Thorax 1987;**42**:1–10

Review articles

Solute permeability of the alveolar capillary barrier

In 1857 Claude Bernard[1] introduced curare into the airways of a dog and discovered that the drug produced muscular paralysis. He also concluded that the walls of the bronchi were far less permeable than the alveolar walls. Since this original observation a great deal of interest has been shown in the ability of soluble compounds to cross the alveolar-capillary barrier, and this interest has accelerated in recent years with the development of new techniques using radiolabelled materials. Increasingly the inspiration behind this research has been the hope of elucidating the nature of changes occurring in the alveolar-capillary membrane in the course of disease. This article outlines some salient features of the anatomy and physiology of the barrier between gas and blood in the lung, summarises methods available to assess solute permeation of this barrier, and concentrates particularly on the technique using measurement of the rate of pulmonary clearance of technetium labelled diethylene triamine penta-acetate (^{99m}Tc-DTPA).

Anatomy and physiology

The histological detail of the alveolar-capillary barrier remained inaccessible to anatomists until the advent of the electron microscope. This has permitted Weibel and others[2] to demonstrate most beautifully the fine structure of the lung at the alveolar level. This is illustrated schematically in figure 1. Capillaries can be seen lying in a slightly asymmetrical fashion within alveolar walls, so that the alveolar-capillary membrane is on one side relatively thick and on the other side thin. The latter measures less than 0·5 μm in cross section, and comprises an epithelial layer of type I alveolar cells, capillary endothelium, and between these two cell types a single basement membrane, which is a continuation of the alveolar basement membrane.[3] This delicacy of structure reflects the need for rapid gas transfer. On the other (thicker) side of the capillary, endothelium adheres to the capillary basement membrane, but there is a space between this and the alveolar basement membrane with its adherent epithelium. This connective tissue space, or interstitium, is eventually drained by lymphatics, and in early pulmonary oedema can accommodate a moderate increase in lung water while permitting adequate gas exchange. Under such conditions the thin side of the alveolar-capillary barrier remains dry.[4 5]

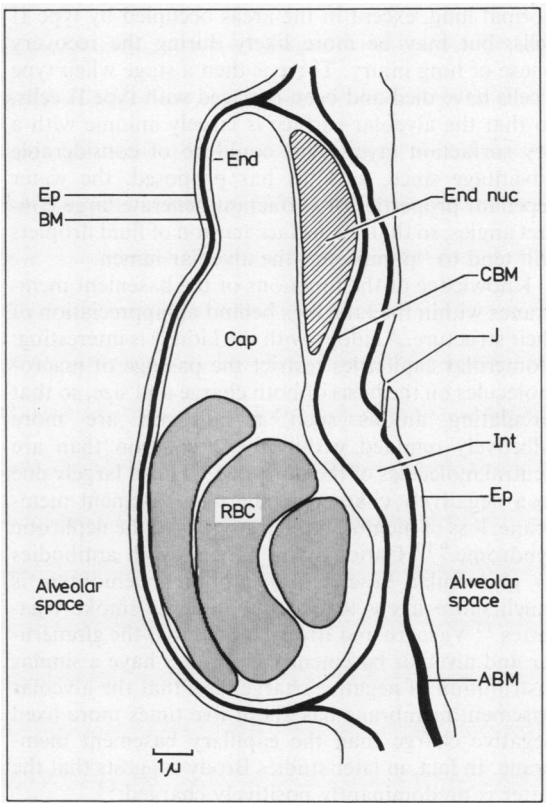

Fig 1 *Schematic cross section of a capillary lying within the alveolar wall. To the left the alveolar-capillary barrier is relatively thin, and comprises an epithelial cell layer (Ep), a single basement membrane (BM), and the endothelial cell layer (End). To the right the barrier is much thicker, and between the two cell layers with their respective basement membranes there is an interstitial space (Int). The alveolar basement membrane (ABM) is anionic, while the capillary basement membrane (CBM) is cationic. Cap—capillary lumen; End nuc—nucleus of endothelial cell; J—interepithelial cell junction; RBC—red blood cell. (After Weibel[2]).*

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Recent ultrastructural studies indicate a remarkable difference in the nature and distribution of fixed electrical charge on the various components of the alveolar-capillary barrier, and this may be important in the transport of molecules across the barrier. The endothelial cells have homogeneous anionic surface sites, whereas the luminal surface of the type I alveolar cell has none.[6] Type II cells show extensive anionic sites but, because they make up only 5–10% of the surface area of pulmonary epithelium, this is for the most part a non-anionic surface. This lack of charge may be relevant to the hypothesis, put forward by Hills,[7] that (cationic) surfactant binds to the alveolar surface, providing an essentially dry layer without an aqueous subphase. This state is perhaps unlikely in the normal lung, except in the areas occupied by type II cells, but may be more likely during the recovery phase of lung injury. There is then a stage when type I cells have died and been replaced with type II cells, so that the alveolar surface is largely anionic with a dry surfactant layer. This could be of considerable advantage since, as Hills has proposed, the water repellant properties of surfactant generate large contact angles, so that the surface tension of fluid droplets will tend to "pump out" the alveolar lumen.

Knowledge of the functions of the basement membranes within the lung lags behind an appreciation of their structure. Analogy with the kidney is interesting: glomerular capillaries restrict the passage of macromolecules on the basis of both charge and size, so that circulating anions such as albumin are more effectively retained within the circulation than are neutral molecules of the same size.[8] This is largely due to a negatively charged glomerular basement membrane; loss of membrane charge leads to the nephrotic syndrome.[9 10] Curiously, in patients with antibodies to glomerular basement membrane haemoptyis is much more likely to occur if patients smoke cigarettes.[11] Vaccaro and Brody[3] noted that the glomerular and alveolar basement membranes have a similar distribution of negative charge, and that the alveolar basement membrane has about five times more fixed negative charge than the capillary basement membrane. In fact, in later studies Brody suggests that the latter is predominantly positively charged.[12]

The alveolar basement membrane is thus likely to inhibit diffusion of anionic molecules into the alveolar space, whereas the capillary basement membrane should facilitate the diffusion of such molecules into the interstitial space. The passage of such anions from plasma to lung lymph in normal lung is easier than is the passage of similarly sized neutral molecules.[13 14] In damaged lungs charge effects are also prominent[12]: under control conditions in experimentally perfused lungs neither cationic nor anionic ferritin was seen to leave capillaries and enter the interstitial space. Lung injury, however, induced by the administration of α naphthylthiourea, caused pulmonary oedema, with rapid migration of anionic ferritin into the interstitial and alveolar spaces but with no migration of cationic ferritin from the capillary.

The practical implications of these findings are not immediately apparent. Albumin is anionic with a molecular size about half that of ferritin (3·5 nm radius compared with 6·2 nm). In fact, most plasma proteins are negatively charged at body pH.[15] Conceivably, the administration of cationic macromolecules may be of therapeutic value in increased pulmonary capillary permeability as a means of plasma expansion and maintenance of a favourable plasma oncotic pressure. There may, however, be extensive binding to anionic surface charges.[16] Moreover, within the interstitial space the charges, exclusive of the capillary basement membrane, are predominantly negative, so that negatively charged proteins are likely to be repelled from interstitial structures with enhancement of their movement into lymph.[12 17] Histological appearances also suggest that the alveolar basement membrane is a more important physiological barrier than the capillary basement membrane, in which case the use of cationic molecules might promote alveolar flooding.

In contrast to gases, which are able to diffuse throughout the whole of the alveolar-capillary surface area, the diffusion of hydrophilic solutes is thought to be restricted to the much smaller surface area occupied by the intercellular junctions. The different junctions between epithelial cells and between endothelial cells explains the dramatic difference in the solute permeability of these two parts of the barrier. Some studies have shown that solute permeation of epithelial junctions is about 10 times less than that of the endothelium; the equivalent pore radii calculated from physiological studies are around 0·6–1·0 nm for the epithelium, and around 4·0–5·8 nm or more for the endothelium.[14 18–21] This low permeability of the epithelium and its ability to actively transport sodium[22 23] explain the very large osmotic pressures (several atmospheres) that can be developed across the epithelial part of the barrier. There is, however, evidence that the high resistance of the epithelium may suddenly break down under the mechanical stress of distension with fluid.[24 25] Therefore the rate of diffusion of compounds from alveoli to blood, or vice versa, is much more likely to be determined by the epithelial than by the endothelial component of the alveolar-capillary barrier. It is not known whether endothelial permeability can increase without a change in epithelial permeability, neither is it clear which is more "important" in various circumstances. For example, electron microscopy shows that the initial site of damage in oxygen exposed lungs is the

endothelium, whereas epithelial disruption is not notable until death is imminent.[5]

Clinical investigation of barrier permeability to solutes

There is no single method that fully characterises solute permeability of the components of the barrier, although several recent approaches have proved useful. These techniques may be divided into those that will assess primarily either endothelial or epithelial permeability (fig 2).

ENDOTHELIAL PERMEABILITY

Chinard[26] developed a method requiring multiple arterial samples after intravenous injection of a range of low molecular weight tracer molecules, allowing calculation of their transit time and distribution volume during a single pass through the pulmonary vasculature. Results are naturally dependent on the distribution of pulmonary blood flow, and there are technical difficulties in taking the necessary rapid sequential arterial samples in the clinical setting, which limits the usefulness of this approach. This method, however, has been used to study conditions such as postoperative respiratory insufficiency[27] and endothelial permeability in renal failure in man.[28] Brigham has more recently been the principal exponent of this technique, and preliminary findings from his group indicate that the analysis of lung uptake of labelled urea can provide a useful measure of increased capillary permeability in the adult respiratory distress syndrome.[29 30]

Since many workers see the clinical problem as that of diagnosing and quantifying increased protein flux from blood to interstitium, there have been many approaches based on external detection of the movement of radiolabelled protein. Sugarman and colleagues[31] have injected radiolabelled albumin and, using a gamma camera, subsequently calculate the ratio of activity over a region of lung to that over the heart. When they observe a rising ratio, they attribute this to lung injury and increasing protein flux into the lung. Pritchard *et al*[32] have developed a technique for experimental purposes that measures lung water in addition to labelled albumin flux. More recent developments have been based on the approach popularised by Gorin and coworkers,[33] who injected indium labelled transferrin (molecular weight 76 000), and also red blood cells labelled with technetium to allow a correction for changes in blood volume in the field of a single scintillation detector over the lung. Basran and colleagues[34] have further refined this technique by introducing a second scintillation detector over the heart. Their index of protein accumulation in the lungs of patients with respiratory distress syndrome was significantly different from that in normal subjects.

The most rigorous scientific evaluation of this type of approach has been undertaken by Dauber *et al*[35] in animals. They have found (*a*) an increase in protein "leak" in thiourea induced lung injury before the development of pulmonary oedema; (*b*) the importance of the blood corrections; and (*c*) the increased error that results from shortening the study period to less than 150 minutes. Their index of protein leakiness was not affected by hydrostatic pulmonary oedema.

This is potentially a very useful method that may allow an early diagnosis of increased endothelial permeability. We believe that this technique should be developed to determine the optimum molecular size and charge of the probe molecule, which may vary in different clinical conditions.

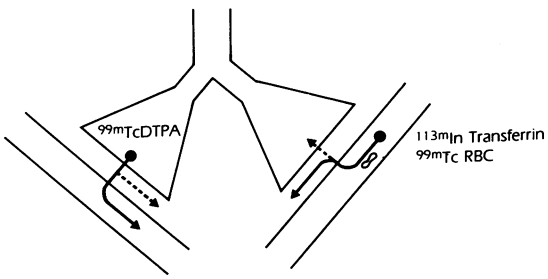

Fig 2 *Two minimally invasive methods for measuring indices of solute permeability of the alveolar-capillary barrier. The clearance from lung of technetium labelled diethylene triamine penta-acetate (DTPA) given by aerosol measures principally epithelial permeability. The accumulation within the lung of indium labelled transferrin (or albumin), standardised for pulmonary blood volume, provides an index of protein leak of the endothelium and eventually the epithelium. In both methods either a gamma camera or a scintillation probe is used to measure changing lung radioactivity. RBC—red blood cells.*

EPITHELIAL PERMEABILITY

Many techniques have been developed to measure epithelial permeability in animal studies. Schanker and colleagues[36] instilled drugs into the lungs of rats, and after a certain time determined drug concentration in excised lungs, so that they were able to calculate the half time of drug absorption from the lung. Valimaki[37] injected iodinated polyvinylpyrrolidone intravenously and observed that in rats exposed to high concentrations of oxygen more tracer was recoverable by endobronchial washing. Egan developed a different approach, instilling radiolabelled tracer molecules into an isolated, saline filled lung segment, and sampling this saline at intervals as well as blood. Normal alveolar epithelium was shown to be relatively impermeable to protein during haemo-

dynamic pulmonary oedema,[38] and to become permeable after the administration of alloxan[39] (a lung damaging agent).

In man, if pulmonary oedema fluid is sufficiently profuse, the ratio of the concentration of endogenous protein in this fluid to its ratio in serum allows the distinction to be made between cardiac and non-cardiac causes.[40 41] Sibbald and colleagues[42] have also achieved this by injecting radiolabelled albumin and other tracers intravenously, and then measuring their rate of appearance in suctioned pulmonary secretions. Although these groups used standard suction catheters, bronchoalveolar lavage via the fibreoptic bronchoscope is becoming increasingly popular.

A major concern with this type of technique is that filling an alveolus with saline may profoundly alter the properties of the alveolar-capillary barrier. Certainly this is so if Hills's concept[7] of the way in which surfactant waterproofs the alveolus is correct. Furthermore, the invasive nature of the procedures may result in damage and increased solute flux across the lung. Introduction of radiolabelled tracer into the airspaces as an aerosol, and measurement of the rate of removal by external radiation detection, renders the technique virtually non-invasive. This procedure was first developed over 20 years ago as a diagnostic procedure for estimating regional ventilation. Various solutes have been used, but over the past decade DTPA has become established as the most widely used compound. It forms stable chelates with most metals and is therefore readily labelled with gamma emitting radionuclides such as indium, chromium, and technetium.[43]

This property led to interest in its use in human plutonium poisoning, given either by intravenous injection or by aerosol to assist excretion of plutonium after inhalation exposure.[44] The plutonium-DTPA complex was known to cross readily into the blood and be excreted in urine. The calcium salt of DTPA is used for this therapeutic purpose. Giving unchelated DTPA may be damaging, since calcium is an important determinant of barrier function, and chelation of extracellular calcium causes retraction of adjacent epithelial and endothelial cells away from one another.[45] This causes an increase in permeability and a fall in electrical resistance. Restoration of a normal calcium concentration restores mechanical and electrical function.[46]

Taplin and Effros with their colleagues in Los Angeles used ^{99m}Tc DTPA in man initially as a means of imaging the lungs, and also measured clearance after inhalation of an aerosol to detect alterations in permeability of the alveolar-capillary barrier in patients with interstitial lung disease.[47 48] Simultaneously, Jones and his colleagues at Northwick Park Hospital, Harrow, were evaluating the use of a closely related compound, chromium labelled EDTA, as a means of diagnosing lung injury after acid aspiration,[49] although in later studies ^{99m}Tc DTPA was used as the radioaerosol.[50] There is now a substantial body of experience in the use of ^{99m}Tc DTPA to study alveolar-capillary barrier function in man and animals.

The pertinent characteristics of a tracer such as ^{99m}Tc DTPA used to study epithelial permeability in this way may be considered as follows. Firstly, it must have an extremely low lipid solubility, so that its diffusion is limited to aqueous pores.[51] This ensures that a second important property, overall molecular size and shape, is a major determinant of transepithelial flux. ^{99m}Tc DTPA has a molecular weight of 492 daltons, with a radius of 0·6 nm, which is similar to the calculated pore size of this cell layer. Thirdly, molecular charge is assumed to be an influence: as discussed above, the uniformly negative alveolar basement membrane is likely to impede the passage of anionic molecules. Electrophoresis indicates that ^{99m}Tc DTPA is anionic (authors' unpublished observations). Fourthly, there should be no active transport system, and none is known to affect ^{99m}Tc DTPA. Finally, the radiolabel should remain securely attached to an inert molecule. Although the stability of binding of technetium to DTPA is easily assessed in vitro by thin layer chromatography, it has been suggested that dissociation may occur in vivo. If this does occur, free technetium (as the pertechnetate ion) should be detectable. Since this is concentrated in the thyroid,[52] it should have been seen on a gamma camera image of the thorax. DTPA also appears not to be metabolised, and if metabolism were to occur, as happens with radiolabelled vasoactive intestinal peptide,[53] it would result in an unexpectedly rapid clearance rate.

EXPRESSING THE RESULTS OF ^{99m}Tc DTPA CLEARANCE TESTS

Results from the aerosol method for measuring epithelial permeability have been expressed in several ways. Most commonly, a plot of radioactivity on a logarithmic scale against time on a linear scale yields a line whose negative slope is the clearance rate, commonly referred to as the k value. We prefer to express our results as a clearance halftime from lung to blood ($T½$), which is the time taken for the initial number of counts to fall by half. The $T½$ is a more widely used and understood measure than the k value. The two are, however, very simply related:

$$T½ = \frac{0{\cdot}693}{k}$$

The k value is often arbitrarily expressed as a per-

centage by multiplying it 100 times. A correction should be made for radioactive decay of the radionuclide being used, which may be performed on individual data points or on the resulting curve. This was frequently omitted in earlier studies.

A bald statement of the halftime with no indication of the variation in the data from which it was calculated allows little reliance to be placed on that result. The correlation coefficient is a commonly quoted statistic, but this indicates only the linearity of the plot of radioactivity versus time. Calculation of the regression coefficient (slope) together with its standard error is of greater value, since this enables confidence limits for a particular halftime clearance rate to be calculated.[54] We have obtained satisfactory results after initial chest counts of 20–40 000/min. The longer the period of recording and the higher the count rate, the lower is the standard error and the greater the reliance that can be placed on the result, although this imposes a greater radioactive load on the patient.

DETERMINANTS OF PULMONARY CLEARANCE OF ^{99m}Tc DTPA

Site of deposition

Most studies using ^{99m}Tc DTPA have used aerosols with a particle size in the range 0·5–2 μm aerodynamic mass median diameter (AMMD), so that deposition is maximised in respiratory bronchioles and alveoli. This minimises the effects of ciliary clearance and absorption through conducting airway epithelium, although some deposition on the conducting airways is inevitable.

In animals the permeability of the conducting airway epithelium to hydrophilic solutes is less than that of the alveolar epithelium because absorption of radiolabelled DTPA deposited in the nasopharynx, trachea, or alveoli was quite different, being 16%, 33%, and 100% respectively.[55] Furthermore, the dog shows a much slower rate of absorption of DTPA from the conducting airways than from the alveoli.[56] Elwood *et al*,[57] however, do not support these differences in man. After rapid inhalation of a large particle (6·3 μm AMMD) aerosol of ^{99m}Tc DTPA to maximise deposition in the central airways they studied clearance rate and found no significant difference between the $T½$ values in their subjects and our previous observations of $T½$ in normal subjects with particles of 2 μm AMMD particles. A potential source of error was their assumption that mucociliary clearance would not influence the lung-blood clearance of ^{99m}Tc DTPA. Elwood *et al*[57] assumed a half life retention of insoluble tracer in the whole lung of 23 hours. The retention in the lung, however, depends on the site of deposition: insoluble particles deposited in conducting airways have a very much shorter retention time than those deposited in peripheral airways. Barrowcliffe *et al*[58] used aerosolised 5 μm labelled polystyrene microspheres to quantify mucociliary clearance and then 5 μm particle size aerosolised ^{99m}Tc DTPA to measure regional lung-blood clearance. They found that when mucociliary clearance was taken into account the lung-blood clearance of ^{99m}Tc DTPA from the conducting airways was very much longer than that from terminal airways. Part of this slow permeation of the conducting airways may be due to binding of ^{99m}Tc DTPA to mucus. Marriott (personal communication) has observed that the diffusion coefficient of ^{99m}Tc DTPA through human mucus is significantly lower than that of tritiated water, and binding of ^{99m}Tc DTPA to mucus occurs at high affinity sites. This implies that, even over several hours, a significant amount of ^{99m}Tc DTPA is unlikely to cross mucus layers of physiologically observed thickness.

Brown and Schanker[51] examined the clearance of a large range of molecules delivered to rat lungs either as a small bolus or as an aerosol. They found a considerable difference in $T½$, with the aerosol delivery consistently resulting in clearance that was about twice as rapid as that after bolus delivery. This implies that tracer delivered as a bolus is more likely to be held up in central airways, and its slower clearance reflects the lower permeability in this region.

Rizk *et al*[59] studied the effect of bronchial and pulmonary arterial occlusion on pulmonary clearance of aerosolised ^{99m}Tc DTPA, but were unable to measure permeability of conducting airway epithelium, since bronchial artery occlusion cannot distinguish between lack of bronchial deposition and low permeability of bronchial epithelium. They did, however, demonstrate that in the absence of pulmonary blood flow, bronchial artery blood flow was sufficient for ^{99m}Tc DTPA clearance to continue at a nearly normal rate. Thus the clearance rate should not be much influenced by variations in pulmonary blood flow.

Lung distension

Lung distension has been shown in man and animals to cause an acute increase in solute flux from lung to blood.[59–61] The clearance rate in animals is inversely related to the molecular weight of the tracer solute, and when the lung is distended the increase in clearance remains proportional to molecular weight.[62] The change occurs whether lung volume is increased by positive or by negative pressure breathing and the underlying mechanism is not known. Possible causes include an increase in lung surface area, change in lung surface properties, widening of intercellular junctions, and recruitment of "leakier" lung units. Some investigators have found a more rapid increase in clearance from the top than from the bottom of the

lung, suggesting that greater stretching of lung units at the top may be responsible.[47 63]

Background radioactivity

North American workers have tended to ignore "background" accumulation of ^{99m}Tc DTPA during the period of study. Clearance of tracer from lung during the first 7–10 minutes after inhalation was calculated by drawing a line of best fit through the data points obtained with the gamma camera,[60] as it is suggested that at this time there is little tracer in the background and thus no need to perform background corrections. When half time clearance rates are rapid, however, this may not be a valid assumption. Furthermore, the pharmacokinetics of ^{99m}Tc DTPA indicate that after intravenous bolus injection the compound is rapidly cleared by a biexponential process, which reflects distribution in the extracellular space, and renal excretion. The relative sizes of pulmonary blood and interstitial compartments for ^{99m}Tc DTPA can be estimated from studies in which ^{99m}Tc DTPA has been used as an indicator to calculate the interstitial fluid volume of the lung.[64] This gives an interstitial space volume of 30 ml/100 g lung—which, with an intravascular pool also around 30 ml/100 g lung, represents a large sink within the counting field. Thus ^{99m}Tc DTPA absorbed across respiratory epithelium will distribute to the interstitial fluid space of the lung, and in subjects with pulmonary oedema this may be a potent source of error because of the increased size of these spaces[65] and because of a larger amount of radioactivity leaking from alveoli into blood and interstitium.

In 1980 Jones and colleagues[50] described a technique whereby a correction might be made by means of a scintillation probe over the thigh and an intravenous injection of ^{99m}Tc DTPA that enabled the determination of the relative sensitivities of the lung and the thigh probes to tracer appearing via this route. Thus a proportion of the thigh activity could be subtracted from the chest activity, to derive activity within the chest corrected for ^{99m}Tc DTPA that had already been absorbed across respiratory epithelium. This method assumes that ^{99m}Tc DTPA activity measured by the leg detector is proportional to activity due to distribution of ^{99m}Tc DTPA to vascular and other tissues within the counting field of the chest detector, and that a bolus of ^{99m}Tc DTPA via a peripheral vein is distributed in the same way as is ^{99m}Tc DTPA absorbed after deposition on permeable respiratory epithelium. In this respect it is important that sufficient time is allowed for distribution to tissues after the bolus injection before extrapolation and calculation of the requisite correction factor, since the aim of the background correction method is to correct not simply for bloodborne, recirculating ^{99m}Tc DTPA but also for distribution in the parenchyma of lung and in chest wall.

O'Doherty *et al*[66] have adapted this method and, instead of recording with a second detector over the thigh, have used a gamma camera region of interest over the shoulder, suggesting that this provides a suitable measure of background activity, although a calibrating intravenous injection is still necessary. They also found that the correction factor increased if the lungs were leaky.

Gellert *et al*[67] have used a region of interest between the kidneys as a site for measurement of background activity. They thereby claim to have developed a technique that requires no intravenous injection since, when such an injection was given in preliminary studies, the increase in activity in the interrenal region was similar to that in various lung regions. In the absence of any means for the generation of suitably sized regions of interest in the lung, however, this finding would appear to represent a happy coincidence, and requires further explanation.

Other groups have advanced reasons why no background correction may be necessary. Jefferies *et al*,[68] for example, refer to studies on two neonates with no ventilation to their left lungs, in whom the chest wall, 30 minutes after administration of an aerosol of ^{99m}Tc DTPA, contributed only 6% to total thoracic counts. This low figure may result from the thinness of the neonatal chest wall and hypoplasia of underlying lung, reducing the amount of ^{99m}Tc DTPA in the background. Of course, these two neonates may have had non-leaky lungs with little ^{99m}Tc DTPA in the background. Extrapolation from the data of O'Doherty *et al*[66] shows that in adults with non-leaky lungs the background at 30 minutes contributes 4–5% to whole lung counts, while the corresponding figure for leaky lungs is about 30%.

Oberdorster *et al*[69] examined pulmonary clearance of inhaled ^{99m}Tc DTPA in the dog, and found that 30 minutes after inhalation *blood* activity of ^{99m}Tc DTPA accounted for less than 2% of total thoracic activity recorded with a gamma camera. These workers, however, used an aerosol with a mass median aerodynamic diameter of 4·4 μm, which is likely to have resulted in considerable deposition of the aerosol on conducting airways as well as alveoli. Since conducting airways are less permeable to ^{99m}Tc DTPA than alveoli (and Oberdorster's own data confirm this view), the presence in the airways of a mass of ^{99m}Tc DTPA that can be only slowly cleared will naturally depress the contribution of circulating ^{99m}Tc DTPA to total thoracic activity. This invalidates extrapolation of data obtained in this way to studies directed at accurately measuring alveolar permeability.

Background correction not only is useful for increasing accuracy, but also permits a longer

duration of measurement of lung clearance of ^{99m}Tc DTPA without including background artefact. Clearance curves may then be resolved into different half time clearance rates that are likely to represent non-homogeneous solute permeability in different regions of the lung. Disadvantages of the correction are the increased complexity and the increased dose of radioactivity. Moreover, there is the possibility that if the detector over the thigh is insensitive this may increase the scatter within the corrected chest counts.

Smoke inhalation

Symptomless subjects who smoke cigarettes have a significantly faster ^{99m}Tc DTPA clearance than non-smokers.[50 66] The clearance half time bears a hyperbolic relation to cigarette smoke inhalation as estimated by the carboxyhaemoglobin level.[70] This effect can be induced in non-smoking subjects within a few days of their taking up smoking,[71] and resolves towards normal at a rate dependent on the degree of initial increase.[72] Rabbits acutely exposed to cigarette smoke show an immediate increase in clearance.[73] In non-cigarette smoking firefighters who were chronically exposed to dense particulate fire smoke there was an increase in permeability unassociated with an increase in carboxyhaemoglobin.[74] This suggests a relationship between lung injury and the particulate phase of smoke rather than the vapour phase of smoke, which has been confirmed by experiments in rats exposed to unfiltered smoke.[75]

Chronic lung diseases

Patients with various interstitial lung diseases have an increased clearance of ^{99m}Tc DTPA from the lung[60] and, in contrast to symptomless cigarette smokers, their clearance curves are multiexponential.[67 76] About half of patients with pulmonary sarcoidosis have increased clearance rates.[60 77]

An increase in ^{99m}Tc DTPA clearance has been reported after inhalation of a histamine aerosol,[78] which has been attributed to stimulation of either H_1 or H_2 receptors.[78 79] The tenacious binding to mucus, however, already referred to, means that ^{99m}Tc DTPA is an unsuitable tracer to test Hogg's hypothesis[80] that bronchial hyperreactivity is associated with increased mucosal permeability. Patients with asthma or chronic obstructive pulmonary disease have mono-exponential clearance curves, with no evidence of increased ^{99m}Tc DTPA clearance.[60 81 82] Inhalation of an aerosol of water ("fog") increases the rate of pulmonary absorption of aerosolised ^{99m}Tc DTPA,[83] and provokes bronchoconstriction in asthmatic subjects. Similarly, earlier studies in our laboratory showed increased absorption of solute from alveoli when hypotonic saline was instilled into lungs.[49] This probably represents not increased permeability due to epithelial damage but the phenomenon of "solvent drag",[84] whereby water flowing paracellularly along an osmotic gradient carries solutes along with it.

Pulmonary infection

Since pneumonia is associated with a protein rich exudate into the pleural and air spaces, it would not be surprising if the transepithelial flux of ^{99m}Tc DTPA were facilitated in infected areas of lung. We have observed that the ^{99m}Tc DTPA clearance rate is considerably more rapid in non-smoking patients suffering from pulmonary tuberculosis than in normal subjects (for example, $T½$ of six minutes compared with 60 minutes). There are two recent reports of increased clearance in immunosuppressed patients with *Pneumocystis carinii* pneumonia,[85 86] which suggest that the technique may sometimes be helpful for both diagnosis of infection and monitoring of the response to treatment.

Respiratory distress syndromes

The detection of increased alveolar-capillary barrier permeability by the use of a molecule as small as ^{99m}Tc DTPA, in preference to a molecule the size of a protein such as albumin, should allow detection of changes in epithelial barrier properties before a stage of injury characterised by increased protein flux across the epithelium. The adult respiratory distress syndrome is associated with a considerable increase in the clearance rate of ^{99m}Tc DTPA.[23 43 87] The clearance curve of ^{99m}Tc DTPA in this syndrome tends to be multi-exponential rather than monoexponential, the fast component having a $T½$ of two to three minutes. During resolution of the disorder there is a gradual reduction in size of the lung compartment with the very rapid half time.[43] Similar changes have been reported in neonates with the infant respiratory distress syndrome.[68] The patchy nature of lung damage is the likely cause of the multiexponential pattern of clearance.

Marks *et al*[61] have discussed the likely distribution of aerosol particles on the alveolar surface. They suggest that an aqueous droplet that lands on surfactant is promptly internalised beneath the surface film and spreads rapidly in the aqueous subphase. If this is correct, the solute concentration, and thus the gradient for diffusion across the alveolar-capillary barrier, would then depend on the volume of fluid lining the alveoli. This volume is thought to be normally extremely small, but it is likely to be larger in oedematous lungs. The surfactant layer may, however, be an important barrier to the diffusion of solute from alveoli to blood. Conditions such as neonatal hyaline membrane disease, in which surfactant is deficient, are associated with greatly increased solute clearance.[68 88] An accompanying inflammatory dis-

order could explain the increased solute permeability that was found, but Robertson *et al*[88] describe a reduction in solute permeability after surfactant replacement. Possibly the greater mechanical forces on the alveolar walls in the surfactant depleted lung may be responsible for increased solute permeation.

Conclusion

Some 130 years after Claude Bernard's original observation, it is evident that the absorption rate of radiolabelled water soluble compounds may provide useful information about physiological and inflammatory processes in human lung. A new phase of research has begun where the effects of various mediators of lung injury may be studied not just in terms of the cellular and biochemical response but with reference to one of the cardinal signs of inflammation, the permeability of the alveolar-capillary barrier.

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