Studies of Partial Liquid Ventilation in a Rabbit Model of Acute Lung Injury

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Declaration

I declare that this thesis was written entirely by myself. The investigational work detailed within this thesis was performed collaboratively by myself and my colleague Dr Ben Stenson, Consultant Neonatologist, Neonatal Unit, The Simpson Memorial Maternity Pavilion, Royal Infirmary of Edinburgh. The work was performed in the Animal Laboratory of Edinburgh University. Collaboration of more than one researcher was necessary as adequate rest periods are required to comply with Home Office regulations for the care of anaesthetised animals. I greatly acknowledge Dr Stenson's help particularly with the static lung compliance measurements and data collation.

The data and discussion presented herein are unique to this thesis and were prepared by myself.

This thesis has not been submitted in candidature for any other degree, diploma or professional qualification.

Signed

Date 16/10/2003

Contents

Dedication

To my parents James and Euphemia, and my sister Lesley.

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Abstract

Introduction; Acute respiratory distress syndrome (ARDS) is a major cause of death on intensive care units. The syndrome is characterised by severe hypoxia, widespread atelectasis and decreased lung compliance, much of this impairment due to depletion and inhibition of surfactant. Therapy is supportive. For research purposes, many of the features of ARDS may be mimicked by a saline lavage model of lung injury.

Perfluorocarbons have many characteristics similar to natural surfactants and have been used as an experimental means of supporting the injured lung, applied either by special liquid ventilators or as a hybrid technique with conventional gas ventilators known as partial liquid ventilation. Although nebulization is an accepted means of drug delivery, Perfluorocarbons had never been applied by a nebulized route to support the injured lung.

Surfactant therapy has also been used to support the lung in the presence of ARDS. Surfactant preparations can be split into two broad categories; artificial surfactants containing no surfactant proteins and natural, but more expensive protein containing surfactants. There may be additional benefits of combining surfactant with Perfluorocarbons.

Methods; This study examined the differences between saline lavaged lung injured rabbits, in the following treatment groups;

i) control,

ii) partial liquid ventilation i.e. poured perfluorocarbon PF 5080;

iii) nebulized PF 5080,

iv) the artificial surfactant Pumactant used in isolation,

v) the natural porcine surfactant Curosurf used in isolation,

vi) Pumactant used in combination with partial liquid ventilation and

vii) Curosurf used in combination with partial liquid ventilation.

The following end points were examined;

a) Survival in saline lavaged rabbits to 12 hours,

b) Differences in oxygenation between the treatment groups to 6 hours,

c) Differences in dynamic compliance between the treatment groups to 6 hours, and static compliance between control, the Pumactant and the Curosurf groups,

d) The appearance of computed tomography densities between the control, nebulized and partial liquid ventilation groups in frozen, ex- vivo lung preparations.

Results; Survival to twelve hours in this study was greatest in the partial liquid ventilation alone or the combination of Pumactant with partial liquid ventilation. Oxygenation was improved by partial liquid ventilation, the natural surfactant Curosurf, or the combination of either Pumactant, or Curosurf with partial liquid ventilation.

Dynamic compliance improved after partial liquid ventilation, Curosurf in isolation or the combination of Curosurf with partial liquid ventilation.

Static compliance improved after treatment with Curosurf but not after treatment with Pumactant.

There was a significant difference in density distributions in the CT scanning studies between the partial liquid ventilation and nebulized perfluorocarbon, and the control

and partial liquid ventilation groups, but not between control and the nebulized groups implying little perfluorocarbon is delivered to the lungs by this route.

Comments; In summary PF 5080 can be used for partial liquid ventilation in this model of lung injury to improve survival, oxygenation and lung mechanics. Using this method, nebulization of PF 5080 cannot be supported as it seems to have little effect on these end-points and may not be delivered in any significant amount to the respiratory tract.

Curosurf is superior to Pumactant in improving oxygenation, and lung mechanics in this model of acute lung injury. There seems to be little difference between Curosurf and poured PF 5080 in terms of these endpoints.

Whether partial liquid ventilation becomes more widespread in the support of the injured lung will depend on further research applications including means of application and trials in humans.

List of Abbreviations

ANOVA	Analysis of Variance
ARDS	Acute Respiratory Distress Syndrome
°C	Degree Celsius
Cdyn	Dynamic compliance of the respiratory system
cc	Cubic Centimetres
cm	Centimetre
cm H_2O	Centimetres of water pressure
CO_2	Carbon Dioxide
Crs	Static compliance of the respiratory system
CT	Computer Tomography
CVP	Central Venous Pressure
DPPC	DiPalmitoyl Phosphatidyl Choline
Dyn(e)/cm	Units of surface tension
ECMO	Extra Corporeal Membrane Oxygenation
ELA	Extra Corporeal Lung Assist
F_IO_2	Fractional inspired oxygen concentration
FRC	Functional Residual Capacity
g	Gram(s)
HFV	High Frequency Ventilation
HU	Hounsfield Unit(s)
Hz	Hertz
ITU	Intensive Therapy Unit

kg	Kilogram
kPa	Kilopascal
kV	Kilovolt
mA	Milliampere
ml	Millilitre
mm	Millimetre
mm Hg	Millimetre of mercury
MOF	Multi-Organ Failure
mol	Mole
O_2	Oxygen
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
PaO_2/ F_IO_2	Ratio of arterial partial pressure of oxygen to fractional inspired oxygen concentration
PAGE	Perfluorocarbon Associated Gas Exchange
PEEP	Positive End-Expiratory Pressure
PFC	Perfluorocarbon(s)
PIP	Peak Inspiratory Pressure
PLV	Partial Liquid Ventilation
pMDI	Pressurized Metered Dose Inhaler
SD	Standard Deviation
SEM	Standard Error of the Mean
SP-A	Surfactant Protein A
SP-B	Surfactant Protein B

SP-C	Surfactant Protein C
SP-D	Surfactant Protein D
T_I	Inspiratory time
TLV	Total Liquid Ventilation
VILI	Ventilator Induced Lung Injury

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Chapter 1

Introduction

ARDS

The acute respiratory distress syndrome (ARDS) is a major cause of death in intensive care practice. The syndrome has an incidence in the United Kingdom [2] of 4.5 cases per 100 000 population and in the same study accounted for 2.5% of ITU (intensive therapy unit) admissions. This may be an underestimate. The clinical course is variable, but once on the ITU patients with ARDS represent a significant workload, as the mean duration of mechanical ventilation is 10-14 days, whilst 10-20% of these patients remain ventilator dependent for more than 3 weeks [3].

The multiplicity of underlying causes has led to a wide range of quoted mortality of 10-90%[4], although a frequently quoted mortality is 50-60% [4;5]. Prognosis is worsened by risk factors such as advanced age, pre-existing diseases and sepsis [5].

If one accepts a mortality of approximately 50-60%, then the mortality for this syndrome has remained essentially unchanged since Ashbaugh et al's original description in 1967 [6]. Krafft et al found no trend towards a reduction of mortality over time in 16 major studies encompassing a total of 1790 patients between 1979 and 1994 [4]. Milberg et al found rather more cause for optimism in his group's analysis of their institute's survival rates for ARDS between 1983-1993, particularly the latter years of this study and in those less than 60 years old and with sepsis

syndrome as their cause for ARDS[7]. The exact reason for this recent reduction in mortality is unclear.

Be that as it may, ARDS, although rare in the population overall, represents a considerable burden to intensive care units, and a patient diagnosed as having "ARDS" has a substantial chance of dying of this condition.

Definition of ARDS

One reason for this variable mortality may have been the laxity of definition of ARDS. In broad terms, ARDS is characterised by hypoxaemia, pulmonary oedema due to leakage from pulmonary capillaries which appears as infiltrates on chest radiograms, and poorly compliant lungs, associated with certain underlying conditions. Most commonly these other conditions are sepsis syndrome, aspiration, primary pneumonia and multiple trauma [8].

In an attempt to standardise definitions for the purposes of epidemiology and future clinical trials, an American-European consensus conference on ARDS, held in October 1992 defined ARDS more exactly at the severe end of an acute lung injury spectrum as follows [8];

i) the timing of ARDS must be acute and persistent, lasting days to weeks. The acute lung injury/ARDS must be associated with one or more known risk factors, characterised by arterial hypoxaemia resistant to oxygen therapy alone and associated with diffuse radiologic infiltrates;

ii) there must be an impairment of oxygenation defined as a $PaO_2/F_IO_2 \leq 200mmHg$;

iii) a pulmonary artery wedge pressure ≤ 18 mmHg when measured, or no clinical evidence of left atrial hypertension.

Although associated with several underlying conditions such as multiple trauma or sepsis, the exact cause of ARDS remains unknown. There is therefore no known single point in the disease process where one might successfully intervene and consequently treatment remains supportive.

Respiratory Supportive Strategies

Previous supportive respiratory strategies had strived assiduously to achieve "normal" arterial partial pressures for oxygen and carbon dioxide[9].

Unfortunately this supportive strategy can cause damage in its own right. In order to achieve a normal PaO_2 or $PaCO_2$, tidal volumes of 10-15ml/kg body weight were used. In the presence of diseased lungs, with poor general compliance, high airway pressures had to be used in order to generate these large tidal volumes. This pressure induced damage (barotrauma) may be seen in the most extreme circumstances as pneumothorax, pneumomediastinum, pneumopericardium and bronchiolar rupture, but also more subtly as pulmonary interstitial emphysema, perivascular haemorrhage, and perialveolar haemorrhage [10].

There is however evidence to suggest that the damaging factor is not so much pressure *per se* but the forcing of too great a volume into the lungs (overdistention) - so called "volutrauma [11;12]. This volutrauma during mechanical ventilation has been termed ventilator induced lung injury (VILI)[13 14].

Ventilator Induced Lung Injury (VILI)

As mentioned above, mechanical ventilation can itself damage the lungs. There has been a recent growing body of evidence that modes of ventilation which lead to repeated opening and closing of airways and large pressure amplitudes,[15] not only cause lung parenchymal damage but may also promote inflammatory mediator production and bacterial translocation from the lung. This may contribute to multiple system organ failure [13]. The corollary of this is that means of supporting ventilation which limit derecruitment and overdistention of lungs may be less damaging [16 17] and may demonstrate a reduced inflammatory response [18 19]and possibly reduced mortality [20].

There have been a number of concepts to deal with these problems of abnormal lung mechanics, complicated by ARDS representing a non-homogenous disease [21]. Gattinoni et al [22] described the acutely injured lungs as consisting of three zones; zone H (healthy) representing normal lung; zone R (recruitable) representing atelectatic lung which could be opened up by therapeutic manoeuvres such as positive end-expiratory pressure (PEEP) to become functional lung units once more, and zone D (diseased) which were resistant to such therapeutic manoeuvres. Those areas of lung which are atelectatic do not allow areas of gas exchange and represent shunt, and hence significantly contribute to hypoxaemia. Thus if one considers the amount of actual lung available for gas exchange, which may be as little as 20-30% of total units, it is as if the patient has a "baby" or shrunken lung [22]; [12].

Positive End-Expiratory Pressure (PEEP)

PEEP increases transpulmonary pressure and increases lung volume at end-expiration i.e. increases functional residual capacity (FRC). This reduces shunt.[23]. PEEP also helps reduce formation of pulmonary oedema in various ways e.g. by haemodynamic effects [24] such as reducing cardiac output [25]. An appropriate amount of PEEP i.e. neither too little nor too much, may also prevent damage to surfactant [26] [27]. In addition to improving gas exchange and decreasing pulmonary oedema, PEEP will oppose tidal opening and collapse of distal airways, lessening lung injury and may decrease release of inflammatory mediators and cytokines from the injured lung. [13]

However PEEP may have undesired effects such as the previously mentioned decrease in cardiac output (by limiting venous return) [25;28] and it has been associated with barotrauma [29]. So although some PEEP may be desirable, too much may be harmful [30].

Several methods have been used to try to determine optimal or "best PEEP". Methods used have included;

a) lung mechanics, i.e. the PEEP which gives the maximum improvement in total static compliance [31],

b) PEEP which reduces shunt fraction to less than 15% of the cardiac output [32],

c) the setting which permits the reduction of inspired oxygen tensions to minimal levels (consistent with acceptable oxygenation) [33],

d) the level which demonstrates the greatest recruitment of lung units on CT imaging [34].

Some of the strategies above present significant risks or logistical problems. Gallagher et al's adherence to increasing the PEEP to ensure a reduction in shunt fraction to less than 15% of cardiac output, no matter which level that may be, resulted in some spectacularly high PEEP settings [32]. In a case series of 59 patients requiring greater than 10 cm H_2O of PEEP (range 15-65 cm H_2O) with a mean of 27 (standard deviation 7 cm H_2O). They quote an incidence of pneumothorax of 10% in their series. Certainly a PEEP level of 65 cm H_2O would seem to be against current opinion of aiming to keep end-inspiratory static (plateau) pressures of less than 30-40 cm H_2O [35].

Gattinoni's group used CT scanning as a means of monitoring the effect of PEEP [34]. This is obviously not a test that can be performed at the bedside, and requires that patients with often perilous oxygenation status be transferred to CT scanners.

A consensus opinion based on lung mechanics and recommended by the American-European Consensus Conference on ARDS was a total PEEP which obliterated the lower inflexion zone (Pflex) of the inspiratory static pressure volume curve of the respiratory system [35], representing the re-opening of collapsed areas of lung. This attempted to ensure near complete recruitment and the consensus was that this was a level of 10-15 cm H_2O in most instances. In terms of recruiting more alveoli by increasing mean alveolar pressure, there was no consensus as to whether the best means to do this was by adding more PEEP or by extending inspiratory time. They made the general comment that the least mean airway pressure should be used which accomplishes acceptable arterial oxygenation at a non-toxic concentration of inspired oxygen (implying this might be an F_IO_2 of 0.65, although without specifically stating what acceptable oxygenation was) [35].

One question could be where best to apply PEEP? In another CT scan study, Gattinoni's group examined the effect of the distribution of tidal volume and recruitment of lung tissue in patients with ARDS [36]. This study looked at the various regional (ventral to dorsal) effects of PEEP with varying plateau pressures (i.e. end inspiratory pressures), whilst keeping the tidal volume constant. They found that increasing PEEP decreased the tidal volume distribution to the most ventral (non-dependent) portions of the lung, whilst significantly increasing the tidal volume to the dorsal (dependent) parts of the lung. This finding they explained as the worsening of compliance in the ventral parts of the lung as these non-dependent portions were increasingly overstretched, coupled with the improving compliance as atelectatic areas in the most dependent parts of the lung were held open. Increasing plateau pressures increased recruitment ventrally to dorsally, but no difference was seen between plateau pressures of 21 through to 46 cm H_2O. Their third conclusion was that increasing PEEP from 0 to 20 cm H_2O increased lung recruitment ventrally to dorsally.

These findings should be taken in conjunction with the findings of the same group from the CT scanning study in 1993 which demonstrated that the hydrostatic pressure of oedematous overlying lung can cause compression atelectasis in the most dependent parts of the lung which may be eliminated by applying an appropriate amount of PEEP [34].

In short, in ARDS some PEEP is needed in the most dependent (most atelectatic) sections of the lung. But a consequence of the heterogeneity of the injured lung is that what is an adequate amount of PEEP for the atelectatic regions may cause overdistention in the non-dependent or healthy regions of the lung. There is therefore a very great risk of overdistention of these areas of lung [12]. Furthermore, the forcing of a tidal volume intended for the whole lung, into an area of only 20% of the whole lung may lead to overdistention and ventilator induced lung injury (VILI).

What would be ideal is a mode of maintaining end-expiratory pressure in a graded manner applying it preferentially where it is most needed.

Prior attempts at applying these respiratory supportive strategies in clinical practice have been varied. Aside from PEEP (as mentioned above), investigators have tried permissive hypercapnia (in an attempt to limit the size of tidal volume and consequently the degree of overdistention of the lung)[37]; High Frequency Ventilation (HFV) [38] and extracorporeal lung assist [39;40] in an attempt to rest the lung.
None had shown undisputed reductions in mortality. The failure of traditional ventilatory strategies to prevent end-expiratory collapse may have contributed to lung injury[41] and interfered with the surfactant system in the lungs [27 42].

Alveolar Surface Tension and the Role of Surfactant

Even under normal circumstances, surface tension arises at the gas/ alveolar fluid interface. This is because the forces between adjacent molecules of the fluid lining the curved surface of the alveolus are much greater than those between the liquid and

gas interface. This forces the liquid surface area to be as small as possible[43]. Thus the surface of a "bubble" (for bubble read alveolus) forms a sphere which is the smallest surface area of the bubble for a given volume. The surface tension generates a pressure which could be predicted from the Law of Young and Laplace [44;45].

The Law of Young and Laplace[45]

$\Delta P = 2\gamma/r$

where p= pressure; γ= surface tension; r= radius

In plain English this means if surface tension, particularly in small alveoli, were not reduced, pressure within these alveoli would rise, which would force gas out of these alveoli into neighbouring lower pressure (larger) alveoli, making these small alveoli smaller still. A vicious circle of alveolar collapse would ensue[46].

Under normal circumstances surfactant lowers the surface tension within alveoli at the gas/ fluid interface. This is because phospholipid molecules in surfactant, particularly DiPalmitoyl Phosphotidyl Choline (DPPC) have hydrophobic acyl chains orientated towards the gas, and hydrophilic polar portions orientated towards the liquid surface [47]. These molecules align along the alveolar surface. As the alveolus becomes smaller towards the end of expiration, the intermolecular repulsive forces of the phospholipids oppose the normal forces of attraction between the surface molecules responsible for surface tension[43] . During surface compression the phospholipids consisting mostly of DPPC forms a rigid monolayer [47]. So alveoli are maintained open and stabilised by the presence of surfactant.

Additionally any group of alveoli which develop a tendency to collapse will to some extent be held open by the stable surrounding alveoli- so called interdependence[43;48]. A factor contributing to the generally poor compliance of the lungs in ARDS is the deficiency in endogenous surfactant function [49 44 50].

Surfactant also keeps the alveoli relatively dry. In a similar way that surface tension has a tendency to collapse alveoli, there is also a tendency for surface tension to suck fluid from the interstitium into the alveoli. By reducing surface tension this tendency is reduced[43;45]. Hill postulates that there may be a "water repellency" component i.e. that surfactant forces fluid into pockets or "pools" within the alveoli from which the fluid is returned to the interstitium[51]. Others claim the traditional view[52], that there is a lining layer of liquid, deeper in some areas such as at corners on which the surfactant sits, and that the surfactant reduces surface tension. This helps to balance forces such as the hydrostatic pressure in the alveolar liquid layer. It is acknowledged however that there is some evidence for both the traditional and Hill's view [52].

A summary of some of the functions of pulmonary surfactant is shown in Table 1 [45].

Table 1 A summary of some of the functions of pulmonary surfactant.

Biophysical functions of surfactant
Prevents collapse of the alveoli and lungs during expiration.
Supports inspiratory opening of the lungs.
Prevents lung oedema formation by balancing hydrostatic filtration forces.
Stabilises and keeps small airways patent.
Improves mucociliary transport.
Translocates particles <6microns into the hypophase of the epithelial lining fluid. and facilitates removal of particles and cellular debris from the alveoli into the large airways.
Immunological, nonbiophysical surfactant functions.
Phospholipids suppress proliferation, immunoglobin production and cytotoxicity of lymphocytes, and also inhibit endotoxin stimulated cytokine release from macrophages.
SP-A & SP-D modulate phagocytosis, chemotaxis and oxidative bursts of macrophages.
Neutralization of endogenous mediators like radicals and reactive oxygen species.
SP-A & SP-D opsonize various micro-organisms for easier phagocytosis
Binding and capture of bacterial toxins by SP-A and SP-D.

Thus surfactant helps both keep alveoli patent and reduces the pressure required to re-expand them, and consequently work of breathing [44;53]. Furthermore endogenous

surfactant reduces alveolar lung water, and may have an important role in deactivating inflammatory mediators, and have cytoprotective properties [54].

Normal Structure and Metabolism of Surfactant

Structure

Surfactant is normally produced by the type II pneumocytes in the lungs, and is composed of the following major components; neutral lipids (mainly cholesterol which constitutes 6-8% of total lipids) and phospholipids (80-90% of total lipids, the majority of which (75%) is DiPalmitoyl Phosphatidyl Choline -DPPC). The other major phospholipids are phosphotidylglycerol (10%), phosphotidylethanolamine (5%), phosphotidylserine and phosphotidylinositol (5%) with less than 5% sphingomyelin. There are also four different surfactant specific proteins called SP-A, SP-B, SP-C and SP-D [47;55 45].

The surfactant components are stored in multilayered membraned bodies known as lamellar bodies in the Type II pneumocytes. After secretion they are converted to a lattice like tubular lipid double layer called tubular myelin (vide infra in Metabolism section; see Figure 1)[44;47]. Tubular myelin is the main intra-alveolar reservoir of surfactant. It may be that the surface film is also formed by intra-alveolar membrane structures[47]. For formation of these structures and for transitions to occur between them other surfactant components, particularly the surfactant proteins are required[47].

SP-A is a hydrophilic highly glycosylated multimeric protein weighing approximately 650kD. SP-A binds phosphatidylcholine, and is felt to promote formation of myelin like tubules as well as promoting adsorbtion. It is also thought

to be involved in the release of surfactant from type II pneumocytes [53] and in immunoregulation within the lung[44 45].

SP-B and SP-C are hydrophobic and help in the adsorbtion and spreading of surfactant at the gas-liquid interface. They also constitute some degree of resistance to inactivation in the presence of acute lung injury [56 47].

SP-D is a hydrophilic protein composed of four subunits each of approximately 43 kiloDaltons in molecular mass. Its functions are less well understood. It is thought to contribute little to the surface active properties of surfactant, but may enhance the production of oxygen radicals in alveolar macrophages and may have a role in the host defences of the lung [47].

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Metabolism[44;47];

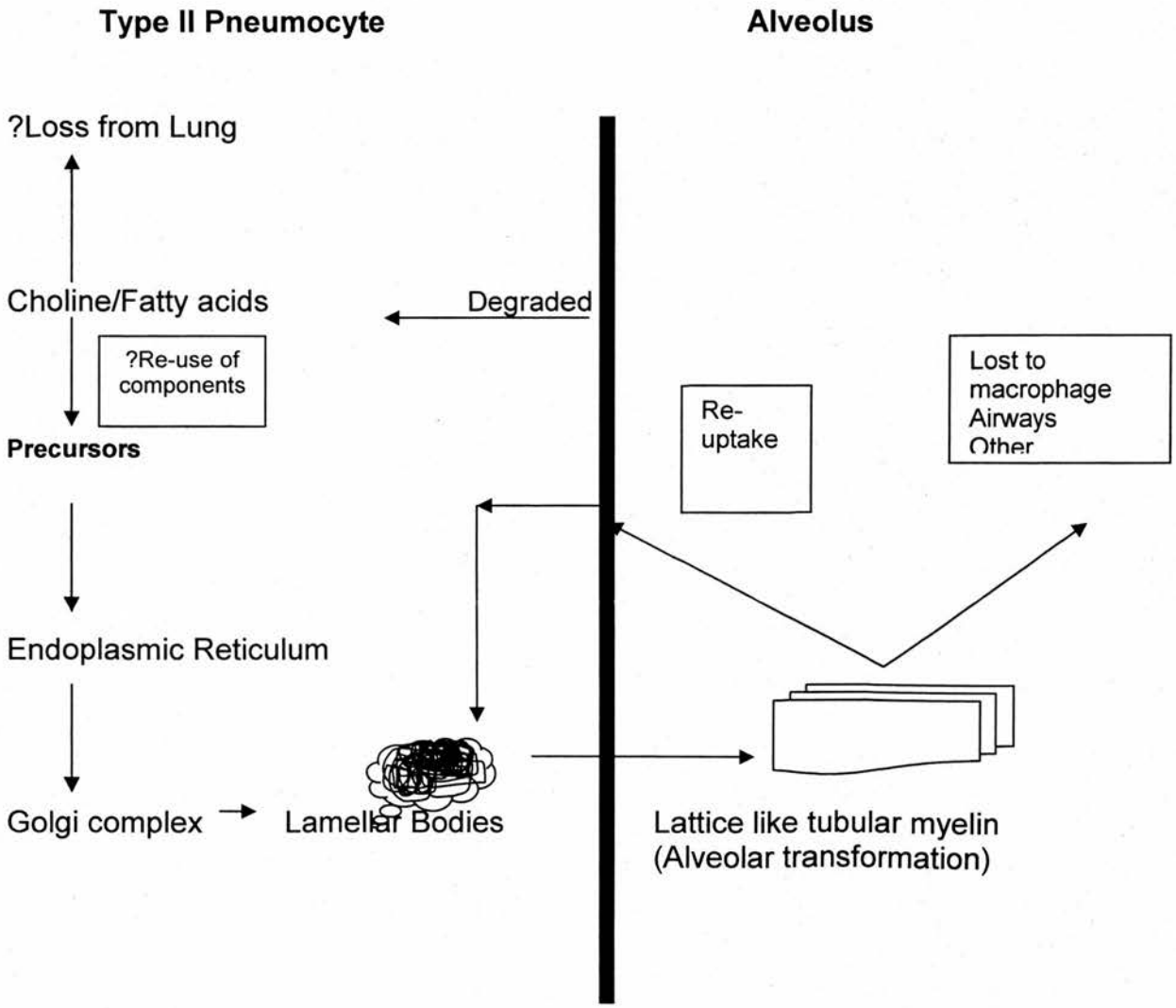
Surfactant phopsholipid is synthesised in the endoplasmic reticulum of Type II pneumocytes, before being transferred to the lamellar bodies prior to secretion via the Golgi complex (see Figure 1 which depicts a schematic representation of major pathways of surfactant phospholipids metabolism). Surfactant proteins are also produced in the endoplasmic reticulum, but are transferred to the lamellar bodies via multivesicular bodies. The lipid and protein components of surfactant are first fused in the lamellar bodies. Secretion of surfactant into the alveoli seems to be regulated by several factors including increased ventilation, acetylcholine, $ß_2$ agonists and prostaglandins. There may even be a feed-back mechanism associated with SP-A. During inspiration spreading of phospholipids from tubular myelin must occur rapidly in order to spread over the expanding surface. The composition of phospholipids may help, the unsaturated phospholipids being squeezed out leaving

only a stiff DPPC monolayer behind. This process may be enhanced by surfactant proteins, particularly SP-B and SP-A (in the presence of SP-B).

The monolayer thus formed has a turnover time variously cited as approximately 3-11 hours, though some cite clearance of surfactant from the alveolar space to occur with a half-life of approximately 20 hours[53].

Surfactant lipids and proteins are removed by alveolar macrophages and the more important route of re-uptake into Type II pneumocytes. Whether the phospholipids are further degraded is unclear, as is the exact means of recognition of these phospholipids and proteins by macrophages and Type II cells.

Figure 1. Schematic representation of major pathways of surfactant phospholipid metabolism [44]



Pulmonary Surfactant in the Presence of Acute Lung Injury.

The importance of an adequate amount of normally functioning surfactant was illustrated as long ago as 1959 by Avery and Mead who highlighted the respiratory distress syndrome of the newborn [46]. Although a differing syndrome from ARDS, there were many similar features, including reduced lung compliance, intrapulmonary shunting, atelectasis and decreased functional residual capacity. Indeed abnormalities of surface tension of minced lungs at autopsy were recorded in Ashbaugh et al's original description of ARDS[6].

The exact nature of the deficit in acute lung injury/ARDS is unclear. There is evidence of an absolute deficiency of total surfactant [57], refuted by others [58 59]. The poor function of surfactant in the face of ARDS may be due to the presence of inhibitors [59 50] or abnormally constituted surfactant [49 58]. Despite these differences (perhaps due to varying experimental models and extraction techniques), as Holm and Matalon note, the surfactant system is in some way compromised, and although this may not be the primary pathogenic factor this should not imply that the effect on the surfactant system is merely of secondary importance[53]. Any surfactant deficiency, whatever its underlying nature, will have an important role in the pathophysiology of acute lung injury, and attempts to rectify this deficiency warrant further investigation.

Surfactant therapy for infants requiring mechanical ventilation for respiratory distress syndrome has been standard therapy since 1990 [60].

However, studies assessing the effects of surfactant in adult human acute lung injury have been few [61] [62;63] and there had been no direct comparisons between preparations of apoprotein containing surfactant (such as Curosurf) and non-apoprotein containing surfactants (such as Pumactant) in adult human ARDS.
The few attempts at trying to rectify this deficit by administration of exogenous surfactant in humans have to date been disappointing [62;63], although there have been procedural criticisms of these studies. There are only two randomised controlled trials of surfactant administration to patients with ARDS; Anzuetto et al for The Exosurf Acute Respiratory Distress Syndrome Study Group, and Gregory et al .

The Exosurf Acute Respiratory Distress Syndrome Study Group[62]

These investigators administered Exosurf, a synthetic surfactant consisting of predominanatly dipalmitoylphosphatidylcholine, and free of apoproteins, to 364 patients (361 controls) with ARDS due to sepsis. They found no statistically significant difference between treated and control groups in terms of oxygenation, duration of mechanical ventilation, length of ITU stay or survival at 30 days.
The calculated average administered dose of Exosurf was 112mg/kg body weight per day (which may in its own right be inadequate) but further admitted that as little as 4% of the nebulized dose could have actually reached the lungs.
The authors also acknowledged that the lack of apoprotein could have contributed to the drug's apparent lack of effect.
They also recognised that ARDS may be the respiratory manifestation of a systemic upset, and that Exosurf was only treating one component of that disorder.

One point mentioned in the methodology, but not stressed in the discussion was the large amount of saline in which the surfactant was dissolved. The dilution was 13.5mg DPCC to 1ml 0.45% saline. Thus, assuming the average administered dose of 112mg/kg/day this would mean 8.3ml/kg/day of 0.45% saline applied to the lungs (or approximately 530ml in a 70 kg patient). It is easy to imagine that this would have a deleterious effect in a patient who already has precarious oxygenation.

The Bovine Surfactant Beractant (Survanta) in Patients with Acute Respiratory Distress Syndrome[63].

These investigators studied the effect of the bovine surfactant, Beractant in a randomised prospective controlled open-labelled clinical study to obtain preliminary information with regard to safety and efficacy. Survanta, the form of Beractant produced by Abbott Laboratories, in addition to containing phospholipids, acid lipids and fatty acids, also contains surfactant proteins B and C. The study was design to determine whether this preparation given early in the course of ARDS could alter PaO_2/F_IO_2, decrease peak inspiratory pressure and decrease PEEP, and increase the quasistatic pulmonary compliance. There were 4 groups totalling 59 patients; control; a group who received 8 doses of 50mg/kg; a group who received 4 doses of 100mg/kg; and a group who received 8 doses of 100mg/kg. The doses were based on previous animal work. Administration of Beractant was made via a catheter inserted into the endotracheal tube just proximal to the carina. F_IO_2 was decreased at 120 hours only for the group treated with 4 doses of 100mg/kg. The group administered 100mg/kg for 8 doses showed more cardiovascular system failure on day 2, thought to be due to the volume of surfactant administered. They concluded

that the results were encouraging and were good justification for further multi-centre trials.

Requirements of a Supportive Therapy in Acute Lung Injury/ ARDS

Thus if one were describing the required properties of a means of ventilatory support for the acutely injured lung, this description may be as follows;

i) in the first instance as an absolute *sine qua non* it must be able support gas exchange i.e. oxygen must be delivered to the blood and carbon dioxide (CO_2) removed from it;

ii) it must make the optimum use of available gas exchange units, by recruiting atelectatic areas;

iii) it must not contribute to iatrogenic lung damage i.e. gas exchange must be achieved with the minimum of volutrauma/ barotrauma to the lung;

iv) it should go some way to rectifying the abnormalities of surface tension caused by the change in lung surfactant;

v) it should alter the progression of the natural history of the disease

vi) it should be non-toxic.

Although these points are contentious, there is evidence that many of these requirements are fulfilled by liquid ventilation , more specifically by partial liquid ventilation with perfluorocarbon(s) [PFC] [64-67].

Review of Liquid Ventilation

The ability to sustain mammalian respiration through a liquid medium is generally accredited to Kylstra et al who described the total immersion of mice using hyperbaric salt solutions [68]. The hyperbaric conditions were required so that sufficient oxygen could be dissolved to sustain cell respiration. As an illustration, a few of these mice were subjected to pressures of 160 atmospheres, equivalent to ambient pressure 1 mile below the surface of the sea.

Unfortunately many of these animals died from what was thought to be respiratory acidosis due to the increased work of breathing.

Clark and Gollan described total immersion techniques of cats and mice but at atmospheric pressures [69]. The agents used were either perfluorocarbons (PFC) or silicone oils. The silicone oils proved to be too toxic for use with living tissues. This left only the PFC, which remain the only agents available for use in this method of respiratory support in either animal models or human subjects.

The Perfluorocarbons

The perfluorocarbons (PFC) are organic compounds in which the hydrogen ions have been replaced by halogens (predominantly fluoride ions). The common production techniques are electrochemical fluorination, by heating the organic product with cobalt trifluoride or careful direct fluorination with gaseous fluoride [70]. Non medical uses of PFC include their use as cooling fluids, insulators and as constituents for products of the cosmetic industry. They were originally developed

during the production of the atomic bomb, in an attempt to find agents which would not react with the unstable uranium isotopes [71;72].

They have also found medical applications as contrast agents during magnetic resonance scanning, as sensitising agents during radiotherapy, and as possible intravenous oxygen carrying agents [73-75]. The presence of the fluoride ion makes the compound more radio-opaque.[70]

Possible Modes of Action

PFCs are potentially of use in liquid ventilation as they are stable inert compounds which do not react with living tissues. Although immiscible both with hydrophobic and hydrophilic fluids, they can dissolve large quantities of gases. As a general rule, gas solubility in a PFC decreases in the order CO_2 >> oxygen> carbon monoxide> nitrogen. Linear PFCs such as Perflubron are said to dissolve oxygen more effectively than cyclic PFCs such as Perfluorodecalin [76]. However oxygen solubility is also inversely proportional to the molecular weight and directly related to the number of fluorine ions present [76]. It should be stressed that oxygen solubility in PFC is purely a passive process unlike the binding and release of oxygen to haemoglobin in the blood.

There is an inverse relationship between vapour pressure of a PFC and molecular weight i.e. the lower the molecular weight, the higher the vapour pressure [75].

Some examples of PFCs which have been used for liquid ventilation are shown in Figure 2. Their physical properties are shown in Table 2. As can be seen, many of the characteristics are fairly similar such as molecular weights (416-499 Daltons), oxygen solubility (approximately 50ml of oxygen per 100ml of PFC, when equilibrated against 100% oxygen at 25 °C) and carbon dioxide solubility

(approximately 200ml CO_2 per 100ml PFC at 1 atmosphere pressure and at body temperature).

Furthermore, PFC have a low surface tension approximately 12-18 dyne/cm, and may to some extent compensate for deficient surfactant function. The low surface tension is due to weak intermolecular forces of the PFC [77].

PFC are nearly twice as dense as water, and if applied to the lungs will gravitate to the most dependent parts of the lung. This function has been compared to the application of PEEP (which some investigators have termed "PEEP in a bottle"), where PEEP is needed most, that is in the collapsed, dependent areas of the lung [78-80]. This may be a means of optimising ventilation and perfusion.

Indeed optimisation of ventilation/ perfusion matching, diverting blood away from dependent poorly ventilated alveoli has been suggested to be one reason why oxygenation improves with liquid ventilation in lung injury[81]. However liquid ventilation does not improve oxygenation in normal mammals[82]. It has also been suggested that this is due to interference with (the already optimal) V/Q matching, increased shunt and possibly diffusion impairment [83].

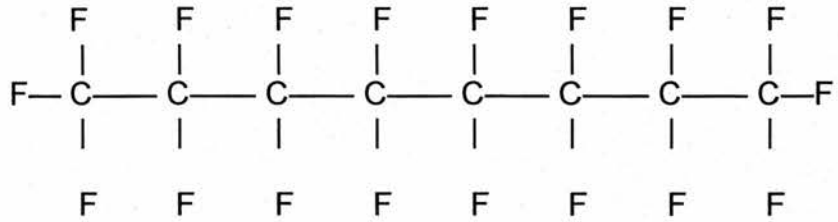
Despite high density, PFC have a viscosity similar to water.

They have also been said to reduce the inflammatory response to lung injury [84 85].

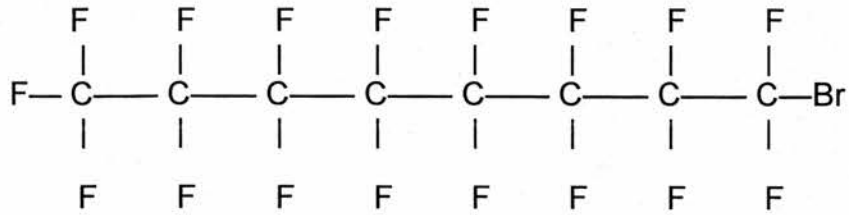
Figure 2

Structure of Selected Perfluorocarbons

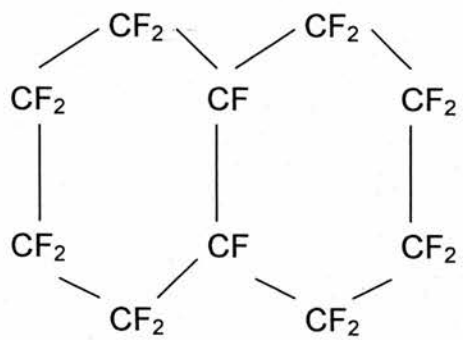
FC 3280/ Also the structure of PF 5080 (C_8F_{18})



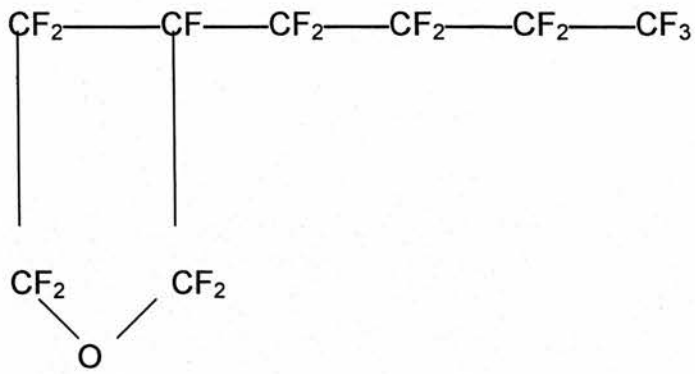
Perflubron ($C_8F_{17}Br$)



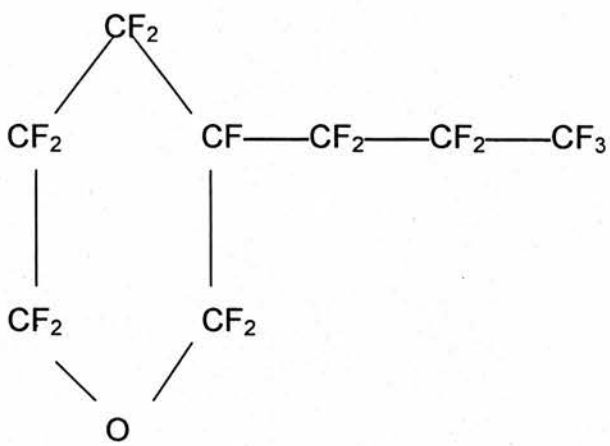
Perfluorodecalin ($C_{10}F_{18}$)



FC 77; 50/50 Mix of the two following isomers of $C_8F_{16}O$

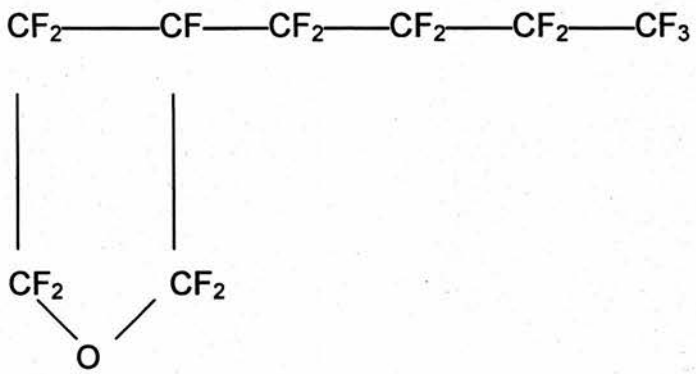


+



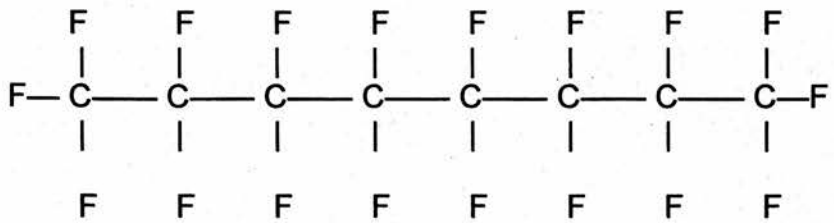
FC 75; 40/20/40 mix of the following PFCs;

40%



+

20%



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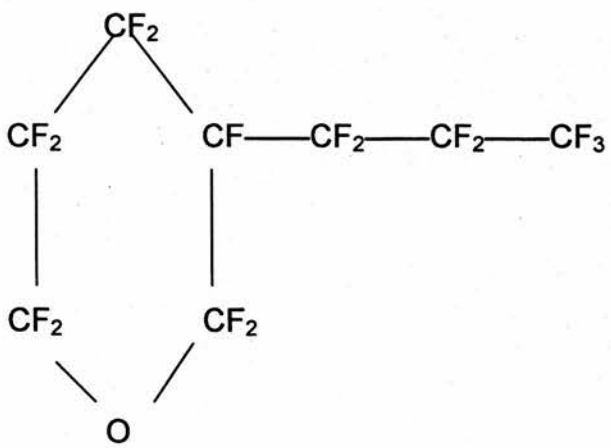
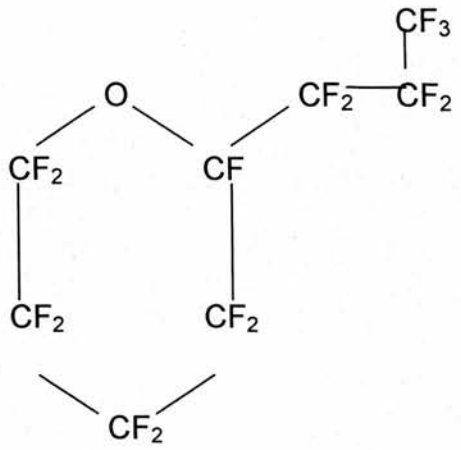
40%



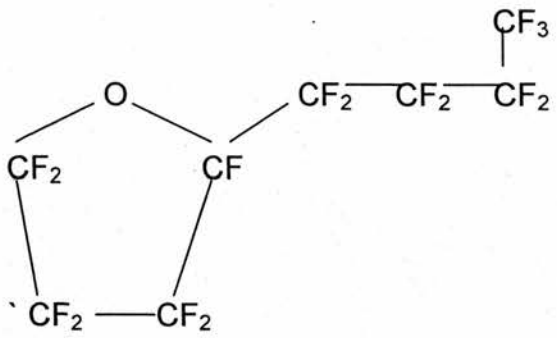
RIMAR 101 ($C_8F_{16}O$)

Structure of Predominant Isomers of RIMAR 101
Perfluoro propyl tetrahydropyran



+

Perfluoro butyl Tetrahydrofuran



Structure of Perflubron and Perfluorodecalin [72]. Structure of PF 5080, FC 3280, FC 75 and FC 77, data on file with Company, (3M Chemical Group, Zwijndrecht, Belgium). Structure of RIMAR 101, data on file with Company (Miteni SPA, Vicenza, Italy).

Choice of Perfluorocarbon

Theoretically, to be useful for partial liquid ventilation, a PFC should possess the following properties; it should have the ability to contain sufficient amounts of dissolved oxygen and carbon dioxide at body temperature. It should have a lower surface tension than the surface tension of injured lungs (often cited as 20-25 dyne/cm) [6 53].

It should be denser than body tissues, so that it will sink to the most dependent regions of the lung but not so viscous that the resistance of the fluid in the airways becomes problematic [65], or that CO_2 removal is inhibited. The CO_2 removal problem can to some extent be compensated for by the ability to dissolve very large amounts of CO_2 [86].

The boiling point and saturated vapour pressure are characteristics important for a number of reasons. A low boiling point and consequently high saturated vapour pressure means that the PFC will evaporate off quickly, and therefore require more frequent replenishment (with cost and labour implications). However as the main route of elimination for PFC used in liquid ventilation is by evaporation [70 75 64], it will be eliminated from the lungs more quickly when the process of weaning from partial liquid ventilation is required. A balance of these two facets is therefore required.

With regard to this thesis, surface tension and viscosity have a bearing on the ease of ultrasonic nebulization. The lower the surface tension, the more drug can be delivered; the greater the viscosity, the less drug can be delivered [87]. However, at the

time of designing this study there was no readily available information on how any specific PFC would behave when nebulized for treating lung injury.
The PFC should be non-toxic, safe and from a practical point of view be readily obtainable and cheap.

Until recently the only PFC licensed for (human) use as a medium to support respiration by the Food and Drug Administration in the United States is Perflubron, the tradename of which is *LiquiVent* ® (Alliance Pharmaceutical Corporation, San Diego, USA). The access to this agent is restricted to a small number of centres, and many studies both in humans and animal models have used other agents on an *ad hoc* basis.

Table 2

Physical Properties of Selected PFC Liquids used in studies to date.

	FC- 77*	Rimar 101	FC - 75*	Perfluoro-decalin	Per-flubron	FC 3280*	PF 5080*
Chemical Formula	50/50 Mix of 2 iso-mers of $C_8F_{16}O$ see Fig 2	$C_8F_{16}O$	Mix 40/20/ 40 $C_8F_{16}O$ C_8F_{18} $C_8F_{16}O$ See Fig 2	$C_{10}F_{18}$	$C_8F_{17}Br$	C_8F_{18}	C_8F_{18}
Molecular Weight (Daltons)	Av. 416	416	Av. 420	462	499	438	438
Boiling Point (°C)	97	101	102	142	143	102	102
Density @ 25 °C (g/cc)	1.78	1.77	1.78	1.95	1.93	1.76	1.76
Kinematic Viscosity (centi-stokes @ 25 °C)	0.80	0.82	0.82	2.90	1.1	0.8	0.8
Vapour Pressure mmHg @ 37 °C	85	64	63	14	11	Approx. 51	Approx. 51
Surface Tension (dyne/cm @ 25 °C	15	15	15	15	18	15	15
O_2 Solubility @ 25 °C(ml gas/100ml liquid)	50	52	52	49	53	Approx 48	Approx 48
CO_2 solubility @ 37 °C(ml gas / 100ml liquid)	198	160	160	140	210	Approx 176	Approx 176

*Data on file 3M Belgium NM, Zwijndrecht, Belgium. Remainder of table [70]

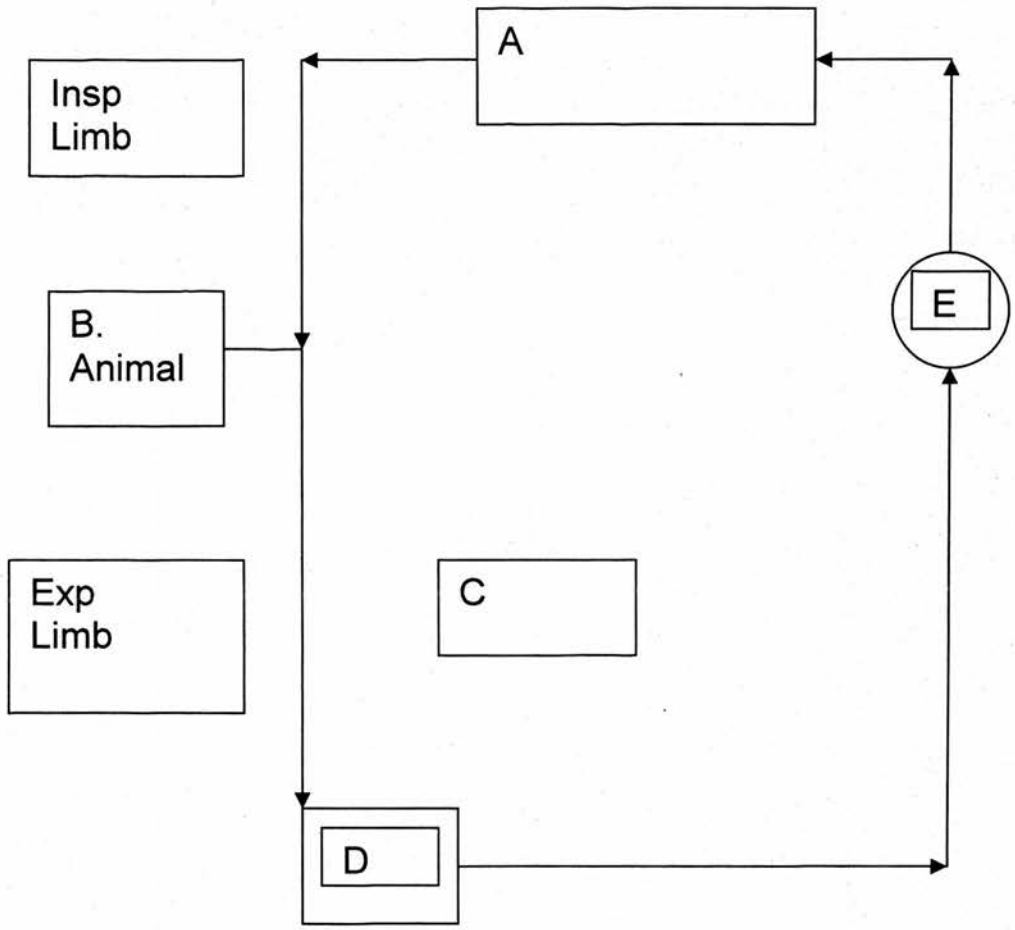
RIMAR 101 [88].

Application of the liquid medium.

As mentioned above, the early experimentation with liquid ventilation involved the total immersion of small animals. For larger animal models of liquid ventilation an extracorporeal circuit was required (see Figure 3).

Figure 3

Schematic Representation of a Liquid Ventilator (for Total Liquid Ventilation) From Hirschl et al [89]



Key

A= Heat Exchanger/ Membrane Oxygenator and Oxygen inlet

B= Animal connected to an endotracheal tube and extracorporeal circuit

C= Pinch valve connected to timer and occlusive roller pump

D= Reservoir

E= Occlusive roller pump

This was connected to the respiratory tract via an endotracheal tube. The lungs were then filled to functional residual capacity (FRC) and tidal volumes of liquid introduced during an "inspiratory" phase and let out of the lungs during an "expiratory" phase. This method where the lungs are filled to FRC and then ventilation is conducted with tidal volumes of liquid is known as *total* liquid ventilation [88;90 89 91].

In the ensuing thirty years, work has continued on the use of liquid ventilation in a number of animal models, utilising normal, premature and lung injured animals [92 93 70;94 95].

The major disadvantages of this technique are that it requires complex and expensive pieces of apparatus such as an extracorporeal circuit [96 89]. Additionally the extracorporeal circuit itself has had developmental problems, particularly the tendency of the expiratory valve to jam.

Fuhrman et al described the first use of partial liquid ventilation in 1991, although this group called the technique perfluorocarbon associated gas exchange (PAGE)[82]. This was a hybrid technique combining filling the lungs of normal piglets to FRC with the perfluorocarbon FC 77, but superimposing gaseous tidal volumes with a standard mechanical ventilator upon this.

Liquid Ventilation in Humans

The first human use of a liquid ventilation technique had been by Greenspan et al in 1989 [97]. This was however not strictly partial liquid ventilation, as Greenspan's

description was in a 28 week gestation neonate who had her (failing) standard ventilation interrupted to allow 2 three minutes periods of total liquid ventilation (separated by 15 minutes). This was achieved with an ad hoc apparatus allowing drainage of oxygenated PFC into the lungs from a burette suspended above the patient. Then gas ventilation was resumed. The lungs had been filled to FRC (30ml/kg) then ventilated with 15ml/kg liquid tidal volumes allowing gravity assisted drainage into a vessel below the patient. Each tidal volume was held within the lungs for 15 seconds and the patient was "ventilated" with a liquid respiratory frequency of 2-3 "breaths" per minute. There was a sustained increase in PaO_2 and a decrease in $PaCO_2$ which was maintained for about 2 hours post procedure. There was an accompanying increase in compliance and a reduction in airways' resistance. Despite this the patient died 19 hours later.

The same centre reported this patient along with two others in a case series in 1990, who were ventilated using a similar technique [98]. All were 23-28 weeks gestation in whom conventional therapy for severe respiratory distress had failed. Although all died within 19 hours, it was felt that this reflected the severity of the underlying lung disease before initiation of liquid ventilation, and demonstrated the potential of this treatment for pulmonary dysfunction in the pre-term neonate.

Partial Liquid Ventilation (PLV)

The first recorded use of partial liquid ventilation in humans is accredited to Hirschl et al who described their experience of PLV in a group of 19 mixed adult, paediatric and neonatal patients [99]. This was an uncontrolled study to evaluate the safety and efficacy of PLV. These patients were being supported with extracorporeal lung assist (ELA) and so had the back up of an extracorporeal means of oxygenation and

removing CO_2 should there have been a deterioration of respiratory function on initiation of PLV. During periods off ELA, the patients demonstrated an improvement in alveolar-arterial oxygen difference and also an improvement in static pulmonary compliance. Fourteen of the patients were successfully weaned from ELA and eleven survived, which was the expected survival for patients who were so gravely ill. Causes of death were cited as irreversible lung disease (4 patients), cerebrovascular accident (1 patient), ischaemic encephalopathy after cardiac arrest (1 patient), and multi-organ failure (2 patients). The authors concluded that, "*partial liquid ventilation can be safely used in patients with severe respiratory failure and may improve lung function*".

Papers have subsequently been published describing PLV in adult [100], paediatric [101] and neonatal practice in a population of premature infants with respiratory distress syndrome [102].

There are currently a number of phase III trials of PLV in ARDS being conducted in North America and Europe.

There had been no studies at the time of originally drafting this thesis investigating the efficacy of introducing PFC into the respiratory tract for the purposes of partial liquid ventilation by means of nebulization.

Potential Problems with Partial Liquid Ventilation.

Pneumothorax

Hirschl et al's described pneumothoraces in 9 of their 19 patients [99]. However, 6 of these patients had had pneumothoraces prior to initiation of partial liquid ventilation. It is therefore difficult to differentiate the side effect of the treatment from a known association of the disease. Despite this, the suspicion of an association between PLV

and pneumothorax persists. Verbrugge and Lachmann give a number of explanations for this [66]. At end expiration alveoli will collapse if insufficient PEEP is applied. This may be as "gas" PEEP or "liquid" PEEP. If insufficient gas PEEP is applied to support the non-PFC filled alveoli, shear force will cause pneumothorax. Secondly, with regard to this level of gas PEEP, the gas PEEP required will depend on the disease state of the individual alveolus. In those alveoli unable to reduce surface tension to less than the PFC (usually PFC used for liquid ventilation have surface tensions of approximately 15-18 dyne/cm-see Table 2) the level of gas PEEP required during PLV will be less than required during conventional ventilation. However in those alveoli with a still functioning surfactant system, coating the alveolus with a thin layer of PFC with a higher surface tension than the "healthy" alveolus will necessitate an increase in PEEP for these areas.

Another problem with PLV is uncertainty as to where the gas is actually going. If the gas is going to those parts of the lung not fully filled with liquid this could cause severe lung overdistention and may result in a high rate of pneumothorax. It is therefore suggested by these authors that "fluid PEEP" i.e. PLV be combined with pressure controlled modes of ventilation in which the pressure level in any alveolus should never exceed the pressure level set on the ventilator to prevent dangerous alveolar overdistention.

Haemodynamics;

Filling the thorax to approximately functional residual capacity with a dense, non - compressible fluid may reasonably be expected to have major effects on the circulation.

Evidence from Houmes et al [103] showed that there were no deleterious effects on the circulation even when large animals (sheep) with an average thoracic antero-posterior diameter of 24 cm were filled with a total of 25ml/kg Perflubron. Several reasons were postulated why this theoretical worry should prove less of a problem in practice.

Some of the earlier studies suggesting that there was haemodynamic compromise were in TOTAL liquid ventilation models i.e. where there was an uninterrupted column of dense PFC. This does not happen with PARTIAL liquid ventilation. Indeed some investigators go as far as to say that "PLV has no known side effects on the cardiovascular system" [64].

One might also have worried that a dense incompressible fluid may increase pulmonary vascular resistance (PVR) and strain on the right side of the heart. Effects upon PVR may in fact be a balance of deleterious and positive effects. On the negative side, the PFC may compress blood vessels increasing pulmonary arteriole pressure and resistance. Set against this is the reduction in hypoxic pulmonary vasoconstriction caused by better oxygenation.

Lactic Acidosis [104]

Despite studies indicating generally well preserved haemodynamic status, a metabolic acidosis has been noted in some animal studies[94 104]. This has been ascribed to changes in regional organ blood flow during mechanical ventilation with positive end-expiratory pressure, even if general global cardiovascular status was maintained. However, this has by no means been universally reported [103]. This may be due to redistribution of blood flow [105] or an ineffective vascular volume [106].

Weaning back to gaseous ventilation.

Many researchers report successful re-conversion to gaseous ventilation after a period of liquid ventilation [90]. However a temporary impairment of arterial oxygenation which took up to several days to return to pre-liquid breathing levels has also been noted [90 107]. This effect has been subscribed to residual PFC remaining in the lungs, causing a diffusion defect, low ventilation/ perfusion areas or decreasing alveolar PaO_2. Reversible changes in lung mechanics have been noted after liquid ventilation [108 107].

As a net amount of PFC is allowed to evaporate off and is not replaced during the conversion phase from liquid to gaseous ventilation, it may be necessary to increase PEEP to prevent atelectasis[109]. This is to compensate for the loss of the fluid bulk of PFC helping to splint open the alveoli at end expiration.

A worrying fact is that occult pneumothoraces may only become obvious when PFC is allowed to evaporate [80].

Interpretation of Standard Chest Radiograms

The development of occult pneumothoraces underscores the point that the radiodensity of PFC may hinder interpretation of radiograms. Although said to be mostly a problem with radiodense atoms such as iodine and bromine[70], even PFC which do not contain these atoms may hinder interpretation of x-rays. As an example, a plain chest film of a rabbit undergoing PLV with PF 5080 (C_8F_{18}) and a control rabbit are shown towards the end of this thesis (Figure 22).

Blocking of the Endotracheal Tube

Hirschl's group reported mucous plugging in one of their 19 patients which compromised gas exchange and required aggressive suctioning and bronchoscopy [99].

This would be in keeping with the finding reported later in the same paper that exudate in the peripheral airways and alveoli could be effectively lavaged to the central airways whence it could be removed by suctioning.

CO_2 clearance

Ineffective elimination of CO_2 was particularly a problem with inadequate settings of total liquid ventilators [90 108]. The difficulty has been ascribed to the high viscosity of PFC compared with gas together with the low CO_2 diffusion coefficient. This problem could be reduced by choosing the appropriate (total liquid) ventilator settings [93].

Elimination and Toxicity

This problem may be largely theoretical. The studies looking at the metabolism of PFC record slow clearance from the body. Elimination even when administered intravenously is largely via the lung and little if any metabolism takes place. Perfluorocarbons are however taken up by the reticular endothelial system.[74]. Reports of perfluorochemicals used as intravenous oxygen carriers cite half-lives of as long as 500 days [75]. Even when applied only to the respiratory tract, traces have been found three years after a 1 hour exposure to liquid ventilation. Admittedly there seems to be no inflammatory reaction to these substances which are regarded as innocuous and chemically inert [75], and the authors of the paper which found trace amounts present three years later still concluded that the PFC (Caroxin-F) could "*be breathed without residual deleterious effects*" [110]. Others have found no effects on the function of surfactant when extracted from dogs five days after exposure [111]. This should also be set against a background of the high mortality of ARDS i.e. a high chance of death in the next few hours to days compared with an unproven

theoretical risk of an indeterminate side effect years in the future. However there is caution in using agents which may be retained in the body for so long even if they appear to do no harm.

Nebulization

Nebulization is an accepted means of delivery for agents such as the B_2 agonists, steroids, ribavirin and surfactants [112;113 114]. Could it be an effective means of delivery for PFC, compared to a similar dose poured into the respiratory tract?

If the administration of PFC is to translate from the laboratory to clinical practice, it must be seen to have practical benefits. The practical clinical advantage of being able to administer PFC by a method (i.e. nebulization) more readily familiar to staff working on an intensive therapy unit is obvious. If the nebulized route were found to be equally effective as pouring PFC into the respiratory tract, it could allay fears about cardiovascular compromise due to a thorax full of PFC impairing venous return [105], even if some would argue that these fears are unfounded [103].

There are three commonly used methods of introducing nebulized agents into the respiratory tract; pressurised Metered Dose Inhalers (pMDI), jet nebulizers and ultrasonic nebulizers [112]. Factors felt to influence the efficacy of delivery are the presence of a holding chamber, siting on the inspiratory limb of a ventilator, absence of humidity and reducing the respiratory rate to increase inspiratory time, all of which increase delivery to the subject.

There had been no previous attempts at trying to administer PFC to the lungs as a means of respiratory support. Pressurised metered dose inhalers consequently were not produced for PFC and jet nebulizers may upset the settings of mechanical

ventilator. In terms of this study, if PFC were to be delivered to the respiratory tract by a nebulized technique, then this would have to be done by means of a ultrasonic nebulizer. There is also the theoretical benefit of delivering a greater dose if this technique is used.

As mentioned above, there was no information available on optimal nebulizer settings specifically for PFC use in ARDS. There was also no information on the distribution pattern of PFC used therapeutically in ARDS. As a general theoretical comment, in order to deposit the PFC in the alveoli, the settings on the ultrasonic nebulizer should be chosen to deliver particles less than 5 microns. Larger than this may result in the droplets being deposited in the larger conducting airways [87].

Radiographic imaging and ARDS

Traditionally radiographic assessment of patients with ARDS has been done by portable chest radiograms [6;115]. There are however problems with this means of assessment. Poor patient mobility and lack of co-operation combined with limited access due to presence of equipment on the ICU has reduced the quality of films. Also, the requirement to use a portable x-ray apparatus is associated with several problems. The poorer power output of these portable units means that longer exposure times must be used (which causes movement artefact). There is also the well documented problem of apparent magnification of the heart due to the shorter film-to-focus distance and the antero-posterior projection of the supine patient. Consistency of film exposure is therefore difficult. Attempts to circumvent this problem have been made with storage phosphor technology. The incident energy is trapped in the phosphor plate as a latent image. This is then subsequently read by

laser, and the detected signal transformed by an analogue-to-digital converter to digital data. This can be presented in a format which resembles a conventional film, but with the advantages that there is a reliable consistency of optical densities between serial films and the ability to post process films and enhance difficult areas [115].

The radiological-pathological co-relations of ARDS can be thought of as follows;
Stage 1 (0-24 hours post initiating cause) in which there is early exudation of fluid but may be "silent" by standard radiographic techniques.
Stage 2 (24-36 hours post initiating cause) is where fluid leakage into the interstitium and alveolar space becomes more obvious with alveolar collapse. There may be a "ground glass" appearance to the chest x-ray.
Stage3 (c. 72hours onwards) histologically the fibrotic process starts and this may begin to manifest itself as persistence of the ground glass appearance with reticular shadowing [115].

Computed Tomography in ARDS

Computed Tomography (CT) imaging measures radiographic density of small discrete parts of the body (pixels) displayed as two dimensional images. The radiographic density is measured in Hounsfield Units (HU) and is represented by varying shades of grey; less dense images (air –1000 HU) are black through to the most dense media (e.g. bone, +1000 HU) which are white. Thus each CT image is a collection of small squares each with a discrete "grey" value which represents the mean radiographic density of a 3 dimensional cylinder/ volume of tissue called a

voxel [116;117]. Dense tissue adjacent to or within the lung may influence this average density ("partial volume effect").

CT imaging of the thorax is becoming an increasingly routine investigation in patients with ARDS with the advent of fast scanners. It has been used extensively to help in the understanding of the pathophysiology [34;117] of ARDS as well as detecting lesions which would have been missed using standard x-rays such as abscesses emphysema and mediastinal disease, and occult pneumothorax[115]. CT scanning has been vital in underscoring the non-uniform heterogenous nature of ARDS, with patches of uninvolved lung interspersed with areas involved with ARDS.
However CT imaging of the lung during liquid ventilation is limited to only a few papers[118-121], and has not been used to compare the distribution patterns of poured versus nebulized PFC in partial liquid ventilation.

Purpose of the Project

I had previous research experience of partial liquid ventilation in a porcine model of ARDS while I had been a visiting Research Fellow at the Virchow Klinik in Berlin (1995/6) [119;122;123]. I wished to consolidate this experience with a further research project. The only PFC approved by the FDA for use as a partial liquid ventilation agent at the time of drafting this project (late 1997) was Perflubron, but this was only available to a few centres. The PFC of which I had previous personal experience in Germany, FC 3280 (3M Chemicals, Bracknell, Berks, UK) had ceased to be produced by the time I was preparing the early planning of this study. However, a similar compound produced by 3M, and called PF 5080 had many similar characteristics to agents such as Perflubron (see Table 2) and FC 3280, and was readily available to me. Thus I chose PF 5080 as the liquid medium which had both reasonable physical characteristics and a ready source of supply.

A second intent was to compare the efficacy of nebulized PFC to a similar dose which was poured into the respiratory tract. The nebulized route is of course well used for administration of drugs such as $Beta_2$ adrenergic agonists on intensive therapy units. However by 1997, it had never been used as a route of administration for the study of partial liquid ventilation. If nebulization were to prove an equally efficacious way to apply PFC as pouring PFC it would have a major benefit in translating this into clinical practice. That is, most ITU staff are familiar with nebulized drugs; most are not familiar currently with (partial) liquid ventilation.

There is less potential for causing lung damage. The simplicity of administration would make the therapy more widely available. All these statements have subsequently been recognised [124].

The primary outcome measure was to be short-term survival (to twelve hours), with secondary outcome measures of gas exchange and lung mechanics, where obtainable.

There are superficial similarities between some of the properties of surfactants and PFC. However, as they may work by differing means, I therefore wished to see if any additional benefit of short-term survival, gas exchange and lung mechanics could be obtained by combining PF 5080 with surfactant. As the efficacy of the surfactant used may depend on its ability to withstand inactivation, could a difference in effect be seen between an expensive apoprotein containing surfactant and a substantially cheaper artificial apoprotein free surfactant? I chose to compare the apoprotein containing surfactant Poractant-alpha, more commonly known by its tradename Curosurf (Serono Laboratories, Welwyn Garden City, UK which cost approximately £334 per 100mg at the time of the project) with the apoprotein free surfactant, Pumactant (Britannia Pharmaceuticals Limited, Redhill, UK produced under the tradename, ALEC, which cost approximately £150 per 100mg). These were preparations available at the time in the UK, and for which I had managed to obtain a supply.

Curosurf is a natural porcine surfactant containing polar lipids mainly phosphatidylcholine which constitutes 70% of total phospholipid content and circa 1% specific low molecular weight hydrophobic proteins SP-B and SP-C. Pumactant is a 7;3 mixture by weight of Dipalmitoyl Phosphotidylcholine (DPPC) and

unsaturated Phosphotidylglycerol (PG) [125]. I chose to administer 100mg/kg, to be free from the criticism of having underdosed the subjects. This dosage has been used commonly in other studies investigating surfactant [126;127 128 1;129-131 132].

The concept of combining the surfactant preparations was based on a number of premises. As mentioned above, as there may be a number of mechanisms of action for both PLV and surfactants used in lung injury, their use may be additive. Further, surfactants are expensive, PFC relatively cheap. The cost of 1 kilogramme of PF 5080 (568ml) was approximately £30 from 3M Chemicals, Bracknell, Berks, UK. Could the combination of PFC/ surfactant show an additive effect? PFC may have a "surfactant sparing" effect i.e. cause a small dose of (expensive) surfactant to go a long way.

Furthermore, the project would allow the direct comparison of a less expensive surfactant protein free preparation (Pumactant) to be compared with an expensive surfactant protein containing preparation (Curosurf).

In addition to investigating the potential difference in mortality, gas exchange and lung mechanics parameters between poured and nebulized PFC, the effect of these modes of administration on the distribution of PF 5080 were examined on CT scanning of the excised lungs. Therefore arrangements were made to perform CT scanning of the excised lungs of 3 groups of animals; viz 10 controls, 6 poured PFC and 10 nebulized PFC. These numbers were chosen to minimise the use of animals in each group. These lungs were frozen in liquid nitrogen immediately post excision. This meant that an extra control group were prepared in liquid nitrogen rather than formalin.

Regarding Histological Analysis

The main end points for this study were intended to be the survival, oxygenation, compliance and CT scanning data. Due to the suspected difference in survival times between treatment groups (which subsequently proved well founded) histology was never intended to be a primary outcome, as it could be argued that a difference seen in animals who died several hours after inducing lung injury, compared with animals who died shortly after inducing lung injury was merely due to the effects of the prolonged mechanical ventilation. In order to compare like with like, one would have to choose a separate arm of the study in which all the groups were repeated but the animals were killed at (for example) 2 hours i.e. at a time when significant numbers of the animals in all treatment groups would be expected to still be alive. As one of the basic tenets of this project was to study the effects of longer term survival (to twelve hours- the maximum allowed under Home Office regulations), then this would have meant effectively doubling the size of this project i.e. a study as conducted with seven treatment groups to observe the parameters above to twelve hours, and an additional study with the same seven study groups terminated shortly after inducing lung injury purely for the purpose of obtaining histological data. This would have exceeded the budget available from the funding bodies. It was however suggested by the funding bodies that some limited histological analysis be done so that evidence of lung injury is demonstrated. This was discussed with members of the Pathology Department of Edinburgh University. There was some debate as to how best to prepare the rabbit lungs including whether simple en-bloc dissection and immersion in formalin be performed, as used in some studies including Lachman's original work [110;122;133-135], or whether infusion of formalin through the lungs via the

pulmonary artery post mortem, but prior to excision should be conducted as cited by others [85;136]. Another consideration was which groups should be studied. For simplicity I was advised to undertake the former i.e. block dissection and immersion, and was finally advised that samples should be taken from the control group only (as the purpose of this histological examination was merely to ascertain that the method above of saline lavage and mechanical ventilation establishes lung injury). Final analysis was done by another member of the Directorate of Pathology Edinburgh University Medical School (Dr William Wallace) to whom I am extremely grateful. The details of the histological examination, and pictures of haematoxylin and eosin slides are shown from representative samples (taken from the control group) in Chapter 3. This is to demonstrate that lung injury has occurred in animals subjected to the saline lavage and mechanical ventilation in the conditions used in our laboratory, and as representation of what the lungs would have look like should no active treatment have been given. Further conclusions regarding the effects of the various treatments upon lung histology are outwith the scope of this project, for the reasons just given. These conclusions should not be drawn from this project, but perhaps should be considered in later studies specifically designed to investigate the histological effects of PF 5080 on lung injury.

Thus the lungs were preserved post autopsy either in liquid nitrogen (for the CT scanning studies), and stored at -70°C (10 control animals, 10 animals treated with nebulized PF 5080, and six animals treated with poured PF 5080) or 10% buffered formalin (all other animals, including a further 6 treated by poured PF 5080).

Statistical Analysis and Power Calculation

The statistical package used for this project was *GraphPad Prism™ Version 2.0 and 4.0* (GraphPad Software Inc., San Diego, California, USA). Normally distributed data were analysed between the groups using Analysis of Variance (ANOVA). The test for goodness of fit to a normal distribution was the Kolmogorov-Smirnov test available on this program, using Dallal and Wilkinson's approximation to Lilliefor's method [137;138]. Data which did not correspond to a normal distribution were analysed using the Kruskal-Wallis test. Survival data were illustrated with Kaplan-Meier survival curves and analysed using the Logrank test [139;140]. Further details are given in the individual chapters. Sample size and power calculations were performed with the aid of the computer program *PS Power and Sample Size Calculations* (Copyright 1997 by W.D. Dupont and W.D. Plummer) [141;142].

Power Calculations for the Project

The accepted chance of a type I error (false positive) was the conventionally taken 1 in 20 (i.e. $P<0.05$). The accepted chance of a type II error (false negative) was the conventionally taken chance of 0.2 (thus power of the test 1-0.2 = 0.8) [143;144]. Information for power calculations was sought from references in the published literature at the time of planning the project, and tried to draw upon references using rabbits, and the saline lavage model of lung injury. Only Mrozek et al's reference had compared combinations of surfactant and PLV in treatment groups of 8 animals (saline lavaged rabbits) [145]. These researchers however had no untreated controls. Evidence from this paper suggested that in order to detect a difference in oxygenation between surfactant alone (the bovine surfactant Survanta) and the combination of Survanta and PLV (with Perflubron) of 13.4kPa (standard deviation

± 7.2kPa) there would have to be 6 animals in each treatment group (assuming P<0.05 and power of 0.8). Calculations were made using the computer program *PS Power and Sample Size Calculations.*

With regard to changes in lung compliance (and again assuming P<0.05 and power of 0.8) Mrozek's researchers [145] found a significant difference in compliance between Survanta alone and Survanta combined with PLV of 0.26 ml/cmH_2O (standard deviation ± 0.11 ml/cmH_2O), in favour of the combination. This suggested that I would have to have a minimum of 4 animals in each group to detect a difference with the Type I and Type II errors quoted above.

Survival data were harder to assess. The conventional assessment of power for a survival study would be based upon the median survival time [146 147 140]. However no previous study had studied the survival of saline lavaged rabbits to 12 hours, in the context of partial liquid ventilation. There were thus no median survival values quoted. Other surrogate guidelines were taken. Kaisers et al using a saline lavage porcine model, showed a significant improvement in survival comparing PLV to control. There were six animals in each group. Survival times for the control group were 0.1-4 hours (mean 1.8 hours ± 1.4 SD.) compared to a range of 4-16 hours (8.2 hours ± 4.5 SD) for the treatment group filled to half functional residual capacity with FC 3280 [122].

Final Size of Treatment Groups.

Taking a combination of the survival, blood gas and lung mechanic results in the available literature, as well as allowing for the originally intended differing histological preparations, and with a small arbitrary but feasible safety margin, it was

decided that the study should consist of 20 control animals, 12 PLV animals and 10 animals in each of the other treatment groups.

Thus the arms of the study were as follows;

1) Control (10 animals); formalin preparation at autopsy,

2) Control (10 animals); liquid nitrogen preparation at autopsy, then CT scanning,

3) Partial Liquid Ventilation i.e. poured PFC (12 animals); (6 animals post mortem liquid nitrogen preparation then CT scanning; 6 animals post mortem 10% buffered formalin preparation),

4) Nebulized PFC (10 animals); liquid nitrogen preparation then CT scanning,

5) Pumactant surfactant (10 animals);

6) Curosurf surfactant (10 animals);

7) Pumactant and poured PFC (10 animals);

8) Curosurf and poured PFC (10 animals).

Chapter 2

Methods

Introduction

This chapter will describe the methodology used during this project. It will include a description of the model of lung injury chosen, as well as descriptions of the important pieces of monitoring apparatus.

Mode of Experimental Lung Injury

Various species of animals have been used in the study of lung injury. They include rat [132], rabbit [84;114;148;149], cat, dog, sheep and pig [88 150 119;123;151;152]. Just as variable is the variety of methods used to induce lung abnormality or induce lung injury such as application of acid [153 154] directly into the respiratory tract, or the induction of lung injury by the injection of an intravenous agent such as oleic acid[91 136 155 156]. Septic models of ARDS have used infusions of endotoxin [151 152].

These methods are all effective but some may result in a speciously severe lung injury, perhaps causing death within 2 hours of initiation.

A further possibility of inducing lung injury is by saline lavage. A saline lavage model of lung injury in the rabbit seemed to offer excellent reproducibility and stability, not merely depleting the lung of surfactant, but also initiating some of the early inflammatory changes of acute lung injury. This has been used as an established means of causing lung injury for some decades [148 133] and has been cited as " *a reliable model of severe ARDS, with similar histological &*

pathophysiological changes (to ARDS)"[80]. Lewis & Jobe quoted saline lavage as not just causing surfactant depletion but also neutrophil influx, atelectasis, alveolar damage and hyaline membrane formation [44], all changes seen in ARDS [157]. Lewis and Jobe also cite lavage as " *a convenient way to cause consistent lung injury across species"* for factors such as "*modes of surfactant delivery, distribution patterns of exogenous surfactants and physiologic responses to different delivery techniques"*. The saline lavage model has been shown to produce acute hypoxaemia in an otherwise haemodynamically stable animal. Thus any haemodynamic instability seen within treatment groups may reasonably be attributed to a factor associated with the treatment group itself [133;148;152]. I had also had practical experience of a (porcine) saline lavage model while I was a Research Fellow in Germany [122 119;123].

At the time of planning the study, there had been no comparative study comparing saline lavage against acid instillation, intravenous infusion of endotoxin or oleic acid infusion.

A study published subsequently by Rosenthal et al in 1998[152] compared saline lavage with hydrochloric acid instillation, endotoxin infusion or oleic acid infusion. They found that instillation of HCl or saline lavage resulted in significant hypoxaemia but no cardiovascular instability. Endotoxin infusion did not result in hypoxaemia, but caused significant decreases in systemic mean arterial pressure and significant increases in pulmonary artery pressure and pulmonary vascular resistance. Oleic acid infusion caused marked hypoxaemia, a pronounced increase in mean pulmonary artery pressure and pulmonary vascular resistance, as well as a

markedly reduced systemic mean arterial pressure, cardiac output and mixed venous PO_2. None of the four methods however in this study caused a significant increase in the cytokine Tumour Necrosis Factor. So each of these various methods had advantages and disadvantages, and none perfectly mimics ARDS.

I wanted to study the effects of PFC upon the lung, and while acknowledging that ARDS does not often occur as an isolated lung injury, I did not want the confounding elements of an unnaturally severe systemic upset. Furthermore, the intended study was designed to see if there was any effect on the respiratory factors contributing to short term mortality. Inducing a falsely severe lung injury which may kill the animals within 1-2 hours would not allow these respiratory factors to be detected. I wanted to reflect an animal model of reasonable size without the species become too cumbersome, and hence chose the rabbit.

In short, saline lung lavage is an accepted means of inducing acute lung injury, of which I already had practical experience.

Methodology for the Study

A total of 82 young adult female New Zealand white rabbits (weight range 2.31-4.27 kg; mean 3.46kg; standard deviation ± 0.40kg) were studied during these investigations. Anaesthesia was induced with 6ml/kg over 5 minutes using a 25% solution of the long acting anaesthetic/analgesic agent urethane. On veterinary advice a repeat dose of 3ml/kg was administered 7 hours later to those animals still alive. A tracheostomy was fashioned with a 3.0 mm internal diameter plain endotracheal tube, while the animals were supine and breathing spontaneously. The endotracheal tube was tied tightly to prevent any gas leakage round the tube. A bolus of the neuromuscular blocking agent pancuronium was administered (0.2mg/kg) and

an infusion of pancuronium commenced at 0.15mg $kg^{-1}hour^{-1}$. An infusion of maintenance fluids was also administered (7ml $kg^{-1}hour^{-1}$ of 0.45%saline/5% dextrose). A 4 French gauge carotid artery catheter and internal jugular venous catheter were inserted for measurement of arterial and venous pressures. Additionally these cannulae facilitated obtaining arterial blood gas samples (via the arterial line) and administration of fluid or drugs (in the case of the central line). An SLE 250 paediatric pressure controlled, time cycled ventilator was used for all experiments (Specialised Laboratory Equipment, Croydon, Surrey, UK). This was a ventilator readily and freely available to me. All animals were ventilated in the supine position, with an inspiratory oxygen fraction (F_IO_2) of 1.0. Initial ventilator settings were chosen to be similar to settings used in previous studies of lung injury induced by saline lavage in the rabbit [104;122;158]. The settings were; the pressure limit was set to generate a tidal volume of 10ml/kg, measured by a Ventrak 1550 respiratory mechanics monitoring system (Novametrix Medical Systems, Wallingford, Connecticut, USA), with an initial respiratory rate of 30 breaths per minute. The duration of the inspiratory phase was 1 second (T_I 1.0). Four centimetres water pressure of PEEP (4cm H_2O) were applied as standard.

Baseline haemodynamic variables (Mean Arterial Pressure, CVP, heart rate where obtainable) were obtained as were respiratory variables (tidal volume, respiratory rate, mean airway pressure, total respiratory system dynamic compliance, arterial blood gas values). It was decided to monitor arterial oxygenation as an index of effect of treatment on the lungs, rather than indirect measurements of delivery of oxygen to tissues such as mixed venous oxygen saturation or lactate.

The reasons are as follows.

Mixed venous saturation is conventionally obtained from the pulmonary artery and some argue that central venous blood does not correlate well with true mixed venous samples in any case[159]. Pulmonary artery catheter placement is associated with complications (some cite incidences as high as 20%)[160]. It was felt that correct placement *in vivo* of a pulmonary artery catheter in a rabbit would be technically difficult, a fact subsequently acknowledged [161].

With regard to lactate, although it is used as a means of monitoring adequate delivery of oxygen to tissues of the whole body, it may take several hours for a change in lactate levels to develop. The change in lactate levels may occur relatively slowly[162] and many investigators monitor lactate levels over several hours e.g. 24 hour time periods[163;164]. Although lactate is a reasonable marker to follow progress of a disease on the ITU, the duration of the study was to be a maximum of 12 hours after lung injury. It would also have involved the purchase of a relatively expensive analyser.

Continuous monitoring of oxygenation was achieved by a pulse oximeter probe applied to the rabbit's tongue using an Ohmeda Biox 3700 pulse oximeter (Datex-Ohmeda, Stirling, Scotland). Arterial blood gases were checked on a CIBA Corning 238 blood gas analyser (Chiron Diagnostics, Halstead, Essex, England), calibrated for PaO_2, $PaCO_2$ and pH against standard calibration solutions provided by the company.

Measurement of total respiratory system dynamic compliance with the Ventrak 1550 respiratory mechanics monitoring system.

Dynamic compliance of the respiratory system (Cdyn) was measured by using a Ventrak 1550 respiratory mechanics monitoring system (a fixed orifice, differential pressure flowmeter), with a flow and pressure transducing head fixed to the top of the endotracheal tube (Novametrix Medical Systems, Wallingford, Conn., USA)[165]. The Ventrak is a custom designed flowmeter intended to minimise the effects of condensation on flowmeter performance. The output pressure versus flow relationship for the Ventrak head is non-linear. The non linearity of the Ventrak flow head is corrected in the signal processing.

The linearity of the flowmeter of the Ventrak was tested with 100% oxygen using a Timeter Calibration Analyser Series RT 200 and an FCO14 Micromanometer, Furness Controls Ltd, Bexhill UK. The results of these assessments are shown in Table 3 and Figure 4 which appears at first glance alinear.

However the output pressure from the Ventrak flow head is further processed by the Ventrak system and the integrated volume from the Ventrak was used in calculations.

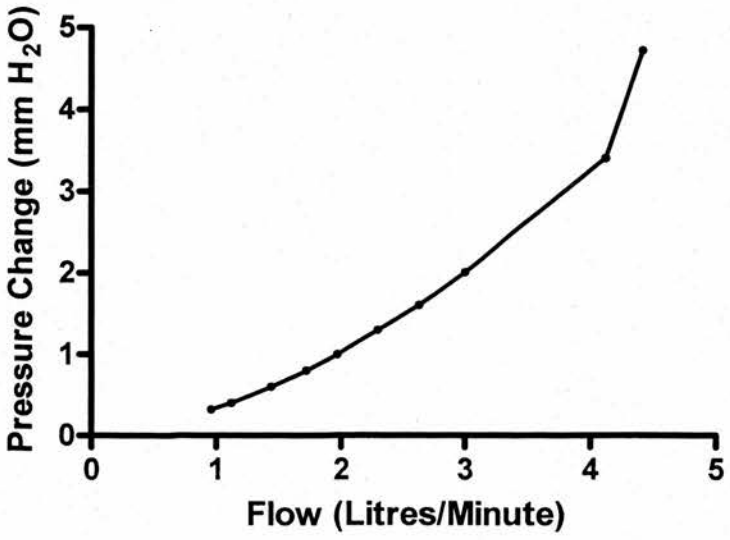
To assess the linearity of the combined Ventrak flow and volume measurement system, the output of the device to a standard volume delivered at different speeds was assessed, using a four-fold difference in time to deliver a known volume. A Hans Rudolph 5510 Series Calibration Syringe was set to 40ml and the volume was delivered through the transducer head. The output signal representing volume from

the Ventrak was measured by capture into a Cambridge Electronic Design Micro 1401 signal acquisition system (C.E.D., Cambridge, England). The approximate range of flow rates for rabbit weights 2.3-4.3 kg (thus tidal volumes 23-43ml) respiratory rates of circa 30/ minute, and inspiratory times of circa 1 second is approximately 1.3-2.7 litres/minute. Therefore flow rates over this range were used to assess the linearity of the volume output. The results of this assessment are shown in Table 4 and Figure 8.

The flow signal appears to have been adequately linearised. Over these flow ranges, the change in output amounts to 1.5ml for a mean volume of 34.3ml i.e. a variation of 4.4%. This relationship was considered satisfactory for estimates of Cdyn where the changes expected were between 10 and 20%, particularly since the flow range for each animal would be less than this imposed range, and the variation within each animal would be less.

The dynamic compliance was calculated by dividing the inspired tidal volume (obtained from the Ventrak) by the difference between the peak inspiratory pressure (obtained from the Ventrak) and the end-expiratory pressure at points of zero flow and was corrected for body weight (giving units of ml cm H_2O^{-1} kg^{-1}). The inspired tidal volume was chosen for standardisation, because if expiratory tidal volume had been chosen in some groups there would have been the confounding element of exhaled PF 5080 vapour.

Figure 4. Pressure-flow relationship of Ventrak (top) and H-R Pneumotachograph (bottom).



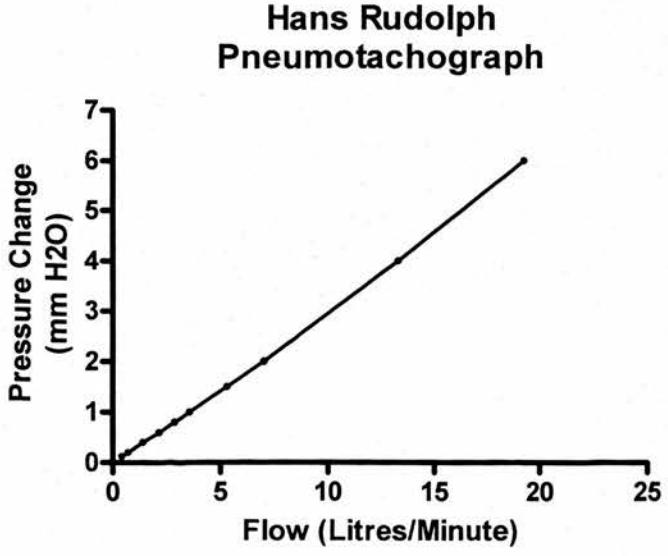


Table 3a. Linearity of Ventrak (Inspiratory) measurement system

Pressure Change (mmH_2O)	**Flow (Litres/Minute)**
0.325	0.96
0.4	1.12
0.6	1.44
0.8	1.72
1.0	1.97
1.3	2.30
1.6	2.63
2.0	3.0
3.4	4.11
4.72	4.4

Table 3b. Linearity of Hans-Rudolph Pneumotachograph

Pressure Change (mm H2O)	**Flow (Litres/Minute)**
0.12	0.42
0.2	0.71
0.4	1.41
0.6	2.17
0.8	2.88
1.0	3.58
1.5	5.31
2.0	7.0
4.0	13.3
6.0	19.2

The viscosity and density of PFC vapour interferes with flow measurement devices such as the pneumotachograph and the Ventrak flow head. This is recognised by other researchers [166]. In the group given nebulized PF 5080, administration was stopped briefly whilst Cdyn was determined.

Baseline recordings of dynamic compliance (Cdyn) were made after adequate lung injury had been achieved and the randomisation process had taken place (vide infra).

Measurement of total respiratory system static compliance using the Hans Rudolph 4500B Pneumotachograph..

Total respiratory system static compliance (Crs) was measured using a modified single breath technique[167].

Figure 5a [167]
Schematic Representation of the Hans Rudolph Pneumotachograph.

(A) The occlusion device is inserted into the ventilator circuit. The pneumotachograph is excluded from the circuit. Gas flow shown by arrows

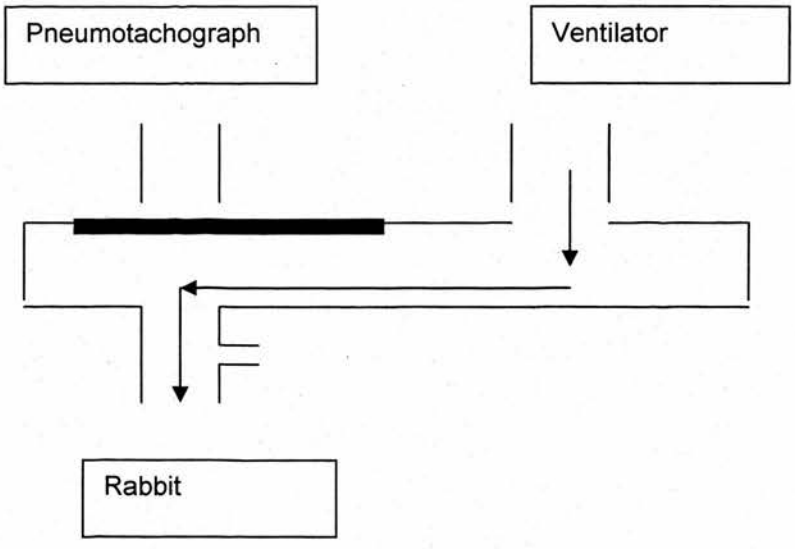


Figure 5b

(B) The airway is occluded at peak inflation, trapping a breath in the rabbit's lungs. The proximal airway pressure is sampled.

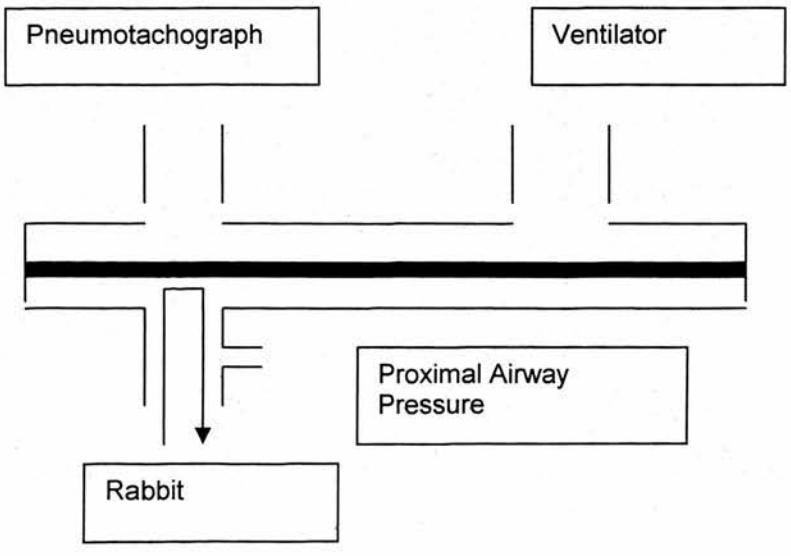
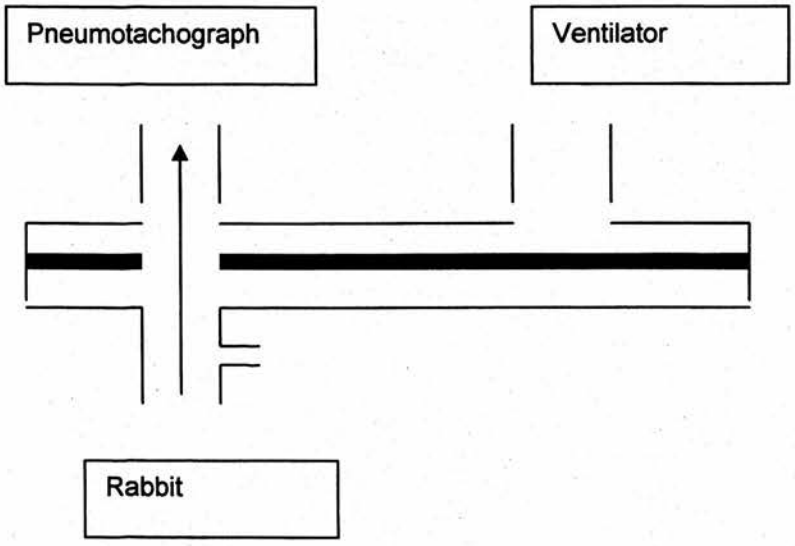


Figure 5c

(C) The occlusion is released. Passive expiratory flow occurs through the pneumotachograph.



There was a rotatable valve which enabled different channels to be opened or closed. The resistance of the pneumotachograph and occlusion device combined was essentially that of the pneumotachograph. With the device inserted into the respiratory circuit the rabbit could be ventilated normally. The device had a side port for sampling proximal airway pressure using a Furness Controls differential pressure transducer (0-10 kPa).

The airway was briefly occluded at end inspiration using a two-way occlusion device (see Figure 5). Proximal airway pressure was measured. The occlusion device was then switched allowing the animal to exhale completely to atmospheric pressure through a Hans Rudolph 4500B pneumotachograph connected to a Validyne MP45 differential pressure transducer (±2 cm H2O). Once the breath had been sampled the occlusion device was switched back, and mechanical ventilation with positive end-expiratory pressure was resumed. During measurements, airway pressure and expiratory flow were sampled to an online personal computer at 250 Hz. The airway pressure was inspected to ensure that a stable plateau pressure (approximately 200ms) had been obtained following the airway occlusion. Crs was calculated as the volume exhaled to atmospheric pressure divided by the plateau pressure and corrected to body weight (ml cm H_2O^{-1} kg^{-1}). Each Crs value was taken as the average Crs obtained from at least five different breaths. To obtain an indication of the consistency of measurement, the coefficient of variation [(the standard deviation of the value of breaths/ their mean) x100] of each average Crs value was calculated. The distribution of these values is shown in Figure 6.

The mean coefficient of variation was 2.7% (SD 1.4%) with a minimum of 0.6% and a maximum of 8.5%. This suggests that the measurements were of an acceptable consistency. The total number of estimates of coefficient of variation was 147.

As gases of differing viscosity affect the pressure drop across the pneumotachograph, it was not possible to use the pneumotachograph for the following treatment groups;

i) Poured PF 5080

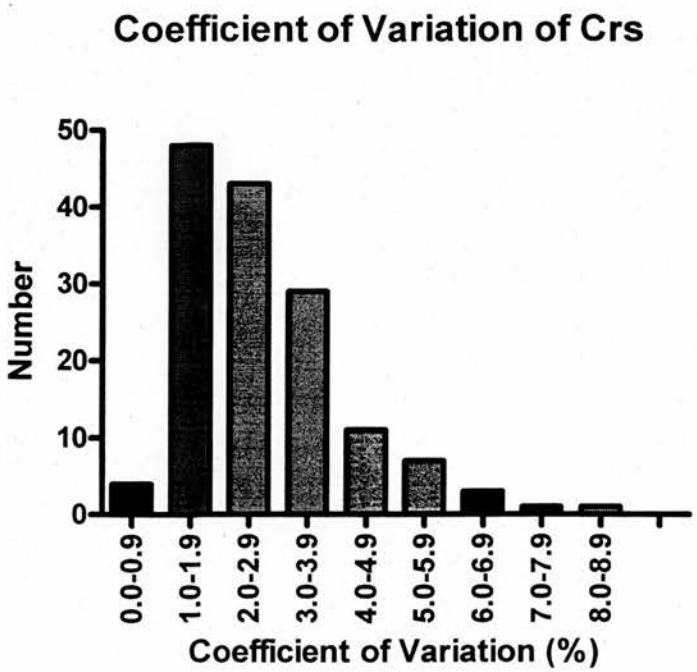
ii) Nebulized PF 5080

iii) Pumactant combined with poured PF 5080

iv) Curosurf combined with poured PF 5080.

Thus Crs measurements are considered only from animals that were not treated with PFC.

Figure 6. Bar chart showing distribution of Coefficient of Variation obtained for Crs estimates made with the Hans Rudolph pneumotachograph. This sample is for all the measurements of Crs noted in all animals (n=147).



Reproducibility of measurements of static compliance of the respiratory system. The airway pressure transducer and the pneumotachograph were tested repeatedly against a known pressure of 20 cm H_2O with a water manometer and a known volume of 50 ml against a Hans Rudolph 5510 Series 50ml calibration syringe, using 100% oxygen before each animal was measured (see Figures 7a and 7b). Pressure calibration gave a mean pressure of 19.8 cm H_2O (SD 0.2 cm H_2O; n=26). Volume calibration gave a mean volume of 50.1ml (SD 0.3ml; n=27).

Figure 7a Scatter plot showing reproducibility of volume measurements for Hans Rudolph Pneumotachograph. Volume calibration against a Hans Rudolph 5510 Series 50ml calibration syringe. Mean volume shown 50.1 ml (SD 0.3 ml). Minimum 49.5 ml; Maximum 50.6 ml.

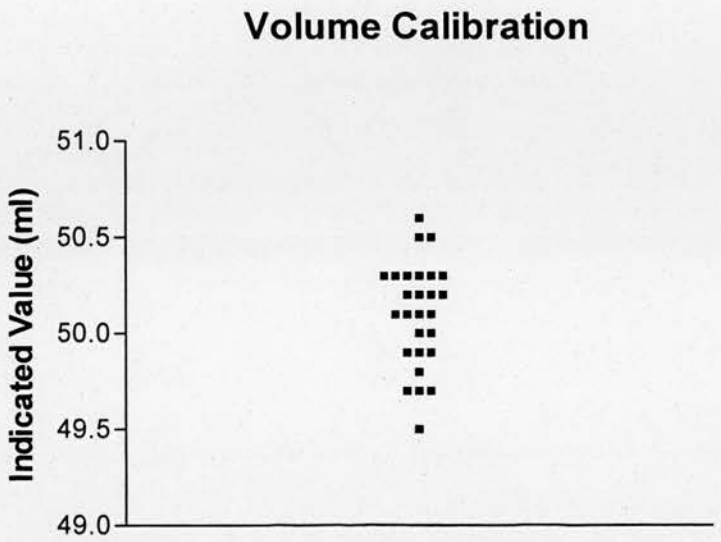


Figure 7b. Scatter plot showing reproducibility of pressure measurements for Hans Rudolph pneumotachograph. Pressure calibration gave a mean pressure of 19.8 cm H_2O with a minimum of 19.5 cm H_2O (SD 0.2 cm H_2O) and a maximum of 20.5cm H_2O.

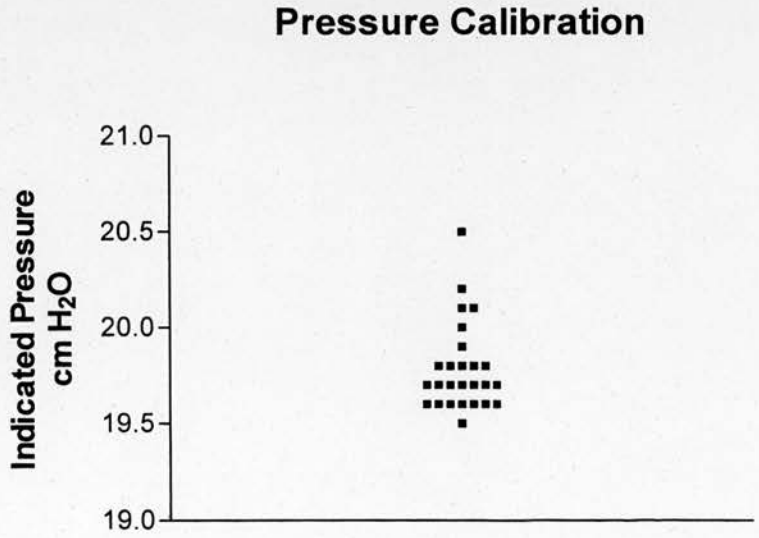
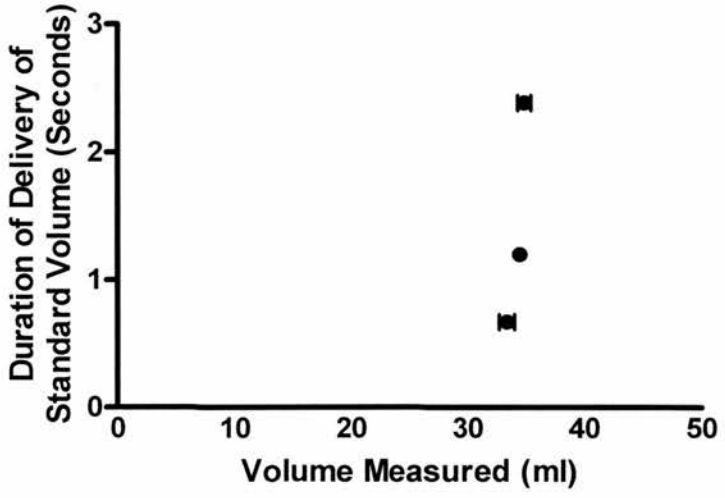


Table 4. Table of assessment of linearity of volume measurements. Ten measurements at each delivery speed. Values are means (SD).

Duration of Flow in Seconds.	Volume Measured in ml.
0.67 (0.06) n=10	33.4 (0.67)
1.2 (0.22) n=10	34.5 (0)
2.39 (0.43) n=10	34.9 (0.55)

Figure 8 Linearity of volume measurement system of Ventrak



Dynamic Compliance versus Static Compliance

Dynamic compliance is simpler and less time consuming to measure than the static compliance measurement technique described above. However differences may occur between values obtained for Crs and Cdyn, due to difference in either tidal volume or end expiratory pressure [168 131]. In this thesis the ventilator settings were adjusted to generate a tidal volume of 10ml/kg by altering peak inspiratory pressure thereby keeping tidal volume constant. However the technique for measuring Cdyn above measures the set level of PEEP whereas the technique for measuring Crs allows the rabbit to exhale to atmospheric pressure. This may allow the rabbit compliance to be sampled at differing points on the pressure-volume relationship i.e. to have differing compliances.

Dynamic compliance is frequency dependent and includes resistive and viscoelastic components in its measurement [169]. Therefore it was important for a standardised respiratory rate to be used in the ventilatory protocol.

Some investigators advocate that the effects of surfactant therapy are best characterised by changes in maximum compliance measured by static not dynamic compliance [168].

Mode of inducing lung injury

The mode of inducing lung injury was by repeated warmed 0.9% saline lavage similar to that described by Lachmann et al[133;148]. The rabbit has a functional residual capacity of approximately 20ml/kg[149 170]. Thus 20ml/kg was repeatedly introduced to the respiratory tract at 5 minute intervals with a dwell time of 1 minute in the supine position. The animal was then turned so that the saline reached all areas

of the lung. In order to generate an adequate tidal volume of gas, during the saline lavage, peak inspiratory pressure (PIP) was increased to 24cm H_2O.

Lung injury was standardised as follows. Oxygenation was monitored with a peripheral pulse oximeter attached to the tongue, and checked by arterial blood gas analysis. An adequate amount of lung injury was deemed to have occurred when the arterial partial pressure of oxygen (PaO_2) was less than 13.3 kPa (100mmHg)[122;134] based on 2 blood gas analyses 15 minutes apart, at a peak inspiratory pressure of 24 cmH_2O and 4cm H_2O PEEP.

At this point the animal was randomised to one of seven treatment groups, by random selection of unmarked sealed envelopes.

1) The Control Group (20 animals)

The peak inspiratory pressure was adjusted to generate a tidal volume of 10ml/kg and a respiratory rate of 30 breaths per minute as described below. No further treatment interventions were given other than the administration of maintenance fluids. At the end of the experiment, 10 animals had their lungs preserved by immersion in formalin, and 10 by immersion in liquid nitrogen.

2) The Poured PFC Group i.e. PLV (12 animals).

These animals were given an FRC (20ml/kg) dose of warmed PFC (PF 5080), or until a meniscus was seen in the endotracheal tube at sternal level with PEEP temporarily switched off so the level could be checked. Evaporative losses were replaced throughout the study by infusion of PF 5080 into the side arm of an endotracheal tube connector such that a meniscus was maintained at this level, checked intermittently at zero PEEP. If required, further top-up boluses were

administered to maintain the meniscus. At end of the experiment, 6 sets of lungs were immersed in formalin, and 6 immersed in liquid nitrogen.

3) The Nebulized PFC Group (10 animals)

This group were given a similar initial loading ("FRC") dose of 20ml/kg PF 5080, to that administered in the Poured PFC group, but this dose was administered by an ultrasonic nebulizer (Devilbiss Ultra-Neb 2000, Sunrise Medical, UK) set at maximal output (1.63Megahertz). As nebulized PFC had not previously been administered as a means of treating the injured lung, there was no specific information available on this technique. The general principles adhered to are shown below.

An ultrasonic nebulizer was used for the reasons discussed above, and the Devilbiss Ultra-Neb used was the ultrasonic nebulizer available for the project. The setting was chosen to be in the range most likely to deliver particles to the alveoli (<5 microns)[87] as discussed previously.

The size of the particles generated had been bench tested prior to the study in collaboration with colleagues at the Institute of Occupational Medicine, using a Hiac/Royco 4100/1200 particle counter[171] (Menio Park, California, USA; threshold 0.3 microns) and the SLE 250 ventilator. The nebulization chamber was 30 cm from the test lung as suggested as the optimal distance for ultrasonic nebulization[112], and the particle counting was performed immediately prior to the test lung. Various volumes for nebulization ("Charges") were tested. The results from this are shown in Table 5.

Table 5 Particle Distribution for PF 5080 nebulized by Devilbiss Ultra-Neb 2000 ultrasonic nebulizer.

	Charge 10mls (counts per minute)	**Charge 20mls (counts per minute)**	**Charge 30mls (counts per minute)**	**Charge 40mls (counts per minute)**
Particle size 0.3-0.5 microns	85 773	56 203	8 366	7 389
0.5-1.5 microns	68 578	22 731	3 584	348
1.5-3.0 microns	51 580	2654	437	6
3-5 microns	2616	18	21	0
5-10 microns	4	0	0	0
>10 microns	0	0	0	0

On the basis of this, nebulization was performed in 10ml aliquots i.e. if a total of 80ml were to be nebulized, it was nebulized as 8 aliquots of 10mls.

The estimated hourly maintenance requirements to replace evaporative PFC losses were calculated as follows (after the formula suggested by Salman et al)[109].

Assuming a tidal volume of 10ml/kg, hourly ventilation was

Tidal Volume	x Resp. Rate	x 60 minutes/hour
10ml/kg	e.g. 30 breaths/min	x60
i.e ventilation		= 18 000 ml $kg^{-1}hour^{-1}$

Vapour pressure of PF 5080 at 37°C = 6.8 kPa. Thus 6.8 volumes % of ventilation /hour

Thus 6.8/100 x	18 000	1224 ml kg^{-1} $hour^{-1}$

At Standard Temperature and Pressure 1 mol. of gas occupies 22 400ml. Thus 1224ml will contain 1224/22400 ml = 0.05mol

Molecular weight PF 5080	= 438 Daltons
So 0.05mol	= 21.9g

Thus 21.9g PF 5080 evaporated $kg^{-1}hr^{-1}$

Density of PF 5080= 1.76g/ml

Thus approximately 13ml PF 5080 evaporated $kg^{-1}hr^{-1}$

Due to the flow bias of the SLE 250 ventilator this does not necessarily imply that all of this was delivered to the lungs. This point is dealt with later in the discussion sections.

All the lungs were immersed in liquid nitrogen at the end of the experiment.

4) <u>The Pumactant only Group (10 animals)</u>

This group were given 100mg/kg of the apoprotein free surfactant (Pumactant). This is the dose of phospholipid most often used in animal studies of exogenous surfactants [126]. Pumactant was injected into the trachea via the endotracheal tube as a single bolus. PIP was adjusted to generate an inspiratory tidal volume of 10ml/kg.

No other interventions were made. All animals had formalin histology preparation post mortem.

5) The Curosurf only Group (10 animals)

This group was given 100mg/kg of the apoprotein containing surfactant Curosurf, injected into the trachea via the endotracheal tube as a single bolus. PIP was adjusted to generate an inspiratory tidal volume of 10ml/kg. No other interventions were made. All these lungs were immersed in formalin at the end of the experiment.

6) Combined treatment with Pumactant/PLV (10 animals)

This group was given 100mg/kg of the apoprotein free surfactant Pumactant, then poured PFC as for the PLV group i.e. PF 5080 in an FRC (20ml/kg) dosage of warmed PFC (PF 5080), or until a meniscus was seen in the endotracheal tube at sternal level with PEEP temporarily switched off. Evaporative losses were replaced throughout the study such that a meniscus was maintained at this level, checked intermittently at zero PEEP. All these lungs were immersed in formalin at the end of the experiment.

7) Combined treatment with Curosurf/PLV (10 animals)

This group was given 100mg/kg of the apoprotein containing surfactant Curosurf 100mg/kg, then poured PF 5080 as above. All these lungs were immersed in formalin at the end of the experiment.

After randomisation the animals were returned to ventilation with an inspiratory tidal volume of 10ml/kg (maximum inspiratory pressure 45 cmH_2O) for 15 minutes prior to administering the randomised treatment. Lung mechanics, haemodynamic and gas exchange parameters were then checked at hourly intervals. The $PaCO_2$ was kept

within the approximate normal range (4-6kPa) for the rabbit[172], by adjusting the respiratory rate. If a respiratory rate greater than 35 was required then the inspiratory time was reduced.

Blood gas, Cdyn and where appropriate Crs parameters were measured hourly until death or until twelve hours after lung injury criteria were achieved. Surviving animals were killed at this point by an overdose of anaesthetic.

The lungs were then clamped in end-expiration and the lungs removed en-bloc. They were then either immersed in liquid nitrogen (if randomised to be in the CT scanning arm of the study as further described in chapter 6 below) or otherwise 10% buffered formalin.

As additional proof of adequacy of lung injury, at the end of the study, 10 of the twenty control animals had representative lung samples taken for histological analysis (7 samples per case). Rabbit 2's lungs had been immersed in liquid nitrogen first (from the Control CT data group) and after the CT data had been obtained, was immersed in 10% buffered formalin. All others were directly immersed in 10% buffered formalin. These samples were prepared by Dr William Wallace, Consultant Pathologist, Edinburgh University Medical School. The data on these samples are given in Table 9 (Chapter 3). Photographs of the haematoxylin and eosin stained microscope slides from some of these samples are shown in Figure 10 in Chapter 3. Further details with regard to the separate arms of the study are detailed and emphasised in the individual chapters below.

Chapter 3

The effect of Partial Liquid Ventilation with PF 5080 on survival times; a randomised, controlled comparison of PF 5080 alone, and with and without combinations of Curosurf or Pumactant.

Summary

The aim of this chapter was to investigate any differences in survival to twelve hours after achieving lung injury, between the seven treatment groups referred to in the previous chapter.

Results were analysed by comparing Kaplan-Meier survival curves using a Logrank test.

There was a significant difference between the groups treated with partial liquid ventilation and the control group, and between the combination of Pumactant and partial liquid ventilation and control. There was a trend towards an improved survival between the Curosurf alone group and control, which just failed to reach statistical significance.

Introduction.

Partial liquid ventilation may improve chances of survival in the context of acute lung injury. Some previous animal studies of acute lung injury had dealt with very short term survival (to 3-4 hours)[134] and none had used the PFC as the preparation PF 5080 in a rabbit model of acute lung injury. This study wished to test the effects of PLV with PF 5080 on survival over a longer period of time (to twelve hours post lung injury, the maximum period allowed under the Home Office Licence for the study).

A further hypothesis was to be tested. Surfactant deficiency, and surfactant inactivation are well recognised features of ARDS [44;49;53;56;57] and may well be responsible for the early changes of compliance seen during the initial stages of development of the syndrome. The airway atelectasis and loss of FRC with increase in right to left shunt leads to the hypoxaemia. Thus modes of therapy which help maintain an adequate FRC and improve oxygenation may stop the slide towards continuing hypoxaemia and premature death.

Methods

A total of 82 young adult female New Zealand white rabbits (weight range 2.31-4.27 kg; mean weight 3.46 kg; standard deviation ± 0.40kg) were studied during these investigations. Anaesthesia, preparation and induction of lung injury were as described in chapter 2. As there were no previous studies on survival to twelve hours in this experimental model, for these treatment groups, power calculations were made on the basis of similar though not identical studies, as discussed in chapter 1.

After achieving adequate lung injury (PaO_2 <13.3 kPa) the animals were randomised to one of seven treatment groups, again as described in chapter 2;

i) Control (20 animals);

ii) Partial liquid ventilation i.e.poured PF 5080 (12 animals);

iii) Nebulized PF 5080 (10 animals);

iv) Pumactant 100mg/kg body weight (10 animals);

v) Curosurf 100mg/kg body weight (10 animals)

vi) Pumactant then partial liquid ventilation with PF 5080 (10 animals);

vii) Curosurf then partial liquid ventilation with PF 5080 (10 animals).

After randomisation the animals were returned to ventilation with an inspiratory tidal volume of 10ml/kg. After 15minutes lung mechanics, haemodynamic and gas exchange parameters were rechecked, and repeated at hourly intervals, time zero being taken as the time that lung injury criteria were achieved. The randomised

treatment intervention was made and hourly blood gas parameters were checked thereafter.

The $PaCO_2$ was kept within the normal range by adjusting the respiratory rate. If a respiratory rate greater than 35 was required then the inspiratory time was reduced. Hypotension thought to be due to hypovolaemia was treated with boluses of fluid, in addition to the background intravenous fluid infusion (7ml $kg^{-1}hour^{-1}$).

Those animals still alive 12 hours after achieving lung injury criteria were euthanased by an overdose of general anaesthetic. The lungs were then clamped in end-expiration and excised *en bloc.*

The excised lungs were either prepared for histological examination by liquid nitrogen or formalin immersion. Histological analysis is included merely to demonstrate some evidence of lung injury, and otherwise does not form part of this thesis.

Results

Kaplan-Meier survival curves [140] are shown for the respective treatment groups (Figure 9). Comparison between pairs of groups was made using the Logrank test [139;140], on the statistics program of *GraphPad Prism™ Version 4.0* (GraphPad Software Inc., San Diego, California, USA). Analysis of the Logrank test gave the following results.

All comparisons made with the control group. A P value of <0.008 was taken as significant (Bonferroni's correction for 6 comparisons i.e. 0.05/6).

Partial liquid ventilation i.e. poured PF 5080 demonstrated a significantly improved survival compared with control ($P = 0.0019$)

Pumactant and partial liquid ventilation combined demonstrated a significantly improved survival compared with control ($P = 0.0048$)

The other comparisons to control yielded the following P values, all of which were not statistically significant at $P > 0.008$;

Nebulized PF 5080	($P = 0.6061$)
Pumactant	($P = 0.2014$)
Curosurf	($P = 0.0092$)
Curosurf and poured PF 5080	($P = 0.0520$)

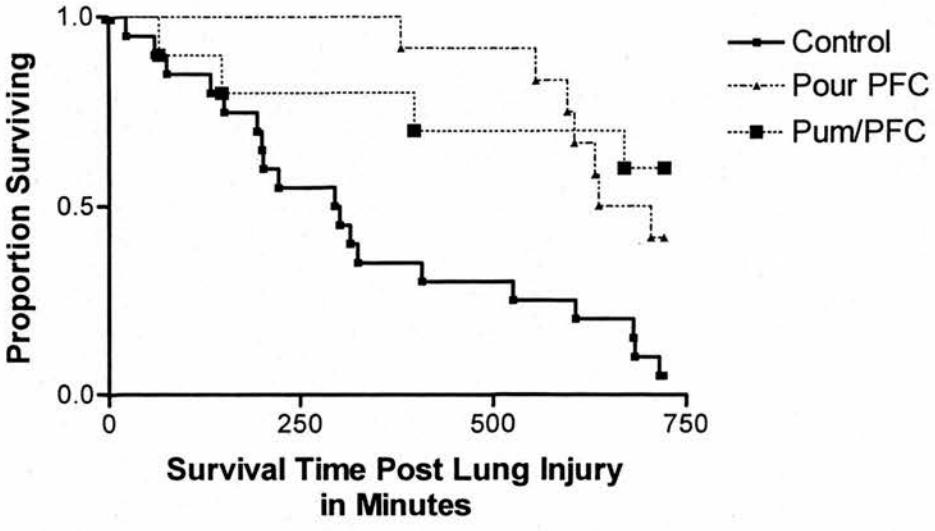
Survival times and number of animals with evidence of pneumothorax are shown in Table 6. The incidence of pneumothorax between groups compared by a χ^2 Test gave the result $X^2= 12.32$; $0.05<P<0.1$ i.e. not significant.

The amount of PF 5080 administered to the respective groups is displayed in Table 7. The nebulized group were given significantly more PF 5080 (although this does not imply that it all reached the alveoli). Additional fluid requirements for each group are shown in Table 8. No group was given significantly more fluid than any other group.

Table 9 describes the histological findings of representative samples from the control group of animals (in terms of acute interstitial inflammatory infiltrate, hyaline membranes and histological confirmation of acute lung injury as well as survival time in minutes). Figure 10 demonstrates examples of histology slides from some of these animals.

Figure 9

Kaplan-Meier Survival Curves of Lung Injured Rabbits



Kaplan-Meier Survival Curves of Lung Injured Rabbits

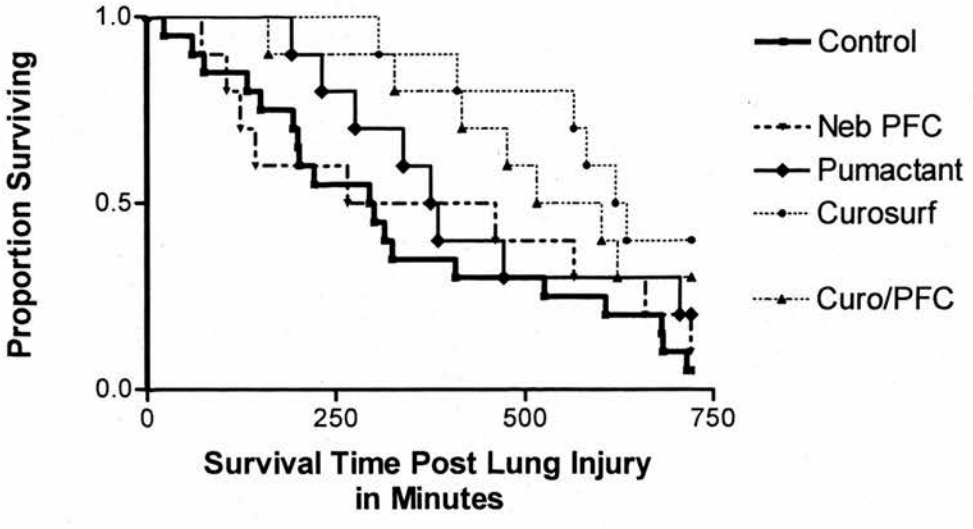


Table 6; Survival Times and number of animals with pneumothorax present.

	Number of Animals	Number of Animals with Pneumo-thoraces	Minimum survival Time (Minutes)	25% Percentile (Minutes)	Median Survival Time (Minutes)	75% Percentile (Minutes)	Maximum Survival Time (Minutes)
Control	20	4	23	172.5	297	565.5	720
Poured	12	4	380	598.5	669	720	720
Neb. PFC	10	2	73	134	363	611.5	720
Pumactant	10	0	192	306.5	379	588.5	720
Curosurf	10	5	306	571.5	626.5	720	720
Pum/Pour	10	4	66	532.5	720	720	720
Curo/Pour	10	6	161	445.5	557.5	671	720

Table 7

Amount of PF 5080 administered per minute of survival. Data are means (with standard deviations).

Group	**Amount of PF 5080 administered (ml/min)**
Partial Liquid Ventilation (n=12)	0.67 (0.12)
Nebulized PFC (n=10)	0.89 (0.20)
Curosurf + PLV (n=10)	0.72 (0.16)
Pumactant + PLV (n=10)	0.70 (0.14)

Comparison between groups by ANOVA with Newman-Keuls post hoc test.

Statistically significant differences between;

Nebulized and PLV ($P<0.01$)

Nebulized and Curosurf/PLV combination ($P<0.05$)

Nebulized and Pumactant/PLV combination ($P<0.05$)

All other comparisons were not statistically significant.

Table 8. Additional fluid requirements in millilitres

	Minimum Value	25% Percentile	Median	75% Percentile	Maximum Value
Control (n=20)	0.0	10.0	49.0	96.0	300.0
Pour (n=12)	20.0	60.0	102.5	130.0	170.0
Neb. PFC (n=10)	0.0	45.0	60.0	70.0	180.0
Pumactant (N=10)	0.0	35.0	100.0	135.0	240.0
Curosurf (n=10)	0.0	0.0	30.0	73.0	150.0
Pum/Pour (n=10)	0.0	60.0	105.0	120.0	135.0
Curo/Pour (n=10)	40.0	92.5	115.0	180.0	210.0

Groups compared by non-parametric Kruskal-Wallis test with Dunn's post test (as values do not correspond to a normal distribution). No significant difference between any group ($P > 0.05$).

Histology

Ten samples were taken from the lungs of control rabbits to assess the degree of lung injury. The pathologist reported that 9 of 10 rabbits showed "unequivocal evidence of acute lung injury with variable interstitial and airspace infiltration by neutrophils and hyaline membrane formation". The 10th case (the first rabbit in the study) was more equivocal but showed at least "mild focal infiltrates". A summary of the pathologist's findings is shown in Table 9, and pictures of representative haematoxylin and eosin microscope slides are shown in Figure 10. Inflammatory cells, haemorrhage, hyaline membrane formation and destruction of lung architecture

including necrosis are shown on the photographs, all features consistent with acute lung injury [157].

Table 9. Histology findings of representative samples from Control group animals.

Animal Number	Acute Interstitial Inflammatory Infiltrate	Hyaline Membranes	Histological Confirmation of Acute Lung Injury	Survival Time in Minutes	Comment
Rabbit 1	Mild Focal	No	Doubtful	200	
Rabbit 2	Mild-moderate diffuse	Diffuse	Yes	300	
Rabbit 7	Mild/moder-ate diffuse	Diffuse	Yes	294	See Pictures
Rabbit 23	Mild/moder-ate focal	Focal	Yes	23	
Rabbit 27	Mild/moder-ate focal	Focal	Yes	133	
Rabbit 55	Mild diffuse	Diffuse	Yes	683	
Rabbit 59	Mild-intense	Diffuse	Yes	715	See pictures*
Rabbit 65	Mild-moderate diffuse	Diffuse	Yes	606	
Rabbit 68	Mild diffuse	Focal	Yes	202	
Rabbit 70	Mild diffuse	Focal	Yes	314	

- Rabbit 59 In comment section. "Focal injury very severe with possible early necrosis".

- Key to arrows on photographs of histology slides (Figure 10).

A = Necrosis

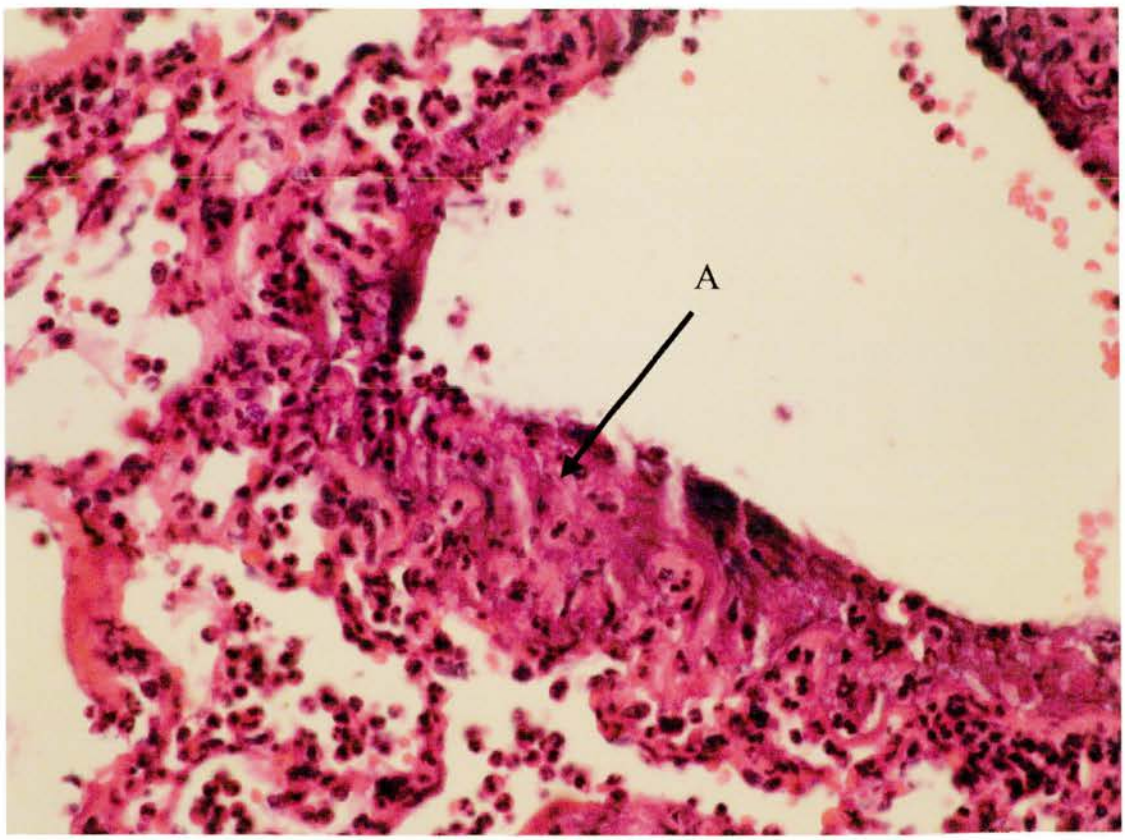
B = Intraluminal neutrophils

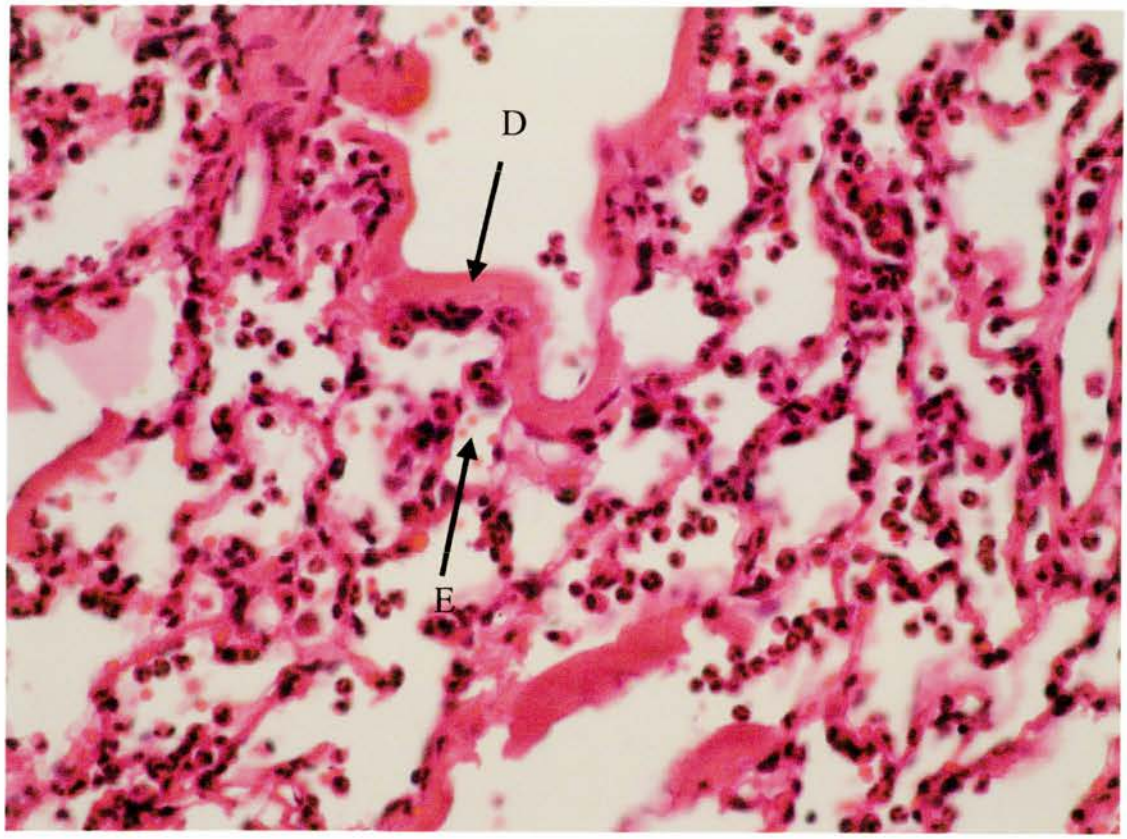
C = Interstitial neutrophils

D = Hyaline membrane

E = Red blood cells

The first picture showing necrosis is taken from Rabbit 59. The other examples are taken from Rabbit 7.


A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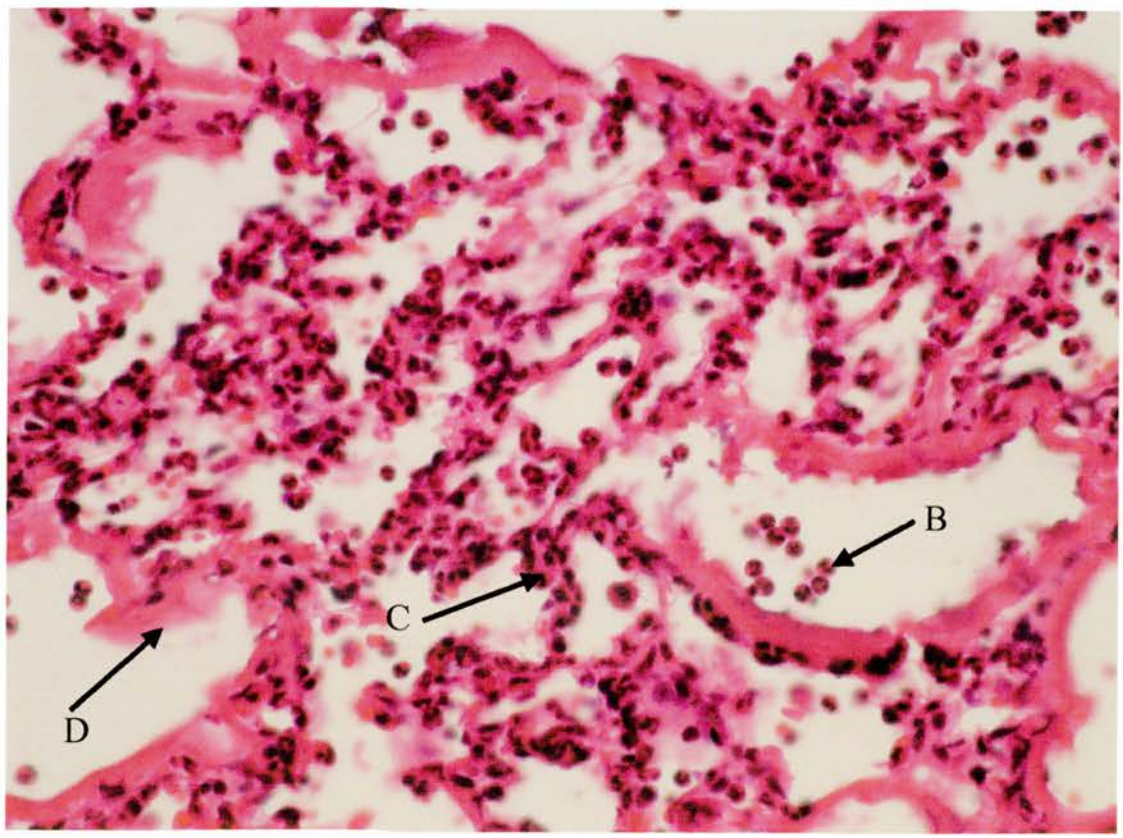
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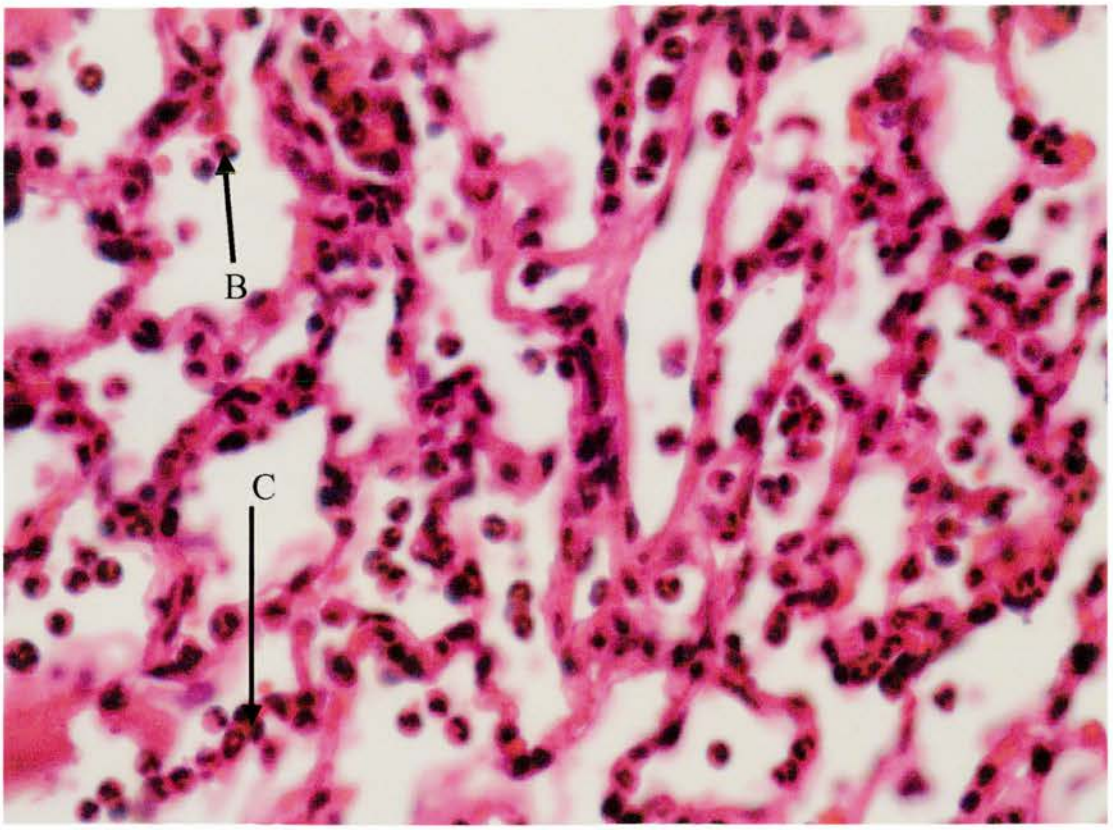
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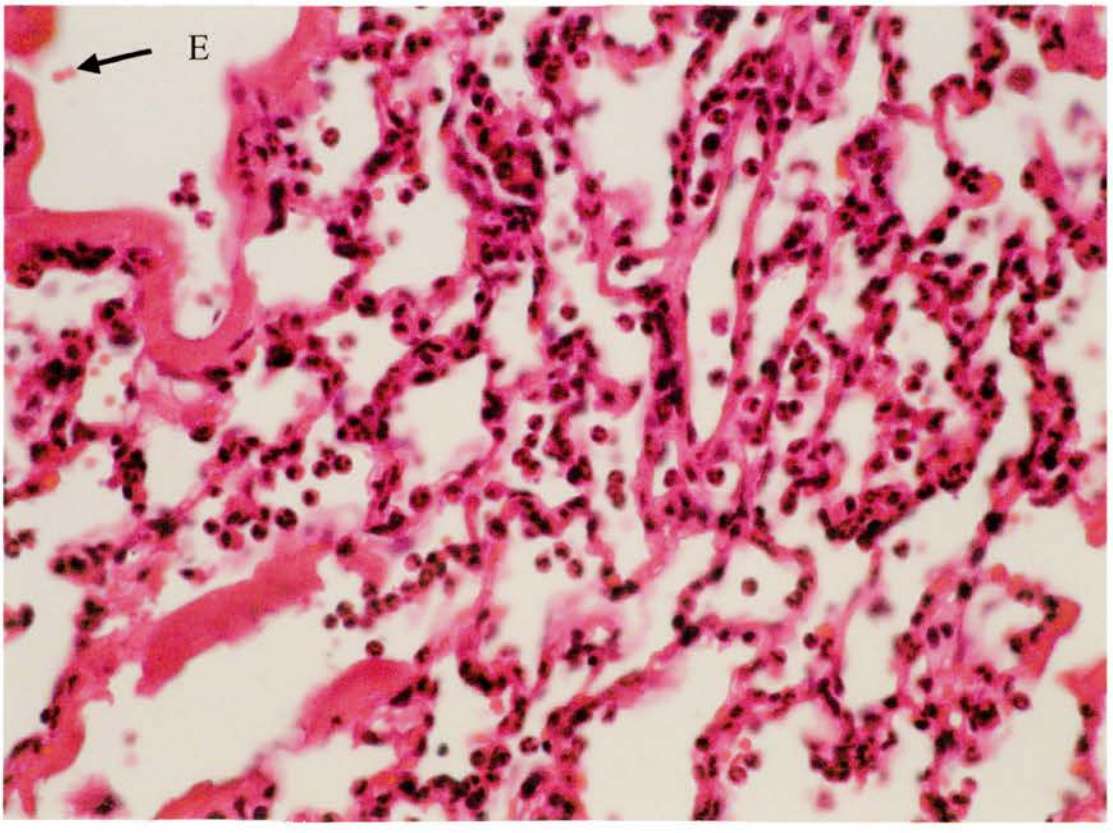
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Discussion

The relative merits of the various common means of inducing experimental lung injury, and the reasons why the saline lavage method was chosen for this study were discussed in chapter 2.

The lung injury caused by saline lavage in this study was graded from mild to severe (widespread hyaline membranes, inflammatory infiltrates and foci of necrosis). However in some animals the acute interstitial inflammatory infiltrate was graded as either mild or mild to moderate. In part the limited inflammatory infiltratration may be caused by the limited time from injury to death.

Five of the ten animals showed diffuse hyaline membrane formation with a further four showing focal hyaline membranes.

In nine of the ten control animals, acute lung injury was confirmed to be present on histological examination. All animals from all groups had to have an arterial PaO_2 less than 13.3 kPa with an F_IO_2 of 1.0, to confirm an adequate and standardised hypoxaemia had been caused by the saline lavage process. It is possible that some of these animals had a not particularly severe insult on histological evidence. However all were lavaged to a similar severity of hypoxaemia, and were then randomised. Thus there should have been an equal distribution of less severely injured animals across treatment groups.

This is the first study of partial liquid ventilation (PLV) with PF 5080 to determine the effect on survival in a rabbit model of ARDS. Other studies have considered fewer animals and generally over a shorter period of study [122;134]. It was found that

PLV and the combination of pumactant and PLV prolonged survival in comparison to controls. With regard to the number of animals in each group, and the multiple comparisons which were made, the study had sufficient power to detect substantial differences only. Although none of the other treatments were associated with a significant prolongation of survival, inspection of the Kaplan-Meier survival curves suggests that some of the other treatments, particularly Curosurf or the combination of Curosurf and PLV, may have prolonged survival. A type II error cannot be excluded. For example, assuming a type I error of 0.05 and assuming a power of the test of 0.8 in order to detect a difference between control (median survival 297 minutes) and Curosurf (median survival 626.5 minutes), there would have to have been 32 animals in the Curosurf group (*PS Power and Sample Size Calculations* ;Copyright 1997 by W.D. Dupont and W.D. Plummer [142]). These median survival values were only available at the end of the study, and had not previously been published, as mentioned in chapter 1. Trying to discern differences between other groups where there appears to be less of a difference in effect on inspection of the Kaplan-Meier survival curves would have required vast numbers of subjects outwith the scope of this project. Again for example, to determine a difference between control and Pumactant assuming the type I + II errors above would require 257 animals per group.

Possible causes of death in these animal studies of ARDS have been cited as pneumothorax and intractable hypoxaemia[134].

The number of animals who developed a pneumothorax is shown in Table 6. The differences in proportions between the groups were not statistically significant (χ^2 Test). Pneumothoraces were frequently observed in all study groups except those

animals treated with Pumactant alone. However, the median (interquartile range) time post randomisation to diagnosis of pneumothorax was 564 (356-628) minutes, by which time 7 of the 10 rabbits in the Pumactant only group had died. Pneumothoraces may not always be diagnosed close to their time of onset. Some of the pneumothoraces were only diagnosed post-mortem when the abdomen was incised and the diaphragms inspected at autopsy. Furthermore, the study protocol did not allow for the treatment of any pneumothorax diagnosed ante-mortem by what would be the normal clinical practice of inserting an intercostal drain. Some authors have suggested that in animals treated with PLV pneumothoraces do not become apparent until PFC starts to evaporate off. Prior to this, the lung can be splinted by the volume of liquid it contains [80]. The non-dependent lung may particularly be prone to overdistention during partial liquid ventilation [1].

Administration of nebulized PFC as a means of supporting the injured lung had not been described previously. There was no meniscus to ascertain degree of filling of the lung with PFC. The amount of PFC administered per minute of survival post randomisation is noted in table 7.

The amount of nebulized PF 5080 actually deposited in the lungs would have been difficult to ascertain in vivo. Bench testing prior to performing these studies (see Table 5) suggested that the optimal setting for the ultrasound nebulization of PF 5080 to deliver to the end of the inspiratory limb of the ventilator was at "maximal" on this make of nebulizer. This is approximately a frequency of oscillation of 1.63 Megahertz. Indirect evidence that the PFC was being delivered to the endotracheal tube was the immediate change in the inspiratory volume shown by the Ventrak

analyser sited directly proximal to the endotracheal tube. This implied the presence of an additional substance. From table 7 the nebulized PF 5080 group appear to have been given a greater amount of PFC. However this does not imply that the substance was delivered to the lower parts of the respiratory tract. One conclusion for the failure of nebulized PF 5080 to affect survival is that no significant amounts of nebulized PFC actually reached the lungs.

One other interpretation is that if given by a nebulized route the PF 5080 would only reach the well ventilated (i.e. less severely affected) parts of the lungs and little reached the atelectatic parts of the lung most in need of treatment.

Surfactant had no effect on survival in this study. The study protocol allowed for only 1 dose of surfactant, where the PF 5080 groups were treated continuously with PFC. There may have been a greater effect on survival if re-dosing of surfactant had been allowed. Alternatively, there may have been a type II error, which would not have been present if resources had allowed a larger study as noted above. The difficulty in making power calculations for survival for this specific group, where no direct previous references were available, was discussed in chapter one.

Preparations of surfactant which do not contain apoproteins such as Pumactant appear to be inferior to those which do in the presence of acute lung injury[132 129]. Apoprotein free preparations are more susceptible to inactivation by inhibitory proteins.

The failure of the combination of Curosurf with partial liquid ventilation to have any additional survival advantage over control or indeed over the individual therapies alone could be explained as follows. Natural surfactants have the ability to reduce

surface tension to near normal values. PF 5080 has a fixed surface tension (15dyne/cm from 3M data sheet). Thus coating the lungs with an agent with a poorer surface tension reducing properties (replacing Curosurf with PF 5080) may lead to a poorer outcome. The inhomogenous nature of ARDS may means that there were some areas of lung able to achieve very nearly a normal surface tension (Curosurf coated) and areas with a substantially higher surface tension (PF 5080 coated) generating shearing forces [15 48] and consequent adverse events.

The rapid action of apoprotein containing surfactant [173] may have restored FRC to near normal [168]. Partial liquid ventilation with relatively large tidal volumes at near FRC may be dangerous. Cox and colleagues found that 10 from 13 of their saline lavaged rabbits ventilated with tidal volumes of 15ml/kg on top of 18ml/kg of Perflubron, developed pneumothoraces within 2 hours.[80] Tutuncu would argue that a ceiling effect is seen in terms of improving compliance with only 3ml/kg [158]. To minimise lung overdistention some authors have recommended that PLV be used only with a pressure controlled mode of ventilation.[66]

One interpretation of the improved survival of the combination of Pumactant/PFC is that the Pumactant contributed little in the way of activity as it was inactivated by inflammatory mediators within the alveoli. There was no marked contrast in surface tension between (Pumactant) surfactant coated areas and PFC coated areas as there could be in the Curosurf/PFC group and thus less shearing forces. Hence the improved survival of the combination of Pumactant/PFC was essentially that contributed by the PFC.

In summary partial liquid ventilation with either PF 5080, or the combination of Pumactant and partial liquid ventilation with PF 5080 improves survival to 12 hours in rabbits after saline lavage lung injury.

Chapter 4

The effect of Partial Liquid Ventilation (PLV) with PF 5080, nebulized PF 5080, surfactant preparations and combinations of PLV with surfactants on arterial oxygenation.

Summary

The aim of this chapter was to investigate any differences in oxygenation between the seven treatment groups.

The effect of PLV with PF 5080 alone on arterial oxygenation was compared with combinations of surfactants given alone or in combination with PF 5080, as well as with nebulized PF 5080, on the population of 82 lung injured female New Zealand white rabbits described in chapters 2 and 3. After inducing lung injury by repeated saline lavage, the animals were randomized to the seven groups described in chapter 2

Arterial oxygenation was measured immediately prior to treatment and at 1, 3 and 6 hours after achieving adequate lung injury. Statistical analysis compared to control was done at each time point by ANOVA with Newman-Keuls test for multiple comparisons.

Oxygenation was improved in the animals treated with PLV, Curosurf and the combinations of Pumactant/PLV and Curosurf/PLV at 1, 3 and 6 hours. There was no difference in the oxygenation between these four groups.

Introduction

One of the defining features of ARDS is a severe hypoxaemia.[8] Hence a question for any method of supporting the injured lung is, can the patient or subject be oxygenated adequately? PF 5080 had never been used for this purpose in a rabbit model of acute lung injury. Additionally, could nebulization of PF 5080 lead to a better oxygenation than pouring the PF-5080 into the respiratory tract?

Although surfactants had been used in animal models of ARDS they had been used with variable success [129;132;150;151;174], and no direct comparison had previously been made between the effects of Pumactant and Curosurf on oxygenation in an animal model of ARDS.

Lastly would an additional effect on oxygenation be seen by combining the varying surfactant preparations with PF 5080 during PLV?

This part of the project was designed to answer these questions.

Methods

The animals were anaesthetised and prepared, and lung injury was induced as described in chapters 2 and 3. When adequate lung injury had been achieved (PaO_2 < 13.3kPa) the animals were randomised to the groups described previously. After randomisation ventilator settings returned the tidal volume to 10ml/kg and 15 minutes later baseline arterial blood gas measurements were made immediately prior to the treatment, and at time points 1, 3 and 6 hours after achieving adequate lung injury. Measurement were made to 6 hours only as there were too few survivors in some groups (control, nebulized PF 5080 and Pumactant groups) beyond this time for further valid comparisons to be made.

Statistical analysis was performed with Analysis of Variance (ANOVA), having checked that the values corresponded to a normal distribution using the Kolmogorov-Smirnov test. The statistical package used for this was *GraphPad Prism™ Version 2.0* (GraphPad Software Inc., San Diego, California, USA).

Results

Results are shown below.

Table 10 shows PaO_2 values for the seven treatment groups, and shows no difference between groups prior to injuring the lungs.

Table 11 shows the PaO_2 for each treatment group after lung injury (but before treatment was given) and at the 1, 3 and 6 Hour time points. There was no difference between the groups immediately after lung lavage.

The results show a highly statistically significant improvement in oxygenation compared to control for the PLV, Curosurf and combination groups at 1 and 3 hours ($P<0.001$), and for the Pumactant/PLV combination at 6 hours ($P<0.001$). There was also a statistically significant improvement in the poured PF 5080, Curosurf and combined Curosurf/ PF 5080 at 6 hours ($P<0.01$). There was no statistically significant difference between PLV, Curosurf or either of the combination groups when compared to each other.

The groups treated with Pumactant or nebulized PF 5080 showed no difference compared to control. There was no difference between Pumactant and nebulized PF 5080 when compared to each other.

Figure 11 shows a scatter diagram of theses values.

Table 10. PaO_2 Values Pre-lung injury.

Treatment Group	PaO_2 kPa mean ± standard deviation
Control n=20	73.45 ± 12.55
Pour n=12	72.96 ± 6.46
Nebulized n=10	74.55 ± 5.81
Pumactant n=10	75.95 ± 5.77
Curosurf n=10	74.49 ± 4.86
Pumactant/PLV n=10	77.42 ± 5.45
Curosurf/PLV n=10	73.99 ± 4.18

Values normally distributed (Kolmogorov-Smirnov test). ANOVA shows no significant difference in PaO_2 prior to inducing lung injury.

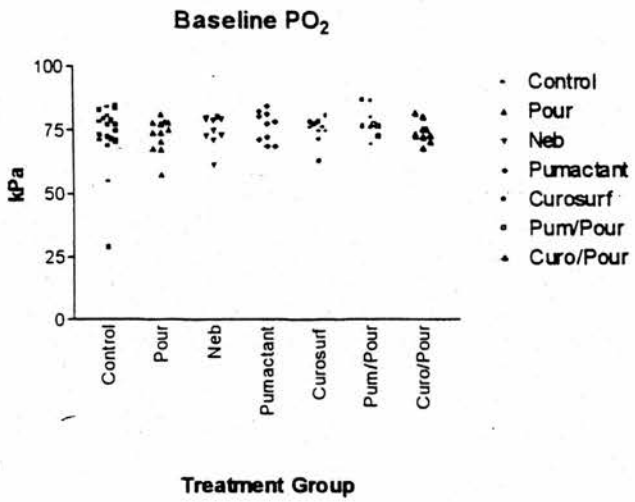
Table 11. PaO_2 values after lung injury.
Values shown are means ± standard deviation (kPa)

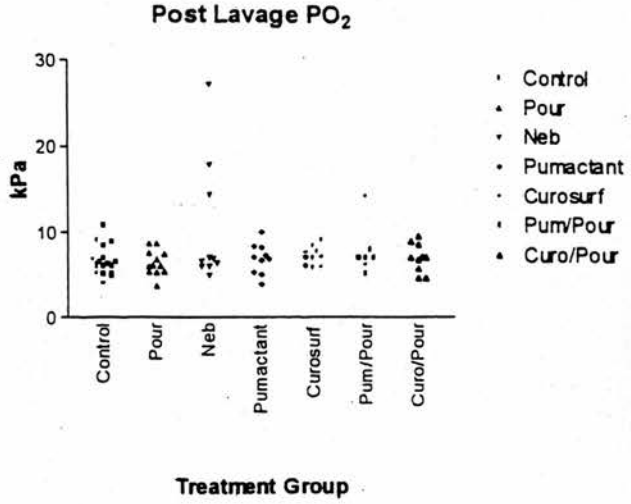
	Time 0 Post Lavage (i.e. Pre Rx)	**1 Hour**	**3 Hours**	**6 Hours**
Control Initial n=20	6.58 ± 1.70	8.71 ± 9.36 (n= 19)	6.76 ± 2.00 (n= 14)	8.6 ± 3.12 (n= 7)
PLV Initial n=12	6.44 ± 1.48	49.03** ± 15.36 (n= 12)	39.7** ± 21.10 (n= 12)	39.93* ± 22.59 (n= 12)
Nebulized PFC Initial n=10	10.3 ± 7.24	6.62 ± 0.89 (n= 10)	7.22 ± 3.24 (n= 6)	6.64 ± 1.28 (n= 5)
Pumactant Initial n=10	6.86 ± 1.78	7.65 ± 3.60 (n= 10)	8.77 ± 8.41 (n= 10)	11.80 ± 9.99 (n= 6)
Curosurf Initial n=10	7.11 ± 1.06	51.62 ** ± 9.83 (n= 10)	43.25** ± 19.89 (n= 10)	42.72* ± 25.76 (n= 9)
Pumactant & PLV Initial n=10	7.56 ± 2.62	48.53 ** ± 10.61 (n=10)	46.50** ± 19.61 (n= 8)	52.80 ** ± 14.49 (n= 8)
Curosurf & PLV Initial n=10	6.96 ± 1.68	46.86 ** ± 11.04 (n= 10)	49.23** ± 15.07 (n= 9)	47.18 * ± 18.62 (n= 8)

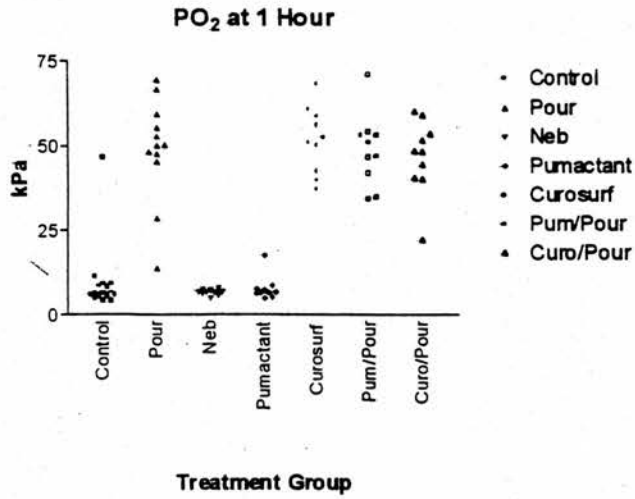
Compared with control group at each time point. Statistical test was ANOVA with Newman-Keuls test for multiple comparisons. * denotes $P<0.01$.

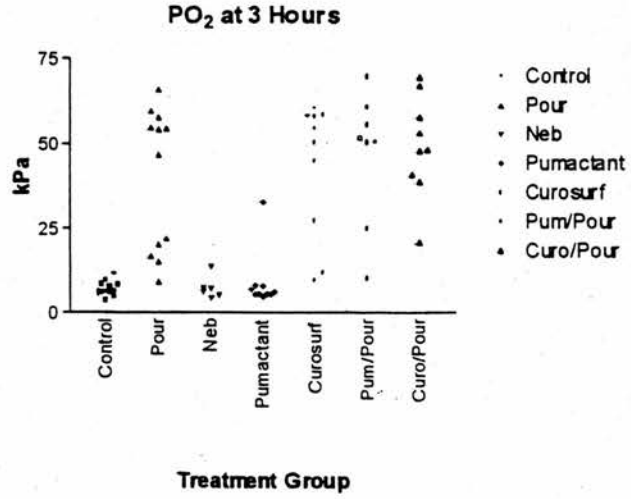
** denotes $P<0.001$.

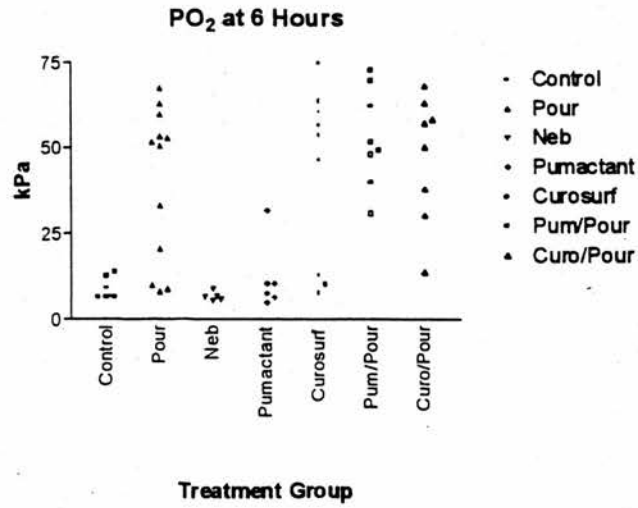
Figure 11. Scatter Diagram of Oxygenation Values











Discussion

The treatments can be broadly split into two categories ; those which demonstrated a difference compared to controls (PLV with PF 5080, Curosurf alone and the combination groups) and those which did not (nebulized PF 5080 and Pumactant alone).

A common factor in the successful treatments is that they are all means of increasing the functional residual capacity, or rather preventing end-expiratory collapse [42].

There were no statistical differences between the four effective treatments when compared to each other. A larger study may have discerned smaller differences.

There are several possible explanations for the failure of nebulized PF 5080 to have an effect. First, it is uncertain how much PFC was actually delivered to the lungs. As shown in the previous chapter, although the mean amount of PF 5080 per minute of survival placed in the nebulization chamber was greater than the amount poured into the lungs in the poured or combination groups, the continuous flow bias in the ventilator circuit (10 l/minute) was approximately 10 times the animals' minute ventilation. This means that no more than 10% of the PFC would actually have entered the animals' respiratory tracts. It is unclear how much would actually be delivered to alveolar level. Also presumably the nebulized PFC would reach only well ventilated (i.e. non-atelectatic lung regions) where it is not required and little if any would reach collapsed alveoli. During PLV, the lungs can be filled to a greater extent, approximating a full liquid FRC.

On bench testing prior to commencement of this study the ultrasonic nebulizer was found to generate 50% of the particles less than 1 micron in diameter (see Table 5, in Chapter 2). This is essentially vaporization. The lack of effect of this route on oxygenation differs from that found by Bleyl et al [155] who found that administering the PFC perfluorohexane to oleic acid injured sheep improved oxygenation and pressure volume characteristics sustained over a two hour period. It is hard to account for these differences, though they were different species and models of lung injury. They used a circle system of administration rather than a continuous bias flow ventilator and claim that 20% of their vaporised PFC was retained in the lungs. It is also possible that perfluorohexane is not biologically inert and in some way altered pulmonary haemodynamics.

Pumactant the apoprotein free surfactant had no effect on oxygenation. This may be due to the apoprotein free surfactants being particularly susceptible to inhibition by protein present in alveoli in acute lung injury [175] [176].

In summary, partial liquid ventilation with PF 5080, Curosurf alone and the combination therapies of Pumactant/partial liquid ventilation with PF 5080 and Curosurf/ partial liquid ventilation with PF 5080, were all associated with a prompt and sustained improvement in oxygenation over 6 hours in this model. There were no statistically significant differences between these groups. In order to discern any differences larger number of animals would have to have been used.

The administration of pumactant alone or nebulized PF 5080 had no significantly different effect on oxygenation compared with control animals.

Chapter 5

The effect of Partial Liquid Ventilation with PF 5080, nebulized PF 5080, Pumactant, Curosurf and combinations of surfactants and Partial Liquid Ventilation with PF 5080, on dynamic and static lung compliances.

Summary

The aim of this chapter was to investigate the effects on lung compliance between the treatment groups.

The effect on dynamic lung compliance (Cdyn) of PLV with PF 5080, nebulized PF 5080, the surfactant preparations Pumactant and Curosurf individually and combinations of surfactant and poured PF 5080 was studied in the population of 82 lung injured female New Zealand white rabbits described in chapters 3 and 4, one three and six hours after achieving adequate lung injury. Additionally, the effect on static lung compliance (Crs) was studied for control animals and those treated with the surfactants Pumactant alone and Curosurf alone at timepoints 1, 3 and 6 hours after achieving lung injury.

Dynamic compliance improved after PLV, Curosurf and the combination of Curosurf/PLV, although there was no difference between groups and control by 6 hours.

Static compliance improved after Curosurf though not after Pumactant. This effect was sustained to six hours.

Introduction

Impaired surfactant function is a feature of ARDS [50 45 58]. Exogenous surfactant administration may have a role in correcting this deficit [177 54 63]. The efficacy of the surfactant treatment may depend upon the preparation used [1]. There had never previously been a comparison of Curosurf with Pumactant in a model of lung injury. Partial liquid ventilation has also been used to improve lung mechanics in the presence of lung injury [91;99;100] but the mode of action may differ from surfactant administration. There may be some rationale in the co-administration of these agents to see if there is an additive effect.

Lastly lung compliance may be measured as dynamic or static compliance, as described in chapter 2. The less cumbersome dynamic compliance technique may generate results differing from the static compliance technique [131;168]. Consequently where possible both methods were assessed.

Methods

The 82 animals described in the previous chapter were anaesthetised and prepared, and lung injury was induced as above. When adequate lung injury had been achieved (PaO_2 < 13.3kPa) the animals were randomised to the groups described in chapters 3 and 4.

After randomisation the pressure level of the ventilator was adjusted back to generate tidal volumes of 10ml/kg. After a further 15 minutes, baseline measurements of dynamic lung compliance (Cdyn) were made immediately prior to the treatment and at time points 1, 3 and 6 hours after achieving adequate lung

injury. These measurements were made in all groups using a Ventrak 1550 respiratory mechanics monitoring system (Novametrix Medical Systems, Wallingford Connecticut, USA) with a neonatal flow sensor. Cdyn was calculated as the tidal volume divided by the difference between the peak inspiratory pressure and the end-expiratory pressure at points of zero flow and corrected to body weight (ml $cmH_2O^{-1}kg^{-1}$). For the nebulized PF 5080 group the nebulizer was switched off temporarily so that readings could be made. It was found that the Ventrak recorded falsely high tidal volumes if readings were made while the nebulizer was running.

Static lung compliance (Crs) measurements were made for control, Curosurf alone and Pumactant alone groups only by using the modified single breath test as described in chapter 2. Due to the PF 5080 in exhaled gas it was not possible to use this technique to measure any animal treated with PF 5080.

Measurement were made to 6 hours only as there were too few survivors in some groups (control, nebulized PF 5080 and Pumactant groups) beyond this time for further valid comparisons to be made.

Results are shown below. The statistical package used for this was *GraphPad Prism™ Version 4.0* (GraphPad Software Inc., San Diego, California, USA).

Statistical analysis for the Cdyn and Crs data was performed with Analysis of Variance (ANOVA) at each time point, having checked that the values corresponded to a normal distribution using the Kolmogorov-Smirnov test.

The Kruskal-Wallis test with Dunn's multiple comparison test was performed on the respiratory rate data of each group at the time points pre-lavage, post lavage, at 1 hour, at 3 hour and at 6 hours.

Results

Results are given below.

Table 12 shows baseline dynamic (Cdyn) and static compliance of the respiratory system (Crs) pre-injury. Data followed a normal distribution for C Dyn. ANOVA with Newman-Keuls post test shows no significant difference between groups. Similarly, data followed a normal distribution for Crs. ANOVA with Newman-Keuls post test shows no significant difference between groups. Thus there was no intrinsic difference between groups prior to inducing lung injury.

Table 13 shows Cdyn from randomisation (i.e. post injury but pre-treatment) and at the 1, 3 and 6 hour timepoints. There was no difference between groups after lung injury but before treatment. PLV, Curosurf and the combination Curosurf/ PLV improved Cdyn compared to control at the timepoint 1 hour ($P<0.001$). The same three groups showed a significant effect compared to control at the 3 hour timepoint ($P<0.01$ for PLV and and the combination Curosurf/ PLV; $P<0.05$ for the Curosurf alone group). By 6 hours, no statistically significant effect could be demonstrated by any treatment over control.

Table 14 shows the Crs immediately pre-treatment and at time points 1, 3 and 6 hours for the groups Control, Pumactant and Curosurf. There was no difference between groups after lung injury but before treatment. Curosurf showed a significant improvement compared to either control or Pumacatant at the 1, 3 and 6 hour timepoints ($P<0.001$). By contrast Pumacatant was not statistically different than control at any timepoint.

Table 15a-15e shows respectively the respiratory rates before lung injury, after lung injury and at the 1, 3 and 6 hour time points for all groups.

Figure 12 shows Crs from randomisation (pre-treatment but after injury) to 6 hours after injury for the groups Control, Pumactant and Curosurf.

Figure 13 shows a scatter diagram of the respiratory rates after injury and at the 1, 3 and 6 hour time points.

Figures 14 (a)-Figure 14 (n) show examples of flow, pressure and volume traces measured by the Ventrak. These are at the timepoints "baseline" (post injury) and "1 hour" after treatment for sample animals from the respective 7 treatment groups.

Figures 15 (a) - Figures 15 (f) show the flow-volume curves (post lung injury and 1 hour after treatment) for a sample animal in the Control, Pumactant and Curosurf groups.

Table 12. Baseline dynamic (C Dyn) and static compliance of the respiratory system (Crs) pre- lung injury. Means are shown ± standard deviations.

	Control n=20	**Pour n=12**	**Neb n=10**	**Pumactant n=10**	**Curosurf n=10**	**Pum/ PLV n=10**	**Curo/ PLV N=10**
C Dyn ml/ cmH_2O/kg	0.89 ± 0.20	0.92 ± 0.19	0.81 ± 0.14	0.85 ± 0.18	0.81 ± 0.07	0.75 ± 0.14	0.88 ± 0.20
Crs ml/ cmH_2O/kg	1.14 ± 0.32	1.16 ± 0.22	0.96 ± 0.20	0.98 ± 0.12	1.07 ± 0.08	1.02 ± 0.20	1.12 ± 0.22

Cdyn and Crs data followed a normal distribution.

ANOVA with Newman-Keuls post test for both Cdyn and Crs data showed no significant difference between groups.

Table 13. Dynamic compliance (ml $cmH_2O^{-1}kg^{-1}$) from randomisation to 6 hours post-treatment. Data are means (Standard Deviations in brackets) Number of rabbits at each respective time point denoted by n= number

Group	Pre treatment	1 Hour	3 Hours	6 Hours
Control	0.47	0.45	0.39	0.37
Initial n= 20	(0.04)	(0.06)	(0.07)	(0.06)
		(n= 19)	(n= 14)	(n= 7)
PLV	0.46	0.55*** (0.06)	0.49**	0.45
Initial n= 12	(0.05)	(n= 12)	(0.07)	(0.11)
			(n=12)	(n= 12)
Nebulized PFC	0.45	0.46	0.41	0.39
Initial n= 10	(0.03)	(0.07)	(0.05)	(0.03)
		(n= 10)	(n= 10)	(n= 5)
Pumactant	0.46	0.44	0.39	0.36
Initial n= 10	(0.05)	(0.05)	(0.05)	(0.06)
		(n= 10)	(n= 9)	(n= 6)
Curosurf	0.47	0.56*** (0.06)	0.47*	0.45
Initial n= 10	(0.02)	(n= 10)	(0.04)	(0.03)
			(n= 10)	(n= 9)
Pumactant + PLV	0.44	0.50	0.46	0.52
Initial n= 10	(0.06)	(0.09)	(0.08)	(0.19)
		(n= 10)	(n= 7)	(n= 8)
Curosurf + PLV	0.47	0.58*** (0.06)	0.50**	0.52
Initial n=10	(0.04)	(n=10)	(0.07)	(0.07)
			(n= 9)	(n= 8)

Regarding Table 13,Compared with control group at each time point. Statistical test was ANOVA with Newman-Keuls test for multiple comparisons.

*Denotes P<0.05 . **Denotes P<0.01

***Denotes P<0.001

Table 14. Static lung compliance group means with standard deviations in brackets. Values are ml cmH_20^{-1} kg^{-1}. n= number of rabbits measured at each respective time point.

	Time 0 (After injury)	**1 Hour**	**3 Hour**	**6 Hour**
Control (Initial n= 20)	0.47 (0.08)	0.44 (0.07) (n=14)	0.36 (0.04) (n= 12)	0.36 (0.05) (n= 6)
Pumactant (Initial n= 10)	0.42 (0.06)	0.40 (0.05) (n= 10)	0.36 (0.03) (n= 9)	0.33 (0.04) (n= 5)
Curosurf (Initial n= 10)	0.46 (0.07)	0.69 (0.11) (n= 10)	0.56 (0.08) (n= 10)	0.52 (0.07) (n= 8)

Groups compared by ANOVA with Newman-Keuls post test for multiple comparisons. At time zero, no difference between groups.

Curosurf versus Pumactant $P<0.001$ at time 1,3 and 6 hours

Curosurf versus Control; $P<0.001$ at time 1,3 and 6 hours.

Pumactant versus Control; no statistical difference at any time point ($P>0.05$).

Table 15(a). Respiratory Rate; Pre-lavage.

	Control	PLV	Neb	Pumactant	Curosurf	Pum/ PLV	Curo/ PLV
Number of Animals	20	12	10	10	10	10	10
Minimum	21	29	20	20	20	20	28
25% Percentile	30	30	30	30	30	30	30
Median	30	30	30	30	30	30	30
75% Percentile	30	30	30	30	30	30	30
Maximum	30	30	30	40	30	30	30

Kruskal-Wallis Test with Dunn's multiple comparison test shows no significant difference in respiratory rate between any treatment group pre-lavage.

Table 15(b) Respiratory Rate; Post lavage.

	Control	PLV	Neb	Pumactant	Curosurf	Pum/ PLV	Curo/ PLV
Number of Animals	20	12	10	10	10	10	10
Minimum	20	25	30	25	30	30	30
25% Percentile	30	30	30	30	30	30	30
Median	30	30	30	30	30	30	30
75% Percentile	30	30	30	30	30	30	30
Maximum	35	30	30	37	30	30	30

Kruskal-Wallis Test with Dunn's multiple comparison test shows no significant difference in respiratory rate between any treatment group post lavage.

Table 15(c). Respiratory Rate; 1 Hour.

	Control	PLV	Neb	Pumactant	Curosurf	Pum/ PLV	Curo/ PLV
Number of Animals	19	12	10	10	10	10	10
Minimum	20	25	30	25	30	30	25
25% Percentile	30	30	30	30	30	30	30
Median	30	30	30	30	30	30	30
75% Percentile	30	30	30	30	30	30	30
Maximum	40	35	30	35	31	30	30

Kruskal-Wallis Test with Dunn's multiple comparison test shows no significant difference in respiratory rate between any treatment group at 1 hour.

Table 15 (d). Respiratory Rate at 3 Hours

	Control	PLV	Neb	Pumactant	Curosurf	Pum/ PLV	Curo/ PLV
Number of Animals	14	12	6	10	10	8	9
Minimum	25	22	27	22	25	15	20
25% Percentile	30	29	20	29	28	22	30
Median	30	30	30	30	30	26	30
75% Percentile	30	30	30	30	30	30	30
Maximum	30	32	35	35	30	30	30

Kruskal-Wallis Test with Dunn's multiple comparison test shows no significant difference in respiratory rate between any treatment group at 3 Hours.

Table 15(e). Respiratory Rate at 6 Hours.

	Control	PLV	Neb	Pumactant	Curosurf	Pum/ PLV	Curo/ PLV
Number of Animals	7	12	5	6	9	8	8
Minimum	25	20	30	22	25	20	18
25% Percentile	28	24	Not applicable	28	27	22	28
Median	30	30	30	29	30	26	30
75% Percentile	30	30	Not applicable	30	30	30	30
Maximum	30	38	35	30	35	30	31

Kruskal-Wallis Test with Dunn's multiple comparison test shows no significant difference in respiratory rate between any treatment group at 6 Hours.

Figure 12: Static compliance (ml/cmH_2O/kg) from randomisation to 6 hours post-treatment. Data are means (standard deviations)

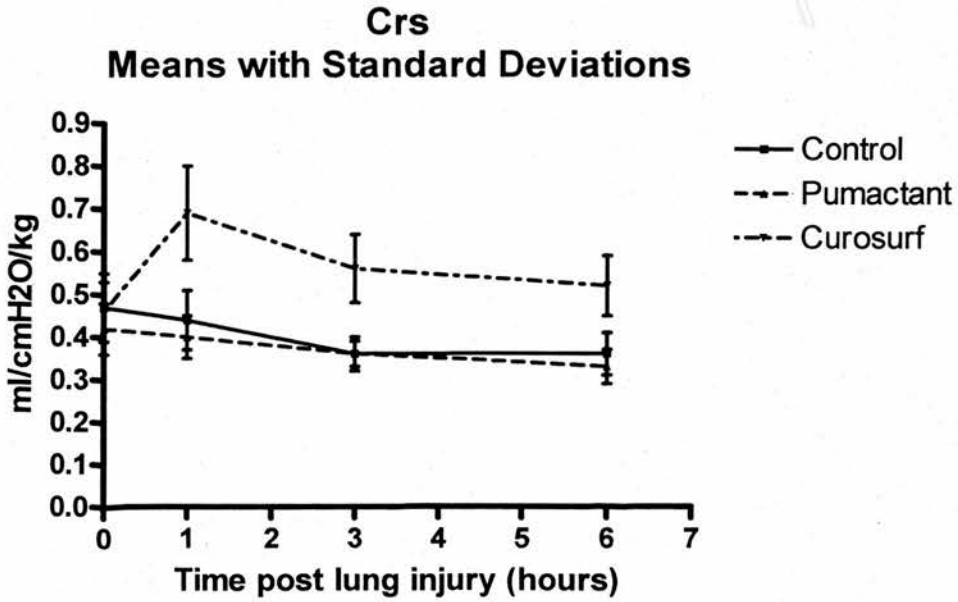
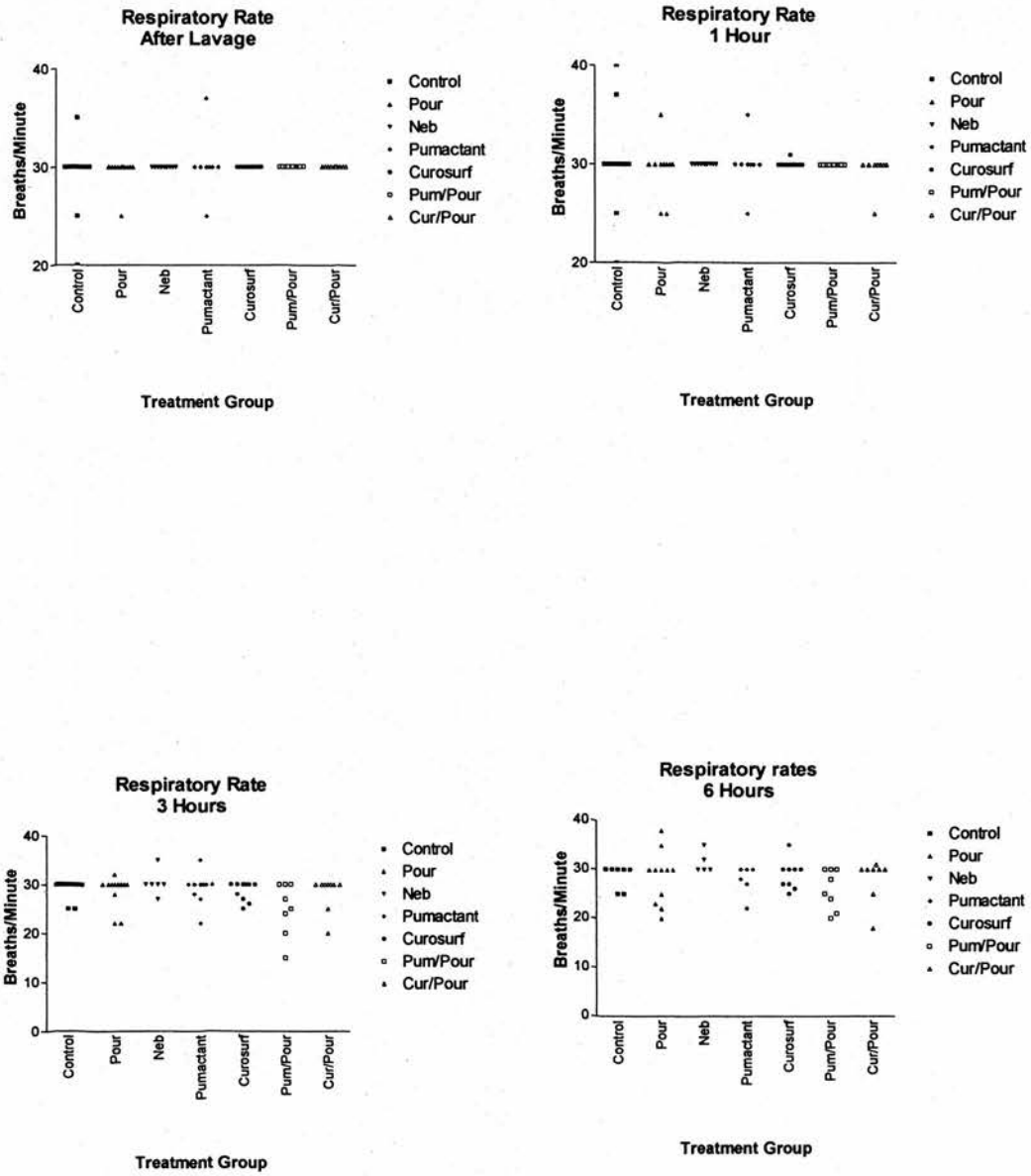


Figure 13. Respiratory Rates at selected timepoints.



The following pages show traces taken from the Ventrak monitoring system (Figure 14(a) - 14(n). The upper trace is the the flow trace, the middle the pressure and the lower trace represents volume. Inspiratory flow is shown above the x axis, and expiratory flow below. Regarding the pressure and volume traces, in each case an upward deflection represents inspiration and downward deflection expiration. Note particularly the effect of PFC vapour on the expired volume.

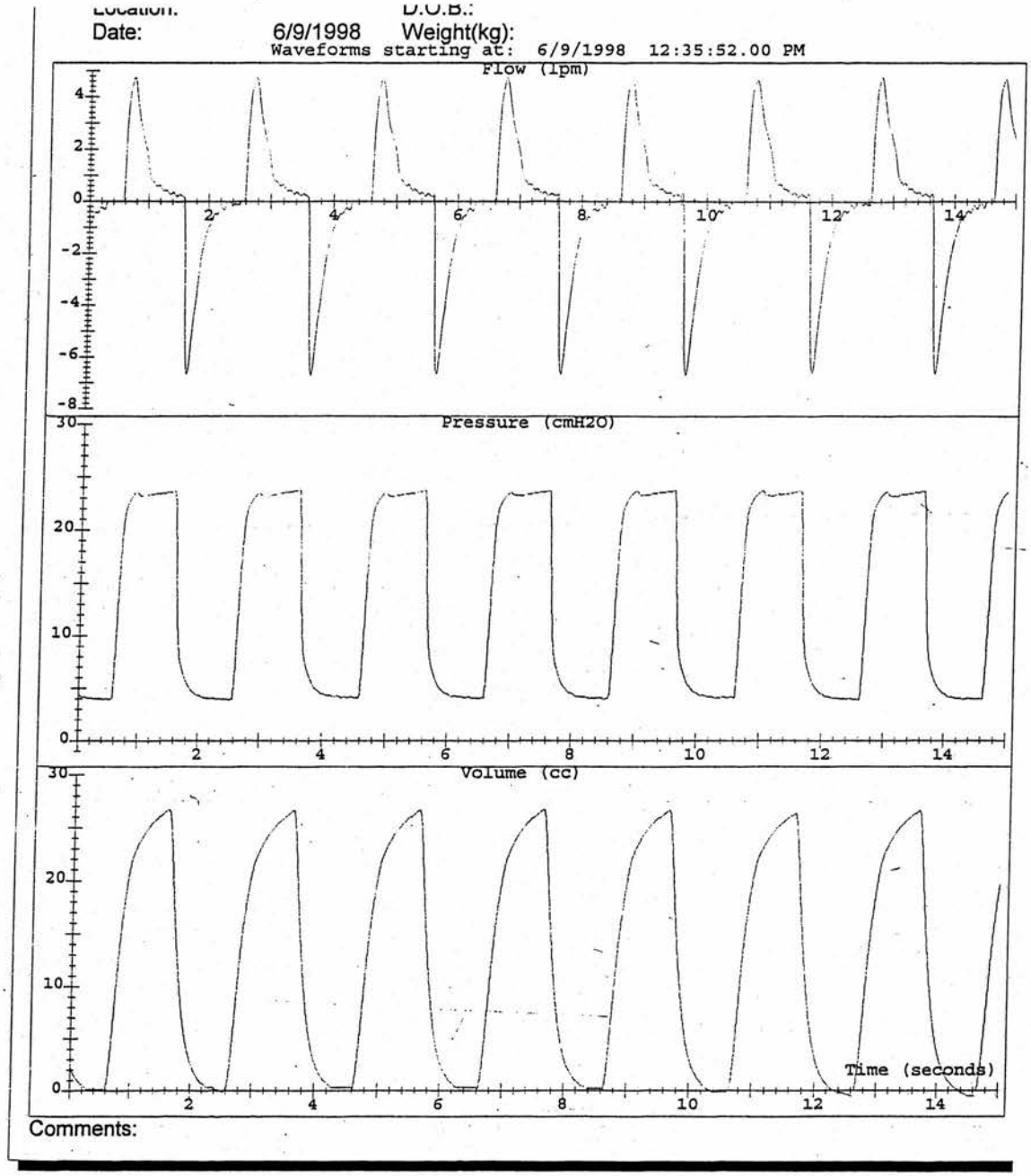


Figure 14(a). Rabbit 15 (Control Group). Post lung injury

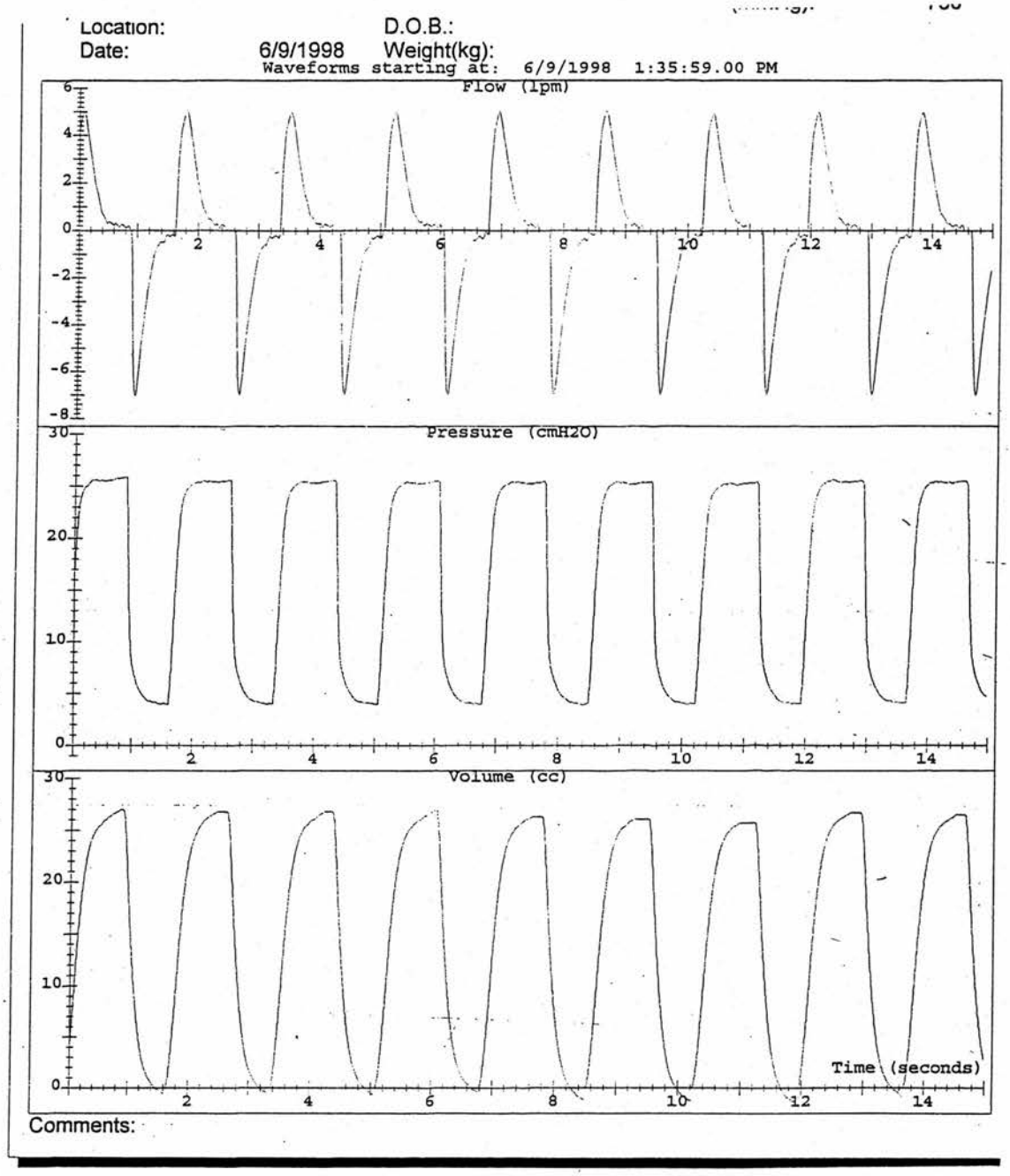


Figure 14(b) Rabbit 15 (Control Group) 1 hour after achieving lung injury.

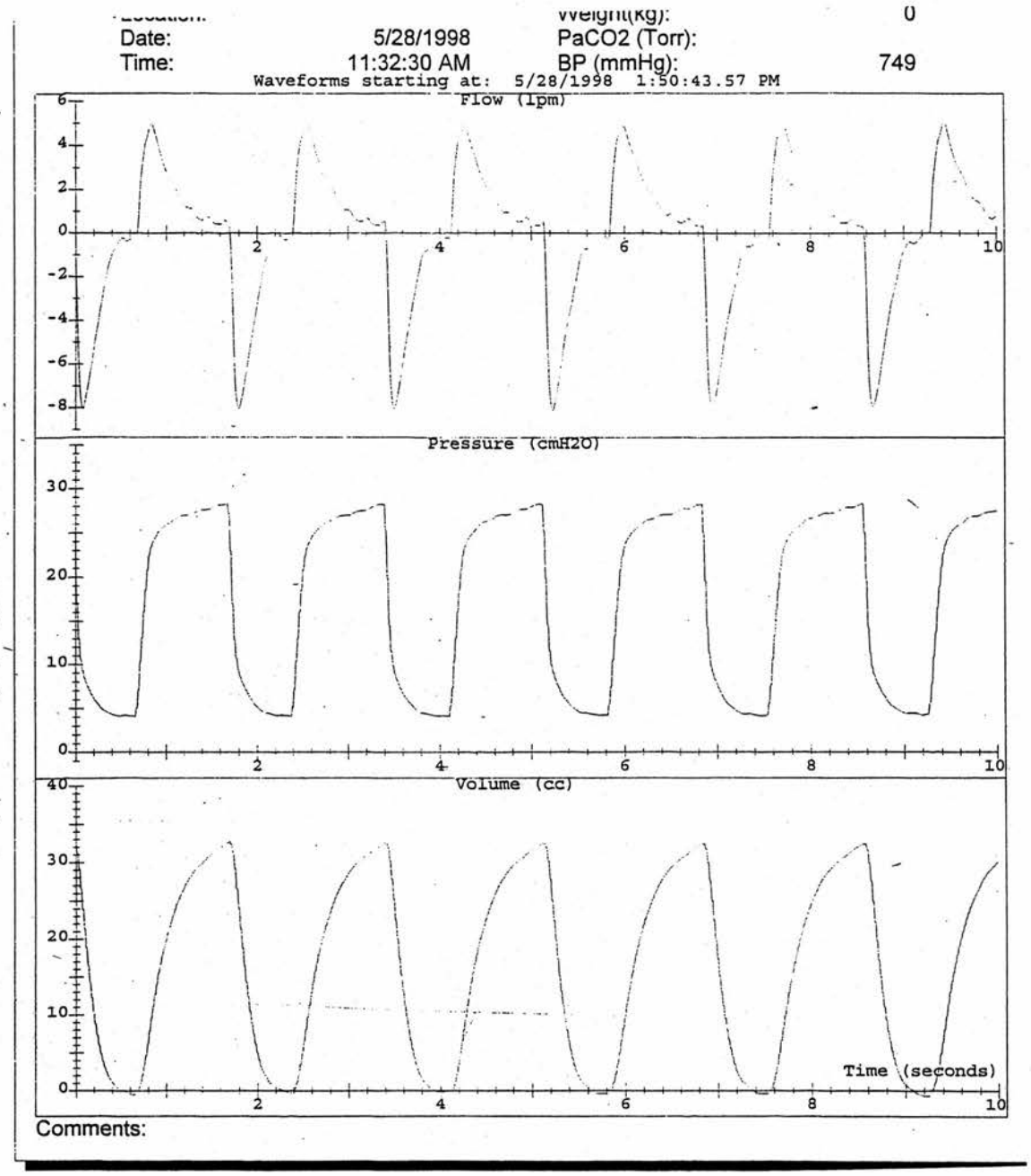


Figure 14(c) Rabbit 10 (PLV Group) Post Lung injury.

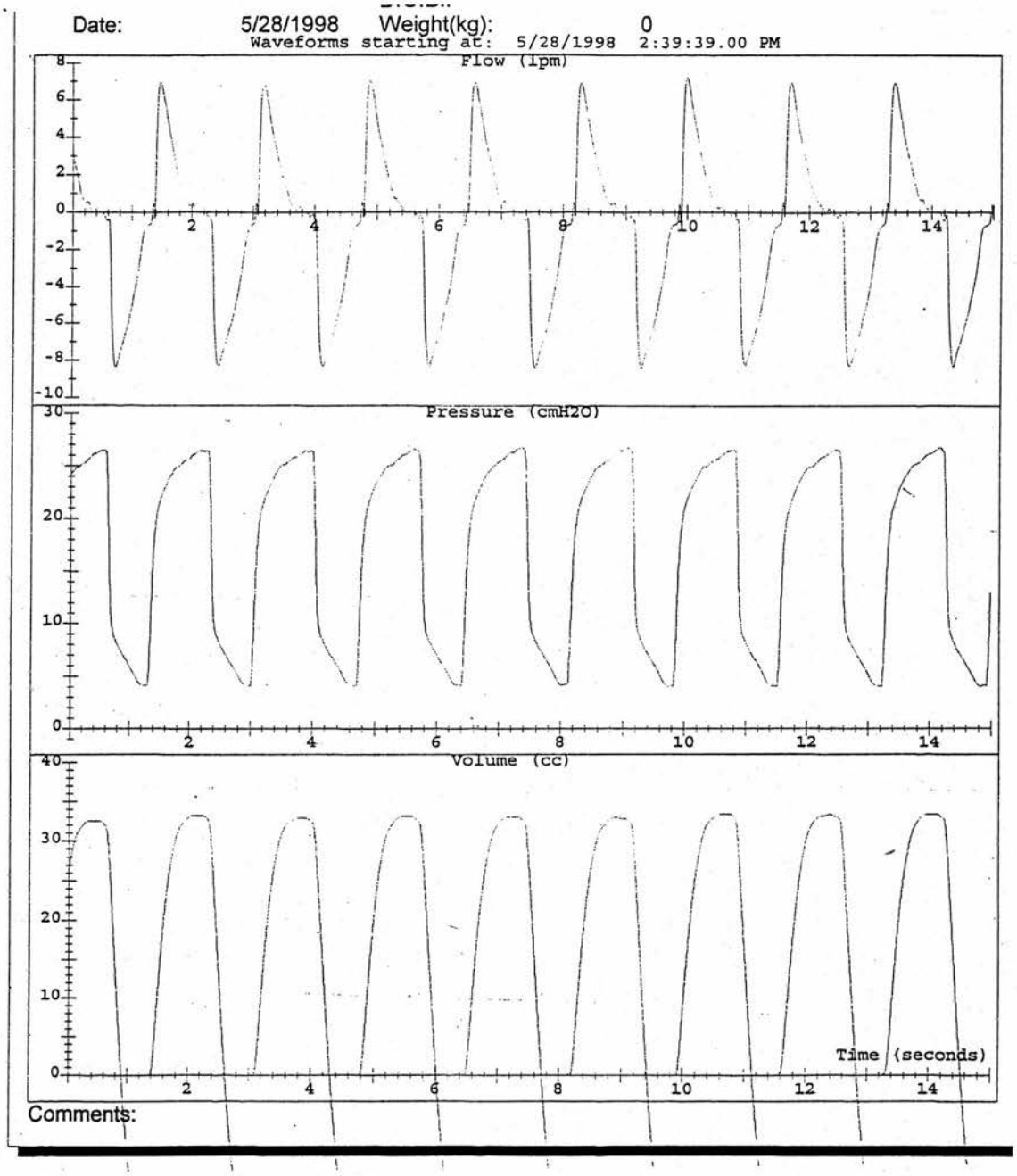


Figure 14(d) Rabbit 10 (PLV Group) 1 Hour timepoint.

The expired volume is artefactually greater because of the influence of PF 5080 vapour on the pneumotachograph measurement.

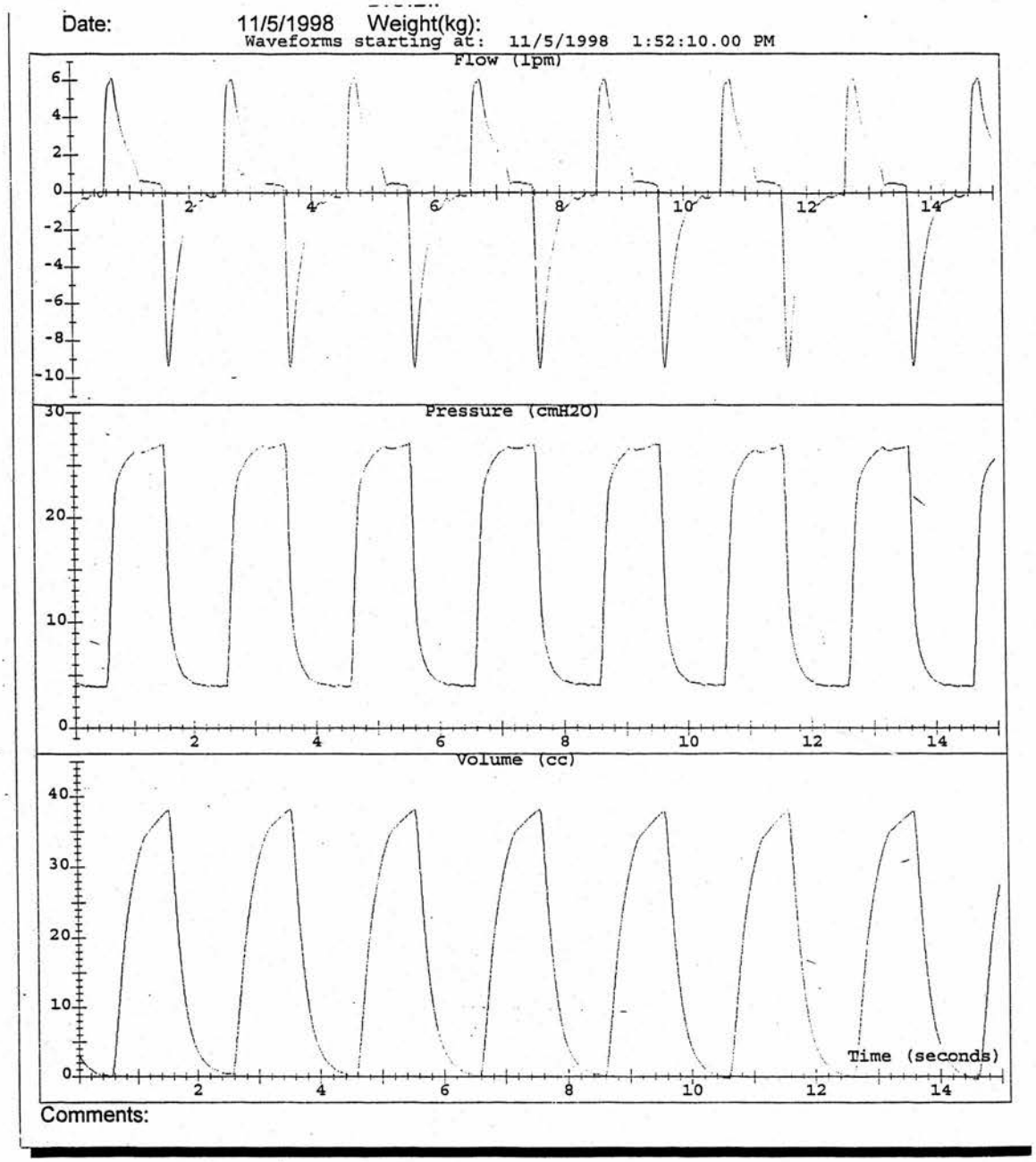


Figure 14(e) Rabbit 49 (Nebulized Group) Post Lung Injury

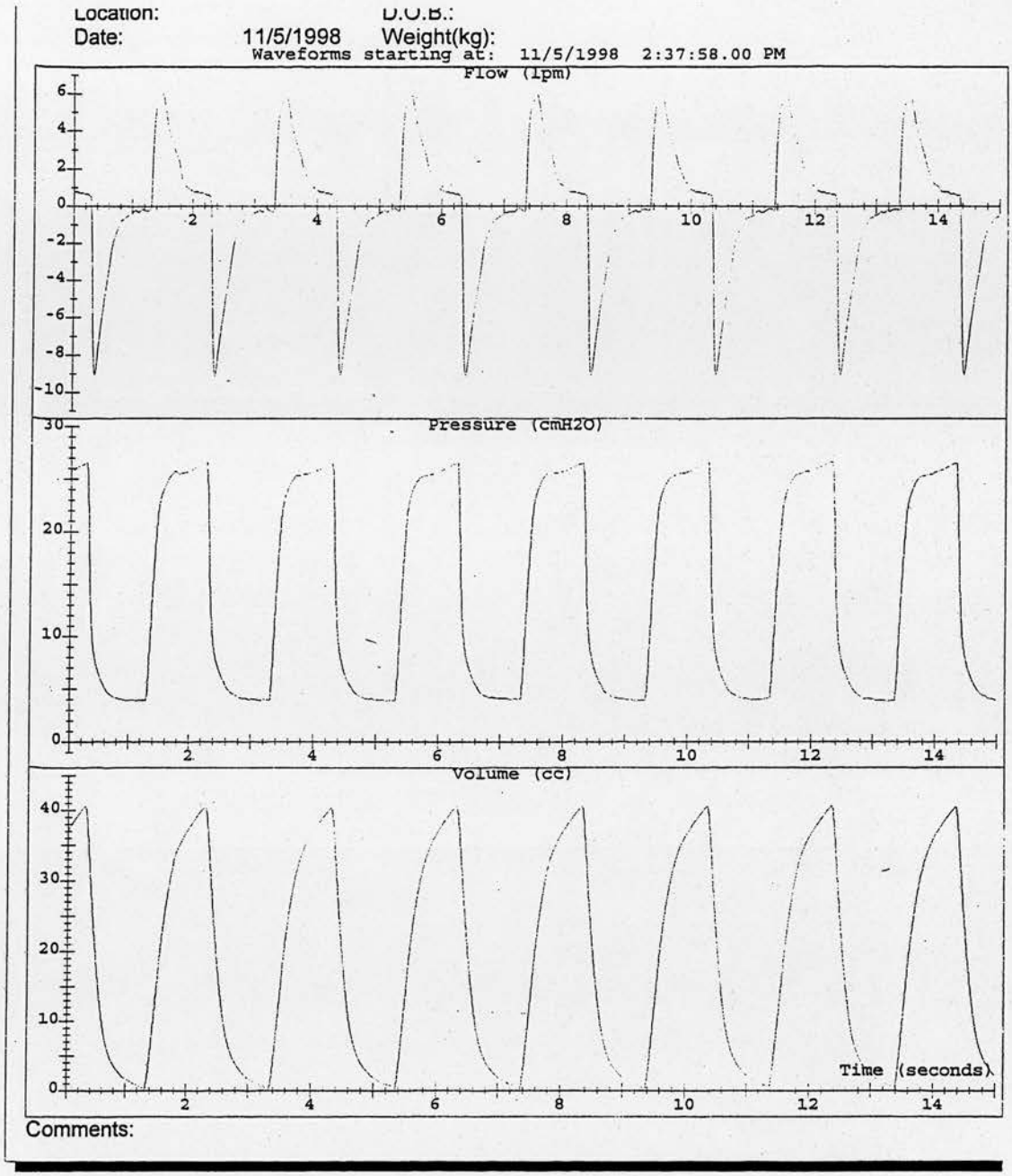


Figure 14(f) Rabbit 49 (Nebulized Group) 1 hour. The absence of the overshoot in the expiratory volume present in all other groups treated with PFC is because the nebulization was stopped transiently to allow the measurement to be performed.

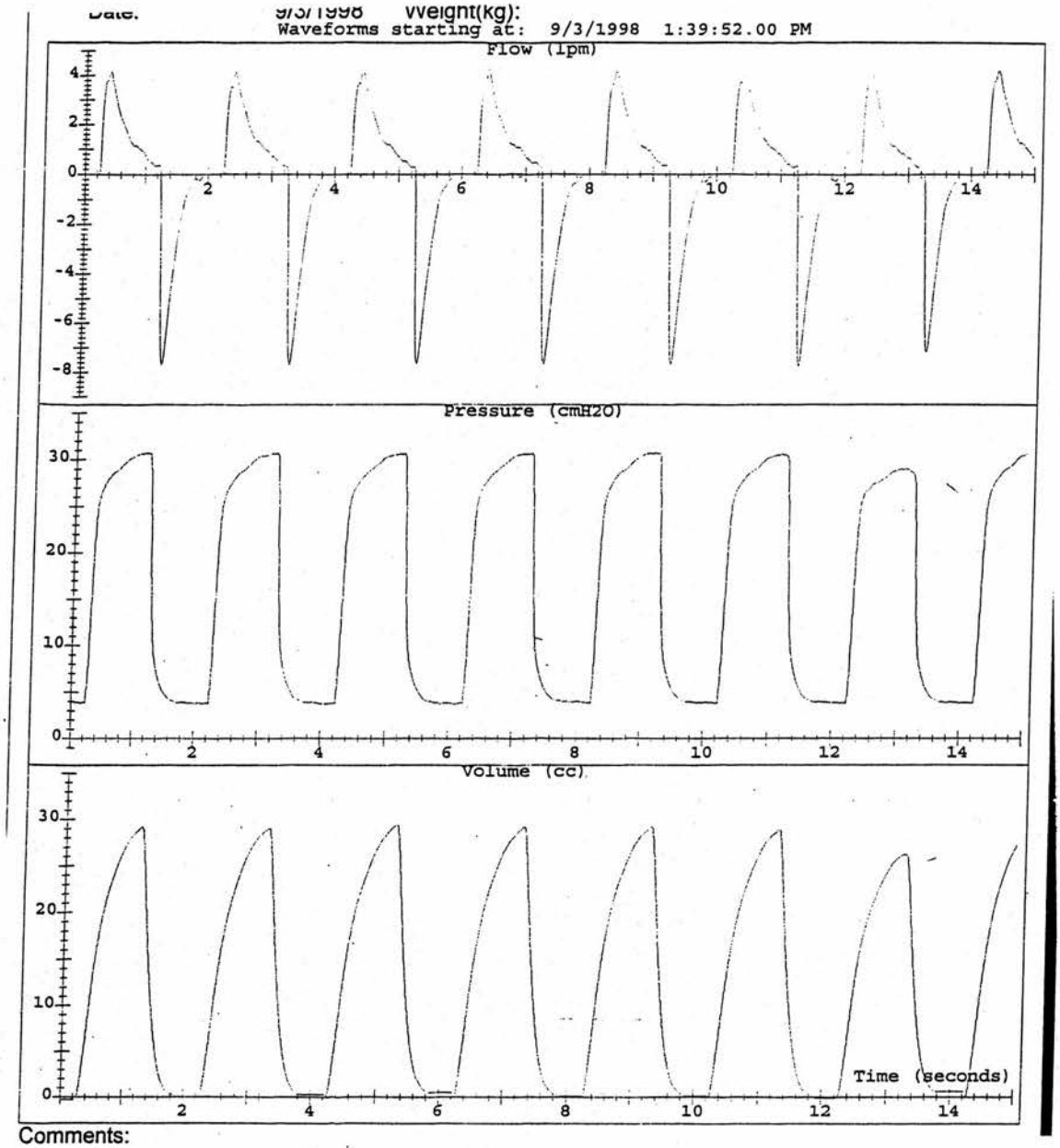


Figure 14(g) Rabbit 35 (Pumactant Group) Post Lung Injury.

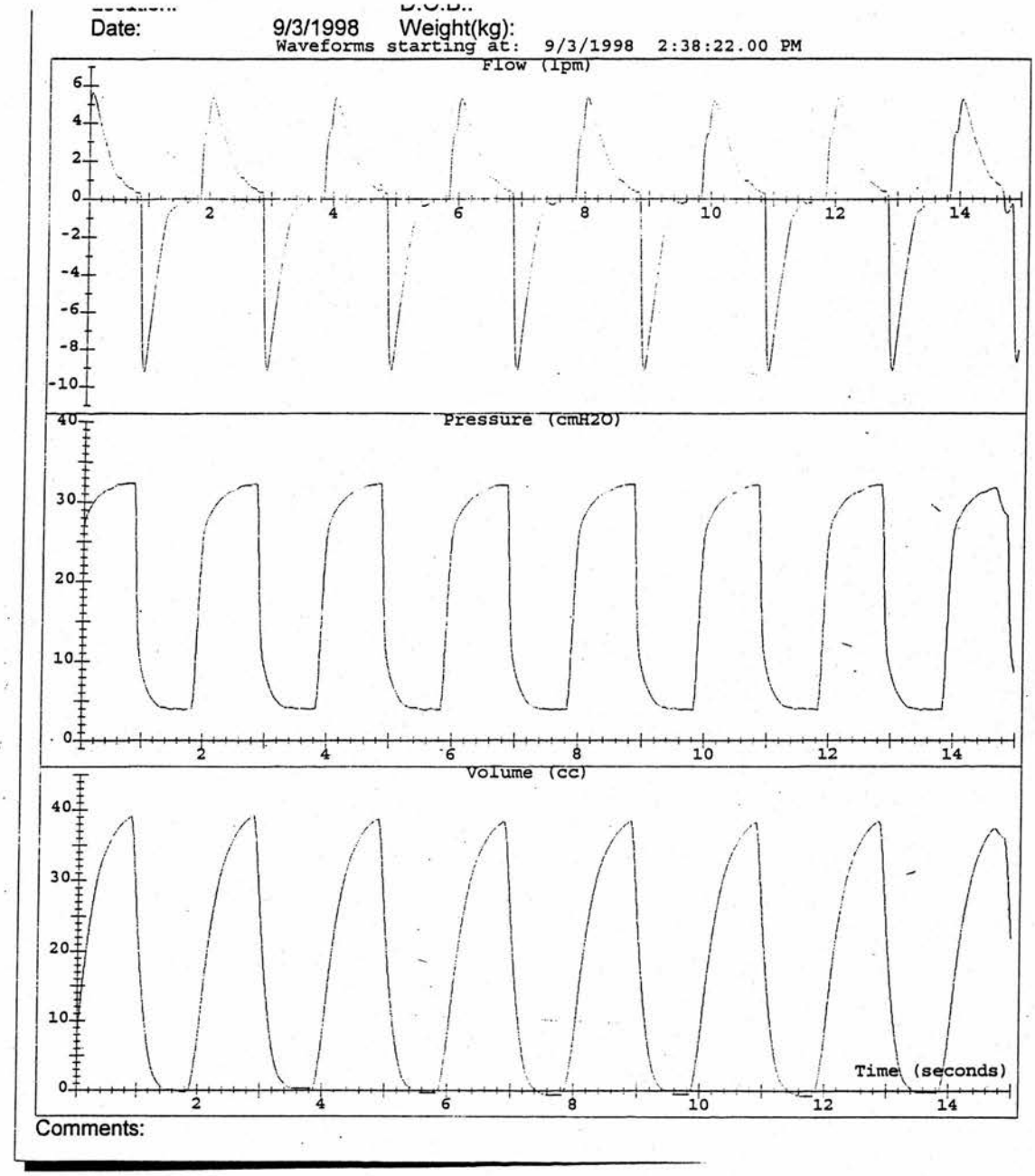


Figure 14(h) Rabbit 35 (Pumactant Group) 1 Hour.

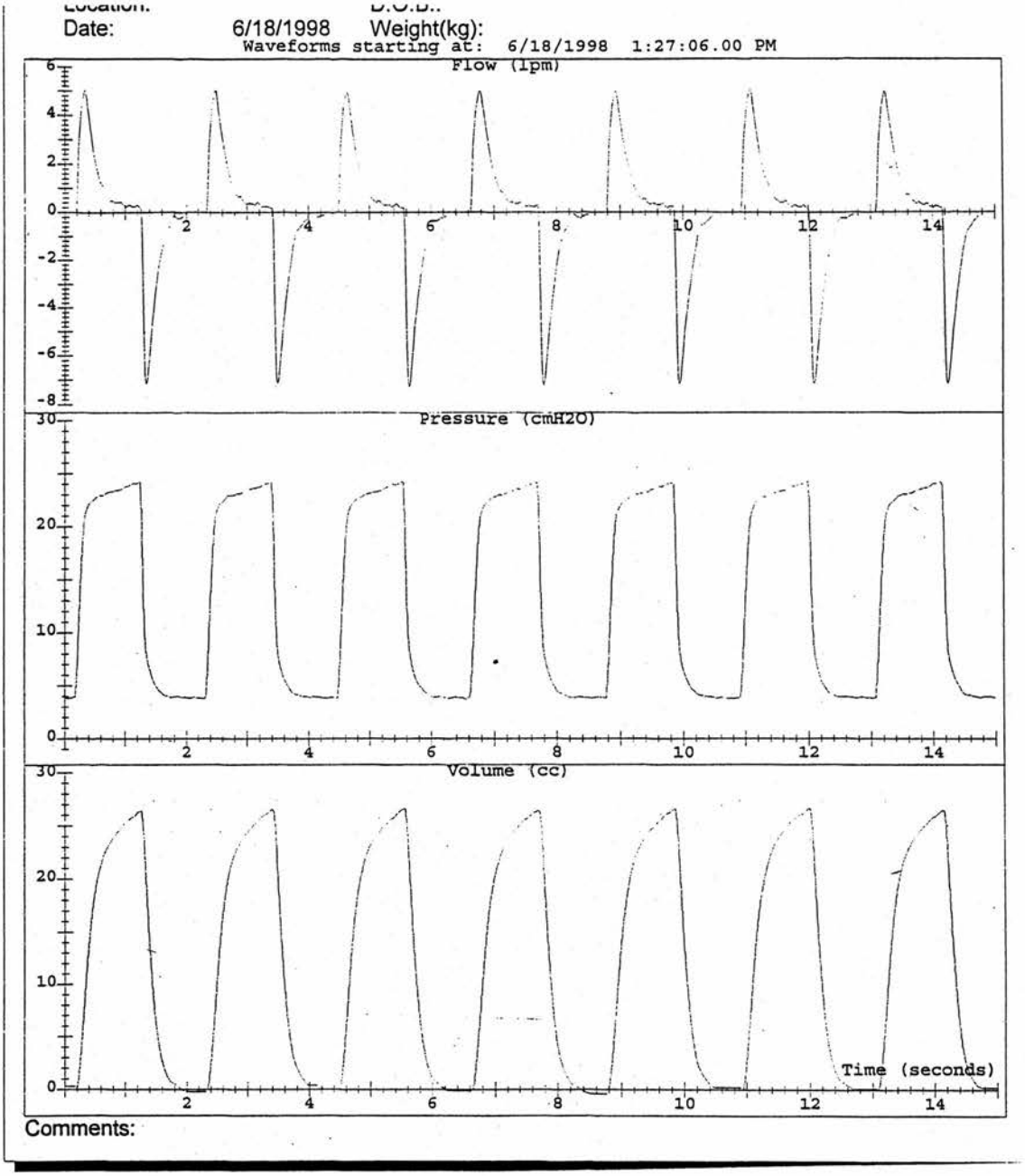


Figure 14(i) Rabbit 20 (Curosurf Group) Post Lung Injury.

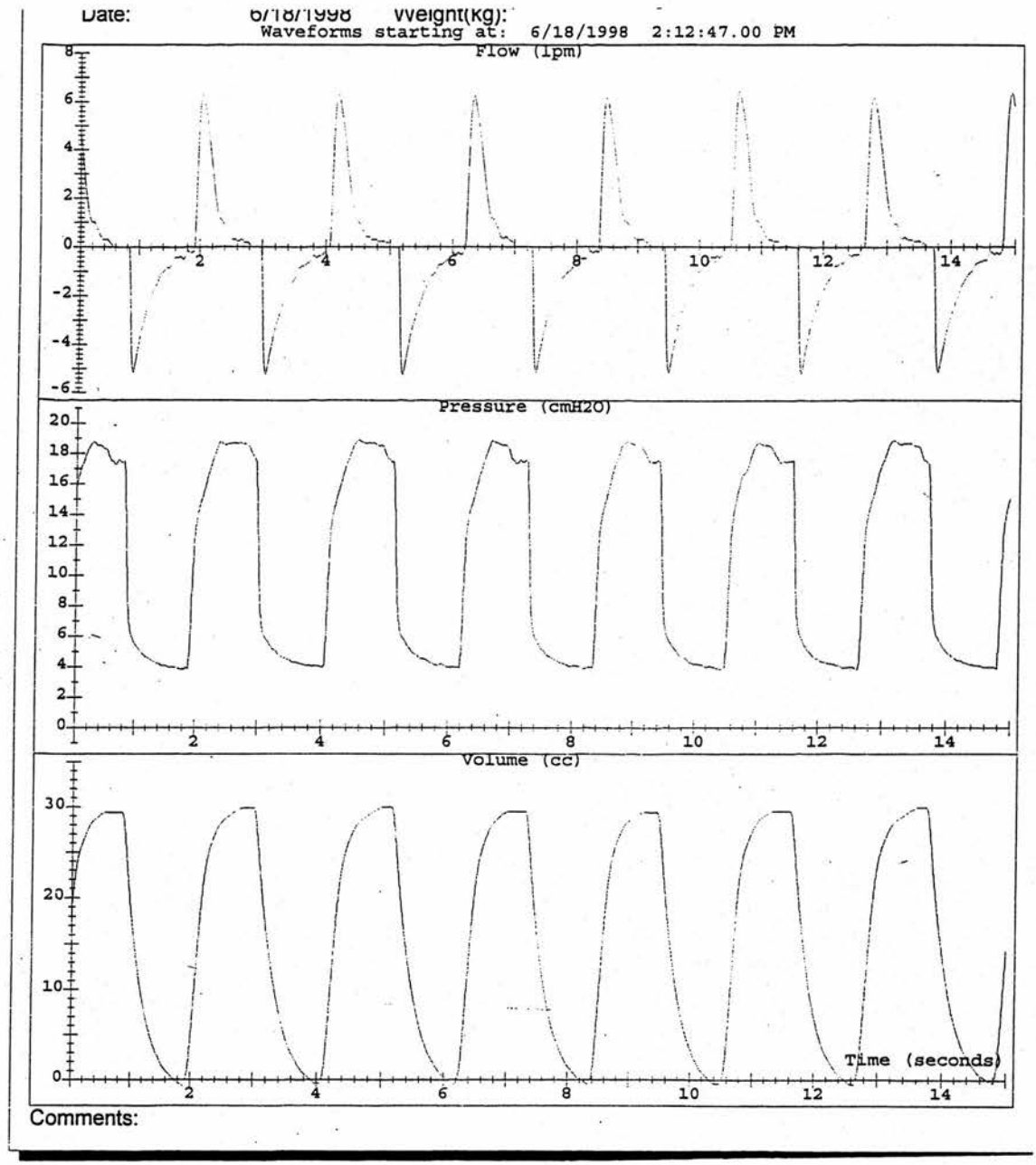


Figure 14(j) Rabbit 20 (Curosurf Group) 1 Hour.

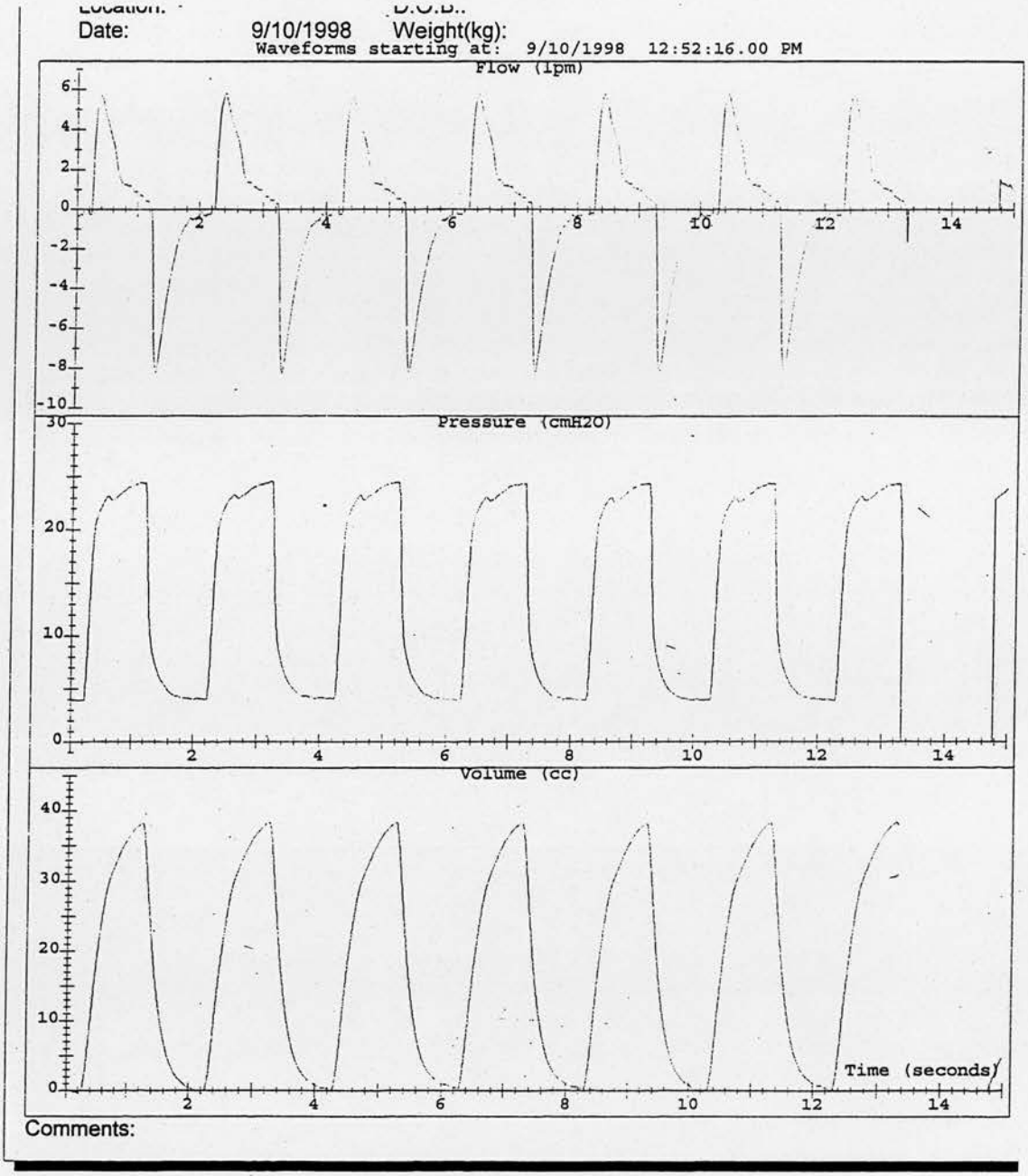


Figure 14(k) Rabbit 39 (Pumactant/PLV Group) Post Lung Injury.

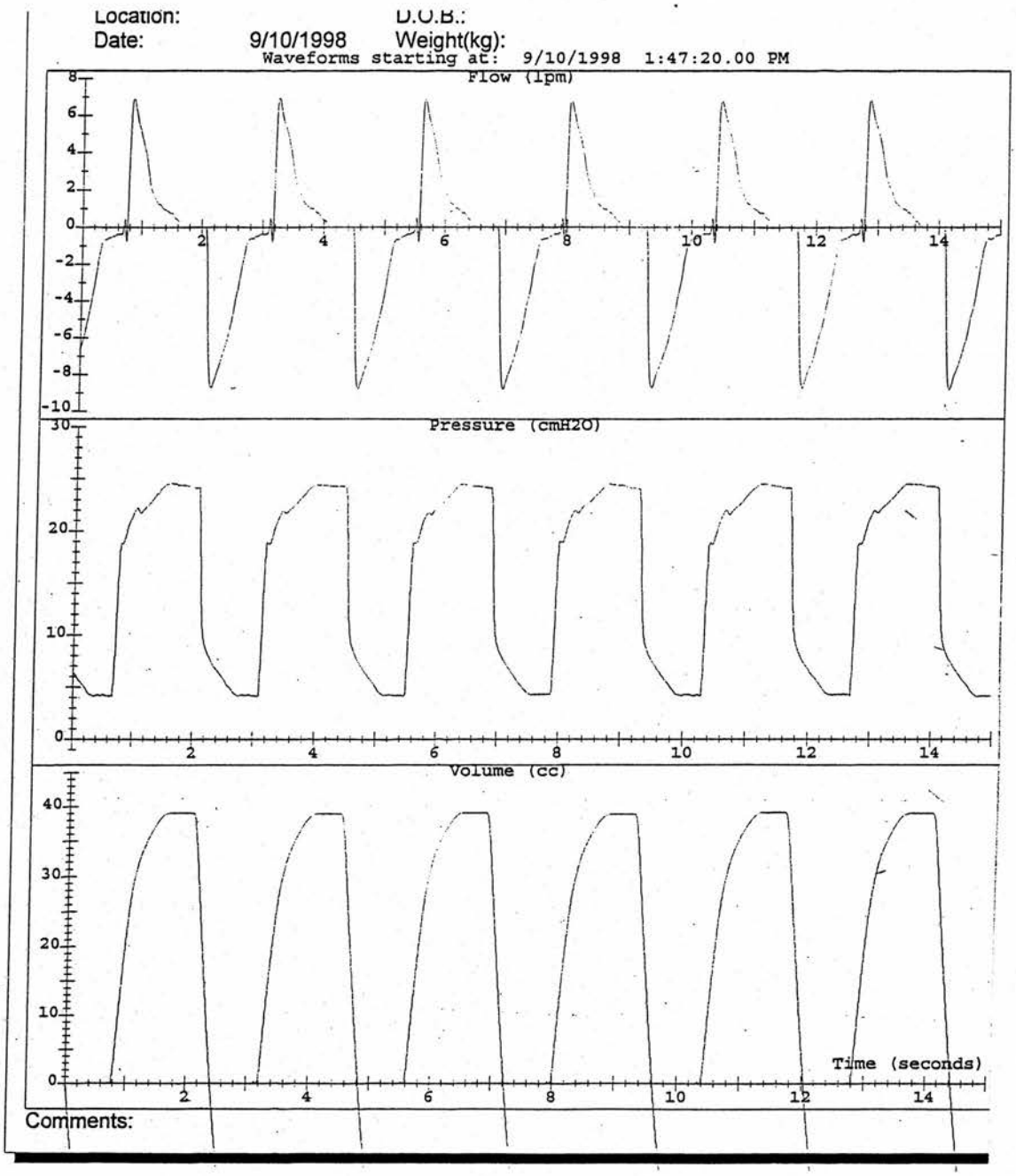


Figure 14(l) Rabbit 39 (Pumactant/PLV Group) 1 Hour.

Once again note the effect of PFC in the exhaled gas.

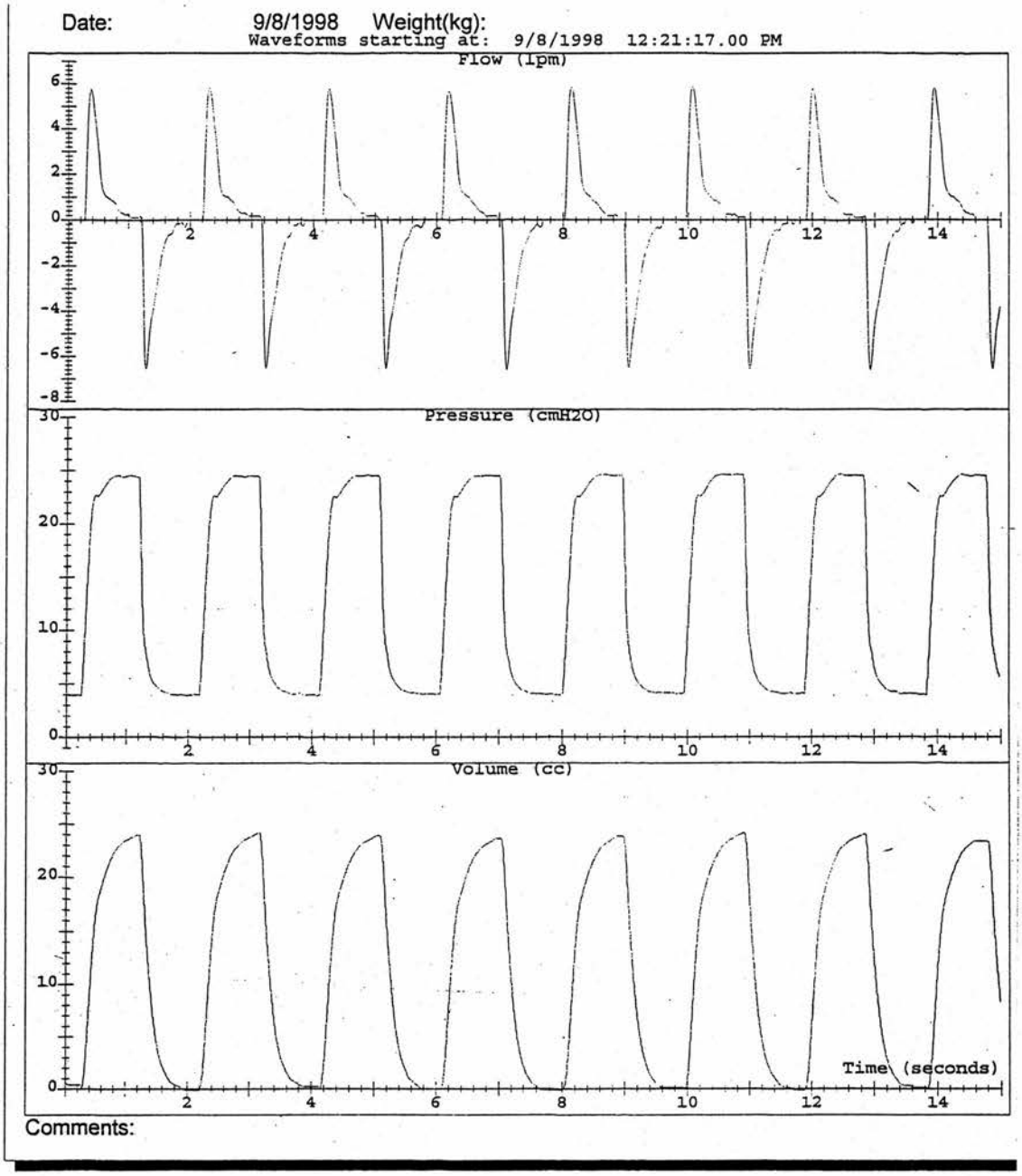


Figure 14(m) Rabbit 37 (Curosurf/ PLV Group) Post Lung Injury

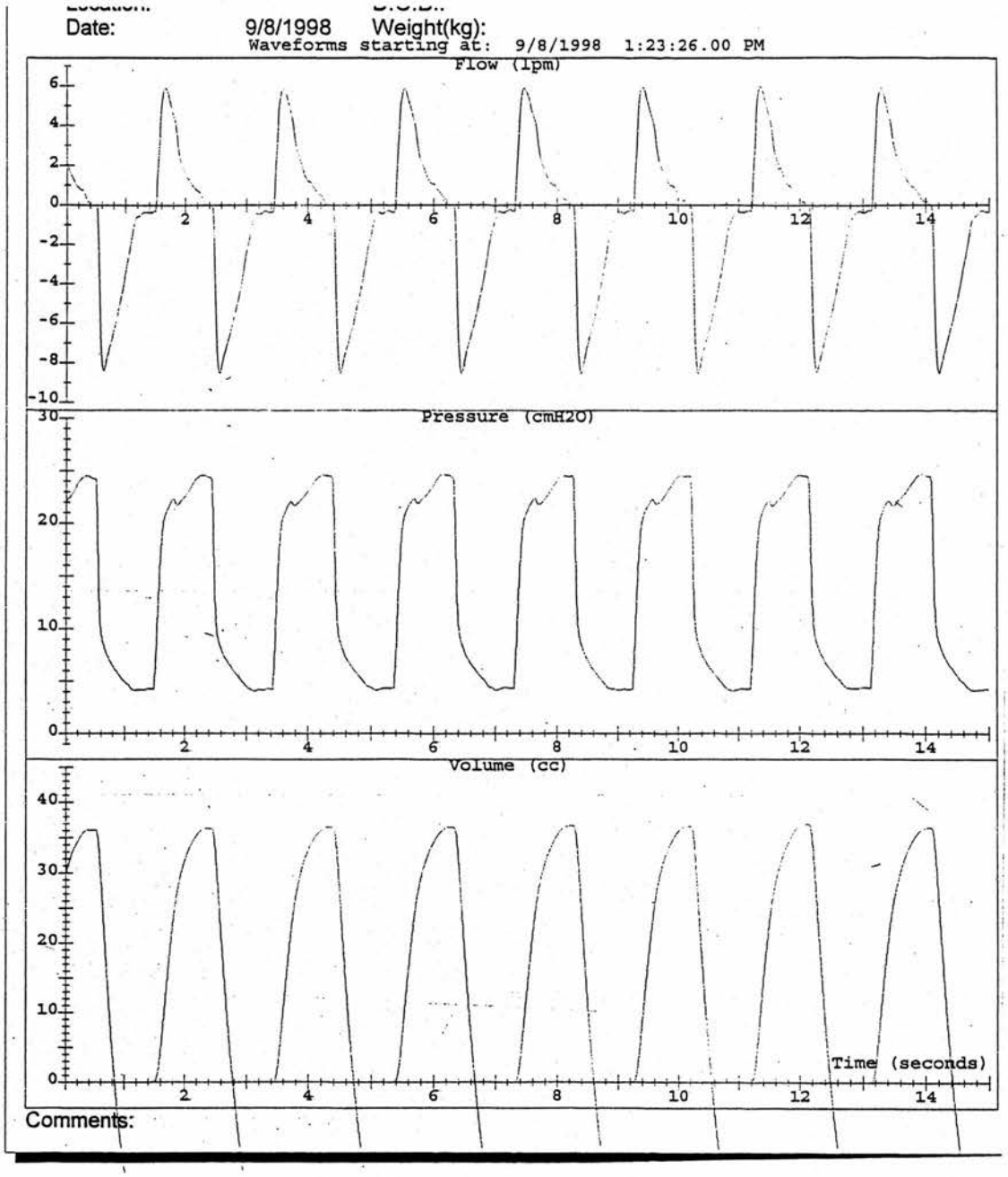


Figure 14(n) Rabbit 37 (Curosurf/ PLV Group) 1 Hour

Similar to Rabbits 10 & 39, note the presence of PFC in the exhaled gases.

The following pages show Flow/ Volume curves for Crs measurements from sample animals in the Control, Pumactant and Curosurf Groups (Figure 15 (a)- Figure 15(f)). For each animal two curves are shown; the upper curve is post lung injury, the lower is 1 Hour after the treatment intervention. It is to be stressed that the value obtained for the exhaled volume is the directly measured volume (not the value extrapolated from the straight line). The airway pressure was measured with a differential pressure transducer as described in Chapter 2.

Figure 15(a) Rabbit 74 (Control Group) Post Lung Injury and Figure 15(b) Same animal 1 Hour later.

Figure 15(c) Rabbit 38 (Pumactant Group) Post Lung Injury and Figure 15 (d) Rabbit 38 1 hour after Pumactant administration.

Figure 15 (e) Rabbit 73 (Curosurf Group) Post Lung Injury and figure 15 (f) Rabbit 73 1 hour after Curosurf administration.

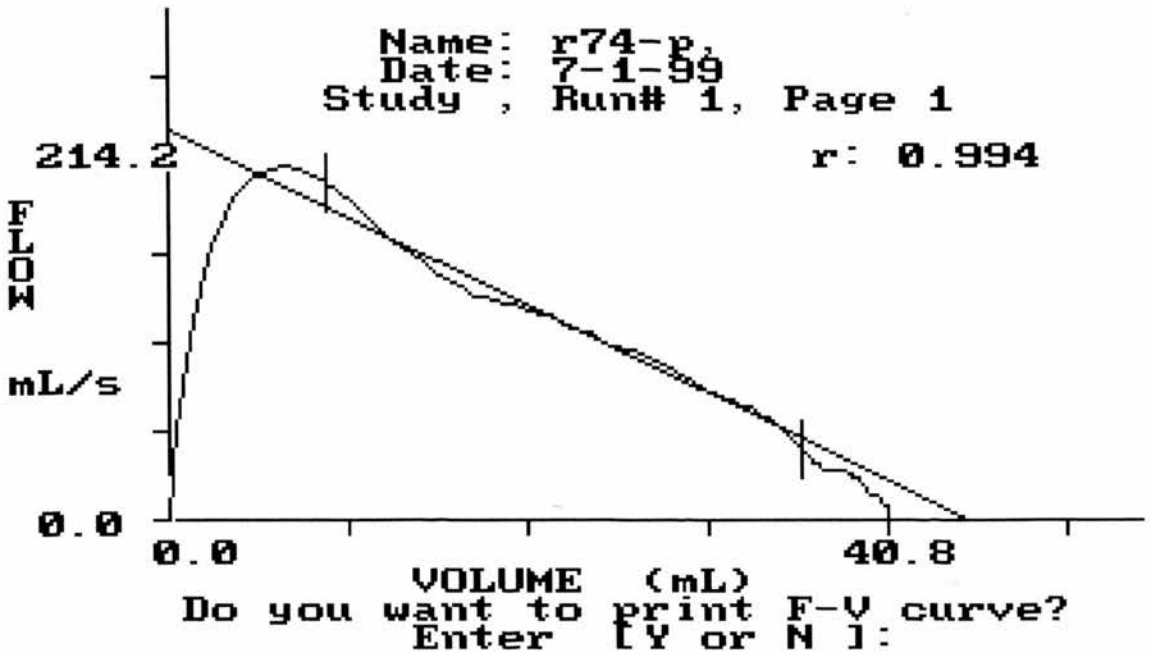


Figure 15 A. Rabbit 74 control group post lung injury

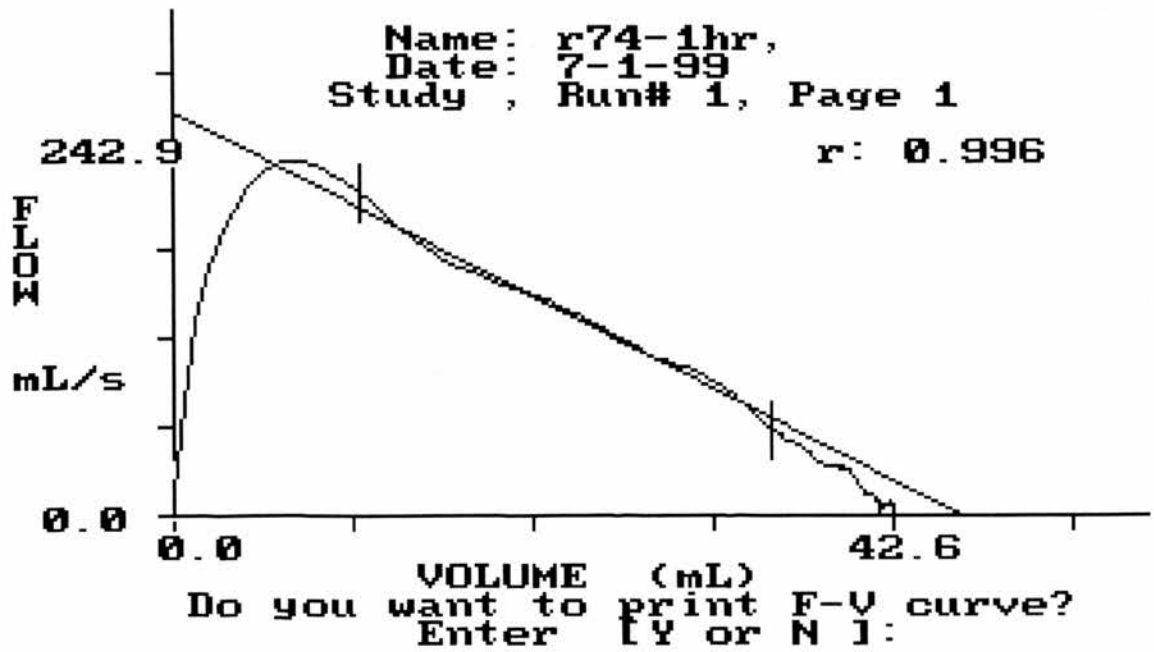


Figure 15 B. Rabbit 74 control group 1 hour later

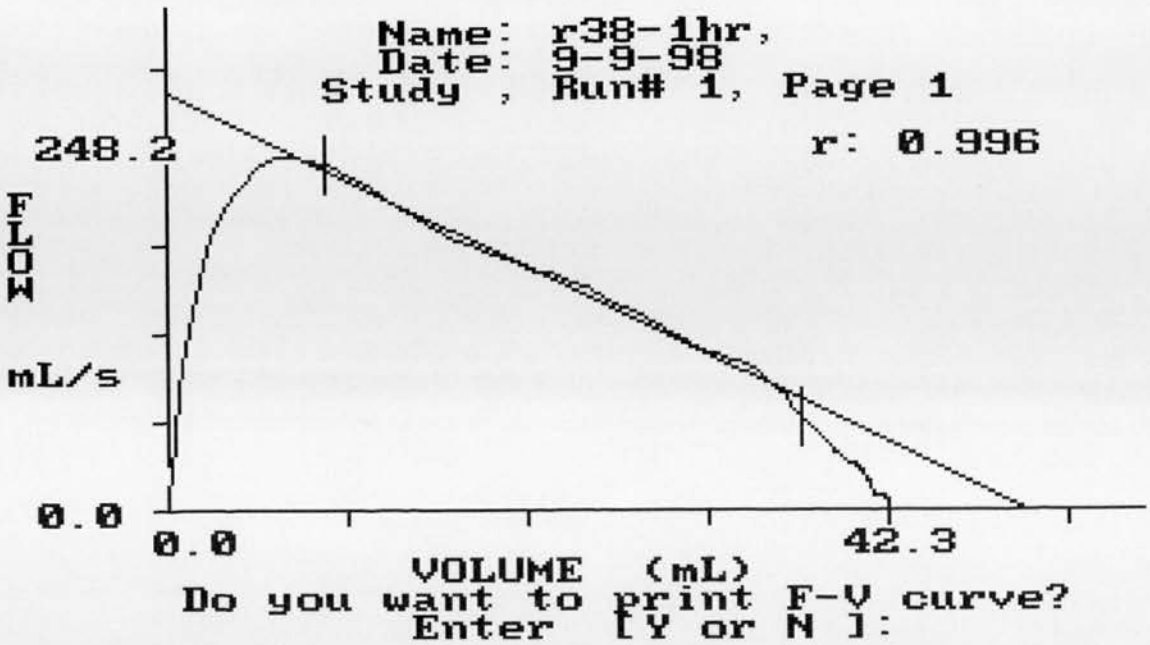


Figure 15 C. Rabbit 38 pumactant group post lung injury

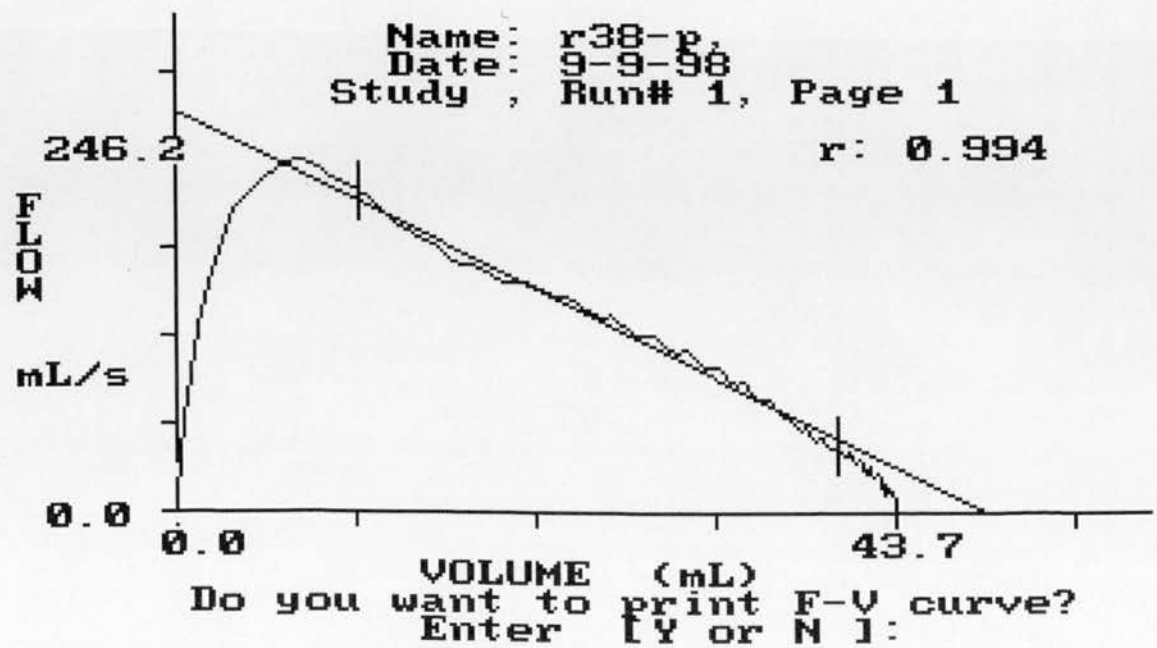


Figure 15 D. Rabbit 38 pumactant group 1 hour after pumactant administration

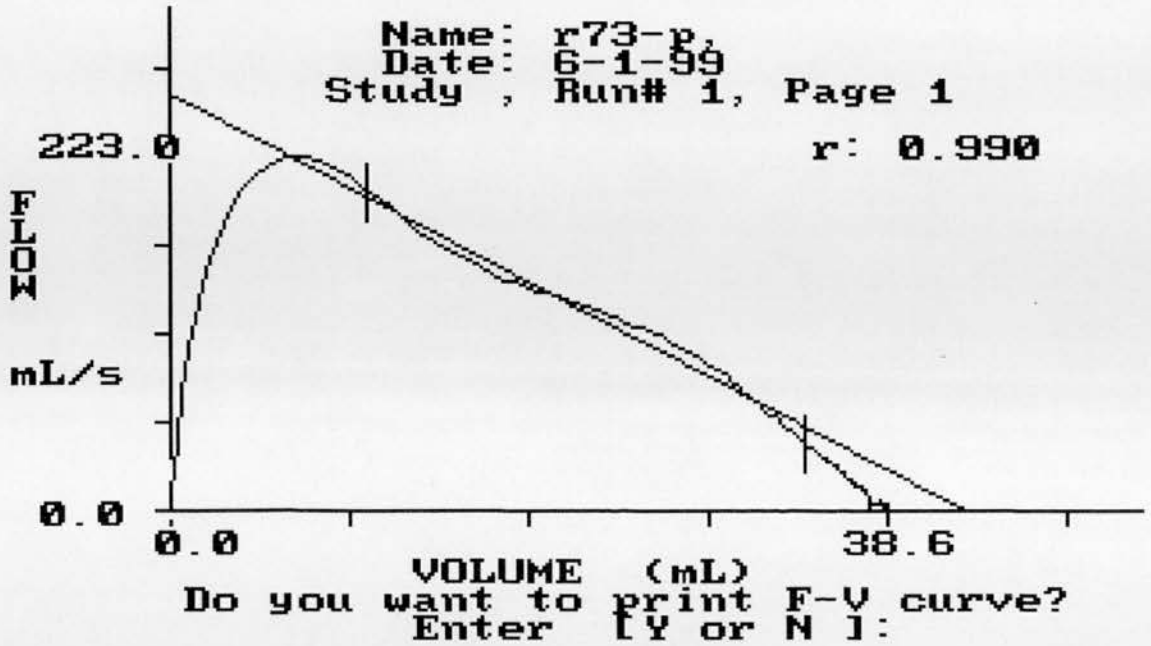


Figure 15 E. Rabbit 73 curosurf group post lung injury

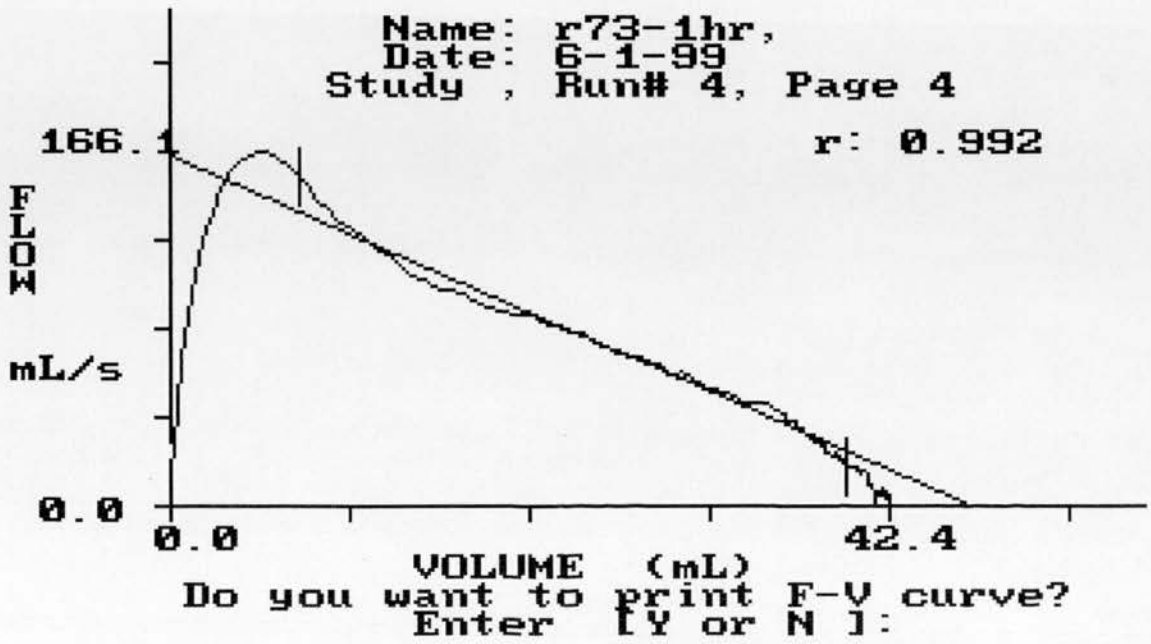


Figure 15 F. Rabbit 73 curosurf group 1 hour after curosurf administration

Discussion.

There was no difference between respective groups for Cdyn or Crs immediately after achieving adequate lung injury. There was also no significant difference between the respiratory rates of the treatment groups either immediately after lung injury or at the 1, 3 or 6 hour time points. If respiratory rates had been markedly different, this could confound any specific change in Cdyn. In many circumstances particularly in disease, dynamic compliance is frequency dependent [169]. A change in respiratory frequency could affect measurements of Cdyn. However after lung injury the respiratory rates of the different treatment groups were similar. It is unlikely that any change in respiratory rate could have concealed any systematic change in Cdyn.

One and three hours after treatment animals who had received PLV with PF 5080, Curosurf alone or the combination of Curosurf/ PLV with PF 5080 had a significantly greater Cdyn than control animals. None of these three treatments were superior to the others. The combination of Pumactant/PLV improved Cdyn at 1, 3 and 6 hours compared to controls, but failed to reach statistical significance at all three timepoints. Cdyn 1, 3 and 6 hours after treatment in animals treated with Pumactant alone or nebulized PF 5080 was not statistically different from controls.

After 6 hours Cdyn was similar in all groups. Evolving occult pneumothoraces may have cancelled out the improvement in Cdyn caused by the treatment intervention.

Of course Curosurf was given as a once only dose. It is possible that Curosurf's initial effect on Cdyn would have been maintained had repeat doses been administered. This is supported by Mrozek and colleagues study in saline lavaged

pigs [145]. They found that the transient initial improvement in compliance and oxygenation diminished over two hours after treatment with the protein containing surfactant, Survanta.

Static compliance values in animals treated with Curosurf were significantly greater than controls and Pumactant treated animals at all time points. This is in keeping with other findings comparing surfactants in animals and human infants[129]. The sustained improvement over a six hour period in Crs between Pumactant and Curosurf, which is not noticed in the 6 hour Cdyn figures may be due to the differing measurement techniques as discussed in chapter 2 [168] [131]. Although there was a sustained greater Crs with the Curosurf treatment, there was no difference with the Cdyn measurements. This may be because Cdyn measures different components of lung mechanics. Cdyn includes resistive and viscoelastic components, and these may have masked the changes in elastic properties shown by the measurement of Crs [169]. Another technical point to consider is that the respective measuring techniques used in this project meant that Cdyn was measured with PEEP still applied, where the technique for measuring Crs allowed the animal to breathe out to atmospheric pressure. Consequently measurements were made at differing parts of the pressure-volume relationship of the respiratory system. It is likely that compliance measured from FRC would be different from that measured at 4cm H_2O PEEP. It is possible that measurements starting from FRC were below an inflection point of the pressure volume curve and consequently the compliance would be less. However despite this possibility Crs measurements generally approximated Cdyn measurements. In the case of the Curosurf group Crs values were generally greater than Cdyn

measurements. This is because dynamic factors such as airway resistance and viscoelastic properties affect the Cdyn [169].

The Pumactant preparation had to be reconstituted with saline and formed an opaque viscid solution that was difficult to administer down the tracheostomy tube. The reasons for its failure to improve survival and oxygenation due to inactivation are noted in the previous two chapters. The viscid Pumactant may have blocked some airways, which would be reflected in poorer Cdyn and static compliance.

Leach et al also found that the artificial, apoprotein free surfactant Exosurf combined with PLV lead to a slightly worse Cdyn than giving PLV alone to preterm lambs.[178] . They administered Exosurf prior to PLV with Perflubron, comparing this group with PLV alone, Exosurf alone and a control group. They had previously compared the effects of mixing Perflubron *in vitro* with various preparations of surfactants (Exosurf, and also two apoprotein containing surfactants Survanta and Infasurf. They found no interference by Perflubron in any of the surfactant surface tension reducing properties. They chose Exosurf as being the more readily available preparation at the time of the study). Further evidence that the artificial surfactants may block access of the PFC to the lungs was demonstrated by a 15 minute delay in the effect of Perflubron following the administration of the Exosurf.

Tarczy-Hornoch and colleagues [1] in their work on excised premature lamb lungs found an improvement in compliance when pre-treating with the surfactant Survanta followed by PLV with Perflubron although an even greater improvement was seen with total liquid ventilation. They attributed this to there being additional sources of surface tension in the surfactant/PLV group (PFC/lung + gas/PFC + gas/lung albeit

that a layer of surfactant helped to reduce surface tension at each of these interfaces) compared with only PFC/lung in the TLV group. They were also are cautious to make the point that findings may be specific to the PFC, surfactant and animal model studied.

In summary, partial liquid ventilation with PF 5080, Curosurf and the combination of Curosurf/PLV with PF 5080 improves dynamic respiratory system compliance in saline lavaged rabbits for up to three hours.

Static total respiratory system compliance is improved by Curosurf but not by Pumactant. This is sustained over a six hour period. The difference in Crs between Pumactant and Curosurf at 6 hours, which is not noticed in the 6 hour Cdyn figures may be due to the differing measurement techniques.

These findings may be specific to the surfactant and PFC studied and care should be exercised before extrapolating this to human use.

Chapter 6

Computerised tomography studies of Partial Liquid Ventilation; density distributions on CT scanning comparing the nebulized route to Partial Liquid Ventilation or control.

Summary

The aim of this chapter was to examine any differences on CT density distribution between the poured and nebulized routes of administration and that of control animals.

The CT images of the excised lungs of rabbits, whose lungs had been injured by saline lavage as previously described, were compared for three treatment groups; control, partial liquid ventilation with PF 5080 and nebulized PF 5080. The lungs were divided into three slices cranially to caudally ("apical slice", "middle slice" and "caudal slice") and also into three zones anteriorly to posteriorly (A-P zones "anterior", "middle" and "posterior"). The mean attenuation number in Hounsfield units (HU) was noted for a region of interest (ROI) taken from each area. A comparison was also made to two normal rabbits that had been neither lung injured nor intubated and mechanically ventilated.

The radiological density of the normal lungs was less than all three other study groups. There was also a difference between the control and partial liquid ventilation groups, as well as the partial liquid ventilation and nebulized groups, but no difference between the nebulized and control group.

The CT results confirm the impression that little PF 5080 can be delivered by the nebulized route using this apparatus. There is a suggestion that PF 5080 is prevented from reaching the alveoli even by the poured route.

When lung regions within the three major treatment groups were compared, no significant difference was found. This suggests less of a gravitational and regional difference than some other studies.

Introduction.

CT scanning is an established method of assessment and investigation of the lungs in ARDS [34;115;117]. There has been limited use of CT scanning to study the effects and distribution of perfluorocarbon during liquid ventilation in animal models of ARDS[179; 119].

There had been no CT investigation of nebulized PFC in lung injury, until this present study.

Methods

A total of 26 young adult female New Zealand white rabbits (10 control rabbits, 6 of the rabbits treated with PLV and the 10 treated with nebulized PFC) were anaesthetised, prepared and randomised as described in chapters 3, 4 and 5. At the end of the procedure, surviving animals were killed with an overdose of general anaesthetic. For comparison two normal New Zealand white rabbits were killed without any instrumentation of the airway or inducing lung injury. These were animals who were left at the end of the study. Under Home Office Regulations such animals must be humanely killed at the end of a project. Rather than let this tissue be wasted, the excised lungs of these animals were prepared by immersing in liquid nitrogen. The original project did not allow financially for a separate control arm of the study with ten further animals to be included, and the original comparison was to be between true "control" (but lung injured) PLV and nebulizer treated animals. Thus some information is included from these normal animals for interest. However it should be borne in mind that they are a small group of two non-randomised animals. No more conclusion than that should be drawn from these two animals.

For simplicity's sake (and due to limited storage for the frozen specimens) it was decided to compare only control, poured and nebulized groups rather than the additional combination groups, which may be investigated in further studies at a later date.

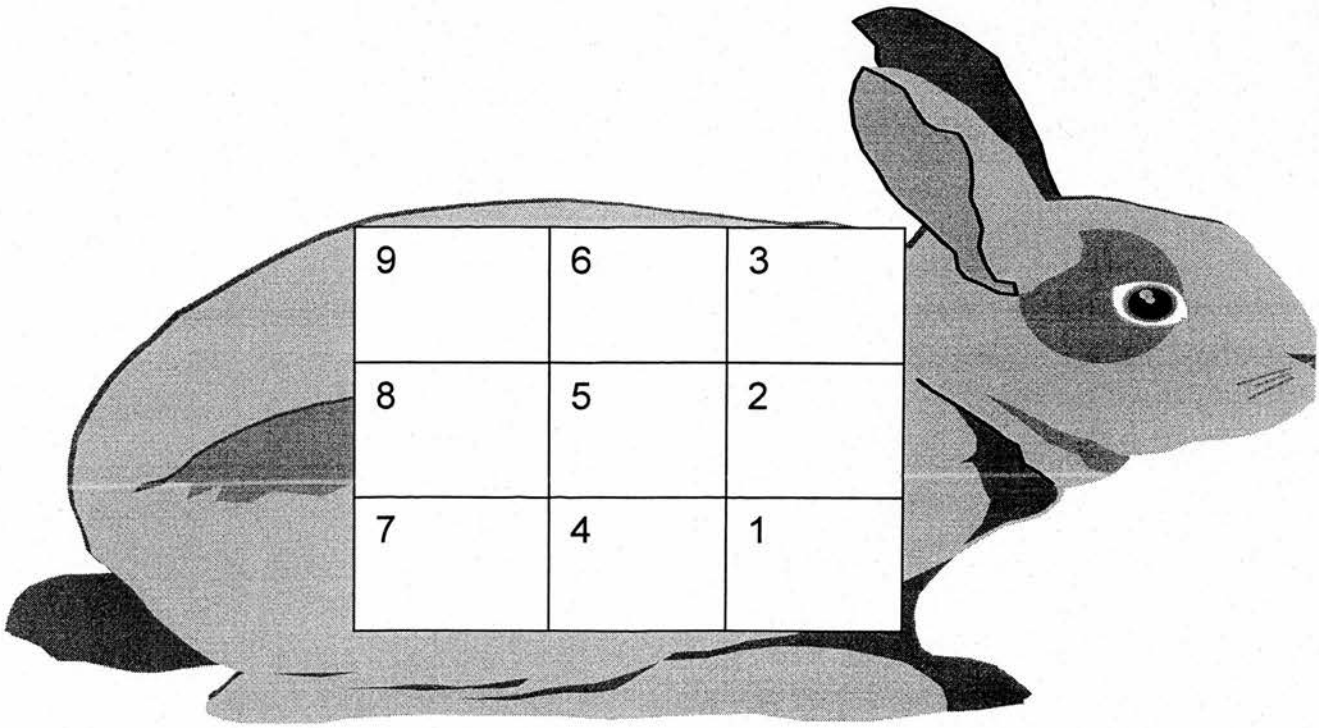
Immediately after death, the trachea was clamped at end-expiration whilst maintaining the previously applied 4cm H_20 PEEP. The lungs were removed en bloc and immediately immersed in liquid nitrogen. They were then stored at –70°C until CT scanning.

CT scanning was done with an IGE Medical Systems hi-speed advantage rapid processing CT scanner (IGE Medical Systems, Milwaukee, USA) using the following settings: 100kV, 100mA, pixel size 0.64mm x 0.64mm, 1 mm slice thickness (thus voxel size 0.64 x0.64 x 1mm), 5mm gaps, 1 second exposure using a bone algorithm (high resolution) axial acquisition.

Nine control animals, six partial liquid ventilation animals and nine animals given nebulized PF 5080 yielded suitable specimens for CT examination as well as the two normal animals.

The excised lungs were scanned in groups of two or three while stored on ice and referred to only by reference numbers i.e. blinded to which group they came from. The radiographer who performed the scanning took no other role in the study.

Figure 16. Key to slices and zones of CT study



Key; Animal in supine position for experiment. Hence
1 Apical slice; Anterior zone
2 Apical slice; Middle zone
3 Apical slice; Posterior zone

4 Middle slice; Anterior zone
5 Middle slice; Middle zone
6 Middle slice; Posterior zone

7 Caudal slice; Anterior zone
8 Caudal slice; Middle zone
9 Caudal slice; Posterior zone

For each animal representative areas were selected from three anterior to posterior zones; in the apical slice anterior zone, in the apical slice middle zone, and in the apical slice posterior zone. The mean radiographic densities within these areas of the respective anterior, middle and posterior zones were recorded in Hounsfield units (HU). This process was repeated for the middle and caudal slices (see Figure 16). To assess the reproducibility of the study, further representative areas were selected at similar levels twice (designated Reading 2 and Reading 3) several months after the first review was performed. This was with no knowledge of what the first results had been.

The mean HU attenuation of a sample of PF 5080 measured on six occasions on the above scanning settings, was found to be 606 HU (SD 13 HU; n=6).

Results;

The mean HU values of each region of interest (ROI) were noted for each of the 9 areas (apical slice, middle slice, caudal slice each with an anterior, middle and posterior zone) for each of the 3 study groups and the two normal animals. Due to fissuring artefact one specimen from the control group and one specimen from the nebulized group were too badly damaged for meaningful analysis to be performed. The data from these two animals is therefore not included in the following results. This left 9 control, 6 partial liquid ventilation i.e. poured , 9 nebulized and 2 normal animals each with CT densities measured in 9 areas (giving 81, 54, 81 and 18 data points respectively). This information is shown in Table 16 for the 1st reading. As the data did not approximate to a normal distribution using the Kolmogorov-Smirnov test, the Kruskal-Wallis test was used to compare the groups. The statistical

package used for this was *GraphPad Prism™ Version 2.0* (GraphPad Software Inc., San Diego, California, USA). Intergroup comparison was made using the Kruskal-Wallis non parametric test, with Dunn's multiple comparison test. This yielded the following results; there was a statistically significant difference between Control and PLV, Control and Normal, PLV and Nebulized, PLV and Normal and Nebulized and Normal. There was no statistically significant difference between the Control and Nebulized groups.

Within group comparisons made for Control, PLV and Nebulized groups yielded no statistically significant differences between any of the lung areas (using the Kruskal-Wallis non-parametric test with Dunn's multiple comparison test). This implies no gravitational effect in either the cranial-caudal or anterior to posterior directions.

The information for the 1^{st}, 2^{nd} and 3^{rd} readings is shown in Table 17.

Table 18 shows the differences between the 1^{st} & 2^{nd} readings, and the 2^{nd} & 3^{rd} readings. The Wilcoxon signed rank test (paired) shows that there was no statistically significant difference between $1^{st}/2^{nd}$ readings compared with $2^{nd}/3^{rd}$ reading.

Figure 17 is a scatter diagram of the CT densities at the first reading.

Figure 18 shows a scatter diagram of the distribution of CT densities of all 3 readings for the control, poured PFC, nebulized PFC and normal animals, as well as a scatter diagram of the differences between the $1^{st}/2^{nd}$ and $2^{nd}/3^{rd}$ readings.

Figure 19 shows the differences between 1^{st} and 2^{nd} readings as well as the 2^{nd} and 3^{rd} readings in bar chart format.

Figure 20 shows the differences in CT densities plotted against mean values for the 1st versus 2nd readings then the 2nd versus 3rd readings.

Examples of the CT images on ice from animals in each group are shown in Figures 21a-c (Control), 21d-f (Poured), 21g-i (Nebulized) and 21j-l(Normal).

Table 16. CT densities 1st Reading

	Control	PLV i.e. Poured	Nebulized	Normal
Number of data points	81	54	81	18
Minimum value (HU)	-228	-80.0	-118	-326
25% Percentile (HU)	-71	-14.5	-65	-274
Median (HU)	-57	8	-57	-201
75% Percentile (HU)	-46	71.50	-47	-156.5
Maximum (HU)	-24	449	55	-103

Intergroup comparison was made using the Kruskal-Wallis non parametric test, with Dunn's multiple comparison test. This yielded the following results;

Control vs Partial liquid ventilation (P<0.001)

Control vs Nebulized (P>0.05)

Control vs Normal (P<0.001)

Partial liquid ventilation vs Nebulized (P<0.001)

Partial liquid ventilation vs Normal (P<0.001)

Nebulized vs Normal ($P<0.001$)

Within group comparisons were also made for the three study groups (Control, PLV and Nebulized). This compared each lung area with each other again using the Kruskal-Wallis non-parametric test with Dunn's multiple comparison test. This found no gravitational effect in either the cranial-caudal or anterior to posterior directions.

*Table 17. CT densities of Regions of Interest taken from 1st, 2nd and 3rd readings of treatment groups. * denotes one area, one animal not possible to obtain a distinct third reading.*

	Number of Values	Minimum Value (HU)	25% Percentile (HU)	Median Value (HU)	75% Percentile (HU)	Maximum Value (HU)
Control 1st Reading	81	-228	-71	-57	-46	-24
Control 2nd Reading	81	-224	-54	-44	-36	-22
Control 3rd Reading	81	-164	-52	-43	-34	-18
Poured 1st Reading	54	-80	-14.5	8	71	449
Poured 2nd Reading	54	-100	-24	18	72.5	483
Poured 3rd Reading	53*	-68	-15	26	61	409
Nebulized 1st Reading	81	-118	-65	-58	-47	-11
Nebulized 2nd Reading	81	-85	-59	-51	-45	-12
Nebulized 3rd Reading	81	-77	-58	-50	-37	-5
Normal 1st Reading	18	-326	-274	-201	-156.5	-103
Normal 2nd Reading	18	-353	-260	-192.5	-150	-94
Normal 3rd Reading	18	-334	-259.5	-200.5	-169.5	-103

For accompanying scatter diagram to Table 17, see Figure 18.

Table 18. Difference between 1st/ 2nd and 2nd/3rd Readings (Also see Figures 16, 17 & 18).

	Differences between 1st versus 2nd Reading	**Differences between 2nd versus 3rd Reading**
Number of Values	234	234
Minimum Value (HU) Of Differences	-153	-218
25% Percentile (HU) of differences	-18.5	-13
Median (HU) Value of Differences	-6	-2.5
75% Percentile (HU) of Differences	4.0	5.0
Maximum Value (HU) Of Differences	127	207

i.e. Median difference of a value between 1st and 2nd reading was –6 HU. Median difference between 2nd and 3rd reading was –2.5 HU. Distribution of differences not Gaussian (according to Kolmogorov-Smirnov test). Therefore non-parametric used. Wilcoxon signed rank comparing test differences between Readings 1 & 2 with differences between Readings 2 & 3.

Wilcoxon signed rank test (paired) to test null hypothesis that there is no significant difference between $1^{st}/2^{nd}$ readings compared with $2^{nd}/3^{rd}$ reading

P=0.087

No statistically significant difference.

Figure 17. Distribution of CT densities; 1st reading. Scatter diagram

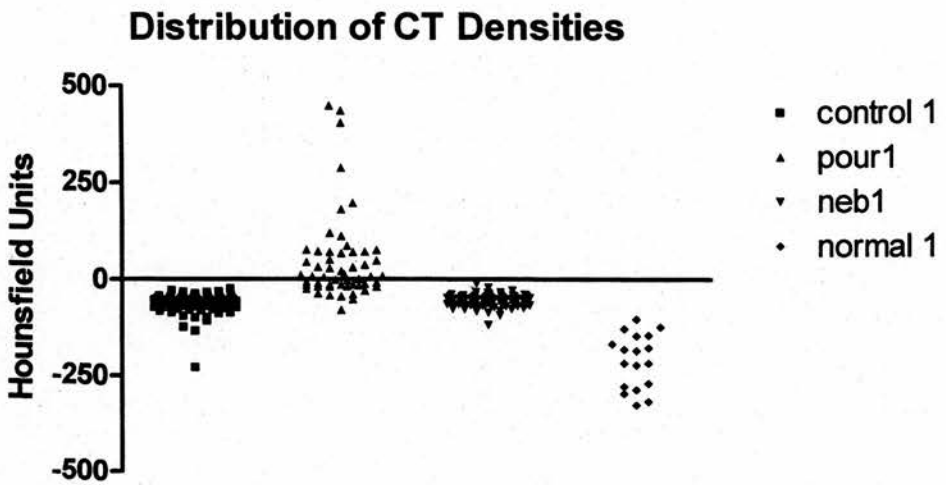
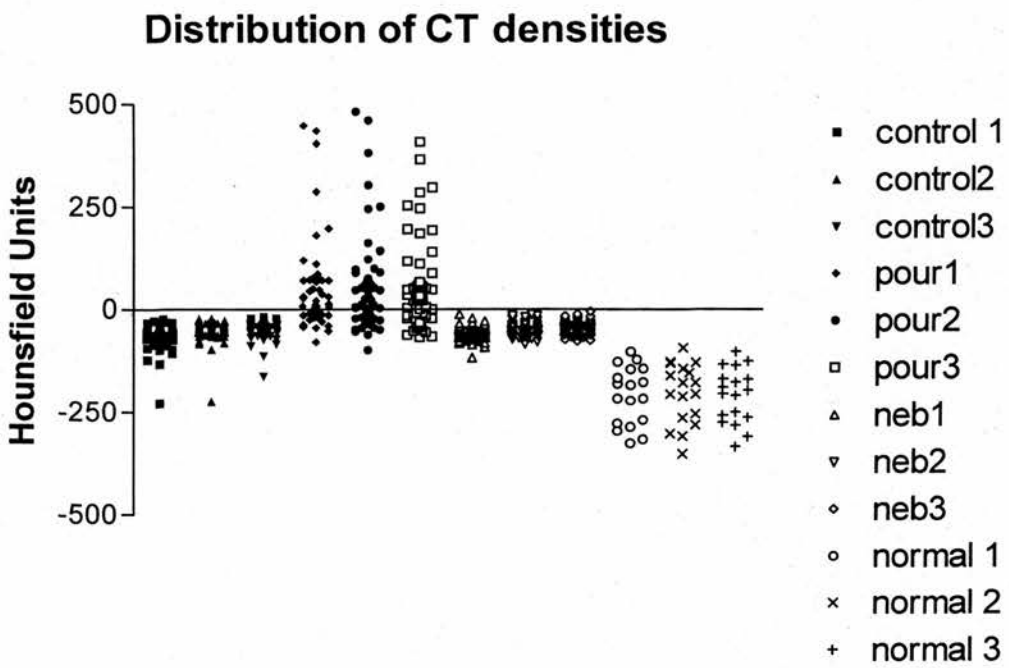


Figure 18; Distribution of CT Densities 1st, 2nd and 3rd readings. Scatter diagram.



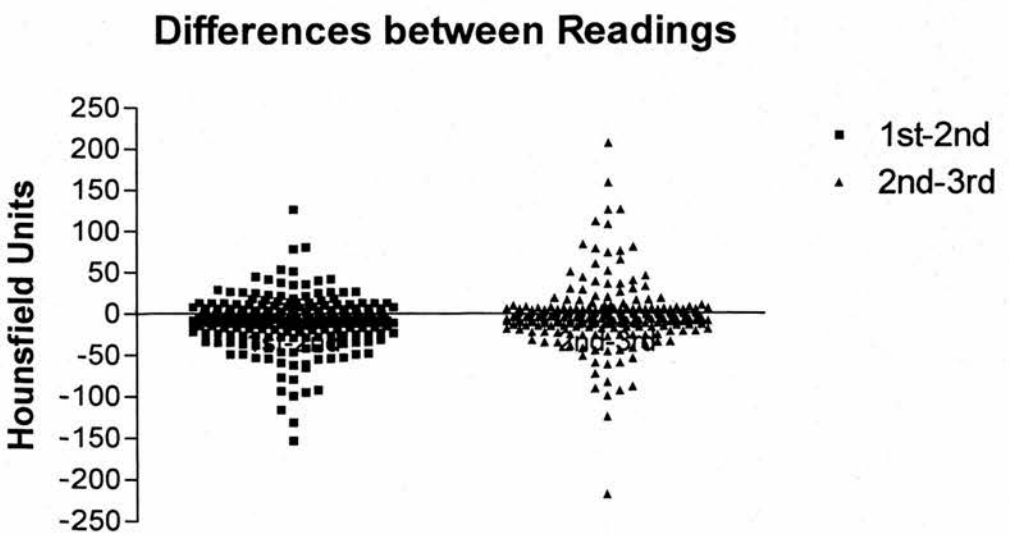
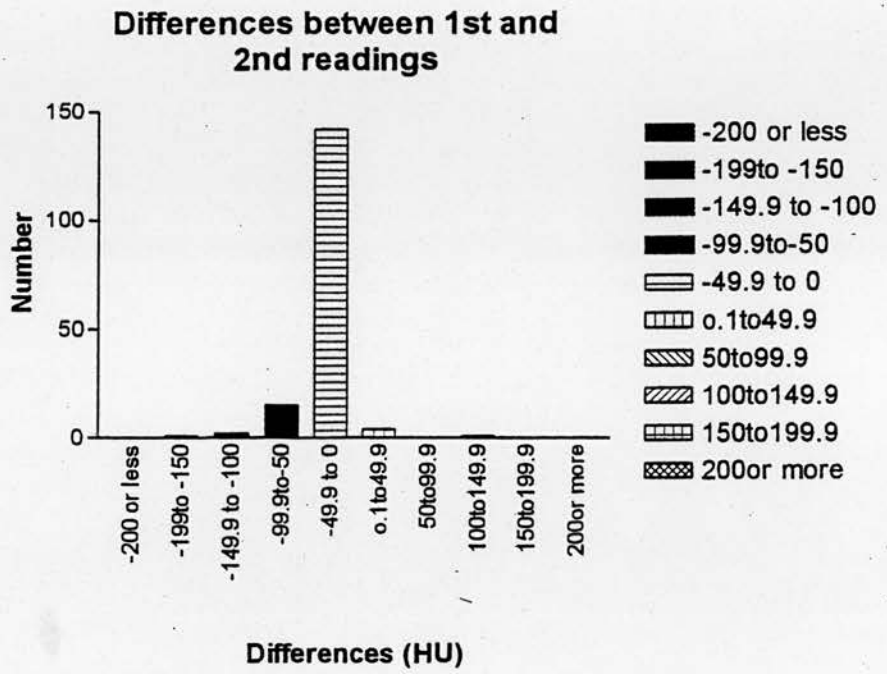


Figure 19. Differences between CT densities 1st & 2nd readings and 2nd & 3rd readings; bar chart format.



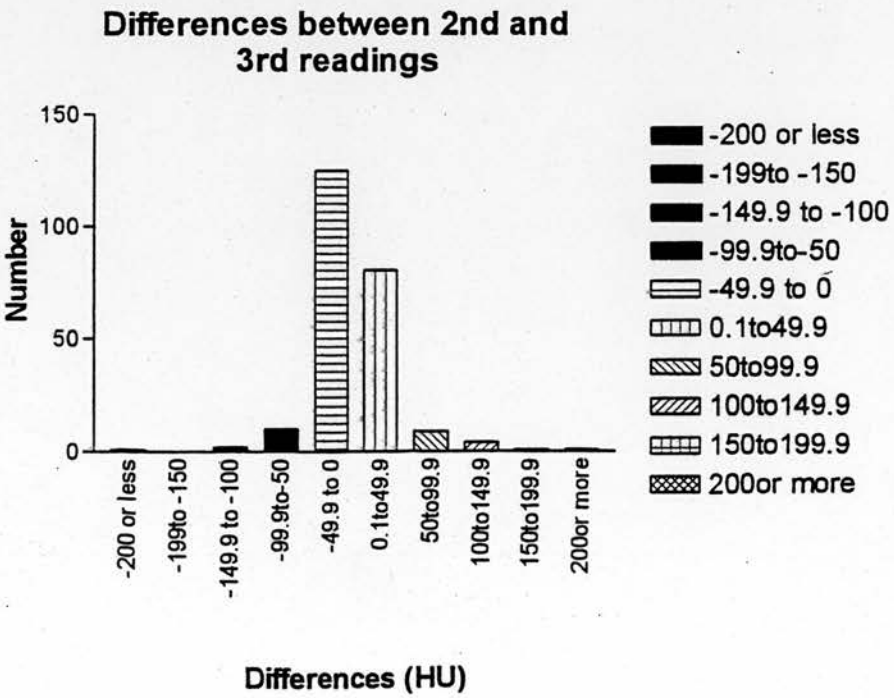
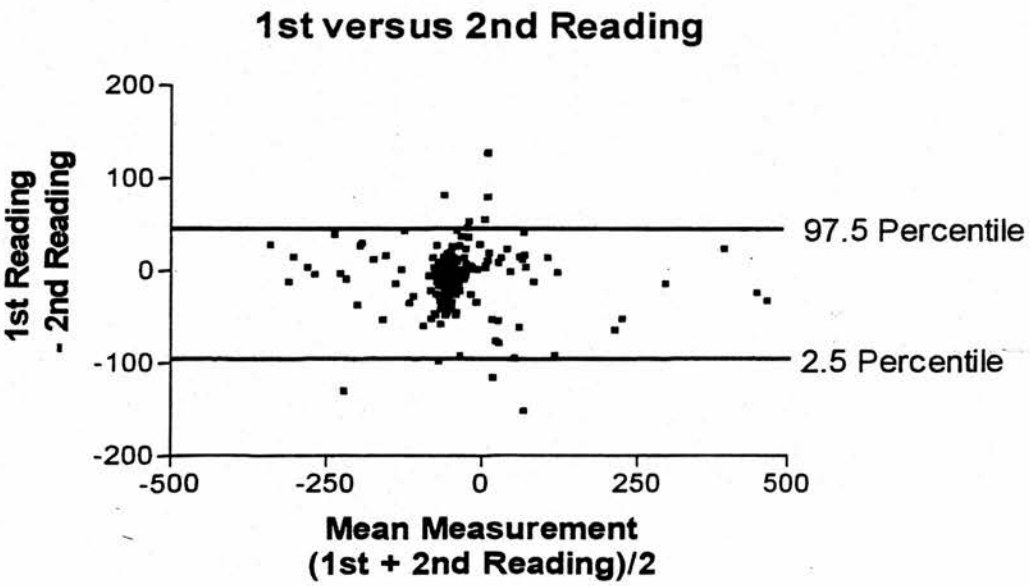
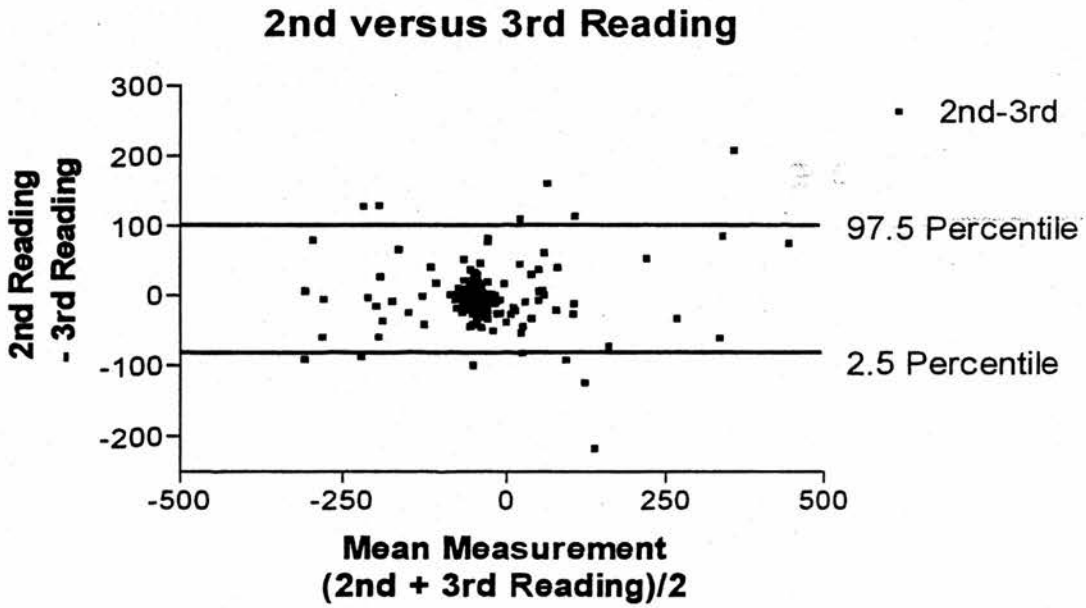


Figure 20. Differences in CT densities plotted against mean values (in HU) after Bland and Altman[180].





Examples of the CT scans are shown in Figures 21a-c (Control) 21d-f (Poured) 21g-i (Nebulized) and 21j-l (Normal).

As an additional aside, an example of standard chest x-rays from an animal in the PLV group is shown for comparison with one from an animal in the control group (Figure 22). They were taken on similar settings (50kV, 2mA). Despite the fact that PF 5080 contains no bromine or iodine, the atoms said to convey radiodensity[70], the presence of PF 5080 obscures the appearance of lung fields. This is shown merely to underscore the point discussed in chapter 1, that diagnosing other underlying features such as pneumothoraces or intravenous catheters using standard x-rays, is difficult through a PFC medium.

Control Group Apical Slice

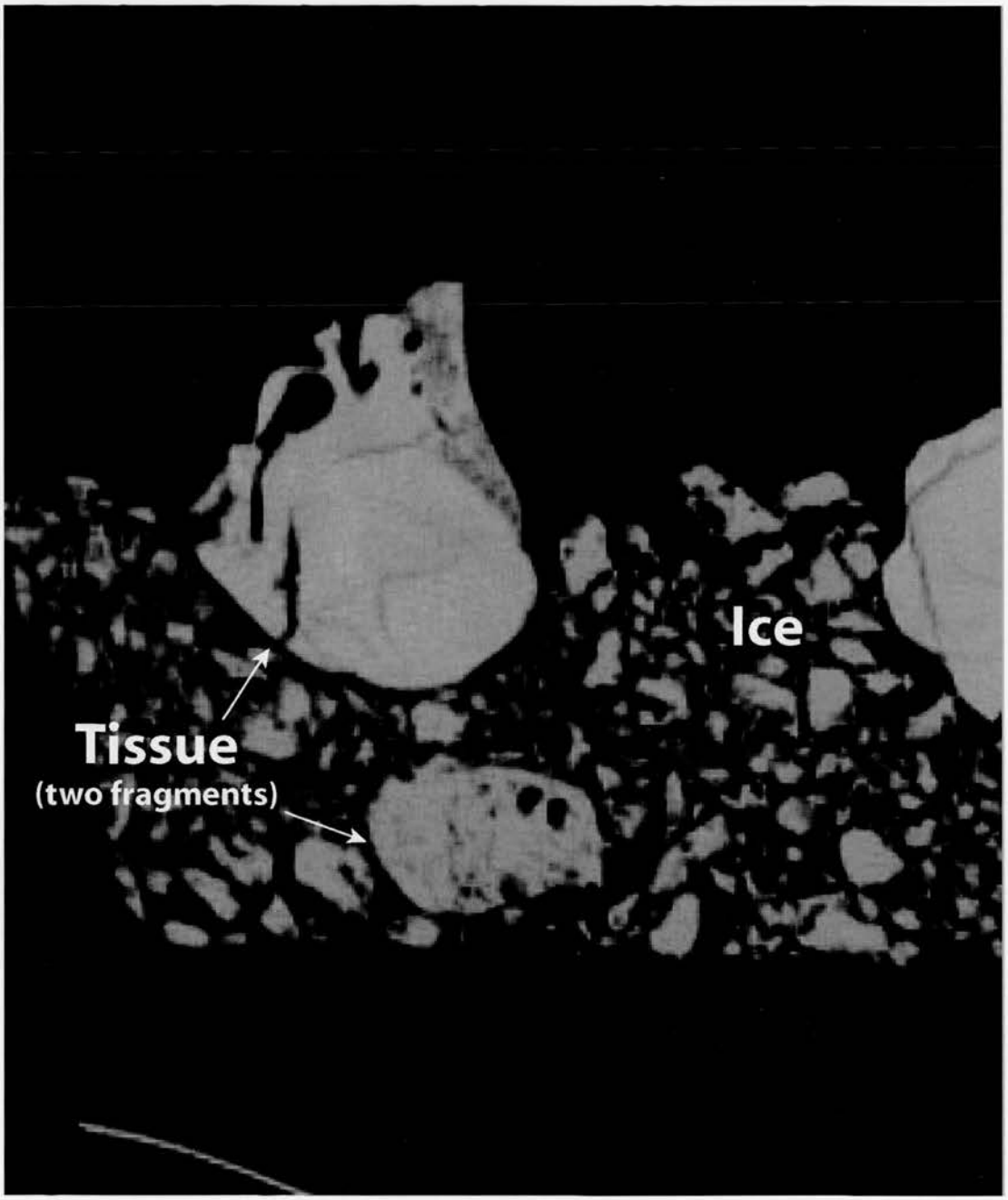


Figure 21 a
Note tissue in two fragments in surrounding ice.

Control Group Middle Slice

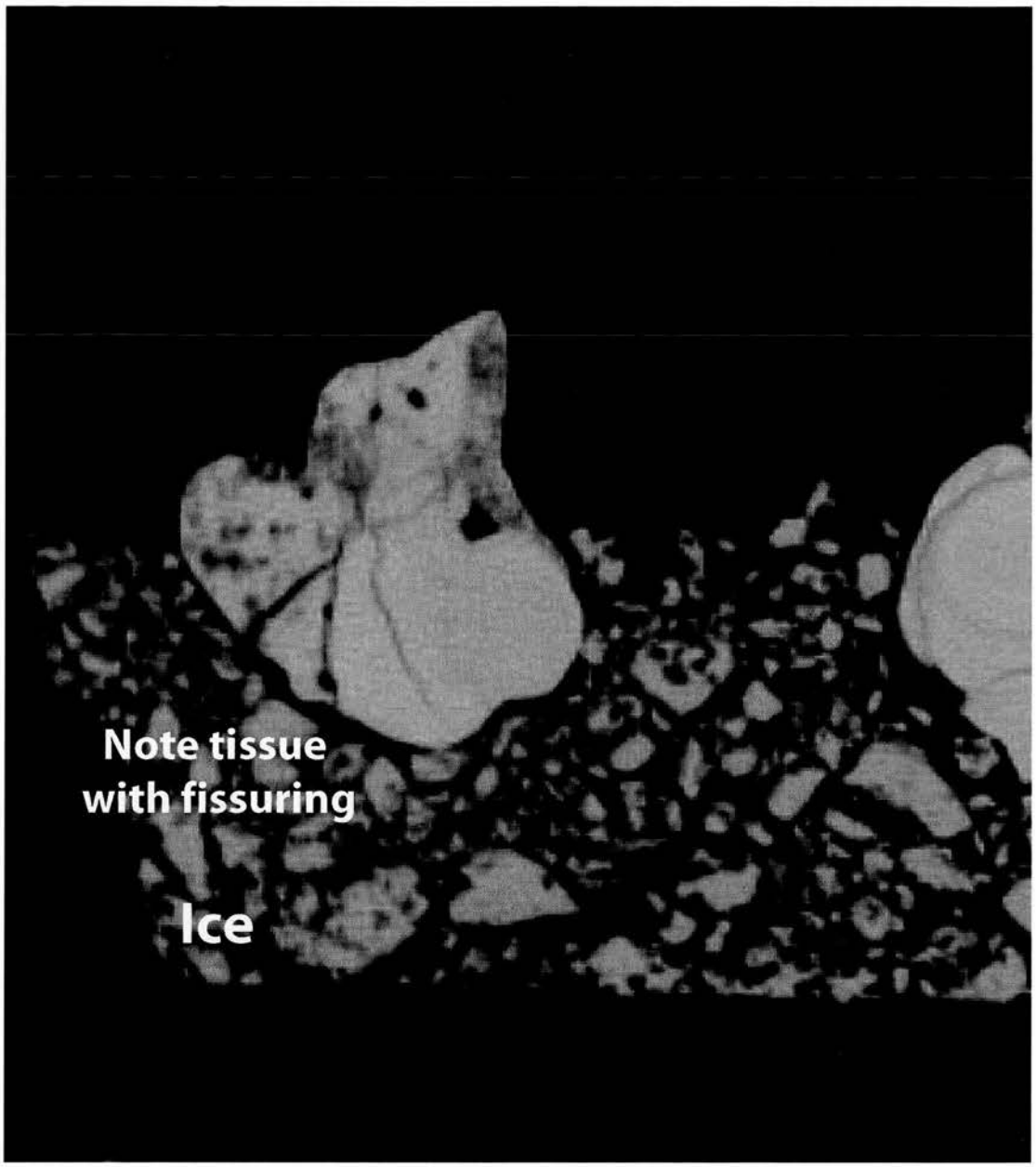


Figure 21 b

Control Group Caudal Slice

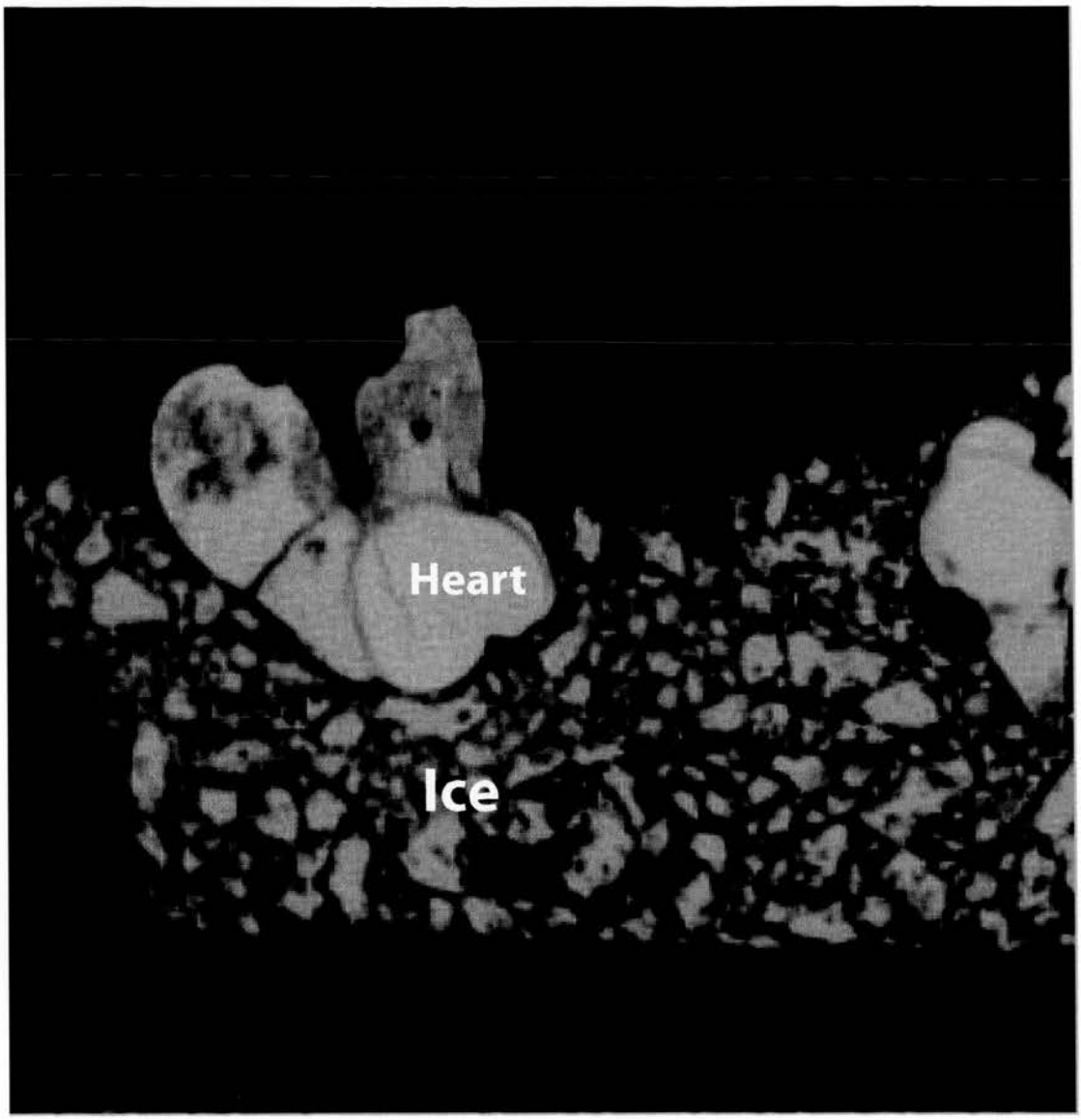


Figure 21 c

Poured Group Apical Slice

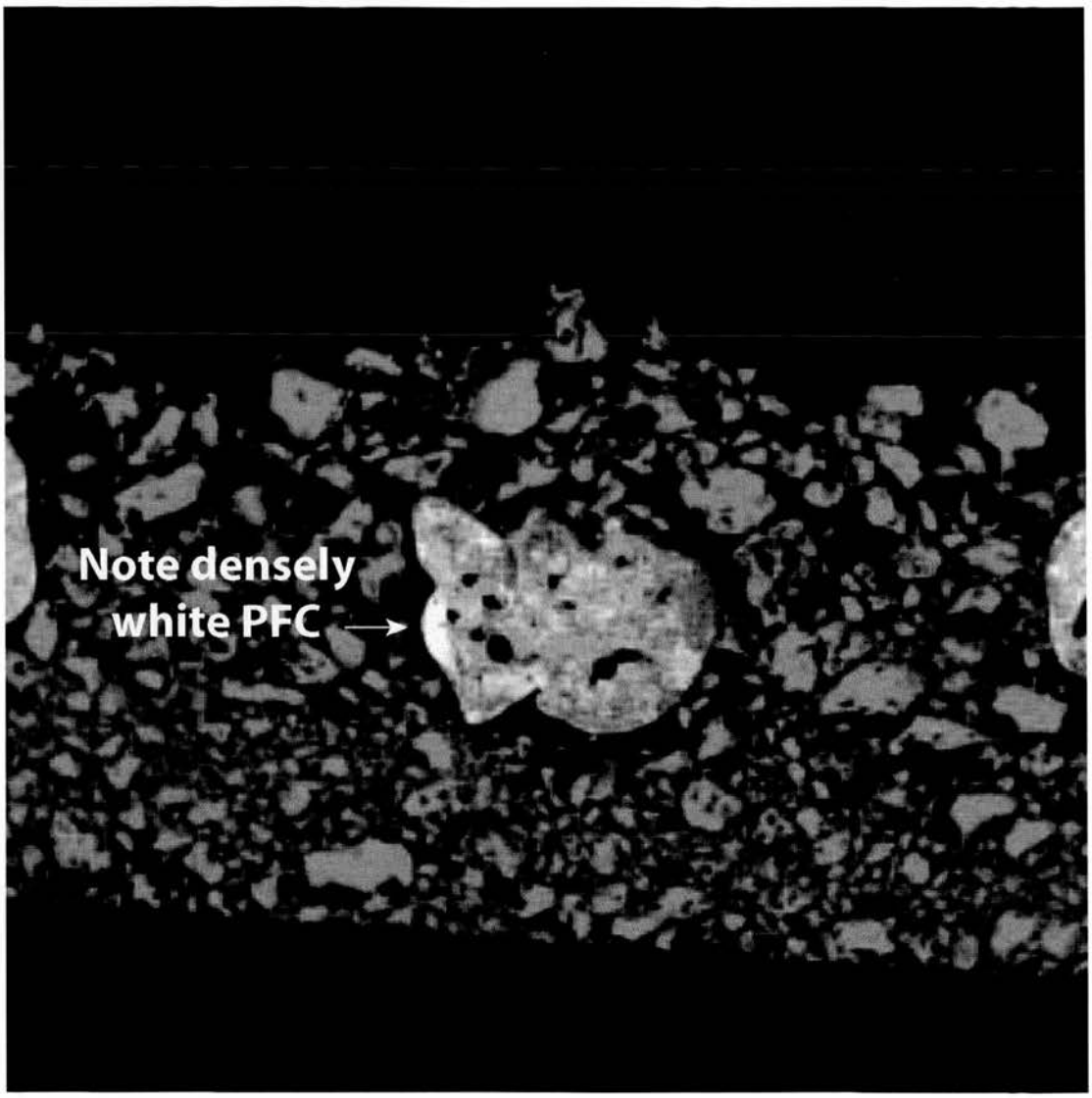


Figure 21 d
Note the intense white density of the PFC on CT scanning.

Poured Group Middle Slice

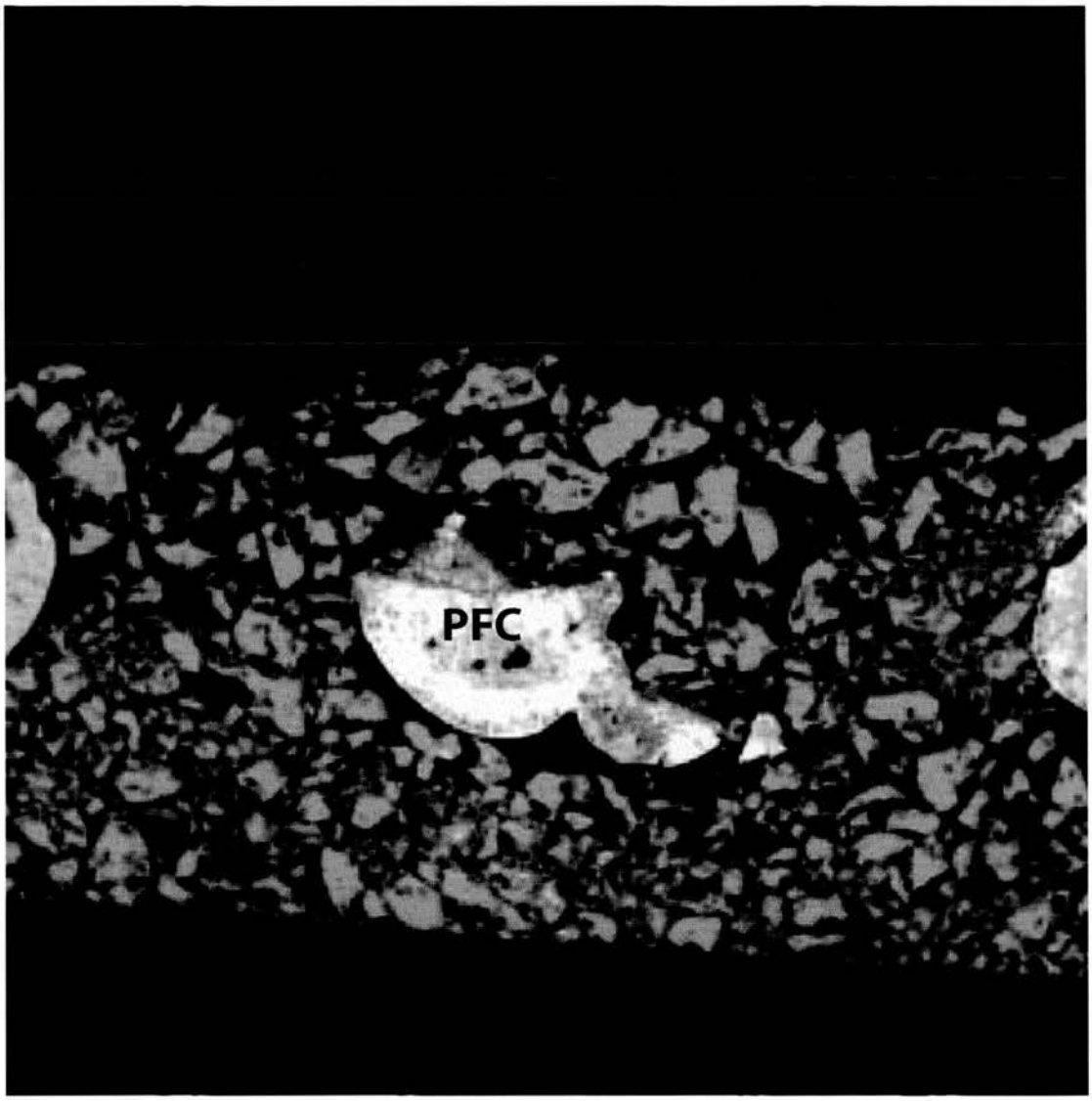


Figure 21 e

Poured Group Caudal Slice

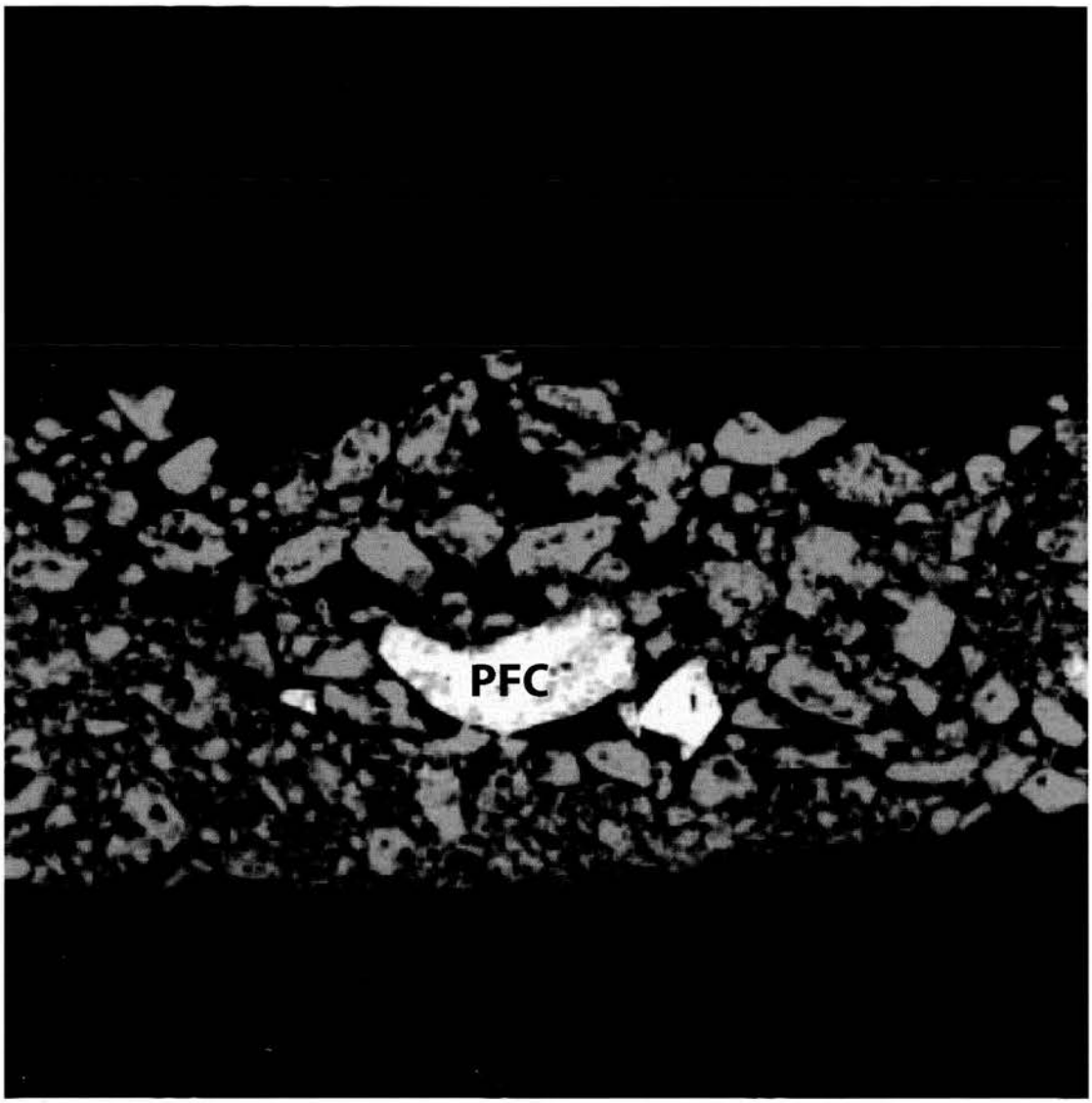


Figure 21 f

Nebulized Group Apical Slice

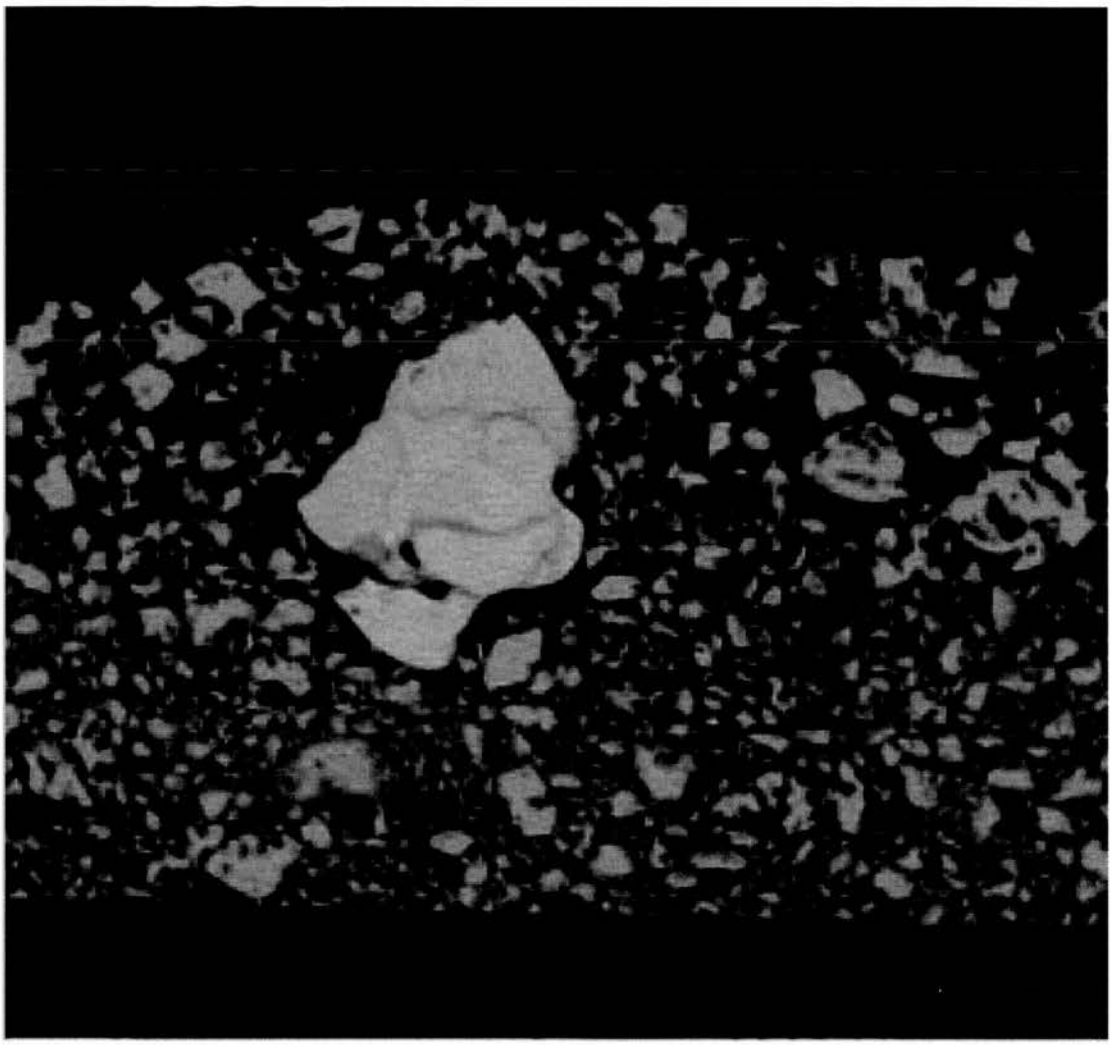


Figure 21 g
Note the difference in appearance of nebulized slices compared with the previous 3 slices from the 'poured' group.
Note the similarity to the control group.

Nebulized Group Middle Slice

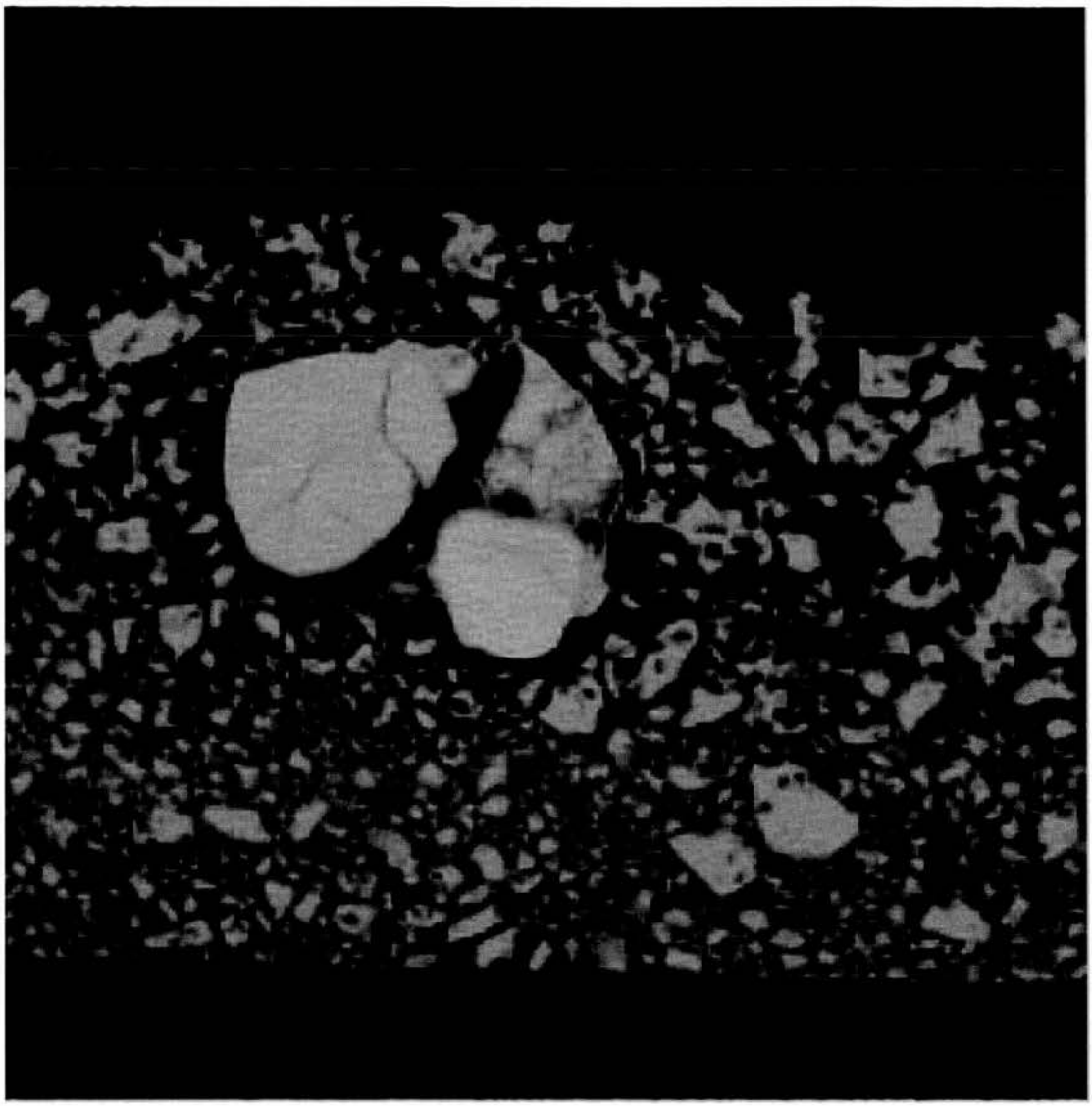


Figure 21 h
Note the fragmentation.

Nebulized Group Caudal Slice

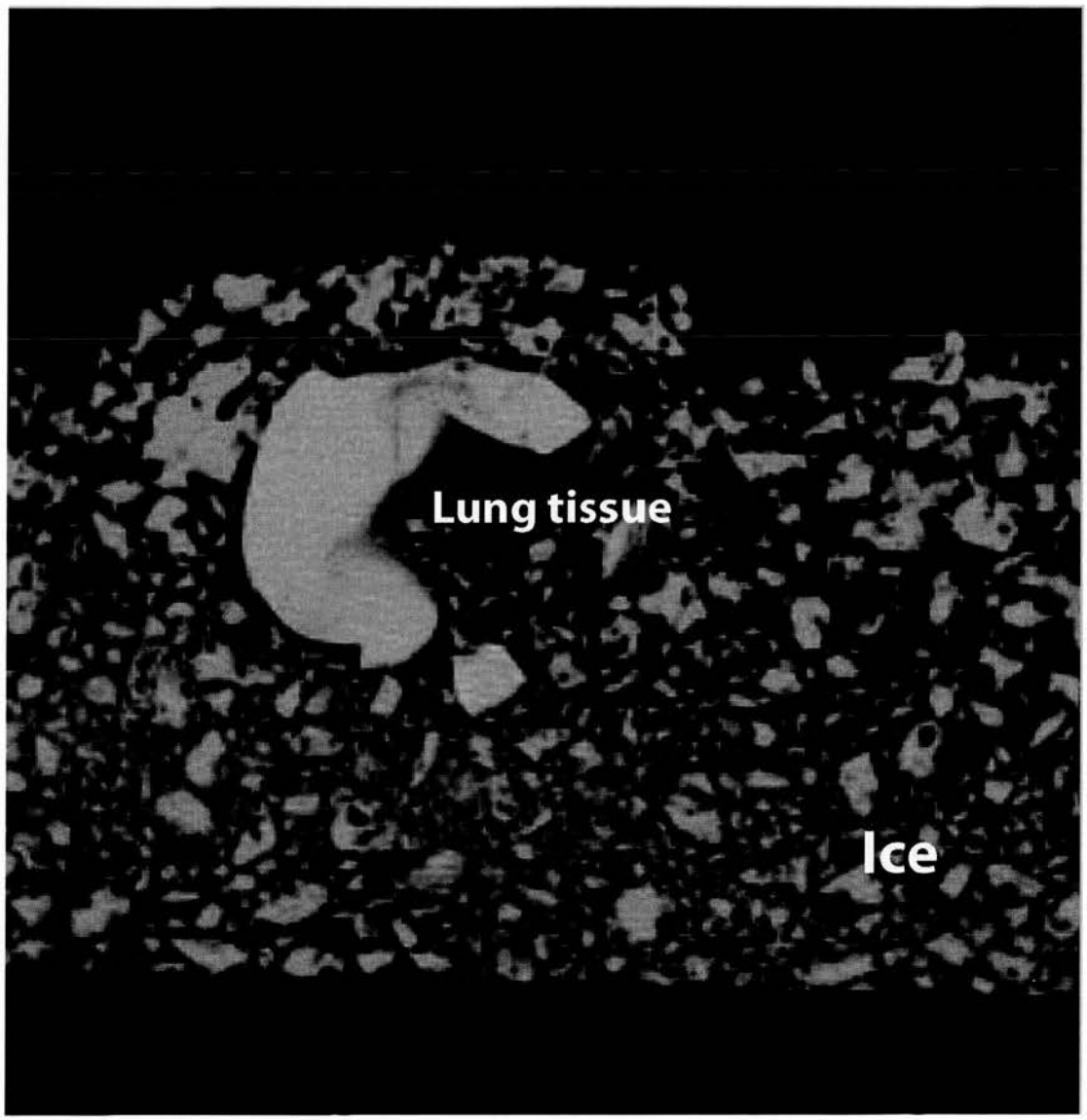


Figure 21 i

Normal Group Apical Slice

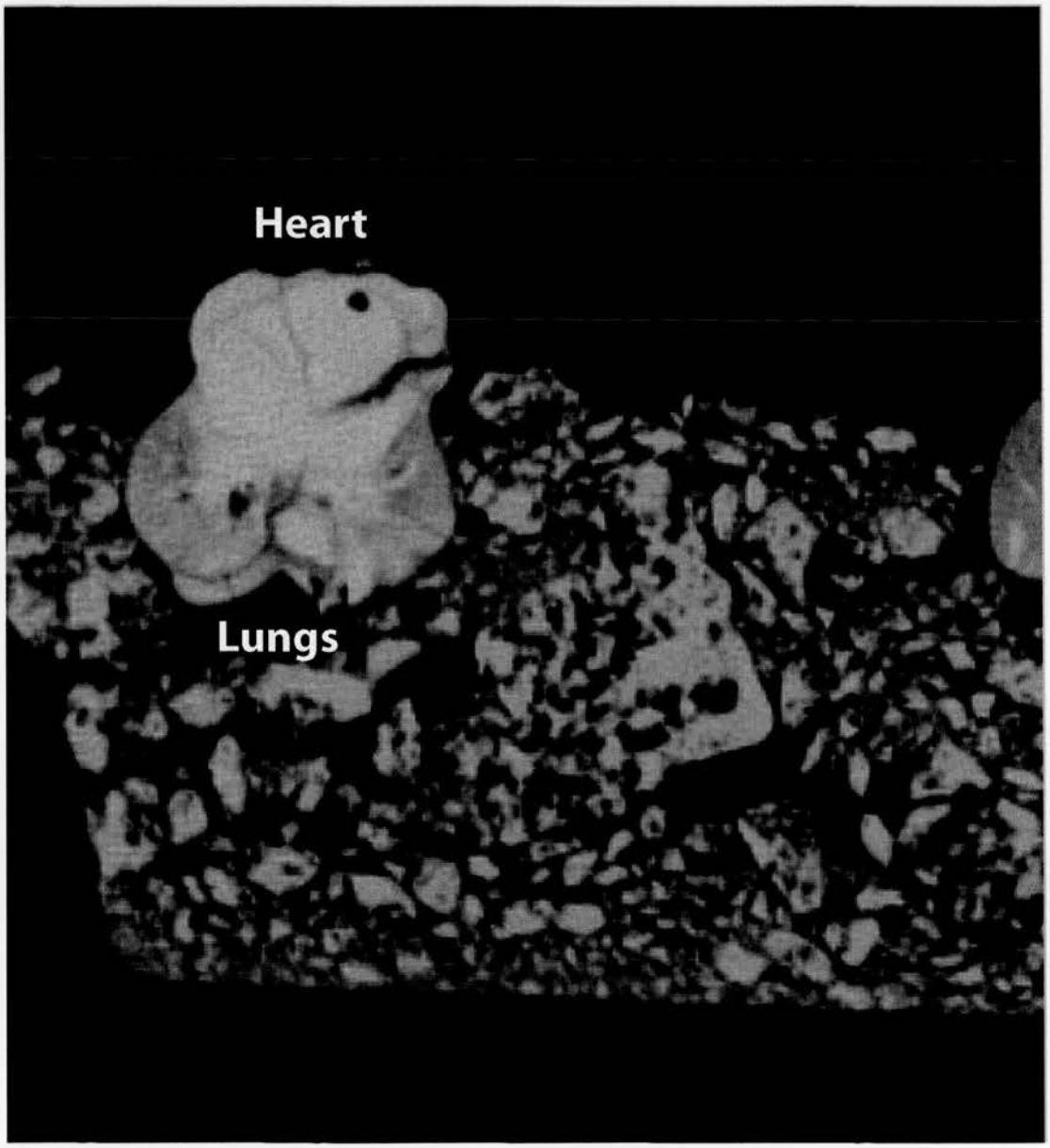


Figure 21 j
Note the generally less dense appearance of the uninjured lungs.

Normal Group Middle Slice

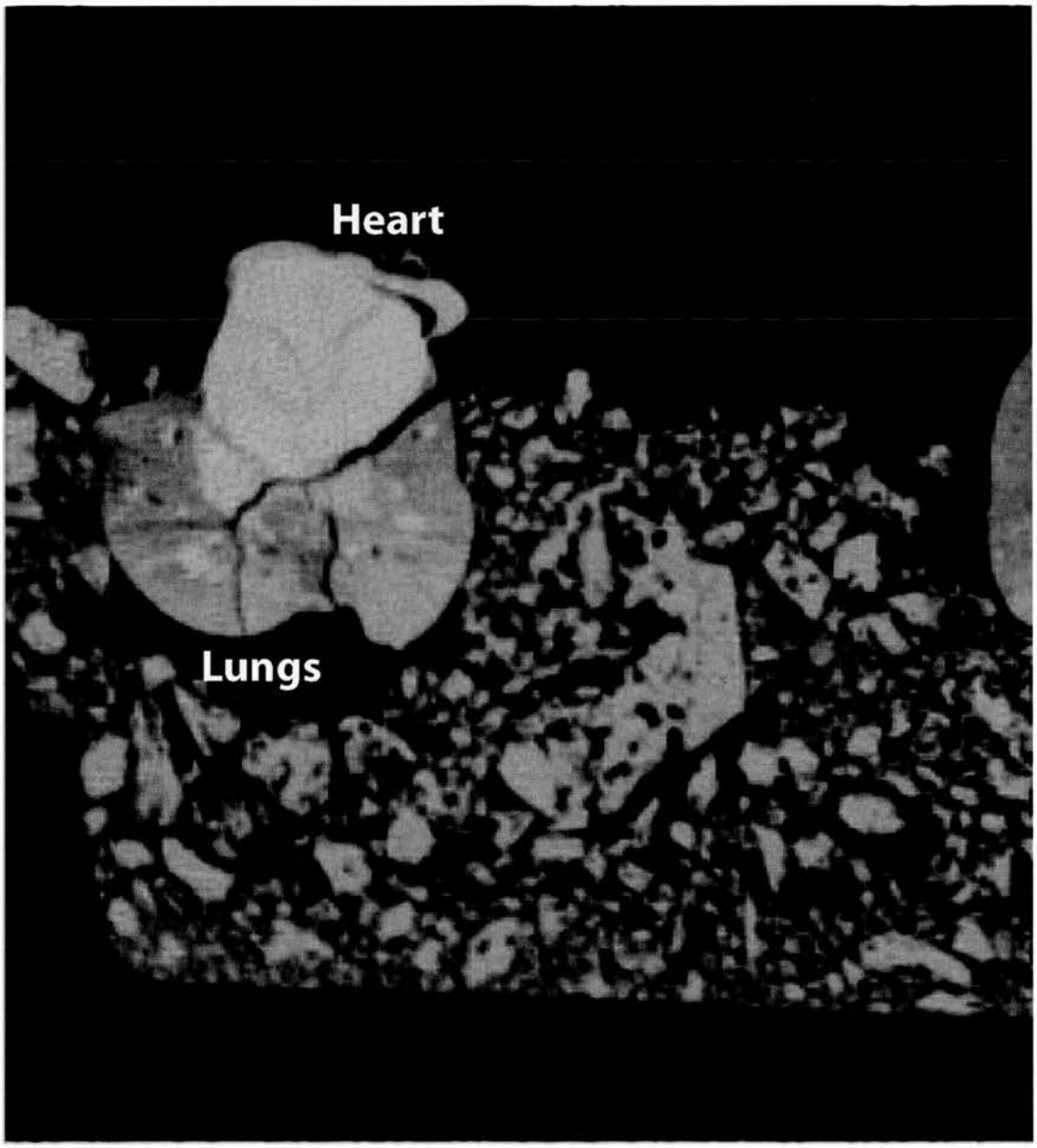


Figure 21 k

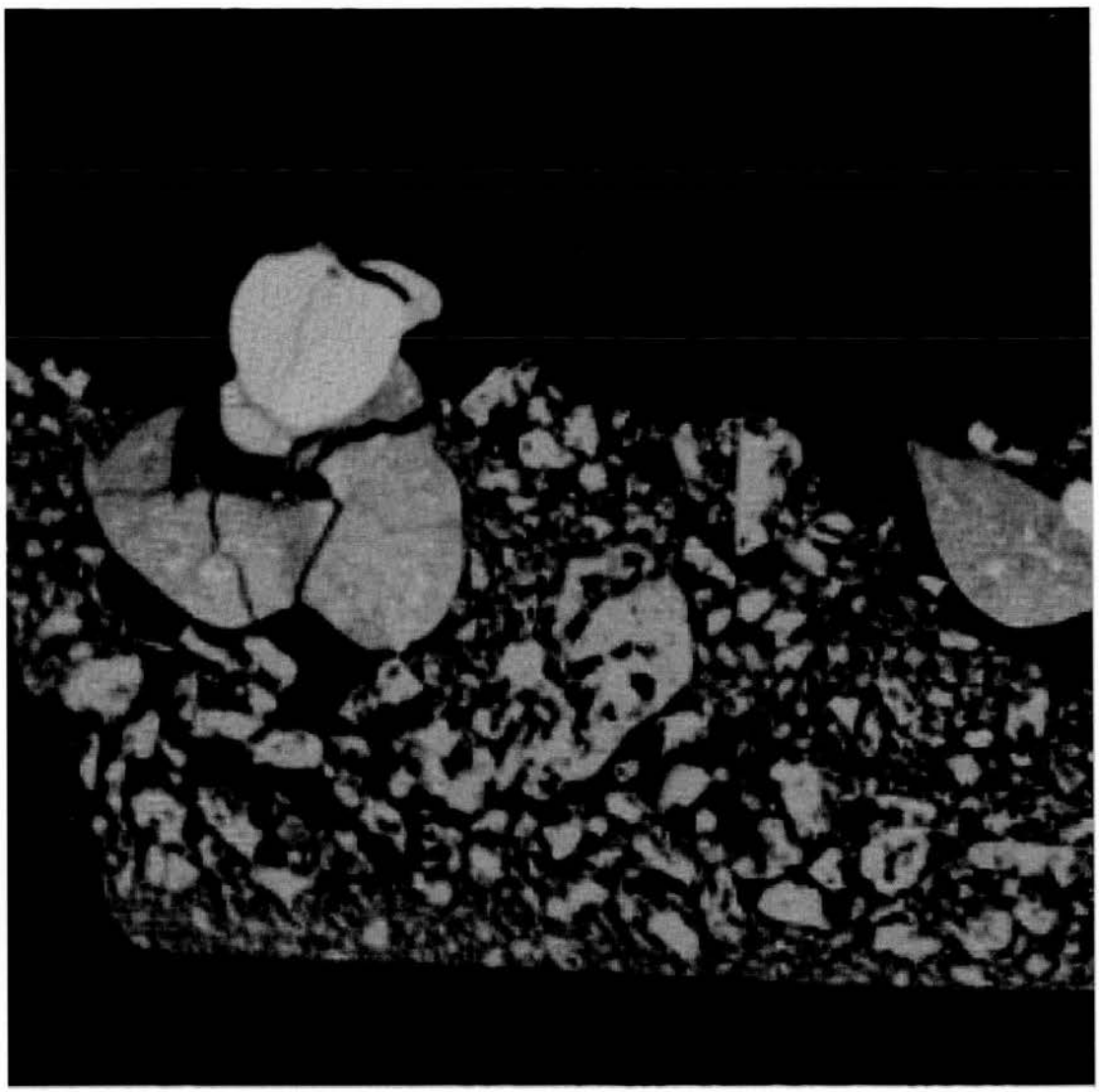
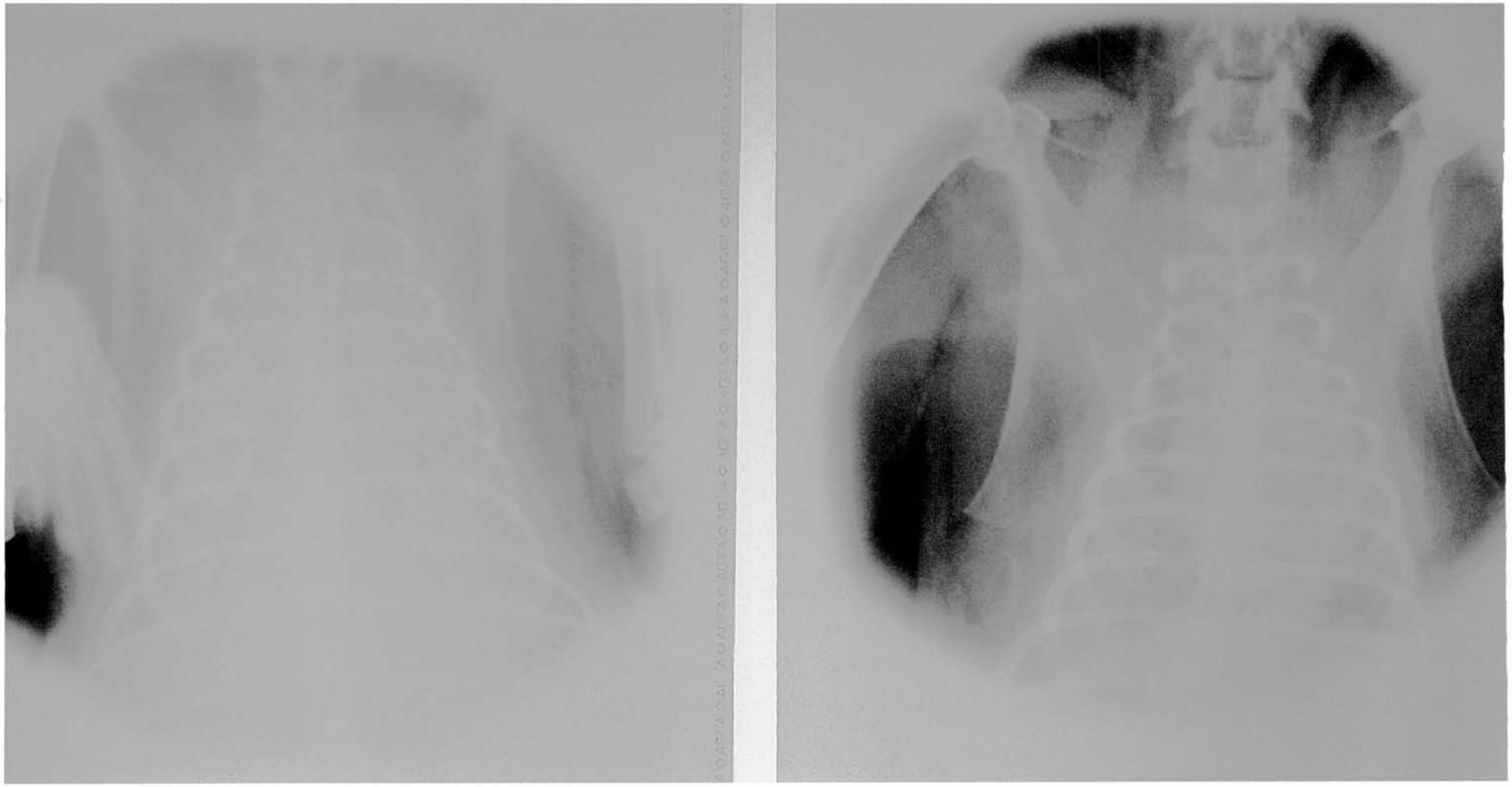


Figure 21 l
Note fissured lung tissue.

Figure 22 Chest X-Rays of Rabbits



PLV

Control

Discussion

First, there was a large capacity for artefact in preparation of samples for this study. The limited number of other studies which described performing CT scanning on lung injured animals undergoing PLV, have done their studies in vivo[118;119]. However there were insurmountable logistical reasons why this could not be done here. Home Office regulations allow animal experimentation to be conducted in only Home Office approved centres. The animal laboratory to which I had access had no CT scanner in situ. Therefore it was not possible to perform the CT scanning on intact animals either in life or after being killed but still ventilated.

Furthermore, logistically it would have been prohibitively expensive to perform the CT scanning at the end of each procedure on fresh lungs. Thus the lungs were frozen in liquid nitrogen and scanned en masse at the end of the project.

This did introduce the possibility of preparation artefact particularly fissuring of the lung on introduction to the liquid nitrogen. For this reason one specimen from the control group and one specimen from the nebulized group, which were both too badly fissured to subject to CT scanning, were not included in the above results.

As the lungs were frozen in one phase of respiration (end–expiration) it is not possible to comment on whether there was any cyclical variation in the distribution of the PFC [118 119]. Furthermore, the process of excising the lungs and lowering them into liquid nitrogen could have introduced artefact or that the process of freezing itself introduces some change of distribution.

Second and third readings taken from approximately similar areas of lung were taken to test agreement (e.g. how much the 1st reading differs from the 2nd or the 2nd from

the 3rd) and repeatability of the method (degree of variation on repeated measures on the same subject)[180]. As the raw CT density distribution as well as the difference against mean plots demonstrate, (Figures 17, 18 and 19) there seems to be no significant difference. The median difference comparing similar areas in the same rabbit between 1st and 2nd readings was –6HU. The median difference comparing similar areas in the same rabbit between 2nd and 3rd readings was –2.5 HU i.e. very small in both cases. There was also no statistically significant difference demonstrable on the Wilcoxon test. Thus the areas selected demonstrated acceptable agreement and repeatability.

There was no difference in densities between the control group and the nebulized PFC. This suggests that negligible amounts of PFC are deposited in the lungs with this method of administration.

In contrast, there was a significant difference between the poured and nebulized groups.

The CT density of pure PF 5080 is approximately 606 HU (standard deviation ± 13) on the scanner settings above. For comparison air has an attenuation value in HU of –1000, water of 0 HU and bone +1000 HU [117]. The CT number can be considered as a function of the physical density and electron density [181]. This may differ slightly from the weight/volume density e.g. physical density of PF 5080 in terms of weight/volume is 1.76g/ml (3M's data sheet). According to Mull [181] an approximation of the physical density of a substance can be calculated by computed tomography by the following formula;

CT number $=K([\mu-\mu_w)]/\mu_w$ where

K is a magnifying constant usually set at 1000

μ is the linear attenuation coefficient of the pixel (or voxel) being studied

and μ_w is the linear attenuation coefficient of water. Then for PF 5080 with a CT number of 606, and assuming a density for water of 1, the formula can be rearranged as follows;

μ= 606/1000 + 1= 1.6.

Thus the linear attenuation coefficient of PF 5080 is 1.6. Therefore, the radiological density of a voxel 1/3 filled with gas, 1/3 filled with tissue or oedema, and 1/3 filled with PF 5080 would be

Gas	0.33	x	0 gram mass	= 0
Fluid/Tissue	0.33	x	1 gram mass	= 0.33
PF 5080	0.33	x	1.6 gram mass (as far as electron absorbing capacity is concerned)	= 0.42
Total mass (electron absorbing capacity)				= 0.75 gram
Thus HU in this voxel				=1000 [0.75-1/1)]
				= -250 HU

(See Figure 23a and 23b)

Figure 23a

Schematic representation of a voxel completely filled with PF 5080

CT attenuation = 606 HU.

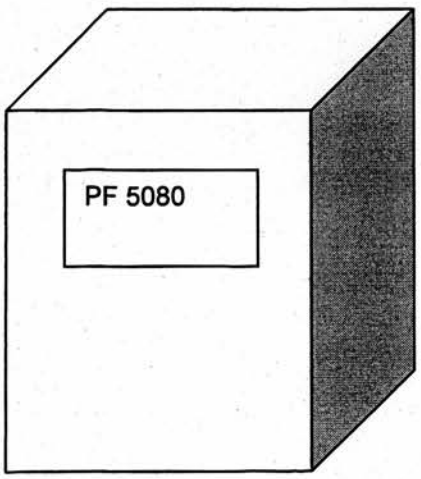
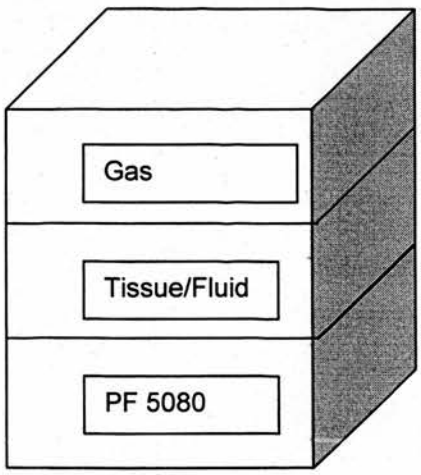


Figure 23b. Schematic representation of a voxel 1/3 filled with gas, 1/3 filled with tissue/fluid, and 1/3 filled with PF 5080. Total CT number for this voxel =-250 HU (see text).



In short, a small amount of a substance with such a drastically different CT attenuation number than PF 5080, can radically alter the CT attenuation number of a lung region or voxel. As the distribution of the original values in Figure 17 and also in Tables 16 and 17 demonstrate, the poured group had the greatest (449 HU). This is in the group whose lungs had been supposedly filled to FRC with PF 5080. This is much less than expected and implies that there is the presence of some other less radiodense substance in the alveoli "diluting" the attenuation of the PFC such as tissue or fluid, or gas. This would be consistent with oedema or gas lying within the alveoli. This is essentially a partial volume effect "in reverse"[116], where instead of a dense substance elevating the mean HU attenuation within a voxel, there is a less dense substance reducing the HU attenuation. In Kaiser et al's study investigating the effects of PEEP superimposed upon PLV in vivo, opacities representing a combination of PFC and oedema/lavage fluid were clearly seen in the airways [119]. With time the aqueous fluid will appear to float on top of the denser PFC, as noted in other studies [120]. If an airway were to be obstructed, this would inhibit access of PF 5080 to the lung periphery. The lung region beyond this obstruction would presumably reflect the HU attenuation of collapsed lung (circa 1 HU) rather that of PF 5080.

Alternatively it may be that the lungs had not been completely filled due to pockets of gas trapped "under" the level of meniscus seen in the tracheostomy tube. Fuhrman suggested in early work on partial liquid ventilation that a bubble of gas may be left at the top of the alveoli at end-expiration[82]. The exact distribution which PFC takes up within alveoli is still debated. A possible elaborate explanation has been given by Tarczy-Hornoch's group (see Figure 24a and 24b) [1].

They suggest that the distribution within the alveolus may be the net effect of surface tension vectors determined by the interface between PFC/lung, gas/PFC and gas/lung i.e whether the PFC has a lentiform structure or whether it forms a lining layer within the alveolus.

Figure 24a. PFC forms lens shaped bubble within alveolus[1]

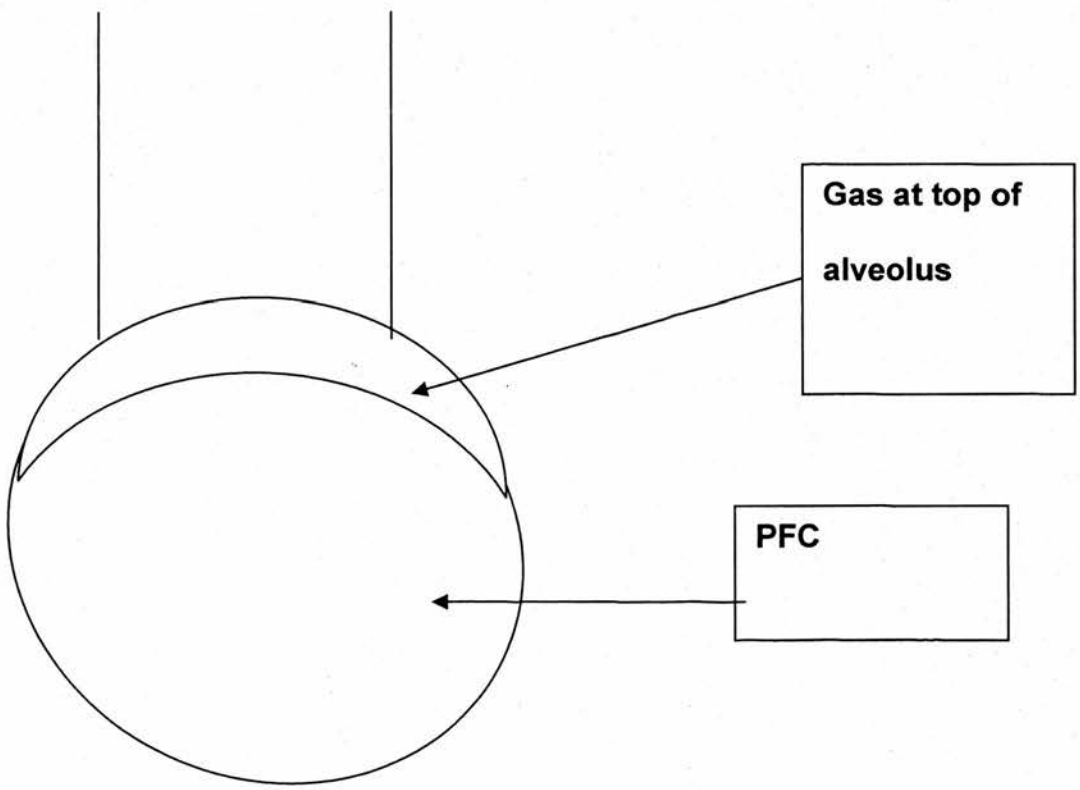
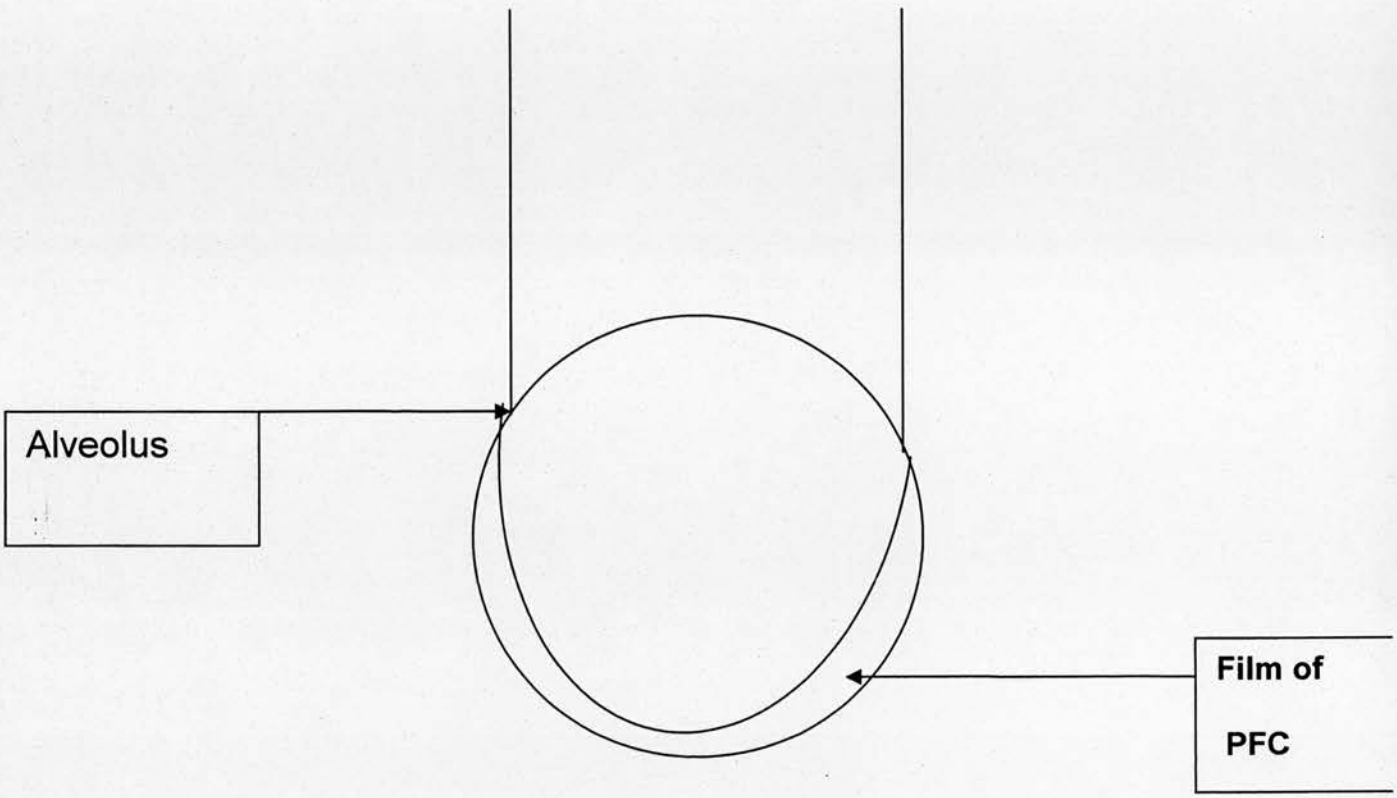


Figure 24b Interaction of surface tensions forces PFC into a rim lining the inside of the alveolus.



A further explanation would be that the quoted figures for the FRC of rabbits [149;170;182] were underestimates, although this would not explain the clearly seen menisci of PFC in the endotracheal tube at zero PEEP.

The study also demonstrated no gravitational effects within study groups, something which rather contradicts Gattinoni et al's work on CT scanning of lungs in ARDS, as well other radiological studies (both CT and conventional radiograms) in the presence of PLV [120;121].

There are some differences between these other studies and the present study which may explain these findings. First, Gattinoni's group studied intact patients with ARDS, removing the confounding preparation artefacts compared with this study. Second, this study dealt with small animals in comparison to either patients or larger animals with correspondingly larger thoraces. It may be that relatively little collapse is induced by the weight of overlying lung when the dimensions of the thorax are of the order of 5cm compared with several times larger in the sheep[118] or humans. Alternatively more animals may be required to make this difference obvious although the number of subjects in the study groups in this study were comparable to those in the other studies.

In summary this ex-vivo CT scanning study of density distributions showed no difference in the density distributions of control animals and animals given PF 5080 by ultrasonic nebulizer. There was a significant difference in CT attenuation values between poured and nebulized PF 5080, and PLV and control animals. The study also implies there is incomplete filling of the lungs with PF 5080 even when poured volumes equivalent to the FRC of the rabbit are administered.
Within the individual groups, no difference was seen between the differing nine lung areas.

Chapter 7

Summary and General Discussion

The work described in this thesis was one of the first attempts to give PFC by nebulisation to treat acute lung injury. When the project was being drafted no other group had attempted this, although in the interim there have been groups who have published work reporting vaporised or aerosolized PFC [155;161;183].

Summary with Future Possibile Directions of Research

The broad outcomes studied in this project were survival, oxygenation, lung mechanics and the CT density distributions. These may be interrelated as follows. It is reasonable to assume that unalleviated hypoxia may cause shortened survival[134]. Four therapies (Poured PFC i.e. PLV, Curosurf, Pumactant/PLV and Curosurf/PLV) caused significant improvement in oxygenation. This was reflected in an improvement in 12 hour survival for Poured PFC and the combination of Pumactant/PLV. The other two therapies could arguably be showing a trend towards improved survival hidden by the relatively small numbers or the conservative nature of the Bonferroni test. However it may be that the rapidity of action of Curosurf either alone, or in combination may have deleterious effects negating some of the positive effects of improved oxygenation. This is discussed in greater detail below. The same four therapies which improved oxygenation, had broadly similar effects on improving Cdyn. Three of these therapies (PLV, Curosurf and the combination of Curosurf /PLV) caused statistically significant improvements. Pumactant/PLV showed a trend towards improvement but was not statistically significant. This may

reflect as yet undetermined interactions between the differing surfactant preparations and PF 5080 which affect oxygenation and lung mechanics in differing ways.

Further work to discover the modes of action of PFC is worthwhile since there does seem to be a difference in the maximum effect for improvement in lung mechanics compared with the improvement in oxygenation[158]. This lends credence to the theory that PFC is coating the alveoli [124;155] and once it has coated the alveoli no further improvement in lung mechanics may be obtained, as PFC has a constant surface tension. In contrast, improvement in oxygenation is caused either by maintaining alveoli open[79], or by an effect on ventilation/ perfusion ratios[81]. As more alveoli are maintained open, a greater improvement in oxygenation occurs. However there may be differing effects on ventilation/ perfusion matching which depend upon the PFC used. This requires clarification.

Some of these therapies, particularly those containing Curosurf, may have worked too well (or more exactly too rapidly)[173] resulting in overdistention of lung and pneumothorax, detracting from their positive effects. This could explain an improvement in compliance but not in survival.

It could perhaps be argued that the Pumactant/PLV group's less significant improvement in lung mechanics was an ideal compromise, allowing a good response in oxygenation but not causing such a spectacular (and damaging) distention of lung.

Another interpretation of the failure of Pumactant/PLV to improve Cdyn, yet apparent success in improving survival and oxygenation was that the Pumactant had no effect on at all, and the improvement in oxygenation and survival seen was purely

due to the PLV. The Pumactant may have inhibited PF 5080 from reaching some airways as alluded to in chapter 5. This would explain why there was no statistically significant improvement in Cdyn.

The fact that there was no statistically significant difference in the incidence of pneumothorax between groups (chi squared test) may just reflect relatively small numbers, or in some groups death rapidly from hypoxia before pneumothorax could develop.

In addition to studying the effects of combinations of PF 5080 with the surfactants Pumactant or Curosurf mentioned above, this work also studied the effects of the artificial surfactant Pumactant or the natural surfactant Curosurf given as a solitary treatment on survival, oxygenation, Cdyn, and Crs.

Pumactant failed to have any effect on survival, oxygenation and either Cdyn or Crs in the current study.

A trial published while this project was being completed, compared Pumactant with Curosurf for the treatment of respiratory distress syndrome of the newborn was stopped prematurely due to an excess of deaths in the Pumactant group[184].

It is recognised that the natural surfactants have a more rapid onset of action than the synthetic non-surfactant protein containing surfactants[173;185]. This very rapid onset may cause undesirable effects as discussed above. Pumactant may have shown a greater effect if this study was repeated but over a longer period of time, such as 24 hours. However this presupposes a change to Home Office regulations, and that the animals would survive a prolonged period with critical oxygenation.

Alternatively the saline lavage model may be an inappropriate model to use when studying Pumactant.

The difference between Curosurf's improvement in oxygenation, Cdyn and Crs and Pumactant's failure may be explained by the presence of surfactant proteins and their increased resistance to inactivation in the presence of injured lung[129;132;175;176].

Certainly there is scope for further work to investigate what actually happens to exogenously administered surfactant in humans with acute lung injury.

The thesis also investigated the effects of nebulized PFC on survival, oxygenation, Cdyn and CT density distributions.

This project found that nebulized PF 5080 had no effect on these four outcomes. This would be consistent with no PFC actually being delivered to the alveoli. This is in contrast to the positive effects seen in subsequently published papers using other apparatus.

It would be interesting to repeat this study with a differing mechanical ventilator, perhaps one using a circle system which re-circulates fresh gases (as used by Bleyl's group)[155] or an aerosol jet injector (similar to that used by Kandler et al)[183]. My project used the SLE 250 which has a continuous flow bias, because of its ready availability to me. This may waste a large proportion of the fresh gas flow. A further study may perhaps look at the proportion of PFC deposited in the lungs by a different method (perhaps by radio-labelling). Alternatively another form of nebulizer may be used.

Another explanation of the differing outcomes is that Bleyl and Kandler's studies used other PFC compounds (Perfluorohexane and Perflubron). Projects designed to study the differing effects of PFC (particularly with differing vapour pressures if a nebulized or vaporised route is considered) should continue to find the most appropriate PFC to use.

The CT density arm of the study did not just yield interesting results for the nebulized group. Another noteworthy outcome was that PFC did not seem to reach all parts of the lung apparently submerged under PF 5080 in the PLV group. This

was presumed due to atalectatic lung preventing PFC reaching these areas of collapse.

Future Uses of PLV with Perfluorocarbons.

One must be cautious about implying that results that occur in animal models in the research laboratory will translate directly into clinical practice. The future of partial liquid ventilation will be largely determined by the results of the current on-going trials.

PF 5080 was substantially cheaper than a single dose of either preparation of surfactant but seemed better than Pumactant at improving oxygenation and lung mechanics. It is equally as good as the more potent surfactant (Curosurf) at improving oxygenation and lung mechanics, and better than both surfactants in improving survival, at least over the relatively short period of the study.

After further studies PFC may prove to be cheaper ways of applying surface active agents in ARDS.

This study did not concern itself with the disease altering potential of PFC. A study specifically designed to explore the effects of PFC on lung inflammation and histology (as mentioned in chapter 2) would be timely.

The place for liquid ventilation may be in specialist centres for the group of patients who are failing to improve on "best conventional" management or for whom permissive hypercapnia is (relatively) contraindicated (such as raised intracranial pressure or ischaemic heart disease) or in whom other novel therapies such as surfactant administration have failed. PLV has been suggested as a means of delivering drugs to the respiratory tract [186] [187]. There has been a small amount of

work comparing PLV use in combination with other treatments used in ARDS (such as inhaled nitric oxide)[123].

PLV can improve oxygenation and lung mechanics and it is worth preserving as part of the armamentarium of novel therapies in ARDS for selected patients. Delivery as either an aerosol or by vapour is as yet an underinvestigated field worth exploring.

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