Validation of Technegas Easy Breather Accessory (TEBA)

A New Concept in Lung Ventilation Scintigraphy

by

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A thesis submitted for the degree of

Doctor of Philosophy

School of Medical Radiation Science

RMIT University, Melbourne, Victoria, Australia

2010

Abstract

Vita Medical Lt approached the principal researcher at Bankstown Hospital Nuclear Medicine department, to examine the possibility of using positive ventilation for patients unable to undergo the conventional method of lung scintigraphy. In consequence, a report and a manufacturing study were produced for Vita Medical to substantiate the use of positive ventilation – TEBA - for the above patient groups. As a result of these findings and initial research work, this was the first time in Australia's field of nuclear medicine such equipment was used for the diagnosis of Pulmonary Embolism. Learning how TEBA apparatus works and conducting several research studies enabled this knowledge to be passed onto other medical radiation scientists.

The Technegas system is in common use for lung ventilation scintigraphy but cannot easily be used in Chronic Airways Limitation or CAL patients due to their moderate to severe dyspnoea, hence the need for the Technegas Easy Breather Accessory or TEBA apparatus.

The findings of this thesis represent a novel aspect in lung ventilation imaging involving a series of experiments with this new apparatus known as TEBA. The investigations were undertaken with both normal and Chronic Airways Limitation (CAL) volunteers and with Intubated Intensive Care patients.

This study centred around four experiments:

Experiment 1	TEBA with CAL patients and normal volunteers
Experiment 2	TEBA with Pulmonary Embolism (PE) patients and normal volunteers.
Experiment 3	TEBA with Intensive Care patients
Experiment 4	Using Technegas with an electron microscope to study patients with known lung disease who had lung ventilation scan prior to a bronchoscopy.

The findings provide important new information about the effectiveness of TEBA and the action of Technegas at the cellular level. Significantly, the results have the potential to change the current thought on Technegas ventilation imaging and its distribution throughout the nuclear medical community.

The data presented in this research were collected from 144 patients in total. In experiment 1 there were 25 normal volunteers who underwent conventional ventilation imaging and 25 normal volunteers who underwent TEBA assisted ventilation imaging, while in experiment 2, there were 25 volunteers with CAL for conventional ventilation and 25 for TEBA; in experiment 3, 25 Intensive Care Patients only had TEBA as they were intubated and therefore required the assistance of positive pressure for their lung ventilation; moreover these patients were too ill to undergo two parts. For experiment four, 20 patients volunteered to have a Technegas ventilation completed prior to their bronchoscopy procedure.

The major findings are presented in **experiments one and two**. These demonstrated that TEBA was effective in distinguishing normal lung ventilation

from ventilation defects found in Chronic Airways Limitation (CAL) and Pulmonary Embolism (PE).

Experiment three demonstrated both normal and PE ventilation patterns of uptake in a group of Intensive Care Patients (ICU) who were not able to undergo the TEBA procedure. This experiment with ICU showed that TEBA ventilation imaging could be used on ventilated and unconscious patients. This has significant implications for the diagnosis and treatment for patients in ICU who formerly could not undergo this procedure. The results of this experiment will have implications for improving the diagnosis and treatment of patients in critical care.

The final **experiment four** studied the action of Technegas at the cellular level and was mainly focussed on the chemical composition of Technegas and its physical structure.

The findings of this thesis provide further evidence for the first line role of lung scintigraphy in the diagnosis of Pulmonary Embolism (PE). This is of vital importance to the large number of patients who are at risk from the life-threatening diseases of PE and Deep Venous Thrombosis (DVT). This is because timely lung ventilation imaging in the diagnosis and follow-up phase of these diseases will reduce the clinical uncertainty in directing treatment and consequently lower health care costs.

Declaration

I certify that except where due acknowledgement has been made, the work is that of the author alone; the work has not been submitted previously, in whole or in part, to qualify for any other academic award; the content of the thesis is the result of work which has been carried out since the official commencement date of the approved research program; any editorial work, paid or unpaid, carried out by a third party is acknowledged; and, ethics procedures and guidelines have been followed.

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Christene Anne Therese Leiper

29th .....day of March 2010

Acknowledgments

The content of the research described in this thesis could not have been completed without the help and support of a great number of people.

I would like to thank my supervisors, Assoc. Prof. S Cowell (RMIT University, Melbourne), Professor R. Benati (Sydney University), Dr B. Elison (Director of Nuclear Medicine, Wollongong Hospital, NSW Australia) and Dr. A. Davison (Sydney University), for their thoughtful advice and careful guidance throughout this project. I am also most grateful to many others; Prof. G. Drummond (Department of Anaesthetics Royal Infirmary, Edinburgh), Robin Henderson (Research and Statistics Royal Infirmary, Edinburgh), Stuart McKenzie (Western General Hospital Edinburgh - Electron Microscopy Unit), John Davies (Department of Nuclear Medicine New Royal Infirmary, Little France, Edinburgh), and Valerie Kidd for her editing.

In particular, much practical help and useful advice was given by Assoc. Prof. Simon Cowell, Robin Henderson, and Stuart McKenzie. The clinical aspects of this work, the energy and enthusiasm would not have been possible without these people. I would like to thank the Medical Radiation Scientists in the Department of Nuclear Medicine at Bankstown – Lidcombe Hospital, Sydney and Dr G Afeaki, Intensive Care Bankstown and Wollongong Hospital.

In addition, I wish to acknowledge the financial support given by Dr Barry Elison that enabled the Trans Electron Microscopy research to be conducted in both Sydney and Edinburgh. Finally, I am grateful for the generosity of the volunteers, who must for ethical reasons remain anonymous.

Dedications

This thesis is dedicated to my family. This research could not have been conducted without their help and support. Firstly to Stephen, who is my closest and longest standing collaborator, best friend and husband. Sarah, who passed away from undiagnosed Pulmonary Embolism (PE), and was the best daughter anyone could have hoped for and who herself achieved academically in education. And my late parents Brenda and Ernest, and family for their gift of optimism and faith in my achievements. I dedicate the findings of this thesis to them all with love, and thanks to everyone.

Abbreviations and Glossary of Terms

Important terms to know in the Intensive Care Unit

Tidal Volume (Vt)	Volume of air inspired or expired during normal breathing (Hess 1996 Essentials of Ventilation)
Respiratory Rate	Mandatory or fixed breath rate is the total frequency of breaths per minute. Spontaneous or unprompted breath rate is dependent on patient's own effort (Fahey 1995 ICU Liverpool Health Service)
Minute Volume	Inspiratory rate x Vt in one minute. Minute volume is utilized to set the lower and upper minute volume alarm limits. Adjusting either tidal volume or respiratory rate will affect Partial Pressure of Carbon Dioxide PaCO2. This is an important measure, as an increase may cause Acute Respiratory Acidosis while a decrease may cause Acute Respiratory Alkalosis (Fahey 1995)
Fraction of inspired oxygen (FIO2)	Sets oxygen concentration in the gas delivered to the patient. The normal setting range is from 21% (room air) to 100% (SW Area Health Service ICU Guideline May 2000)
Sensitivity	Patient effort or triggering device. Also known as amount of negative pressure or flow change patient must generate from circuit to initiate assisted breath within SIMV or intermittent mandatory ventilation (Hess 1996 Essentials of Mech. Ventilation)
Inspiratory Time	Total inspiratory time measured per breath in secs (Fahey 1995)
Inspiratory pause	Apneustic episode of 15 seconds or more. Apneusis or apneustic breathing is an abnormal breathing pattern characterized by a prolonged pause at full inspiration. Also called inspiratory hold, end inspiratory pause and plateau, maintains air in lungs at the end of inspiration (Fahey 1995)
Expiratory time	Total expiratory time measured per breath in secs (Fahey 1995)
Inspiratory: Expiratory (I: E) ratio	Comparing inspiration to expiration as fraction or ratio (Fahey 1995)
Inverse I: E ratio	Reciprocal of I: E ratio (Fahey 1995)
Peak Flow / Flow Rate	Peak flow rate is the speed with which the tidal volume is delivered. It is measured in litres per minute (Hess 1996 Essentials of Mechanical Ventilation)
Pressure Support	Defines inspiratory pressure during pressure-supported breath

Level	(Hillman Liverpool Health Service-Sydney in Critical Care)
Inspiratory pressure	Defines inspiratory pressure during a pressure-controlled breath (Hillman Liverpool Health Service)
Peak Inspiratory Pressure	Maximum airway pressure reached during the delivery of gas into the patient circuit and is dependent on lung compliance, airway resistance, tidal volume and flow pattern (Hillman Liverpool Health Service)
PEEP – Positive End Expiratory Pressure	The aim of mechanical/artificial ventilation is to improve gas exchange, and thus reduce the work of breathing. Used to set the desired level of PEEP in cm H2O (SW Area Health Service ICU Guideline May 2000)
Pressure Limit	The ventilator delivers a breath until set pressure is reached then vents remaining gas (SW Area Health Service ICU Guideline)
Accelerating Waveforms	Section of waveform where flow rate is increasing with time. Flow gradually accelerates in a linear fashion to already set peak flow rate (SW Area Health Service ICU Guideline).
Square Waveform	Reaches its peak flows rapidly and maintains this rate until breath is delivered. Peak flow rate is delivered immediately at onset of inspiration, maintained throughout inspiratory phase, and abruptly terminated at onset of expiration. This is the most commonly used wave pattern (SW Area Health Service ICU Guideline).
Sine Waveform	The sine waveform was designed to match the normal flow waveform of a spontaneously breathing patient. Inspiratory flow rate gradually accelerates to peak flow and then tapers off. (SW Area Health Service ICU Guideline)
Weaning a patient from ventilation	Weaning means that a patient will go through a gradual transition from mechanical ventilatory support to spontaneous breathing. (Laghi 1995 Weaning: Current opinion in Critical Care 1: p.71-76)

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CHAPTER 1 LITERATURE REVIEW

1. 1 Introduction

According to Van Beek, 1996, Pulmonary Embolism (PE) is responsible for more deaths than any other single disease yet it is often under diagnosed. Beek suggests PE will only be diagnosed in thirty percent (30%) of cases, while Faiad and associates (1997) report that “Pulmonary Embolism” is the leading cause of death in all age groups. A good clinician who suspects their patient may have PE, actively pursues a definitive and prompt diagnosis, because the correct diagnosis and treatment can dramatically reduce the mortality of the disease (Faiad 1997).

PE is seen as such a serious life-threatening illness because it can cause permanent damage to part of the lung due to the lack of blood supply to lung tissue, resulting in cyanosis from low blood oxygen levels or leading to damage of other vital organs from lack of oxygen (Howarth, 1999). Acute respiratory consequences of pulmonary embolism include increased alveolar dead space, pneumoconstriction, hypoxia and hyperventilation. Prolonged exposure to lack of perfusion includes regional loss of surfactant and subsequent pulmonary infarction (Kamanger, 2010).

If a large enough clot is left untreated, PE will cause death. Of the many patients who die every year from PE, most die within thirty (30) to sixty (60) minutes after symptoms commence (Howarth, 1999).

PE is one of the most common causes of death in hospitalised patients who must remain immobilised. The greatest risk of PE occurs in patients with

previous deep vein thrombosis (DVT) or a history of previous PE (Clinical Imaging, 1994). In addition, the risk of PE doubles every ten (10) years after the age of sixty-five (65) (Faiad, 1997). Therefore, in order to effect, timely and appropriate treatment of this disease, it is important to gain an accurate diagnosis of PE as quickly as possible. There are various types of diagnostic tools used in the clinical diagnosis of PE. These tools are:

- Physical examination of the patient
- Chest Radiograph (CXR)
- Computerised Tomography (CT)
- Pulmonary Angiography
- Computed Tomographic Angiography (CTA)
- Lung Scintigraphy

1.2 Examining patients

1.2.1 Physical examination

According to Faiad (1999), PE can be so lethal that the diagnosis should be sought actively in any patients presenting with chest symptoms. These symptoms may be chest wall and shoulder pain, wheezing, palpitations, and in the relatively small number of patients who present with haemoptysis, suggesting an embolus.

Thus the physical examination is of vital importance to the diagnosis of PE. The majority of PE patients demonstrate an increased respiratory rate and present with rates audible on auscultation. An increased second heart sound is also common (Ramzi, 1994). Other physical signs of PE may include tachycardia,

increased temperature, physical evidence of phlebitis, cardiac tamponade, diaphoresis, oedema, cardiac murmur and cyanosis (Ramzi, 1994). In addition, patients with PE often present with primary or isolated complaints of seizure, syncope, productive cough, fever, new onset of a reactive airway disease known as “Adult Asthma” and/or atrial fibrillation as well as an array of other symptoms (Faiad 1997). It is therefore not surprising that the clinical diagnosis of PE is fraught with error. Signs and symptoms serve only to raise the suspicion of PE.

1.2.2 Chest Radiograph

The chest radiograph (CXR) is often the first imaging procedure obtained as the patient presents with dyspnoea. Many studies have reported that a normal CXR occurs only in the minority of cases of PE. However in most patients with a positive diagnosis of PE the CXR is often abnormal. For example, a study by Galvin and associates in 1994 of patients with PE and no pre-existing cardiac or pulmonary disease reported only fourteen percent (14%) of the chest radiographs were normal.

Thus the standard CXR on its own may display densities that could indicate the presence of a number of pulmonary disorders. For example, areas of Atelectasis have been found in the lower lobe and reported as areas of parenchymal density (Rays, 1996). In addition, after twenty-four (24) to forty - eight (48) hours, one third of the patients with proven PE develop focal infiltrates and signs of pneumonia on their CXR. To further complicate the use of CXR results, most of the densities that appear in the CXR are caused by

pulmonary haemorrhaging and oedema and can be confused with infectious infiltrates or malignant masses (Rays, 1996).

Pleural effusions are commonly reported on the CXR and are mostly unilateral despite the fact that most clots are bilateral (Faiad, 1997). In many cases the diaphragm may be elevated, reflecting volume loss in the affected lung and the central pulmonary arteries may be prominent either from pulmonary hypertension or the presence of a clot in those arteries (Faiad, 1997).

As a result of the variable findings on the CXR, in isolation from other imaging procedures, the CXR is not able to provide a positive diagnosis for PE (Rays, 1996). Therefore, although a useful first line of attack in the diagnosis of PE the CXR can only assist in evaluating an alternative diagnostic consideration (Clinical Imaging, 1994).

1.2.3 Computerised Tomography (CT)

In contrast to the CXR, the faster scan times in current generation CT scanners enable CT to provide a more detailed assessment of the lung parenchyma (Madan, 2000). For example, CT is able to readily identify a bulging appearance at the boarders of the lungs due to the affected lobule being filled with blood and oedematous fluid (Van Beek, 1996). CT is also able to resolve a linear strand at the apex that usually represents a distal pulmonary artery filled with a clot, while low attenuation areas on CT usually represent viable lung tissue (Faiad, 1997).

Since the clinical presentation of PE is non-specific, the findings on CT are often the first clinical indication that the patient may be suffering from PE (Murray, 1988). In addition to visualising the area of infarction, CT can see the

exact location of the clot. CT has also been shown to be useful in patients suffering from CAL, dyspnoea, and known pulmonary artery hypertension (Clinical Imaging, 1994). These diseases are all difficult to diagnose.

Recent improvements in the resolution of CT have enabled this modality to image very small PE. Therefore one may ask what is the risk-to-benefit ratio of anticoagulating these patients with small sub-segmental PE who have no other clot and no ongoing risk factors for venous thromboembolism (Rays 1996). Despite these improvements in technology, a major consideration particularly for patients receiving multiple CT scans, is the significant radiation burden that CT delivers to the patient (Clinical Imaging, 1994).

1.2.4 Pulmonary Angiography

Until the advent of high resolution helical (spiral) computed tomographic angiography (CTA), pulmonary angiography was known as the “gold standard” imaging procedure for identifying emboli that lodge in segmental or larger arteries. Pulmonary angiography has the major disadvantage of being highly invasive and not without risks to the patient as it requires the placement of a catheter from either the femoral or a peripheral vein through the right - sided cardiac structures including the right atrium, tricuspid valve and right ventricle into the main pulmonary artery. The catheter is then directed into either the right or left pulmonary artery using the ventilation perfusion scan as a “road map”. The intravenous administration of contrast media in pulmonary angiography provides a more detailed view of the pulmonary vasculature (Madan, 2000).

The most reliable signs of PE in a pulmonary angiogram include an intraluminal filling defect and an abrupt termination of a branch vessel. In addition,

pulmonary angiography is most accurate for perfusion defects in segmental and larger sized arteries. However, the reproducibility of readings in sub-segmental and smaller vessels is poor (Madan, 2000).

The majority of patients with a negative angiogram have a good prognosis (Ramzi, 1994), hence the former “gold standard” status of this imaging modality. However, pulmonary angiography is an invasive procedure with minimal though measurable levels of patient morbidity and mortality (Madan, 2000).

1.2.5 Computed Tomographic Angiography (CTA)

CTA is less invasive than pulmonary angiography as it does not require pulmonary artery catheters and is more sensitive than pulmonary angiography (Clinical Imaging, 1994). Recent reports indicate that CTA’s absolute sensitivity and specificity is evolving over time so that much smaller emboli are able to be identified even in distal lung vessels (Faiad, 1997). However CTA, due to its high cost, is found only in major hospital centres. In addition, CTA gives a high radiation dose to the patient plus it cannot easily distinguish between PE, CAL and other overlying respiratory conditions.

1.2.6 Lung Scintigraphy

Lung scintigraphy, also known as the V/Q scan is performed in two parts, the ventilation and the perfusion study (Taylor, 2000). The ventilation scan (V) assesses the airflow to the lungs. In this procedure, patients are asked to inhale a radioactive aerosol or gas. Images can then be taken at multiple angles around the chest. The perfusion lung scan (Q) assesses the blood flow to the lungs (Taylor, 2000). Following the intravenous injection of a radioactive tracer

such as Macro Aggregated Albumin or MAA, patients are imaged around the chest using the same angles as the ventilation images (Taylor, 2000).

- ***Perfusion Lung scans***

The perfusion scan evaluates blood flow within the lungs. In the case of suspected pulmonary embolus (PE), both ventilation and perfusion scintigraphy can be performed either simultaneously or one immediately after the other. If ventilation is normal but perfusion is abnormal, a “mismatch” is said to exist. This mismatch is often indicative of a PE (Taylor, 2000).

- ***Lung Perfusion Radiopharmaceuticals***

The principle of the perfusion scintigraphy is simple. It relies on the virtually complete removal of intravenously injected radioactive particles by arteriolar capillary blockade. Provided these particles are mixed homogeneously in blood in the right side of the heart, and are almost entirely extracted from the pulmonary circulation during the first passage, their appearance in the lung will provide images of regional pulmonary perfusion (Taylor, 2000, Murray, 1998).

- ***Particles used for Lung Scintigraphy***

Red blood cells are 7 ± 1.5 μm in diameter and are able to pass through the smallest capillaries which are 8.2 $\pm$ 1.5 μms in diameter (Cheng, 2000). The pre-capillary arterioles are 25 $\pm$ 10 μm in diameter, compared with the much larger distribution arteries 80 $\pm$ 20 μm (Cheng, 2000). Injected particles in the perfusion lung scan are larger than particular vessels and are therefore

selectively filtered out almost entirely on the first pass of blood through the lungs (Murray, 1988).

The most commonly used particulate material used in perfusion lung scans is known as Macro Aggregated Albumin or MAA. The particles used in MAA contain the following size distribution:

10 - 20 μm	66%
20 - 40 μm	26%
40 - 60 μm	6%
60 - 80 μm	2%

Therefore most of the particles found in MAA lodge in the pre-capillary arterioles and not in the capillaries (Taylor, 2000). The human lung contains about 200 to 300 x 10^6 pre-capillary arterioles so that a lung scanning MAA dose of 1 x 10^6 particles would block only 0.3 to 0.5% of these vessels (Zöphel et al., 2009). Perfusion lung scanning has a reputation of great safety and can be used in severely ill patients without apparent ill effect (Murray, 1988).

When macroaggregates of albumin are used, less than 1mg of protein is administered. A considerably larger quantity of protein must be given experimentally before any effects are noted (Habibian, 1999). It is usually accepted that the adult human dose should contain 200,000 - 400,000 particles. Doses much below 100,000 particles may produce poor scans. It is therefore important that the amount of activity added to the kits should not greatly exceed the manufacturer's specifications (Murray, 1988). The TC-99m MAA vial should be agitated prior to withdrawing the dose. Immediately prior to injection, the syringe should be gently inverted a few times to ensure re-suspension of the particles (Habibian, 1999). The radiopharmaceutical should be injected with

patient supine. The patient should breathe deeply during the injection and blood must not be drawn back into the syringe as this could cause “clumping” if re-injected into the patient (Taylor, 2000).

- ***Ventilation Scans***

The ventilation scan is used to evaluate the ability of air to reach all portions of the lungs, whereas the perfusion scan measures the supply of blood through the lungs (Danjan, 1994). A ventilation and perfusion lung scintigraphy (V/Q) scan is most often performed to detect a PE but it is also used to evaluate lung function in people with advanced pulmonary disease such as CAL, Chronic Bronchitis and to detect the presence of shunts (abnormal circulation) in the pulmonary blood vessels (James, 1995). In a ventilation scan a mask is placed over the nose and mouth, and the patient is asked to breathe the gas while lying supine on the gantry table outside the scanner. Six standard images are acquired post ventilation (Isawa, 1991); these include anterior, posterior, right and left anterior and posterior oblique images.

1.2.7 Ventilatory Systems

There are four types of ventilatory systems used for intubation. Every patient will be different regarding which classification and mode of ventilation will be required, and this will change at different stages of their disease. In addition, Intermittent Positive Pressure (IPPV) has two distinct pathways, either volume controlled ventilation or pressure controlled ventilation; from both these groups,

depending on both the pathological and physical condition of the patient, there will be varying modes.

1) Pressure cycled ventilation

Here the ventilator is manually set to reach an upper pressure limit; once the patient reaches this level, inspiration ceases and expiration commences. The inspiratory time, inspiratory flow rate, and tidal volume vary with every breath, depending on a patient's resistance and compliance, according to Hess (1996).

2) Volume cycled ventilation

In the volume cycled system, the ventilator is set to reach a set tidal volume, once this tidal volume is reached, inspiration ends and expiration commences. The airway pressure attained, inspiratory flow rate, and inspiratory time vary with each breath, depending on the patient's resistance and compliance.

3) Time cycled ventilation

If using time cycled ventilation, inspiration ends and expiration begins after a predetermined time interval has been reached. Generally an inspiratory time, a respiratory pause or plateau, or an I-E ratio (inspiration/expiration) are set, depending on the specifications of the ventilator used, to determine time cycling. The airway pressure attained, the inspiratory flow, and the tidal volume vary with every breath, depending on the patient's resistance and compliance.

4) Flow cycled ventilation

Flow cycled ventilation uses the principle that inspiration ends and expiration begins when the flow rate drops to a predetermined percentage of its peak value. The tidal volume and inspiratory time vary with every breath, depending on the patient's resistance and compliance (Fahey, 1995).

1.3 Aerosols in Nuclear Medicine

Aerosols were discovered by Burch in 1965 and are described as very small particles suspended in air (Burch, 1965). Since that time research has shown (Atkins, 2004) that images do not really measure regional ventilation but rather airway patency and the relationship between ventilation and aerosol particle deposition (Kuni, 2000).

With the introduction of smaller and more uniform aerosol particles it is seen that >70% of the deposited activity is located in the lung parenchyma (James, 2002).

In Chronic Airways Limitation or CAL patients, aerosol droplets of particles <2.0μm are deposited in the central airways in greater amounts, roughly in proportion to the airways obstruction. In moderate CAL the aerosol will show some central deposition, with reasonable penetration of activity to the periphery of the lungs. Severe CAL has marked central deposition with much impaired or absent peripheral penetration of the labelled aerosol (James, 2002).

Aerosols that have been used in Nuclear Medicine lung scintigraphy include:

^{133}Xe Xenon,
^{127}Xe Xenon
Krypton-81m
Tc-99m Diethylene triamine-pentacetic acid (DTPA)
Tc 99m Technegas.

1.3.1 ^{133}Xe Xenon

Since its introduction in 1965, ^{133}Xe had been the most widely used radionuclide for assessing pulmonary ventilation (Cherng, 2000). More recently, ^{127}Xe also

has been used (Cherng, 2000). Since Xenon is physiologically inert, the relatively long physical half-lives of ^{133}Xe (5.3 days) and ^{127}Xe (36.4 days) do not contribute to patient radiation exposure. This is because most of the gas is removed from the body by expiration. The usual activity administered for a single ^{133}Xe wash-out procedure ranges between 370 and 740 MBq. Ventilation scanning is undertaken by using radioactive Xenon, for which the patient breathes through a closed gas delivery system (Cherng, 2000).

Upon injection of Xenon gas into the delivery system, it starts to be taken into the lungs. This process of “wash in” is recorded. Further imaging is done during equilibrium and finally the system is opened to room air during inhalation which leads to the “wash-out” of the radioactive Xenon (Cherng, 2000). When Tc-labelled aerosols are used, the agent sticks to the bronchiolar and alveolar walls once inhaled, thus allowing the measurement of uptake and equilibrium distribution, but not the wash-out (Cherng, 2000).

Xenon-133 (gamma energy lower than Tc-99m, dosage 40 MBq) and aerosol ventilation scans (dosage 800 - 1,200 MBq = 22 -32 mCi, of which only about 10% will actually be used) have to be acquired prior to perfusion scanning, as the perfusion agent remains in the lung after perfusion scanning and therefore also appears in the ventilation image (Cherng, 2000).

While Xe-133 scans are usually done using a dorsal projection, aerosols are imaged with the projections given above. Aerosols are given in a dose which is so small that the remaining radioactivity of the ventilation study will not interfere with the radioactivity from the perfusion study (Cherng, 2000).

1.3.2 ^{127}Xe Xenon

Xe-127 (dosage 40 MBq), has a gamma energy higher than that of Tc-99m, and can be used in post-perfusion ventilation scanning (Hannah, 1992). The advantage of this technique is that the projection most clearly showing a perfusion defect can also be imaged during ventilation, but has the disadvantages of high cost and limited availability (Cherng, 2000).

1.3.3 Krypton-81m

Krypton-81m is a poorly soluble inert gas with a 13 second half-life. It decays to Krypton-81m by isomeric transition, emitting 190 KeV gamma rays and internal conversion electrons (Cherng, 2000). The gas is obtained by eluting a generator containing its 4.7 hour half-life parent Rubidium-81m; this is a cyclotron produced positron emitter, hence has limited availability (Cherng, 2000). During a ventilation scintigraphy scan, patients breathe at their own pace from a reservoir that stores the continuously eluted Krypton 81m between inspirations. The short time required for imaging Krypton 81m, its simplicity, and the low absorbed radiation dose enable images of ventilatory flow to be obtained in multiple views (Cherng, 2000).

1.3.4 Tc-99m DTPA Aerosol

Tc-99m Diethylene triamine-pentacetic acid (DTPA) is administered via a nebulizer of which the patient should receive an administered activity not exceeding 80MBq in the lungs (Clinical Imaging, 1994). The amount of administered activity should be reduced if the perfusion study is to be performed immediately after ventilation study. As both agents are labelled Tc-99m, the

count rate of the second study must be at least four times the count rate of the first study (Clinical Imaging, 1994). The aerosol should be administered supine. DTPA aerosol from a nebuliser has been found to be a useful marker for interstitial lung disease as well as for PE (Rays, 1996). However, delivering the aerosol to a patient whose breathing was already compromised, is not easy nor always successful.

Lung ventilation imaging is frequently performed with Tc-99m DTPA, derived from a commercial nebuliser. Airborne contamination is a significant problem with this procedure; it results in exposure of staff to radiation and can reduce gamma camera performance when the ventilation is performed in the camera room. It is essential for the patient to have his/her mouth securely around the mouthpiece as this is where leakage of DTPA occurs when the lips are not tightly secured.

1.3.5 Tc-99m Technegas

A serendipitous experience by Dr Bill Burch in 1986, when he accidentally inhaled an ultra-fine mist of technetium “vapour”, led to the development of a ventilation agent that became known as Technegas (Burch et al, 1986). Technegas can be produced readily from standard sodium pertechnetate. On inhalation, it diffuses and adheres to the alveolar walls, enabling multiple conventional views. The production method involves the use of a Technegas Generator in which 800 - 1000 MBq of Tc-99mO_4^- is evaporated to dryness in a graphite crucible (Lemb, 1993). The crucible is heated to 2500^oC in an atmosphere of pure Argon for 15 seconds. The resulting vapour and Argon mixture are inhaled by the patient via a Patient Administration Set (PAS). The

Technegas produced is a dispersion of carbon particles containing Tc-99m atoms, either trapped within the core or between overlapping sheets. These clusters have a maximum size of 0.017 μm and the carbon mass inhaled by a patient in a Technegas study was measured as 5 micrograms (±20%) (Lemb, 1993).

Technegas, the radioaerosol used for ventilation, should not show any significant clearance from lungs or redistribution for the duration of image acquisition, allowing multiple images to be taken (Senden, 1997). Technegas with effective half-time of 335 minutes has an advantage over Xenon 133 as it remains within the lung to allow multiple planar and/or Single Photon Emission Computed Tomography (SPECT) imaging to be done.

This 335 minute half-time is nearly equivalent to the half – life due to physical decay alone (361 minutes), therefore only a minimal dose of Technegas is required for lung scintigraphy, resulting in a relatively low incidence of side effects (Burch, 1986).

Thus aerosol deposition patterns in the lungs are helpful in the differential diagnosis of chronic obstructive lung diseases such as CAL and diagnosing PE, (Burch, 1984). Technegas being an ultrafine aerosol, is probably more useful for ventilation studies than conventional aerosols produced by a nebulizer. Besides respiratory lung function, pulmonary nuclear medicine techniques have made it possible to study non-respiratory lung function such as mucociliary clearance mechanisms.

1.4 Interpretation of Images

The interpretation of lung images will give information on the regional perfusion and ventilation of the lung. The physiological change caused by PE is decreased perfusion with normal ventilation or V/Q mismatch. Most cases of PE are multiple and reported as high probability scan in which two or more segments of V/Q mismatches have occurred. Other scanning reports will have varying diagnostic yields. High probability of PE shows perfusion defects of greater than ninety percent (90%), intermediate 30 to 40 percent (30% - 40%), low probability is less than ten percent (10%) and normal lung scan is zero (0) (Danjun, 1994).

Repeat scintigraphy is requested in seven days for a positive result of PE to assess response to anticoagulant therapy. The objectives of treatment for PE are to prevent death and recurrent DVT and PE and to prevent the post – phlebotic syndrome (damage to valves within the deep veins). Anticoagulant drugs constitute the mainstay of treatment, and graduated compression stockings (for up to 24 months) significantly decrease the incidence of the post-thrombotic syndrome (Taylor, 2000).

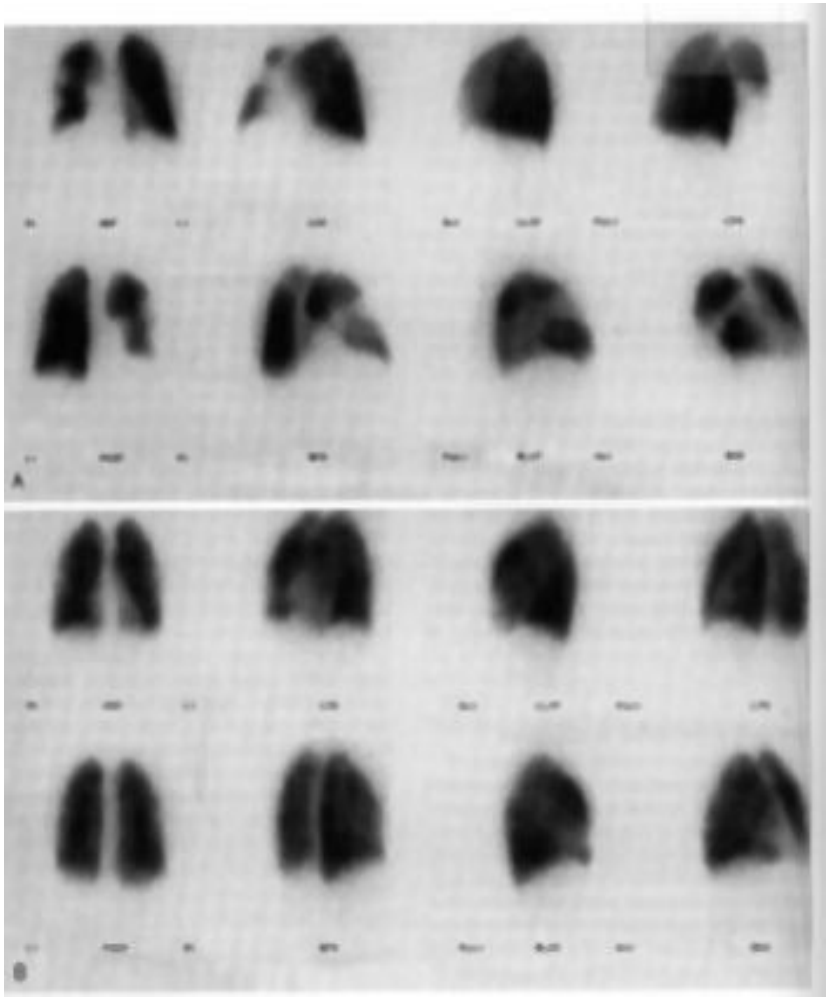
The problems in clinical diagnosis of PE relate to the non-specificity of clinical signs and symptoms. Other problems relate to the invasive nature and increased costs associated with diagnostic procedures (Prospective Investigation of Pulmonary Embolism Diagnosis, PIOPED, 1990). These include CXR, Pulmonary Angiography, Spiral CT, MRI and V/Q Scans. The decision about the right combination of tests to investigate PE is subject to various debates. Smith et al. 2000 believed Spiral CT was the only way to fully observe intraluminal filling defects, as the pulmonary arterial phase and venous phase

can be clearly separated after injection with intravenous contrast medium. However, in a completely occluded vessel, estimation of the degree of location of perfusion defects is far better seen with lung scintigraphy (Early, 1995).

Economic evaluations of performing lung scintigraphy in nuclear medicine require a report of the analysis as well as the way in which the study has been performed. A direct specification of at least two alternative programs involves economic evaluation, an outcome measure, method of costing, benefits, and an allowance for uncertainties e.g. by implementing a sensitivity analysis (PIOPED,1990). Decision analysis can be based on clinical trials, retrospective (PIOPED,1990). Decision analysis can be based on clinical trials retrospective studies or databases. The outcome measure is therefore the key to an overall economic evaluation (PIOPED, 1990). In nuclear medicine, cost effectiveness analysis benefits are frequently used. Effectiveness is applied to the number of studies performed, survival probability, morbidity, and quality of life etc. (EJNM, 2004). To diagnose deep venous thrombosis (DVT) is the first step in patient selection as it has a high incidence and significant mortality rate (Taylor, 2000). Patients with symptoms of chest pain, dyspnoea and tachypnoea with absent cardiorespiratory disease should be investigated for underlying PE (James, 1995). The concern for time, patient selection and various procedures can be overcome by integrating results in ‘synergism’ thus together or in unison, therefore an added value for the patient and diagnostic evaluation. Teamwork of specialists from different disciplines is necessary to integrate their competencies and attain established objectives (Taylor 2000). Advantages of risk stratification for diagnosis of patients suspected of having PE have been shaped primarily by diagnostic accuracy studies. Much more meaningful to the

practice of clinical medicine are outcome studies. There are risks and expense associated with anticoagulation treatment, therefore a certain degree of offsetting benefit of treatment. In high probability patients treatment has been well established what is still uncertain is whether there are patients who have insignificant PR (Taylor, 2000).

Figure 1.1 **Perfusion lung scan demonstrating inhomogeneous flow pattern in a patient with primary pulmonary hypertension.** Nuclear Medicine, Bankstown, Lidcombe, Sydney



This image (figure 1.1) is a ventilation/perfusion scan demonstrating multiple defects consistent with thromboemboli in a patient with chronic pulmonary hypertension.

PE may include subtle abnormalities, which are diagnosed as PE and are false positives. However as diagnostic equipment improves, smaller emboli will be detected, and then we should ask when emboli would be regarded as clinically significant (James, 1995). Emphasis must therefore be placed on patient outcome research looking at thromboembolic disease.

Patients who had a low probability scan / diagnosis for PE were not treated and a prospective study outcome by Wells in 1998 concluded that: “management of patients with suspected PE on the basis of pre-test probability and the results of ventilation-perfusion scanning are safe” (PIOPED).

Pre-test probability has been assessed using a defined algorithm that considered signs and symptoms, and that an alternative diagnosis was responsible for patients’ symptoms and presence of risk factors (Rays, 1996). A more realistic approach that would result in better diagnosis and management for patients would be to use risk stratification. This would involve categorizing patients into high risk, intermediate and low probability of pulmonary embolism. Risk stratification requires pre-test probability and taking a thorough patient history for evidence of underlying cardio-respiratory disease, the presence of PE and whether there is potential for future emboli occurring (Rays, 1996).

Both lung scintigraphy and spiral CT play a potential role in the risk stratification and assessment of PE. However, it is still uncertain whether it is better to use spiral CT to observe intraluminal filling defects or lung scintigraphy for regional perfusion abnormalities. In patients with an increased pulmonary vascular resistance, which can be fatal, there has been considerable argument (Clinical Imaging, 1994) as to whether the assessment of ventilation and perfusion defects with lung scintigraphy has a more prognostic significance in risk

stratification than the presence of intraluminal filling defects using spiral CT. The Wells (1998) report provided satisfactory evidence that non-invasive tests, such as lung scintigraphy, can safely be used to identify low risk patients.

There is little evidence to suggest spiral CT can be used to risk-stratify patients. A study carried out by Rathbun and associates in 2004, concluded that use of helical CT in the diagnosis of PE has not been adequately evaluated as the safety of not administering anticoagulants to patients with negative results on spiral CT is uncertain (Clinical Imaging, 1994).

Lung scintigraphy still plays an important first line role in the diagnosis of PE. The efficacy of lung scintigraphy in diagnosis and treatment monitoring played a significant role in reducing the clinical uncertainty, in directing treatment and in lowering health care costs in PE (Kobzik, 2000).

Henry Royal (1999) suggested lung scintigraphy is a useful tool in stratifying patients suspected of PE. “Sometime in the future, lung scans may be in need of life support, but today, lung scans are alive and well.” (European Economic Evaluation 1999).

However, a major limitation in lung scintigraphy is the high amount of intermediate or indeterminate probability lung scans still being reported. Nonetheless, the number of lung scintigraphy results in these categories has declined due to patient clinical pre-selection and improved scanning procedures (Rays, 1996). Lung scintigraphy itself remains pivotal in the diagnosis of PE even though there is divided opinion on the choice of interpretive criteria for lung scintigraphy reporting. Further study is therefore needed to help identify patients with suspected PE who may benefit from additional diagnostic

procedures after low or intermediate probability lung scintigraphy (Howarth,1999).

1.4.1 Technegas study at the Cellular level

Studying Technegas at the cellular level will allow both the physical characteristics of the Technegas aerosol particles and their behaviour to be studied. Investigating this area links both the properties of chemistry and the physical nature of Technegas together as they are related since the behaviour of the particles in the lung and appearance depends on their physical properties (Burch, 1993).

Technegas particles have a uniform deposition in the lungs, as particles are much smaller than conventional radioaerosols thus making these Technegas particles penetrate deeper into the lungs (Strong, 1989). Small particles of Technegas give better penetration into the lung but are more difficult to produce and do carry less mass of the prepared aerosol. Particle size distribution and inhalation methods may allow us to have a better knowledge of the method of Technegas delivery to different sections of the lung (Burch, 1993).

Trans Electron Microscopy, known as TEM, is used for research of Technegas as both the resolving power and resolution are specified using the minimum resolvable distance. To-date research done in this area of cellular structure of Technegas post ventilation on humans has been minimal. There has, however, been a great deal of information on Technegas at the chemical level and its physical structure, looking mainly at the biochemical side of Technegas and pertechnetate.

Examining bronchial washings and sputum under TEM may provide information on the Technegas particle and its characteristics (Strong, 1989). Studies of particle size have also suggested that lung scintigraphy in CAL patients is often non-uniform, of poor quality, and the lung deposition is degrading the end result of the diagnostic test (James, Brown, 2002).

1.5 Diseases of the Lung and Respiratory System

1.5.1 Perception of Chronic Airways Limitation (CAL)

When asked to imagine a patient who presents with a 'disabling disease of the airways', most people think of an asthmatic, possibly a child, in the throes of an acute exacerbation, desperately searching for their metered-dose inhaler for relief (American Lung Association, March 2002). Compare this with the CAL patient, usually over 60 years old with breathlessness, wheeze and productive cough, whose disease is probably self-inflicted by smoking and who responds relatively poorly to treatment. No wonder there is such a chasm between these two images in the public perception and that of healthcare workers (FDA, 1999).

The good news is that research has raised the profile of CAL to the extent that international bodies responsible for respiratory care are now producing guidelines and they, too, are attempting to raise the profile and awareness of CAL.

CAL is not a single entity but a collection of conditions that share the features of chronic obstruction of expiratory flow. As a diagnostic label, it encompasses many previously used clinical descriptions including chronic bronchitis,

emphysema, chronic obstructive airways disease, chronic airflow obstruction and some cases of chronic asthma which have resulted in irreversible lung destruction (FDA, 1999). CAL is one of the five most lethal diseases in the world. Both its mortality and its frequency are increasing. The disease is closely associated with smoking. Studies have shown that smoking is associated with up to a 20–fold increase in the risk of death from CAL. Smoking can lead to the two most common forms of this disease, emphysema and chronic bronchitis. CAL is largely a disease of the elderly. Although symptoms may begin to occur in the 40s, the disease is generally not diagnosed until the patient has reached his or her 60s. There is no known cure for CAL, so it is very important to learn how to effectively manage the disease. Strategies for managing CAL include making lifestyle changes (e.g. quitting smoking) and taking medications such as bronchodilators (Lippincott, 1991).

In more severe cases, oxygen replacement therapy in which patients breathe oxygen from either oxygen cylinders or electric concentrators that extract oxygen directly from air. In extreme cases surgery to reduce the lung volume or even lung transplantation is recommended. Researchers are studying experimental medications and surgeries in the hope of finding a more effective treatment for CAL and, ideally, a cure (American Lung Association, 2002).

1.5.2 What is Chronic Airway Limitation

Chronic Airway Limitation (CAL) is a chronic, progressive disease of the lungs that gradually reduces airflow. It is characterized by phlegmy coughing, wheezing and dyspnoea. As the disease progresses, quality of life may be

severely compromised. The key features of CAL are of a slowly progressive condition characterised by marked airways obstruction, which does not change markedly over time. Under the microscope, pathological changes can be seen in the large airways, small bronchi and bronchioles, and in the lung tissue itself as well as lung blood flow (Hannah, 1992).

Hypersecretion of mucus and airway inflammation occurs primarily in the large airways. The small airways are the sites of increased airways resistance. The alveoli are also destroyed and this is described as emphysema. In CAL patients radioaerosol images with airways limitation are often of poor quality, with considerable particle deposition in the major airways and minimal penetration to the lung parenchyma. Attempts have been made to improve particle size and improve image quality. Agnew in 1984 quantified the pattern of non-uniform deposition and studied the depth of penetration. This study reported a relationship with the extent of airways limitation. Agnew discussed CAL in respect to this disease and suggested it may originate in the lesser airways and migrate to the larger airways.

1.5.3 Key diseases when reporting on CAL

Bronchiectasis: is an inflammation with infection causing damage to the airways with an alteration to the lining of the airways, becoming distorted and enlarged (Fogelman 1988).

Chronic Cough: prolonged non – productive cough lasting for more than six (6) weeks.

Common Cold: includes Rhinoviruses which are seldom serious, para-influenza and respiratory syncytical virus, produce mild infection in adults but can precipitate into a severe lower respiratory infection in children. The coronoviruses are believed to cause a large percentage of all adult colds.

Cystic Fibrosis (CF): a chronic, progressive and frequently fatal genetic disease of the body's mucous glands. Patients with CF have a lifespan of about thirty (30) years.

Tuberculosis: infectious disease caused by a bacterium called mycobacterium tuberculosis. This bacterium affects the lungs and is contagious and spreads by inhaling the bacterium that has been sprayed by droplets into the air by the person with the active disease that coughs (FDA, 1999).

1.5.4 Mechanisms Underlying CAL

Most CAL patients have smoked for at least 20 years and commonly present in their sixties with a productive cough or an acute respiratory complaint. During their sixties onwards, exertional dyspnoea is usually a feature and intervals between acute exacerbations become shorter as the disease progresses. In its earlier stages, slow, laboured expiration, plus wheezing on forced expiration may be apparent. A worsening in airflow obstruction is associated with hyperventilation and a gradual increase in the antero-posterior diameter of the chest. The underlying causes of CAL have still to be fully elucidated, nevertheless cigarette smoking is felt to be generally the most important. Recent interest has seen recognition of the significant morbidity associated with CAL, greater understanding of the disease process, advent of new effective

treatments, together with a gradual appreciation of the degree of misdiagnosis, have served to focus attention on clearly defining and characterising CAL (Brostoff, 1999).

Interest has also been promoted by a change in emphasis in the way the efficacy of available treatments is assessed. Reduced useful lung volumes and slow forced emptying of the lungs are characteristics of CAL, hence the current policy of assessing patients' lung function by measuring Forced Expiratory Volume (FEV_1). However, many researchers feel such measurements of lung function may not be the best means of assessing prospective management strategies (Brostoff, 1999).

What is now felt to be clinically relevant is improvement in the patient's quality of life. Quality of life measurements have previously been ignored, due to their poor correlation with FEV_1. However, they are now incorporated into the new CAL management guidelines for this very reason, and are part of most new studies into CAL. The shift in the 'burden of financial responsibility' from secondary to primary medicare must also have helped sharpen doctors' interest in tackling CAL. Limited resources must be effectively targeted and CAL eats up a lot of medicare resources, particularly in hospital costs (Girodo, 1992).

1.6. Anatomy and Physiology of the Lung

The upper respiratory tract (URT) includes the mouth, nares (within the nasal cavity), paranasal sinuses and nasopharynx. During inspiration and expiration, gases traverse through the URT and enter the lower respiratory tract, beginning

at the larynx. This eventually bifurcates forming the bronchi, bronchioles and terminal bronchioles. Distal to the terminal bronchioles are the respiratory bronchioles, alveolar ducts and alveolar sacs, which lead to the alveoli. The alveoli form the majority of lung tissue, where gaseous exchange occurs between the inspired air and circulating blood.

The alveoli have a single layer of epithelial cells encased in a capillary network suitable for gaseous exchange; in normal human lungs there are between two hundred and fifty (250) and three hundred (300) million alveoli present (Roussos, 1995). The pulmonary artery bifurcates into branches forming the segmental artery supplying blood to the capillary network in the alveoli.

During inspiration, the most important muscle is the diaphragm, which inserts into the lower ribs. On contraction of the diaphragm, abdominal contents are forced downward and forward, and the vertical dimensions of the chest cavity are increased due to inspiratory intercostal muscles (Feselman, 1988). The ribs are lifted and moved out, causing an increase in the diameter of the thorax (Roussos, 1995).

Breathing is usually involuntary but voluntary breathing is necessary when the person is performing activities such as walking, talking etc. (Roussos, 1995). In these cases homeostatic changes in ventilatory rate and volume are adjusted automatically by the nervous system to maintain normal gas exchange. Breathing is controlled and regulated by neural and chemical balances within the body. Sensors and receptors form a highly complex ventilatory system.

The medulla oblongata of the central nervous system is responsible for both inspiratory and expiratory neurons. Within the pons are the apneustic and pneumotaxic centres (these centres are in direct relationship with the medulla),

give the rhythmic quality to respiration (Roussos, 1995). Chemosensitive regions are also housed within the medulla and are important, as they are sensitive to levels of carbon dioxide and hydrogen ion concentrations in cerebrospinal fluid (CSF). Peripheral chemoreceptors known as aortic and carotid bodies, respond to chemical changes in the blood, particularly in arterial oxygen tension (Early, 1995). Hypoxia stimulates the peripheral chemoreceptors, which in turn stimulate the respiratory centres to increase ventilation. These receptors are also sensitive to a reduction in arterial oxygen tension (Early, 1995).

The Hering-Breuer reflex (Garbe 1986) is well known as it aids in the control of respiration due to inflation and deflation of the lungs. Receptor sites are located in the respiratory tract, mainly the bronchi and bronchioles. These reflexes are activated by either stretching or a non-stretching and compression of the lungs. The inflation reflex inhibits inspiration preventing further inflation; as expiration begins the receptors are no longer stretched and therefore impulses are no longer sent, and inspiration commences (Garbe, 1986).

The external pressure exerted on the thorax is atmospheric, at sea level it is 100 mmHg (Garbe, 1986). When the lungs are resting with no airflow the intrapulmonic pressure is also atmospheric. There are different types of pressure changes in the lungs, which occur with different types of respiration. For air to flow in the lungs, intrapulmonic pressure has to be negative or less than atmospheric so that a pressure gradient can be set up between the atmosphere and the alveoli.

Intra pleural pressure exists between the pleural spaces, which is subatmospheric [-5mmHg] (Taylor, 1989). This is caused by the elasticity of the

lungs, which recoil from the thoracic cage, creating a vacuum between the visceral and pleural space (West, 1990). If air enters this intrapleural space, the "pull" is lost and a pneumothorax or lung collapse will occur, and the thoracic cage will expand. On inspiration, it is the elastic recoil between the thorax and lungs that causes chest expansion (Taylor, 1989). As the thorax expands during inspiration, intrapleural pressure becomes more negative (-8 mmHg), or subatmospheric, and inflation of the alveoli occurs as air moves from a point of high to low pressure (West, 1990).

The lungs are innervated by the autonomic nervous system (ANS) (West, 1990). Fibres of the sympathetic division in the lung, branch from the upper thoracic and cervical ganglia of the spinal cord, while fibres of the parasympathetic division are carried in the vagus nerve, which is important in the regulation of ventilation (Taylor, 1989). The respiratory centres in the brain stem control involuntary ventilation by transmitting impulses to the respiratory muscles causing them to contract or relax (Taylor, 1989).

1.6.1 Air Movement

Even the movement of air through the lungs requires a complex set of control mechanisms originating from the central nervous system (CNS), which can be affected by a range of physical boundaries (West, 1990).

For example, the pneumotaxic centre in the upper pons functions to maintain rhythmic respiration's sending inhibitory signals to the inspiratory centre causing inspiration and expiration (West, 1990). Strong foci from the pneumotaxic centre result in shorter inspiration, and mild stimuli results in longer inspirations (West, 1987). The apneustic centre sends stimuli to the respiratory centre

prolonging inspiration. The pneumotaxic centre usually overrides the apneustic centre causing receptors to respond to physical changes in oxygen or carbon dioxide concentrations. This is achieved through both the sympathetic and parasympathetic divisions of the ANS and respiratory centres in the brain stem (West, 1987).

Dimensions of the airway tree influence ventilatory flow of air in a number of ways (West, 1990). Airflow velocity is reduced along the airway tree as the total cross – sectional area of the airways increases with every generation of which there are 23 generations in total (West, 1990).

In the smaller airways, oxygen transport is slower than diffusion as molecules move through air at a velocity of approximately 5 cm per second. The airway size also determines the resistance to airflow and albeit small; it is the reciprocal of the ratio of ventilatory air - flow to the pressure difference between the mouth and the alveoli, normally no greater then 1cm of water (West, 1990). It is, however, significant enough to potentially affect the distribution of ventilation to the numerous gas exchange units. Poiseuille's Law describes the resistance (R) of a bronchiole with radius "r" and length "l": $R = k .l/r^4$ (West, 1987).

Therefore, if you double the airway diameter, the resistance will be reduced sixteen (16) fold (West, 1987). In a normal lung, the distribution of airflow to the periphery occurs, whereas in CAL patients, distribution is greatly disturbed, with airway resistance requiring greater work to ventilate the lung (West, 1990). Airway resistance to mass airflow is seen in the conducting airways and falls rapidly toward the periphery (West, 1987).

As the diameter of the airways decreases, one would assume an increase in resistance towards the periphery, however this does not occur due to airways having a low resistance because of flow velocity being rapid as the airways branch. Further, thin walled bronchioles become widened as the lung expands on inspiration. Airway resistance is therefore seen to fall as the lung volume increases (West, 1987).

1.6.2 Gas Exchange

Oxygen flow rate is dictated by the amount of oxygen in the mitochondria and the amount of mitochondria in the working muscle sets the limit for oxygen flow or VO2; also the mitochondrial volume is proportional to VO2. This is due to oxidative phosphorylation in the mitochondria, which is the only pathway for adenotriphosphate or ATP production, by oxidative metabolism (West, 1990). However, the driving forces of oxygen flow are affected by homeostatic regulations.

Pulmonary diffusing capacity is the diffuse transfer of oxygen from alveolar air to blood which meets with resistance albeit small, therefore the diffusing capacity is an estimate of the global conductance or the reciprocal of the total resistance to the diffusion of oxygen being offered as a gas exchanger (West, 1990).

The alveolar surface of the lung is immense therefore the barrier is extremely thin and this is why the diffusing capacity is so large, or the resistance so low. The alveolar capillary membrane is the perfect medium for oxygen exchange. This is due to the large total surface area, being about 70 to 100m squared and its thinness, 0.5 micrometer. Also the concentration of oxygen molecules

(PaO2) is greater in alveolar gas than in capillary blood, which promotes rapid movement down the concentration gradient from the alveolus (West, 1990).

The partial pressure of oxygen or oxygen tension in mixed venous or pulmonary artery blood is about 40 mmHg as it enters the capillary, and alveolar oxygen tension (PaO2) is 100 mmHg at sea level. Therefore, the pressure gradient facilitates diffusion of oxygen from the alveolus into the capillary. Blood remains in the pulmonary capillary for a shorter period than needed for oxygen concentration to equalize across the alveolocapillary membrane (West 1987, Taylor 1989).

Therefore, oxygen has less time to diffuse into the blood, even during increased cardiac output which speeds bloodflow, shortening the time blood remains in the capillary. As oxygen diffuses across the alveocapillary membrane, it dissolves in the capillary blood where it produces pressure, which is the partial pressure of oxygen in arterial blood or PaO2. As PaO2 rises, oxygen moves from the plasma into the red blood cells (erythrocytes) and binds with haemoglobin molecules till these are saturated. Oxygen then continues to diffuse across the semipermeable membrane until it equilibrates, eliminating the pressure gradient across the alveolocapillary membrane. Here diffusion ceases (Taylor, 1989).

As mentioned previously, air enters the lungs via the nose and the mouth where it is humidified and filtered. Pulmonary gas exchange involves inspired oxygen (O2) to be exchanged at the alveolar level with carbon dioxide (CO2). The oxygen then binds to Haemoglobin (Hgb) in the pulmonary capillaries. Pulmonary gas exchange is primarily dependent upon three processes, ventilation, diffusion and perfusion (West, 1990).

Ventilation occurs when air moves into the alveoli; the inspiratory muscles contract generating a force, which expands the chest wall and the lungs to overcome the resistance and inertia of the respiratory system. Together, the respiratory muscles, lung parenchyma, airways and chest wall will determine the volume of gas that will reach the alveoli (Taylor 1989, West, 1990). The amount of air that enters the lungs is known as the tidal volume (TV), and the amount of gas retained in the lungs when fully expanded is the total lung capacity (TLC). The maximum volume a person can exhale is the vital capacity (VC) and remaining gas is the residual volume (RV). At the end of a normal breath, the amount left in the lungs is the functional residual capacity (FRC) (Taylor 1989, West 1990).

Structural hierarchy of the airways is important as lung structure is defined through the hierarchial properties of the airways. The acinus is the complex of all the airways distal to the terminal bronchiole and is known as the first respiratory order respiratory bronchiole. This therefore means it is the largest unit from which all airways participate in gas exchange (West, 1987).

Diffusion is the movement of molecules from a region of high concentration to a region of lower concentration. In the lung, oxygen moves by diffusion from alveolar gas into the pulmonary blood. In patients with CAL oxygen diffusion will be impaired (West, 1987).

Perfusion describes the route of blood through the lungs. The right ventricle in the heart pumps blood into the pulmonary artery. The branches of this artery supply the alveolar capillaries, which drain through the pulmonary veins into the left atrium. Instances of obstruction, as is the case with PE or lung parenchyma (as seen in bronchitis, emphysema, and fibrosis) cause arterial hypertension. In

both scenarios obstruction and vascular resistance to blood flow increases (West, 1987, 1990).

Ventilation and perfusion in an ideal pair of lungs would be supplied with equal volumes of air and would have a uniform gas composition during inspiration. Also, all the alveoli would be supplied with the same flow of mixed venous blood. Therefore ventilation and perfusion would be optimally matched, with optimal gas exchange between blood and alveoli would take place (West, 1990).

In real lungs the above does not occur per unit of lung volume, ventilation and perfusion are both greater at the bases of the lungs compared to the apices. The ratio of ventilation to blood flow, the ventilation / perfusion (V/Q) ratio varies by a small amount throughout the lungs (West, 1990).

Pulmonary capillaries are influenced by air pressure in the alveoli. If blood pressure within the capillary is less than the pressure of a gas in the alveoli adjacent to it, there is a tendency for the pressure in the alveoli to remain close to atmospheric during quiet breathing but may become positive during artificial ventilation or heavy breathing (West, 1990).

Pulmonary capillaries are influenced by air pressure in the alveoli that surround them. If blood pressure within a capillary is less than the pressure in the alveoli, this will lead to pressure in the alveoli compressing the capillary and limiting blood flow through it. Alveolar gas pressure may have an effect on the distribution of pulmonary blood flow (West, 1987 & 1990).

Arterial oxygenation is the process of delivering oxygen to the cells, and depends on several factors. These include cardiac output, the amount of haemoglobin present, oxygen saturation, and the oxygen binding capacity. This

is normally 1.34 mililitres of oxygen, per one (1) gram of haemoglobin. Therefore, patients with life-threatening conditions should receive supplemental oxygen (West, 1987 & 1990).

Once oxygen is delivered to the cells, it needs to be utilized by the “Krebs Cycle“. Oxygen failure is a respiratory crisis in which the primary problem is hypoxaemia, PaO2< or = 60 mmHg. Hypoxia and hypoaemia are often confused or used interchangeably, but there is a difference. Hypoxaemia (PaO2) is defined as inadequate oxygen in arterial blood and hypoxia is decreased oxygen supply to the cells or tissues (Taylor, 1989).

Hypoxaemia occurs when there is a decreased arterial blood saturation of oxygen (PaO2), and can occur anywhere from when oxygen is inspired to when it reaches the mitochondria, the powerhouse of the cell. This may be due to a decrease in inspired oxygen, alveolar hypoventilation, diffusion problems, ventilation/perfusion mismatch, or shunt and increased oxygen consumption (Taylor, 1989).

Hypoxia is the decreased oxygen supply to cells or tissues, Hillman and associates (1996) reported both PaCO2 and SaO2 measuring adequate oxygenation of the body. Hypoxia is a better indicator of an oxygen delivery problem. The most common cause of hypoxia in acute respiratory failure is a ventilation / perfusion mismatch. Mismatching affects the exchange of oxygen and carbon dioxide. In pulmonary embolism there is a decrease in perfusion in relation to ventilation. This results in dead space or wasted ventilation. Hypoxia may be improved with one hundred percent (100%) oxygen for ten (10) to fifteen (15) minutes to wash out all the nitrogen in the alveoli, leaving only carbon dioxide (CO2) and oxygen (O2) (Hillman, 1996).

Pulse oximetry is used to measure oxygen (O2) saturation. It is important to maintain saturation at least greater than ninety percent (90%). A PaO2 of at least 80 mmHg indicates an adequate saturation. Measuring a patient's arterial blood gas will measure pH, PaO2, PaCO2, HCC3 (bicarbonate) levels. This will assist in the regulation of oxygen therapy, determine the severity of respiratory / metabolic disorders, and may reflect local disturbances (Hillman, 1996, James, 1992).

1.7 Pathophysiology of the Lungs

1.7.1 Balance between perfusion and ventilation

In a normal human, we aim to be in a state of homeostasis whereby a physiological balance between perfusion and ventilation exists. Respiratory diseases lead to imbalances between these two functions (Taylor, 1989).

Musculoskeletal deformities of the thorax are among the most common causes of respiratory failure. Generally, total lung capacity will be reduced in all deformities of the thorax, and most of the reduction in the lung is due to a decrease in chest wall compliance. However, within the total lung capacity, different subdivisions will demonstrate slight variations that help identify the individual mechanical disorders characteristic of that type (James, 1995).

Except for fibrosis and bronchiectasis, most thoracic deformities are not characterized by airway obstruction or intrinsic lung disease. Ventilation-perfusion abnormality therefore is attributable to local deflation and poor ventilation of the lung. To support this there has been much research in thoracic

deformity of regional ventilation and perfusion by radioisotopic methods (James, 1995). PE is usually multiple occurring in the lower lobes of the lung.

Thoracic function can be evaluated indirectly by measurements of the function of the lungs, which reflect movements of the chest wall. However, such measurements are non-specific, as lung function tests reflect not only disorders of the thorax, but the lung themselves. All subdivisions of lung volume, including total lung capacity (TLC), vital capacity (VC), residual volume (RV), and functional residual capacity (FRC), are determined by the mechanical function of the chest wall and lung (Danjun, 1994, Roussos, 1995).

Complete inspiration and expiration require vigorous muscular efforts and therefore, TLC, VC, and RV are influenced by inspiratory and expiratory muscle strength. FRC in healthy people is a balance of passive elastic forces generated by the lungs and chest wall. Chest wall recoil is influenced by the tone in the muscles of the rib cage (Danjun, 1994, Roussos, 1995).

Primary infections such as bronchitis, broncho pneumonia and other forms of pneumonia are seen in clinical and pathologic practice. Smoking and air pollution, chronic bronchitis and emphysema have greatly increased within our society. Lung malignancy, seen on autopsy, shows some degree of pulmonary oedema, atelectasis, or broncho pneumonia (Fogelman, 1988). Bronchogenic carcinoma causes a decrease or absence in pulmonary blood flow to the affected segment of lung (Danjun, 1994, Fogelman, 1988).

Obstructive lung diseases are characterized by increased resistance to airflow (Danjun, 1994, Fogelman, 1988). Acute diseases include asthma or bronchitis; these affect the conducting airways and involve ciliary dysfunction.

1.7.2 Chest Wall and Respiratory Muscles in CAL Patients

CAL patients have an increased airflow resistance and reduced dynamic pulmonary compliance, making respiratory muscles work constantly against an increased load (Taylor, 1989). Inspiratory muscles therefore generate more pressure to move air into the lung. Ventilation is increased in CAL patients compared to healthy people. CAL patients have an increase in functional residual capacity (FRC) so inspiratory muscles operate at shorter lengths than normal and have a reduced ability to lower intrathoracic pressure (Taylor, 1989).

Analysis of respiratory muscle function in CAL requires an understanding of "length-tension" relationship of muscle (West, 1990). Active tension, which occurs during contraction, is a function of resting length of muscle. The relaxed length of muscle increases active tension increases until a maximum length is reached (West, 1990). Passive tension results from the elastic forces of connective tissue, the sarcolemma, blood vessels etc. Passive tension continues to rise as resting length is increased (Taylor, 1989, West, 1990). Thus the length tension relationship determines the influence of hyperinflation of the diaphragm (Taylor, 1989).

Arora (1987) suggested studies of structures of the diaphragm of CAL patients at autopsy have a loss of diaphragm weight and thickness, which might be due to general muscle wasting observed in these patients.

End expiratory volume is considerably larger in CAL patients as they may not have time to expel all the air through their airways (Taylor, 1989). A considerable reduction in maximal inspiratory pressure has to be present for a significant fall in vital capacity to occur. Generally, volume measurements are

less variable and better standardized than maximum pressures, and most patients with respiratory symptoms from skeletal abnormalities or muscle weakness have a distinctive restrictive ventilatory defect.

Weakness in CAL patients is reflected in the shape of their maximum flow-volume curves (Taylor, 1989). Airflow depends on effort such as the maximum expiratory flow and maximum inspiratory flow, which are independent on effort and are most affected by CAL (Taylor, 1989, West, 1990). In both normal and CAL patients the shape of the inspiratory flows over much of the vital capacity are larger than expiratory flows. Therefore FIV_1 (forced inspiratory volume from RV) is greater than FEV_1 (Taylor, 1989).

CAL patients with acute respiratory failure due to underlying PE will have symptoms of hypoxaemia present in varying degrees (James, 1992). Treatment for CAL patients includes controlled low flow oxygen, moisture delivered to the airways and methods to improve alveolar ventilation with or without mechanical means. Finally for severe CAL, intubation and ventilatory management are options for patients requiring supportive care (James, 1992).

1.8 Pulmonary Embolism

1.8.1 Causes and Diagnosis

Pulmonary Embolism (PE) is not only the impaction of thrombus within the pulmonary arteries but may be caused by a variety of reasons e.g. fat embolus (Roussos, 1995). The thrombi usually originate from systemic veins, in the Ileofemoral system. However, the aetiology of venous thrombosis is mainly from venous stasis, trauma to veins and abnormalities of coagulation. Venous stasis

can occur due to patient immobilization, heart failure (reduced cardiac output), venous pressure and compression of calf muscles. Other ways venous blood flow can diminish include obesity, varicose veins, and inactivity (Danjun, 1994, Roussos, 1995).

Differential diagnosis of PE is varied and depends upon the clinical scenario (Taylor, 1991). This includes pneumonia, pneumothorax, pulmonary oedema, pericaritis, rib fracture, myocardial infarction, and septicaemia. Key diagnostic investigations may include lung scintigraphy, chest x-ray, ultrasound, spiral CT, Pulmonary Angiography, Magnetic Resonance Imaging (MRI), and objective tests for proximal DVT.

Objective testing for DVT is useful in patients with suspected PE, particularly those with non-diagnostic lung scan results, therefore intermediate, intermediate or low probability categories, as a negative result do not exclude PE (Taylor, 1991). If a patient has adequate cardiorespiratory reserve, ultrasound imaging may be used as an alternative to pulmonary angiography (Rumack, 1998). The rationale is that the clinical objective in such patients is to prevent recurrent PE, which is unlikely in the absence of proximal vein thrombosis (Rumack, 1998). For patients with inadequate cardiorespiratory reserve, the clinical objectives are to prevent death and morbidity from an existing embolus and to allow further investigations for the presence or absence of PE (Howarth, 1999, Palla, 1988).

1.8.2 Hypercoagulable states

Pregnancy can cause venous thrombosis as the enlarging uterus obstructs the inferior vena cava. Economy Class Syndrome has been attributed to prolonged periods of inactivity in long flights causing venous stasis (James, 1995).

Other hypercoagulable states include antithrombin$_{111}$ deficiencies, defective fibrinolysis and obesity, recent surgery, and oral contraceptives contribute to primary DVT.

1.9 Clinical Signs of Pulmonary Embolism

The majority of patients with pulmonary emboli have an underlying clinical predisposition (James, 1995). Less than 10% of the patients have no discernible cause for deep venous thrombosis at the time of presentation (Murray, 1998).The common predispositions found in patients with pulmonary embolus include immobilization, surgery, fracture, malignancy, thrombophlebitis, trauma, oestrogen therapy, obesity, myocardial infarction, and stroke (Murray, 1988).

Regrettably, the clinical diagnosis of PE is unreliable as the symptoms and signs of PE are non-specific. The classical clinical triad of haemoptysis, pleuritic chest pain and dyspnoea occurs in only 20% of patients (Botnar, 2004).

The greater majority of patients who are proven to have a PE present with dyspnoea and chest pain are more often pleuritic when the clots are peripheral (James, 1995). Cough is another non-specific sign of lung disease and presents in slightly less than half of the patients (James, 1995). It is also a well established fact that the clinical evidence of DVT is usually absent in patients

with lower extremity clots (James, 1992). Haemoptysis, which often suggests a diagnosis of PE, is only present in the minority of cases. Palpitations, wheezing and angina – like pain, are all seen in a small number of cases (Fedullo, 2003, Botnar, 2004.

Physical examination is important in diagnosing PE (Fedullo, 2003). The majority of patients will present with an increased respiratory rate and have audible rates on auscultation, and an increased second heart - beat is common (Botnar, 2004). The remainder of clinical signs include: increased heart rate, elevated temperature, physical evidence of phlebitis, cardiac gallop, diaphoresis, oedema, cardiac murmur and cyanosis and are found in less than 50% of the patients who present with PE.

It is therefore not surprising that the clinical diagnosis of PE is fraught with error; the signs and symptoms serve only to raise the suspicion of PE (Botnar, 2004). Those patients with PE have essentially the same symptoms as those who were initially suspected of having an embolic event, but did not. Clinical assessment alone will be unreliable and further investigation is required to reach the correct diagnosis (Early 1995, Botnar, 2004).

The pathophysiologic response of PE depends on the extent to which the pulmonary artery blood flow is obstructed, size of occluded vessels, number of emboli and the release of thromboxane from platelets that accumulate at the site of the thrombus (West, 1987). The two pathophysiological consequences of emboli are respiratory compromise (non perfusion of ventilated segment), and haemodynamic compromise (increased resistance to pulmonary blood flow), (West, 1987). If a patient's PO2 is 60% or below depending on their pathology (therefore patient history of CAL, pneumonia etc.), and if the patient is hypoxic,

a differential diagnosis of PE will be made until further confirmation. The normal PO2 is 80 - 100% (James, 1992).

Consideration needs to be given to conditions that predispose the patient to DVT and PE. Sonographic evidence of DVT is only reported in fifteen (15%) of patients with diagnosed intermediate probability post lung scintigraphy, therefore a negative Doppler ultrasound was reported in eighty-five (85%) (Rumack, 1998). This should not deter attention away from making a diagnosis of PE. Sonographic evidence of DVT is seen in only twenty-three (23%) of patients with angiographically confirmed PE (Leiper et al., 1998).

Patients with massive PE usually have a dramatic presentation with a sudden onset of severe shortness of breath (SOB), hypoxaemia and right ventricular failure. Symptoms include central chest pain, often like angina, severe dyspnoea and frequently syncope, confusion and less frequently coma. The patient on examination will also have severe tachypnoea, cyanosis and hypotension. The increase in pulmonary vascular resistance will lead to acute right ventricular failure (Habibian 1999, Rubinstein, 1988).

With pulmonary hypertension, there is a marked right ventricular dilatation with a shift in the patient's septum, decreasing cardiac output, and therefore further decreasing coronary perfusion, which may result in cardio-respiratory arrest. If patients with a massive PE survive, they are at great risk of further thromboembolism (Rubinstein, 1988).

Emergency management of severe PE which could prove fatal must include intravenous heparin, oxygen, mechanical ventilation, volume resuscitation and the use of inotropic agents or even vasodilators (Rubinstein, 1988). In addition to these supportive measures, if the patient survives a severe, life-threatening

PE, the patient must have thrombolysis, pulmonary thrombectomy, with or without coronary bypass (CABG), travenous catheter embolectomy or clot dissolution, and the insertion of an inferior vena cava filter (Rubinstein, 1988).

1.10 Diagnostic Tests to detect PE

Tests to exclude PE and DVT include angiography, lung scintigraphy, venography and Doppler ultrasound (Rays, 1996). Although angiography is definitive for PE, it comes with a risk, albeit small, of anaphylactic reactions, as well as morbidity associated with the contrast injections, which makes it high enough to warrant alternative, less accurate but much safer diagnostic procedures, which are also preferred (Rays, 1996, Clinical Imaging 1994).

Effective cost (EC) of each diagnostic test represents the amount spent per unit of diagnostic information and is defined as the ratio of the expected direct cost (EDC) compared to its diagnostic performance (DU representing the test sensitivity and specificity) (PIOPED, 1990). EDC refers to the direct cost for performing the test and the estimated cost is the morbidity and mortality that can be incurred while having the procedure. With this methodology, allowances are made for comparison of individual procedures as well as determination of the maximum cost and minimum performance characteristics for any new procedure to be competitive with those that already exist (EJNM, 1999, PIOPED 1990).

As PE and DVT account for many hospitalizations annually, prevalence of DVTs and PEs increase with age, and as our aging population will be more prone to this condition; an accurate diagnosis is critical (Botnar, 2004). Physicians must

learn to be skilled in risk stratification of their patients with suspected PE. Recent advances in medicine have entailed improvement in the method of diagnosis and in clinical management of PE and DVT (Freeman, 1999).

Lung scintigraphy is a noninvasive, reliable method of diagnosing patients who present with suspected PE (Murray, 1988). Lung scintigraphy entails a low amount of radiation exposure to the patient and remains one of the primary forms of investigating PE; this is based on 35 years of reliable clinical experience in the diagnosis of PE (James, 1995, Murray, 1988, Taylor, 2000).

In a controversial article in 1977, Robin published a paper titled "Overdiagnosis and overtreatment of pulmonary embolism: the emperor may have no clothes" which highlights pulmonary embolism as being overdiagnosed in previously healthy patients due to the inappropriate use of diagnostic studies (PIOPED, 1990). Since then, clinical investigations have established the diagnostic role of ventilation-perfusion scintigraphy, pulmonary angiography, impedance plethysmography, and compression ultrasound evaluation of lower extremities for venous thromboembolism (PIOPED, 1990).

Clinical signs alone are unreliable in the diagnosis of both acute pulmonary embolism and deep venous thrombosis, underscoring the importance of diagnostic testing (Freeman, 1999, Howarth 1999).

Robin’s article reviews the clinical utility of current diagnostic modalities (pulmonary angiography, ventilation-perfusion scintigraphy, and ultrasound) and of new diagnostic procedures (spiral computed tomography (CT), D-dimer assays, and magnetic resonance imaging (MRI) in the diagnosis of venous thromboembolism.

Robin (1977) placed some of the blame for over-diagnosis and over-treatment of PE in previously healthy patients on ventilation-perfusion imaging. Retrospective diagnostic accuracy studies of ventilation-perfusion imaging in patients with suspected PE led to funding of a prospective multi-centre diagnostic accuracy study PIOPED, 1990.

There was a flaw with the PIOPED 1990 study as it was a diagnostic accuracy study rather than a patient outcome study. The results of ventilation-perfusion imaging were compared with the results of pulmonary angiograms, and the treatment of patients based primarily on the results of pulmonary angiograms. Therefore, for lung scans to survive, and for the diagnosis and treatment of patients suspected of PE to improve, these two widely held, overly simplistic views of PE must change (PIOPED, 1990).

Ventilation scans improve the specificity of the perfusion scan by locating areas of non ventilated lung, which is necessary for diagnosis of PE in regions of absent perfusion and identifying ventilation perfusion mismatches or segmental perfusion defects (Taylor, 2000). Lung perfusion images can provide a quantitative estimate of total lung perfusion as they mimic regional blood flow (Palla et al, 1988). An interpretation criterion which is based on mismatched defects seen on perfusion scintigraphy is low, intermediate or high probability for PE based on the PIOPED study.

Scintigraphy is an important diagnostic first line adjunct in diagnosing patients with suspected PE (Danjun, 1994). There are several diagnostic tests available to assist in the diagnosis, including: ultrasound, spiral CT, angiography, arterial blood gases and chest radiographs (CXR). CXR acquired within 24 hours of

lung scintigraphy can be inconclusive or of minimal value to the physician due to its insensitivity for PE (Rays, 1996).

Ultrasound, using Doppler is non- invasive and has a proven high accuracy rate in diagnosing DVT. Physiological methods rely on detecting altered venous flow haemodynamics (Rumack 1998). Duplex Doppler and Colour Flow Doppler provide objective anatomic information similar to venography as well as physiologic information of venous haemodynamics (Taylor, 2000, Rumack, 1998). Doppler sonography includes both quantitative duplex spectral analysis and qualitative colour flow Doppler sonography (Rumack, 1998).

The addition of colour flow Doppler is a useful modification of the standard compression study. In normal veins, colour should fill the vessel lumen and venous flow augmentation is usually necessary to completely fill the vessel lumen from wall to wall (Rumack, 1998).

Examination of the calf veins is considered by some to be controversial. In some centres the lower leg is not evaluated, as it is rare for the isolated calf DVT to cause significant PE. However in other departments the calf is routinely evaluated due to a 20% incidence of proximal clot propagation (Rumack, 1998).

The gray-scale compression sonographic findings of DVT are based on direct visualization of a thrombus and lack of venous compressibility (James, 1995, Rumack, 1998). There is a negative for using Doppler ultrasound; the potential for false positives is real due to the inability to visualize central veins and reliance on blood flow haemodynamics to detect venous occlusions (James, 1995, Rumack, 1998).

While Doppler venous flow detection only provides information about blood flow, real-time ultrasound provides a two-dimensional, cross-sectional representation

of the lower extremities (Rays, 1996). Compression ultrasound with venous imaging (real-time B-mode imaging) has a sensitivity of 89% to 100% and a specificity of 86% to 100% for detection of proximal deep venous thrombosis in symptomatic patients (Rays, 1996, Clinical Imaging 1994). In addition, compression ultrasound with venous imaging is widely available, non-invasive, and accurate for the diagnosis of symptomatic proximal deep venous thrombosis (Rumack, 1998).

The combination of Doppler venous flow detection and real-time B-mode imaging is called duplex scanning (Rays, 1996). New technology has led to colour display of Doppler frequency, called colour duplex scanning. Duplex scanning is highly accurate for diagnosis of proximal deep venous thrombosis in symptomatic patients; both sensitivity and specificity are greater than 95% (Rumack, 1998, 26). In addition, the Doppler analysis allows differentiation of fresh thrombi from older thrombi (Rumack, 1998).

Tapson and associates (1999) studied the diagnostic accuracy of compression ultrasound and reported that it was limited in patients with deep venous thrombosis of the calf (Rumack, 1998). Compression ultrasound with venous imaging, duplex ultrasound, and colour duplex studies all vary in technique, cost, availability, and operating characteristics (Rumack, 1998). Tapson's 1999 article suggests despite these differences, as long as compression is used, no single technique has demonstrated an advantage over another in prospective clinical trials for the detection of proximal deep venous thrombosis in symptomatic patients (Tapson, 1999). For asymptomatic patients, ultrasound techniques are not sensitive enough to exclude a diagnosis of deep venous thrombosis (Rumack, 1998).

Spiral CT is another method of diagnosing PE, providing parallel projections and x-ray images without distortion. Following intravenous bolus injection of contrast medium, the pulmonary arterial phase and venous phase are clearly separated. Spiral imaging has introduced the concept of volumetric imaging with ionizing radiation (Early, 1995).

Spiral CT has taken CT from what was primarily an anatomic tool to one that offers physiologic and pathogenic information (Rays, 1996). Greater lesion detection (ability to reconstruct overlapping cross-sectional images) creates high quality multiplanar 3D reformatted images (Annals of Emergency Medicine,1999). Spiral or helical CT uses 'slip-ring' technology eliminating the old step and shoot phase allowing uninterrupted scanning during continuous patient advancement through the gantry. Once a volume of data is acquired, images can be reconstructed at any point along that volume (Annals of Emergency Medicine, 1999).

The creation of overlapping slices can lead to studies of greater sensitivity since small lesions which otherwise would have fallen between continuous slices may now be detected on the overlapping images (Clinical Imaging 1994). The actual time taken to acquire a complete Spiral CT to the production of interpretable images is essentially a sum of the two components: the time it takes to carry out the scan (scan time), and the time necessary to reconstruct the individual 2-D images from 3-D data (image reconstruction time) (Annals of Emergency Medicine, 1999).

The latter is longer, seconds versus minutes. An extra sharp filter is used for reconstructing a Spiral CT (Clinical Imaging 1994). Spiral CT enables an exact demonstration of thrombosis if there are severe, centrally localized emboli. In

completely occluded vessels, estimation of the degree of location of perfusion defects is far better with scintigraphy (Clinical Imaging 1994). The conclusion, based on clinical results and technical equipment necessary to run CT, is that lung scintigraphy remains the first choice for diagnosing PE (Annals of Emergency Medicine, 1999).

Pulmonary emboli are sometimes seen on conventional contrast-enhanced CT scans. However, such a finding is usually serendipitous and depends on catching the intravenous contrast bolus at the exact time it arrives in the pulmonary vasculature (Rays, 1996).

Spiral CT scanning (also known as helical CT) was introduced in the early 1990s and involves an x-ray beam and a coupled array of detectors rotating continuously in a spiral manner (Rays, 1996). This spiral technique results in reduction in examination time compared with conventional CT scanning. During a single breath-hold, spiral CT is capable of imaging nearly the entire thorax. Therefore, intravenous contrast can be timed to arrive in the pulmonary vasculature to give vascular opacification (Rays, 1996, Clinical Imaging, 1994).

Since its introduction, spiral CT scanning has gained popularity for diagnosis of pulmonary embolism (Rays, 1996). In studies comparing spiral CT scanning with pulmonary angiography as the diagnostic standard, spiral CT scanning had a sensitivity of 64% to 93% and a specificity of 89% to 100% (Rays, 1996). The greatest sensitivity and specificity were achieved when a pulmonary embolism involved the main, lobar, or segmental pulmonary arteries (Howarth, 1999).

Given the limitations of ventilation-perfusion scanning, it is not surprising that spiral CT scanning has gained popularity (Annals of Emergency Medicine, 1999). Spiral CT scanning actually visualizes the clot, whereas ventilation-

perfusion scanning only displays the secondary effects of the pulmonary emboli on the pulmonary vasculature bed (Annals of Emergency Medicine, 1999). In addition, the majority of patients undergoing ventilation-perfusion scanning have non-diagnostic results and require further testing (Early, 1995).

Other key advantages of spiral CT over ventilation-perfusion scanning include the ability to identify other disease states that can mimic pulmonary embolism (e.g., lung tumours, pleural disease, pericardial disease) (Rays, 1996). In one study, 11% of patients undergoing spiral CT scanning for suspicion of pulmonary embolism had unequivocal abnormalities of lung parenchyma that could explain the clinical presentation (Clinical Imaging, 1994). Given that most patients being evaluated for pulmonary embolism do not have emboli, spiral CT can sometimes provide an alternative diagnosis (Clinical Imaging, 1994). Although evidence suggests that spiral CT may be cost-effective, information is insufficient to make firm recommendations at this time (EJNM, 1999). In terms of cost, a ventilation-perfusion scan is about one and a half times and a pulmonary angiogram is six to eight times as expensive as a spiral CT scan (EJNM, 1999).

There has been widespread discussion amongst radiologists and nuclear physicians suggesting the selective substitution of spiral computed tomography (CT) for lung scintigraphy for the diagnosis of PE (Annals of Emergency Medicine, 1999). Radiologists suggest spiral CT is more accurate than combining ventilation and perfusion lung scintigraphy and the nuclear physicians “best judgement” (Annals of Emergency Medicine, 1999). This is due to the fact nuclear medicine physicians classify lung patients in groups according to their probability of having PE, whereas a thrombus is visible using

spiral CT (Rays, 1996). Lung scintigraphy research has been undertaken over many years and as yet a large- scale research project has not been conducted on spiral CT on these criteria (Annals of Emergency Medicine, 1999). Most referring physicians are aware of the strengths and limitations of an assessment that relies primarily on lung scintigraphy, as this has the backup of many years research. Spiral CT will have an interesting role to play in patient evaluation for the diagnosis of PE (Annals of Emergency Medicine, 1999).

The negative aspects of spiral CT are the high radiation doses received by the patient and the increasing number of x-rays ordered by clinicians (Rays, 1996). The absorbed dose in tissues from CT is high and is within the range of 10 – 100 mGy (Annals of Emergency Medicine, 1999). Clinicians therefore need to justify patients having x-ray examinations and ask if these tests will affect patient management. Reducing patient doses should remain a priority; CT doses are quite high and have not reduced over time, as has conventional radiology (Clinical Imaging, 1994). Computerized Tomography (CT) and magnetic resonance have a limited ability to detect occlusion of the distal pulmonary arterial branches that exclude PE (Clinical Imaging, 1994).

Despite being a sound diagnostic tool, spiral CT scanning is not without limitations. The main limiting feature is the inability of spiral scanning to detect pulmonary embolism in subsegmental pulmonary arteries (Rays, 1996). Using data from six studies, Mullins et al. (2000) found a sensitivity of 29% for detection of subsegmental pulmonary embolism by spiral CT, compared with pulmonary angiography (Annals of Emergency Medicine, 1999).

The question arises as to the prevalence of subsegmental emboli. Data from the PIOPED (1990,1995) study showed that 5.6% of patients had isolated

subsegmental emboli. Other studies indicated this number was likely to be 5% to 36% (Van Beek, 1996, Fedullo, 2003). The clinical significance of isolated subsegmental emboli is not clearly known, but they may be markers for larger clots residing in the lower extremity veins (Fedullo, 2003). In addition, treatment of isolated subsegmental emboli in patients with poor cardiopulmonary reserve can result in clinical improvement.

1.11 Clinical Guidelines

To date, there are insufficient multi-centre studies to support the use of spiral CT as an initial diagnostic technique for detection of venous thromboembolism (Annals of Emergency Medicine, 1999). Although the exact role of spiral CT in the diagnostic algorithm for pulmonary embolism is still not clear, a few key observations can be made to help guide clinical judgment.

Given the wide range of sensitivities of spiral CT for diagnosis of pulmonary embolism, it should be used as a "rule-in" modality, rather than a "rule-out" procedure (Rays, 1996). Hence a normal spiral CT scan does not exclude pulmonary embolism with certainty. If the spiral CT is normal and the clinical suspicion of pulmonary embolism is high, further testing is strongly recommended (compression ultrasound, D-dimer assay, or pulmonary angiography) (Fedullo, 2003, Botnar, 2004).

Since the investigation of venous thromboembolism can be tedious, invasive, and expensive, there has been an increased focus on D-dimer assays, which are rapid, noninvasive, and inexpensive. Fibrin is the main component of

thrombus formation, and degradation of cross-linked fibrin results in fibrin degradation products including D-dimers (Clinical Imaging, 1994).

D-dimers are commonly found in the circulation when venous thromboembolism is present. However, this finding lacks specificity, since D-dimers are also found in other disease states, including cancer, congestive heart failure, and inflammatory conditions (Clinical Imaging, 1994).

Tapsen and associates in 1999 used two general methods of measuring D-dimers. The original enzyme-linked immunosorbent assay (ELISA) methods have been well studied and in general have a 95% negative predictive power (Rays, 1996). In 1998, Janssen et al. studied the limitations associated with classic ELISA methods including cost and inability to perform the test rapidly. Janssen developed rapid ELISA assays (VIDAS DD [Biomerieux, France] and Instant IA DD [Stago, Asniere, France]). The VIDAS DD has been extensively studied and has a sensitivity of 94% to 100% and a negative predictive value of 92% to 100% (Rays, 1996, Clinical Imaging, 1994).

The other method of measuring D-dimer fragments is latex agglutination testing, which is both rapid and economical (Clinical Imaging, 1994). However, the latex testing methods are subjective and can be difficult to read. Most of the latex methods have inadequate sensitivity and negative predictive value for clinical use (Clinical Imaging, 1994). New latex tests have been introduced with sensitivities and negative predictive values approaching classic ELISA methods such as Simple Red [Agen Diagnostics Limited, Australia] and Tinaquant [Boehringer, Mannheim, Germany]. Janssen's results for the Simple Red assay show a sensitivity of 89% to 100% and a negative predictive value of 95% to

100%. The Tinaquant assay has a sensitivity of 99% and a negative predictive value of 93% (Botnar, 2004).

Evidence supporting use of D-dimers in clinical practice is rapidly emerging. Perrier and colleagues (1997) evaluated the role of an ELISA D-dimer assay in 198 patients suspected of having a pulmonary embolism. Using a cut-off value of less than 500 g/L as normal, these investigators reported that of the 198 patients with a normal D-dimer value, only one patient had a pulmonary embolism and one was lost to follow-up (Rays, 1996). The negative predictive values of the D-timer test in this study were 99% (196 of 198 patients).

Elevated D-dimer fragments are too non-specific for diagnosis of venous thromboembolism by themselves (Rays, 1996). With negative predictive values close to 100%, certain D-dimer assays have the potential to be the only screening test necessary to rule out venous thromboembolism (Rays, 1996). However, many authors and experts have been reluctant to propose such a diagnostic strategy (Clinical Imaging, 1994).

The American Thoracic Society's clinical practice guidelines (1999) on acute venous thromboembolism reaffirm the emerging value of D-dimers assays but currently do not endorse widespread use (Rays, 1996). At present, assay performance varies widely, and different assays cannot be clinically applied interchangeably (Rays, 1996).

If D-dimer assays are to be used in a diagnostic strategy, the details of the assay should be known, including type (latex or ELISA), operating characteristics (sensitivity and negative predictive value), and outcomes of clinical studies supporting the particular assay (Clinical Imaging 1994). Until

further studies emerge, testing for D-dimers should be restricted to patients in whom clinical suspicion of venous thromboembolism is low or moderate (Rays, 1996, Clinical Imaging, 1994).

Given that the majority of patients with abnormal chest x-ray studies have a nondiagnostic ventilation-perfusion scan, spiral CT scanning is an option for initial study in these patients (Annals of Emergency Medicine, 1999). Finally, if an alternative diagnosis is being considered in addition to pulmonary embolism, spiral CT scanning can provide new information that a ventilation-perfusion scan cannot (Clinical Imaging, 1994). Further prospective, multi-institutional investigations can help clarify the role of spiral CT for the diagnosis of pulmonary embolism.

Newer equipment, however, will mean reduced patient dosage, as anatomical based on-line adjustment of exposure factors should be used (EJNM, 1999). Management of dose means a reduction of mA (tube current), which is determined by the inherent characteristics of the scanner, size of the patient, the anatomical region for investigation and technique used (EJNM, 1999). Therefore, absorbed dose should be sufficient to meet all the aforementioned criteria (Madan, 2000). Patient dose depends on the radiation of the x-ray beam, and is linearly related to the product of the tube current (mA) and scans time(s) (Clinical Imaging, 1994).

Scan length also controls the volume of patient irradiated, unfortunately with new fast scanners there has been a tendency to increase scan length so that scans of the thorax, abdomen and pelvis are common. Effort should be made to restrict the areas of scanning to those clinically essential (Madan, 2000).

In the future there may be another scintigraphic approach to diagnose more directly thromboembolic disease and PE (Leiper, 1998). Research indicates the 99mTc peptide imaging of activated platelet receptors may help in the detection of acute DVT and PE. Lung scintigraphy however, will continue to be widely used to assist with the diagnosis of PE, as it is cheap, easy, non-invasive and readily available for use (Leiper, 1998). Arterial Blood Gases (ABG) assist in the diagnosis of PE, and other associated conditions such as respiratory acidosis/alkalosis or metabolic acidosis/alkalosis (Fahey, 1995). ABGs do not only identify a specific problem but can also help identify possible strategies to correct for these imbalances (Fahey, 1995). The oxyhaemoglobin curve shows the relationship between percentage of haemoglobin saturation (SaO2) and partial pressure of arterial oxygen (PaO2) (Hess, 1996).

A normal oxyhaemoglobin curve assumes certain parameters. These are a pH of 7.4; temperature of 37°C and PaCO2 of 40 mmHg (Earis & Pearson, 1995). At the arterial end of the curve the slope is gentle, reflecting the alveolar-capillary O2 transfer site. PaO2 drops markedly with little change in SaO2 at this point. At the steeper portion of the curve, venous disassociation occurs as O2 leaves the haemoglobin and is transferred to the cells (Earis & Pearson, 1995).

At this site SaO2 drops dramatically with little change in PaO2. Oxygenation at the cellular level depends on the ability of O2 to bind with and be released from the Haemoglobin (Hb) in the blood (Adams, 1984, Earis & Pearson, 1995).

Thus pH is the measurement of acidity of a solution (Hydrogen ion concentration). The optimal pH is 7.4 (Range 7.36 - 7.45) (Hillman, 1996). Death can result when the pH is less than 6.8 or greater than 7.8. Buffers in the

body will always try to maintain homeostasis and restore the pH to normal (Earis & Pearson, 1995).

This process is called compensation and is controlled by the use of buffers (Earis & Pearson, 1995). Buffers are weak acids or bases that prevent sudden change in pH. Examples of buffer systems are: phosphate buffer system, Hb/OxyHb system (Habibian 1999). Hb releases oxygen and attracts H+ ions. There is a formula which summarizes the buffer system:

> (Carbon dioxide+water =carbonic acid = hydrogen + bicarbonate) e.g. CO2 + H2O Lungs = H2CC3 = H^+ + $HCC3^-$ Kidneys (Adams, 1984).

Magnetic Resonance Imaging MRI or MRV for magnetic resonance venography may be considered a complementary diagnostic tool to the less expensive venous sonography (Rays, 1996). It is the complementary use of MRV techniques at the level of the pelvis and mediastinum that offers the greatest cost benefit and part of a patient's diagnostic workup (Rays, 1996). A major consideration of DVT is the mortality and morbidity associated with PE (Earis & Pearson, 1995).

The long-term sequelae of DVT include the development of chronic venous disease, venous insufficiency and associated trophic changes. The incidence of upper extremity venous disease has increased in part due to the better use of central venous lines as they serve as a nidus for thrombus formation (Rumack, 1998).

MRV images the central veins such as the inferior and superior vena cava, the iliac, brachiocephalic as well as peripheral veins. The future of MRI in the investigation of PE will depend on how integrated it can become with other non-

invasive technologies (Clinical Imaging 1994). MRV pulse sequences can be differentiated into either T1 or T2 weighted spin echo images as well as flow sensitive sequences (Rays, 1996, Clinical Imaging, 1994). The shift in MRI today is to use rapid flow sequences; saturation pulses attenuate the signal intensity of moving blood within the veins (Rays, 1996). Contrast infusions of Gadolinium (^{153}Gd) with concentrations of 0.1 - 0.2 mmol/kg can be used to increase the signals arising from moving blood in the vein lumen. However there is no real justification for using Gadolinium (Rays, 1996).

The presence of a filling defect in the lower extremities can be confirmed with flow sensitive sequences (Rays, 1996). With DVT and total obstruction of the veins, T2 weighted sequences can confirm the presence of inflammatory changes in the soft tissues due to a response associated with the presence of DVT. The negative aspect, however, is the high cost on a per patient basis and with respect to the diagnosis of isolated calf DVT, there is little data to justify the use of MRV, although it has a definite advantage at pelvic level (Rays, 1996, Clinical Imaging, 1994).

Forced Expiratory Volume or FEV_1 is another useful diagnostic measurement, as FEV_1 evaluates air resistance in the larger airways by using a spirometer. FEV_1 is decreased in Chronic Airways Limitation (CAL) patients, as there is an increased airway resistance (Hillman, 1996). Forced Vital Capacity (FVC) divided by FEV_1 is a ratio used as it is independent of variations in lung volumes due to age, stature and restrictive lung disease (Hillman, 1996, Adams, 1984).

Lung function studies may support or exclude a diagnosis, but cannot make a diagnosis (Adams, 1984). Early lung function tests may help both in the control of the condition and in the diagnosis. Patients with CAL will undergo these tests

regularly and early diagnosis and treatment of respiratory defects can significantly enhance and extend the life of large numbers of people (Adams, 1984, Earis & Pearson, 1995).

Vital Capacity (VC) is the volume change at the mouth between the position of full inspiration and full expiration (West, 1987). It is usually performed slowly from a position of maximum inspiration to full expiration. Some patients may have a degree of broncho-spasm. Increased values will be obtained from patients with CAL; a reduction in VC indicated a pattern of restriction (West, 1987).

Forced Vital Capacity (FVC) represents the volume of gas which can be "squeezed" from the lung when maximum effort from a position of full inspiration (West, 1987). Sometimes FVC can be less than VC as the forced manoeuvre can raise inter-pleural pressure causing premature closing of the peripheral airways, called air trapping (FDA, 1999, West, 1987).

Forced Expiratory Volume (FEV_1) is the amount of gas expelled from the lung over a timed period from a position of maximum inspiration with the person making a maximum effort with a usual time interval of one second (West, 1987). A reduced measurement indicates airway obstruction occurring in the larger airways from which the air is first expelled (West, 1987).

1.12 Lung Scanning

Ventilation lung scans provide images of regional ventilation (Habibian, 1999). Ventilation scintigraphy involves the use of a radioactive aerosol inhaled by the

patient using a commercially available Patient Administration Set (PAS), which is connected to the Technegas Generator (TcG) (Leiper, 1998). In a Technegas ventilation study these radioactive particles are smaller than one micro metre (μm) in diameter and adhere to alveolar structures without appreciable movement for approximately forty (40) minutes (Isawa et al, 1996). Once the aerosol is administered, lung scintigraphy commences and provides both quantitative and qualitative information for studying lung physiology (Habibian 1999). A concise explanation is given later. Adequate ventilation is sought to optimise images and to provide high specificity for the diagnosis of PE (James, 1995, Habibian 1999).

The ventilation-perfusion scan has long been considered the pivotal test for diagnosis of pulmonary embolism (Habibian 1999). Most of the information regarding ventilation-perfusion scanning comes from PIOPED (1990, 1995), a prospective multi-institutional effort, which compared ventilation-perfusion scanning with the standard diagnostic criterion of pulmonary angiography.

1.12.1 Oxygen Saturation

Due to transient hypoxia during the first inhalation of Technegas and Argon there is a brief instance of anoxia when oxygen levels may fall by varying degrees in patients for a few seconds prior to returning to normal levels (James, 1992).

It is anticipated that the majority of patients will undergo some temporary changes in oxygen saturation levels during the administration of Technegas (Leiper, 1998). This is a common occurrence, not associated with any harmful symptoms. Therefore, oxygen via nasal prongs is used for volunteer CAL

patients who need supplementation to keep their oxygen saturation levels within normal range (99 - 100 %), making hypoxia less significant (Leiper, 1998).

The first spectrophotometric measurements of blood were made in the 1930s (James, 1992). In the 1950s, a spectrophotometer was used for measuring haemoglobin and its derivatives. Specific instrumentation for measuring oxygen saturation was developed in the 1960s. The use of ear and finger oximeters for continuous estimates of arterial saturation arose from aviation studies in both Germany and America during World War 2. Widespread use of pulse oximeters developed in the 1980s (James, 1992).

The oxygen saturation monitor provides continuous oxygen saturation readings, which are produced by measuring the absorption of different wavelengths of light (James, 1992). At certain known wavelengths both oxygenated haemoglobin (HbO2) and reduced haemoglobin (Hb) absorb light (Hillman, 1996). The continuous pulsation from the digital artery modulates light, which is passed through the probe thereby enabling calculation of the delivered oxygen saturation levels while ventilating the patient. The digital display from the monitor is updated every twenty-five (25) seconds, the range is 0 - 100% (Hillman, 1996, James, 1992).

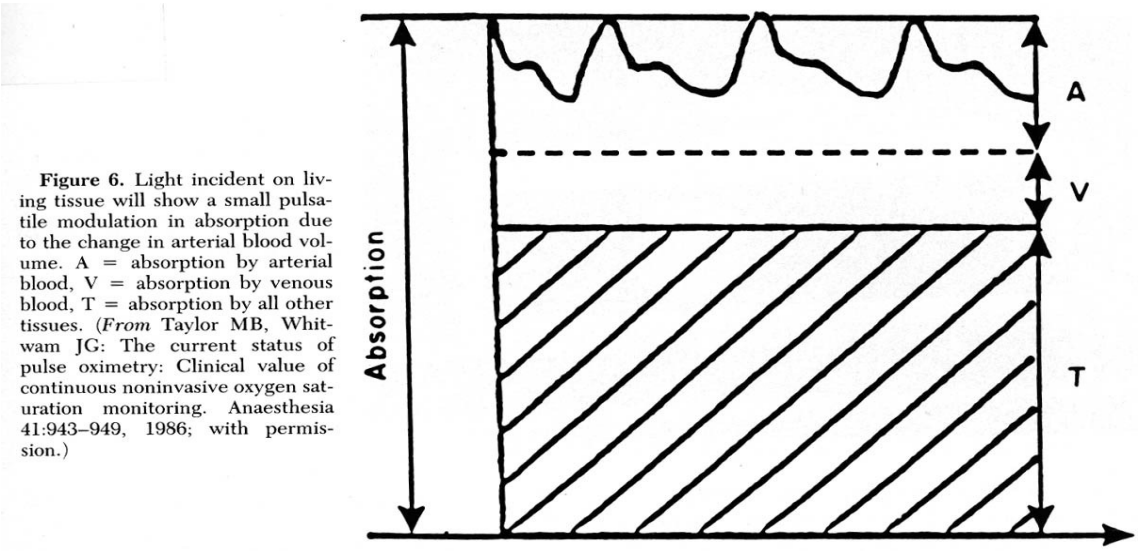


Figure 6. Light incident on living tissue will show a small pulsatile modulation in absorption due to the change in arterial blood volume. A = absorption by arterial blood, V = absorption by venous blood, T = absorption by all other tissues. (*From* Taylor MB, Whitwam JG: The current status of pulse oximetry: Clinical value of continuous noninvasive oxygen saturation monitoring. Anaesthesia 41:943–949, 1986; with permission.)

Figure 1.2 **Non-invasive Monitoring of Oxygen**

Nickerson et al. (1988) studied the reproducibility of oximeter measurements in normal volunteers and suggests variations of 1% in the range of saturations above 90%, and 2% in the range below 90%, indicating that a clinical change has occurred.

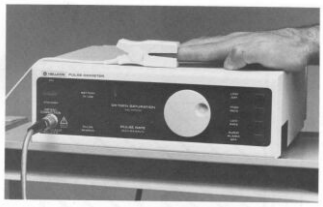


Figure 1.3 **The Nellcor pulse oximeter**

Routine monitoring of oxygen saturation will help prevent, although rare, any side effects, and ensure the safety of all patients who may be at risk (Leiper, 1998). Using the Nellcor oximeter, oxygen saturation levels are monitored at the beginning, trough (middle) and end of the ventilation stage. Valuable information can be obtained by reading patients' saturation levels prior to ventilation. The initial saturation is important, as it is the duration and degree of hypoxia induced and the patient's ability to withstand changes in oxygen saturation levels that will require monitoring (Hillman, 1996, Leiper, 1998).

Historically there are two basic ways to measure haemoglobin oxygen saturation levels in blood: 1) gasometrically and 2) spectrophotometrically (Adams, 1984, Earis & Pearson, 1995). The development of spectrophotometric methods dates back to Isaac Newton's studies of light in the 1600s. Research undertaken by Lambert in 1760 and Beer in 1852 resulted in the Beer-Lambert Law that describes the transmission/absorption of light as a logarithmic function of the concentration of the absorbing molecules in solutions.

1.12.2 Measured Saturation

An oximeter is a spectrophotometer designed to measure blood oxygen saturation (Fahey, 1995). Each type of haemoglobin molecule has its own light absorption spectrum. Oximeters contain light sources at selected wavelengths that correspond to the absorption spectra of the haemoglobin molecules to be measured (Hillman, 1996). Therefore, a basic oximeter that can measure saturated oxygen needs to determine the absorption at only two wavelengths, one for haemoglobin and one for oxyhaemoglobin. Pulse oximeters use two wavelengths that can be transmitted through the skin (e.g., a finger, ear or toe), allowing non-invasive monitoring of saturation (Adams, 1984). However, two–wavelength oximeters can give misleading estimates of the oxygen content of blood in the presence of elevated levels of Carboxyhaemoglobin (COHb) and Methaemoglobin (MetHb) (Adams, 1984, Earis & Pearson, 1995).

1.12.3 Chest X-Ray and PE in Nuclear Medicine

The chest x-ray is often the first imaging screen obtained when a patient is admitted to accident and emergency with dyspnoea (Fogelman, 1988). In

patients that are positive for PE, their chest x-ray will be abnormal. Much research has been carried out to establish that a normal chest x-ray only occurs in a minority of cases of PE (Fogelman, 1988, Early, 1995).

In the chest x-ray, atelectasis and parenchymal densities are quite common. The areas of atelectasis are more common in the lower lobe of the lung as are the areas of parenchymal density (Wade, 1982). Most of these densities are caused by pulmonary haemorrhage and oedema and may be confused with infectious infiltrates or malignancies (Early, 1995 and Wade, 1982). These effusions are usually visible when the patient is examined, mostly small and occupying less than fifteen percent (15%) of a hemithorax and rarely increase in size after the third day. Any size increase should raise a suspicion of pulmonary infection or re-embolization.

Pleural-based opacities with convex medial margins are known as Hampton's Hump, which maybe an indication of a lung infection (Roussos, 1995, Fogelman, 1988). The rate of resolution of these densities is a good method of judging if lung tissue has infracted. Areas of pulmonary oedema and haemorrhage resolve in a few days to a week (Roussos, 1995). Linear scars are found when the infarcted lung has decreased over weeks to months (Fogelman, 1988 and Early, 1995).

If the central pulmonary artery is prominent, this is either from pulmonary hypertension or the presence of a clot in those arteries (Adams, 1984). Cardiomegally is a non-specific finding but may imply an enlarged right ventricle as seen in patients who present with large bilateral pulmonary emboli (Earis & Pearson, 1995). In conclusion, the chest x-ray can be normal in a minority of

patients and abnormalities when present are often non-specific (Earis & Pearson, 1995).

1.13 PE and Nuclear Medicine

The early 1970s were indeed a turning point for nuclear medicine, bringing to mind Whitehead's statement: "It is a well founded historical generalization that the last thing to be discovered in any science is what the science is really about" (taken from Taylor, 1989).

Approximately one patient in three hospitalised will have a nuclear medicine diagnostic procedure performed in which a radioactive isotope or tracer has an essential role (Murray, 1988). One example is lung scintigraphy, when a decreased blood flow to the lungs is observed, which may indicate the presence of PE. The use of the radioactive tracers in diagnostic and therapeutic medicine has become so widespread that nuclear medicine is a "speciality" department (Habibian 1999, Isawa, 1991).

1.13.1 Indications for Lung Scintigraphy

Indications for both ventilation and perfusion lung scintigraphy are to evaluate possible pulmonary embolism (PE) and the evaluation of pulmonary flow post heparin for patients with confirmed PE (Murray, 1988). PE is usually multiple and produces more than two scintigraphic perfusion defects (a defect being a reduction in perfusion) accompanied by a normal ventilation scan and clear chest x-ray (Habibian 1999, Murray, 1988). Both lung ventilation and perfusion scintigraphy have a high specificity for PE.

The typical pattern seen in PE is of perfusion defects with normal ventilation, whereas matching perfusion and ventilation defects are typical of other respiratory diseases (Taylor, 2000). In patients with CAL, their image quality is poor, showing considerable particle deposition in the major airways and little penetration to the lung parenchyma (Taylor, 2000). Attempts have been made to improve aerosol production systems in order to reduce the particle size and therefore improve image quality (Taylor, 2000). The use of a positive pressure device known as TEBA assists CAL patients as Technegas is gently "pushed" into the patients' lungs, requiring no effort from the patient and giving a more uniform image for interpretation (Leiper, 1998).

Technegas used for lung ventilation should not show significant clearance or redistribution over the time of image acquisition (Murray, 1988). Technegas has the distinct advantage that it remains fixed in the lung, having an effective half – life of 335 minutes, which is equivalent to the half – life due to physical decay (Burch, 1986).

1.13.2 How Lung Scans are interpreted in Nuclear Medicine

Perfusion defects of PE are wedged shaped and pleural based; therefore seeing a centrally located defect "stripe sign" is evidence against PE. Segmental perfusion defects are important characteristics of PE. Defects of irregular shape are unlikely to be due to PE. The size of the perfusion defect is also an important clue to the diagnosis of PE (Early, 1995). Defects are usually large and occupy more than seventy-five percent (75%) of a segment. Twenty-five to seventy five percent (25 - 75%) are moderate and segmental, whereas

defects less than twenty-five percent (25%) are usually not caused by PE (PIOPED, 1990).

Lung perfusion should not be used solely as a diagnostic modality. A good medical history, recent chest x-rays and a ventilation study must also be sought (Botnar, 2004).

1.13.3 Why Nuclear Medicine procedures are useful

The single greatest value of nuclear medicine procedures is that they provide the unique ability to apply the tracer technique to humans; that is, to be able to track the course of labelled molecules or cells as they travel throughout the human body (Murray, 1988).

We can examine both the site and rate of important biological processes and detect abnormalities as regions of dysfunction or decreased perfusion (Howarth, 1999). If we say that the first principle of nuclear medicine is the tracer or isotope, the second is the “homogeneity principle” which states that the function of many body organs is relatively homogeneous (Murray, 1988). Disease can manifest itself as a region of local dysfunction that may occur prior to the overall function of the organ becoming impaired, which often results in an earlier diagnosis.

Nuclear physicians use a combination of applied physiology and biochemistry and make direct measurements of regional function. This is known as a “working hypotheses”, in other words a means of predicting the outcome and optimum care for the patient (Habibian 1999). The patient’s physiological and biochemical measurements often provide the essential information for the diagnosis to be made (Murray, 1988).

With the use of radio isotope scanning, it is not necessary to achieve the high concentration necessary to produce opacity to X-rays. It is the relative concentration within the organ e.g. lung with respect to its surroundings that is important in nuclear medicine rather than simply the absolute concentration (Taylor, 1989).

The use of radioactive tracers in nuclear medicine has made possible an improvement in perception and conceptualisation of disease (Roussos, 1995). Nuclear medicine gives us symbolic representations of patterns and changes in the spatial and temporal distribution of chemicals that make up living organisms (Roussos, 1995, Fogelman, 1988, Early, 1995).

Limiting our perception to static images is equivalent to assuming that nothing ever changes, therefore our images have to be concerned with time, with the order of events and duration (Taylor, 2000). When we report on an image, we are investigating what is happening in that patient's body and looking at the spatial and temporal distribution of isotope in nuclear medicine (Taylor, 2000, Garbe, 1986).

Approximately one hundred percent (100%) of the diagnostic studies performed with radioactive tracers in nuclear medicine use a radionuclide (Murray, 1988). Together with the use of the gamma camera and computer, technetium – 99m has played a major role (Palla et al, 1988). There are several reasons for its widespread use: (1) its short half–life and mode of radioactive decay keep the radiation dose to the patient low (ALARA); (2) it is readily available as a daughter product of the radionuclide, molybdenum – 99, with a 67 hour half-life; (3) it forms chemically stable complexes with a wide variety of molecules.

Most radioactive drugs are administered to provide diagnostic information rather than to produce a therapeutic effect, although the use of radioactive iodine to suppress thyroid function is an important exception (Habibian 1999). In a nuclear medicine study, diagnostic information is encoded in gamma rays, a type of electromagnetic radiation that has the ability to penetrate tissues of the body and be detected by the gamma camera also known as an Anger Scintillation camera (Habibian 1999, Taylor, 2000). In the case of therapeutic radiation, the gamma rays transfer energy to the cells being irradiated in order to reduce their function or kill them, as is the case for cancer therapy (Taylor, 2000).

In diagnostic studies, the aim is to maximise the number of photons being recorded while minimizing the associated radiation dose to the patient. From the viewpoint of minimizing the latter, the shortest possible half – life, which permits measurement of the diagnostic information, is desired (Taylor, 2000).

Most biochemicals that we investigate consist of carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur (Behrens, 1959). Hydrogen, phosphorus and sulphur have no suitable gamma emitting isotopes. Carbon – 11, nitrogen – 13, and oxygen – 15, decay by emitting positrons, which combine with electrons to emit two 511keV photons in almost exactly opposite directions (Behrens, 1959). Their production requires the availability of a particle accelerator known as a cyclotron, which is only available at major centres (Murray et al, 1988).

Advances in nuclear medicine over the years have been along three lines: better chemicals, better instruments and better quantification (Subramanian, 1975). With the introduction of emission tomography, quantification improved significantly (Rays, 1996). There are two types of emission tomography: Single

emission tomography known as SPECT and positron emission computed tomography or PET. The latter permits a more accurate quantification, and the former provides significant improvement over planar imaging, where the three dimensional distribution of radioactivity is projected onto a single plane (Rays, 1996). Whether imaging performed with the planar or SPECT technique, the positive predictive value of a high-probability scan is sufficiently high (96%), and that of a low-probability scan in the setting of low clinical suspicion is sufficiently low (4%), that many patients can be managed with V/Q scans alone (Maki, 2011).

1.13.4 Non-specific signs and symptoms

The problems in clinical diagnosis of PE relate to the non-specificity of clinical signs and symptoms: the most common symptoms reported are unexplained dyspnoea, light-headedness, and chest pain (Botnar, 2004). A routine anterior / posterior chest X-ray will also be acquired within the first 24 hours after admission. This X-ray will accompany the patient having a lung scan which will assist in the diagnosis along with the acquired images from the lung scintigraphy (Rays, 1996).

Other diagnostic tests may be requested if the lung scintigraphy report is intermediate and the chest x-ray non specific for PE. The “Gold Standard” is Spiral CT, which may give a definitive diagnosis for PE. The decision about which combination of tests to investigate this disease is subject to various debates (Rays, 1996, Clinical Imaging, 1994).

Since the clinical presentation of PE is usually non-specific, the findings on CT are often the first clinical indication that the patient may be suffering from PE. In

addition to being able to visualise the infarction, the area where the clot is located can also be visualised (Clinical Imaging, 1994).

1.14 Lung Scintigraphy

Lung scintigraphy continues to be a useful diagnostic tool and is a logical imaging investigation to obtain post chest radiography (Clinical Imaging, 1994). The lung scintigraphy report will be categorized as low, intermediate or high probability for PE (PIOPED, 1990). A normal perfusion scan is characterized by even distribution of radiotracer throughout both lungs. It is also important to note that the accuracy of any diagnostic investigation is dependent on its reproducibility (Taylor, 2000, Clinical Imaging, 1994). A high probability perfusion scan is represented by multiple segmental or larger defects with normal ventilation in at least one area of abnormal perfusion. This is known in nuclear medicine as a ventilation / perfusion mismatch. A lung scan report with high probability may show multiple segmental defects and normal ventilation (Rays, 1996, Clinical Imaging, 1994).

Various forms of the PIOPED criteria have been used since the 1990s by nuclear physicians as a reliable method of diagnosing PE. It is important to recognise that a minority of patients with a PE have a high probability scan and that this pattern of events is not pathogenomic for acute embolism especially if there is a history of PE. A minority of emboli do not resolve and the perfusion study may not return to normal (Clinical Imaging, 1994).

If a ventilation / perfusion scan does not fit into the normal or high probability category, then it is a non–diagnostic report for PE. The majority of patients fall into this category due to the presence of subsegmental defects, or defects of any size matching abnormalities seen on a chest x-ray, and further investigations may be necessary (Clinical Imaging, 1994).

A low probability category is not particularly reliable as suggested by PIOPED data. Disagreement amongst experienced nuclear physicians is common when perfusion defects are small. Lung scans are sensitive investigations that eliminate the diagnosis of PE when they are normal. Patients with high probability lung scans can be treated without further often more invasive investigations (Rays, 1996).

1.14.1 Heparinise prior to a lung scan

It is interesting to see how many doctors pre-heparinise their patients prior to diagnostic lung scanning. Pre-heparinising patients may lead to misdiagnosis as the patient has already commenced anticoagulant treatment. Faced with an inconclusive lung scan result of intermediate probability, the next step may be a contrast enhanced spiral CT or pulmonary angiogram. Most doctors would probably agree that the costs of additional diagnostic tests to diagnose PE outweigh the benefits of basing anticoagulant therapy on a well-founded diagnosis.

The incidence depends largely on the population sampled. Patients with CAL are more likely to have an intermediate probability for PE, or non-diagnostic scans. The almost nonexistent morbidity and relatively low cost compared with spiral CT and pulmonary angiography make the V/Q lung scan the most

desirable first line diagnostic test for PE, after the CXR, which may identify pathology and which is needed to correlate with the lung scan (EJNM, 1999).

The risk of morbidity for the patient commencing anticoagulation therapy is low, but in contrast morbidity from anticoagulant therapies is high (EJNM, 1999). There is also the medico-legal risk to the doctor in the event that the patient dies on anticoagulant treatment before a diagnostic test is done. Haemorrhage is a side effect of heparin-induced thrombocytopenia. All patients commenced on anticoagulant therapy face months of treatment, which creates a cost burden and inconvenience to the patient (EJNM, 1999, PIOPED, 1990).

Most doctors, besides ordering a lung scan, also like to look at duplex ultrasound to diagnose DVT prior to commencing anticoagulation therapy when a lung scan is intermediate or the scan is delayed for twelve hours until the morning. A large number of patients with PE have positive ultrasound studies for DVT (Rumack, 1998).

Complications of heparin therapy include bleeding, thrombocytopaenia and osteoporosis (Taylor, 2000). Patients at risk of bleeding include those who have had recent surgery or trauma or who have other clinical factors that predispose to bleeding, such as an ulcer, liver disease or age > 65 years. The management of bleeding will depend on its severity, and the risk of recurrent venous thromboembolism (Taylor, 2000).

Heparin induced thromocytopaenia is a well recognised complication, which may occur 5 - 10 days post heparinization (West, 1987). Approximately 1 - 2% of patients receiving unfractionated heparin will experience a fall in their platelet count to less than the heparin on platelets and is of no consequence (West, 1987). This mild to moderate thrombocytopaenia may be a direct effect of

heparin on platelets and is of no consequence. However, 0.1 - 0.2% of patients may develop an immune thrombocytopaenia that can also be accompanied by arterial or venous thrombosis, which may lead to death or limb amputation (West, 1987).

Osteoporosis has been reported when a patient received unfractionated heparin in doses of 20,000 U per day (or more) for more than six (6) months.

Demineralisation of bone can progress to fracture of the vertebral bodies or long bones (Adams, 1984).

1.15 The History of Technegas

1.15.1 How Technegas was invented

Up till the 1980s, the radioaerosol used in lung scintigraphy for the prompt diagnosis of Pulmonary Embolism (PE) was Xenon-133, an inert radioactive gas. At the first Asia and Oceania Congress of Nuclear Medicine held in Sydney in1976, Taplin's paper on "Lung Imaging in Pulmonary Disease" highlighted the mismatch between the quality of the perfusion agent and different other ventilation agents; this eventually led to Burch et al. discovering a superior ventilation tool.

Burch and colleagues introduced Technegas in November 1984 and described it as a sub-set of nano-encapsulated carbon composites or aggregates. Technegas is produced in a Technegas Generator, which is purpose built for lung scintigraphy. Its production requires an apparatus capable of heating a

graphite crucible to 3000°C, thereby eliminating the concern of inhalation of tungsten oxide. It was concluded after several experiments, that all the technetium–99m absorbed on the surface of the graphite crucible and simultaneously volatilised to become coated with graphite above 2200°C. The structure of Technegas consists of hexagonal flat crystals of technetium metal encapsulated within a carbon capsule to be within a range of 30 – 60 nm in size. Prior to ventilation, these technetium-99m particles coagulate into aggregates with a median diameter of 100 to 160 nm. Depending on the time of Technegas generation and ventilation, the longer the time, the larger the particles. These particles can be as large as 225 nm measured at 8.5-minute generations (Burch 1996). Technegas particle size and the amount of deposition in the lung will determine the mucociliary clearance rate of technetium (Lloyd 1997).

In studying the structure of Technegas, Senden et al. (1997) reported Technegas was a coalescence of metal atoms which are encapsulated with intercolating hexagons of carbon on a pure graphite surface.

1.15.2 Technegas Characteristics

Technegas is an aerosol consisting of a gaseous continuous phase and a discontinuous phase of individual particles. The discontinuous phase can be either solid, liquid or both. There are many terms used to denote different types of aerosols. One term used by Lloyd (1994) is smoke, which is solid and derived from a combustion process. Another term used is mist, which is a liquid process as used in Technegas.

The behaviour of an aerosol is dependent on size, shape and composition and the collective properties that include: size distribution, number density, etc.

Previous research work on the chemical nature of Technegas proposed that Technegas ultra fine aerosol particles range in size from a few nanometres to a few hundred μm. Lloyd (1994) suggests that the lower end of the range of aerosol particles represents the transition from molecules to particles, whereas particles of the upper end of the scale do not remain airborne for long enough to form an aerosol. The largest useful size for studies of the lung is approximately 10 μm; inhaled particles greater than this will deposit in the upper airways.

Although the nature of Technegas is still uncertain, it has proved to be a satisfactory imaging agent for ventilation lung studies (Taylor 2000). Technegas is a structured suspension of ultra fine carbon particles which have been labelled with technetium, and has both the characteristics of an aerosol and a gas. Particle size ranges from 5 – 25 nm with good alveolar deposition, minimal central deposition and no mucociliary clearance (James 2002). Distribution of Technegas is determined by regional ventilation, therefore providing an insight into lung physiology. Lemb and Oei (1992) suggest that the combination of ventilation and perfusion scintigraphy has been shown to produce a high sensitivity and specificity in the diagnosis of PE.

This radioaerosol is in the form of a mist, which is a liquid aerosol. Aerosols are dependent both on particular properties such as size, shape and composition, and collective properties which include size distribution, density etc. The size of Technegas particles ranges from a few nanometres to a few hundred μm, the

lower end representing the transition from molecule to particle (Lemb & Oei 1993).

Aerosol particle size distributions are mostly skewed and generally cover a large range of sizes, and so geometrical progression of size intervals is useful. A continuous size distribution function, n(r) is obtained and the integral of this function will give the number of particles in that range (Lloyd 1997). Various measures are used for the average size and spread of sizes. The modal diameter is the size associated with the peak in size distribution and the arithmetic mean is used for the sum of all diameters divided by the number of particles (Lloyd 1997).

Deposition of Technegas particles in the lung has been of interest to the nuclear medicine community over the years (Lloyd 1997). Measurement of deposition is relatively straightforward but there are different clearance rates and multifarious pathways of exit from the lung (Lloyd 1997). In a study by Howarth (1999), Technegas was seen to have the characteristics of both an aerosol and a gas and mimic the regional distribution of a gas. According to research by Burch (1993), the hexagonal shape of Technegas particles undergoes significant structural change post inhalation. However, the reasons for this change are not well known.

Burch and associates (1986) suggest that although Technegas is frequently referred to as an ultra-fine aerosol, inferences based on studies with regard to the behaviour of ultra fine particles in the respiratory tract should be made with caution.

There is also a distinction between patient dose and ventilation dose, a medium diameter of 20 nm versus 200 nm being quite significant (Senden 1997).

Particle size affects the site and amount of particle deposition as well as the clearance of Technegas. Kim et al. (2000), reported that where deposition occurs, particle size will be affected as the lung shifts proximally with decreasing particle size below 100 nm. This proximal shift in deposition affects both tissue dose to the airways as well as the mucociliary clearance rate, which has a shorter path length for clearance from the lung. Deposition fraction or DF of aerosol is measured in the lung by: DF = 1 - A_{EX} / A_{IN} where A_{EX} and A_{IN} are exhaled and inhaled activity respectively. James and Brown (2002) found patients with Chronic Airway Limitation (CAL) have an increased deposition relative to healthy subjects, some of which occurs in the airways, although most deposition is thought to occur in the parenchymal lung.

Given that CAL subjects have heterogeneous ventilation within their lungs, they may receive a dose to their parenchymal lung that is many times greater than that occurring in the normal lung. James (2002) found no evidence of rapid pulmonary clearance for carbon ultra fine particles into the circulation. An increase in the parenchymal dose may therefore be attributable to an exacerbation of airway inflammation but not due to rapid movement of a significant number of insoluble particles in the circulation.

1.15.3 Technegas equipment and potential in diagnostics

The Technegas Easy Breather Accessory (TEBA) has been designed as an accessory to the Technegas Generator. The generator is a mobile, microprocessor controlled device. TEBA provides positive pressure while the generator provides the correct atmospheric conditions for the production of

Technegas. The system delivers Technegas, to frail-aged, ventilated patients or patients who are otherwise unable to undertake a normal ventilation study.

The Technegas Generator is a micro-processor operated electromechanical device that provides the correct atmospheric conditions for the production of Technegas. The NSC 800/810 series carries out checks for the generation procedure, including:

Front plate assembly

- Chamber cap assembly
- Front plate assembly
- Main circuit board
- Display circuit board
- Transformers
- Final assembly items
- Drawer Assembly
- Front frame and control
- Rear box and cover

Operation

The graphite crucible is positioned between the electrodes located at the base of the drawer assembly. The crucible is then loaded with pertechnetate (500 – 900 MBq) to a maximum volume of 110µl. It is important not to overload the crucible as a flat surface or meniscus is required to prevent splashing of radioactive material. Air bubbles are trapped in the graphite crucible, which will expand and ascend to the surface, causing the eluant to expand, spill and possibly splash out of the crucible. Therefore overfilling the crucible may

actually result in less radioactivity being simmered and burnt (Vita Medical Manual).

The drawer is closed and for safety precautions, a two-handed operation is required. A prompt appears on the display panel to initiate the simmer. The Technegas Generator checks for the presence of Argon gas and a routine check for gas leaks are done. Gas leaks are found by monitoring the pressure in the chamber over time and this program allows for a 50% drop in pressure. The generator will not proceed to the next step with any fall below this level. The unit will open and close the inlet valves if all the checks are done satisfactorily, and simmer/ purge stage commences. The simmer/ purge stage dries the pertechnetate onto the walls of the well in the graphite crucible. This is achieved by a small current passing through the crucible, increasing the temperature to seventy 70°C and Argon gas is pushed across the top of the crucible to disturb the surface of the pertechnetate. Concurrently, the chamber is purged of any air leaving an inert atmosphere. The simmer / purge stage lasts six minutes. When finished the generator will recheck for Argon gas and the prompt display appears. At this stage the burn generation is initiated (Vita Medical Manual).

After the start, a high voltage current passes through the crucible raising the temperature to 2500°C, lasting fifteen (15) seconds, vaporizing the dried pertechnetate off the crucible wall. An ultra fine dispersion of labelled carbon is produced, which is the Technegas. The purge process is now initiated to clean out the system for the next patient.

Once Technegas is produced, the user is instructed to disconnect the main power and Argon gas supply. The Technegas is stored in the chamber until

delivery to the patient. The user has ten minutes to deliver the gas to the patient. Technegas particles aggregate due to collisions and become too large to be useful; consequently the patient valve is locked with the solenoid, ten minutes after generation. The purge process is initiated to clean out the system for the next patient (Vita Medical Manual).

Technegas ventilation studies play a significant role in the diagnosis of pulmonary disease however, a percentage of the patient population, especially patients with CAL and unconscious patients, pose a number of problems in obtaining ventilatory images of reasonable diagnostic quality. The main problems relate to the dead space in the manifold of the Technegas generator. The delivery of Technegas through the manifold becomes variable, leading to turbulence and deposition of the aerosol in the pharynx or central airways (West 1999). There are also other factors of contamination, leakage and undue radiation exposure to the operator if the delivery of Technegas is obstructed (Murray 1998).

Previous research by Leiper (1998) Technegas Easy Breather Accessary (TEBA): A New Concept in Lung Ventilation Scintigraphy for Nuclear Medicine, evaluated the efficacy and safety of the device known as the Technegas Easy Breather Accessory (TEBA). These results found image interpretability for intubated patients using TEBA was good or adequate. Peripheral distribution was adequate, while central deposition was mild to moderate. Image quality was based on mean central deposition, peripheral distribution and image interpretability, and was generally superior for intubated patients. TEBA improved interpretability of ventilation images and consequently increased the reporting physician's confidence in diagnosing PE. The TEBA system will

provide images as good as or better than conventional images in a relatively sick population of patients at risk of PE.

The positive attributes of TEBA such as ease of set-up and application may indicate its potential to allow intubated unconscious patients to undergo Nuclear Medicine lung scintigraphy, whereas in the past, this procedure would not have been attempted on these patients who are perhaps in greatest need of determining the diagnosis of PE.

Ian Tetley, co-founder of Technegas with Christene Leiper, Principal Investigator (right) and her assistant (left).

1.16 Aims of this research

Lung scintigraphy in particular ventilation scanning in its present form has been shown to have limitations because some CAL patients with moderate to severe dyspnoea cannot use the conventional method for lung ventilation. This study examines whether positive pressure ventilation techniques will overcome these limitations for CAL patients.

The achievement of optimal ventilation studies on all patients, especially those with pulmonary disease, such as CAL, has the potential to improve the overall specificity of lung scintigraphy for the diagnosis of PE.

This research also aims to demonstrate that the TEBA system will provide images as good as or better than conventional images in a relatively sick population of patients at risk of PE.

The following five studies and their objectives comprise this research:

1. ***How well does Technegas Easy Breather Accessory (TEBA) perform with normal volunteers and Chronic Airways Limitation (CAL) patients***
   - To determine whether TEBA could provide ventilation images as good as or better than conventional ventilation scans in a group of normal volunteers and also in a group of patients with CAL.

2 ***Using TEBA for Pulmonary Embolism***
   - To determine whether TEBA could provide ventilation images as good as or better than conventional ventilation scans in a group of patients with Pulmonary Embolism (PE).

3 ***Positive Pressure Ventilation for Intubated Intensive Care Patients***

- To determine whether TEBA can allow ICU patients to undergo a V/Q scan
- To determine whether TEBA has a role to play in the management/diagnosis of PE or COAD in intubated / ICU patients
- To verify/assess the quality of images of ICU patients using TEBA.

4 ***Technegas at the Cellular Level***

- To analyze the technegas particle.

5 ***Sputum and Bronchial Washings at the Cellular Level***

- To analyze the sputum cells and determine if they have the same characteristics as Technegas from bronchial lavage washout specimens
- To measure activity of Technegas remaining in sputum post bronchoscopy
- To investigate under EM the structure of Technegas in sputum.

The findings from these experiments will increase our present knowledge in Nuclear Medicine.

CHAPTER 2 EXPERIMENT 1

How well does Technegas Easy Breather Accessory (TEBA) perform with normal volunteers and CAL patients

2.1 Introduction

A significant publication from the British Thoracic Society's Audit of February 2005 stated, "…more than one in ten patients with CAL admitted to hospital are dead within ninety days of admission, and over one in three are re-admitted during that time…" (Nilsson et al 2005). Due to the high prevalence of this chronic condition, which affects a large proportion of the working population, more attention needs to be paid to improving the diagnosis of CAL. While TEBA has been used successfully in a number of clinical settings (Cook & Leiper 1998), few formal studies have been published that demonstrate the usefulness, effectiveness, and efficacy of TEBA in normal volunteers or in patients with a range of respiratory pathologies such as Pulmonary Embolism (PE) or CAL. Let us first look at the symptoms and how we report on CAL, Deep Vein Thrombosis (DVT) and PE.

2.2 Understanding Chronic Airways limitation (CAL

Chronic Airways Limitation has had many names in the past including: Chronic Obstructive Airways Disease (COAD), Chronic Obstructive Pulmonary Disease (COPD) and Chronic Airflow Obstruction (CAO). CAL comprises two related

diseases, chronic bronchitis and emphysema, one rarely occurring without a degree of the other (Lippincott 1991 and Miller, 1997).

Lung imaging is an accurate, non-invasive way of evaluating ventilation and perfusion. Ventilation and perfusion to broncho-pulmonary segments is matched in a healthy individual, in CAL matched ventilation and perfusion, defects occur. In diagnosing PE, segmental reduction in perfusion occurs with maintenance of normal ventilation. This leads to the mismatch of perfusion and ventilation in the broncho-pulmonary segment. In acute infection seen with CAL patients, the ventilation defect may exceed the perfusion defect (Van Beek & Cate, 1996).

In CAL patients, airway quality is poor as seen on lung scintigraphy images (see Chapter 4), at the time of reporting due to particle deposition in the major airways and minimal penetration to the lung parenchyma. Refining the Technegas particle size may assist in improving the overall image quality of the ventilation scan for CAL patients (Strong & Agnew, 1989).

The ability to image lung ventilation using technetium 99m labelled Technegas is well documented (Murray et al, 1988, and Taylor, 2000). In particular Technegas has been used to advantage, being able to distinguish between patients with no lung disease and patients with CAL (Strong et al, 1989). However, like any ventilation imaging study, Technegas is limited by the ability of the patient with lung disease to inhale and exhale. Unfortunately many of these patients have difficulty with respiration thus limiting their ability to meet ventilation lung scan requirements.

As discussed in chapter 1, Technegas Easy Breathing Accessory (TEBA) was developed to overcome the imaging problem inherent in ventilating patients with

lung diseases (Leiper, 1999). TEBA has the potential to enhance the quality of ventilation imaging as demonstrated in initial studies (Howarth & Lan, 1993).

2.3 Varying Reporting Methods

Deep Venous Thrombosis (DVT) and Pulmonary Embolism (PE) are separate but related aspects of the same disease process known as venousthromboembolism (VTE). Even with heparin given prophylactically, VTE is a medical issue with VTE remaining constant at 1 event per 1000 persons per year since 1979 and 1 event per 100 persons for those aged 85 and over (Gray 2002 USA).

In 2002, Seminars in Nuclear Medicine published Gray's article, which investigated the Prospective Investigation of PE Diagnosis II or PIOPED II. This was a prospective multi-centre study and PIOPED II was used as a composite reference test for DVT that is based on lung scintigraphy, bilateral Doppler ultrasound of the lower extremities, digital subtraction pulmonary angiography, and contrast venography in various combinations to obtain a diagnosis of PE status in the patient.

2.3.1 PIOPED I & II lung ventilation/perfusion scans using the same criteria

The PIOPED study database has been extremely useful because it contains precise descriptions of a large number of ventilation-perfusion scans and their corresponding andiograms. The criteria for this experiment will be:

1. A single moderate perfusion defect is categorized as intermediate, rather than as low probability.
2. Extensive matched V/Q abnormalities are categorized as low probability, provided that the chest x-ray (CXR) is clear.
3. Two segmental mismatches or more may be considered for high probability, and in some cases two mismatches should be considered for intermediate probability. However, due to the small number of cases with this finding, no definite, statistically founded recommendation can be made (Gottschalk 1993).

2.4 Aims

The aim of this experiment is:

- To determine whether TEBA could provide uniform ventilation, which was as good as or better than conventional ventilation in a group of normal volunteers and also in a group of patients with CAL. Refer to section 2.7 of this chapter.

The research was designed to achieve optimal ventilation studies, as CAL patients tend to have varying degrees of abnormal distribution of Technegas within their lungs due to poor breathing patterns and varying degrees of dyspnoea, which will be tested in Chapter 3 and compared.

The purpose of this study was to evaluate the use of positive ventilation for CAL patients using TEBA and verify how the qualities of images produced by this system compare to CAL conventional ventilation images (see figures in Chapter 4). Adequate ventilation images are desirable to improve the specificity of lung scintigraphy in the diagnosis of PE.

2.5 Methods

2.5.1 Protocol for conventional lung scintigraphy

During conventional ventilation / perfusion scintigraphy, the medical radiation scientist rehearses the patient with the Patient Administration Set (PAS), independent of the Technegas Generator, to allay the patient's concern. The volunteer has the test in two phases: ventilation (V) and perfusion (Q). When ventilating, the volunteer breathes in radioactive aerosol or gas (refer Chapter 1). Images of lungs are acquired using six projections for both ventilation and perfusion phases. In the perfusion phase, technetium 99m-labelled macro-aggregated albumin (MMA) was injected into a peripheral vein. These tiny aggregates then lodge in the lung pre-capillaries and provide a high-quality representation of the lung perfusion. Images in the same six projections as for the ventilation scan were then acquired. The V/Q scan gives images of regional perfusion and ventilation (see image 2.1). Pulmonary Embolism reported on

lung scintigraphy is seen as decreased perfusion with normal ventilation, known as a “V/Q mismatch”.

2.5.2 Inclusion and Exclusion Criteria

Pregnant or breast-feeding females, under the age of eighteen years (18) of age were excluded from the study. Other exclusion criteria included:

- The ability to understand directions given.
- Ability to use the TEBA system i.e. no contraindications such as:

1) Claustrophobia, 2) Low oxygen saturation levels, 3) Unwillingness to participate.

Informed Consent was obtained from each volunteer following an explanation of the purpose of the aims of the research, expected duration of participation, and a full description of the procedure, which included a small amount of radiation to the volunteer patient. Ethics was approved (see Appendix B). This study included 25 volunteers (13 healthy males and 12 healthy females with a mean age of 54.5 years with no lung disease) and 25 volunteers with CAL (16 males and 9 females with a mean age of 62.5 years). The method of recruitment consisted of posters placed in Bankstown Hospital Sydney, word of mouth, and actively asking people both within and outside the hospital to enrol.

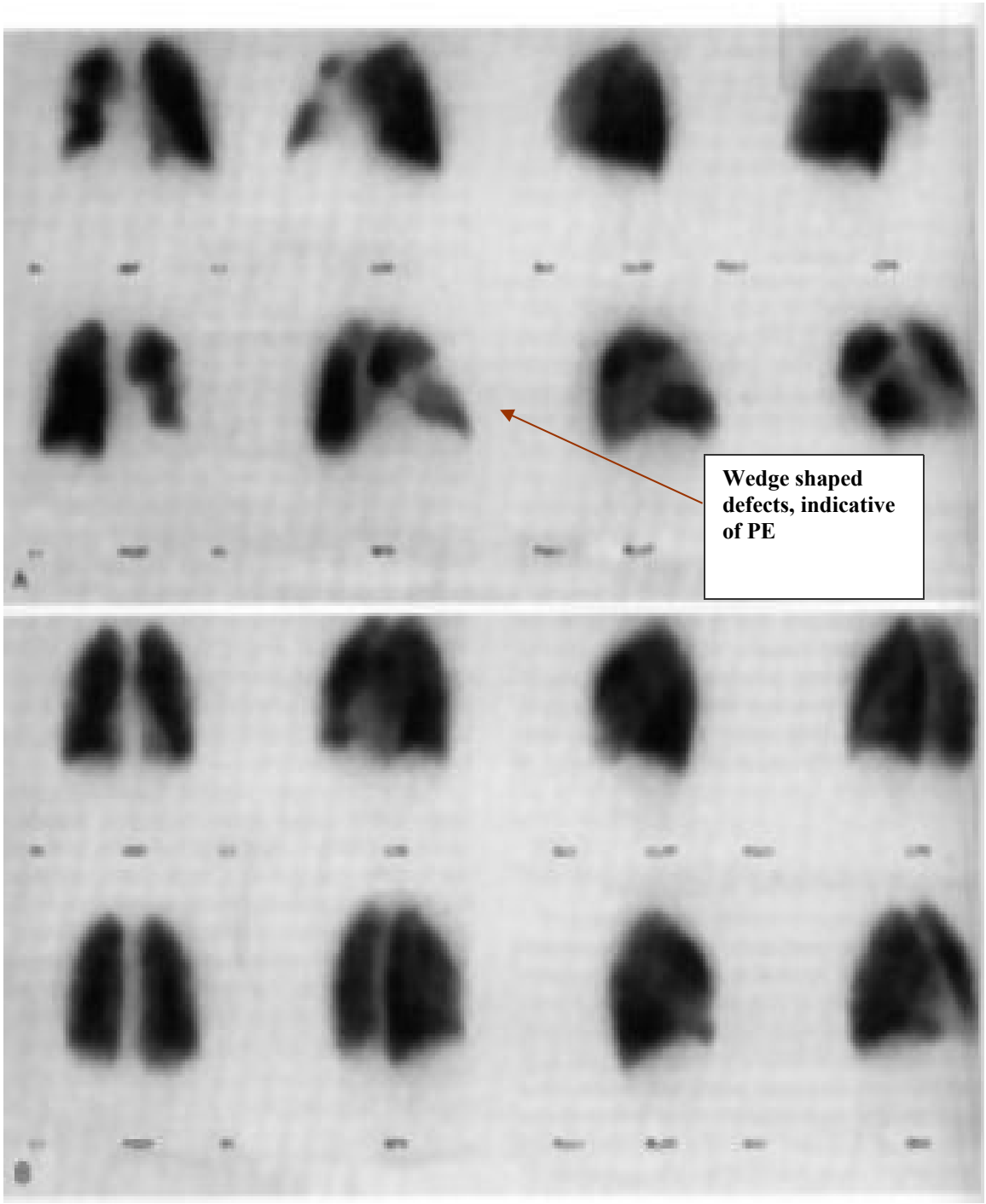


Figure 2.1 **VQ (Ventilation / Perfusion) Mismatch,**
Nuclear Medicine, Bankstown, Sydney

The image of a ventilation/perfusion scan shows multiple defects (see figure 2.1, arrow points to PE) consistent with Pulmonary Emboli in a patient with chronic pulmonary hypertension. It illustrates inhomogeneous flow pattern in a patient with primary pulmonary hypertension.

2.5.3 Patient positioning for lung scintigraphy

The patients are positioned supine or with two pillows to assist with dyspnoea, for both the inhalation and the MAA injection phase of the lung scan procedure, and for image acquisition. The reasons for laying the patient supine include:

1. The patient will relax, which in turn minimizes movement resulting in better image quality for interpretation between ventilation and perfusion studies.
2. Diaphragmatic breathing occurs when the patient is positioned supine leading to better registration of the lung fields and the medical radiation scientist can accurately measure how much Technegas has been inhaled.
3. Need for consistent position since posture affects ventilation distribution in the lungs (Krieg et al., 2007)
4. Ventilation continued until count rate on the gamma camera reached 2000 counts/s, corresponding to about 37 MBq inhaled activity. All volunteers had a conventional Technegas ventilation scan and perfusion with technetium 99m MAA lung scintigraphy scan followed 5 to 7 days later by a TEBA ventilation scan only. Volunteers were scanned in random order. Therefore, the 25 healthy volunteers with no history of CAL and second group of 25 with CAL served as their own control.

2.5.4 Acquisition for TEBA lung ventilation

1. The volunteers were asked to lie supine on a gantry bed with two pillows if needed for both the ventilation and perfusion lung scan. The normal healthy and CAL volunteers were instructed in the same way by the medical radiation scientists on the method for TEBA ventilation breathing

technique by demonstrating with TEBA PAS equipment independent of the Technegas Generator. Once volunteers understood what they needed to do, a facemask was placed over their nose and mouth, making sure there was a good seal so no radiation would leak into the room during the ventilation procedure.

2. When TEBA commenced, volunteers continued to breathe at their own pace as positive pressure was “pushed” into their lungs in synchronicity with the individual volunteer’s breathing pattern. Ten litres of wall oxygen were connected to the reservoir bag to assist with the ventilation.

3. A single breath was not usually sensitive enough for the detection of subsegmental ventilatory defects as it was essential to have enough count rate from the radioactive tracer inhaled into the lungs for acquisition. At Bankstown Nuclear Medicine Department, the minimum recommended end point count-rate was 2000 counts/s.

4. Areas of minimally impaired ventilation may be masked by inherent inhomogeneous distribution of Technegas. Therefore re-breathing was necessary until the required rate of 2000 counts/s was achieved. The number of breaths, activity and time taken to ventilate each volunteer were noted.

5. Both healthy and CAL volunteers were connected to the Nellcor oxygen saturation apparatus, so any changes in oxygen saturation could be monitored. The Nellcor pulse oximeter is a medical device that is non-invasive and provides continuous information on the percent of oxygen combined with haemoglobin. This precaution was designed to check for

hypoxia during ventilation and to monitor any changes during ventilation imaging procedures.

6. Lung ventilation scintigraphy consisted of 6 static images acquired for both ventilation and perfusion imaging: anterior, posterior (this view provides the largest assessment of lung tissue), right posterior oblique, right anterior oblique, left posterior oblique and left anterior oblique. These planar ventilation/perfusion images as well as the SPECT study were performed sequentially. The ventilation images were acquired first, then an intravenous injection of Tc-99m MAA was administered for the perfusion scan and the same six images as described above were acquired. After static images were completed, a SPECT perfusion acquisition was obtained for each healthy as well as CAL volunteer. SPECT acquisition was done using a triple head gamma camera with low energy all-purpose (LEAP) collimators, 40 projections at 15 seconds per view on a 64 x 64 matrix with a total acquisition time of 10 minutes. SPECT results in increased diagnostic accuracy over planar imaging because background activity and overlapping tissues interfere far less with activity from the target structure when tomographic techniques are used (Early & Sodie, 1995).

Before each volunteer left the nuclear medicine department, images were checked by the researcher to ensure all were completed correctly. Images were also checked by the nuclear medicine physicians as part of the regular quality control of the procedure. This was to clarify that the volunteers' scans were able to identify central and peripheral deposition of the Technegas. Once the

conventional and TEBA studies were completed, a comparison for concordance of the quality of both sets of images was sought from the nuclear medicine physicians.

TEBA delivery requires two medical radiation scientists, one at the distal end of the Technegas generator, the other at the front. By squeezing the resuscitator bag in synchronicity with the patient's breathing pattern, Technegas is gently delivered into the lungs. TEBA has been approved for use on ventilated patients and the elderly and is undergoing clinical investigation for use in paediatrics (Vita Medical Ltd. 2002).

2.5.5 Generation of Technegas

During the simmering phase, technetium eluant is evaporated onto the carbon crucible (Hannan, 1982). The crucible is loaded with pertechnetate ($TcO4^-$) until a meniscus / flat surface occurs and evaporates to dryness. Then the carbon crucible is heated to 2500º under an atmosphere of high purity Argon (99.99%) for fifteen (15) seconds. The carbon crucible is composed of high purity graphite only. At this temperature, carbon atoms aggregate into small clusters, each entailing a technetium atom. Each particle is a single, flat crystal of technetium metal encapsulated in carbon. The total particle output of one Technegas dose is in the range of 3.4×10^6 particles per cc. This corresponds to less than one (1) micron of carbon inhaled per Technegas administration (Burch, 1986).

This heating procedure is also similar to the production of C-60 molecules named "buckminsterfullerene" and was originally thought that TcG may consist of "bucky balls" with a diameter of 5 nm or less (Burch, 1986). The prolonged storage of Technegas, once produced, promotes aggregation to form larger

particles with an initial small particle loss rate of about 20% per four minutes. Accordingly, its use is preferred within ten minutes of preparation to ensure reproducible studies (Strong, 1989 and Burch, 1986).

In an inert atmosphere of high purity Argon, the technetium generator eluate is evaporated to dryness in a graphite crucible or “boat”. Autoradiography has confirmed that only one technetium atom is usually present per initial carbon cluster (Lemb, 1993).

The mass of carbon inhaled by the patient in a Technegas study, has been measured as fifty micrograms (± 20%). Based on resting tidal volumes, an inhalation of Technegas is more than two orders of magnitude below the permitted 24 hour pollution level (in the USA atmospheric pollution level is 75 micro gray continuous) which itself is set to account for chemically noxious vapours from industry and motor vehicles (Vita Medical Manual, 1999).

On the basis of an infinite residence time for Technegas particles in the lung, paryenchymal radiation exposure can be calculated to be 4500 microsieverts for 37 MBq inhaled activity. This should be included in any rationale that requires a perfusion dose to be “flooded” with Technegas when necessary. Therefore in ventilation perfusion scintigraphy, ventilation is performed ensuring a four to one ratio with the perfusion (Taylor, 1991).

The recommended activity of sodium pertechnetate (Tc-99m) to be deposited in the crucible is between 250 – 700 MBq. For adults, adequate images are obtained after approximately 40 MBq of Technegas has been inhaled; however the activity present in the lungs will vary between patients (Vita Medical Manual, 1999).

Technegas size, measured with a screen diffusion battery averaged a diameter of 5-25 nm. This analysis of particle size was also clarified by photon correlation spectrometer amplitude of the size distribution corresponded to a particle range between 60 – 225 nm (Lemb, 1993). Prior to ventilation these primary particles can be as large as 225 nm depending on the time between aerosol generation and ventilation delivery. Inhaled radioaerosol is linked to particle size (patient with CAL); inhaled Technegas may penetrate to the alveolar regions of the lung. The carbon crucible or “boat” is prepared by “wetting” it with absolute alcohol, and drawing back the excess ethanol with the same syringe, leaving the crucible wet. Methylated alcohol is not used as it may leave residues on the evaporation process, which may lead to pyrolysis in the gas generation stage. While using forceps to hold the graphite crucible, it is aligned between the left and right contacts. Pushing the lever on the side of the Technegas Generator opens the contacts (Vita Medical Manual, 1999).

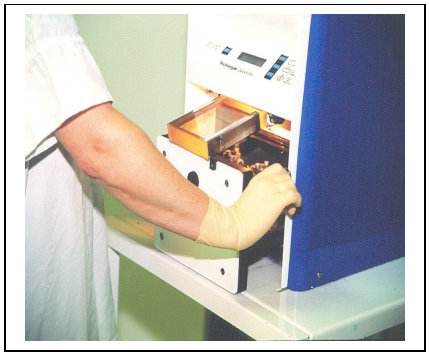


Figure 2.2 **Loading the Crucible**
(Vita Medical Limited)

500 – 600 MBq of pertechnetate is loaded into the crucible. On days when the generator activity is low, two or three simmers may be necessary to reach the amount of radioactive concentration necessary for achieving an adequate ventilation count-rate. This is achieved by interrupting the generation cycle

following the evaporation phase, refilling and re-evaporating the crucible (Vita Medical Manual, 1999).

Technegas has a power supply of two hundred and forty (240) volts and withstands a fifteen-second power surge of 20A while the carbon crucible is heated. It is important to use only 'pure' (> 99% pure) Argon. If Technegas is burnt in an oxygen/Argon mixture, "pertechnegas," a by product is produced. Gloves are worn as the lever and internal components of the Technegas are radioactive or "hot" during Technegas generation. The start button is pressed which checks for sealing, then simmering and purging commences. This cycle lasts six minutes. "Double" simmering as mentioned earlier can commence at four minutes by interrupting the cycle. With every fifty (50) patients, the contacts and filters must be changed. The "start" button on the generator begins a sequence of events, which raises the crucible temperature above 2500° C for the first 15 seconds. Technegas is now prepared for the patient to begin ventilation. The display reads "verifying burn", which holds for 3 seconds, then "disconnect mains" i.e. the Argon and power. Ten (10) minutes are allowed for Technegas ventilation of the patient after generation (Vita Medical Manual).

2.5.6 Technegas Generator

Repair and maintenance of the Technegas Generator must be done on a regular basis by a qualified technician. Since the Technegas Generator is used in conjunction with radioactive isotopes, maintenance should be performed after decay of residual radioactivity to minimize exposure.

2.6 Reporting criteria

The three senior nuclear medicine physicians involved in this study have been trained in their speciality area of nuclear medicine to look at both conventional and TEBA lung scintigraphic images as well as SPECT studies and had greater than 20 years of combined reporting expertise. The reporting criteria used for this research was examined by the three nuclear medicine physicians for correctness and for “ease” of use in reporting lung research studies and they considered the criteria covered all necessary areas for this study. A formal report from the physicians for each volunteer was not sought as this was a research study.

The reporting criteria below provide a detailed description of the areas of reporting for both conventional and TEBA studies undertaken in this research.

2.6.1 Unblinded reporting

The nuclear physician on duty viewed the research ventilation scans for any abnormality before the volunteers or patients left the department. The TEBA assisted ventilation images were viewed and compared with the baseline ventilation scan.

2.6.2 Blinded reporting

Coded images of baseline ventilation and TEBA assisted ventilation scintigraphy were studied by the nuclear physicians in a blinded fashion with particular attention to visual scoring of central and peripheral distribution of Technegas, and image interpretability. Both conventional and TEBA images were read without prior knowledge of which method of ventilation created the

image. The primary objective was to compare conventional and TEBA ventilation images with respect to overall image quality, the occurrence of central deposition and the ability to clearly define lung margins, thus peripheral penetration and any lung ventilation defects. Also evaluation of the safety of TEBA was made by considering any adverse affects and oxygen saturation levels.

The secondary objective was to compare ventilation lung scintigraphy using conventional versus TEBA for concordance of quality of the image, peripheral penetration, definition of lung defects if any, and the occurrence of central deposition in various disease settings. Readers are Nuclear Medicine Physicians who are blinded to the images. Prior to reading the scans, the blinded readers agreed on the appropriate method for experimental evaluation of both conventional and TEBA images. This included identifying the anatomical divisions which define the lung regions, reviewing the critical criteria for determining lung scan diagnosis, and defining the criteria for comparing overall image quality and lung margin definition of the studies.

Blinded assessment studies included a comparison of both normal and CAL images using the following four factors:

1. Concordance of the quality of the images
2. Peripheral penetration
3. Definition of lung defects, and
4. The occurrence of central deposition in conventional versus TEBA.

Image Quality Assessment was rated by the three blinded readers on a scale of 0 - 2 for overall clinical quality of the image. Image quality was assessed as a

comprehensive evaluation of the scans' ability to delineate ventilation defects, the occurrence of central deposition, and the peripheral penetration of the tracers.

0 = image quality is poor

1 = image quality is acceptable

2 = image quality is excellent

For assessment of ventilation abnormalities, blinded readers were asked to evaluate each of the images independently for possible abnormalities in divided segments of the lungs. These segments were each rated as defined below:

0 = No ventilation abnormality present (low probability)

1 = Possible abnormality (intermediate probability), mild, diffuse irregularity, or small/equivocal irregularity.

2 = Defined abnormality (high probability)

Blinded readers also assessed the degree of central deposition found in each lung image.

0 = High degree of central deposition that hinders the reading of the image

1 = Some central deposition present

2 = No central Deposition present

To assess peripheral penetration, blinded readers were asked to independently determine if the scans defined the margins of the lungs.

0 = Poor peripheral distribution of the lung ventilation agent

1 = The ventilation image accurately defines the lung margins, but the edges are not crisply defined

2 = The ventilation accurately defines the lung margins.

2.6.3 Reviewers' Assessment

Reviewers are nuclear medicine physicians who review the image after the reader. The reader is a nuclear medicine physician, who is blinded to the experiment images. Reviewers were asked to assess the quality of the image, whether the images define the margins of the lungs concordantly, the degree of central deposition present, and whether both images define any lung defects effectively. They were then asked to assess whether or not the images were concordant for both ventilation and perfusion.

The reviewers addressed the following questions for each study:

Quality: Are both conventional and TEBA images useful and of diagnostic quality?

Yes or No. If no, which image is not diagnostic? A or B, or Neither.

Peripheral Penetration: Do both conventional and TEBA images define the lung margins accurately?

Yes or No. If no, which image more accurately defines the lung margin? A or B or Neither.

Central Deposition:

Are both conventional and TEBA images concordant with regard to the presence and amount of central deposition?

Yes or No. If no, which image contains a greater quantity of central deposition?

A or B or Neither.

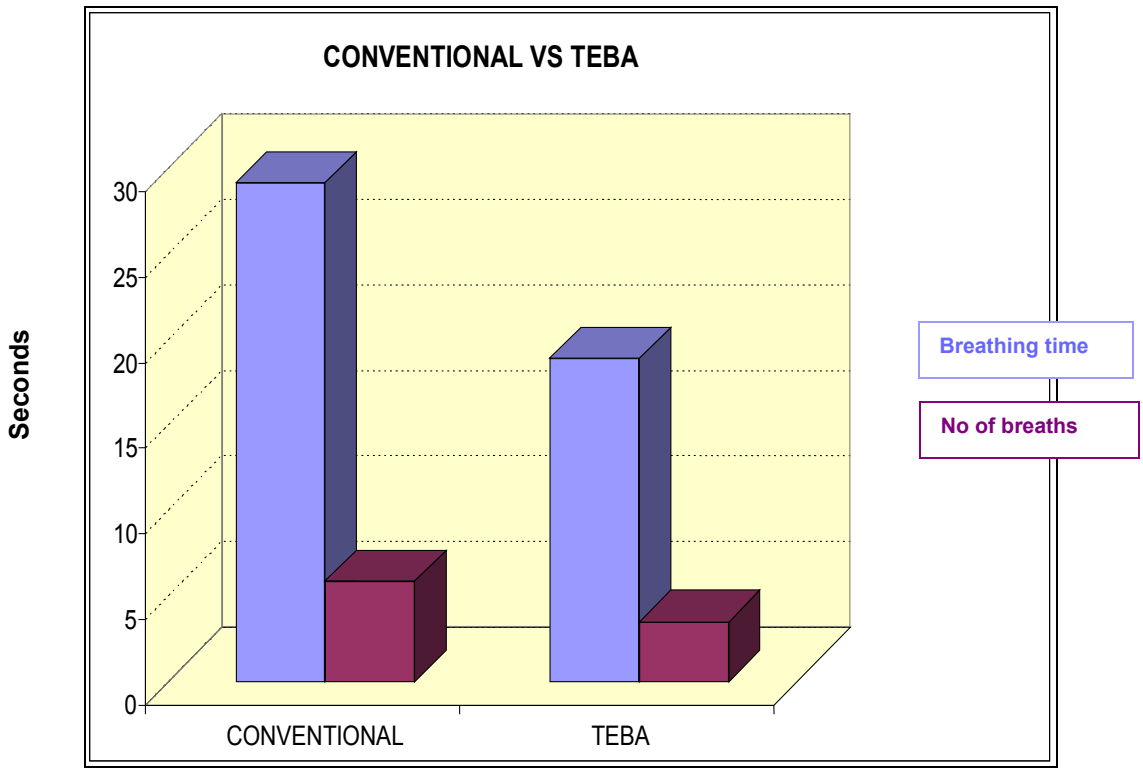
Lung Defects: Both conventional and TEBA images define lung defects clearly. Yes or No or Neither image. If no, which image does not define lung defects clearly? - A or B or Neither.

Volunteers' breathing time was collected by counting the number of breaths taken for ventilation and recording this information (see section 2.7, *Results)*. The data consisted of: number of breaths, time taken for ventilation, activity loaded, and oxygen saturation. From these figures, tables show the results.

2.7 Results

Results are presented in the following order: CAL volunteers for both conventional and TEBA lung scans (see Figure 2.1). Results for other parameters such as conventional breathing times are shown in Figure 2.3.

Figure 2.3 **Bar Graph showing normal (conventional) and CAL (TEBA) Breathing Time in blue and Number of Breaths in purple**


CONVENTIONAL VS TEBA
Seconds
30
25
20
15
10
5
0
CONVENTIONAL
TEBA
Breathing time
No of breaths

The table below shows that both breathing times and the number of breaths are significantly less ($p<0.05$) for TEBA than the conventional method for PE. The final count rate and activity loaded show no significant difference.

Table 2.1**: PE versus TEBA**

	PE	**TEBA**	**P VALUE**
BREATHING TIME (S)	21.64 ± 8.53	15.96 ± 9.57	P = 0.037
NUMBER OF BREATHS	4.88 ± 1.83	2.92 ± 2.58	P = 0.0026
FINAL COUNT RATE (k COUNTS)	2.47 ± 0.99	2.03 ± 1.17	P = 1.1712

Table 2.2**: Normal versus PE**

	Normal Subject	**PE**	**P VALUE**
BREATHING TIME (S)	16.00 ± 9.50	21.64 ± 8.53	P = 0.01
NUMBER OF BREATHS	2.89 ± 2.50	4.88 ± 1.83	P = 0.01
FINAL COUNT RATE (k COUNTS)	2.00 ± 1.09	2.47 ± 0.99	P = 0.01

Table 2.3 **One-Sample t-test : Breathing Times for CAL volunteers**

VARIABLE	Mean	St. Dev	Se Mean	95% CI	P value
BREATHING TIMES	13.8000	8.4311	1.6862	10.3198,17.2802	0.000

Table 2.4 **One-Sample t-test: Number of Breaths for CAL volunteers**

Test of mu = 5.538 vs not = 5.5

VARIABLE	St.Dev	SE Mean	95% CI	P value
No. BREATHS	1.35647	0.27129	2.00008, 3.11992	0.000

2.8 Discussion

We will now discuss how our research demonstrated conventional ventilation versus positive pressure ventilation in healthy and CAL patients, showing the difference in number of breaths taken. The time taken for healthy patients to ventilate is equivalent or sometimes more than positive pressure TEBA patients (as shown in above tables).

2.8.1 Healthy Volunteers

Healthy volunteers were reported as having a relatively even distribution of Technegas throughout their lung fields for both conventional and TEBA ventilations. Several healthy volunteers experienced some slight hypoxia due to the anoxic nature of Technegas and Argon in the first few seconds of ventilation (see Figure 2.1). SPECT studies of the healthy volunteers showed good contrast and edge definition, and a good target to background ratio.

2.8.2 CAL Volunteers

CAL volunteers in the study were reported as having varying degrees of abnormal distribution of Technegas throughout their lung fields as reported by the nuclear medicine physicians in their overall image interpretation at the end of scanning. Peripheral deposition had improved using TEBA in areas that normally would be reported as poorly ventilated. According to West (1990) poor ventilation of the lungs is a characteristic of CAL. The clearance ratio is slower in CAL volunteers in relation to normal lung scan clearance. Technegas is not distributed as evenly in CAL due to the limited airflow into the lungs and filling volume of CAL patients (West 1990). Some CAL patients experienced Hypoxia

and CAL images had varying degrees of abnormal distribution reported in their lung scans.

SPECT reported improved contrast, edge definition and target to background ratio. Breathing times improved in CAL TEBA ventilation as they could breathe in Technegas at their own pace as Technegas is “pushed” into the lungs in synchronicity with the volunteers breathing rate.

2.8.3 Number of Breaths

As this experiment involved a comparison of two measurements from the same ventilation imaging study, conventional ventilation imaging versus TEBA ventilation imaging, the paired t-test was used where the mean differences fell within a normal range. If the mean differences departed from normality the non-parametric Koch’s adaptation of the Wilcoxon-Mann-Whitney rank sum test would be used. The two sets of data can be obtained at different times and under a different set of conditions, which suited this experiment as TEBA used a slightly different procedure for the ventilation inhalation process (Gosling, 1998). The most common test to explain a statistical inference is the two sample paired test which compares two independent samples. The results from the One Sample t-tests (see Table 2.2) for breathing times, number of breaths and final k-counts the p value is 0.000 implying that the null hypothesis proved that there was no difference in TEBA and the conventional lung scintigraphy images, both were satisfactory, therefore the null hypothesis was accepted. Observing both the conventional and breathing times in table 2.2, p-value was 0.000, which implies that the null hypothesis (Ho) is true and therefore in the acceptance region of the test. The number of breaths for the conventional method for lung

ventilation and TEBA also fell into this category of acceptance. Therefore we can say that there was little or no change and the null hypothesis was accepted. With the healthy volunteers, CAL volunteers and high probability PE patients it was not possible to take arterial blood gas studies (ABGs) as this would have been invasive and was not necessary to verify the usefulness of the positive ventilation, TEBA. However, the oximeter results provided continuous oxygen saturation readings throughout the lung scintigraphy (Table 2.3).

CAL patients had an increased deposition rate relative to healthy volunteers, some of which occurs in the airways, although mostly occurred in the parenchymal lung.

Given that CAL patients have heterogeneous ventilation within their lungs, they may receive a dose to their parenchymal lung that is many times greater than that occurring in the normal lung. James (2002) found no evidence of rapid pulmonary clearance for carbon ultra fine particles into the circulation. An increase in the parenchymal dose may therefore be attributable to an exacerbation on their airway inflammation but not due to rapid movement of a significant number of insoluble particles into the circulation (Brown, 2002).

2.8.4 CAL Limitations

As most CAL patients have an underlying predisposition to PE due to being immobilized, they are vulnerable both physically and emotionally from the beginning of the procedure. This is due to the patients experiencing moderate to severe chest pain and dyspnoea and they may feel as though they cannot tolerate a mouthpiece or nose peg as is used for the conventional method of

lung ventilation because they find it difficult to inhale with a neutral ventilation pressure.

In this study CAL TEBA patients may use air instead of oxygen for patients with CO2 retention (ref Table 2.3). This minimized the risk of respiratory depression secondary to suppression of hypoxic drive had pure oxygen been used to provide positive pressure. For CAL patients the likely outcome of using TEBA showed significant improvement in the ventilation count rate over the baseline, conventional method. Breathing time was significantly shorter and compliance enhanced with the utilization of TEBA.

In about 90% of patients (including CAL) referred for lung scintigraphy, the lung scan will either confirm or exclude PE. However, in 10 to 15% of patients, the lung scintigraphy will be reported as non diagnostic or intermediate. According to Annals of Medicine 33(5) 1999, the incidence of PE in these patients was documented on pulmonary angiography as 30 to 35%. An intermediate result should raise the importance of further investigation and that there may still be a real risk of PE. There is concern that positive pressure ventilation may create false positive diagnoses of PE, by improving peripheral penetration of tracer in poorly ventilated areas associated with reflex vasoconstriction and hypo-perfusion, not due to vascular obstruction.

When inhaling Technegas, there may be a transient lowering of oxygen saturation levels. This is a common occurrence, not associated with any harmful symptoms. Leiper, 1999 demonstrated that patients may experience transient dizziness, but this does not appear to correlate with age, sex, smoking history, number of inhalations required, or a history of respiratory disease. Therefore it is difficult to determine which volunteers, if any, will experience this fall in

oxygen saturation. Despite transient falls in oxygen saturation, there has been no published data of any serious reactions following Technegas administration since it began in 1986. Normal lungs will have a relatively even distribution throughout; however, in cases of lung disease or CAL, varying degrees of abnormal distribution are seen (Isawa et al., 1991).

2.9 Conclusion

The results show that TEBA provides quality uniform ventilation for both normal volunteers and patients suffering from CAL. In addition, the results for both groups of patients showed close concordance between TEBA ventilation and conventional ventilation. Thus, we were able to achieve the aim of the experiment that TEBA could provide ventilation which was as good as or better than conventional ventilation in a group of normal volunteers and a group of patients with CAL. This result could potentially broaden the number of CAL patients who may benefit from using this method. TEBA has the potential to provide a more accurate diagnosis and to broaden the number of potential CAL patients who could benefit from a lung scintigraphy study.

Experiment Two includes patients with a possible diagnosis of PE to further test the utility of TEBA in the clinical context. It also looks at concordance with conventional PE volunteers and TEBA volunteers.

The finding of better quality images in both healthy and CAL volunteers suggested that hypothesis I (null hypothesis (H_o): TEBA could provide ventilation as good as or better than conventional ventilation in a group of normal volunteers and a group of patients with CAL) should not be rejected. Finally, the close concordance of both TEBA and conventional ventilation suggests that hypothesis II (alternate hypothesis H_a: there will be no difference in the quality of the scan between TEBA CAL or the healthy volunteers' conventional lung scintigraphy) is supported by the data.

CHAPTER 3 EXPERIMENT 2

Using TEBA for Pulmonary Embolism

3.1 Introduction

As PE accounts for many hospitalisations per year, and this number is increasing apace with the aging population, an accurate diagnosis is critical. It is well documented that the majority of patients presenting with PE have an underlying clinical predisposition. However, the clinical diagnosis of PE is unreliable and signs and symptoms are non-specific (Wade, 1982), therefore it is not surprising that clinical diagnosis is fraught with error. Recent advances have been needed to improve the method of diagnosis and the TEBA positive pressure device may prove to be an invaluable tool.

3.2 Detecting Pulmonary Embolism

Nuclear Medicine physicians must acquire skills in risk stratification for patients suspected of PE (Dietlein, 1999). In general, lung scintigraphy scans for PE are interpreted using the parameters established by the Prospective Investigation of Pulmonary Embolus (PIOPED, 1980, Gottschalk et al., 1993, Worsley & Alavi,1995). The PIOPED interpretation criteria are based on matched defects seen on perfusion lung scintigraphy as being low, intermediate or high probability for PE, based on the original paper by Biello (1979) and PIOPED (1995).

All participants in this study presented with moderate to severe dyspnoea accompanied with varying degrees of pleuritic chest pain and were subsequently referred for a lung scintigraphy scan as PE was suspected. Most patients with minimal dyspnoea are able to tolerate the conventional method of ventilation (see chapter 1 on Technegas) but moderate to severe patients found the nose-peg and mouthpiece too difficult to tolerate. The conventional method of administering Technegas for a ventilation scan requires the utilisation of the breath hold for five seconds was also a problem as patients with moderate to severe dyspnoea could not manage this technique.

We will now look at the various symptoms in:

1) Conventional PE patients
2) PE patients having a lung scan
3) Intensive Care Patients

PE may present as no symptoms. However, when the patient does have symptoms of PE, the challenges of obtaining a scan are comparable with the same symptoms experienced by PE patients and Intensive Care patients.

3.2.1 Conventional PE Patients

The clinical features of PE can be diverse and confusing, ranging from no symptoms to sudden death. The symptoms, when present, depend upon the extent of pulmonary arterial occlusion. The most common symptoms are dyspnoea and chest pain. Some patients may experience sudden apprehension of impending doom with tachypnoea and elevation of right heart pressures with or without syncope, which usually indicate massive PE. (James J., Nucl. Med. Comm 1995). The fear and uncooperativeness is thought to be related to the dyspnoea becoming worse.

Positive pressure administration of Technegas using the TEBA has the potential to allay the patients' fears as they do not have to take breaths or have a nose peg or tube in their mouth. When using TEBA a face - mask is placed over the patient's nose and mouth and they continue to breathe with air or oxygen.

The amount of the radionuclide injected into the vein for the perfusion study or inhaled into the lungs for the ventilation procedure is so small that there is no need for precautions against radioactive exposure. Allergic reactions to the radionuclide are rare, but may occur. For some patients, having to lie still on the scanning table for the length of the procedure may cause some discomfort or pain as lying supine is difficult for patients suffering from breathlessness.

3.2.2 PE Patients having a Lung Scan

There are groups of patients in whom ventilatory images of diagnostic quality are difficult to obtain. These include PE patients with moderate to severe dyspnoea or those who are distressed, frail and unable to cooperate fully. PE patients develop a moderate or severe dyspnoea and high temperatures. Dsypnoea is most noticeable during any physical activity and can be heightened during a conventional lung ventilation scan as some PE patients who may be distressed know they will not be able to breathe with a peg on their nose and a mouthpiece, thus causing apprehension.. TEBA presents the possibility to deliver sufficient levels of Technegas for optimal image acquisition while at the same time reducing patient anxiety and breathlessness. This is because TEBA's use of positive pressure with a face - mask over the patient's nose and mouth ensures the patient receives the required air or oxygen during the administration of the Technegas for each ventilation scan.

3.2.3 Intensive Care Patients

Physical examination is often difficult in the intensive care unit (ICU) setting and for many years has been complemented by the portable chest radiograph (CXR) and the portable 300GE gamma camera. The gamma camera was mainly used for cardiac work and not for lung scanning. The interpretation of portable ICU radiographs and Nuclear Medicine cardiac images may also be difficult. This is due to the limitations of applying optimal radiographic and nuclear imaging technique in the ICU setting, as well as the patient's condition and the presence of monitoring and other devices (either in or on the patient) that might obscure portions of the chest. Thus it was unheard of for the intubated ICU patient to be transported to the nuclear medicine department for a diagnostic lung scan. However, by using TEBA, a positive pressure device, this has changed as unconscious ICU patients can be momentarily disconnected from their ventilators and connected to the TEBA apparatus.

There is minimal discomfort to the patient as they are receiving 100% oxygen for the entire ventilation procedure after which the patient is reconnected to their portable ventilator. The only discomfort noted is that the patient’s arms must remain above their head for the entire procedure; this is to allow the ventilation tubing and many intravenous leads to be placed distal to the patient so the gantry can be pushed into the gamma camera for imaging.

Factors such as increased dead space from the connection to the Technegas chamber, variable efficiency of inhalation and variable delivery flow rates, may lead to turbulence and deposition of the agent in the upper aero-digestive tract and central airways. TEBA avoids the problem of introducing dead space by its location distal to the Technegas generator. TEBA is safe and well tolerated by PE patients and other

spontaneously breathing patients. TEBA has been successful for ventilating the unconscious patient. TEBA requires less time to achieve an adequate count rate, and improved diagnostic quality of ventilation images.

3.3 Aim

The aim of this experiment was: to determine whether TEBA could provide ventilation as good as or better than conventional ventilation in a group of patients with Pulmonary Embolism (PE). This experiment aimed to test two hypotheses:

TEBA has the potential to provide a more accurate diagnosis in patients with no lung disease (Null hypothesis, H_o). There will be no difference in the lung scintigraphy image quality for TEBA assisted patients and healthy volunteers without TEBA. If this hypothesis is correct, this could potentially broaden the number of patients who may benefit from lung scintillation scan. The alternate hypothesis (H_a) evaluates whether TEBA is better than conventional lung scintigraphy in patients.

3.4 Materials and Methods

Clinical diagnosis of PE has to address the non-specificity of clinical signs and symptoms, therefore it is usual to have several diagnostic procedures such as a chest X-ray (CXR) and a lung scan. The CXR is typically the first imaging procedure performed when a patient presents with dyspnoea and a lung scan is usually the second diagnostic test.

The CXR accompanies the patient to the nuclear medicine department as an aid in diagnostic reporting. A CXR acquired within 24 hours of lung scintigraphy can be inconclusive or of minimal value to the nuclear medicine physician due to its low sensitivity for PE (Taylor, 2000).

This experiment recruited 50 volunteer participants, [25 normal subjects and 25 patients with known PE]. The participants were asked to lie in a supine position on the gantry bed, to allow uniform distribution of Technegas throughout both lung fields. The subjects were monitored for their oxygen saturation using a Nellcor N180 throughout the procedure. Normal lung scintigraphy using 6 projections was obtained for ventilation and perfusion imaging: anterior, posterior, right posterior oblique, right anterior oblique, left posterior oblique and left anterior oblique. These planar ventilation / perfusion images as well as a SPECT study were performed sequentially. TEBA was administered between 5 to 7 days post conventional scan with no accompanying perfusion scan.

SPECT acquisition used the triple head gamma camera with low energy all - purpose (LEAP) collimators, 40 projections at 15 seconds per view on a 64 x 64 matrix, acquisition time of 10 minutes.

3.4.1. Stages of Preparation for Technegas Generation

The crucible was removed from its package using gloves and forceps; the crucible well was filled with 95% or absolute ethyl alcohol and drawing back the excess ethanol with a 1ml syringe, leaving the crucible wet. Methylated alcohol was not used as it may leave residues from the evaporation process, which could lead to pyrolysis in the gas generation stage (Tetley Medical Ltd. Users Manual 1999). The syringe was drawn back so there was no excess alcohol left in the crucible. The crucible was positioned so that one end was at the left hand contact and the other end was aligned with the right hand contact of the Technegas machine. There must be good contact or a low yield of Technegas may result, and rotation of the crucible is required to make sure the well cavity is in the upright position. Pushing the lever on the side of the Technegas Generator opened the contacts to enable proper mounting of the crucible.

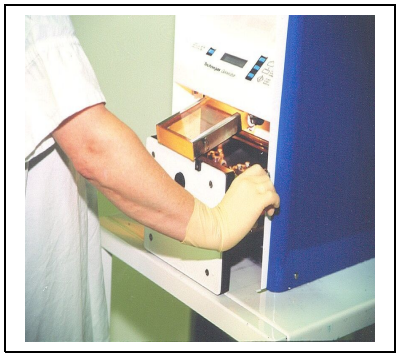


Figure 3.1 **Loading the Crucible** (Vita Medical Limited)

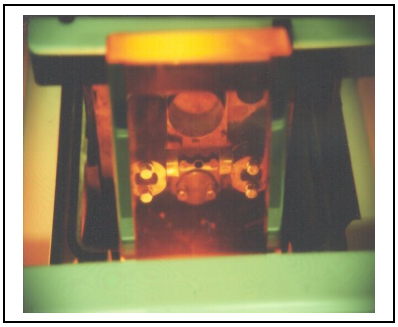


Figure 3.2 **The crucible or "boat" and contacts**

Overfilling the crucible may cause air bubbles to become trapped in the graphite crucible. Any unwanted bubbles will expand and ascend to the surface, causing the

eluant to spill and possibly splash out of the crucible. Overfilling the crucible may actually result in having less radioactivity being simmered and burnt.

Approximately 700 – 900 MBq of technetium-99m pertechnetate in a 1ml syringe in lead glass syringe shield was injected into the crucible. The crucible was filled to a meniscus, concave or flat but never convex. On days when the generator activity was low, two or three simmers of the crucible was sometimes necessary to reach the amount of radioactive concentration necessary for achieving an adequate ventilation count-rate. This re-simmer was achieved by interrupting the Technegas generation cycle following the evaporation phase, refilling and re-evaporating the crucible. The drawer was closed and for safety precautions, a two handed operation was required to close the door of the Technegas generator, allowing the start to be pressed to initiate the simmer phase.

The Technegas Generator automatically checked for the presence of Argon gas or any gas leaks. The Technegas Generator detects gas leaks by monitoring the pressure in the chamber over time allowing a tolerance range of up to a 50% drop in pressure. Once all checks were completed satisfactorily, the generator commenced the simmer/purge stage, which dried the technetium pertechnetate onto the walls of the well in the graphite crucible. This was achieved by a small current passing through the crucible, increasing the temperature to 70°C and then pushing a flow of Argon gas across the top of the crucible to disturb the surface of the pertechnetate. Concurrently, the chamber was purged of any air, ensuring an inert atmosphere was created. The simmer/purge stage lasted six minutes. When finished, the generator rechecked for Argon gas contamination and then the burn generation was initiated.

During the simmering phase, technetium eluant was evaporated onto the carbon crucible composed of high purity graphite, which was heated to 2500º under an

atmosphere of high purity argon (99.99%) for15 seconds. At this temperature, carbon atoms aggregate into small clusters each entailing a technetium atom. Each particle was a single, flat crystal of technetium metal encapsulated in carbon. The total particle output of one Technegas dose was in the range of 3.4×10^6 particles per cc. This corresponded to less than one (1) micron of carbon inhaled per Technegas administration (Tetley Medical Ltd. Users Manual 1999).

The Technegas generator had a power supply of 240 volts and withstands a 15 second power surge of 20A while the carbon crucible is heated. It was important to use only 'pure' (>99% pure) Argon. If Technegas is burnt in an oxygen / Argon mixture, "pertechnegas," a by-product is produced. Gloves were worn as the lever and internal components of the Technegas were radioactive or "hot" during Technegas generation.

The generator then checked for sealing, then simmering and purging commenced. This cycle lasted six minutes. "Double" simmering as mentioned earlier can commence at four minutes by interrupting the cycle. The simmering/purging circle raised the crucible temperature above 2500° C for the first 15 seconds. Technegas was then available for the patient to begin ventilation. When the generator display verified the burn, the Argon and power were disconnected. The generator allowed 10 minutes for Technegas ventilation of the patient after generation.

Technegas ventilation levels in each patient/subject were monitored until a count rate of between 1000-2000 counts per second has been reached. When Technegas ventilation was commenced, each subject was asked to take in a breath and hold it for 5 seconds, representing total lung capacity, then breathe out (Isawa, 1991). A single breath was not usually sensitive enough for the detection of subsegmental ventilatory defects because it essential to ensure a minimum count rate of 1000

counts per second from the radioactive tracer inhaled into the lungs. Also, areas of minimally impaired ventilation may be masked by inherent inhomogeneous distribution of Technegas (Leiper & Cook, 1998).

The major components of the positive pressure ventilation delivery system or TEBA included a 1.6 litre, self-inflating resuscitator bag that was mounted distal to the Technegas Generator. The Patient Administration Set (PAS) connected the patient to the front of the Technegas Generator. A pneumatic control line was connected between the PAS and check valve assembly in TEBA. Depressing the valve opened the Technegas flow to the patient via the PAS in a series of one-way valves, which prevented the patients' exhaled air from returning to the unit. An anaesthetic mask was fitted to the patient's nose and mouth, depressing the valve; this opened the Technegas flow to the patient via the PAS (Tetley Medical Ltd.1999).

The 1.6 litre bag was squeezed with one hand in synchronicity with the patients' breathing cycle, thus pushing oxygen from the bag to the patient. As the bag was squeezed, control valves opened and / or closed from the pressure of squeezing the bag. The same pressure from the bag also operated the PAS check valve. TEBA avoided the problem of introducing dead space by its location distal to the Technegas Generator.

The rapid re-inflation of the resuscitation bag required reduced ventilatory time which in turn improved patient compliance. Patient discomfort was minimized, as assisted ventilatory time was generally less than one minute. The risk of Barotrauma (the sequelae of a ventilator–induced lung injury, a mismatch between the volume of gas

delivered and the amount of lung available) was also reduced due to the smaller volume of oxygen or air required during TEBA operation (Earis & Pearson, 1995).

According to Hillman (1996) air may be used with PE volunteers if their inherent CO2 retention is required to maintain their usual oxygen saturation, and reduce severity of Technegas induced Hypoxia. Air reduces the risk of respiratory depression secondary to suppression of the hypoxic drive if pure oxygen is used to provide positive pressure (Hillman, 1996).

3.4.2 Image Quality Ranking

The image quality ranking of the lung scintigraphy scans were reported by the nuclear medicine physicians using the same criteria and methodology as described in chapter 2 of this thesis. The nuclear medicine physicians had no prior knowledge of which method of ventilation created the images. The physicians considered TEBA images to be as good and in some cases superior to the conventional scans.

Unblinded reporting: nuclear medicine physicians reported on each subject's scan.

Blinded reporting: we used coded images of conventional and TEBA scans. The primary objective is for nuclear medicine physicians to pay particular attention to the visual scoring of central deposition, the ability to define lung margins and peripheral penetration of Technegas, and image interpretability. The secondary objective was to look at concordance of quality of lung scans, peripheral penetration, definition of lung defects if any, and occurrence or central deposition in various disease states (PE).

3.5 Inclusion and Exclusion Criteria

Exclusion:

Exclusion criteria included:

- Pregnant or breast-feeding females, or anyone with:
- An inability to understand directions or use the conventional ventilation PAS
- Contraindications for the study included claustrophobia
- Hypoxia or unwillingness to co-operate.

Inclusion:

Written consent was obtained from each subject prior to the commencement of the Lung TEBA ventilation and perfusion scan. Adults who have read the Informed Consent form explaining the purpose of the aims of our research, expected duration of participation and a full description of the procedure which includes a small amount of radiation to the volunteer patient.

Ethics:

Ethics approvals can be found in Appendix B.

Study Cohort:

The study group totalled 50 participants who met the criteria; 25 volunteers (13 males and 12 females), with a mean age of 55.6 years with no lung disease and 25 volunteers (15 males and 10 females) with a mean age of 60.5 years with PE. The volunteers returned 5 to 7 days after the conventional scan and SPECT to undergo TEBA ventilation scan. The volunteers were scanned in random order.

3.6 Results

Clinical Investigation of Positive Ventilation Delivery System – TEBA on Normal subjects and patients with PE – for results see Table 3.1. There are 25 conventional subjects and 25 TEBA patients in this table. We used a sample size of 50 as too many can result in unnecessary exposure of humans to risk; and to underestimate the sample size will lead to no “clear cut” result at the conclusion of the study. Having too many or too few participants also requires re-adjusting of the p-values or significance levels.

We were also interested in the breathing times, number of breaths and final count rates of both the conventional and TEBA patients. Review of breathing times and number of breaths seen in table 3.1 show the utilization of TEBA to be significant. This may be due to the positive pressure we used to deliver the Technegas to the lungs. Final count rate was similar for both conventional and TEBA. Adequate ventilation images are desirable to improve the specificity of lung scintigraphy in the diagnosis of PE.

Table 3.1 **Breathing times, number of breaths and final count rate for both conventional and TEBA ventilation**

Breathing time		No. of breaths		Final count rate	
Conventional	TEBA	Conventional	TEBA	Conventional	TEBA
24	14	4	3	2.0	2.2
23	12	4	2	1.8	2.1
18	14	3	2	2.0	2.2
22	16	3	2	1.8	1.9
20	18	3	2	1.7	2.1
23	16	3	3	1.1	2.2
26	15	2	2	2.0	2.0
18	7	2	2	1.7	2.3
12	9	3	2	1.5	2.0
16	16	3	1	2.1	2.2
32	22	2	1	1.8	2.1
18	7	3	2	2.1	2.3
14	8	2	2	1.7	1.9
17	14	3	2	1.0	1.9
10	8	2	2	1.8	1.9
6	6	2	2	2.0	2.2
5	12	2	2	2.6	2.2
22	10	10	2	2.3	2.1
13	10	5	3	2.0	2.1
10	8	6	3	2.3	2.2
9	7	7	3	2.0	2.3
6	6	4	3	2.0	2.0
5	4	3	2	2.1	2.3
12	11	2	2	1.2	2.0
14	12	2	2	2.0	2.1

Table 3.2 **Mean Breathing, Number of Breaths and Final Count rate**

	CONVENTIONAL	TEBA	Value p=(0.05)
Breathing Time (S)	Mean =15.8	Mean = 11.28	0.005
Number Of Breaths	Mean = 3.4	Mean = 2.16	0.005
Final Counts (K Counts)	Mean =1.86	Mean = 2.1	0.005

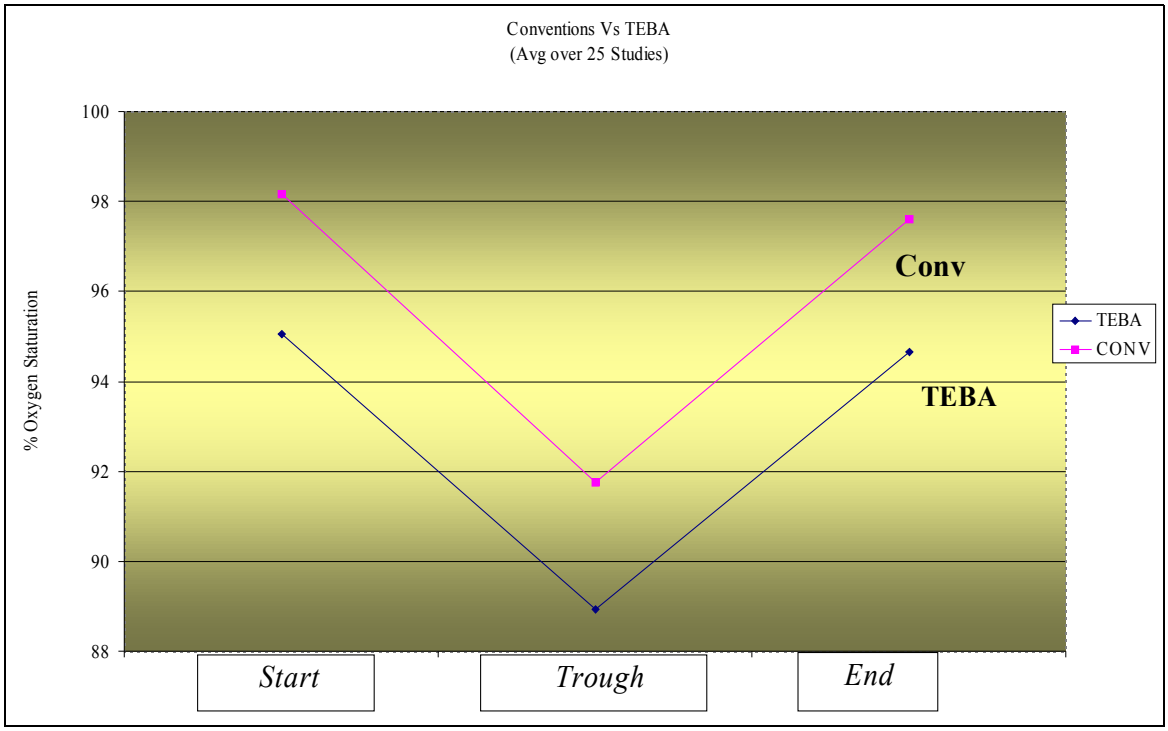
Table 3.3 **Activity loaded and oxygen saturation rates for conventional and TEBA**

Activity	Activity	Start	Start	Trough	Trough	End	End
C	T	C	T	C	T	C	T
850	800	99	99	98	96	99	99
800	870	98	99	94	96	98	99
840	890	97	99	93	96	99	99
860	890	98	99	96	96	98	99
800	850	98	98	96	91	98	98
850	880	97	94	94	97	97	98
900	920	97	100	95	97	98	100
800	850	97	99	96	94	98	99
850	840	98	99	94	96	98	99
850	860	96	97	92	95	97	99
800	870	98	97	96	99	98	99
860	980	98	99	96	98	98	99
880	860	99	98	98	96	99	99
800	990	99	100	96	97	98	99
850	960	98	99	95	96	98	98
900	830	97	96	96	96	98	99
850	890	99	99	98	98	99	99
900	920	98	99	96	97	98	98
900	930	98	97	93	96	98	99
850	990	98	99	93	98	98	100
900	960	99	100	92	96	99	100
900	970	97	99	96	96	98	99
850	890	98	98	93	96	98	99
800	880	99	99	98	98	99	99
850	900	99	99	96	97	99	100

Activity loaded in the crucible for the conventional (C) and the TEBA ventilation is 800 – 950 MBq sodium pertechnetate (Tc-99m). For adults, adequate images are obtained after approx. 40 MBq of Technegas is inhaled; however activity in the lungs varies between participants (Kim, 2000). Oxygen saturation is important to measure especially with TEBA, as this introduces positive pressure into lung fields. As seen in

the table above, there is no significant difference in the baseline. The trough, however, shows saturation levels decreased then returned to their initial value within a short period. Oxygen saturation reached a trough after first and second inhalation, which may be associated with inhaling the anoxic Technegas mixture. The use of oxygen to drive the positive pressure without CO2 retention assisted in reducing the severity of the Technegas induced hypoxia. It is important to maintain saturation levels above 90% as red cells must carry sufficient oxygen through the arteries to all internal organs in order to sustain life (West, 1987). Usually, when red blood cells pass through the lungs, 95 - 100% of them are saturated with oxygen. If there is lung disease or other lung conditions (PE), fewer red blood cells will be carrying their normal load of oxygen and therefore oxygen saturation will be reduced.

Figure 3.3 **Oxygen Saturation in both conventional and TEBA patients**



The initial oxygen saturation may be a predictor of how well the subject can withstand a change in oxygen saturation and the duration and degree of hypoxia. The table shows that most participants will undergo some temporary changes in oxygen saturation during the administration of Technegas due to temporary hypoxia.

Figure 3.4 **Line Graph using a different interpretation to show Oxygen Saturation**

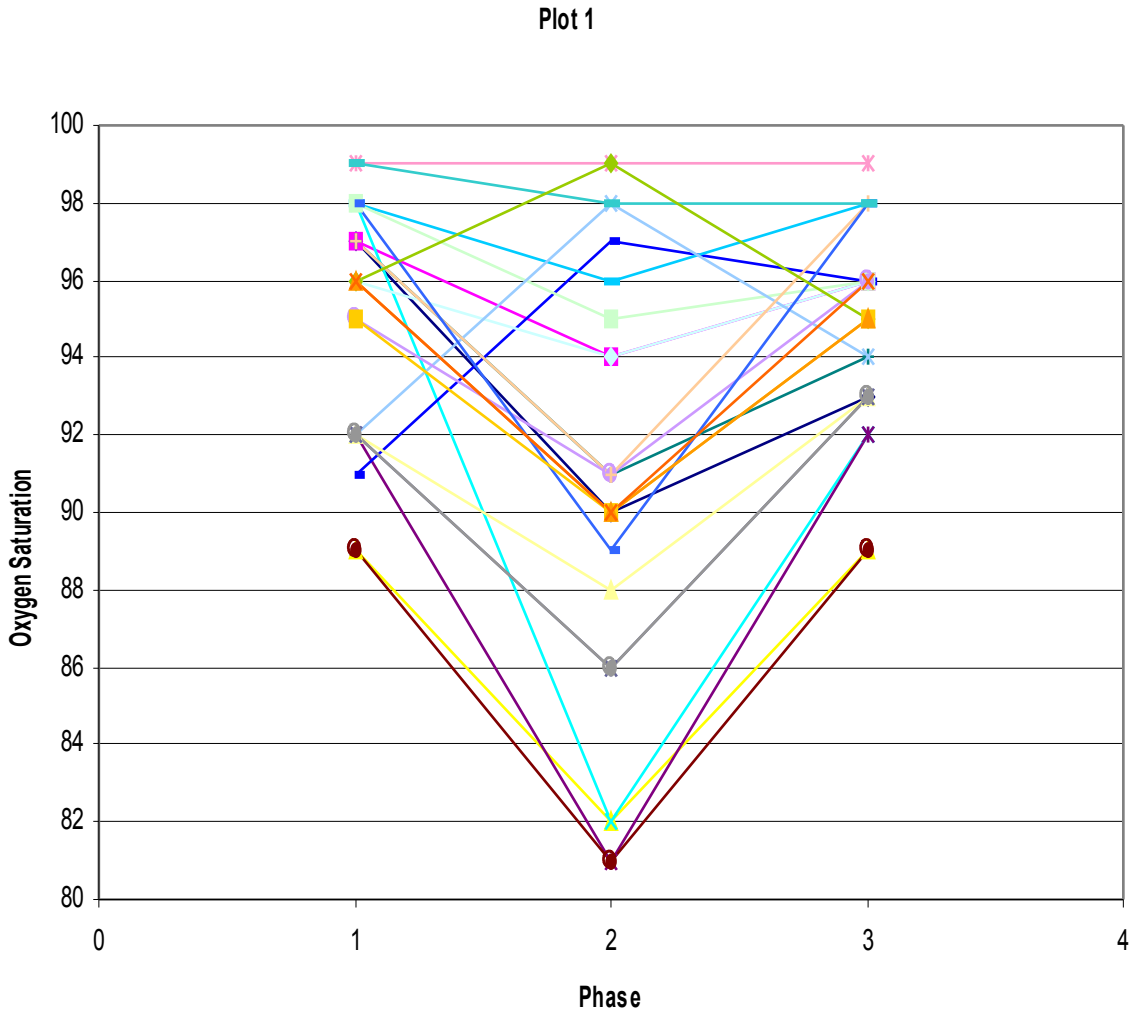
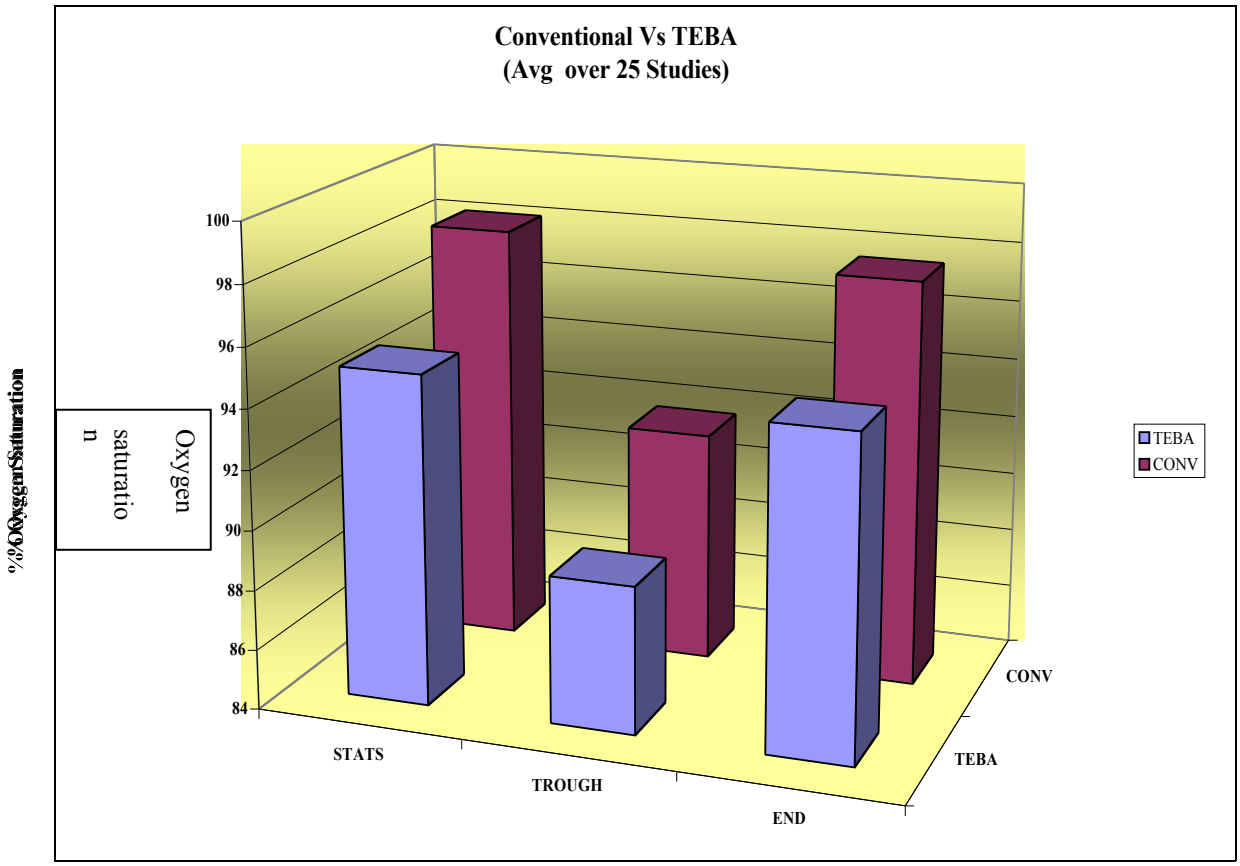


Figure 3.5 **Conventional versus TEBA Oxygen Saturation**



The graphs show the percentage of oxygen saturation during Technegas administration. It is clear that during lung ventilation at midpoint or trough, oxygen saturation may dip quite low. This is due to hypoxia, which is transient, then oxygen saturation returns to normal. Hypoxia is improved by 100% oxygen administration to assist in washing out all the nitrogen in the alveoli, leaving only carbon dioxide and oxygen (West 1990). Using oxygen to drive the positive pressure in participants without CO_2 retention helped reduce the severity of the Technegas induced hypoxia. Blue bar columns, TEBA show mean values for oxygen saturation during a lung scintigraphy at start, trough (middle) and end of scan. This can then be compared to the conventional scintigram, where bar columns are purple. The vertical axis signifies oxygen saturation and horizontal axis the number of subjects.

Figure 3.6 **Breathing Times for both Conventional and TEBA**

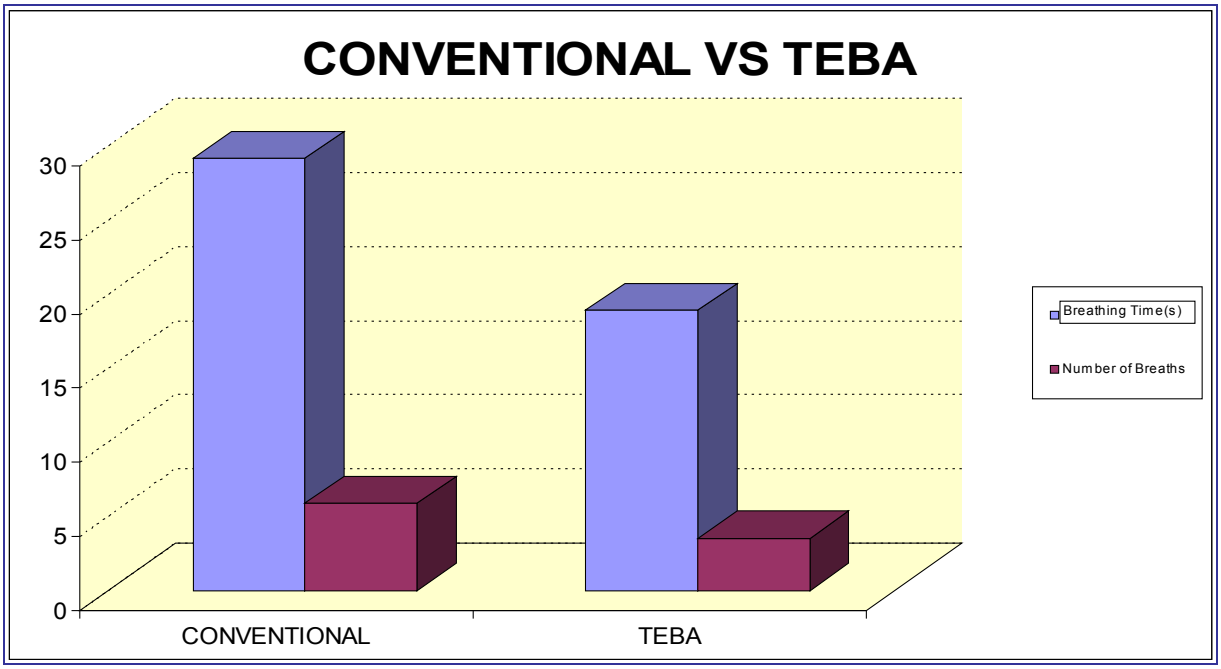
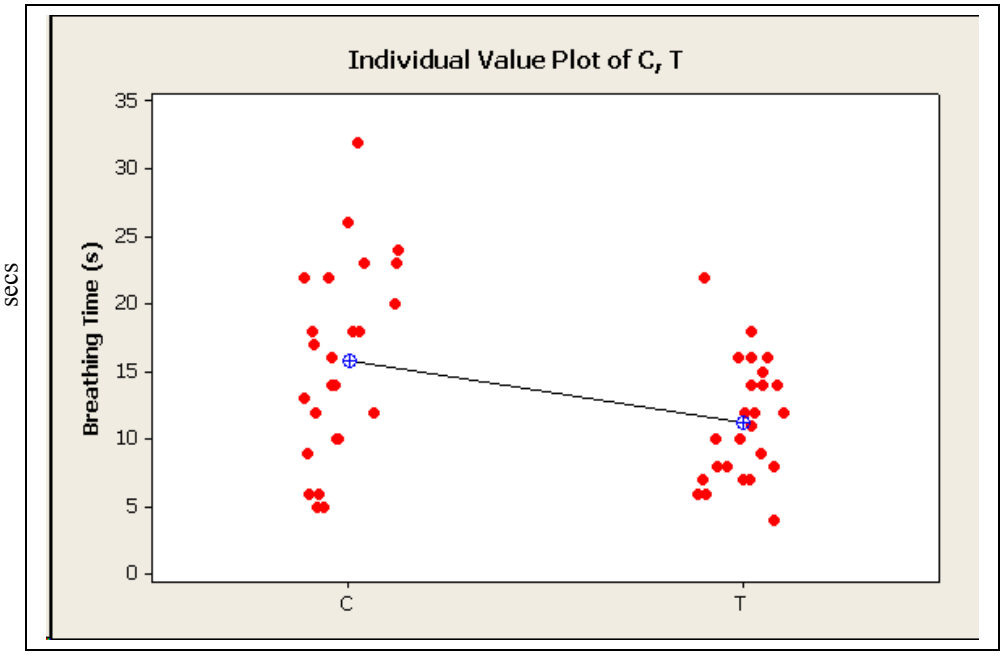


Figure 3.7 Drop Plot showing Breathing Times for Conventional and TEBA



These two charts above give a good representation of using TEBA. The breathing in figure 3.6 (large blue column) shows conventional breathing time is longer than

TEBA. The smaller purple column identifies the number of breaths. TEBA uses fewer breaths to achieve an adequate count rate for lung scintigraphic acquisition.

The advantage of using a drop-plot graph in figure 3.7 is that it is clear from c (conventional) and T (TEBA) that we are plotting two variables. We can see from the cluster and height of the conventional dots that breathing time measured in seconds is increased compared to TEBA.

3.7 Discussion and Conclusion

Lung scintigraphy plays a first line role in the diagnosis of PE. Previous studies (EJNM, Vol 26; 1999) showed its efficacy in the diagnosis and follow-up progress in reducing clinical uncertainty, in directing treatment and in lowering health care costs (EJNM, Vol 26; 1999).

Normal lungs have a relatively even distribution of Technegas throughout, however in this experiment, PE showed varying degrees of abnormal distribution. Adequate ventilation of patients is sought to optimise images and to provide high specificity for the diagnosis of PE.

There are many reasons why PE may not be clinically significant, such as subtle abnormalities, which were diagnosed as PE and were subsequently found to be false positives. However, in the future, as diagnostic equipment improves, smaller PE may be detected.

TEBA for both normal volunteers and volunteers with PE showed breathing times and number of breaths were less than the conventional method (see above tables and graphs in results section which support this). TEBA has shown to provide ventilation images which are as good and in some patients superior to conventional images in patients with PE and normal volunteers.

Therefore the alternate hypothesis (Ha) is seen to be true as lung scintillation quality in some patients is superior for TEBA assisted PE patients and conventional subjects. As the alternate hypothesis proved to be correct, this potentially means we would see an increase in the number of PE patients having lung scintillation scan using TEBA.

CHAPTER 4 EXPERIMENT 3

Positive Pressure Ventilation for Intubated Intensive Care Patients

4.1 Introduction

There is not an objective definition of clinically important Deep Vein Thrombosis (DVT) in critically ill patients because, to our knowledge, no studies have systematically correlated clinical or radiographic characteristics of acute venous thrombosis with patient outcomes. Despite the lack of such data, the concept of a clinically important DVT in the critically ill is vital as an assessment can determine whether it should be treated and could prove useful in future clinical research.

Routine thromboprophylaxis is provided to critical care patients based on an individual assessment of their thrombosis and bleeding risks but without definitive diagnostic tests to confirm venous thromboembolism (VTE). This is due to the immobility of the intubated unconscious patient, who is not able to have a lung scan to confirm or deny the diagnosis of Pulmonary Embolism (PE). Accordingly, the level of anti–coagulant therapy in this group is high, resulting in an increased incidence of anti-coagulant side effects. Positive ventilation with TEBA can be successfully used to diagnose PE in intubated patients.

4.2 Predisposing factors for DVT

Venous thromboembolism (VTE), which includes both deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common complication of critical illness. Critically ill patients harbour many coincident risk factors for DVT, such as the need for surgery, catheters, immobility and use of sedatives and paralytic agents (Hillman, 1996). As in the non-critically ill population, it is likely that most episodes of DVT are asymptomatic and confined to the deep veins of the calf. However, in time, 20–30% of untreated calf vein thrombi extend proximally into the thigh, where they pose a 40–50% risk of PE (Fahey, 1995). According to Van Beck (1996), early studies of the natural history of PE suggest that untreated PE has a mortality rate of at least 25%.

Geerts (2006) suggests the prevention of VTE in patients who are unconscious and intubated recovering from critical illness is often challenging. Neither D-dimer levels nor tests of hypercoagulability (activated protein C resistance ratio, Prothrombin 20210A gene mutation, levels of protein C, protein S, or antithrombin, anticardiolipin antibody titer, and lupus anticoagulant) had any predictive value for DVT in critically ill patients.

Thrombotic risk factors that may be acquired during the ICU stay include immobilization, pharmacologic paralysis, central venous lines, surgical procedures, sepsis, mechanical ventilation, vasopressor use, and hemodialysis. These patient groups share a high risk for VTE, they often have at least a temporary high bleeding risk, and relatively few thromboprophylaxis trials have been carried out with these specific intubated unconscious patients.

The TEBA apparatus enables the intubated patient to be attached to the Technegas generator in safety, allowing ventilation for the lung scan and acquisition to continue for a definitive diagnosis. The risk of haemorrhage (and associated complications) is the major adverse effect of VTE prescribing anticoagulation to the unconscious intubated patient in the ICU without having a diagnostic test (Van Beek, 1996).

A lung scintigraphy scan was ordered for these critically ill patients due to the amount of intubated, unconscious patients in ICU that were commenced on anticoagulant therapy without the use of diagnostic aids in the past.

4.3 PE not always detected

One large study by Faiad in 1996 found PE at autopsy in 59 out of 404 hospitalized patients (14.6%). Among the 20 patients who died from PE, this diagnosis was not suspected in 14 (70%). In a 25-year longitudinal study by Hillman (1996), 9% of patients had autopsy evidence of PE and again the diagnosis of PE was not suspected in 84% of patients before autopsy. A study by Moser and associates (1994), further emphasised this problem in critical care practice, where 13 of 34 (38%) of ICU patients with known DVT who had no symptoms of PE, were diagnosed with PE by ventilation-perfusion scans.

It is possible that many mechanically ventilated patients with sudden episodes of hypotension, tachycardia, or hypoxia may have undetected PE as reported by Fedullo 2003. Unsuspected PE may also contribute to difficulty in weaning patients from mechanical ventilation (Laghi and Tobin, 1995). Douketas and associates in 1998 suggested that in critically ill patients with impaired cardiopulmonary reserve, a

small PE, which might be of minimal clinical importance in patients who are less ill, might have severe or fatal consequences. Thus, the clinical consequences of DVT has the potential to be particularly serious, but may be unrecognized in the ICU. It is also possible that ICU patients undergoing treatment for DVT are more likely than other patients to suffer serious complications from anticoagulant therapy, according to a report by Douketas (1995).

Earis and Pearson (1995) suggested ICU critically ill patients cannot reliably communicate their symptoms of DVT as clinically important episodes are defined as symptomatic events, and intensivists rarely use the reference standard of venography to diagnose DVT. Furthermore, physical signs such as unilateral leg oedema, which are important clinical features of DVT outside the ICU, are uncommon in the ICU because many critically ill patients are supine and frequently have severe bilateral oedema. These observations suggest that the classic definition of a clinically important DVT is not suitable in the ICU setting.

4.4 Comparing TEBA with conventional methods

Lung scintigraphy is a well established diagnostic tool for the diagnosis of PE. However, it has not previously been utilized for intubated intensive care patients, as these patients are critically ill, intubated and not able to be easily transported to the nuclear medicine department for lung scintigraphy.

As discussed in chapter 1 of this thesis, the use of TEBA with ICU patients has the potential to minimize discomfort and lessen the risk of Barotrauma (Leiper, 1998) as

ventilatory time due to the rapid re-inflation of the reservoir bag, is less than one minute. Barotrauma is the sequelae of a ventilator induced lung injury resulting from a mismatch between the volume of gas delivered and the lung volume available (Hillman, 1996). This is due to the smaller volume of oxygen or air required for respiration during the TEBA operation.

4.4.1 Four types of ventilatory equipment for Intubation

There are four types of ventilatory systems used for intubation.

1) Pressure cycled ventilation

In this type, the ventilator is manually set to reach an upper pressure limit; once the the patient reaches this level, inspiration ceases and expiration commences. The inspiratory time, inspiratory flow rate and tidal volume vary with every breath, depending on the patient's resistance and compliance according to Hess (1996).

2) Volume cycled ventilation

In the volume cycled system, the ventilator is set to reach a set tidal volume, once this tidal volume is reached, inspiration ends and expiration commences. The airway pressure attained, inspiratory flow rate, and inspiratory time vary with each breath, depending on the patient's resistance and compliance.

3) Time cycled ventilation

If using time cycled ventilation, inspiration ends and expiration begins after a predetermined time interval has been reached. Generally an inspiratory time, a respiratory pause or plateau, or an I-E ratio (inspiration / expiration) are set, depending on the specifications of the ventilator used, to determine time cycling. The

airway pressure attained, the inspiratory flow, and the tidal volume vary with every breath, depending on the patient's resistance and compliance.

4) Flow cycled ventilation

Flow cycled ventilation uses the principle that inspiration ends and expiration begins when the flow rate drops to a predetermined percentage of its peak value. The tidal volume and inspiratory time vary with every breath, depending on the patient's resistance and compliance (Fahey, 1995).

Every patient will be different regarding which classification and mode of ventilation will be required, and this will change at different stages of their disease. In addition, Intermittent Positive Pressure (IPPV) has two distinct pathways, either volume controlled ventilation or pressure controlled ventilation; from both these groups, depending on both the pathological and physical condition of the patient, there will be varying modes. The following section outlines the four main types of mechanical ventilation systems.

4.4.2 Mechanical ventilation systems

Mechanical ventilation includes the following four systems:

1. **CMV and ACMV**
2. **IMV**
3. **SIMV**
4. **CPAP and PEEP**

1. Controlled Mechanical Ventilation and Assist Controlled Mechanical Ventilation (ACMV) is available in both pressure control or volume control ventilation depending on the type and mode of the ventilator available. In this system a preset tidal or inspiratory volume is delivered to the patient, who in turn receives a mandatory or assisted breath.

2. Intermittent Mandatory Ventilation (IMV). For this system the patient receives a pre-set rate and tidal volume. In between these mandatory breaths, the patient may initiate spontaneous breaths, but there is no synchrony between patient and ventilator. The volume of the spontaneous breath is dependent on the respiratory muscular effort that the patient is able to generate.

3. Synchronized Intermittent Mandatory ventilation (SIMV) – (includes PSV and PEEP) is available in both pressure-controlled, and volume-controlled ventilation depending on the type and model of the ventilators available. The patient receives a preset rate and tidal volume or inspiratory pressure. In between mandatory breaths the patient may initiate spontaneous breaths in synchrony with the ventilator.

SIMV is different to IMV as it uses a window of time in which a breath is due and will look to deliver this breath within the timing window. The ventilator delivers the

mandatory breath simultaneously as it senses the patient's inspiratory effort (Laghi, 1995).

When using SIMV systems the patient's level and type of breathing must also be assessed and considered to ensure the most appropriate ventilation technique is used. There are three different types of breaths during SIMV:

1) ***Mandatory breath*** *is used*, when the patient does not initiate sufficient inspiratory effort within the timing window. The mandatory SIMV breath is delivered at the scheduled time and the ventilator will then reset to respond to the next spontaneous inspiratory effort.

2) ***Assisted breath***, is used when a mandatory SIMV breath is due as determined by the rate control, the assist or timing window opens and waits for the patient's inspiratory effort. Upon sensing the patient's inspiratory effort, the ventilators deliver the preset tidal volume or inspiratory pressure in synchrony with the patient. As soon as the mandatory breath has been triggered the assist window closes. Once the assist breath has been delivered subsequent patient effort results only in a third type of breathing known as spontaneous or pressure breath.

3) ***Spontaneous or pressure breaths***, occur until the next mandatory breath is due. Hess (1996) suggests that the intensive care patient may initiate a spontaneous breath in synchronicity with the ventilator. Spontaneous breathing will be dependent on the respiratory muscular effort that the patient can generate.

Advantages of SIMV

The advantages of using SIMV as suggested by Laghi (1995), are that synchrony improves the patient's comfort level and reduces the competition between ventilator and patient. SIMV prevents breath stacking and barotrauma and decreases respiratory muscle atrophy; the patient uses the ventilatory muscles to a greater degree than with CMV or ACV. The haemodynamic effects of IPPV are less with SIMV than CMV or ACV. Using this method will also decrease the amount of sedation required and makes the weaning process easier.

A pressure support level must always be set while the patient is in the SIMV mode. If the patient wakes and tries to take a breath without the assistance of pressure support, he / she will feel as though they are suffocating, and become distressed.

Pressure support ventilation (PSV) is only functional when the patient is spontaneously breathing. Pressure support is active only during inspiration and is triggered by the sensitivity control, of the ventilator.

According to Hess (1996) ventilators are classified as either flow or pressure generators, depending on their mode of operation. A preset flow of gas is delivered to flow generators, regardless of any opposing forces; any impedance of the total respiratory system will be seen as a difference in airway pressure.

As outlined earlier in this chapter ventilators can operate in many different ways. In the control mode, the ventilator controls the volume and timing of ventilation, in the assist mode inspiratory efforts trigger the start of inflation. Intermittent mandatory ventilation (IMV) is when the patient performs most of the work of breathing. The mechanical inflation of the lungs occurs at various intervals and is predetermined during spontaneous breathing. If the IMV is not applied in the correct way severe

muscle fatigue will result. When synchronous intermittent mandatory ventilation (SIMV) is used the lung inflation's during IMV are synchronized to coincide with the patient's own inspiratory efforts (Hillman, 1996).

4. Continuous Positive Airway Pressure (CPAP) and Positive End Expiratory Pressure (PEEP)

Fahey (1995) referred to continuous airway pressure (CPAP) as the addition of a fixed amount of positive pressure throughout a spontaneous breath. The pressure is never allowed to return to atmospheric pressure, by contrast positive end expiratory pressure (PEEP) specifically refers to the application of a fixed amount of positive pressure during expiration only. Positive End-Expiratory Pressure (PEEP) can be used in conjunction with mechanical ventilation. Some patients spontaneously have an increased end expiratory pressure and lung volume due to the activation of inspiratory muscles or through adduction of the vocal chords. Endotracheal intubation may lead to a loss of the PEEP and a reduced end expiratory lung volume (Hess 1996).

CPAP and PEEP are not modes of ventilation, as they do not provide ventilation. Rather, PEEP is used in conjunction with other modes of ventilation and CPAP is used during spontaneous breathing to improve oxygenation and decrease the work of breathing. According to Fahey (1995) the main advantage of using PEEP and CPAP is that they provide positive pressure at end expiration, improve alveolar ventilation and increase functional residual capacity (FRC). Increasing the FRC increases oxygenation and decreases the work of breathing (Fahey, 1995).

Hess (1996), reported that PEEP can be used to improve arterial oxygenation. The end expiratory lung volume is a decreased in patients with acute respiratory failure. In these patients closure of the small airways is associated with a low lung volume, especially in the dependent lung regions. Lung regions that have closed airways are not ventilated, and perfusion of these regions results in arterial hypoxaemia. PEEP can hold the airway open throughout the respiratory cycle and may restore ventilation to previously perfused but unventilated regions, therefore improving oxygenation.

A disadvantage of PEEP is hyperinflation of previously expanded alveoli, which can damage perfusion, resulting in an increase dead space. PEEP can also lower arterial PO_2 levels results from increased perfusion to the lung regions with low ventilation to perfusion ratios. This happens as PEEP causes blood flow to move from well-ventilated non-dependant lung regions to the less ventilated regions.

PEEP also decreases tissue oxygenation by decreasing cardiac output; this is caused by a lowering in venous return. The intraventricular septum can also be shifted with high levels of PEEP; the septum becomes flat, reducing the left ventricular size. If this occurs an increased pressure is required to fill the left ventricle to a given volume (Hess, 1996).

Continuous positive airway pressure or CPAP is the positive end expiratory pressure administered during spontaneous breathing. CPAP is administered to the patient via an endotracheal tube or appropriate facemask. When CPAP is used, resistance to gas flow in the inspiratory circuit should be low enough to ensure inspiratory effort does not lower the airway pressure. Gas flow to the patient should be kept in excess of the patient's need; therefore, the physiological effects of CPAP are similar to PEEP (Hillman, 1996).

4.5 Physiology cardiac/respiratory systems for ventilated patients

Both the cardiac and the respiratory systems are affected by mechanical ventilation. Increased airway pressure is accompanied by an increase in alveolar gas and pleural pressures during the inspiratory phase of mechanical ventilation. Airway and alveolar gas pressures during the expiratory phase decrease to normal levels. Venous return to the heart can become impeded with any rise in intra-thoracic pressure during mechanical ventilation, patients with a normal blood volume and nervous system can re-establish the necessary pressure gradient by increasing the peripheral venous pressure (West, 1990). Patients with low blood volumes cannot utilize this compensatory mechanism to increase their peripheral venous pressure as they are already constricted. This means that in these patients mechanical ventilation decreases cardiac output.

During spontaneous breathing in a healthy patient, gas distribution remains uniform when the lungs are passively inflated. However, in ICU ventilated patients, intrapulmonary distribution of inspired gas is different during mechanical ventilation than during spontaneous breathing. Ventilation of dependent lung regions is greater than non-dependent regions of spontaneous breathing patients when inspiration has been initiated from lung volume equal to or greater than the Functional Residual Capacity (FRC). During mechanical ventilation under general anaesthesia, the respiratory muscles are usually paralysed, and gas distribution is altered in all but the prone position (Laghi 1995).

During spontaneous breathing, the movement of the dependant portion of the diaphragm is greater than the non-dependant portion of the diaphragm. This

alteration in gas distribution is seen during mechanical ventilation, which is associated with a different pattern of diaphragmatic motion.

Conversely, the nondependent portions of the diaphragm move more than the dependent portions during mechanical ventilation in a paralysed patient. The addition of the rib cage to the tidal volume is also changed during mechanical ventilation. In the recumbent position, the patient's relative contribution of the rib cage is greater during mechanical ventilation than during spontaneous breathing (Hess, 1996).

The balance between the regional muscular expanding forces and the regional impeding forces determines the pattern of chest wall breathing in spontaneous patients. The alveolar gas pressure used for mechanical ventilation means that the forces are no longer reliant on muscle contraction, therefore the pattern of motion during mechanical ventilation is constant by the distribution of "regional elastances" which are reciprocal of compliance within the lung and chest wall (Hess, 1996).

Gas distribution therefore will be altered in non-uniform lung disease by mechanical ventilation. The altered gas distribution during mechanical ventilation provides evidence that the operation of the respiratory muscles is an important determinant of inspired gas distribution during spontaneous breathing. Furthermore, the observation that body position alters gas distribution during mechanical ventilation maybe an indicator that gravity is a determinant of gas distribution in normal respiration.

4.6 Additional considerations in caring for ICU patients

The care of patients in the ICU requires a specialised team of nurses and medical staff with expertise in the use of both a wide range of equipment and the monitoring

and appropriate treatment of unconscious critically ill patients. Therefore intervention of these patients, such as performing lung scintigraphy outside the ICU, requires additional precautions and constant monitoring to ensure the patient's health is not compromised. These activities are generally undertaken by ICU nursing staff but all staff involved in performing lung scans on ICU patients need to be cognisant of the detailed monitoring requirements. Church (1998) summarised the major activities that must be maintained while a patient is outside the ICU. The ICU nurse and Registrar must remain with patient for the duration of the scan to:

- Continuously monitor patient's oxygen saturation levels, blood pressure and pulse
- Monitor airway pressure for any sustained increase to prevent barotrauma
- Observe amount and type of airway secretions and document
- Make sure the scanning room has wall oxygen and suction for the patient before commencement of the test
- Ensure a filter is used on the inspiratory and expiratory limb to protect the patient and equipment. It is only during the ventilation stage using Technegas that this filter is removed. After this procedure it is replaced
- Assess the patient for pain relief and / or sedation requirements
- Ensure that the stomach has been deflated; placement of a gastric tube either nasal or oral is required post intubation
- Ensure ETT tapes (tapes to secure oral endotracheal tubes) are firmly tied; a tube falling out or becoming dislodged is a medical emergency. All tubing is supported so as to reduce damage to oral / nasal mucosa and prevent displacement. (Church, 1998).

4.6.1 Cuff Pressures

All intensive care patients should have the pilot tube cuff inflated at all times to prevent an air leak unless specially requested by the physician. The cuff pressure is routinely checked and is rechecked if a cuff leak is heard (pressures range from 20 to 30 mmHg). Tracheal ischaemia can occur if the cuff pressure exceeds or approaches capillary pressure, which is approximately 32 mmHg. Using low-pressure cuffs has reduced the incidence of tracheal ischaemia, but it is still necessary to routinely observe the patient (Hess, 1996).

If the cuff is under-inflated, the patient will experience loss of ventilatory volumes and PEEP, and possible aspiration of gastric contents. Over-inflation will cause necrosis and ulceration of the trachea, with possible development of the tracheosophageal fistula.

4.6.2 Arterial Blood Gases in the ICU

Arterial blood gases (ABG) are routine in the ICU, especially on intubated patients to measure their outcome (see Table 4.3 in Results section). ABGs assist in the diagnosis of PE and associated conditions such as respiratory acidosis/alkalosis or metabolic acidosis/alkalosis. ABGs can also help identify possible strategies to correct for these imbalances. The oxyhaemoglobin curve in Figure 4.1 shows the relationship between percent of haemoglobin saturation (SaO2) and partial pressure of arterial oxygen (PaO2). At this site SaO2 drops dramatically with little change in PaO2. Habibian (1999) suggests that oxygenation at the cellular level depends on the ability of O2 to bind with and be released from haemoglobin (Hgb) in the blood.

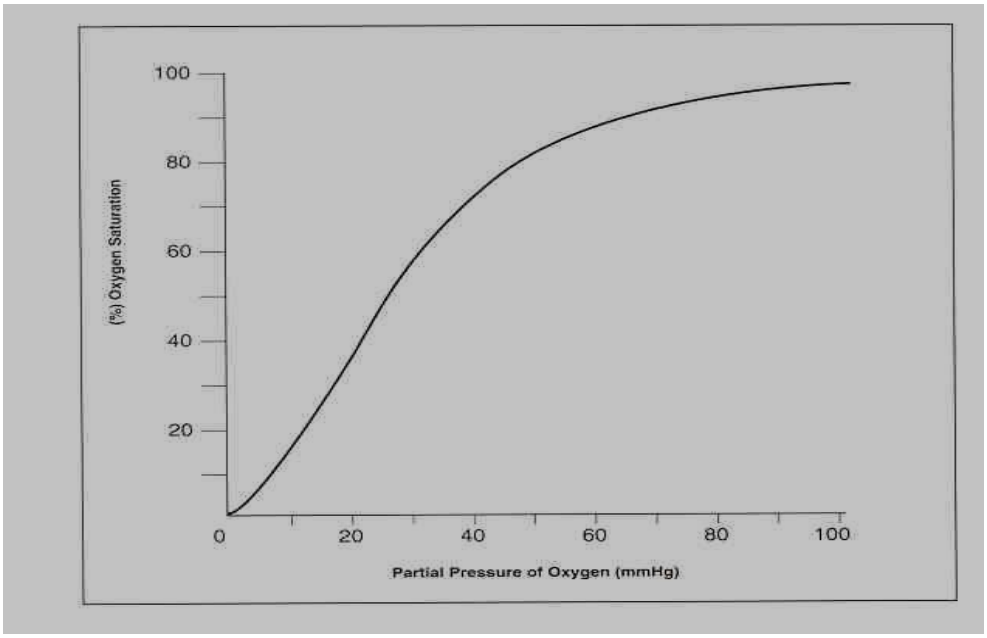


Figure 4.1 Oxyhaemoglobin Dissociation Curve (Morgan 1999)

Earis and Pearson (1995), suggest that the Oxyhaemoglobin dissociation curve (ODC) is a graphic relationship between haemoglobin oxygen saturation and the partial pressure of oxygen in the blood. The affinity of haemoglobin for oxygen produces an S-shaped curve representing the way oxygen normally loads onto, and releases from, the haemoglobin molecules. The flat upper portion represents oxygen loading of haemoglobin as blood passes through the lungs. Hillman (1996) suggests that when the partial pressure of oxygen is high, oxygen binds with the haemoglobin molecule.

However, because most haemoglobin molecules are already saturated, additional loading of oxygen onto haemoglobin will not significantly increase as partial pressure continues to increase. The steep lower portion of the curve represents the relationship at the tissue level. Haemoglobin molecules are not well saturated because they have already lost some of their oxygen to tissues. Even with minor reductions in the partial pressure of oxygen, large amounts of oxygen are off-loaded

from haemoglobin molecules (Earis and Pearson, 1995). These include a blood pH of 7.4 PaCO2 of 40 mmHG, temperature of 37^{o}C, and normal levels of 2, 3-DPG.

Transient hypoxia may occur but is typically not associated with any harmful symptoms to the patient.

As described by Hess (1995), ICU patients are strictly monitored while ever out of the intensive care environment. While in the department of Nuclear Medicine the patients' intravenous medications assist in keeping the patient stable while out of the intensive care unit, these patients are therefore more conducive to have a TEBA lung scan. The most common indication for using ventilatory support is reversible respiratory failure caused by either hypoxaemic or hypercapnoeic failure according to Hess (1995).

4.7 Aims

The aims of the experiments in this chapter are:

- To determine whether TEBA can allow ICU patients to undergo a V/Q scan
- To determine whether TEBA has a role to play in the management/diagnosis of PE or COAD in intubated / ICU patients
- To verify/assess the quality of images of ICU patients using TEBA (see Figure 4.3).

Consent for the Unconscious Patient:

The patient's relative (next of kin) or the Director of Intensive Care who has read the Informed Consent form explaining the purpose of the aims of our research, expected duration of participation and a full description of the procedure which included administering a small amount of radiation to the volunteer patient.

Ethics: See Appendix B

Study Cohort:

There were 25 unconscious intubated ICU patients in the study, including 18 males and 7 females with a mean age of (60.88 years).

4. 8 Materials and Methods

In this experiment 25 intubated ICU patients, (18 males and 7 females with a mean age of 60.88 years), were required to undertake a ventilation / perfusion (V/Q) lung scan using the TEBA positive ventilation system. The project aimed to verify the quality of images produced by this system using, the same V/Q lung scan procedure described in chapter 2 of this thesis, compared to conventional CAL images post ventilation. For this experiment ICU patients had only one TEBA lung scintigraphy scan as they were too ill to return to the nuclear medicine department. However, if they had a high probability lung scan, they returned for a second scan within 5-7 days to check progress after being treated with anticoagulants.

As discussed in chapter 1 of this thesis positive pressure ventilation or TEBA is used for intubated patients because the conventional method of ventilation is not the best method to use for the unconscious patient.

Two medical radiation scientists, with specific knowledge on how to use the TEBA apparatus, were required to perform the ventilation/perfusion lung scans on each ICU patient and a registrar and nurse from the intensive care unit were present throughout each lung scan to observe the patient.

As all patients in this experiment were unconscious they were all at risk of developing Hypoxia within the first few seconds of ventilation, (Fahey, 1995). Therefore, oxygen saturation measurements were taken on each patient throughout the V/Q scan.

The main objectives of ventilation are to support pulmonary gas exchange for these intubated patients, thereby improving arterial oxygenation and augmenting alveolar ventilation and correcting acid / base levels as PaCO2 and pH of the patient. West implied that improving the functional residual lung capacity will improve lung volume

and reduce the work of breathing (West, 1990) While minimizing the work of breathing and promoting gas exchange, several changes will occur as a patient changes from breathing spontaneously to breathing by mechanical means.

For the ventilation perfusion lung scan, the routine six projections of anterior, posterior, right posterior oblique, left posterior oblique, right anterior oblique and left anterior oblique were acquired. Each patient was required to have their arms raised above their head onto a pillow to minimise attenuation by the arms when acquiring images. This proved quite difficult as the patients were unconscious or sometimes semi-conscious, so arms had to be held onto the pillow by another staff member or ICU nurse. From the patient perspective this position was difficult for a number of reasons, the main being tiredness and discomfort for the patient who could not communicate.; also these patients had multiple sets of intravenous lines in both arms as well as central lines in their neck for arterial pressure measurements and administration of drugs. Furthermore, each patient was required to have intubation tubing connected to a Positive End-Expiratory Pressure (PEEP) system to ensure continuous ventilation throughout the time of the V/Q scan.

4.8.1 Inclusion and Exclusion Criteria

All intubated ICU patients were included in our study. Not included were:

- ICU patients weaned off their ventilators
- Children
- pregnant women or breast feeding mothers.

4.9 Results

Lung scintigraphy plays a major role in interpreting patients suspected of PE. The lung scan is most useful when it presents as either low or high probability of PE in conjunction with corresponding likelihood of PE. A normal or low probability scan is associated with 4% prevalence of PE and a high probability of 97% when clinical suspicions are high. However, an intermediate probability lung scan (IPLS) representing an uncertain diagnosis of PE occurs in more than one third of all lung scans. There is also an inter-observer variability between nuclear medicine physicians must also be considered. Additional diagnostic tests are recommended for V/Q scans with an intermediate probability of PE as 30-40% of pulmonary angiograms have been positive for PE (Fedullo, 2003, Wong, 2001).

Each V/Q lung scan in this experiment was examined for the presence of PE by two nuclear medicine physicians using the following criteria. Important characteristics of PE on the lung scan included the finding of wedge-shaped segmental and /or pleural based perfusion defects. While perfusion defects of irregular shape were unlikely to be due to PE. Patients with multiple perfusion defects that covered more than 75% of a lung segment were considered to be high probability for PE; a single defect that covered more than 25% of the lung segment was considered to have an intermediate probability of PE and were thought to require more diagnostic intervention. Whereas patients with one perfusion defect that affected less than 25% of a lung segment was considered to have a low probability of PE (Early 1995).

Table 4.1 shows the age and gender of the twenty-five patients who participated in the study.

Table 4.1 **Overview of Age and Gender** showing the number of ICU patients and their overall age groups

1	Male	63 years
2	Female	79 years
3	Male	82 years
4	Male	67 years
5	Female	68 years
6	Male	75 years
7	Male	78 years
8	Female	56 years
9	Male	44 years
10	Female	55 years
11	Female	73 years
12	Male	60 years
13	Male	84 years
14	Female	72 years
15	Male	69 years
16	Male	74 years
17	Male	75 years
18	Female	68 years
19	Male	72 years
20	Male	75 years
21	Male	73 years
22	Male	69 years
23	Male	62 years
24	Male	79 years
25	Male	65 years
		Total 25 mean = 60.8

4.9.1 Ventilation Perfusion Scan Results for ICU patients

A major outcome for this experiment was the significant achievement that 25 intubated ICU patients successfully underwent a V/Q scan after being transported from the ICU to the nuclear medicine department at Lidcombe/Bankstown Hospital.

As shown in Table 4.2, seven patients (28%) were found to have a high probability of PE and were placed in anticoagulant therapy, while eleven patients (44%) had an intermediate probabitly of PE and these were also placed on anticoagulant therapy. The remaining seven patients (28%) were found to have a low probability of PE so anticoagulant therapy was not prescribed for this third group.

Table 4.2 ICU Patients Lung Scan Diagnosis and treatment showing that a high percentage of anticoagulation therapy may be unnecessary

Probability	Total Number (/%)	Anticoagulant Therapy Yes or No
High	7 (28%)	Yes
Intermediate	11 (44%)	Yes
Low	7 (28%)	No

These results show that, anticoagulant therapy should only be commenced on patients with a definitive diagnostic result, to stop unnecessary risk of bleeding and placing patient on long term treatment when this may have been avoided. Significantly:

- **7 low probability who would not need to commence anticoagulant therapy**
- **11 intermediate who would have commenced anticoagulation therapy**
- **7 high probability who commenced treatment and would have returned in 5-7 days for repeat TEBA lung scintigraphy to assess anticoagulation therapy if they were stable enough to return to the nuclear medicine department.**
- **7 of 25 would have been commenced on anticoagulation therapy in ICU if not transported to nuclear medicine for lung scan & diagnosed as low probability for PE**

The following images illustrate the difference in ventilation scan quality between a TEBA lung scan on an intubated ICU patient (figure 4.2) and a conventional ventilation scan on a patient with CAL (figure 4.3). The scan of the TEBA assisted ICU patient (figure 4.2) shows an even distribution of Technegas throughout the lung fields. There is good edge definition and peripheral penetration with only minimal central deposition of the Technegas. By comparison, the conventional scan of a patient with CAL (figure 4.3) shows non-uniformity, clumping of Technegas.

Figure 4.2 **ICU TEBA Lung Scan Image on an intubated patient**

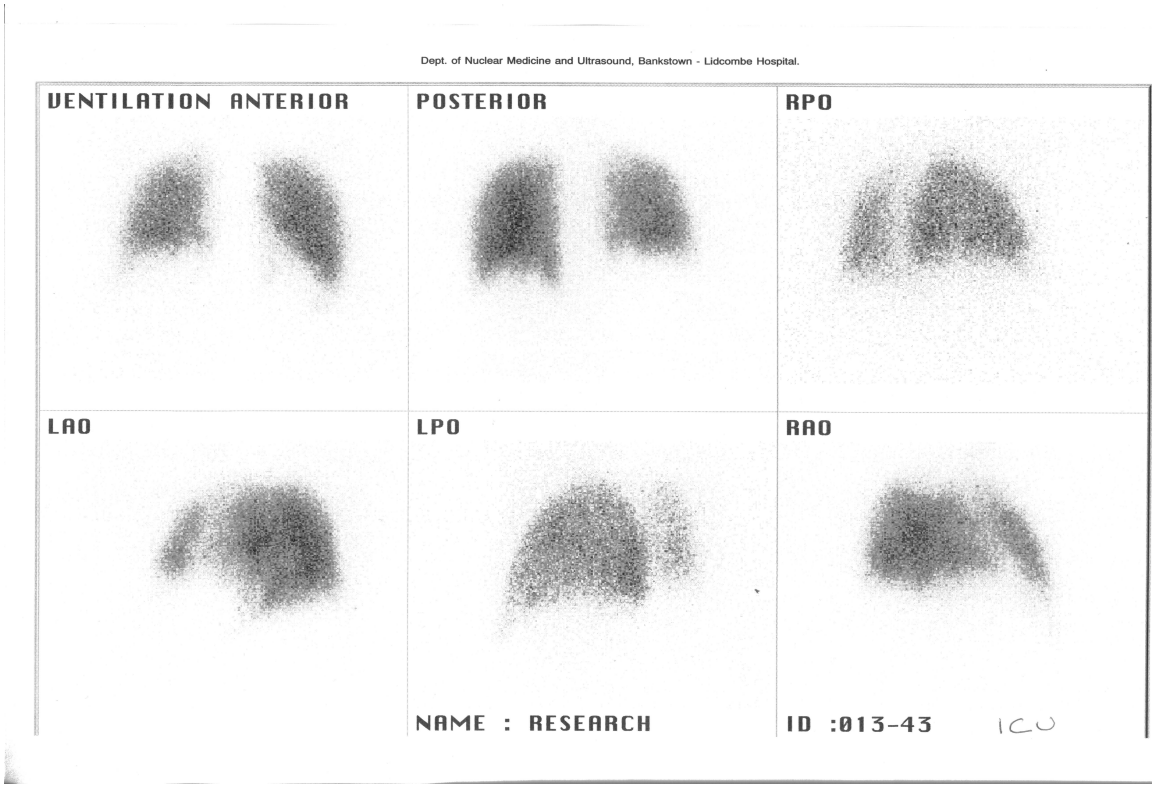
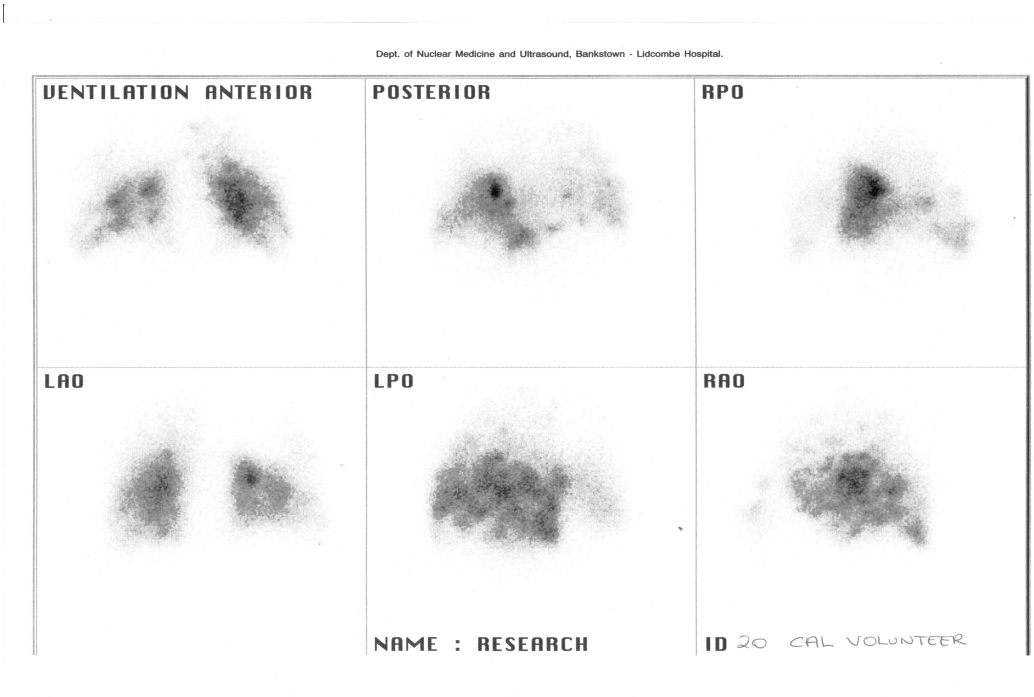


Figure 4.3 **CAL conventional lung ventilation shows non-uniformity, clumping of Technegas**



Blood Gas Results for ICU Patients

The blood gas results are displayed in Table 4.3 and include pH levels and whether $PaCO_2$, PaO_2 and HCO_3 were normal, raised or below normal. The results showed that only three of patients, (numbers 10, 15 and 24) had normal blood gas results at the time of the V/Q scan. The poor respiratory function of the majority of patients (21 out of 25) in the experiment demonstrates the difficulty faced by the researchers in conducting a study on critically ill patients for the ICU.

Table 4.3 **ICU Blood gas results**

Patient	**pH**	**$PaCO_2$**	**PaO_2**	HCO3	Diagnosis
1	7.36	⇧ hypercapnia	⇧	⇧	Acidosis fully compensating
2	7.40	⇧	Normal	⇧	Respiratory Acidosis fully compensating
3	7.3	Slight ⇧	Normal	Normal	Pt. Has slight hypercapnia
4	⇩ 7.21 acidosis	Slight ⇧	⇧	⇧	Severe respiratory acidosis but compensating
5	7.3	⇩	Normal	⇩	Respiratory alkalosis and compensating.
6	7.5	Normal	⇧	⇧	Metabolic alkalosis slight to partial compensating
7	7.4	⇩	⇧	⇧	Respiratory Alkalosis fully compensated
8	⇩7.2	⇧	⇧	⇧	Respiratory Acidosis, hypoxia and partial compensation
9	7.4	⇩	⇩	⇩	Hypoxia
10	7.4	Normal	Normal	Normal	Normal Blood gases
11	7.45	⇩	⇩	⇧	Metabolic Alkalosis and partial compensation
12	7.5	⇧	⇩	⇧	Metabolic Alkalosis compensated
13	⇩ 7.2	⇧	⇧	⇧	Severe metabolic acidosis
14	7.3	⇩	⇩	⇩	Metabolic acidosis with hypoxaemia
15	7.4	Normal	Normal	Normal	Normal blood gases
16	⇩ 7.2	⇩	Normal	⇩	Respiratory acidosis with hypoxia
17	7.4	⇩	⇩	⇧	Compensated respiratory acidosis
18	⇩ 7.2	⇩	⇩	⇧	Metabolic acidosis
19	⇩ 7.1	⇧	⇩	⇩	Respiratory Acidosis
20	7.3	⇩	Normal	□	Respiratory alkalosis, fully compensating
21	7.5	⇩	⇩	⇧	Metabolic alkalosis with partial compensation
22	⇩ 7.2	⇩	⇩	Normal	Metabolic acidosis
23	7.4	⇩	⇩	⇩	Respiratory acidosis
24	7.3	Normal	Normal	Normal	Normal blood gases
25	7.3	⇩	⇩	⇩	Metabolic acidosis with hypoxaemia

4.9.2 Blood Gas Variations

There are several factors that can change a blood gas level. These include:

1. Flushing the patients' arterial lines prior to sampling avoids contamination, otherwise, the pH and electrolytes will be affected.
2. Extremely high white cell count rates will cause falsely low pO2 level and falsely elevated haematocrit.
3. Haemolysed samples will cause falsely elevated potassium levels.
4. Intravenous fluids can affect sodium results.
5. Blood gas analysis must be performed immediately for best results.
6. Other key sources of pre-analytical error is sample handling, including the choice of syringe, needle size, and anticoagulants, as well as air bubbles in the line, temperature and sample mixing (Ref. Hillman 1996 and Easis 1995).

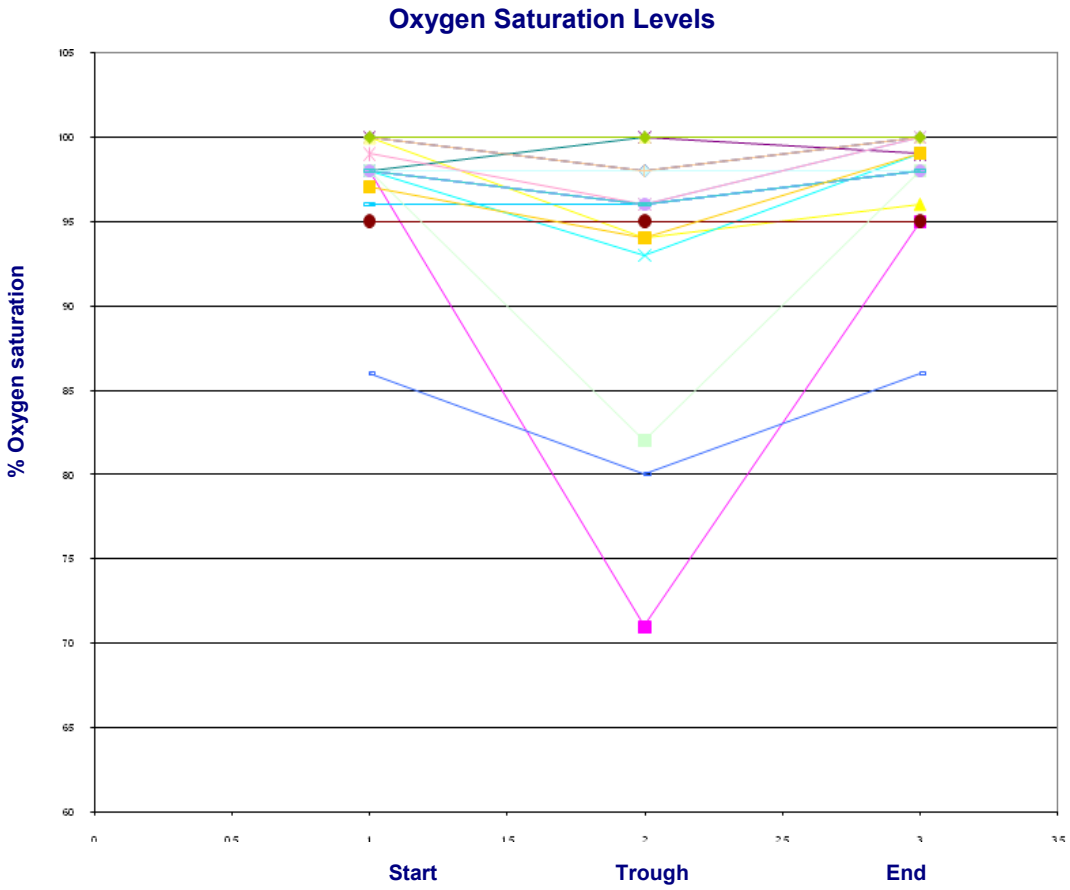
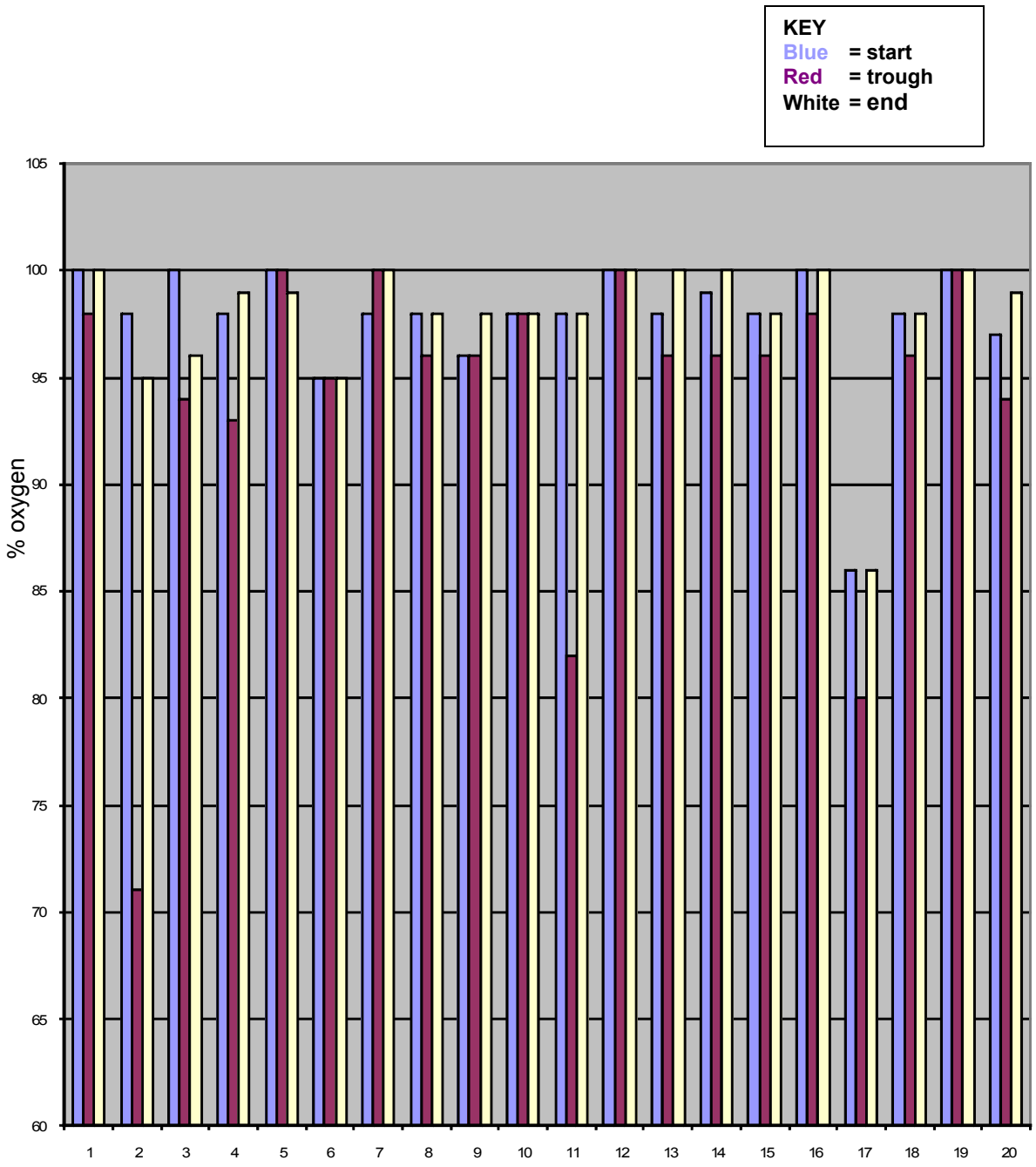


Figure 4.4 **Line Graph showing Oxygen Saturation Levels in ICU patients during TEBA ventilation with start, trough and end**

Figures 4.4 and 4.5 show blood gas results for the 25 patients in the study. The vertical axis in Figure 4.4 represents oxygen saturation, horizontal number of breaths. Three distinct dips or troughs are shown where the patient has become hypoxic in the middle of the ventilation process. Hypoxia can occur and only lasts a few seconds then returns to normal. These ICU patients were on 100% continuous oxygen.

Two patients recorded 80 and 72% oxygen saturation midway through the ventilation showing the need for the medical radiation scientist to have a basic understanding of the immediate care for the intubated unconscious patient. These incidents also highlight the necessity for the intensivist and ICU nurse to be present throughout the scan.

Figure 4.5 **ICU Intubated Patients' Oxygen Saturation**



4.10 Discussion and Conclusions

After much research and support from the Intensive Care Department and Nuclear Medicine Departments at Bankstown–Lidcombe Hospital Sydney, a protocol was developed so that intubated ICU patients could be transported to the Nuclear Medicine Department for lung scintigraphy. There were limitations to using TEBA on the ICU patients as it required two people to use this system; the principal researcher and one other medical radiation scientist trained by the principal researcher in the use of TEBA with ICU patients.

The aim in chapter 4 was to determine whether TEBA can allow ICU intubated patients to undergo a lung scan in the nuclear medicine department. This aim was achieved after much planning to implement the safe transport and management of the unconscious patient. In addition to the two specially trained medical radiation scientists who performed the TEBA assisted V/Q scans, a medical intensivist and an ICU nurse also accompanied each patient throughout the study.

While each patient was mechanically ventilated, there was a risk of Barotrauma from high inflation pressures and/or volutrauma from high tidal volumes. While both these conditions cause respiratory failure as intrathoracic pressure changes (Hess, 1996) to date none of these conditions have been reported using TEBA.

In addition, TEBA assisted ventilation also has the potential to induce increased intrathoracic pressure, decreasing venous return to the heart, in turn leading to a fall in cardiac output. This can be evidenced by increased pulse rate and decreased blood pressure during TEBA ventilation of the intubated unconscious patient. This did occur in two critically ill patients (nos 11 & 17) who were involved in this experiment

(see Figure 4.5). The research team provided appropriate treatment instantaneously ensuring the oxygen levels of both patients returned quickly to within acceptable limits.

In this experiment, oxygen supplementation was administered continuously up to 10L/min to ICU patients with an oxygen saturation level between 91-99%. In the final report ICU patients were concordant with the other groups ventilated, however there is a concern that positive pressure ventilation may create a falsely positive diagnosis of PE as it can improve peripheral penetration of tracer in poorly ventilated areas that are associated with reflex vasoconstriction and hypoperfusion not due to vascular obstruction. Clearly, further investigation is required to clarify this issue, as the numbers of subjects in this research were too small to make any definite conclusion.

TEBA assisted ventilation can be a difficult procedure and without being properly trained in the use of this apparatus, no medical radiation scientist should attempt to disconnect an intensive care patient from his or her ventilator. Once a medical radiation scientist is trained and has a good understanding of the intubated critical care patient's management, TEBA can provide a reliable result for the patient. Images from the V/Q scans were reported to be as good as and in some cases better than conventional images for these ICU patients.

TEBA has played an important part in diagnosing PE in ICU patients, who previously would have been commenced on anticoagulant therapy without the aid of a lung scintigraphy scan. TEBA provided the group of critically ill intubated patients in this experiment a ventilation image that was at least as good as and in some cases better than those obtained from the conventional ventilation methods.

In conclusion, the ICU intubated patients only had one lung ventilation/ perfusion scintigraphy scan using TEBA as they were critically ill and this research could not justify these patients returning for a second time. By using TEBA connections and removing the filter from the tracheostomy tube, the intubated patients could be ventilated with no significant discomfort or complications. Pneumothorax or barotraumas were reduced due to the smaller volume of oxygen or air used during TEBA operation. However, TEBA required two medical radiation scientists who were both fully trained in the use of TEBA for ventilation scintigraphy.

TEBA has a definite role to play in the management and diagnosis of PE in ICU patients.

Chapter 5 Experiment 4: Technegas at the Cellular Level

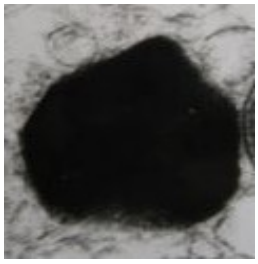


Image of Technegas particles inhaled in lung ventilation, taken on the JEOL100CX11 Electron Microscope at Western General Hospital, Edinburgh, Scotland.

Studying Technegas at the cellular level is like
"trying to find a needle in a haystack"

5.1 Introduction

Previous experiments have looked at the chemical nature of Technegas, but to date no-one has looked at Technegas when inhaled by humans. In order to give the nuclear medicine community a better insight into the nature of Technegas, we will investigate specimens of lung washings taken from volunteer patients who were booked in for a routine bronchoscopy. We will look at Technegas at the cellular level, measuring the particles inhaled in lung ventilation. At the same time, we will delve into the physics and electron microscopy procedures used to study Technegas.

To examine Technegas at the cellular level proficiently, we need to take a step back and examine both the physics surrounding the use of Technegas and the particles of Technegas at the microscopic level, in order to give us a broader picture of this ultra-fine aerosol particle. The work in this chapter will cover both discarded methods used as well as potential methods to try to measure the number of Technegas particles inhaled into the lung post ventilation, lung deposition and the physics behind Electron Microscopy (EM). Chapter 5 is an extension of previous work undertaken by other researchers in this field and was intended to provide new research findings in the field of Nuclear Medicine.

We wish to be able to visualise, using EM, the hexagonal particles of Technegas by setting up our own experiment using human lung washings from volunteer patients. We will also try to study the effectiveness of carbon vapour, which coats smaller quantities of technetium seen on our EM microtomes. Finally using EM, we will examine Techegas ultrafine particles, showing the thickness of carbon film.

5.2 What is Technegas

Technegas is an aerosol consisting of a gaseous continuous phase and a discontinuous phase of individual particles. The discontinuous phase can be either solid, liquid or both. There are many terms used to denote different types of aerosols. One term used by Lloyd (1994) is smoke, which is solid and derived from a combustion process. Another term used is mist, which is a liquid process as used in Technegas.

The behaviour of an aerosol is dependent on size, shape and composition and the collective properties that include: size distribution, number density, etc.

Previous research work on the chemical nature of Technegas proposed that Technegas ultra fine aerosol particles range in size from a few nanometres to a few hundred microns. Lloyd (1994) suggests that the lower end of the range of aerosol particles represents the transition from molecules to particles, whereas particles of the upper end of the scale do not remain airborne for long enough to form an aerosol. The largest useful size for studies of the lung is approximately 10 μm; inhaled particles greater than this will deposit in the upper airways.

5.3 Aims

In this chapter, we are focussing on lung ventilation, deposition of the Technegas particle at both microscopic level, and the physics involved to attempt to measure this ultrafine particle. Regarding Technegas ventilation, we will cover the following topics:

- **uniformity of Technegas**

Uniformity is the dispersion of Technegas throughout the lungs when a patient is ventilated.

- **number of particles**

The aim of this work was to measure the number of particles, size distributions, and regional lung deposition of the Technegas. The Technegas size distribution was approximately lognormal with an activity median diameter of 158 nm and a geometric standard deviation of ±1.5. The median size increased with the number of simmers and with the time from generation. The increase in size with the number of simmers is thought to be due to the increased salt content in the crucible prior to the "*burn*". It is also of significance to try to estimate the number of Technegas particles inhaled into the lungs. The predicted lung deposition is 37% in the alveolar region and 5% in the bronchial region. Significant changes in deposition are not predicted over the range of particle sizes measured.

- **shape of Technegas particles**

Senden's research (1997) suggested the active particle of Technegas was a hexagonal platelet of metallic technetium contained within a thin layer of graphite

carbon. The average size of the hexagonal platelets was seen to be approximately 30-60 nm.

- **electron density of Technegas**

Graphite is formed by flat hexagonal layers of carbon atoms. The bonding energy of two atoms located in the same layer exceeds the same energy for different layers. It is interesting to note that the bonding energy for the graphite between atoms in the same layer is as strong as the bonding in a diamond structure, although the solidity of graphite, determined by interlayer bonding, is very low. All the carbon nanostructures known now could be constructed from an ordinary hexagonal graphene layer. The production method of “Technegas”, discovered in 1984, appears to be very similar to the production method of the fullerenes; it has large (from ten to one hundred nanometers) carbon nanocrystals with the metastable technetium atoms inside. Consequently, this substance was the first commercial application of the filled carbon structures *before* the discovery of the fullerene in 1985.

- **amount of Technegas remaining in the lung**

This refers to the residual amount remaining post ventilation using Technegas.

Studying Technegas under normal light microscopy and EM

Technegas cannot be visualised under normal light microscopy due to both its electron density and the size of the Technegas particle.

- **Technegas remaining in the sputum post bronchoscopy**

It is of particular interest to study Technegas in human volunteers who were already consented for a routine bronchoscopy. Prior to their bronchoscopy, the volunteers

will have a Technegas lung ventilation scan and have Technegas “onboard”. Post bronchoscopy, these lung washings and a sputum specimen will be examined and prepared for EM. To date, previous research looked at the chemical and physiological nature of Technegas. However, our work is distinctive in that we studied Technegas using human subjects. Our study will incorporate physics to try to estimate the number of inhaled Technegas particles into the lungs, study lung deposition, and look at the discarded and potential methods involved.

5.4 Summary of purpose of this research

- to determine the pathway of a commonly used radioactive gas; Technegas post inhalation into the lungs.
- to be able to investigate the shape and cellular structure of Technegas.
- Histopathology aims to visualize the body’s extensive complex systems and their interactions as well as various disease states that occur within the human body. By examining Technegas at the cellular level, this research aims to ascertain the cellular structure of the Technegas particle and density, and will measure the number of Technegas particles.
- Electron Microscopy (EM) is used for our research of the Technegas ultra fine particle as both the resolving power and resolution specified use the minimum resolvable distance.

5.5 Relevant Research

In 1997 in the Journal of Nuclear Medicine, Senden and Burch et al. had written a paper on “The Physical and Chemical Nature of Technegas” and the structure of inhaled technetium 99m. The results of this paper showed that the active particle Technegas had been identified as hexagonal platelets of metallic technetium contained within a thin layer of graphite carbon. The average size of the Technegas platelet was 30 to 50 nm. This paper is significant as it gives an explanation for the physical structure and approximate size of Technegas.

In 1993 Lemb et al. wrote a paper for the European Journal of Nuclear Medicine on particle structure, size and distribution of Technegas. There were many papers written at this time, all looking at the chemical and physical nature of Technegas but not the localisation of these particles in human lung tissue. My research was intended to provide evidence for this localisation. EM gives detail at the cellular level, which is necessary to study the localisation of Technegas particles in lung washings taken post bronchoscopy.

5.6 Funding

To enable this study to continue, Dr B. Elison donated funding for the use of the Electron Microscope (EM) at St. George Hospital, Sydney and Western General, Edinburgh. EM work of any nature is an extremely costly service.

In the first instance, Electron Microscopy (EM) preparation of samples and Light Microscopy was achieved at Wollongong Hospital and EM only undertaken at St. George Hospital, Sydney. More extensive EM work was later carried out at Western General Hospital, Scotland, which included both preparation and microscopy in the one unit. Any funds that were left over from St. George went towards funding the research at Western General in Scotland.

5.7 Inclusion / Exclusion Criteria

The study will include twenty (20) adults, eleven (11) male and nine (9) female with a mean age of ±48.5 years who were scheduled for a regular bronchoscopy, but will exclude children and females who are either pregnant or breastfeeding.

Ethics: See Appendix B.

The only differences between patients in the present study and other patients undergoing bronchoscopy will be:

- an additional ventilation lung scan
- one additional bronchoscopy sample
- a sputum sample.

I have considered the radiation dose implications and am aware that naturally occurring background radiation is typically 2-3 mSv per year. The ventilation study involves exposure to a very small amount of radiation, estimated at 0.6 mSv (IRCP 80), which is a fraction of the background dose. To date there have been no studies of the radiation dose to the lungs post ventilation of Technegas. In an effort to quantify this dose, the use of microscopy is required to examine the bronchial and sputum samples post ventilation and the bronchoscopy procedure.

Ethical considerations necessary for this research include: Informed Consent with a statement that includes an explanation of the purpose of the research, the expected duration of the patient's participation, and a description of the procedure which includes a small amount of radiation, an additional lung washing/lavage sample at bronchoscopy, and a sputum sample post bronchoscopy.

5.8 Physics behind EM and Technegas Particles

In order to study EM, we need to look at the structure of the Electron Microscope and the physics it entails. In this section, we will look in more depth at the relationship of Technegas encompassing particle structure, measurement, deposition, and particle clearance under the following headings:

1. What is Electron Microscopy
2. X ray generation and emission in thin films
3. Spatial resolution and EM
4. Detection of X-rays in EM

5. Estimation of number of particles

6. Lung Deposition

7. Measuring particles of Technegas

8. Senden & Burch's study on formation of Technegas

5.8.1. **What is EM**

Figure 5.1 **An Electron Microscope**

Electron Microscopy (EM) involves the passage of a high-velocity electron beam through our specimens that are thin enough to transmit at least fifty percent of the incident electrons. The emergent beam of transmitted electrons is then refracted by a system of lenses to form a magnified, two-dimensional image of our specimens.

A tungsten filament or cathode in an evacuated tube is heated, thus producing the electron beam. Electrons being attracted to the anode pass through a small hole, causing an increase in energy known as kinetic energy. Electromagnets focus the electron beam onto a specimen causing a small amount of kinetic energy to be lost

due to the interaction with the specimen. As the electron beam passes through the specimen, electrons encounter the electromagnetic fields of many extra electromagnets, directing the electron beam onto a fluorescent plate. When beta particles (electrons) bombard a specimen, electrons are emitted and this beam is analogous to a diffracted light ray, whose intensity is a direct function of the electrons' kinetic energy (Slayter & Slayter, 1992).

The major systems in EM are: an illuminating system, comprising of a source of radiation, a condenser lens assembly that focuses the "illuminating beam" onto the plane of our specimens, and after passing through the specimen, the beam enters the imaging system. This consists of a number of lenses, which together produce the final magnified image. The lenses are known as the objective lens, which produces the intermediate image, and the projector lens, which produces the final magnified image of the specimen. The images can then be viewed and recorded photographically; this represents the images translated by EM (Chescoe & Goodhew, 1984).

EM is used in our study to examine lung and sputum samples using high-energy electrons; this feature makes microanalysis of small known volumes straightforward. EM uses electrons instead of light to visualize small objects; therefore the resolution is increased relative to the shortness of the wavelength of the electron beams. Instead of lenses, electrons are focused by electromagnetic fields and form an image on a fluorescent screen (Chescoe & Goodhew, 1984, Conn,1990).

The tissue surface of the lungs once resolved is approximately two hundred microns (200 μm), which is seen as a stereo view due to the large depth of focus (Conn,1990). Taking two images from different angles gives better images; also tilting the specimen of lung or sputum may give greater detail in the depth of concavity.

The lung, as it is an organ of many shapes, is ideally suited for EM and direct visualization of a three dimensional ultra structure is helpful for a patient's diagnosis. EM depends either on determining the intensity of a secondary effect excited within the specimen or on the measurement of some change in the primary beam of electrons after it has interacted with the specimen (Koehler, 1973). A primary electron beam of energy may excite Auger electrons or x-rays from the specimen and subsequent beams below the specimen will contain electrons which have lost a characteristic amount of energy. Also excited from the specimen will be secondary electrons which could be used for Scanning Electron Microscopy (SEM) imaging (Rios et al., 1992).

In EM, the radiation source is a beam of electrons, which is produced by an electron gun. This consists of a tungsten filament, enclosed in a metal casing called the cathode shield, and an anode plate. Both the cathode shield and the anode plate have central apertures, which are centrally aligned with the tungsten filament tip. Electrons are produced by passing heated current through the filament. The small difference in voltage between the filament and the cathode shield deflects electrons away from the walls of the shield and they form a cloud of electrons near the cathode shield aperture. Electrons are propelled down the column by the vast difference in voltage between the filament (-20 to-100kV) and the anode plate is a zero potential. This difference in voltage is called the "accelerating voltage". The lenses are electro magnetic and a current passing through the coil creates a magnetic field, which has the ability to deflect electrons. To focus an electron beam onto a given plane, the current passing through the coil is changed (Slayter & Slayter, 1992).

In EM the absorption of electrons by a specimen of usable thickness is very small, and the image is formed partly by electrons that have passed through the specimen,

and partly by electrons being scattered by the specimen. The majority of electrons pass through the specimen without deviation but some are deflected by electrons orbiting atoms in the specimen and within the atomic nucleus itself. This all forms a pattern, which is transformed into an image on the fluorescent viewing screen, as electrons bombarding the screen produce a visible fluorescence, and areas which have been deflected remain dark (Stradling,1990).

5.8.2 Apparatus used in EM

EM consists of three main parts which are the Vacuum system, the Electrical System and the microscope column. We will discuss these separately:

a) Vacuum system

This is necessary because electrons can only travel a few millimetres in air before they collide with gas molecules. As most electron microscope columns are approximately a metre in length, the air must be evacuated. The vacuum maintained in an electron microscope is approximately 1×10^{-4} mmHg and at this vacuum the electrons can travel about 2.5 metres. In low vacuum conditions, the life of the filament is reduced due to oxidation of the tungsten atoms; this oxidation also decreases the efficiency with which the filament can emit electrons (Somlyo, 1986).

To obtain constantly high vacuum, rotary oil diffusion pumps are used. A series of air locks is introduced into the system at key areas to prevent long periods of time being spent on ventilating and re-evacuating the column. These air locks are usually located around the specimen and camera areas. During operation, the oil diffusion pump generates a great deal of heat so is cooled by a water jacket cooling system (Somlyo,1986, Stradling,1990).

b) Electrical System

This consists of a high-tension (HT) unit, a lens current supply unit, and voltage and lens current stabilizing units. The high - tension unit usually operates at between –20kV and –100kV. This unit is used to accelerate the electrons. The lens current supply unit provides power to the electro-magnetic lenses and the current must be stable because any fluctuation in results will mean a loss in resolution. The voltage and current stabilizers maintain this degree of stability. The power for the auxiliary supplies vacuum pumps and switch circuits.

c). Microscope column

This consists of an evacuated metal tube in which are aligned, one under another the filament enclosed in the cathode shield, the anode plate, various electro-magnetic lenses, viewing screen and a photographic plate.

The first magnetic lens focuses a beam of electrons emerging from the filament; the condenser lens reduces the diameter size of the electron beam from approximately fifty microns to approximately one micron and this small diameter beam is projected onto a specimen by the second condenser lens. Below the two condensers lenses is the specimen holder, which can be manipulated by external controls in two directions, giving access to any part of the grid (Stradling,1990).

The objective lens transforms the electrons, which have been scattered by the specimen into an image. The objective lens also determines the resolution of the microscope, and it is at this intermediate stage that electron optical aberrations such as astigmatism, diffraction and distortion are significant since they will be greatly magnified as the final image is formed. The depth of focus for the objective lens is

constant and varying the voltage current through the lens only serves to focus the specimen (Slayter & Slayter,1992).

The “projector lens” now magnifies the image. Increasing the current through this lens increases the magnification. Most modern transmission electron microscopes operate with a three- lens projector system, a diffraction lens, an intermediate lens and a projector lens. The projector lens current is kept constant and varying the current to the other two lenses determines the magnification. Switching the intermediate lens off and varying the current of the diffraction lens adjusts the magnification from 3,000 to 18,000. Turning the current of the diffraction lens to maximum and varying the current to the intermediate lens increases the magnification from 20,000 to 500,000. The projector lens has a depth of focus of several metres, so the positions of the viewing screen and the photographic plate are not critical (Slayter & Slayter,1992).

The viewing screen is coated with a fluorescent material which when bombarded with electrons, fluoresces in the visible range. An image of the specimen is produced onto the screen. Due to the grain size of the fluorescent material, the best resolution achieved on the screen is about 35 microns. Photographs are generally taken because more detail is recorded on fine grain photographic material than on the viewing screen. Photographs are recorded by lifting the viewing screen and allowing the electrons to bombard the electron–sensitive photographic emulsion. Resolution achieved with modern transmission has been approximately 0.2 nanometres using crystalline material and between 1 and 2 nanometres is the upper limit for biological material (Slayter & Slayter,1992).

5.8.2 X-Ray Generation and Emission in Thin Films

The spectrum of x-rays emitted from a thin specimen which is irradiated with a high energy (>10KeV) beam of electrons will consist of a series of sharp peaks superimposed on a non flat background of continuous radiation known as Bremsstrahlung (Slayter & Slayter,1992, Rios et al, 1992). For a thin film, very little energy is lost in the specimen and we can assume that the ionization cross–section, which is the probability that an electron will eject a particular inner shell electron, is consistent throughout the specimen thickness. This is known as “the thin film criterion” and from this we can deduce the intensity of a characteristic peak from element A (Chescoe & Goodhew, 1984, Conn, 1990). In order to calculate the number of X-ray photons per incident electron, we will use the following formula:

X-ray photons per incident electron $I_A = pBN \frac{C^A}{A_A} \omega_K Q$

Parameters:

ω	=	Fluorescence yield
Q	=	Ionization cross-section, small area for high energy electrons
I_A	=	Measured characteristic x-ray intensity
P	=	Density
B	=	Backscatter
N	=	Avogadros Number
C_A	=	Concentration of element A by weight
A_A	=	Atomic weight of element A
K	=	Correction factor

Using low voltage, EM may improve the contrast of our bronchial washings and sputum specimens as increased inelastic scattering at lower energies increases the contrast at the same time. This method is accomplished by decelerating electrons before they impinge on our specimen, then accelerating the electrons again after scattering past the specimen (Koehler,1973).

Electrons interacting with Technegas bronchial washing specimens and sputum will be scattered over an angular range depending on the mass and density of each specimen. To prevent the electrons recombining, an objective aperture is preset, thereby stopping the electrons.

It has been observed that beyond a certain level of enlargement, the useful magnification is exceeded, therefore the main purpose of microscopy is to obtain detail not perceived by an untrained eye. Specifically, resolution is achieved when two or more points can be distinguished as separate points, also known as spatial resolution (Crang & Komparens,1988).

5.8.3 Spatial Resolution and EM

There have been limited experimental results to date to enable comparisons. It has been seen that scattering of electrons within the specimen will give rise to beam spreading. What is clear, is how to define the magnitude of beam spreading, and what proportion of electrons or x-rays generated are to be included. This is still unresolved; however Goldstein et al. (1977) assume that all scattering takes place at mid plane of the specimen (Chescoe & Goodhew,1984). To minimize the effect of spreading, we need a very thin sample of low atomic number and density, and analysis should be at the highest practicable voltage.

5.8.4 Detection of X-rays in EM

In EM, the operator must maximize the collection efficiency in order to improve the detection limits for a small concentration of an element in a specimen of lung or sputum. The closest distance for the detector in EM is about 20 mm, giving a collection efficiency of less than one percent, assuming x-rays are emitted isotropically. When the electron beam is being scattered by the lung or sputum sections, the main effects are:

1) Backscattering of the electrons onto the lower condenser pole piece.
2) Fluorescence by x-rays generated onto the specimen at the region being studied.

5.8.5 Estimation of number of particles

Particle size and the number of particles do affect the site and amount of particle deposition in the lungs as well as the subsequent clearance times, which is why it is important to include this measurement in our research.

According to Senden and Burch in 1997, the behaviour of aerosols is dependent on particle size, shape, composition and the collective properties which include size distribution, number density etc. The size of a Technegas particle ranges from a few nanometres to a few hundred microns. The lower end signifies the transition from molecules to particles, whereas the upper end does not remain airborne long enough to form an aerosol. According to James and Brown in 2002, 10 μm is the largest useful size for studies and anything greater than this deposits itself in the upper airways.

Research by Amis and Crawford et al. (1990) suggests particle distribution is a statistical progression and the number of particles has to be large enough to achieve the minimal variance. If the particles are randomly located, the density is Poisson distributed and the variance is equal to the number of particles counted. Activity concentration may also be measured.

This chapter reviews the nature and characterisation of Technegas and its deposition into the lungs. We study the structure of Technegas with the aid of Electron Microscopy and observe the clearance from the lungs. This work has also investigated the number of particles in Technegas, a technetium -99m ultra fine aerosol. Brown (2002) reported that dosimetry research of ultrafine particles and particle numbers in humans has being very poorly characterised. Determining the number of particles may give an idea of how much aerosol is inhaled by healthy subjects compared to subjects with obstructive lung disease.

Brown (2002) studied both the deposition and clearance rate of aerosol in both healthy and CAL subjects and reported that relative to healthy subjects, patients with moderate to severe CAL received a higher dose from ultrafine particle exposure. Brown (2002) also noted that lung clearance did not statistically differ between healthy and CAL subjects.

An article by Weibert in 2006 further suggests that there is a fast and substantial uptake of particles in the lung. Weibert studied 15 healthy subjects who inhaled Technegas particles of 100 nm in size. Radioactivity over the lung was studied for a period of 70 hours. The clearance of these ultrafine particles from the lungs was also measured. Lung retention at 46 hours was mean SD ± 99.4% and cumulative leaching of 99m technetium from the particles 2.6 ± 0.96% at 70 hours. Weibert

concluded that there is no evidence of quantitatively important translocation of 100nm particles to the systemic circulation from the lungs.

According to Lloyd (1994), an aerosol is a two phase system consisting of the gaseous or continuous phase and a discontinuous phase of individual particles. The characterisation of an aerosol is therefore dependant on both the individual particle properties and the collective properties such as size distribution, number density etc.

A Technegas aerosol particle ranges in size from a few nanometres to a few hundred microns. Senden and Burch (1996) identified the size of Technegas particles or aggregates to be a log-normal distribution ranging from 30 – 60 nm in width and approximately 5 nm in thickness, with 80% of particles being below 100 nm.

5.8.6 Lung deposition

The lung deposition (DF = 1- exhaled activity divided by inhaled activity, respectively) affects both the tissue dose to the airways as well as the mucociliary clearance rate, the normal being 3 mm/ml/minute (Lloyd 1996). Half life (the biological half-life is the time taken for half of a radioactive material present in a body as a result of inhalation to be eliminated) for mucociliary clearance studies depends on the site of particle deposition; approximately an hour for central airways and three hours for peripheral deposition. Due to a number of factors such as non–specificity of the measurement of particle sizes for different diseases, variables in particle size and inhalation techniques, mucociliary clearance studies are not widely used in clinical practice. In retrospect, mucociliary clearance studies have been used for a variety of respiratory diseases as sarcoidosis, pneumoconiosis etc. and therefore papers on the usefulness of clearance studies have been published.

There is a notable decrease in the speed and clearance capacity of mucous as the airways narrow towards the lung periphery due to the diminished cilia in this region. Therefore the rate at which mucociliary clearance occurs will depend on the rate of inhalation from the patient, the site of deposition of the particles and their size. CAL and patients with a respiratory disease will have a reduced clearance due to excess mucous production and limited cilia motility as Technegas does not diffuse through the alveolar capillary barrier and the only limiting factor for image acquisition is the half- life.

Mucociliary clearance is impaired in stable, acutely ill patients with no airway manipulation and correlates with simple markers of underlying disease severity. Mucociliary dysfunction may help to explain the increased susceptibility of hospital-acquired respiratory infection in critically ill patients (Laghi, 1995).

Dose rate of technetium: this is calculated by DR = DF multiplied by the minute ventilation multiplied by concentration which is a measurement of 10$\mu g/m^3$; it is used for health epidemiological studies to aid in ambient particulate exposure increments of ultra fine particulates (Lloyd 1996). Dose rates are calculated to give an estimate of the ultra fine particle mass that would be deposited per hour in the lungs. Browns' research paper, *Ultrafine Particle Distribution and Clearance in Healthy and Obstructed Lungs* (2002), reported that 54% higher dose rate was found in Chronic Airway Limitation (CAL) patients than in healthy subjects ($p=0.02$).

Previous research has shown that pulmonary particle clearance is similar between CAL and healthy subjects. In 2002 Brown estimated 85% particle retention at 24 hours post deposition. Isotope leaching would not cause differences between the two groups of CAL and healthy patients. According to Miller and associates in 1995, ventilation is reduced to obstructed regions while healthier regions receive the

balance of ventilation and associated particle exposure. The pattern therefore is less uniform in CAL subjects than in healthy ones. Miller and associates assumed that a quarter of the parenchymal lung in CAL patients might receive all the deposition normally distributed across the full parenchymal lung. By using this approximation and the dose rate, the parenchyma in CAL patients would receive a 600% greater dose than the tissues seen in the normal healthy lung. The increased surface dose in CAL subjects may elicit an inflammatory response with systemic effects as described by Seaton, et al. (1995).

Other research has proposed that ultra fine particles in the circulation may accumulate in other organs such as the liver. Brown (2002) found no accumulation in the liver or other organs. Any activity seen in the liver may be due to scatter from the lung and can also include overlap of the lung parenchyma in the liver region.

To measure particles in Technegas aerosol, a large number of particles have to be **"measured"** not **calculated** and placed into size-range intervals to produce a size distribution. A size histogram may be used where the height of each bar represents the number of particles, n_1, in each size (diameter) range, r_1. Aerosol particle size distributions are regularly skewed and cover a large range of sizes and so a geometrical progression of size intervals is often used. If there is a limit of a small size interval, a continuous size distribution function, n(r), is acquired. If a log scale is used, the ordinate scale is transformed to represent the number of particles in each log size interval (dn/dlogr). Therefore particle size distribution can be measured by using the average size and spread of sizes. If a size distribution is normal, then it can be completely described by the mean and standard deviation (Gosling, 1998).

Technegas aerosol distributions are almost certainly skewed; having a longer tail at the larger particle size and so using a log distribution will result in a reasonable fit

(see Poisson Distribution in figure 5.2). Fitting these functions to size distributions provides a suitable means to summarise the distribution. In normal distributions, particles lie within 95% within 2 standard deviations of the mean. In normal distributions, the aerosol approaches monodispersity as i approaches 0, however log–normal distribution of a monodisperse aerosol is defined as: $i\, l_g = 1$.

When investigating a radio – aerosol activity size distribution, a(r) is significant. This is derived as the integral divided by the known size interval, giving the fraction of the total activity contained within the size range. Size distributions are defined as da/dlogr (r), giving the fractional activity per unit log size interval as a function of size or diameter (Gosling, 1998). The activity median diameter separates the activity size distribution into halves; half being contained within the larger particles and the other half within the smaller particles. If the relationship between Technegas and the particle size is known, the distribution can be measured from the number size distribution.

Sites of deposition of inhaled Technegas are important to study for diagnostic purposes. Technegas particle deposition on airway surfaces occurs by inertial impaction, gravitational sedimentation and Brownian diffusion. For effective delivery of Technegas to the lungs, detailed knowledge of Technegas deposition is necessary within the lung. Deposition measurement of Technegas is complex, and assumptions are made that the proportion deposited in the alveoli is distinguishable from that deposited in the airways, as this is a slow cleared alveolar or bronchial compartment.

Technegas particles with sizes between 0.05 - 2 μm have a somewhat low bronchial deposition and reasonably higher alveolar deposition, making this level suitable for lung scintigraphy. Deposition of smaller particles (1 - 2 μm) will be due to the effect of impaction and susceptible to airway diameter and the velocity of the patient's

Technegas inhalation technique. Particles of 0.1 μm or less will be deposited by the process of diffusion and are therefore far less sensitive to alteration.

Gosling (2002) suggests that a patient's changing inhalation pattern of Technegas and image differences seen with a wide variety of disease patterns, mathematical models have the advantage of being able to see differing figures for the deposition of particles at each stage of division of the bronchial tree.

5.8.7 Variations in deposition with particle size

A semi-empirical model of deposition in the respiratory tract as presented by Stahlhofen et al. in 1989 is based on a wide ranging survey of experimental data and predictions from theoretical models. The model yields analytical expressions for deposition in different functional regions. This allows extrapolation of experimental data to particles of any size and density and any breathing pattern. We have used this model to predict the variation in deposition with particle size, assuming an inhalation pattern typical for the conventional breathing method (a slow deep inspiration from functional residual capacity with a breath hold; inhaled volume 2.5 litres, inhalation rate 400 cc/sec with a 5 second breath hold).

Lloyd (1994) explains the modelled deposition in the alveolar, bronchial and extrathoracic regions as a function of particle size (assuming spherical particles of unit density). It can be seen that particles with sizes between approximately 0.05-2μm have a relatively low bronchial deposition and a reasonably high alveolar deposition. This range should therefore be suitable for ventilation imaging, and outside this range, unacceptable bronchial deposition may occur. The model by Stahlhofen (1989) was derived from studies in normal subjects as it is important to

consider the effect of airways narrowing in disease. Deposition for particles in the 1-2μm range will be largely due to impaction and therefore site deposition is likely to be sensitive to changes both in the airway diameter and in inhalation velocity. Particles of 0.1μm and less will deposit mainly by diffusion and should be less sensitive to changes.

5.8.9 Formation of Technegas (Senden & Burch)

Since Technegas invention in 1986, there have been several papers written on investigations of this agent, studying particle size and composition of Technegas. The study conducted by Senden and Burch et al. in 1996 investigated the formation of Technegas and is based on the chemistry and structure of the particle.

Method: 0.1ml of solution that ranged in concentration from 10^{-2} to 5 x 10^{-6} mol/ L^{-1} was added to the crucible. Aerosol was collected by electrostatic precipitation on a copper EM grid supporting holey carbon films immediately post generation. Residue was also scraped off the surface of the crucible and the entire gray residue within the crucible removed.

This powder was transferred as a suspension under ethanol to reduce any dispersal of the powder occurring. The suspension was then transferred to a quartz plate and moistened with five (5%) percent ethylene glycol in ethanol to act as a binder. Both aerosol and powder from the residue left in and outside the crucible were examined, using different techniques such as diffraction studies as well as SEM, EM and energy–dispersive x-ray analysis EDXA.

Results: Aerosol results from the investigators' experiments showed hexagonal platelets. EM showing particles larger than a few hundred nanometers were rare and

most appeared to be less than one hundred nanometers (100 nm) as this experiment used higher crucible loadings than in a clinical situation. In this case the distribution was around thirty to sixty nanometers (30 - 60nm) in diameter. This size also corresponds to a study by Lem and Oei in 1993. Electron diffraction proved the Technegas particle consists of a native metal. Crucible residue under EM showed half micron sized technetium particles distributed over the interior of the crucible with some concentration around the rim of the crucible. The particles were spherical which included a technetium-rich centre surrounded by carbon. The carbon is thin enough for the electrons to penetrate and scatter from the metal centre.

Conclusion: Senden and Burch's research showed that the formation and stabilization of the active particle in Technegas is dependent on: a chemical reductant, a volatile substrate and component, and surface passivation of the resultant aerosol. The reduction of carbon occurs at the crucible, temperatures above the melting point for technetium - 2250°C, cause the vapour pressure in the metal to increase. Once in the vapour phase, technetium will condense and collect in the chamber as a metallic aerosol. It is at this stage that technetium particles form the hexagonal platelets.

The effectiveness of carbon to passivate the available metal surfaces at high crucible loadings indicates that there is carbon vapour present to coat the smaller quantities of technetium present in clinical loads. Carbon film thickness is on average a few nanometers, seen on EM images; the carbon therefore appears to form a coherent layer around the whole metal surface. The carbon film also protects the condensed technetium metal from reoxidation.

Some researchers have thought that Technegas contained radio-labelled fullerenes or "bucky balls", such as C_{60} and C_{70}. Research by Senden and Burch (1997) has

shown this not to be the case, as technetium aerosol can be accounted for as a hexagonal platelet. The comparative volumes of a fullerene with an average platelet would mean that a fullerene species would have to be the active Technegas species and it would have to exist in populations around a million times the number of observed platelets. Secondly, according to Senden and Burch (1997), the conditions are far from optimal for the efficient production of fullerenes. Particle size, if spherical in shape, can be defined by its diameter or radius. Spherical particles are usually found in aerosols, whereas solid particles have different shapes as plate shaped. Agglomerations or clusters of Technegas particles make different shapes, which can be very complex where differences in size of the particles are seen. If particles have a well-defined shape we can then determine surface area and volume, and if density is known, particle mass can then be defined.

5.9 History of Microscopy

In ancient Roman times, people used magnifying glasses to observe an image. It was not until 1590, that two magnifying glasses were used in succession to enable better visualization of an object. From the 1590s until the 1930s, a microscope was described as an apparatus in which light was focused by using curved glass surfaces or mirrors to form enlarged images (Slayter & Slayter, 1992).

De Brogli, Schrodinger, Ruska, Avagadro and Rutherford made a dynamic impact on science and pre-empted the introduction of Electron Microscopy (EM) in the 20th century. EM has increased in popularity from the 1950s onwards as specimens could

be resolved in the order of atomic dimensions. Only in recent times has its popularity declined due to the cost of using EM.

Ernst Ruska designed the first electron microscope in 1933 and studied the flow of electrons in a sealed vacuum. Ruska proved that electrons would flow in a straight path if a sealed vacuum was maintained to stop electrons moving around. Electrons were projected onto a screen, below which the image was formed so that specimens could be examined.

Later in 1941, American engineers advanced the microscopic magnification ten thousand (10,000) times. EM images produced detailed structures of the specimen by using a short wavelength of electron beams measuring approximately 5 nm to increase the resolving power, as is practiced today in the 21st century (Saxton,1978).

In 1924 DeBroglis theory demonstrated material particles in the form of waves, and today we use DeBroglis theory for the electron beam in terms of ray paths. Later, Schrodinger's Cartesian coordinates (x, y, z) were seen to measure electrons moving in a linear momentum but Schrodinger neglected to mention the existence of spin or the angular momentum of electrons at the time of his research (Slayter & Slayter,1992).

Rutherford's "Scatter Law" was calculated by the fraction of incident electrons scattered through a thin film of thickness, enabling us to determine Avagadros number, which is the number of atoms per cubic centimetre exposed to the electron beam. It is clear that to produce EM, we must have high velocity electrons passing through a thin specimen, enough to transmit at least 50% of the incident electrons. The emerging electrons are refracted by a series of electromagnets, forming a two-dimensional image of the specimen. The resolving property is an instrument property,

specifying the smallest point a microscope can resolve. The related quantity resolution is the amount of detail in the image of the specimen and requires a satisfactory resolving power, which is dependent on contrasting a combination of properties of both the microscope and the specimen (Slayter & Slayter,1992).

5.10 Physiology of the Lung

To gain a better understanding of the physiology taking place in the lungs, we will study both upper and lower respiratory tracts and the dynamics involved.

5.10.1 Upper Respiratory Tract (URT)

As mentioned in earlier chapters, respiratory gas exchange occurs in the lungs, which are always in a state of partial inflation, achieved by the rigidity of the chest wall. Negative pressure occurs between the outermost surface and inner wall of the thorax. Visceral fluids coat the two surfaces, the viscera and pleura, thus achieving movement of the lungs (Barnes & Stockley, 1994).

The main purpose of the respiratory passages is to deliver inspired air to the gas exchanging or respiratory area of the lung. Exchange of gases between air and blood commences in the lung acinus which is made up of bronchioles and alveolar ducts (Ramzi et al, 1994). The trachea, as discussed, penetrates each lung leading onto the mainstream bronchus which branch by “dichotomy”, progressively reducing their dimension.

Airways work to a structural hierarchy directly related to ventilation and gas exchange. The acinus is the complex of all airways lying distal to the terminal

bronchiole and served by a first order respiratory bronchiole. In gas exchange, this is the largest unit. Branching of airways commences with a parent branch, which in turn gives rise to two smaller daughter branches. These branches are known as functional zones, which are the number of branches which progressively by generation travel down the inverted tree. In all, there are 23 levels, the trachea being zero, bronchi – bronchioles – terminal bronchioles consist of 16 levels. The respiratory bronchioles – alveolar ducts – alveolar sacs are located at levels 17 to 23. The number of branches used is always twice the number seen in the parent generation, a consequence of dichotomy (Barnes & Stockley, 1994).

The pseudo – stratified ciliated columnar epithelium lining the nasal passages is also known as respiratory-type epithelium. The nasal vestibule contains keratinizing stratified squamous epithelium. Squamous epithelium dominates in the oropharynx, vocal cords and anterior margins of the epiglottis.

Mucous functions mainly to warm and humidify air, and acts as an immunologic defence and a ciliary cleansing mechanism against infective and inert particles. Lastly, via the olfactory epithelium, mucosa provides a sense of smell.

The conchae or mucoperiosteum are contiguous with underlying bone. The respiratory mucosa is modified so the lamina propria or subepithelial tissues are filled with fibrous and elastic tissue mixed with seromucous glands and dilatable vascular sinuses that contribute to the warming of air. Mucous is made up of viscous fluid containing glycoproteins which line the respiratory tract to the distal bronchus. Cilia moves mucous in the direction of the pharynx, where it is swallowed or expectorated. The serous component contains immunoglobulins, lysosomes and enzymes directed against bacteria. Defence mechanisms of the URT are lymphoid tissue seen as

single cells of focal collections containing lymphoid follicles (Barnes & Stockley, 1994).

The larynx is cartilage, its upper end being the epiglottis. Within the lumen of the larynx, the vocal cords are situated, also known as folds, covered by stratified squamous epithelium also seen on the lingual and laryngeal surfaces of the epiglottis. All the surfaces are of pseudostratified ciliated columnar epithelium. Elastic cartilage forms the basis of the epiglottis and provides elastic recoil of the organ post-swallowing.

Hyaline cartilage of the trachea assists in keeping the airways open. Posterior ends consist of smooth muscle, which complete the encirclement of the lumen (Barnes & Stockley, 1994).

During forced respiration, the fibroelastic tissues located between the cartilage and smooth muscle allow tracheal movement in both diameter and length.

The major feature of the tracheal mucosa or respiratory epithelium is the ciliated columnar cells and mucous secreting goblet cells. Brush cells with apical micro-villi, and roundish brush cells represent stem cells or cells containing submucosa with elastic fibres. The respiratory epithelium of the trachea does not participate in gas exchange but does coat the surface with a viscous film produced by the goblet cells and excreted by ducts emptying the submucous glands. This fluid contains mucins, immunoglobulins, lysozymes and antiproteases, which disable bacterial functions. All other inhaled particles are moved via ciliary action towards the pharynx. Where the trachea bifurcates the primary bronchi are formed, and their structure is similar to the trachea (Barnes & Stockley, 1994).

5.10.2 Lower Respiratory Tract (LRT)

The lower respiratory tract (LRT) causes the gradual reduction in diameter and multiple branching of the bronchi and bronchioles. Both have clusters of alveoli, and depending on location, there may be between 8 and 25 generations of branching within the bronchial tree. A healthy pair of lungs has a combined volume of approximately 2.5 litres at rest of which 6 litres accounts for the total amount expandable with maximum inspiration. Lung histology shows a sponge-like structure, of which all the blood vessels and bronchial tree amount to 10% of the total lung volume. The remainder is dedicated to respiratory function (Barnes & Stockley, 1994).

Lung histology commences in the bronchus and terminates at the alveolus. The bronchi contain cartilage while bronchioles lack cartilage, instead comprising smooth muscle walls. Blood vessel inner lining is squamous endothelium and respiratory bronchioles have out-pockets of alveoli. Alveoli are clusters of sacs arranged along the respiratory bronchioles (Barnes & Stockley, 1994, Davies & Moores, 2003). Squamous cells line the alveoli commencing with epithelial lining of the bronchial tree following simplification and reduction in height.

The bronchus is made up of respiratory mucosa and islands or cusps of hyaline cartilage, similar to the trachea. Elastic fibres are found in the lamina propria and submucosa; seromucous glands empty secretions into the bronchial epithelium via collecting ducts. On the epithelium these secretions mix with mucin secreted from the epithelium goblet cells. The mucoid layer produced contains immunoglobulins and antibacterial substances. Mucosa is kept wet and traps particulate matter. Lymphoid cells occur in the lamina propria and submucosa. Oxygen molecules reach

the alveoli by convection or mass air-flow and molecular diffusion within the air phase (Barnes & Stockley, 1994, Davies & Moores, 2003).

The bronchioles are the final conducting portions of the airways and lie between the larger airways containing submucosa, cartilage and alveoli, where gas exchange occurs. Bronchioles are usually less than one millimetre in diameter and lack cartilage and submucosal glands. Rather incomplete bundles of smooth muscle form a circle around the mucosa. The epithelial lining is simply columnar cells becoming cuboidal as the bronchioles decrease in size; ciliated cells remain and goblet cells disappear (Barnes & Stockley, 1994, Davies & Moores, 2003).

Clara cells begin as non-ciliated elements. Clara cells secrete proteins and thus reduce the stickiness of the mucus produced by the large diameter airways to produce lysozyme and immunoglobulins. As bronchioles are very small, with lack of cartilaginous support, blockage and closure can occur individually. This could be due to hypersecretion of mucous observed in chronic bronchitis, hyperplasia and contraction of smooth muscle seen in congestive airways limitation (CAL) and asthma, which can reduce airflow, in some instances becoming fatal.

The main unit of lung function is the acini, which includes all components capable of facilitating gas exchange namely respiratory bronchioles, alveolar ducts and alveoli. Transitional airways are respiratory bronchioles as they conduct air and participate in an exchange being “alveolar like”. They are able to expand and contract as they are lined by cuboidal epithelium containing clara cells. Alveoli are distributed along their length forming the alveolar duct, structures which are tube-like branching and terminating into one or more sacs lined by alveoli, with small amounts of smooth muscle present in some walls of the ducts.

Bronchioles are not easily occluded as they possess a small lumen and have spasmodic contractions of smooth muscle in the walls of the ducts. Paraffin sections show alveoli to be honeycomb arrangements of empty spaces bordered by the thin walls forming open sacs or closed polygons (Koehler, 1973). The pores of Kohn, which are small openings of 5 -10 μm in diameter, provide a potential route for communication between alveoli. Alveoli air pressure is equivalent to atmospheric pressure, so that the only way more air can fill the alveoli, is for the whole lung to expand in volume, thereby decreasing pressure to allow more air to be inspired. Within the adjacent alveolar walls are capillaries of the pulmonary circulation supported by collagen and elastic fibres also containing lymphoid cells and macrophages. There are 500 million alveoli with a surface area estimated to be 80-140 metres squared depending on body size (Barnes & Stockley, 1994, Davies & Moores, 2003).

Diffusion of gases occurs through capillaries, which have a total length of 1600 kilometres or 1000 miles and contain only 100 millilitres of blood. It can be seen how thinly this volume would be spread over the total alveolar surface allowing for rapid gas exchange.

The histology of alveolar epithelium has various cell types:

type 1 alveolar cells which are squamous epithelium and cover most of the alveolar wall

type 2 alveolar cells which are cuboid and account for less than ten percent of the alveolar surface area and macrophages.

The pleura are serous membranes covering the surface of the lungs except the hilum and the inner thorax. The surface of the lungs supports squamous or cuboidal

mesothelial cells, deep within which is supporting tissue with elastic fibres and blood vessels. The watery fluid enables the two membranes to slide over one another but resists separation. The visceral pleura contribute to the elastic recoil of the lungs. (Barnes & Stockley, 1994, Davies & Moores, 2003).

5.11 Materials and Methods

In this section we will firstly review the broncoscopy procedures and outcomes. We also need to measure the number of Technegas particles inhaled. In doing so, we used several methods, including discarded and potential methods until we ultimately found a mathematical method of describing the number of particles inhaled at ventilation. Thus this section reviews:

- What bronchoscopy entails
- bronchoscopy set-up
- broncoscopy procedure
- post bronchoscopy procedure
- discarded methods
- potential methods
- potential outcomes

5.11.1. What bronchoscopy entails

A bronchoscopy (see diagram in figure 5.2 below) is an examination where doctors pass a thin, flexible tube, called a bronchoscope, down your throat and into the main bronchi (airways) of the lungs. With the bronchoscope your doctors are able to do the following:

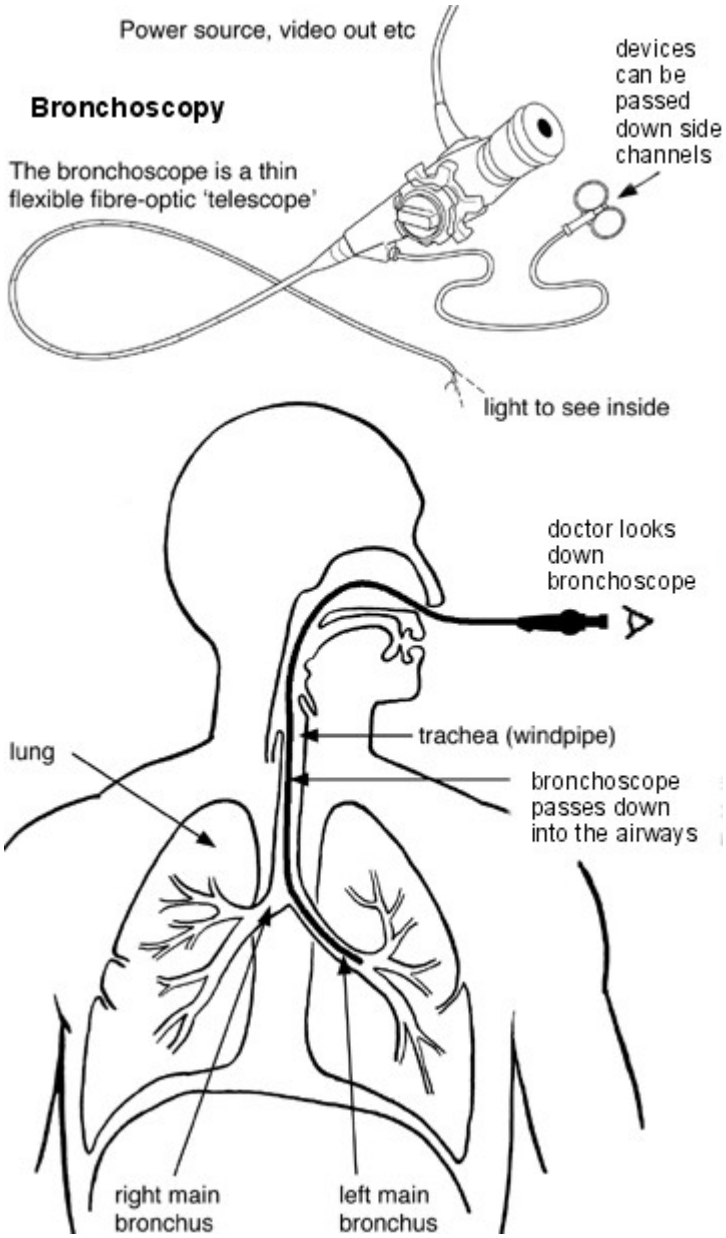
- look at the lining of the air passages leading into the lungs
- take photographs of what they see

- take 'washings' (lavages) of the surface of any suspicious looking area so that the cells can be looked at under the microscope (this is called a cytological test)
- take a small piece of tissue from any suspicious area so that it can be examined under the microscope (this is called a biopsy).

Lung cancers most often arise from the surface of the lining of the bronchi and so a bronchoscopy often confirms the diagnosis of lung cancer. Sometimes if a growth is deep inside the lung, then a bronchoscopy may fail to detect it.

Bronchopscopy

Figure 5.2 **Depicting the bronchoscopy procedure and location of bronchoscope**



Source: Patient UK EMIS Feb.2006

The tip of the bronchoscope will be inserted into a nostril and then gently guided round the back of your throat into your trachea. The bronchoscope can also be passed via the mouth rather than via the nose if you have narrow nasal passages.

The bronchoscope inspects the lining of the trachea and main bronchi and bronchoscopes transmit pictures through a camera attachment onto a TV monitor for real time viewing during the procedure to aid in diagnosis.

Today bronchoscopy is widely considered a safe and invaluable diagnostic tool and should be utilized by more physicians for a definitive diagnosis and subsequent treatment for their patients. In 1967 fibre-optic bronchoscopy was introduced for clinical use. Prior to this time bronchoscopes were rigid and required a full surgical procedure including the use of a general anaesthetic. With the introduction of the fibre-optic scope, bronchial pathology was visualized and sampled peripherally.

5.11.2 Bronchoscopy set-up

Prior to the patients' bronchoscopies, a lung ventilation study was acquired in the Nuclear Medicine Department, Wollongong Hospital Sydney, Australia. The lung study only involved exposure to a very small amount of radiation, estimated to be 1.5 mSv. Naturally occurring background radiation is 2-3 mSv, and so the radiation risk to these patients was minimal and also to the staff in the Bronchoscopy Unit.

The bronchoscopies were carried out at Wollongong Hospital and the lung washings / lavages were prepared in the pathology department and made ready for transportation to the EM department at St. George Hospital, Sydney.

I was given extensive training in Cytology and Histopathology over a period of several months which included preparation techniques for lung washings and sputum preparation.

All the lung study samples were bronchial washings; trans-bronchial samples would only be taken if required by the specialist. Trans-bronchial specimens are preferred

for this study as ideally, the closer a sample is taken to the terminal bronchi, the more alveoli sampling would be obtained and the better the study results would be. However, due to the specialist's decision regarding our patient's risk of haemorrhage, all our samples were taken from the left lower lobe of the lung. This would ultimately limit our findings.

5.11.3 Bronchoscopy procedure

For our research patients, the bronchoscope was inserted via the mouth with nasal prongs delivering a steady stream of two to three litres of oxygen to prevent hypoxia occurring during the procedure. All bronchoscopes were in the day theatres under sterile operating conditions.

In a routine bronchoscopy, respiratory specialists are able to choose from two sampling techniques: Lavage and /or biopsy, depending on the provisional diagnosis of the patient. The sampling techniques used in this present study would be the routine procedure as chosen by the respiratory specialist for each patient.

Prior to the bronchoscope being inserted, Xylocaine 1% an anaesthetizing spray was introduced into the back of the mouth and tongue for local anaesthesia. At this point a cannula was inserted ready for "light" anaesthesia. A short acting benzodiazepine, Medazolam was used to suppress anxiety. If coughing was a problem, an opiate was used to suppress the cough and minimize anxiety. The patients were positioned supine with the specialist proximal to the patient's head. Laying them supine means the patient is less likely to experience vaso-vagal attacks, as blood will not have time to "pool" in the abdomen, causing a vaso–vagal reaction.

While the bronchoscope is inserted, 20 mls of lignocaine 2% mixed with normal saline are introduced via a side channel. The amount of lignocaine administered depended on the amount of patient discomfort while the bronchoscope is being inserted. As the tube is positioned in the bronchi, the worst part of the test is over. Examination usually takes approximately fifteen to twenty minutes.

During the bronchoscopy, the pathway taken allows direct visualization of the trachea, bronchi and as far as possible into the lung. For all our research volunteers this was the left lower lobe of the lung. This is possible as the bronchoscope is a long flexible tube, with a light at the distal end allowing direct viewing of the respiratory system. All samples taken were lavage washings from the left lower lobe of the lung. The specialist had made the decision not to take lung biopsies from these patients due to the increased risk of haemorrhage.

5.11.4 Post bronchoscopy procedure

As soon as the patient was “awake” in the post-op ward, I asked them to take a deep cough and to “expectorate” into a sterile jar which was counted on a gamma camera to measure the amount of activity left in the lungs post bronchoscopy. Technegas had been inhaled, so there would be activity left within the lung post bronchoscopy.

The remaining fraction of administered activity should be uniformly distributed within the lungs, where it is thought to remain for an extensive period of time. Lloyd et al (1995) had studied radiation to the lungs post ventilation, looking mainly at biochemical cellular reactions to Technegas and pertechnetate. After measuring the activity left in the sputum post bronchoscopy these specimens were taken with the lung washings for EM preparation.

The patient is not allowed to eat or drink for two hours until the swallowing reflex has returned to normal. Patients usually remain in the day-only ward until their swallowing reflex returns to normal, approximately three hours post bronchoscopy.

5.11.5 Discarded Methods

When trying to estimate the number of Technegas particles inhaled into the lungs, a number of possible methods were considered but discarded at the outset of our study. We thought the ideas, however, should be included in our paper although considered not viable or valid to our research. These methods included:

1 Trying to calculate the net activity produced in a Technegas chamber measuring activity pre and post burn, after decay correcting.
2 Calculating the number of multi-carbon molecules produced.
3 Finding out the labelling efficiency of the Technegas particle.
4 Calculating the total activity in the lungs by drawing a region of interest or ROI and attenuate and decay correct to time of burn.
5 Calculating the number of radioactive Technegas particles in the lung.

As Technegas cannot be calculated only measured, this rules out the above methods. The reason for only measuring relates to: the activity in an aliquot of pertechnetate – 99m, which does not reflect the total amount of technetium in the solution, also remembering that the particles formed from technetium particles are in the gas phase.

According to Lemb and Oei (1993), you cannot measure the loss of carbon from a crucible, as it is too unreliable. Measuring the output of Technegas has proven to be

too difficult, as one would need to invest “x” amount of dollars in equipment, and it still would not be easy to run this experiment.

5.11.6 Potential Methods

There is a concept that may work for this experiment on finding the number of particles inhaled, bearing in mind that the distribution of particles is quite wide and that the activity size distribution is log-normal with median 160 nm and Graded Standard Deviation (GSD) is ± 1.5. The next step would be to adjust the vertical scale of the distribution so that the integral equals the activity in the patient.

Assumptions may be made about the relationship between particle size and activity and one would have to assume a spherical particle with activity proportional to the mass. From this number we could derive size distribution.

The calculation for spherical particles would be: A (r) = k pi /6 r*r*r n (r)

Where A (r) is the activity distribution

K = the activity per unit mass

n (r) = the number distribution.

The work that follows provides a potential method for the estimation of the number of Technegas particles from knowledge of total activity.

Let X denote particle diameter. Lloyd (1994) established that a reasonable model is provided by the log-normal distribution with probability density function given by:

$$f(x) = \frac{\exp(-(\ln x - \eta)^2/(2\times\sigma^2))}{1.5x\sqrt{2\pi}} \quad x \geq 0$$

and parameters median $\eta = 160\text{nm}$ and geometric standard deviation $\sigma = 1.5\text{nm}$.

This establishes the distribution of particle diameters. In particular, the proportion of particles in the size range x to x + dx, where dx is small, is given approximately by f(x)dx. If there are N particles in total, we would thus expect Nf(x)dx to have diameters in range x to x+dx. On the assumptions that particles are spherical, with activity proportional to mass, we have, for a particle of diameter x:

$$\text{Activity } A(x) = k\rho \times \text{ParticleVolume} = k\rho \times \frac{4}{3}\pi x^3$$

where k is Activity per Unit Mass and ρ is particle density.

Given that there are N particles, Lloyd (pers. comm.) states that Total Activity will be given by :

$$\int_0^\infty A(x)\times Nf(x)dx = Nk\rho \int_0^\infty (\text{Particle Volume})\times f(x)dx$$

Each particle of diameter x produces activity A(x). Since dx is small, we can reasonably say that all the particles in the size range x to x + dx will produce activity

A(x) approximately. Since there are Nf(x)dx of them approximately, the total activity from particles in the size range x to x + dx will be A(x)xNf(x)dx. We must then total across the size range, i.e. from:

x = 0 to x = ∞, and mathematically this is achieved by integration. The integration could not be performed analytically so a standard technique from elementary numerical analysis, the trapezoidal rule, was used.

The integral on the right hand side was evaluated using the trapezoidal rule for numerical integration to be 4494210 (Henderson, 2006). Thus, we have the relationship:

$$\text{Total Activity} = 4494210 N k \rho$$

$$\Rightarrow N = \frac{\text{Total Activity}}{4494210 \times k \times \rho}$$

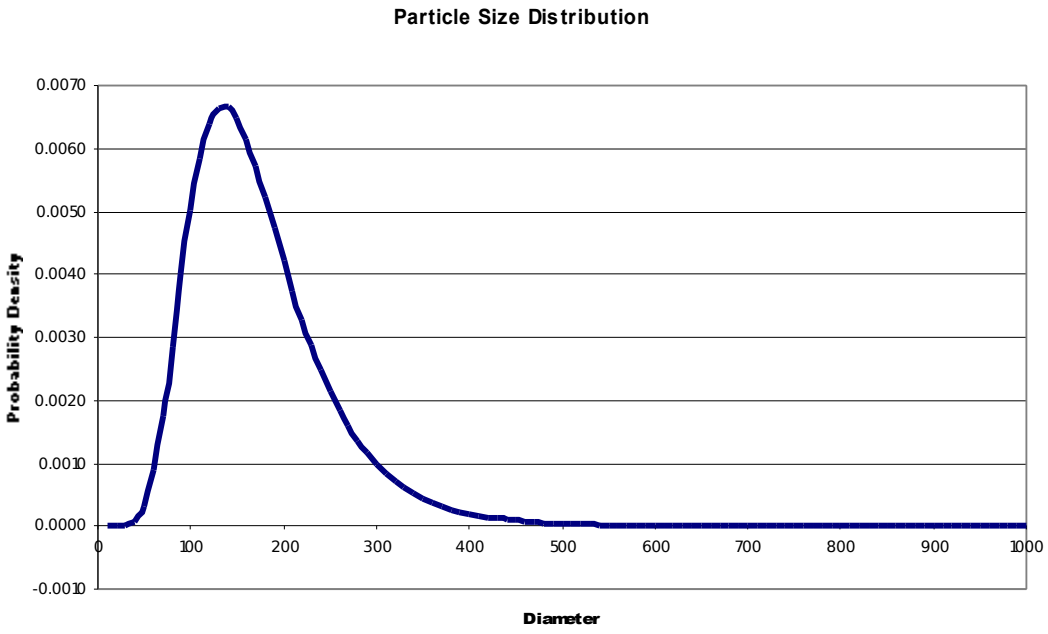
The density value is known. Thus knowledge of an estimate of the constant k, i.e. of the Activity per Unit Mass, would permit an estimate of N to be calculated.

By measuring the activity of Technegas we can estimate:

1. the amount of activity inhaled into the lungs.
2. how much residual activity is left in the crucible.

Primary particles that make up the structure of Technegas range in size from 5 – 30 nm. Senden & Burch (1996) observed the hexagonal platelets of metallic technetium wrapped within a carbon capsule to be within a range of 30-60nm. Prior to ventilation, these technetium 99m particles coagulate into aggregates with a median diameter of 100 to 160 nm. Depending on the time of Technegas generation and ventilation, the longer the time, the larger the particles; these particles can be as large as 225nm measured at 8.5-minute generation. Technegas particle size and the amount of deposition in the lung will determine the clearance rate of technetium.

Figure 5.3 Poisson Distribution



A Poisson distribution is used to describe the number of events per unit of a continuum. In our experiment, it is common for aerosol distributions to be skewed (having a long tail at the larger particle sizes) and a log normal distribution is often a reasonable fit.

5.11.7 Potential outcomes

The information obtained from lung washings/lavage of the main bronchioles was used to observe the structure, distribution, and the mechanism of clearance from the lung.

Initial investigations included:

- The migration of Technegas
- The alignment of Technegas within lung tissue
- Distinguishing between Technegas particles and lysosomes under EM as both cellular structures are electron dense.
- Cellular damage

The bronchial lung lavage/washings were brought to the pathology department. Peracchia and Mittler (1972) introduced the idea of fixing tissue for EM using a gradually increasing pH value. For our research we used a Cocodylate Buffer system for our fixation process. Care had to be taken that Cocodylate did not react with the fixative as this reduces the buffering power and fixation ability.

Washing in buffer served several purposes, the main one being to remove any excess primary fixative prior to post fixation. Cocodylate was also used for our freeze storage at 4 °C of our specimens before being transported to another hospital for final EM preparations.

5.12 Method for examining the specimens

The following items examine the different stages of preparation needed for EM investigation of samples:

- Transportation of prepared specimens for EM
- Fixation procedures
- Initial preparation steps for EM
- Transitional Fluids

- Final preparation steps for EM
- Microtomes
- Results of EM

5.12.1 Transportation of prepared specimens for EM

The specimens were transported from Wollongong to the EM unit at St.George Hospital. Unfortunately there are only one or two runs per week from Wollongong Hospital to St. George Hospital, Sydney. There were problems with the handling of the specimens in the EM unit and also with the time taken for preparation of the specimens and reporting. St.George's EM unit had other researchers using its facilities and they kept our specimens for months as other projects were ahead of ours. We waited a total of 18 months for the final specimens that came with no accompanying report to be forwarded to Scotland as I had emigrated overseas during this waiting period.

On arrival of the specimens in Scotland, I was working at the Western General Hospital in Edinburgh. I made arrangements in the first instance to view the specimens in the EM unit, having asked the microscopist if he would oversee my reporting of the findings from Wollongong, which was agreed. On examination of the sent specimens, it was found that most of the EM specimens had not been prepared properly and others were damaged in transit. Thus the only thing to do was to try and salvage what we could from the specimens and re-run the EM from scratch. This meant I had to study EM again and with it enough Histology and Cytology for me to be able to prepare the specimens from scratch and report on them under the supervision of the EM specialist at Western General. The process took another year and many hours of my own time to achieve this goal.

I was trained in Electron Microscopy on the JEOL 100CX11 1983 model to the stage where I could read and report on my samples and my supervisor would check my reporting. This training was invaluable for my research. Within this research I needed to complete smaller experiments in order to answer the questions posed.

5.12.2. Fixation Procedures

“The knowledge of fixation dates back to 4000 BC; Hippocrates knew something of the biological effects of mercury and its salts, as did the Persians of that time.” (Bancroft 1982).

The aim of fixation is to prevent autolysis (a process by which biological cells self-destruct) and bacteria from occurring on the specimen. There also should be no change in the specimen’s shape or volume and the specimen should be left as close to its living status as possible with no small molecules being lost (Koehler,1973). Buffers adjust the hydrogen ion concentration in fixatives as the pH value varies. Fixation occurs between a pH of six and eight.

Cocodylate buffer wash removed excess primary fixative prior to fixation. Glutaraldehyde is used commonly in tissue blocks and can react with osmium tetroxide producing an electron dense precipitate of reduced osmium, which may be deposited onto the tissue specimen.

Our study used 1mm cubes of tissue, which are fixed at four (4°C) for between 4 - 16 hours in 3.1% glutaraldehyde in 0.1M sodium cocodylate buffer. The fixative is carefully decanted and replaced with cold 0.1M sodium cocodylate buffer. The specimens are rinsed for thirty minutes. Post fixation at four (4°C) for two hours in

pre-cooled one percent osmium tetroxide is decanted and the specimen is given a rinse in 0.1M sodium cocodylate buffer.

A dehydration method was used in our specimens to remove all free water from the fixed tissue and replace it with a solution that is miscible with the embedding medium. Most embedding media are not miscible with water so an organic solvent, usually ethanol, can be used. Dehydration has to be done as quickly as permissible, as all these chemicals will extract lipid from tissue. The time must be long enough to make sure all the water is removed from the lung and sputum specimens as incompleteness at this stage will cause incomplete dehydration, resulting in inadequate infiltration and polymerization of the embedding medium.

The main point of using a fixative for cytology is to preserve the necessary cytomorphologic characteristics and the diagnostically necessary cytochemical elements of the cell. According to Keebler, (1997) the appropriate fixative for cytodiagnosis involves:

- Penetrating cells rapidly
- Minimizing cell shrinkage
- Maintaining morphologic integrity
- Inactivating autolytic enzymes
- Replacing cellular water
- Allowing permeability of dyes across cell boundaries
- Permitting cell adhesion to a glass surface
- Matching for the subsequent staining method used
- Being bactericidal
- Being reproducible
- Representing a permanent cellular record.

In former times, Papanicolaou used ether and 95% ethanol (1:1) as the fixative. The most common fixatives are ethanol 95%, isopropanol 80%, methanol 100% and 95% denatured alcohol as well as spray fixatives which have replaced ether and ethanol as the two primary fixatives of choice. When using fixatives, the cytologist will see slightly altered cell appearance and some various artefacts will be present. Cytologists are familiar with these artefacts that occur from using various fixatives.

There are two wet fixation methods according to Keebler (1997):

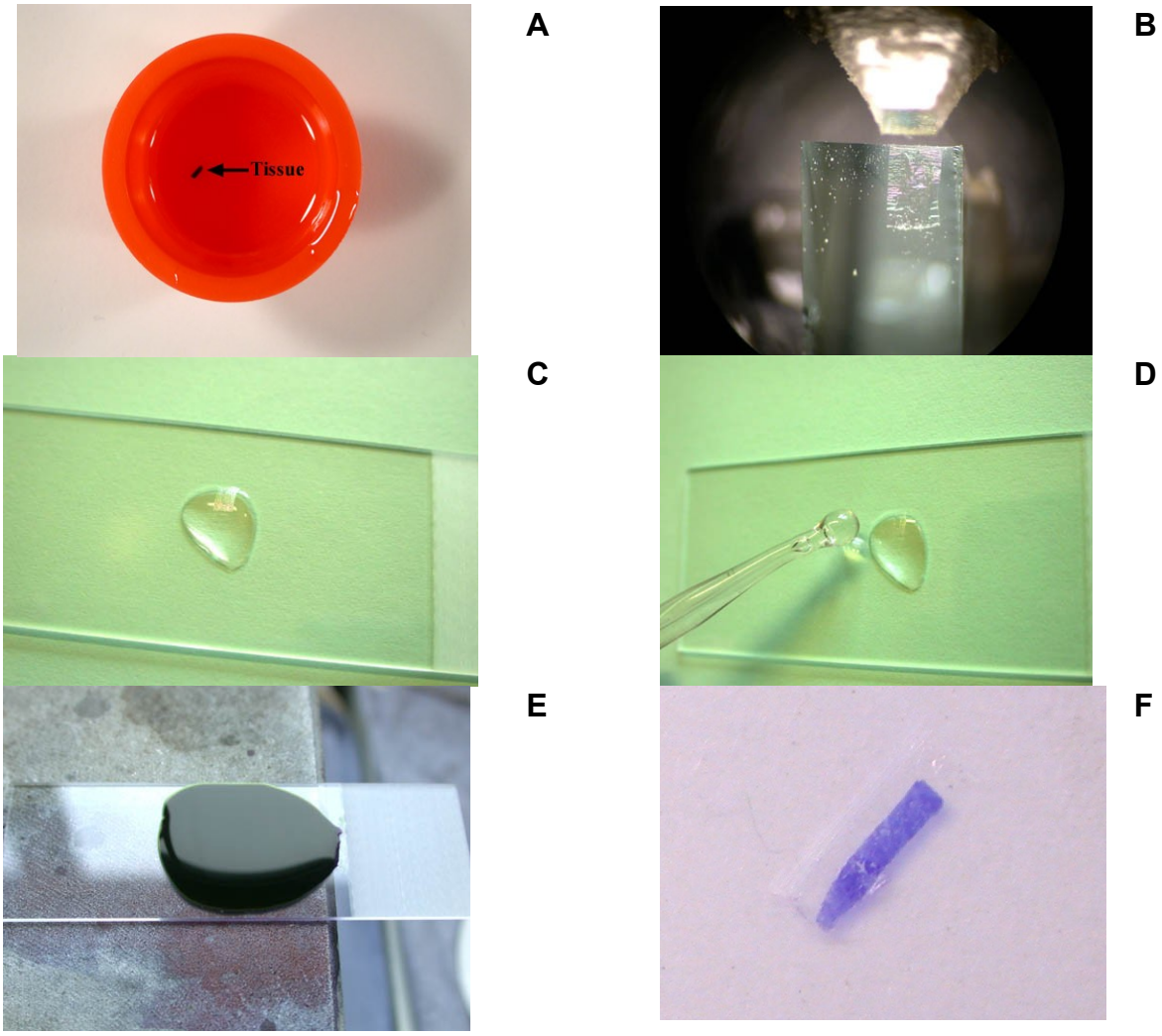
1. **Wet fixation**: this is the instant immersion of the wet cell sample into a fixative solution, these cells are not exposed to air and remain in the fixing solution until they are numbered and stained.
2. **Wet fixation** with air-drying: immediate submersion of the cell samples into a fixative, then after a specified time the samples are removed and air – dried. After air-drying, the samples can be placed into a container for transport to a laboratory. This was also the method chosen for our research.

When the samples arrive at the laboratory, the cell samples are immersed into a Coplin Jar containing 95% ethanol.

Spray Fixation: A spray fixative is used immediately onto the wet cell sample. This is then allowed to air dry, and then placed into a container for transportation to the laboratory. Immediate fixation is the goal no matter what type of fixative used. Fixation occurs while the sample is wet to preserve the cellular fixation as the specimens are easier to fix, stain, cover slip and screen. Cellular adherence to a glass slide will be highly dependant on what part of the anatomy the sample has

originated from. Cells taken from fluid adhere less (bronchial washings) to the slide than sputum (taken post bronchoscopy), which adheres well. Sprays for fixation purposes may be needed to achieve better results than just placing the sample in 95% ethanol. Mayer's albumin may be added to fluid samples prior to centrifugation or onto the glass slide to assist with cellular adherence.

Figure 5.4**: Preparatory steps for EM**



A = Impregnation
B = Semi Thin Section
C = Semi Thin Section Glass Slide
D = Semi Thin Section Xylene Vapour
E = Toluidine Blue
F = Solid Semi Thin Section Toluidine Blue
G = Pyramid

5.12.3 Transitional fluids

Where the dehydration agent and the embedding media are miscible, the penetration of the embedding media is facilitated by the introduction of a transitional fluid. The main transitional fluids available are 1:2 epoxy propane (propylene oxide), xylene and toluene.

Sectioning the lung and sputum tissue is accomplished using a diamond knife, prepared from industrial diamonds, machined and then polished to a very high cutting edge. The edge is cleaned using a cotton bud and running it parallel not at right angles. Collection of debris on the diamond knife will result in a poor sample section.

Section cutting of the specimens for EM was carried out the next day. From the resin block 50-60 nanometre sections were cut using a diamond knife and water bath. Sections that were cut were spread over the surface of a finely perforated copper grid approximately 3mm in diameter. The sections adhered to the grid by surface forces.

5.12.4 Preparation for EM

The method of preparing for EM entails using ultra thin sections as the electron beam can only penetrate matter for a short distance. After embedding the specimen, a suitable mounting medium is required. The specimen is cut with a diamond knife, and once sectioned portions are floated in water, picked up and placed on a wire grid for viewing. The preparation of tissue specimens for EM involves the same basic steps as light microscopy; however, EM uses special fixatives. For my research “cocodylate” buffer and glutaraldehyde are used since the greater resolving power of EM requires finer and more specific cross linking of proteins (Keebler, 1997, Koehler,1973).

Buffered solutions of glutaraldehyde are also used for EM fixation, enabling examination of the specimen within the electron beam; generally tiny sections of samples less than that used for light microscopy are infiltrated in copious amounts of fixative. The tissue blocks used were no longer than 1mm cubed, and epoxy resin was chosen as the embedding medium for this research.

Cytology is based on cell samples prepared in various ways, and although the actual methods of fixation do vary in laboratories, their methods follow basic concepts. The goal is to reduce artificial cell changes while giving an optimal diagnostic cell sample (Keebler, 1997, Koehler,1973), see also figure 5.4: Preparatory steps for EM.

5.12.5 Microtomes

The microtomes used for thin sectioning are designed to strict tolerance. Machines are mostly semi-automatic. The block must advance towards the knife and each successive stroke gives sections of about 50 - 60 nanometres. This is achieved by a mechanical advance machined micrometer screw and lever system.

Glass and diamond knives are provided with a trough, which allows a fluid reservoir to lie behind the cutting edge so that sections when cut will float on the surface. The sections from the bronchial washings and sputum when viewed in refracted light, give the colour of the section as it is cut, which indicates its approximate thickness. Our sections are best seen when they appear silvery gold (Ultramictrotome Section Colour Reference Chart published by Drukker International, Netherlands). Compression during sectioning is removed by exposing the sections to xylene vapour as they lie in the water trough (see EM preparation set-up (figure 5.4) and Microtomes (figure 5.5).

5.12.6 Microtomes

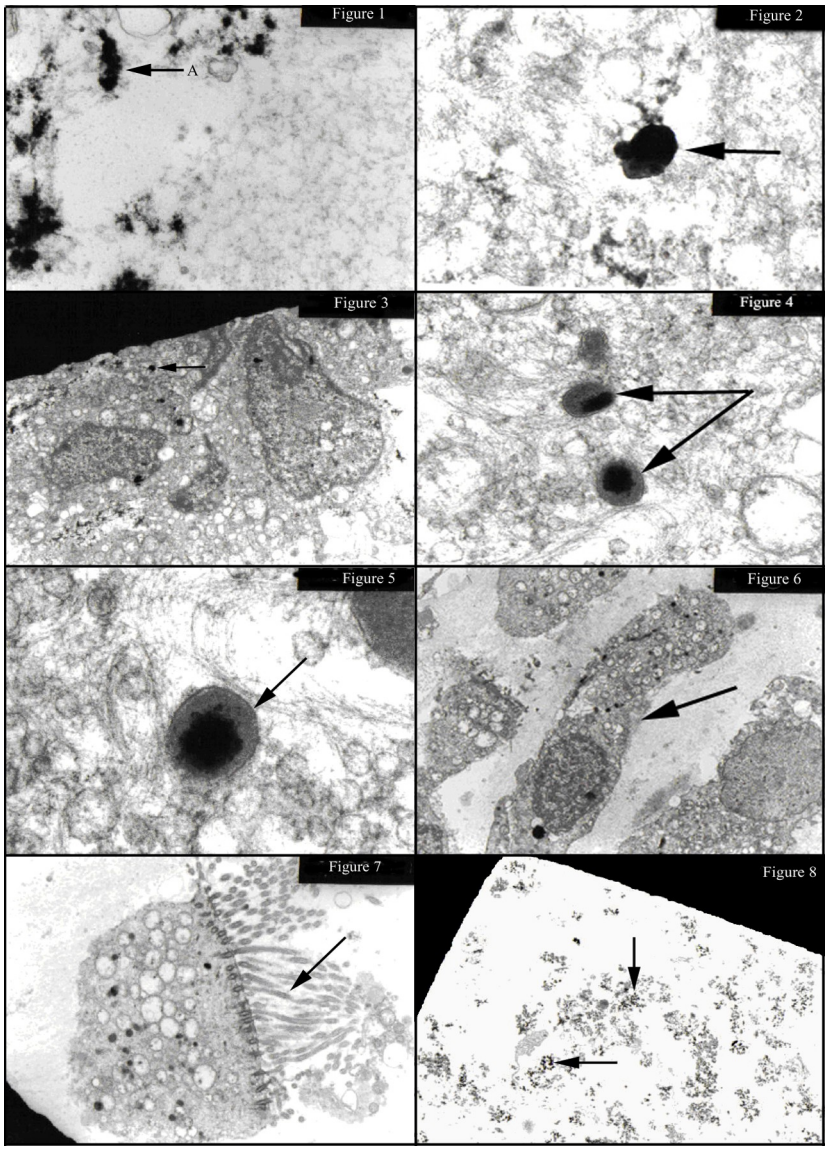


Figure 5.5: **Microtomes**

Image 1: Carbon Particles from Technegas in sputum

Image 2: Technegas Particle

Image 3: Technegas particles & Macrophage

Image 4: Lysosome

Image 5: Single Lysosome

Image 6: Macrophage/Epithelial Cell

Image 7: Epithelial cells & Cilia

Image 8: Carbon particles at low magnification

5.13 Results of Technegas and EM research

Our investigations produced images of the different parts of the cell under electron microscopy. There were some interesting cells visualised on EM that we salvaged from our damaged samples. We decided to list these as part of our research material observed under EM for Technegas at the cellular level (see list below at 5.13.1).

The microanalysis of thin samples from the lung and sputum is limited by both the specimen preparation and the microscopic technique and conventional preparation used for our research provided useful morphological information. However, due to chemical fixation, dehydration and embedding procedures, contamination of the specimens may occur and elements also bound by lipids, proteins etc. may be removed during the preparation phase.

Slight modifications to the fixing procedures can help to retain certain elements; in our research we used glutaraldehyde as a fixative. According to Karnovsky, M.J. (1965), glutaraldehyde solution of 0.1% to 1.0% concentration may be used for system disinfection and as a preservative for long term storage. Glutaraldehyde kills cells quickly by cross-linking their proteins and is usually employed alone or mixed with formaldehyde as the first of two fixative processes to stabilize specimens such as bacteria, plant material, and human cells. Fixation is followed by dehydration of the tissue in ethanol or acetone, followed by embedding in an epoxy resin or acrylic resin. Safety: as a strong disinfectant, glutaraldehyde is toxic and can cause severe eye, nose, throat and lung irritation, along with headaches, drowsiness and dizziness. It is a main source of occupational asthma among health care providers.

To minimize elemental loss, we froze the specimens, which had to be transported from one hospital to another for final preparation and reporting. Apart from the elemental losses during preparation, irradiation by the electron beam can induce further redistribution of the elements within the specimen.

A tissue section for EM should not be any larger than 80 nanometres. Epoxy resin was used as the embedding medium as it polymerizes uniformly with the lung and sputum specimens. By using epoxy resin we found the specimens to be more stable in the electron beam and were seen to have a slow rate of sublimation or transition. Microtomes using Technegas EM slides. In this experiment we observed only one electron dense hexagonal structure of Technegas. It was then decided to add all microscopic images found of interest in this summary below as these images were seen in all 20 subjects under the electron microscope. The total result was disappointing as only one clear specimen was gained. This was due in part to damage of the original specimens both in preparation by St.George EM Unit and the method in which the specimens were transported to Scotland. The results below were those prepared by myself and reported on in Scotland under supervision at the Western General, Edinburgh. Due to the limitations of our experiments, which will be discussed later in further detail, the following structures were observed as add-ons to our studies:

5.13.1 Investigational structures seen on EM

1) Technegas particle
2) Centrioles
3) Eosinophils

4) Epithelial Cells

5) Endoplasmic Reticulum

6) Golgi Complex

7) Lysosomes

8) Mitochondria

9) Nucleus

10) Phagocytosis and Pinocytosis

11) Artefacts

1) Technegas Particle

An electron dense hexagonal particle was observed of metallic technetium which is encapsulated within a layer of graphitic carbon. No particle observed was larger than 100 nm. There was a great deal of technetium scattering throughout the EM grid plates.

2) Centrioles

Stubblefield and Brinkley's 1967 studies of centrioles showed that centrioles and basal bodies are two structures, which are morphologically identical. In our study, centrioles were seen as short cylindrical bodies lying at right angles to each other. Collectively these centrioles comprise the diplosome which lies partly surrounded by the Golgi Complex. The region in which the centrioles lie is known as the centrosome, and the line joining the centrosome to the centre of the nucleus is known as the cell axis.

3) Eosinophils

These were readily distinguished by their large size, a bi-lobed nucleus, and large granules in the cytoplasm adjacent to the nucleus. The cytoplasm was observed to be a grayish colour under EM.

4) Epithelial Cells

Epithelial cells with cilia were seen. The epithelium is found in the upper airway so this is a common cell for us to study. The cilia seen were cut so well that they where worth mentioning. The cells appeared to be in several layers due to their nuclei being situated at different levels throughout. All cells seemed to be in contact with the basement membrane, but the cells were of all different shapes and heights and none reached the “free” surface. The epithelium therefore would not be stratified but pseudo-stratified.

There were some deeply placed nuclei belonging to the short basal cells; the more superficial are those of the columnar ciliated cells. Interspersed goblet cells were also seen. The short cilia were numerous and seen close together. Each cilia arises from a basal body which seemed to be lined up adjacent to each other and at first sight has the appearance of a continuous membrane.

5) Endoplasmic Reticulum

The endoplasmic reticulum (ER) is usually difficult to see when using EM. However, on examining our sectioned specimens, there were quite a few. The endoplasmic reticulum observed consisted of a continuous system of membrane–bound cavities that ramify throughout the cytoplasm. The endoplasmic reticulum also has a loose meshed-like irregular network of branching and anastomosing tubules. Endoplasmic

reticulum appeared to be confined to the main mass of cell cytoplasm. The organelle does often extend close to the cell membrane consisting of a system of inter-connected cavities and passages, not a series of isolated sacs or vesicles.

There are two types of endoplasmic reticulum: the granular or rough endoplasmic reticulum, and the agranular or smooth endoplasmic reticulum, into which category our specimens fall. The rough has a cytoplasmic surface studded with loops, rows or spirals of ribosomes called polyribisomes. The smooth endoplasmic reticulum or granular is not associated with ribosomes.

6) Golgi Complex

In our examination of the bronchial washings and sputum, golgi complexes consisting of flattened sacs, also vacuoles and vesicles were observed (see figure 5.6: Golgi Complex). Golgi are composed of a smooth membrane and have a characteristic organization of their elements. Using EM to examine our specimens, we could see that Golgi are composed of three basic elements, the most characteristic being a stack of flattened sacs, the ends of which are slightly dilated. Vesicles lie on the convex or outer face of the stack and these vesicles arise from “budding” out of the endoplasmic reticulum. Golgi complexes are seen especially well in secretary epithelia, sputum. The functions of the golgi are many and quite varied; golgi are involved with the secretary process, production of nearly every exocrine and endocrine secretion, cartilage and synovial membranes.

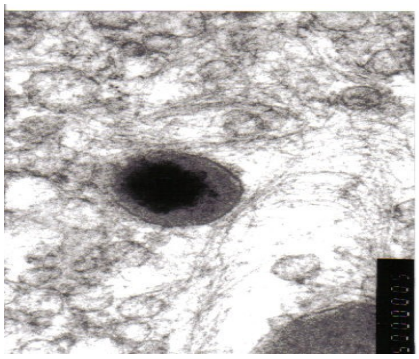


Figure 5.6 **Golgi Complex seen in sputum**

7) Lysosomes

Lysosomes in our specimens had contents that were medium to low electron dense so they could not be unequivocally distinguished by morphological features alone from other vesicular structures in the cell. It was only when increasing the light intensity and magnification that a single membrane could be visualized. In some reporting sessions it was difficult to distinguish if these electron dense structures were in fact hexagonally shaped Technegas particles or not, as a membrane is not obvious.

Lysosomes in general are a group of membrane bound particles containing acid hydrolases, meaning rich in lytic enzymes. The primary lysosome is a vesicle containing many acid hydrolases, while some may contain mitochondria, pigment masses and other dense bodies.

“Myelin bodies” are described as lysosomal bodies by Hruban, (1972). Myelin bodies are produced in tissues fixed with glutaraldehyde especially when fixation lasts for many hours or days. Myelin figures can be found in the cytoplasmic matrix and mitochondria. Myelin bodies are found in alveolar cells referred to as cytosomes or multi-lamellar bodies, which represent the secretary granuoles normally produced by

the cells. Myelinoid bodies are also occasionally seen in cells, which engage in erythrophagocytosis and are pleomorphic, therefore showing diversity in form.

8) Mitochondria

When studying our EM specimens it was clear that the mitochondria had many variations in size, structure and shape and remained the most distinguishable from all other cell organelles in EM. The mitochondria reported were granular, thread-like components of the cytoplasm, also called bioblasts. Some mitochondria had extended diagonally across a field when seen using EM.

The morphological features seen included a double membrane bound body containing matrix, a system of cristae or folds and some granuoles. The mitochondria remain the major source of cellular ATP (adenotriphosphate), known as the "powerhouse of the cell" (Racker, 1968). In 1902 Benda described mitochondria as thread–like or granular components seen in cells. These organelles in living cells are seen to move slowly and change their shape and size. Mitochodria also appear to be able to divide and recombine.

9) Nucleus

In our research of bronchial washings and sputum, light microscope studies of the mitotic nucleus and its chromosomes have shown more than is seen with thin sections used for EM. In fact EM does not elucidate satisfactorily the structure of chromatin, and the variations that may underlie cell differentiation, function and neoplastic transformation.

Using EM, the nuclei seem to show some irregularity due to the thinness of the bronchial washing and sputum sections which were seen as two-dimensional images. Thus EM shows that many nuclei, instead of being smooth and round or oval in shape, may be irregular depending on the type of cells for example macrophages that have invaginations as seen in our specimens.

10) Phagocytosis and Pinocytosis

Phagocytosis was named by Metchinikoff in 1883 to describe the process whereby cells ingest food. Metchinikoff also looks at the concept of microphages (neutrophils) and macrophages and their roles in defence mechanisms.

In our investigations we saw evidence of macrophagic activity within the cells. Bronchial washings and sputum will always have macrophages present within.

Phagocytosis and pinocytosis are derived from the Greek roots for “eating” and “drinking” respectively. The distinction between particulate uptake (eating) and fluid uptake (drinking) relate more to the resolving power of the microscope, be it light or electron microscopy for the final analysis of the particulate (Koehler,1973).

Macrophages were seen, including some macrophages, with carbon particles scattered throughout. As a patient coughs, free carbon particles are dispersed throughout. These carbon particles were viewed using 5k x magnification. Free macrophages were seen and had a rounded appearance, with the cell outline being slightly irregular. Macrophages have various shapes when seen under EM.

The nucleus is rich in chromatin, and also appears to show a slightly acidophilic cytoplasm when viewed under light microscopy.

Alveolar macrophages travel within the cell and exist in the non–ciliated lung regions. The macrophage will ingest foreign material by phagocytosis, after which it will migrate to the ciliated surface where it is removed by the mucociliary clearance mechanism. The half-life for mucociliary clearance by this method is days to months. Some particles that are not engulfed by the macrophage may infiltrate the lymphatic system or interstitial tissue, making clearance a longer process (Brown & Zenman, 2002).

11) Artefacts

As our lung washings and sputum specimens needed to be prepared for EM, artefacts may be easily introduced. An artefact is something artificially produced, and not indicative of the specimen we are examining. It is accepted that artefacts will be introduced during the various preparation stages for EM, however, with good techniques these can be minimized. As such, artefacts can be accepted as being consistent as long as the microscopist can identify them as such. Artefacts that may occur in our research preparation for EM:

a) When preparing pellets for our research made from a suspension, centrifugation may cause isolated cells to undergo distortion and dislocation of sub-cellular components if the lung and sputum specimens are centrifuged prior to fixing.

b) Our bronchial lavage samples may cause stratification of the particles within the pellet. Systemic sampling is taken from the pellet to prevent artefacts occurring, as a pellet has depth and therefore sampling should be taken from top to bottom of the specimen.

c) There are numerous limitations which must be imposed on these thin lung and sputum specimens for microanalysis.

d) Contamination is usually seen when the diameter of the beam is reduced below 100nm.

e) Micro diffraction patterns from inelastic scattering caused by the presence of a thin layer of carbon, which “washes out” the diffraction pattern. This is one of the most sensitive ways of detecting contamination in the microscope (Crang & Komparens,1988).

There are numerous sources of contamination and its effect on analytical sensitivity is dependent on the microscope, specimen and user combination.

Technegas was not visualized in every patient's washing and sputum specimens, although carbon was scattered throughout. This may be for a number of reasons:

- Macrophages "gobbling" up the contents of the cell including Technegas.
- Technegas diffusing across the alveolar membrane and into the blood stream.
- As the specimens were not immediately fixed in the operating theatre, exponential decay is continuing to occur from the time of the lung ventilation study to the time of fixation process, which can be up to half an hour.
- Another consideration is that although Technegas is fixed and stained, it may continue to decay over time.

The only quick method to see Technegas in both specimens may be to view them with a wet preparation technique instantaneously, thus exponential decay is still occurring but the time factor is much less.

5.14 Our Conclusions

We have answered some of our objectives by examining all lung washings from the left lower lobe of the lungs from volunteer subjects. Trans-bronchial specimens were not performed due to the risk of haemorrhage as indicated by the specialist.

A limiting factor of this study was that we were unable to take trans-bronchial specimens from any of our subjects and so we were compromised at the outset. If we had sampled more alveoli, we would have obtained a better study result. Ideally, the closer a sample is taken to the terminal bronchi, the more alveoli sampling would be obtained and the better the results. Some patients haemorrhaged during their bronchoscopy procedure, so red blood cells and some lymphocytes were also seen under EM. The most common particles observed were carbon scattered throughout each specimen as the patient inhales this from the Technegas.

Other limitations were that Technegas did not seem to migrate and remained within the lung. Moreover, some nuclei seemed to be dying due to a delay in immediate fixation.

There was cellular damage seen only from the sectioning methods used by St. George Hospital, Sydney for EM preparation and also damage to the actual specimens during the transportation process from St. George Hospital Sydney to Scotland. Due to this damage, all patients' specimens were prepared again at the Western General Hospital, Edinburgh, Scotland.

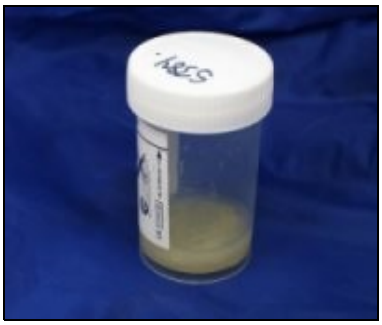
Technegas was seen to be very electron dense as were the lysosomes. At times, a single hexagonal particle of Technegas was difficult to distinguish, although the lysosome has a double membrane surrounding it (see previous images). Artefacts

were also very electron dense but easily distinguished from Technegas as they had round shape walls.

As we did not use autoradiography due to the cost of this study and also suggestions that we may not need to use this method from specialists in microscopy at St. George, in hindsight, statistically trying to find Technegas in each sample was like “finding a needle in a haystack”. For future researchers interested in pursuing these studies, a better and less cost effective way to conduct this research may be to use a fluorescent tag on the technetium under microscopy.

Chapter 6 Experiment 5:

Sputum and Bronchial Washings at the Cellular Level



Sputum Expectorate at Wollongong Hospital, Sydney, Australia

6.1 Introduction

Predominantly expectorate is made up of mucous with cellular and non-cellular material produced by the host, and also contains substances that have been inhaled. Expectorant consists mainly of squamous epithelium from the oral cavity and pharynx containing columnar cells. These cells exfoliate from the tracheobronchial tree and also contain goblet cells (Taylor, 1991). Sputum was examined and expected to contain a small amount of radioactivity still present post ventilation with Technegas and post bronchoscopy. Sputum was studied to see if the sputum cells had the same characteristics as Technegas from bronchial lavage washout specimens.

6.2 Aims

- measure activity of Technegas remaining in sputum post bronchoscopy
- investigate under EM the structure of Technegas in sputum.

6.3. Methods and Materials

Sputum expectorate was collected immediately post bronchoscopy into a sterile sealed jar in the recovery area. The sputum is expected to contain a small amount of radioactivity still present post ventilation with Technegas prior to the bronchoscopy.

The specimen was obtained from a deep cough and with no immediate fixation, brought to the Nuclear Medicine Department for counting using the same dual head gamma camera used for the ventilation. Blue sheets were laid on top of the gamma camera collimator and the sputum specimen in the sealed jar laid on top of the collimator to take a reading of the remaining counts. All twenty patients' count rates had similar readings post bronchoscopy of between 40 and 45 MBq remaining in the sputum. Once counted, the specimen is brought to the laboratory for examination and fixing. This entailed a gross examination of expectorate as seen with the naked eye and results recorded as follows:

a) Using a wooden stick, a small section of expectorate is collected and smeared onto the cover – slip.

b) Sputum is fixed for four hours in 3.1% glutaraldehyde. The fixative is carefully decanted and replaced with 0.1M cacodylate buffer. The sputum is then rinsed. Post fixation, the sputum specimen is left at four (4°C) for two hours in pre–cooled 1% osmium tetroxide in 0.1M sodium cacodylate buffer. The osmium tetroxide is decanted and the tissue given a rinse in 0.1M sodium cacodylate buffer.

c) Dehydration removed all the free water in the sputum specimen and this was replaced with a solution, which was miscible with the embedding media-ethanol. The ethanol is used to extract any lipid from the sputum. Incomplete removal of water from the sputum will result in polymerization of the embedding media.

d) Araldite, which is a resin, was used in combination with a hardener and an accelerator to polymerize slowly in an oven at sixty (60°C) overnight.

6.3.1 Inclusion and Exclusion Criteria

***Exclusion*:** No females who are pregnant or breast- feeding.

***Inclusion*:** Adults who have read the Informed Consent form explaining the purpose of the aims of our research, expected duration of participation and a full description of the procedure which includes a small amount of radiation to the volunteer patient. Ethics approvals can be found in Appendix 9. The study will include twenty (20) adults, eleven (11) male and nine (9) female with a mean age of 48.5 years scheduled for a regular bronchoscopy.

6.3.2 Preparation for cross-sectional specimens of sputum for EM

The preparation of thin specimens remains one of the most significant aspects of EM. The preparation of a good specimen does require a certain skill, which I am barely perfecting, as it takes years to achieve. It comprises the following procedures:

Section Cutting: EM demands sections of 50 - 60 nanometers (500 - 600 angstroms) from the block, as the electron beam is unable to penetrate thicker sections sufficiently to allow details of structures to be clearly seen. Ordinary microtome knives are not suitable for cutting such thin sections since it is impossible to polish a metal edge finely enough to obtain good sections, so therefore glass or diamond knives are used instead.

“Thick sections” of between 0.5 – 1 micron thick are mounted on a glass slide for light microscopy. The section is dried onto the slide on top of a hot plate then stained with toluidine blue in order to localize areas of interest for subsequent EM. The block surface is then trimmed down to include only those structures of interest. This area is called the pyramid.

Next, the sections for EM are spread over the surface of a finely perforated copper grid approximately three (3) millimetres in diameter. The cross bars of the grid give sufficient support to the section without the need for an intervening layer. The sections adhere to the grid by surface forces.

Diamond knives are set into a block of metal and then inserted into a specially built holder, which is used as a floating-out trough. Between 10 and 20% solution of ethanol is used as the floating-out fluid as this has a lower surface tension than water and the cutting edge wets more readily. Ethanol can assist the specimen to expand a little and therefore counteracts the compression which the specimen undergoes during sectioning.

It is important to fill the trough correctly when the specimen is “floated out”. Too high and the block face will pick up liquid as it passes the cutting edge and the section will be dragged over the knife edge. Too low, the sections will not float out properly and result in a pile-up on the cutting edge. The correct height is to fill the trough until the knife-edge is wet.

Block trimming: ultramicrotomes and a block are clamped into a “chuck”. Trimming is carried out with a razor blade under a binocular microscope. Once the tissue is exposed, the block face is squared off and trimmed into the shape of a trapezium. The slides of the block are cut so that they incline at approximately 50 degrees.

Contrast Enhancement: if we do not prepare the bronchial washing and sputum specimens any further, the contrast in the specimens will be so low that little detail can be distinguished. Contrast in the electron microscope relies on the differential absorption and scattering of electrons, which depends on the presence in the section of material of high atomic weight. Fixation with osmium tetroxide adds some contrast, but exposing the specimens to a solution of heavy metal salts as acetate further increases contrast. Membranes, granules and other distinctive structures take up the heavy metal preferentially. A common practice is to stain with acetate in ethanol followed by lead citrate. The alcohol is used to facilitate the entry of the heavy metal into the substance on the thin section.

Araldite: consists of resin (CY 212) used in combination with a hardener (DDSA dodecenyl succunic anhydride), a plasticiser (dibutyl phthalate), and an accelerator (BDMA benzyl dimethylamine).This mixture polymerizes slowly at room temperature but can be accelerated by increasing the temperature. Blocks were polymerized at sixty (60°C) for two to three days. The amount of accelerator added to the final solution is critical and affects both polymerization and cutting properties. Too much accelerator produces a dark amber coloured block, which is much too brittle to cut; well-cured blocks are a pale straw colour.

The other types of epoxy resin are Epon, Spurr, Maraglas 655, Polyester Resins, Water Soluble Solutions, Glycol Methacrylate, Aquon and Durcapan.

6.4 Quality Assurance for the Electron Microscope

Before starting the operating procedure once our specimens are prepared, it is important to run a daily check on the EM as older microscopes have a tendency to "shift".

My specimens were reported on the Jeol 100CX11 Electron Microscope (EM) at the Western General Hospital Edinburgh, Scotland. The Jeol EM was made in 1983. It is worth noting that the microscope is cooled by a water jacket and the temperature should be kept between 18 and 24°C. In Scotland, EM has to have a built-in safety device for temperature so if the temperature falls below 8°C, therefore if there is frost or snow on the ground, an EM must not be switched on.

Magnification ranges:

Normal range x 500 – x 450,000

Lower range x1 00 – x 600

Start-up Checks:

1) Mains water on
2) Isolator switch on
3) Microscope key to start

Close Down Checks:

1) The filament emission should be turned fully anticlockwise
2) Accelerating Voltage has to be switched down to & including high tension
3) Key off
4) EM will shut down after thirteen (13) minutes
5) Mains water off

Gun Alignment:

1) Objective aperture out

2) Specimen holder out

3) Condenser aperture always at position 2

4) Check vacuum panel. EM should be at required vacuum and read approximately forty (40) micro-amps on vacuum gauge after half an hour

5) Switch on high-tension

6) Use the priming button next to the desiccator, move up through the voltages until 60kV has been reached

7) Saturate the filament by turning filament tension knob clockwise. The beam current micro-chip reading should also be 60; adjust the gun bias if this reading is too high or low. Re-saturate the filament with the filament emission knob and set the point with the stop on the back of the filament emission knob

8) Using condenser knob, make a small spot with the beam. On spot size 1 and using the gun alignment, trans centre the beam

9) Change the spot size to 3 and make the beam spot as small as possible. Re–centre the beam using alignment

10) Repeat steps until the beam is centre in both spot sizes.

Gun Alignment Tilt:

1) Spot size 1; make a small spot using the condenser
2) Turn the filament anti-clockwise until a filament is seen
3) Sharpen image with a fine condenser control
4) If filament image is not central in the spot, centralize using gun alignment.

6.5 Insertion of Holey Carbon Grid to view specimens

The grid should be placed into the specimen holder face down (dull surface face up).

Insert the specimen holder into the column and the red light appears. Thereafter:

1) Wait approximately thirty seconds (30) and the red light will go out
2) Turn the specimen rod clockwise to ninety degrees (90)
3) Hold on to the specimen tightly, because at this stage, the column vacuum starts to pull the specimen holder into the column
4) If the specimen holder is released too quickly, the ruby bearing at the end of the specimen holder could possibly be damaged and is very expensive to replace.

6.5.1 Objective Aperture Insertion

After adjustment of the current centre:

1) Select a low magnification 2k, spot size 1 and make a small spot with the condenser
2) Insert objective aperture to preferred position (for Araldite sections, position 2 is used)
3) Centre the aperture with aperture controls, after proceeding with the objective aperture astigmatism, condenser alignment and image alignment then the microscope will be ready to use.

6.6 Results

6.6.1 Sputum results aid diagnosis of lung cancer

Collecting both expectorate and bronchial material aids the diagnosis of lung cancer, seen in many of the volunteer subjects who had a bronchoscopy. It is interesting that expectorate is equal if not higher than bronchial material in the percentages of lung cancers diagnosed, hence the importance of both specimens being taken to further aid in diagnosis.

Expectorate cytologic specimens may be divided into: a) epithelial cells, b) macrophages, c) leukocytes, d) mucous, e) intrinsic non cellular components, f) extrinsic non cellular components, and g) living organisms (Wield, 1997).

Wet fixation with air-drying was the method used for sputum with the immediate submersion of the cell samples into a Coplin Jar containing 95% ethanol, a fixative.

After a specified time, the samples are removed and air – dried, this was the chosen method of fixation for our research. Cellular adherence to a glass slide was good as the sample had originated from sputum and was viscous and sticky. Cells taken from fluid adhere less (bronchial washings) to the slide than sputum (taken post bronchoscopy), which adheres well.

6.6.2 Results of Micro-analysis

The microanalysis of thin samples from the sputum is limited more by the specimen preparation than the microscopic technique. Conventional preparation used for our research provided useful morphological information. Due to chemical fixation, dehydration and embedding procedures, contamination of the specimens may occur but if this does happen, the artefacts will be known by the person reporting on the specimen and allow for this.

Unfortunately, the majority of results of the sputum samples taken were destroyed in transit and only one sample retrieved, so one result was reported, see Figure 5.5, Carbon Particles from Technegas (sputum).

Using EM to examine our previously damaged specimens was disappointing. Scattered technetium was seen, and one single sample of Technegas was viewed. Golgi were commonly reported in sputum and are composed of three basic elements, the most characteristic being a stack of flattened sacs, the ends of which are slightly dilated. Vesicles lie on the convex or outer face of the stack and these vesicles arise from “budding” out of the endoplasmic reticulum. Golgi complexes are seen especially well in secretary epithelia, sputum. The functions of the golgi are many

and quite varied; golgi are involved with the secretary process, production of nearly every exocrine and endocrine secretion, cartilage and synovial membranes.

EM does not elucidate satisfactorily the structure of chromatin and the variations that may underlie cell differentiation, function and neoplastic transformation.

Using EM, the nuclei seem to show some irregularity, this being due to the thinness of the sputum sections which were seen as two-dimensional images. Therefore, EM shows that many nuclei instead of being smooth and round or oval may be irregular depending on the type of cell Macrophages seen, including some with carbon particles scattered throughout. As a patient coughs, free carbon particles are dispersed throughout. These carbon particles were viewed using 5k x magnification.

Free macrophages were seen and had a rounded appearance, with the cell outline being slightly irregular. Macrophages have various shapes when seen under EM. The nucleus is rich in chromatin, and appears to show a slightly acidophilic cytoplasm when viewed under light microscopy.

For future researchers the technetium found within the sputum should be tagged with a monoclonal antibody or a fluorescence agent so that it can be seen more distinctively under EM and differentiated by its hexagonal configuration and electron density.

CHAPTER 7 MAJOR FINDINGS AND CONCLUSIONS

7.1 Novel aspect of lung scanning

This thesis involved 50 participants, 25 conventional subjects and 25 TEBA patients. The patients involved in these experiments were those who were inpatients in the Intensive Care Units, and those with CAL and PE. Recent advances have been needed to improve the method of diagnosis and the TEBA positive pressure device may prove to be an invaluable tool to those working in Nuclear Medicine.

There is concern that TEBA or positive pressure ventilation may create a falsely positive diagnosis of PE by improving the peripheral penetration of tracer in poorly ventilated areas that are associated with reflex vasoconstriction and hypoperfusion not due to vascular obstruction. Clearly, more research is required to clarify this issue, as the number of subjects in these experiments was too small to make a definite conclusion. **This thesis investigated a novel aspect in ventilation lung imaging, involving a series of experiments on normal volunteers, chronic airways limitation or CAL patients, and unconscious, intubated intensive (ICU) care patients, and patients with Pulmonary Embolism (PE).The fourth experiment studied Technegas at the cellular level using Electron Microscopy (EM). This** experiment was unique as it used bronchoscopy to obtain human lung washings/lavages from volunteers following the administration of Technegas for a ventilation lung scan. This was in considerable contrast to previous studies by Isawa et al. (1996), Lemb et al. (1993,1994), Senden et al. (1997) that used samples of Technegas obtained directly from the Technegas generator and studied both their chemical and physical properties. Twenty volunteers, who were already booked in for a routine bronchoscopy procedure, agreed to participate in the experiment that only required one extra sample to be taken during the procedure. In order to evaluate Pulmonary Embolism (PE) in compromised patients, it was necessary to examine the use of positive ventilation procedures in lung scintigraphy.

7.2 Major benefits of TEBA

The series of experiments in this thesis showed that using positive pressure known as TEBA for lung ventilation was as good as or better than conventional lung

scinitigraphy for patients with CAL and for unconscious patients from an Intensive Care Unit. The results from experiment three found that TEBA can be an effective tool in the management of ICU patients with a possible diagnosis of PE. TEBA has the potential to play an important role in diagnosing PE in ICU patients, who previously may have been commenced on anticoagulant therapy without the aid of lung scintigraphy. TEBA was able to provide a subjectively superior ventilation image for these critically ill, intubated patients.

TEBA in most cases showed an improvement in ventilation count rate over the baseline, conventional method of ventilation (see Table 3.2) for normal volunteers and patients with CAL or PE. When compared to conventional ventilation delivery, breathing time with the utilization of the TEBA was significantly shorter (see table 3.1 and figure 3.3). The mean number of breaths in both TEBA and CAL group to achieve the required counts was approximately 3 breaths.

No volunteers or patients reported any significant discomfort or complications attributable to the device. The temporary lowering of oxygen saturation associated with inhaling the anoxic Technegas and Argon mixture was common for all three groups within this research (Figure 3.4). The use of oxygen to drive the positive pressure in patients without CO2 retention helped reduce the severity of Technegas induced hypoxia. This in turn may reduce the theoretical risk of tissue infarction in patients with severely impaired coronary or cerebral circulation in the presence of severe hypoxia, although such complications have not been reported despite the large number of ventilation studies using Technegas to date.

7.3 Significant results of the experiments

Investigations into the use of TEBA with normal volunteers, patients with CAL and PE and inpatients from the ICU of a hospital were conducted in three experiments involving 124 patients and normal volunteers. A further investigation, involving the use of EM with human lung tissue samples, was undertaken with 20 patients who were due to undergo bronchoscopy. This research using human samples is a first in Australian Nuclear Medicine Lung research.

A limitation to performing a TEBA V/Q scan on an ICU patient, is that two trained nuclear medicine staff are required to complete the procedure: one to operate the PAS and the other to “squeeze” the resuscitation bag in synchronicity with the subject’s breathing (Leiper 1998). If the operators do not maintain an adequate ventilation technique to deliver the Technegas to the patient, radioactive tracer leakage and contamination of the scanning room may occur unless specially trained nuclear medicine personnel are available. Therefore while the ICU patient is in the department, an intensivist and a nurse specialist must be present.

The requirement for specialised training of either ICU or nuclear medicine staff has both economic and radiation safety implications. Nuclear Medicine centres that plan to offer V/Q studies to ICU must address these issues as part of their imaging.

Technegas in human lung tissue, at the cellular level

The final experiment in this thesis studied Technegas in human lung tissue, at the cellular level. Previous research using Electron Microscopy (EM) reported the physical and chemical nature of Technegas by directly studying samples of Technegas. The uniqueness of experiment four related to the method of obtaining the Technegas samples. In this experiment the Technegas came from lung biopsy

samples and washings of human volunteers who had undergone a Technegas ventilation study. This was the first EM investigation of Technegas within the lung tissue.

The experiment aimed to identify the location and structure of Technegas in the lung tissue samples using electron microscopy. Analysis of the EM data showed a range of cellular structures, however, only one hexagonal Technegas particle was seen under EM. This hexagonal particle was found to be of metallic technetium encapsulated within a layer of graphitic carbon, thus demonstrating for the first time that it is possible to observe Technegas particles from human lung tissue samples.

It was disappointing that no further particles were observed. However, it is likely that a modified form of lung sample collection, with samples taken from trans-bronchial specimens rather than from the left lower lobe of the lung, would prove fruitful in gaining greater understanding of the action and location of Technegas within human lung tissue.

7.4 Conclusions

This is the first study to report the successful application of TEBA assisted ventilation/perfusion lung scintigraphy, with a range of subjects, including normal volunteers, patients with CAL and PE as well as unconscious, intubated patients from the Intensive Care Unit.

Better management of PE and CAL - These observations have led to new suggestions about the use of TEBA in the management of PE and CAL. New suggestions are:

1. Introducing oxygen saturation analysis using a pulse oximetry as a routine procedure during lung ventilation. This provides a continual monitoring of the patient should they become hypoxic.
2. Prior to any anticoagulation, tests such as PT, PTT (prothrombin time), or D-dimer tests to determine a baseline of patient status in ICU should be performed as a routine procedure.
3. An algorithm should be developed as a medical standard diagnostic tool to investigate the symptoms of each patient depending on their initial presentation.

This data confirms the potential role of TEBA in the management of ICU patients with lung disease and has significance for reducing the incidence of complications arising from unnecessary use of anti-coagulant medication. Further, this research indicates that having the potential to successfully transport an intubated, unconscious patient to the nuclear medicine department for a V/Q scan may result in different management regimes for ICU patients in the future with regard to commencing anticoagulant therapy. For example, it is now possible to diagnose patients prior to commencing treatment, thus reducing the risk of haemorrhage.

Improved Diagnostic quality of ventilation - The results demonstrated that TEBA assisted ventilation is as good as or better than conventional ventilation in a group of patients with varying levels of lung function. The data also provides evidence that compared to conventional ventilation lung scintigraphy, TEBA requires less time to achieve an adequate count rate, giving the potential for improved diagnostic quality of ventilation and management of patients with life threatening lung disease.

A first in nuclear medicine - This EM research was the first to use human bronchial washings/lavage specimens to study Technegas at the cellular level. No other nuclear medicine research to date has looked at this aspect, all other researchers studied the chemical and physical nature of Technegas.

Appendix A: Preparation and Use of Technegas

Overview

Technegas is produced in a Technegas Generator, which is purpose-built for lung scintigraphy. Its production requires an apparatus capable of heating a graphite crucible to 3000°C, thereby eliminating the concern of inhalation of tungsten oxide. It is concluded, after several experiments, that all the technetium–99m is absorbed on the surface of the graphite crucible and simultaneously volatilised to become coated with graphite above 2200°C. The crucible is heated to 2500°C in an atmosphere of pure Argon for 15 seconds. The resulting vapour and Argon mixture are inhaled by the patient via a Patient Administration Set (PAS). The Technegas produced is a dispersion of carbon particles containing Tc-99 atoms, either trapped within the core, or between overlapping sheets. These clusters have a maximum size of 0.017 microns and the carbon mass inhaled by a patient in a Technegas study is measured as 5 micrograms (± 20%) (Lemb, 1993).

The structure of Technegas consists of hexagonal flat crystals of technetium metal encapsulated within a carbon capsule to be within a range of 30-60 nm in size. Prior to ventilation, these technetium 99m particles coagulate into aggregates with a median diameter of 100 to 160 nm. Depending on the time of technegas generation and ventilation, the longer the time, the larger the particles. These particles can be as large as 225 nm measured at 8.5-minute generations (Burch 1996). On inhalation, it diffuses and adheres to the alveolar walls, enabling multiple conventional views. Technegas particle size and the amount of

deposition in the lung will determine the mucociliary clearance rate of technetium (Lloyd 1997).

Preparation of Technegas conducted during experiments:

The carbon crucible is removed from its package using gloves and forceps; the crucible well is filled with 95% or absolute ethyl alcohol (ethanol) and drawing back the excess ethanol with a 1ml syringe, leaving the crucible wet. *Methylated alcohol is not used*, as it may leave residues from the evaporation process, which could lead to pyrolysis in the gas generation stage (Tetley Medical Ltd. Users Manual 1999). The syringe is drawn back so there is no excess alcohol left in the crucible. The crucible is positioned so that one end is at the left hand contact and the other end is aligned with the right hand contact of the Technegas machine. There must be good contact or a low yield of Technegas may result. Rotation of the crucible is required to make sure the well cavity is in the upright position. Pushing the lever on the side of the Technegas Generator opens the contacts to enable proper mounting of the crucible.

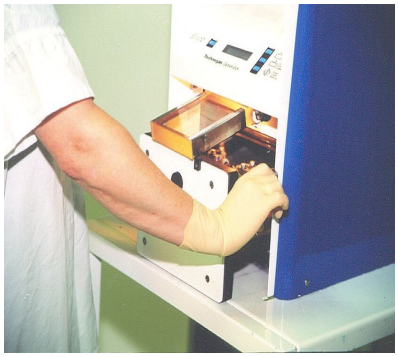


Figure A1 **Loading the Crucible**
(Vita Medical Limited)

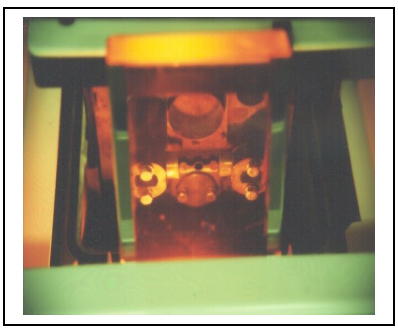


Figure A2 **The crucible or "boat" and contacts**

Overfilling the crucible may cause air bubbles to become trapped in the graphite crucible. Any unwanted bubbles will expand and ascend to the surface, causing the eluant to spill and possibly splash out of the crucible. Overfilling the crucible may actually result in having less radioactivity being simmered and burnt.

Approximately 700 – 900 MBq of technetium 99m pertechnetate in a 1ml syringe (in lead glass syringe shield) is injected into the crucible. The crucible is filled to a meniscus, concave or flat but never convex. On days when the generator activity is low, two or three simmers of the crucible is sometimes necessary to reach the amount of radioactive concentration necessary for achieving an adequate ventilation count-rate. This re-simmer is achieved by interrupting the Technegas generation cycle following the evaporation phase, refilling and re-evaporating the crucible. The drawer is closed and for safety precautions, a two handed operation is required to close the door of the Technegas generator, allowing the start to be pressed to initiate the simmer phase.

The Technegas Generator automatically checks for the presence of Argon gas or any gas leaks. The Technegas Generator detects gas leaks by monitoring the pressure in the chamber over time allowing a tolerance range of up to a 50% drop in pressure. Once all checks are completed satisfactorily, the generator commences the simmer/purge stage, which dries the technetium pertechnetate onto the walls of the well in the graphite crucible. This is achieved by a small current passing through the crucible, increasing the temperature to 70°C and then pushing a flow of Argon gas across the top of the crucible to disturb the surface of the pertechnetate. Concurrently, the chamber is purged of any air ensuring an inert atmosphere is created. The simmer/purge stage lasts six minutes. When

finished, the generator rechecks for Argon gas contamination and then the burn generation is initiated.

During the simmering phase, technetium eluant is evaporated onto the carbon crucible composed of high purity graphite, which is heated to 2500ºC under an atmosphere of high purity Argon (99.99%) for 15 seconds. At this temperature, carbon atoms aggregate into small clusters each entailing a technetium atom. Each particle is a single, flat crystal of technetium metal encapsulated in carbon. The total particle output of one Technegas dose is in the range of 3.4×10^6 particles per cc. This corresponded to less than one (1) micron of carbon inhaled per Technegas administration (Tetley Medical Ltd. Users Manual 1999).

Appendix B: Ethics Submission

Ethics:

Ethics approved by:

- Bankstown Hospital for thesis (approval no. 6663)
- Wollongong Hospital, Sydney, Electron Microscopy

  (approval no. HEO3/023)
- University of Sydney, Human Research & Ethics Committee

  (approval no. 6333).
- Electron Microscopy Unit Edinburgh Western General Hospital.

  Verbal approval granted for me to have training and learn Electron Microscopy to be able to report on my specimens with a supervisor.

The above ethics committees have given their approval for the various experiments to take place in their institutions for my research thesis (C Leiper).

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