Determination of Local Oxygen Consumption

by

Healthy and Diseased Lungs

in

a Rabbit Model

GU Jia-Shi

A Thesis Submitted in Partial Fulfillment

of the Requirements for the Degree of

Master of Philosophy

In

Medicine

©The Chinese University of Hong Kong

June 1999

UL

Abstract

Objective: To evaluate in a rabbit model the hypothesis that (1) the lung consumes a significant proportion of the total body oxygen consumption (VO_{2wb}), and (2) the pulmonary oxygen consumption (VO_{2pul}) increases with acquired lung injuries.

Method: The oxygen consumption (VO_2) of the animal was measured simultaneously using two different methods: (1) indirect calorimetry which measures the whole body VO_2 (VO_{2wb}), and (2) estimation from the cardiac output based on the Fick principle (cVO_2). Cardiac output was determined by the thermodilution method, since the cVO_2 does not include the oxygen consumption of the lungs (VO_{2pul}), VO_{2pul} could be calculated from the difference between VO_{2wb} and cVO_2 ($VO_{2wb} - cVO_2 = VO_{2pul}$). 3 groups of rabbits were studied: (a) healthy controls (n=21); (b) rabbits with acute lung damage (AD) induced by subcutaneous N-nitroso-N-methylurethane (NNNMU) (n=14) and (c) rabbits with chronic lung damage (CD) induced by intratracheal bleomycin.

Results: In the healthy controls, Mean $\pm$ SD, VO_{2wb} was 26.7 ± 5.6 ml/min, and cVO_2 was 25.8 ± 5.5 ml/min; In the AD group, VO_{2wb} was 27.3 ± 6.1 ml/min, and cVO_2 was 24.8 ± 6.1 ml/min; In the CD group, VO_{2wb} was 30.2 ± 8.0 ml/min, and cVO_2 was 24.2 ± 7.4 ml/min. The VO_{2pul} in the control group was $3.3 \pm 2.2\%$ of the VO_{2wb}. In AD and CD groups, VO_{2pul} increased to 9.7 ± 5.2 % and $20.4 \pm 6.3\%$ of the VO_{2wb} respectively. Histopathological examination confirmed the presence of acute inflammation and chronic lung damage in the animal in AD and CD group respectively.

Conclusion: The lungs normally consumed about 3% of VO_{2wb}. This increased to 9.7% in acute and 20.4 % in chronic lung injury. The increase in pulmonary VO_2 was likely due to increased local metabolism caused by pulmonary inflammation.

(288 words)

摘要

目的：探討局部肺耗氧量。

方法：建立用 N-nitroso-N-methylurethane (NNNMU)及博來霉素誘發之急、慢性肺損傷對照大兔動物模型，並使用非直接熱量計和 Fick 方法測得量度耗氧量(mVO_2)及計算耗氧量(cVO_2)。因 Fick 方法在計算中未有包括肺部耗氧量(VO_{2pul})，故肺耗氧量可從 mVO_2 及 cVO_2 之差求得($mVO_2 - cVO_2 = VO_{2pul}$)。

假設：(1)全身耗氧量(VO_{2wb})有相當部份由肺耗用；

(2)肺耗氧量隨肺損傷增加。

結果：急性損傷組(27.3 ± 6.1 ml/min)和慢性損傷組之 mVO_2(30.2 ± 8.0 ml/min)與對照組(26.7 ± 5.6 ml/min)相若；而 cVO_2 在急性損傷組，慢性損傷組與對照組分別為 24.8 ± 6.2 ml/min, 24.2 ± 6.2 ml/min 及 25.8 ± 5.4 ml/min。肺耗氧量佔全身耗氧量在對照組為 3.3 ± 2.2 %，而在急性損傷組和慢性損傷組為 9.7 ± 5.2 % 及 20.4 ± 6.3 %。

結論：正常肺部耗用全身耗氧量之 3.3 ± 2.2%，而因急性或慢性損傷，肺耗氧量則會增至 9.7 ± 5.2 % 及 20.4 ± 6.3%，根據組織病理的資料提示，耗氧量增加是由於肺部炎症反應的發生；由於 VO_{2pul} 上升與炎症病變程度相關，故治療有慢性肺病變的病童時，應重點針對肺部炎症的處理。

Acknowledgments

Hysan Clinical Research Laboratories, Animal House, Technical Services Unit, Department of Anatomical & Cellular Pathology, Department of Surgery, Department of Orthopeadiatrics & Trauma, and the Prince of Wales Hospital, for providing me with research facilities for this study.

Finally I would also like to express my deepest gratitude to my parents and my brother, for their patience, everlasting love and unfailing support.

Statement of Originality

This study was carried out in the Department of Paediatrics, the Chinese University of Hong Kong. The animal experiments and histological studies were mainly performed by myself in the Lee Hysan Laboratory, the Prince of Wales Hospital. The histopathological staining and scoring were performed with the help of Professor Kai Fai To, and staff of the Department of Anatomical & Cellular Pathology. The final data analysis was performed with the help of my supervisor, Professor Tai Fai Fok.

List of Abbreviations

%	Percentage
$\geq$	Less or equal to
μ	Micron
$^{\circ}C$	Celsus
μl	Microlitre
$>$	More than
$\pm$	Plus or minus
AD	Acute damage
AD group	Acute damage group
ARDS	Adult Respiratory distress syndrome
BLM	Bleomycin
BPD	Bronchopulmonary dysplasia
CaO_2	Arterial oxygen content
CD	Chronic damage
CD group	Chronic damage group
CLD	Chronic lung disease
CO	Cardiac output
CO_2	Carbon dioxide
CON group	Control group
cVO_2	Calculated oxygen consumption
CvO_2	Mixed-venous oxygen content
Ee	Energy expenditure
F_{ECO2}	Mixed expiratory carbon dioxide concentration

F_{EO_2}	Mixed expiratory oxygen concentration
F_{ICO_2}	Inspired carbon dioxide concentration
$F_{IO_2} - F_{EO_2}$	Oxygen concentration difference between inspiratory and mixed expiratory concentrations
F_{IO_2}	Inspired oxygen concentration
$F^*_{CO_2}$	Mixed inspiratory carbon dioxide concentration
g/dl	Gram per decimal liter
H&E	Hematoxylin-esoin
Hb	Hemoglobin
HbO_2	Oxyhemoglobin
Kg	Kilogram
L	Liter
L/min	Liter per minute
M	Mole
mg	Milligram
min	Minute
ml	Milliliter
ml/L	Milliliter per liter
ml/min	Milliliter per minute
MT	Masson Trichrome
mVO_2	Measured oxygen consumption
N group	Control (Normal) group
NICU	Neonatal Intensive Care Unit
NNNMU	N-nitroso-N-methylurethane
O_2	Oxygen
PaO_2	Partial pressure of oxygen in arterial

	blood
PO_2	Oxygen dissolved in blood
Q	Constant flow
RDS	Respiratory distress syndrome
RER	Respiratory exchange ratio
RQ	Respiratory quotient
RR	Respiratory rate
SaO_2	Oxygen saturation in arterial blood
SD	Standard deviation
SvO_2	Oxygen saturation in venous blood
T_{blood}	Blood temperature in degrees °C
T_{inj}	Injectate temperature in degrees °C
TM	Trade mark
U	Unit
VCO_2	Carbon dioxide production
V_{inj}	Volume of injectate in liters
VO_2	Oxygen consumption
VO_{2b}	Body oxygen consumption
VO_{2wb}	Oxygen consumption of the whole body
VO_{pulm}	Pulmonary oxygen consumption

List of Figures

List of Tables

Tables of Contents

Section One : **Introduction & Literature Review**

Introduction & Objective

Introduction

Bronchopulmonary dysplasia (BPD) is a chronic lung disease (CLD) that results from the unresolved or abnormally repaired lung damage. This condition occurs most commonly in premature babies who have been exposed to high concentrations of inspired oxygen and positive-pressure ventilation. Infants with CLD always have increased oxygen consumption (Masrkestad *et al*, 1981; Kao *et al*, 1988) and energy expenditure (Weinstein *et al* 1981), and very often with decrease of growth rate (Vohr *et al*, 1982; Yu *et al*, 1983; Kurzner *et al*,1988). For a long time, these phenomena was believed to be due to the abnormalities impaired mechanical function of the damaged lung, which resulted in increase in the work of breathing.

However in 1988, Kao and colleagues observed that resting oxygen consumption of CLD patients remained unchanged, even after their pulmonary mechanics had been improved by the use of bronchodilators (Kao *et al*, 1988).

Oxygen consumption is commonly measured by: (1) Indirect calorimetry; and (2) thermodilution technique, applying the Fick principle. The former involves direct measurement of the inspired and

expired oxygen, and the latter involves calculation of oxygen from the measured cardiac output and oxygen saturation of arterial and central venous blood.

In previous studies, a difference between measured and calculated oxygen consumption was always found (Keinanen *et al*, 1997). Furthermore, the measured oxygen consumption was always higher than the calculated oxygen consumption and the difference became more obvious when there were lung injuries or pneumonia. (Jolliet *et al*, 1996; Light, 1988).

Since the thermodilution method measures the cardiac output through the pulmonary circulation, before entering the lung, it can be presumed that the oxygen content difference between arterial and mixed venous blood does not include the effect of oxygen uptake of the lung.

Based on the above observation, I believe that the amount of oxygen consumed locally by the lung contributes a significant part of total body oxygen consumption, this might become more significant in damaged lung.

Objective the present study

To evaluate the hypothesis that: (1) oxygen consumption by the lung contributes significantly to the total oxygen consumption of the body; and (2) acquired lung injury increases the local oxygen consumption by the lung.

Literature Review

Chapter 1 A Review of

Chronic Lung Disease (CLD)

1. *BPD – an example of CLD*

Bronchopulmonary dysplasia (BPD) was first named and described in 1967, when Northway and associates reported the abnormal clinical, radiological and pathological findings of the lungs of a group of premature newborns who had been subjected to prolonge ventilation through endotracheal intubation with high pressures and high oxygen concentration (Northway, 1967).

It is a common chronic lung disease (CLD), found mostly in preterm newborns that require prolonged mechanical ventilation and oxygen therapy. Infants with CLD are characterized clinically by chronic respiratory distress, hypoxemia and, hypercapnia. Characteristic patchy collapse is usually shown on the chest radiograph.

In most of the cases, BPD is preceded by respiratory distress syndrome (RDS), but also the condition may follow other acute pulmonary disorders (Philip, 1975; Banerjee *et al*, 1972; Pusey *et al*, 1969; Davis & Rosenfeld, 1994). Wilson-Mikity syndrome, pneumonia, apnea of prematurity, and meconium aspiration have all been observed before the onset of BPD (Pusey *et al*, 1969).

The definition of BPD has been subjected to much discussion, and different definitions have been suggested by different workers. (Bancalari *et al*, 1979; O'Brodovich & Mellins, 1985; Shennan *et al*, 1988; HiFi Study Group, 1989,1990; Hack *et al*, 1991;Hoekstra *et al*, 1991; Liechty *et al*, 1991; Corcoran *et al*, 1993; Rojas *et al*, 1995). As a result, the reported incidence of BPD was very variable, ranging between 5 to 80% of babies who had received mechanical ventilation (Berg *et al*, 1975; Johnson, 1976; Edwards, 1983; Farrel & Palta, 1986; Boynton, 1988; Shennan *et al*, 1988; Hack *et al*, 1991; Campbell & McIntoch, 1997).

2.   *Pathological change & Clinical presentations*

BPD is a disease of scarring and repairing of lung tissue, which affects its growth. Abnormalities of airways, pulmonary vasculature, and pulmonary function are found. In CLD neonates, there are muscular hypertrophy, bronchospasm, mucosal edema of the bronchioles, and excessive airway mucus. These cause luminal narrowing, and increase in airway resistance. (Morray et al, 1982; Gerhardt et al, 1987; Kao et al, 1988; Mallory et al, 1991; Abman et al, 1994; Baldari et al, 1997). Moreover, decrease in lung volume, lung compliance, and reduction of

maximal forced expiratory flow at functional residual capacity are also noticed in infants with CLD (Watt et al, 1977; Godfrey et al, 1983).

3. *Clinical sequel of CLD infants*

Besides chronic respiratory distress and hypoxia, infants with CLD have elevated oxygen consumption and energy expenditure, and a decrease in growth rate (Markestad & Fitzhardinge, 1981; Weinstein *et al*, 1981; Vohr *et al*, 1982; Yu *et al*, 1983; Berman *et al*, 1986; Kao *et al*, 1988; Kurzner *et al*, 1988; Yeh *et al*, 1988; Kalhan & Denne, 1990; Casey *et al*, 1991; Piedboeuf *et al*, 1991; Westerterp *et al*, 1991; Brownlee *et al*, 1992; De Gamarra *et al*, 1992; Abman & Groothius, 1994; Knopper *et al*, 1994; Van Goudoever *et al*, 1994; Meer *et* al, 1996).

3.1 *O₂ consumption of CLD infants*

Though the results of clinical studies varied, a number of studies have demonstrated a raised oxygen consumption in infants with CLD (Kurzner *et al*, 1988). Weinstein (Weinstein *et al*, 1981) and Kao (Kao *et al*, 1988) reported a 25% higher resting VO_2 in infants with CLD when compared with those in the control group.

Pulmonary edema and interstitial fibrosis restrict the elasticity of the lung tissues and increase the work of breathing, apparently due to the elevated airway resistance and decreased lung compliance (Morray *et al*, 1982; Kao et al, 1983,1988; Gerhardt *et al*, 1987; Abman & Groothius, 1994). This further leads to an increased in oxygen consumption.

3.1-1 Oxygen consumption

Oxygen consumption is the total amount of oxygen consumed by the body per minute. By estimating arterial oxygen transport (total amount of oxygen carried to the tissues), the amount of oxygen consumed by the body per minute can be determined (Nunn, 1993).

3.1-2 Oxygen transportation

O_2 diffuses from the alveoli to the blood in two forms: dissolved oxygen in plasma, or in combination with hemoglobin.

3.1-2a Dissolved O_2

After entering the lung, oxygen moving across the alveolar capillary membrane immediately dissolves in plasma. Clinically, it is measured as the partial pressure of O_2 in arterial blood (PaO_2). Factors that influence PaO_2 level include the gas volume of the lung, the adequacy

of alveolar ventilation, the function of inspired oxygen (F_{IO2}), together with the physical characteristics of the affinity of hemoglobin for oxygen.

3.1-2b   *Haemoglobin*

Hemoglobin molecule is formed from the heme, an iron-porphyrin compound. It is joined to the protein globin, which consists of four polypeptide chains. The chains are of two types: alpha and beta. The differences in their amino acid sequences give rise to various types of human hemoglobin. Oxygen dissolved in plasma is transported in reversible chemical combination with the hemoglobin molecule. Oxygenation transforms hemoglobin to oxyhemoglobin.

$$O_2 \quad + \quad Hb \quad \longleftrightarrow \quad HbO_2$$

Deoxygenated Hb   Oxyhemoglobin
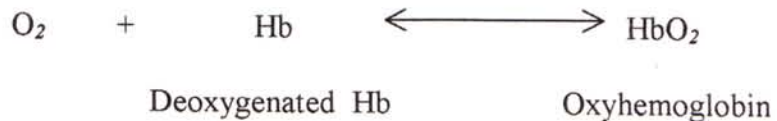
Oxyhemoglobin accounts for about 97 to 98 percent of the oxygen that is transported to the tissues and about 17 to 20 ml of oxygen in 100ml of blood.

3.2   *Energy expenditure of CLD infants*

In 1988 Kurzner hypothesized that an increase in mechanical work of breathing (and associated phenomena such as sympathetic activity) results in extra energy expenditure in CLD infants. The inefficient gas exchange and increased dead space at the level of the alveoli may result in an increased minute ventilation. Hence, increases in respiratory rate and energy cost per breath could result in a disproportional increase of mechanical work of breathing in CLD infants. (Kurzner *et al*, 1988; Meer *et al*, 1996).

Several controlled studies have shown an increased energy expenditure (Ee) in infants with CLD (Kurzner *et al*, 1988; Weinstein *et al*, 1988; Yeh *et al*, 1988; De Gamarra *et al*, 1992). Weinstein (Weinstein *et al*, 1981) observed that the mean resting caloric expenditure of CLD infants was 11kcal/kg/day higher than that of the control group. Piedboeuf and co-workers (Piedboeuf *et al*, 1991) found a 30% higher metabolic rate in CLD patients when compared to newborn infants of similar age with normal respiratory function. Kao (Kao *et al*, 1988) documented a 25-50% increase in caloric requirements by the BPD infants. With the doubly labeled water technique, Meer and colleagues (Westerterp *et al*, 1991; Meer *et al*, 1996) reported a 16% increase in the

energy expenditure in BPD infants which closely agreed with the 25% increase measured by respiratory calorimetry (Yeh *et al*, 1988; Kalhan & Denne, 1990; De Gamarra *et al*, 1992).

3.3   *Growth rate of CLD infants*

Poor growth or even growth failure is a common problem of infants with CLD. However, whether the growth failure is related to the increased work of breathing remains controversial (Kao *et al*, 1988; Kurzner *et al*, 1988). Although body weight is the most affected parameter, growth in length (Berman *et al*, 1986; Kurzner, *et al*,1988; Casey *et al*, 1991; Abman & Groothius, 1994), and skeletal growth velocity can also be impaired (Yu *et al*,1983; Brownlee *et al*, 1992; Knopper *et al*, 1994; Van Goudoever *et al*, 1994). It has been observed that somatic growths parameters of CLD children are generally along the 10th to 25th percentile (Kurzner *et al*, 1988; Casey *et al*, 1991; Abman & Groothius, 1994).

In 1981, Markestad and Fitzhardinge found that the mean weight at postterm of 26 infants with CLD was between the third and tenth percentile; mean height was at about twenty-fifth percentile in the first 2 years (Markestad & Fitzhardinge, 1981). Vohr speculated that growth

failure in infants with CLD might be related to poor nutritional intake or prolonged intravenous feedings (Vohr *et al*, 1982).

It was generally believed that growth failure in CLD can be placed in one of two categories: inadequate nutritional intake or inadequate oxygenation (Kurzner *et al*,1988; Abman & Groothius, 1994; Moyer-Mileur *et al*, 1996).

The oxygen consumption and caloric requirements of BPD patients are somehow proportional to the severity of the lung disease. In 1988, Kurzner observed a significant lower lung compliance, higher respiratory rate and minute ventilation in CLD infants with growth failure, in comparison to CLD infants without growth failure. A careful review of caloric intake has been proposed as an important step in examining a CLD patient with impaired growth. (O'Brodvich & Mellins, 1985; Abman & Groothius, 1994).

4.   *Treatment & Management of CLD infants*

General management for CLD infants includes O_2 therapy, fluid restriction, diuretic therapy, bronchodilator and corticosteroid (Ng *et al*, 1993).

Bronchodilators and diuretics have been proved to have additive effects on the improvement of pulmonary function in infants with BPD (Kao *et al*, 1983,1987; Patel *et al*, 1985; McCann *et al*, 1985; Engelhardt *et al*, 1986). Bronchodilators and diuretics have been used in the treatment of CLD to decrease the amount of excess lung fluid and total body water (Rooklin *et al*,1979; Kao *et al*,1983,1984,1987; McCann *et al*, 1985; Logvinoff *et al*, 1985).

4.1 *Diuretics*

Diuretic drugs commonly used in CLD patients are furosemide, chlorothiazide, and spironolactone. Both furosemide given intravenously and chlorothiazide given orally have been shown to improve pulmonary function in infants with CLD (Kao *et al*, 1983,1984,1987; Najak *et al*, 1983; Logvinoff *et al*, 1985; McCann *et al*, 1985; Engelhardt *et al*, 1986; Mammel *et al*, 1987; Rush *et al*, 1990; Ehrenkaranz & Mercurio, 1992).

Besides increasing urine and solute excretion, diuretics also have their beneficial effects on lung function. For instance, furosemide and some thiazide drugs exert a nitrate-like effect on vascular smooth muscle (Davila & Davila, 1981; Mironneau *et al*, 1981); furosemide given by inhalation affects chloride transport in tracheal epithelium (Widdicmobe *et al*, 1983) and dilates constricted airways (Bianco *et al*, 1988). The pulmonary effect of furosemide seems to be independent of its diuretic effect, since the medication is able to reduce lung edema even in anephric animals (Bland *et al*, 1978).

4.2   Bronchodilators

Theophylline, caffeine, albuterol, and terbutaline have all been shown to provide acute therapeutic relief of bronchospasm in CLD babies. Theophylline given symptomatically improved airway resistance and dynamic compliance, and decreased the work of breathing in ventilator-dependent infants with CLD (Rooklin *et al*, 1979). After administration of inhaled β-agonist bronchodilators, such as albyterol, infants with CLD exhibited improvement in pulmonary function test results (Davis *et al*, 1990; Abman & Groothius, 1994; Fok *et al*, 1998).

4.3   *Corticosteroids*

Studies on the use of dexamethasone have authenticated improvement of lung function and compliance in CLD infants within days; this effect appeared to facilitate weaning the infants from assisted ventilation and intubation (Najak *et al*, 1983). The benefits of dexamethasone seemed to be immediate but short lived. The duration of mechanical ventilation appeared to be shortened, but not the duration of O_2 supplementation (Korones, 1996). There was no effect on mortality or the duration of hospital stay, (Avery *et al*, 1985; Cummings, D'Eugenio & Gross 1989; Harkavy *et al*, 1989; Collaborative Dexamethasone Trial Group 1991). The use of dexamethasone was associated with a number of adverse effects, such as infection, hyperglycemia, hypertension, intestinal perforation, and suppression of the adrenal glands.

As a result of the short-term benefits, the postnatal use of dexamethasone for the prevention or the amelioration of CLD has become widespread in NICUs (Farrell & Fiascone, 1997)

5. *Interpretations of the observed phenomena, why does CLD impair growth?*

5.1 *The traditional view*

It has been widely accepted that the raised oxygen consumption, energy expenditure and decreased growth rate noticed in CLD patients are due to their increased work of breathing (Wolfson *et al*, 1984; *Kurzner et al*, 1988; Meer *et al*, 1996).

The total work of breathing is the sum of the mechanical work done in moving the lungs, the respiratory gases, the thorax, the diaphragm, and the chest wall during the breathing cycle. Mechanical work of the lungs may be greatly increased in obstructive airway, and in diffused pulmonary fibrosis. When the mechanical work of breathing increases, the oxygen (O_2) requirement of the respiratory muscles rises, leading to greater total body oxygen consumption (VO_2).

Since both diuretics and bronchodilators improved the pulmonary function, it was expected that a reduction of VO_2 could be achieved in CLD infant by treating them with these medications.

5.2 *Disagreement with the traditional view*

In 1988, Kao and colleagues studied the effect of bronchodilator treatment on VO_2 in a group of CLD infants (Kao *et al*, 1988). To their surprise, the treated infants showed a significant improvement in the lung mechanics but no change in VO_2. This important discovery overthrew the assumption that increased VO_2 and energy expenditure were due to increased work of breathing, and led to the need for the exploration of an alternative explanation.

Chapter 2   Measurement of

Oxygen Consumption

Oxygen consumption may be measured either as the loss of oxygen from a closed rebreathing system or, by the subtraction of the quantity of oxygen exhaled from the amount inhaled. Methods usually used in measuring oxygen consumption include invasive (e.g. Fick O$_2$ consumption method; dye-dilution method; thermodilution method) and non-invasive techniques (e.g. Indirect calorimeter, Doppler ultrasonography; Transthoracic Electrical Bioimpedance).

1.   *Invasive measurement of VO$_2$*

By using the Fick principle, O$_2$ consumption could be estimated using the amount of oxygen transported in the patient's blood as an indicator. VO$_2$ is then calculated as the difference between the arterial and mixed venous oxygen contents multiplied by the patient's cardiac output.

1.1   *Cardiac output*

Cardiac output is the quantity of blood delivered by the heart to the systemic circulation over time, that is, the volume of blood pumped by the left ventricle into the aorta each minute. It is the product of stroke volume (the amount of blood ejected by the heart per beat) multipled by heart rate.

Cardiac output is an important measurement in the overall assessment of cardiovascular function and is essential to the calculation of stroke volume, blood oxygen transport, and intrapulmonary shunt as well as pulmonary and systemic vascular resistance. It is affected by a number of factors including preload, afterload, myocardial contractility, and muscular synchrony.

1.2 *Fick method*

The Fick principle is one of the classical methods for the determination of cardiac output. Because the tissue oxygen consumption cannot be directly measured, so it can only be calculated, taking into account cardiac output, hemoglobin level, and arterial and mixed venous oxygen saturation. The rationale of the Fick principle is that if the amount of oxygen transported to tissues, and the amount returning to the heart are known, the amount of oxygen consumed can be determined.

In 1870 Adolph Fick, the German physiologist stated that: "During any interval of time, the amount of a substance entering a given compartment in the inflowing blood must be equal to the quantity of the substance being removed from the blood by the compartment plus the

quantity of the substance leaving in the outflowing blood." (Fick, 1870)
(*Fig 1.2-1*)

The Fick principle can be applied to calculate oxygen consumption as
follows:

$$\text{Cardiac output (CO) (l/min)} = \frac{\text{O}_2 \text{ consumption (VO}_2)(\text{ml/min})}{\text{Difference between arterial and mixed venous oxygen content (ml/l)}}$$

$$\text{CO} = \frac{\text{O}_2 \text{ consumed (VO}_2)}{[(\text{arterial O}_2 \text{ content})-(\text{mixed venous O}_2 \text{ content in the right atrium})]}$$

or,

$$\text{CO} = \frac{\text{VO}_2}{\text{CaO}_2 - \text{CvO}_2} \quad \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots[1]$$

where CaO_2 = arterial O$_2$ content, CvO_2 = mixed venous O$_2$ content

Since,

O$_2$ content (ml/L) = {[O$_2$ saturation (%)] × [amount of O$_2$ per ml

combined with Hb (ml/g/L)]

+ (O$_2$ per ml dissolved in plasma (ml/L),

or

O$_2$ content = [(O$_2$ saturation) × (1.36*Hb)] + 0.003PO$_2$,

Since each gram of human Hb is combined with 1.36ml of O$_2$ when fully

saturated (Greogory, 1974), and the amount of oxygen dissolved in

plasma is 0.003ml/L/Kpa,

therefore ,

Arterial O$_2$ content (CaO$_2$) = (SaO$_2$×1.36Hb) + 0.003PaO$_2$

Mixed-venous O$_2$ content (CvO$_2$) = (SvO$_2$×1.36Hb) + 0.003PvO$_2$

Thus

$$CO = \frac{VO_2}{[(SaO_2 \times 1.36Hb)+0.003PaO_2]-[(SvO_2 \times 1.36Hb)+0.003PaO_2]} \cdots [2]$$

By rearranging equation [1] and [2]:

VO$_2$ = CO × (CaO$_2$ − CvO$_2$)

or,

VO$_2$ = CO x {[(SaO$_2$ x 1.36Hb) + 0.003PaO$_2$]

−[(SvO$_2$ x1.36Hb) + 0.003PvO$_2$]}

1.3   *Advantages & Disadvantages of Fick method in estimating VO₂*

When the procedure is performed correctly, The Fick method is the most accurate way of measuring cardiac output, especially when the cardiac output is low, and is widely accepted as a "gold standard" for cardiac output determination.

However, this method has a number of limitations (Hillis *et al*, 1985; Mahutte *et al*, 1994). It is invalid in the presence of intracardiac or intrapulmonary shunts. The patient must be in stable states, so that a steady metabolic state is achieved, and the method is not suitable in critically ill patients. The procedure requires highly skilled personnel to perform, is time-consuming, and requires meticulous technique. Moreover as blood withdrawal from an arterial line and a central venous line is required, there is considerable risk of infection. This method does not provide easily repeatable or continuous cardiac output measurements and the results are not readily available for immediate clinical intervention.

Because of the limitations, the method is usually used in the laboratory or research settings, and rarely as a clinical investigation.

1.4   *Measurement of cardiac output by thermodilution*

Using the Fick method to estimate VO$_2$ requires assessment of cardiac output. One of the techniques for measuring cardiac output is the thermodilution method. It was first described by Fegler in 1954 (Fegler, 1954), and was first used in human patients by Branthwaite and Bradley at St. Thomas' Hospital in 1968 (Branthwaite & Bradley 1968). However, it did not gain widespread acceptance until the development of the thermodilution pulmonary artery catheter, by Ganz and coworkers in 1971 (Ganz *et al*, 1971).

There are three slightly different thermodilution techniques in clinical use: cold thermodilution, warm thermodilution and transpulmonary thermodilution, all of which use a thermal indicator (cold or warm) injected into the right side of the heart, and any change in temperature of the blood is detected either in the pulmonary arterial or in the aorta.

During the cold and warm thermodilution, a catheter, with a temperature sensor at its tip such as the Swan Ganz catheter is inserted via a central vein (usually the internal jugular of subclavian) into the right atrium. The tip of the catheter containing the thermistor should lie inside

a branch of the pulmonary artery, and the side hole allowing exit of the injectate should lie inside the right atrium. Cold or warm normal saline or dextrose is injected rapidly via one port of the catheter into the atrium through a side-hole near the tip of the catheter. The cold or warm injectate mixes with the blood in the right atrium and ventricle before passing into the pulmonary artery where the change in temperature is sensed by the thermistor. The passage of the blood mixed with the injectate through the measurement site creates a curve representing the change in temperature over time. The area bound by the curve is inversely proportional to the volume of blood passing through the site (*fig 1.2-2*). Cardiac output is then calculated form the temperature-time curve.

For the transpulmonary thermodilution, the injection catheter is placed inside the right atrium. A fine wire thermistor is inserted through the femoral or carotid artery into the proximal part of the descending aorta, where any change in temperature following injection is detected. This method is particularly suitable for studying small animals.

The thermodilution method is believed to be a "gold standard" for cardiac output measurement in subjects without intracardiac or intrapulmonary shunts (Riedinger & Shellock, 1984). Previous studies

reported very good agreement and high correlation between thermodilution cardiac output and other techniques, including the indocyanine green dye method (Fischer *et al*, 1978), Doppler echocardiography (Christie *et al*, 1987), the direct Fick method (Hillis *et al*, 1985), and more recently, impedance cardiography (Gothshall *et al*, 1989).

1.4-1   Advantages and Disadvantages of Thermodilution Method

The thermodilution procedure can be easily and quickly performed repeatedly by just one trained person. It also has the advantage of not requiring any blood sampling.

However, this method does not provide continuous cardiac output measurement, and its accuracy decreases while the cardiac output is low (Imai *et al*, 1997) or when there is presence of intracardiac or intrapulmonary shunts, tricuspid regurgitation, and possibly severe mitral regurgitation (Nishikawa & Dohi, 1993). To insert a catheter into the heart poses an infection risk, and the risk of damaging the carotid or subclavian arteries. The procedure might also be complicated by pneumothorax, dysrrhythmias including ventricular fibrillation, perforation of the atrium or ventricle, damage to the tricuspid or

pulmonary valves, knotting of the catheter, catheter transection and endocarditis. (Buchbinder & Ganz, 1976; Barash *et al*, 1981; Boyd *et al*, 1983; Durand *et al*, 1995; Eidelmann *et al*, 1994; Horst *et al*, 1984; Gore *et al*, 1987; Kinirons & MacSullivan, 1996; Robin, 1985,1987,1988; Sykes, 1992; van Doorn *et al*, 1994).

2. *Non-invasive measurement of VO₂*

By subtracting the quantity of O_2 exhaled from the amount inhaled, VO_2 could be measured non-invasively by a metabolic analyzer—the Detatrac II metabolic monitor (Datex Instrumentarium Corp., Helsinki, Finland).

2.1 *Metabolic analyzer---Deltatrac^{TM} II*

The Deltatrac is an open system indirect calorimetry designed for measurements of VO_2 and VCO_2 in both spontaneously breathing and mechanically ventilated subjects (Meriläinen, 1987). The difference between the inspired and expired oxygen fractions (F_{IO2}, F_{EO2}) is measured with a fast-response paramagnetic differential oxygen sensor (OM-101, Datex/Instrumentarium) (Meriläinen, 1988).

2.2   *Paramagnetic sensor*

Oxygen has a magnetic susceptibility, which is 100 times higher than other common respiratory gases. This has been used to construct analyzers first by Pauling and colleagues, (Pauling *et al*, 1946) and later by Hummel (Hummel *et al*, 1968). Since O_2 molecules in a non-homogeneous field tend to move to where the field strengths are higher, this principle can be used to measure O_2 concentrations with a shorter response time (150ms).

3.   *Measured and calculated oxygen consumption*

Indirect calorimetry measure VO_2 directly (mVO_2) and the VO_2 estimated by the Fick method is indirect, being calculated from cardiac output and the difference between arterial and mixed venous oxygen consumption (cVO_2).

3.1   *Difference between mVO₂ and cVO₂*

Despite very carefully controlled experimental conditions, differences between mVO_2 and cVO_2 have always been noticed with mVO_2 being consistently greater than cVO_2. The differences recorded by various previous workers were: Keinanen (Keinanen & Takala, 1997): 33

ml/min; Jolliet (Jolliet *et al*, 1996): 25 ml/min.m^2; Brandi (Brandi *et al*, 1992) : 5 ml/min.m^2; Myburgh (Myburgh *et al*,1992) : 24 ml/min and Smithies (Smithies *et al*, 1991) : 36 ml/min.

Furthermore, a larger difference between mVO$_2$ and cVO$_2$ had been observed where there was damage in the lung such as pneumonia (Light *et al*, 1988) or acute lung injuries (Jolliet *et al*, 1996). Patients after cardiac bypass operation and patients with adult respiratory distress syndrome have also been found to have significant difference between mVO$_2$ and cVO$_2$ (Takala *et al*, 1989; Gill *et al*, 1994).

4.   *Summary*

Oxygen consumption can be measured by the thermodilution method applying the Fick principle (calculated oxygen consumption, cVO$_2$); or by an indirect calorimetry, (measured oxygen consumption, mVO$_2$). However, in spite of the reliability of both methods, a consistent difference between mVO$_2$and cVO$_2$ was always detected, and the mVO$_2$ was always greater than the cVO$_2$. The difference was appeared to be more marked when there were injuries in the lung.

Researcher(s)	Year	Subject of study	$VO_{2pulm} : VO_{2wb}\%$
Fritts *et al*	1961	Human /Patient	12
Fritts *et al*	1963	Human /Control	0.7
Fritts *et al*	1963	Human /Patient	0.95-11.6
Levinson *et al*	1987	Human /Patient	16
Light	1988	Dogs/ Pneumonia	13-15
Takala *et al*	1989	Human /Patient	16-24
Chopin *et al*	1990	Human /Patient	10
Smithies *et al*	1991	Human /Patient	13
Bizouarn *et al*	1992	Human /Patient	22
Myburgh *et al*	1992	Human /Patient	8
Smithies *et al*	1992	Human /Patient	27
Becq *et al*	1992	Human /Patient	22-40
Oudemans-van Straaten *et al*	1993	Human/Patient	27-32
Chioléro *et al*	1994	Human /Patient	13
Loer *et al*	1997	Human /Patient	5

Table 1.3-1 VO_{2pulm} reported in previous literature
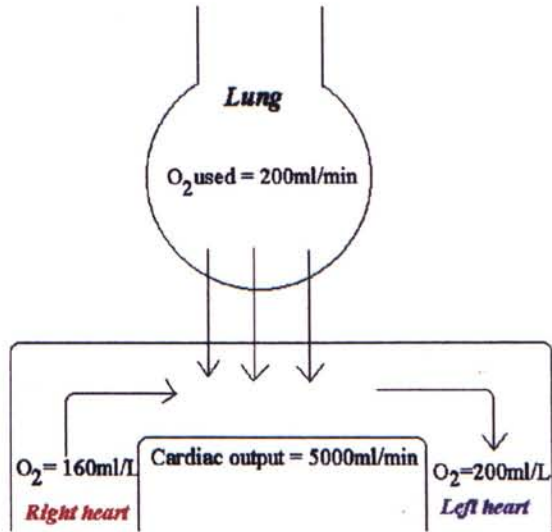
Figure 1.2-1. Fick principle for determining cardiac output
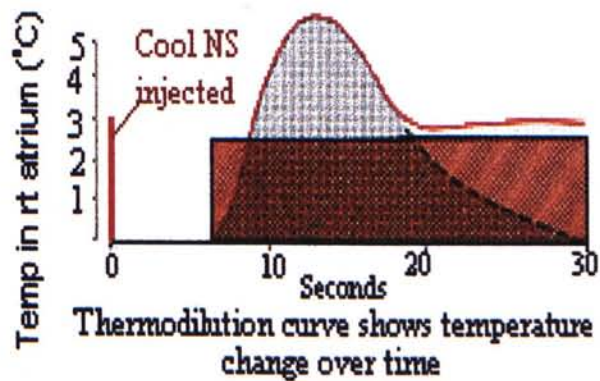
Figure 1.2-2. Thermodilution curve shows temperature change over time

Chapter 3   Hypothesis

1. *Possible explanations for the difference between mVO$_2$ & cVO$_2$*

1.1 *Measurement variabilies and Mathematical errors*

There are various interpretations for the difference between mVO$_2$ and cVO$_2$. Among the explanations is technical error in the measurements. It has been suggested that up to 14% of the total body oxygen consumption could result from the variability of the methods used (Nunn, 1996). One source of the artifact is mathematical coupling of shared variables with the use of Fick method, particularly the cardiac output. Variation in cardiac output may also be caused by drugs with inotropic effect used in some of the subjects (Russell & Phang, 1994).

Nevertheless, measurement variability and mathematical errors should be random and could not explain the consistent observations of a higher mVO$_2$ than cVO$_2$.

1.2 *Oxygen consumption of the lung*

Since the Fick method measures the oxygen content of the blood in the right atrium before entering the lung, it can be assumed that the oxygen content difference between arterial and mixed venous blood does not include the effect of local oxygen uptake by the lung tissue. In contrast, mVO$_2$ estimated by indirect calorimetry measures VO$_2$ of the

entire body including the lungs. Thus the difference between mVO_2 and cVO_2 represents the intrapulmonary oxygen consumption (Keinanen & Takala , 1997). Moreover, the exaggerated difference between mVO_2 and cVO_2 in subjects with lung damage might be due to an increased metabolic rate of the injured lung (Fritts *et al*, 1961; Fritts *et al*, 1963; Levinson *et al*, 1987; Light *et al*, 1988; Takala *et al*, 1989; Chopin *et al*, 1990; Smithies *et al*, 1991; Bizouarn *et al*, 1992; Myburgh, 1992; Smithies *et al*, 1992; Nunn *et al*, 1993; Jolliet *et al*, 1996) (*Table 1.3-1*).

In 1987, Nunn proposed that the oxygen consumption of the lung (VO_{2pulm}) could be assessed by the difference between whole-body VO_2 (VO_{2wb}) measured by indirect calorimetry and the values calculated by the Fick method. He estimated that lung used up 1-4% of the whole-body VO_2 (VO_{2wb}) (Nunn, 1987).

Loer and co-workers reported that the lung depleted about 5% of total body oxygen consumption in humans. They measured the pulmonary oxygen consumption before and during total cardiopulmonary bypass (CPB). As the pulmonary circulation is separated from systemic blood flow during this procedure, the contribution of systemic (bronchial) blood

flow to pulmonary gas exchange during cardiopulmonary bypass was assessed (Loer *et al*, 1997).

1.3 VO_{2pul} *with lung damage*

Many studies have demonstrated an increase in body oxygen consumption could occur in the presence of pulmonary infection (Fritts *et al*, 1961, 1963; Levinson *et al*, 1987; Takala *et al*, 1989; Chopin *et al*, 1990; Smithies *et al*, 1991,1992; Bizouarn *et al*, 1992; Myburgh *et al*, 1992; Becq *et al*, 1992; Oudemans-van *et al*, 1993). Extensive research has shown that infants in whom BPD developed could be distinguished from those without BPD by the present of an ongoing inflammatory process within the lung that augments and perpetuates lung damage (Zimmerman, 1995). If the enhanced VO_2 of infected lungs is real, it is possible that inflammatory cells such as neutrophils and macrophages might play a part.

1.4 *Response of Neutrophils, Macrophages and oxygen consumption*

Both the neutrophils and macrophages undergo oxidative metabolism. Inflammatory tissues might have increased oxygen consumption due to accumulation of the cells and increased phagocytosis.

Moreover, the oxygen consumption of neutrophils increases enormously during the "oxygen burst" associated with the formation of oxygen-derived free radicals.

Premature infants often require supplemental oxygen as well as mechanical ventilation, which may cause inflammatory lung change with influx of large number of neutrophils (Ogden, 1984; Merritt *et al*, 1981,1983; Bruce *et al* 1982; Zimmerman, 1995). After the first week, the number of neutrophils is greatly reduced in the lungs of babies who recovered from RDS completely, but elevated numbers persist in those who progress to CLD (Merritt *et al*, 1981,1983; Ogden, 1984; Bruce *et al* 1992; Zimmerman, 1995). The presence of inflammatory cells and reparative process in CLD may result in excessive consumption of oxygen the lung.

2. *Hypothesis*

Based on the observations previously described, I have derived the following hypothesis:

(1) Oxygen consumption by the lung contributes significantly to the total oxygen consumption of the body.

(2) Acquired lung injury increases local oxygen consumption by the lung.

Methods & Materials

Chapter 1.   Animal Model

This study is approved by the Ethics Committee of the Chinese University of Hong Kong. All personnel involved in this animal experiments are holders of the Animal Licence issued by the Department of Health, Hong Kong Special Administrative Region. Animal experiments in this study were performed in the Lee Hysan Clinical Research Laboratory of the Prince of Wales Hospital, Shatin, Hong Kong.

White New Zealand rabbits were divided into three groups at random: healthy control (N), acute lung damage (AD), and chronic lung damage (CD) groups. The weight and age of each animal were recorded. Lung injuries in AD group were induced with a single subcutaneous dose of N-nitroso-N-methylurethane (NNNMU) (12mg/kg) 2-3 days before experiment. In the CD group, bleomycin was given intratracheally through an endotracheal tube 8 weeks before experiment. In each rabbit, oxygen consumption was measured simultaneously by indirect calorimetry using a metabolic analyzer (mVO_2) and by the thermodilution technique applying Fick principle (cVO_2). Since the thermodilution method samples the oxygen concentration of the blood from the right atrium before entering the lung, the difference of mVO_2 and cVO_2 gives the local tissue oxygen consumption of the lung (VO_{2pul}).

Chapter 2. Materials

1.   *Animals*

44 White New Zealand rabbits were bred and raised in the Animal House of the Chinese University of Hong Kong. At 9-32 weeks when they were weighing 1.7-4.1 kg, the rabbits were randomized into 3 groups (1) healthy control group (N), (2) acute lung damage (AD), and (3) chronic lung damage group (CD).

2.   *Chemicals used for inducing lung damage*

2.1   *Acute damage group*

2.1-1   *N-nitroso-N-methylurethane (NNNMU)*

N-nitroso-N-methylurethane (NNNMU)   (Pfaltz & Bauer Inc., Waterbury) is a potent alkylating agent with marked carcinogenic effect on animal tissues. Tumors of the stomach and esophagus have been induced by a single oral administration of the compound in varies species of animals (Schoental *et al*, 1960). A single subcutaneous injection consistently results in acute lung injury in various species of animals including rabbit. The damaged lung shows alterations of the endogenous surfactant system resembling those in patients with ARDS (Ryan *et al*, 1981; Lewis *et al*, 1990).

2.1-2   *Administration to rabbits*

In adult rabbits, progressive lung injury over time was noticed after a single subcutaneous injection of NNNMU at a dose of 12mg/kg. Severe respiratory distress and cyanosis was obvious by 3 to 4 days, and a 90% mortality by 4 days was observed (Godwin *et al*, 1991). Changes of ARDS symptoms were evident as early as 1 to 2 days after NNNMU injection (Ito *et al*, 1997).

On histological examination, the lungs of these animals showed microatelectasis, interstitial and alveolar edema, together with hyaline membrane formation. There was necrosis of alveolar epithelial cells during the acute phase and evidence of regeneration during recovery (Ryan *et al*, 1976, 1983). Disaturated phosplatidylcholine (DSPC) and phosphatidylglyceral (PG) in alveolar lavage were observed during the early phase of injury (Ryan *et al*, 1981; Liau *et al*, 1984). In electron microscopic studies, there was necrosis of both types of alveolar epithelial cells without significant injury to the capillary endothelium (Ryan *et al*, 1981).

A major physiological consequence of this injury was a marked lessening in lung compliance together with a reduction in lung volume (Barrett *et al*, 1979). This decrease in CL and increase in surface tension may be due to a quantitative reduction or qualitative change in lung in surfactant, or to a combination of both. Qualitative changes in surfactant may be directly caused by the NNNMU, or may be the result of denaturation by edema fluid protein (Ryan *et al*, 1978).

The lung injury induced by NNNMU has been used as a model for ARDS (Ryan *et al*, 1976; Richardson *et al*, 1986). In comparing to more acute injury models, the NNNMU lung injury model may provide information regarding ARDS with a slow evolution of the clinical syndrome in contrast to a direct chemical or physical type of injury, such as blunt chest trauma or aspiration (Pappert *et al*, 1996).

2.2   *Chronic damage group*

2.2-1   *Bleomycin (BLM)*

BLM consists of a group of antibiotics isolated from *Streptomyces verticillus*. It is used primarily in the treatment of lymphomas, squamous cell carcinomas, and testicular tumors (Yagoda *et al*, 1972). Although the

agent is highly effective in the treatment of certain malignancies, skin and pulmonary toxicity limits its tolerance by many patients. These adverse effects have been noted in both clinical trials (Ichikawa, Nakano & Hirokawa, 1969) and animal studies (Thompson *et al*, 1972; Adamson & Bowden, 1974).

2.2-2   *Pulmonary toxicity of Bleomycin*

BLM-induced pulmonary damage is an established model for the study of interstitial pneumonitis and pulmonary fibrosis. Whether administered subcutaneously (Lazo, Catravas & Gillis, 1981), intraperitoneally (Adamson & Bowden, 1974), or intravenously (Thompson *et al*, 1972), bleomycin induces pulmonary fibrosis in many animal species, including humans (Adamson & Bowden, 1974; Comis, 1978). Intratracheally administered BLM has been shown to induce a rapid and predictable pneumonitis, progressing to fibrosis in many species of animals (Snider *et al*, 1978).

2.2-3   *Administration to animals*

In mice, hamsters and rabbits, administration of bleomycin was followed by initial phase of alveolitis eventually progressing to a chronic

inflammatory process charactered by fibrosis (Adamson & Bowden, 1974; Aso, Yoneda & Kiklawa, 1976; Comis, 1978). There was an early phase of exsudative injury with hyaline membranes formation, interstitial and alveolar edema, followed by a chronic phase of diffuse interstitial fibrosis (Bellet-Barthas *et al*, 1983).

Intratracheal bleomycin (BLM, 10 mg/kg) causes chronic lung fibrosis in rabbits. The evolution of fibrosis in the rabbit is slower than in other experimental animals. There is cellular and fibrocellular lesions 4 weeks after BLM administration. Changes in lung content of collagen, protein and DNA are evident at 8 weeks after BLM. O'Connor (O'Connor *et al*, 1986) pointed out that the rabbit model has advantages over others for the serial study of lung fibrosis, because of the continuing active inflammation and slow progress of fibrosis induced by intratracheal BLM.

In 1988, Shen and coworkers demonstrated a wide spectrum of lung injuries in New Zealand white rabbits by using 5U/kg or 10U/kg of intratracheal bleomycin with and without oxygen supplementation (Shen *et al*, 1988). The bleomycin-induced interstitial lung disease in the rabbit

causing acute lung injury as well as chronic inflammation and fibrosis was found on Day 12 and Day 56 respectively (Jellinek *et al*, 1997).

Chapter 3. Instruments

1. *Measurement of VO_2 & VCO_2 -- DeltatracII^{TM} Metabolic analzyer*

The DeltatracII^{TM} metabolic monitor (Datex Instrumentarium Corp., Helsinki, Finland) (*fig 2.3-1*) is an open system indirect calorimetry device designed for the measurement of VO_2 and VCO_2 in both spontaneously breathing and mechanically ventilated patients (Stothers *et al*, 1979). The paramagnetic analyzer of the equipment (OM-101, Datex) (*fig 2.3-2*) detects any difference in inspired and mixed expired oxygen partial pressures, from which VO_2 is calculated. The expired CO_2 fraction (F_{ECO2}) is measured with an infrared CO_2 sensor. Energy expenditure is calculated from the measured VO_2 and VCO_2. A microcomputer controls a set of magnetic valves for the automatic control of the absolute F_{IO2}, F_{ICO2}, and gas analyzer baselines. During mechanical ventilation the device is connected to the outlet port of the ventilator and expired air is collected through a built-in mixing chamber, The mixed expiratory gases are then diluted in the mainstream of the constant flow generator (*fig 2.3-3*). F_{IO2} and F_{ICO2} are measured from the ventilator's inspiratory limb and F_{ECO2} and F_{ECO2} from the mixing chamber. The fraction of CO_2 in the diluted expiratory gases, downstream from the flow generator is also measured. VCO_2 is calculated in both canopy and

respirator measurements as the product of the constant flow (Q) and fraction of CO_2 in the diluted expiratory flow (*fig 2.3-3 & 2.3-4*):

$$VCO_2 = Q \times F_{ECO2}$$

With the canopy, the VO_2 is calculated using the Haldane transformation (Meriläinen, 1987):

$$VO_2 = (Q / [1 - F_{IO2}]) \times (F_{IO2} - F_{EO2} - F_{IO2} \times [F_{ECO2} - F_{ICO2}])$$

where Q is the constant gas flow, and F_{EO2} and F_{ECO2} the fractions of oxygen and CO_2 in the mixed expiratory gas flowing through the monitor (*fig 2.3-3*). During respirator measurement, the RQ is calculated with the Haldane transformation from the gas fractions:

$$RQ = (1 - F_{IO2}) / \{[(F_{IO2} - F_{EO2}) / F_{EO2}] - F_{IO2}$$

where the F_{EO2} and F_{ECO2} are measured from the mixing chamber and then form a constant flow and the concentration of CO_2 down stream from the mixing chamber in a manner identical to the canopy measurement. The VO_2 is subsequently calculated from the RQ as:

$$V\ O_2 = VCO_2/\ RQ$$

The effect of humidity is excluded by balancing all measured gases, including the calibration gas, with Nafion tubing (Perma Pure Products, Tom's River, NJ). All gas exchange results are expressed in STPD.

2.   *Measurement of cardiac output -- Cardiomax II model 85*

The Cardiomax II model 85 (Columbus Instruments, Ohio) is a thermodilution cardiac output computer (*fig 2.3-5*). During its operation, a known volume of ice-cold Normal Saline is introduced into the right atrium through a central venous catheter. A microprobe (temperature sensor) is placed in the subject's aorta to monitor the changes in

temperature brought about by the dilution of the injectate in the blood. The computer records the temperature changes over time and integrates the curve bounded by the dilution curve and the baseline. Cardiac output is computed using the following equation:

$$\text{Cardiac output} = \frac{(T_{blood} - T_{inj}) \times V_{inj}}{\text{Integral of curve}}$$

where: T_{blood} is the blood temperature prior to injection (degrees °C); and T_{inj} the injectate temperature (degrees °C) at the time of injection; V_{inj} is the volume of injectate (milliliters). Integral of curve is the area bound by T_{blood} and the recorded change in temperature over the time required for the blood to re-establish T_{blood}.

Injectate temperature is measured with a platinum probe. It is the method of temperature measurement used by the National Bureau of Standards.
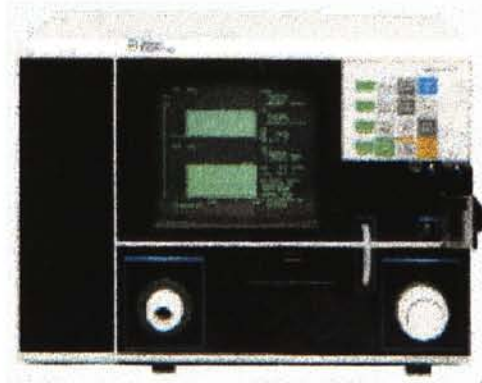
Figure 2.3-1. DeltatracII™ metabolic monitor
(Datex Inst. Corp., Helsinki, Finland)
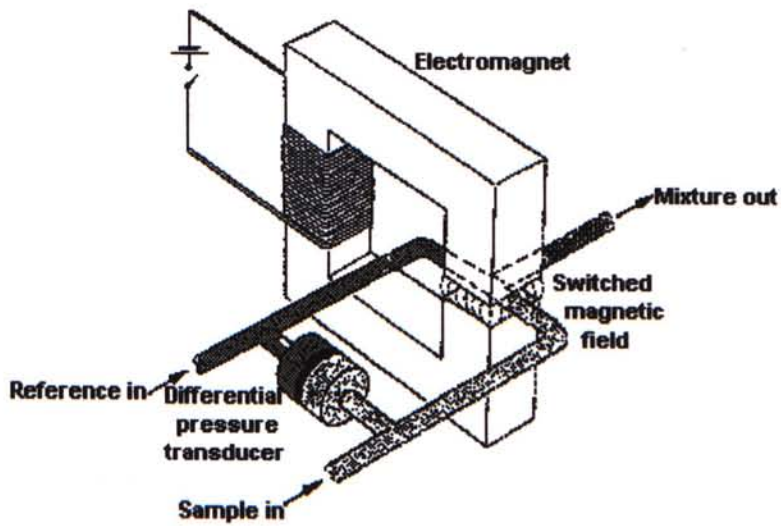
Figure 2.3-2. Paramagnetic analyzer (OM-101, Datex)
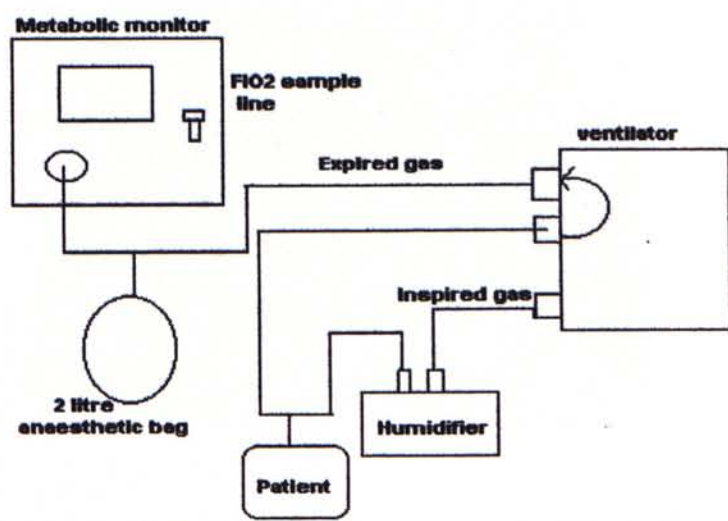
Figure 2.3-3. Operating conditions for the respiratory mode
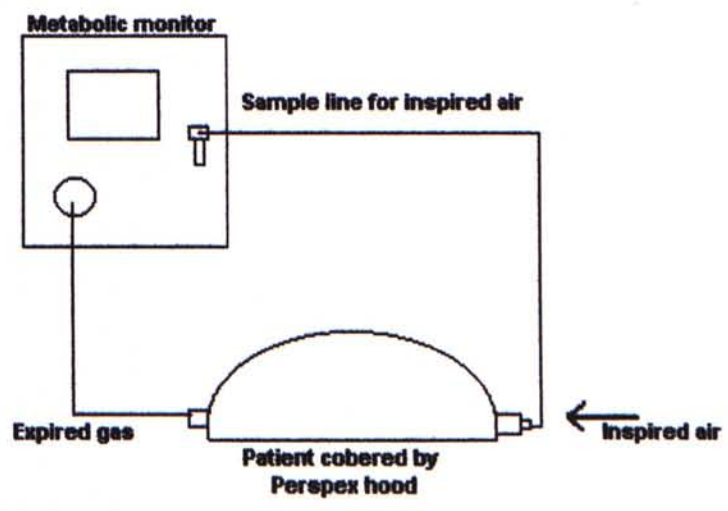
Figure 2.3-4 Operating conditions for the canopy mode
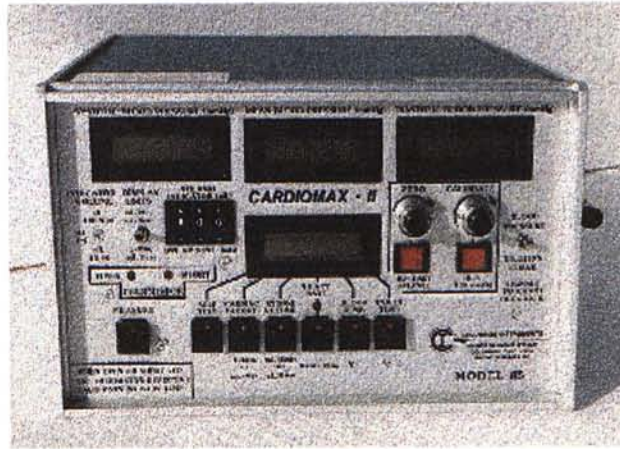
Figure 2.3-5   Cardiomax-II Model 85 (Columbus Instruments, Ohio, USA)

Chapter 4. *Methods*

1.   *N-nitroso-N-methylurethane (NNNMU) Preparation*

0.1 ml of 97% NNNMU solution (Pfaltz & Bauer Inc., Waterbury) was first diluted in 0.9ml of 0.15M sodium chloride (NaCl) and then further diluted in 6ml of 0.15M sodium chloride to a concentration of 138.57mg/ml in 7 ml NNNMU solution.

2.   *Bleomycin Preparation*

5ml of 0.15M sodium chloride solution was drawn into a syringe, and injected into an ampoule of bleomycin to dissolve its content. The resultant solution was drawn into the syringe before use.

3.   *2.5% pentobarbitone Preparation*

10gm of 25M pentobarbitone powder was weighed and dissolved in 100ml of double deionized water.

4.   *Animal Preparation*

Adult White New Zealand rabbits were bred and raised in the Animal House of the Chinese University of Hong Kong. Thirty adult rabbits (19.33 ± 6.34 weeks of age) rabbits of either sex weighing 3.28 ±

0.92 kg were randomized into 3 groups: (1) healthy control group (C), (2) acute lung damage group (AD) and chronic damage (CD) group. Their weight and age were recorded.

4.1   Control (Normal) group

Rabbits in this group were obtained directly from the Animal House of the Chinese University of Hong Kong shortly before experiment without any prior treatment.

4.2   Acute lung damage group

Two to three days before experiment, lung injury in this group was induced by a single subcutaneous injection (15mg/kg) of N-nitroso-N-methylurethane (NNNMU) (Pfaltz & Bauer Inc., Waterbury) (Godwin *et al*, 1997; Ito *et al*, 1997) at a shaved area of the back. The rabbits were kept at the Animal House until the day of experiment.

4.3   Chronic lung damage group

In the CD group, intratracheal instillation of Bleomycin (Bleocin™, Nippon Kayaku, Japan) was given 56 days before experiment,

at a dose of 10U/kg (Berend *et al*, 1985; Bigby *et al*, 1985; O'Connor *et al*, 1986).

Animals were anesthetized with xylazine and ketamine prior to intubation and instillation of bleomycin into the lungs via a cuffed endotracheal tube (Mallinckrodt medical, Athlone). During instillation, the rabbit was rolled from side to side to ensure even distribution of the drug in the lungs, and the cuff of the endotracheal tube was left inflated for at least 30 min to prevent tracheal clearance of the bleomycin. The rabbit was allowed to breathe humidified 100% oxygen for 5 min after the intratracheal instillation (Shen *et al*, 1988). The rabbit was returned to the Animal House after it had awakened from anesthesia. They were kept in the Animal House until the experiment.

5. *Preparation of the animals for VO$_2$ measurement*

Each animal was anaesthetized with 1-1.5ml of ketamine and xylazine (7:1) given intramuscularly. A 24-gauge angio-catheter was then inserted into the artery of one of the external ears. Anesthesia was subsequently maintained by injections of ketamine and xylazine mixture at a dose of 0.10-0.15 ml/kg through the ear artery as required. After

shaving of the skin, a longitudinal mid-line incision was made along the neck to expose the right jugular vein. A 3.5 French catheter (UNO Metric, Demark) flushed with heparin (CP pharmaceuticals, Wrexham UK) was introduced into the vein for about 6-8 cm. By repeated trials and errors in animal not used for this study, I have confirmed that this maneuver would place the catheter tip in the right atrium. The left carotid artery was then exposed and the microprobe containing a temperature sensor (Columbus instruments, Ohio, USA) was inserted through the carotid artery into the aorta. Again, by repeated trials and errors in practicing sessions, insertion of the catheter through a standard point at the carotid artery will place the tip of the microprobe at the proximal part of the descending aorta. The catheter in the right atrium was used for injection of cold saline and blood sampling for determination of mixed venous Hb and oxygen saturation. The arterial catheter of the external ear was used for blood sampling for estimation of arterial Hb and SaO_2, and also for continue infusion of 10% dextrose at a rate of 1ml per hour. 5-10 injections were performed for each rabbit. Less than 10% of thermodilution was excluded before calculating the mean cVO_2. Single arterial and venous blood samples were withdrawn for blood gases on each rabbit. Blood losses due to operation and sampling were replaced with the same volume of Normal Saline (*fig 2.4-1*).

6. *Measurement of oxygen consumption*

Oxygen consumption was measured twice, firstly by indirect calorimetry using the DeltatracII^{TM} metabolic monitor (the results represented total body oxygen consumpiton including that by the lung [VO_{2wb}]). And secondly by the thermodilution method and Fick principle using the Cardiomax II cardiac output computer (the results should represent the oxygen consumption of the body excluding that of the lungs [VO_{2b}]).

6.1 *VO_{2wb} measurement*

All the measurements were obtained with the rabbit breathing spontaneously, the rabbit was placed in a specially designed air-tight plastic box measuring 55cm x 17.5cm x 15cm (*fig 2.4-2*). Inspiratory gas from the metabolic analyser entered the box through an entry port at the head panel and returned to the analyser through an exit port at the end panel. Two side-ports near the head end allowed passage of the peripheral arterial catheter, the central venous catheter, and the microprobe wire so that measurement of cardiac output and blood sampling could be performed without opening the box. The side-ports were sealed by several

layers of cling film wrapped tightly around the catheters and wire so as to prevent any gas leakage.

Whole body oxygen uptake, CO_2 excretion, and the respiratory quotient were determined in 1-min intervals with the DeltatracII™ metabolic monitor for 15 minutes and the mean value obtained. The accuracy of the metabolic monitor in measuring VO_2 had been validated before and the relative error was only $\pm$ 1.9-4% (Phang *et al*, 1990; Takala *et al*, 1989).

The calorimeter was calibrated before each study with a high-accuracy calibration gas (mixture of 95% oxygen and 5% CO_2) (Datex Inst. Corp., Helsinki, Finland).

6.2   *VO_{2b} measurement*

During the measurement of VO_{2wb}, cardiac output was determined simultaneously by the thermodilution method using the Cardiomax Cardiac Output Monitor (Cardiomax II model 85, Columbus instruments, Ohio, USA). The temperature of aortic blood measured by the microprobe

was recorded in degree $^{\circ}$C as a baseline temperature. Subsequently, 0.5-0.8 ml of ice-cold isotonic saline was introduced into the right atrium using an automatic injector (Injector 400, Columbus instruments, Ohio, USA) immediately before it was drawn into the syringe of the injector. The temperature of the saline was monitored with a thermosensor connected to the monitor. To ensure that there was no change in temperature of the saline during its passage through the catheter, the entire length of the catheter up to its entry into the body box was immersed in ice water. The length of the catheter inside the box was wrapped in ice by an inner layer of gauze and an outer layer of cling film up to its entry into the internal jugular vein. The automatic injector was interfaced with the cardiac output monitor to signal the monitor of the onset of injection. Immediately after injection of the saline, the change in temperature in the aortic blood was continuously monitored and recorded by the cardiac output monitor. A temperature dilution curve integrating the temperature changes over time and the calculated cardiac output were displayed on screen. Only curves that showed an initial fast attack and then smoothly roll into an exponential decay were accepted (*fig 1.2-2*). At the same time, arterial and mixed venous blood samples were withdrawn from the ear artery and the right atrium respectively. The oxygen saturation of the sample was immediately estimated with a blood gas

analyser (Chiron Diagnostics, USA). An EDTA sample of each of the arterial and venous blood was sent immediately to the Haematology Laboratory for measurement of Hb level. Oxygen consumption (VO_{2b}) was calculated from the measured values as previously described (see: *Ch.2, 1.2 Fick method p22.*).

7. *Histopathology*

At the end of the experiment, the rabbit was sacrificed with an overdose of intravenous pentobarbital sodium. Postmortem chest X-ray was performed to confirm that the arterial catheter and the microprobe were in good position. The lung was dissected and persevered in 10% formalin.

Lung tissue was embedded in paraffin, and multiple section of 5 µm thickness was prepared. The sections were stained with Hematoxylin-eosin and Masson trichrome, and examined under the light microscope. Histologic lung injuries were scored using a semiquantitative scoring system (King *et al*, 1995) by a pathologist blinded to the grouping of the animals. At magnification x 63 and 250, the degrees of atelectasis, congestion, edema, number of neutrophils, hemorrhage hyaline

membrane formation and fibrosis formation were separately estimated from sections of both interstitium and in the air space of alveolar using the following scoring system: none injury = score of 0; mild injury = score of 1; moderate injury = score of 2; severe injury = score of 3. These scores were averaged as the histologic lung injuries score for that animal.

8.   *Statistics*

All continuous variables are presented as means ± SD and compared among the groups using analysis of variance (ANOVA). When a difference is shown, the Bonferroni's test is used for Pairwise Comparison. Discrete variables were compared by X^2 or Fisher exact test. Differences among the groups were considered significant when the α error was 5% or less ($p \leq 0.05$).

Microprobe, placed in aortic arch

Carotid artery

Jugular Vein

NS injection

Catheter to
Right atrium
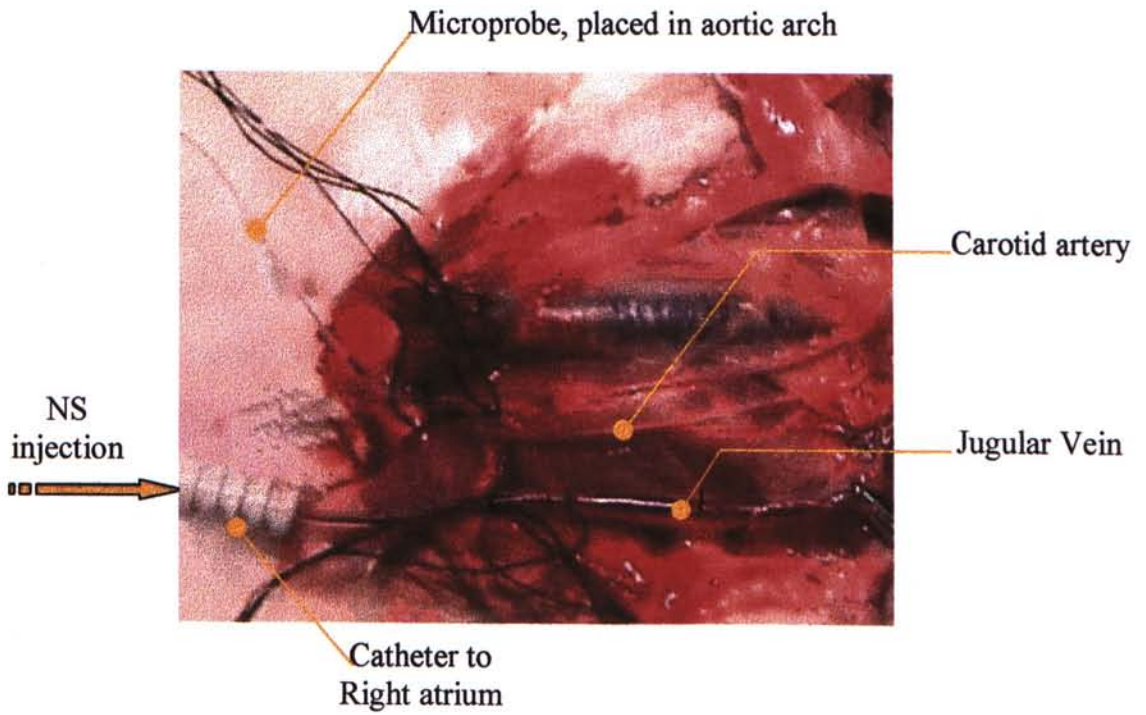
Figure 2.4-1. Vessels cannula on rabbit model
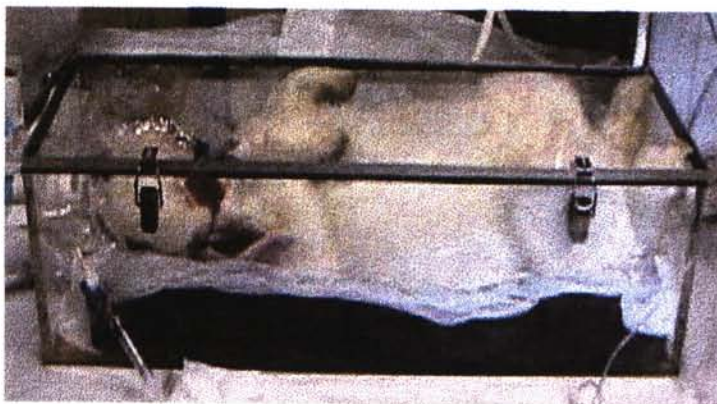
Figure 2.4-2. Air-tight plastic box for measuring mVO_2

Results

A total of forty-four rabbits were entered in this study. There were

34 male and 10 female rabbits, with Mean (SD) body weight 3.3 (0.5) kg,

body length 40.3 (2.4) cm, age 19.3 (3.2) weeks. (*Table3.1-1*)

1.   *Healthy (Control) group*

A total of twenty-one rabbits were entered in this group. There

were twenty male and one female rabbits, with body weight 3.2(0.3) kg,

body length 39.7(1.8) cm, age 18.3(1.7) weeks. The whole body oxygen

consumption measured by the Deltatrac (i.e. mVO_2) was 26.7(5.6)

ml/min. During the estimation of VO_{2wb}, their cardiac output measured by

thermodilution was 664.1(111.1) ml/min. Arterial oxygen saturation and

haemoglobin concentration were 93.6(7.6)% and 11.2(1.2) g/dl

respectively. Mixed venous oxygen saturation and haemoglobin

concentration of the right atrial sample were 67.5(7.7)% and 11.2(1.2)

g/dl respectively. The calculated oxygen content was 145.3(21.0) ml/L in

the artery and 105.5(17.5) ml/L in the right atrium. Oxygen consumption

of the body (VO_{2b}) estimated by the Fick method was 25.8(5.5) ml/L; the

ratio of VO_{2pulm} to the whole body VO_2 was 3.3(2.2)%.

1.1 Pulmonary histology

No pathological changes were noticed in the sections of the lung tissues. Normal alveolar structure was seen under light microscope. (*Table 3.3-1*) (*Fig 3.1-1-3*)

2. Acute lung damage group

A total of fourteen rabbits were entered in this group. There were eight male and six female rabbits, with body weight 3.3 (0.6)kg, body length 40.9(2.0) cm, age 19.0(3.4) weeks. The whole body oxygen consumption measured by the Deltatrac (i.e. mVO_2) was 27.3(6.1) ml/min. During the measurement of VO_{2wb}, their cardiac output measured by the thermodilution method was 674.6(135.6) ml/min. Oxygen saturation and haemoglobin concentration were 95.8(3.1)% and 11.2(1.6) g/dl respectively in the artery, and 71.4(5.8)% and 11.5(1.9) g/dl respectively in the right atrial sample. The oxygen content was 149.2 (21.6) ml/min and 111.5 (21.8) ml/min in arterial and right atrial sample, respectively. Oxygen consumption of the body (VO_{2b}) estimated by the Fick method was 24.8(6.1) ml/L. The ratio of VO_{2pulm} to the whole bodyVO_2 was 9.7(5.2) %.

2.1   *Pulmonary histology*

The lung histology of the animals in this group showed the development of acute interstitial edema with infiltration of neutrophils. Examination of representative section shows diffused congestion, edema and the formation of hyaline membranes in the alveolar space. (*Table 3.3-2*) (*Fig 3.2-1~4*)

3.   *Chronic lung damage group*

A total of nine rabbits were entered in this group. There were six male and three female rabbits, with body weight 3.6(0.5) kg, body length 40.7(3.6) cm, age 22.1(4.1) weeks. The whole body oxygen consumption measured by the Deltatrac (i.e. mVO_2) was 30.2(8.0) ml/min. During the measurement of VO_{2wb}, their cardiac output was estimated to be 639.6(118.3) ml/min by thermodilution. Oxygen saturation and haemoglobin concentration were 85.5(12.1)% and 13.3(1.2)g/dl respectively in the arterial sample, and 64.8(14.1)% and 13.1(1.4) g/dl respectively in the right atrial sample. The oxygen content calculated was 158.1(31.06) and 119.1(30.9) ml/L in the arterial and the right atrial samples, respectively.   Oxygen consumption of the body (VO_{2b}) as

estimated using the Fick principle was 24.2(7.4) ml/L. The ratio of VO_{2pulm} to the whole bodyVO_2 was 20.4(6.3)%.

3.1   *Pulmonary histology*

Marked pathological changes were seen in the lungs of this group of animals. There were patchy atelectasis with areas of normal tissue alternating with areas of very active inflammation, congestion and fibrosis. Other abnormalities included abnormal alveoli with intra-alveolar accumulation of inflammatory cells. Fibrosis of the parenchymal matrix was recognized when the lung sections were stained with Masson trichrome (*Table 3.3-3*) (*Fig 3.3-1~3*).

4.   *Comparison of the pulmonary oxygen consumption among the three groups*

The pulmonary oxygen consumption (VO_{2pulm}), as calculated from the difference between VO_{2b} measured by the Fick method and VO_2 measured by the Deltatrac metabolic Monitor was 0.9(0.6) ml/min in the healthy controls, 2.5(1.2) ml/min in the acute lung damage group, and 6.0(2.0) ml/min in the chronic lung damage group. The differences among the three groups were statistically significant, as compared by

ANOVA ($P<0.0001$). Multiple Pairwise comparison using the Bonferroni's method showed the VO_{2pul} of both the AD and CD group was significantly greater than that in the healthy control group.

When expressed as a proportion of the total body oxygen consumption, the VO_{2pulm} was 3.3(2.2) % in the healthy controls, 9.7(5.2)% in the acute lung damage group, and 20.4(6.3) % in the chronic lung damage group. The differences among the three groups were again statistically significant ($p<0.0001$, ANOVA). Multiple Pariwise comparison using the Bonferroni's method again showed that VO_{2pul} constituted a significantly larger proportion of total body VO_2 in the AD and CD group when compared to the healthy controls.

Unit VO_2 expressed as pulmonary oxygen consumption per kilogram (VO_{2pulm}, ml/kg/min), was calculated from the difference between VO_{2wb} and VO_{2b} divided by the body weight of rabbits. This was 0.3(0.2) ml/kg/min in the healthy controls. 0.8(0.4) ml/kg/min in the acute lung damage group, and 1.7(0.6) ml/kg/min in the chronic lung damage group. Multiple Pairwise comparison using the Bonferroni's method showed the VO_2 per kilogram of both the AD and CD group was significantly greater than that in the healthy control group (*Table 3.1-1*).

Figure 3.1-   Rabbit lung in healthy control group:

Figure 3.1-1: (Hematoxylin-esoin, x 63)

Figure 3.1-2: (Masson Trichrome, x 63)

Figure 3.1-3: (Hematoxylin-esoin, x 400)

Sections show normal pulmonary parenchyma. The alveolar septa is thin without
significant inflammatory cell infiltrate. No increased in interstitial fibrosis is seen
in the Masson Trichrome stain (*figure3.1-2*).
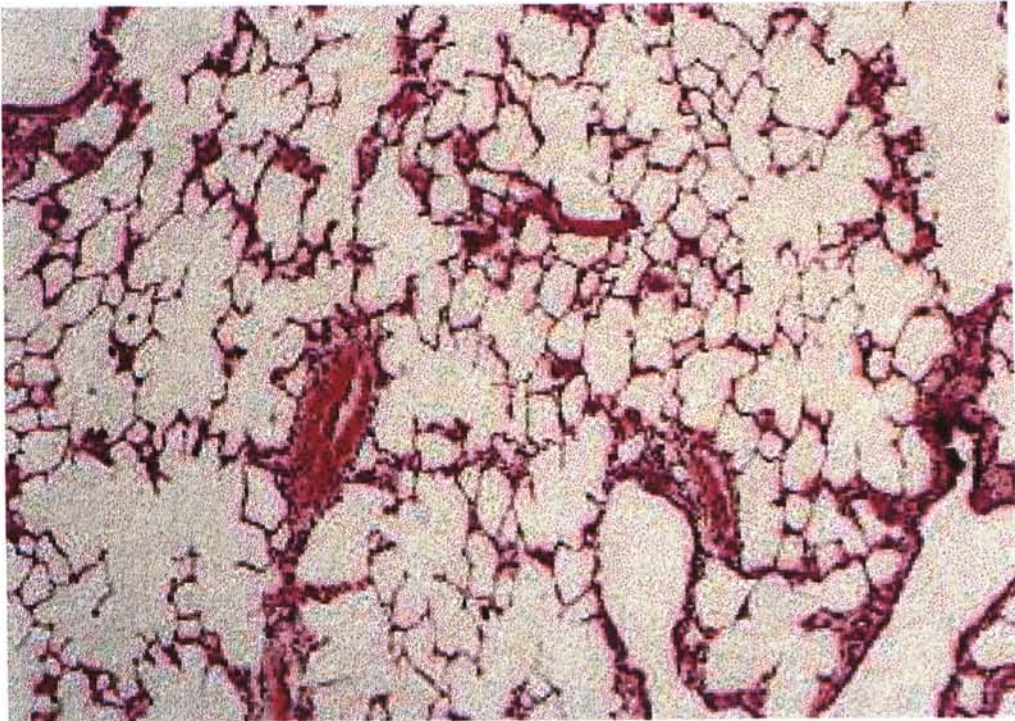
Figure 3.1-1   (Hematoxylin-esoin, x 63)
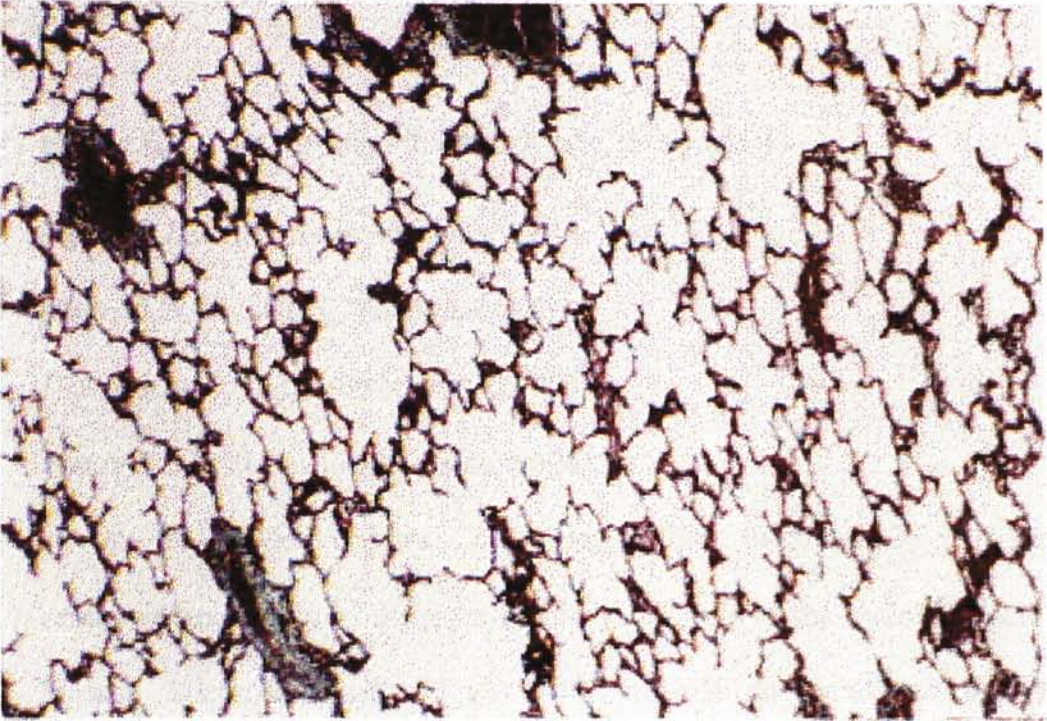
Figure 3.1-2 (Masson Trichrome, x 63)
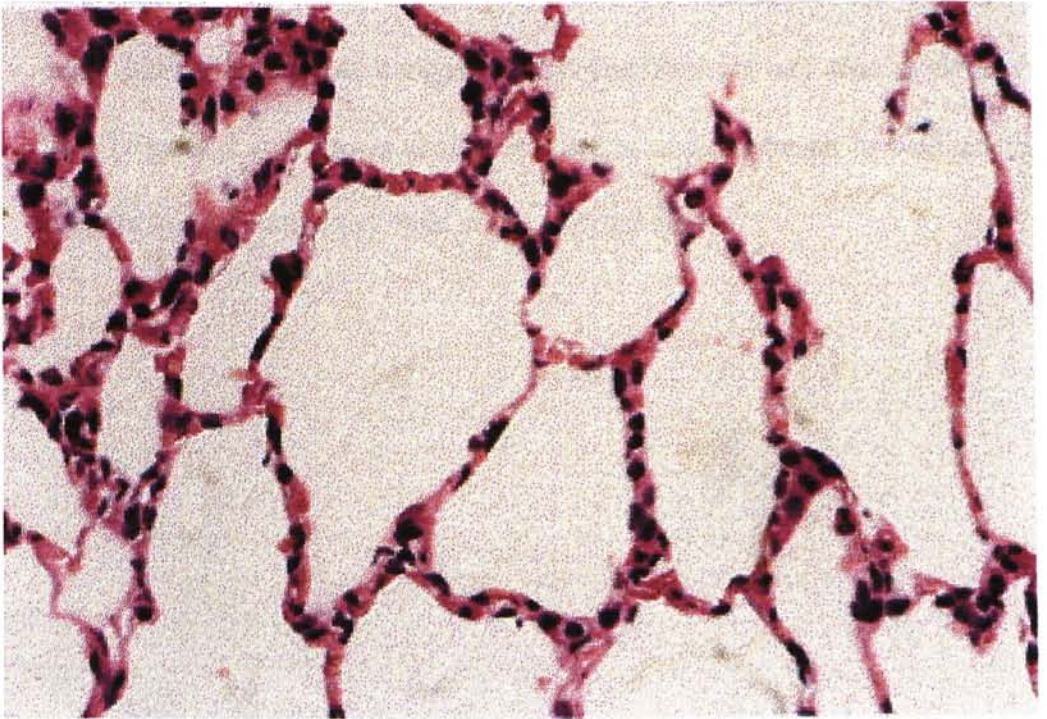
Figure 3.1-3. (Hematoxylin-esoin, x 400)

Figure 3.2- Rabbit lungs in acute damage group (AD).

Figure 3.2-1: (Hematoxylin-esoin, x 63)

Figure 3.2-2: (Masson Trichrome, x 63)

Figure 3.2-3: (Hematoxylin-esoin, x 400)

Figure 3.2-4: (Hematoxylin-esoin, x 400)

The low power view (*Figure 3.2-1*) reveals diffuse infiltration by inflammatory cells, with expansion of the septa and focal collapse of the alveolar spaces. The Masson Trichrome stain (*Figure 3.2-2*) reveals no significant increase in interstitial fibrosis. High power view demonstrates marked increased in inflammatory cells consisting of both polymorphs and mono-nuclear inflammatory cells. Interstitial edema, congestion and haemorrhage are also evident. Another field (*Figure 3.2-4*) shows similar features of acute damage, as well as fibrinious exudate and inflammatory cells in the alveolar spaces.
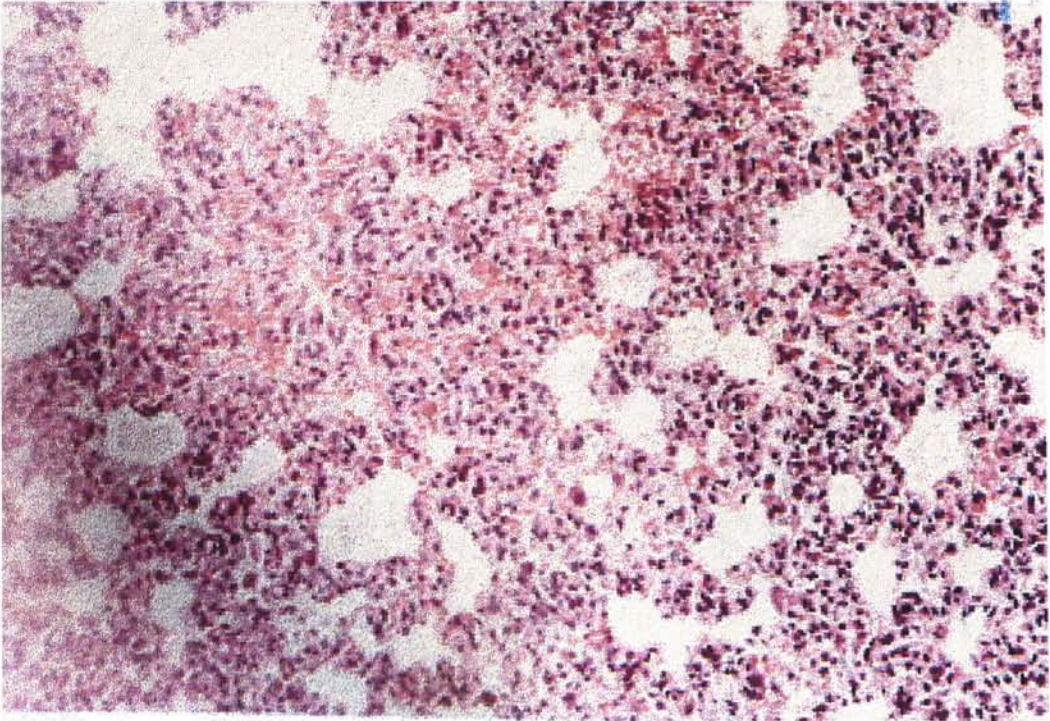
Figure 3.2-1 (Hematoxylin-esoin, x 63)
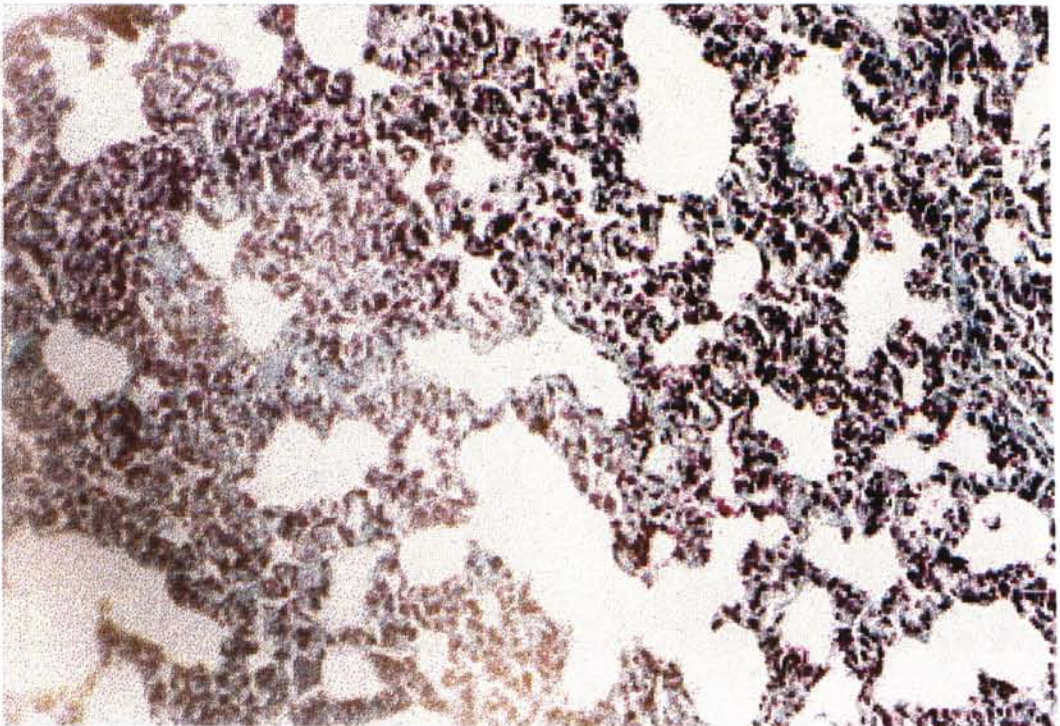
Figure 3.2-2 (Masson Trichrome x 63)
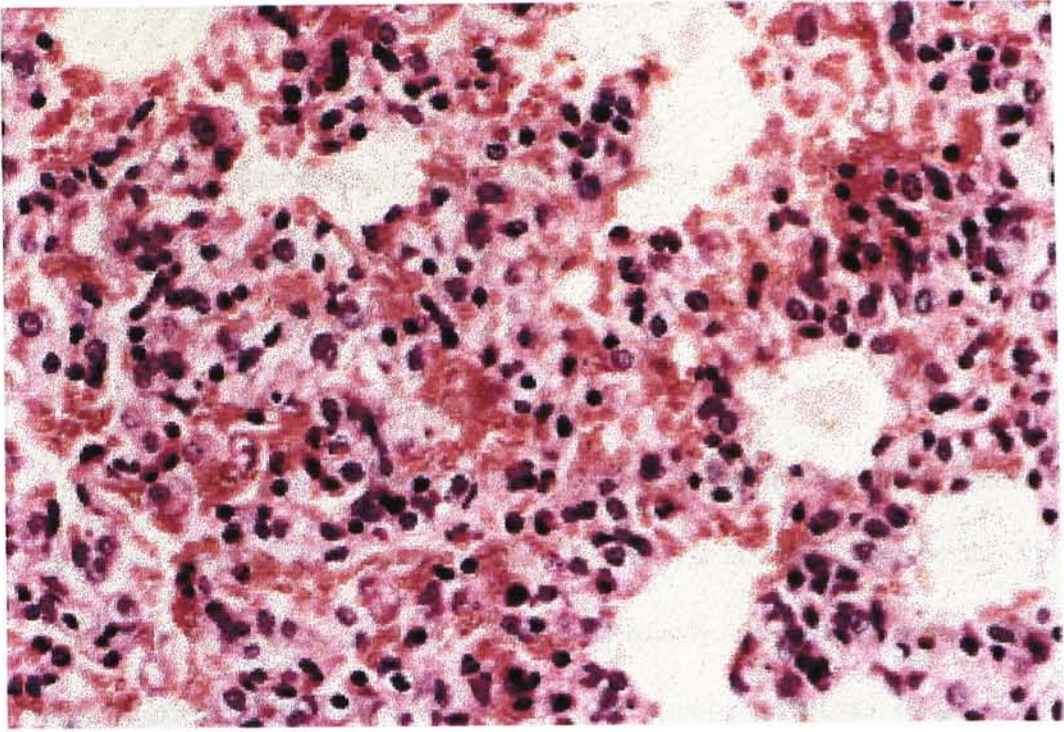
Figure 3.2-3 (Hematoxylin-esoin, x 400)
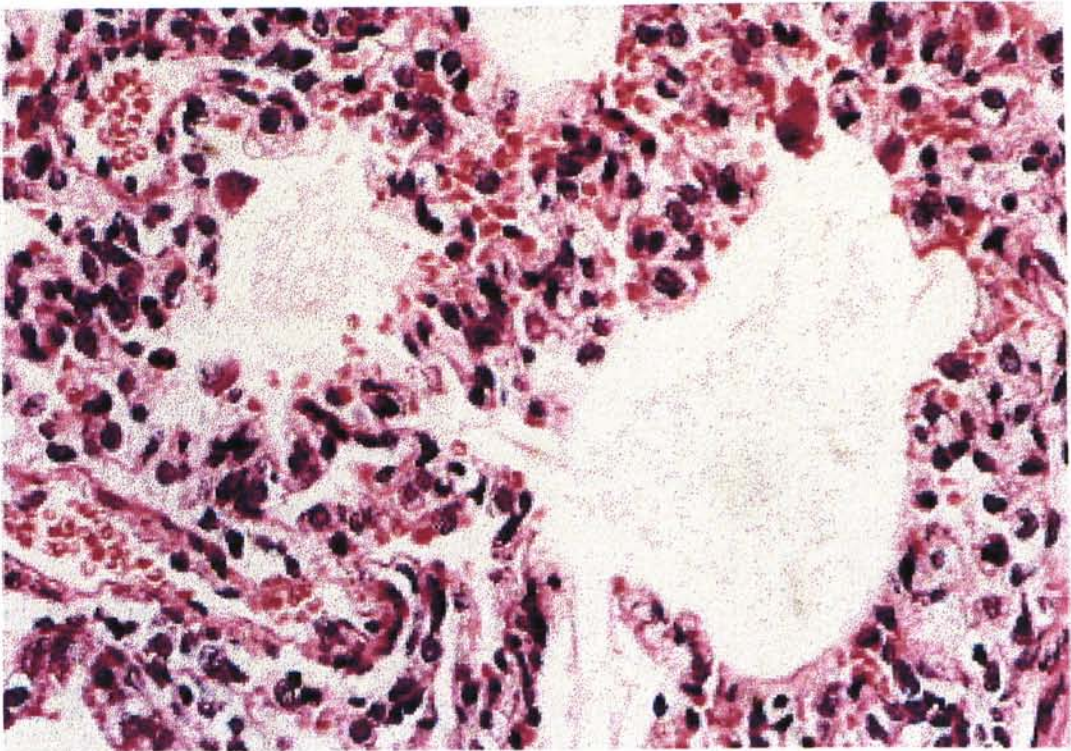
Figure 3.2-4 (Hematoxylin-esoin, x 400)

Figure 3.3-   Rabbit lung in chronic damage (CD) group.

Figure 3.3-1: (Hematoxylin-esoin, x 63)

Figure 3.3-2: (Masson Trichrome, x 63)

Figure 3.3-3: (Hematoxylin-esoin, x 400)

The low power view (*Figure 3.3-1*) demonstrates patchy interstitial fibrosis with focal increased in inflammatory cells. The fibrosis is highlighted by the Masson Trichrome stain (*Figure 3.3-2*). Medial hyperplasia is also noted in the pulmonary artery (arrow). The inflammatory cells are mainly composed of mono-nuclear inflammatory cells (*Figure 3.3-3*).
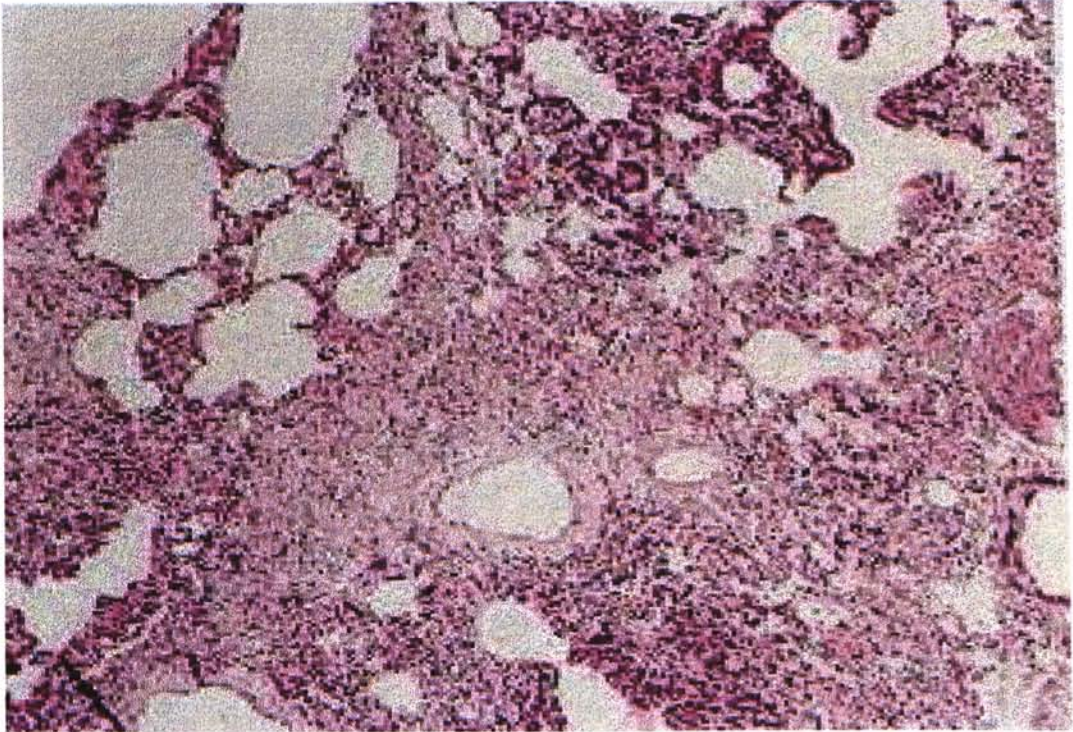
Figure 3.3-1   (Hematoxylin-esoin, x 63)
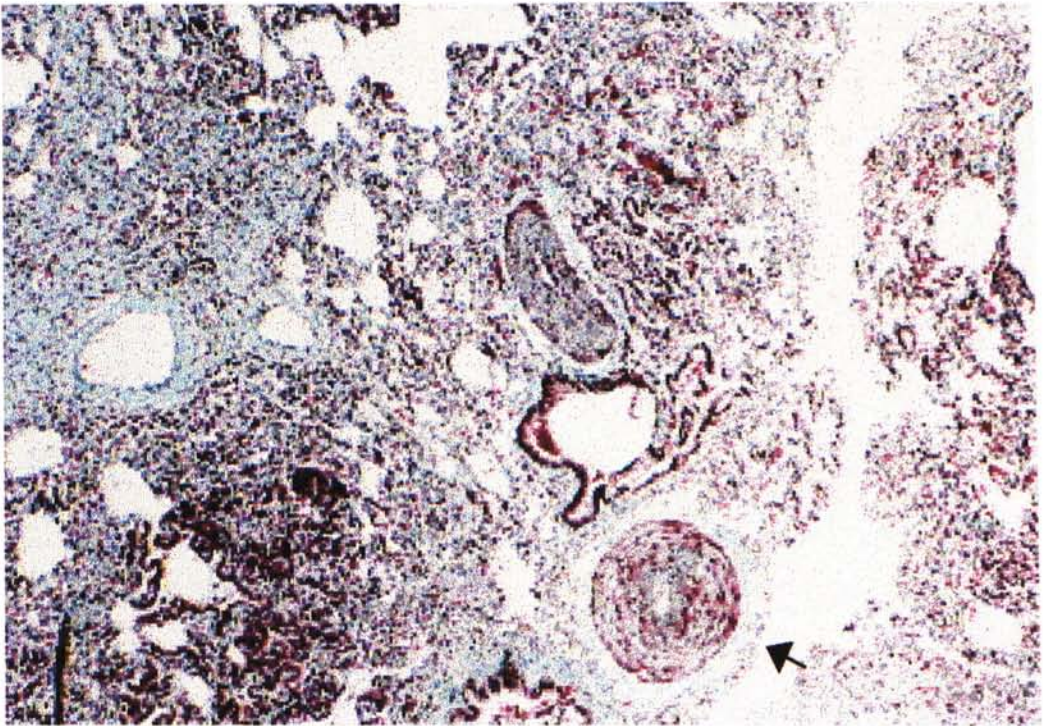
Figure 3.3-2   (Masson Trichrome, x 63)
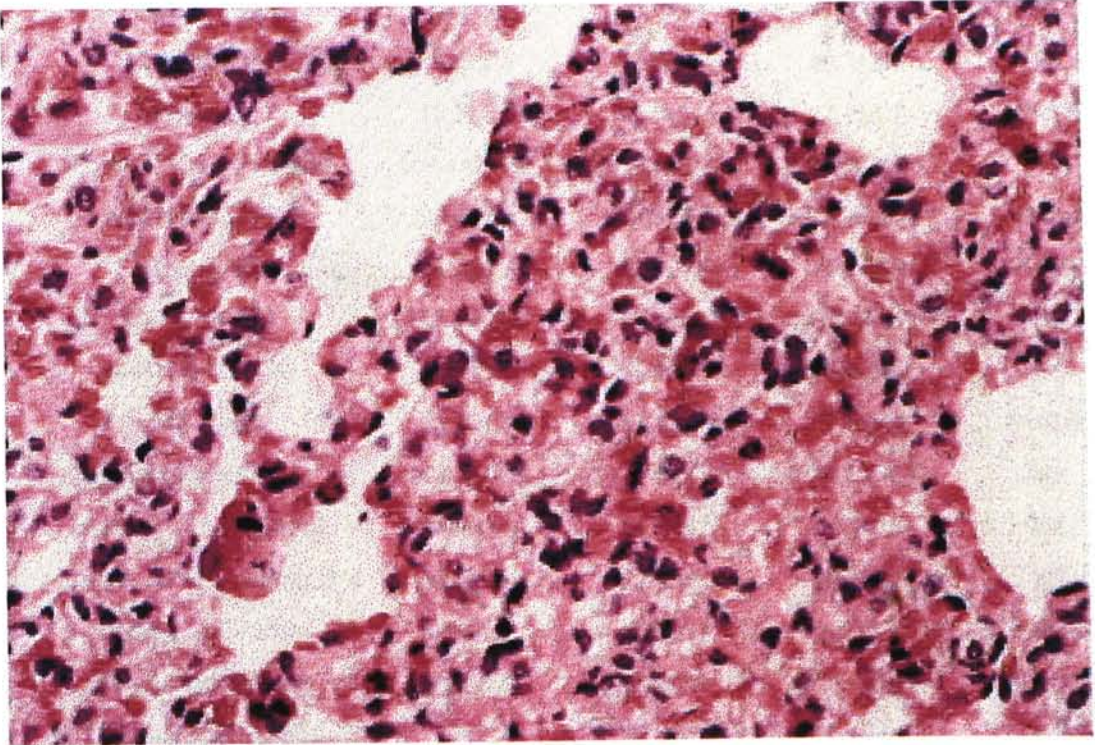
Figure 3.3-3   (Hematoxylin-esoin, x 400)

	Control group	AD group	CD group	P value
Weight *(kg)*	3.2±0.3	3.3±0.6	3.6±0.5	*$P<0.05$
Length *(cm)*	39.7±1.8	40.9±2.0	40.7±3.6	NS
Age *(wk)*	18.3±1.7	19.0±3.4	22.1±4.1	*$P<0.05$
Mean CO $_{TD}$ *(ml/min)*	664.1±111.1	674.6±135.6	639.6±118.3	NS
mean SaO$_2$ *(%)*	93.6±7.6	95.8±3.1	85.5±12.1	*$P<0.05$
aHb *(g/dl)*	11.2±1.2	11.2±1.6	13.3±1.2	‡$P<0.05$
CaO$_2$ *(ml/L)*	145.3±21.0	149.2±21.6	158.1±31.0	NS
mean SvO$_2$ *(%)*	67.5±7.7	71.4±5.8	64.8±14.1	NS
vHb *(g/dl)*	11.2±1.0	11.2±1.9	13.1±1.4	‡$P<0.05$
CvO$_2$ *(ml/L)*	105.5±17.5	111.5±21.8	119.1±30.9	NS
mean cVO$_2$ *(ml/min)*	25.8±5.5	24.8±6.1	24.2±7.4	NS
mean mVO$_2$ *(ml/min)*	26.7±5.6	27.3±6.1	30.2±8.0	NS
VO$_{2pul}$ *(ml/min)*	0.9±0.6	2.5±1.2	6.0±2.0	*$P<0.05$
VO$_{2pul}$ *(ml/kg/min)*	0.3± 0.2	0.8±0.4	1.7±0.6	†$P<0.001$
VO$_{2pul}$: VO$_{2wb}$ *(%)*	3.3±2.2	9.7±5.2	20.4±6.3	*$P<0.05$
Lung : Body weight *(%)*	0.4±0.1	0.4±0.1	0.4±0.0	*$P<0.05$

Values are Means ± SD; n=21 for control group, n=14 and n=9 for AD and CD groups respectively.
*$P< 0.05$, CD > Control group.
‡$P< 0.05$, CD group > AD and Control group.
†$P<0.001$, AD and CD groups > Control group.
*$P<0.05$, CD> AD> Control group.
NS: not significance.

Table 3.1-1   Comparison of measurements obtained by metabolic monitor and Fick method in the Control, acute lung damage and chronic lung damage groups.

Case	N01	N02	N03	N04	N05	N06	N07	N08	N09	N10
Sex	F	M	M	M	M	M	M	M	M	M
Weight (kg)	4	3.05	2.75	2.9	2.8	3.1	2.9	3.5	3.5	3
Length (cm)	45	39	39	38	38	40	39	40	40	38
meanCO$_{TD}$ (ml/min)	857.3	539	847.3	559.3	615	617.8	554	602	761.3	639.7
mean SaO$_2$ (%)	96.7	93.2	68.4	93.4	98.6	98.3	97.2	98.0	96.8	81.7
aHb (g/dl)	11	11.9	10.5	11.3	11.5	11.6	11.5	13.1	12.8	12.4
CaO$_2$ (ml/L)	147.9	154.2	99.8	146.7	157.6	158.5	155.4	178.5	172.2	140.8
mean SvO$_2$ (%)	73.9	68.8	54.1	61.0	71.5	74.4	75.6	79.6	74.2	59.4
vHb (g/dl)	11.2	11.1	10.9	11.6	11.3	11.6	11.5	13.1	12.7	12.7
CvO$_2$ (ml/L)	115.1	106.2	82.0	98.4	112.3	119.9	120.8	144.9	131.0	104.8
mean cVO$_2$ (ml/min)	28.1	25.9	15.1	27.0	27.9	23.9	19.2	20.2	31.0	23.1
mean mVO$_2$ (ml/min)	28.8	26.7	15.7	27.5	28.5	24.5	19.5	20.8	32.8	23.3
$mVO_2 > cVO_2$ (ml/min)	0.7	0.8	0.6	0.5	0.64	0.6	0.3	0.6	1.4	0.2
VO$_{2pul}$ (ml/kg/min)	0.2	0.3	0.2	0.2	0.2	0.2	0.1	0.2	0.4	0.1
VO$_{2pul}$: bodyVO$_2$ (%)	2.3	3.1	4.0	1.7	2.2	2.5	1.6	3.03	4.18	1.0
Lung : Body weight (%)	0.3	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

Table 3.2-1 Characteristics of rabbits included in the control group

(To be continued)

Case	N11	N12	N13	N14	N15	N16	N17	N18	N19	N20	N21
Sex	M	M	M	M	M	M	M	M	M	M	M
Weight (kg)	3.2	3	3	3	3.4	3.4	3.1	3.2	2.9	2.9	3.6
Length (cm)	39	39	39	36	40	42	40	41	40	40	42
meanCO$_{TD}$ (ml/min)	747	824	644	487.7	785	592.3	788.5	662	635.8	528	659.3
mean SaO$_2$ (%)	95.8	90.7	98.3	98.6	93.4	95.0	81.0	97.9	96.8	98.1	97.7
aHb (g/dl)	9.9	7.6	9.7	10.8	10.2	11.8	11.1	12	10.9	10.8	12.3
CaO$_2$ (ml/L)	131.8	95.8	132.5	148.0	132.4	155.8	125.0	163.3	146.7	147.3	167.0
mean SvO$_2$ (%)	70.2	58.7	74.3	53.7	61.8	67.5	59.4	73.6	72.0	61.8	72.6
vHb (g/dl)	10.6	9.2	9.7	10.6	10.1	11.4	11.2	11.3	10.5	10.8	12.1
CvO$_2$ (ml/L)	103.4	75.1	100.2	79.1	86.8	107.0	92.5	115.6	105.1	92.8	122.1
mean cVO$_2$ (ml/min)	21.2	17.1	20.8	33.6	35.8	28.9	25.6	31.6	26.4	28.8	29.6
mean mVO$_2$ (ml/min)	23.3	18.1	21.2	34.2	37.4	30.8	26.4	33.55	28	28.85	29.95
mVO$_2$ > cVO$_2$ (ml/min)	2.09	1.0	0.4	0.6	1.6	1.7	0.8	2.0	1.6	0.1	0.2
VO$_{2pul}$ (ml/kg/min)	0.7	0.3	0.1	0.2	0.5	0.5	0.3	0.6	0.5	0.0	0.1
VO$_{2pul}$: bodyVO$_2$ (%)	9.0	5.5	1.7	1.8	4.2	6.0	2.9	5.9	5.6	0.3	0.8
Lung : Body weight (%)	0.6	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4

Table 3.2-1   Characteristics of rabbits included in the control group (*con't*)

Case	AD01	AD02	AD03	AD04	AD05	AD06	AD07	AD08	AD09	AD10
Sex	M	F	M	M	M	F	M	M	M	M
Weight *(kg)*	1.7	3.2	3.55	3	2.6	3.7	3.4	3.4	3.3	3.3
Length *(cm)*	37	43	42	39	39	40	41	41	39	39
meanCO$_{TD}$ *(ml/min)*	831.8	746	843	742.5	707.7	644.3	574	423	654.5	502
mean SaO$_2$ *(%)*	99.0	98.2	96.0	91.8	94.4	98.8	90.5	92.6	92.2	98.7
aHb *(g/dl)*	7.8	11.2	13.6	10.1	10.4	13.3	13.3	12.5	10.3	10.2
CaO$_2$ *(ml/L)*	107.3	152.9	181.5	128.9	135.5	182.7	167.3	160.9	132.0	139.9
mean SvO$_2$ *(%)*	75.0	70.2	76.6	69.1	72.4	81.7	69.7	69.8	64.0	68.3
vHb *(g/dl)*	7.5	10.9	14.7	10.5	10.7	13.3	13.1	12.9	10.4	10
CvO$_2$ *(ml/L)*	78.2	106.4	156.5	100.9	107.7	151.0	126.9	125.2	92.5	94.9
mean cVO$_2$ *(ml/min)*	24.3	34.7	21.0	20.8	19.7	20.4	23.2	15.1	25.8	22.6
mean mVO$_2$ *(ml/min)*	26.5	36	23.1	22.3	22.6	25.2	25.4	18.8	26.3	25
mVO_2 > cVO_2 *(ml/min)*	2.3	1.3	2.0	1.5	3.0	4.8	2.2	3.7	0.4	2.4
VO$_{2pul}$ *(ml/kg/min)*	1.3	0.4	0.6	0.5	1.1	1.3	0.7	1.1	0.1	0.7
VO$_{2pul}$: bodyVO$_2$ *(%)*	8.5	3.6	8.9	6.7	13.0	19.2	8.7	19.6	1.6	9.6
Lung : Body weight *(%)*		0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.5	0.5

Table 3.2-2 Characteristics of rabbits included in the AD group

(To be continued)

Case	AD11	AD12	AD13	AD14
Sex	F	F	F	F
Weight (kg)	3.7	3.7	3.7	3.7
Length (cm)	43	43	43	43
meanCO$_{TD}$ (ml/min)	751	755	801	468.5
mean SaO$_2$ (%)	96.5	99.	95.4	99.3
aHb (g/dl)	10.1	10.1	11.9	12.1
CaO$_2$ (ml/L)	135.5	139.0	157.8	167.0
mean SvO$_2$ (%)	69.2	83.0	66.8	63.8
vHb (g/dl)	9.9	9.4	11.9	12.1
CvO$_2$ (ml/L)	95.2	108.5	110.5	107.3
mean cVO$_2$ (ml/min)	30.2	23.1	37.9	28.0
mean mVO$_2$ (ml/min)	31.5	25.7	41.9	31.9
$mVO_2 > cVO_2$ (ml/min)	1.3	2.6	4.0	3.9
VO$_{2pul}$ (ml/kg/min)	0.3	0.7	1.1	1.1
VO$_{2pul}$: bodyVO$_2$ (%)	4.0	10.3	9.6	12.3
Lung : Body weight (%)	0.4	0.4	0.4	0.4

Table 3.2-2   Characteristics of rabbits included in the AD group *(con't)*

Case	CD1	CD2	CD3	CD4	CD5	CD6	CD7	CD8	CD9
Sex	F	F	F	M	M	M	M	M	M
Weight (kg)	4.1	3.9	3.9	3.3	3.3	2.5	3.7	3.7	3.9
Length (cm)	45	43	43	38	38	34	43	43	39
meanCO$_{TD}$ (ml/min)	619.3	691.3	602.3	576	575.7	450	846.7	614.5	781
mean SaO$_2$ (%)	84.5	67.6	63.0	95.2	97.7	90.7	88.3	94.3	86.6.
aHb (g/dl)	13.6	11.7	12.1	13.1	13	15.9	12.8	13	14.1
CaO$_2$ (ml/L)	159.8	109.9	106.0	173.4	176.5	200.5	157.1	170.4	169.7
mean SvO$_2$ (%)	51.6	52.1	39.8	79.8	81.8	64.4	69.5	70.8	73.0
vHb (g/dl)	13.4	11.3	11.8	13	13	16	12.9	12.5	14.4
CvO$_2$ (ml/L)	96.1	81.8	65.3	144.2	147.8	143.2	124.6	123.0	145.9
mean cVO$_2$ (ml/min)	39.4	19.4	24.5	16.8	16.5	25.8	27.5	29.1	18.6
mean mVO$_2$ (ml/min)	45.88	27.9	33.7	22.6	19.9	32.2	31.1	35.3	23.1
mVO$_2$ > cVO$_2$ (ml/min)	6.47	8.53	9.20	5.81	3.36	6.45	3.60	6.18	4.50
VO$_{2pul}$ (ml/kg/min)	1.6	2.2	2.4	1.8	1.0	2.6	1.0	1.7	1.2
VO$_{2pul}$: bodyVO$_2$ (%)	14.1	30.6	27.3	25.7	16.9	20.0	11.6	17.5	19.5
Lung : Body weight (%)	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4	0.3

Table 3.2-3   Characteristics of rabbits included in the CD group

Table 3.3-1 Results of lung scoring in Control group

Case	Atelectasis	Interstitium					Air-space				Peribronchial Infiltration
		Congestion	Acute		Chronic		Edema	Acute		Hyaline membrane	
			Edema	Neutrophil	Chronic infiltration	Fibrosis		Haemorrhage	Neutrophil		
N01	0	0	0	0	0	0	0	0	0	0	3
N02	0	0	0	0	0	0	0	0	0	0	0
N03	0	0	0	1	1	0	0	0	0	0	1
N04	0	0	0	0	0	0	0	0	0	0	0
N05	0	3	0	0	0	0	0	0	0	0	0
N06	1	1	0	0	1	0	0	0	0	0	0
N07	0	0	0	0	0	0	0	0	0	0	0
N08	0	0	0	0	0	0	0	0	0	0	0
N09	0	0	0	0	0	0	0	0	0	0	0
N10	0	1	0	1	0	0	0	0	0	0	1
N11	0	3	0	1	0	0	0	0	0	0	1
N12	0	0	0	1	1	0	0	0	0	0	0
N13	0	0	0	1	1	0	0	0	0	0	0
N14	0	0	1	1	1	0	0	0	0	0	2
N15	0	0	0	0	0	0	0	0	0	0	0
N16	0	0	0	0	0	0	0	0	0	0	0
N17	0	0	0	0	0	0	0	0	0	0	1
N18	0	0	0	1	1	0	0	0	0	0	0
N19	0	0	0	0	0	0	0	0	0	0	0
N20	0	0	0	0	0	0	0	0	0	0	0
N21	0	2	0	0	0	0	0	0	0	0	0

Where: 0=none, 1=mild, 2= moderate, 3= severe.

Table 3.3-2 Results of lung scoring in AD group

Case	Atelectasis	Interstitium					Air-space				Peribronchial Infiltration
		Acute		Neutrophil	Chronic		Edema	Acute		Hyaline membrane	
		Congestion	Edema		Chronic infiltration	Fibrosis		Haemorrhage	Neutrophil		
AD01	0	1	1	3	2	0	2	2	1	1	1
AD02	0	2	1	2	1	0	1	0	0	1	0
AD03	0	1	1	1	1	0	0	0	0	0	0
AD04	0	1	0	2	1	0	0	0	0	0	0
AD05	0	1	0	2	0	0	0	0	0	0	0
AD06	0	1	0	1	0	0	0	0	0	1	0
AD07	0	0	0	0	0	0	0	0	0	1	0
AD08	0	0	0	0	0	0	0	0	0	1	0
AD09	0	3	1	0	0	0	1	0	0	0	0
AD10	0	1	0	1	0	0	0	0	0	0	0
AD11	1	1	0	1	0	0	0	0	0	0	0
AD12	1	1	0	1	0	0	0	0	0	0	0
AD13	0	1	0	1	0	0	0	0	0	0	0
AD14	0	1	0	1	0	0	0	0	0	0	0

Where: 0=none, 1=mild, 2=moderate, 3= severe.

| Case | Atelectasis | Interstitium | | | | | Air-space | | | | Peribronchial |
| | | Acute | | Chronic | | | | Acute | | | |
		Congestion	Edema	Neutrophil	Chronic infiltration	Fibrosis	Edema	Haemorrhage	Neutrophil	Hyaline membrane	Infiltration
CD01	1	1	0	1	1	0	0	0	0	0	0
CD02	0	1	0	0	1	0	0	0	0	0	0
CD03	1	1	0	1	1	2	0	0	0	0	1
CD04	0	2	0	0	0	0	0	0	0	0	0
CD05	1	1	0	0	1	0	0	0	0	0	0
CD06	1	1	0	1	2	1	0	0	0	0	0
CD07	0	1	0	0	1	0	0	0	0	0	0
CD08	1	1	0	0	1	1	0	0	0	0	0
CD09	1	1	0	0	1	1	0	0	0	0	0

Where: 0=none, 1=mild, 2= moderate, 3= severe.

Table 3.3-3   Results of lung scoring in CD group

Group	Atelectasis	Interstitium					Air-space				Peribronchial Infiltration
		Acute			Chronic		Acute				
		Congestion	Edema	Neutrophil	Chronic infiltration	Fibrosis	Edema	Haemorrhage	Neutrophil	Hyaline membrane	
Con : AD	NS	$P=0.004$	$P=0.052$	$P=0.003$	NS	NS	$P=0.029$	NS	NS	$P=0.004$	NS
Con : CD	$P=0.000$	$P=0.003$	NS	NS	$P=0.002$	$P=0.001$	NS	NS	NS	NS	NS
AD : CD	$P=0.012$	NS	NS	$P=0.019$	$P=0.012$	$P=0.007$	NS	NS	NS	$P=0.047$	NS

Mann Whitney test, significance if $P<0.05$
Where, NS: not significance

Table 3.3-4 Pathological changes of rabbit lungs in different groups.
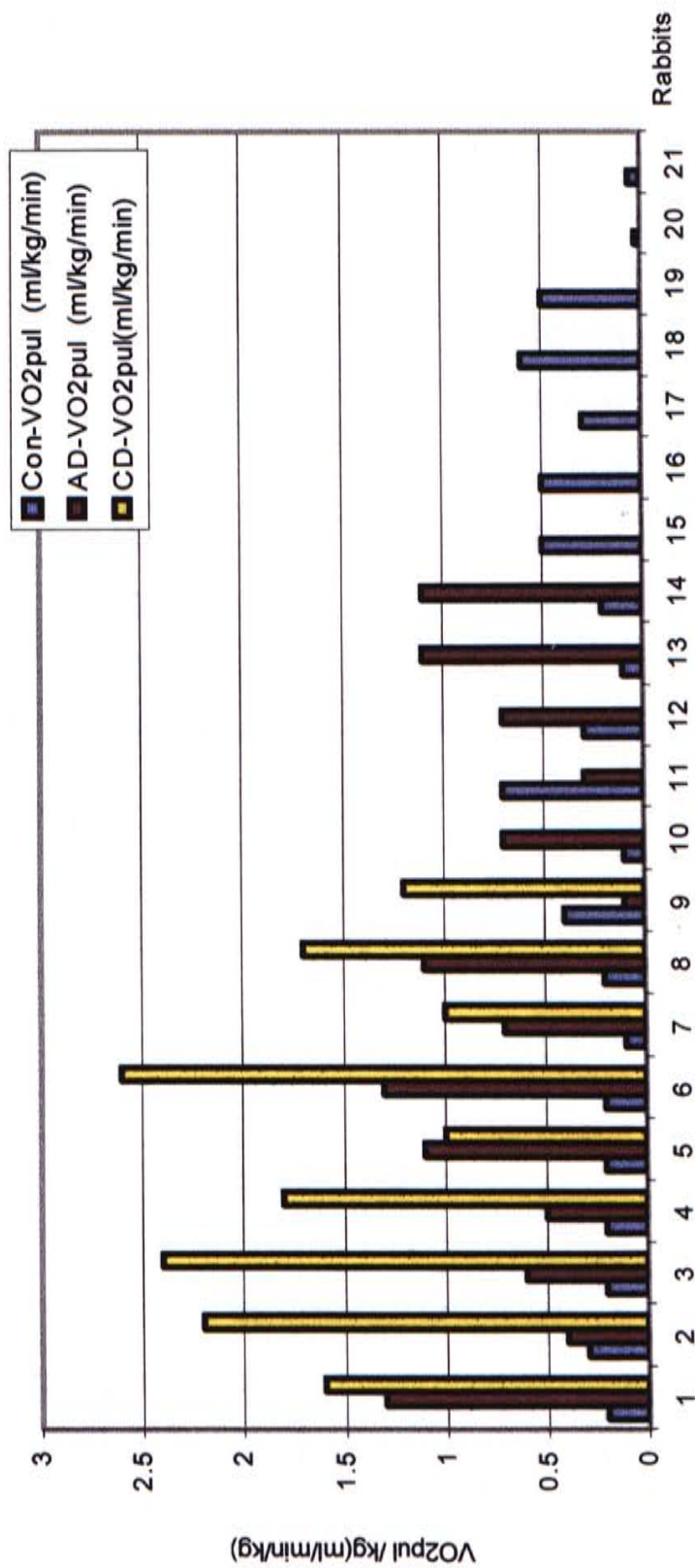
92

Figure 3.4-1 VO2pul in Control, AD & CD group
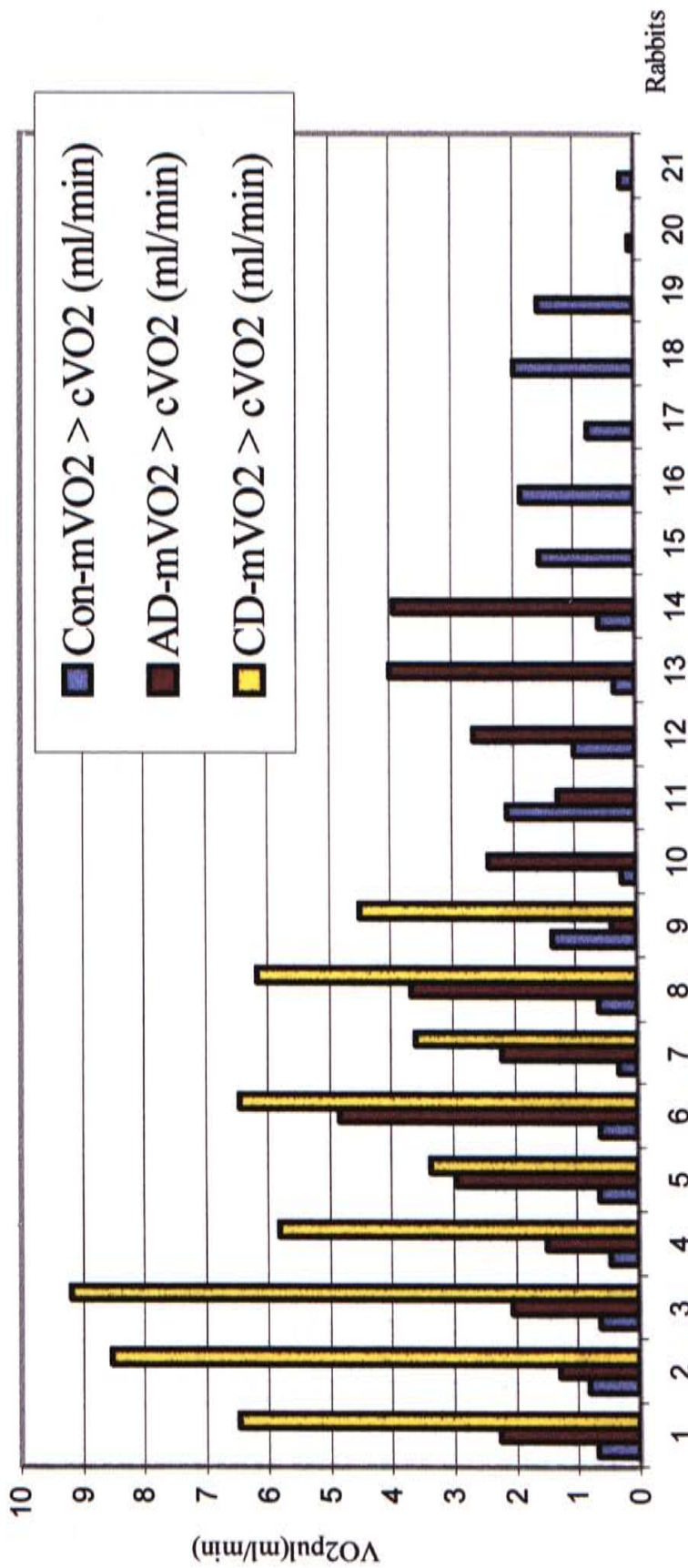
Figure 3.4-2   VO$_{2pul}$ in Control, AD and CD group

94
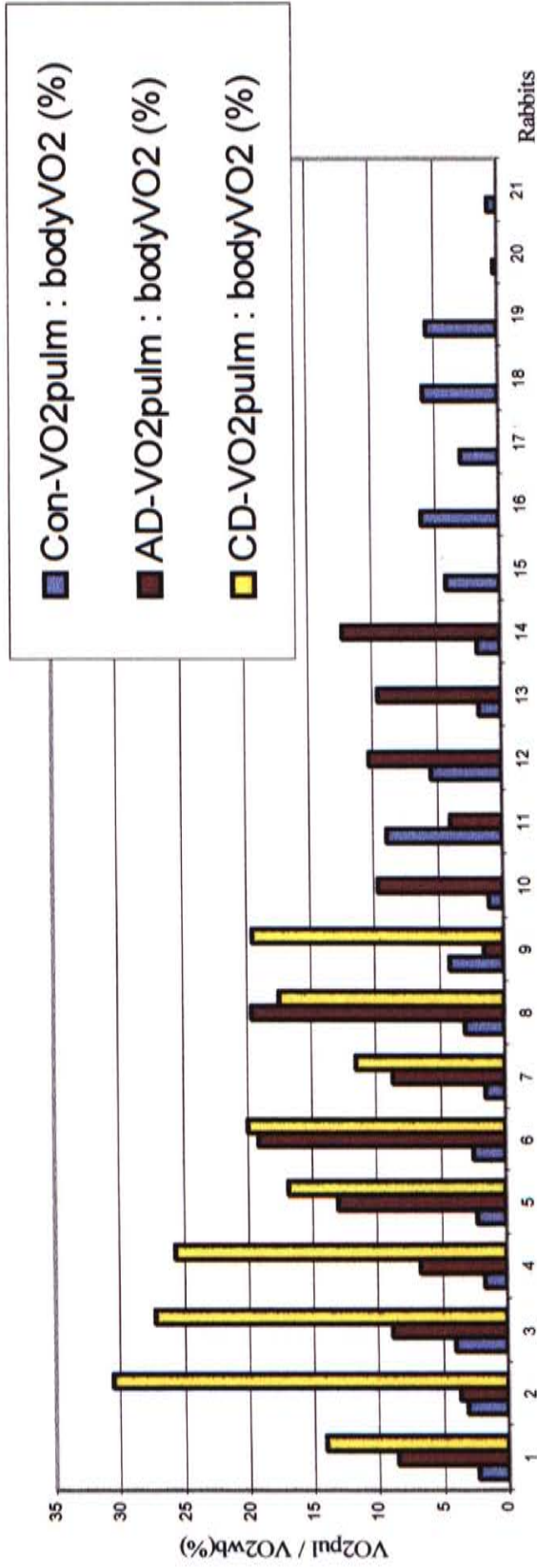
Figure 3.4-3 VO2pul in Control, AD and CD group

95
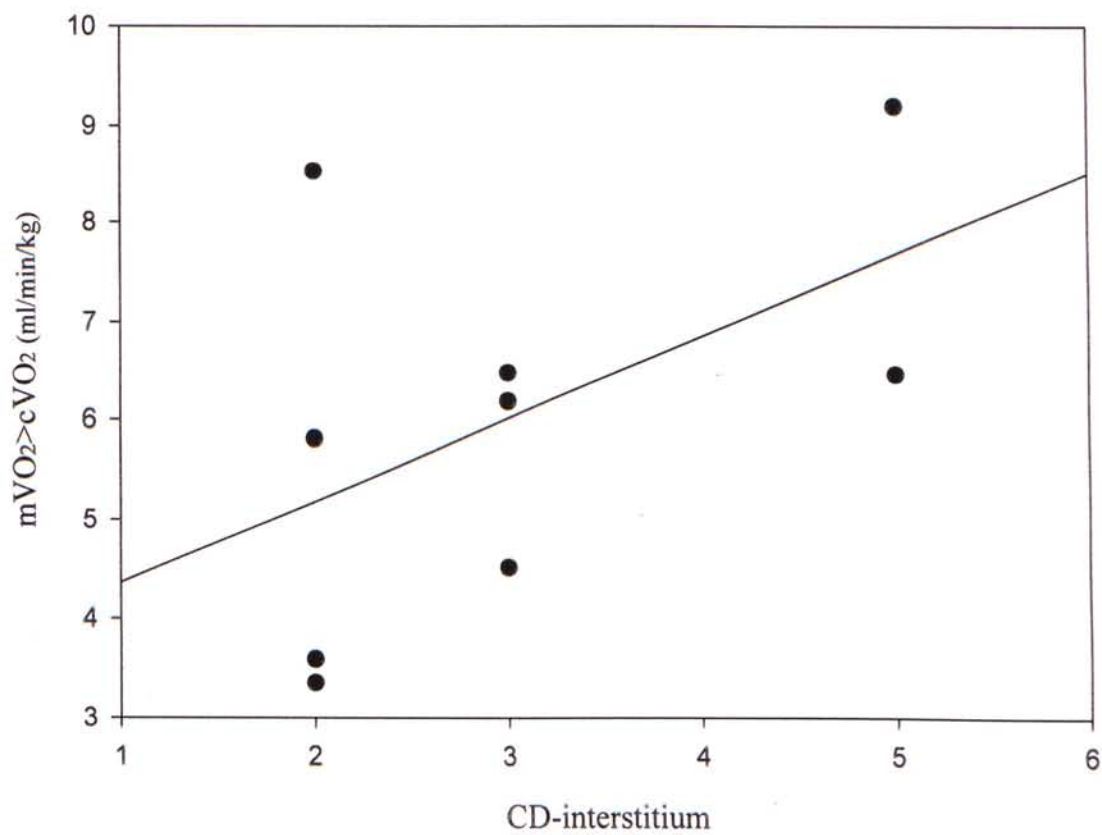
Figure 3.4-4 Relationship between VO_{2pul} (ml/min/kg) and severity of interstitial damage of the lung in CD group

Discussion

The present study is the first study that compares the local oxygen consumption by the lungs in healthy subjects, and subjects with acute and chronic lung injury. The findings of this study show that (1) mVO_2 (VO_{2wb}) is greater than cVO_2 (VO_{2b}) in all these groups of rabbits, including the healthy controls, and (2) the difference between VO_{2wb} and VO_{2b} which represents the local oxygen consumption by the lungs, is significantly greater in the lung injury groups than in the healthy controls. These findings support my hypothesis that (1) oxygen consumption by the lung contributes significantly to the total oxygen consumption of the body, and (2) lung injury increases local oxygen consumption.

The determination of VO_2 by the Fick method has many potential sources of errors because it relies on the measurement of several parameters: cardiac output by thermodilution, as well as pulmonary and systemic arterial oxygen contents. Because no simple technique can be considered a gold standard for cardiac output determination under clinical conditions, the problem of assessing the errors of measurement using the thermodilution method is complex (Levett & Replogle, 1977; Thys, 1987).

In *in-vitro* study, under strictly controlled conditions, the accuracy of the thermodilution cardiac output measurement ranges between ± 7 and ± 13% (Thys, 1987). Under clinical conditions, the error in accuracy is considered similar, averaging ± 10% (Levett & Replogle, 1977; Thys, 1987), and relies principally on the accuracy of the injectate volume and the use of correct computation constants (for temperature and volume), as well as the care of injectate manipulation (e.g. avoiding heating of injectate). The use of an automatic injector, the volume and temperature of injectate, and the timing of injection in the respiratory cycle may all influence precision.

The Fick method makes the assumption that 1 gram of hemoglobin combines with 1.39 ml of oxygen. This assumption is based on the molecular weight of hemoglobin (Braunitzer, 1963) and assumes that all hemoglobin is in the active form. However, this may not be entirely true, since the international standard of hemoglobin estimation is based on iron content, not on its actual oxygen-combining capacity, and not all of the iron content is in the form of active hemoglobin. Since some of the iron is likely to be in the form of haemochromogens, it is not altogether surprising that the observed oxygen-combining capacity is less than the theoretical value of 1.39ml/g. The oxygen-combining power of

hemoglobin has been exhaustively studied by Greogory (Greogory, 1974), who proposed the values of 1.306 ml/g for human adult blood and 1.312ml/g for fetal blood. But if the value of 1.31 was used instead of 1.39 in the previous studies, the values of VO_2 measured by the reversed Fick procedure would be reduced by about 6% and the difference between mVO_2 and cVO_2 values would be further enhanced.

We used 1.39 in the present study because it has been accepted for New Zealand White rabbit that 1 gram of hemoglobin combines with 1.39ml of oxygen (Hindman, 1995). On the other hand, the known maximum oxygen binding capacity of hemoglobin in rabbit has been found to be 1.34-1.39ml/g hemoglobin (Hindman, 1995). In contrast, if 1.39 is replaced by 1.34 in my study, the values the of cVO_2 are lessened by about 3.6% and the difference between mVO_2 and cVO_2 values would become even greater (Smithies *et al*, 1991) and thus would not alter the conclusion of my study.

Another potential error in using the thermodilution method to determine cardiac output is the asynchrony in the timing of injection of the ice-cold saline into the right atrium and the commencement of

temperature measurement by the intra-aortic temperature sensor. In my early practice sessions, I injected the saline manually and the cardiac output measured fluctuated widely with poor reproducibility. The problem was solved by using the automatic injector supplied by the manufacturer of the cardiac output monitor. The automatic injector was interfaced with the monitor electronically. Temperature measurement was activated by injection of the injector, resulting in perfect synchrony of the two procedures.

Warming of the injectate during its passage through the catheter might also introduce significant measurement error to the thermodilution method. This was overcome by immersing the entire length of the catheter in ice water up to its entry into the body box. By wrapping the length of the catheter inside the body box in ice further prevented any loss in temperature of the injectate.

On the other hand, two-third of the rabbits in the CD group were scored to have atelectasis with congestion, so the lung pathology in the CD group might contribute to the presence of intrapulmonary shunts, and tricuspid insufficiency. In clinical settings, tricuspid regurgitation

frequently occurs in chronic lung problems including that of bronchopulmonary dysplasia. This is secondary to the occurrence of pulmonary hypertension. The abnormal mixing of blood with injectate solution and indicator temperature loss to adjacent tissue secondary to bi-directional flow from the right ventricular cavity might be the proposed cause of inaccuracy. If surrounding tissue fails to release this cold injectate prior to the completion of the thermodilution curve integration, indicator will not be detected by the thermistor and cardiac output would be subsequently overestimated, it seems more likely occurred in the rabbits in CD group rather than in AD group. This could be one of possible factors that contribute to the elevation of the VO_{2pulm} in the rabbits with chronic lung disease.

Former studies have reported that the accuracy of various commercial indirect calorimeters generally have a level of accuracy that is acceptable for clinical purposes (Braun *et al*, 1989; Takala *et al*, 1989; Westenskow, Roberts & Pace, 1989; Makita, Nunn & Royston, 1990; Svenson, Sonander & Stevqvist, 1990; Smithies *et al*, 1991). The metabolic monitor used in my experiment is a precision instrument with a mean error of only 1.9% - 4% in oxygen consumption measurements (Takala *et al*, 1989; Phang *et al*, 1990).

In performing the measurement, I have meticulously minimised the error caused by air-leak by using a specially designed air-tight body box instead of the canopy provided by the manufacturer. The body box has an internal volume similar to that of the neonatal canopy and allows blood taking, injection of the injectate, and temperature measurement without opening its lid or disturbing the animal.

With the above precautions to minimise measurement error, I have shown that the healthy rabbit lung consumes about 3% of the total-body oxygen uptake for its own metabolic requirements. This is very similar to former reports that the lung uses 0.7-5% of the total body oxygen consumption in human (Frittes *et al*, 1963; Nunn, 1987; Loer *et al*, 1997)(*Table 1.3-1*).

The systematic difference between VO_2 measured in our animals by metabolic monitor and the reversed Fick method could only be explained by the VO_2 of the lung.

The lung receives its blood supply from both the bronchial circulation derived from aortic blood and the pulmonary circulation.

Bronchial blood flows constitutes about 1-2% of the output of the left ventricle. It has a small contribution to pulmonary capillary blood flow and gas exchange (Deffebach *et al*, 1987; Levitzky, 1995). The major portion of this blood supplies the bronchial walls and the visceral pleura. Pulmonary blood flow constitutes the entire output of the right ventricle, which supplies the lung with mixed venous blood draining from all the tissues of the body. This is the blood that undergoes gas exchange with the alveolar air in the pulmonary capillaries. Therefore, it is not surprising that the alveolar walls derive oxygen chiefly from the alveolar air, whereas the bronchi, the smaller air passages, and the major portions of the visceral pleura utilise the oxygen carried by the bronchial flow (Bloomer *et al*, 1949; Lilker & Nagy, 1975). The cVO_2 calculated by the Fick principle measures oxygen uptake by the body before its blood enters the lungs and the VO_2 estimated represents the oxygen consumption of the body (VO_{2b}) without the lung. On the other hand, since the mVO_2 is measured by way of the respiratory gases, the measured volume represents VO_2 of whole body (VO_{2wb}), including the lungs. Therefore, the difference between mVO_2 and cVO_2 should indicate the amount of oxygen consumed by the lungs (VO_{2pulm}):

$$mVO_2 - cVO_2 = VO_{2pulm}$$

One of the studies performed by Loer and coworkers estimated the lung oxygen consumption in patients undergoing open heart surgery for coronary bypass grafting or valvular replacement. During total cardiopulmonary bypass, all blood from the caval veins was drained into the cardiotomy reservoir and the caval veins were occluded by banding so that pulmonary artery flow ceased completely. The left ventricle was vented. Blood was oxygenated and regulated by a membrane oxygenator and a roller pump respectively. Systemic hypothermia of 28°C was induced by blood cooling and maintained for at least 30 minute. During this period, the lungs were ventilated with low tidal volumes and positive end-expiratory pressure. Oxygen consumption was estimated by respiratory gas analysis, assuming that pulmonary gas exchange was completely separated from body gas exchange during measurement (Loer *et al*, 1997). Total lung oxygen consumption might have been underestimated because of the unknown magnitude of the bronchial circulation under such circumstances. The absolute quantity of transpleural diffusion varies with the magnitude of the concentration gradients, and Loer used 50% oxygen for both ventilating the lung and oxygenating the blood during total cardiopulmonary bypass. Hence the transpleural partial pressure gradient for oxygen was minimised, limiting the gas exchange with ambient air. Moreover, Loer's study consisted

mainly of elderlies with marked cardiac disease, requiring different type of open-chest operation and positive end-expiratory pressure ventilation, which might have affected the oxygen consumption of the lung. Atelectasis of the lung during open-chest operation, who had an significant effect on the lung's metabolism and oxygen requirement (Hachenberg *et al*, 1994). Thus, the finding of this study were difficult to interpret as they certainly did not represent the normal healthy subjects or subjects with any particular type of lung decreases. In contrast, our animal study provided assessments of VO_{2pul} *in vivo* without any major external factors that might affect lung metabolism, and the finding were unlikely to have been affected by measurement errors due to the contributions by transpleural diffusion, bronchial circulation, development of atelectasis or the absence of pulmonary artery flow.

Our finding provide the first direct comparison of the oxygen consumption of the healthy and diseased lungs. Compared to the healthy control group, the pulmonary oxygen consumption was significantly increased from 3.3± 2.2% to 9.7 ± 5.2 % and 20.4 ± 6.3 % in the acute and chronic lung damage groups respectively. The among of pulmonary oxygen consumption by diseased lungs was similar to that reported in previous studies on both human and animals with pulmonary injuries

(Fritts *et al*, 1963; Takala *et al*, 1989; Becq *et al*, 1992; Bizouarn *et al*, 1992; Loer, 1997).

In a canine model of experimental pneumococcal pneumonia, Light (Light, 1988) demonstrated that the VO_{2pul} was 13-15% of VO_{2wb}. A number of human studies, mostly in adult patients with ARDS (Kox & Chirst, 1991), sepsis and respiratory failure (Smithies *et al*, 1991), cardiac surgeries (Oudemans-van *et al*, 1993; Takala *et al*, 1989), or acute lung injury (Jolliet *et al*, 1996), have all provided evidence that VO_{2pul} could constitutes a significant proportion of body oxygen consumption when there is significant lung injury (Fritts *et al*, 1961, 1963; Levinson *et al*, 1987; Takala *et al*, 1989; Chopin *et al*, 1990; Smithies *et al*, 1991, 1992; Becq *et al*, 1992; Bizouarn *et al*, 1992; Myburgh *et al*, 1992; Oudemans-van *et al*, 1993; Chioléro *et al*, 1994). In these studies VO_{2pul} varied from 14 - 40% of VO_{2wb}. The most likely reason for the increase of VO_{2pul} in lung-injured subjects is the presence of pulmonary inflammatory response (Kirklin *et al*, 1983; Oeveren *et al*, 1985). In our lung-damaged animals, lung section showed an abundance of neutrophils and macrophage in the interstitium. Both neutrophils and macrophages undergo oxidative metabolism, which increases when the cells perform phagocytosis during inflammations. Moreover, neutrophils show an enormous increase in

oxygen consumption during the "oxygen burst" associated with the formation of oxygen-derived free radicals. Thus inflammation in the lungs inevitably increase the organ's oxygen consumption, as observed in our animals in the lung- damaged groups (Nunn, 1996).

In both our AD and CD group of animals, histopathological examination revealed inflammatory changes of moderate to severe degree damage. The histopathological changes in our AD group of rabbits consisted of acute inflammatory responses in both the interstitium and the air space including congestion, edema, infiltration of neutrophils and hyaline membrane formation. Their changes were similar to those reported previously for NNNMU-induced lung damage (Finley, Swenson & Comroe, 1962; Ryan, Loomis & Barrett, 1976; Ryan *et al*, 1981; Haselton, 1983; Liau *et al*, 1987; Harris *et al*, 1989; Harriset *et al*, 1989; Lewis, Ikegami & Jobe, 1990). The changes closely resembled those in the lungs of patients with early stages of ARDS, with profound alveolar damage (Ashbaugh *et al*, 1967; Petty *et al*, 1977, 1979; Mason, 1987; Petty, 1990; Gregory *et al*, 1991).

The morphologic changes in the lungs of the CD group displayed marked chronic changes in the interstitium with atelectasis, infiltration by inflammatory cells, and fibrosis. The changes were similar to those observed in rodents that displayed chronic inflammatory process and interstitial pneumonitis featured by fibrosis (Adamson & Bowden, 1974; Aso, Yoneda & Kiklawa, 1976; Comis, 1978).

In my experiments, despite the increase in VO_{2pul}, the total body VO_2 of animals in the AD and CD group were higher than that of the control group although the difference was not statistically significant. Their body weight and growth rate did not appear to be impaired.

Theoretically, organ injuries should increase oxygen requirement to satisfy the need for tissue repair and recovery. In canines with pneumonia, Light (Light, 1988) reported that the whole body oxygen consumption, measured by collection of expired gas was considerably increased.

The lack of statistically significant difference is VO2 between our healthy and diseased rabbits could be due to the relatively small and lack

of power of the sample size. One other possible explanation for this discrepancy may be the mechanism of lung damage. In Light's study, lung injury was induced in dogs by infecting the lungs with *Streptococcus pneumoniae*. The oxygen uptake by the bacteria, along with the destruction of the lung, could have contributed to the increase in VO_{2wb}. The lung damage in our animals was induced by chemical destruction without the use of bioactive agents. This might have contributed to a lesser degree of increment in overall oxygen consumption of the body. Moreover, it has been previously reported that both the growth rate and the body weight had remained unchanged in rabbits receiving as much as 17U/kg bleomycin intratracheally (Laurent *et al*, 1981; Hesterberg *et al*, 1981).

Conclusion

Growth failure is a common and difficult management problem in infants with chronic lung disease. Current treatment consists mainly of nutritional supplementation, and the use of bronchodilators to reduce the work of breathing. My study provides evidence that a significant increase in the oxygen consumption and energy expenditure by the inflamed lungs might be an important contributing factor. Therapies aiming at suppressing lung inflammation might be a logical approach in managing CLD infants with growth failure.

My study showed that in the healthy state, adult rabbit lung consumed about 3% of the total oxygen consumption of the body. Oxygen consumption by the lung was increased significantly in the presence of either acute or chronic lung injury. This was likely to be related to the result of inflammatory changes such as cellular infiltration.

In my experiment, a new animal model not considered in previous studies was introduced. We have demonstrated that there were reflections of morphological damages with acute and chronic inflammatory changes, which might be potentially comparable to preterm human infants with chronic lung disease, for example, bronchopulmonary dysplasia (BPD).

Further works need to be done to clearly characterize these pathological alterations, in order to introduce a most suitable model for the investigations of BPD, and other CLDs in premature infants.

Future Studies

As already discussed, this is the first study compares the local oxygen consumption by the lung in healthy subjects and subjects with acute and chronic lung injury. Based on the findings and the established methodology of this study, a number of areas could be considered for further exploration.

Though we have demonstrated the morphological damages with acute and chronic inflammatory changes, more works need to be done to clearly identify the precise pathway of the pathological alterations. In particular, the surfactant and cytokines involved, as well as the comparability of this model to the preterm human infants with BPD are all need to be further investigated. This is especially important because with the improvement of the technology and facilities of the NICUs, the survival rate of prematurity infants is increasing, as well as the incidence of BPD. Similarly, it represents an area that could be exploited for the utilization of a new animal model.

In addition, it is worthwhile to see whether administration of steroid or other anti-inflammation medications to subjects with lung damage (e.g. CLD) would reduce their oxygen consumption and improve their growth. Non-steroid anti-flammatory drugs are of

particular interest since unlike steroid, they do not have any know

pharmacological effect on somatic growth or metabolism.

Bibliography

Abman SH, Groothius JR. (1994) Pathophysiology and treatment of bronchopulmonary dysplasia. *Pediatr Clin North Am* 41:177-315.

Adamson IYR, Bowden DH. (1974) The pathogenesis of bleomycin-induced pulmonary fibrosis in mice, *AM J Pathol* 77:185-98.

Ashbaugh DG, Bigelow BD, Petty TL, Levine BE. (1967) Acute respiratory distress in adults. *Lancet* 2:319-323.

Aso Y, Yoneda K, Kiklawa Y. (1976) Morphologic in biochemical study of pulmonary changes induced by bleomycin in mice. *Lab Invest* 35:558-68.

Avery GB, Fletcher AB, Kaplan M et al. (1985) Controlled trial of dexamethasone I n respirator- depend infants with bronchopulmonary dysplasia. *Pediatrics* 75:106-11.

Avery ME, Tooley WH, Keller JB, et al (1987) Is chronic lung disease in low birthweight infants preventable? A survey of eight centers. *Pediatrics* 79:26.

Babior B (1978) Oxygen-dependent microbial killing by phagocytes. *N Engl J Med* 298:659-668; 721-725.

Bailey D & Morgan KM. (1996) *Organonitrogen chemistry*.

Baldari E Filippone M, Trevisanuto D et al (1997) Pulmonary function until two years of life in infants with bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 155:149-55.

Bancalari E, Abdenour GE, Feller R, et al. (1979) Bronchopulmonary dysplasia: clinical presentation. *J Pediatr* 95:819-23.

Banerjee CK, Girling DJ, Wiggleswoth JS (1972): Pulmonary fibroplasia in newborn babies treated with oxygen and artificial ventilation. *Arch Dis Child* 47:509.

Barash PG, Nardi D, Hammond G Walker- Smith G, Capuano D, Laks H, Kopriva CJ, Baue AE, Geha AS (1981). Catheter-induced pulmonary artery perforation: mechanisms, management, and modifications. *Journal of Thoracic an Cardiovascular Surgery* 82: 5-12.

Barrett CR, Bell ALL, Ryan SF (1979) Alveolar epithelial injury causing respiratory distress in dogs. *Chest* 75: 705-11.

Becq MC, Mangalaboyi J Chopin C (1992) The difference between the oxygen consumption estimated by Fick and expired gas methods increases in pneumonia. *Intensive Care Med* 18:s41.

Bellet-Barthas M. Le Roy JP. Barthelemy L. Bellet M. (1983) Pulmonary toxicity of bleomycin in the rabbit: histophysiological and anatomo-radiological correlations. Toxicological European Research - *Recherche Europeenne en Toxicologie.* 5(3): 113-6, May.

Berend N. Feldsien D. Cederbaums D. Cherniack RM. (1985) Structure-function correlation of early stages of lung injury induced by intratracheal bleomycin in the rabbit. *American Review of Respiratory Disease.* 132(3):582-9, Sep.

Berg TJ, Pagtakhan RD, Reed MH, et al (1975): Bronchopulmonary dysplasia and lung rupture in hyaline membrane disease: influence of continuous distending pressure. *Pediatrics* 55:51.

Berman W, Katz R, Yabek SM, et al. (1986) Long-term follow-up of bronchopulmonary dysplasia. *J Pediatr* 109:45-50.

Bhutani VK, Abbasi S. (1992) Long-term pulmonary consequences in survivors with bronchopulmonary dysplasia. *Clin Perimatol* 19:649-71.

Bianco S, Vaghi A, Robuschi M, and Pasargiklian M. (1988) Prevention of exercise-induced bronchoconstriction by inhaled furosemide. *Lancet* 7:252.

Bigby TD. Allen D. Leslie CG. Henson PM. Cherniack RM. (1985) Bleomycin-induced lung injury in the rabbit. Analysis and correlation of bronchoalveolar lavage, morphometrics, and fibroblast stimulating activity. *Am Rev of Resp Dis.* 132(3):590-5, Sep.

Bizouarn P, Soulard D, Blanloeil Y, Guillet A, Goarin Y (1992) Oxygen consumption after cardiac surgery- a comparison between calculation by Fick's principle and measurement by indirect calorimetry. *Intensive Care Med* 18: 206-209.

Bland RD, McMillan DD & Bressack MA. (1978) Decreased pulmonary transvascular fluid filtration in awake newborn lambs after intravenous furosemide. *J Clin Invest* 62:601.

Bloomer WE, Harrison W, Lindskog GE, Liebow AA (1949): Respiratory function and blood flow in the bronchial artery after ligation of the pulmonary artery. *Am J Physiol* 157:317-28.

Bogovski P & Bogovski S. (1981) Animal species in which N-nitroso compounds induce cancer. *Int J cancer* 27,417-474.

Boyd KD, Thomas SJ, Gold J, Boyd AD (1983) A prospective study of complications of pulmonary artery catheterizations in 500 consecutive patients. *Chest* 84:245-249.

Boynton BR (1988): The epidemiology of bronchopulmonary dysplasia. *In* Merritt TA, Northway WH, Jr, Boynton BR (eds): *Bronchopulmonary Dysplasia.* Boston, Blackwell Scientific Publications.

Brandi LS, Grana M, Mazzanti T, Giunta F. (1992) Energy expenditure and gas exchange measurements in postoperative patients: Thermodilution versus indirect calorimetry. *Crit care med* 20: 1273-1283.

Brandi LS, Oleggini M, Lachi S,et al. (1988) Energy metabolism of surgical patients in the early postoperative period : A reappraisal. *Crit Care Med* 16: 18-22.

Branthwaite MA, Bradley RD (1968) Measurement of cardiac output by thermodilution in man. *Journal of Applied Physiology* 24:434-438.

Braun U, Zundel J, Freiboth K et al., (1989) Evaluation of methods for indirect calorimetry with a ventilated lung model. *Intensive Care Med* 15:196.

Braun U, Zundel J, Freiboth K, Weyland W, Heidelmayer CF and Hellige G. (1989) Evaluation of methods for indirect calorimetry with a ventilated lung model. *Intensive Care med* 15:196-202.

Braunitzer G (1963) Molekware struktur der hamoglobine. *Nova Acta Leopoldina* 26:113.

Brownlee KG, Ng PC, Henderson MJ, Smith M, Green JH, Dear PRF. (1992) Catabolic effect of dexamehtasone in the preterm baby. *Arch Dis Child* 67:1-4.

Bruce M, Boat T, Martin R, et al. (1992) *Chest* 81(suppl 5):44S-5S.

Buchbinder N, Ganz W (1976) Hemodynamic monitoring: invasive techniques. *Anesthesiology* 45:146-155.

Calabresi P, Parks Re Jr.(1980) Chemotherapy of nepotistic diseases. In: Gilman AG, Goodman LS, Gilman A, eds. *The pharmacological basis of therapeutics*. New York: Macmillan, 1256-1314.

Campbell AGM, McIntoch N (1997) *Forfar and Arneil's textbook of Peadiatrics* 5th ed., Churchill Livingstone pp193-195.

Capderou A Douguet D, Losay J. (1997) Comparison of indirect calorimetry and thermodilution cardiac output measurement in children.   *Am Rev Respir crit care med* 155:1930-1934.

Casey PH, Kraemer HC, Bernbaum J et al. (1991) Growth status and growth rates of a varied sample of low birth weight, preterm infants: a longitudinal cohort study from birth to three years of age. *J Pediatr* 119:599-605.

Catravas JD,Lazo JS, Dobuler KJ, Mills LR, Gillis CN. (1983) Pulmonary endothelial dysfunction in the presence or absence of interstitial injury induced by intratracheally injected bleomycin in rabbits. *Am Rev respir dis*128:740-746.

Chang AC, Kulik TJ, Hickey PR, Wessel DL. (1993) Real-time gas-exchange measurement of oxygen consumption in neonates and infants after cardiac surgery. *Crit Care Med* 21:1369-1375.

Chopin C, Mehdaoui H, Boniface B, Mangalaboyi J, Chambrin MC, Lestavel P, Rimé A, Fourrier F (1990) Transport, consommation et extraction de l'oxygèn au cours des é tats septiques graves. *Rean Soins Intensive Med Urg* 6:147-153.

Christie J, Sheldahl LM, Tristani FE, et al. (1987) Determination of stroked volume and cardiac output during exercise: Comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution. *Circulation* 76:539-547.

Collaborative Dexamethasone Trial Group (1991). Dexamethasone therapy in chronic neonatal lung disease: an international placebo controlled trial. *Pediatrics* 88:421-7.

Comis RL (1978) *Bleomycin pulmonary toxicity. In* Carter SK, Crooke ST, Umezawa H, eds. Bleomycin: current status and new developments. *New York: Academic Press* 279-92.

Cooke RWI (1991): Factors associated with chronic lung disease in preterm infants. *Arch dis child* 66:776.

Cooper JAD, White DA & Matthay RA. (1986) Drug-induced pulmonary disease Part 2: Noncytotoxic drugs. *Am Rev respir dis*133: 488-505.

Cooper JAD, White DA & Matthay RA. (1986) Drug-induced pulmonary disease Part 1: Cytotoxic Drugs. *Am Rev respir dis*133: 321-340.

Corcoran JD, Patterson CC, Thomas PE, Halliday HL. (1993) Reduction in the risk of bronchopulmonary dysplasia from 1980-1990: results of a multivariate logistic regression analysis. *Eur J of Pae* 152:671-681.

Cummings JJ, D'Eugenio DB, Gross SJ.(1989) A controlled trial of dexamethasone in preterm infants at high risk for bronchopulmonary dysplasia. *N Engl J Med* 320:1505-10.

Davies G, Jebson P & Hess D. (1986) Continuous Fick cardiac output compared to thermodilution. *Crit Care Med* 14; 881-5.

Davila D & Davila T. (1981) Thiazide diuretics inhibit contractions of isolated smooth muscles. *Pharmacology* 22:108.

Davis JM, Rosenfeld WN (1994): *Chronic lung disease.* In Avery GB, Fletcher MA, *The Newborn.* 4th ed. Philadelphia, J. B. Lippincott Co., p453.

Davis JM, Sinkin RA, Aranda JV. (1990) Drug therapy for bronchopulmonary dysplasia. *Pediatr Pulmonol* 8:117-25.

De Gamarra E. (1992) Energy expenditure in premature newborns with bronchopulmonary dysplasia. *Biol Neonate* 61:337-344.

Deffebach ME, Charan NB, Lakshinarayan S, Butler J. (1987) the bronchial circulation: Small, but a vital attribute of the lung. *Am Rev Respir Dis* 135:463-81.

Durand M, Laval J, Feretti G, Perea I, Wollschlager K, Blin D, Girardet P. (1995) cases of pulmonary artery false aneurysm induced by Swan-

Ganz catheter: treatment by early embolisation. *British Journal of Anaesthesia* 74, suppl. 2:10

Edwards DK (1983): Bronchopulmonary dysplasia today. *In* Milunsky A, Friedman EA, Gluck L (eds) (1983): *Advances in Perinatal Medicine.* Vol.3. New York, Plenum Medical Book Co., p117.

Ehrenkaranz RA ,Mercurio MR. (1992) Bronchopulmonary dysplasia. In Sinclair JC, Bracken MB(eds): *Effective Care of the Newborn Infant.* Oxford, Oxford University Press. p399.

Eidelmann LA, Pizov R, Sprunh CL. (1994) Pulmonary artery catheterization-At the crossroads? *Critical Care Medicine* 22:543-545.

Engelhardt B, Elliott S, Hazinski TA. (1986) Short- and long term effects of furosemide on lung function in infants with bronchopulmonary dysplasia. *J Pediatr* 109:1034.

Farrel PA and Fiascone JM. (1997) Bronchopulmonary dysplasia in the 1990s: A review for the pediatrician. *Curr probl pediatr* 27: 133-163.

Farrell PM & Palta M (1986): *Bronchopulmonary dysplasia*: Report of the 90th Ross Conference on Pediatric Research. Columbus, OH, Ross Laboratories, p3.

Fegler G. (1954) Measurement of cardiac output in anesthetized animals by thermo-dilution method. *J Exper Physiol* 39:153.

Fick A. (1870) Uber die Messung des Blutquantums in den Herzventriklen. Reprinted and translated in Hoff HE, Scott HJ. (1948) Physiology. *N Engl J Med* 239:120.

Finley TN, Swenson EW, Comroe JH. (1962) The cause of arterial hypoxemia at rest in patients with alveolar-capillary block syndrome. *J Clin Invest* 41:618-622

Fischer AP, Benis AM, Jurado RA, et al. (1978) Analysis of errors in measurement of cardiac output by simultaneous dye and thermal dilution in cardiothoracic surgical patients. *Cardiovasc Res* 12:190-199.

Fisher AB and Forman HJ (1985) Oxygen utilization and toxicity in the lungs. *Handbook Physiol.*section 3, 1,231.

Fishman AP, Macklem PT, Mead J, Geiger SR. (1986) Mechanics of breathing. Bethesda, Md. *Handbook of Physiology* vol 3 American Physiology Society.

Fok TF, Lam K, Ng PC, So HK, Cheung KL, Wong W, So KW. (1998) Randomised cross-over trial of salbutamol aerosol delivered by metered don inhale, jet nebuliser and ultrasonic nebuliser chronic lung disease. *Arch Dis Child* 79: F100-4.

Fritts HW, Richards DW, Cournand A (1961) Oxygen consumption of tissues in the human lung. *Science* 133: 1070-1072.

Fritts HW, Strauss B, Wichern W, Cournand A (1963) Utilization of oxygen in the lungs of patients with diffuses, non-obstructive pulmonary disease. *Trans Assoc Am Physicians* 76: 302-311.

Ganz W et al. (1971) A new technique for measurement of cardiac output by thermodilution in man . *Am J Cardiol*, 27:392.

Gerhardt T,Hehre D,Feller R, et al. (1987) Serial determination of pulmonary function in infants with chronic lung disease. *J Pediatr* 110:448-56.

Gill RS, Cunningham DG, Sharpe Md. (1994) Oxygen consumption in sepsis: contribution of the lung [Abstract]. *Am J Respir Crit Care Med* 149:A414.

Glauser FL, Falls RK, Mathers JAL, and Millen JE. (1981) Pulmonary microvascular and alveolar epithelial permeability characteristics in N-nitroso-N-methylurethane-injected dogs. *Chest* 79:217-221.

Godfrey S, Bar-Yishay E, Arad I, Landau LI, Taussig LM. (1983) Flow-volume curves in infants with lung disease. *Pediatrics* 72:517.

Godwin CA,Brown DT, MasurH, Gil VJ, Ognibene FP. (1997) Sputum induction: a rapid and efficient technique for diagnosing pneumocystis carinii pneumonia in immunosuppressd patients. *Respir Care* 36:33-9.

Gore JM, Goldberg RJ, Spodick DH, Alpert JS, Dalen JE. (1987) A community- wide assessment of the use of pulmonary artery catheters in patients with acute myocardial infraction. *Chest* 92: 721-727.

Gothshalll RW, Wood VC, Miles DS. (1989) Comparison of two impedance cardiographic techniques for measuring cardiac output in critically ill patients. *Crit care Med* 17:806-811.

Gregory IC. (1974) The oxygen and carbon monoxide capacities of foetal and adult blood. *J Physiol* (Lond) 236:625.

Gregory TJ, Longmore WJ Moxley MA Whitsett JA, Reed CR, Fowler AA, Hudson LD, Maunder RJ, Crim C, Hyers TM. (1991) Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. *J Clin Invest* 88:1976-1981.

Hachenberg T, Tenling A, Hansson HE, Tyden H, Hedenstierna G. (1994) Ventilation-perfusion inequality in patients undergoing cardiac surgery. *Anesthesiology* 80:509-19

Hack M, Horbar JD, Malloy MH, et al. (1991) Very low birth weight outcomes of the National Institute of Child Health and Human Development Neonatal Network. *Pediatrics* 87:587-97.

Hallman M, and Epstein BL. (1982) Myoinositol decreases N-nitroso-N-methylurethane induced lung surfactant deficiency. *Life Sci* 31: 175-180.

Hammarlund K. (1984) Measurement of oxygen consumption in preterm infants: assessment of a method using a mass spectrometer. *Clin Physiol* 4:519-531.

Harkavy KL, Scanlon JW, Parveen PK, et al. (1989) Dexamethasone therapy for chronic lung disease in ventilator and oxygen-dependent infants: controlled trial. *J Pediatr* 115:979-83.

Harris JD , Jackson F Jr, Moxley MA, Long more WJ (1989) Effect of exogenous surfactant instillation on experimental acute lung injury. *J Appl Physiol* 66:1846-1851.

Haselton PS. (1983) Adult respiratory distress syndrome: a review. *Histopathology* 7:307-32.

Hesterberg TW, Garriets JE, Reister KM, Jackson AC, Cross CE, Last JA. (1981) Bleomycin-induced pulmonary fibrosis: correlation of biochemical, physiological and histological changes. *Toxicol Appl Pharmacol* 60:360-7.

HiFi Study Group. (1989) High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants. *N Engl J Med* 320:88-93.

HiFi Study Group. (1990) High-frequency oscillatory ventilation compared with conventional mechanical ventilation in the treatment of respiratory failure in preterm infants: assessment of pulmonary function at 9 months corrected age. *J Pediatr* 116:933-41.

Hillis LD, Firth BG, Winniford MD. (1985) Analysis of factors affecting the variability of Fick versus indicator dilution measurements of cardiac output. *Am J Cardiol* 56:764-768.

Hindman BJ, Dexter F, Smith T & Cutkomp J. (1995) Pulsatile versus nonpulsatile flow-no difference in cerebral blood flow or metabolism during normothermic cardiopulmonary bypass in rabbits. *Anes* 82; 241-250.

Hindman BJ, Dexter F, Cutkomp J & Smith T. (1995) Diaspirin cross-linked hemoglobin does not increase brain oxygen consumption during hypothermic cardiopulmonary bypass in rabbits. *Anes* 83:1302-1311.

Hoekstra RE, Jackson JC, Myer TF, et al. (1991) Improved neonatal survival following multiple doses of bovine surfactant in very

premature neonates at risk for respiratory distress syndrome. *Pediatrics* 88:10-8.

Horst HM, Obeid FN, Vij D bivins BA. (1984) The risks of pulmonary artery catheterization. *Surgery Gynecology and Obstetrics* 159:229-232.

Hummel H. (1968) Ein neuer magnetischer Sauerstoffmesser mit sehr kurzer Einstellziet und hoher Selektivität. *Chem. Ing Techn.* 40:947.

Ichikawa T, Nakano I, Hirokawa I. (1969) Bleomycin treatment of the tumours of penis and scrotum. *J Urol* 102:699-707.

Imai T, Takahashi K, Fukura H, Morishita, Y (1997) Measurement of cardiac output by pulse dye densitometry using indocyanine green: a comparison with the thermodilution method. *Anesthesiology* 84:816-822.

Ito Y, Veldhuizen RAW, Yao LJ. (1997) Ventilation strategies affect surfactant aggregate conversion in acute lung injury. *Am Rev Respir crit care med* 155:493-499.

Jansen NJG, Oeveren W van, Broek L vd, Oudemans-van Straaten HM, Stoutenbeek CP, Chang Njiek Hhoen M, Roozendaal KJ, Eysman L, Wildevuur CRH (1991) Inhibition of dexamethasone of the reperfusion phenomena in cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 102:515-525.

Jellinek H,Krafft P, Hiesmayr M (1997) Measurement of right ventricular performance during apnea in patients with acute lung injury. *J of Trauma: inj, inf, crit care*(42) 6;1062-1067.

Johnson JD (1976): Therapeutic misadventures. *In* Moor TD (ed): *Iatrogenic Problems in neonatal intensive care*: Report of the 69th Ross Conference on Pediatric Research. Columbus, OH, Ross Laboratories, p21.

Johnston RB (1988) Monocytyes and macrophages. *N Engl J Med* 318: 747-752.

Jolliet P, Theorens JB, Pichard C. (1996) Relationship between pulmonary oxygen consumption, lung inflammation, and calculated venous admixture in patients with acute lung injury. *Intensive care med* 22:277-285.

Kalhan SC, Denne SC. (1990) Energy consumption in infants with bronchopulmonary dysplasia. *J Pediatr* 116:662-664.

Kao LC, Durand DJ, Nickerson BG. (1988) Improving pulmonary functon does not decrease oxgen consumption in infants with bronchopulmonaty dysplasia. *Pediatr* 112:616-21.

Kao LC, Durand DJ, Phillips BL, Nickerson BG. (1987) Oral theophylline and diuretics improve pulmonary mechanics infants with bronchopulmonary dysplasia. *J Pediatr* 111-439.

Kao LC, Warburton D, Cheng MH, Cedeno C, Platzker ACG, Keens TG. (1984) Effect of oral diuretics on pulmonary mechanics in infants with

chronic bronchopulmonary dysplasia: results of a double-bind crossover sequential trial. *J Pediatr* 74: 379.

Kao LC, Warburton D, Platzker ACG, Keens TG. (1984) Effect of isoproterenol inhalation on airway resistance in chronic bronchopulmonary dysplasia. *J Pediatr* 73:509.

Kao LC, Warburton D, Sargent CW et al. (1985) Furosemide acutely decreases airway resistant in chronic bronchopulmonary displasia. *J Pediat* 103:624.

Kao LC, Warburton D, Sargent CW, Plataker ACG,Keens TG. (1983) Furosemide acutely decreases airways resistance in chronic bronchopulmonary dysplasia. *J Pediatr* 103:624.

Keinanen O & Takala J. (1997) Calculated versus measured oxygen consumption during and after cardiac surgery. Is it possible to estimate lung oxygen consumption? *Acta Ana Scand* 41:803-9.

Kennedy TP, Tao NV, Hopkins C Pennington L, Tolley E, Hoidal JR (1989) Role of reactive oxygen species in reperfusion injury of the rabbit lung. *J Clin Invest* 83:1326-1335.

Kharazmi A, Andersen LW, Baek L, Valerius NH, Laub M, Rasmussen J (1988) Endotoxinemia and enhanced generation of oxygen radicals by neutrophils from patients undergoing cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 98:381-385.

King J, DeBoisblanc BP, Mason CM, Onofrio JM, Lipscomb G, Mercante DE, Summer WR, Nelson S. (1995) Effect of granulocyte

colony-stimulating factor on acute lung injury in the rat. Am J *Respir Crit Care Med* 151: 302-9.

Kinirons B, MacSullivan R. (1996) Valve perforation associated with pulmonary catheter insertion. *Anaesthesia* 51:293 (letter).

Kirklin JK, Westaby S, Black EH, Kirklin JW, Chenoweth DE, Pacifico AD (1983) Complement and the damaging effects of cardiopulmonary by pass. *J Thorac Cardiovasc Surg* 86:845-847.

Knopper DC, Mackanjee HR. (1994) Current strategies in the management of bronchopulmonary dysplasia: the role of corticosteroids. *Neonatal Netw* 13:53-60.

Ko W, Hawes AS, Lazenby D, Calvano SE, Shin YT, Zelano JA, Antonacci AC, Isom SE. Krieger KH (1991) Myocardial reperfusion injury. *J Thorac Cardiovasc Surg* 102:297-308.

Korones SB (1996) Complications: Bronchopulmonary dysplasia, air leak syndromes, and retinopathy of Prematurity. *Assisted ventilation of the neonate.* 3rd ed. Ch.18 p.338.

Kox WJ, Chirst F (1991) Oxygen transport pattern in hemorrhagic and septic patients. In : Vincent JL (ed) *Update in intensive and emergency medicine.* Springer, Ber lin Heidelberg New York Tokyo, pp113-119.

Kreybig T (1968) *Experimentelle Pranatal-Toxikologie*, Aulendorf, Editio Cantor.

Kurzner SI. Garg M, Bautista DB. (1988) Growth failure in bronchopulmonary dysplasia: elevated metabolic rates and pulmonary mechanics. *J Pediatr* 112:73-80.

Laurent GJ, McAnulty RJ, Corrin B, Cockerill P. (1981) Biochemical and histological changes in pulmonary fibrosis induced in rabbits with intratracheal bleomycin. *Eur J Clin Invest* 11: 441-8.

Lazo JS, Catravas JD, Gillis CN. (1981) Reduction in rabbit serum and pulmonary angiotensin converting enzyme after subacute bleomycin treatment. *Biochem Pharmacol* 18: 2577-84.

Lee KH, Rico P, Boujoukos AJ. (1995) Measurement of lung oxygen consumption in a patient after double-lung transplant. *J of Thoracic & cardio Sur* 110 (6) 1764-1765.

Levett JJ, Replogle RL. (1977)Thermodilution cardiac utput: a critical analysis and review of the literature. *J Surg Res* 27:392-404.

Levinson MR, Groeger JS, Miodownik S. (1987) Indirect calorimetry in the mechanically ventilated patient. *Crit care med* 15:144-147.

Levitzky MG. (1995) Blood flow to the lung. *Pulmonary physiology* 4th edt pp89.

Lewis JF, Ikegami M, Jobe AH. (1990) Altered surfactant function and metabolism in rabbits with acute lung injury. *J Appl Physiol* 69:2303-2310.

Liau DF, Barrett CR Jr, Bell ALL Jr., Cermamsky G., and Ryan SF. (1984) Diphosphatidylglycerol in experimental acute alveolar injury in the dog. *J Lipid Res* 25:678-83.

Liau DF, Redington Barrett C, Bell AL., and Ryan SF. (1987) Functional abnormalities of lung surfactant in experimental acute alveolar injury in the dog. *Am Rev Respir Dis* 136:395-401.

Liechty EA, Donovan E, Purohit D, et al. (1991;) Reduction in neonatal morality after multiple doses of bovine surfactant in low birth weight neonates with respiratory distress syndrome. *Pediatrics* 88:19-28.

Light RB. (1988) Intrapulmonary oxygen consumption in experimental pneumococcal pneumonia. *J Appl Physiol* 64:2490-2495.

Lijinsky W & Andrews AW. (1979) The mutagenicity of nitrosamides in Salmonella typhimurium. *Mutat Res* 68,1-8.

Lilker ES, Nagy EJ. (1975) Gas exchange in the pulmonary collateral circulation of dogs. *Am Rev Respir Dis* 112:615-20.

Lister G, Hoffman IE and Rudolph AM. (1974) Oxygen uptake in infants and children: a simple method for measurement. *Pediatrics* 53:656-662.

Lister G, Hoffman IE and Rudolph AM. (1977) Measurement of oxygen consumption: assessing the accuracy of a method. *J Appl. Physiol* 3:916-917.

Loer SA, Scheeren TWL,Tarnow J. (1997) How much oxygen does the human lung consume? *Anesthesiology* 86:532-7.

Logvinoff MM, Lemen RJ, Taussig LM,Lamont BA. (1985) Bronchodilators and diuretics in childdren with bronchopulmonary dysplasia. *Pediatr Pulmonol* 1:198.

Lübbe N, Seitz W, Bornscheuer A & Verner L. (1989) Erste klinische Erfahrungen mit dem S&W Kaloximet R, einem Gerät zur O2 Verbrauchsmessung in Anästhesie ung intensivmedizin. *Anästhesie* 38:157.

Mahutte CK, Jaffe MB, Chen PA, Sassoon CS (1994) Oxygen Fick and modified carbon dioxide Fick cardiac outputs. *Crit Care Med* 22:86-95.

Makita K, Nunn Jf, Royston B. (1990) Evaluation of metabolic measuring instruments for use in critically ill patients. *Crit Care Med* 18:638.

Malech HL. Gallin JL (1987) Neutrophils in human disease. *N Engl J Med* 317:687-694.

Mallory GB, Chaney H, Mutich RL, et al. (1991) Longitudinal changes in lung function during the first three years of premature infants with moderate to severe bronchopulmonary dysplasia. *Pediatr Pulmonol* 8-14.

Mammel MC,Fiterman C, Coleman M et al. (1987)   Short-term dexamethasone therapy for bronchopulmonary dysplasia: acute effects and one-year follow up. *Dev Pharmacol Ther* 10:1.

Margaret wood & Alastair JJ Wood. (1990) *Drugs and anesthesia Pharmacology for anesthesiologists 2nd ed.* p592

Mason RJ (1987) Surfactant in adult respiratory distress syndrome. *Eur J Respir Dis* 71 [Suppl]: 229-236.

Masrkestad T, Fitzhardinge PM. (1981) Growth and development in children recovering from bronchopulmonary dysplasia. *J Pediatr* 98:597-602.

McCann EM, Lewis K, Deming DD, Donovan MJ, Brady JP. (1985) Controlled trial of futosemide therapy in infants with chronic lung disease. *J Pediatr* 106:957.

McGuinness K and Childs C. (1991) Development of an indirect calorimeter for use in infants and young children. *Clin Phys Physiol Meas* 12:343-351.

Meer K, Westerterp KP, Houwen RHJ, Brouwers HAA, Berger R, Okken A. (1997) Total energy expenditure in infants with bronchopulmonary dysplasia is associated with respiratory status. *Eur J Ped*156:299-304.

Meriläinen PT. (1987) Metabolic monitor. *Int J Clin Monit Comp* 4:167.

Meriläinen PT. (1988) A fast differential paramagnetic O_2 sensor. *Int J Clin Monit Comput* 1987; 4:167.

Merritt TA, Cochrane CG, Holcomb K, et al. (1983) Elastase and alpha1-proteinase inhibitor activity in tracheal, aspirates during respiratory distress syndrome:role of inflammation in the pathogenesis of bronchopulmonary dysplasia. *J Clin Invest* 72:656-66.

Merritt TA, Stuard LD, Puccia J et al. (1981) Newborn tracheal aspirate cytology: classification during respiratory distress syndrome and bronchopulmonary dysplasia. *J Pediatr* 98:949-56.

Mironneau J,Savineau JP, & Mironneau C. (1981) Compared effects of indapamide, hyfrochlorothiazide and chlorthalidone on electrical and mechanical activities in vascular smooth muscle. *Eur J pharmacol* 75:109.

Montesano R & Bartsch H (1976) Mutagenic and carcinogenic N-nitroso compounds: possible environmental hazards. *Mutat Res* 32,179-228.

Morray JP, FoxWW, Kettrick RG, Downes JJ. (1982) Improvement in lung mechanics as a function of age in the infant with severe bronchopulmonary dysplasia. *Pediatr* 16:290.

Moyer-Mileur LJ, Nielsen DW, Pfeffer KD, et al. (1996) Eliminating sleep-associated hypoxemia improves growth in infants with bronchopulmonary dysplasia. *Pediatrics* 98:779-83.

Myburgh JA, Webb RK, Worthley LI. (1992) Ventilation/perfusion indices do not correlate with the difference between oxygen consumption measured by the Fick principle and metabolic monitoring systems in critically ill patients. *Crit care med* 20:479-482.

Najak ZD, Harris EM, Lazzara A et al. (1983) Pulmonary effects of furosemide in preterm infants with lung disease. *J Pediatr* 102:758.

Neale S (1976) Mutagenicity of nitrosamide and nitrosamidines in micro-organisms and plants. *Mutat. Res* 32,229-266.

Neuhof H and Wolf H. (1978) Method for continuously measured oxygen consumption and cardiac output for use in critically ill patients. *Crit care med* 15:951.

Newburg LH, Johnson MW, Wiley FH, Sheldon JM, Murrill WA. (1937) A respiration chamber for use with human subjects. *J Nutr* 613:193-201.

Ng PC. (1993) The effectiveness and side effects of dexamethasone in preterm infants with bronchopulmonary dysplasia. *Arch Dis Child* 68:330.

Nishikawa T & Dohi S. (1993) Errors in the measurement of cardiac output by thermodilution(Review). *Can J Anaesth* 40:2 142-53.

Northway WH Jr, Rosan RC, Porter DY. (1967) Pulmonary disease following respirator therapy of hyaline-membrane disease. *N Engl J Med* 276:357-68.

Nunn JF and Pouliot JC (1962) The measurement of gaseous exchange during nitrous oxide anaesthesia. *Br J Anaesth* 34,752.

Nunn JF. (1987) Non-respiratory function of the lung. *Applied Physiology.* 3th ed. London, Butterworths, pp285.

Nunn JF. (1993) Non-respiratory function of the lung. *Applied Physiology.* 4th ed. Oxford: Butterworths-Heinemann Ltd 306-317.

Nunn JF. (1996) Pulmonary oxygen consumption. *Int care med* 22:275-276.

O'Brodovich HM, Mellins RB. (1985) Bronchopulmonary dysplasia unresolved neonatal acute lung injury. *Am Rev Respir Dis* 132:694-709.

O'Connor CM. O'Brien A. Sweeney EC. FitzGerald MX. (1986) Progress of bleomycin induced lung fibrosis in rabbits. *British Journal of Experimental Pathology.* 67(4): 461-71, Aug.

Oeveren W van, Kazatchkine MD, Descamps-Latscha B, Maillet F, Fischer E, Carpentier A, Wildevuur ChRh (1985) Deleterious effects of cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 89:888-899.

Ogden BE, Murpht SA, Saunders GC, et al. (1984) Neonatal lung neutrophils and elastrase/proteinase inhibitor imbalance. *Am Rev Respir Dis* 130:817-21.

Oudemans-van Straaten HM, Scheffer GJ. (1993) Oxygen consumption after cardiopulmonary bypass-implications of different measuring methods *Intensive care med* 19: 105-110.

Patel H, Yeh TF, Jain R, and Pildes R. (1985) Pulmonary and renal responses to furosemide in infants wwith stage III-IV bronchopulmonary dysplasia.   *Am J Dis child* 139:917.

Pauling L, Wood RE & Studevant JH. (1946) Oxygen meter. *J AM Chem Soc* 68:795.

Pennock BE and Donahoe M. (1993) Indirect calorimetry with a hood: flow requirements, accuracy, and minute ventilation measurement. *J Appl Physiol* 74:485-491.

Petty TL (1990) Acute respiratory distress syndrome (ARDS). *DM* 36:9-58.

Petty TL, Reiss OK, Paul GW, Silvers GW, Elkins ND(1977) Characteristics of pulmonary surfactant in adult respiratory distress syndrome associated with trauma and shock. *Am Rev Respir Dis* 115:53-536.

Petty TL, Silvers WG, Paul WG, Stanford RE. (1979) Abnormalities in lung elastic properties and surfactant function in adult respiratory distress syndrome. *Chest* 75:571-574.

Phang PT, Rich T, Ronco J. (1990) A validation and comparison study of two metabolic monitors. *J Par Ent Nu* 14(3): 259-6, May~Jun.

Philip AGS (1975) Oxygen plus pressure plus time: the etiology of bronchopulmonary dysplasia. *Pediatrics* 55:44.

Piedboeuf B, Chessex P, Hazan J,Pineault M,Lavoie JC. (1991) Total parenteral nutrition in the newborn infant: energy substrates and respiratory gas exchange. *J Pediatr* 118:97-102.

Pusey VA, MacPherson RI, Chernick V. (1969) Pulmonary fibroplasia following prolonged artificial ventilation of newborn infants. *Can Med Assoc J* 100:451.

Revelly JP, Chioléro. (1997) Relationship between pulmonary oxygen consumpiton, lung inflammation, and calculated venous admixture in patients with acute lung injury. *Int care Med* 23 (4) 481-2.

Richardson P, Bose CL, Dayton V, and Carstrom JR. (1986) Cardiopulmonary function of cats with respiratory distress induced by N-nitroso-N-methylurethane. *Pediatr Pulmonol* 2:296-302.

Riedinger MS, Shellock FG. (1984) Technical aspects of the thermodilution method for measuring cardiac output. *Heart Lung* 13:215-222.

Robin ED. (1985) The cult of the Swan-Ganz catheter- use and abuse of pulmonary flow catheters. *Annals of Internal Medicine* 103:445-449.

Robin ED. (1987) Death by pulmonary artery flow-directed catheter: time for a moratorium? (editorial). *Chest* 92:727-731.

Robin ED. (1988) Defenders of the pulmonary artery catheter. *Chest* 93: 1059-1066.

Roco JJ, Phang PT. (1991) Validation of an indirect calorimeter to measure oxygen consumption in critically ill patients. *J Crit Care* 6: 36-41.

Rojas MA, Gonzalez A, Bancalari E, et al. (1995) Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. *J Pediatr* 126:605-10.

Rooklin AR, Moomjian AS, Shutack JG, Schwartz JG, Fox WW. (1979) Theophylline therapy in bronchopulmonary dysplasia. *J Pediatr* 95:882.

Rush MG, Engelhardt B, Parker RA, et al. (1990) Double-blind, placebo-controlled trial of alternate-day furosemide therapy in infants with chronic bronchopulmonary dysplasia. *J Pediatr* 117:112.

Russell JA, Phang PT. (1994) The oxygen delivery/consumption controversy. *Am J Respir Crit Care Med* 149:533-537.

Ryan SF, Barrett CR, Lavietes MH, Bell ALL, Rochester DF. (1978) Volume-pressure and morphometric observations after acute alveolar injury in the dog from N-nitroso-N-methylurethane. *Am Rev Respir Dis* 118:735-45.

Ryan SF, Bell ALL Jr, Barrett CR Jr. (1976) Experimental Acute alveolar injury in the dogs: Morphologic and mechanical correlations. *Am J Pathol* 82:353-72.

Ryan SF, Liau DF, Bell ALL. (1981) Correlation of lung compliance and quantities of surfactant phospholipids after acute alveolar injury from N-nitroso-N-methylurethane in the dog. *Am Rev Respir Dis* 123:200-204.

Ryan SF, Loomis Bell AL Jr and Barrett CR Jr. (1976) Experimental acute alveolar injury in the dog. *Am J Pathol* 82:353-64.

Ryan SF. (1983) The acute infiltrative lung diseases and the role of the open lung biopsy in their diagnosis. *Pro Surg Pathol* 5:209-42.

Schoental R: Nature 188,420(1960); *Acta Unio Repts.* (U.S.) 52,637 (1937)

Shen AS, Haslett C, Feldsien DC. (1988) The intensity of chronic lung inflammation and fibrosis after Bleomycin is directly related to the severity of acute injury. *Am Rev respir dis*137: 564-571.

Shennan AT, Dunn MS, Ohlson A,et al. (1988) Abnormal pulmonary outcomes in premature infants: predictions from oxygen requirements in the neonatal period. *Pediatrics* 82:527-32.

Sibille Y, Reynolds HY. (1990) Macrophages and polymorphonuclear neutrophils in lung defense and injury. *Am Rev Respir Dis* 141:471-501.

Smithe KM, Mrozek JD, Simonton SC. (1997) Prolong partial liquid ventilation using conventional and high-frequency ventilatory techniques: Gas exchange and lung pathology in an animal model of respiratory distress syndrome. *Crit care med* 25(11):1888-97 Nov.

Smithies MN, & Bihari DJ. (1992) Problems with oxygen consumption in the ICU. *Intensive care med*18:s41.

Smithies MN, Royston B, Makita K, Konieczko K.& Nunn JF. (1991) Comparison of oxygen consumption measurements: Indirect calorimetry versus the reversed Fick method. *Crit care med* 19:1401-1406.

Snider GL, Celli BR, Goldstein RH, O' Brien JJ, Lucey EC. (1978) Chronic interstitial pulmonary fibrosis produced in hamsters by endotracheal bleomycin. *Am Rev Respir Dis* 117:289-97.

Stähelin H, Karnovshy ML, Suter E. (1957) Studies on the interaction between phagocytes and tubercle bacilli. III. Some metabolic effects in

guinea pigs associated with infection with tubercle bacilli. *J Exp Med 105*: 265-277.

Stähelin H, Suter E, Karnovsky ML. (1956) Studies on the interaction between phagocytes and tubercle bacilli. I. Observations on the metabolism of guinea pig leukocytes and the influence of phagocytes. *J Exp Med* 104:121-136.

Stother JK, Warner RM. (1979) Effect of feeding on neonatal oxygen consumption. *Arch Dis Child* 54:415-20.

Svenson KL, Sonander HG, Stevqvist O. (1990) Validation of a system for measurement of metabolic gas exchange during anesthesia with controlled ventilation in an oxygen consuming lung model. *Br J Anaesth* 64:311.

Sykes MK. (1992) Clinical measurement and clinical practice. *Anaesthesia* 47: 425-432.

Takala J, Keinanen O, Vaisanen P and Kari A. (1989) Measurement of gas exchange in intensive care: Laboratory and clinical validation of a new device. *Crit Care med* 17:1041-1047.

Taylor FB, Abrams ME. (1996) Effects of surface active lipoproteins on clotting and fibrinolysis, and of fibrinogen on surface tension of surface active lipoprotein. *Am J Med* 40: 346-50.

Thompson GR, Baker JR, Fleischman RW, Rosenkrantz H, Schaeppi UH, Cooney DA. (1972) Preclinical toxicologic evaluation of bleomycin (NSC125066), a new antitumor antibiotic. *Toxicol Appl Pharmacol* 22:544-55.

Thys DM. (1988) Cardiac output. *Anesthesiol Clin North Am* 6:803-24

Tran PL, Weinbach J,Opolon P, et al. (1997) Prevention of bleomycin-induced pulmonary fibrosis after adenovirus-mediated transfer of the bacterial bleomycin resistance gene. *Journal of Clinical Investigation*.99 (4): 608-17, Feb 15.

van Doorn CAM, lyons G, Swindells S, Unnikrishnan R. (1994) Perforation of the right ventricle and cardiac tamponade: the use of a pulmonary artery catheter. *British Journal of Intensive Care* 4: 66-67.

Van Goudoever JB, Wattimena JDL, Carmielli VP, Sulkers EJ, Degenhart HJ, Sauer PJJ. (1994) Effect of dexamethasone on protein metabolism in infants with bronchopulmonary dysplasia. *J Pediatr* 124:112-118.

Vickers MD, Schnieden H, Wood-Smith FG. (1984) *Drugs in anaesthetic practice 6*th *ed.* p102-3.

Vohr BR, Bell EF, Oh W. (1982) Infants with brochopulmonary dysplasia: growth pattern and neurologic and developmental outcome. *Am J Dis Child* 136:443-7.

Watt JL, Ariagno RL, Brady JP. (1977) Chronic pulmonary disease in neonates after artificial ventilation: distribution of ventilation and pulmonary interstitial emphysema. *Pediatrics* 60:273.

Wechsler W. (1973) Carcinogenic and teratogenic effects of ethyl-and methyl-mirrosourea during pregnancy in the rat. Tomatis, L. & Mohr, U., eds, *Transplacental carcinogenesis* (IARC Scientific Publications

No.4), Lyon, *International Agency for Research on Cancer*, pp.127-142.

Weinstein MR, Oh W. (1981) Oxygen consumption on infants with bronchopulmonary dysplasia. *J Pediatr* 99:958-61 301.

Weiss SJ. (1989) Tissue destruction by neutrophils. *N Engl J Med* 320:365-376.

Westenskow DR, Roberts SE, Pace NL (1989) Evaluation of replenishment type oxygen consumption monitor. *Crit care Med* 17:798.

Westerterp KP, Lafeber HN, Sulkers EJ, Sauer PJJ. (1991) Comparison of short term indirect calorimetry and doubly labelled water method for the assessment of energy expenditure in preterm infants. *Biol Neonate* 60:75-82.

Widdicomb JH, Nathanson IT, and Highland E. (1983) Effects of "loop" diuretics on ion transport by dog tracheal epithelium. *Am J physiol* 245(5):c388.

Wolfson MR, BhutaniVK, Shaffer TH, Bowen FW. (1984) Mechanics and energetics of breathing helium in infants with bronchopulmonary dysplasia. *J Pediatr* 104:752.

Wyse SD, Pfitzner J, Rees A, Lincoln JCR and Branthwaite MA. (1975) Measurement of cardiac output by thermal dilution in infants and children. *Throx* 30:262-265.

Yagoda A, Mukherji B, Young C, et al. (1972) Bleomycin, an antitumor antibiotic: clinical experience in 274 patients. *Ann Intern Med* 77:861-70.

Yeh TF, McClenan DA, Ayahi OA, Pildes RS. (1988) Metabolic rate and energy balance in infants with bronchopulmonary dysplasia. *J Pediatr* 112:73-80.

Yu VYH, Orgill AA, Lim SB, Bajuk B, Astbury J. (1983) Growth and development of very low birthweight infants recovering from bronchopulmonary dysplasia. *Arch Dis Child* 58:791-794.

Zimmerman JJ. (1995) Bronchoalveolar inflammatory pathophysiology of bronchopulmonary dysplasia. *Clin perinatol* 22:429-56.