) are very restricted. However Karnovsky (1968) and others have evidence for the existence in capillaries of an additional set of larger but fewer pores of about 2,000 Å wide. These larger pores could be of considerable importance in capillary fluid exchange because they would allow plasma proteins to leave the vascular compartment, causing a fall in the plasma osmotic pressure. It was suggested by Lendis (1964) that there might be a greater number of large pores in venules than in true capillaries, which could explain the gradient of increasing permeability along the capillary first described by Rous et al in 1930.

Some features of structure and physiology specific to fluid exchange in the lung

The pulmonary capillary bed is the most concentrated in the body and its surface area in adults has been estimated to be between 65-80 square metres (Weibel, The Pulmonary Circulation and Interstitial Space, Univ. Chicago Press, pp. 9-25, 1969). This tremendous surface area is primarily/...

primarily important for efficient gas exchange which is further facilitated by the very thin blood/gas barrier (mean thickness 0.5 μ). The endothelial cells, which form an uninterrupted lining to the capillaries, have pores of 100-200 Å in diameter between adjacent cells (Schneeberger and Karnovsky, J. Cell Biol. 37 : 781-703, 1968). In young sheep lung capillaries Boyd et al (J. Physiol. 201 : 567-588, 1969) found evidence of pores of 260 Å mean diameter. Large pores of up to 1,000 Å wide, first described by Majno and Palade (1961) in muscle capillaries, may also be present in lung capillaries. Schneeberger and Karnovsky further demonstrated the existence of numerous, densely arranged pinocytotic vesicles in the pulmonary capillary endothelium, which were responsible for some transport of small protein molecules across the capillary wall.

Whereas the pericapillary interstitial space is normally small, being between 100 Å - 5 μ wide (Cottrell et al, 1967), in pulmonary oedema it becomes distended by accumulated fluid. Mean alveolar wall thickness was shown by Staub et al (1967) to increase to as much as 8 μ in the presence of oedema. It is of interest, however, that the increase in thickness of the blood/gas barrier did not reduce the diffusing capacity of carbon monoxide, suggesting gas exchange was unimpaired. In most cases of clinical pulmonary oedema the ultimate cause is an elevated capillary hydrostatic pressure bringing about excessive filtration. Salt water drowning is an extreme form of pulmonary oedema brought about by the high concentration of sodium chloride in the water filled alveoli. There is massive movement of water out of the vascular compartment resulting in a rapid increase in concentration of the blood constituents. Fresh water drowning is also brought about by a large concentration gradient across the blood/gas barrier, but in this case water movement is into capillaries, causing haemodilution and lysis of red cells. Therefore, in the rapid fluid exchange involved in drowning, all the advantages of the blood/gas barrier become disadvantages.