PULMONARY CLEARANCE OF ^{99m}Tc-DTPA: ROLE OF THE PULMONARY SURFACTANT SYSTEM

PULMONALE ^{99m}Tc-DTPA KLARING EN DE ROL VAN HET SURFACTANT SYSTEEM

PROEFSCHRIFT

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CHAPTER 1

PREFACE AND GENERAL INTRODUCTION

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PREFACE

It is established that in a wide variety of acute and chronic lung diseases the integrity of the alveolar-capillary membrane is altered. Increased permeability of the barrier is thought to cause increased filtration of fluids and proteins into the alveoli and increased absorption of inhaled substances into the blood stream. This can eventually lead to impaired gas exchange and, therefore, deterioration of tissue oxygenation.

Attempts to elucidate the nature of changes occurring in the alveolar-capillary barrier has led to increasing research with radio-labelled tracer molecules, such as technetium-99m-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) and technetium-99m-labelled human serum albumin (^{99m}Tc-HSA), in order to measure the permeability of this membrane.

The alveolar-capillary membrane consists of four layers, namely; the alveolar surfactant layer, the alveolar epithelium, the interstitium with basement membrane, and the vascular endothelium. It is generally agreed that the epithelium is the main rate-limiting factor in determining permeability and, therefore, limiting the ability of different solutes to transfer across this barrier.

Results of recent research indicate that the alveolar surfactant layer may also be a rate-limiting factor for solutes and, therefore, is of importance to the permeability of this membrane. This thesis focuses on the significance of the surfactant layer on permeability measurements with radio-labelled materials and also investigates how different medical interventions, such as artificial ventilation and inhalation anaesthetics, influence permeability of the alveolar-capillary barrier when measured with the described techniques. In addition, the effects of cigarette smoking on surfactant function and ^{99m}Tc-DTPA clearance was studied in humans.

The thesis comprises seven manuscripts: one review describing the effects of artificial ventilation on the pulmonary surfactant system and six manuscripts presenting original work.

OVERVIEW OF THE STUDY

This thesis consists one review and six studies in which the role of the pulmonary surfactant system on clearance measurements of pulmonary deposited ^{99m}Tc-DTPA and ^{99m}Tc-HSA is investigated.

In **chapter 2**, on-going research concerning the effects of artificial ventilation on lung mechanics in general and the pulmonary surfactant system in particular is reviewed. Special attention is given to those ventilator settings which might damage the lungs and the alveolar surfactant layer and a possible pathophysiological mechanism is presented.

It is thought that ventilator settings which prevent large pressure-volume swings during the breathing cycle might preserve the lungs, the surfactant system and prevent the occurrence of ventilation-induced damage. This becomes more crucial whenever already damaged and stiff lungs are mechanically ventilated. **Chapter 3** describes a study performed in rabbits with surfactant deficient, uncompliant lungs in which high frequency jet ventilation (HFJV) at 2 and 15 Hz is investigated. These two ventilator settings are compared to a standard method of ventilation in healthy and sick animals.

Chapter 4 presents a study in which the influence of an experimentally increased surfactant content on ^{99m}Tc-DTPA clearance measurements is investigated. This was achieved by intravenous administration of Ambroxol® or exogenous natural surfactant instillation in healthy animals. Both methods are accepted techniques to increase alveolar surfactant content. At the same time, the effects of standard ventilator settings are compared with settings involving large tidal volumes.

The sensitivity of ^{99m}Tc-DTPA clearance measurement is well known and this technique was compared with blood gas tension measurement. The purpose of this

study was to investigate whether blood gas tension measurement under conditions of surfactant deficiency and artificial ventilation sufficiently characterises lung damage and to compare this technique with ^{99m}Tc-DTPA clearance rate measurement (**chapter 5**).

Although ^{99m}Tc-DTPA is often used for permeability measurements, it has been stated that ^{99m}Tc-DTPA is too small a molecule to be able to discriminate between intermediate and severe lung damage. This is why it has been advocated to use larger tracer molecules, as for instance, albumin. This study describes the effects of lung lavage and subsequent tracheal exogenous surfactant replacement on permeability measurements in rabbits using ^{99m}Tc-labelled-human serum albumin (^{99m}Tc-HSA), instead of ^{99m}Tc-DTPA (**chapter 6**).

Patients (especially COPD patients) anaesthetised with inhalation anaesthetics are susceptible to post-operative complications as, for instance, atelectasis, decreased lung compliance and decreased total lung volume, which may in turn necessitate prolonged mechanical ventilation. The hypothesis is that these anaesthetics influence the integrity of the alveolar-capillary membrane and the surfactant system in particular. In this study halothane anaesthesia was compared with barbiturate anaesthesia under two different oxygen concentrations with the ^{99m}Tc-DTPA clearance method for measuring alterations in permeability of the alveolar-capillary membrane (**chapter 7**).

Chapter 8 comprises a study performed in humans on the pulmonary clearance of ^{99m}Tc-DTPA in non-smokers and smokers. Clearance of the tracer is known to be increased with smoking but the exact mechanism is not yet known. In this study,the technique of ^{99m}Tc-DTPA clearance measurement is compared with other methods of testing alveolar-capillary permeability and correlated to certain parameters involving the pulmonary surfactant system.

INTRODUCTION TO THE TECHNIQUE

The alveolar-capillary barrier, consisting of four layers (alveolar surfactant layer, alveolar epithelium, interstitial space with basement membranes and vascular endothelium), is important in allowing free diffusion of gases and restricting the transfer of water, solutes and proteins across the barrier. The integrity of the alveolar-capillary membrane can thus be assessed by measuring its permeability to molecules. This feature can easily be explored by using radio-labelled solutes and monitoring their transfer across the membrane into the blood stream and their subsequent clearance from the lung.

Current evidence indicates that the epithelium (rather than the endothelium and interstitium) is primarily responsible for restricting solute transport across the membrane. Its resistance is at least ten times greater for solutes with a molecular weight ranging from 10 - 100,000 dalton 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) [Gorin and Stewart, 1979; Taylor and Gaar, 1970; Staub, 1974] and permeability measurements with solute clearance reflect mainly epithelial permeability.

A non-invasive, accurate method for measuring permeability of the alveolar-capillary barrier was developed by Rinderknecht and Taplin [Rinderknecht et al, 1977] and involved pulmonary clearance rate measurements of aerosolised and subsequently inhaled and deposited technetium-99m-labelled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA). In brief: a radio-labelled tracer molecule is administered to the lung in an aerosol and deposited activity over the lungs is subsequently measured by a sodium iodide scintillation probe or gamma camera. The clearance rate of the tracer from the lungs reflects the permeability of the alveolar-capillary barrier.

Clearance rate calculation

After establishing sufficient activity in the lungs, the count rate can be measured by using, for instance as in our studies, a gamma camera. Gamma camera images are obtained in successive 1-minute frames for the collecting period, stored in 64 x 64 images matrix and in a computer (Digital PDP 11/34, Maynard, USA). Data from the clearance measurement can subsequently be analysed by selecting a region of interest, which may be only a part of the lung, or both lungs. A time-activity curve is generated, being a plot of radioactivity on a logarithmic scale against time on a linear scale. This yields a line whose negative slope is the clearance rate, commonly referred to as the *k* value. The course can be described according to the equation:

$$N = N_0 \times e^{-kt}$$

where N is the count rate at any time t, and N_0 is the count rate at time t = 0. The rate constant *k* (min^{-1}) for the clearance of ^{99m}Tc-DTPA from the lungs can easily be converted to a half-life (T½) of the tracer in the lung, which is the time taken for the initial number of counts to fall by half. The T½ is more widely used and understood than the *k* value. The conversion equation is as follows:

$$T½ = 0.693/k \ (= \ln 2/k)$$

The clearance rate *k* as a percentage per time is obtained simply by multiplying *k* by 100%. A correction should be made for radioactive decay of the radionuclide during the study time, which may be performed on individual data points or on the resulting curve.

The need for correction of intravascular and tissue accumulation of ^{99m}Tc-DTPA during pulmonary clearance measurements is a point of controversy between different investigators. Some have stated that background correction is absolutely necessary and constitutes a significant refinement of the technique [Huchon, 1986;

Barrowcliffe and Jones, 1987 and 1988; Jones et al, 1980]. The correction factor is determined from relative changes in activity in an area of the thigh or shoulder and in the chest after an intravenous injection of ^{99m}Tc-DTPA.

Others, however, state that the transfer rates of ^{99m}Tc-DTPA from the blood into and out of muscular tissue are not necessarily the same as those from blood into and out of the lung tissue. Therefore, changes in blood or thigh activity are not accurate indices of the contribution of thoracic blood and tissue to the background activity observed in the lung fields [Oberdörster et al, 1986]. Some showed that background activity constituted only a small percentage of the total amount of activity and concluded that background correction is not necessary [Rizk et al, 1984; Oberdörster et al, 1984; O'Brodovich and Coates, 1987]. The influence of background corrections, however, becomes greater at higher clearance rates.

A consensus, however, seems to be reached at the latest NHLBI workshop [Staub et al, 1990] where it was stated that background corrections are not necessary when the data collecting period does not exceed 20-30 min after aerosol inhalation. Data collecting periods longer than 30 min may require background correction.

The duration of insufflation or inhalation of the aerosol should be minimised (3-4 min maximum) lest an initial fast component of the clearance be masked. In normal lungs, this factor is not a problem where clearance rates follow a monophasic course and are relatively slow (independent of duration of monitoring). In abnormal lungs, however, clearance curves can have biphasic characteristics and curve-stripping procedures have to be applied in order to obtain a more detailed mathematical analysis. In some pulmonary disorders bi-phasic curves are produced when monitoring for more than 10 min. Biphasic (biexponential) clearance curves for ^{99m}Tc-DTPA indicate that different lung regions have different clearance rates and bi-phasic clearance curves are considered to be almost certainly abnormal.

Studies have shown that radioaerosol clearance rates from the larger airways, bronchi, trachea and nasal epithelia are significantly slower than that of the alveolar-capillary membrane and thus can influence clearance rate measurements on alveolar level [Barrowcliffe et al, 1987; Greiff et al, 1990]. In part this phenomenon could be explained by the fact that ^{99m}Tc-DTPA can bind to the mucus layer overlining the broncho-tracheal epithelium [Cheema et al, 1988] or by the physically greater diffusion distance of the tracer to the blood [Oberdörster et al, 1986].

REFERENCES

Barrowcliffe MP, Jones JG, Agnew JE, Francis RA, Clarke SW. (1987) The relative permeabilities of human conducting and terminal airways to ^{99m}Tc-DTPA. *Eur. J. Respir. Dis.*, 153, 68-73.

Barrowcliffe MP, Jones JG. (1987) Solute permeability of the alveolar capillary barrier. *Thorax*, 42, 1-10.

Barrowcliffe MP, Otto C, Jones JG. (1988) Pulmonary clearance of ^{99m}Tc-DTPA: influence of background activity. *J. Appl. Physiol.*, 64, 1045-1049.

Brain JD, Valberg PA. (1979) State of the Art: deposition of aerosol in the respiratory tract. *Am. Rev. Respir. Dis.*, 120, 1325-1375.

Cheema MS, Groth S, Marriott C. (1988) Binding and diffusion characteristics of ^{14}C EDTA and ^{99m}Tc-DTPA in respiratory tract mucus glycoprotein from patients with chronic bronchitis. *Thorax*, 43, 669-673.

Coates G, O'Brodovich H. (1987) Extrapulmonary radioactivity in lung permeability measurements. *J. Nucl. Med.*, 28, 903-906.

Gorin AB, Stewart PA. (1979) Differential permeability of endothelial and epithelial barrieres to albumin flux. *J. Appl. Physiol.*, 47, 1315-1324.

Greiff L, Wollmer P, Erjefält I, Pipkorn U, Persson CGA. (1990) Clearance of ^{99m}Tc-DTPA from guinea pig nasal, tracheobronchial, and bronchoalveolar airways. *Thorax*, 45, 841-845.

Huchon GJ. (1986) La clairance respiratoire des solutés. *Bull. Eur. Physiopathol. Respir.*, 22, 495-510.

Jones JG, Lawler P, Crawley JCW, Minty BD, Hulands C, Veall N. (1980) Increased alveolar epithelial permeability in cigarette smokers. *The Lancet*, 1, 66-68.

Jones JG, Minty BD, Royston D. (1982) The physiology of leaky lungs. *Br. J. Anaesth.*, 54, 705-721.

Jones JG, Royston D, Minty BD. (1983) Changes in alveolar-capillary barrier function in animals and humans. *Am. Rev. Respir. Dis.*, 127, S51-S59.

Lourenço RV, Klimek MF, Borowski CJ. (1971) Deposition and clearance of two micron particles in the tracheobronchial tree of normal subjects-smokers and non-smokers. *J. Clin. Invest.*, 50, 1411-1420.

Lourenço RV, Loddenkemper R, Carton RW. (1972) Patterns of distribution and clearance of aerosols in patients with bronchiectasis. *Am. Rev. Respir. Dis.*, 106, 857-866.

O'Brodovich H, Coates G. (1987) Pulmonary clearance of ^{99m}Tc-DTPA: a noninvasive assessment of epithelial integrity. *Lung*, 165, 1-16.

Oberdörster G, Utell MJ, Weber DA, Ivanovich M, Hyde RW, Morrow PE. (1984) Lung clearance of inhaled ^{99m}Tc-DTPA in the dog. *J. Appl. Physiol.*, 57, 589-595.

Oberdörster G, Utell MJ, Morrow PE, Hyde RW, Weber DA. (1986) Bronchial and alveolar absorption of inhaled ^{99m}Tc-DTPA. *Am. Rev. Respir. Dis.*, 134, 944-950.

Rinderknecht J, Krauthammer M, Uszler JM, Taplin G, Effros RM. (1977) Solute transfer across the alveolar capillary membrane in pulmonary fibrosis [Abstract]. *Am. Rev. Respir. Dis.*, 115, 156.

Rinderknecht J, Shapiro L, Krauthammer M, Taplin G, Wasserman K, Uszler JM, Effros RM. (1980) Accelerated clearance of small solutes from the lungs in interstitial lung disease. *Am. Rev. Respir. Dis.*, 121, 105-114.

Rizk NW, Luce JM, Hoeffel J, Price DC, Murray JP. (1984) Site of deposition and factors affecting clearance of aerosolised solute from canine lungs. *J. Appl. Physiol.*, 56, 723-729.

Staub NC, Hyde RW, Crandall E. (1990) NHLBI Workshop summary: workshop on techniques to evaluate lung alveolar-microvascular injury. *Am. Rev. Respir. Dis.*, 141, 1071-1077.

Valberg PA, Brain JD, Sneddon SL, LeMott SR. (1982) Breathing patterns influence aerosol deposition sites in excised dog lungs. *J. Appl. Physiol.*, 53, 824-837.

Waldman DL, Weber DA, Oberdörster G, Drago SR, Utell MJ, Hyde RW, Morrow TE. (1987) Chemical breakdown of technetium-99m-DTPA during nebulization. *J. Nucl. Med.*, 28, 378-382.

CHAPTER 2

EFFECT OF DIFFERENT VENTILATOR SETTINGS ON LUNG MECHANICS: WITH SPECIAL REFERENCE TO THE SURFACTANT SYSTEM

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INTRODUCTION

Since its introduction for clinical routine use some 40 years ago, artificial ventilation has proven to be a life-saving method or therapy in intensive care but also remained a topic of much discussion and controversy because artificial ventilation involves a disturbance to normal cardiovascular and respiratory function [1, 2].

It is also established that artificial ventilation can lead to decreased lung compliance and dysfunction of gas exchange. Even more important is that artificial ventilation, which is generally considered to be a supportive therapy, can by itself lead to formation of atelectasis, pulmonary oedema, pneumonitis and fibrosis (for review: see Table). To date, no adequate explanation of the pathophysiologic basis of all these changes due to artificial ventilation has been documented. However, there is evidence that some of these changes are induced by alterations to the surfactant system. These latter findings have stimulated ongoing extensive research in this area in an attempt to explain the side effects of artificial ventilation and to find improved methods of artificial ventilation in which these effects are minimised.

This paper gives a review of studies in which surfactant or surfactant-dependent functions have been investigated in combination with artificial ventilation, and what the immediate effects are of the ventilator setting used.
Firstly, the normal surfactant functions and the methods used for *in vivo* characterization of the surfactant system will be briefly described; secondly the influence of artificial ventilation on these functions will be discussed.

SURFACTANT FUNCTIONS

The normal physiological functions of the pulmonary surfactant system include:

a) Mechanical stabilization of lung alveoli

The integrity of the surfactant system of the lung is a prerequisite for normal breathing with the least possible effort. Surfactant produces this effect by decreasing the surface tension of the interface between the alveoli and air. This provides an explanation as to why we have to generate a pressure of only 4-8 cm H_2O during each inspiration, whilst at the air-liquid interface only the surface tension of the plasma is present and then a pressure of 25-40 cm H_2O (depending on the radius of the alveoli) has to be generated for each inspiration. This is a well-known symptom in immature newborns with respiratory distress syndrome (RDS) and in adults with respiratory failure. In alveoli of different radii, an equal lowering of surface tension would not, however, produce stabilization of the alveolar system. It would, according to the law of Laplace ($P = 2\sigma/r$; P = pressure in the bubble, σ = surface tension, r = radius of the bubble), lead instead to the collapse of the smaller bubble or alveoli, and to their emptying into the larger ones. Since alveoli *in vivo* do not exhibit such behavior, one may conclude that the second remarkable quality of the alveolar lining layer is that it can change the surface tension, dependent on the size of the alveoli.

b) Stabilization of small airways

As early as 1970, Macklem and coworkers drew attention to the significance of stabilization of the peripheral airways by surfactant and hinted that its lack might cause airway obstruction or collapse of the small bronchioli with air trapping [3]. This was shown in an animal model in our laboratory in which the bronchial surfactant was damaged and it was demonstrated that the pressure needed to open up collapsed bronchioli is approximately 20 cm H_2O. In the same study it was also demonstrated that severe impairment of bronchial surfactant can be successfully treated with exogenous surfactant replacement [4]. Besides its role in mechanical stabilization, bronchial surfactant also has a transport function for mucus and inhaled particles. This has been proven, *in vitro* in a study showing that particles on a surface film move only in one direction if the surface film is compressed and then dilatated - comparable to the compression and expansion during inspiration and expiration [4,

5]. Further, bronchial surfactant also acts as an antiglue factor, preventing the development of large adhesive forces between mucus and the bronchial wall [6].

c) Protection against lung oedema

Another function of the pulmonary surfactant system is stabilization of the fluid balance in the lung and protection against lung oedema [7]. In general, alveolar flooding will not occur as long as the suction force in the pulmonary interstitium exceeds the pressure gradient generated by surface tension in the alveolar air-liquid interface. Since the pressure gradient is inversely related to the radius of the alveolar curvature there is, for each combination of interstitial resorptive force and average surface tension, a critical value for surface tension and alveolar radius, below which alveolar flooding occurs.

FUNCTIONAL CHANGES DUE TO "DISTURBED" SURFACTANT SYSTEM

When considering the physiologic functions of the alveolar-bronchial surfactant system it can easily be understood that alteration in its functional integrity will lead to [8]:

- decreased lung distensibility and thus to increased work of breathing and increased oxygen demand by the respiratory muscles
- atelectasis
- transudation of plasma into the alveoli with decreased diffusion for oxygen and CO_2
- enlargement of functional right-to-left shunt due to perfusion of non-ventilated alveoli (the v. Euler-Liljestrand reflex does not "work" in surfactant deficient alveoli) resulting in hypoxemia and acidosis.

METHODS FOR IN VIVO INVESTIGATION OF THE SURFACTANT SYSTEM

The methods used to investigate the integrity of the pulmonary surfactant system *in vivo* include: pulmonary compliance measurements, Technetium-99m-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) clearance rate measurement and, indirectly,

via monitoring of blood gases.

Pulmonary compliance measurements

The fact that the surfactant system is responsible for a half to two-thirds of the total retraction force of the lungs, makes lung mechanic studies very important. Moreover, recording of pressure-volume (P-V) diagrams is the only direct *in vivo* test for characterization and indication of damage to the surfactant system. To quantify these changes in the P-V diagram, different indices for both the inspiratory loop and expiratory loop were introduced [9, 10, 11]. However, in some pathological changes in the lung the expiratory loop cannot be used for characterization of the surfactant system e.g. if there is a combination of surfactant deficiency with a massive alveolar oedema, part of the oedema fluid may block the airways during expiration, resulting in air trapping: this can mistakenly be interpreted as a high expiratory stability.

Furthermore, the P-V diagram enables differentiation between surfactant deficiency, pulmonary oedema, pneumonia and fibrosis. For example, when it is established that the cause of decreased lung compliance is due to surfactant deficiency, it is still possible to open up the lungs by application of a very high inspiratory pressure and keep them stable during expiration by a high positive end-expiratory pressure (PEEP). A similar situation arises in lungs with oedema or pneumonia, but due to the fact that these lungs are filled by plasma or exudate, the total volume which can be forced in at a certain pressure will be less than that of a purely surfactant-deficient lung. To differentiate between different types of restrictive lung diseases eg. pneumonia and fibrosis, it is necessary, additionally, to evaluate blood gases at different intra-pulmonary pressures. An increase of PEEP or mean airway pressure in a pneumonia lung will always be followed by an increase in PaO_2 whereas in case of fibrosis PaO_2 will decrease or will not change.

^{99m}Tc-DTPA clearance measurement

^{99m}Tc-DTPA is a small (molecular weight 492 dalton) water-soluble molecule which, after deposition on the alveolar surface, is transferred into the blood stream across various biological barriers. These barriers are: the surfactant layer, the alveolar epithelium, the basal lamina layer and the endothelium. Clearance of ^{99m}Tc-DTPA from the lung measures the overall permeability of these layers. When ^{99m}Tc-DTPA is administered via an air jet nebulizer as an aerosol whose particles favour alveolar deposition, a gamma camera is used to measure the amount of activity in the lungs over a certain period of time. After measurement, clearance curves can be analysed in freely-selected lung regions. This ^{99m}Tc-DTPA clearance technique has proven a very sensitive and non-invasive method to study the integrity of the pulmonary surfactant system.

It has been demonstrated that the ^{99m}Tc-DTPA clearance rate depends mainly on the functional integrity of the surfactant system: lungs with a damaged surfactant system are known to have a very high clearance rate [12, 13, 14].

Surfactant system and arterial oxygen tension (PaO_2)

Measurement of PaO_2 is an easy to obtain indirect parameter from which conclusions can be drawn about the integrity of the surfactant system. If a connection is suspected between presence of hypoxemia and decreased function of the pulmonary surfactant system, it can be established by letting the patient breathe 100% oxygen. In healthy lungs, PaO_2 should increase to at least 500 mm Hg; whereas with a damaged surfactant system PaO_2 will be < 500 mm Hg, due to atelectasis and intrapulmonary shunting. If the application of continuous positive airway pressure (CPAP) or PEEP leads to an improvement of PaO_2 and this improvement is dependent on the height of the end-expiratory pressure it means that collapsed terminal airways and/or alveoli, due to the damaged surfactant system, have been re-opened. This also means that each tendency of alveolar collapse and atelectasis that can be compensated for by a counter pressure (e.g. CPAP, PEEP) indicates a

surfactant deficiency. In other words: every application of CPAP or PEEP with the intention to increase functional residual capacity (FRC), decrease intrapulmonary shunt and improve gas exchange, can only be interpreted as compensating for the increased retraction forces due to a damaged surfactant system. The degree of damage to this system is proportional to the application of CPAP or PEEP required.

OVERVIEW OF EFFECTS OF ARTIFICIAL VENTILATION ON LUNG MECHANICS AND SURFACTANT FUNCTION

Soon after the introduction of artificial ventilation in medical routine, side-effects of this therapy became obvious which led to extensive research in this area. Different ventilator modes were developed in an attempt to diminish these side-effects. Numerous clinical and experimental studies were performed to investigate e.g. the influence of PEEP, tidal volumes, frequency, inspiration/expiration ratios, inflations etc. during ventilation.

Table 1 Summary of studies investigating surfactant fuctions in combination with artificial ventilation; conventional mechanical ventilation (CMV); high frequency ventilation (HFV).

CMV

Investigated parameters	**Reference**	**Conclusions**
Tidal volume, frequency, inspiratory pressure	15 - 36	Artificial ventilation can decrease lung compliance, total lung volume and surface activity of lung extracts. Normoventilation does not have a great influence on lung compliance etc, whereas hyperventilation (large tidal volumes) caused great changes to the ventilated lungs (abnormal X-rays and grossly abnormal lungs [30]). The changes induced by these changes are directly related to the size of the tidal volume used. Whenever large tidal volumes and/or high peak inspiratory pressures are used microvascular damage due to volume overdistension can develop, leading to high permeability oedema, epithelial damage and hyaline membranes [27].

Investigated parameters	Reference	Conclusions
End-expiratory pressure	17, 22, 24, 25, 34, 37-47	The end-expiratory pressure used during ventilation is of great importance because the use of PEEP can prevent a decrease in lung compliance, total lung volume surface activity of lung extracts. These beneficial effects of PEEP are directly related to the level of PEEP used. The mechanism by which PEEP prevents ventilator induced changes could be that PEEP prevents alveolar collapse and keeps the end-expiratory volume of the alveoli above a critical level of the static state of the surfactant film. PEEP should be cautiously applied because PEEP always results in cardio-vascular depression and can sometimes result in extravascular fluid retention in the lung.
Duration	16, 19	The longer a non-optimal ventilator setting is used, the greater the change in lung compliance etc, will be.
Inspiration/ expiration ratio	48 - 56	Pressure-controlled inversed ratio ventilation (IRV) which covered 80 % of the ventilator cycle when compared to volume-controlled IRV showed reduced recoil pressure, improved blood gases and caused less morphological damage to surfactant deficient lungs.
Inflation/sigh	21, 22, 39, 57 - 69	An occasional sigh or inflation during artificial ventilation can restore the decreased lung compliance (due to the ventilation) to normal. This effect can in part be attributed to a release of surfactant in the alveoli (stretch-effect). Inflations with high pressures or large volumes can, however, induce alterations in solute-permeability of the alveolar-capillary barrier, eventually resulting in protein influx into the alveoli. This could reduce surfactant activity. Some authors state that sighs or inflations are not necessary in patients ventilated with large tidal volumes. In lungs with established disease, sighs/inflations did not seem to have great influence on gas exchange and compliance.
HFV	70 - 92	Most studies showed no large difference in effects between conventional ventilation and HFV on gas exchange at the same mean airway pressures. In some studies, however, oxygenation was better with HFV than with CMV. This difference could be due to the fact that HFV maintains larger mean lung volumes due to gas trapping and auto-PEEP. The risk of barotrauma during HFV is decreased due to the smaller volume/pressure changes during the ventilation cycle with HFV when compared to CMV with large tidal volumes [76, 84]. Thus always preventing huge loss of surfactant during ventilation.

All these studies employed various investigative methods including, for example, lung mechanics, blood gases, biochemistry, morphology, surface tension measurements of lung extracts, and epidemiology to characterise the effects of artificial ventilation on surfactant and lung mechanics. These studies are summarised in the table and some general conclusions concerning the effects of the different parameters are presented.

HOW ARTIFICIAL VENTILATION AFFECTS THE SURFACTANT SYSTEM

Lung compliance, FRC and total lung volume are decreased in respiratory failure and can also decrease during anesthesia when artificial ventilation is applied to the patient. This is due to the formation of atelectatic areas in the ventilated lung. To prevent these changes from occurring, ventilator settings with large tidal volumes and high peak inspiratory pressures are used. Studies have shown, however, that these ventilator modes with large tidal volumes and high peak inspiratory pressures during artificial ventilation affect the pulmonary surfactant system [24, 29, 32, 43]. Moreover, these changes are directly related to the tidal volume used and the duration of the mechanical ventilation [see Table]. The exact mechanism by which the surfactant system is affected by artificial ventilation is not yet entirely clear. One factor is that the surfactant in the alveolar lining is actively removed from the alveolus towards the larger airways; this can lead to a shortage of surfactant at the alveolar level causing changes in surface tension characteristics in the lung, seen during or after prolonged periods of mechanical ventilation.

In normal spontaneous breathing, during end-expiration the surfactant molecules covering the alveolar epithelium are compressed on the small alveolar area (leading to low surface tension or a high surface pressure) thus preventing the alveoli from collapse. If the surface of the alveolus is smaller than the surface occupied by the surfactant molecules, the molecules are squeezed out of the surface and forced towards the airways. These surfactant molecules are then 'lost' for the alveoli and are eventually cleared from the lungs. During the following inflation of the alveoli, the surface is replenished with surfactant molecules coming from the underlying hypophase where surfactant molecules in micelles are 'stored' for later use. During the next expiration, the mechanism repeats itself and again surfactant molecules are forced out of the alveolus and subsequently replenished from the hypophase; this is a continuing cycle (Figs. 1 and 2).

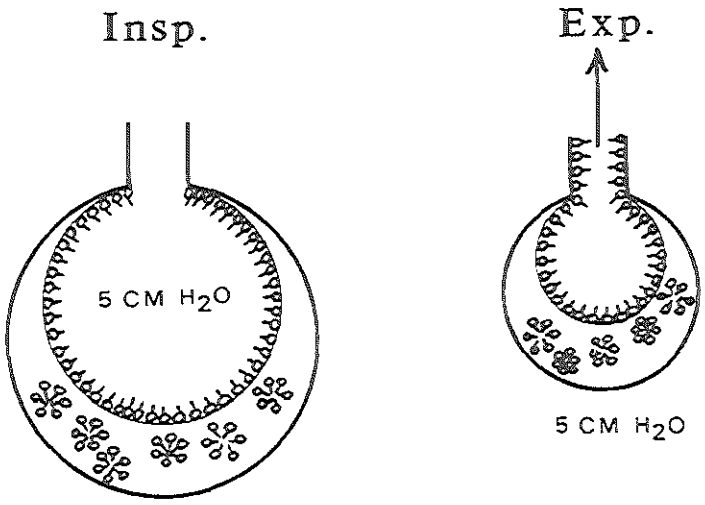

Fig. 1 Balance between synthesis, release and consumption of surfactant in the healthy lung. The pressure values given represent the intrapulmonary pressure needed to open up this alveolus. At the surface and in the hypophase (micelles), there are sufficient molecules of surfactant. These micelles deliver the surfactant necessary to replace the molecules squeezed out during expiration.

The amount of surfactant that must be produced and subsequently secreted by the alveolar type II cells is proportional to the loss of surface active molecules during the breathing cycle. When production and secretion of new surfactant molecules keep pace with consumption, no surfactant deficiency can occur, as in a normal healthy lung.

It becomes clear that the rate of loss of surfactant is greater when the phasic volume changes of the alveolus are larger during the breathing cycle. When the alveoli are collapsed, for instance in atelectatic lung areas, the surfactant molecules are highly compressed on the surface and a significant part is forced out of the alveolus. The smaller the airspace, the lower the surface tension must be to retain airspace stability.

If the collapsed alveolus is subsequently highly inflated, replenishment from the hypophase must accordingly be larger than normal; even more so when a combination of high peak inspiratory pressures and large tidal volumes are used to open up collapsed airspaces. In these situations consumption is greater than normal and production and secretion of surfactant must increase to prevent a surfactant-deficient state.

Rhythmic compression (expiration) and decompression (inspiration) of the alveolar lining during the breathing cycle thus causes loss of surfactant during artificial ventilation, especially when compression is far below the static state of the surfactant layer, which is normally equal to or just above the normal FRC level. The use of negative end-expiratory pressure (NEEP) or ventilation of a lung with an open thorax, exacerbates this process and thereby increases the loss of surface active molecules [37, 47].

The application of PEEP during mechanical ventilation prevents a decrease in lung compliance and surface activity of lung extracts [For review: 93]. This can be explained by the fact that PEEP prevents alveolar collapse and thus keeps the end-expiratory volume of the alveoli above a critical level of the static state of the surfactant film, thereby preventing excessive loss of surfactant during expiration.

It is established that during normal spontaneous breathing humans do occasionally sigh. Therefore, it was suggested that the addition of large inflations or sighs during mechanical ventilation could prevent the formation of atelectasis and subsequent decrease of lung compliance [21, 64]. Studies have shown that these periodic hyperinflations during mechanical ventilation are able to reopen collapsed airways and alveoli in normal healthy lungs [see Table]. Findings by Bendixen and Laver showed that a decrease of lung compliance can be reversed by a single large hyperinflation [94]. This reversal can, in part, be due to the fact that after hyperinflation the lamellar bodies (surfactant store) of type II cells are decreased in

volume and density (stretch-effect) which indicates extra release of surfactant by type II cells [59].

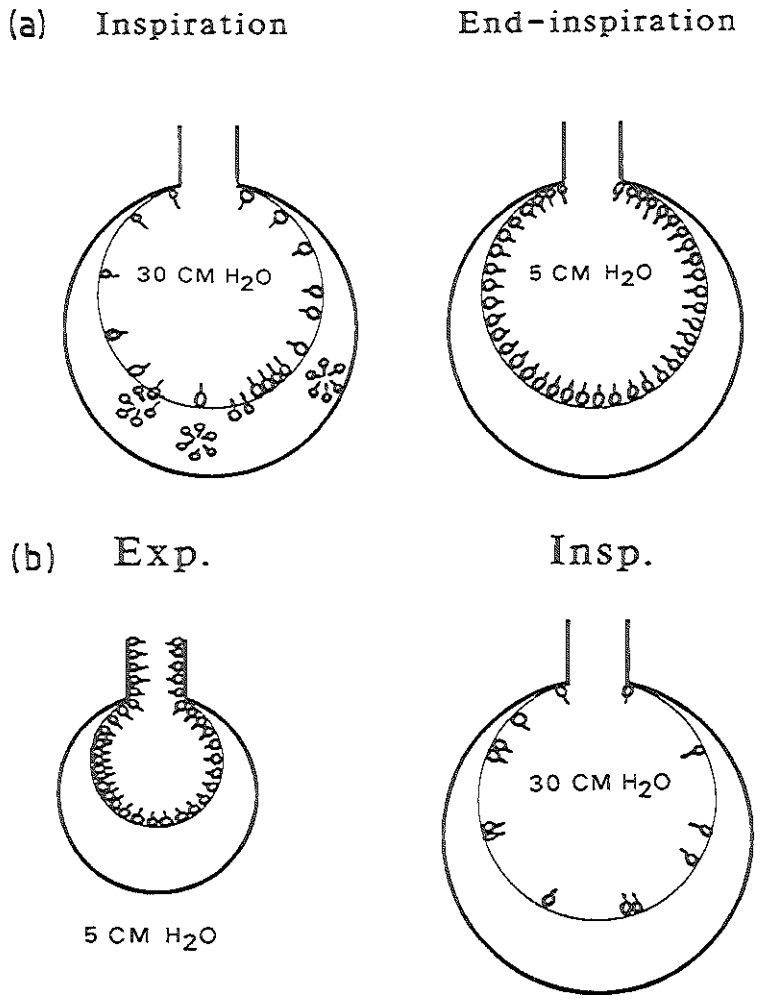

Fig. 2 **A:** Imbalance between synthesis, release and consumption of surfactant due to artificial ventilation. At the beginning of inspiration, there exists an apparent deficiency of surfactant molecules but there is a respreading of molecules stored in the hypophase to the surfactant layer. At the end of inspiration there is, in principle, enough surfactant on the surface.
B: With the next expiration, surface active molecules are further squeezed out and no surface active molecules are left in the hypophase for respreading, creating the situation where a serious surfactant deficiency follows.

Another factor that might be of importance for deterioration of lung mechanics during ventilation is that mechanical ventilation, especially in non-homogenous lungs, creates severe shear forces between open and closed airways and possible overstretch of the epithelium during the breathing cycle, resulting in necrosis and desquamation of bronchiolar and alveolar epithelium [95, 96, 97]. Overstretch of the intercellular junctions of the epithelium leads to increased permeability with a resulting influx of various kinds of plasma proteins, like albumin and fibrinogen etc, which are known to inhibit surfactant function [98]. Impaired surfactant function will lead to decreased lung distensibility and collapse of alveoli and small airways leading to atelectasis, enlargement of the right-to-left shunt due to perfusion of non-ventilated alveoli, hypoxemia and acidosis [99, 100, 101]. Furthermore, the surfactant system itself is essential for stabilization of the fluid balance in the lung and for prevention of pulmonary oedema [102, 103, 104, 105]. Inhibition of the surfactant function by oedema constituents therefore accelerates the formation of pulmonary oedema and the development of respiratory failure resulting in a vicious circle of surfactant inactivation and loss of pulmonary function. The ventilator mode which is then necessary to ventilate these lungs is potentially more damaging than the ventilator settings that caused it.

Summary of studies investigating the different aspects and parameters of ventilation on lung mechanics provides the rationale for optimal ventilator settings. These optimal ventilator modes should open up the whole lungs and should keep them totally open during the ventilatory cycle. All ventilator settings which keep the alveoli inflated during end-expiration, and thus above the critical level of the static state of the surfactant film, would prevent the non-physiologic consumption rate of surfactant due to artificial ventilation. Further, in the totally open lung there are no, or minimal, shear forces during ventilation which could damage the epithelium and indirectly damage the surfactant system as described above. A ventilator mode which fulfills these prerequisites is, for example, pressure controlled inversed ratio ventilation (IRV).

CONCLUSION

Although artificial ventilation remains one of the major breakthroughs in modern health care it is clear that it should be applied cautiously. From the reviewed studies, which are largely different as to intention and methods used, the same conclusion can be drawn, namely: that when lungs are ventilated with large volumes and pressure changes the pulmonary surfactant system becomes depleted or in some other way damaged. This results in decreased pulmonary compliance which in turn creates the condition in which secondary lung oedema can develop. This leads to further damage and inactivation of the alveolar surfactant system. The ventilator mode which is then necessary to ventilate these lungs is potentially more damaging than the ventilator setting that caused it. In a diseased lung these pathophysiologic changes are probably more pronounced and severe than in healthy ventilated lungs. The results of these studies provide the rationale for optimal ventilator settings (aiming at adequate gas exchange and minimal cardiocirculatory interference) which should produce smaller pressure-volume swings during the ventilatory cycle and keep the lung volume equal to or just above FRC level, to prevent a significant depletion of surface active material. One may also speculate that ventilator-induced changes in lung mechanics may be prevented by instillation of exogeneous surfactant.

REFERENCES

1. Price HL, Connor EH, Dripps RD. Some respiratory and circulatory effects of mechanical respirators. J Appl Physiol 1954;6:517
2. Cournand A, Motley HL, Werko L and Richards DW. Physiological studies of the effects of intermittent positive pressure breathing on cardiac output in man. Am J Physiol 1948; 152: 162-174.
3. Macklem PT, Proctor DF, Hogg JC. The stability of peripheral airways. Respir. Physiol 1970;8:191-203.
4. Lachmann B. Possible function of bronchial surfactant. Eur J Respir Dis 1985;67:49-61
5. Rensch H, von Seefeld H. Surfactant - mucus interaction. In: Robertson B., van Golde LMG, Batenburg JJ (eds). Pulmonary Surfactant. Elsevier, Amsterdam, 1984;203-214.
6. Reinfenrath R. Surfactant action in bronchial mucus. In: Cosmi EV, Scarpelli EM (eds) Pulmonary Surfactant System. Elsevier, Amsterdam, 1983;339-347.
7. Guyton AC, Moffatt DS, Adair TA. Role of alveolar surface tension in transepithelial movement

of fluid. In: Robertson B, Van Golde LMG, Batenburg JJ. (eds.) Pulmonary Surfactant, Elsevier, Amsterdam, 1980;171-185

8. Lachmann B. Surfactant replacement. Appl Cardiopulm Pathophysiol 1989; 3:3-11.

9. Clements JA, Hustead RE, Johnson RP, Gribnetz I. Pulmonary surface tension and alveolar stability. J Appl Physiol 1961;16:444-450

10. Gruenwald PA. A numerical index of the stability of lung expansion. J Appl Physiol 1963;18:665-667.

11. Rüfer R. Der Einfluß oberflächenaktiver Substanzen auf Entfaltung und Retraktion isolierter Lungen. Pflügers Arch ges Physiol 1967;298:170-184.

12. Evander E, Wollmer P, Jonson B, Lachmann B. Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. J Appl Physiol 1987;62: 1611-1614

13. Wollmer P, Evander E, Jonson B. Assessment of surfactant function by measurement of the pulmonary clearance of inhaled ^{99m}Tc-DTPA. In: Lachmann B (ed.) Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome. Berlin-Heidelberg-New York: Springer-Verlag, 1989a; 66-76.

14. Wollmer P, Evander E, Jonson B, Lachmann B. Pulmonary clearance of inhaled ^{99m}Tc-DTPA and surfactant function: In: Ekelund L, Jonson B, Malm L (eds.). Surfactant and the Respiratory Tract: Amsterdam/New York/Oxford, Elsevier Science Publishers, 1989b;97-102

15. Evander E, Wollmer P, Jonson B. Pulmonary clearance of ^{99m}Tc-DTPA: effects of ventilatory pattern. Clin Physiol 1990;10:199-209.

16. Faridy EE, Permutt S, Riley RL. Effect of ventilation on surfactant forces in excised dogs' lungs. J Appl Physiol 1966;21:1453-1462

17. Faridy EE, Effect of distension on release of surfactant in excised dogs' lungs. Respir. Physiol. 1976a; 27:99-114

18. Forrest JB. The effect of hyperventilation on pulmonary surface activity. Brit J Anaesth 1972; 44:313-319

19. Greenfield LJ, Ebert PA, Benson DW. Effect of positive pressure ventilation on surface tension properties of lung extracts. Anaesthesia 1964;25:312-316

20. McClenahan JB, Urtnowski A. Effect of ventilation on surfactant and its turnover rate. J Appl Physiol 1967; 23:215-220

21. Mead J, Collier C. Relation of volume history of lungs to respiratory mechanics in anesthetised dogs. J Appl Physiol 1959; 14:669-678

22. Thet LA, Clerch L, Massaro GD, Massaro D. Changes in sedimentation of surfactant in ventilated excised rat lungs. J Clin Invest 1979;64:600-608.

23. Thet LA, Alvarez H. Effect of hyperventilation and starvation on rat lung mechanics and surfactant. Am Rev Respir Dis 1982; 126:286-290.

24. Webb H, Tierney DF. Experimental pulmonary oedema due to intermittent positive pressure ventilation with high inflation pressures. Protection by positive end-expiratory pressure. Am Rev Respir Dis 1974;110:556-565.

25. Wyszogrodski I, Kyei-Aboagye K, Taeusch Jr HW, Avery ME. Surfactant inactivation by hyperventilation: conservation by end-expiratory pressure. J Appl Physiol 1975;38:461-466

26. Bowton DL, Kong DL. High tidal volume ventilation produces increased lung water in oleic acid-injured rabbit lungs. Crit Care Med 1989; 17:908-911

27. Dreyfuss D, Basset G, Soler P, Saumon G. Intermittent positive-pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. Am Rev Respir Dis 1985; 132:880-884

28. Hernandez LA, Peevy KJ, Moise AA, Parker JC. Chest wall restriction limits high airway pressure-induced lung injury in young rabbits. J Appl Physiol 1989; 66:2364-2368.

29. Kolobow T, Moretti MP, Fumagalli R, Mascheroni D, Prato P, Chen V, Joris M. Severe impairment in lung function induced by high peak airway pressure during mechanical ventilation. Am Rev Respir Dis 1987; 135:312-315

30. Mascheroni D, Kolobow T, Fumagalli R, Moretti MP, Chen V, Buckhold D. Acute respiratory failure following pharmacologically induced hyperventilation: an experimental animal study. Intensive Care Med 1988; 15:8-14

31. Nicholas TE and Barr HA. The release of surfactant in rat lung by brief periods of hyper-ventilation. Respir Physiol 1983; 52:69-83.

32. Peevy KJ, Hernandez LA, Moise AA, Parker JC. Barotrauma and microvascular injury in lungs of nonadult rabbits: effects of ventilation pattern. Crit Care Med 1990; 18:634-637

33. Suter PM, Fairley HB, Isenberg MD. Effect of tidal volume and positive end-expiratory pressure on compliance during mechanical ventilation. Chest 1978: 73:158-162

34. Thornton D, Ponhold H, Butler J, Morgan T and Cheney FW. Effects of pattern of ventilation on pulmonary metabolism and mechanics. Anesthesiology 1975; 42:4-10.

35. Oyarzùn MJ, Clements JA. Ventilatory and cholinergic control of pulmonary surfactant in the rabbit. J Appl Physiol: Respirat Environ Exercise Physiol 1977;43:39-45

36. Oyarzun MJ, Clements JA, Baritussio A. Ventilation enhances pulmonary alveolar clearance of radioactive dipalmitoyl phosphatidylcholine in liposomes. Am Rev Respir Dis 1980;121:709-721.

37. Benzer H. Respiratorbeatmung und Oberflächenspannung in der Lunge. In: Frey R, Kern F, Mayrhofer O. (eds). Anaesthesiologie und Wiederbelebung, 38. Berlin-Heidelberg-New York: Springer-Verlag. 1969.

38. Ennema JJ, Reijngoud DJ, Wildevuur ChRH, Egberts J. Effects of artificial ventilation on surfactant phospholipid metabolism in rabbits. Respir Physiol 1984a;58:15-28

39. Wauer R, Lachmann B. Die Ventilette, ein Notfallbeatmungsgerät für Neugeborene und Säuglinge - ein Erprobungsbericht. Pädiatrie und Grenzgebiete. 1972;11:411-422.

40. Argiras EP, Blakeley CR, Dunnill MS, Otremski S, Sykes MK. High PEEP decreases hyaline membrane formation in surfactant deficient lungs. Br J Anaesth 1987; 59:1278-1285.

41. Caldini P, Leith JD, Brennan MJ. Effect of continuous positive-pressure ventilation (CPPV) on oedema formation in dog lung. J Appl Physiol 1975; 39:672-679.

42. Demling RH, Staub NC, Edmuns jr LH. Effect of end-expiratory airway pressure on accumulation of extravascular lung water. J Appl Physiol 1975; 38:907-912.

43. Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary oedema. Respective effects of high airway pressure, high tidal volume, and positive end-expiratory pressure. Am Rev Respir Dis 1988; 137:1159-1164

44. Hopewell PC, Murray JF. Effects of continuous positive-pressure ventilation in experimental pulmonary oedema. J Appl Physiol 1976; 40:568-574

45. Toung TJK, Saharia P, Mitzner P, Permutt S, Cameron JL. The beneficial and harmful effects of positive end expiratory pressure. Surg Gynecol Obstetr 1978; 147:518-524

46. Sandhar BK, Niblett DJ, Argiras EP, Dunnill MS, Sykes MK. Effects of positive end-expiratory pressure on hyaline membrane formation in a rabbit model of the neonatal respiratory distress syndrome. Intensive Care Med 1988; 14:538-546

47. Perna AM, Brawley RK, Bender HW, Gott VL. Fatal respiratory distress syndrome after prolonged mechanical ventilation: its pathogenesis and prevention. J Surg Res 1971; 11:584-589.

48. Lachmann B, Freyse EJ, Grossmann G, Robertson B. Lung-thorax compliance in the artificially ventilated premature rabbit neonate in relation to variations in inspiration:expiration ratio. Pediatr Res 1981; 15:833-838.

49. Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe distress syndrome. Crit Care Med 1982; 10:724-732.

50. Berman LS, Downs JB, van Eeden A, Delhagen D. Inspiration:expiration ratio. Is mean airway pressure the difference? Crit Care Med 1981; 9:775-777.

51. Boros SJ. Variations in inspiratory:expiratory ratio and airway pressure wave form during mechanical ventilation: the significance of mean airway pressure. Pediatrics 1979; 94:114-117.

52. Gurevitch MJ, van Dyke J, Young ES, Jackson K. Improved oxygenation and lower peak airway pressure in severe adult respiratory distress syndrome. Chest 1986; 89:211-213.

53. Herman S, Reynolds EOR. Methods for improving oxygenation in infants mechanically ventilated for severe hyaline membrane disease. Arch Dis Child 1973; 48:612-617.

54. Lain DC, DiBenedetto R, Morris SL, Nguyen AV, Saulters R, Causey D. Pressure control inverse ratio ventilation as a method to reduce paek inspiratory pressure and provide adequate

ventilation and oxygenation. Chest 1989; 95:1081-1088.

55. Reynolds EOR. Effect of alterations in mechanical ventilator settings on pulmonary gas exchange in hyaline membrane disease. Arch Dis Child 1971; 46:152-159.

56. Spahr RC, Klein AM, Brown DR, MacDonald HM, Holzmann SR. Hyaline membrane disease. A controlled study of inspiratory to expiratory ratio in its management by ventilator. Am J Dis Child 1980; 134:373-376.

57. Faridy EE. Effect of ventilation on movement of surfactant in airways. Respir Physiol 1976b; 27:323-334

58. Hildebran JN, Goerke J, Clements JA. Surfactant release in excised rat lung is stimulated by air inflation. J Appl Physiol: Respirat Environ Exercise Physiol 1981;51:905-910.

59. Massaro GD, Massaro D. Morphologic evidence that large inflations of the lung stimulate secretion of surfactant. Am Rev Respir Dis 1983;127:235-236

60. Balsys AJ, Jones RL, Man SFP, Wells A. Effects of sighs and different tidal volumes on compliance, functional residual capacity and arterial oxygen tension in normal and hypoxemic dogs. Crit Care Med 1980; 8:641-645

61. Egan EA. Lung inflation, lung solute permeability, and alveolar oedema. J Appl Physiol: Respirat Environ Exercise Physiol 1982; 53:121-125

62. Egan EA. Response of alveolar epithelial solute permeability to changes in lung inflation. J Appl Physiol: Respirat Environ Exercise Physiol 1980; 49:1032-1036

63. Egbert LD, Laver MB, Bendixen HH. Intermittent deep breathing and compliance during anesthesia in man. Anesthesiology 1963; 24:57-60

64. Ferris BG, Pollard DS. Effect of deep and quiet breathing on pulmonary compliance in man. J Clin Invest 1960; 39:611-614

65. Housley E, Louzada N, Becklake MR. To sigh or not to sigh. Am Rev Respir Dis 1969; 101:611-614

66. Kim KJ, Crandall ED. Effects of lung inflation on alveolar epithelial solute and water transport properties. J Appl Physiol: Respirat Environ Exercise Physiol 1982; 52:1498-1502

67. Laver MB, Morgan J, Bendixen HH, Radford EP. Lung volume, compliance, and arterial oxygen tensions during controlled ventilation. J Appl Physiol 1964; 19:725-733

68. Novak RA, Shumaker L, Snyder JV, Pinsky MR. Do periodic hyperinflations improve gas exchange in patients with hypoxemic respiratory failure? Crit Care Med 1987; 15:1081-1085

69. Levine M, Gilbert R, Auchincloss JH. A comparison of the effects of sighs, large tidal volumes, and positive end expiratory pressure in assisted ventilation. Scand J Resp Dis 1972; 53:101-108

70. Delemos RA, Coalson JJ, Gerstmann DR, Null DM, Akerman NB, Escobedo MB, Robotham JL, Kuehl TJ. Ventilatory management of infant baboons with hyaline membrane disease: the use

of high frequency ventilation. Pediatr Res 1987;21:594-602

71. Ennema JJ, Reijngoud DJ, Egberts J, Mook PH, Wildevuur ChRH. High-frequency oscillation affects surfactant phospholipid metabolism in rabbits. Respir Physiol 1984b;58:29-39.

72. Man GCW, Ahmed IH, Logus JW, Man SFP. High-frequency oscillatory ventilation increases canine epithelial permeability. J Appl Physiol 1987;63:1871-1876

73. McCulloch PR, Forkert PG, Froese AB. Lung volume maintenance prevents lung injury during high frequency oscillatory ventilation in surfactant-deficient rabbits. Am Rev Respir Dis 1988;137:1185-1192

74. Obara H, Maekawa N, Hamatani S, Iwai S. Effect of combined high-frequency oscillatory ventilation on rabbit alveolar type II cells. Crit Care Med 1987;15 :692-695

75. Solimano A, Bryan C, Jobe A, Ikegami M, Jacobs H. Effects of high-frequency and conventional ventilation on the premature lamb lung. J Appl Physiol 1985;59: 1571-1577

76. Bos JAH, Schairer W, Schaffers JT, Tenbrinck R, Tenhave-Opbroek AAW, Bakker WH, Wollmer P and Lachmann B. Effect of high frequency jet ventilation (HFJV) on the pulmonary clearance of 99mTc-DTPA in respiratory failure in rabbits. Brit J Anaesth 1989;63:59S-64S

77. Frantz III ID, Stark AR, Davis JM, Davies P, Kitzmiller TJ. High frequency ventilation does not affect pulmonary surfactant, liquid, or morphological features in normal cats. Am Rev Respir Dis 1982;126:909-913

78. Barzilay E, Lev A, Ibrahim M, Lesmes C. Traumatic respiratory insufficiency: comparison of conventional mechanical ventilation to high-frequency positive pressure with low-rate ventilation. Crit Care Med 1987; 15: 118-121.

79. Bell RE, Kuehl TJ, Coalson JJ, Ackermen NB, Null DM, Escobedo MB, Yoder BA, Cornish JD, Nalle L, Skarin RM, Cipriani CA, Montes M, Robotham JL, DeLemos RA. High-frequency ventilation compared to conventional positive-pressure ventilation in the treatment of hyaline membrane disease in primates. Crit Care Med 1984; 12:764-768.

80. Eyal FG, Hayek Z, Armengol J, Jones RL. Comparison of high-frequency negative-pressure oscillation with conventional mechanical ventilation in normal and saline-lavaged cats. Crit Care Med 1986; 14:724-729.

81. Gallagher TJ, Boyse PG, Davidson DD, Miller JR, Leven SB. High-frequency percussive ventilation compared with conventional mechanical ventilation. Crit Care Med 1989; 17:364-366.

82. The HIFI study group. High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants. N Engl J Med 1989; 320:88-93.

83. Jibelian G, Lachmann B. Gas exchange during conventional and high-frequency pulse ventilation in the surfactant-deficient lung: influence of positive end-expiratory pressure. Crit

Care Med 1984; 12:769-773.

84. Kolton M, Cattran CB, Kent G, Volgyesi G, Froese A, Bryan AC. Oxygenation during high-frequency ventilation compared with conventional mechanical ventilation in two models of lung injury. Anesth Analg 1982; 61:323-332.

85. Kudoh I, Soga T, Numata K. Effect of high-frequency ventilation on extra-vascular lung water volume in dogs. Crit Care Med 1987; 15:240-242.

86. Kumar BS, Beney K, Jastremski, Nieman G, Bredenberg C. High-frequency jet ventilation versus conventional ventilation after surfactant displacement in dogs. Crit Care Med 1984; 12:738-741.

87. Mansel JK, Gillespie DJ. Oxygenation during ventilation by high-frequency oscillation in dogs with acute lung injury. Crit Care Med 1986; 14:955-959.

88. Mautone AJ, Condorelli S, Scarpelli EM. Ventilation and pulmonary mechanics during high-frequency oscillation of immatture lungs at birth. Crit Care Med 1987; 15:233-239.

89. Murray IP, Mikhail MS, Banner MJ, Modell JH. Pulmonary embolism: high-frequency jet ventilation offers advantages over conventional mechanical ventilation. Crit Care Med 1987; 15:114-117.

90. Quan SF, Militzer HW, Calkins JM, Sobony RE, Waterson CK, Otto CW, Conahan TJ. Comparison of high-frequency jet ventilation with conventional mechanical ventilation in saline-lavaged rabbits. Crit Care Med 1984; 12:759-763.

91. Rehder K, Schmid ER, Knopp TJ. Long-term high-frequency ventilation in dogs. Am Rev Respir Dis 1983; 128:476-480.

92. Walsh MC, Carlo WA. Sustained inflation during HFOV improves pulmonary mechanics and oxygenation. J Appl Physiol 1988; 65:368-372.

93. Tyler DC. Positive end-expiratory pressure: a review. Crit Care Med 1983; 11:300-308.

94. Bendixen HH, Hedley-Whyte J, Laver MB. Impaired oxygenation in surgical patients during general anesthesia with controlled ventilation. N Engl J Med 1963; 269:991-996.

95. Nilsson R, Grossmann G, Robertson B. Lung surfactant and the pathogenesis of neonatal bronchiolar lesions induced by artificial ventilation. Pediat Res 1978;12:249-255.

96. Finlay-Jones J-M, Papadimitriou JM and Barter RA. Pulmonary hyaline membrane: light and electron microscopic study of the early stage. J Path 1974; 112:117-124.

97. Nilsson R, Grossmann G and Robertson B. Bronchiolar epithelial lesions induced in the premature rabbit neonate by short periods of artificial ventilation. Acta Path Microbiol Scand Sect A 1980; 88:359-367.

98. Seeger W, Stohr G, Wolf HR, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. J Appl Physiol 1985; 58:326-338.

99. Bredenberg CE. Surface tension and alveolar changes during ventilation. In: Prakash O, ed.

Applied physiology in clinical respiratory care. The Hague: Martinus Nijhoff, 1982;191-206.

100. Lachmann B. Surfactant replacement in acute respiratory failure: animal studies and first clinical trials. In: Lachmann B, ed. Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Berlin Heidelberg: Springer-Verlag, 1988;212-223.

101. Holm BA, Matalon S, Notter RH. Pulmonary surfactant effects and replacements in oxygen toxicity and other ARDS-type lung injuries. In: Lachmann B, ed. Surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Berlin Heidelberg: Springer-Verlag, 1988; 224-244.

102. Clements JA. Pulmonary oedema and permeability of alveolar membranes. Archs Envir Hlth 1961; 2:104-107.

103. Albert RK, Lakshminarayan S, Hildebrandt J, Kirk W, Butler J. Increased surface tension favors pulmonary oedema formation in anesthetised dogs' lungs. J Clin Invest 1979; 63:1015-1018.

104. Nieman GF, Bredenberg CE. Pulmonary oedema induced by high alveolar surface tension. Prog Resp Res 1984; 18:204-207.

105. Guyton AC, Moffatt DS, Adair TA. The role of alveolar surface tension in transepithelial movement of fluid. In: Robertson B, van Golde LMG, Batenburg JJ, eds. Pulmonary surfactant. Amsterdam: Elsevier, 1984: 171-185.

CHAPTER 3

EFFECTS OF HIGH FREQUENCY JET VENTILATION (HFJV) ON THE PULMONARY CLEARANCE OF ^{99m}Tc-DTPA IN RESPIRATORY FAILURE IN RABBITS

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EFFECTS OF HIGH FREQUENCY JET VENTILATION ON THE PULMONARY CLEARANCE OF ^{99m}Tc-DTPA IN RESPIRATORY FAILURE IN RABBITS

SUMMARY

The effects of high frequency jet ventilation (HFJV) at 2 and 15 Hz on the pulmonary clearance of technetium 99m diethylene triamine pentaacetate (^{99m}Tc-DTPA) were compared with conventional volume-controlled (VC) ventilation with positive end-expiratory pressure (PEEP), in a model of respiratory failure induced by two lung lavages in adult rabbits. In group 1 the lungs were ventilated with HFJV at 2 Hz, and in group 2 ventilation was with HFJV at 15 Hz; group 3 underwent VC conventional ventilation. Group 4 also had conventional ventilation, but without previous lung lavage and functioned as a control group. In all groups, mean airway pressure was maintained at a value to ensure PaO_2 > 25 kPa. The measured half-life time (T½) of the ^{99m}Tc-DTPA (mean (SD)) was: group 1, 28 (7.8) min; group 2, 73.5 (7.9) min; group 3, 56.5 (12.4) min and group 4, 92.6 (13.2) min. Assuming that conventional VC with PEEP ventilation causes no additional harm to surfactant depleted lungs, it is concluded that HFJV at 2 Hz leads to further damage of the lungs, whereas HFJV at 15 Hz improves reparative processes, by keeping the lungs constantly inflated.

INTRODUCTION

High frequency jet ventilation (HFJV) is known to deliver continuous positive airway pressure (CPAP) and opens atelectatic lung units. Adequate oxygenation and elimination of carbon dioxide may be maintained with tidal volumes less than the anatomical dead space in animals and patients with respiratory failure [1, 2]. There is evidence that damage to the alveolar-capillary membrane is a consequence of large intra-pulmonary pressure-volume excursions [3]. Especially repeated closing and reopening of the alveoli may lead to damage of the alveolar-capillary membrane because of high shear forces [4, 5]. It is assumed that HFJV reduces pulmonary damage by keeping the lung inflated and avoiding shear forces. The amplitudes of the pressure excursions during HFJV are known to be inversely related to the

frequency used. It is possible, therefore, that HFJV at low frequency may lead to more lung damage than HFJV at high frequency.

A sensitive index of the integrity of the alveolar-capillary barrier is the pulmonary clearance of inhaled ^{99m}technetium-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) [6]. The purpose of this study was to assess the effect of HFJV on this clearance rate in a model of respiratory failure induced by depletion of surfactant. The pulmonary clearance of ^{99m}Tc-DTPA was measured, therefore, in surfactant depleted rabbits during conventional mechanical ventilation and during HFJV at two different frequencies. Structural abnormalities of the lung were examined histologically.

MATERIALS AND METHODS

The experiments were performed in 24 adult rabbits (2.5-3.5 kg body weight) allocated to four groups. The animals were anaesthetised with pentobarbitone 50-60 mg kg^{-1}. Tracheotomy was performed and a tube for applying high frequency ventilation (Hi-Lo jet, Mallinkrodt, U.S.A.) was inserted into the trachea. A carotid artery was cannulated for continuous monitoring of arterial pressure and blood sampling. Neuromuscular block was induced with pancuronium 0.3-0.4 mg kg^{-1} i.m. All animals underwent ventilation with a Servo Ventilator 900 C (Siemens-Elema AB, Sweden). Initially, the ventilation frequency (*f*) was 30 b.p.m, The inspiratory/ expiratory (I:E) ratio was 1:2, FiO_2 was 1.0 and minute ventilation was set to maintain $PaCO_2$ at 4-5 kPa.

Respiratory failure was induced in 17 animals by performing lung lavage [7, 8]. In brief, lung lavage was performed with volumes of isotonic saline at 37 °C equal to the gas volumes needed to inflate the healthy lungs to 40 cm H_2O. Each volume of saline was administered in two instillations through the tube at a pressure not exceeding 40 cm H_2O [7]. Two such lavages were performed. After induction of respiratory failure, the animals were allocated to three groups with different patterns of ventilation. In all three groups, the mean airway pressure (MAP) was adjusted so

that PaO_2 would exceed 25 kPa.

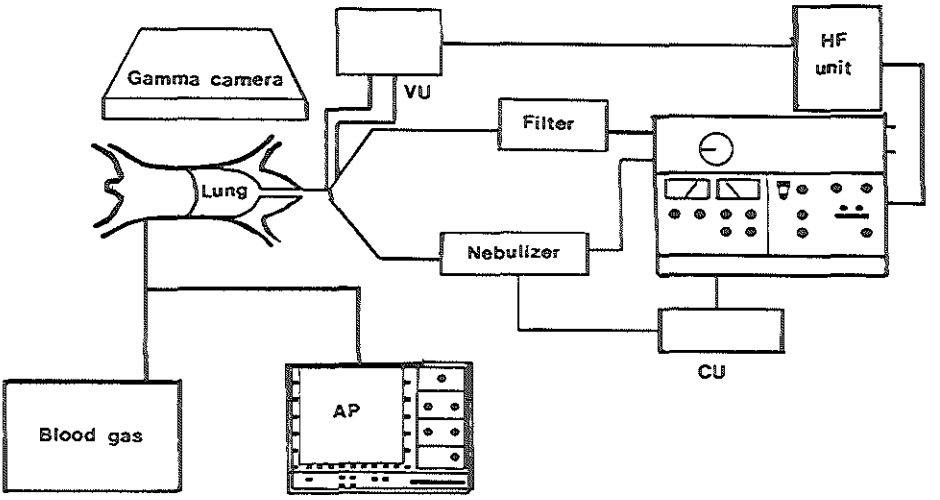

Fig. 1 Schematic representation of the experimental apparatus. VU = valve unit; AP = arterial pressure; CU = control unit.

In group 1 (n = 7) ventilation comprised HFJV at 2 Hz and in group 2 (n = 5) it was HFJV at 15 Hz. The duty cycle was 20% in both groups. HFJV was applied by a high frequency jet ventilator (Servo HFV 970, Siemens-Elema AB, Sweden) via the high frequency channel of the tube, whereas the main lumen was open. Group 3 (n = 5) underwent ventilation with conventional volume controlled (VC) ventilation (tidal volume 20 ml kg^{-1}, I:E ratio 1:2 and *f* 30 b.p.m.) with a positive end-expiratory pressure (PEEP) of 0.3-0.4 kPa. A control group of animals (group 4, n = 7) underwent ventilation with VC PEEP ventilation without surfactant depletion.

The patterns of ventilation were applied for 60 min. A solution of ^{99m}Tc-DTPA was nebulized into the inspiratory line of the ventilator using an air jet nebulizer (Ultravent, Mallinkrodt Medical, The Netherlands). This type of nebulizer produces an aerosol with fine particles (0.6-2μm), favouring alveolar deposition. The supply of pressurized air to the nebulizer was controlled by a pneumatic valve which was connected to the

Servo Ventilator via an electronic circuit. The nebulizer operated only during expiration, filling the inspiratory line with aerosol in order to administer the particles with the ensuing insufflation. Any particles not retained in the lungs of the animal were trapped in a filter placed in the expiratory line of the ventilation circuit (fig. 1).

Before each run of the nebulizer, the system was checked carefully for air leaks. The nebulizer and the filter were protected with heavy lead shielding. During nebulizing, in all groups pressure controlled ventilation was used with an insufflation pressure of 2 kPa, an I:E ratio of 1:1 and a frequency of 100 b.p.m. in order to reach a high activity in a short time. When a count rate of approximately 200 counts per second (c.p.s.) over the lungs was reached (after 2-3 min), the aerosol administration was stopped, the experimental ventilation mode resumed and the clearance measurement started. Gamma camera images were obtained in successive 1-min frames for 20 min and stored in a 64 x 64 images matrix in a computer (Digital PDP 11/34, Maynard, U.S.A.). The data from the clearance measurement were analysed by selecting a region of interest, which was both lungs in all animals, and generating a time-activity curve. A mono-exponential function was fitted to the experimental data and a half-life (T½) of the tracer in the lungs calculated.

When the clearance measurement was completed, the animal was killed with an overdose of pentobarbitone and the lungs were prepared for histological examination. Before fixation, the lungs were expanded with air for 5 min at a pressure of 2.94 kPa. This pressure was reduced to 0.98 kPa, which was maintained while the lungs were perfused for 60 min via the pulmonary artery with a solution of buffered 1% glutaraldehyde at a pressure of 6.37 kPa. The lungs were stored in 3.5% formaldehyde. Staining was with haematoxylin and afloxin. The sections were examined by light microscopy.

Statistical analysis

All data are reported as mean (SD), unless stated otherwise. Differences between the means were tested by the Mann-Whitney-Wilcoxon test for unpaired samples.

RESULTS

The distribution of the inhaled ^{99m}Tc-DTPA aerosol was uniform in the lungs of all animals. The T½ of ^{99m}Tc-DTPA in the lungs in the different groups of animals are shown in table I. The reference group had the longest T½, 93 (13) min. In group 3, which was lavaged but had similar ventilation to the control group, the T½ was reduced significantly, to 57 (12) min. The animals undergoing ventilation with HFJV at 2 Hz (group 1) had the shortest T½ 28 (7.8) min, whereas group 2 (15 Hz) had the longest T½ of the lavaged animals (73.5 (7.9) min), although this was not significantly greater than that of group 3.

Table I Mean (SD) PaO_2 and $PaCO_2$, mean airway pressure (Pawp), mean arterial pressure (MAP) and half-life (T½) in the different groups. Significant differences ($P<0.05$, Mann-Whitney-Wilcoxon test for unpaired samples): # groups 1 v. 3, 1 v. 4, 2 v. 4, 3 v. 4; ǂ all groups comparisons; * groups 1 v. 2, 1 v. 3, 1 v. 4, 2 v. 4, 3 v. 4

	n	#PaO_2 (kPa)	ǂ$PaCO_2$ (kPa)	*Pawp (kPa)	MAP (kPa)	T½ (min)
Group 1 (2 Hz)	7	28.16 (18.04)	2.12 (0.57)	1.49 (0.39)	13.56 (1.41)	28 (7.8)
Group 2 (15 Hz)	5	38.64 (13.07)	6.90 (1.53)	0.81 (0.1)	14.88 (1.32)	73.5 (7.9)
Group 3 (0.5 Hz)	5	55.73 (6.57)	4.50 (0.61)	0.79 (0.1)	14.07 (1.14)	57 (12)
Group 4 (0.5 Hz, no lavage)	7	70.20 (4.70)	4.20 (0.6)	0.38 (0.02)	14.90 (1.47)	93 (13)

PaO_2 greater than 25 kPa could be maintained in the lavaged animals with all patterns of ventilation. Conventional VC PEEP ventilation (group 3) provided the best oxygenation and kept $PaCO_2$ within a normal range with the lowest mean airway pressure (P_{awp}) (table I). P_{awp} was greatest in group 1; this was necessary to obtain a PaO_2 greater than 25 kPa and led to hyperventilation (see $PaCO_2$). In group 2, however, the opposite results were found; for adequate oxygenation, alveolar ventilation was too small to maintain the $PaCO_2$ in the physiological range (table I).

Arterial pressure was stable at a normal value throughout the study in all groups. Under light microscopy, all lung samples from the lavaged animals showed evidence of pneumonitis, pulmonary oedema, bronchial and bronchiolar epithelial desquamation and necrosis as described previously [7]. The degree of pneumonitis, pulmonary oedema, bronchial and bronchiolar epithelial damage varied between the three groups (figs 2-4).

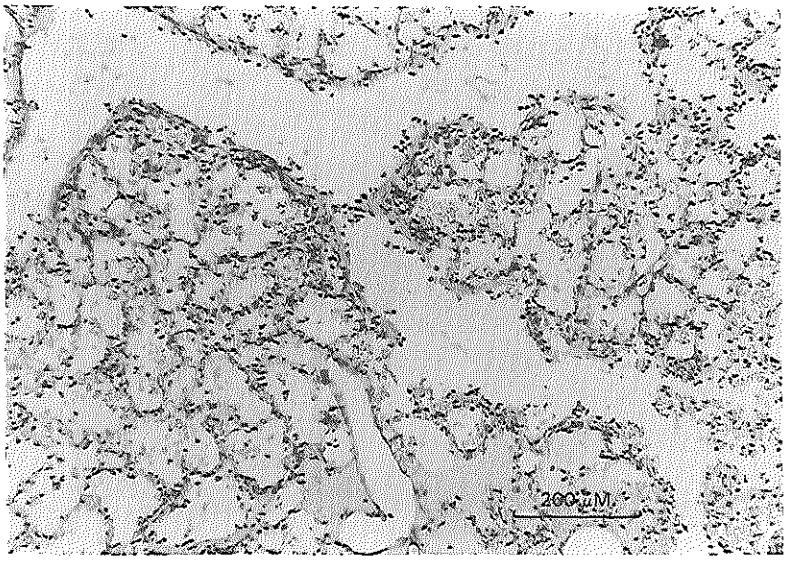

Fig. 2 Representative example of a rabbit in group 1. There is necrosis and desquamation of bronchiolar epithelium, pulmonary oedema and alveolar collapse. Haematoxylin and afloxin.

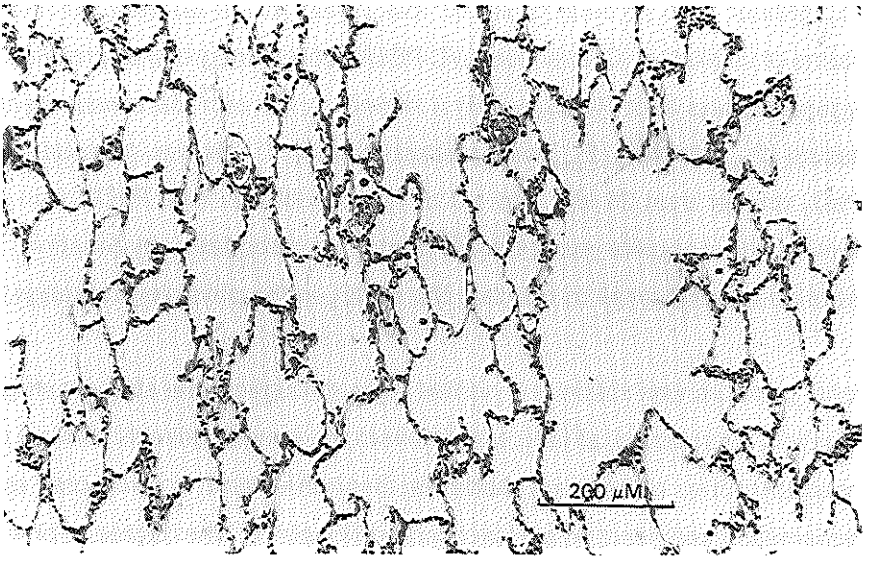

Fig. 3 Representative example of a rabbit in group 2. The lungs are well inflated. The alveoli are equally distributed. This is very similar to unlavaged lungs. Haematoxylin and afloxin.

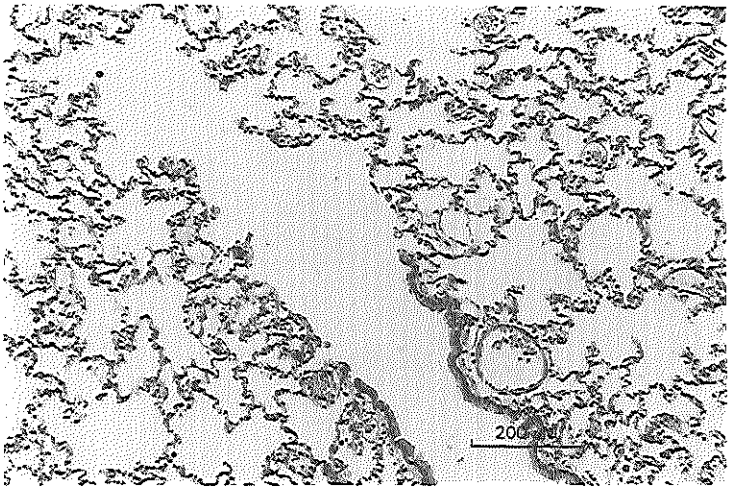

Fig. 4 Representative example of a rabbit in group 3. The lungs are unequally ventilated with areas of good ventilation and areas of collapsed alveoli. The histological examination showed further damage to the alveolar epithelium which was, in degree, between the damage shown in the lungs of groups 1 and 2. Haematoxylin and afloxin.

In group 1, the lungs showed widespread atelectasis, alternating with areas of well-expanded or even hyper-expanded alveoli. Necrosis and desquamation of airway epithelium were striking findings, especially in poorly expanded areas, combined usually with accumulation of oedema fluid in bronchioles and alveoli, and formation of hyaline membranes. The lungs showed minor foci of non-specific pneumonia. Also, small areas of interstitial and intra-alveolar haemorrhage were found. These histological results were confirmed by the measurements of the clearance rate of ^{99m}Tc-DTPA (table I) in this group. A typical example of this group is shown in figure 5.

The histological changes were less marked in group 3 and least obvious in group 2 results which were also confirmed by the clearance rate measurements of ^{99m}Tc-DTPA. Typical examples of these groups are shown in figures 6 and 7. In group 4 the epithelium was intact in all airways and no evidence of pneumonitis was found.

DISCUSSION

Surfactant depletion by lung lavage leads to increased alveolar surface tension and reduced lung compliance. The lavage procedure *per se* is not considered generally to cause substantial structural damage to the lung [8], but mechanical ventilation of the poorly compliant lung may cause severe structural damage [7]. The purpose of this study was to evaluate two modes of HFJV in the surfactant-depleted lung with respect to alveolar-capillary barrier integrity and structural changes. Conventional VC PEEP ventilation was included for comparison.

The pulmonary clearance of ^{99m}Tc-DTPA reflects the transfer of the tracer from the alveoli to blood and hence the properties of the alveolar-capillary barrier. The rate of transfer of ^{99m}Tc-DTPA is often interpreted as representing the solute permeability of the alveolar epithelium [6, 9] but it has been suggested recently that it is also dependent on the functional integrity of the pulmonary surfactant system [10-13]. In normal humans and animals, the rate of pulmonary clearance of ^{99m}Tc-DTPA is

related to lung volume [14, 15]. The rate of clearance of ^{99m}Tc-DTPA increases if lung volume is increased by voluntary hyperinflation or application of PEEP. No information on lung volume was obtained in this study, but the MAP may be used to obtain an estimate of lung expansion, even if this is not accurate [16]. Increased rate of ^{99m}Tc-DTPA in the lavaged animals in this study may be caused by surfactant depletion *per se*, by derangement of the alveolar-capillary structure related to the effects of mechanical ventilation of the surfactant-depleted lung, or by differences in the patterns of ventilation. As the animals underwent ventilation for 1 h after lavage, the effect of mechanical ventilation was not limited to causing lung injury, but also provided the possibility of restoration of lung function.

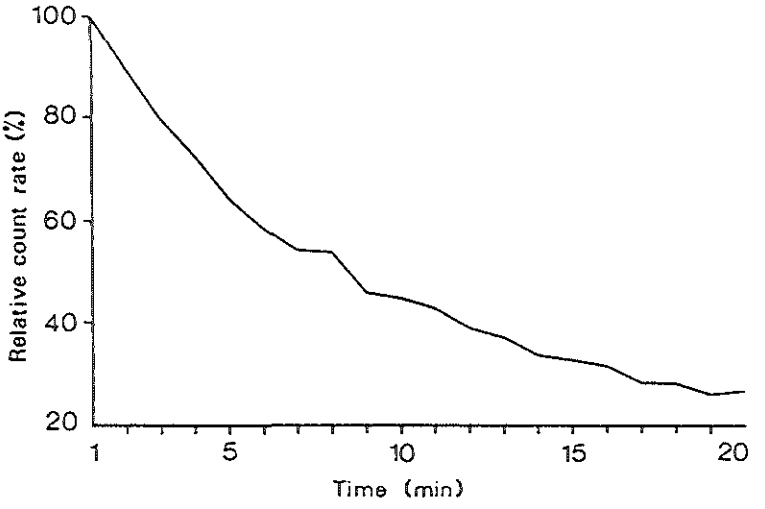

Fig. 5 Clearance curve of ^{99m}Tc-DTPA of rabbit lungs shown in figure 2. The clearance half-life (T½) in this animal with severe lung damage was 10.5 min.

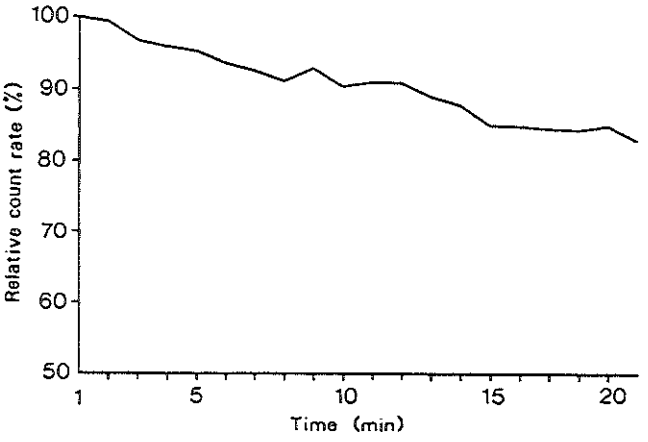

Fig. 6 Clearance curve of ^{99m}Tc-DTPA of rabbit lung shown in figure 3. The clearance half-life (T½) in this animal was 68.5 min.

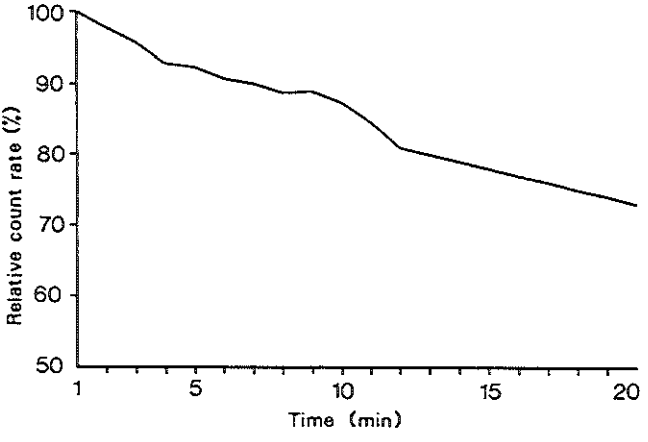

Fig. 7 Clearance curve of ^{99m}Tc-DTPA of lung of rabbit shown in figure 4. The clearance half-life (T½) in this animal was 42.2 min.

The difference in pulmonary clearance of ^{99m}Tc-DTPA between groups 3 and 4 illustrates the effects of surfactant depletion and possible structural lesions caused by VC mechanical ventilation [14]. HFJV at 2 Hz caused the fastest clearance of ^{99m}Tc-DTPA which may indicate that this mode of ventilation caused more structural damage to the alveolar-capillary unit than conventional VC PEEP ventilation or HFJV at 15 Hz. This is confirmed by our histological data. As the MAP was highest in group 1, increased lung volume may also have contributed to the increased rate of ^{99m}Tc-DTPA clearance. The animals undergoing ventilation with HFJV at 15 Hz (group 2) had the lowest rate of clearance of the lavaged animals, even though the difference from the conventional VC ventilation was not significant. The finding of a lower clearance rate in group 2 than in group 3 may indicate that HFJV at 15 Hz caused less structural damage to the lung or provided better conditions for restoration of lung morphology (or both) than conventional VC ventilation.

REFERENCES

1. Froese AB, Bryan AC. High frequency ventilation, state of the art. *American Review of Respiratory Disease* 1987; 136: 1363-1374.
2. Rouby JJ, Simonneau G, Benhamou D, Sartene R, Sardnal F, Deriaz H, Duroux P, Viars P. Factors influencing pulmonary volumes and CO_2 elimination during High Frequency Jet Ventilation. *Anesthesiology* 1985; 63: 473-482.
3. Hamilton PP, Onayemi A, Smyth JA, Gillan JE, Cutz E, Froese AB, Bryan AC. Comparison of conventional and high-frequency ventilation: Oxygenation and lung pathology. *Journal of Applied Physiology* 1983; 55: 131-138.
4. Lachmann B, Danzmann E, Haendly B, Jonson B. Ventilator settings and gas exchange in Respiratory Distress Syndrome. In: Prakash O, ed. *Applied Physiology in Clinical Respiratory Care.* The Hague, Boston, London: Martinus Nijhoff, 1982; 141-176.
5. Hughes JMB, Hoppin FG, Mead J. Effect of lung inflation on bronchial length and diameter in excised lungs. *Journal of Applied Physiology* 1972; 32: 25-35.
6. Barrowcliffe MP, Jones JG. Solute permeability of the alveolar capillary barrier. *Thorax* 1987; 42: 1-10.
7. Lachmann B, Jonson B, Lindroth M, Robertson B. Modes of artificial ventilation in severe respiratory distress syndrome. *Critical Care Medicine* 1982; 10: 724-732.
8. Lachmann B, Robertson B, Vogel J. In-vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiologica Scandinavica* 1980; 24: 231-236.

9. Jones JG, Royston D, Minty BD. Changes in alveolar-capillary barrier function in animals and humans. *American Review of Respiratory Disease* 1983; 127: 51-58.

10. Wollmer P, Evander E, Jonson B. Assessment of surfactant function by measurement of the pulmonary clearance of inhaled ^{99m}Tc-DTPA. In: Lachmann B, ed. *Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome.* Rotterdam: Springer-Verlag, 1989; 66-76.

11. Wollmer P, Evander E, Jonson B, Lachmann B. Pulmonary clearance of inhaled Tc-99m-DTPA and surfactant function. In: Ekelund L, Jonson B, Malm L, eds. *Surfactant and the Respiratory tract.* Amsterdam, New York, Oxford: Elsevier Science Publishers, 1989; 97-102.

12. Evander E, Wollmer P, Jonson B, Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. *Journal of Applied Physiology* 1987; 62: 1611-1614.

13. Jefferies AL, Kawano T, Mori S, Burger R. Effect of increased surface tension and assisted ventilation on ^{99m}Tc-DTPA clearance. *Journal of Applied Physiology* 1988; 64: 562-568.

14. Marks JD, Luce JM, Lazar NM, Wu JNS, Lipavsky A, Murray JF. Effect of increases in lung volume on clearance of aerosolised solute from human lungs. *Journal of Applied Physiology* 1985; 59: 1242-1248.

15. Cooper JA, Van der Zee H, Line BR, Malik AB. Relationship of end-expiratory pressure, lung volume and ^{99m}Tc-DTPA clearance. *Journal of Applied Physiology* 1987; 63: 1586-1590.

16. Simon BA, Weinmann GG, Mitzner W. Mean airway pressure during high frequency ventilation. *Journal of Applied Physiology* 1984; 58: 1069-1078.

CHAPTER 4

PULMONARY CLEARANCE OF ^{99m}Tc-DTPA AND EXPERIMENTALLY INCREASED ALVEOLAR SURFACTANT CONTENT IN RABBITS

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PULMONARY CLEARANCE OF ^{99m}Tc-DTPA AND EXPERIMENTALLY INCREASED ALVEOLAR SURFACTANT CONTENT IN RABBITS

SUMMARY

We measured clearance of ^{99m}Tc-DTPA in rabbits with experimentally increased alveolar surfactant content. In one group of animals, surfactant production was increased by treatment with ambroxol, another group was treated with tracheal instillation of natural surfactant. A group of untreated control animals and animals with instillation of saline was also studied. Clearance of ^{99m}Tc-DTPA was measured during standard conditions of mechanical ventilation and during ventilation with large tidal volumes. In ambroxol and surfactant treated groups, clearance rate was reduced compared to untreated control animals. In contrast, clearance rate increased after saline instillation. The differences were observed at both modes of ventilation. The findings indicate that the pulmonary surfactant system is a rate-limiting factor for clearance of ^{99m}Tc-DTPA and that the volume dependence of clearance is not explained by stretching of the alveolar wall only.

INTRODUCTION

The alveolar-capillary barrier allows free diffusion of gases but restricts the diffusion of water and solutes. The permeability of the alveolar-capillary barrier to small solutes can be studied by measurement of the pulmonary clearance of ^{99m}Technetium-labeled diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) [6, 13, 15, 27]. The radioactive tracer is administered as an aerosol and, subsequently, the radioactivity is measured over the lungs with a detector. A clearance curve is obtained, which reflects the transfer of the tracer across the alveolar-capillary barrier.

^{99m}Tc-DTPA is considered to be transported by simple diffusion via the paracellular route. Increased rate of clearance in disease states is often interpreted to represent damage to the alveolar epithelium but, recently, the pulmonary surfactant system as

a rate-limiting factor for the alveolar-capillary transfer of ^{99m}Tc-DTPA has gained increasing attention [5, 22, 24, 29]. The pulmonary clearance of ^{99m}Tc-DTPA increases dramatically after surfactant depletion by lung lavage [11] and after administration of synthetic detergent, which interferes with surfactant function [9, 12, 14]

The pulmonary clearance of inhaled ^{99m}Tc-DTPA is known to increase in response to increases in lung volume [1, 3, 10, 19, 21, 23, 25] e.g. by the application of positive end-expiratory pressure (PEEP) or by ventilation with large tidal volumes.

The purpose of this study was to examine in a rabbit model, whether increased alveolar surfactant content affects the pulmonary clearance of ^{99m}Tc-DTPA under standard conditions of mechanical ventilation and during ventilation with large tidal volumes.

MATERIAL AND METHODS

We studied two models of increased alveolar surfactant content; stimulation of surfactant production by treatment with ambroxol and tracheal instillation of natural surfactant. New Zealand White adult rabbits (CPB, Zeist, The Netherlands) were used in this study.

Experimental groups of animals:

A group of eight animals with a mean body weight (BW) of 3.3 ± 0.6 kg (SD) served as untreated controls.

A second group of eight rabbits with a mean BW of 3.2 ± 0.5 kg were injected intravenously with five doses (50 mg/kg per dose) of Ambroxol® (Mucosolvan, Dr Karl Thomae GmbH, Biberach an der Riss, FRG) with 12 h intervals, also prior to the clearance measurements.

A third group of five rabbits with a mean BW of 2.6 ± 0.1 kg were treated with

instillation of natural surfactant. The surfactant used in this study is a natural surfactant isolated from bovine lungs in basically the same manner as previously described by Metcalfe et al [20]. Each animal was given 100 mg surfactant phospholipids/kg BW suspended in 5 ml of 0.6 % saline. The surfactant preparation was instilled into the tracheal cannula while the animal was breathing spontaneously. Three ml was instilled with the animal in the supine position. Three minutes later, the animal was placed in the prone position and another 2 ml was instilled. The animal continued to breathe spontaneously for another 3 min before being connected to the ventilator.

A fourth group of four animals with a mean body weight of 2.4 ± 0.2 kg were treated with tracheal instillation of 5 ml of saline, in the same manner as for instillation of surfactant.

Animal preparation and ventilation:

The animals were anaesthetised with nembutal 50 mg/kg intravenously followed by additional doses as required to maintain anaesthesia. They were then tracheotomised and a neuromuscular block was induced with pancuronium bromide (0.3-0.4 mg kg^{-1} i.m.). The animals were connected to a Servo Ventilator 900C (Siemens-Elema AB, Solna, Sweden) and ventilated with pure oxygen. Ventilation was volume-controlled with an inspiratory time of 25% of the respiratory cycle. A ventilation frequency (f) of 50 min^{-1} was maintained during preparation and administration of the ^{99m}Tc-DTPA aerosol and for the first 20 min of the clearance measurement. The ventilation frequency was then changed to 20 min^{-1}, which was maintained for the following 20 min of the clearance measurement. The end-tidal partial pressure for CO_2 (P_ECO_2) was monitored with a CO_2 analyser 930 (Siemens-Elema AB) and inspiratory pressure was adjusted to keep P_ECO_2 within a physiological range. A positive end-expiratory pressure (PEEP) of 2 cm H_2O was applied to prevent atelectasis. Peak and mean airway pressures as well as expired tidal volume were recorded from the pressure and flow transducers located in the Servo Ventilator. A carotid artery was cannulated

for blood pressure monitoring.

Aerosol generation and administration:

A solution of ^{99m}Tc-DTPA was prepared from a commercial kit (Technescan DTPA, Mallinckrodt Medical, Petten, The Netherlands) and put in an air jet nebulizer (Ultravent, Mallinckrodt Medical). The mass median diameter of the aerosol particles produced was 1.7 μm as measured with a laser light scattering technique. The aerosol was administered via the ventilation circuit as described by Dahlbäck et al [4]. The nebulizer was placed in the inspiratory line of the ventilation circuit. Supply of pressurized air to the nebulizer was controlled by a pneumatic valve which, in turn, was connected to the Servo Ventilator via an electronic circuit. During the period of aerosol administration, the nebulizer was operating during expiration only, filling the tubing in the inspiratory line with aerosol. The particles thus produced were administered with the ensuing insufflation. Aerosol was administered until a count rate of approximately 300 counts/s had been reached (after 1-2 min). The clearance measurement was then immediately started. Gamma camera images were obtained in one-minute frames for 40 min and stored in a 64 x 64 image matrix in a computer.

Experimental protocol:

All animals quickly reached a stable condition after the preparation as well as after the instillation of surfactant and saline. The ^{99m}Tc-DTPA aerosol was administered with the initial mode of ventilation (ventilation frequency 50 min^{-1}). The clearance measurement was then immediately started. The initial pattern of ventilation was maintained for the first 20 min of the clearance measurement. The ventilation frequency was then changed to 20 min^{-1} and the inspiratory pressure increased with the intention of keeping P_ECO_2 in the physiological range. This pattern of ventilation was maintained for the remaining 20 min of the clearance measurement. In one animal treated with surfactant instillation, clearance was measured during 20 min only. Airway pressures and tidal volume were recorded at each pattern of ventilation. When the clearance measurement was completed, the animal was killed with an

overdose of pentobarbital.

Data analysis:

We analysed the clearance measurements by selecting a region of interest over both lungs and generating a time-activity curve (Fig. 1). Mono-exponential functions were then fitted to the experimental data from 1-20 min and from 21-40 min and the half-life (T½) time of the tracer in the lungs calculated. The correlation coefficient for the fit ranged from 0.92 to 0.99.

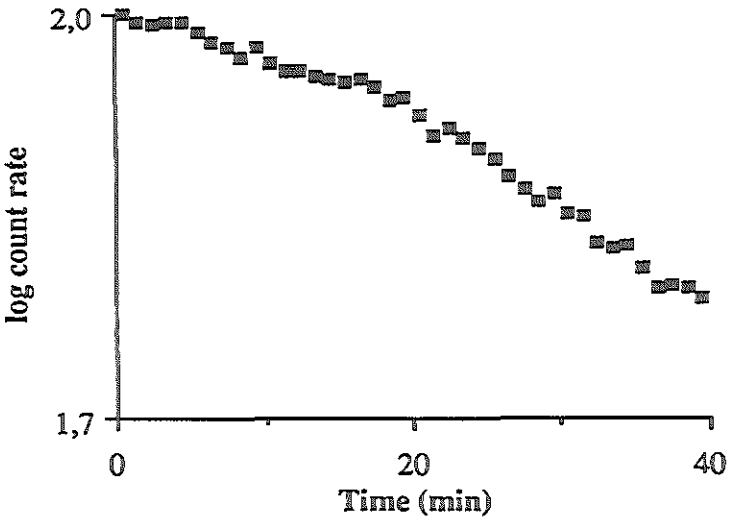

Fig. 1 Time-activity curve obtained over the lungs of a control animal. Count rate was expressed as a percentage of the initial value. A ventilation frequency of 50 min^{-1} was maintained for the first 20 min. The frequency was then changed to 20 min^{-1} and the inspiratory pressure increased. Clearance rate increases in response to the change in ventilation pattern.

Differences in P_ECO_2 and T½ with respect to treatment and pattern of ventilation were analysed with analysis of variance using an additive model for the factors experimental group, animals within group and ventilation frequency.

RESULTS

All animals could be kept under stable respiratory and circulatory conditions throughout the experiment. There was no significant difference in P_ECO_2 between the experimental groups, but P_ECO_2 was somewhat lower during ventilation with a frequency of 20 min^{-1} than during ventilation with 50 min^{-1} ($p<0.05$, Table 1). The tidal volume was about twice as high during ventilation with a frequency of 20 min^{-1} than during ventilation with a frequency of 50 min^{-1}. Tidal volume as well as airway pressures were slightly higher in the animals treated with surfactant or saline than in the other groups.

Table 1 Ventilatory parameters registered during measurement of pulmonary clearance of ^{99m}Tc-DTPA in ambroxol treated animals and in the control group. Values are means ± SEM. P_ECO_2, end tidal partial pressure for carbon dioxide; P_{peak}, peak airway pressure; P_{mean}, mean airway pressure; V_T, tidal volume.

	P_ECO_2 (kPa)	P_{peak} (cm H_2O)	P_{mean} (cm H_2O)	V_T (ml/kg)
f=50 min^{-1}				
Controls	4.2 ± 0.2	10.5 ± 0.4	3.9 ± 0.1	9.0 ± 0.6
Ambroxol	4.0 ± 0.1	10.0 ± 0.4	4.0 ± 0.1	8.5 ± 0.3
Surfactant	5.0 ± 0.6	13.8 ± 1.0	4.2 ± 0.2	11.1 ± 0.8
Saline	3.8 ± 0.1	13.2 ± 1.1	4.3 ± 0.3	14.8 ± 1.0
f=20 min^{-1}				
Controls	3.8 ± 0.2	14.3 ± 0.4	5.3 ± 0.1	20.9 ± 1.4
Ambroxol	3.7 ± 0.1	14.9 ± 0.8	5.3 ± 0.1	20.3 ± 1.0
Surfactant	4.2 ± 0.4	17.6 ± 0.6	6.1 ± 0.1	24.6 ± 0.5
Saline	4.0 ± 0.2	15.8 ± 1.1	6.0 ± 0.2	24.5 ± 0.4

The mean (± SEM) T½ for the pulmonary clearance of ^{99m}Tc-DTPA in the control group was 90 ± 5 min at f=50 min^{-1} and fell to 69 ± 7 at f=20 min^{-1} (Fig. 2). The T½ was longer in the ambroxol treated animals at both ventilation frequencies, mean values being 132 ± 9 and 102 ± 9 min. The results were similar in the animals treated with surfactant instillation, the T½ being 134 ± 13 min at f=50 min^{-1} and 125

± 15 at f=20 min^{-1}. In the animals treated with saline instillation, however, the T½ was shorter than in the controls at both ventilation frequencies; 61 ± 4 min at f=50 min^{-1} and 56 ± 4 min at f=20 min^{-1}. The analysis of variance showed significant differences between experimental groups ($p<0.001$) as well as between modes of ventilation ($p<0.001$).

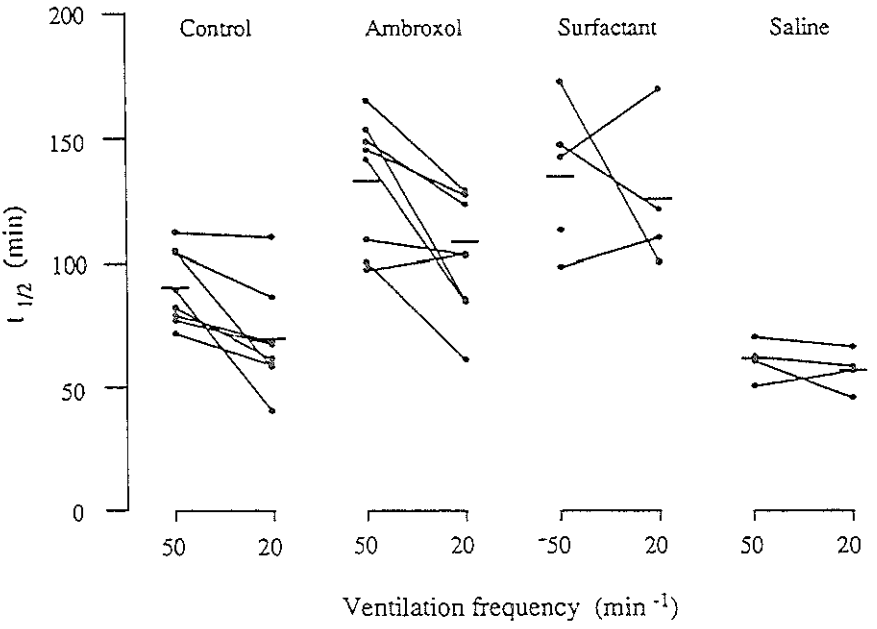

Fig. 2 Half-life of ^{99m}Tc-DTPA in the lungs in the different experimental groups at the two modes of ventilation. Closed symbols indicate individual animals and horizontal lines mean values.

DISCUSSION

We have shown that ambroxol treatment and surfactant instillation reduce the rate of pulmonary clearance of ^{99m}Tc-DTPA in normal rabbits. The reduced clearance rate is observed both under standard conditions of mechanical ventilation and during ventilation with large tidal volumes.

Ambroxol is known to increase the incorporation of palmitate into the phospholipid fraction of lung homogenates and of alveolar lavage fluid in rabbits and rats [7, 8, 28]. The amount of phospholipids recovered from the alveoli by lavage is also

increased in ambroxol treated rabbits and guinea pigs [16, 26]. In guinea pigs treated with the same dose of ambroxol as used in this study, the yield of phospholipids was increased by 60% [16]. Tracheal instillation of natural surfactant is known to increase alveolar phospholipid content and is frequently used in the treatment of idiopathic respiratory distress syndrome in newborns and also in a few cases of adult respiratory distress syndrome (ARDS) [17]. Both models used in this study thus represent experimentally induced states of increased alveolar surfactant content.

The rate of pulmonary clearance of ^{99m}Tc-DTPA increases with lung volume, and the conditions of mechanical ventilation therefore need to be carefully controlled. In this study, there were only trivial differences between experimental groups in the insufflation pressures used and the tidal volumes recorded. Increased alveolar surfactant content may affect lung compliance, i.e. the relation between distending pressure and volume. A previous study has shown that ambroxol in the dose used in this study does not affect lung mechanics in guinea pigs [16]. There is no indication in this study of any substantial difference in compliance of the respiratory system between the three groups of animals. Furthermore, the most likely effect of a moderate increase in alveolar surfactant content would be an increase in lung compliance, which would tend to increase lung volume at a given distending pressure. The reduction in the rate of pulmonary clearance of ^{99m}Tc-DTPA in the experimental groups relative to the control group is not likely to be explained by differences in lung volume.

The rate limiting factors for the diffusion of ^{99m}Tc-DTPA across the alveolar-capillary barrier are incompletely understood. The pulmonary surfactant system has been proposed as one rate limiting factor. This suggestion has largely been based on experimental derangement of surfactant function. Evander et al. [11] showed that the pulmonary clearance of ^{99m}Tc-DTPA increased after surfactant depletion by lung lavage, and that this effect was partly reversed by surfactant replacement after lavage. Evander et al. [9] and Jefferies et al. [14] used the synthetic detergent dioctyl

sodium sulfosuccinate to disturb surfactant function and found that the pulmonary clearance of ^{99m}Tc-DTPA increased dramatically. Subsequently, Evander et al. [12] also demonstrated a relation between the dose of synthetic detergent administered and the effect on clearance. The present finding of reduced rate of pulmonary clearance of ^{99m}Tc-DTPA in two experimental models of increased alveolar surfactant content provides further evidence that the pulmonary surfactant system is a rate limiting factor for the alveolar-capillary transfer of ^{99m}Tc-DTPA. Although the two models of increased alveolar surfactant content are different, the changes observed in pulmonary clearance of ^{99m}Tc-DTPA are remarkably similar.

Effros [5] has reviewed several mechanisms whereby surfactant could influence the pulmonary clearance of ^{99m}Tc-DTPA. Surfactant could affect the spreading of the tracer in the epithelial lining fluid. If permeability varies within the alveolus, e.g. is greater in the corners, this would affect the clearance rate. If excess alveolar surfactant increases the thickness of the epithelial lining fluid, this would reduce clearance rate. Tubular surfactant may obstruct interepithelial diffusion pathways and, finally, ^{99m}Tc-DTPA may bind to some component of surfactant.

It is well established that pulmonary clearance of ^{99m}Tc-DTPA increases with lung volume [1, 3, 10, 19, 21, 23, 25]. The mechanism is generally considered to be stretching of the alveolar epithelium with widening of intercellular junctions [1, 3, 19, 21, 25]. Increased alveolar volume has been suggested to reduce the thickness of the alveolar lining fluid and thereby increase clearance rate [5]. Ventilation with large tidal volume has, however, also been suggested to affect the surfactant system, probably by greater loss of surfactant into the airways [2, 10]. We found the clearance rate to be lower in the two experimental groups than in the control group at both modes of ventilation. The clearance rate was somewhat slower during ventilation with large tidal volumes in the treated groups than during ventilation with small tidal volumes in the control group. Assuming similar compliance of the respiratory system, the time weighted average of lung volume should be higher

during ventilation with large tidal volume than during ventilation with small tidal volume. The findings in this study therefore indicate that simple stretching of the alveolar wall is unlikely to be the only mechanism for increased pulmonary clearance of ^{99m}Tc-DTPA during ventilation with large tidal volumes.

We found the relative change in clearance rate in response to the change in ventilation pattern to be similar in ambroxol treated animals as in controls and only slightly lower in animals treated with surfactant instillation. If pulmonary surfactant were the dominating limiting factor for alveolar-capillary transfer of ^{99m}Tc-DTPA, the response to a change in ventilation pattern might be expected to be attenuated in the experimental groups of animals. It appears difficult to explain the volume dependence of the pulmonary clearance of ^{99m}Tc-DTPA on the basis of alveolar wall stretching, or effects on the pulmonary surfactant alone.

In animals treated with saline instillation, the T½ was lower than in any other group. Lung lavage is a well established model for inducing surfactant dysfunction and respiratory distress [18]. The present findings suggest that surfactant function may be compromised by introduction of as little as 5 ml of saline into the airways. This is supported by the finding that pulmonary clearance of ^{99m}Tc-DTPA may increase dramatically without any change in dynamic compliance of the respiratory system or arterial oxygenation when surfactant dysfunction is induced by detergent administration [9].

In summary, this study has shown that the rate of pulmonary clearance of ^{99m}Tc-DTPA is reduced in rabbits treated with ambroxol and in rabbits given natural surfactant relative to untreated controls. The difference applies to standard conditions of mechanical ventilation and to ventilation with large tidal volumes. The findings indicate that the pulmonary surfactant system is a rate limiting factor for the alveolar-capillary transfer of ^{99m}Tc-DTPA. The study also indicates that the volume dependence of the pulmonary clearance of ^{99m}Tc-DTPA is not explained by stretching of the

alveolar wall only.

REFERENCES

1. Barrowcliffe, M.P., G.D. Zanelli, and J.G. Jones. Pulmonary clearance of radiotracers after positive end-expiratory pressure or acute lung injury. *J. Appl. Physiol.* 66: 288-294, 1989.
2. Bos, J.A.H., and B. Lachmann. Effects of artificial ventilation on surfactant function. In: *Neue Aspekte zur respiratorischen Insuffizienz*, edited by E. Rügheimer. Berlin:Springer-Verlag, 1990, in press.
3. Cooper, J.A., H. van der Zee, B.R. Line, and A.B. Malik. Relationship of end-expiratory pressure, lung volume and ^{99m}Tc-DTPA clearance. *J. Appl. Physiol.* 63: 1586-1590, 1987.
4. Dahlbäck, M., P. Wollmer, B. Dregfeldt, and B. Jonson. Controlled aerosol delivery during mechanical ventilation. *J. Aerosol Med.* 2:339-347, 1989.
5. Effros, R.M. Permeability of blood-gas barrier. In: *The Lung: Scientific Foundations,* edited by R.G. Chrystal and J.B. West. New York: Raven Press; 1991, p. 1163-1176.
6. Effros R.M., and G.R. Mason. Measurements of pulmonary epithelail permeability in vivo. *Am Rev Respir Dis* 127:S59-S65, 1983.
7. Elemer, G., and Y. Kapanci. Effect of ambroxol on pneumocyte type II cell. A morphological and biochemical study. *Curr. Probl. Clin. Biochem.* 13:47-55, 1983.
8. Elemer, G., and Y. Kapanci. Incorporation of palmitate into pulmonary surfactant under influence of ambroxol. *Prax. Klin. Pneumol.* 37:723-724, 1983.
9. Evander, E., P. Wollmer, and B. Jonson. Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effect of the detergent dioctyl sodium sulfosuccinate in aerosol. *Clin. Physiol.* 8:105-111, 1988.
10. Evander, E., P. Wollmer, B. Jonson. Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of ventilation pattern. *Clin. Physiol.* 10:189-199, 1990.
11. Evander, E., P. Wollmer, B. Jonson, and B. Lachmann. Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. *J. Appl. Physiol.* 62:1611-1614, 1987.
12. Evander, E., P. Wollmer, S. Valind, L. Sörnmo, and B. Jonson. Biphasic pulmonary clearance of ^{99m}Tc-DTPA induced by detergent aerosol. Submitted for publication.
13. Huchon, G.J., J.W. Little, and J.F. Murray. Assessment of alveolar-capillary membrane permeability of dogs by aerosolization. *J. Appl. Physiol.* 51:955-962, 1981.
14. Jefferies, A.L., T. Kawano, S. Mori, and R. Burger. Effect of increased surface tension and assisted ventilation on ^{99m}Tc-DTPA. *J. Appl. Physiol.* 64:562-568, 1988.
15. Jones, J.G., B.D. Minty, and D. Royston. The physiology of leaky lungs. *Brit. J. Anaesth.* 54:705-721, 1982.
16. Lachmann, B. The effect of ambroxol in newborn and adult animals with surfactant deficiency.

In: *Pulmonary Surfactant System*, edited by E.V. Cosmi and E.M. Scarpelli. Amsterdam: Elsevier Science Publishers, 1983, p237-248.

17. Lachmann, B. Surfactant replacement. *Appl. Cardiopulm. Pathophysiol.* 3:3-11, 1989.

18. Lachmann, B., B. Robertson, and J. Vogel. In vivo lung lavage as experimental model of respiratory distress syndrome. *Acta Anaesthesiol. Scand.* 24:231-246, 1980.

19. Marks, J.D., J.M. Luce, N.M. Lazar, J.N.S. Wu, A. Lipavsky, J.F. Murray. Effect of increases in lung volume on clerance of aerosolised solute from human lungs. *J. Appl. Physiol.* 59:1242-1248, 1985.

20. Metcalfe, I.L., G. Enhorning, and F. Possmayer. Pulmonary surfactant-associated proteins: their role in the expression of surface activity. *J. Appl. Physiol.* 49:34-41. 1980.

21. Nolop, K.B., D.L. Maxwell, D. Roston, and J.M.B Hughes. Effect of raised thoracic pressure and volume on ^{99m}Tc-DTPA clearance in humans. *J. Appl. Physiol.* 60:1493-1497, 1986.

22. O'Brodovich, H., and G. Coates. Pulmonary clearance of ^{99m}Tc-DTPA: a noninvasive assessment of epithelial integrity. *Lung* 165:1-16, 1987.

23. O'Brodovich, H., G. Coates, M. Marrin. Effect of inspiratory resistance and PEEP on ^{99m}Tc-DTPA clearance. *J. Appl. Physiol.* 60:1461-1465, 1986.

24. Peterson, B.T. Pulmonary clearance of aerosolised ^{99m}Tc-DTPA and the lung epithelium. *J. Aerosol Med.* 2:315-328, 1989.

25. Peterson, B.T., H.L. James, and J.W. McLarty. Effects of lung volume on clearance of solutes from the air spaces of lungs. *J. Appl. Physiol.* 64:1068-1075, 1988.

26. Prevost, M.C., G. Soula, and L. Douste-Blazy. Biochemical modifications of pulmonary surfactant after bromhexine derivative injection. *Respiration* 37:215-219, 1979.

27. Rinderknecht, J., M. Krauthammer, J.M. Uszler, G. Taplin, and R.M. Effros. Solute transfer across the alveolar capillary membrane in pulmonary fibrosis. *Am Rev Respir Dis* 115:156, 1977.

28. Von Wichert, P., U. Bavendamm, M. von Teichmann, G. Muller, E. Thalheim, A. Wilke, and U. Wiegers. Increased incorporation of fatty acids into phospholipids of lungs and livers of rabbits under the influence of bromhexine and ambroxol. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 297:269-273, 1977.

29. Wollmer, P., E. Evander, and B. Jonson. Assessment of surfactant function by measurement of the pulmonary clearance of inhaled ^{99m}Tc-DTPA. In: *Surfactant Replacement Therapy in Neonatal and Adult Respiratory Distress Syndrome*, edited by B. Lachmann. Berlin:Springer-Verlag, 1988, p.66-76.

CHAPTER 5

PULMONARY ^{99m}Tc-DTPA CLEARANCE: A VERY EARLY INDEX FOR PERMEABILITY CHANGES IN THE ALVEOLAR-CAPILLARY BARRIER IN RABBITS

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PULMONARY ^{99m}Tc-DTPA CLEARANCE: A VERY EARLY INDEX FOR PERMEABILITY CHANGES IN THE ALVEOLAR-CAPILLARY BARRIER IN RABBITS

SUMMARY

A method for fast and accurate evaluation of the permeability of the alveolar-capillary membrane is by measurement of pulmonary clearance of radiolabeled hydrophylic solutes, such as Technetium-99m-diethylene triamine pentaacetate (^{99m}Tc-DTPA). Using this technique we report a study in rabbits which recovered from a mild respiratory insufficiency after a period of 140 min of mechanical ventilation with 100% O_2 (group 1). Respiratory insufficiency in group 1 was induced by two lung lavages. As controls we used animals which were mechanically ventilated with the same settings as group 1 but not lavaged (group 2), and animals which were neither ventilated nor lavaged (group 3). During mechanical ventilation for 140 min blood gas values of group 1 restored to normal compared to those of controls in groups 2 and 3. Pulmonary clearance, however, was still abnormally high in group 1 compared to the two other groups, indicating a greatly disturbed permeability of the alveolar-capillary barrier. It is concluded that pulmonary clearance of ^{99m}Tc-DTPA is a very sensitive technique for detection of any disturbance of the alveolar-capillary membrane.

INTRODUCTION

The adult respiratory distress syndrome (ARDS), as first described by Ashbaugh and colleagues more than 20 years ago [1], is a syndrome commonly found in intensive care units nowadays. Because of its complex pathogenesis and high mortality rate, it represents one of the major problems in modern intensive care medicine, both from the therapeutic and financial viewpoint. Although ARDS may have many different pathophysiological causes, the timely onset of treatment could be one of the most important factors influencing the final outcome [2]. The earlier therapeutical measures are taken, the better the prognosis for the patient. This means that early diagnosis of a possible developing ARDS is extremely important.

For normal function of the respiratory system, the integrity of the alveolar-capillary barrier is an important factor in that it normally allows rapid exchange of oxygen and carbon dioxide between the blood and alveolar air. It also restricts, however, the diffusion of large molecules, such as albumin and other blood proteins, from the lung capillaries into the alveoli. When the integrity of this barrier is altered, as in ARDS, permeability is increased allowing high permeability oedema to develop. This results not only in deterioration of blood gas tensions but also leads to inhibition of the pulmonary surfactant system by the presence of various plasma proteins [3, 4].

Surfactant is normally responsible for mechanical stabilisation of the alveoli and small airways during the ventilatory cycle and protects against formation of lung oedema [5, 6]. Thus an intact pulmonary surfactant system is vital for normal gas exchange. Being part of the alveolar-capillary barrier, it is obvious that functional integrity of the surfactant system also plays a role in maintaining normal permeability of this barrier.

An indication of the permeability of the alveolar-capillary barrier may be of major diagnostic and, possibly, therapeutic importance. A method for fast and accurate evaluation of the permeability of the alveolar-capillary membrane is by measurement of pulmonary clearance of radio-labelled hydrophylic solutes. A much-used solute is ^{99m}Technetium-labelled-diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) which allows non-invasive assessment of the integrity of the alveolar-capillary barrier [7, 8, 9].

When one part of the alveolar-capillary barrier is damaged as, for example, by removing the alveolar surfactant lining by lung lavage, blood gas tensions are known to deteriorate [10]. The purpose of this study was to compare the sensitivity of pulmonary clearance of ^{99m}Tc-DTPA with that of blood gas analysis to characterise mild respiratory insufficiency in rabbits under conditions of mechanical ventilation.

MATERIALS AND METHODS

Twenty-one New Zealand adult rabbits (CPB, Zeist, The Netherlands) (2.5 ± 0.5 kg body weight) were used. Permission was obtained from the institutional animal investigation committee. All animals were anaesthetised with pentobarbital (50-60 mg kg^{-1} i.v.) with additional doses, as required, to maintain anaesthesia. Following tracheotomy a carotid artery was cannulated for arterial blood pressure monitoring and blood sampling. Neuromuscular block was induced with pancuronium (0.3-0.4 mg kg^{-1} i.m.). All animals were ventilated using a Servo Ventilator 900 C (Siemens Elema AB, Solna, Sweden) with, initially, the following ventilator settings: frequency (f) was 30 b.p.m, the inspiratory:expiratory (I/E) ratio was maintained at 1:2, FiO_2 was 1.0 and minute ventilation was set to maintain $PaCO_2$ between 30-40 mm Hg.

Animals were divided into three groups (7 per group). In group 1, a very mild respiratory failure was induced by lung lavage according to Lachmann [10]. In brief, lavage was performed with 30 ml kg^{-1} of isotonic saline at 37°C equal to the gas volume needed to inflate the healthy lungs to about 40 cm H_2O. Each volume of saline was administered through the tube at a pressure not exceeding 40 cm H_2O. Two such lavages were performed. After induction of respiratory failure, group 1 was volume controlled ventilated with the following ventilator settings: tidal volume (V_T): 10-12 ml kg^{-1} and positive end-expiratory pressure (PEEP) of 3 cm H_2O. Group 2, with no lavage, was also ventilated for 2 h with the same settings as group 1 and served as healthy controls. To investigate any possible deleterious effects of the 2 h period of mechanical ventilation on the integrity of the alveolar-capillary barrier a third group was not lavaged and not ventilated for 2 h, and clearance measurements began immediately after preparation of these animals (group 3).

During the period of mechanical ventilation, blood gas tensions were measured (ABL-330, Radiometer, Copenhagen, Denmark) at 15, 60 and 140 min. At 120 min clearance measurements of ^{99m}Tc-DTPA started.

Clearance Measurements

A solution of ^{99m}Tc-DTPA, prepared from a commercial kit (Technescan DTPA, Mallinckrodt Medical, Petten, The Netherlands), was nebulized into the inspiratory line of the ventilator using an air jet nebulizer (Ultravent, Mallinckrodt Medical, The Netherlands). This type of nebulizer produces an aerosol with fine particles (mean size 1.7 μm, as measured by laser light scattering), favouring alveolar deposition [11]. The supply of pressurized air to the nebulizer was controlled by a pneumatic valve connected to the ventilator via an electronic circuit (for set-up see Fig. 1).

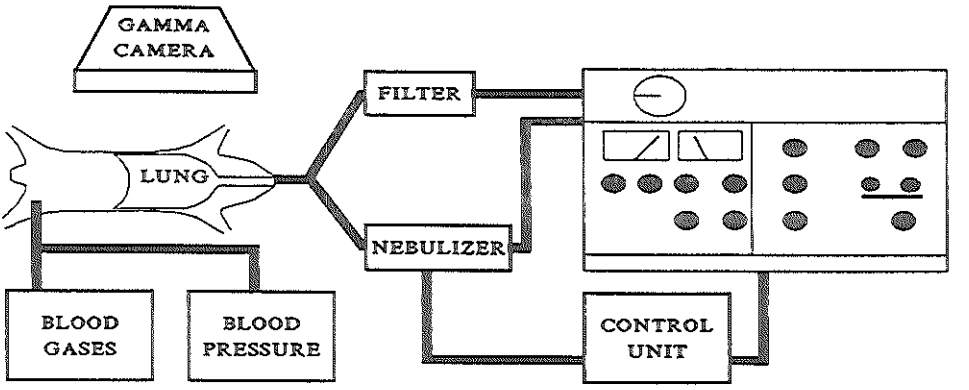

Fig. 1 Experimental set-up. In contrast to the schematic, blood was drawn from the carotid artery.

The nebulizer operated only during expiration, filling the inspiratory line of the ventilation circuit with particles; this in order to administer the particles with the ensuing insufflation. Any particles not retained in the lungs of the animal were trapped in a lead-shielded filter placed in the expiratory line of the ventilation line [12].

Before each run of the nebulizer, the ventilatory system was checked carefully for air leaks. During aerosol administration, all groups were pressure controlled ventilated with an insufflation pressure of 20 cm H_2O, an I/E ratio of 1:1 and a frequency of 30 b.p.m in order to reach a high activity over the lungs in a short time. When approximately 200-300 counts per second over the lungs was reached (after 2-3 min),

aerosol administration was stopped, the initially used mechanical ventilation was resumed and clearance measurement started. Gamma camera images were obtained in successive 1-min frames for 20 min and stored in a 64x64 images matrix in a computer (Digital PDP 11/34, Maynard, USA).

Data from the clearance measurements were analysed by selecting a region of interest, including both lungs of the animals, and generating a time-activity curve. A mono-exponential function was fitted to the experimental data and the half-life (T½) of the tracer in the lungs calculated. When the clearance measurement was completed, animals were sacrificed by an i.v. overdose of pentobarbital.

Statistical analysis

Values are given as mean ± SD. Differences between the means were tested by the Mann-Whitney-Wilcoxon test for unpaired samples. $P = < 0.05$ was considered to be statistically significant.

RESULTS

Table 1 P_aO_2 and P_aCO_2 values in mm Hg. Significant differences ($P<0.05$, Mann-Whitney-Wilcoxon test for unpaired samples): # group 1 versus groups 2 and 3. Values are mean ± SD.

Group	1		2		3	
	P_aO_2	P_aCO_2	P_aO_2	P_aCO_2	P_aO_2	P_aCO_2
Control	537	31	549	32.2	550	35
	± 18	± 4.9	± 13	± 6.3	± 16	± 5
Time (min)						
15	379#	33.2	557	33.4	564	36
	± 148	± 6.5	± 20	± 5.2	± 13	± 4
60	435#	34.1	576	38.4		
	± 180	± 5.7	± 34	± 3.4		
140	579	31.3	587	36.7		
	± 34	± 4.0	± 19	± 4.5		

Surfactant depletion by two lung lavages in group 1 resulted in a mild respiratory insufficiency characterised by a statistically significant decrease in PaO_2 (Table 1). During the course of the study period, blood gas values in these animals returned to normal and after 140 min there was no statistically significant difference between the three groups.

However, permeability of the alveolar-capillary barrier, as assessed by clearance rates of ^{99m}Tc-DTPA in the groups, showed a statistically significant difference. The calculated T½ of ^{99m}Tc-DTPA was: group 1, 19 ± 4.7 min; group 2 (no lavage, but ventilated for 2 h) 93 ± 13 min; and group 3 (no lavage and not ventilated for 2 h) 90 ± 15 min. The clearance curves of the three groups are shown in Figure 2. Arterial blood pressure remained stable in all animals of all groups during the entire study.

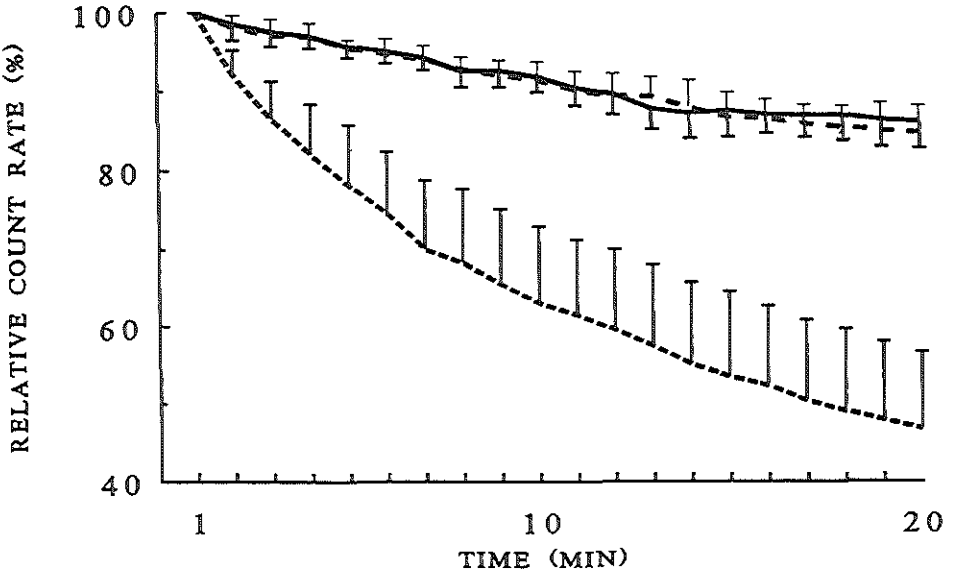

Fig. 2 Time-activity curves (means) of ^{99m}Tc-DTPA in animal lungs.
(dashed line: group 1; broken line: group 2; solid line: group 3)

DISCUSSION

The measurement technique for pulmonary clearance of ^{99m}Tc-DTPA is a relatively new, easy to perform, non-invasive method for assessment of the permeability of the alveolar-capillary barrier. This barrier, consisting of several structural layers, plays a major role in gas exchange in that it enables optimal conditions for the process of oxygen and carbon dioxide transport.

Although the technique has been well investigated over the last years, the mechanisms which influence or dictate the clearance rate of ^{99m}Tc-DTPA are not yet fully understood [for review see 9]. Most theories focus only on the integrity of the alveolar epithelium as the main factor of influence for the diffusion rate across the alveolar-capillary barrier. The mechanism behind this theory is that the hydrophilic solute molecule must pass through the aqueous pores between the alveolar epithelial cells, the so-called intercellular junctions. These pores have a median diameter of 0.6-1.0 nm. When compared to the median diameter of a ^{99m}Tc-DTPA molecule (± 0.57 nm) it is highly probable that the main route of diffusion of the tracer across the alveolar-capillary layer is through these pores.

Studies by Evander and Wollmer, however, show that not only the alveolar epithelium dictates the diffusion rate but also the pulmonary surfactant system [13, 14]. In a rabbit model they demonstrated that after removal of the surfactant layer in the alveoli by lung lavage, the clearance rate of ^{99m}Tc-DTPA across the alveolar-capillary membrane increases greatly. That this increase in clearance rate could not be attributed to damage of the alveolar epithelium by lavage was demonstrated by the fact that surfactant replacement restored the pulmonary clearance rate of ^{99m}Tc-DTPA to almost normal values. Another indicator of the importance of the surfactant system is the greatly increased pulmonary clearance rate in patients with ARDS and neonates with hyaline membrane disease [15, 16]. One established pathophysiological factor in both these syndromes is a significant surfactant deficiency with its concomitant results [17]. In experimental ARDS models, e.g., by infusion of intravenous oleic acid

or by prolonged ventilation with 100% oxygen resulting in oxygen toxicity, results also show an increased diffusion rate [18, 19]. The increased permeability of the alveolar-capillary barrier, as measured by the ^{99m}Tc-DTPA clearance technique, is also seen in other acute lung diseases e.g. pneumocystis carinii pneumonia [20]

In a study by Evander and coworkers to further investigate the possible rate-limiting influences of surfactant, a detergent (dioctyl sodium sulfosuccinate) was aerosolised and administered to the lungs of rabbits [21]. This detergent does not cause any structural damage to the alveolar epithelium, does not increase microvascular membrane permeability to macromolecules, and acts only on the surfactant system [22]. At 30 min after administration of the detergent, blood gas tensions and lung mechanics had not changed, but the clearance rate of ^{99m}Tc-DTPA from the lungs had increased greatly. The authors conclude that this effect must be due to the fact that the detergent acts on the surfactant layer, interfering with both function and integrity. That blood gas tensions did not change significantly, indicates that the detergent's interference with the surfactant layer was minimal and this underlines the high sensitivity of the clearance technique for permeability assessment of the alveolar-capillary barrier.

These findings provided the rationale for this investigation on the diagnostic sensitivity of ^{99m}Tc-DTPA clearance in a mild respiratory insufficiency. The pulmonary clearance measurements of ^{99m}Tc-DTPA in this study show that permeability of the alveolar-capillary barrier of group 1 which was partly depleted from the alveolar surfactant is abnormally high compared to groups 2 and 3. This difference in clearance of the tracer molecule cannot be attributed to the 2 h period of mechanical ventilation because no differences in clearance measurements exist between groups 2 (no lavage but ventilated for 2h) and 3 (no lavage, not ventilated for 2h). Thus, the greatly increased permeability of the alveolar-capillary barrier in group 1 can only be attributed to the lung lavage procedure in this group.

The procedure of lung lavage induces mild respiratory insufficiency by surfactant depletion which may lead to minor atelectasis, followed by intrapulmonary shunting and intrapulmonary fluid accumulation with subsequent deterioration of blood gas tensions [6]. Damage to the surfactant system in the whole lung, however, was so mild that, with the applied ventilator settings, blood gases of group 1 were restored to normal after 140 min. Moreover, that after 2 h of mechanical ventilation the mild respiratory insufficiency was restored to normal at least suggests that the pulmonary surfactant system was also normalised. However, the finding that the pulmonary clearance of ^{99m}Tc-DTPA is still abnormally high indicates that the surfactant system in group 1 has not yet fully restored to normal.

The proven sensitivity of pulmonary clearance measurement of ^{99m}Tc-DTPA enables to use this technique for detection of a damaged and more permeable alveolar-capillary barrier and, in particular, possible very early detection of a disturbed pulmonary surfactant system. Moreover, that alteration of therapeutic measures for lung diseases characterised by surfactant deficiency only on the basis of blood gas values can neglect the fact that normal blood gas values measured during mechanical ventilation (including PEEP) does not necessarily mean that the permeability of the alveolar-capillary barrier, and thus the integrity of the surfactant system, has also restored to normal. This finding indicates that additional care is needed in this area of lung diseases and that ^{99m}Tc-DTPA clearance measurements could have a role in assessment of the permeability of the alveolar-capillary barrier.

REFERENCES

1. Ashbaugh DG, Bigelow DB, Petty TL and Levine BE (1967) Acute respiratory distress in adults. Lancet 2: 319-323.
2. Wiener-Kronish JP, Gropper MA and Matthay MA (1990) The adult respiratory distress syndrome: definition and prognosis, pathogenesis and treatment. Brit J Anaesth 65: 107-129.
3. Holm BA, Notter RH, Finkelstein JN (1985) Surface property changes from interactions of albumin with natural surfactant and extracted lung lipids. Chem Phys Lipids 38: 287-298.

4. Seeger W, Stohr G, Wolf HRD, Neuhof H (1985) Alteration of surfactant function due to protein leakage: Special interaction with fibrin monomer. J Appl Physiol 58: 326-338.

5. Van Golde LMG, Batenburg JJ and Robertson B (1988) The pulmonary surfactant system: biochemical aspects and functional significance. Physiol Rev 68: 374-455.

6. Lachmann B (1987) The role of pulmonary surfactant in the pathogenesis and therapy of ARDS. In: Vincent JL (ed) Update in Intensive Care and Emergency Medicine, Berlin, Springer-Verlag, 123-134.

7. O'Brodovich H and Coates G (1987) Pulmonary clearance of ^{99m}Tc-DTPA: a noninvasive assessment of epithelial integrity. Lung 165: 1-16.

8. Barrowcliffe MP and Jones JG (1987) Solute permeability of the alveolar capillary barrier. Thorax 42: 1-10.

9. Hunter DN, Morgan CJ, Evans TW (1990) The use of radio-nuclide techniques in the assessment of the alveolar-capillary membrane permeability on the intensive care unit. Intens Care Med 16: 363-371.

10. Lachmann B, Robertson B and Vogel J (1980) In-vivo lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 24: 231-236.

11. Brain JD and Valberg PA (1979) Deposition of aerosol in the respiratory tract. Am Rev Respir Dis 120: 1325-1373.

12. Dahlbäck M, Wollmer P, Drefeldt B and Jonson B (1989) Controlled aerosol delivery during mechanical ventilation. J Aerosol Med 2: 339-347.

13. Evander E, Wollmer P, Jonson B and Lachmann B (1987) Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. J Appl Physiol 62: 1611-1614.

14. Wollmer P, Evander E, Jonson B and Lachmann B (1989) Pulmonary clearance of inhaled Tc-99m-DTPA and surfactant function. In: Surfactant and the Respiratory tract. Ekelund L, Jonson B and Malm L (eds). Elsevier Science Publishers, 97-102.

15. Jefferies AL, Coates G and O'Brodovich H (1985) Pulmonary epithelial permeability in hyaline membrane disease. N Engl J Med 311: 1075-1080.

16. Jones JG, Royston D and Minty BD (1983) Changes in alveolar-capillary barrier function in animals and humans. Am Rev Respir Dis 127: S51-S60.

17. Avery ME, Mead J (1959) Surface properties in relation to atelectasis and hyaline membrane disease. Am J Dis Child 97: 517-523.

18. Huchon GJ, Little JW and Murray JF (1981) Assessment of alveolar-capillary membrane permeability of dogs by aerosolization. J Appl Physiol: Respir Environ Exercise Physiol 51: 955-962.

19. Matalon S and Egan EA (1981) Effects of 100% O_2 breathing on permeability of alveolar epithelium to solute. J Appl Physiol: Respir Environ Exercise Physiol 50: 859-863.

20. Jones DK and Higenbottam TW (1985) Pneumocystis pneumonia increases the clearance rate of inhaled ^{99m}Tc-DTPA from lung to blood. Chest 88: 631-632.

21. Evander E, Wollmer P and Jonson B (1988) Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effect of the detergent dioctyl sodium sulfosuccinate in aerosol. Clin Physiol 8: 105-111.

22. Nieman GF, Bredenberg CE. (1985) High surface tension pulmonary edema induced by detergent aerosol. J Appl Physiol 58: 129-136.

CHAPTER 6

PULMONARY ^{99m}TC-HUMAN SERUM ALBUMIN CLEARANCE AND EFFECTS OF SURFACTANT REPLACEMENT AFTER LUNG LAVAGE IN RABBITS

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PULMONARY CLEARANCE OF ^{99m}Tc-HUMAN SERUM ALBUMIN AND EFFECTS OF SURFACTANT REPLACEMENT AFTER LUNG LAVAGE IN RABBITS

SUMMARY

Pulmonary clearance of technetium-labelled human serum albumin (^{99m}Tc-HSA) was measured in order to investigate whether the surfactant layer is also a rate-limiting factor when measuring the permeability of the alveolar-capillary membrane using ^{99m}Tc-labelled albumin. Three groups of rabbits were studied: group 1 received natural surfactant after lung lavage; group 2 underwent lung lavage only, whereas group 3 served as untreated, healthy controls. All animals of group 2 had bi-exponential clearance curves of the tracer, the half-life (T½) of the fast and slow compartments being 14.6 ± 6.7 min and 459.8 ± 167 min, respectively. In group 1, all animals except one also had bi-exponential clearance curves; T½ of the fast compartment being 35.9 ± 6.4 min and T½ of the slow compartment, 847.5 ± 143.5 min. The animals of group 3 had mono-exponential clearance curves with T½ of 618 ± 241 min; after applying high airway pressure ventilation, T½ decreased to 236 ± 121 min. These results indicate that the surfactant layer is also for ^{99m}Tc-HSA clearance measurements a rate-limiting factor. It is also concluded that the use of ^{99m}Tc-HSA does not provide many advantages above the use of ^{99m}Tc-DTPA as tracer molecule.

INTRODUCTION

Techniques using radio-labelled solutes for permeability measurements of the alveolar-capillary membrane have been a subject of extensive research over the last decade. Introduced by Rinderknecht and others [Rinderknecht et al, 1977; Huchon et al, 1981], most workers administer the tracer molecule directly to the lung and monitor the clearance rate of the tracer molecule from the lungs. The most commonly used tracer is technetium-99m-labelled diethylene triamine pentaacetate (^{99m}Tc-DTPA).

This hydrophillic solute (molecular weight 492, radius ± 0.6 nm) is thought to diffuse through the intercellular junctions between the alveolar epithelium. Although several factors are known to influence the clearance rate of ^{99m}Tc-DTPA across the alveolar-capillary barrier, the integrity of the pulmonary surfactant system seems to be one of the most important rate-limiting determinants [Evander et al, 1987, 1988]. Pulmonary clearance of ^{99m}Tc-DTPA has been shown to increase after inducing surfactant deficiency by lung lavage or hydrochloric instillation and to decrease after experimentally increasing alveolar surfactant content [Evander et al, 1987; Jefferies et al, 1984a; Bos et al, 1991a]. Clinical indications for the importance of the surfactant system for ^{99m}Tc-DTPA clearance rate have been shown in studies in humans: increased clearance rates of ^{99m}Tc-DTPA were observed in disease states where surfactant deficiency is a pathophysiological factor as, for instance, in hyaline membrane disease and the adult respiratory distress syndrome (ARDS) [Braude et al, 1986; Jefferies et al, 1984].

Pulmonary clearance rate measurements of ^{99m}Tc-DTPA have proven to be a very sensitive method for detecting alterations in the alveolar-capillary membrane whilst other more standard methods, such as blood gas tension measurements and lung mechanics, did not reflect any changes [Evander et al, 1988]. Some workers, however, have queried the sensitivity of the technique based on the fact that, even in healthy lungs, pulmonary clearance of ^{99m}Tc-DTPA can increase to levels found in severe ARDS by physiological factors, such as increase of lung volume, or even by cigarette smoking [Jones et al, 1980; Nolop et al, 1986; Cooper et al, 1987]. This led to the hypothesis that ^{99m}Tc-DTPA is a too small molecule to 1) allow discrimination between intermediate damage and severe damage to the membrane and 2) to allow discrimination between increase in permeability resulting from damage to the membrane and increase due to physiological factors. This provided the rationale for using radio-labelled solutes which have a greater molecular weight and radius as, for instance, human serum albumin (^{99m}Tc-HSA) (± 69,000 dalton, 3.5 nm [Egan et al, 1983]) so that influence on clearance from physiological factors, like lung volume,

would be largely eliminated.

The purpose of this study was to investigate whether the alveolar surfactant layer is also a rate-limiting factor in the pulmonary clearance of ^{99m}Tc-HSA and whether subsequent exogenous surfactant instillation after lung lavage would restore the rate of clearance to almost normal values, as is achieved with ^{99m}Tc-DTPA clearance rates.

MATERIALS AND METHODS

Twenty New Zealand adult rabbits (CPB, Zeist, The Netherlands) (2.5 ± 0.5 kg bodyweight) were used. Approval of the protocol was obtained from the institutional Animal Investigation Committee. The animals were anaesthetised with nembutal 50 mg kg^{-1} intravenously. Following tracheotomy, neuromuscular blockage was induced with pancuronium bromide 0.3-0.4 mg kg^{-1} i.m. A carotid artery was cannulated for blood gas measurements and blood pressure monitoring (Fig. 1).

Initially, all animals underwent pressure controlled ventilation using a Servo Ventilator 900 C (Siemens-Elema AB, Solna, Sweden). The ventilator settings were accordingly: frequency (*f*) of 30 min^{-1}, inspiratory/expiratory ratio of 1:2, FiO_2 was 1.0 and a positive end-expiratory pressure (PEEP) of 2 cm H_2O was applied in order to prevent atelectasis formation during preparation. The minute ventilation was set to keep $PaCO_2$ between 30 - 40 mm Hg. Peak, mean airway pressures and expired tidal volumes were recorded from the pressure and flow transducers located in the ServoVentilator 900 C.

Respiratory failure was induced in 13 animals by performing lung lavage [Lachmann et al, 1982]. In brief, lung lavage was performed with 30 ml kg^{-1} of isotonic saline at 37° C. Each volume of saline was administered through the tube at a pressure not exceeding 40 cm H_2O. The lungs were lavaged until PaO_2 fell below 100 mm Hg with the following ventilator settings: *f* was 30 min^{-1}, peak inspiratory airway pressure at

26 cm H_2O, FiO_2 of 1.0 and a PEEP of 6 cm H_2O. Animals were randomly assigned to group 1, 2 or 3. In group 1 (n=7) natural surfactant was instilled via the tracheal tube after the lavage procedure. Group 2 (n=6) received no surfactant. Another group of seven animals served as untreated, healthy controls (group 3). Group 3 was ventilated for 60 min according to the initial settings during preparation. After 60 min, the ventilator settings were changed to the same settings as in groups 1 and 2. This in order to exclude any possible effect of ventilation on clearance of the solute.

The surfactant used in this experiment is a natural surfactant isolated from bovine lungs in basically the same manner as previously described by Metcalfe and colleagues [Metcalfe et al, 1980]. Each animal was given 100 mg surfactant phospholipids kg^{-1} bw suspended in 0.6% saline (25 mg phospholipids ml^{-1} saline).

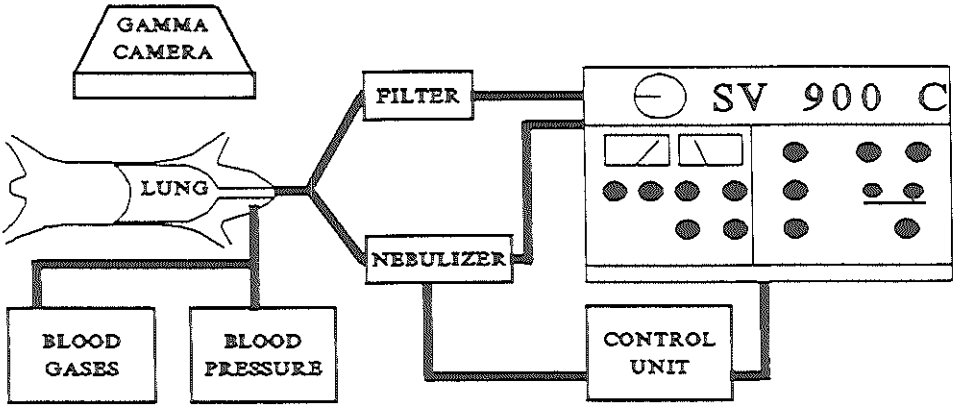

Fig. 1 Schematic representation of the experimental apparatus.

A solution of ^{99m}Tc-HSA was prepared using a commercial kit (Technescan DRN 4361, Mallinckrodt Diagnostica, Petten, The Netherlands) and placed into an air jet nebulizer (Ultravent, Mallinckrodt Diagnostica, Petten, The Netherlands). Before and after nebulizing, the binding percentage of the ^{99m}Tc-label with the HSA-molecule was tested using thin layer chromatography.

The aerosol was then administered via the ventilation circuit as earlier described by Dahlbäck [Dahlbäck et al, 1989]. In brief: the nebulizer was placed in the inspiratory line of the ventilation circuit. The supply of pressurized air to the nebulizer was controlled by a pneumatic valve which, in turn, was connected to the ServoVentilator via an electronic circuit. During the period of aerosol administration, the nebulizer was operating during expiration only, filling the tubing in the inspiratory line with aerosol. The particles thus produced were administered with the ensuing insufflation. Aerosol was administered until a count rate of approximately 300 counts/s had been reached (after 1-2 min). The clearance measurement was then immediately started. Gamma camera images were obtained in one-minute frames for 120 min and stored in a 64 x 64 image matrix in a computer (Digital PDP 11/34, Maynard, U.S.A.).

After 110 min, a small dose of ^{99m}Tc-HSA was injected intravenously in all animals in order to correct for blood background activity as described by Barrowcliffe and colleagues [Barrowcliffe et al, 1988].

During the study period, blood gas tensions were measured at 0, 30, 60, 90 and 120 min. When the clearance measurement was completed, the animal was killed with an overdose of nembutal. After the study period, lung fluid was collected (whenever possible) from the animals of groups 1 and 2 for total protein measurement.

Data analysis:

Clearance measurements were analysed by selecting a region of interest over both lungs and generating a time-activity curve (Fig. 2). Each corrected time-activity curve

was analysed by fitting a mono- (eq. 1) as well as a bi-exponential (eq. 2) equation to the experimental data.

$$A(t) = A(0)\, e^{-kt} \qquad \text{(eq. 1)}$$

$$A(t) = A_F(0)\, e^{-k(F)\, t} + A_S(0)\, e^{-k(S)\, t} \qquad \text{(eq. 2)}$$

A(t) is the radioactivity in the lung at any time t, A(0) the radioactivity in the lung at time zero and k the decay constant. In the bi-exponential analysis, $A_F(0)$ and $A_S(0)$ represent the amount of radioactivity eliminated with the fast (k_F) and slow (k_S) clearance components. Clearance was expressed as the half-life time (T½ - ln2/k) for the single clearance component in the mono-exponential and for the fast (T½ f) and slow (T½ s) clearance components in the bi-exponential analysis. The relative amount of tracer cleared by the fast clearance component (f_F) was calculated as $A_F(0)/(A_F(0) + A_S(0))$.

The desired quantities (i.e. A(0), k, $A_F(0)$, $A_S(0)$, k_F and k_S) were obtained by minimization of the sum of squares by using the so-called Nelder-Mead simplex method [Dennis and Woods, 1987; Nelder and Mead, 1965]. This is a method for multidimensional minimization which does not rely on the use of gradient information. Different initial values were used to investigate the convergence properties of the method; in this study, similar estimates were obtained for widely different initial values. A lower bound on the standard error for each parameter estimate was calculated on the basis of the Fisher information matrix. The F-test was used for detecting differences between the mono- and bi-exponential fits.
Mono-exponential functions were fitted to the experimental data of group 3 from 1-60 min and from 60-120 min and the T½ of the tracer in the lungs during this period was calculated.

Statistical analysis of the mean half-lives between the groups was performed using

the Mann-Whitney-Wilcoxon test for unpaired samples. Differences were considered statistically significant when $p<0.05$.

RESULTS

All animals could be kept under stable circulatory conditions throughout the experiment. The pulmonary distribution of ^{99m}Tc-HSA was uniform in all animals. There was a distinct difference in clearance of the tracer between the groups. The clearance curve of the controls (group 3) was monophasic of character with a statistically significant increase in clearance after applying - after 60 min - the same ventilator settings as used in groups 1 and 2 (Fig. 2). The measured half-lives were 618 ± 241 min and 236 ± 121 min, respectively. All clearance curves of ^{99m}Tc-HSA of the lavaged animals were of a bi-exponential character with bi-exponential curve fitting statistically significantly better than mono-exponential in all animals. The T½ of the fast compartment was 14.6 ± 6.6 min (mean ± SD) and of the slow compartment, 460 ± 167 min. The f_F value was 0.27 ± 0.06. Analysis of the surfactant treated animals of group 1 resulted in a T½ of the tracer for the fast compartment of 36.0 ± 6.4 min (mean ± SD); slow compartment, 847.5 ± 143.5 min. These values are statistically significantly different from the values of group 2. The relative amount of tracer cleared by the fast compartment (f_F) in these animals was 0.32 ± 0.04. In one animal of group 1, however, the monophasic equation analysis fitted better than the biphasic equation and the T½ was 515 min.

Lung lavage decreased blood gas tensions to below 13.3 kPa in all lavaged animals (groups 1 and 2). The number of lavages necessary for this decrease was on average 12 - 14. Subsequent surfactant replacement restored blood gas tensions to almost normal values. In group 1 after 120 min blood gas tension values were not statistically different from the initial blood gas tension values in this group. There was, however, a significant difference in blood gas tension values between groups 2 and 3 (controls) after 120 min.

Table 1 Blood gas tensions of the three groups during the study period.Values are mean ± (SD). Statistical analysis was performed using the Mann-Whitney-Wilcoxon test for unpaired samples. $P<0.05$ was considered statistically significant. L: lavage; S: surfactant treatment; H: healthy.

Group	1		2		3	
Time (min)	P_aO_2 (kPa)	P_aCO_2 (kPa)	P_aO_2	P_aCO_2	P_aO_2	P_aCO_2
-5	71.13	4.33	71.75	4.51		
	(2.4)	(0.4)	(2.0)	(0.5)		
	L + S	L + S	L	L	H	H
	10.46	5.03				
	(2.7)	(0.6)				
0	62.72	5.35	9.89	4.69	73.19	4.07
	(11.7)	(0.5)	(2.6)	(0.5)	(2.2)	(0.6)
30	61.34@	5.17	23.54#	4.57	75.14	4.81
	(1.3)	(0.6)	(7.7)	(0.7)	(2.7)	(0.6)
60	61.97@	5.40	28.57#	4.77	76.79	5.0
	(13.5)	(0.52)	(14.2)	(0.7)	(3.8)	(0.4)
90	62.61@	5.39	24.6#	5.27	76.93	5.11
	(15.8)	(0.9)	(13.7)	(0.6)	(5.2)	(0.2)
120	66.66@	5.15	23.8#	5.51	78.75	5.04
	(10.8)	(0.5)	(11.6)	(0.6)	(4.3)	(0.4)

#: statistical significance: group 1 (surfactant treated) compared with group 2 (lavaged).
@: statistical significance: group 3 (controls) compared with group 1

DISCUSSION

In animals the lung lavage model initially represents a pulmonary surfactant deficiency at the alveolar level [Lachmann et al, 1982]. The surfactant layer in the alveoli and airways is washed away by the warmed saline, leading to decreased lung compliance, atelectatic areas and deterioration of blood gas tensions. When mechanical ventilation is applied to these lungs, high peak airway and end-expiratory pressures are needed to open up the alveoli and stabilise them during the breathing cycle. Many studies have shown that non-optimal ventilation settings often result in

epithelial damage on alveolar and bronchiolar levels, leading to further damage and deterioration of normal lung function [For review Bos and Lachmann, 1991b; Kolobow et al, 1987; Mascheroni et al, 1988; Nilsson et al, 1978]. This lavage model has also been shown to increase alveolar-capillary membrane permeability when measured with radio-labelled solutes as, for instance, ^{99m}Tc-DTPA [Evander et al, 1987]. Exogenous surfactant instillation, however, can restore lung functions and mechanics to almost normal when administered immediately after the last lung lavage [Kobayashi et al, 1984]. It also restores permeability of the alveolar-capillary barrier, as measured by ^{99m}Tc-DTPA, indicating that (at least for ^{99m}Tc-DTPA clearance measurements), the alveolar surfactant layer is an important rate-limiting factor, as well as the alveolar epithelium [Evander et al, 1987, 1988; Jefferies et al, 1988; Nieman et al, 1990; Bos et al, 1991a].

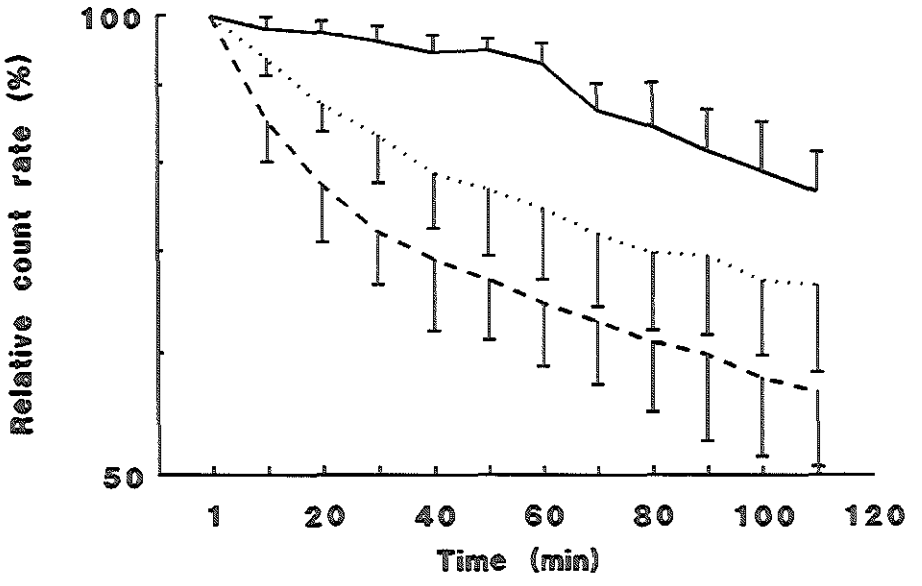

Fig. 2 Time-activity curves of the different groups during the study period. Values are given as mean ± (SD). Dotted line represents group 1 (surfactant treated animals), dashed line group 2 (lavaged animals) and solid line group 3.

Recent reports state that epithelial permeability measurements could be better performed using larger molecules whose transfer across the membrane is less influenced by physiological factors, as lung volume [Peterson et al, 1989; Barrowcliffe et al, 1989]. Barrowcliffe and colleagues showed that ^{99m}Tc-albumin is a suitable tracer molecule for measuring epithelial permeability [Barrowcliffe et al, 1989].

The control group (group 3) in our study showed a mono-exponential clearance curve for ^{99m}Tc-HSA, which has also been demonstrated by other workers [Peterson et al, 1989, Barrowcliffe et al, 1989]. The rate of clearance is so slow that one can not exclude mechanisms of transport other than diffusion across the alveolar-capillary barrier. This transport, for instance, could be due to an active vesicle transport system. Passive diffusion through the intercellular pores in the alveolar epithelium is not likely as the diameter of these pores is too small for the albumin molecules to pass through. The application of high airway pressure ventilation, in order to be able to monitor the effects of surfactant treatment, enables passive diffusion to occur and clearance rate increases. A possible mechanism could be that the ventilator setting after 60 min produced larger pressure-volume swings during the breathing cycle (which was confirmed by our finding of an increased expired tidal volume with this mode), leading to damage to the epithelium and/or disruption of the normal integrity of the pulmonary surfactant system [Bos et al, 1991a; for review: Bos and Lachmann, 1991b]. Thus the integrity of the whole alveolar-capillary membrane is altered and permeability of the barrier increases.

The finding of a bi-phasic clearance curve in the lavaged animals (group 2) (see Fig. 2) is probably caused by a combined effect of the lung lavage procedure inducing alveolar surfactant deficiency and the non-optimal mechanical ventilation mode. Firstly, lung lavage could affect individual alveoli and/or lung regions to a varying degree with differing degrees of damage. There may be two (or more) pools of radioactivity which clear the tracer with different half-lives. This would then be the same condition as is caused by oleic acid administration which also results in

formation of regions of severe lung injury and high epithelial permeability [Peterson et al, 1989; Huchon et al, 1988].

Secondly, lung lavage could have a transient effect on epithelial permeability. The increased permeability, due to a surfactant deficit, quickly returns to normal after a few hours with a return to normal surfactant function. This scenario is, however, not likely because after 2 h with the applied ventilator settings blood gas tensions had not returned to normal. Lowering of PEEP after the study period immediately resulted in further deterioration of lung function and blood gas tensions, indicating that there was no stabilisation of the lung at end-expiration and surfactant function had therefore not restored to normal.

Thirdly, clearance rates of labelled albumin slowed due to the large exudation of plasma fluid into the alveoli. Whenever the membrane is damaged (e.g. by surfactant deficiency and/or epithelial damage), complications due to a patho-physiologically high permeability can develop with subsequent influx of fluid with a high protein concentration, loss of oncotic pressure gradient across the epithelium and subsequent formation of pulmonary oedema, limiting diffusion of albumin either way [Egan et al, 1977]. Oedema is accompanied with physical thickening of the alveolar-capillary membrane due to fluid accumulation in the interstitial space of the barrier, thereby lengthening the diffusion path. These latter mechanisms could be of some importance in this study because in all animals of group 2, 3 - 8 ml of haemorrhagic fluid with a high protein content could easily be extracted from the lungs after 2 h of artificial ventilation, indicating at least presence of oedema fluid in the larger airways. As has been stated in many reports, the resistance of the epithelium to diffusion of all kinds of solutes is ten times that of the endothelium, meaning that the presence of oedema fluid can be interpreted as substantial damage to the epithelium [Gorin and Stewart, 1979; Staub 1974; Taylor and Gaar, 1970].

Another cause for an initial fast clearance of radioactivity from the lungs which has

to be considered is dissociation of the label from the albumin molecule. Testing of the binding percentage of the labelling showed that before nebulization 98 ± 0.4% and after nebulization 99 ± 0.1% of the label was connected to the albumin molecule. This indicates that at least no dissociation has taken place in vitro. In vivo dissociation of the label, however, can not be entirely excluded although pertechnetate cumulates in the gastric mucosa, salivary glands and the thyroid. No activity was found in these areas in our study. Peterson and colleagues stated that the increase in ^{99m}Tc-HSA clearance observed after oleic acid injection was not caused by dissociation of the label [Peterson et al, 1989].

Several workers have shown that exogenous surfactant instillation in lungs reduces permeability of the alveolar-capillary membrane [Jobe et al, 1983, 1985; Robertson et al, 1985]. Surfactant thereby restricts formation of pulmonary oedema and the influx of water and proteins into the intra-alveolar air spaces. For these permeability measurements, intravenously injected iodine-labelled albumin was used and permeability was assessed by measuring the amount of labelled albumin recovered in lung lavage fluid. These studies confirmed that the alveolar surfactant layer is of major importance to the permeability of the alveolar-capillary barrier. These studies also showed that although the epithelium is of greatest importance in limiting free diffusion of water and solutes into the alveolar air space, without the surfactant layer high permeability of the membrane exists. This indicates that the phospholipid-protein layer has a critical sealing-off function. But sofar in all these studies investigating the influence of surfactant on permeability of the barrier with radio-labelled albumin, the route of transfer was from blood to alveolar air space.

The finding that surfactant restores normal permeability is, in part, confirmed by this study. The clearance rates in the surfactant treated animals are smaller than in the non-treated group. The clearance curves of almost all animals in group 1, however, are of a bi-exponential character, indicating still possible abnormal permeability and transfer characteristics of the tracer through the membrane. The initial fast

compartment clearance rate of the surfactant treated group is, however, significantly slower than that of lavaged animals. The reason behind the multi-exponential clearance curves in the surfactant treated animals could be that between the first lung lavage and the moment of exogenous surfactant administration, ventilation-induced damage already developed. Studies by Nilsson showed that epithelial disruption takes place after only a few breaths in case of surfactant deficiency combined with mechanical ventilation [Nilsson et al, 1978]. Grossmann showed that this damage could largely be prevented by direct surfactant instillation to the lungs [Grossman et al, 1986]. This leads us to hypothesise that the fast compartment clearance of the tracer in group 2 is caused by epithelial disruption induced by mechanical ventilation, which can not be completely restored by the administered surfactant. Surfactant instillation, however, probably prevented further damage to the epithelium with subsequent, at least partial, restoration of normal integrity of the alveolar-capillary membrane. Inhomogeneity of surfactant distribution in the lungs may also have caused differences in clearance rates, indicating that alveoli that received no surfactant had a higher permeability and those receiving sufficient surfactant probably had normal or even decreased permeability for the tracer molecule [Bos et al, 1991a]. This feature might enable the evaluation of different surfactant preparations, in which the more effective ones will spread more homogeneously in the lungs leading to restored monophasic clearance characteristics.

The slow compartment clearance rate in animals of group 2, however, was almost normal despite the presence of a high permeability alveolar oedema and severe alveolar surfactant deficiency. This finding makes it hard to discriminate between existence of normal permeability due to a normal integrity of the alveolar-capillary barrier and a normal clearance due to the probable presence of intra-alveolar oedema fluid.

The data of this study shows that surfactant replacement after lung lavage generally restores normal permeability of the alveolar-capillary barrier when using ^{99m}Tc-HSA

as tracer molecule. Whether ^{99m}Tc-HSA is a better tracer than ^{99m}Tc-DTPA remains to be seen because the clearance rate of ^{99m}Tc-HSA also increases sharply after applying ventilator modes which increase lung volume. Also the role of oedema formation in the lungs, with subsequent protein influx into the alveoli, has still to be resolved.

REFERENCES

Barrowcliffe M.P., Otto C., Jones J.G. (1988) Pulmonary clearance of ^{99m}Tc-DTPA: influence of background activity. *J. Appl. Physiol.*, **64**, 1045-1049.

Barrowcliffe M.P., Zanelli G.D., Jones J.G. (1989) Pulmonary clearance of radiotracers after positive end-expiratory pressure or acute lung injury. *J. Appl. Physiol.* **66**, 288-294.

Bos J.A.H., Wollmer P., Bakker W.H., Hannappel E., Lachmann B. (1991a) Pulmonary clearance of ^{99m}Tc-DTPA and experimentally increased alveolar surfactant content in rabbits. *J. Appl. Physiol.*, accepted for publication.

Bos J.A.H., Lachmann B. (1991b) Effect of different ventilator settings on lung mechanics: with special reference to the surfactant system. *Appl. Cardiopulmon. Pathophysiol.*, in press.

Braude S., Nolop K.B., Hughes J.M.B., Barnes P.J., Royston D. (1986) Comparison of lung vascular and epithelial permeability indices in the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.*, **133**, 1002-1005.

Cooper J.A., Van der Zee H., Line B.R., Malik A.B. (1987) Relationship of end-expiratory pressure, lung volume and ^{99m}Tc-DTPA clearance. *J. Appl. Physiol.*, **63**, 1586-1590.

Dahlbäck M.P., Wollmer P., Drefeldt B., Jonson B. (1989) Controlled aerosol delivery during mechanical ventilation. *J. Aerosol. Med.*, **2**, 339-347.

Dennis Jr J.E., Woods D.J. (1987) New computing environments: microcomputers in large-scale computing (ed. Wouk A.) *SIAM* pp. 116-122.

Egan E.A. (1983) Fluid balance in the air-filled alveolar space. *Am. Rev. Respir. Dis.*, 127, S37-S39.

Egan E.A., Nelson R.M., Gessner I.H. (1977) Solute permeability of alveolar epithelium in acute hemodynamic pulmonary edema in dogs. *Am. J. Physiol.*, 233, H80-H86.

Evander E., Wollmer P., Jonson B. (1988) Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effect of the detergent dioctyl sodium sulfosuccinate in aerosol. *Clin. Physiol.*, **8**, 105-111.

Evander E., Wollmer P., Jonson B., Lachmann B. (1987) Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. *J. Appl. Physiol.*, **62**, 1611-1614.

Gorin A.B., Stewart P.A. (1979) Differential permeability of endothelial and epithelial barriers to albumin flux. *J. Appl. Physiol.*, **47**, 1315-1324.

Grossmann G., Nilsson R., Robertson B. (1986) Scanning electron microscopy of epithelial lesions induced by artificial ventilation of the immature neonatal lung: the prophylactic effect of surfactant replacement. *Eur. J. Pediatr.*, **145**, 361-367.

Huchon G.J., Little J.W., Murray J.F. (1981) Assessment of alveolar-capillary membrane permeability of dogs by aerosolization. *J. Appl. Physiol: Respirat. Environ. Exercise Physiol.* **51**, 955-962.

Huchon G.J., Montgomery A.B., Lipavsky A.J.A., Hoeffel J.M., Murray J.F. (1988) Pulmonary clearance of three aerosolised solutes in oleic acid-induced lung injury. *J. Appl. Physiol.*, **64**, 1171-1178.

Jefferies A.L., Kawano T., Mori S., Burger R. (1988) Effect of increased surface tension and assisted ventilation on ^{99m}Tc-DTPA clearance. *J. Appl. Physiol.*, **64**, 562-568.

Jefferies A.L., Coates G., Webber C.E., O'Brodovich H.M. (1984a) Measurement of pulmonary clearance of radioaerosol using a portable sodium iodide probe. *J. Appl. Physiol.*, **57**, 1908-1912.

Jefferies A.L., Coates G., O'Brodovich H. (1984b) Pulmonary epithelial permeability in hyaline-membrane disease. *N. Engl. J. Med.*, **311**, 1075-1080.

Jobe A., Ikegami M., Jacobs H., Jones S., Conaway D. (1983) Permeability of premature lamb lungs to protein and the effect of surfactant on that permeability. *J. Appl. Physiol.*, **55**. 169-176.

Jobe A., Jacobs H., Ikegami M., Berry D. (1985) Lung protein leaks in ventilated lambs: effect of gestational age. *J. Appl. Physiol.*, **58**, 1246-1251.

Jones J.G., Lawler P., Crawley J.C.W., Minty B.D., Hulands C., Veall N. (1980) Increased alveolar epithelial permeability in cigarette smokers. *Lancet*, **1**, 66-68.

Kobayashi T., Kataoka H., Ueda T., Murakami S., Takada Y., Kokubo M. (1984) Effects of surfactant supplement and end-expiratory pressure in lung-lavaged rabbits. *J. Appl. Physiol.*, **57**, 995-1001.

Kolobow T., Moretti M.P., Fumagalli R., Mascheroni D., Prato P., Chen V., Joris M. (1987) Severe impairment in lung function induced by high peak airway pressure during mechanical ventilation. *Am. Rev. Respir. Dis.*, **135**, 312-315.

Lachmann B., Jonson B., Lindroth M., Robertson B. (1982) Modes of artificial ventilation in severe respiratory distress syndrome. *Crit. Care Med.*, **10**, 724-732.

Mascheroni D., Kolobow T., Fumagalli R., Moretti M.P., Chen V., Buckhold D. (1988) Acute respiratory failure following pharmacologically induced hyperventilation: an experimental animal study. *Intens. Care Med.*, **15**, 8-14.

Metcalfe I.L., Enhorning G., Possmayer F. (1980) Pulmonary surfactant-associated proteins: their role in the expression of surface activity. *J. Appl. Physiol.*, **49**, 34-41.

Nelder J.A., Mead R. (1965) A simplex method for function minimization. *Computer Journal*, 7, 308.

Nieman G., Ritter-Hrncirik C., Grossman Z., Witanovski L., Clark Jr W., Bredenberg C. (1990) High alveolar surface tension increases clearance of technetium 99m diethylene triamine pentaacetic acid. *J. Thorac. Cardiovasc. Surg.*, **100**, 129-133.

Nilsson R., Grossmann G., Robertson B. (1978) Lung surfactant and the pathogenesis of neonatal bronchiolar lesions induced by artificial ventilation. *Pediatr. Res.*, **12**, 249-255.

Nolop K.B., Maxwell D.L., Royston D., Hughes J.M.B. (1986) Effect of raised thoracic pressure and volume on ^{99m}Tc-DTPA clearance in humans. *J. Appl. Physiol.*, **60**, 1493-1497.

Peterson B.T., Dickerson K.D., James H.L., Miller E.J., McLarty J.W. and Holiday D.B. (1989) Comparison of three tracers for detecting lung epithelial injury in anaesthetised sheep. *J. Appl. Physiol*, **66**, 2374-2383.

Rinderknecht J., Krauthammer M., Uszler J.M., et al. (1977) Solute transfer across the alveolar capillary

membrane in pulmonary fibrosis [Abstract]. *Am. Rev. Respir. Dis.*, **115**, 156.

Robertson B., Berry D., Curstedt T., Grossmann G., Ikegami M., Jacobs H., Jobe A., Jones S. (1985) Leakage of protein in immature rabbit lung: effect of surfactant replacement. *Respir. Physiol.*, **61**, 265-276.

Staub N.C. (1974) Pulmonary oedema. *Physiol. Rev.*, **54**, 678-811.

Taylor A.E., Gaar K.A. (1970) Estimation of equivalent pore radii of pulmonary capillary and alveolar membranes. *Am. J. Physiol.*, **218**, 1133-1140.

CHAPTER 7

PULMONARY CLEARANCE OF ^{99m}Tc-DTPA DURING HALOTHANE ANAESTHESIA

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PULMONARY CLEARANCE OF ^{99m}Tc-DTPA DURING HALOTHANE ANAESTHESIA

SUMMARY

We studied the integrity of the alveolar-capillary barrier during different forms of anaesthesia by measuring the pulmonary clearance of inhaled ^{99m}Tc-DTPA. We studied four groups of rabbits. Groups I and II were anaesthetised with nembutal only and the fractional concentration of inspired oxygen (FiO_2) was 0.30 and 1.00, respectively. Groups III and IV were anaesthetised with 1% halothane and FiO_2 was 0.30 and 0.99, respectively. ^{99m}Tc-DTPA was administered as a fine aerosol and the clearance of the tracer from the lungs was subsequently measured with a gamma camera. The mean half-life of the tracer in the lungs in Groups I-IV was 60, 58, 59, and 26 min, respectively. The rapid pulmonary clearance of ^{99m}Tc-DTPA in Group IV indicates that halothane in combination with high oxygen concentration increases the permeability of the alveolar-capillary barrier. This may be due to effects on the pulmonary surfactant system and/or the alveolar epithelium.

INTRODUCTION

The alveolar-capillary barrier allows rapid diffusion of gases but forms a barrier against diffusion of water and solutes. In recent years, non-invasive techniques have been developed for the assessment of the integrity of the alveolar-capillary barrier [1]. One such technique entails measurement of the pulmonary clearance of technetium-99m-labelled diethylene triamine pentaacetic acid (molecular weight 492 dalton), ^{99m}Tc-DTPA [2]. The radioactive tracer is administered as an aerosol and, subsequently, the radioactivity is measured over the lungs with a detector. A clearance curve is obtained, which reflects the transfer of the tracer across the alveolar-capillary barrier.

The permeability of the alveolar-capillary barrier to ^{99m}Tc-DTPA is known to increase after exposure to various inhaled substances, e.g. smoke [2, 3], aerosolised detergent [4] and prolonged exposure to high concentrations of oxygen [5].

Many inhalation anaesthetics are highly lipophilic and have been thought to affect the permeability of the biological membranes to water and ions [6]. The purpose of this study was to examine the acute effect of halothane and oxygen on the permeability of the alveolar-capillary barrier to ^{99m}Tc-DTPA.

MATERIAL AND METHODS

We studied 20 rabbits under different forms of anaesthesia. Anaesthesia was always induced with nembutal 50 mg kg^{-1}. The animals were then tracheotomised and a carotid artery was cannulated for blood pressure monitoring. After the preparation, anaesthesia was maintained with nembutal in Groups I (n=4) and II (n=6) and with halothane (1%) in Groups III (n=4) and IV (n=6). All animals were ventilated with a Servo Ventilator 900C (Siemens-Elema, Solna, Sweden) at a frequency of 40 min^{-1} and tidal volumes of approximately 15 ml kg^{-1}. Inspiratory time was 33% of the respiratory cycle, and there was no inspiratory pause. In Groups I and III, the fractional concentration of inspired oxygen (FiO_2) was kept at 0.30, whereas in Groups II and IV it was kept at 0.99-1.00.

In the animals given halothane, blood pressure consistely fell. To compensate for this, an infusion of dopamine was started soon after the administration of the halothane. The infusion rate was adjusted so that blood pressure was kept at the control level, a typical dose rate being 0.3 mg kg^{-1} h^{-1}. The animals were placed under gamma camera and were kept under anaesthesia for a minimum of 15 min before the measurement of the pulmonary clearance of ^{99m}Tc-DTPA.

A solution of ^{99m}Tc-DTPA was prepared using a commercial kit (Technescan DTPA, Mallinkrodt Diagnostica, Petten, The Netherlands). For administration of the radioactive tracer, we nebulized the solution into the inspiratory line of the breathing circuit as described previously [7] using an air jet nebulizer (UltraVent, Mallinkrodt Diagnostica). The mass median aerodynamic diameter of the particles was 1.7 μm as measured with a laser light scattering technique. When a count rate of

approximately 400 counts s^{-1} was reached over the lungs (after 1-2 min), the aerosol administration was stopped and the clearance measurement started. Gamma camera images were obtained in 1-min frames for 20 min and stored in a 64 x 64 image matrix in a computer. When the clearance measurement was completed, the animal was sacrificed with an overdose of nembutal.

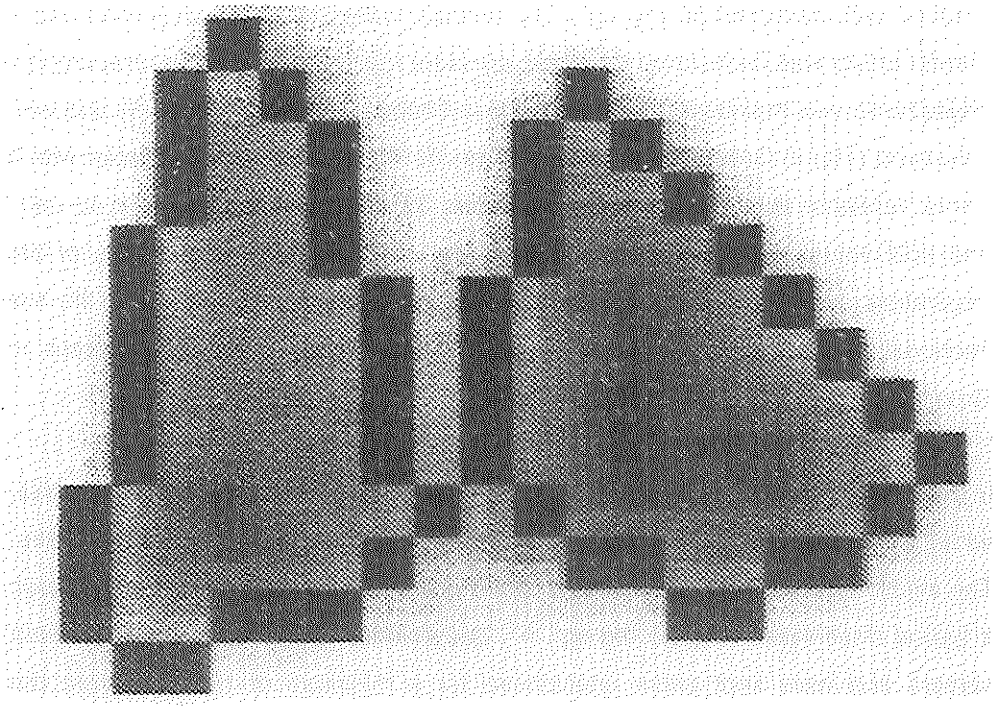

Fig. 1 Gamma camera image of a rabbit anaesthetised with nembutal and ventilated with pure oxygen. There is uniform distribution of the radioactivity in the lungs. The selection of the region of interest is indicated.

We analysed the data from the clearance measurement by selecting a region of interest over both lungs (Fig. 1) and generating a time-activity curve. A mono-exponential function was then fitted to the experimental data and the half-life (T½) of the tracer in the lungs calculated.

The T½ in the four groups was compared with the non-parametric Kruskal-Wallis statistics for comparison of multiple groups.

The study was approved by the local animal investigation committee.

RESULTS

All animals could be kept under deep anaesthesia throughout the experiment. In the animals anaesthetised with halothane, arterial blood pressure could be maintained at a normal level with dopamine infusion.

In all animals, the distribution of inhaled aerosol was uniform, and there was little particle deposition in the large airways (Fig. 1). During the measurement, a diffuse tissue background radioactivity appeared, and radioactivity could be seen to accumulate in the kidneys and the bladder, especially in the animals in Group IV. No other site of accumulation of radioactivity was seen.

Composite clearance curves from the four groups of animals are shown in Fig. 2. The clearance curves obtained in Groups I-III are virtually identical, while the animals in Group IV have considerably faster pulmonary clearance of ^{99m}Tc-DTPA. Excellent exponential fits were obtained with correlation coefficients ranging from 0.98-1.00. The calculated half-life for the clearance in Groups I-IV was 60, 58, 59 and 26 min, respectively (Fig. 3). The Kruskal-Wallis statistical analysis showed a significant difference between the groups ($P<0.02$).

DISCUSSION

We have shown that the pulmonary clearance of ^{99m}Tc-DTPA is similar during barbiturate anaesthesia with an FiO_2 of 0.30 or 1.00 and halothane anaesthesia and $FiO_2=0.30$, but considerably increased during halothane anaesthesia in pure oxygen. Owing to the limited number of animals studied, small effects of oxygen and halothane alone have been overlooked. Such effects would, however, be trivial

compared to the combined effect and also compared to the changes in clearance rate observed in disease states [8]. The clearance rate observed in the first three groups is similar to that previously found in normal rabbits studied under similar conditions [9, 10].

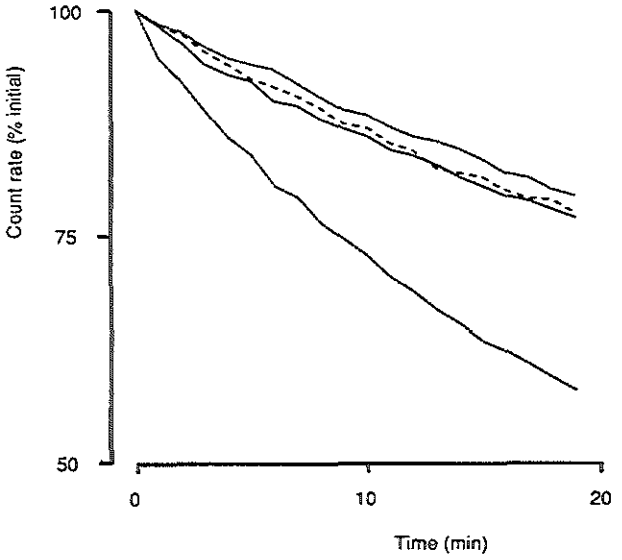

Fig. 2 Composite time-activity curves measured over the lungs in rabbits under different forms of anaesthesia. Group I (—) was anaesthetised with nembutal , and FiO_2 was 0.30. Group II () was also anaesthetised with nembutal, but FiO_2 was 1.00- Group III (....) was anaesthetised with halothane in 30% O_2 and Group IV (-,-) with Halothane in 99% O_2. The curves follow a monoexponential course in all groups (note linear scale on the ordinate).

Provided that the ^{99m}Tc-DTPA is administered in an aerosol with sufficiently small particles, the tracer will reach the terminal airways and the alveoli and will largely reflect solute transport in the gas exchanging region of the lungs. The pulmonary clearance of ^{99m}Tc-DTPA measured by external detection reflects the rate of transfer of the tracer from the air space to the blood. The clearance measurement is insensitive to large changes in pulmonary blood flow [11], and only small amounts of the tracer are cleared via the lymphatics [12]. It has been suggested that the ^{99m}Tc-DTPA could be oxidised in the lung to form free $^{99m}TcO_4^-$, which has a considerably

faster clearance than ^{99m}Tc-DTPA [13]. The fates of the two chemical forms of the ^{99m}Tc in the body are quite different: ^{99m}Tc-DTPA is rapidly eliminated by glomerular filtration, whereas ^{99m}Tc-O_4^- accumulates in the salivary glands, the thyroid and the gastric mucosa. The distribution of the radioactivity during measurements clearly indicates that ^{99m}Tc-DTPA is stable under the conditions used in this study. The increased rate of pulmonary clearance of ^{99m}Tc-DTPA during anaesthesia with halothane in oxygen can therefore be interpreted to represent increased permeability of the alveolar epithelium to water-soluble molecules [8]. The rate of pulmonary clearance of inhaled ^{99m}Tc-DTPA has also been suggested to reflect the functional integrity of the pulmonary surfactant system [4, 9, 14, 15].

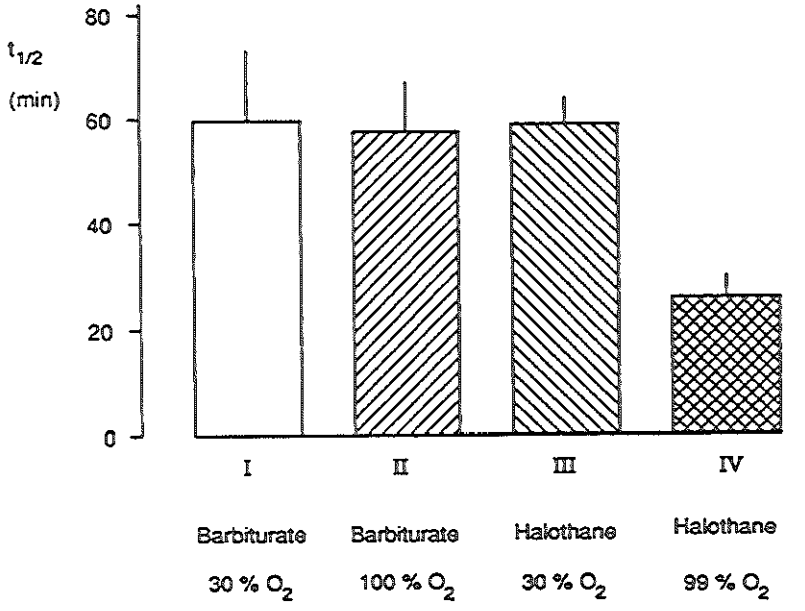

Fig. 3 Mean half-life (T½) for ^{99m}Tc-DTPA in the lungs for the four groups of animals. The T½ is much shorter in the animals anaesthetised with halothane in 99% O_2 than in the other groups.

Pulmonary surfactant consists mainly of phospholipids, which form a monomolecular layer at the air-liquid interface in the distal airways of the lung. Pulmonary surfactant is believed to be essential for the stability of the alveoli by reducing surface tension as the alveolar area is compressed during expiration. Without surfactant, small alveoli

would tend to empty into larger ones, and atelectasis would ensue.

The pulmonary clearance of inhaled ^{99m}Tc-DTPA has been shown to increase greatly after experimental perturbation of the surfactant system. Thus Evander et al [9] showed that clearance increased after surfactant depletion by lung lavage and that the effect could be partly reversed by surfactant replacement. The pulmonary clearance of ^{99m}Tc-DTPA also increases after administration of an aerosol containing a synthetic detergent which interferes with surfactant function [4].

Halothane has been reported to destabilise surfactant monolayers in vitro, but only in a high (20%) concentration [16]. The relation between surfactant function in vivo and the surface activity measured in vitro is not obvious, and it appears possible that halothane might have effects on the surfactant layer at lower concentration in vivo. We could not use as high concentrations of halothane in vivo as used in vitro, and do not know whether they would not affect the pulmonary clearance of inhaled ^{99m}Tc-DTPA. Anaesthesia with 1% halothane and 30% oxygen did not affect the pulmonary clearance of inhaled ^{99m}Tc-DTPA.

Exposure to pure oxygen causes lung damage pathologically characterised by atelectasis, oedema, alveolar haemorrhage and hyalinization of alveolar membranes [17]. The pathogenesis is not completely understood, but may involve increased formation of oxygen radicals, leading to peroxidation of surfactant and membrane lipids [17, 18]. Exposure to pure oxygen reduces the amount and impairs surface activity of pulmonary phospholipids, and also reduces incorporation of radiolabelled precursors into pulmonary phospholipids [19-22]. Measurements of pulmonary phospholipids and surface activity have generally been made after at least 48 h exposure to oxygen, and we are not aware of any studies of the acute effect. The permeability of the alveolar epithelium to cytochrome c (molecular weight 12,523 daltons) is increased after 48 h of exposure to pure oxygen, before there is any evidence of oedema formation [23]. No information concerning shorter periods of

exposure was given in this study. Several drugs are known to augment oxygen toxicity in general [17] and specifically the adverse effects on phospholipid synthesis [18].

In a previous study of the effects of oxygen on the pulmonary clearance of inhaled ^{99m}Tc-DTPA, Griffith et al [5] found an increased clearance rate in normal subjects after exposure to 50% oxygen for 48 h. The short exposure to pure oxygen in the animals anaesthetised with barbiturate in the present study did not result in increased pulmonary clearance of ^{99m}Tc-DTPA. This is in agreement with previous findings [4, 9, 10]. Exposure to pure oxygen only thus appears to have no acute effect on the transfer of small solutes across the alveolar-capillary barrier.

The increased rate of pulmonary clearance of ^{99m}Tc-DTPA during anaesthesia with 1% halothane in oxygen is likely to be caused by combined effects of the two gases on the pulmonary surfactant and/or alveolar epithelium. To the extent that the increased clearance of ^{99m}Tc-DTPA reflects damage to the surfactant system or the epithelium, this may be a pathogenetic mechanism in the development of several per- and postoperative complications, e.g. atelectasis and pulmonary oedema. Further studies of the effects of various anaesthetics and oxygen on the alveolar-capillary barrier therefore seem warranted.

In conclusion, we have shown that the pulmonary clearance of ^{99m}Tc-DTPA is unaffected by the short-term ventilation with pure oxygen during barbiturate anaesthesia. Anaesthesia with 1% halothane and an FiO_2 of 0.30 does not affect the clearance rate, but when halothane is given in pure oxygen, the clearance rate increases markedly. The effect of halothane and oxygen on the pulmonary clearance of ^{99m}Tc-DTPA may reflect adverse effects of the gases on the pulmonary surfactant system and/or the alveolar epithelium.

REFERENCES

1. Jones JG, Minty BD, Royston D. The physiology of leaky lungs. *Br J Anaesth* 1982: **54**: 705-721.
2. Jones JG, Minty BD, Lawler P, Hulands G, Crawley JCW, Veall N. Increased alveolar epithelial permeability in cigarette smokers. *Lancet* 1980: **1**: 66-68.
3. Minty BD, Royston D, Jones JG, Smith DJ, Searing CSM, Beeley M. Changes in permeability of the alveolar-capillary barrier in firefighters. *Br J Indust Med* 1985: **42**: 631-634.
4. Evander E, Wollmer P, Jonson B. pulmonary clearance of inhaled 99m-Tc-DTPA: effect of the detergent dioctyl sodium sulfosuccinate in aerosol. *Clin Physiol* 1988: **8**: 105-111.
5. Griffith DE, Holden WE, Morris JF, Min LK, Krishnamurthy GT. Effects of common therapeutic concentrations of oxygen on lung clearance of 99-Tc-DTPA and bronchoalveolar lavage albumin concentration. *Am Rev Respir Dis* 1986: **134**: 233-237.
6. Featherstone RM, Muehlbaecher CA. The current role of inert gases in the search for anaesthesia mechanisms. *Pharmacol Rev* 1963: **15**: 97-121.
7. Dahlbäck M, Wollmer P, Drefeldt B, Jonson B. Controlled aerosol delivery during mechanical ventilation. *J Aerosol Med* 1989: **2**: 339-347.
8. Barrowcliffe MP, Jones JG. Solute permeability of the alveolar capillary barrier. *Thorax* 1987: **42**: 1-10.
9. Evander E, Wollmer P, Jonson B, Lachmann B. Pulmonary clearance of inhaled 99m-Tc-DTPA: effects of surfactant depletion by lung lavage. *J Appl Physiol* 1987: **62**: 1611-1614.
10. Evander E, Wollmer P, Jonson B. Pulmonary clearance of inhaled ^{99m}Tc-DTPA: effects of ventilation pattern. *Clin Physiol* 1990: **10**: 189-199.
11. Rizk NW, Luce JM, Hoeffel JM, Price DC, Murray JF. Site of deposition and factors affecting clearance of aerosolised solute from the canine lungs. *J Appl Physiol* 1984: **56**: 723-729.
12. Coates G, O'Brodovich HM. The contribution of lymphatic drainage to the clearance of inhaled 99m-Tc-DTPA from the lungs. *Clin Invest Med* 1986: **9**: 15-20.
13. Nolop KB, Braude S, Royston D, Maxwell DL, Hughes JMB. Positive end-expiratory pressure increases pulmonary clearance of inhaled ^{99m}Tc-DTPA in non-smokers but not in healthy smokers. *Bull Europ Physiopathol Respir* 1987: **23**: 57-60.
14. O'Brodovich H, Coates G. Pulmonary clearance of ^{99m}Tc-DTPA: A non-invasive assessment of epithelial integrity. *Lung* 1987: **165**: 1-16.
15. Wollmer P, Evander E, Jonson B. Assessment of surfactant function by measurement of the pulmonary clearance of inhaled Tc-99m-DTPA. In: Lachmann B, ed. surfactant replacement therapy in neonatal and adult respiratory distress syndrome. Berlin: Springer-Verlag, 1988: 66-76.
16. Enhorning G, Pototschnik R, Possmayer E, Burgoyne R. Pulmonary surfactant films affected

by solvent vapors. *Anesth Analg* 1986: **65**: 1275-1280.

17. Deneke SM, Fanburg BL. Normobaric oxygen toxicity of the lung. *N Engl J Med* 1980: **303**: 76-86.

18. Haagsman HP, van Golde LMG. Lung surfactant and pulmonary toxicology. *Lung* 1985: **163**: 275-303.

19. Giammona ST, Kerner D, Bondurant S. Effect of oxygen breathing at atmospheric pressure on pulmonary surfactant. *J Appl Physiol* 1965: **20**: 855-858.

20. Beckman DL, Weiss HS. Hyperoxia compared to surfactant washout on pulmonary compliance in rats. *J Appl Physiol* 1969: **26**: 700-709.

21. Gross NJ, Smith DM. Impaired surfactant phospholipid metabolism in hyperoxic mouse lungs. *J Appl Physiol* 1981: **51**: 1198-1203.

22. Holm BA, Notter RH, Siegle J, Matalon S. Pulmonary physiological and surfactant changes during injury and recovery from hyperoxia. *J Appl Physiol* 1985: **59**: 1402-1409.

23. Matalon S, Egan EA. Effects of 100% O_2 breathing on permeability of alveolar epithelium to solute. *J Appl Physiol* 1981: **50**: 859-863.

CHAPTER 8

INTEGRITY OF THE ALVEOLAR-CAPILLARY BARRIER AND ALVEOLAR SURFACTANT SYSTEM IN SMOKERS

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INTEGRITY OF THE ALVEOLAR-CAPILLARY BARRIER AND ALVEOLAR SURFACTANT SYSTEM IN SMOKERS

SUMMARY

The purpose of this study was to examine the integrity of the alveolar-capillary barrier in smokers and non-smokers. Alveolar-capillary solute transfer was measured with ^{99m}Tc-DTPA. Transudation of albumin and urea was measured in bronchoalveolar lavage fluid. The integrity of the alveolar surfactant system was assessed by measurement of the surface activity and by the measurement of the yield of phospholipids in alveolar lavage fluid. The mean decay constant for the pulmonary clearance of ^{99m}Tc-DTPA was 0.028 min^{-1} in the smokers and 0.009 min^{-1} in the smokers ($p<0.001$). The recovery of albumin and urea in alveolar lavage fluid were, however, very similar in the two groups. The surface activity of alveolar lavage fluid as well as the yield of phospholipids was significantly reduced in the smokers relative to the non-smokers. The rate constant for the pulmonary clearance of ^{99m}Tc-DTPA correlated with variables reflecting surfactant function. The study shows that the increased alveolar-capillary transfer of ^{99m}Tc-DTPA in smokers is not accompanied by increased transudation of small or large molecules into the alveoli. The findings support the hypothesis that increased clearance of ^{99m}Tc-DTPA in smokers is related to surfactant dysfunction.

INTRODUCTION

Alveolar air is separated from the pulmonary capillary blood by a delicate barrier, the integrity of which is crucially important for the liquid homeostasis in the lung. The alveolar-capillary barrier comprises the alveolar surfactant system, the alveolar epithelium, basement membrane and the capillary endothelium. The functional integrity of the barrier can be studied by measurement of the transfer of solutes from the alveolus to the blood or the transudation of solutes from the capillaries to the air space.

The most common technique for studies of solute transfer from the air space to the blood is the measurement of pulmonary clearance of ^{99m}Tc-labelled diethylene triamine pentaacetate (^{99m}Tc-DTPA) [1]. This hydrophillic molecule has a molecular weight of 492 dalton. The tracer is administered in aerosol form and subsequently the clearance of the tracer from the lungs is measured by external counting over the lungs. The rate of clearance reflects the transfer of the tracer across the alveolar-capillary barrier. Several groups have shown that the rate of pulmonary clearance of ^{99m}Tc-DTPA is much faster in smoking than in non-smoking subjects [2-6]. The mechanism for the increased alveolar-capillary barrier permeability in smokers is not clear. It has been suggested to reflect damage to the alveolar epithelium [1] but also abnormal surfactant function in the alveoli [7].

Indications of transudation of solutes from the capillaries into the alveoli can be obtained by analysis of solutes in bronchoalveolar lavage fluid [8]. The yield of albumin in lavage fluid has thus been measured in smokers in efforts to assess the properties of the alveolar-capillary barrier [9-13]. It has recently been shown that urea (mw=60 dalton) readily diffuses from the blood and the interstitial space into lavage fluid during the procedure [14-16]. This suggests that the yield of urea in lavage effluent could be a suitable indicator of the alveolar-capillary barrier permeability, provided the dwell time of fluid in the alveoli is controlled. We are not aware of any studies of the yield of urea at bronchoalveolar lavage in smokers nor of any attempt to correlate measurements of pulmonary clearance of ^{99m}Tc-DTPA with indicators of increased alveolar-capillary barrier permeability at bronchoalveolar lavage in smokers. It is well established that the yield of surfactant phospholipids in bronchoalveolar lavage effluents is reduced in smokers [12, 17]. The rapid pulmonary clearance of ^{99m}Tc-DTPA has been suggested to be related to impaired surfactant function, but there are no studies of clearance in relation to measurements of phospholipids in lavage fluid.

The purpose of this study was to relate measurements of pulmonary clearance of

^{99m}Tc-DTPA to indicators of alveolar-capillary barrier permeability and to indices of surfactant function obtained at bronchoalveolar lavage in normal smokers and non-smokers.

MATERIALS AND METHODS

SUBJECTS

We studied 13 current smokers and nine life long non-smokers. None of the smokers had a history of chronic bronchitis. The physical characteristics of the two groups of subjects were similar (table 1). Physical examination showed nothing abnormal in any of the subjects.

SPIROMETRY

A spirometry including vital capacity (VC) and forced expiratory lung volume (FEV_1) was performed with a spirometer based on pneumotachography (Vitalograph Compact, Vitalograph Ltd., Buckingham, England). The results were related to predicted values [18].

PULMONARY CLEARANCE OF ^{99m}Tc-DTPA

A solution of ^{99m}Tc-DTPA was prepared from a commercially available kit (Pentetate II, Amersham International, Amersham, U.K.) and nebulized with an air jet nebulizer (UltraVent, Mallinckrodt Diagnostica, Petten, the Netherlands). The mass median diameter of the particles was 1.7 μm. The subjects inhaled the aerosol by quiet tidal breathing for 1 to 2 minutes whilst seated in front of a gamma camera (Maxicamera 400T, General Electric Co., Milwaukee, Wisconsin, USA) until a count rate of approximately 2000 counts/s had been reached. The subjects were then immediately placed in the supine position on a thin couch and the gamma camera placed under the couch so as to obtain an image of the lungs in the posterior view. The gamma camera was interfaced with a computer system and the measurement of radioactivity over the chest were stored in one minute frames for 30 minutes in a 64 x 64 image matrix. After approximately 20 minutes, a small amount of ^{99m}Tc-DTPA was injected

intravenously to enable a correction for non-pulmonary radioactivity to be performed.

The measurements were analysed by selecting a region of interest enclosing both lungs and generating a time-activity curve. The correction for non-pulmonary radioactivity was performed as described by Barrowcliffe and colleagues [19] using a back-ground region selected over the great vessels in the abdomen. An exponential function

$$A = A_o \times e^{-kt},$$

where A_o is the count rate at time zero and A is the radioactivity at any time t was fitted to the experimental data from 1 to 20 min. The slope was expressed as the decay constant (k).

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage was performed as previously described in detail [20]. After premedication with morphine and scopolamine and local anaesthesia with lidocaine, the fiberoptic bronchoscope (Olympus BF 1 T 10) was loosely wedged in an anterior subsegment of the right middle or lower lobe. Three 50 ml aliquots of tepid sterile saline were sequentially infused and then gently aspirated and collected in two separate containers. The first aliquot was considered to represent to a great extent a bronchial wash. The following two aliquots were pooled and considered more to represent alveolar wash. The lavage procedure was standardised as far as possible and the time from infusion to aspiration of saline (dwell time) recorded. The lavage fluid was kept on ice until centrifuged (4° C, 200g) for ten minutes. The supernatant was removed from the cell pellet and kept frozen at -70° C until analysed.

MEASUREMENT OF UREA AND ALBUMIN IN LAVAGE FLUID

The concentrations of urea and albumin were measured in bronchial wash fluid as well as in bronchoalveolar lavage fluid. In the bronchial wash fluid, urea and albumin

concentrations were measured in six non-smokers. Urea and albumin concentrations were measured in unconcentrated lavage fluid [21, 23]. The total amount of urea and albumin in the lavage fluid was calculated by multiplying the concentration with the volume of aspirated fluid. The plasma concentration of urea and albumin were also measured in venous blood samples.

MEASUREMENT OF PHOSPHOLIPIDS IN LAVAGE FLUID

The concentration of phosphatidyl choline was measured in bronchoalveolar lavage fluid. Lipids were extracted twice with two fold volume of chloroform : methanol (2:1) and once in chloroform. The extract was evaporated to dryness at 40°C in a rotary evaporator under vacuum. The residue was dissolved in 2.0 ml of chloroform: methanol (2:1).

Phospholipids were separated by thin layer chromatography on 250 μm thick gel plates (Kieselgel 60 F_{254}, Merck). The solvent systems used were chloroform / methanol / water (65/25/4) [24, 25] and chloroform/methanol/acetic acid/water (100/60/16/8) [25, 26]. To visualise the phospholipid fractions, the chromatogram was exposed to iodine vapour. Phosphatidyl choline (PC) was localised in the chromatogram with the help of side standards of dipalmitoyl phosphatidyl choline (DPPC; Sigma Chemical Company, St Louis, MO, USA) spotted on each plate. Three standards of 10, 20 and 30 μg of DPPC were used. Spots in the chromatograms corresponding to DPPC were quantified using a densimeter coupled with an integrator (wave length 366 nm). The PC recovered from smokers and non-smokers was essentially DPPC as confirmed by gas-liquid chromatography (contained >73% palmitic acid).

MEASUREMENT OF SURFACE ACTIVITY OF LAVAGE FLUID

The ability of bronchoalveolar lavage fluid to lower surface tension at an air-water interface was measured on a Wilhelmy balance (Wilhelmy-Tensiometer, Biegler Electronic, Mauerbach, Austria). 0.8 ml of lavage fluid was added to the saline filled

trough and surface tension recorded with a platinum plate while the area was altered by continuous cycling from 100 to 20% at a cycle speed of 0.33 min^{-1}. Maximum ($gamma_{max}$) and minimum ($gamma_{min}$) surface tension were recorded during the fifth cycle.

STATISTICAL ANALYSIS

Differences between groups were investigated with Student's t-test. Relation between variables was assessed by linear regression analysis. The level of significance was set at $p=0.05$.

RESULTS

There were no substantial differences in physical characteristics between smokers and non-smokers (table 1). There was a wide range in the cumulative tobacco consumption of the smokers. All subjects were essentially normal at spirometry, and there were no significant differences between the two groups.

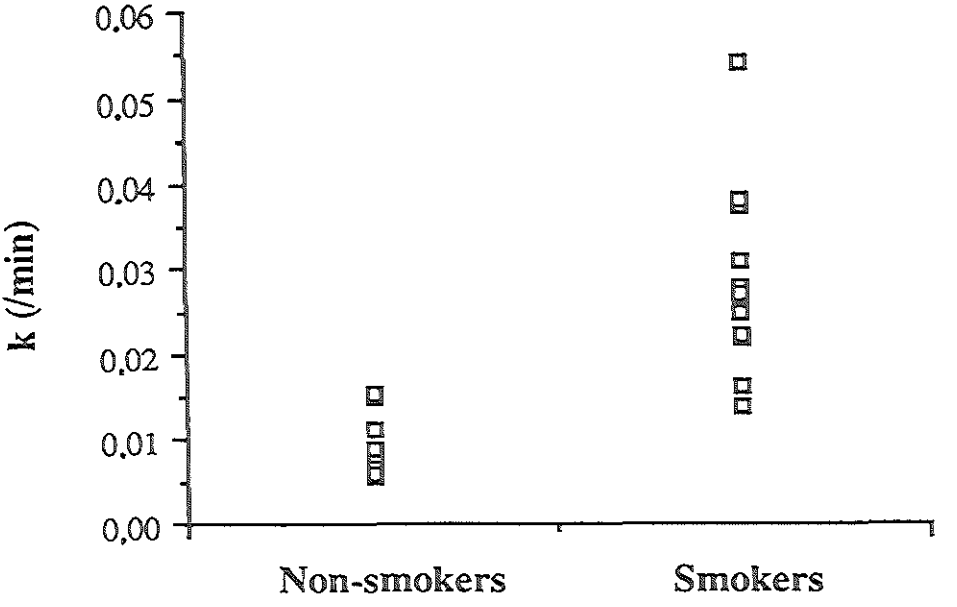

Fig. 1 Rate constant for the pulmonary clearance of ^{99m}Tc-DTPA in the two groups of subjects.

Table 1 Characteristics of subjects studied, results of carboxyhaemoglobin measurements and results of spirometry.

	Non-smokers	Smokers
n	9	13
Age (years)	37 (11)	35 (12)
Height (m)	1.79 (0.07)	1.82 (0.07)
Weight (kg)	72 (10)	79 (12)
Smoking history (pack years)	0	17 (12)
COHb (%)	0.62 (0.30)	2.58 (1.1)
VC (% pred.)	103 (14)	96 (13)
FEV_1 (% pred.)	106 (14)	100 (10)

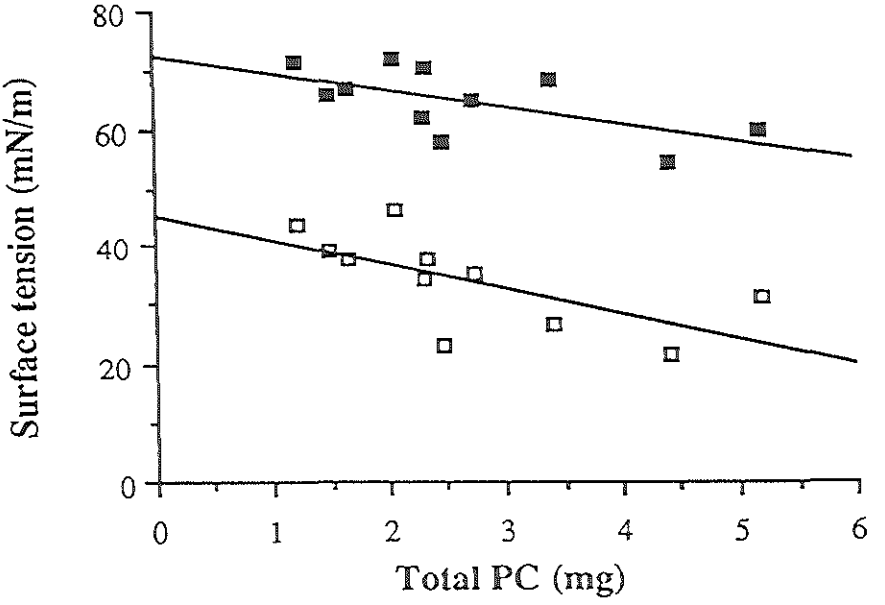

Fig. 2 Maximum (filled symbols) and minimum (open symbols) surface tension of alveolar lavage fluid versus total yield of phospholipids in alveolar lavage fluid. There was a significant correlation for maximum ($r=-0.63$) as well as for minimum ($r=-0.66$) surface tension.

Pulmonary clearance of ^{99m}Tc-DTPA was much faster in the smokers than in the non-smokers (fig. 1). The decay constant ranged from 0.014 to 0.054 min^{-1} with a mean of 0.028 min^{-1} in the smokers compared to a range from 0.006 to 0.016 min^{-1} and a mean of 0.009 min^{-1} in the non-smokers ($p<0.001$). There was thus a small overlap between the groups.

Dwell time and recovery of lavage fluid were very similar in the two groups of subjects (table 2). In the bronchial wash fluid, the concentration as well as the total amount of urea was higher in smokers than in non-smokers, but the difference did not quite reach statistical significance. The concentration of albumin in bronchial wash fluid was very similar in the two groups. In bronchoalveolar lavage fluid, the concentration of both urea and albumin were virtually identical in non-smokers and smokers. The plasma concentrations of urea and albumin were also similar in non-smokers and smokers; urea 4.8 ± 0.8 mmol l^{-1} and 4.4 ± 0.7 mmol l^{-1}, respectively and albumin 0.68 ± 0.03 mmol l^{-1} and 0.65 ± 0.04 mmol l^{-1}, respectively.

The concentration of phosphatidyl choline in alveolar lavage fluid was measured successfully in six non-smokers and in 10 smokers. Measurements of surface activity were obtained in six non-smokers and in 11 smokers. The yield of phospholipids in bronchoalveolar lavage fluid was approximately twice as high in non-smokers as in smokers (table 3). The concentration ($p<0.01$) as well as the total amount ($p<0.05$) of phosphatidyl choline was significantly greater in non-smokers than in smokers. The surface activity of alveolar lavage fluid was also lower in non-smokers than in smokers (table 3). Both $gamma_{min}$ and $gamma_{max}$ were significantly lower in non-smokers than in smokers.

Table 2 Urea and albumin concentration measurements in lavage fluid.

	Non-smokers	Smokers
Bronchial wash		
Recovery (ml)	15 (5)	17 (6)
Dwell time (min)	1.8 (0.5)	1.6 (0.5)
Urea concentration (mmol/l)	0.03 (0.01)	0.06 (0.04)
Total urea (mmol)	0.51 (0.26)	1.19 (1.19)
Albumin concentration (μmol/l)	1.7 (0.5)	1.8 (0.6)
Total albumin (μmol)	31 (12)	32 (12)
Alveolar wash		
Recovery (ml)	69 (17.4)	68 (13.8)
Dwell time (min)	3.7 (0.9)	3.2 (1.1)
Urea concentration (mmol/l)	0.08 (0.03)	0.08 (0.04)
Total urea (mmol)	5.04 (1.52)	5.21 (2.2)
Albumin concentration (μmol/l)	1.7 (0.6)	1.7 (0.8)
Total albumin (μmol)	116 (54)	119 (57)

Measurements of the yield of phosphatidyl choline and measurements of surface activity of the bronchoalveolar lavage fluid can be regarded as indicators of the functional integrity of the surfactant system, and we were interested in possible correlations between the measurements. When the lavage PC concentration was correlated with measurements of surface activity, a non-smoker with a PC concentration of 100.0 mg ml^{-1} was a clear outlier. If this value was excluded, PC concentration correlated inversely with $gamma_{max}$ ($r=-0.68$, $p<0.05$) and $gamma_{min}$ ($r=-0.84$, $p<0.01$). In all subjects, the total amount of PC recovered correlated inversely with $gamma_{max}$ ($r=-0.63$, $p<0.05$) and $gamma_{min}$ ($r=-0.66$, $p<0.05$, fig.2).

Table 3 Phospholipid (PC) concentration measurements and surface activity measurements in lavage fluid.

	Non-smokers	Smokers
PC concentration (mg/ml)	60 (31)	(30 (9)
Total PC (mg)	3.86 (0.12)	2.08 (0.65)
g_{max} (mN/m)	62.1 (6.0)	67.2 (3.2)
g_{min} (mN/m)	28.6 (5.2)	37.9 (5.2)

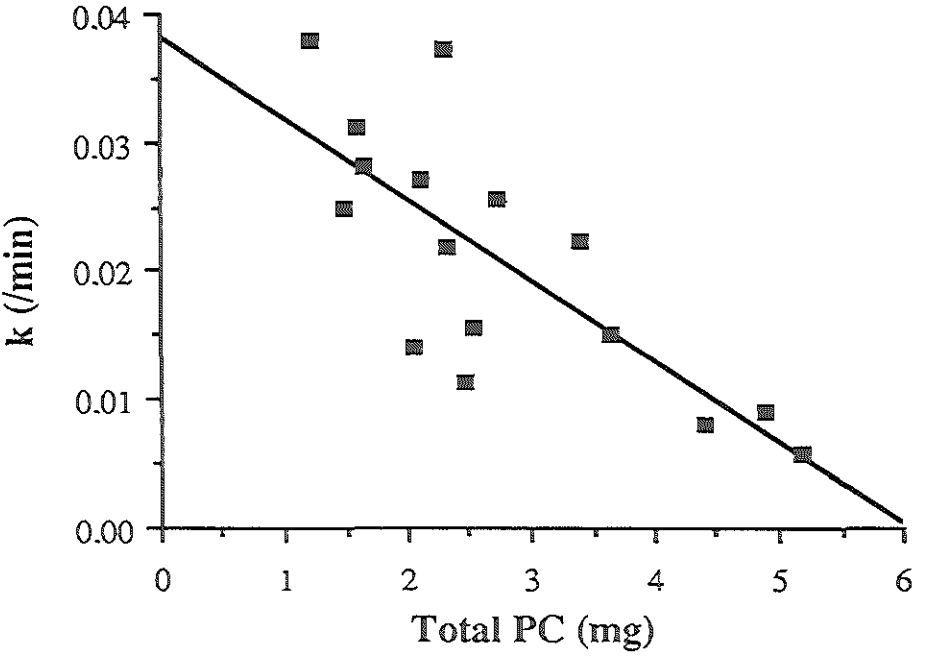

Fig. 3 Rate constant for the pulmonary clearance of ^{99m}Tc-DTPA versus the total yield of phospholipids in alveolar lavage fluid.

We found several correlations between the rate of pulmonary clearance of ^{99m}Tc-DTPA and variables reflecting the properties of the surfactant system. The rate constant k thus correlated inversely with the lavage PC concentration ($r=-0.62$, $p<0.05$) as well as the total amount of PC recovered ($r=-0.77$, $p<0.01$, fig. 3).

DISCUSSION

The alveolar-capillary barrier comprises the alveolar surfactant system, the alveolar epithelium, the basement membrane and the capillary endothelium. In studies of fluid filled lungs the alveolar epithelium has been shown to be much less permeable to small water soluble molecules than the capillary endothelium, whether the tracer substance is introduced into the alveolar space or into the blood [26]. ^{99m}Tc-DTPA is a hydrophillic compound and is considered to be transported by simple diffusion via the paracellular route. The increased rate of clearance observed in smokers is often, in accordance with studies of solute transfer in fluid filled lungs, interpreted to represent a high permeability of the alveolar epithelium to ^{99m}Tc-DTPA. Since the alveolar epithelium is the major barrier to solute flux also from the blood into the alveoli [14, 26], increased permeability of the epithelium might be expected to cause increased transfer of solutes into bronchoalveolar lavage fluid. When a tracer such as ^{99m}Tc-DTPA is introduced into the alveoli in aerosol form, the alveolar surfactant system is functionally intact, in contrast to the situation in the fluid filled lung. The pulmonary clearance of ^{99m}Tc-DTPA is increased in experimental states of surfactant dysfunction [27, 28] and reduced by experimentally increased alveolar content [29]. Recent speculations have suggested the rapid clearance in smokers to be related to abnormal surfactant function [7]. This study was designed to investigate the relation between pulmonary clearance of ^{99m}Tc-DTPA and indices of solute transfer from the pulmonary capillaries and alveolar surfactant function obtained at bronchoalveolar lavage. We found that while the transfer of ^{99m}Tc-DTPA from the alveoli to the blood is much faster in smokers than in non-smokers, there is no indication of increased leakage of urea or albumin from the pulmonary circulation to the alveoli during bronchoalveolar lavage in smokers. Indices of alveolar surfactant function are abnormal in smokers and correlate with the rate of pulmonary clearance of ^{99m}Tc-DTPA.

The yield of urea and albumin in bronchoalveolar lavage fluid have long been used as indicators of the permeability of the air-blood barrier [8]. Urea is a small molecule

and is able to diffuse through the intact cell membranes [30]. It is well established that urea diffuses into lavage fluid in appreciable amounts during a few minutes of fluid dwell time in the alveoli [14, 15, 16, 31]. The concentration of urea in alveolar lavage fluid has previously been shown to be increased in patients with interstitial lung disease [15, 20]. We found the alveolar lavage fluid concentration of urea to be approximately 2% of the serum concentration in both smokers and non-smokers. A first approximation of the rate of transfer of urea from blood and interstitial tissue can be obtained from a simple two compartment model assuming first order kinetics (Appendix). The calculations result in similar rate constants in smokers and non-smokers. Furthermore, the rate constants for urea are of the same of magnitude as the rate constants for ^{99m}Tc-DTPA found in the non-smokers. The main barrier for the transport of urea from blood to the fluid filled alveolus is the alveolar epithelium [14, 26]. If the main mechanism for the increased rate of alveolar-capillary transfer of ^{99m}Tc-DTPA in the smokers were increased permeability of the alveolar epithelium, it appears likely that the rate of transfer of urea from blood to air space would also be increased.

The rate of transfer of albumin from blood into fluid filled alveolus is much lower than that of urea [14, 16, 26] and very little albumin diffuses into alveolar lavage fluid under normal conditions. Unless there are gross changes of the permeability of the alveolar-capillary barrier, the use of albumin concentration in lavage fluid as an index of permeability relies on changes in the amount of albumin in the alveoli at steady state. The yield of albumin at bronchoalveolar lavage in smokers has been studied with conflicting results. Low and colleagues [12] found the ratio between the concentration of albumin in plasma and lavage fluid to be significantly reduced in smokers compared to non-smokers. Bell and colleagues [32] found the total amount of protein recovered by lavage to be considerably increased in smokers. In contrast, Reynolds and colleagues [9] and Warr and colleagues [10] found no differences in the yield of albumin between smokers and non-smokers, and Banks and colleagues [13] found a tendency for the yield of albumin to be reduced in smokers. Thompson

and colleagues [11] measured albumin concentration in bronchial and alveolar lavage fluid separately in normal smokers and non-smokers as well as in patients with chronic bronchitis. They found no differences between normal smokers and non-smokers. In the patients with chronic bronchitis, albumin concentration was increased in the bronchial but reduced in the alveolar lavage fluid. Our finding of similar albumin yield in normal smokers and non-smokers at bronchoalveolar lavages thus agrees with most previous studies and give no indication of increased transudation of albumin from the pulmonary capillaries.

Low yield of surfactant phospholipids from smokers was first described by Finley and Ladman [17] and subsequently by Low and colleagues [12]. In an experimental study, Le Mesurier et al. [33] showed the lavage yield of phospholipids to be reduced in rats exposed to cigarette smoke. Abnormal surface activity of lavage fluid from smokers has been described by Cook and Webb [34]. In general agreement with these studies, we found a lower yield of phospholipids and reduced surface activity of alveolar lavage fluid in smokers than in non-smokers. Surface tension, as measured in this study, may reflect quantitative and/or qualitive abnormalities of alveolar surfactant. Correlation analysis showed the variance in phospholipid concentration to explain some 40% of the variance in maximum and minimum surface tension of lavage fluid. This may indicate that there are also qualitive differences in the properties of alveolar surfactant between smokers and non-smokers. Some support for this concept is provided by the finding that cigarette smoke impairs surface activity of lavage fluid in vitro [35].

The rate constant for the alveolar-capillary transfer of ^{99m}Tc-DTPA correlated with both measurements of phospholipid concentration and the surface activity of lavage fluid. These correlations do not, of course, prove a causal relationship between surfactant dysfunction and the pulmonary clearance of ^{99m}Tc-DTPA. Taken together with the findings of normal lavage fluid concentrations of urea and albumin they do, however, support the hypothesis that abnormal clearance of ^{99m}Tc-DTPA in smokers could be

more related to surfactant function than to permeability of the alveolar epithelium. There are several possible mechanisms whereby surfactant dysfunction could influence the absorption of ^{99m}Tc-DTPA [36]. Surfactant could affect the spreading of the tracer in the aqueous hypophase in the alveolus. If permeability varies within the alveolus, e.g. is greater in the corners, this would affect the clearance rate.

In summary, we found increased rate of alveolar-capillary transfer of ^{99m}Tc-DTPA in smokers relative to non-smokers, but no indication of increased transfer of urea or albumin from the blood into the alveoli at lavage. The rate of pulmonary clearance of ^{99m}Tc-DTPA correlated with indices of surfactant function obtained at alveolar lavage, supporting the hypothesis that increased clearance of ^{99m}Tc-DTPA in smokers is related to surfactant dysfunction.

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APPENDIX

The rate of diffusion of urea from blood and interstitial fluid into alveolar lavage fluid was estimated in a simple two compartment model. Urea is assumed to diffuse from a body water compartment with a constant concentration of urea corresponding to the plasma concentration into an alveolar lavage fluid compartment. Since the concentration of urea in lavage fluid amounted to only 2% of the plasma concentration, the change in concentration gradient was neglected. The increase in urea concentration of lavage fluid (U_l) with time (t) is described by

$$U_l = U_p (1-e^{-kt}),$$

where U_p is the plasma concentration of urea and k the rate constant. Using the mean values of lavage and plasma concentration of urea found in non-smokers yields a rate constant of 0.0047. The corresponding value for smokers is 0.0057.

REFERENCES

1. Barrowcliffe MP, Jones JG. Solute permeability of the alveolar capillary barrier. *Thorax* 1987;**42**:1-10.
2. Jones JG, Lawler P, Crawley JCW, Minty BD, Hulands G, Veal N. Increased alveolar epithelial permeability in cigarette smokers. *Lancet* 1980;**1**:66-8.
3. Dusser DJ, Minty BD, Collignon MAG, Hinge D, Barritault LG, Huchon GJ. Regional respiratory clearance of aerosolised ^{99m}Tc-DTPA: posture and smoking effects. *J Appl Physiol* 1986;**60**:2000-6.
4. Huchon GJ, Russel JA, Barritault LG, Lipavsky A, Murray JF. Chronic air-flow limitation does not increase respiratory epithelial permeability assessed by aerosolised solute, but smoking does. *Am Rev Respir Dis* 1984;**130**:457-460.
5. Kennedy SM, Elwood RK, Wiggs BJR, Paré PD, Hogg JC. Increased airway mucosal permeability in smokers. *Am Rev Respir Dis* 1984;**129**:143-8.
6. O'Byrne PM, Dolovich M, Dirks R, Roberts RS, Newhouse MT. Lung epithelial permeability: relation to nonspecific airway responsiveness. *J Appl Physiol* 1984;**57**:77-84.
7. Wollmer P. Transfer of ^{99m}Tc-DTPA, lung surfactant and lung injury: a review of the literature. *Appl Cardiopulmon Pathophysiol* 1991. in press.

8. Reynolds HY. Bronchoalveolar lavage. *Am Rev Respir dis* 1987;**135**:250-263.

9. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974;**84**:559-573.

10. Warr GA, Martin RR, Sharp PM, Rossen RD. Normal human bronchial immunoglobulins and proteins. Effect of cigarette smoking. *Am Rev Respir Dis* 1977;**116**:25-30.

11. Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis. *Am Rev Respir Dis* 1989;**140**:1527-1537.

12. Low RB, Davis GS, Giancola MS. Biochemical analysis of bronchoalveolar lavage fluids of healthy human volunteer smokers and nonsmokers. *Am Rev Respir Dis* 1978;**118**:863-875.

13. Banks DE, Morgan JE, Deshazo RD, Weissman D, Rodrigues FH, Barkman HW, Salvaggio JE. Reliability of cell counts and protein determinations in serial bronchoalveolar lavage procedures performed on healthy volunteers. *Am J Med Sci* 1990;**300**:275-282.

14. Effros RM, Mason GR, Silverman P, Reid E, Hukkanen J. Movements of ions and small solutes across endothelium and epithelium of perfused rabbit lungs. *J Appl Physiol* 1986;**60**:100-107.

15. Van der Graaf EA, Jansen HM, Weber JA, Koolen MGJ, Out TA. Influx of urea during bronchoalveolar lavage depends on the permeability of the respiratory membrane. *Clin Chimica Acta* 1991;**196**:27-40.

16. Schmekel B, Venge P. The distribution of myeloperoxidase (MPO), eosinophil cationic protein (ECP), albumin and urea in sequential bronchoalveolar lavage. *Eur Respir J.* in press.

17. Finley TN, Ladman AJ. Low yield of pulmonary surfactant in cigarette smokers. *N Engl J Med* 1972;**286**:223-227.

18. Berglund E, Birath G, Bjure J, Grimby G, Kjellmer I, Sandqvist L, Söderholm B. Spirometric studies in normal subjects. I. Forced expirograms in subjects between 7 and 70 years of age. *Acta Med Scand* 1963;**173**:185-192.

19. Barrowcliffe MP, Otto C, Jones JG. Pulmonary clearance of ^{99m}Tc-DTPA: influence of background activity. *J Appl Physiol* 1988;**64**:1045-9.

20. Schmekel B, Wollmer P, Venge P, Linden M, Blom-Bulow B. Transfer of ^{99m}Tc-DTPA and bronchoalveolar lavage findings in patients with asymptomatic extrinsic allergic alveolitis. *Thorax* 1990;**45**:525-529.

21. Gutman I. Urea. In: Bergmeyer HU, ed. *Methods of enzymatic analysis.* Verlag Chemie Weinheim, 1977:1791.

22. Lizana J, Hellsing K. Polymer enhancement of automated immunological nephelometric analysis as illustrated by determination of urinary albumin. *Clin Chem* 1974;**20**:415-420.

23. Woelk H, Goracci G, Gaita A, Pocellati G. Phospholipase A1 and A2 activities of neuronal and glial cells of the rabbit brain. *Hoppe-Seylers Z Physiol Chem* 1973;**354**:729-736.

24. Balzer H, Khan AR. The different fatty acid composition of lecithins in the sarcoplasmatic

reticulum vesicles and the membranes of adrenal chromaffin granules. *N-S Arch Pharmacol* 1975;**291**:319-333.

25. Madeira VMC, Antunes-Madeira MC. Chemical composition of sarcolemma isolated from rabbit skeletal muscle. *Biochem Biophys Acta* 1973;**298**:230-238.

26. Staub NC. Pulmonary edema. *Physiol Rev* 1974;**54**:678-811.

27. Evander E, Wollmer P, Jonson B, Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA: effects of surfactant depletion by lung lavage. *J Appl Physiol* 1987;**62**:1611-1614.

28. Evander E, Wollmer P, Jonson B. Pulmonary clearance of ^{99m}Tc-DTPA: effect of the detergent dioctyl sodium sulfosuccinate in aerosol. *Clin Physiol* 1988;**8**:105-111.

29. Bos JAH, Wollmer P, Bakker WH, Hannappel E, Lachmann B. Clearance of ^{99m}Tc-DTPA and experimentally increased alveolar surfactant content. Submitted for publication.

30. Macey RI. Transport of water and urea in red blood cells. *Am J Physiol* 1984;**246**:C195-C203.

31. Van der Graaf EA, Jansen HM, Weber JA, Koolen MGJ, Out TA. Influx of urea during bronchoalveolar lavage depends on the permeability of the respiratory membrane. *Clin Chim Acta* 1991;**196**:27-40.

32. Bell DY, Haseman JA, Spock A, McLennan G, Hook GER. Plasma proteins of the bronchoalveolar surface of the lungs of smokers and non-smokers. *Am Rev Respir Dis* 1981;**124**:72-79.

33. Le Mesurier SM, Stewart BW, Lykke AW. Injury to type-2 pneumocytes in rats exposed to cigarette smoke. *Environ Res* 1981;**24**:207-217.

34. Cook WD, Webb WR. Surfactant in chronic smokers. *Ann Thorac Surg* 1966;**2**:327-333.

35. Webb WR, Cook WA, Lanius JW, Shaw RR. Cigarette smoke and surfactant. *Am Rev Respir Dis* 1967;**95**:244-247.

36. Effros RM. Permeability of the blood-gas barrier. In: Crystal RG, West JB, eds. *The Lung: Scientific foundations*. New York: Raven Press, 1991:1163-1176.

SUMMARY AND CONCLUSION

This thesis focuses on the importance of an intact pulmonary surfactant system and investigates the ways in which this system is influenced by various components of modern medicine, including mechanical ventilation and anaesthetic procedures. Further as permeability of the alveolar-capillary membrane is a reliable and sensitive indicator of the functional integrity of the surfactant system, clearance rates of radio-labelled solutes were measured in order to establish the effects of mechanical ventilation, anaesthetic procedures and smoking on surfactant function.

It is established that an optimally functioning surfactant system is crucial for maintenance of normal gas exchange in human lungs. The alveolar surfactant layer is an integral part of the alveolar-capillary membrane which prevents alveolar collapse and allows rapid diffusion of oxygen and carbon dioxide but restricts transfer of proteins and other molecules into the alveolar airspace. Alterations in the integrity of the surfactant layer therefore influences the functional integrity of the whole barrier. There are various methods for testing the integrity of the surfactant layer and the pulmonary surfactant system in general. These include: pulmonary compliance measurements, biochemical and biophysical analysis of lung extracts and pulmonary ^{99m}Tc-DTPA clearance measurement. This thesis focuses on the latter technique in assessing the state of the pulmonary surfactant system. **Chapter 1** presents a general introduction to the technique.

In all experimental studies, the animals were anaesthetised and paralysed so that the use of artificial ventilation was imperative. Due to the unphysiological nature of mechanical ventilation, use of these techniques has direct/indirect effects on the structure of the lung and on the pulmonary surfactant system. The influences of different modes of ventilation on the surfactant layer is discussed in **chapter 2** and a possible mechanism of how artificial ventilation disrupts the surfactant system is presented. The mode of ventilation used under conditions of pulmonary surfactant deficiency is even more crucial than in normal healthy lungs. Different modes can

cause widely differing effects on the restoration processes of the surfactant system in the lungs. In **chapter 3**, this hypothesis was tested using the pulmonary ^{99m}Tc-DTPA clearance technique in rabbits. The studied modes were high frequency jet ventilation (HFJV) at 2 and 15 Hz and volume controlled PEEP ventilation at 0.5 Hz in surfactant deficient and healthy lungs. The results show that HFJV at 15 Hz has the lowest clearance rate for ^{99m}Tc-DTPA and it appears that HFJV at 15 Hz provides the best conditions for restoration of the pulmonary surfactant system. HFJV at 2 Hz proved to be highly deleterious to the lungs: histology evidenced extensive damage to the lungs and the clearance rate of ^{99m}Tc-DTPA was the fastest of all studied modes indicating a totally disrupted alveolar-capillary membrane.

The hypothesis that the outcome of these clearance measurements is dependent on the integrity of the surfactant system provided the rationale for the study described in **chapter 4**. Whenever the alveolar surfactant layer is removed, depleted or damaged, the clearance rate of ^{99m}Tc-DTPA increases dramatically. The opposite should occur when the alveolar surfactant content is experimentally increased. This was achieved by administration of Ambroxol® intravenously or exogenous natural surfactant intratracheally. The results of this study led to the conclusion that an increased alveolar surfactant content does indeed slow the clearance rate of ^{99m}Tc-DTPA.

Blood gas tensions are one of the most important parameters on which to base or adjust ventilatory strategy, especially in the intensive care unit (I.C.U.) But the question rises whether it is the most sensitive parameter to indicate the beginning of acute respiratory insufficiency, or are other lung function tests available. Under these conditions, ^{99m}Tc-DTPA clearance measurements were compared with blood gas tension measurements. This study, described in **chapter 5**, shows that blood gas tensions are not always sensitive enough to accurately describe the status of the surfactant system under mechanical ventilation. Whereas blood gases had restored to normal, permeability measurements of the alveolar-capillary membrane using

^{99m}Tc-DTPA still showed a highly abnormal situation. The fact that ^{99m}Tc-DTPA clearance measurements are so sensitive to changes in the surfactant layer could be of importance in the management of ventilated patients in the I.C.U.

The proven sensitivity to changes in the permeability of the alveolar-capillary membrane is at the same time reason to question the usefulness of ^{99m}Tc-DTPA to perform permeability measurements. It is suggested that ^{99m}Tc-DTPA is too small a molecule to enable discrimination between intermediate and severe damage to the alveolar-capillary membrane and therefore the use of larger molecules as, for instance ^{99m}Tc-labelled human serum albumin (HSA), is advocated. In **chapter 6**, a study is presented in which the dependency of the pulmonary clearance of ^{99m}Tc-HSA is investigated in rabbits receiving, and not receiving exogenous surfactant replacement therapy after lung lavage. It was concluded that the pulmonary clearance of ^{99m}Tc-HSA is at least partially dependent on the integrity of the surfactant system. The study also showed that high airway pressure ventilation significantly alters permeability for ^{99m}Tc-HSA and that the formation or existence of pulmonary oedema has some unknown influences on the diffusion rate of ^{99m}Tc-HSA across the alveolar-capillary barrier. This leads us to suggest that the use of ^{99m}Tc-HSA does not provide many advantages over the use of ^{99m}Tc-DTPA and that for each molecule there probably is a specific situation for application.

Another extensively used feature of modern medicine that may have a deleterious effect on the pulmonary surfactant system is the use of volatile anaesthetic vapours as, for instance, halothane. In **chapter 7**, the effects of 1.0% halothane with 30% and 99% oxygen on the permeability of the alveolar-capillary membrane was investigated by measuring the ^{99m}Tc-DTPA clearance rate. Halothane mixed with pure oxygen significantly increased the permeability of the alveolar-capillary membrane, suggesting that this combination of gases altered the structure of the alveolar epithelium and/or the integrity of the alveolar surfactant layer. This could be a factor which contributes to the development of various per- and postoperative complications, such as lung

atelectasis and pulmonary oedema which, in severe cases, could necessitate prolonged mechanical ventilation after anaesthesia.

In the study presented in chapter 8 the integrity of the alveolar-capillary membrane is examined in smokers and non-smokers. Pulmonary ^{99m}Tc-DTPA clearance rate was measured, as well as transudation of albumin and urea in bronchoalveolar lavage fluid. The integrity of the pulmonary surfactant system was assessed by surface tension measurements and phospholipid yield measurements in lavage fluids. The latter measurements were clearly reduced in smokers. The rate constant for the pulmonary clearance of ^{99m}Tc-DTPA correlated with variables reflecting surfactant function. The increase in clearance rate of ^{99m}Tc-DTPA was not accompanied by an increase in transudation of large and small molecules, e.g. albumin and urea, into the alveoli. These findings support the hypothesis that increased clearance of ^{99m}Tc-DTPA in smokers is related to surfactant dysfunction.

The studies presented in this thesis indicate the importance of the pulmonary surfactant system for pulmonary clearance rate measurements using ^{99m}Tc-labelled molecules as, for instance DTPA and HSA. This noninvasive and simple technique could therefore, with certain limitations, be an important new tool to assess the integrity of the surfactant system.

SAMENVATTING EN CONCLUSIES

Dit proefschrift richt zich op het belang van een intact long surfactant systeem en onderzocht wordt in hoeverre dit systeem beïnvloed wordt door verschillende medische behandelingen, inclusief kunstmatige beademing en anesthesie procedures. Daar de permeabiliteit van de alveolus-capillair membraan een betrouwbare en gevoelige parameter is voor de functionele integriteit van het surfactant systeem, is deze gemeten d.m.v. de klaring snelheden van radio-actief gemaakte stoffen.

Een optimaal functionerend surfactant systeem is cruciaal voor de gas uitwisseling in de longen. De surfactant laag in de longalveolus maakt deel uit van de alveolus-capillair membraan en voorkomt het samenklappen van de alveolus en maakt snelle diffusie van zuurstof en kooldioxyde mogelijk. Tegelijkertijd echter beperkt het de transfer mogelijkheden van eiwitten en andere moleculen van de bloedbaan naar de alveolaire luchtruimte. Veranderingen in de integriteit van de surfactant laag bepaalt daardoor de integriteit van de gehele barriere. Om de functionele staat van de surfactant laag te testen zijn verschillende technieken beschikbaar: Long compliantie metingen, biochemische en biophysische analyse van long extracten en long ^{99m}Tc-DTPA klaring metingen. Alle onderzoeken m.b.t het surfactant systeem in dit proefschrift zijn uitgevoerd d.m.v deze laatste techniek. In **hoofdstuk 1** wordt een algemene introductie gegeven van deze methode.

In alle experimenten waren de konijnen onder anesthesie en verlamd zodat kunstmatige beademing noodzakelijk was om de dieren in leven te houden. Gezien het on-fysiologische karakter van kunstmatige beademing is het logisch te veronderstellen dat gebruik van deze technieken directe of indirecte effecten zal hebben op de structuur van de long en het surfactant systeem in het bijzonder. De invloeden van de verschillende beademingstechnieken wordt besproken in **hoofdstuk 2**. In dit hoofdstuk wordt ook een mogelijk mechanisme aangegeven over hoe beademing het surfactant systeem verstoort. De keuze van beademing wordt nog belangrijker als het longsurfactant systeem al beschadigd is en het is mogelijk dat de

diverse methoden verschillende mogelijkheden bieden voor restauratie processen. In **hoofdstuk 3** is deze hypothese getoetst met behulp van konijnen. De bestudeerde beademingsmethoden waren: Hoogfrequentie jet beademing (frequentie van 2 en 15 Herz) (HFJV) en volume gecontroleerde PEEP beademing met een frequentie van 0.5 Hz. Deze beademingstechnieken zijn gebruikt in surfactant deficiente en gezonde longen. De resultaten van de experimenten geven aan dat bij HFJV van 15 Hz de alveolus-capillair membraan de laagste doorlaatbaarheid voor ^{99m}Tc-DTPA heeft. Het lijkt erop dat juist deze manier de beste condities schept voor restauratie van het beschadigde surfactant systeem. Daarentegen bleek dat HFJV van 2 Hz uiterst schadelijk was voor de longen.

Wanneer het longsurfactant systeem is beschadigd, dan is de permeabiliteit van de alveolus-capillair membraan verhoogd voor ^{99m}Tc-DTPA. Wanneer er een duidelijke relatie is tussen de gemeten permeabiliteit van de alveolus-capillair membraan en het surfactant systeem zou het zo moeten zijn dat een toegenomen surfactant concentratie in de alveolus een verlaagde gemeten permeabiliteit zou moeten opleveren. Deze hypothese is onderzocht in de studie beschreven in **hoofdstuk 4**. De surfactant concentratie is verhoogd d.m.v intraveneuze Ambroxol® toediening of intratracheale surfactant toediening. De resultaten van deze studie laten inderdaad zien dat een toegenomen surfactant concentratie in de alveoli een verlaagde klaring van ^{99m}Tc-DTPA te zien geeft.

Bloedgassen zijn belangrijke parameters waarop de arts vaak zijn respiratoire beleid afstemt op de I.C.U. Het is natuurlijk de vraag of deze parameters de meest gevoelige zijn om een beginnende respiratoire insufficientie of een herstel daarvan weer te geven. De studie beschreven in **hoofdstuk 5** laat zien dat m.b.v bloedgassen bij kunstmatige beademing het surfactant systeem niet adequaat beschreven kan worden. Terwijl de bloedgassen zich genormaliseerd hadden was de gemeten permeabiliteit van de alveolus-capillair membraan nog steeds hoogst abnormaal.

De bewezen gevoeligheid van de pulmonale ^{99m}Tc-DTPA klaring methode voor veranderingen in de alveolus-capillair membraan is aan de andere kant ook reden voor vraagtekens om deze techniek voor permeabiliteitsmetingen te gebruiken. Er wordt gesuggereerd dat het ^{99m}Tc-DTPA-molecuul te klein is om discriminatie mogelijk te maken tussen intermediaire en zware schade aan de alveolus-capillair membraan. Grotere moleculen, zoals ^{99m}Tc-gelabelde humaan serum albumine (^{99m}Tc-HSA) zouden daarvoor beter geschikt zijn. In **hoofdstuk 6** wordt een studie beschreven die de pulmonale klaring van ^{99m}Tc-HSA beschrijft in konijnen en waarin permeabiliteitsmetingen met ^{99m}Tc-HSA worden gerelateerd aan de integriteit van het surfactant systeem. Geconcludeerd wordt dat de pulmonale doorlaatbaarheid voor ^{99m}Tc-HSA gedeeltelijk afhankelijk is van het surfactant systeem; dat de doorlaatbaarheid voor dit molecuul verhoogd is na applicatie van hoge drukken beademing bij deze dieren en dat de vorming cq aanwezigheid van longoedeem nog onbekende gevolgen heeft voor dit soort metingen. Het gebruik van ^{99m}Tc-HSA in plaats van ^{99m}Tc-DTPA heeft althans in dit stadium nog geen duidelijke voordelen en waarschijnlijk is er voor elk molecuul een specifieke gebruikssituatie.

Een routine-handeling in de hedendaagse geneeskunde in het toedienen van anaesthesie bij patienten. Hierbij wordt veelvuldig gebruik gemaakt van zgn volatiele anaesthesie gassen zoals bijvoorbeeld halothaan die bekend staan om hun mogelijk negatieve invloed op het surfactant systeem. In **hoofdstuk 7** worden de effecten van 1.0 % halothaan vermengd met 30% en 99% zuurstof gemeten op de permeabiliteit van de alveolus-capillair membraan. Halothaan vermengd met puur zuurstof laat de permeabiliteit van deze membraan toenemen, daarmee aangevende dat de combinatie van deze twee gassen de structuur van de alveolaire epitheel aantast en/of de integriteit van de surfactant laag aanwezig in de alveoli. Dit proces zou een rol kunnen spelen bij de ontwikkeling van verschillende per- en post-operatieve long complicaties zoals atelectasis en pulmonale oedeemvorming.

In **hoofdstuk 8** wordt een studie beschreven bij rokers en niet-rokers, waarin de

integriteit van de alveolus-capillair membraan wordt onderzocht d.m.v. zowel de ^{99m}Tc-DTPA klaring techniek als metingen van de transudatie van albumine en ureum in de bronchoalveolaire lavage vloeistof. Het longsurfactant systeem is ook onderzocht door metingen van de oppervlakspanning en fosfolipidgehalte in de long lavagevloeistof. Het bleek dat met name bij de laatste twee methoden de waarden verminderd waren ten opzichte van gezonde niet-rokers. De gemeten ^{99m}Tc-DTPA klaring snelheid correleerde met variabelen van de surfactantfunctie. De toename in doorlaatbaarheid van de alveolus-capillair membraan voor ^{99m}Tc-DTPA werd niet aangetoond voor albumine en ureum. Deze resultaten ondersteunen de hypothese dat de toegenomen permeabiliteit voor ^{99m}Tc-DTPA in rokers samenhangt met veranderingen in de functionele integriteit van het aanwezige surfactant systeem.

De studies in dit proefschrift tonen het belang aan van het pulmonale surfactant systeem voor permeabiliteitsmetingen verricht met ^{99m}Tc-gelabelde moleculen zoals DTPA en HSA. Deze non-invasieve en simpel uit te voeren techniek is daarom een belangrijke nieuwe techniek voor testen van de integriteit van het surfactant systeem.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd geboren op 31 augustus 1966 te Rotterdam. In 1984 behaalde hij het VWO-diploma aan de scholengemeenschap "Calland" te Rotterdam. Aansluitend begon hij in 1984 met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam, alwaar hij in 1988 het doctoraalexamen geneeskunde behaalde. Gedurende zijn studie werd hij student-assistent op de afdeling Experimentele Anaesthesiologie van de Erasmus Universiteit onder leiding van Prof. Dr. B. Lachmann. Na zijn doctoraalexamen trad hij in dienst bij de Erasmus Universiteit als wetenschappelijk onderzoeker alwaar tot begin 1992 dit proefschrift werd bewerkt onder begeleiding van Prof. Dr. B. Lachmann.

LIST OF PUBLICATIONS

Bos JAH, Schairer W, Schaffers JT, Tenbrinck R, TenHave-Opbroek AAW, Bakker WH, Wollmer P and Lachmann B. Effects of high frequency jet ventilation on the pulmonary clearance of ^{99m}Tc-DTPA in respiratory failure in rabbits. Brit J Anaesth 1989; 63:59S-64S.

Bos JAH, Armbruster S, Mohajer M, Hurrell M and Lachmann B. "EUROPA" eine allgemeine Hard- und Softwarelösung zur Datenerfassung und Verarbeitung in der Anaesthesie. Der Anaesthesist, 1989; 38 Supplement 1:358 (Abstract).

Wollmer P, Schairer W, Bos JAH, Bakker WH, Krenning EP and Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA during halothane anaesthesia. Acta Anaesthesiol Scand, 1990; 34:572-575.

Bos JAH, Scheiter W, Gumbrecht W, Montag B, Eijking EP, Armbruster S, Erdmann W and Lachmann B. Development of a micro transmission cell for in vivo measurement of SaO_2 and Hb. Adv Exp Med Biol. 1990, 47-52.

Eijking EP, Bos JAH, Armbruster S, Schelter W, Gumbrecht W, Erdmann W and Lachmann B. On-line measurement of SaO_2, Ht and Hb using a micro transmission cell. Adv Exp Med Biol, 1990, 53-58.

Schelter W, Gumbrecht W, Montag B, Bos JAH, Eijking EP and Lachmann B. A micro transmission cell for monitoring of oxygen saturation and hemoglobin concentration. Sensors and Actuators, 1990, B1, 495-498.

Gumbrecht W, Schelter W, Montag B, Bos JAH, Eijking EP and Lachmann B. Monitoring of blood PO_2 with a thin film amperometric sensor. IEEE, Transducers, 1991, Digest of technical papers, 85-87 (in press)

Bos JAH, Lachmann B. Surfactant function: is it influenced by artificial ventilation? In: The surfactant system in the lung. Cosmi EV, Di Renzo GC, Anceschi MM (eds.), Houndmills and London, Macmillan academic and proffesional LTD, 1991, 96-107.

Bos JAH, Wollmer P and Lachmann B. Effects of increased alveolar surfactant content on Tc-99m-DTPA clearance. Eur Respir J, 1991; 4:225s.

van Daal GJ, Gommers D, Bos JAH, van Golde PHM, van der Kamp R and Lachmann B. Huan recombinant surfactant is as effective as commercially available natural surfactants. Eur Respir J, 191; 4:492s.

Bos JAH, Van Daal GJ, Wollmer P and Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA: a very sensitive index for permeability changes in the alveolar-capillary barrier in rabbits. Adv Exp Med Biol, 1991, in press.

Bos JAH, Lachmann B. Effects of artificial ventilation on the pulmonary surfactant system. In: New Aspects in Respiratory Insufficiency. (E. Rügheimer ed.), 1991, in press.

Bos JAH, Wollmer P, Bakker WH, Hannappel E, Lachmann B. Pulmonary clearance of ^{99m}Tc-DTPA and experimentally increased alveolar surfactant content in rabbits. J. Appl. Physiol, 1991, in press.

Tenbrinck R, Sluiter W, Silveri F, Bos AP, Eijking EP, Go ATJI, Bos JAH, Tibboel D, Lachmann B. Effect of artificial ventilation on pulmonary antioxidant enzyme activities in a congenital diaphragmatic hernia rat model, Adv Exp Med Biol, 1991, accepted for publication.

Van Daal GJ, Bos JAH, Eijking EP, Gommers D, Hannappel E, Lachmann B. Surfactant replacement therapy improves lung compliance and functional residual capacity in endstage influenza A pneumonia in mice. Am Rev Respir Dis, 1991, accepted for publication.

Lachmann B, Tütüncü AS, Bos JAH, Faithfull NS, Erdmann E. Perfluorooctylbromide (perfluobron) instillation combined with mechanical ventilation: an alternative treatment of acute respiratory failure in adult animals. Adv Exp Med Biol, 1991, accepted for publication.

Bos JAH, Wollmer P, Eijking EP, Gommers D, van Gelder W, Bakker WH, Lachmann B. Pulmonary ^{99m}Tc-human serum albumin (^{99m}Tc-HSA) clearance and effects of surfactant replacement after lung lavage in rabbits. 1991, submitted for publication.

Schmekel B, Bos JAH, Kahn R, Venge P, Wohlfart B, Lachmann B, Wollmer P. Integrity of the alveolo-capillary barrier and alveolar surfactant in smokers. 1991, submitted for publication.

Gommers D, Vilstrup C, Bos JAH, Larsson A, Werner O, Hannappel E, Lachmann B. Effects of exogenous surfactant therapy on lung function cannot be assessed by dynamic compliance. 1991, submitted for publication.

Vilstrup C, Gommers D, Bos JAH, Hannappel E, Lachmann B, Larsson A, Werner O. Surfactant instilled in premature lambs improves lung volume and ventilation homogenity within five minutes. 1991, submitted for publication.

Bos JAH, Lachmann B. Effects of different ventilator settings on lung mechanics: with special reference to the surfactant system. 1991, submitted for publication.