TOWARDS LUNG VOLUME MEASUREMENT BY A REBREATHING TECHNIQUE

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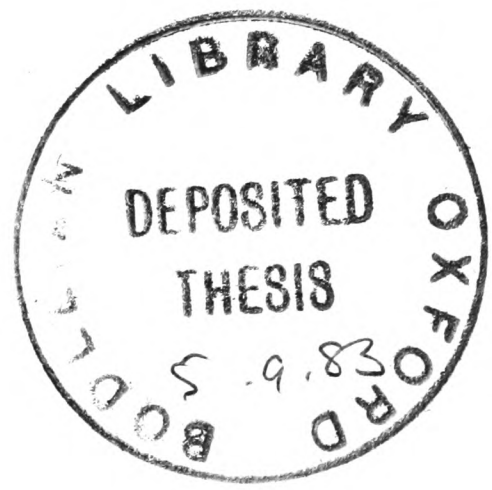
Doctor of Philosophy

by

IAN LAURENCE SCOTT

Linacre College, Oxford



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To Mum and Dad and Susan.

Abstract

IAN LAURENCE SCOTT, LINACRE COLLEGE.
D.Phil., Hilary term.

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The work contained in this thesis was concerned with rebreathing methods of measuring lung volume. In particular, one novel rebreathing technique which uses oxygen as the indicator gas was assessed. This technique appeared methodologically simple and readily applicable in a clinical environment. In essence, it relied on a graphical extrapolation of the time related changes in oxygen concentration to allow for oxygen uptake. This technique has been tested using a mass spectrometer which enabled nitrogen and argon as well as oxygen to be simultaneously used as indicator gases.

Although the lung volumes as measured by the different indicator gases should have been the same, these were found to be different. These discrepancies were related to the concentration of the indicator gas which existed in the bag and lung prior to rebreathing. A hypothesis explaining these inconsistancies was formulated. This was based on an initial but non-sustained output of carbon dioxide into the bag-plus-lung system.

A numerical model of idealised rebreathing showed that the hypothesis was sufficient to explain the discrepancies observed. A correction procedure was devised which performed successfully in the model. This correction was incorporated into an on-line computing procedure for calculating real lung volume. When tested in normal subjects this gave consistent results for lung volume, irrespective of indicator gas employed. The corrected lung volumes were unaffected by the initial gas compositions in the bag and lung, and were also independent of non-sustained gas exchange, whether this was due to carbon dioxide and/or nitrous oxide. This technique could, therefore, be use under anaesthetic conditions, since the uptake or output of nitrous oxide no longer upsets the calculation of lung volume.

The use of more than one indicator gas, within the same manoeuvre, was shown to provide a valuable indication of the presence of errors in the system. When this approach was applied to more conventional rebreathing techniques of lung volume measurement, it also highlighted the presence of inaccuracies.

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PREFACE

When research for this thesis commenced in 1979, the central problem was understanding the phenomenon known as the "hyperoxic shunt" (see later). To this end three lines of work were pursued concurrently. The first was the development of a rebreathing technique for measuring lung volume. The second was the study of deliberately induced pulmonary dysfunction in dogs, and the third was the modification of commercial intravascular oxygen sensors. Each line inevitably revealed problems which demanded individual solutions. One such problem in the measurement of lung volume became the dominant research topic, and the subject of this thesis. It is impossible, as yet, to reunite the three lines of work, and so in the interests of a cohesive thesis only the work on the measurement of lung volume is presented. However, all three bodies of work have provided a variety of problems for research training, and together form the basis for a more sophisticated approach to the original problem. The three original lines of research will now be briefly discussed in the context of the original aim.

Dr. J. Kerr (Kerr, 1975) working in the Nuffield Department of Anaesthetics observed that many patients with various degrees of pulmonary dysfunction had levels of venous admixture which increased progressively as the alveolar oxygen partial pressure rose from 100 to over 600 mm Hg. This observation has since been confirmed in other centres and conflicts with classical expectations.

One possible explaination is "absorption atelectasis" consequent on the removal of the "splinting" effect of intra-alveolar nitrogen. This possibility promoted the study of lung volume, under the premise that this atelectasis might be measurable as a decrease in lung volume.

An alternative hypothesis, based on hypoxic pulmonary

vasoconstriction, prompted the study of deliberately induced pulmonary dysfunction in dogs. Previous unpublished work in the Nuffield Department of Anaesthetics had suggested that in dogs with undamaged lungs, the venous admixture rose with rising alveolar oxygen concentrations, though the effect was small. It seemed a reasonable first step to ascertain whether increasing the mean level of venous admixture, by deliberately induced lung damage, would amplify this hyperoxic shunt effect. It was hoped that this would lead to an animal model of hyperoxic shunt. Although a stable preparation with pulmonary dysfunction was established, this did not demonstrate a hyperoxic shunt. The results of these experiments did, however, provide some interesting information concerning sources of error in the determination of venous admixture. This has been prepared for publication elsewhere.

The time course of changes in shunt, following a change in inspired oxygen concentration, was expected to help distinguish between an explanation based on absorption atelectasis and one based on a pulmonary vascular response. This prompted investigation into improved methods of following the oxygen tension in the arterial and venous blood. For this, the technique of pulsed polarography was applied to existing commercially available intravascular oxygen sensors. These were modified by replacing the original protective membrane by membranes of different characteristics. This has enabled the speed of response of these sensors to be improved without increasing their dependance on fluid flow velocity. This work has been accepted for publication (Scott et al, 1983).

Work is continuing in this laboratory towards developing a method of using a forcing oscillation in inspired nitrous oxide concentration to determine venous admixture. The transmission of this oscillation from the alveolar gas to the arterial and venous blood is influenced by the distribution of ventilation and perfusion, and can theoretically be used to

characterise this distribution. This new method will depend upon the technical advances and experience gained during the course of this thesis, with on-line mass spectrometry and with "fast" time response intravascular polarographic sensors. This approach will be tested in the dog model of pulmonary dysfunction which has been developed. This new approach should provide for a more sophisticated study of the hyperoxic shunt effect than was possible at the outset of this research. It represents the ultimate convergence of the three lines of investigation described in this preface. This will be the subject of my post-doctoral research for the next two years.

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CHAPTER 1

The Measurement of Lung Volume. A Review.

1.1 INTRODUCTION

Of all the absolute lung volumes (see Figure 1.1), the most important is probably the Functional Residual Capacity (FRC). The FRC is the volume remaining in the lungs at the end of a normal expiration. This is the volume that the tidal respirations are ventilating. It is the relaxation volume of the respiratory apparatus. At FRC there is no activity in the respiratory muscles, and therefore, when the glottis is open, there is no pressure difference between an alveolus and the external atmosphere. The magnitude of the FRC depends upon the balance between the inward elastic recoil of the lungs and the outward recoil of the chest wall. The difference between these two generates the negative pressure in the pleural cavity. In functional terms, the FRC is a buffer between the inspired air and the mixed venous blood. Without any FRC, the composition of the alveolar capillary blood would fall to mixed venous levels at the end of expiration and change dramatically again during inspiration. This would result in large fluctuations in the arterial blood gases. In addition, the FRC constitutes about one third of the body's store of oxygen which can be be called upon during periods of apnoea.

1.2 WHY MEASURE LUNG VOLUME?

In the nineteenth century, the measurement of lung volume was pursued in order to understand better the mechanisms of gas exchange in the normal and diseased lung. Hutchinson (1846) believed that the vital capacity (VC) was an important diagnostic tool in pulmonary disease. At this time, Pulmonary Tuberculosis was a prevelant condition and there was no clinical test to allow early diagnosis, since chest X-rays were not availiable. In order to determine an abnormal VC, Hutchinson realised the need to predict the normal VC. From measurements in 1,012 normal subjects, he found a

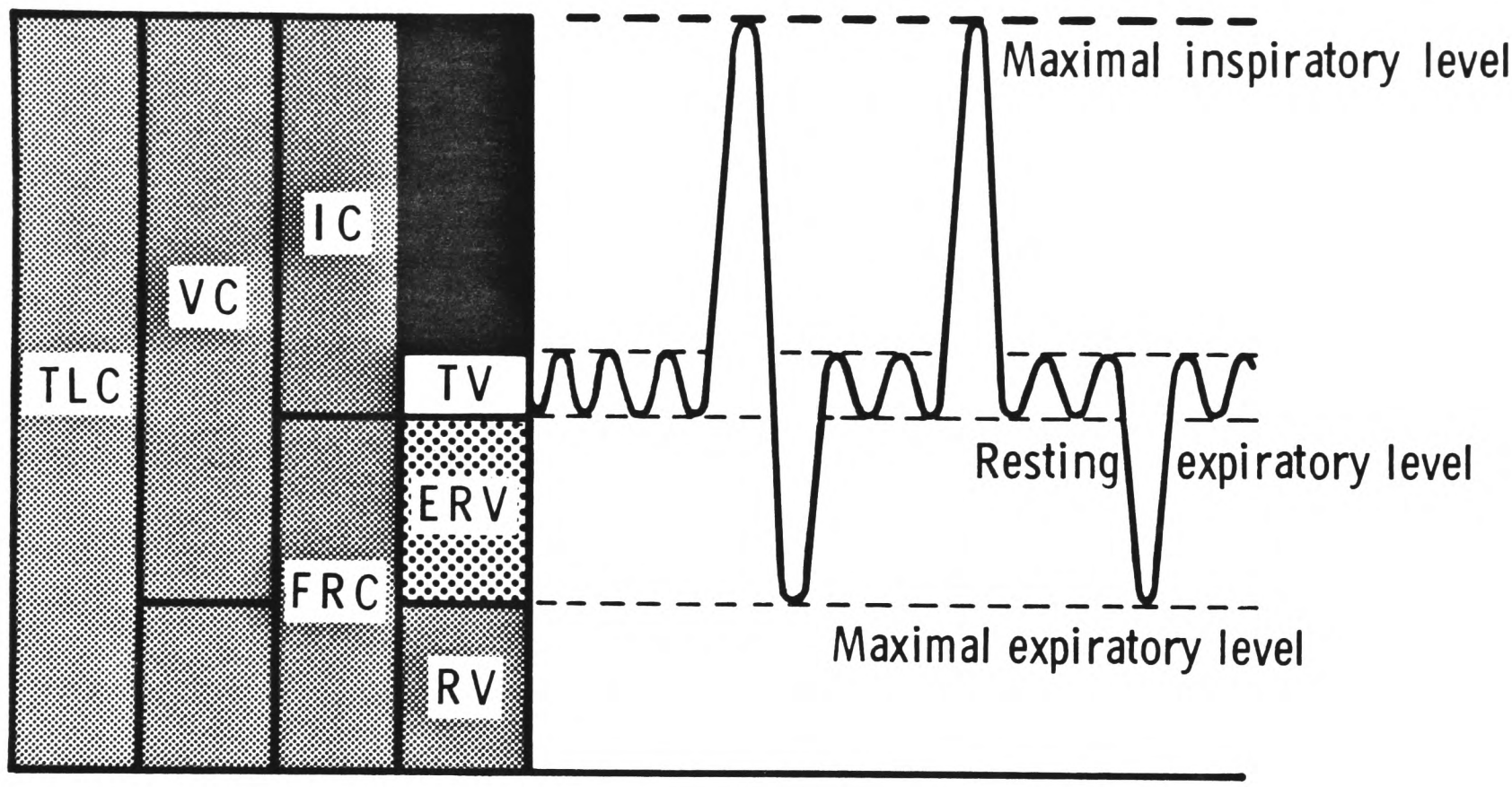


Figure 1.1 Subdivision of lung volume. The abbreviations are those in general use and as used in this thesis. Those volumes which include RV (Residual volume) are absolute lung volumes and cannot be measured by spirometry.
TLC = total lung capacity. VC = vital capacity. IC = inspiratory capacity. FRC = functional residual capacity. TV = tidal volume. ERV = expiratory reserve volume.
(Redrawn from Briscoe, 1965: Fig 1).

normal relationship between vital capacity and height. This relationship was the first assessment of lung function to be applied to patients and it formed the most important aspect of pulmometry for half a century.

At the turn of the century, Bohr (1907) began to object to the use of Hutchinson's "normal" figures for two reasons. Firstly, the VC measured in a patient can vary widely between individual assessments, and secondly, VC excludes the volume remaining the lungs at the end of a maximal expiration, known as the residual volume (RV). Both RV and FRC are absolute lung volumes since they are measurements of true capacities, VC is a relative lung volume and is the difference between two capacities (TLC minus RV, see Figure 1.1). Bohr thought that the variability in VC was more the result of variations in RV rather than variations in total lung capacity (TLC). TLC seemed to Bohr to be the more important diagnostic measurement. Bohr and his pupils (Hasselbalch, Rubow, Siebeck, Bie and Maar) established the relationships between TLC, FRC and RV under normal and pathological conditions. They stressed that the relationships rather than the absolute figures were important. Within an individual, comparisons between one type of lung volume and another show less population variability. In particular, the ratio of the residual volume to the vital capacity is known to provide a useful characterisation of the degree of pulmonary distension.

Bohr believed that the level of FRC was controlled by a respiratory reflex. He supposed that as the demand for oxygen increased so did the FRC. This would cause an increase in the surface area for gas exchange and would facilitate pulmonary blood flow. Bohr's theory was critisised by Hasselbalch (1912) and Krogh and Lindard (1913) but it did spur the clinical interest in the changes of FRC in different morbid states. In the early twentieth century, it was hoped that the measurement of lung volumes would provide a useful diagnostic tool for the elucidation of pulmonary dysfunction. It soon became clear that the inherent population variability

of lung volumes was too large to enable the distinction between normal and pathological states to be drawn. Today, better preventive medicine and public health has resulted in a decline of many serious respiratory diseases. The measurement of lung volume in pulmonary function laboratories is relegated to a lesser position, as just one of a battery of tests. In terms of clinical diagnosis, these tests are complementary to the findings of X-ray and clinical examination. The importance of FRC measurement in current clinical practice is primarily in following the time course of disease in a given patient, and in assessing treatment with respect to its ability to alter FRC in serial measurements.

In respiratory physiology, the measurement of static lung volumes has now been superseded, at the forefront of interest, by more dynamic physiological concepts. However, the measurement of lung volumes is still useful clinically, and of importance in the determination of more subtle indices of pulmonary function which can be derived from a study of gas exchange. The measurement of absolute lung volume is still a prerequisite for some gas exchange approaches to measuring cardiac output, carbon dioxide transfer, tissue volume and a whole range of pulmonary variables. For such techniques, the accuracy of lung volume measurement will affect the accuracy of the measured index.

In some ways, investigation of lung volumes has played an active rather than passive role in other fields of pulmonary research. A divergent pattern is evident in the evolution of techniques for measuring FRC. Reassessment of sources of error has led to the consideration of new aspects of physiology. The consideration of the distribution of ventilation (reviewed by: Fowler, W.S., 1952; Bouhuys and Lundin, 1959; Bouhuys, 1964) is an example, as are the concepts of a slow space (Hickam et al, 1954) and of trapped gas (Bedell et al, 1956). Increasingly, the

assessment of FRC is becoming clinically important in critically ill patients (McClung and Mitchell, 1978; Ozzane et al, 1981a). This is because the relationship of FRC to closing capacity is critical in gas exchange. This will now be discussed.

1.2.1 Closing Capacity

In the normal lung, airways, which are less than 2mm in diameter, have no cartilage to support their wall (Wiebel, 1963). These airways are kept patent by the transmural pressure gradient which is the difference between external and intraluminal pressure. The intraluminal pressure, under conditions of no flow, is the same throughout the respiratory airways and can be recorded at the mouth. The external pressure is not amenable to direct measurement in small airways. The negative intrapleural pressure is believed to be transmitted to the space directly surrounding the small airways. The lung parenchyma acts as a system of "guy ropes" resisting airway collapse. This ~~external~~ transmural pressure is believed to be approximately equal to the intrapleural pressure and this has been shown to be the case in large airways (Hyatt and Flath, 1966). The external pressure is also modified by the tone of the bronchiolar smooth muscle lining the airways.

A change in intrapleural pressure, created by movement of the thoracic cage, will cause the lung volume to alter, as governed by compliance. The changes in lung volume, associated with changes in transpleural pressure, are shown for normal lungs in Figure 1.2. Clearly, as lung volume decreases from total lung capacity to residual volume, the transpleural and the transmural pressure gradients also decrease. As a consequence, the diameter of the terminal airways will be reduced as a function of decreasing lung volume. Regional variations are superimposed on the general transpleural pressure gradient at a given lung volume. Depending on the alignment of the lung with respect to the vertical, the weight of the lung will cause a more negative intrapleural pressure at the

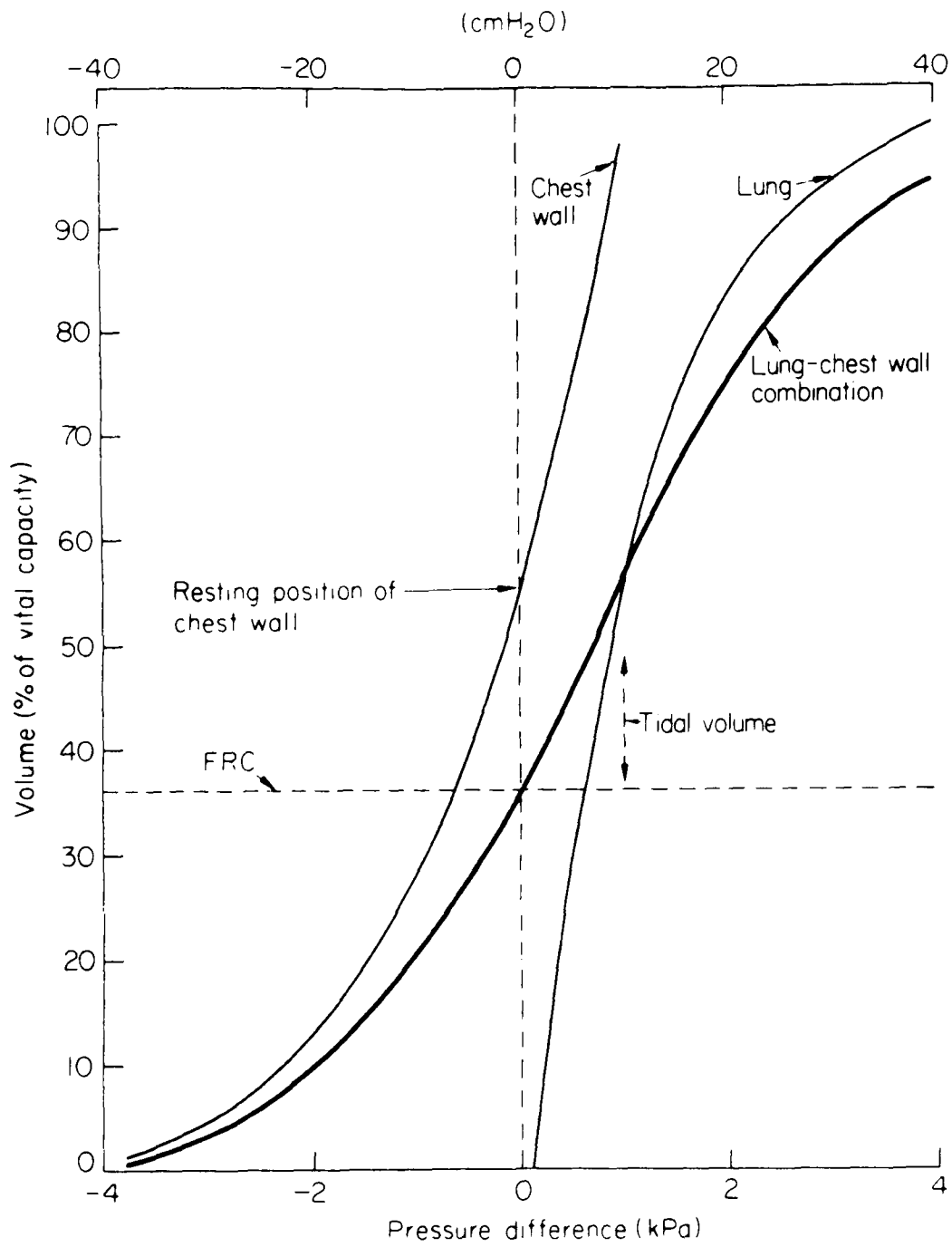


Figure 1.2 Pressure-volume diagram of the lung, chest wall, and lung and chest wall combined. The pressure difference is that between the airways and the outside of the chest, and is required to maintain a given lung volume. Positive pressure differences expand the thorax. The "lung" line shows the relationship for the lung outside of the thorax. The "chest wall" line is derived by subtraction of the "lung" from the "lung and chest wall combined".
(From Sykes, McNicol and Cambell, 1972: Fig 1.3).

upper surface than at the lower. The transmural pressure gradient is, therefore, less in the dependent than in the non-dependent regions. There are also dynamic effects such that, during expiration, the intraluminal pressure will decrease downstream because of flow dependent pressure losses.

The result is that, during expiration, the calibre of the airways is progressively reduced and consequently airway resistance increases (Figure 1.3). If the expiration is prolonged, the transmural pressure continues to fall. Approaching residual volume, some of the airways in the dependent areas of the lung will become severely narrowed or close altogether. The volume of gas remaining in the lungs at the point of detection of the closing of airways is the "Closing Capacity" (CC). The difference between this and the residual volume is known as "Closing Volume". The closing capacity is defined experimentally by the "single breath oxygen test" (Anthonisen et al, 1969) or the "foreign gas bolus test" (Dollfus et al, 1967). These tests produce an inert gas gradient between the dependent and the non-dependent parts of the lung such that there is a higher inert gas concentration in the latter than in the former. A bolus of inert gas is inspired, starting from residual volume, and is preferentially distributed to the non-dependent areas. During subsequent slow expiration, a plot of expired gas concentration versus expired volume shows a rise to an alveolar plateau followed by a further sharp rise of the inert concentration. This is attributed to closure of airways in the dependent region. This results in an increase in the proportion of expirate from non-dependent regions, which contain a higher concentration of inert gas. The concepts surrounding airway closure are well reviewed by Rehder et al (1977) and Lehane (1982).

In normal healthy subjects, CC is less than FRC. In 1965, Nunn et al showed that voluntary breathing of air from RV, for between three to ten minutes, resulted in a significant reduction in arterial oxygen saturation

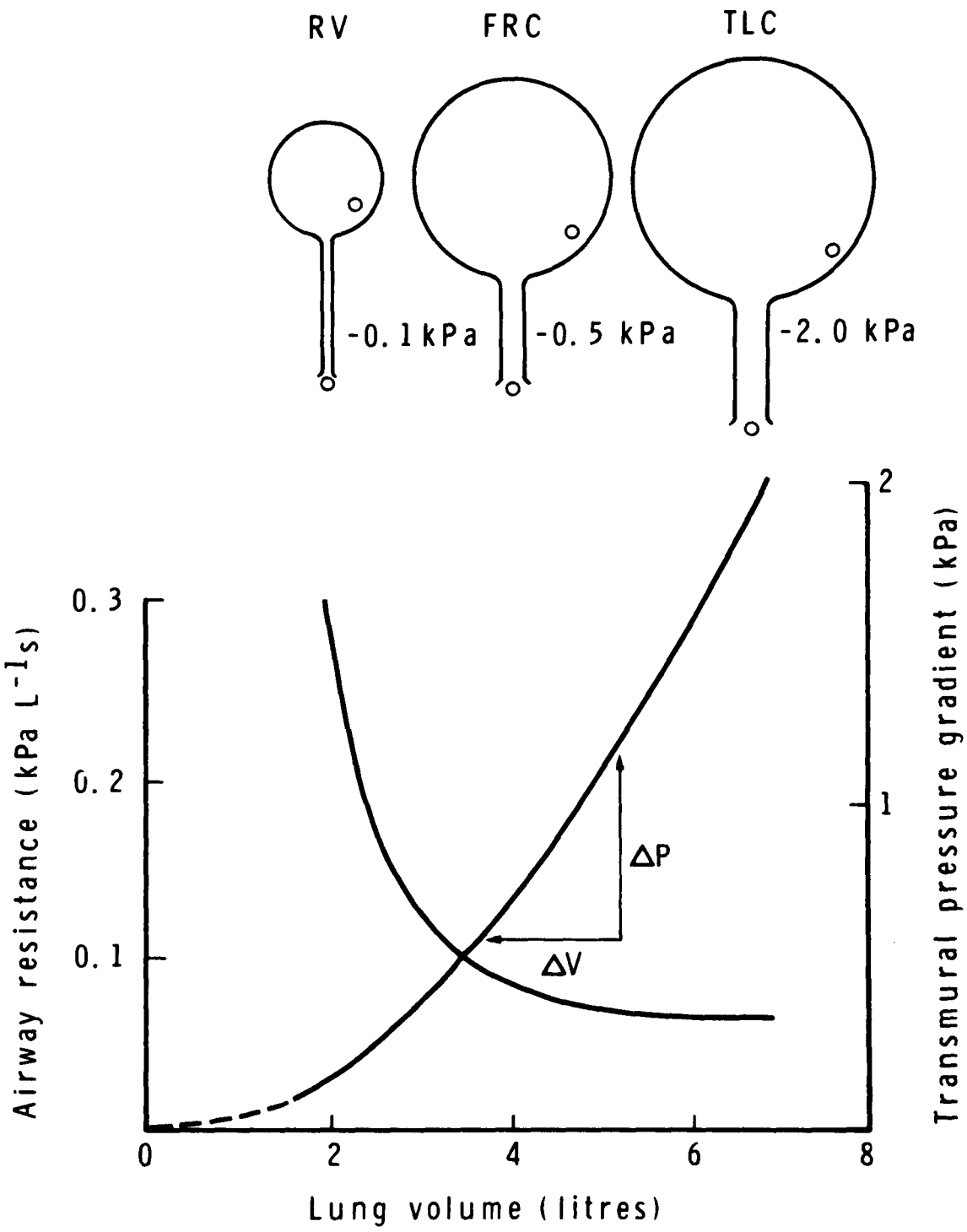


Figure 1.3 Airway resistance and transmural pressure difference as a function of lung volume. The diagramatic alveoli show how the geometry of an alveolus and its airway change with changes in lung volume and, therefore, changes in transmural pressure gradient. These changes in the airway account for the observed airway resistance changes. Pressures shown are related to atmosphere. Slope $\Delta V/\Delta P$ is lung compliance.
(Redrawn from Nunn, 1969: Figs 23 and 43)

in some healthy individuals. When normal end-tidal lung volumes were re-established, the saturation rapidly returned to control level. This was attributed to reversable airway narrowing or closure at low lung volumes, which gave rise to low or zero ventilation to perfusion ratios. This interpretation was confirmed by Morrison et al (1982) and Schonfeld and Ploysongsang (1983). Closing capacity increases with age in such a way that the average CC is the same as average FRC at age 66 years in the upright position and 44 years in the supine (Leblanc et al, 1970). It appears that FRC changes little with age (Ibanez and Raurich, 1982) but is reduced between 500 - 1000 mls in moving from an upright to a supine position. On the other hand, CC increases with age (Anthonisen et al, 1969) and seems to be independent of body position (Craig et al, 1971). Thus, the relationship of FRC to CC may well be responsible for the poor oxygenation observed with age and postural changes in some subjects.

Since Bergman's findings in 1963 it has been well documented that there is a fall in FRC coincidental with the induction of anaesthesia (see Laws, 1968; Don et al, 1970). Closing volume, however, does not seem to change in anaesthesia (Gilmour et al, 1976). It is possible that shunting of pulmonary blood through regions of trapped gas, behind closed airways, is primarily responsible for impaired oxygenation during anaesthesia and in the post-operative period. The maintenance of FRC is, therefore, important during anaesthesia, and can be increased by increasing the end-expired pressure. The use of positive end-expired pressure (PEEP) causes a reduction in cardiac output. Thus, PEEP may improve oxygen carriage to the tissues by increasing the O_2 content per unit of blood flow, but may impair it by reducing blood flow itself. In patients with diseased lungs, the improvement tends to be the predominant effect (Nunn et al, 1965).

A change in the relationship between FRC and CC might be expected in acute respiratory failure (ARF) because of the pronounced decrease in FRC observed in this condition (Pontoppidan, 1968). It has been shown, that once ARF has developed, the most effective way of improving oxygenation is to increase the FRC. This is conventionally achieved by the maintenance of a positive pressure in the airways throughout the respiratory cycle. In ventilated patients, PEEP is applied which allows the lungs to expire the tidal volume but not to collapse any further. Leftwich et al (1973) have shown that PEEP increases FRC and reduces the shunt in such patients. In spontaneously breathing patients, a constant positive airways pressure (CPAP) is applied to maintain FRC. Kumar et al (1970) have shown that CPAP, in spontaneously breathing patients, has a similar effect to PEEP in ventilated patients.

In the light of these observations, it can be seen that the measurement of FRC would provide useful information as to how different patterns of ventilation may improve the oxygenation of patients with ARF.

1.3 MEASUREMENT OF LUNG VOLUME

The investigation of lung volumes was probably the earliest aspect of respiratory physiology in which the methods used by the pioneer workers are comparable to those employed by modern respiratory physiologists.

The great iatrophysicist, G.A.Borelli (1680), is accredited as being the first man to measure a lung volume. He measured his inspiratory volume and called attention to the presence of gas in the lungs after a maximal expiration. Jurin (1718), speculating on Borelli's findings, commented that inspiratory volume would vary between subjects and even in the same subject under different cicumstances. In the late 18th and early 19th centuries, many scientists were measuring lung volumes. Summarising his

observations, Goodwyn (1788) wrote: "....the lungs contain a considerable quantity of air, even after complete expiration, but this volume must vary in different subjects in proportion to the capacity of the thorax."

It was Sir Humphry Davy (1800) who, after discovering hydrogen, used it as the indicator in the first indicator dilution technique to measure what he termed "Residual Air" (now know as the residual volume, RV). Grehant (1860) applied Davy's technique to the measurement of the FRC. Grehant (1887) became interested in how the composition of gas in the lungs is renewed by the inspired gas. He realised that four factors were involved: (i) the volume of gas in the lung before inspiration, FRC, (ii) the volume of the inspiration, (iii) the volume of the inspirate that remained in the lung at the end of the next expiration and (iv) the quality of the mixing between the inspired gas and the FRC.

Indicator dilution continued to be used to measure lung volumes for the succeeding century, with hydrogen or nitrogen as the indicators. Hutchinson (1846) invented the "wet" spirometer and this enabled the accurate measurement of the differences between various lung volumes. Hutchinson's design has been changed only slightly over the years. The divisions of lung volume and their interrelationships are shown in Figure 1.1 along with a representative spirometer trace. The nomenclature has at times been confused and the terms used in this thesis are the result of efforts at standardisation made principally by Christie (1932) and the American Physiological Society (Pappenheimer et al, 1950). Figure 1.1 demonstrates how differences between lung volumes are calculated by spirometry. A less direct technique is needed to give absolute volumes because residual volume is not amenable to spirometry.

1.4 MEASUREMENT OF ABSOLUTE LUNG VOLUMES

In order to measure absolute lung volumes, a technique other than spirometry must be used. The subdivisions of total lung capacity most commonly measured are residual volume and functional residual capacity. Though it seemed to many in the late forties and fifties, that problems in the measurement of lung volume had been settled, and that lung volumes could now be used as a basis for investigations in other fields, this was not the case. Measuring lung volume quickly, simply and reliably still poses a problem today, particularly in anaesthesia and intensive care.

A plethora of methods for measuring absolute lung volumes have been described in the literature. The more important techniques have been reviewed by Christie (1932), Briscoe (1965) and Freedman (1979). There are broadly three types of method: Pneumatometric, Radiographic and Gas Exchange. The first two categories are only of passing interest in the context of this review and will be dealt with rather briefly. The last category contains many divisions, subdivisions and refinements. Only the the general principles and properties of each division will be discussed, except where the details are of direct relevance to this research.

1.4.1 Pneumatometric Methods

1.4.1a Explosive Decompression

Willmon and Behnke (1948) first used this method on six trained divers. They sealed the subjects in a compression chamber at four atmospheres and then rapidly decompressed them to one atmosphere whilst they were in the maximal expiratory position. They measured the amount of gas evolved at the mouth at the lower pressure and using Boyle's Law calculated the residual volume. (Boyle's Law states that, at any one temperature, the product of pressure and volume is a constant). Dejours and Rahn (1953) used a similar procedure on five normal subjects. The

decompression was limited to one atmosphere and lasted for fourteen seconds.

Air emboli did not develop in either study, but this technique would nowadays be considered too stressful for most healthy subjects, let alone sick patients.

1.4.1b Body Plethysmography Methods

The plethysmographic technique was originally described by Pfluger (1882) and also utilises Boyle's Law. This method is often considered the "yard-stick" against which other techniques are jugded. There are two types of Body Plethysmography namely "constant volume" and "constant pressure". The most widely used being constant volume plethysmography. This was developed mainly by DuBois et al (1956) and requires a rigid box large enough to accommodate a man. The subject breathes the air inside the box through a remotely controlled shutter. The subject is instructed to pant (with an open glottis) against the closed shutter. The pressure generated in the lung is measured at the mouth, and the resulting pressure changes within the box are also measured. When these pressures are plotted one against the other, the relative pressure changes in the lung and plethysmograph are inversely proportional to their volumes. Knowing the box volume allows the lung volume, when the shutter was closed, to be calculated. The volume calculated by plethysmography is more correctly known as Thoracic Gas Volume (TGV) since it measures gas volumes requiring no airway to the mouth.

The other technique of plethysmography is a "constant pressure" method (Mead, 1960). In this method, changes in lung volume are measured directly with a sensitive wedge spirometer attached to the box, or with a flow-meter whose output is integrated with respect to time, to give volume.

Boyle's Law only applies under conditions of isothermic gas expansion. This is the case in alveoli since they represent units of high surface area with respect to volume, and during panting the volume change is small (Briscoe and DuBois, 1958). It may not be the case for larger air spaces like cysts, bullae or pneumothoraces in which the gas expansion is likely to be adiabatic (Briscoe, 1965). Plethymography is also influenced by abdominal gas, the exact amount depending on the size of the lung volume being measured (Brown et al, 1978). The application of the technique is limited because of the size of the apparatus and because of the non-reproducibility of results obtained from different trained operators (Lord et al, 1977).

1.4.2 Radiographic Methods

There have been four approaches to measuring lung volume using radiological or other chest measurements. The first was made by Lundsgaard and Van Slyke (1918). It was based on measuring chest dimensions. When compared to a hydrogen dilution technique, it proved unable to predict lung volume although the authors claimed it was a better index for predicting absolute lung volumes than Hutchinson's previous relationship between vital capacity and height.

The second method, used by Hurtado and Fray (1933), was to measure the area of the lung by planimetry on a standard postero-anterior (PA) X-ray. They multiplied this by actual PA diameter and derived a "radiological chest volume" (RCV). This exceeded total lung capacity by about thirty percent but correlated closely enough to be used to predict TLC. Gilson and Hugh-Jones (1949) commented that, although the area on the X-ray could be measured to an accuracy of .05 per cent, the caliper measurement of the PA diameter was accurate to only .5 cm in 25 cm. Measuring the PA diameter from a lateral X-ray (Cobb et al, 1954) gave a

better agreement between RCV and TLC.

A third approach considered the lung to lie in a paraboloid of revolution around a vertical axis, the height and base diameter being taken from the chest X-ray (Kovach et al, 1956). This method gave a rather low correlation coefficient between predicted lung volume and lung volume measured by an open circuit nitrogen washout technique (see section 1.4.3b(i)).

Finally, Barnhard et al (1960) described a method which gave a good correlation with TLC measured by plethysmography. Their technique involved dividing each lung into five elliptical cylindroids, allowance being made for the spine, heart, diaphragm, blood and tissue. After a slight modification, Loyd et al (1966) found an extremely close correlation with plethysmographic TLC (r=.97). A quicker method which made fewer allowances gave almost as good ~~good~~ agreement (Reger et al, 1972).

These techniques are rather time consuming and have not been used a great deal. However, with the advent of computerised axial tomography (CAT), there could be a revival of interest in radiological methods, since these are likely to become faster, more accurate and possibly semi-automated.

1.4.3 Gas Exchange Methods

With two notable exceptions, the gas exchange methods for estimating lung volume measure the volume of distribution of insoluble inert gas or gases. The first exception was the use of oxygen as an indicator in a rebreathing technique (Suwa and Bendixen, 1971). This is of central importance to this thesis and will be discussed subsequently at greater

length. The second exception was in the measurement based on breath by breath net capillary gas exchange for oxygen (Swanson, 1980). This will also be discussed later. Mitchell et al (1982) have recently described an "oxygen wash-in" method for measuring FRC. However, this is effectively a "nitrogen wash-out" technique using the measured O_2 and an assumed CO_2 concentration to calculate the N_2 concentration by subtraction.

The inert gas techniques consist of the closed circuit and open circuit methods. In closed circuit methods, a gas mixture in a spirometer or bag is rebreathed, starting from the lung volume to be measured. The contents of the lung and bag/spirometer are mixed by the rebreathing. Knowing the bag/spirometer volume and the dilution of the inert gas into the whole system, the initial lung volume can be inferred.

Open circuit methods impose a square wave change in the inert gas composition of the inspiratory gas mixture, while the subject breathes through a system of inspiratory and expiratory valves. The volume and inert gas concentration of several expirates or a single expirate is determined. The total amount of inert gas washed out of, or into, the lung is derived. Combining this with the change in lung inert gas fraction gives the initial lung volume.

1.4.3a Closed Circuit Techniques

1.4.3a(i) Forced Breathing

This was first described by Davy in 1800. He used the dilution of hydrogen from a balloon into his lungs to estimate his residual volume. Using just one or two deep respirations from the balloon, Davy deemed the lung/balloon to be system mixed. He was so confident of the rapid mixing of the inspired gas with the residual air that he never tested this assumption: "For if portions of hydrogene were found in the airholder equal to those in the residual gas in the two experiments, it would prove

that a 'uniform' mixture of residual gas with the gas inspired was produced by respiration. That such a mixture must have taken place, appeared however, so evident from analogous facts, that I judged the experimental proof unnecessary." In the succeeding century many investigators (see Christie, 1932) used the hydrogen dilution method, but most felt that between five and seven breaths were required for mixing. They therefore diluted the hydrogen in air to prevent anoxia.

In the oxygen dilution technique of Lundsgaard and Van Slyke (1918), the principle was the same as that described above except that the gas rebreathed was pure oxygen and the inert insoluble gas, whose dilution was measured, was the nitrogen initially present in the lungs. These investigators were aware of problems encountered by others in producing uniform mixing in the bag/lung system. Grehant (1864), Bohr (1907) and Siebeck (1911) had found good mixing after about five breaths. By contrast, Sonne (1918) and Roelsen (1938) had found a non-uniform bag/lung system even after five breaths. This was also observed by Krogh and Lindhard (1913) but to a lesser extent. Lundsgaard and Van Slyke (1918) found no systematic changes in the lung volume calculated with prolonged rebreathing. They recommended five to seven deep breaths but found in practice that "almost constant values of nitrogen in the rubber bag after four or five respirations". This method was used extensively to measure RV in health and disease. Lundsgaard and Schierbeck (1924) studied the method in more detail by measuring the expired nitrogen concentration at each successive maximal breath. The nitrogen concentration fell "parabolically" to the fourth breath, because of mixing, and then slowly rose again and was still rising between the 25th and 30th breaths. They interpreted this accretion of the nitrogen fraction as being due to the diminution of the rebreathing volume as a result of the retention of carbon dioxide in the body (the spirometer contained no CO_2 absorber) accompanied by continued

oxygen uptake. Thus, the system volume diminished and so concentrated the nitrogen. Lundsgaard and Schierbeck also showed that the nitrogen composition of the bag and lung, after mixing, were not the same. This was because the nitrogen was being continually concentrated in the lung and this was transmitted to the bag only via the tidal ventilations. The changes in bag nitrogen composition must, therefore, lag behind those in the lung (see Chapter 6, section 6.2.2a). They concluded that these manifestations could give the impression of incomplete mixing but that they were artifactual. However, in a rebreathing system although mixing may be as complete as possible, the system is never completely homogeneous and a dynamic equilibrium exists between the bag and the lung. Lundsgaard and Schierbeck maintained that, if the minimal nitrogen concentration is taken as the mixed concentration, then lung volume can be calculated.

In an excellent article, Rahn, Fenn and Otis (1949) described a modification of the Lundsgaard and Van Slyke technique. They were the first workers to fully appreciate, and attempt to quantify, the effect of forced rebreathing on the respiratory exchange ratio (R or RQ). They stated: "During the early stages of rebreathing the CO_2 tension is much below normal and CO_2 must be coming off into the lung at a very high rate. The volume of the lung-spirometer system must therefore be actually increasing rather than decreasing during the first few breaths.... Thus the RQ must be well above unity in the first few seconds .... but rapidly diminishes to abnormally low values owing to the large storage capacity of the body". They appreciated that the equation for enumerating lung volume incorrectly assumed a constant rebreathing volume. They went on to show that the system volume returned to its original volume (in their study and in normal subjects) after 3 breaths (9 seconds) so that the net volume change in a sample taken at this time was negligable. Thus, Rahn et al modified the Lundsgaard and Van Slyke technique by taking the mixed sample

after the third breath rather than the fifth. They made a further modification which was to use 80 per cent as an estimate of the initial alveolar nitrogen (in fact the inert gas fraction) and not 79.1 per cent by volume (% v/v), as this could only occur if the R was unity. This technique has been widely used, particulary in field conditions, because it is simple to perform and uses portable equipment. The disadvantage is that it is an empirical approach to a problem and is valid only under restricted conditions. It is invalid, for example, if mixing continues after the isovolumetric point.

In 1974, Nunneley et al modified the Rahn technique by introducing a second inert gas (argon) as the gas present originally in the bag. By using the concentration ratio of the two inert gases, lung volume could theoretically be calculated independently of any volume changes due to carbon dioxide and oxygen exchange. This technique is directly analogous to the one described by Van Slyke and Binger in 1923. This will be discussed in the next section. By being able to monitor all the gases continuously, Nunneley et al were able to show that this technique was sensitive to the small exchanges of the inert gases with body stores. They tried, unconvincingly, to rectify this phenomenon by including a rather contrived correction factor for inert gas exchange.

In 1971 Suwa and Bendixen, in a short abstract, suggested a modification to the "oxygen dilution" method of Lundsgaard and Van Slyke (1918) by using oxygen as the indicator. This technique had previously been used by Cerretelli et al (1970) to estimate lung volume during a rebreathing determination of mixed venous oxygen. Suwa and Bendixen recorded the concentration of oxygen at the mouth with a rapid oxygen electrode whilst the subject forcibly rebreathed from a bag containing 1.4 litres of oxygen. After rebreathing the system contained less oxygen than

at the start. They ~~that~~ suggested that a linear extrapolation of the latter mixed portion of the oxygen concentration trace to the initial instant would allow a mixed concentration independent of oxygen uptake to be estimated. The advantages of this approach lie in its simplicity and its potential applications in intensive care and anaesthesia. This technique provided the impetus for the lung volume studies to be described in this thesis.

1.4.3a(ii) Quiet Breathing

The forced breathing methods, outlined above, may be suitable for healthy subjects but in some sick patients the requirement for rapid mixing cannot be met. This is due either to impaired mixing within the patient's lungs, or to the inability to tolerate the exertion required to achieve mixing. These factors preclude the use of such techniques in many cases where the measurement of lung volume would have been clinically useful. This prompted Van Slyke and Binger (1920, 1923) to develop the first quiet breathing technique. They allowed five minutes for rebreathing with normal respirations in order to achieve mixing. Carbon dioxide absorption was clearly necessary so that rebreathing could continue for long periods without discomfort. Their very elegant technique does not measure the dilution of one gas but the volume ratio of two: a foreign inert gas, present initially in the bag/spirometer (hydrogen) and a resident inert gas (nitrogen). This provides enough information to calculate lung volume, in the presence of a diminishing rebreathing volume, without the need for spirometry. The principles involved will be examined in more detail in the Chapter 6 (section 6.2.2b).

Christie (1932), when reviewing methods of lung volume measurement, commented that the Van Slyke-Binger technique was: "a very definite advance in the technique of lung volume measurement..... and it is suprising how few have availed themselves of this excellent method." Christie himself did

use the method. He considered that the error due to nitrogen elimination and hydrogen absorption into the blood would be 150 - 200 ml. However, Christie didn't regard this as a drawback. He also believed that careful experimenters were not at risk when using hydrogen, which forms an explosive mixture with oxygen and could, in those days, have been contaminated by arsenic during production. However, since these risks had discouraged the use of the Van Slyke-Binger technique, Christie set about modifing the method.

About the same time Sendroy, Hiller and Van Slyke (1932) also concluded that there was a need for a quiet breathing technique which did not require hydrogen. The reduction in system volume was measured directly using a spirometer. This was essentially the same as Christie's technique and the two groups published their findings independently in the same year. In a footnote in his classic article of 1932, Christie criticised Sendroy et al (somewhat churlishly, perhaps) for not providing for maximal reproducibility by familiarising their subjects with the apparatus, and for not making use of spirometric measurements. In consequence, it is Christie who is historically remembered for the introduction of the technique.

Christie recognised the discrepancy between inspired and alveolar nitrogen concentration which arose when R was less than unity (Lundsgaard and Schierbeck, 1924). He considered this process to be essentially the same whether breathing air or rebreathing from the spirometer. Christie reasoned, that since the initial alveolar nitrogen was larger than the nitrogen concentration of air, the final lung nitrogen (after rebreathing) would be larger than the final spirometer nitrogen concentration. By measuring only the initial inspired and the final spirometer concentrations, Christie believed that these errors would nullify one another.

It is interesting to note that this effort to eliminate the use of hydrogen from the original quiet breathing technique (Van Slyke and Binger, 1923) was preceded by a technique which eliminated nitrogen. In Germany, Knipping (1926) had developed a simple electrometric analysis of hydrogen. Anthony (1930), who was aware of the advantages which a quiet breathing technique had to offer, exploited this advance in another version of a quiet breathing technique. He used spirometry to measure the system volume change, and hydrogen as the indicator gas. Aside from the ease of gas analysis, the use of hydrogen offered a further advantage. Since hydrogen was initially absent from the lung, there was no uncertainty about the initial alveolar fraction.

Nevertheless, it was Christie's technique which became the most widely used for the clinical measurement of lung volume, particularly in the USA. It had the advantage of using standard equipment and gas (O_2) and was safe enough for use with patients. In 1937 Lassen, Cournand and Richards provided an important extension of the discovery by Lundsgaard and Schierbeck (1924) of the non-homogeneity of a bag/lung system. They observed that, after "mixing", the spirometer nitrogen on inspiration was consistantly <u>higher</u> than the alveolar nitrogen on the subsequent expiration. This rather suprising finding seemed to imply that nitrogen <u>dilution</u> rather than nitrogen <u>concentration</u> was taking place in the lung. This was at variance with normal concepts of gas exchange. It was explained in terms of a "nitrogen lag effect" where the diminishing system oxygen caused an elevation in the nitrogen fraction, such that the inspired nitrogen concentration never remained constant, but was rising. This rising inspired nitrogen, it was argued, was transmitted to the next expirate after dilution throughout the FRC. The FRC contained gas from the previous expiration (which was of a lower nitrogen concentration than the

inspirate) so that the rise in expired nitrogen lagged behind that of the inspirate. This arguement seems to skirt round the essential feature which is that the inspired nitrogen concentration was larger than the nitrogen concentration in the previous expirate. The reason for this was the presence of a carbon dioxide scrubber in the spirometer. The removal of carbon dioxide reduced the expired volume which concentrated the nitrogen present within the spirometer. The volume shrinkage due to carbon dioxide absorption would be equivalent to the CO_2 output and this would have exceeded the shrinkage which took place in the lungs, due to the excess of oxygen uptake over carbon dioxide output. Thus, the nitrogen in the spirometer became more concentrated than that in the lungs and, therefore, than in the previous expirate. The "nitrogen lag effect", also known as the "oxygen storage effect" (Gilson and Hugh-Jones, 1949), was the result of CO_2 absorption in the spirometer causing the inspired gas to have a higher nitrogen fraction than the gas within the lungs. The nitrogen content of the subsequent expiration was then derived from nitrogen in the inspired and resident lung gas and, depending on the pulmonary gas exchange, was a weighted average of these two concentrations (ie. more than the previous expirate and less than the inspirate). Mathematically, the situation is extremely complicated and the degree of "nitrogen lag" will depend on a number of variables, including FRC, tidal volume, respiratory quotient and spirometer volume. This is considered more fully in the Chapter 6 (section 6.2.2a).

The importance of the observation of Lassen et al remains that the relationship, assumed by Christie between the inspired and expired gas concentrations, was shown to be fundamentally wrong purely because of the absorption of CO_2 in the spirometer.

Lassen et al's modification of Christie's technique was to measure alveolar nitrogen before and after rebreathing, along with the final

spirometer concentration. This entailed two additional gas analyses. Since the gas analysis was by chemical methods, it added considerably to the time taken for a lung volume determination, and effectively made the technique impractical for routine purposes. Lassen et al used their technique to demonstrate that Christie's method could overestimate lung volume by several hundred millilitres, especially if the ratio of lung volume to tidal volume was large.

The realisation that this erstwhile standard technique could be seriously in error initiated three responses. Firstly, there were attempts to develop a closed circuit technique in which the volume of the rebreathing system was kept constant. This began the last stage towards the development of the helium rebreathing technique which is a standard for lung volume measurement today. The English investigators, Herrald and McMichael (1939), first sucessfully developed the constant volume technique. The New York researchers, under Cournand, tried using a constant volume technique with limited success (Cournand et al, 1940), probably because of poor mixing in the spirometer (Briscoe, 1965). The second response, initiated by Cournand and his associates, was to design an entirely new technique in which the experimentally induced change in nitrogen concentration were maximal and the inspired concentration remained constant. This was the "open circuit nitrogen washout technique" (Darling et al, 1940b).

A third response will now be discussed. This came from a body of opinion which felt that the "quiet breathing" approach was not discredited because they either believed that the technique was adequate in a clinical enviroment or could not demonstrate the "nitrogen lag" effect. Anthony (1938), who was using an analogous method to Christie, with hydrogen as the indicator gas, was unable to demonstrate an equivalent "hydrogen lag effect" in his experimental set up. Anthony understood the implications of

carbon dioxide absorption in concentrating the spirometer gas. He also realised that the balance between this and concentrating of nitrogen within the lungs, owing to gas exchange, was very important. He reasoned that a larger system volume would reduce the effect of carbon dioxide absorption in the spirometer and push the balance in favour of the lungs. He concluded that, by using a spirometer with a volume almost twice the four litres used by Lassen et al (1937), and by assisted mixing in the spirometer, he had avoided the errors of the "oxygen storage effect". Birath (1944) subsequently used Anthony's method to study lung volume and mixing efficiency in connection with Pulmonary Tuberculosis therapy. Kaltreider et al (1938) believed the magnitude of the "oxygen storage effect" was negligably small. Their spirometer capacity approached eight litres. They believed that the increased accuracy which would result from collecting alveolar gas samples (as Lassen et al, 1937, had suggested) would not compensate for the problems of this collection, especially in sick patients. They believed the Christie approach was adequate for clinical purposes. Wolfe and Carlson (1950) also favoured the more practical to the more correct approach and wrote: "... clinical adaptability rather than maximum accuracy was the goal in this study ...". Again the spirometer volume was larger than that of Lassen et al, being in excess of ten litres. In the interests of simplicity, Meneely et al (1960) returned to Christie's approach. Once again, using a spirometer volume in excess of eight litres, Meneely et al were able to demonstrate the "oxygen storage effect" but argued that its magnitude did not alter the clinical interpretations. They concluded that an undue amount of effort had been directed towards a more exact measurement of lung volume and had resulted in an over-complex methodology without a corresponding improvement in the quality of results. The effect of this being to make the estimation of lung volume so laborious as to deter its use as a clinical tool. Interestingly, Meneenly had earlier been involved in refining one of the

more meticulous methods, the aforementioned "constant volume technique" (see below).

It would seem that those studies, which found the Christie style technique sufficiently accurate, all use substantially larger spirometer volumes than did Lassen et al. In a later theoretical study (Chapter 6, section 6.2.2a), it will be seen that larger spirometer volumes reduce the nitrogen lag as Anthony predicted.

The constant volume technique was developed by Herrald and McMichael (1939). They obviated the objection to Chrisie's technique by maintaining a constant system volume. They added enough oxygen to compensate for the oxygen absorbed in the lung. As the initial alveolar nitrogen fraction was assumed, only one gas sample was analysed. This was the final mixed N_2 concentration. The analysis was by chemical means. They also used a pump to ensure complete mixing in the spirometer and a katharometer to assess the completion of mixing. In a further improvement, McMichael (1939) employed the foreign gas indicator technique of Anthony (1930) in the guise of a constant volume technique. Using hydrogen as the indicator was ideal since its very high thermal conductivity allowed it to be analysed very accurately by the katharometer. This greatly simplified the method and, since there was no hydrogen initially in the lungs, no assumptions needed to be made about the initial alveolar fraction.

When helium became available from fractionated air, Meneely and Kaltreider (1941 and 1949) and Gilson and Hugh-Jones (1949) separately modified the McMichael method using helium instead of hydrogen. Since helium has about the same thermal conductivity as hydrogen, is very unreactive chemically and is the most insoluble inert gas known, it is ideal.

By this time, physical methods of analysis had been established for

many gases. This allowed gas mixing to be recorded continuously and used to assess ventilatory efficiency (Birath, 1944). Bates and Christie (1950) used the new helium closed circuit method to study mixing efficiency. A new era of gas mixing studies had begun.

1.4.3b Open Circuit Techniques

1.4.3b(i) Multiple-Breath Washout

This method was originally devised by Cournand and associates (Darling et al, 1940b) as a result of their objections to Christie's closed circuit technique (described earlier). The inert indicator gas, nitrogen, was present initially in the subject's lungs. Assuming an initial lung nitrogen of 81% v/v produced errors of less than 100 ml (Cournand et al, 1941). The subject inspired pure oxygen via an inspiratory valve for seven minutes and all the expirate was collected in a Tissot Spirometer. The volume of nitrogen in the spirometer after seven minutes was measured along with the final alveolar nitrogen fraction. Originally, the intention was to exclude the last alveolar sample and assume complete washout of nitrogen. It was retained, however, since a final alveolar concentration of over 2.5% v/v was found to be a helpful indication of pathological unevenness of ventilation. A correction was made for the nitrogen excreted from the blood (Darling et al, 1940a) and this became the method of choice in American Hospitals for the following twenty years. Gilson and Hugh-Jones (1949) found that the standard error of duplicate measurements was 186 ml, which was slightly higher than the closed circuit helium dilution technique which was 164 ml.

Uneven Ventilation

In order to understand the objections to using this technique in certain patients, it is necessary to digress to consider some of the early

work which developed the concepts of uneven ventilation.

In all branches of science, a technical innovation can open a new avenue of investigation. This is as true of respiratory physiology as of any other experimental discipline. Up to about 1940, all gas analysis was performed by chemical methods and all volumes were measured by spirometry. With the advent of more rapid physical methods of gas analysis and with the development of a variety of flow transducers, a multitude of physiological and clinical studies were initiated. A method for rapid hydrogen analysis was developed in 1940 (Aschoff et al), for nitrogen analysis in 1943 (Lilly and Anderson) and one for oxygen in 1946 (Pauling et al). These and other methods of gas analysis made the collection of data less time-consuming than before, so that the interest shifted to a more mathematical treatment of the results, using comparmentalised models of gas exchange. This trend continues today, with the application of respiratory mass spectrometers and microcomputers to the collection and analysis of data. With the arrival of new physical methods of gas analysis (reviewed by Lilly, 1950), came the ability to follow the washout of lung nitrogen (Fowler et al, 1952; Hickam et al, 1958) or previously inspired helium (Hickam et al, 1954; 1958) continuously and in much greater detail than before. It became clear from these studies that emphysematous lungs, in particular, contain what Hickam called a "slow space".

The slow space is made up of alveoli which are very poorly ventilated with respect to their volume and, as a result, gas exchange in these alveoli is retarded in comparison to better ventilated alveoli (fast space). In Figure 1.4 this effect is shown by the presence of two major exponential components in the washout curve. This figure shows how in an emphysematous patient the slow space constituted 74 per cent of the FRC but only contributed 24 per cent of the gas at the end of a forced expiration. In the Cournand technique (Darling et al, 1940b), a final end-tidal sample is used to estimate the amount of nitrogen remaining in the lungs. Ideally,

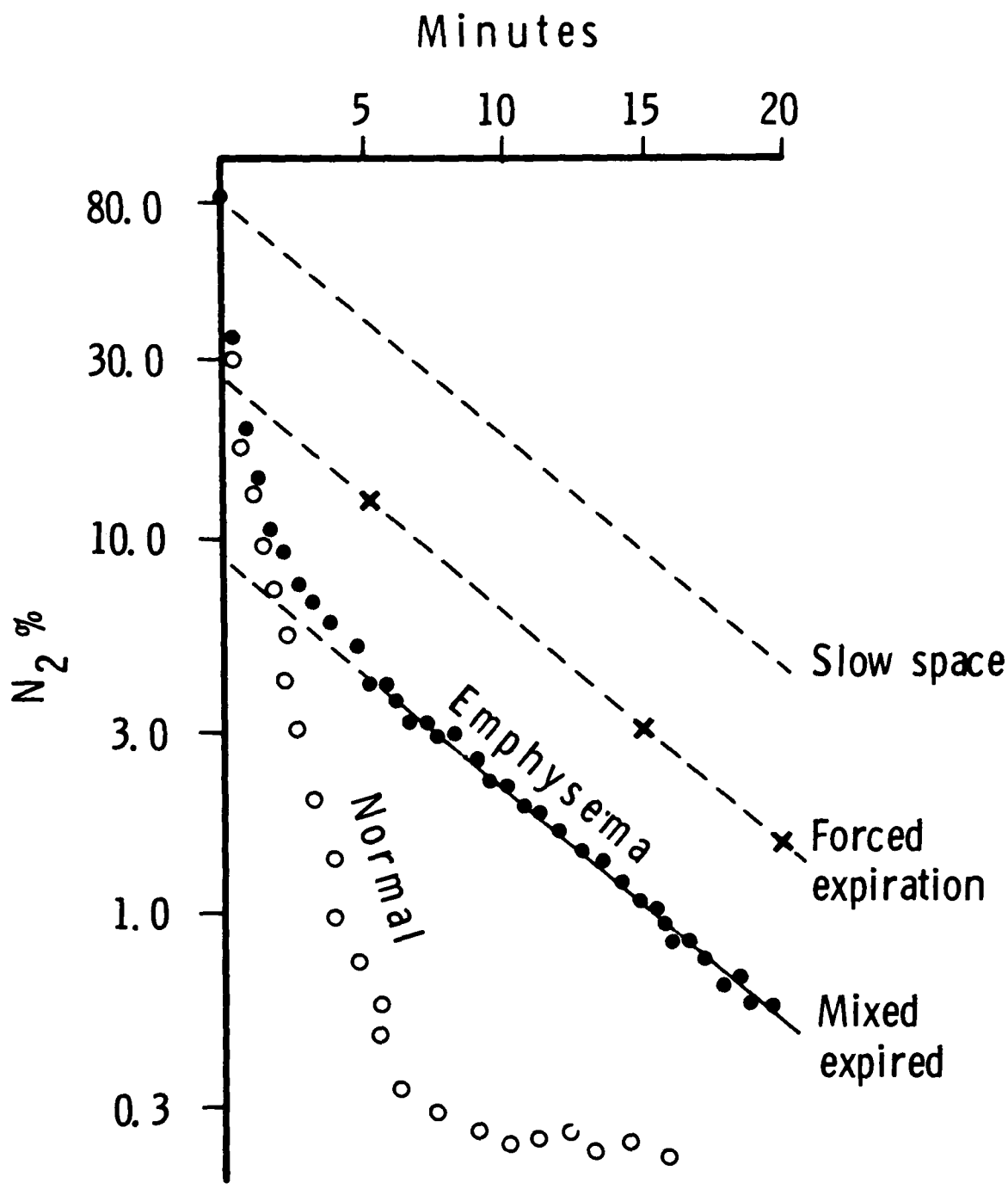


Figure 1.4 Nitrogen washout curves whilst breathing oxygen. ●: are mixed expired nitrogen concentrations of an emphysematous patient. The parallel lines indicate the concentration at the end of a forced expiration and the concentration of the slow space. ○: show data from a normal subject.
(Redrawn from Briscoe, 1965: Fig 3)

this would be an alveolar sample weighted by the volumes containing a given nitrogen concentration. In fact, it is an alveolar sample weighted by the ventilation received by each alveolus. Thus, in emphysema, the forced expiration grossly underestimates the mean alveolar nitrogen concentration. It tends to contain more gas from better ventilated alveoli and this causes the seven minute washout method (Darling et al, 1940b) to underestimate the lung volume in the presence of a slow space.

Emmanuel et al (1961) showed the effect of a slow space on Cournand's technique (Darling et al, 1940b) and devised a method to predict more accurately the final mixed alveolar nitrogen. They collected mixed expired samples every half a minute and measured the volume and concentration of each sample before combining them. This allowed them to follow the trends in the washout. Sampling was continued for fifteen minutes before the amount of nitrogen left in the lungs was predicted by extrapolating the slow component to estimate the amount of nitrogen that would have been washed out if the procedure had been continued indefinitely. This technique gave an FRC which was on average 32 per cent larger than that of Darling et al (1940b) when applied to the same data from patients with chronic pulmonary emphysema. Bedell et al (1956) had found a similar size of discrepancy between the body plethysmographic method and that of Darling et al when applied to emphysema. This led Emmanuel et al (1961) to speculate that both their method, and the body plethysmograph, were measuring the same lung volume, that is thoracic gas volume. Tierney and Nadel (1962) found that the prolonged nitrogen washout of Emmanuel et al, and body plethysmograph gave the same lung volumes in the same emphysematic patients and that these were different to the nitrogen washout technique (Darling et al, 1940b). This work contradicted the theory that, in emphysema at least, there are areas of trapped gas which can be measured by plethysmography but not by washout techniques (Bedell et al, 1956). Thus suggesting that such areas do take part in gas

exchange but some only very slowly or intermittently.

The disadvantages of the multiple breath nitrogen washout are that it is time consuming, that one has to estimate the nitrogen eliminated from the body and that the analysis of the mixed nitrogen concentration is extremely sensitive to errors. In an FRC of three litres, for example, an absolute error of .05% v/v in the determination of the mixed nitrogen fraction, would lead to 300 ml error in FRC estimate.

1.4.3b(ii) Single-Breath Methods

Interest in single breath methods of measuring lung volume was revived when the use of single-breath nitrogen tests to detect uneven ventilation, first described by Fowler, W.S (Fowler, 1949; Comroe and Fowler, 1951), gained widespread clinical popularity in the late 1960's and early 1970's (Mitchell and Renzetti, 1968; Buist and Ross, 1973; Rodarte et al, 1976). In this technique, the subject inspired a breath of pure oxygen from residual volume to vital capacity and slowly breathed out to residual volume again. The nitrogen concentration, recorded at the mouth, was plotted against the expired volume. The volume expired before any nitrogen was detected was taken to be the anatomical dead space. This allowed the volume of the alveolar component of the expirate to be calculated and, thus, the mean alveolar nitrogen concentration was estimated. The lung volume was calculated from the dilution of the initial lung nitrogen fraction by a known volume of oxygen to give the subsequent mean alveolar nitrogen concentration. This technique tended to underestimate lung volume because poorly ventilated spaces contributed little to the expired gas.

This test may also be performed by a single inspirate of any inert gas. The use of helium to measure lung volume is incorporated into the standard single breath carbon monoxide transfer test (Ogilvie et al, 1957) which is a routine clinical measurement of pulmonary diffusing capacity.

1.4.3b(iii) Breath-to-Breath Capillary Gas Exchange

Breath by breath gas exchange as measured at the mouth is buffered from the gas exchange taking place at the capillary level by the FRC. In order to estimate the capillary gas exchange, a correction for the breath by breath changes in the volume of gas "stored" in the lung, at end-expiration, has been used (Auchincloss et al, 1966). Swanson (1980) has shown that it is possible to calculate an effective lung volume (ELV) which minimises the breath-to-breath variations in capillary gas exchange. This assumes that the principal cause of these variations is indeed changes in end-expired volume which vary randomly about some mean value, FRC. Dejours et al (1966) believed that the varations in breath-to-breath gas exchange were the result of changes pulmonary blood flow and composition. The ELV is a nominal value for the volume of distribution of a gas which effectively participates in breath-to-breath gas exchange. It includes gas dissolved in tissue and in the pulmonary capillary blood volume. For oxygen, the ELV will be slightly larger than the FRC because of the low solubility of oxygen in tissue and plasma. Although there is a large amount of oxygen bound to haemoglobin, the characteristics of oxy-haemoglobin dissociation mean that, at non-hypoxic alveolar oxygen tensions, this oxygen is inaccessable to the pulmonary store. The physical solubility of carbon dioxide in tissue and plasma is over twenty times greater than that of oxygen and this is reflected in larger pulmonary stores for carbon dioxide.

Measurement of breath by breath uptakes/outputs of various inert gases would seem to offer an elegant method of studying some of the pulmonary variables which have been traditionally studied by rebreathing or single breath techniques. This approach requires simultaneous flow and concentration measurements with computer data collection and analysis. The attraction of this method is the lack of a perturbation in making the

measurements, so that it is less likely that the variable one wishes to measure will be disturbed. Stout et al (1975) have shown that this technique can be used to yield estimates of pulmonary blood flow and lung tissue volume, by the analysis of any two breaths, with respect to the uptake of nitrous oxide compared to the washout of nitrogen.

1.5 MEASURING FRC DURING ANAESTHESIA

Though methods of measuring lung volume are available, none are particulary convenient for use under anaesthesia. Body plethysmography, though technically difficult, has been applied to anaesthetised and artificially ventilated patients and subjects (Don et al, 1972; Westbrook et al, 1973; Hedenstierna et al, 1981). The main disadvantage of the technique is that it physically obstructs access to the patient. The nitrogen washout and rebreathing/indicator dilution techniques depend on gas exchange. They measure the volume of the lung participating in gas exchange. Although this may be less than the total gas volume in the lungs, it is the volume which is being adequately ventilated and thus most important to the anaesthetist. The nitrogen washout technique has been used successfully in artificially ventilated patients (Paloski et al, 1981; Suter et al, 1981). The method cannot be used during nitrous oxide anaesthesia, because of the need to have a high lung nitrogen fraction. The conventional rebreathing/indicator dilution technique, using helium as the indicator, has been adapted for anaesthesia, (Dery et al,1965; Colgan and Whang, 1967; Laws, 1968; Dobbinson et al, 1973; Hewlett et al, 1974). This demands considerable complexity in the anaesthetic circuit and in the practical technique. The rebreathing system incorporates a CO_2 absorber and the volume of the system is kept constant by titrating the addition of gas to the system against the end-expiratory excursion of a spirometer. If nitrous oxide is used in the anaesthetic, as is commonly the case, it is

desirable to limit the exchange of N_2O between the blood and the rebreathing system by keeping the N_2O partial pressure in the system close to the estimated mixed venous partial pressure. Superimposition of the requirement for artificial ventilation imposes technical complexities which, though not insuperable, have prevented the more widespread application of this technique to anaesthesia.

2.1 INTRODUCTION

Mass spectroscope determinations were first employed in the detection of stable isotopes, the discovery of which provided an explanation for the fractional atomic weights which had been observed (Richards, 1910). Sir J. J. Thompson, in 1912, demonstrated the isotopic nature of neon using "positive ray" analysis. In 1913 Aston constructed a new positive ray apparatus which he called the "mass spectrograph". He used this to determine accurately the mass of isotopes. Shortly after this development, Dempster (1918) constructed an instrument which was fundamentally simpler than Aston's but well suited to measuring the abundance of a particular mass species. This he called a "mass spectrometer". Henceforth, there was to be a division of work on mass analysis between those physicists interested in the measurement of mass, and those interested in measurement of abundance. Dempster's mass spectrometer was adapted and modified and by the end of the thirties it had evolved considerably.

Instruments designed for gas analysis were used primarily in the petrochemical industry for analysis of hydrocarbon mixtures (Leck, 1979), and in research for detection of "marker" isotopes and trace impurities (Nier, 1948). Early mass spectrometers were of the "magnetic sector" type. The sample was presented to the analyser in a sealed low pressure reservoir from which a small amount passed into the ionisation chamber by means of a "leak". The ionisation chamber was highly evacuated and the sample was ionised by bombardment with electrons. The ions produced were accelerated out of the ionisation chamber in the form of an ion beam. This beam travelled through a magnetic field, which was at right angles to the ion beam's direction of travel. This magnetic field deflected the ions causing them to travel through an arc. The radius of the arc was dependent on the mass and charge of the ion species. The beam of ions was spread out in space, forming a mass spectrum. By judicious placement of an ion

collector, the current carried by a particular ion species could be measured and this was proportional to the concentration introduced for ionisation.

2.2 RESPIRATORY MASS SPECTROMETERS

Mass spectrometers had certain characteristics which made them attractive to respiratory physiologists. They were specific, consumed small quantities of gas and could potentially measure several components simultaneously. In order to be used for respiratory work, the mass spectrometer was required to analyse a concentration signal which fluctuated with time. This meant continuously drawing the sample gas into the detector, thus depriving the instrument of a steady state, which was achieved in industrial applications. Continuous sampling adversely affected the performance of the mass spectrometer. However, the performance required for a respiratory instrument was many orders of magnitude less than that of some other applications. In 1947 Siri built the first respiratory mass spectrometer. The sample gas was drawn, with a pump, across a needle valve which acted as the leak. By varying the accelerating voltage 200 times a second, Siri could scan the various ion beams across a single collector. The output was displayed on an oscilloscope and provided a pseudo-continuous mass spectrum. In 1949 Hunter et al, using a similar inlet system, described a different method for simultaneous analysis of several components. They modified a commercial industrial machine by providing individual collectors for nitrogen, oxygen and carbon dioxide. This provided truly continuous analysis. Then, in 1952, a single channel industrial instrument was modified and used for respiratory studies by Comroe.

The first satisfactory respiratory mass spectrometer was designed and

built by Fowler, K.T. (Fowler and Hugh-Jones,1957). He used a fine capillary in conjunction with a "molecular leak" for the inlet system, and this has become the standard inlet system. Fowler built his mass spectrometer to conform to a list of specifications given to him by a group of clinical investigators and respiratory physiologists. These have served as the guide lines for the subsequent development of these instruments:

1. Continuous analysis of inspirate and expirate from the mouth or bronchial tree. In fact, Fowler's machine scanned its mass range 25 times a second, which is near enough to continuous for most applications in applied physiology (West, 1967).
2. A mass range suitable for simultaneous analysis of at least four respiratory gases, including oxygen, nitrogen and carbon dioxide. Fowler (1969) later suggested the range between 15 and 50 atomic mass units (amu) was adequate.
3. Provision of analyses in the form of meter deflections and/or a continuous writeout.
4. Sampling at a rate of no more than 15 ml/min. West (1967) considered this rather too stringent for sampling at the mouth, and Fowler (1969) revised this to 50 ml/min.
5. Complete 90% of the response to a step change in concentration in a tenth of a second or less. This has proved fast enough for the most demanding respiratory work (Fowler, 1969).
6. Sufficient stability to detect 1% changes in concentration.
7. Transportability, requiring only electricity to run. This was accomplished because second world war advances in vacuum technology enabled Fowler to dispense with the running water, liquid nitrogen or dry ice required in older vacuum systems.

The applications for mass spectrometry in respiratory physiology have been discussed by Hugh-Jones (1960), West (1967), Mosharrafa (1970) and

Bushman (1975). Since Fowler, a great many strides have been taken to develop respiratory mass spectrometers. There are three types of machine which differ in the method used to separate the ion beam:- the traditional magnetic sector machines, time of flight machines (discussed by Harrington, 1960) and quadrupole machines. It is the last type of machine that is of interest in this thesis, and the Centronic 200 Medical Gas Analyser (MGA 200, Twentieth Century Electronics L.T.D., Croydon) in particular. Consequently, the workings of this type of machine will be examined with regard to the fate of gas molecules from their passage in the inlet to their detection and the subsequent registering of their concentration. Before this, it is necessary to review some of the properties of gas flow, particularly under the conditions prevailing in mass spectrometry.

2.2.1 Gas Flow

The "mean free path" of a gas molecule is the average distance it can travel without colliding with another gas molecule. If this is small, then the effect of intermolecular forces will dominate the gas mixture's properties, each individual gas species contributing to the viscosity and density of the mixture. As the pressure falls, the mean free path increases and the probability of intermolecular interactions is reduced. Thus, the laws governing gas flow depend upon pressure. Broadly speaking, at pressures above 1 mmHg, gas flow is "viscous" whilst, below 10^{-3} mmHg it is "molecular". Between these two pressures, it is "transitional" (partly viscous and partly molecular) (Buckingham, 1979).

2.2.1a Viscous Flow

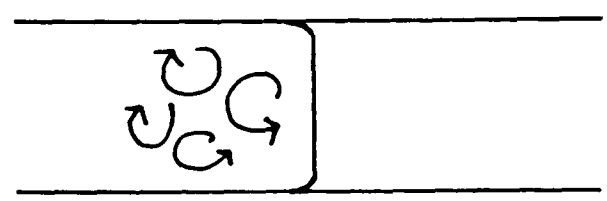
Gas flows from a region of high pressure to a region of lower pressure, the rate of flow depending on the pressure difference and the resistance to flow. At a high flow velocity in tubes which have a large diameter compared to their length, flow is commonly "turbulent". In

turbulent flow, individual molecules have irregular movement superimposed on their progression along the tube. As a result, the concentration front, between a new gas introduced into the tube and the old gas, is square (Figure 2.1a). The resistance of a gas flowing turbulently through a tube of given dimensions depends on the flow rate and the gas density. In tubes in which the flow velocity is moderate and the radius small compared to the length, the flow of gas is "laminar". Laminar or streamline flow was independently demonstrated by Hagen (1839) and Poiseuille (1840). They showed that the resistance to flow for a fluid under these conditions, in a tube of given dimensions, was governed only by the viscosity of the fluid. Therefore, for a constant pressure gradient, the quantity of gas flowing through a tube in unit time is inversely proportional to the viscosity of the gas. The viscosity can be thought of as the resistance of molecules to movement relative to one another, caused by intermolecular attractive forces. In laminar flow the gas molecules travel down the tube in concentric cylindrical layers. These layers slide over one another and the resistance to shearing between the layers is the viscosity. The central annulus travels at twice the mean flow velocity, and the rate of flow decreases closer to the walls, such that the velocity front is parabolic (Figure 2.1b). Thus, if a square wave front of concentration change enters one end of the capillary, the front becomes more parabolic as it moves towards the other end.

In rough or branched tubes, turbulent flow becomes more likely. However, in smooth tubes (such as mass spectrometer capillary inlets), the nature of the flow can be predicted by calculating the non-dimensional Reynold's number. The Reynolds number is given by:

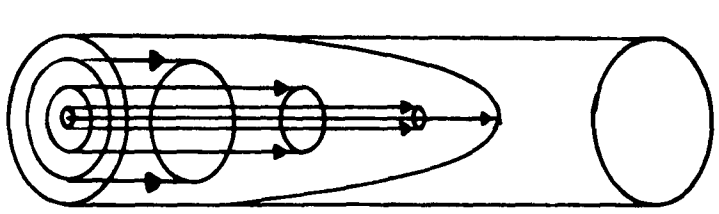
$$\text{Reynolds' No} = \frac{\text{mean linear velocity of gas x tube radius x gas density}}{\text{gas viscosity}}$$

For Reynolds' numbers over 1500, the flow is turbulent. Below 1000 it is



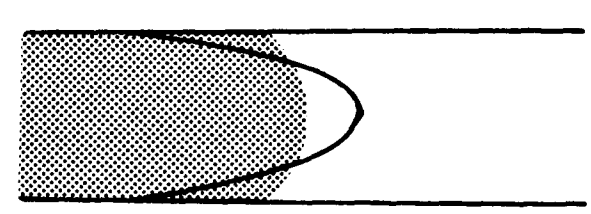
(a)

TURBULENT FLOW



(b)

LAMINAR FLOW



(c)

TAYLORIAN DISPERSION

Figure 2.1 The downstream concentration profiles which result from a step change in concentration upstream. This is shown for three flow conditions. The arrows indicate the movement of gas molecules.

laminar (Cooper, 1961). Between these two, both types of flow co-exist. Under conditions of laminar flow, Taylor (1953) showed (using liquids) that if, the axial advance is slow and the tube diameter is small (capillary), the usual parabolic boundary is ablated by radial diffusion (Figure 2.1c). Therefore, a more diffusible gas will tend to maintain a flatter concentration profile than a less diffusible gas so that the axial advance of the less diffusible gas will be greater. This is known as "Taylorian dispersion" and leads to the separation of different gas species.

2.2.1b Molecular Flow

Knudsen (1909) showed that, if the mean free path of a gas is significantly greater than the tube diameter, molecules will travel as though independent of other gas molecules. The rate of flow per unit partial pressure difference is inversely proportional to the square root of molecular weight of the gas species. Therefore, the rate of flow will vary for different molecular species but, for a given species flow, will be proportional to the partial pressure gradient.

2.3 THE CENTRONIC MGA 200

2.3.1 Description

The aim of the inlet system is to provide a gas concentration in the ionisation chamber proportional to the concentration of gas which was presented at the catheter tip (see Figure 2.2). In most machines this is accomplished in two stages (an exception to this is described by Sodal et al, 1972). In the first stage gas is transported from the capillary tip to the sample chamber. This must occur without degrading the time related concentration gradients set up at the capillary tip. Viscous flow through the capillary would ensure that this is the case (section 2.3.1a). The second stage involves the transport of gas from the sample chamber to the ionisation chamber. The flow of a particular gas into the ionisation

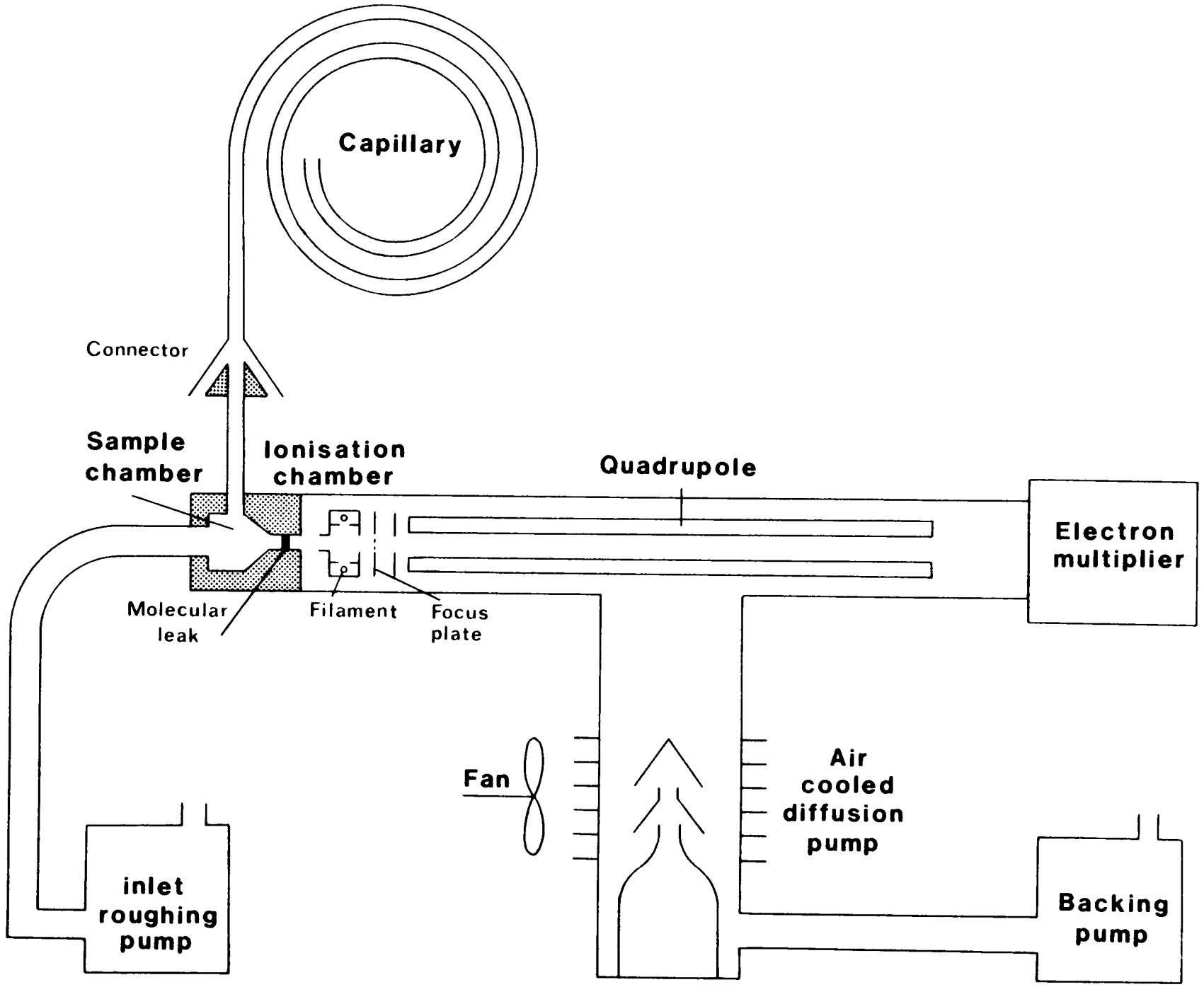


Figure 2.2 A schematic diagram of the Centronic 200 MGA mass spectrometer.

chamber must be proportional to its partial pressure in the sample chamber, and independent of the concentrations of other gases. This will be the case if flow into the ionisation chamber is molecular (section 2.3.1b). Ideally, the two flow regimes (viscous and molecular) would be separated between these two stages, and the transitional flow region would be minimised (Buckingham, 1979).

2.3.1a Catheter System

A respiratory mass spectrometer must provide continous gas sampling at a low flow rate. This is accomplished by pumping the sample through a capillary tube and into the sample chamber. From there a small fraction passes across the leak into the ionisation chamber (Figure 2.2). The length of capillary must be sufficient for convenient connection of the subject to the mass spectrometer. The capillary introduces a "lag time" into the mass spectrometer's response. The lag time is the time taken for the gas to be transported through the inlet system. This is not normally included in the response time (Figure 2.3). Fowler (1969) found that, by using a capillary inlet with an internal diameter 0.25 mm or less the lag times were small, even at low flows. In addition, because of the high resistance, it reduced the pressure at the leak to that required for molecular flow through the leak (see section 2.3.1b). The sample is pumped by a "roughing pump" downstream of the sample chamber, (Figure 2.2, single stage rotary pump, Edwards High Vacuum) such that the pressure is reduced from atmospheric, upstream, to a few mmHg in the sample chamber. These conditions are conducive to laminar flow along the capillary. If a characteristic parabolic flow front (laminar flow) is established in the capillary, a step change in concentration presented upstream will be spread out longitudinally in the capillary. This would result in a shorter lag time but a longer response time (Figure 2.3). However, Fowler (1969) has shown that Taylorian dispersion would be expected to occur over the greater

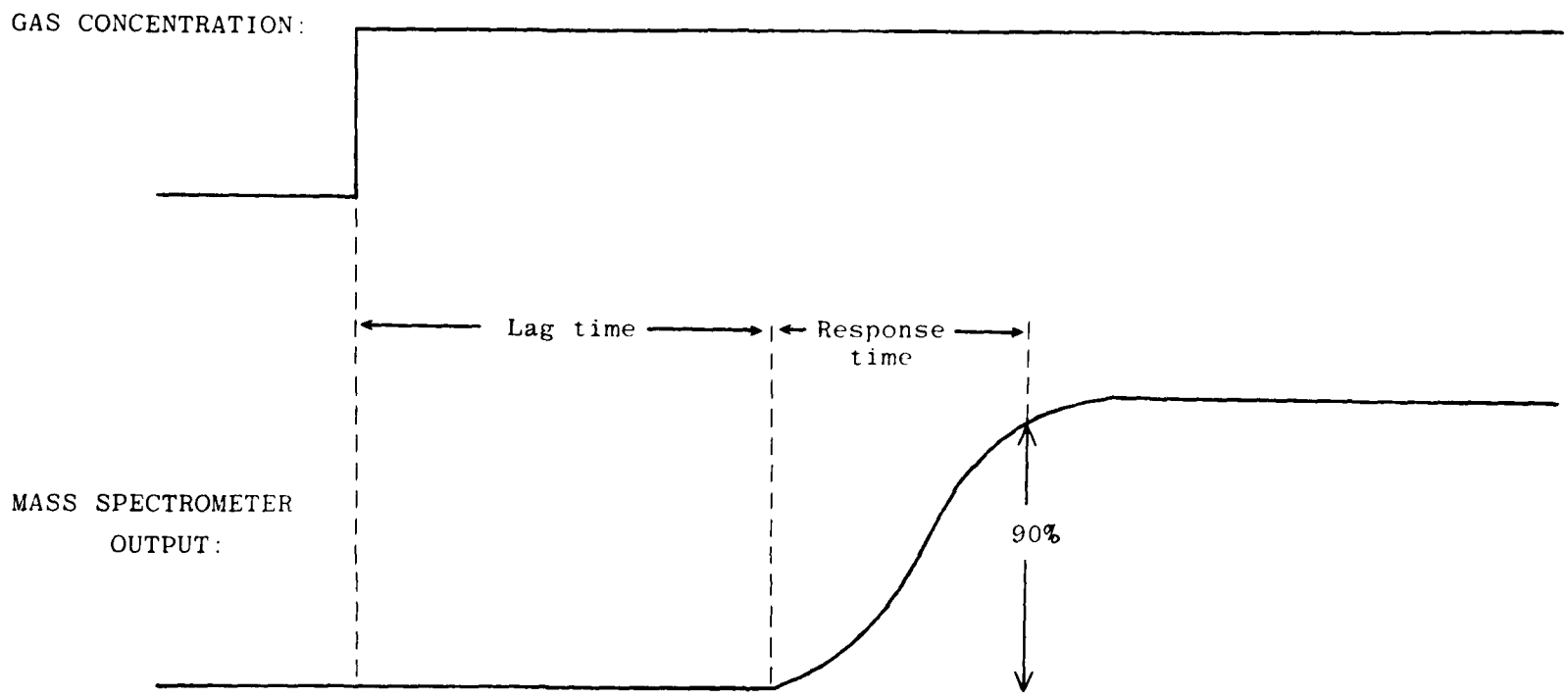


Figure 2.3 The time relationships between a square wave change in concentration, at the capillary tip, and the "lag time", and "response time" of the mass spectrometer.

part of the capillary, thereby minimising the blurring of the concentration profile (Figure 2.1). This may cause a less diffusible gas to travel faster than a more diffusible one. It has been shown experimentally that, for capillaries of less than four metres in length, the flow characteristics within the capillary do not contribute significantly to the response time (Goodwin and Holme, 1975; Goodwin 1979). Gillbe et al (1981) found that in long sampling capillaries the response time was affected by absorption into the capillary material, particularly if this was polyethylene.

Since resistance is proportional to viscosity for laminar flow, changes in the viscosity of the gas mixture cause inverse changes in the flow in the capillary. If the flow from the sample chamber to the sample pump is also viscous, its resistance will change in the same proportion. Thus, the sample chamber pressure will remain unchanged. Transiently, however, the catheter (upstream of the sample chamber) contains gas of one viscosity and the tube (between the sample chamber and the pump) gas of a different viscosity. Under these conditions, the pressure in the sample chamber will change and this will in turn alter the amount of sample which is eventually analysed. The duration of this transient fluctuation of pressure will be the same as the capillary transit time (lag time). By increasing the sampling rate, the lag time can be reduced. If, however, the flow from the sample chamber to the sample pump is not viscous, but molecular, then the resistance to this part of the flow will not change with viscosity. Consequently, changes of sample gas viscosity will cause errors which are not transient but sustained. In order to ensure viscous flow between the sample chamber and pump, a sample chamber pressure of between 10 and 40 mmHg is used together with wide bore tubes between the sample chamber and the pump.

2.3.1b Ionisation Chamber

Once in the sample chamber, most of the sample gas is drawn away by the roughing pump, but a small fraction passes through a leak into the ionisation chamber. In 1945 Honig demonstrated that the gas flow into an ionisation chamber must be molecular. This is in order that the production of ions of a particular species should be proportional to the partial pressure of the gas species in the sample chamber, and independent of the viscosity of the gas mixture. In order to achieve molecular flow in industrial analysers, a small puncture hole in a metal diaphragm was sufficient. In respiratory instruments, the sample chamber pressure is a hundred times higher than that of industrial instruments (because of the need to ensure viscous flow out of the sample chamber, section 2.3.1a). Therefore, a special leak must be used. Fowler and Hugh-Jones (1957) used a porous material called "metrosil". This is used also in the Centronic MGA 200. This is a pellet 0.5 mm thick and 1 mm in diameter made of compressed silicon carbide granules. This "molecular leak" provides numerous leakage paths with such a small pore size that the flow is molecular up to a sample chamber pressure of 100 mmHg (Buckingham, 1979).

In the MGA 200, the molecular leak allows only about one hundredth of the sample, $3x10^{-5}$ $l.mmHg^{-1}.s^{-1}$, to pass into the ionisation chamber. Inside the ionisation chamber, the pressure is about 10^{-5} mmHg. The gas molecules are subjected to bombardment by a beam of thermally produced electrons which are emitted, through a slit, from a tungsten filament heated to 2000^{o}C. Some of the molecules have an outer electron knocked off and become positively charged ions. These ions are accelerated and collimated towards the entrance of the quadrupole mass filter. From here they are attracted through the filter by a negative potential of about four thousand volts distal to the filter.

2.3.1c Quadrupole

The quadrupole filter is inside the dispersion tube which is maintained at 10^{-6}mmHg by an air cooled diffusion pump (Edwards High Vacuum, model E02A) and a single stage rotary pump (Edwards High Vacuum, model EDM2). The rotary pump provides a backing pressure of 0.15 mmHg which is sufficient for the efficient operation of the diffusion pump (see Buckingham and Dennis (1975) for details of the vacuum system). The quadrupole consists of four parallel rods, which are 127 mm long and 6 mm in diameter, arranged in a precise rectangular array. Opposite rods are polarised with the same DC potential and adjacent rods with a potential of the same magnitude but opposite sign. Superimposed on these potentials is an alternating voltage in the radio frequency (RF) range. The ability of an ion to traverse the quadrupole will depend on its mass to charge ratio (m/e) and the combination of potentials applied to the quadrupole. As all the ions of interest have unit positive charge, the m/e ratio is equal to the atomic mass number (amu). In order to understand the passage of ions through the quadrupole, one must consider the pairs of opposite rods separately (Mosharrafa, 1970). A positively charged ion travelling between two positively charged rods will be repelled equally by both and thus tend to remain equidistant from the two rods. If an RF voltage is superimposed on the DC potential such that the potential on the rods is negative for a short time, then an ion will tend to oscillate as it passes through. An ion with a small m/e ratio will be able to respond faster than an ion with a larger m/e ratio. The smaller ion will oscillate with increasing amplitude. It will eventually strike one of the rods, and thus be lost from the ion beam. An ion with a large mass to charge ratio will move much more sluggishly within the electromagnetic field and will not be affected sufficiently by the short period of attraction from the rods. Thus, it will travel the length of the rods without collision. This field configuration is a "high pass mass filter".

Conversely, a pair of rods with a standing negative potential will attract positive ions towards them. If the RF field is superimposed on this, then an ion with a small m/e ratio will respond to the transient repulsive forces sufficiently to avoid collision with the rods before escaping from the other end. An ion with a larger m/e ratio will drift towards the rods and be lost. This is a "low pass mass filter". Thus, the quadrupole is a combination filter in that the two opposite rods, charged at a positive DC voltage act as the high pass mass filter and the two rods, charged at a negative voltage act as a low pass mass filter. The combination creates a "band pass mass filter". By ajusting the ratio of the DC to AC voltage (DC/AC), it is possible to set the width of the band pass and, by doing so, the mass resolution of the quadrupole. In theory, the width of the band pass could be infinitely sharp but, in practice, the trajectory of the ions into the quadrupole is important as is the geometry of the rod assembly. Departures of these factors from the ideal, will result in a loss of resolution (Ball, 1969). For the MGA 200, a DC/AC ratio of 0.168 is optimal. A greater ratio than this will prevent passage of any ions, whilst a lower ratio will allow the passage of ions of more than one m/e, and therefore impair resolution. For each m/e, there will be an optimal AC voltage. Thus, for optimal resolution over a succession of mass numbers, the DC voltage is altered to retain a DC/AC ratio of 0.168. Once through the quadrupole, the ions strike the collector and are registered.

The MGA 200 has eight channels on which to follow concentration with time. Each channel can be set to read a different amu. The access of the eight channels to the quadrupole rods is multiplexed. A field appropriate to one channel is applied to the rods for 2.5 msec before the control of the quadrupole is given to the next channel and so on in succession. All eight mass numbers are scanned in this way fifty times a second. The

collector in the MGA 200 is the first dynode of an electron multiplier. The multiplier magnifies the minute ion current several thousand fold and then provides this as the input to the "head amplifier". The output of the head amplifier is also multiplexed so that the individual peak heights may be determined.

Because of the molecular flow of gas molecules into the ionisation chamber (section 2.3.1a), the current of ions reaching the analyser for each gas species is proportional to its concentration within the ionisation chamber. However, this constant of proportionality need not be the same for each gas species, for a number of reasons:-

1. Under the existing conditions of molecular flow, the flow of molecules into the ionisation chamber will be inversely proportional to the square root of their molecular weight.
2. Once inside the ionisation chamber, the energy required to ionise different gas atoms or molecules varies, so that the proportion of ions produced for a given number of molecules will depend upon the gas species.
3. Some gases undergo fractionation into ions of different mass and/or charge so that, at a particular m/e, only a proportion of the ions produced are detected.

The calibration process recovers the differences caused by these processes. It adjusts the gain on each channel so that the output signal from the head amplifier is related by the same constant of proportionality for all channels to the partial pressures of the gas species read by that channel.

2.4 PERFORMANCE

2.4.1 Automatic sensitivity control

2.4.1a Water Vapour

Water vapour has traditionally been the main problem with respiratory instruments. Water vapour will condense inside unheated capillaries and lower its partial pressure (PH_2O). This concomitantly raises the partial pressures of the other gases. A dry gas mixture will vaporise this water and lower the partial pressures of its component gases. Also, water vapour tends to be adsorbed on to metal surfaces in a vacuum (Stout et al, 1974). A change in the PH_2O introduced into the analyser will require time for an equilibration to be established between the adsorbed water and the vapour (Goodwin, 1979). This can produce response times for water vapour of the order of seconds. Although PH_2O is not usually of interest in respiratory studies, water vapour is still a problem. Since the response time for water vapour is different from that of the other respiratory gases, and since, in unheated capillaries, the water vapour pressure is modified by condensation, the partial pressures of the other gases will be altered by an indeterminate amount. A solution was described by Bargeton et al (1970) for improving long term stability, and adapted by Scheid et al (1971) to stabilise within-breath changes. The solution was to express each gas partial pressure as a fraction of the total dry gas pressure. Thus, concentrations are expressed as dry gas fractional concentrations, and so independent of water vapour changes and of changes in the total gas pressure.

In order to accomplish this correction on-line and sufficiently rapidly to correct for changes occuring within a single breath, Scheid et al (1971) designed an electronic circuit. The output of each dry gas channel was fed into a separate amplifier, the gain of which was controlled by a light sensitive feedback resistor. The output of all these amplifiers was added together, and any deviation of the sum from a fixed value caused

a change in the intensity of a light emitting diode. This altered the gain of the amplifiers by means of their photo-sensitive feedback resistors. If the fixed value was set to unity, then the fractional concentrations were read. If it was set to the total dry gas pressure, then partial pressures expressed at this pressure were obtained. The unit in the MGA 200 is known as the automatic sensitivity control (ASC). This alters the overall gain by feedback to the EHT (extra high tension) on the electron multiplier, and so effectively changes the sensitivity. These "ASC" techniques utilise the mass spectrometer's unique ability to measure all the dry gases in the system simultaneously and with similar lag and response times. The use of the mass spectrometer as a relative fractional concentration instrument, by using the ASC, provides compensation for a number of errors outlined below.

2.4.1b Gas Viscosity

Changes of viscosity in the inlet system cause transient and possibly steady state changes in the sample chamber pressure, which in turn will alter the amount of gas reaching the ioniser (see section 2.3.1a). The effects can be minimised by optimising the design of the inlet system, as described previously, but cannot be eliminated at this stage. By using the ASC, this effect will be removed though at a later stage. Curiously, Scheid et al (1971) state that their ASC device "cannot be used for elimination of artifacts originating in different viscosities of the component gases".

2.4.1c Other benefits of ASC

Clearly any factor which changes the total output of the mass spectrometer, by changing the output of the component gases in proportion to their concentration, will be compensated for by the ASC. For example:

1. Changes in the sample chamber pressure resulting from pressure fluctuations at the capillary tip due to respiratory effort (this

Scheid et al noted as an advantage of their system).

2. Changes in capillary resistance due to viscosity, temperature, or water deposition.
3. Pressure fluctuations in the inlet roughing pump.
4. Fluctuations in the background pressure in the ionisation chamber
5. Changes in the electron emission current.
6. Changes in the electron multiplier or head amplifier gain.

Thus, one would expect that the use of the ASC mode would increase stability. Davis and Spence (1979) found that the use of the ASC increased stability by a least four fold.

2.4.1d Problems with ASC

The problems of using the ASC include the need to measure all the dry gases in the system, or at least those whose concentrations will be changing. In the early experiments (Chapter 3) oxygen, nitrogen and carbon dioxide were the only gases whose concentrations were being experimentally varied. Argon was not measured. Scheid et al (1971) suggest, that when breathing air, the mass spectrometer's ASC should be set so that the sum of the O_2, N_2 and CO_2 concentrations equalled 99.1% v/v. This dispenses with the need to measure the argon by assuming a constant argon concentration of 0.9% v/v. Some of the rebreathing manoeuvres to be described in Chapter 3 entailed breathing room air then rebreathing gas mixtures containing no argon. Thus, 0.9% v/v argon was initially present in the alveolar gas but not in the rebreathing bag. The approach of Scheid et al would thus be invalid during rebreathing and would tend to under-read the true concentrations. In these experiments, a different approach was chosen. The sum of the O_2, N_2 and CO_2 concentrations was set to be 100% v/v on the ASC, even though argon was present in small concentrations. This caused the true concentrations to be over-read. The mass spectrometer effectively ignored argon, just as it ignored water vapour. The lung volume calculated

would need to be corrected by a factor 1/.991. In practice, this factor was taken as unity.

2.4.1d(i) Errors in the ASC

A calibration error on one channel in ASC will produce calibration errors on all the channels entered into ASC. Similarly, a channel with a low signal-to-noise (S/N) ratio will distribute this noise to the other channels by introducing it into the dry gas summation.

Consider two concentration channels, one subject to a calibration error ("1") and the other not ("2"). The true concentrations on each channel are "F1" and "F2". If "E" is a offset error and "e" is an error of gain, then:

F1 + E = apparent concentration in the presence of an offset error

F1.e = apparent concentration in the presence of a gain error

The above errors will affect the totals used to calculate the ASC concentration.

$$1 - F1 + F1 + E = 1 + E = \text{ASC total with offset error.}$$

$$1 - F1 + F1.e = 1 + F1.(e-1) = \text{ASC total with gain error.}$$

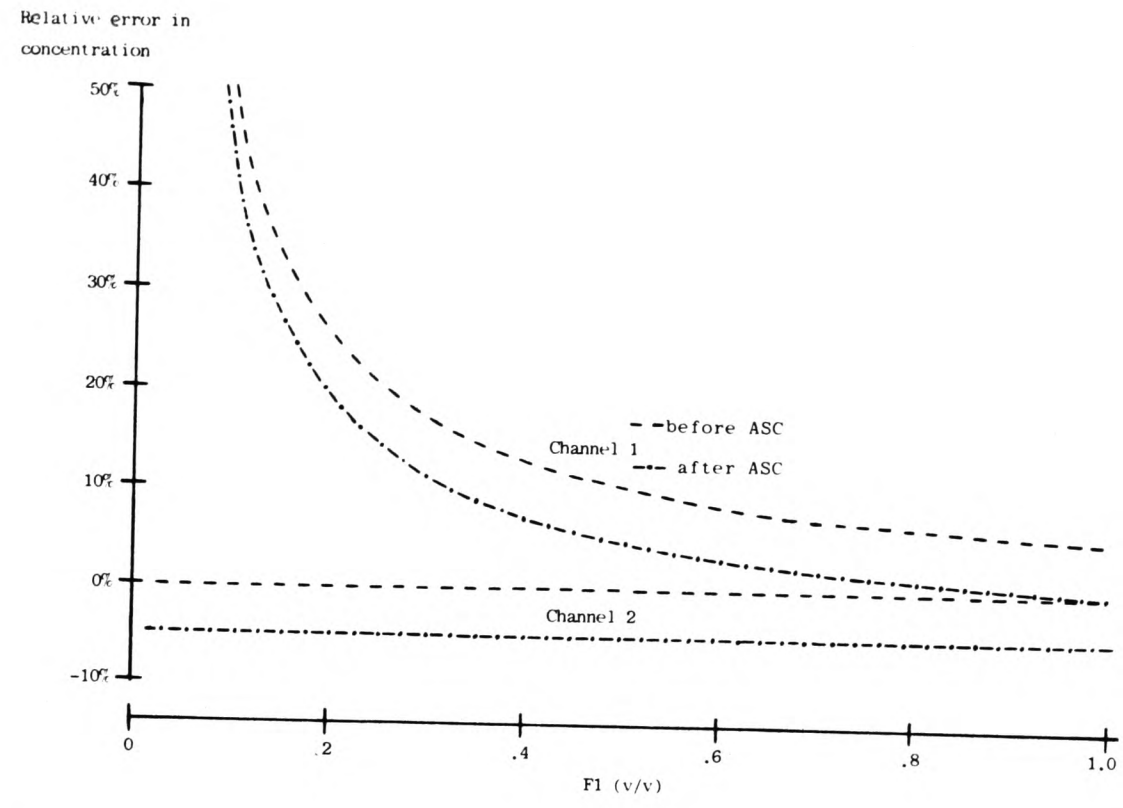
Thus, after ASC, the concentrations for channel 1 are respectively for the two types of error (offset and gain):

$$\frac{F1+E}{1+E} \quad \text{and} \quad \frac{F1.e}{1+F1.(e-1)}$$

After ASC, the concentrations of the correctly calibrated channel (2) are also modified:

$$\frac{F2}{1+E} \quad \text{and} \quad \frac{F2}{1+F1.(e-1)}$$

Figure 2.4 shows how an offset error on channel 1 causes relative errors on channel 2, after ASC. The upper graph illustrates the



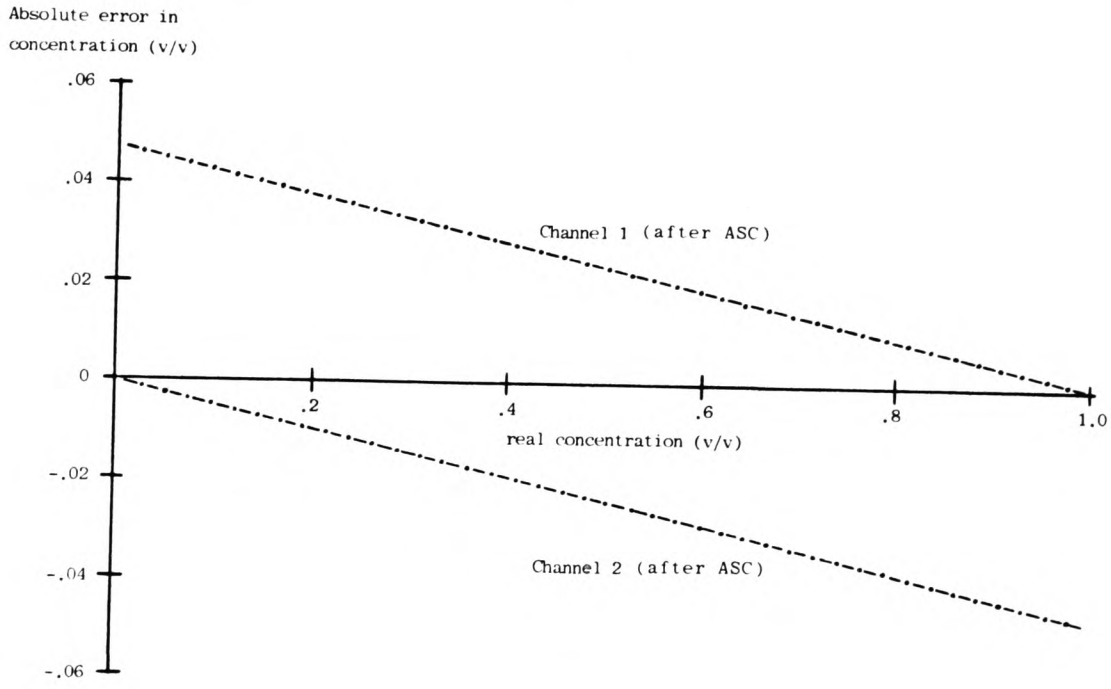
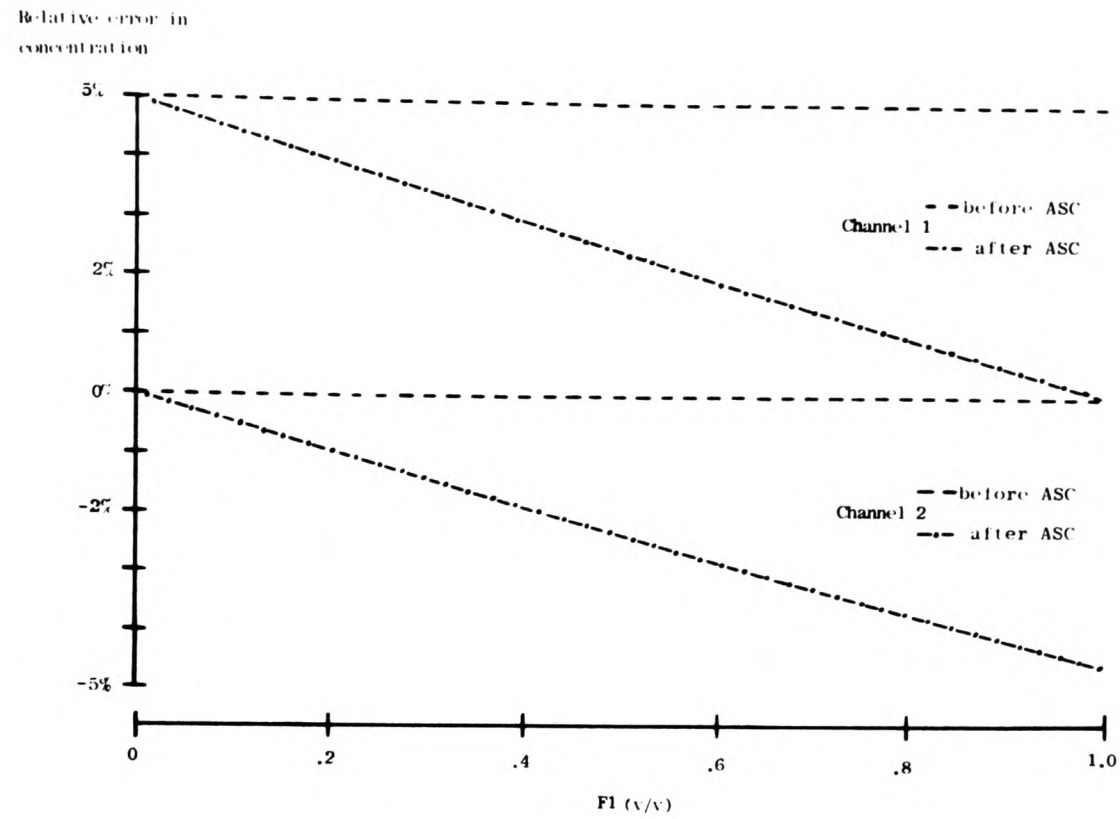


Figure 2.4 The upper graph shows how an offset on channel 1 of + 0.05 v/v causes relative errors (calculated by :- (real-measured/real concentrations), which are dependent upon the real concentration for channel 1 (F1). After ASC this induces a relative error in a correctly calibrated channel (channel 2).
The lower graph shows that the absolute measurement error, after ASC, is linearly related to the real concentration. The line for channel 2 assumes a binary gas system.



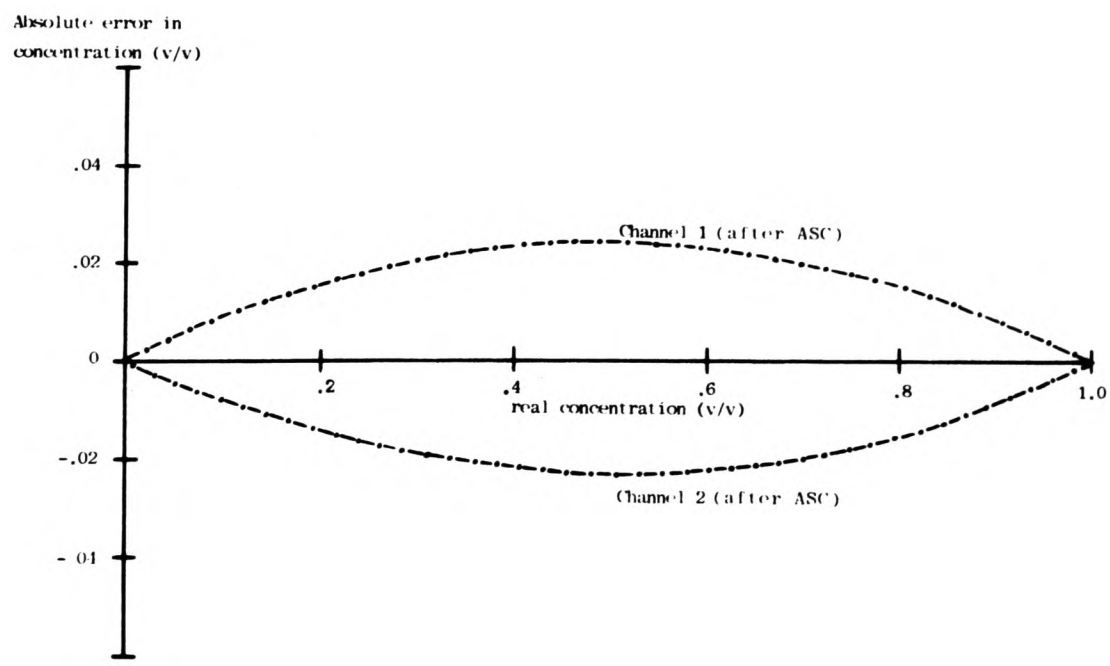


Figure 2.5 The upper graph shows the relative errors (for a range of real concentrations, F1) induced by a gain of 1.1 on channel 1. After ASC this causes an error on channel 2.
The lower graph shows the absolute measurement error after ASC to be non-linearly related to the real concentration. (A binary gas system is assumed).

relationship between the actual concentration of gas 1 (F1) and the size of the error (relative to the signal) on both channel 1 and 2. The lower graph shows the magnitude of absolute errors between the true and observed concentrations, after ASC. A binary gas system is assumed (ie. F1 + F2 = 1). Figure 2.5 illustrates these relationships for a gain error on channel 2. In the presence of both a gain and an offset error, a composite of these two errors will be produced. If errors are present on more than one channel, the resulting errors will be considerably more complex. The important observation is that errors of gain can transform linear calibration lines before ASC into non-linear lines after ASC. Non-linearity of the mass spectrometer will cause problems in lung volume determination (Chapter 4, section 4.6.5).

2.4.1d(ii) Time response of the ASC

The ASC compensation takes a finite time to stabilise a perturbed system. The ASC consists of electronic summing circuitry, which detects a change in output, and a negative feedback of these changes which controls the sensitivity. In a perfect control system, a step change in the concentration of a gas excluded from ASC would have no effect on the concentrations of the gases in ASC. This was tested in the following way. The ASC was set to ignore CO_2 and the mass spectrometer sampled a CO_2/air mixture (1:9). The catheter was then rapidly removed from the gas stream into air. The result was an initial rapid rise (about 10%) in the oxygen and nitrogen concentrations followed by a slower decline to their original concentrations. The total time of this transient was about 600 msec. This response included the response characteristics of the analyser. Repeating this experiment with humid air failed to produce any transient. This demonstrates that, in the presence of the large time responses associated with water vapour changes (section 2.4.1a), the ASC response time is adequate. In a further experiment, a rapid change in the ASC total was

achieved by switching one gas in, or out, of ASC. This provided a square wave stimulus to the ASC control system which was uncomplicated by the analyser response characteristics. Such experiments indicated that the time constant of the ASC control system was 90 msec, and suggest that perturbations with a frequency component greater than 1.8 Hz would not be compensated for effectively.

2.4.2 Spectral Overlap

Certain molecules, when subjected to electron bombardment, break down into their component atoms or combinations of atoms. There is commonly a main peak resulting from the predominantely produced ion and one or more smaller fragmentation peaks. Carbon dioxide for example breaks down to give singly charged carbon monoxide ions. These have an m/e ratio of 28 which is the same as nitrogen. Nitrous oxide splits into components appearing at peaks 28, 32 and 30, the first two being the same peaks as nitrogen and oxygen respectively. The main peak of nitrous oxide is at 44 which is the same as the principal peak of carbon dioxide. The spectral peaks produced by the various gases used in this thesis are shown in Figures 2.6 and 2.7. At first sight, it seems an impossible task to resolve the separate components from the combined peaks. However, if the relative magnitudes of the fragmentation peaks for any one gas remain independent of changes of concentration, it should be possible, by examining the height of subsiduary peaks, to extract the individual components from composite peaks. The solution was described by Davis and Spence (1979). The work in this thesis demanded the measurement of nitrogen (N_2), oxygen (O_2), argon (Ar), carbon dioxide (CO_2) and nitrous oxide (N_2O). A "subtraction unit" was designed and built for use with these gases and the circuit is shown in Figure 2.8. The principal peaks were N_2: 28, O_2: 32, A: 40, CO_2: 44, and N_2O: 44 amu. The first four gases were measured at their principal peaks. N_2O was measured at a secondary peak at 30 amu (see Figure 2.7). The peak

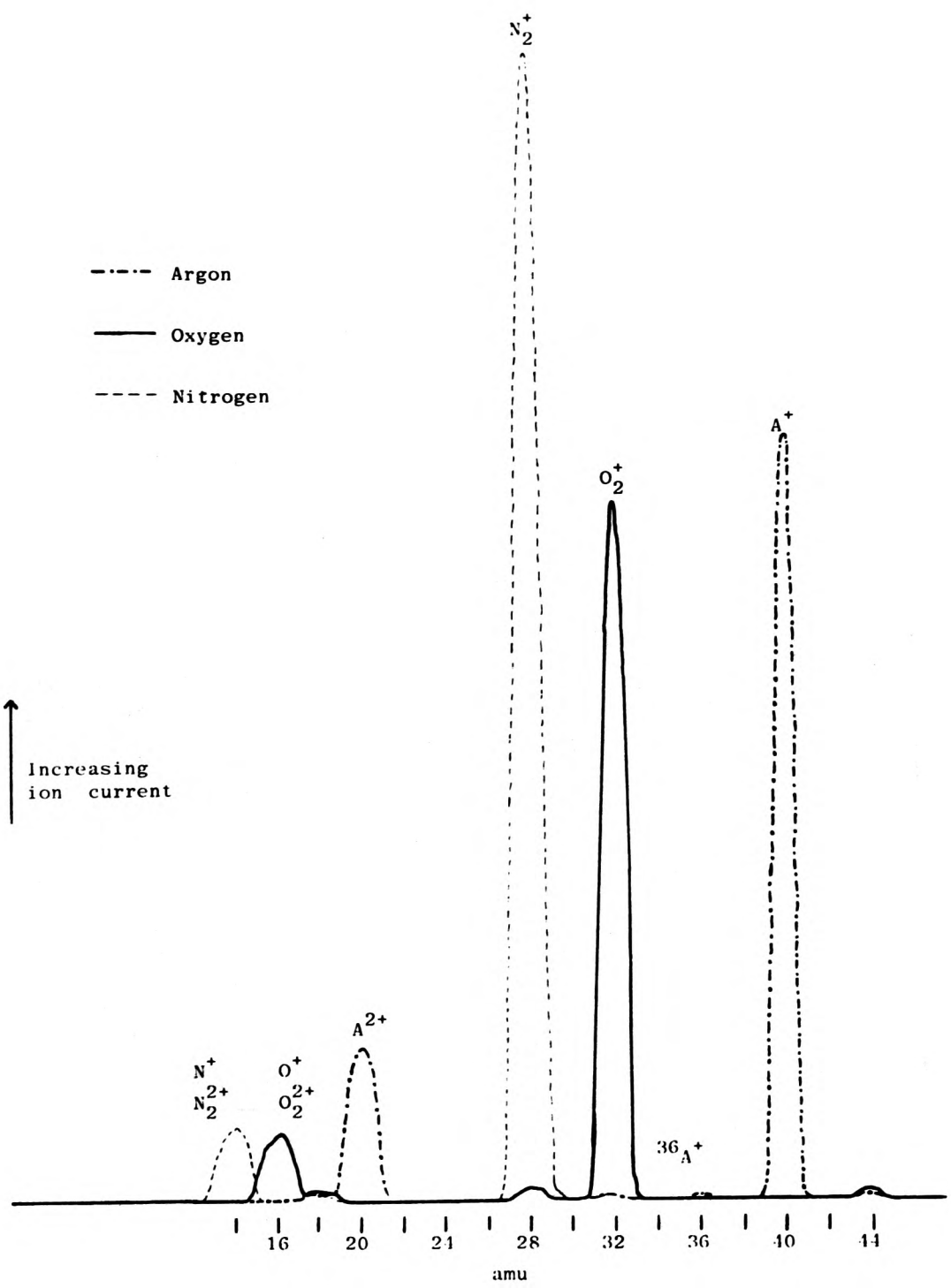


Figure 2.6 The ion currents produce for a range of atomic mass units (amu) when sampling either pure O_2 or pure N_2 or pure Ar. Known as mass spectra.

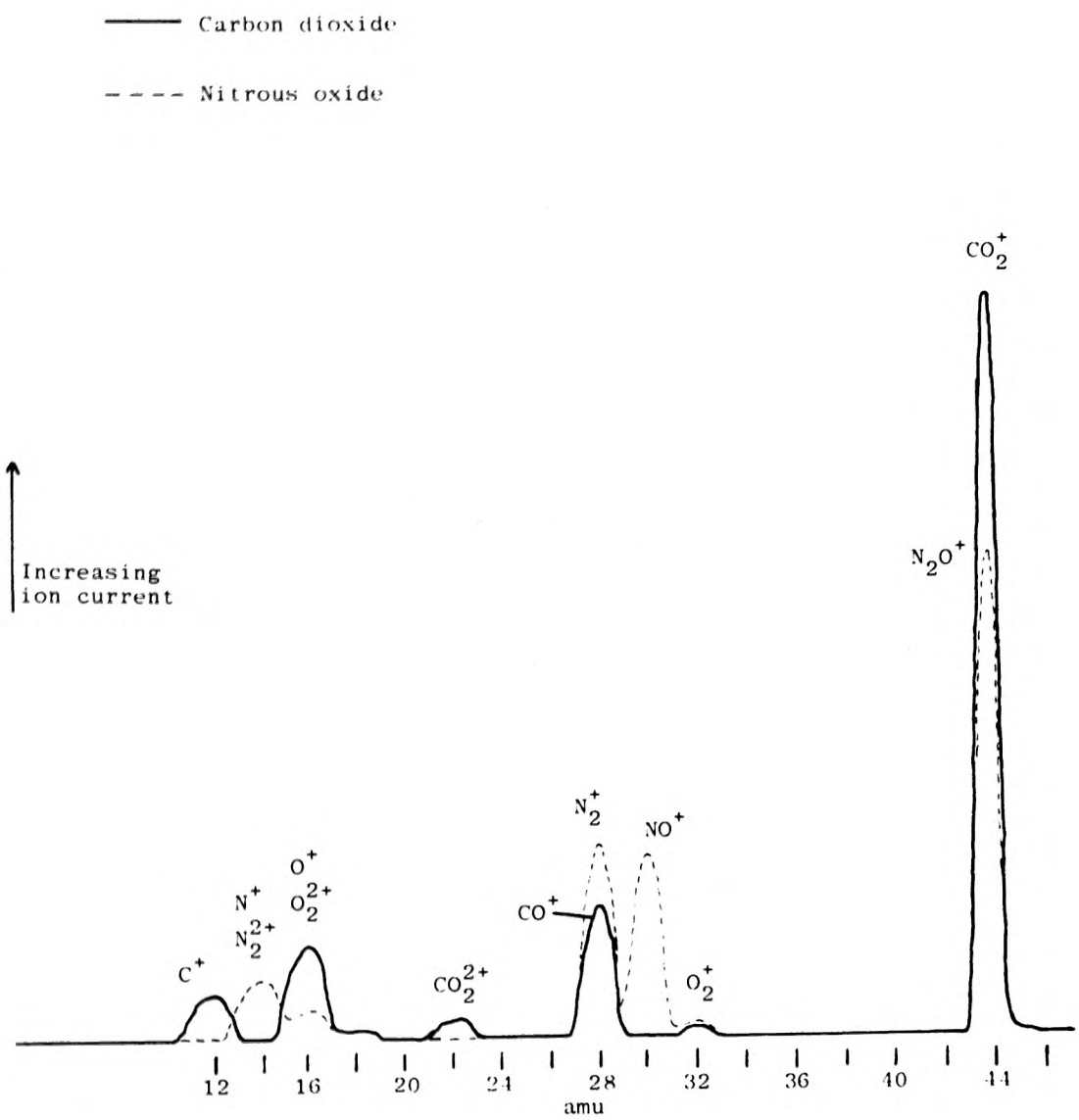


Figure 2.7 The mass spectra produced when sampling either pure CO_2 or pure N_2O.

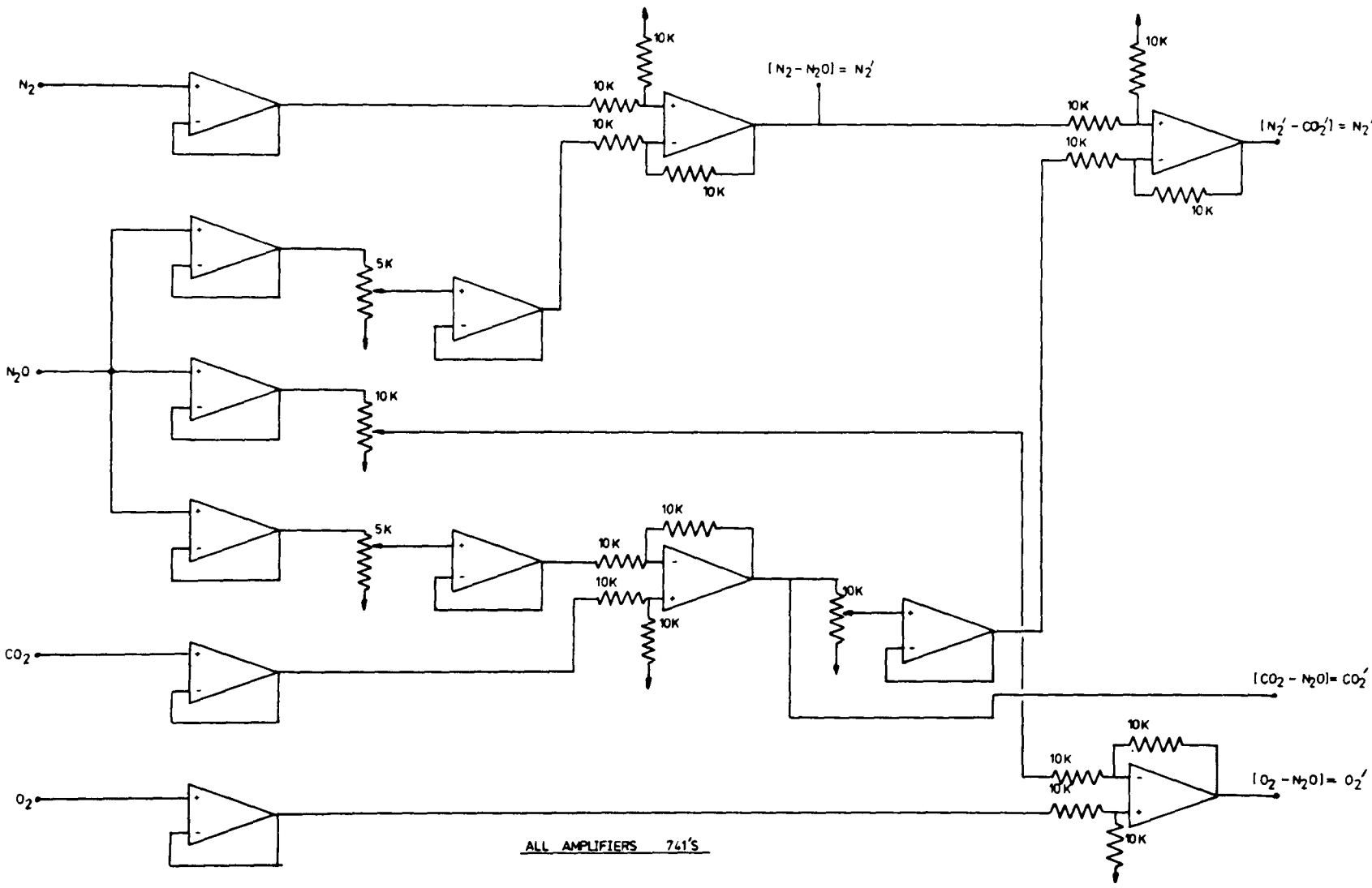


Figure 2.8 Circuit diagram for the subtraction unit.

at 30 amu was unique to nitrous oxide. Thus, N_2O was measured at this peak, and an amount proportional to this subtracted from the height of peak 44 such that, in the absence of CO_2, and, in the presence of N_2O, no CO_2 was inferred at m/e 44. Nitrous oxide also had subsidiary peaks at 28 and to a small extent at 32 amu. A subtraction similar in principle was performed on these peak heights. Carbon dioxide had subsidiary peaks at 28 and 32 (the latter one was so small that it was neglected) but, since the true concentration of CO_2 had been derived from subtraction of the N_2O contribution, the overlap of CO_2 at peak 28 was similarly removed.

The problem with this system was the increased signal to noise ratio for carbon dioxide in the presence of nitrous oxide. The CO_2 concentration in respiratory work is usually less than 7% v/v. This signal was read on top of a signal for N_2O, which in this thesis and anaesthetic applications can be 50% v/v or more. Small errors in the nitrous oxide signal, caused by noise or alinearities at peak 30 (section 2.4.2a), were transferred in absolute terms to the CO_2 signal. For example, if in 50% v/v N_2O there was a relative error of 1% at peak 30 (an absolute error of 0.5% v/v), a ~~factor~~ Fraction of the N_2O signal at m/e 30 had to be be subtracted from the peak at 44. Figure 2.7 shows that the peak at 44 was about 2.5 times the height at peak 30. Thus, a 0.5% v/v error on the peak at 30 is increased 2.5 fold at peak 44 ~~(1.25% v/v)~~. This error will be transmitted to the reading of CO_2 concentration at 44 amu. Since for each per cent CO_2, the peak height is 1.5 times as big as for the same percentage of N_2O at the same peak, the absolute error in CO_2 concentration will be 0.~~8~~3% v/v. If the carbon dioxide concentration was 5% v/v, then this would represent a relative error of ~~17~~6%. There will be a further error if the ratio of N_2O peaks at 30 and 44 amu has not been accurately established or is not constant. Because the proportion of the nitrous oxide that appears at peaks 28 and 32 amu is considerably smaller than that at 44 the signal to noise ratio at these

atomic mass units is not so badly affected.

2.4.2a Problems with Nitrous Oxide

The need to measure N_2O at a subsidiary peak requires the sensitivity of the machine to be increased. This entails increasing the EHT on the electron multiplier. A small peak, such as the N_2O peak at 30 amu, has a relatively ~~high~~ low signal to noise ratio and when in ASC reduces the S/N ratio of the other channels.

The peak at 30 amu occupies a position between the two large peaks of N_2 at 28 amu and O_2 at 32 amu. Many workers have noted a relationship between one or both of these peak heights and N_2O at 30 amu (Gillbe et al, 1981; Graham et al, 1979 and Stout et al, 1974). This has been attributed to the presence of an isotope molecular nitrogen composed of two atoms of isotopic N_2^{15} (Gillbe et al, 1981) and to poor resolution of the base line between the oxygen and nitrogen peaks (Stout et al, 1974). This phenomenon was observed in this work, and the presence of a peak related to N_2 at 29 amu was noted (Figure 2.9), presumably $N^{15} - N^{14}$. This, according to Lilly (1950), accounts for about 0.8% of the nitrogen in the air. Overlap occurs between this peak and the peak at 30 amu, and the high gain needed to measure N_2O at 30 amu can cause an offset of 1.5% v/v. This leads to errors in N_2O estimation, which will also cause errors in CO_2 measurement via the subtraction unit. By increasing the resolution of the quadrupole, and careful mass centering, the effect was reduced to an offset in air of less than 0.5% v/v. Gillbe et al (1981) have suggested that the relative sizes of the fragmentation peaks of N_2O change at higher concentrations.

The problems encountered in measuring nitrous oxide have led some workers to measure nitrous oxide at its principal peak, 44 amu (Ozzane et al, 1981). Carbon dioxide must then be read at a peak unique to itself, at 12 amu. A factor proportional to this is subtracted from the peak at 44 amu. The CO_2 peak at 12 amu is, however, very small (see Figure 2.7), and

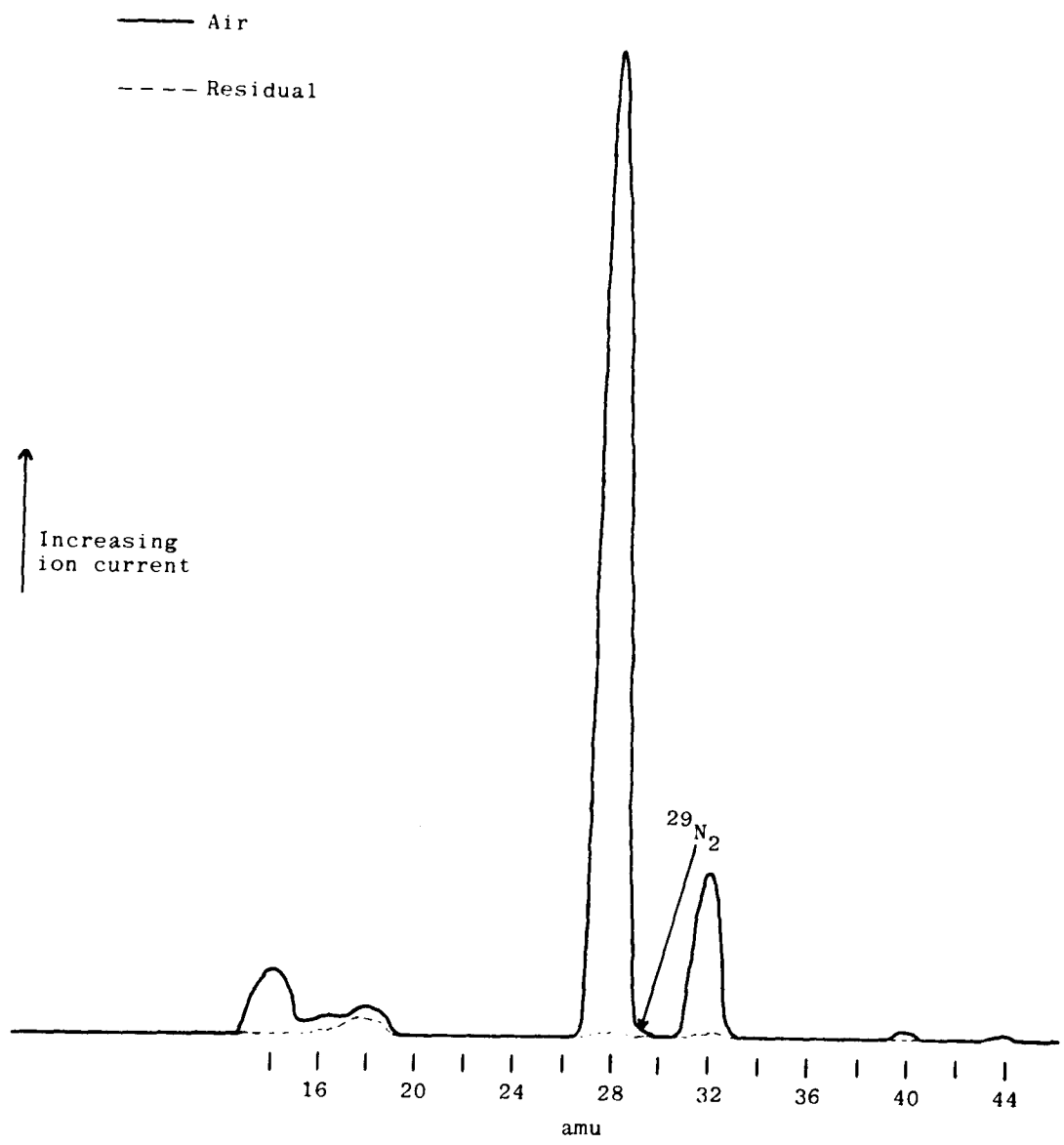


Figure 2.9 The mass spectra produced when sampling air as compared to the residual spectra.

can be expected to be rather noisy. Other investigators have chosen to measure carbon dioxide at peak 12 and nitrous oxide at peak 30 and dispense altogether with the troublesome peak at 44 (Mapleson et al, 1980).

2.4.3 Reactions within the Analyser

A common problem with mass spectrometers is the production of spectral peaks when the inlet is closed. These are known as "background" or "residual spectra" and are produced predominantly from water vapour which sticks to the metal surfaces of the evacuated chambers, and carbon dioxide and other breakdown products of the oil used in the diffusion pump. These can usually be offset during calibration. The residual spectrum observed in the Centronic MGA 200, used in this study, is shown in Figure 2.9.

The reactions of oxygen on heated tungsten filaments are well known (Becker et al, 1961; Singleton et al, 1966). The rate of combination of oxygen is a function of the filament temperature and the oxygen partial pressure (Beatty et al, 1981). The principal product is an oxidised layer on the tungsten although carbon impurities in the filament lead to the production of carbon dioxide and carbon monoxide. Goodwin (1979) believes the contribution of these reactions is small in comparison to reactions occuring on the vacuum chamber surfaces. These reactions occur at low pressures between the gas molecules and on the metal surfaces being bombarded by electrons. The result is a further production of carbon dioxide and carbon monoxide, which causes oxygen dependent offsets at peaks 44 and 28 amu (Figure 2.6). The time taken for these reactions to reach an equilibrium will increase the response time for oxygen (Goodwin, 1979) in a manner partly dependent on the immediate history of oxygen administration.

Pre-exposure of the mass spectrometer to anaesthetic gases has been found to increase markedly the carbon dioxide peaks. This effect increases

as the oxygen concentration is increased and seems to be worst at peak 12 (Graham et al, 1979)

2.4.4 Stability

Stability depends upon a number of factors which includes the ASC, spectral overlap and resolution. The resolution is inversely related to stability (Goodwin, 1979). Stability encompasses both short term effects (ie. noise) and long term effects (ie. drift). The signal to noise ratio in ASC was found to be about 200:1 for O_2, N_2 and Ar, and better than 100:1 for CO_2. When measuring nitrous oxide, the S/N for O_2, N_2 and Ar was greater than 100:1, but the N_2O S/N was about 70:1 and the CO_2 was about 50:1.

The drift of the MGA 200 when operated in the ASC has been estimated as 1% in 24 hours (Davis and Spence, 1979; Gillbe et al, 1981). During the experiments undertaken in this thesis, the calibration of the mass spectrometer was checked periodically during each experimental protocol. The calibration drift was never found to be more than $\pm$ 0.5% v/v between calibration checks, which were separated by between 30 to 60 minutes.

Gillbe et al (1981) define the operating temperature of the MGA 200 as 22-27^{o}C. It has been found, during these experiments, that the stability is adversely affected on warm days. The mass spectrometer itself produces a considerable amount of heat and removal of the back panel has been found to help in keeping it cool.

2.4.5 Response and lag times

The distinction between the "response time" and the "lag time" is illustrated in Figure 2.3.

The response time is the result of three factors:-

1. The inlet system's ability to transmit unaltered concentration signals

to the ionisation chamber. Defects in this are probably small (section 2.3.1a).

2. The response time of the electronic circuitry.

3. The clearance time of the ionisation chamber.

When the concentration in the ionisation chamber is changed the previous gas concentration falls exponentially. The new gas concentration cannot be truly measured until the previous gas has been pumped away. The time constant for the decline of the previous gas concentration is equal to the ionisation chamber volume times the pumping speed (Fowler, K.T., 1969). In order to reduce this time response, Fowler suggested using a "gas tight" ionisation chamber with a volume of only a few millilitres, the only exits being the slits for the electron beam and the hole through which the ions pass. With this arrangement, the pressure outside the chamber is about ten times less than that inside. As a result, most of the ion concentration decays with the fast time constant of the ionisation chamber and the rest with the slower time constant of the dispersion tube. This can mean that the last percent of the response to a new gas concentration can take a second to achieve.

In order to check the time response of the machine used for these studies, the response time was measured using a transient signal recorder (Transcribe 10, Bryans Southern Instruments L.T.D.). While the mass spectrometer sampled from a gas mixture, the catheter was rapidly pulled out of this gas stream into air. A response from 0 to 90% took between 80 and 90 msec for N_2O, Ar and N_2; and between 100 and 110 msec for O_2 and CO_2. When these responses were measured in ASC, a common response time of between 100 and 110 msec was found.

In the work presented in this thesis, the only data points of interest are the end-expiratory and end-inspiratory points. So long as the response is complete before the end of a breath (longer than 1 second), the

response time performance is adequate for this application. Fowler (1969) suggested that a response time of better than 1 second was all that was required for looking at the alveolar plateau, and Severinghaus et al (1978) state that a 700 msec response time is adequate for end tidal sampling.

The lag time is dependent upon the physical characteristics of the inlet system; such as temperature, sample rate, capillary diameter and length. In these studies, a nylon capillary inlet (0.25 mm in diameter and 1.3 m long) was used. The sample flow rate through this capillary was about 70 $ml.min^{-1}$. In an attempt to estimate the lag time a solenoid valve was used to provide a step change in concentration. The outflow from the valve went directly into a luer "Y" piece. The mass spectrometer catheter was connected into one branch of the "Y", so that the capillary sampled adjacent to the solenoid outlet. The other branch was open to atmosphere. The solenoid could be switched between two gas supplies, and the electrical event associated with switching was used to trigger a transient signal recorder. The mass spectrometer output was fed into the same recorder. This allowed the time between the switching of the solenoid and the first response of the mass spectrometer to be determined. This delay was about 180 msec and included the opening time of the solenoid valve.

So far as the experimental work in this thesis was concerned, the absolute lag time was not important. What was important was the difference between the detection of expiration or inspiration by a flow sensor and the detection of the associated concentration changes by the mass spectrometer (Chapter 5). This delay was measured in the apparatus using the transient recorder and was found to be 180-190 msec for inspiration and approximately 280 msec for expiration. These times included the washout of the apparatus and lung dead space.

2.4.6 Linearity

Gillbe et al (1981) decribed non-linearities associated with the MGA 200. They found that, when the concentration of most gases exceeded 80% v/v, the concentrations as given by the MGA 200 did not fall on a linear calibration line made at lower concentrations. Thus, calibration of the machine on zero and 100% v/v gas mixtures would cause errors in the middle range. Once these had passed through the ASC and subtraction circuits, these errors would be modified in a very complex manner (section 2.4.1d(i)). The solution is to avoid exposure to excessively high concentrations of any gas both during calibration and experiments. In early experiments two factors were compounded in causing errors. The first was the use of pure O_2, N_2 and Ar, which have been found to distort the subsequent readings for all other gases (Gillbe et al, 1981). In this study, pure oxygen was observed to augment the subsequent readings for all other gases. The second factor was that all the calibration was performed out of ASC, so that the viscosity of the gas mixture was a contributing factor in the mass spectrometer's output (section 2.4.1b).

Figure 2.10 shows the effect of calibrating the MGA 200 on zero and 100% v/v points for oxygen and testing the calibration at intermediate points. A resulting curvilinear calibration line was found, as predicted as from gain error operating within the ASC mode (section 2.4.1d(i), Figure 2.5). Since calibration problems were blamed for the errors described in Chapter 3, a new calibration procedure was adopted for later studies in an attempt to avoid such errors.

The gas mixtures referred to in this calibration are shown in Table 2.1. It will be noted that a concentration of 100% v/v was never used to set the gain for any gas. A further aim of the new calibration procedure was to calibrate as much as possible within the ASC mode.

The calibration technique is illustrated schematically in Figure

GAS MIXTURES FOR CALIBRATING MASS SPECTROMETRY

	1 (Air)	2	3	4	5	6 (Entonox)
N_2	78.1	27.8	9.4	0	0	0
O_2	20.9	0	37.4	57.7	0	48.4
CO_2	0	0	7.8	11.8	0	0
N_2O	0	0	0	0	25.6	51.6
Ar	0.9	72.2	45.4	30.5	74.4	0

Table 2.1 Composition of the calibration gases in volumes percent.

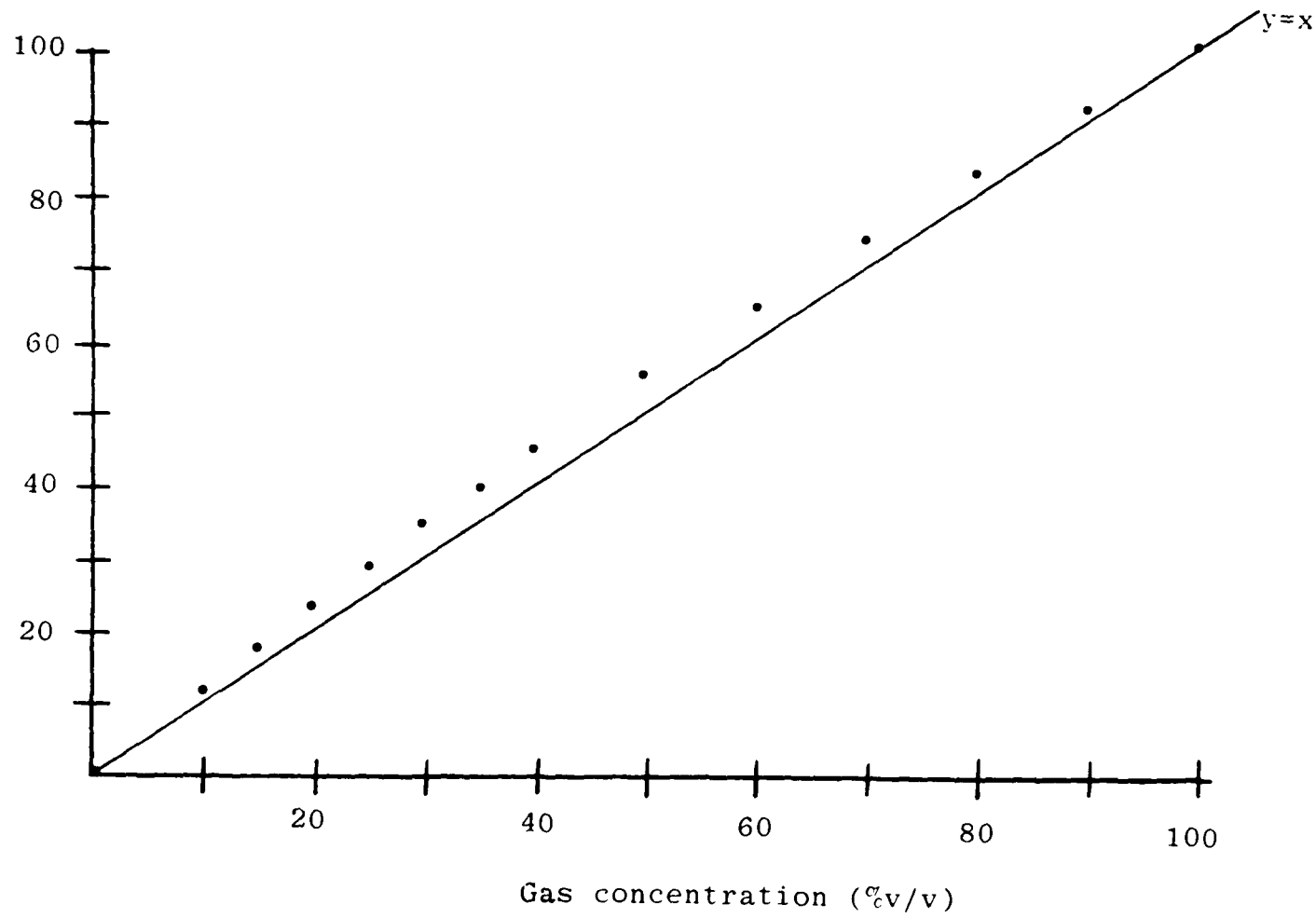


Figure 2.10 The mass spectrometer output verses the true concentration of O_2, after calibration between 0 and 100% v/v. The line of identity is shown for comparison.

2.11. The first step was to calibrate O_2 and N_2 out of ASC. The correct zeros were established for O_2 and N_2 on different gas mixtures but their gains were established on the same gas mixture- air. Therefore, the correct relative gains of O_2 and N_2 were set, independent of factors other than concentration. Their zeros were set on different gas mixtures but care was taken to avoid gases known to induce offsets. Referring to Figure 2.11, the calibration procedure was:

1. The channels required were tuned to the relevant mass peaks. The sum for the ASC was set at unity.
2. Using air (mixture No 1) and a mixture containing no oxygen (No 2) the O_2 channel was spanned from 0 to 20.9% v/v. The N_2 channel was then similarly spanned from 0 to 78.1% v/v. using ~~mixture No 4~~ pure argon and air. These gases were then introduced into ASC.
3. The argon channel was then entered into the ASC. Since the N_2 was calibrated, the use of a N_2/Ar mixture (No 2) allowed the Ar to be calibrated. By altering only the Ar gain and offset between 74% v/v (mixture No 2) and 0.9% v/v (air), argon calibration was achieved inside the stabilising constraints of the ASC.
4. CO_2 was then entered into ASC. Using the O_2, CO_2 and Ar mixture, (No 4) the CO_2 gain and offset between zero (air, No 1) and 11.8% v/v was set. This also provided the opportunity to "back-off" the CO_2 spectral overlap on the N_2 peak, by continually adjusting the subtraction unit as the CO_2 gain was set.
5. The O_2, N_2, CO_2 and Ar channels were all now deemed to be calibrated. This could be checked by sampling mixture No 3.
6. The N_2O channel was then entered into ASC. The use of the N_2O and Ar mixture (No 5) allowed the N_2O gain to be set, using air (No 1) for a zero. At the same time, the spectral overlaps of N_2O on the N_2, O_2 and CO_2 signals were backed-off using the subtraction unit.

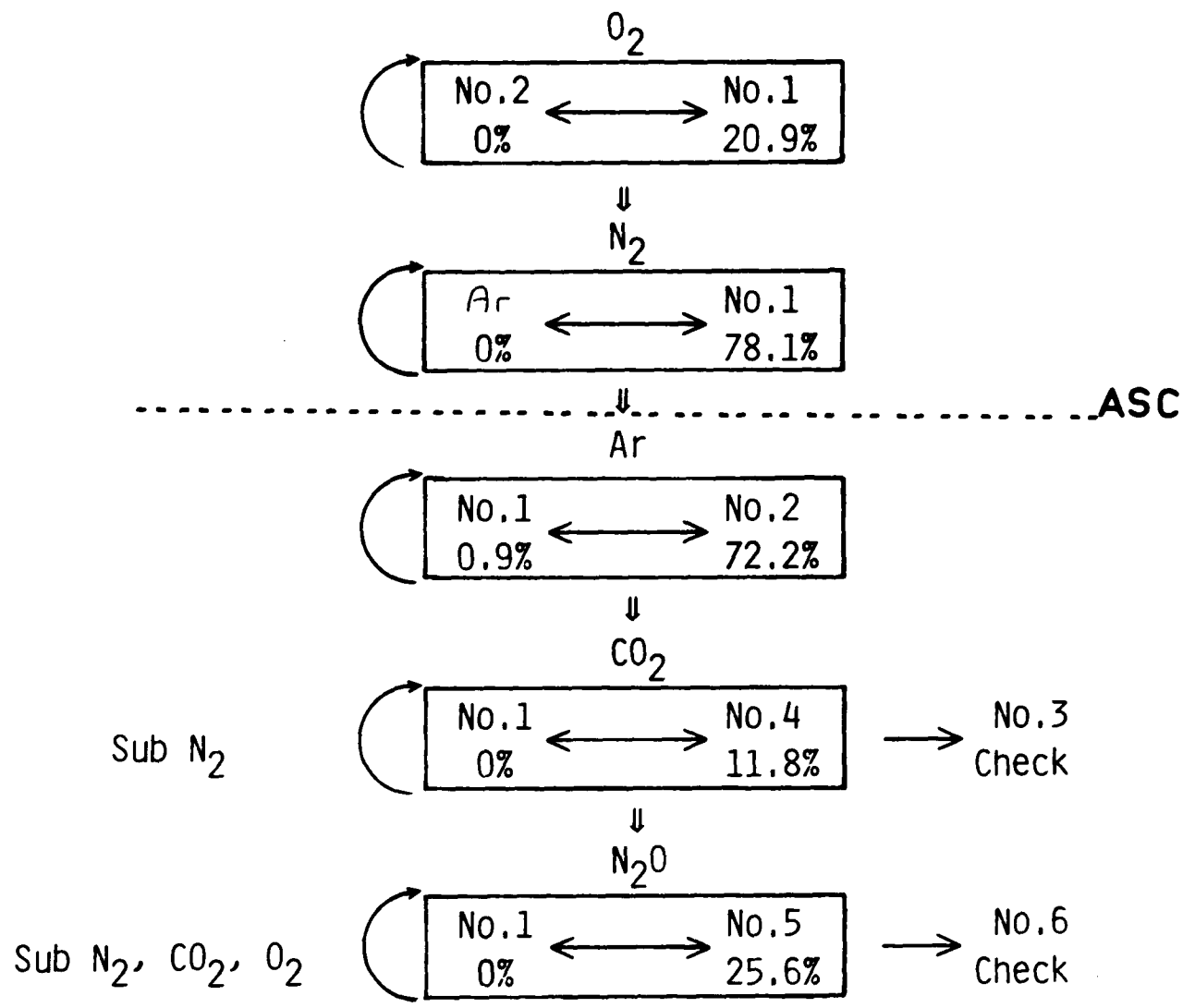


Figure 2.11 Diagramatic representation of the calibration technique (see text).

7. The N_2O calibration and the N_2 and CO_2 subtractions were then checked on the calibrated entonox cylinder (No 6).

When performing the calibration, sufficient time was allowed for a steady state to be achieved, this took several seconds. During calibration, a leeway of $\pm$ 0.2% v/v was allowed between the calibration cylinder concentration and the mass spectrometer reading. After performing this calibration, the mass spectrometer was found to be accurate with an error of less than $\pm$0.5% v/v absolute from a series of mixture, from 0 to 100% v/v, supplied from a gas mixing pump (Wosthoff o.H.G, Bochum). In the absence of N_2O this was improved to $\pm$0.3% v/v absolute. Mosharrafa (1970) suggests that $\pm$ 0.5% v/v is adequate for respiratory work. The error in lung volume measurement caused by these uncertainties will be described in below . The presence of absolute error at low concentrations is indicative of the presence of offsets. Indeed, it could take over a minute for the oxygen to reach zero after pre-exposure to oxygen (section 2.4.3). The CO_2 offset was dependent upon the O_2 concentration and also seemed to be worse in the presence of N_2O (section 2.4.3). Nitrous oxide had an offset in the presence of nitrogen (section 2.4.2a).

2.4.6a Effect of Measurement Errors

It is appropriate to consider the way in which errors in gas concentration measurement will affect the calculation of lung volume. In the next Chapter the equation for calculating lung volume will be shown to be:

$$V_{Lo} = V_{Bo} \cdot \frac{F_{Bo} - F_{Mo}}{F_{Mo} - F_{Lo}} \qquad (2.1)$$

V_{Lo} is the lung volume and V_{Bo} is the rebreathing bag volume. F_{Bo}, F_{Mo} and F_{Lo} are all fractional concentrations which must be measured by the mass spectrometer. A number of properties concerning the above quotient of

concentrations can be derived by inspection.

1. An error in measuring F_{Mo} will affect both the numerator and the denominator, being subtracted from the numerator and added to the denominator. Both will tend to alter the quotient in the same direction. Therefore, the sensitivity to errors of F_{Mo} will be high.
2. Errors in the estimation of F_{Bo} or F_{Lo} only affect either the numerator or the denominator, and thus, the quotient is less sensitive to these errors.
3. If all of the concentrations were in error by the same factor, then this factor would cancel out.
4. If all of the concentrations were subject to the same offset, then these would subtract out.

The last two properties ensure that the measurement of lung volume by such a method is independent of systematic errors in the gain or offset of the measuring device. The corollary of this is that the calculation requires only that the measured concentrations be linearly related to the real concentrations.

Given a linear "calibration line" errors may still result because of random errors about this line. Consider a random measurement error, "e", where each concentration measurement has an uncertainty: $\pm$ e. The worst case of these errors, when introduced into equation 2.1, would be:

$$V_{Lo} = V_{Bo} \cdot \frac{F_{Bo} - F_{Mo} + 2e}{F_{Mo} - F_{Lo} - 2e}$$

By substituting for F_{Mo} from equation 2.1, the resulting error in lung volume measurement is:

$$\frac{V_{Lo} \cdot (F_{Bo} - F_{Lo})/(V_{Bo} + V_{Lo}) + 2e}{V_{Bo} \cdot (F_{Bo} - F_{Lo})/(V_{Bo} + V_{Lo}) - 2e} \cdot V_{Bo} - V_{Lo}$$

Figure 2.12 illustrates the relationship between the absolute difference between the initial bag and lung concentrations for an indicator

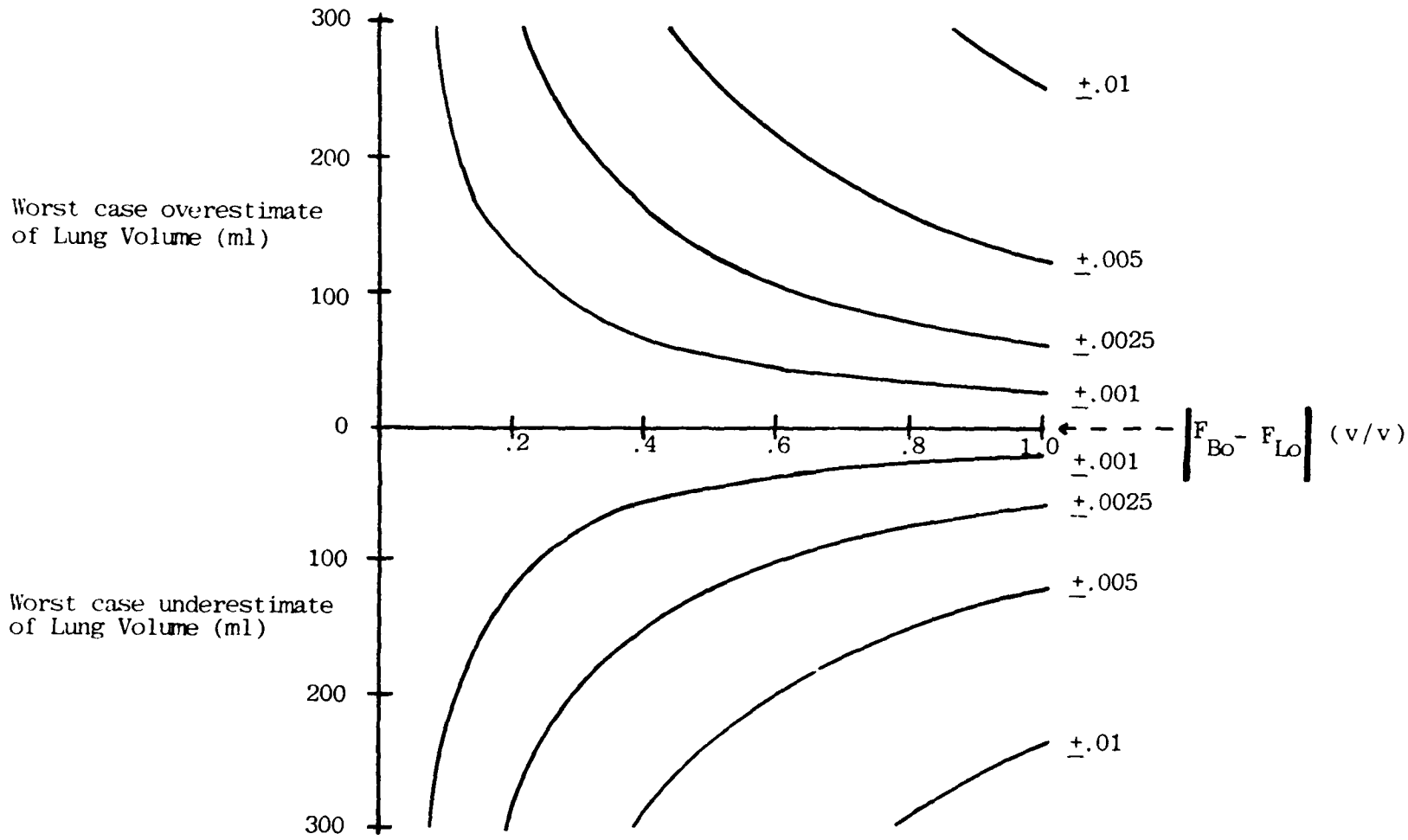


Figure 2.12 The relationship between the absolute difference in the initial bag-lung concentration gradient and the worst case errors of lung volume measurement. This is shown for a number of different measurement errors (listed up the right hand side of the figure).

gas ($|F_{Bo} - F_{Lo}|$) and the above error in the estimated lung volume. This is shown for various absolute measurement errors expressed as fractional concentrations (v/v). In constructing this figure a lung volume of 3 litres and a bag volume of 4 litres were assumed. Thus, a 300 ml error in lung volume determination represents an error of 10% in this calculation. As the concentration difference between the initial bag and lung concentrations becomes smaller the error increases acutely. When the stability and accuracy (as discussed previously) are taken into account, the uncertainty of a concentration measurement with the Centronic MGA 200 is probably about $\pm$ 0.005 (v/v). However, the deviation of the mass spectrometer from a linear calibration line will always, by necessity, be less than or equal to its deviation from one particular calibration line, the "line of identity". Deviations about the line of identity result in errors of accuracy. The deviation from linearity was found to be, in some cases, as little as half the deviation from the "line of identity". Therefore the "e" for the analyser used in this thesis is probably between the $\pm$ 0.0025 and $\pm$ 0.005 lines in Figure 2.12. Briscoe (1965) quotes a standard deviation of lung volume estimation by the constant volume helium method as between 100 and 300 ml, the worst case error would be about three times this. Therefore deviation from a linear response which could result in worst case errors of up to 300 ml are probably acceptable in the context of lung volume measurement. This is compatable with the use of this mass spectrometer to measure lung volume, provided that the initial concentration difference, between the bag and the lung, is over 20% v/v.

CHAPTER 3

Apparent Lung Volumes for Multiple Indicator Gases

3.1 INTRODUCTION

In 1971, Suwa and Bendixen suggested a variant of the forced rebreathing oxygen dilution technique of Lundsgaard and Van Slyke (1918), in which the oxygen rather than the nitrogen was used as the indicator gas. Forced rebreathing methods dispense with the need for volume measurement, carbon dioxide absorption and oxygen replenishment, which are required by most quiet breathing techniques. Numbered amongst the problems of forced breathing methods are the uncertainties concerning changes in the system volume during the manoeuvre. This problem has been tackled, empirically, by Rahn et al (1949), and algebraically, by Nunneley et al (1974). Suwa and Bendixen's proposal was based upon a graphical solution, which was ostensibly simpler than these previous attempts. It entailed using a rapid oxygen analyser with a chart recorder to produce a concentration versus time graph of the manoeuvre. By the time mixing was achieved, the system volume differed by an unknown amount from that present at the start of the manoeuvre, owing to gas exchange. The slope of the later, mixed, portion of the trace, however, reflected the prevailing rate of gas exchange. Suwa and Bendixen's premise was that, by extrapolating from this later part of the concentration trace to the start of rebreathing, one could calculate a hypothetical oxygen concentration ($F_{Mo}O_2$). This is the concentration which would have resulted if the contents of the bag and lung had been mixed at the initial instant of the manoeuvre ("time zero", before any gas exchange had taken place). At "time zero", the amount of O_2 in the system, $(V_{Bo} + V_{Lo}).F_{Mo}O_2$, would equal the sum of the amounts present separately in the bag, $(V_{Bo}.F_{Bo}O_2)$, and lung, $(V_{Lo}.F_{Lo}O_2)$, immediately prior to rebreathing. (V_{Bo} = initial bag volume, V_{Lo} = initial end-expiratory lung volume, $F_{Bo}O_2$ is the initial concentration of oxygen in the rebreathing bag, and $F_{Lo}O_2$ is the initial mean alveolar concentration of O_2.)

By rearrangement,

$$V_{Lo} = V_{Bo}(F_{Bo}O_2 - F_{Mo}O_2)/(F_{Mo}O_2 - F_{Lo}O_2) \qquad (3.1)$$

Since there is no need to measure volume during rebreathing, the apparatus needs to be no more than a rebreathing bag plus a change-over valve. The use of O_2 as the indicator gas allows a rapid and portable polarographic O_2 electrode to be used for gas analysis. At first sight, the extrapolation technique dispenses with the need to use and measure two inert gases (Van Slyke and Binger, 1923; and Nunneley et al, 1974) and avoids the inflexibility of the approach of Rahn et al (1949). The simplicity of the oxygen method, suited the aims of this work, and offered potential advantages in intensive care and, possibly in anaesthesia. The method deserved closer study. Preliminary work, on healthy subjects, used a fast responding polarographic sensor to measure the O_2 concentration during the rebreathing manoeuvre. However, it was found that the values obtained for lung volume varied consistently, but inexplicably, with the change in oxygen concentration imposed at the onset of rebreathing. To examine this more closely, a respiratory mass spectrometer (Centronic 200 MGA) was used to record the concentrations of all gases in the system.

3.2 METHODS

3.2.1 The Rebreathing Valve

Five healthy volunteers were studied. During the studies, they were seated comfortably in a standard position and breathed through a remotely operated switch (Figure 3.1) which brought about the change from non-rebreathing (or "control" conditions) to rebreathing conditions. The device consisted of a central hollowed perspex hub which engaged a rebreathing port (R), an inspiratory port (I), an expiratory port (E), and a mouthpiece port (Black, Scott and Somerset,1980). The inflow through "I" and the outflow through "E" were directed by one way valves (P) taken from

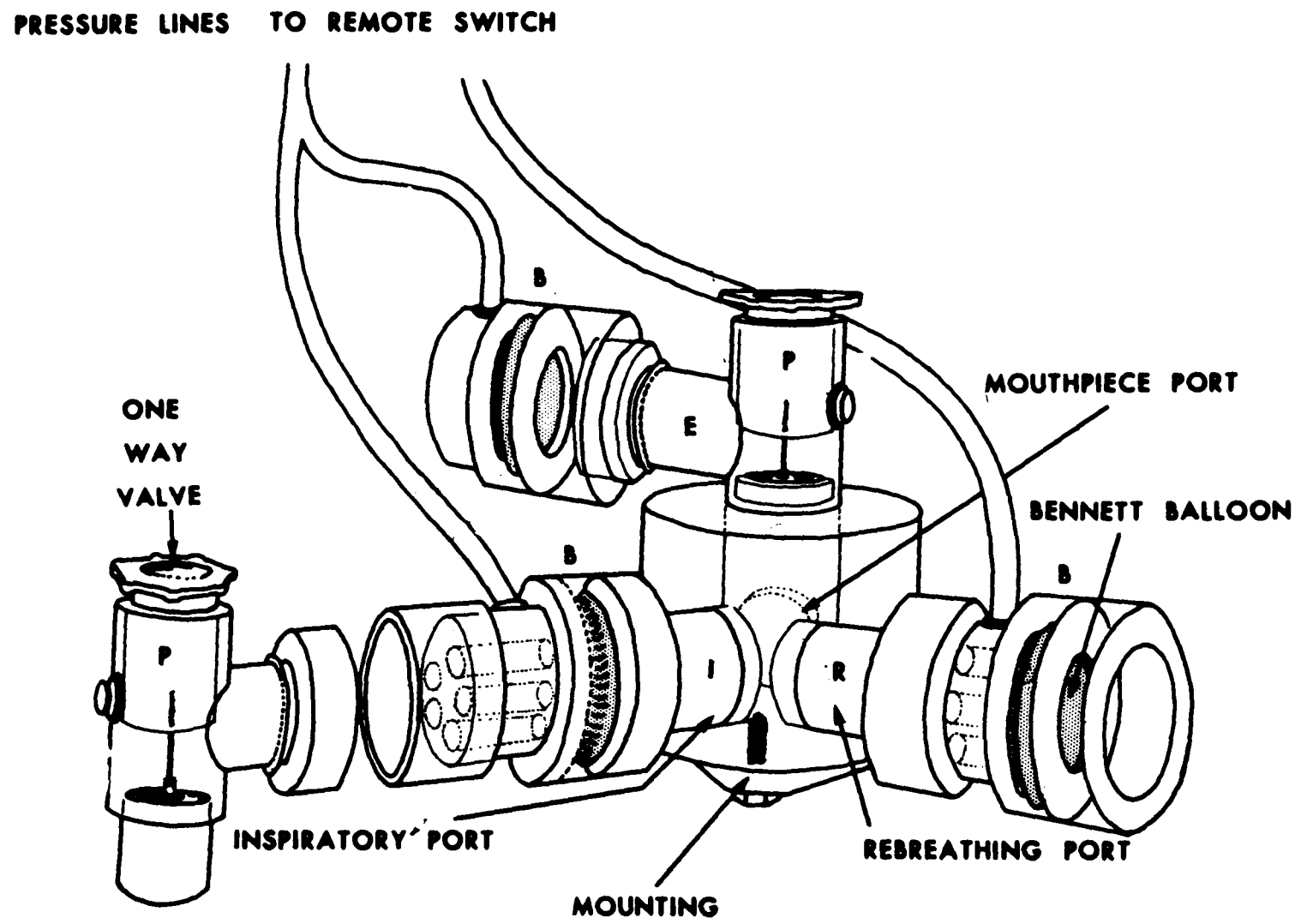


Figure 3.1 The rebreathing switch, see section 3.2.1 for description.

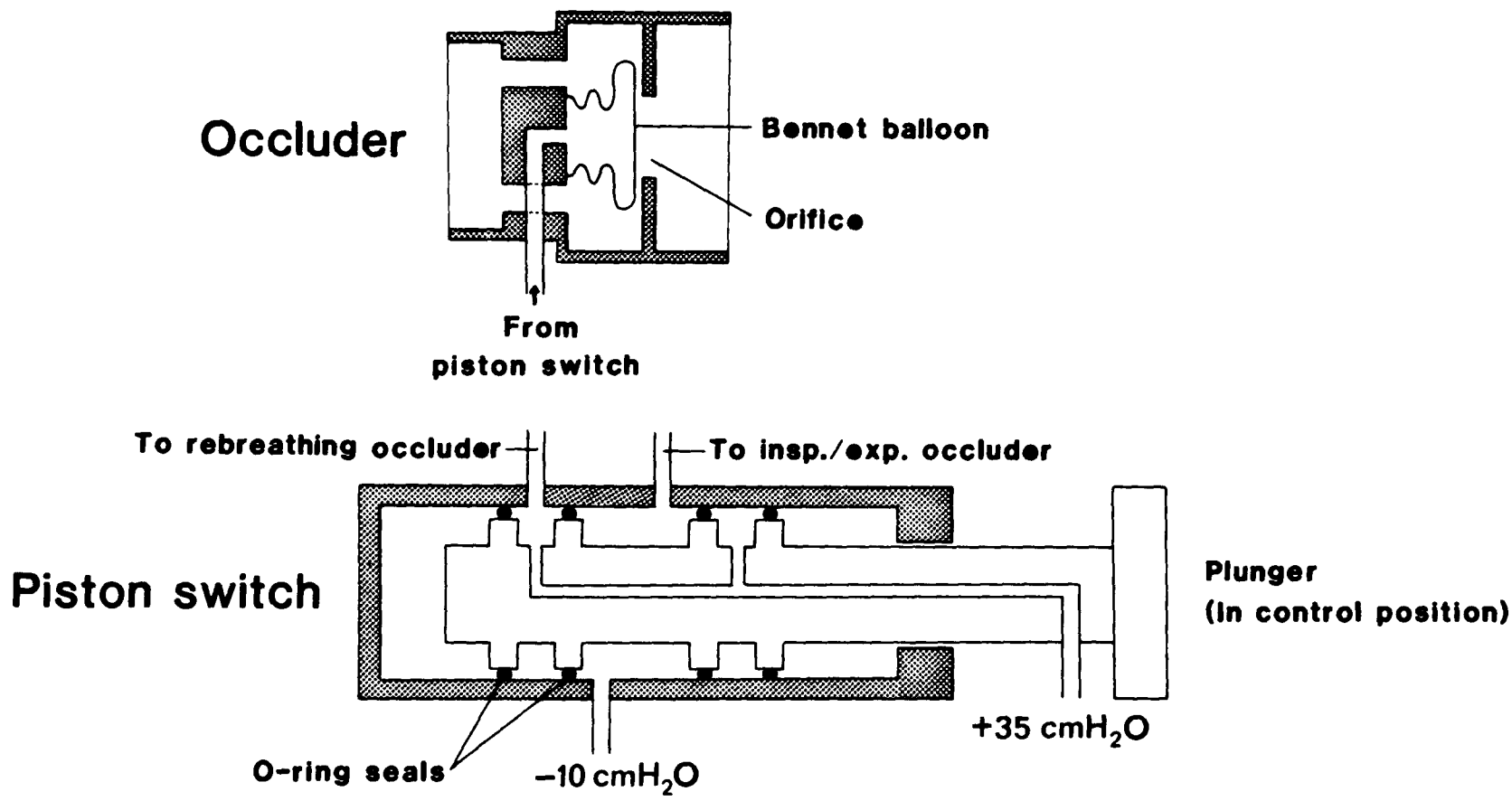


Figure 3.2 Top: An occluding valve which is controlled by the piston switch.
Bottom: Piston switch used to interchange the pressures between the rebreathing occluder and the inspiratory and expiratory occluders.

a Penlon Bain anaesthetic circuit. Each port was guarded by a small balloon (B) (Bennett PR2 ventilator component no. 0705) which could be inflated to occlude or deflated to open the orifices of their adjacent ports. During the control period, the "I" and "E" balloons were kept deflated by connection to a vacuum supply, regulated to provide a pressure of -10 cm H_2O, whilst the "R" balloon was inflated to +35 cm H_2O (derived from a suitably limited compressed air supply). Thus, before rebreathing, the "I" and "E" ports were open and the rebreathing port was closed. To start the rebreathing manoeuvre, the pressure supplied to the "I" and "E" balloons was interchanged for that supplied to the "R" balloon. The interchange was effected rapidly by a remotely operated piston switch (Figure 3.2). This closed ports "I" and "E", whilst simultaneously opening the rebreathing port. Thoughtful orientation of the balloon valves protected the rebreathing circuit from potential leaks around the balloon mounts.

3.2.2 Mass Spectrometry

The mass spectrometer (Centronic 200 MGA) can distinguish up to eight different gases. Three, and later, four gases were used in this study (O_2, N_2, CO_2 and Ar). The separation of the gas species is on the basis of the atomic mass to charge ratio (m/e) of the ion species produced after ionisation within a vacuum. A gas mixture is split into a "mass spectrum" and, by reading the peak heights (proportional to the number of ions produced) at different m/e, the concentration of the component gases is derived. The oxygen peak height was read at mass to charge ratio 32, nitrogen at 28 and Argon, if used, at 40. A proportion of the carbon dioxide, is broken down (upon ionisation) and forms carbon monoxide ions, which interfere, with the reading of N_2 at m/e ratio of 28. A CO_2 concentration of 6% v/v would cause the nitrogen concentration to be overread by 0.5% (see Chapter 2, section 2.4.2). In the experiments

described in this chapter, this error was ignored.

The mass spectrometer sampled the respiratory gases at a rate of 70 ml/minute through a nylon catheter 1.25 m long with 0.5 mm internal diameter. In these initial studies, the mass spectrometer was calibrated for O_2, N_2, and Ar using gas mixtures containing either zero or 100% v/v of these gases. The CO_2 channel was calibrated at zero, in air, and 6%, with a mixture of 6% v/v CO_2 in air, from a BOC certified cylinder. There were later found to be pitfalls in this mode of calibration which will be discussed later, but they cannot be held responsible for the principal observations obtained in this study. Similar observations were repeated in later work undertaken with better understanding of the methodology.

After calibration of each dry gas, the instrument was switched into the "Automatic Sensitivity Control" (ASC) mode. This mode prevents variations in the overall output of the machine by adjusting the gain in such a way that the sum of the fractional concentrations of the dry gases is always unity. This compensates for the changes in actual concentrations brought about by the variation in water vapour content of the sampled gas mixtures. In essence, the mass spectrometer ignores water vapour, if it has not been entered for ASC, and yields "dry gas" compositions, (Scheid et al, 1971)

Since the rebreathing bag was filled with a known volume of <u>dry</u> gas measured at room temperature and the concentrations were measured as dry gases, the lung volume values obtained must also apply to dry gas at room temperature (T_R). This temperature varied little during the course of one experimental session. Correction to BTPS would involve the factor:

$$\frac{310}{273 + T_R} \times \frac{P_B}{P_B - 47}$$

This factor would be the same for all indicators in a single manoeuvre, and essentially the same for all steps of the protocol. Thus, ignoring this factor would not account for differences in volumes of distribution between

indicators or between steps of a protocol. As these differences, rather than the absolute value of lung volume, were the focus of interest, the universal correction to BTPS was not employed.

3.2.3 Rebreathing Technique

During the control period, the subject inhaled through port "I" from ambient air (or from a Douglas bag containing one of a number of gas mixtures) and exhaled, via port "E", through a pneumotachograph which recorded expiratory flow. After a stable control period, and at the end of a normal expiration (signalled by the cessation of flow through the pneumotachograph), the piston switch was manually operated to start the rebreathing manoeuvre. The timing of the change could not be anticipated by the subject but, as soon as it had been effected, he or she was instructed to breathe as deeply and as regularly as possible so as to ensure rapid mixing of the bag and lung contents.

The rebreathing bag was a six litre anaesthetic bag. The orifice and occluding balloon for the rebreathing port comprised a unit which remained permanently attached to the rebreathing bag. All bags were fitted with an identical unit (Figure 3.2) of this sort. For each rebreathing manoeuvre, the rebreathing bag was filled with four litres of a selected dry gas mixture. The mixtures had previously been made up in 100 litre storage bags, using rotameters. The four litre amounts were transfered to the rebreathing bag using a 1 litre gas syringe (P.K. Morgan, Chatham, Kent) and a one way flap valve. After filling of the rebreathing bag, its orifice was occluded by inflating its balloon from the +35 cm H_2O supply. The bag was then transferred and fitted onto the rebreathing valve. After each rebreathing manoeuvre, the remote piston switch was returned to the control position. The "used" bag and its occluder were then exchanged for a "new" bag. The positive pressure sealing the "new" bag was then routed through the piston switch. The apparatus was now set up for a further

experimental run.

This arrangement permitted the study of a succession of rebreathing manoeuvres, separated by only 2-5 minutes, which was the time normally taken for a subject to reach steady state conditions of control breathing. Any one of a number of control gas mixtures could be followed by any one of a number of rebreathing gas mixtures. In the initial studies, the control mixture was air and the rebreathing mixtures were O_2 in N_2. Later Ar was used in both the control and rebreathing mixtures. A variety of gas change protocols were employed. Each complete protocol consisted of between 2 and 4 different concentration regimes between the control and rebreathing gas mixtures. Each regime was repeated 5 times within a randomised sequence of individual determinations.

3.3 RESULTS and DISCUSSION

3.3.1 Interpretation of the Rebreathing Trace

Figure 3.3 illustrates representative concentration traces obtained when a subject rebreathes from a bag, filled with 48.5% v/v O_2 and 51.5% v/v N_2, after a control period breathing air. The switch is operated at end expiration and "time zero" marks the beginning of the first inspiration from the rebreathing bag. During subsequent breaths, the subject inspires steadily to near total lung capacity and expires to near residual volume. The resulting mixing of lung and bag contents causes the bag and lung gas compositions to approach each other progressively. After 4 breaths, a near constant minimal difference of about 1% v/v O_2 remains on the O_2 concentration trace, which reflects the O_2 uptake within each breath. After this time, the bag and lung O_2 concentrations fall in parallel, almost linearly with time, which is also the result of the continuing O_2 uptake from the bag/lung system.

Before describing the details of the trace, three terms must be

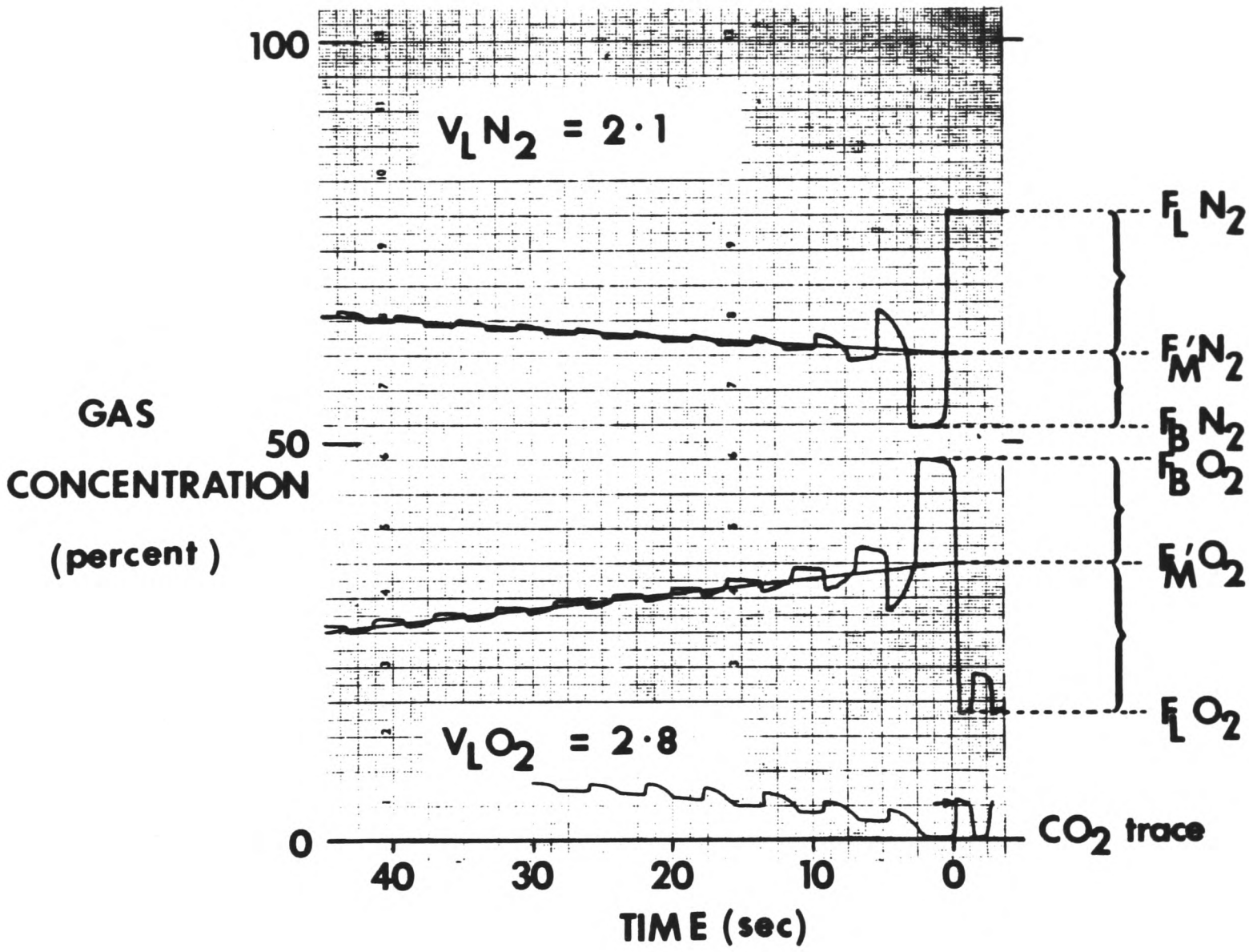


Figure 3.3 Concentration versus time traces for oxygen, nitrogen and carbon dioxide from the mass spectrometer (time runs from ~~left~~ right to ~~right~~ left). This shows a rebreathing manoeuvre in which a subject breathes air before rebreathing from a bag, containing 48.5% v/v O_2 in N_2. The "extrapolated mixing lines" are drawn for O_2 and N_2. These yield the extrapolated mixing concentrations which when entered into equation 3.1 give the apparent lung volumes shown. The significance of the brackets is described in the text.

defined to save confusion:

(i) The part of the trace when the bag and lung concentrations stop converging will be called the "equilibrated portion of the trace".

(ii) The term "mixing line" will be used to refer to a theoretical line which represents the concentration which would be produced if the bag and lung were homogeneious at each instant in time.

(iii) The "extrapolated mixing line" is a line drawn by the experimenter, through the "equilibrated portion of the trace", in an attempt to estimate the path of the "mixing line".

These terms will appear in inverted commas in order to signify their special meaning.

An estimation of the position of the "mixing line" only begins to become discernable after about 10 seconds in this manoeuvre. It is assumed to lie between the excursions of the "equilibrated portion of the trace". By drawing a straight line through this portion to the start of rebreathing the "extrapolated mixing line" is plotted.

A closer inspection of Figure 3.3 reveals some details about the gas exchange occurring before and during the rebreathing.

1. When the inspiratory and expiratory concentrations are sufficiently different, the change from end-expiration to inspiration is not a "square wave". It has a form which reflects the blurring of the distinction between dead space and bag gas as well as time response characteristics of the mass spectrometer. A steady inspiratory level is attained before the end of inspiration. Thus, it is reasonable to assume that the contents of the rebreathing bag are well mixed and that the time response of the mass spectrometer is sufficient for inspiratory and expiratory periods greater than two seconds. The expired CO_2 and O_2 fractions never reach a steady level as they do during inspiration. The concentration of oxygen is falling throughout expiration and that of carbon dioxide is rising. This

was first shown by Krogh and Lindhard in 1914. Until 1961, the accepted interpretation of this was on the basis of inhomogeneities of the gas distribution in the lungs. The work of Rahn, Kim and Farhi (1961) suggested that the above phenomenom was due to gas exchange continuing throughout expiration. Later parts of the expirate having had more time to lose O_2 and acquire CO_2.

2. In order to investigate inhomogeneities of gas distribution, an inert gas or gases must be used. The effects of ventilation-perfusion distributions are not then confused with ventilation inequalities. The first inspiration from, and expiration into, the rebreathing bag is effectively a "single breath" nitrogen washout manoeuvre, similar to that described by Comroe and Fowler (1951) and used to investigate uneven ventilation. The slope on the expired nitrogen trace is known as the "alveolar plateau". This plateau is observed, during expiration, following a step change in the inspired concentration of any, relatively insoluble, inert gas. Krogh and Lindhard (1917) attributed the slope of this plateau, after inspiration of a hydrogen mixture, to incomplete mixing of the inspired gas with the resident gas thereby creating a concentration gradient within the alveolar gas. This has come to be known as the "stratified" or "series ventilation" theory, and it is supported by the work of Sikand et al (1966) and Cummings et al (1966 and 1967). An alternative theory was suggested by the early work of Sonne (1934) and Roelson (1938) and by the theoretical appraisal of Rauwerda (1946, cited by Bouhuys, 1964). This states that each respiratory unit is perfectly mixed, but different units recieve a different proportion of the inspired gas and that sequential emptying of these units produces the sloping alveolar plateau. This theory is the "regional" or "parallel ventilation" theory. Otis et al (1956) showed that a model incorporating terminal airway resistance and alveolar compliance would provide for differential

ventilation and sequential emptying. Ball et al (1962) and Milic-Emili et al (1966) demonstrated the presence of regional differences in ventilation but suggested that these regions did not empty sequentially. There continues to be controversy as to the relative importance of these two types of inhomogeneity but there is no doubt that inhomogeneity does occur. Compounded with any inhomogeneity effects is the continuing gas exchange during expiration (Cotes, 1967). Inert gas later in the expiration has been in the alveoli longer and, therefore, has had more time to be concentrated (by the net uptake of oxygen over carbon dioxide output) than that in the earlier part of the expirate. The work of Cormier and Belanger (1981 and 1982) and Van Liew and Arieli (1981) supports the view that gas exchange contributes to the inert gas alveolar plateau.

3. During the "equilibrated portion of the trace", the difference between inspiratory and expiratory O_2 is considerably less than that which occurred during the control period. If no less O_2 is being extracted per breath, this change reflects the fact that the depth of ventilation is markedly increased. An important corollary to this is that, if hyperventilation was not used whilst rebreathing, the scope for drawing the "extrapolated mixing line" would be considerable. This would make the estimation of the course of the true "mixing line" for O_2 very difficult. There is also a visual artifact which makes the difference between inspiration and expiration appear smaller during rebreathing. When gas concentrations are measured at the mouth, the most pronounced change in composition is the end-expiratory to inspiratory transition ("E/I transition"). The I/E transition, on the other hand, does not appear so abrupt because of the dead space washout and the sloping alveolar plateau. It is, however, this I/E transition that reflects the gas exchange taking place in the lung. When breathing air the I/E and E/I transitions are the same size, because the inspired concentration before and after expiration

is the same. During rebreathing, the inspired concentration is changing and, as a result, the E/I transition (the most "step-like") is reduced because the inspired concentration before the expiration is greater than that after, due to the gas exchange during the breath.

4. The "equilibrated portion" of the O_2 mixing line slopes downwards with time. If this O_2 uptake was matched by CO_2 output, and nitrogen was insoluble, the nitrogen trace would be flat. The rise in the N_2 concentration (as noted by Lundsgaard and Schierbeck, 1924) indicates that one of these assumptions is false. The nitrogen output under the most favourable conditions can be calculated.

Referring to Figure 3.3, it can be seen that after 15 seconds the bag and lung contents are well mixed. Thus, it is a reasonable assumption that, at this time, the lung tissue gas tensions are in equilibrium with the lung gas (Cander and Forster, 1959). As a result, any nitrogen gained by the gaseous system must be supplied by the pulmonary blood flow. After 15 seconds, the nitrogen fraction in the lung is about 63% v/v. If the mixed venous tension is assumed to remain constant and equivalent to a concentration of 80% v/v, then the most generous driving pressure for a nitrogen output is about 15% of the dry ambient pressure. Using the Fick dilution principle with an elevated cardiac output of 10 L/min (due to hyperventilation, Armitage and Arnott, 1949) and an Ostwald blood solubility coefficient (at 37°C) for N_2 of 0.015 (Table 3.1), the expected N_2 output is:

Pulmonary blood flow		Solubility		Correction for H_2O		Concentration gradient
10,000	x	0.015	x	713/760	x	15/100

that is, 21 ml/min. Therefore, between 15^{th} and 45^{th} second of the manoeuvre, the projected nitrogen output is 10.5 ml. This would raise the

system concentration, of nitrogen by only 0.2% v/v. Inspection of the real trace indicates that the actual rise in N_2 concentration in the 30 second period was between 3 and 3.5% v/v. Thus, the expected N_2 output is a minor component in the actual slope of the nitrogen trace. The slope probably reflects the net contraction of the bag/lung volume as a result of the uptake of O_2 in excess of the CO_2 output. If this was the cause of the slope, then the net system volume contraction can be estimated as:

System volume		Concentration rise		Final concentration
6.6	x	0.03	÷	0.66

or 300 mls over 30 seconds. Therefore, oxygen consumption would need to be 600 ml/min (or in STPD, 530 ml/min) over and above the CO_2 output. This large value for volume shrinkage is indicative of two phenomena. Firstly the O_2 uptake is elevated because the cardiac output is higher than at resting levels. Secondly, the the CO_2 output is not elevated but drastically reduced, as proposed earlier.

3.3.2 Discrepancies between the Apparent Volumes of Distribution

The procedure suggested by Suwa and Bendixen, (1971), was to draw a straight line through the "equilibrated portion" of the O_2 trace and extrapolate it to zero time. The O_2 concentration intercept at this time is taken as $F_{Mo}O_2$ and, using equation 3.1, lung volume can be calculated. This approach should be equally applicable for N_2. Thus, the corresponding procedure applied to the "equilibrated portion" of the N_2 trace should yield a corresponding $F_{Mo}N_2$.

From equation 3.1, the lung volume, V_{Lo}, is the product of the initial bag volume, (V_{Bo}, 4 litres), and the ratio of the upper bracket on the O_2 trace, ($F_{Bo}O_2-F_{Mo}O_2$), to the lower bracket, ($F_{Mo}O_2-F_{Lo}O_2$). The result of this calculation is 2.7 litres. The same should apply to the N_2

OSTWALD SOLUBILITY COEFFICIENTS AT 37°C

GAS	WATER	REFERENCE	BLOOD	REFERENCE
ARGON	0.029	Morrison et al, 1954		
CARBON DIOXIDE	0.637	Morrison et al, 1952		
HELIUM	0.0095	Morrison et al, 1954		
HYDROGEN	0.0190	Morrison et al, 1952		
NITROGEN	0.014	Steward et al, 1973	0.015	Steward et al, 1973
NITROUS OXIDE	0.47	Steward et al, 1973	0.47	Steward et al, 1973
OXYGEN	0.0270	Power, 1968	0.0253*	Christoforides et al, 1969

* Haemoglobin inactivated by $NaNO_2$

Table 3.1 The Ostwald solubility coefficients (37°C) for gases commonly used in respiratory research.

of Figure 3.3

trace but it is obvious by inspection that the ratio of the bracket ($F_{Mo}N_2$-$F_{Bo}N_2$) to ($F_{Lo}N_2$-$F_{Mo}N_2$) is smaller than the corresponding ratio for O_2. Calculation of lung volume from the N_2 trace gives a value of 2.1 litres.

Differences between volumes of distribution are to be expected for gases of sufficiently different solubility. They may be used to measure lung tissue volume separately from the gas volume in the lung, (Cander and Forster, 1959; Hall et al, 1972; Sackner et al, 1975; Peterson et al, 1978). The water solubilities of N_2 and O_2 are not sufficiently different to explain this discrepancy (Table 3.1). The solubility of oxygen in lung tissue, is a rather special case. The lung tissue volume includes the resident pulmonary capillary blood volume (Sackner et al, 1964), and haemoglobin has a high affinity for O_2. This affinity is dependent on the partial pressure of oxygen. At a partial pressure of 13 kPa, the haemoglobin is almost fully saturated with oxygen. Increasing the O_2 partial pressure causes only a small increase in the amount of O_2 bound to the haemoglobin. The interest of this thesis is confined to the oxygen distribution volume at alveolar concentrations of about 13 kPa upwards. By using the figures for the standard oxy-haemoglobin dissociation curve (Severinghaus, 1966 and Roughton and Severinghaus, 1973) along with the physical solublity of O_2 in blood of 0.0029 $ml(STPD).mmHg^{-1}.100ml^{-1}$ of blood (Table 3.1), an equivalent solubility of oxygen in nearly saturated blood at at 37°C can be derived (Figure 3.4). The equivalent Ostwald solubility for oxygen from 13 to 70 kPa is only 0.027.

In conclusion, the expected distribution volumes of oxygen and nitrogen, under the conditions of these experiments, are not sufficiently different to explain the observed discrepancy.

The discrepancy was seen in all subjects in rebreathing manoeuvres

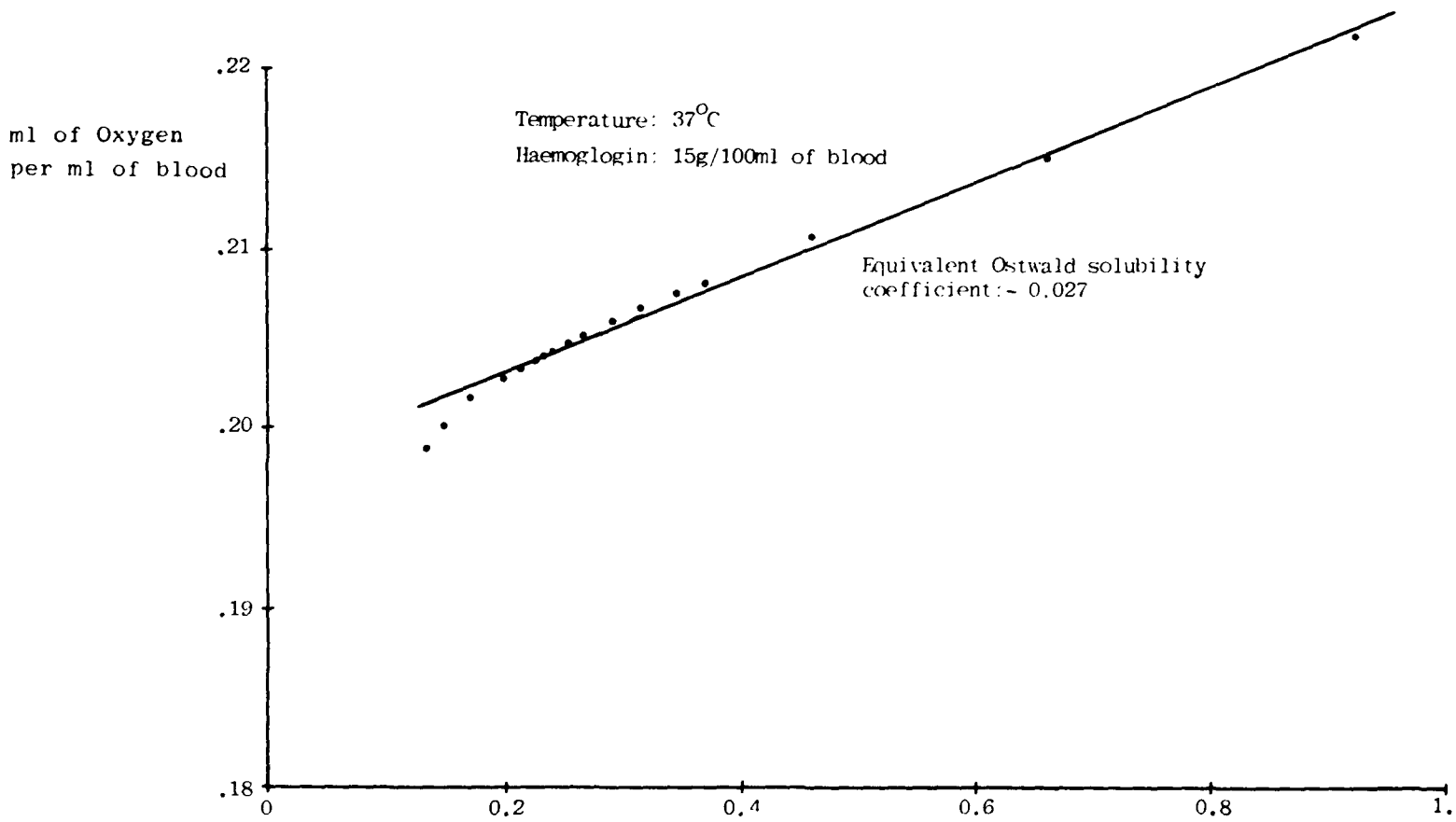


Figure 3.4 A plot of the volume of oxygen (ml) dissolved in each millilitre of blood (at 37°C) versus the partial pressure in standard atmospheres. Data is from Severinghaus (1966) and Roughton and Severinghaus (1973). The slope of this relationship is equivalent to an Ostwald solubility coefficient.

performed at lung volumes ranging from residual volume almost to total lung capacity (Figure 3.5).

The discrepancy varied with the type of concentration change imposed between the control and the rebreathing period. This is shown in Tables 3.2 to 3.5. In these tables, the figures given for lung volume are the means and standard deviations of 5 repetitions. Since this was the exploratory phase of this study, a rigorous statistical analysis is deferred until a more definitive stage. However, if two means differ by more than the sum of their standard deviations, this difference is likely to be statistically significant.

Table 3.2 shows the results of a protocol in one subject in which a control period breathing air was followed by rebreathing from one of four mixtures containing varying concentrations of O_2 in N_2. Although there is no obvious progression of the volumes of distribution of O_2, there is an obvious decrease in the apparent volume of distribution of N_2 with increasing N_2 concentration in the rebreathing bag. Table 3.3 shows that the relative sizes of the apparent volumes of distribution vary with the direction of the concentration change imposed between the control and rebreathing periods. Tables 3.4 and 3.5 demonstrate that N_2 and Ar are qualitatively interchangeable.

3.4 DISCUSSION

3.4.1 The cause of the Apparent Discrepancies

By closer inspection of Figure 3.3, one can confirm that, at any point in time, the sum of the concentrations of the three gases is indeed 100% v/v. If the linearly "extrapolated mixing lines", drawn from the "equilibrated portions of the traces", truly indicate the course of the "mixing line", through pre-mixing portions, summation to 100% v/v should also apply to the concentrations on the "extrapolated mixing line". More especially, it should apply to the intercept of the "extrapolated mixing

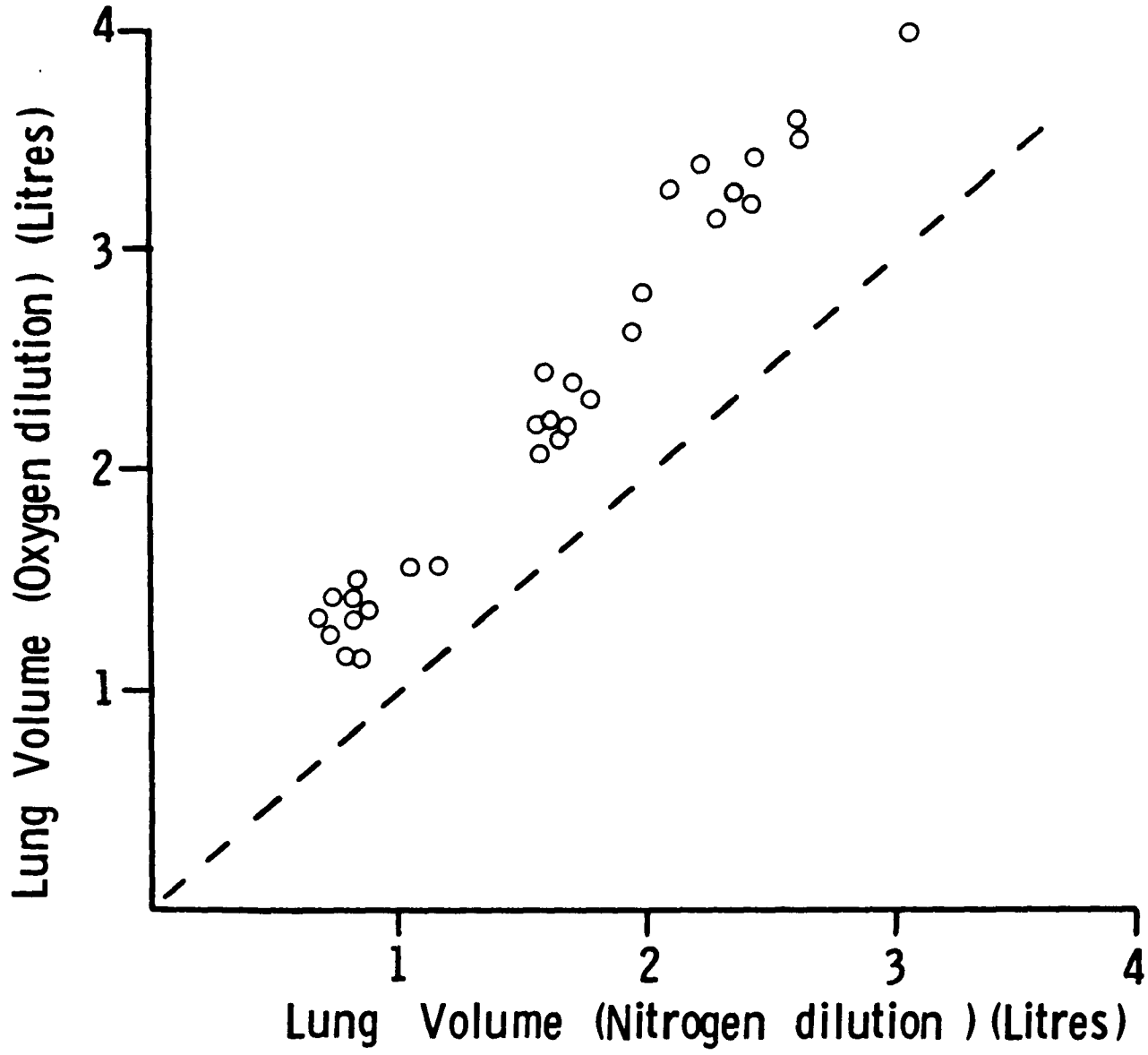


Figure 3.5 The relationship between the apparent lung volumes by O_2 and N_2 dilution over a range of end-expiratory volumes. The results are from a single subject and a single composition change (breathing air then rebreathing 50% v/v O_2 and N_2). The dotted line is the line of identity.

CONTROL	REBREATHING		APPARENT LUNG VOLUME	
	O_2%	N_2%	O_2	N_2
AIR	100	-	2.82 (.10)	2.50 (.08)
	75	25	2.88 (.20)	2.41 (.10)
	50	50	2.84 (.12)	2.14 (.13)
	35	65	2.95 (.26)	1.67 (.18)

Table 3.2 The apparent volumes of distribution (litres) of O_2 and N_2 for a protocol consisting of 4 composition changes (one per row). The subject breathed air in the "control" period and then rebreathed from a bag containing an O_2/N_2 mixture. Each apparent lung volume is the mean of five repetitions, standard deviations shown in brackets.

CONTROL		REBREATHING		APPARENT LUNG VOLUME	
O_2%	N_2%	O_2%	N_2%	O_2	N_2
21	79	50	50	3.20 (.25)	2.09 (.02)
100	-	50	50	2.14 (.20)	2.49 (.15)

Table 3.3 Same format as Table 3.2. In each manoeuvre the apparent volume of distribution is larger for the gas which is initially more concentrated in the rebreathing bag.

CONTROL		REBREATHING		APPARENT LUNG VOLUME	
O_2%	N_2%	O_2%	N_2%	O_2	N_2
21	79	50	50	2.99 (.19)	2.32 (.18)
O_2%	AR%	O_2%	AR%	O_2	AR
21	79	50	50	2.91 (.23)	2.44 (.16)

Table 3.4 Same format as Table 3.2. The first line shows a rebreathing manoeuvre involving O_2 and N_2 and the second a similar manoeuvre with O_2 and Ar. Note the interchangability of N_2 and Ar.

CONTROL			REBREATHING			APPARENT LUNG VOLUME		
O_2%	N_2%	Ar%	O_2%	N_2%	Ar%	O_2	N_2	Ar
21	79	-	50	-	50	2.91 (.13)	2.54 (.11)	2.79 (.14)
21	-	79	50	50	-	2.89 (.27)	2.85 (.16)	2.63 (.17)

Table 3.5 Same format as Table 3.4 but for a triple gas concentration change. Showing the interchangability of N_2 and Ar.

line" at zero time. Therefore, it follows that $(1-F_{Mo}O_2-F_{Mo}N_2)$ should equal $F_{Mo}CO_2$. The value of $(1-F_{Mo}O_2-F_{Mo}N_2)$ is indicated in Figure 3.3 by the level of the arrow on the CO_2 trace at time zero. The $F_{Mo}CO_2$ is the concentration of CO_2 which would result if the bag and the lung had been mixed instantaneously at "time zero". If, for example, the initial lung CO_2 content was 5.5% v/v of a 2.6 litres lung volume, then, when mixed with a four litre bag, the $F_{Mo}CO_2$ will certainly be less than 2.5% v/v. Put another way, the extrapolation of the discernable CO_2 mixing line to zero time must clearly be 1.5 - 2% v/v below the value indicated by the arrow (Figure 3.3). Such an effect would follow if the apparent values of $F_{Mo}O_2$ and $F_{Mo}N_2$ obtained above are, in fact, lower than the true amount. An initial, but not sustained, rate of increase in the volume of the bag/lung system would cause this to happen. (The apparent values are henceforth denoted $aF_{Mo}O_2$ and $aF_{Mo}N_2$). A volume change of the sort just described is, in fact, to be expected under the circumstances in which this manoeuvre is performed. Once rebreathing has begun, the ventilation is greatly increased and this will tend to lower the alveolar PCO_2 whilst raising the PO_2. Because at non-hypoxic oxygen concentrations, the haemoglobin is nearly fully saturated, increasing the alveolar PO_2 does not significantly raise the oxygen uptake, assuming the pulmonary blood flow remains constant. Carbon dioxide output, on the other hand, is directly related to the alveolar PCO_2, and so CO_2 initially leaves the pulmonary blood at an accelerated rate. As the CO_2 accumulates in the rebreathing system, the alveolar PCO_2 rises and the carbon dioxide output, therefore, declines. After about 10 seconds, the CO_2 output would have virtually ceased, if the PCO_2 in the alveoli had risen to be the same as the mixed venous blood. In practice, the output of CO_2 does not cease because the mixed venous CO_2 tension will climb due to recirculation. The slopes of the "equilibrated portions" of the traces for O_2 and N_2 reflect only the later volume change due to the unabated O_2 uptake. They do not reflect the initial volume

increase owing to the CO_2 output since this is virtually complete by the time the mixing lines become discernable. Thus, the initial CO_2 output would already have diluted the O_2 and N_2 concentrations so that their respective "extrapolated mixing lines" would have undergone a parallel downward displacement. Linear extrapolations from the "equilibrated portions" to zero time would, as a result, indicate artificially low values, $aF_{Mo}O_2$ and $aF_{Mo}N_2$.

3.4.2 Historical Context

In 1924, Lundsgaard and Schierbeck showed that the "equilibrated portion" of a nitrogen rebreathing trace exhibits an upward trend with time. They attributed the primary cause of this slope to an uninterrupted oxygen uptake in the face of a near zero CO_2 output. Christie (1932) noted that, if the oxygen concentration was sufficient to ensure complete saturation of the blood, the oxygen consumption would be related to the cardiac output and little affected by the alveolar partial pressure. Carbon dioxide excretion, however, would be impaired as the inspired (spirometer) CO_2 content rose. Christie realised that the level of CO_2 output depended upon the interaction of two opposing factors: hyperventilation, raising CO_2 excretion, and the build up of this CO_2 in the lung, tending to lower the CO_2 excretion. He went on to note that the balance between these factors was difficult to estimate but he thought an error of 34 ml in his lung volume determination could result. Rahn et al (1949) considered the volume changes in two phases: one phase completed before the system was mixed, characterised by an elevated CO_2 output and therefore a large respiratory quotient (R), and the other phase, when mixing had occured, dominated by the O_2 uptake and, therefore, having a low R. Nunneley et al (1974) estimated the error, caused by what they called the "R effect", to be 100 ml in a determination of lung volume. Suwa and Bendixen (1971) seem to have been aware of the problems of an elevated CO_2

output, when they commentated that: "Theoretically using 6% CO_2- O_2 mixture in the bag should improve the accuracy". However they discounted its significance: "..in practice the results were identical to those obtained using pure O_2".

Thus, the phenomena underlying the observed discrepancies have long been recognised. However, they had never previously presented themselves as apparently different lung volumes for different gases. In addition, the magnitude of the discrepancies seen in this study had not been previously observed or predicted. As a result, the phenomenon was rediscovered in a new context.

3.4.3 Correction for the effects of Volume Change

3.4.3a Carbon Dioxide in the Rebreathing Bag

An explanation of the discrepancies between the distribution volumes of O_2 and N_2, based on an initial non-sustained output of CO_2 into the bag/lung system, is consistent with observations made with CO_2 in the rebreathing bag. The addition of carbon dioxide to the rebreathing bag will minimise the fall in alveolar PCO_2, which accompanies the start of rebreathing. If sufficient CO_2 is added, the output of CO_2 could, in theory, be temporarily halted. Table 3.6 shows that the effect of adding CO_2 to the rebreathing bag was to cause significant ($p<.01$, Student t test) reductions in the differences between the oxygen and nitrogen "FRC" before and after CO_2 addition. The fact that approximately mixed venous CO_2 partial pressures in the bag did not produce a complete correction has, in retrospect, been attributed to calibration errors, which are discussed later.

3.4.3bAn Analytical Technique

From equation 3.1, it follows:

$$V_{Lo}.F_{Lo} + V_{Bo}.F_{Bo} = F_{Mo}.(V_{Bo} + V_{Lo}) \qquad (3.2)$$

CONTROL	REBREATHING			APPARENT LUNG VOLUME		DIFFERENCE (O_2 - N_2)	
	O_2%	N_2%	CO_2%	O_2	N_2		
	50	50	-	2.79 (.13)	2.10 (.15)	0.69 (.18)	N = 10
AIR	50	44.5	5.5	2.68 (.16)	2.33 (.12)	0.35 (.15)	N = 8
	50	43.5	6.5	2.50 (.13)	2.34 (.06)	0.16 (.09)	N = 4

Table 3.6 The effect of adding carbon dioxide to the rebreathing bag. The layout is similar to Table 3.2. "N" indicates the number of observations in each mean. A column showing the difference between the O_2 and N_2 apparent lung volume is added. As the initial CO_2 concentration in the rebreathing rises a significant (Student t test: $P<.01$) reduction in this difference is observed.

Where F_{Mo} is defined as the concentration that would have existed if mixing was instantaneous. If the true F_{Mo} has been diluted by addition of CO_2 to the system prior to the completion of mixing then:

$$V_{Lo}.F_{Lo} + V_{Bo}.F_{Bo} = aF_{Mo}.(V_{Bo} + V_{Lo} + Vmiss) \qquad (3.3)$$

Where Vmiss is strictly, the volume change which must be associated with the extrapolated mixing concentration to preserve the mass balance in equation 3.3 (see Chapter 6, section 6.2.1b). If there is an initial volume change, which is exhausted before the "equilibrated portion of the trace", superimposed on a "steady state" volume change, then Vmiss will represent the volume change with the non-sustained rate. This assumes the extrapolation completely accounts for the effects of a steady state volume change (this is reviewed in the discussion). The hypothesis is that Vmiss will approximate to the initial CO_2 output. By combining equations 3.2 and 3.3, a relationship between F_{Mo} and aF_{Mo} is produced:

$$F_{Mo}.(V_{Bo} + V_{Lo}) = aF_{Mo}.(V_{Bo} + V_{Lo} + Vmiss)$$

The above equation will apply to any gas, whose mixing concentration could have been calculated by the extrapolation were it not for the initial CO_2 output. At a simplistic level, this encompasses any gas which is not taken up or given off by the lungs or one whose uptake or output is constant. Thus, two analogous equations can be written one for oxygen and one for nitrogen. Dividing one into the other produces:

$$\frac{F_{Mo}N_2}{F_{Mo}O_2} = \frac{aF_{Mo}N_2}{aF_{Mo}O_2} \qquad (3.4)$$

This simply states that the ratio of the extrapolated, or apparent, mixed concentrations is the same as the ratio of the "true" mixed concentrations. Recalling equation 3.1:

$$V_{Lo} = V_{Bo}.(F_{Bo}O_2 - F_{Mo}O_2)/(F_{Mo}O_2 - F_{Lo}O_2)$$

This equation can be rewritten for N_2, and by combining the two one obtains:

$$\frac{(F_{Bo}O_2 - F_{Mo}O_2)}{(F_{Mo}O_2 - F_{Lo}O_2)} = \frac{(F_{Bo}N_2 - F_{Mo}N_2)}{(F_{Mo}N_2 - F_{Lo}N_2)} \qquad (3.5)$$

There are now two equations (3.4 and 3.5) with the two unknowns: $F_{Mo}O_2$ and $F_{Mo}N_2$ Therefore, one can solve for both of these using simultaneous equations.

This technique is, in fact, analogous to the Van Slyke and Binger (1923) approach and to the more recent approach of Nunneley et al (1974) to changes in the respiratory quotient during rebreathing. The difference is that the previous authors did not use oxygen, but required a further inert indicator gas. In this work, oxygen is transformed into a "pseudo inert" indicator gas by the use of an extrapolation procedure.

3.4.3c An iterative technique

An alternative solution involves using an iterative technique to solve the two equations. By a rearrangement of equation 3.4:

$$\frac{F_{Mo}N_2}{aF_{Mo}N_2} = \frac{F_{Mo}O_2}{aF_{Mo}O_2}$$

Subtracting one from either side:

$$\frac{(F_{Mo}N_2 - aF_{Mo}N_2)}{aF_{Mo}N_2} = \frac{(F_{Mo}O_2 - aF_{Mo}O_2)}{aF_{Mo}O_2} \qquad (3.6)$$

A rearrangement of the above gives an equation relating to ratio of the <u>differences</u> between the "true" and apparent mixing concentrations to the ratio of the apparent mixing concentrations:

$$\frac{(F_{Mo}O_2 - aF_{Mo}O_2)}{(F_{Mo}N_2 - aF_{Mo}N_2)} = \frac{aF_{Mo}O_2}{aF_{Mo}N_2}$$

This forms the basis of the iterative correction procedure in which

(F_{Mo} - aF_{Mo}) is the amount to be added to the extrapolated mixing concentration to give the true mixed concentration. Small increments $\delta F_{Mo}O_2$ and $\delta F_{Mo}N_2$ are chosen such that $\delta F_{Mo}O_2/\delta F_{Mo}N_2$ equals $aF_{Mo}O_2/aF_{Mo}N_2$. These increments are added to $aF_{Mo}O_2$ and $aF_{Mo}N_2$ respectively. The resulting estimates, $*aF_{Mo}O_2$ and $*aF_{Mo}N_2$, are then assessed on their ability to improve the proximity of the lung volume as calculated by O_2 and N_2 when introduced into equation 3.1. Further increments are then added to produce a new estimate, this process is continued until the estimates give identical lung volumes. The assessment of the estimates is accomplished as follows (from equation 3.5):

$$\frac{(F_{Bo}O_2 - F_{Mo}O_2)}{(F_{Mo}O_2 - F_{Lo}O_2)} = \frac{(F_{Bo}N_2 - F_{Mo}N_2)}{(F_{Mo}N_2 - F_{Lo}N_2)}$$

This equality may be rewritten for the approximations to the true mixing concentration ($*aF_{Mo}$), then:

$$\frac{(F_{Bo}O_2 - *aF_{Mo}O_2)}{(*aF_{Mo}O_2 - F_{Lo}O_2)} - \frac{(F_{Bo}N_2 - *aF_{Mo}N_2)}{(*F_{Mo}N_2 - aF_{Lo}N_2)} = e$$

Where "e" is an error produced by discrepancies between the true and approximate mixing concentrations. In fact V_B.e is the difference between the two estimates of lung volume. As the cycle is reiterated, the size of "e" becomes progressively smaller, as the approximations converge on the "true" mixed values and eventually the sign of "e" is reversed. By choosing sufficiently small increments for $aF_{Mo}O_2$ and $aF_{Mo}N_2$, one can iterate to a discrepancy which is vanishingly small. When only two gases are used in the iterative correction procedure, the error, "e", can always be reduced to zero. This is shown graphically for a theoretical manoeuvre in Figure 3.7. The quantity displayed on the x-axis comes from equation 3.6 such that, for any level of Vmiss (dotted lines), this quantity is the same for the two indicator gases. The quantity on the y-axis is the apparent lung volume over the range of levels of Vmiss. The iteration

effectively traces back along the curves plotted for O_2 and N_2 (moving parallel to the x-axis) in an attempt to reduce the error, V_{Bo}.e. When the lines intersect, equation 3.5 is true. At this point, the calculated mixing concentrations, when introduced into equation 3.1, will produce a unique lung volume for O_2 and N_2, as shown on Figure 3.6.

Comparing Figure 3.3 with Figure 3.6, it is clear that the "true" path for the oxygen and nitrogen mixing line curves upwards from the straight line extrapolation. The absolute discrepancy for O_2 ($F_{Mo}O_2 - aF_{Mo}O_2$) is different than that for nitrogen ($aF_{Mo}N_2 - F_{Mo}N_2$) by a factor $aF_{Mo}N_2 / aF_{Mo}O_2$ (from equation 3.6). The sum of these discrepancies is equal to the distance between the two arrows on the CO_2 trace of Figure 3.6.

Applying this correction method to the protocol illustrated in Table 3.1 gave unique volumes of distribution which showed no trend with change in O_2 concentration in the rebreathing bag (Table 3.7).

It should be noted that the correction technique does not require any knowledge of the time-course, or total amount, of the early non-sustained output of CO_2. In addition, it is not dependent upon the volume change being specifically due to CO_2. An early non-sustained output, or uptake, of N_2O would just as easily disturb the volume of the bag/lung system, and so produce discrepancies between the volumes of distribution of two indicators. Provided that two such indicators were present, and that the concentration ratios between the bag and the lung differ, the corrections described above should, in principle, work equally well.

3.4.3d Triple Gas Correction Technique

The correction techniques described above should be applicable to

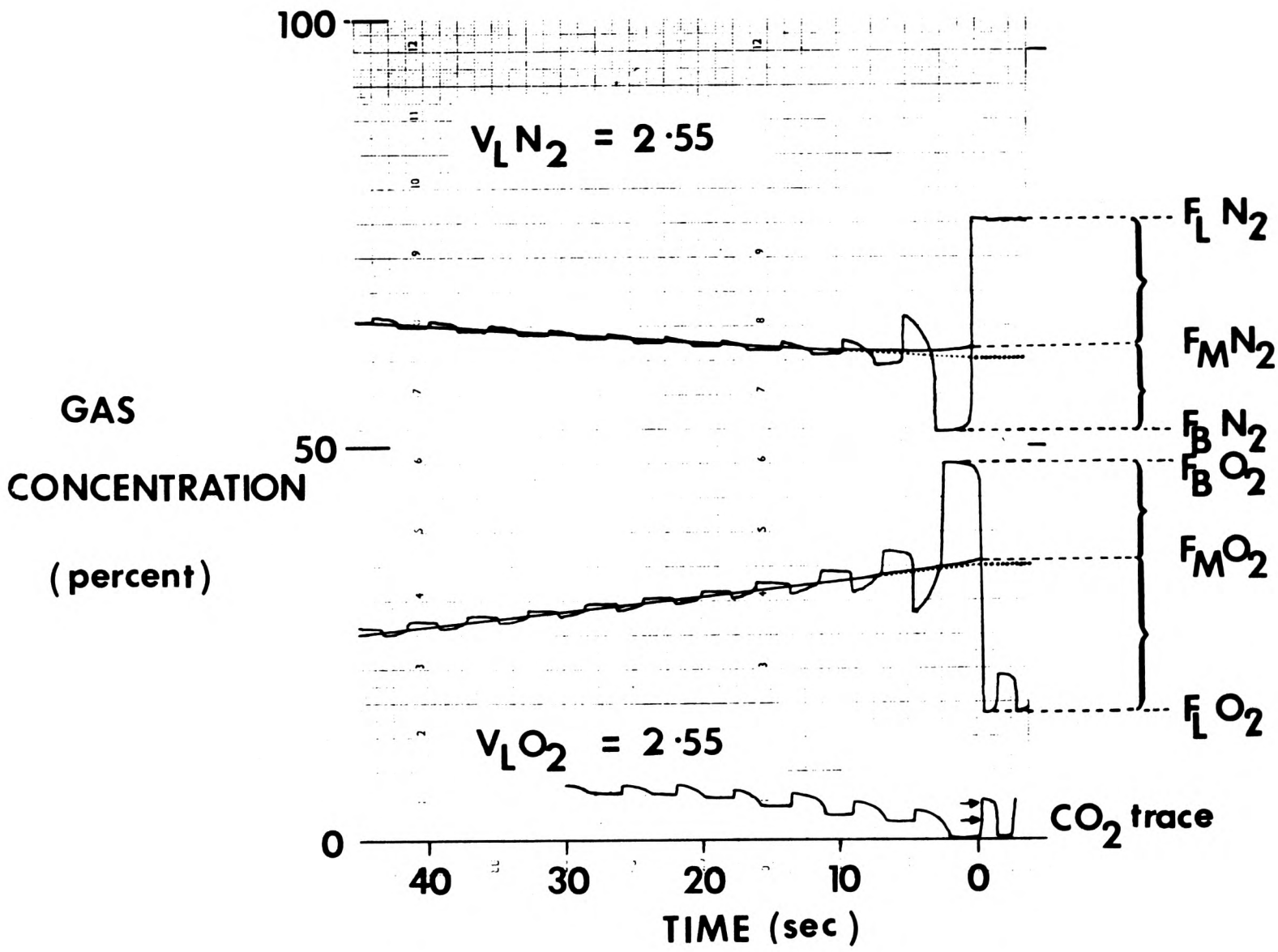


Figure 3.6 The same trace as shown in Figure 3.3 The dotted lines show a projected extrapolation which would give the initial mixing concentrations as calculated by the correction technique. The use of these concentrations in equation 3.1, leads to the calculation of the same lung volume by O_2 and N_2 dilution. The arrows on the CO_2 trace are derived by subtraction of the initial mixing concentrations of O_2 plus N_2 from unity. The upper arrow is before and the lower after correction.

situations where there are more than two indicators of the same notional volume of distribution, as when argon is included as a third gas in a protocol. For the iterative method there are three errors to be collectively minimised.

$$\frac{(F_{Bo}O_2 - {}^*aF_{Mo}O_2)}{({}^*aF_{Mo}O_2 - F_{Lo}O_2)} - \frac{(F_{Bo}N_2 - {}^*aF_{Mo}N_2)}{({}^*F_{Mo}N_2 - aF_{Lo}N_2)} = e1$$

$$\frac{(F_{Bo}O_2 - {}^*aF_{Mo}O_2)}{({}^*aF_{Mo}O_2 - F_{Lo}O_2)} - \frac{(F_{Bo}Ar - {}^*aF_{Mo}Ar)}{({}^*F_{Mo}Ar - aF_{Lo}Ar)} = e2$$

$$\frac{(F_{Bo}N_2 - {}^*aF_{Mo}N_2)}{({}^*aF_{Mo}N_2 - F_{Lo}N_2)} - \frac{(F_{Bo}Ar - {}^*aF_{Mo}Ar)}{({}^*F_{Mo}Ar - aF_{Lo}Ar)} = e3$$

In order to reduce the errors, the estimated mixing concentrations are increased in small increments, of which there are now three ($\delta F_{Mo}O_2$, $\delta F_{Mo}N_2$ and $\delta F_{Mo}Ar$) to be applied in each cycle of the iteration. They are chosen such that

$$\delta F_{Mo}O_2 : \delta F_{Mo}N_2 : \delta F_{Mo}Ar = aF_{Mo}O_2 : aF_{Mo}N_2 : aF_{Mo}Ar.$$

This is shown graphically in Figure 3.8. The results of applying this correction in a three gas system differ from the application to a two gas system in that it is not always possible to correct the three volumes of distribution to an unique value. In this case, sucessive approximations are made until a minimal value is obtained for the sum of squares of the deviations of e1, e2 and e3 from the mean value of the three e's. In an error free system, a solution based on simultaneous equations would give the same answer as the triple iterative and there would now be three unknowns and as many equations. However, the experimental system contains a number of errors concerning both the assumptions and the measurements. Under these conditions, the iterative approach produces the optimal solution but <u>not</u> a unique lung volume from the three indicator gases.

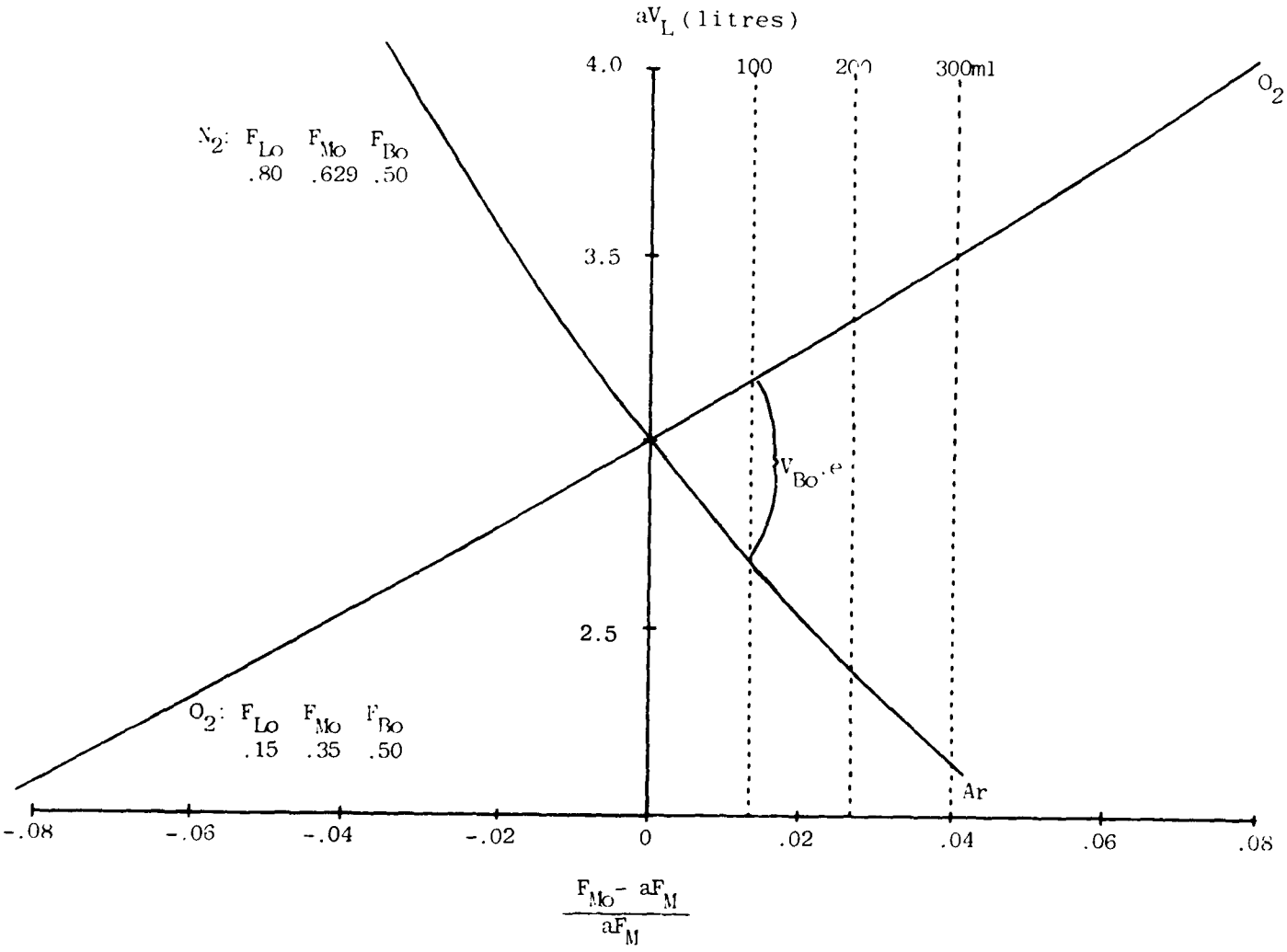


Figure 3.7 A theoretical plot of the apparent lung volumes (for O_2 and N_2) versus the relative difference between the true and apparent mixing concentrations. The dotted lines indicate the volume of gas which must have diluted the true mixing concentrations (Vmiss). Bag volume is 4 litres and the bag, lung and true mixed concentrations for O_2 and N_2 are shown. $V_{Bo}.e$ is described in the text.

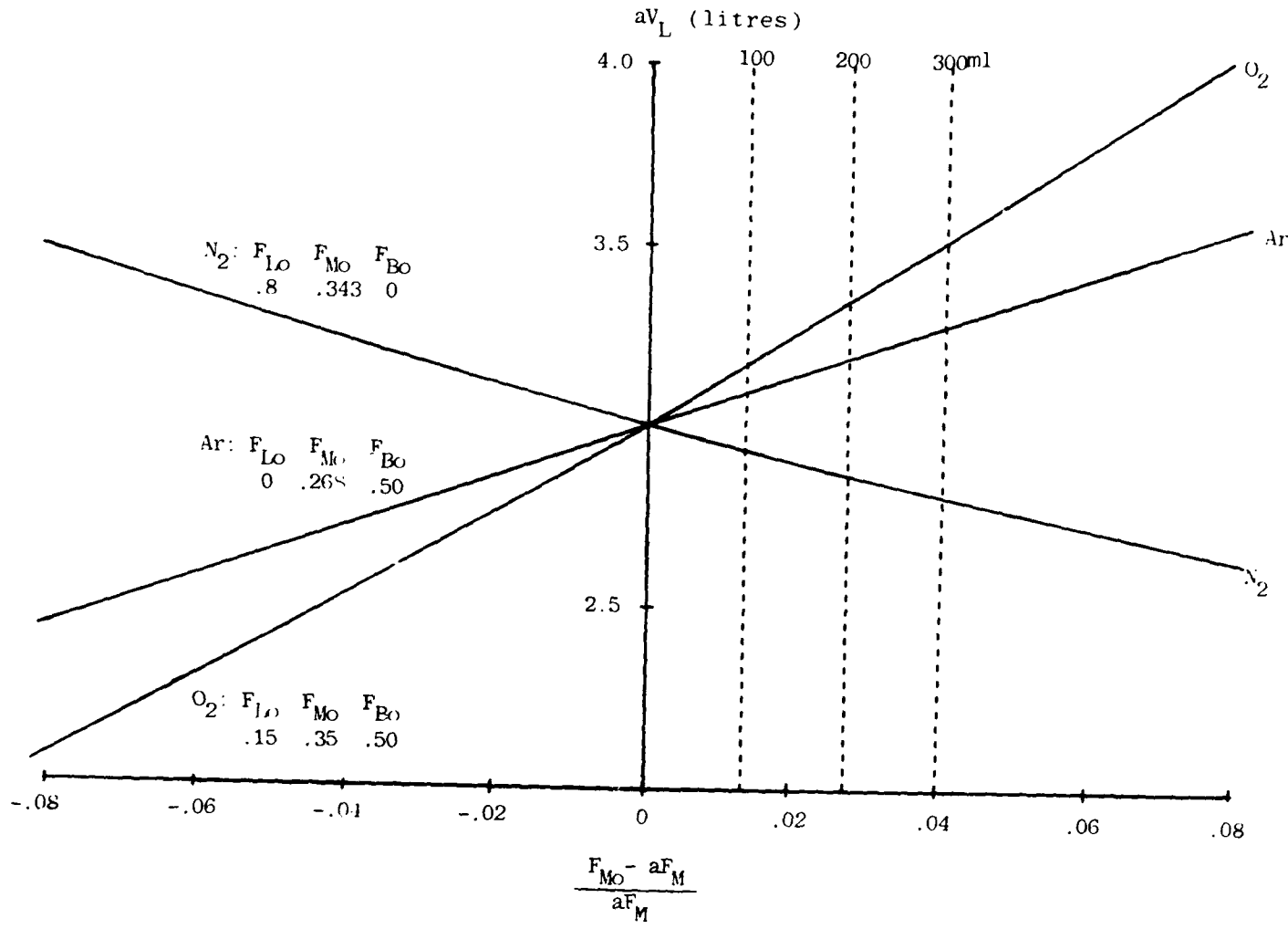


Figure 3.8 Same format as Figure 3.7, except that the apparent lung volumes of three gases are shown. An error in the slope of any one or all of the lines would not result in a unique point of interception.

Table 3.8 shows the results of the triple gas iteration procedure on a simple protocol employing two types of concentration change. A subject breathes 21% v/v O_2 in Ar during the control period and then rebreathes either 35% v/v O_2:15% v/v N_2:50% v/v Ar, or 35% v/v O_2:50% v/v N_2:15% v/v Ar during the rebreathing period. Although the apparent volumes of distribution are closer after correction than before, discrepancies still persist between indicators and between different steps of the protocol for each indicator gas.

On reflection, it is clear that the iteration to a unique value, as illustrated in Table 3.7 for a system with two indicators, is not a guarantee of correctness. If the initial values for the bag, lung and apparent mixing concentrations were erroneous, the errors would be compounded by the iteration. One can deliberately choose a grossly erroneous value for one or more of these values. The iterative procedure applied to a two indicator system will still yield a unique lung volume, although that volume might well be grossly different from a realistic volume. For example, if the slope of one or both of the curves in Figure 3.7 was altered, these two lines would still intersect at a point but this may not correspond to the true lung volume. With an additional indicator, errors in the starting values for the iteration result in residual discrepancies between the "corrected" lung volumes. Referring to Figure 3.8, if the slope of one or other of the lines changes, instead of converging on a unique value for lung volume, there is an irreducible "triangle of uncertainty" between three different estimates. Thus, it is reasonable to consider what unintentional errors might be impairing the correction in the circumstances of these experiments.

CONTROL	REBREATHING				
			APPARENT LUNG VOLUME		CORRECTED LUNG VOLUME
	O_2%	N_2%	O_2	N_2	
AIR	100	-	2.82 (.10)	2.50 (.08)	2.61 (.09)
	75	25	2.88 (.20)	2.41 (.20)	2.63 (.12)
	50	50	2.84 (.12)	2.14 (.13)	2.59 (.12)
	35	65	2.95 (.26)	1.67 (.18)	2.61 (.19)

Table 3.7 The effect of the iterative correction applied to the protocol of composition changes shown in Table 3.2. The volumes of distribution of O_2 and N_2 can be corrected to a unique lung volume which shows no trend between the composition changes.

3.4.4Errors in measuring Bag, Mixed and Lung Concentrations

3.4.4a Errors in Mass Spectrometer Calibration

After the completion of this preliminary work, Gilbe et al (1981) described how the use of 100% v/v O_2 and 100% v/v N_2 in calibrating the Centronix 200 MGA can lead to errors. Pure O_2 causes overreading of the O_2 signal during the exposure and overreading of other gases for a period afterwards. Exposure to pure N_2 causes underreading. Thus, the calibration procedure must have introduced errors in a way governed by the sequence in which the O_2 and N_2 calibrations were carried out, and by the interval between the O_2 and N_2 calibrations. These errors would have been compounded by the operation of the ASC mode. In these initial studies, the accuracy of the mass spectrometry was usually checked, at intervals after the initial calibration, only by observing that the mass spectrometer accurately read the composition of room air. Later, when more complete linearity checks were undertaken, these sometimes revealed non-linearity between the calibration points (see Chapter 2, Figure 2.10). After this discovery, all subsequent calibrations of the machine used a more appropriate calibration procedure, which was described in Chapter 2 (section 2.4.6). This new calibration removed the non-linearities that had been observed. Nevertheless, similar patterns of discrepancy were observed between the apparent volumes of distribution of different indicators. Thus, the gross patterns of discrepancy observed early in this study were not due to errors in the mass spectrometry. It is possible, however, that such errors are reflected in the failure of the iterative correction illustrated in Table 3.8.

Ineffective calibration also provides an insight as to why the use of CO_2 in the rebreathing bag was not completely effective in abolishing the differences between the apparent volumes of distribution of O_2 and N_2 listed in Table 3.6. Even if the presence of CO_2 prevented the volume

CONTROL			REBREATHING			APPARENT LUNG VOLUME			CORRECTED LUNG VOLUME		
O_2%	N_2%	Ar%	O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar
21	-	79	35	15	50	3.10 (.06)	2.48 (.21)	2.21 (.14)	2.72 (.08)	2.29 (.24)	2.57 (.09)
21	-	79	35	50	15	2.94 (.17)	2.64 (.20)	2.38 (.08)	2.58 (.07)	2.38 (.07)	2.55 (.06)

Table 3.8 The effect of the iterative correction applied to a protocol of triple gas composition changes. The iteration fails to correct to a unique lung volume. The apparent lung volume remains dependent upon the type of composition change imposed.

disturbance, non-linearity of the mass spectrometer could prevent two indicators from providing a unique lung volume.

3.4.4b The difference between Alveolar/End-Tidal Concentrations

If a properly calibrated mass spectrometer is used, the initial bag concentration should be faithfully recorded, but there is still scope for error in measuring the initial lung concentrations, since the end-tidal concentrations, particularly for O_2, may not accurately reflect mean alveolar concentrations in an inhomogeneous lung.

There are three factors which would cause the composition of end-tidal gas to be different from the mean alveolar gas. These factors were mentioned earlier in connection with the overall shape of a rebreathing graph (section 3.3.1). The first factor is inhomogeneity in the distribution of ventilation in relation to perfusion. Sikand et al (1966) have shown that, in normal lung, the effect of this type of inhomogeneity does not significantly affect the concentrations recorded at the mouth. The second factor is serial inhomogeneities in gas distribution. This would cause an end-expiratory sample to overestimate the mean alveolar oxygen tension. The third factor is the effect of gas exchange. Gas sampled at the mouth at one instant was "alveolar gas" some time before, this time being the time taken to travel through the conducting airways to the mouth. Therefore, even in a perfectly homogeneous lung, the gas concentrations recorded at the mouth will be the concentrations that had previously existed in the "alveolar space". The effect of gas exchange, in this context, is to cause the end-tidal sample to overestimate the oxygen concentration in the "alveolar space". The inert gas concentration, at the mouth, will only be affected by gas exchange as a result of the net uptake of oxygen, and this is likely to be a small effect.

Figure 3.9 shows the result of panting on the end-tidal oxygen concentration, immediately before a rebreathing manoeuvre. When a maximal expiration is performed, the end-tidal oxygen falls markedly from the end-tidal concentration seen at FRC. This is probably because of stratification of the inspired gas. Okubo and Piiper (1974) calculated that an increase in frequency and decrease in the depth of ventilation should significantly increase the statification in the lungs. Consideration of Figure 3.3 shows that ~~under~~ over-estimating $F_{Lo}O_2$ would have the effect of increasing the apparent volume of distribution of O_2. This is because, in calculating the apparent volume of distribution of O_2 using equation 3.1, the difference, $(F_{Bo}O_2 - F_{Mo}O_2)$, would be divided by an underestimate of the true $(F_{Mo}O_2 - F_{Lo}O_2)$. Since the difference between inspired and alveolar nitrogen is substantially smaller than for oxygen, the calculation of the apparent volume of distribution of N_2 is liable to be less in error than for the O_2. The effect of panting on the lung volumes, as calculated by O_2 and N_2 dilution is shown in Table 3.9. This demonstrates the susceptibility of the O_2, but not the N_2, apparent lung volume to this errror.

Figure 3.10 shows the discrepancy between the apparent volumes of distribution of oxygen and nitrogen measured in one subject over a period of several days. For every estimation, the subject breathed air, in the control period, and 50% v/v O_2 in N_2 for rebreathing. On day "zero", an episode of upper respiratory tract infection began, during which there was appreciable bronchospasm. It is likely that, in the face of bronchospasm, the regional variations in ventilation become larger. Airway closure may occur, over part or the whole normal breathing range, and the end-tidal O_2 concentration would tend to overestimate the mean alveolar fraction. This is because the end tidal sample would contain gas from non-dependent and better ventilated regions since the gas in the dependent regions would be

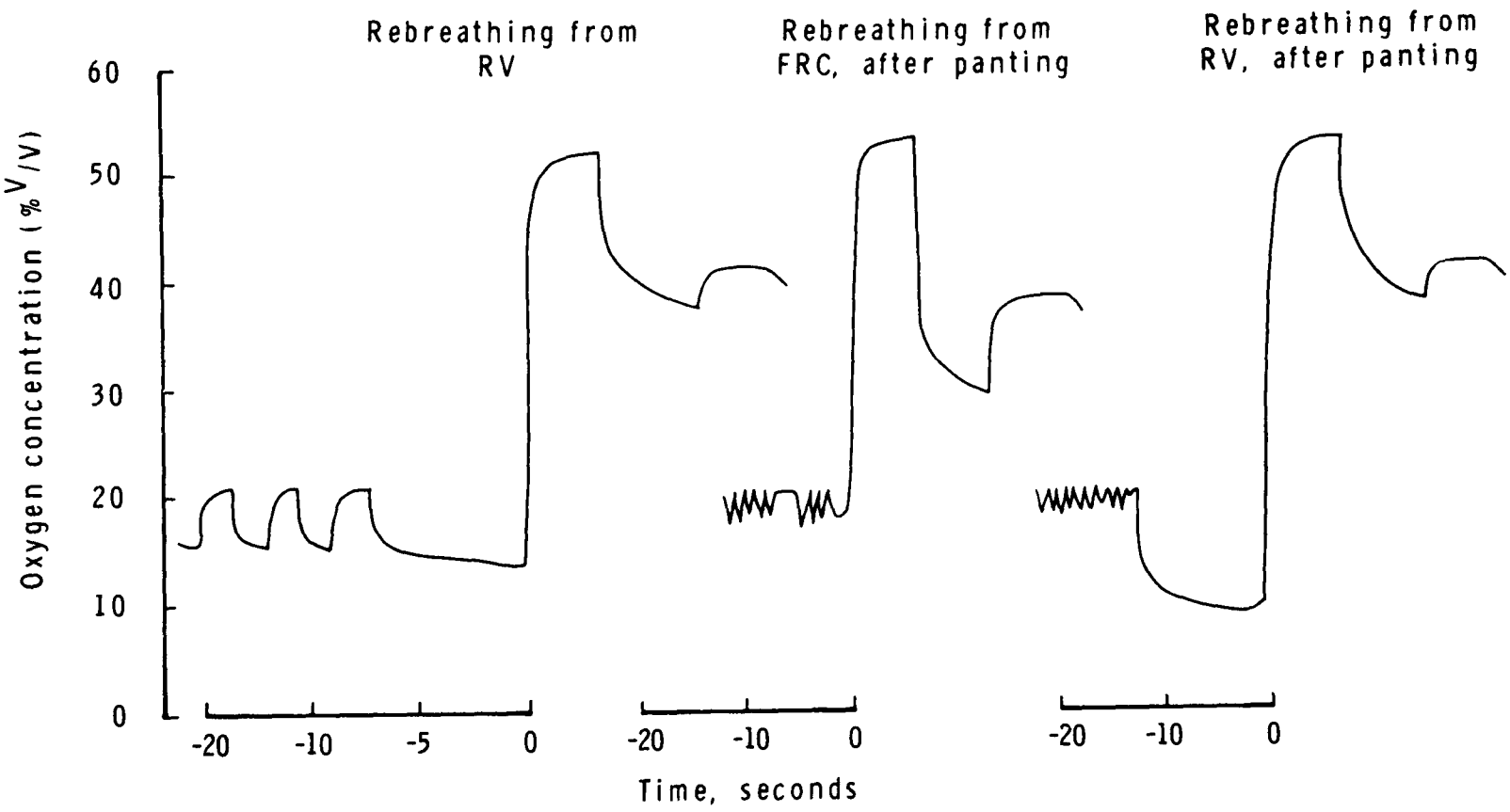


Figure 3.9 The start of three rebreathing manoeuvres. In the first, a residual volume manoeuvre (RV) immediately precedes rebreathing. In the second, a number of panting breaths precede rebreathing. In the third, panting again precedes rebreathing, but the breath immediately before rebreathing is a maximal expiration.

		CONTROL	REBREATHING		APPARENT LUNG VOLUMES	
			O_2%	N_2%	O_2	N_2
1)	R.V. MANOEUVRE:	AIR	50	50	3.08 (0.19)	2.23 (0.11)
		AIR - PANTING	50	50	△ 3.71 (0.17)	2.29 (0.13)
			O_2%	N_2%	O_2	N_2
2.	F.R.C. MANOEUVRE:	AIR	50	50	1.39 (0.09)	0.77 (0.05)
		AIR - PANTING	50	50	1.39 (0.04)	0.79 (0.05)

Table 3.9 The apparent lung volumes for O_2 and N_2 with a normal "control" period as compared to panting. This is shown:
1. When a maximum expiratory manoeuvre immediately precedes rebreathing.
2. When rebreathing commences from the respiratory level maintained during the rest of the "control" period.
The △ indicates a significant difference (Student t test: $p<.01$) in apparent lung volume for O_2, but not N_2, before and after panting

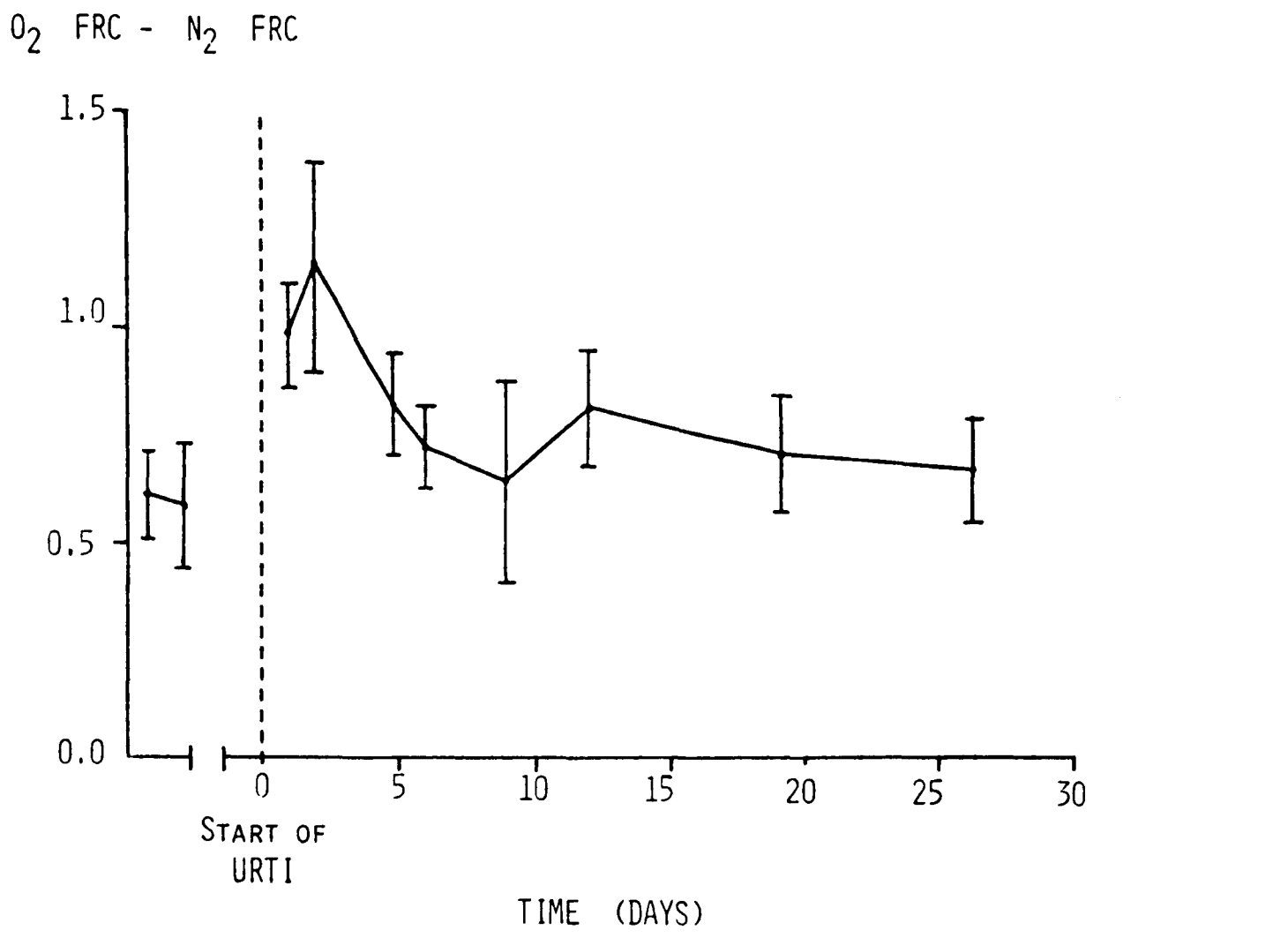


Figure 3.10 The discrepancies between the apparent volumes of distribution of O_2 and N_2 measured in one subject before and during an upper respiratory tract infection (URTI). For each measurement, the subject rebreathed from an initial bag concentration of 50% O_2 in N_2 after a "control" period breathing air.

prevented from having access to the mouth. Once again, the end-tidal N_2 concentration would remain close to the mean alveolar N_2 concentration. Thus the discrepancy between the apparent volumes of distribution of O_2 and N_2 would increase as observed in Figure 3.10.

3.4.4c Errors in the extrapolation

Two assumptions are implicit in the extrapolation procedure: the first is that a linear equation adequately reflects the course of the mixing line, and the second is that the true mixing line lies within an "envelope" described by the bag and lung concentrations on the rebreathing trace.

The theoretical arguments for the equation of the mixing line are presented in the next chapter but the assumption that it is linear is false. Under some conditions, such as in the presence of nitrous oxide or with a small bag volume, the mixing line will be appreciably curvilinear.

During the later "equilibrated portion of the trace", there remains a difference between the end-tidal bag and lung concentrations, owing to the prevailing gas exchange. Assuming that these end-tidal concentrations represent the mean bag and mean alveolar concentrations, the true mixing line will lie between the two, such that the distance between the bag and the mixed line, and the mixed line and the lung, is in the ratio $V_L : V_B$ (Chapter 6, Figure 6.2). Since the hyperventilation employed in the manoeuvre almost envariably changes the end-expiratory volume, the $V_L : V_B$ ratio is likely to change from breath to breath during rebreathing. Depending upon the ventilation, and the concentrations, there may be considerable leeway in placing this mixing line by eye.

The position of the theoretical mixing line is only defined between the bag and lung concentrations at the end of an expiration. In the experimental situation, the recording of the bag concentration is delayed until the end of the next inspiration, when the bag contents are drawn

across the sampling probe. If the inspired and expired concentrations remain separated in the "equilibrated portion of the trace" and if the trace has an appreciable slope, a considerable error in placing the mixing line by eye may result from this delay.

Major ventilatory inhomogeneities will delay mixing of the bag and lung contents. In such a situation the use of a smaller bag volume would reduce the time taken to achieve a reasonable degree of mixing. The "mixing line" will only lie between the bag and lung end-tidal concentrations in a homogeneous lung or in a lung with ventilatory inhomogeneities disposed in parallel and simultaneously ventilated. In lungs with ventilatory inhomogeneities arranged in series, there may be an initial period where mixing within the faster, more proximal, compartments is taking place. At such a time, the mean concentration for the bag/lung system may be outside of the "envelope" created by the bag and lung end-tidal concentrations. This situation is illustrated in Figure 3.11.

3.5 CONCLUSIONS

The validity of Suwa and Bendixen's (1971) proposal that oxygen could be used as the indicator gas for the measurement of lung volume has been examined. This technique measures the dilution of oxygen during rebreathing, employing an extrapolation procedure to calculate the hypothetical initial mixed concentration. A logical test of the validity of this approach was to incorporate the simultaneous measurement of an inert gas or gases, (utilising the same extrapolation procedure). This provided two or more indicators of lung volume within the same manoeuvre. When these indicators were used to determine lung volume, there were discrepancies between their apparent distribution volumes. These artifacts were attributed to an initially large, but non-sustained, rate of CO_2

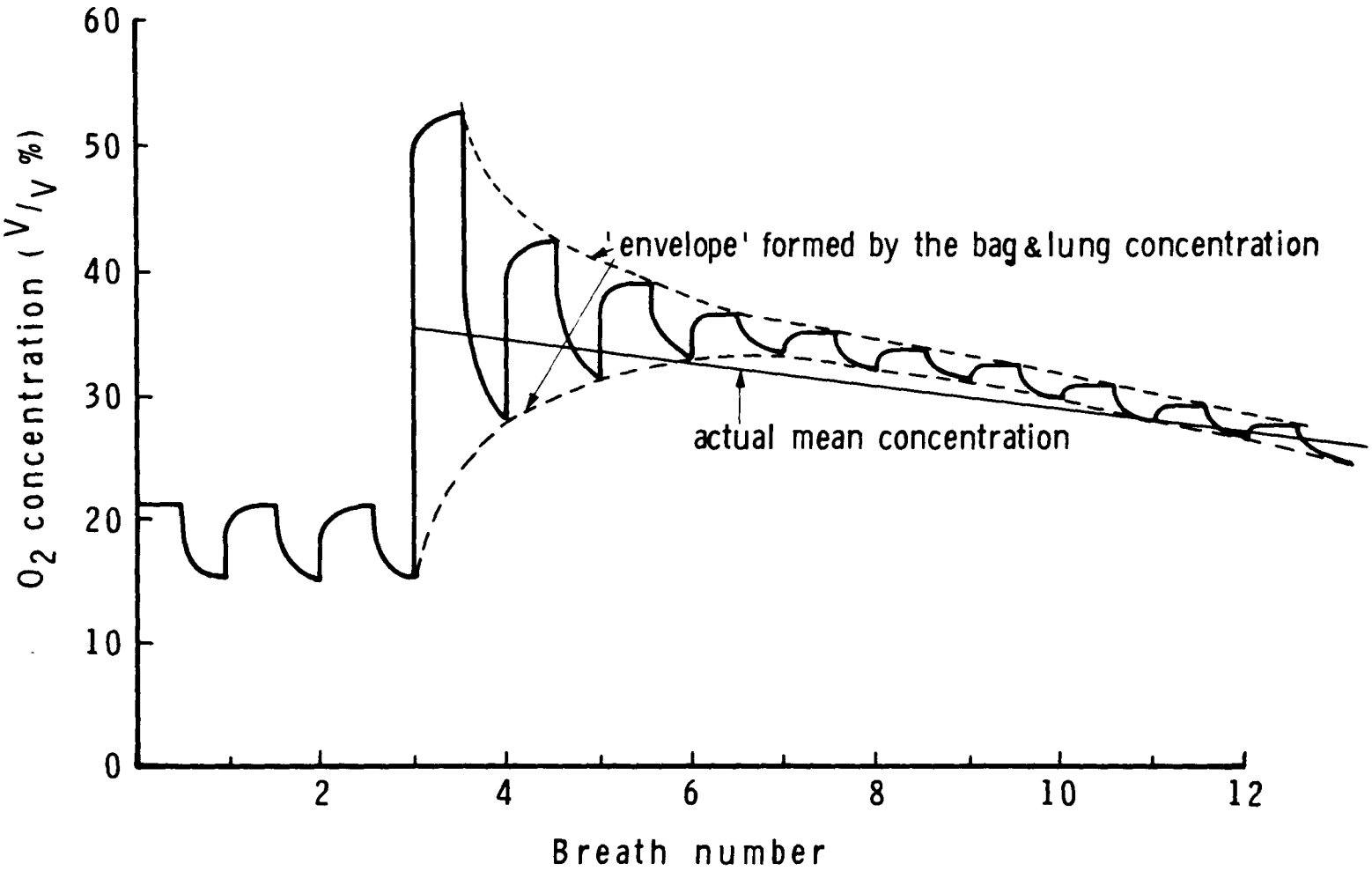


Figure 3.11 The oxygen concentration for a hypothetical rebreathing manoeuvre in the presence of series inhomogeneity. The actual mean O_2 concentration in the system (continuous line) is outside the envelope formed by the bag and lung concentrations (dotted lines), for some of the time.

output into the rebreathing system (Black, Hahn, Maynard and Scott, 1980). The variety of apparent lung volumes observed was governed in some way by the concentration change imposed between control and rebreathing. A correction technique was developed which, in theory, allowed the true lung volume to be calculated. When using three indicator gases, the correction was imperfect and this probably indicated the presence of experimental errors.

CHAPTER 4

A simple Numerical Model of Rebreathing

4.1 INTRODUCTION

In this chapter, a simple numerical model of the rebreathing manoeuvre is used in an attempt to simulate the pattern of discrepancies that were observed in Chapter 3. The aim is to develop and test a means of correcting arithmetically for the uncontrolled and indeterminate changes in the combined volume of the rebreathing bag and lung. This correction is intended to be applicable whether the volume change is produced by the output of CO_2 alone, or by the additional uptake or output of another gas, such as nitrous oxide. The ultimate aim is to develop a method of lung volume measurement, suitable for anaesthesia and intensive care.

4.2 METHODS

4.2.1 Experimental Methods

The apparatus and experimental techniques were similar to those described in Chapter 3. An important difference was that nitrous oxide was use in some of these experiments, and this required the use of the subtraction unit described in Chapter 2. The interference of CO_2 with the reading of N_2, which was ignored in Chapter 3, was also removed using the subtraction unit.

A number of subjects were studied, but the results of only a single subject are presented. These results are divided between three protocols each protocol, consisting of three or four types of composition change. Each composition change was repeated five times in a random sequence.

4.2.2 Numerical Methods

4.2.2a A Model Simulation of Rebreathing

The model was similar in many respects to that used by Petrini et al (1978). The features assumed were:

1. Instantaneous inspiration and expiration.
2. Instantaneous mixing of the inspirate with alveolar gas, and of the expirate with the gas in the rebreathing bag.
3. Values for uptakes and outputs are assumed for the gases under consideration. A gas may be assumed not to undergo any alveolar-capillary exchange (ie. zero uptake or output).
4. If non-zero gas uptakes and outputs occur, they do so during the inspiratory pause. Thus, the composition of the alveolar gas does not change during expiration.
5. A given gas's uptake, or output, may either remain constant or change from breath to breath in an exponential fashion.
6. During rebreathing, there is a constant inspiratory tidal volume, a constant dead space to tidal volume ratio and a constant end-expiratory volume (FRC). Thus, the net uptakes from (or outputs into) the bag/lung system are reflected in breath-to-breath changes in the expired volume, and ultimately in the volume of the rebreathing bag.

The model assumptions, as described above, are required to avoid the use of differential equations. Instead, the model algorithms are built around mass balance equations which apply at end-expiration and end-inspiration, these will be discussed further in Chapter 6. The use of such equations simplifies the modelling because, the number of calculations required are considerably less than in a model based on differential equations, so that the time taken to produce a simulation is reduced. Since the hypothesis being tested involves "between breath" changes in the system volume, the finer details of the "within breath" time courses of gas exchange (which could be provided by a differential equations simulation) are superfluous. The model was written as a computer programme, using "H-P Basic", and was "run" on a Hewlett-Packard 9825A desk top computer. The

computer programme is shown in Appendix 2. The calculator was interfaced to a printer, which allowed the simulated concentration traces to be plotted. The programme allowed rebreathing manoeuvres with up to four gases to be simulated (O_2, N_2, Ar, and CO_2 or O_2, N_2, N_2O and CO_2).

The behaviour of oxygen was mimicked by making one gas have a constant uptake of 25 mls/breath, at a rebreathing frequency of 10 breaths per minute. Two other gases, nitrogen and argon, were designated as inert gases with no uptake or output. The behaviour of CO_2 was imitated by setting up an output which was initially high (usually 60 ml in the first breath), because of the deliberate hyperventilation, but which declined on subsequent breaths. The decline was a geometric progression, the CO_2 output for each breath being 70% of that for the previous breath. Although rather arbitrary, this pattern of CO_2 output was chosen on the basis of producing a reasonable imitation for the profile of the end-expired carbon dioxide fraction (see Figure 4.5), seen in real subjects. When nitrous oxide was simulated in the model, it replaced the argon and was a gas for which there could be initial uptake or output, depending on the relative initial concentrations of N_2O in the bag and lung. A geometric progression governed the decline of the N_2O uptake or output towards zero. It was set at 0.5 ml times the first breath percentage concentration difference between the bag and the lung, and decreased by a factor of 0.7 on each subsequent breath.

Thus, at the start of a simulation, values were assigned to the following variables (as depicted in Figure 4.1):-

1. Initial rebreathing bag volume, (V_{Bo})
2. Functional Residual Capacity, (V_L)
3. The alveolar ventilation, (V_A = tidal volume minus dead space)
4. Initial bag gas composition, ($F_{Bo}O_2$, $F_{Bo}N_2$, $F_{Bo}Ar$, $F_{Bo}N_2O$)
5. Initial alveolar gas composition, ($F_{Lo}O_2$, $F_{Lo}N_2$, $F_{Lo}Ar$, $F_{Lo}N_2O$, $F_{Lo}CO_2$)
6. The first breath uptakes or outputs of oxygen, ($V_{b1}O_2$), carbon dioxide,

($V_{b1}CO_2$) and nitrous oxide, ($V_{b1}N_2O$)

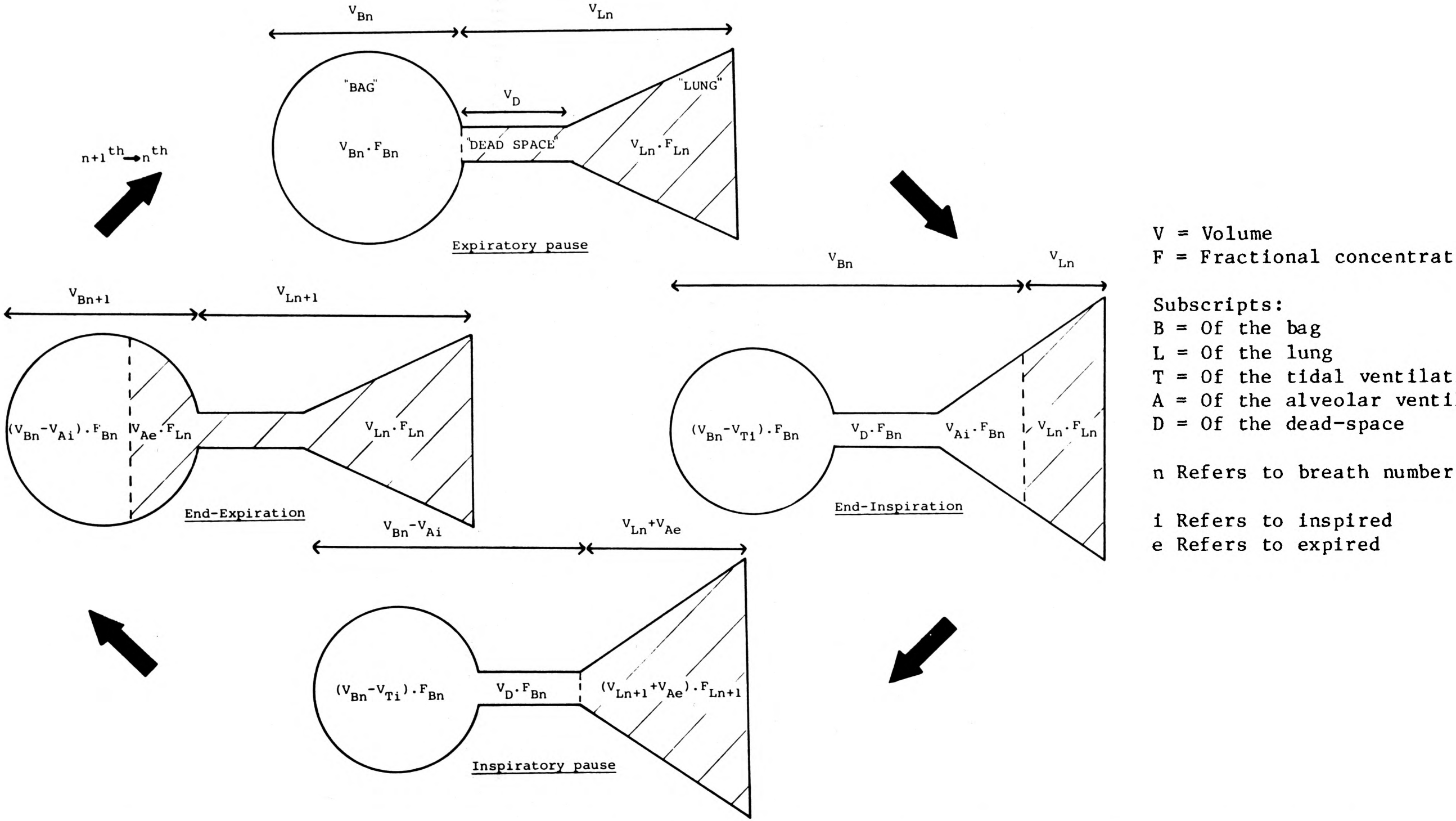
7. The factors by which the above uptakes or outputs changed from breath to breath.

The algebraic sum of $V_{b1}O_2$, $V_{b1}CO_2$ and $V_{b1}N_2O$ is the net volume change in the bag/lung system, (V_{b1}Net), for the first breath. As the FRC was taken to be constant throughout the rebreathing manoeuvre, the net gas uptake term for each breath was decremented from the volume of the rebreathing bag.

Using the above variables in the equations shown in Figure 4.1, the programme calculated, in succession, the dilution of the first inspirate in the alveolar gas to give the composition of the first expirate; then, the dilution of the first expirate in the gas left in the bag, to give the composition of the second inspirate; then, the dilution of the second inspirate in the new alveolar gas, to give the composition of the second expirate; and so on as shown on Figure 4.2. Figure 4.3 shows the sequence in which the calculations of the lung and bag values were made, in relation to the ventilation. The relative timing of the uptakes or outputs is also shown.

For all computations, unless otherwise stated, the volume of the rebreathing bag was set at 4 litres and the "subject's" FRC set at 2.8 litres. A rebreathing bag volume of 4 L was used in making all the real observations, and 2.8 L was an estimate of the FRC of the most extensively studied subject. The respiratory rate was 10 breaths per minute and the tidal volume was 3 L. (The real subject was trained to breathe very deeply and steadily during the manoeuvres). The dead space was assumed to be 300 ml. The alveolar portion of the tidal volume was, therefore, 2.7 L.

An example of a simulation is shown in Figure 4.4, in which the hypothetical subject rebreathed from a 4 litre bag, containing 50% v/v O_2 in N_2, after a control period breathing air. There is a constant oxygen



V = Volume
F = Fractional concentration

Subscripts:
B = Of the bag
L = Of the lung
T = Of the tidal ventilation
A = Of the alveolar ventilation
D = Of the dead-space

n Refers to breath number

i Refers to inspired
e Refers to expired

Figure 4.1 Schematic diagram of the model simulation of rebreathing. The arrows indicate the order of calculation. The shaded area, delimited by a dotted line, shows the boundary between the two homogeneous volumes.

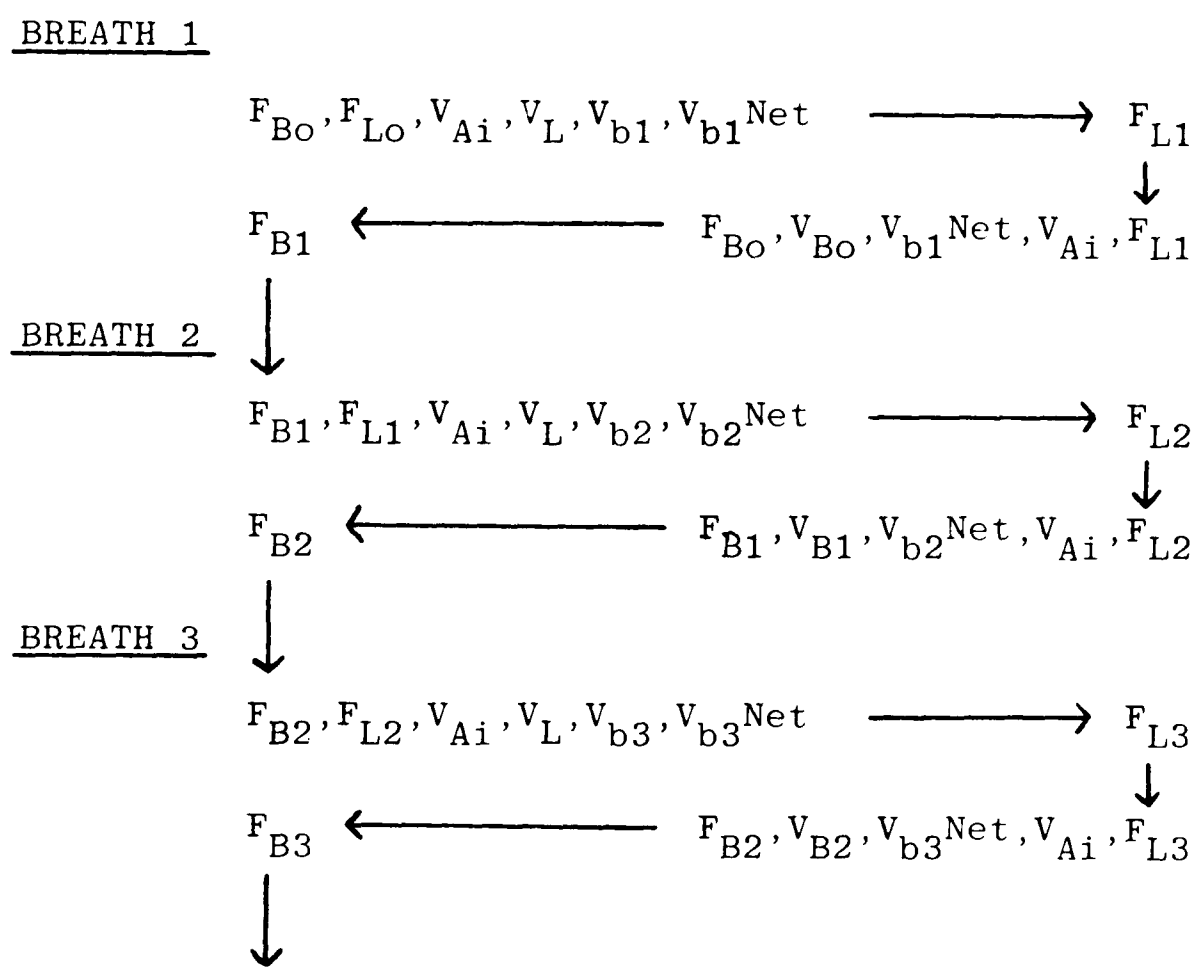


Figure 4.2 Starting with the initial lung and bag concentrations and volumes and the uptakes and outputs, the computer program calculates in succession the lung and bag compositions for the subsequent breaths of the rebreathing manoeuvre.

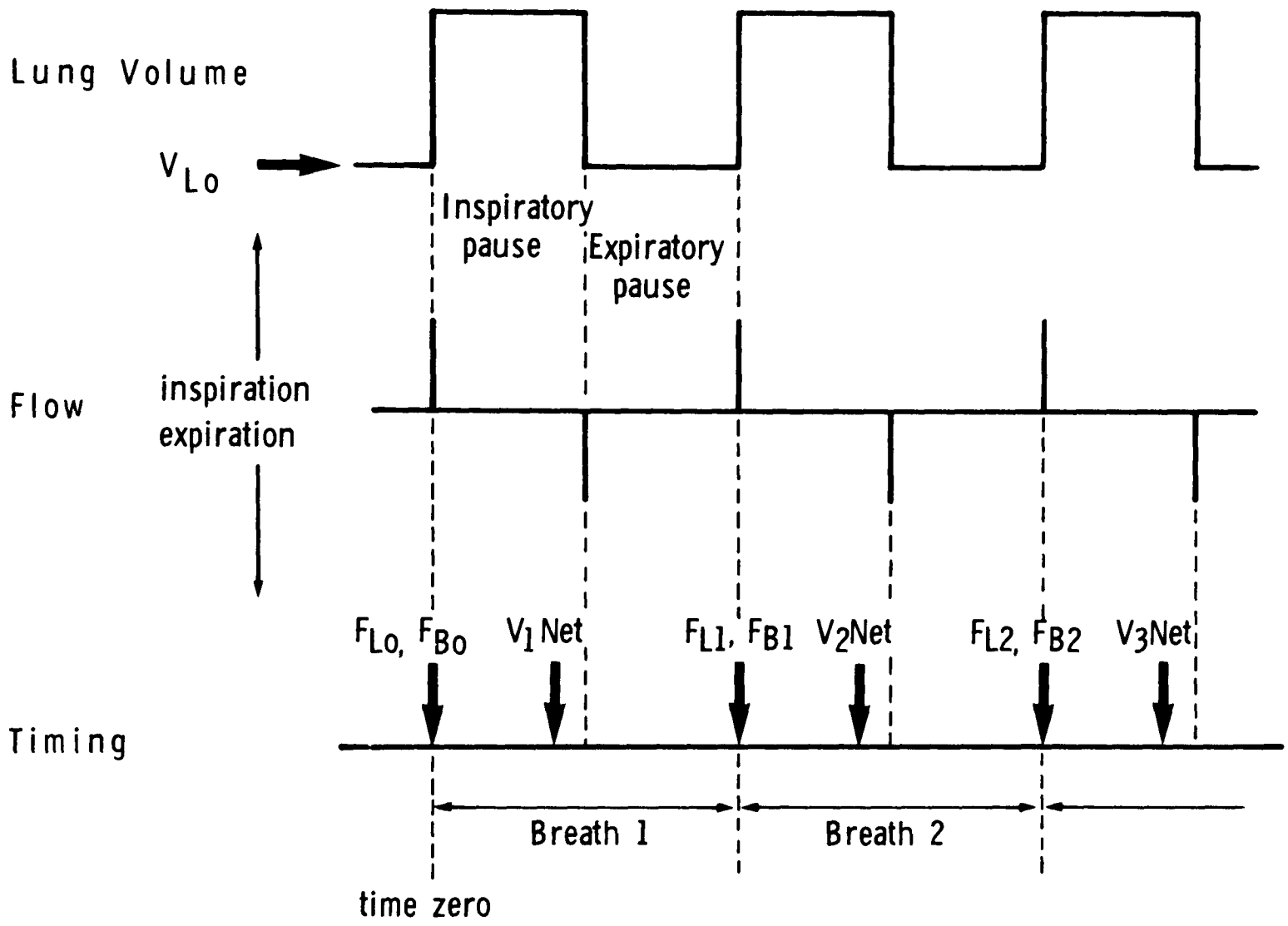


Figure 4.3 The equivalent volume and flow characteristics of the simulation. "Timing" shows the time where the calculations occur relative to the period of each breath.

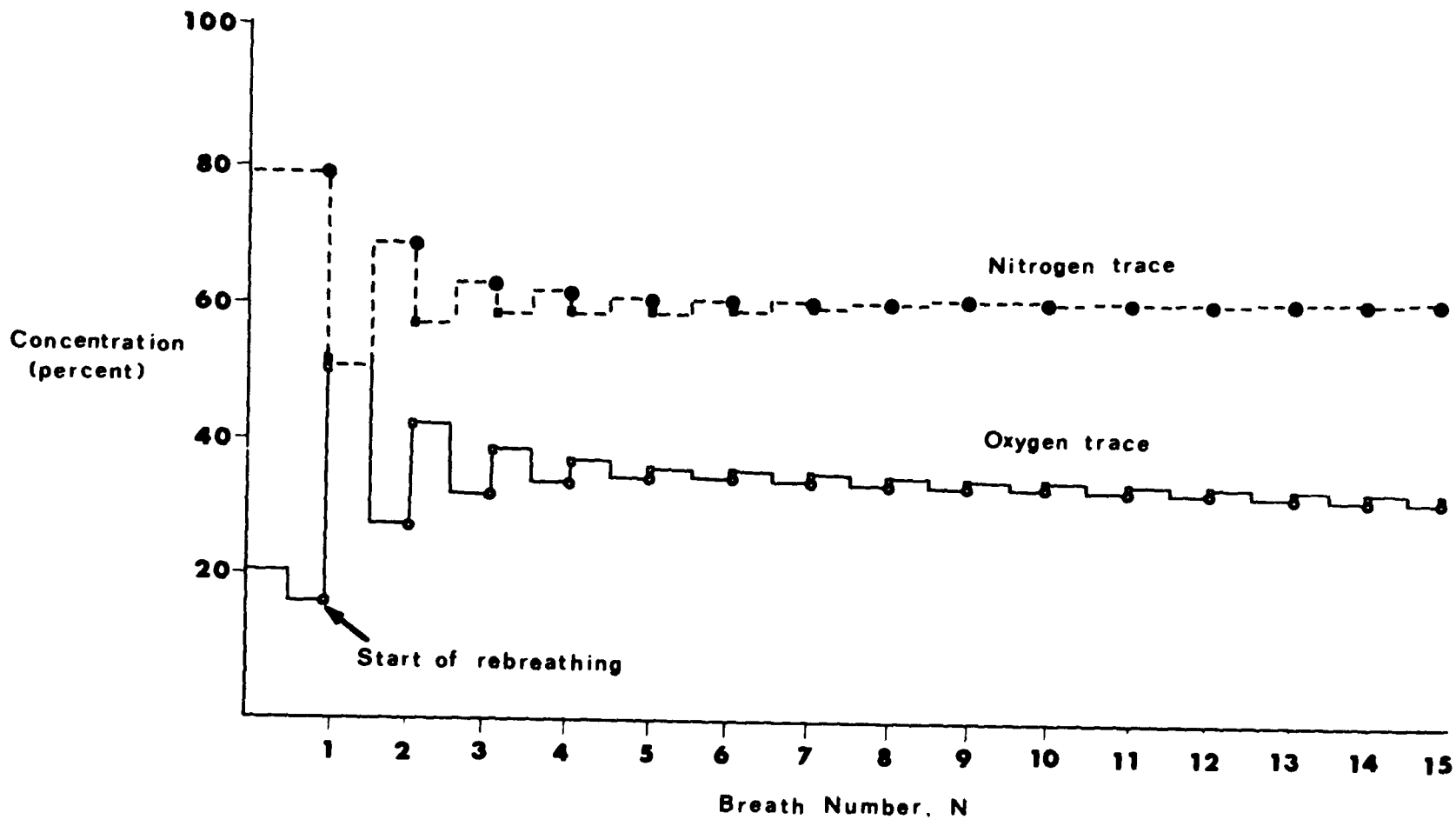


Figure 4.4 A trace of the simulated bag and lung concentrations versus breath number, as generated by the model. The hypothetical subject rebreathes from a bag, initially containing 50% v/v oxygen in nitrogen, after a control period of breathing air. The data points have been joined to produce a square wave trace of the sort which would be generated by the structural assumptions of the model.

uptake of 25 ml per breath and the carbon dioxide output is 60 ml per breath, during the first breath, decreasing by a factor of 0.7 on each subsequent breath. The CO_2 output and O_2 uptake profiles are illustrated in Figure 4.5.

Though the traces in Figure 4.4 are shown as square waves with equal inspiratory and expiratory periods, the I:E ratio is not specified. The model calculates only the pair of bag and lung values, at end-expiration, which is adequate to characterise the trace for the whole breath. These are shown by the symbols placed at the end-expiratory instant of each breath.

For each breath, the model generates a pair of points (bag and lung), and the mixed concentration (F_{Mn}) lies between these two points. In theory (Chapter 6), the true mixed concentration, for each breath, should be a <u>weighted</u> average of the bag and lung concentrations, such that:

$$F_{Mn} = \frac{F_{Bn} \cdot V_{Bn} + F_{Ln} \cdot V_{Ln}}{V_{Bn} + V_{Ln}} \qquad (4.1)$$

After seven breaths, the simulated concentrations are well mixed and a linear regression is fitted to the 9 mixed concentrations calculated for the 7th to the 15th breaths, inclusive. This line is extrapolated to the first instant of the manoeuvre which is called "time zero". The linear extrapolation is intended to allow for the effects of a constant oxygen consumption (Suwa and Bendixen, 1971). Were this procedure valid, the concentration, extrapolated to "time zero" for each gas, would be the hypothetical mixed or mean concentration, (F_{Mo}), which would exist if the bag and alveolar gases had been mixed instantaneously (before the removal of any oxygen from the system). From mass balance (Chapter 3, equation 3.2):

$$F_{Mo} \cdot (V_{Bo} + V_{Lo}) = V_{Bo} \cdot F_{Bo} + V_{Lo} \cdot F_{Lo}$$

By rearrangement:

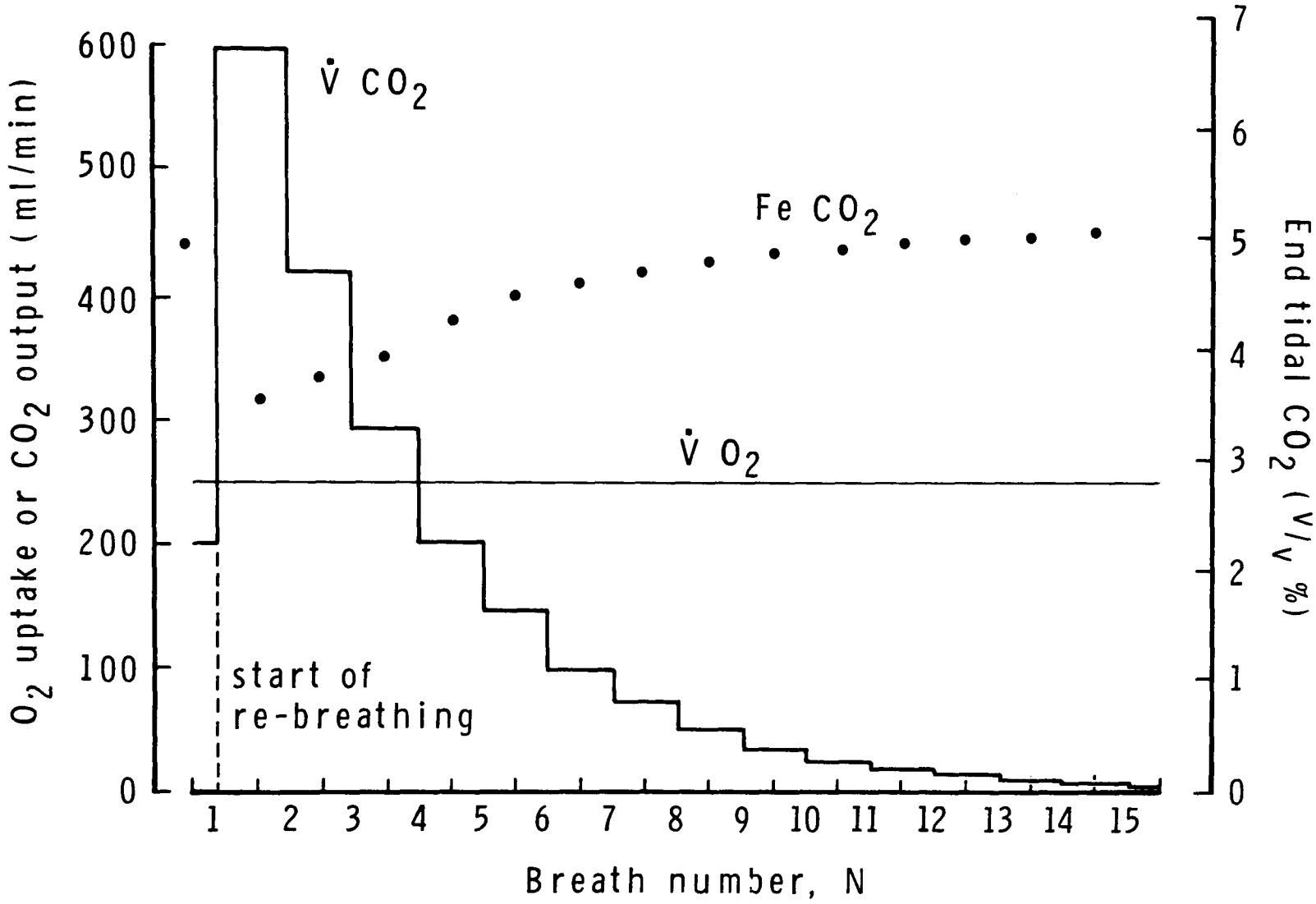


Figure 4.5 The simulated carbon dioxide output and oxygen uptake of the model, for each breath. The effective end-tidal lung CO_2 concentration which these exchanges would produce is shown.

$$\frac{V_{Lo}}{V_{Bo}} = \frac{F_{Bo} - F_{Mo}}{F_{Mo} - F_{Lo}} \qquad (4.2)$$

Figure 4.6 shows the effect on the system volume of the gas exchange profiles shown in Figure 4.5. Figure 4.7 illustrates the effect of this change in system volume on the mixed concentrations of three indicator gases. A linear extrapolation of these mixed concentrations from breaths 7 to 15 does not give the "true" mixed concentration at "time zero" (F_{Mo}) but an "apparent mixing" concentration (aF_{Mo}) which underestimates F_{Mo} (shown by the brackets, Figure 4.7). The relationship of the apparent mixing concentration to the true mixing concentration is described in Chapter 3. The apparent mixing concentrations from Figure 4.4 are 34.9% v/v for O_2, and 60.8% v/v for N_2. If these apparent initial mixing concentrations are used in equation 4.2, the volume of distribution of O_2 appears to be 3.04 litres, whereas that for N_2 appears to be 2.24 litres.

The computer model was used to simulate the performance of the "subject" in a number of protocols of concentration change between control and rebreathing.

4.2.2b The Correction Procedure

The carbon dioxide concentration, per se, is of no interest in the context of lung volume measurement. The disturbing effect of the CO_2 is purely that its volume dilutes the indicator gases. An analogy can be drawn between CO_2 and water vapour. Water vapour can introduce intractable problems into respiratory measurements because its volume is variable and difficult to determine. The conventional approach to these problems is to consider only that portion of the system occupied by dry gas. In such a sub-system, the sum of the fractional dry concentrations is by definition unity. In order to calculate a dry concentration from the corresponding true concentration, the true value is scaled by a factor: $1/(1 - FH_2O)$. This is the same as, 1/(sum of dry gas fractions). The Centronic 200 MGA

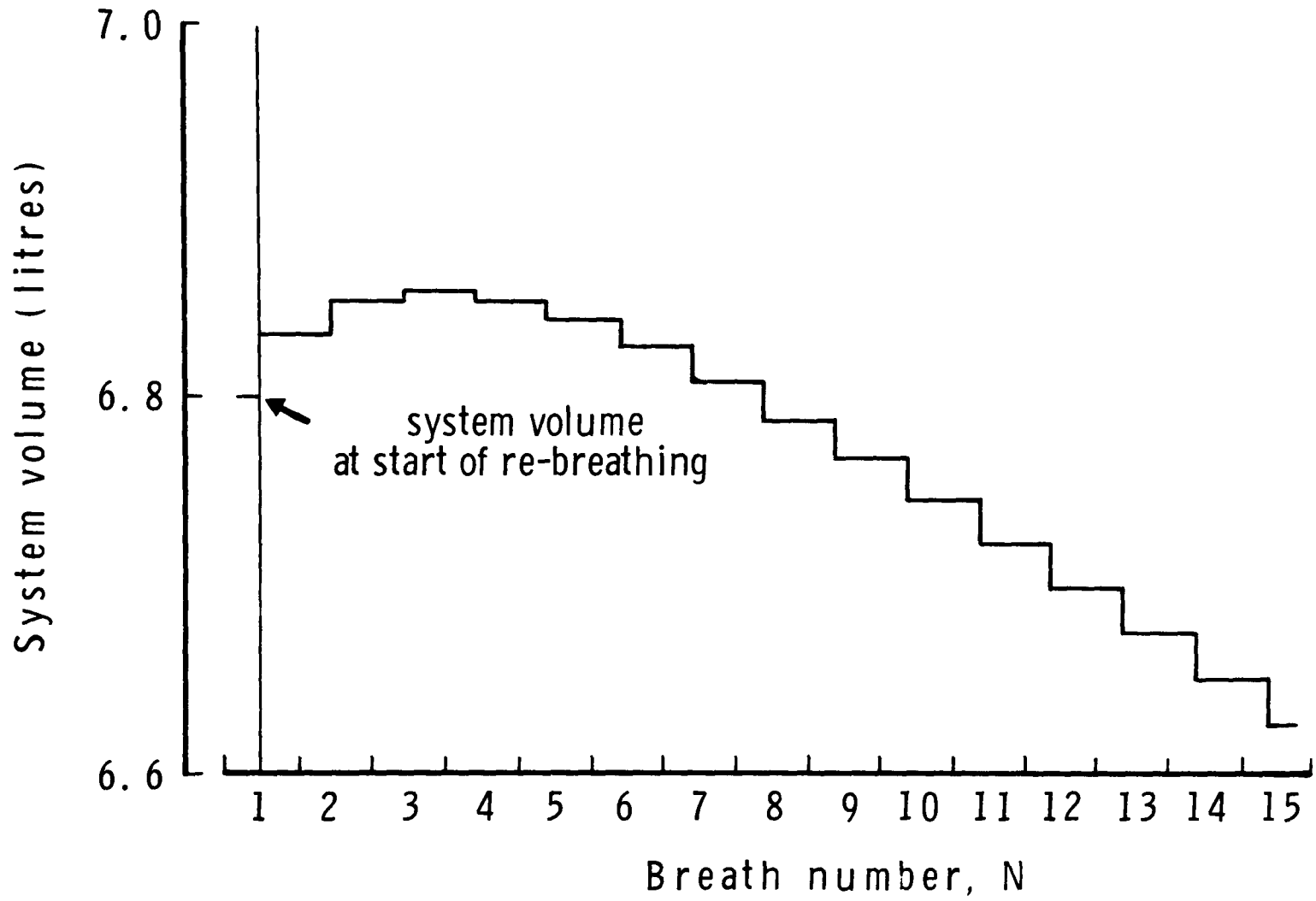


Figure 4.6 The simulated breath by breath changes in system volume which result from the gas exchange shown in Figure 4.5.

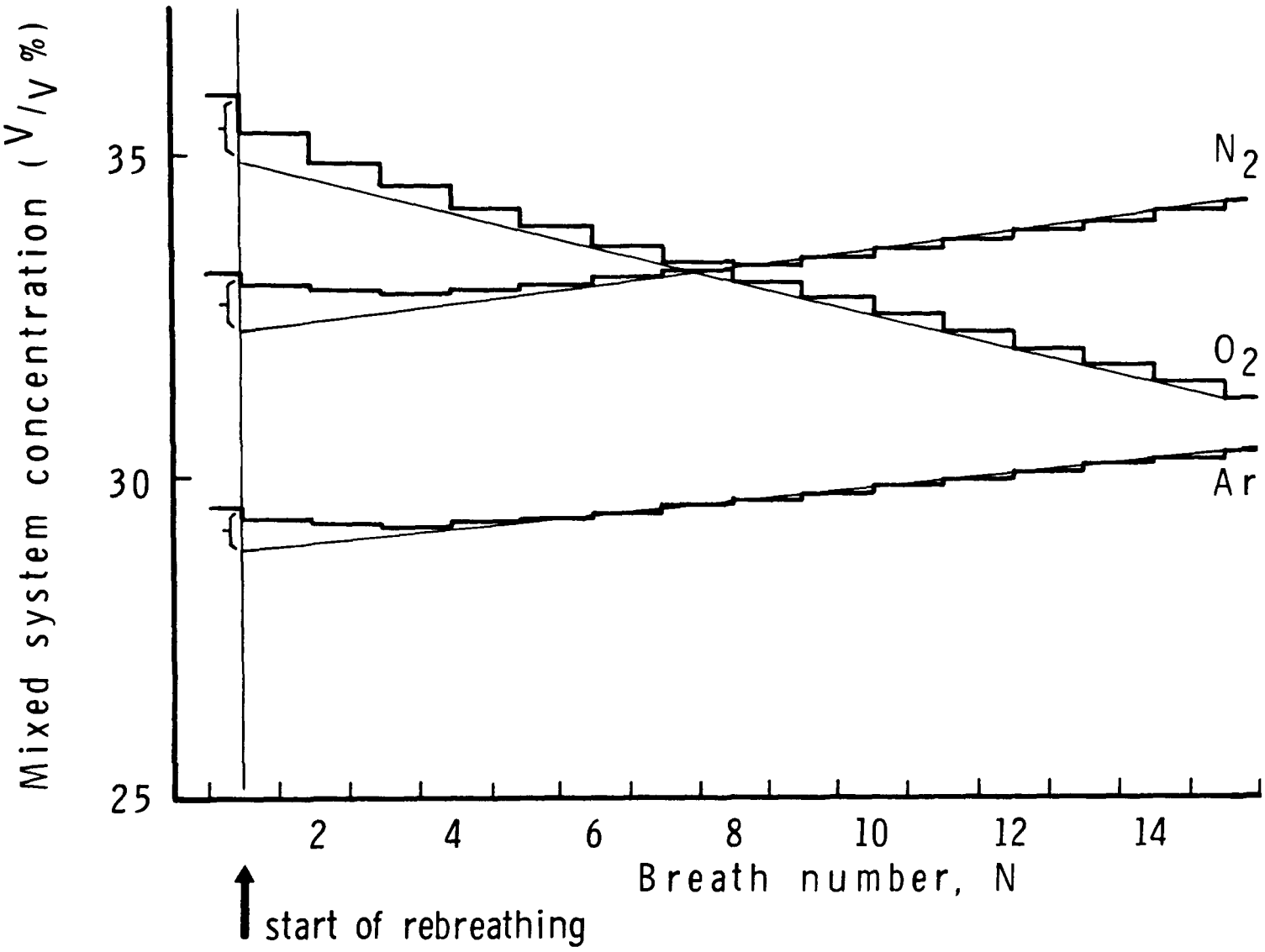


Figure 4.7 The change in mixing concentration (each breath) for the "indicator" gases. The straight lines show the course of a linear extrapolation (breaths 15 to 7). The difference, at the start of rebreathing, between the true and extrapolated mixing concentrations is shown by the brackets.

mass spectrometer employs an Automatic Sensitivity Control mode (ASC) to perform this function "on-line", similar to the one described by Scheid et al (1971). The correction procedure, proposed below, is an extension of this approach applied to carbon dioxide.

Consider a further subdivision of the dry gas portions- namely that notionally occupied by O_2, N_2, and Ar. The remainder of the dry gas portion would be occupied by CO_2 and N_2O. In the model, the volume of that part occupied only by O_2, N_2 and Ar changes at a constant rate, equal to the oxygen consumption (since the alveolar-capillary exchanges of N_2 and Ar are assumed to be zero). The troublesome "non-constant volume changes" affect only the portion occupied by N_2O and/or CO_2. By dividing each of the real concentrations of O_2, N_2 and Ar by their sum (S), the concentrations are "transformed", and the sum of the transformed concentrations (F') will be unity.

$$S = FO_2 + FN_2 + FAr$$

$$\frac{F}{S} = F'$$

$$F'O_2 + F'N_2 + F'Ar = \frac{FO_2 + FN_2 + FAr}{S} = 1$$

This is a process of normalisation with respect to a subdivision of the complete system, containing only the "indicator gases". If the real dry gas composition is 36% v/v O_2, 18% v/v N_2, 18% v/v Argon, 6% v/v CO_2 and 22% v/v N_2O, the sum of the O_2, N_2 and Argon concentrations is 72% v/v. The respective transformed concentrations are 50% v/v, 25% v/v and 25% v/v and the sum of the transformed concentrations is 100% v/v. Performing this transformation on the bag and lung gas compositions for every breath during the simulated rebreathing manoeuvre, yields a transformed concentration trace (Scott et al, 1981).

Clearly, after the exclusion of CO_2 and N_2O as gases with non-constant exchanges, at least two of the other three gases considered, (O_2, N_2 and Ar), must still be present in the bag/lung system. The transformed traces notionally represent the dilution of the gases O_2, N_2 and Ar in each other, in that part of the system which is isolated from the non-sustained uptakes and outputs of CO_2 and N_2O. Oxygen, nitrogen and argon are thus the indicators of the volume of that notional portion of the rebreathing system which is changing at constant rate.

The transformed mixed concentration, for each breath (F'_{Mn}), is a weighted average of the transformed bag (F'_{Bn}) and lung (F'_{Ln}) concentrations.

If V' is defined so that: $V'.F' = V.F$, then from equation 4.1:-

$$F'_{Mn} = \frac{V'_{Bn}.F'_{Bn} + V'_{Ln}.F'_{Ln}}{V'_{Bn} + V'_{Ln}} \qquad (4.3)$$

The terms "V'" refer to the volume occupied by the transformed gases. It is valid to perform the extrapolation, suggested by Suwa and Bendixen, on the transformed traces. Since the effects of CO_2 and N_2O exchange have been eliminated, the extrapolation should adequately account for the constant oxygen uptake. Performing the extrapolation from these "transformed" concentration traces, yields a transformed mixing concentration (F'_{Mo}) and when this is applied in a modification of equation 4.2 gives (since $V'.F' = V.F$):

$$\frac{V'_{Lo}}{V'_{Bo}} = \frac{F'_{Bo} - F'_{Mo}}{F'_{Mo} - F'_{Lo}} \qquad (4.4)$$

Where, $F'_{Bo}O_2$, $F'_{Mo}O_2$ and $F'_{Lo}O_2$ are the initial bag, mixing and lung concentrations as read from the transformed trace. V'_L and V'_{Bo} are those parts of the initial lung and bag volumes occupied by the "indicators":

$$V'_L = V_L \cdot (F_{Lo}O_2 + F_{Lo}N_2 + F_{Lo}Ar) = V_L \cdot S_{Lo}$$

and

$$V'_{Bo} = V_{Bo} \cdot (F_{Bo}O_2 + F_{Bo}N_2 + F_{Bo}Ar) = V_{Bo} \cdot S_{Bo}$$

Thus, S_{Lo} is the sum of the "indicator" concentrations initially in the bag and S_{Bo} is the corresponding sum for the bag. Then:

$$\frac{V'_{Lo}}{V'_{Bo}} = \frac{V_{Lo} \cdot S_{Lo}}{V_{Bo} \cdot S_{Bo}} \qquad (4.5)$$

Substituting equation 4.5 into equation 4.4 gives an equation for calculating the real lung volume from transformed concentrations.

$$V_{Lo} = \frac{V_{Bo} \cdot S_{Bo}}{S_{Lo}} \cdot \frac{F'_{Bo} - F'_{Mo}}{F'_{Mo} - F'_{Lo}} \qquad (4.6)$$

4.3 RESULTS

4.3.1 Simulated Data versus Real Data

The simulations of real data are divided into three sections: 1) without N_2O, 2) with N_2O uptake and 3) with N_2O output. These are shown under the heading "protocol" in Table 4.1. Under the heading of "control", the initial breathing mixture is indicated. Under the heading "rebreathing" (Table 4.1), the initial bag concentrations are shown. Under the heading "subject", the apparent lung volumes (aV_L) produced in a real subject are shown. These aV_L's were observed during the manoeuvres whose concentration changes are shown in the "control" and "rebreathing" columns. Each apparent lung volume is the mean of five observations the standard deviations are left out for clarity, but they were in the range 0.03 to 0.3 L. The patterns of apparent volumes of distribution, within each determination, were always as indicated by the pattern of the means. Under the heading "model", the results of the model simulations are shown. The starting parameters for the model were as described in the methods (section 4.2.2a). When breathing air in the "control" period the initial

PROTOCOL	CONTROL	REBREATHING			SUBJECT Apparent Lung Volume			MODEL Apparent Lung Volume		
(1) Without N_2O		O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar
	AIR	35	65	-	3.18	1.92	-	3.12	1.66	-
		35	45	20	3.03	2.10	2.83	3.12	2.42	2.97
		35	20	45	3.20	2.53	3.08	3.12	2.56	2.97
		35	-	65	3.26	2.75	3.16	3.12	2.68	2.97
(2) With N_2O Uptake		O_2%	N_2%	N_2O%	O_2	N_2	N_2O	O_2	N_2	N_2O
	AIR	35	65	-	3.40	1.38	-	3.12	1.66	-
		35	45	20	3.25	2.16	3.15	3.06	2.41	3.19
		35	20	45	2.82	2.34	3.03	2.98	2.66	3.16
		35	-	65	2.75	2.37	3.05	2.91	2.74	3.13
(3) With N_2O Output		O_2%	N_2%	N_2O%	O_2	N_2	N_2O	O_2	N_2	N_2O
	"ENTONOX"	100	-	-	3.47	-	2.62	3.38	-	2.86
		75	25	-	4.01	2.83	2.30	3.68	3.04	2.86
		75	-	25	3.28	-	1.98	3.54	-	2.55

Table 4.1 The subject verses the model. Three protocols are shown comparing the apparent lung volumes (litres) for a real subject and a model simulation. The first column shows the protocol, the second column the control breathing mixture, the third column the rebreathing mixture, and the last two columns show the subject and model performances.

lung fractions were assumed to be: 15% v/v O_2, 80% v/v N_2 and 5% v/v CO_2. When breathing entonox, the initial lung fractions were: 45% v/v O_2, 50% v/v N_2O and 5% v/v CO_2. The bag fractions are as shown in the "rebreathing" column. The results of each of the three protocols will be considered seperately.

4.3.1a Without nitrous oxide

The first protocol in Table 4.1 shows the performance of a subject and model who, after initially breathing air, rebreathed from a bag containing 35% v/v oxygen, with the balance made up of nitrogen and argon (N_2:Ar, 65:0 or 45:20 or 20:45 or 0:65). Differences between the three estimates of lung volume, in the same manoeuvre, are shown along the rows under the headings "subject" and "model". The subject displays apparent lung volumes in decreasing order of magnitude $aV_L(O_2)$, $aV_L(Ar)$ and $aV_L(N_2)$. This order is imitated by the model simulation. Looking down thecolumns, of the apparent lung volumes, the differences between the aV_L of a given gas, during the four types of concentration change are seen. For the real subject, the apparent distribution volume for oxygen ($aV_L(O_2)$) shows no trend down the column. This is reflected in the simulation, where $aV_L(O_2)$ is the same throughout the four composition changes. The $aV_L(N_2)$ in the subject becomes larger, down the column, as it does in the model. The real $aV_L(Ar)$ appears to increase slightly down the column, ALthough this is not statistically significant (Student's t test, $p<.05$). The model shows a constant aV_L for argon.

4.3.1b With nitrous oxide uptake

The second protocol in Table 4.1 is similar to the first, except that nitrous oxide replaces argon. Since nitrous oxide is fifteen times more soluble than argon, a significant N_2O uptake is expected. In modelling this, the input parameters included a N_2O uptake which was

assumed to depend on the size of the concentration change imposed (as described in the method section, 4.2.2a). The relative magnitudes of the different indicators, in the same manoeuvre (across rows), for the subject are again reproduced in the model. The real subject shows a trend for decreasing $aV_L(O_2)$ and increasing $aV_L(N_2)$ down the columns, which is also imitated in the model simulation. The subject fails to demonstate a clear trend for $aV_L(N_2O)$ down the column. The model does show such a trend but this is small enough to be within the limits of measurement error.

4.3.1c With nitrous oxide output

The third protocol in Table 4.1 shows a subject initially breathing "entonox" (50% v/v O_2 and 50% v/v N_2O). Rebreathing was then from one of three mixtures, as shown in the table. Since the subject was allowed to become nearly saturated with N_2O during the control period, the model was set up to provide a N_2O output during rebreathing. The magnitude of this output was determined in the same way as described for the N_2O uptake. There is, once again, agreement between subject and model in terms of the relative sizes of the different estimates of lung volume in the same manoeuvre (rows). The model also imitates the trends displayed by the subject between manoeuvres (columns).

Comparable simulations with a range of other protocols were also obtained.

4.3.2 The Performance of the Correction in the Model

Table 4.2 illustrates the performance of the correction procedure when applied to the data simulated by the model. The three protocols shown are identical to those in Table 4.1 (except that the apparent lung volumes for N_2O are omitted). In Table 4.2, the headings "lung" and "bag" are analogous to the headings "control" and "rebreathing" used in Table 4.1. However, in Table 4.2, the headings indicate the specific concentration parameters used in the model simulation. Under the heading "apparent lung

PROTOCOL	LUNG			BAG			APPARENT LUNG VOLUME			CORRECTED LUNG VOLUME		
(1) WITHOUT N_2O	O_2%	N_2%	CO_2%	O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar
	15	80	5	35	65	-	3.12	1.66	-	2.73	2.73	-
	15	80	5	35	45	20	3.12	2.42	2.97	2.73	2.77	2.81
	15	80	5	35	20	45	3.12	2.56	2.97	2.73	2.79	2.81
	15	80	5	35	-	65	3.12	2.68	2.97	2.73	2.79	2.81
(2) WITH N_2O UPTAKE	O_2%	N_2%	CO_2%	O_2%	N_2%	N_2O%	O_2	N_2		O_2	N_2	
	15	80	5	35	65	-	3.12	1.60		2.73	2.73	
	15	80	5	35	45	20	3.06	2.41		2.74	2.74	
	15	80	5	35	20	45	2.98	2.66		2.76	2.76	
	15	80	5	35	-	65	2.91	2.74		2.77	2.77	
(3) WITH N_2O OUTPUT	O_2%	N_2%	CO_2%	O_2%	N_2%	N_2O%	O_2	N_2		O_2	N_2	
	45	50	5	100	-	-	3.38	-		-	-	
	45	50	5	75	25	-	3.68	3.84		2.83	2.83	
	45	50	5	75	-	25	3.54			-	-	

Table 4.2 The correction applied in the model. A similar format to Table 4.1, the initial concentrations (bag and lung) used in the model are shown. The table shows the model performance, before and after correction, for the three protocols shown in Table 4.1.

volume", simulated aV_L's are presented and these are duplicated from those shown in Table 4.1 (under the heading "model"). The simulated data are then subjected to the correction procedure and the results are shown under the heading "corrected lung volume". The correction reduces the previous discrepancies to provide lung volume estimates which are all within 100 ml of the true lung volume, namely 2.8 litres.

In all cases, the correction procedure reduced the discrepancy within and between manoeuvres, but failed to correct to the exact lung volume (that is, the one which was put into the model). Repeating the simulation, omitting the CO_2 output, produced the same apparent lung volumes as were produced as a result of the correction procedure.

4.4 DISCUSSION

4.4.1 Simulated Data versus Real Data

The recognisable imitation of the real data provided by the model suggests that the discrepancies seen in real life may be the result of initial volume changes of CO_2 and N_2O which occur at rates which are initially high but later decline.

The simplifing assumptions about the breathing pattern and the timing of intra-breath gas exchange, although necessarily unrealistic, appear to be sufficient to simulate the observed trends. Changes in the simulated pattern of breathing (tidal volume, dead space and rate) exert little influence on the results, provided that the alveolar ventilation is sufficient to ensure that the bag and lung contents are equilibrated by the seventh breath of the manoeuvre.

4.4.2 The performance of the correction in the Model

Regardless of the adequacy of the simulations, the correct procedure should be able to compensate for the effects of the CO_2 output. This is indeed the case, since results of the correction are identical to

simulations with no CO_2 output. A "residual dicrepancy" remains, after correction, both within and between manoeuvres.

In the model, the transformed mixed concentrations can be calculated, for each breath, as a weighted average of the bag and lung concentrations (equation 4.3). In real manoeuvres, this weighting is unknown, since the bag and lung volumes during rebreathing are unknown. The use of an arbitary weighting of 1:1 has been found in the model to cause an error of less than 10 ml in the corrected lung volumes. However, in a situation where the bag and lung concentrations do not approach each other so closely the error in estimating the mixed concentration may be much larger and may become a significant component of the measurement error (see Chapter 6, section 6.3.4c).

Sections 4.5 and 4.6 are concerned with understanding the relationship between the apparent lung volume and the CO_2/N_2O exchange. The cause of the residual discrepancy is then identified.

4.4.3 Application of the Correction

As the correction technique is an extension of the treatment of water vapour, one might be inclined to exclude the CO_2 from the mass spectrometer's ASC, as one does for water vapour. Two examples of this approach are shown in Table 4.3 (Subject A and Subject B). In both cases, the discrepancy between lung volumes (O_2 as compared to N_2 dilution) is reduced (but not abolished) by the removal of CO_2 from ASC. The two estimates should be identical. If "F'" represents the concentrations transformed by the absence of CO_2 from the ASC and "T" is the sum of the transformed fractional concentrations of the gases entered into the ASC (commonly set to unity), and, if O_2 and N_2 are the only two gases in the ASC, it follows that:

			Apparent Lung Volume CO_2 in ASC		Apparent Lung Volume CO_2 out of ASC	
	CONTROL	REBREATHING				
Subject A	O_2%	N_2%	O_2	N_2	O_2	N_2
	50	50	2.81	1.97	2.54	2.44
	"	"	2.86	2.34	2.49	2.46
AIR	"	"	2.92	2.28	2.46	2.40
	"	"	3.00	2.31	2.46	2.30
	"	"	3.00	2.17	2.37	2.37
X			2.92	2.21	2.46	2.39
SD			(.08)	(.15)	(.06)	(.06)
Subject B						
	50	50	2.84	2.24	2.58	2.15
	"	"	2.91	2.08	2.57	2.08
AIR	"	"	2.74	2.22	2.55	2.19
	"	"	2.92	2.30	-	-
	"	"	2.77	2.03	-	-
X			2.84	2.17	2.57	2.14
SD			(.08)	(.11)	(.02)	(.06)

Table 4.3 The effect of leaving carbon dioxide out of ASC on the mass spectrometer. The results are shown for two subjects (Subject A and Subject B). All composition changes involve rebreathing 50% v/v oxygen in nitrogen after a control period breathing air. The five individual assessments with ASC and the three and five assessments with CO_2 out of ASC are shown for each subject. The means and standard deviations are also shown.

$$F'O_2 = T-F'N_2$$

Therefore:

$$\frac{F'_{Bo}O_2 - F'_{Mo}O_2}{F'_{Mo}O_2 - F'_{Lo}O_2} = \frac{(T-F'_{Bo}N_2) - (T-F'_{Mo}N_2)}{(T-F'_{Mo}N_2) - (T-F'_{Lo}N_2)}$$

The T's subtract out from both numerator and denominator, so that:

$$\frac{F'_{Bo}O_2 - F'_{Mo}O_2}{F'_{Mo}O_2 - F'_{Lo}O_2} = \frac{F'_{Bo}N_2 - F'_{Mo}N_2}{F'_{Mo}N_2 - F'_{Lo}N_2} \qquad (4.7)$$

Hence, if the ASC was working perfectly, the lung volumes calculated by the transformed concentrations should be equal.

With water vapour, the change in inspired and expired tension, within the ionisation chamber, is attenuated and sluggish (Goodwin, 1979). With CO_2, there are likely to be rapid changes in the CO_2 tension in the ionisation chamber during the initial part of rebreathing. These will be too fast for the ASC feedback system and will produce transient responses (see Chapter 2, section 2.4.1d(ii)). For this reason, a digital ASC, working arithmetically on the output, is more stable, and is favoured. Another reason for favouring a digital technique is the need to measure accurately the initial bag and lung fractions of CO_2 and/or N_2O, in order to convert the notional measured volume back to a real volume (in the same way as gas volumes are converted to BTPS even though the concentrations are measured dry). In the case of water vapour, one assumes the vapour pressure is dependent only upon temperature. For CO_2 and N_2O, these partial pressures must be measured. Simply excluding the carbon dioxide and nitrous oxide from the mass spectrometer's ASC would greatly compromise the accuracy of their measurement, since the outputs of these channels would fluctuate as the sensitivity of the machine altered.

4.5 RESULTS OF SIMULATIONS EXPLORING THE RESIDUAL DISCREPANCY

Careful inspection of the results in Table 4.1, along with a number of other simulations, indicates that there is a semblance of order in the chaos of apparent lung volumes. In the absence of N_2O, if a gas is initially present in a larger concentration in the bag than in the lung, then the apparent lung volume is larger than the true lung volume. If the lung concentration is larger than in the bag, then the apparent lung volume is less than the true lung volume. The discrepancies widen as the concentration gradients decreases. The situation with N_2O is less easily defined and depends on the size and direction of the N_2O exchange.

Table 4.4 repeats the first protocol shown in Table 4.2, plus an approximate "inverse" of this protocol. In this case, the initial bag nitrogen and argon fractions are exchanged for the initial lung nitrogen and argon fractions. For O_2, however, it is not a complete inversion, because the simulation required 5% v/v CO_2 in the lungs. This, as in reality, is provided at the expense of the oxygen fraction. Thus, although the gradient between the bag and lung oxygen concentration is reversed, it is also halved.

The first thing to note is that, in the second, inverted protocol, the relative order of the calculated lung volumes, within a manoeuvre (rows), is the reverse of the first protocol. The $aV_L(O_2)$ is constant throughout a protocol (columns) but is different between the two protocols. The same is true of the $aV_L(Ar)$. The $aV_L(N_2)$, in the inverted protocol, decreases down the column, whereas it increased before. Table 4.4 indicates that when the initial lung fraction of N_2 or Ar is zero, the $aV_L(N_2)$ or $aV_L(Ar)$ is <u>always</u> 2.97 L, independent of the bag fraction. Conversely, if the initial bag fraction is zero, the $aV_L(N_2)$ or $aV_L(Ar)$ is <u>always</u> 2.68 L. These are clearly two special cases.

PROTOCOL	LUNG				BAG			Apparent Lung Volume			Corrected Lung Volume		
(1) Without N_2O	O_2%	N_2%	Ar%	CO_2%	O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar
	15	80	-	5	35	65	-	3.12	1.66	-	2.73	2.73	-
	15	80	-	5	35	45	20	3.12	2.42	2.97	2.73	2.77	2.81
	15	80	-	5	35	20	45	3.12	2.56	2.97	2.73	2.79	2.81
	15	80	-	5	35	-	65	3.12	2.68	2.97	2.73	2.79	2.81
(2) "INVERSE"	O_2%	N_2%	Ar%	CO_2%	O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar
	30	65	0	5	20	80	-	2.30	4.59	-	2.92	2.92	-
	30	45	20	5	20	80	-	2.30	3.39	2.68	2.92	2.84	2.79
	30	20	45	5	20	80	-	2.30	3.08	2.68	2.92	2.82	2.79
	30	0	65	5	20	80	-	2.30	2.97	2.68	2.92	2.81	2.79

Table 4.4 This shows the first protocol of Table 4.2 (ie "without nitrous oxide") and an "inverse" of this protocol. The apparent and corrected lung volumes are shown for the two protocols. See the text for details.

Table 4.5 focuses on the single concentration change, LUNG: 15% v/v O_2, 80% v/v N_2, 5% v/v CO_2; BAG: 35% v/v O_2, 65% v/v Ar. This composition change exhibits both a zero lung fraction for Ar, and a zero bag fraction for N_2, and therefore contains both the special cases.

The table illustrates two simulated protocols. The first protocol shows the effect of varying the modelled CO_2 output on both the apparent and corrected volumes of distribution. In the first protocol, under the heading "CO_2 output", the total CO_2 output used in the model is presented. The CO_2 output is assumed to be added entirely in the first breath. This means that the CO_2 output is completed before mixing and, therefore, does not affect the slope of the line drawn for the extrapolation. The oxygen uptake is 25 ml/min throughout. As the CO_2 output is increased, the discrepencies between the three apparent lung volumes increase. The increase in $aV_L(Ar)$ between two levels of CO_2 output is exactly equal to the increase in added CO_2. The decrease in $aV_L(N_2)$, however, is less than the added CO_2. The corrected volumes are <u>independent</u> of the amount of CO_2 added.

The second protocol in Table 4.5 shows the effect of varying the oxygen uptake on the apparent and corrected volumes of distribution. The heading "oxygen uptake" indicates the rate of oxygen uptake in ml per minute (10 breaths/min). The CO_2 output is 60 ml in the first breath decreasing by a factor of 0.7 in subsequent breaths. Increasing the O_2 uptake does not modify the apparent lung volumes for the inert gases to any great extent. For oxygen, however, the apparent lung volume is decreased as the oxygen consumed per breath (assumed constant- V_bO_2) is increased, the decrease is more marked at larger levels of oxygen consumption. With regard to the corrected volumes, the absence of a V_bO_2 provides a complete correction whilst a V_bO_2 of greater than zero causes the correction to become progressively worse.

EFFECT OF CO_2 OUTPUT	LUNG O_2%	LUNG N_2%	LUNG CO_2%	BAG O_2%	BAG N_2%	BAG Ar%	CO_2 Output (L)	Apparent Lung Volume O_2	Apparent Lung Volume N_2	Apparent Lung Volume Ar	Corrected Lung Volume O_2	Corrected Lung Volume N_2	Corrected Lung Volume Ar
	15	80	5	35	-	65	0.0	2.74	2.79	2.81	2.73	2.79	2.81
	15	80	5	35	-	65	.05	2.85	2.76	2.86	"	"	"
	15	80	5	35	-	65	.10	2.97	2.75	2.91	"	"	"
	15	80	5	35	-	65	.15	3.03	2.69	2.96	"	"	"
	15	80	5	35	-	65	.164*	3.12	2.68	2.97	"	"	"

EFFECT OF O_2 UPTAKE	LUNG O_2%	LUNG N_2%	LUNG CO_2%	BAG O_2%	BAG N_2%	BAG Ar%	O_2 Uptake (L/min)	Apparent Lung Volume O_2	Apparent Lung Volume N_2	Apparent Lung Volume Ar	Corrected Lung Volume O_2	Corrected Lung Volume N_2	Corrected Lung Volume Ar
	15	80	5	35	-	65	0.0	3.19	2.69	2.97	2.80	2.80	2.80
	15	80	5	35	-	65	.25	3.12	2.68	2.97	2.73	2.79	2.81
	15	80	5	35	-	65	.50	2.93	2.66	3.00	2.52	2.77	2.85
	15	80	5	35	-	65	.75	2.58	2.63	3.06	2.16	2.72	2.91

Table 4.5 Top: the effect of carbon dioxide output. The CO_2 output is all in the first breath. Results are shown for a single composition change, assuming an O_2 uptake of 25 ml/breath. Changes in CO_2 output do not affect the corrected lung volume.
Bottom: The effect of oxygen uptake, in the same protocol as above. A CO_2 output of 164mls is assumed. The level of oxygen uptake markedly affects the correction.

4.6 DISCUSSION

4.6.1 Relationship between aV_L and the CO_2/N_2O exchange

The presence of two special cases is highlighted in Table 4.4. For the given model parameters, if the inert gas (N_2 or Ar) is present initially only in the bag, then its apparent volume of distribution will be 2.97 L, independent of the indicator's concentration. Whereas, if this is reversed so that the indicator is present only in the lung, the apparent volume of distribution is 2.68 L, independent of the indicator's concentration. Table 4.5 shows that the values of the apparent lung volume, in the special cases, depends upon the CO_2 output. When the lung indicator gas concentration is zero, the apparent lung volume is increased by the same amount as the CO_2 output is incremented. The identification of the special cases led to the quest for the derivation of the general case. That is, a relationship between the effective volume change before extrapolation (Vmiss) and the apparent lung volume (aV_L). This will be derived in the Chapter 6 (section 6.2.1b):

$$aV_L = V_{Lo} + \frac{Vmiss}{1 - \frac{F_{Lo}\cdot(V_{Bo} + V_{Lo} + Vmiss)}{F_{Bo}\cdot V_{Bo} + F_{Lo}\cdot V_{Lo}}}$$

Thus, for a given V_{Lo}, V_{Bo} and Vmiss, this size of the difference between the apparent and true lung volumes is related to F_{Lo} and F_{Bo}. This relationship is shown in Figure 4.8 for V_{Lo} of 2.8 L, a V_{Bo} of 4 L and Vmiss of 164 ml. The two special cases are, when F_{Lo} is zero (F_{Bo}/F_{Lo} is infinity):

$$aV_L = V_{Lo} + Vmiss$$

When F_{Bo} is zero (F_{Bo}/F_{Lo} is zero), the F_{Lo}'s cancel:

$$aV_L = V_{Lo} - \frac{V_{Lo}\cdot Vmiss}{V_{Bo} + Vmiss} = V_{Lo}\cdot\frac{V_{Bo}}{V_{Bo} + Vmiss}$$

The quantity Vmiss does not truely equate to the volume of CO_2 added,

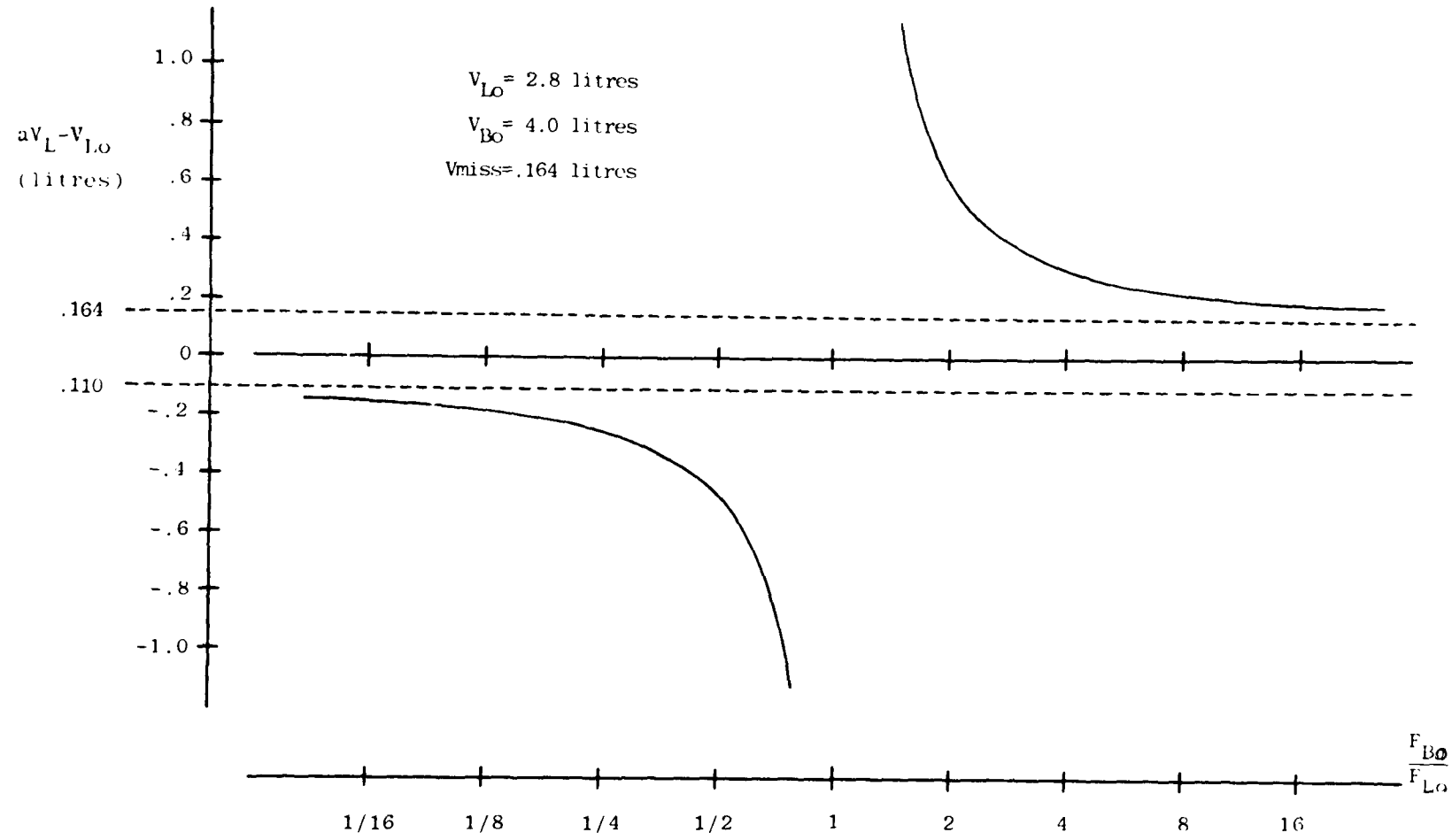


Figure 4.8 This plot shows the relationship between the initial composition change and the error this induces in the estimation of lung volume (for a given Vmiss, V_{Bo} and V_{Lo}). As the difference between F_{Bo} and F_{Lo} becomes smaller, the error ($aV_{Lo} - V_{Lo}$) in lung volume estimation increases hyperbolically.

before the extrapolation, but in the model it is a very close approximation. In the original simulations, 166 mls of CO_2 are added before the extrapolation, and a further 32 mls thereafter. In Table 4.5, the same distribution of apparent lung volumes, as found in the original simulation, is produced by the non-physiological simulation in which 164 ml of CO_2 are added, all within the first breath (see Table 4.5, indicated by asterisk). Therefore, in the original simulation, Vmiss is 164 ml compared to a real volume of added CO_2 of 166 ml. The closeness of this approximation refects the ability of the extrapolation to take account of the continuing oxygen uptake as well as the 32 ml of further carbon dioxide output. In reality, therefore, the term Vmiss will depend on the change in the slope of the extrapolation caused by later gas exchange. If the combined effects of the CO_2 output and O_2 uptake are reasonably linear, during the extrapolation phase, then Vmiss will approximate to the volume of CO_2 added. This rationale can be extended to include the N_2O exchange.

4.6.2 The Residual Discrepancy

The fact that the corrected values are independent of both the size and profile of the CO_2 output, is a good indication that the correction does what it was intended to do:- that is, to compensate for the CO_2 output by ignoring it.

The second part of Table 4.5 shows the effect of varying the O_2 consumption in the presence of an initial CO_2 output. If the extrapolation was appropriate, the size of the oxygen consumption should not affect the corrected results. As it is, increasing the V_bO_2 alters the corrected lung volume for the inert gases and, for the oxygen, the error is more marked. If there is no oxygen uptake then the correction is perfect. Increasing the V_bO_2, seriously compromises the correction accuracy. The reason for this is the rationale for a linear extrapolation, and this will now be analysed.

4.6.2a The form of the mixing line

The correction procedure devised in this model effectively considers a hypothetical system in which there is no CO_2 and, therefore, no CO_2 output. In such a sub-system, the course of the mixing line lies on a line representing the mean O_2 concentration in that part of the bag/lung system devoid of CO_2. The latter is the total amount of O_2 in the system divided by the total volume of the sub-system. Recalling equation 4.3:

$$F'_{Mn}O_2 = \frac{V'_{Bn}.F'_{Bn}O_2 + V'_{Ln}.F'_{Ln}O_2}{V'_{Bn} + V'_{Ln}}$$

The numerator of the quotient is the total amount of O_2 in the system at breath "N" (which is the same as $V_{Bn}.F_{Bn} + V_{Ln}.F_{Ln}$). The denominator is the transformed system volume at breath "N". The amount of oxygen in the system at any breath is the amount initially present minus the total oxygen uptake. The transformed system volume is similarly related to the initial conditions. Thus:

$$F'_{Mn}O_2 = \frac{V_{Bo}.F_{Bo}O_2 + V_L.F_{Lo}O_2 - N.V_b\,O_2}{V'_{Bo} + V'_L - N.V_bO_2} \qquad (4.8)$$

Both the numerator and the denominator of the quotient on the right hand side of equation 4.8 decrease linearly with breath number (N), by an amount equal to the O_2 consumption per breath. Since the denominator is larger than the numerator, this causes the value of the quotient to decrease as a function of breath number (or time). This decrease is more hyperbolic than linear. The non-linearity is small when the volume of the bag/lung system is large compared to the O_2 consumption, but it increases as the former decreases with respect to the latter. Therefore, using a smaller bag volume accentuates the non-linearity of the mixing line for a given O_2 uptake.

Since equation 4.8 is not a true linear function of "N", or time, a linear extrapolation gives different values for the initial mixing

concentration for different oxygen uptakes. This is shown Figure 4.9 for the composition changes illustrated in Table 4.4. The extrapolated mixing concentration values deviate from the true $F_{Mo}O_2$ and yield volumes of distribution for O_2 which deviate from the true value. The deviation at an O_2 uptake of 500 mls/minute is greater than that for 250 mls/minute.

The mean concentrations of the inert indicators, in a hypothetical sub-system without CO_2 or N_2O, are also hyperbolic rather than linear functions of breath number or time. This is shown for N_2 in equation 4.9.

$$F'_{Mn}N_2 = \frac{V_{Bo} \cdot F_{Bo}N_2 + V_L \cdot F_{Lo}N_2}{V'_{Bo} + V'_L - N \cdot V_bO_2} \qquad (4.9)$$

Figure 4.10 shows the error caused by a linear extrapolation of a nitrogen trace at varing oxygen consumptions.

The numerator of the quotient, on the right hand side of equation 4.9, is unaffected by oxygen uptake. This numerator is constant as "N" increases, if the alveolar-capillary exchange of N_2 is assumed to be zero. Thus, if equation 4.9 is inverted, the constant numerator becomes the denominator and the reciprocal of $F_{Mn}N_2$ is a true linear function of "N" or time.

$$\frac{1}{F'_{Mn}N_2} = \frac{V'_{Bo} + V'_L - N \cdot V_bO_2}{V_{Bo} \cdot F_{Bo}N_2 + V_L \cdot F_{Lo}N_2}$$

Figure 4.11 shows the time courses of $1/F_{Mn}N_2$ (for the composition change illustrated in Table 4.5) at each at four different levels of oxygen consumption. The reciprocal extrapolation allows the correct initial mixing concentration to be determined (for inert gases) independent of the of the O_2 uptake. Oxygen is the only other gas in the correction system, its mixing concentration is determined by subtraction of the initial mixing concentrations of the inert gases from one. In the model, the reciprical extrapolation eliminates the residual discrepancy and allows the correct lung volume to be determined.

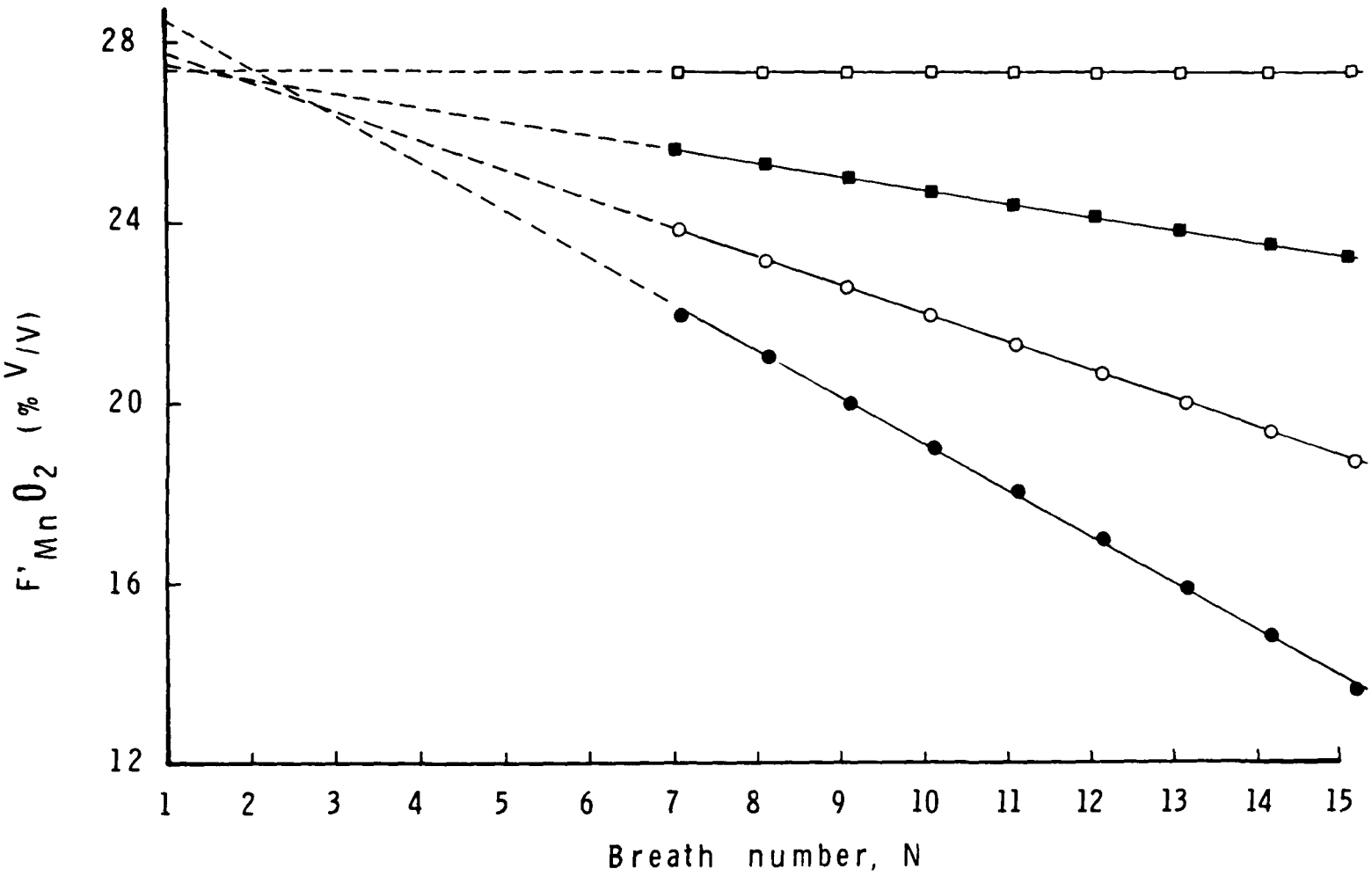


Figure 4.9 The effect of a linear extrapolation of the transformed mixing concentrations for breaths 7 to 15. Note that as the oxygen consumption is increased the error in the extrapolation increases also.
□:zero oxygen uptake, ■:25ml/breath
○:50ml/breath, ●:75ml/breath.

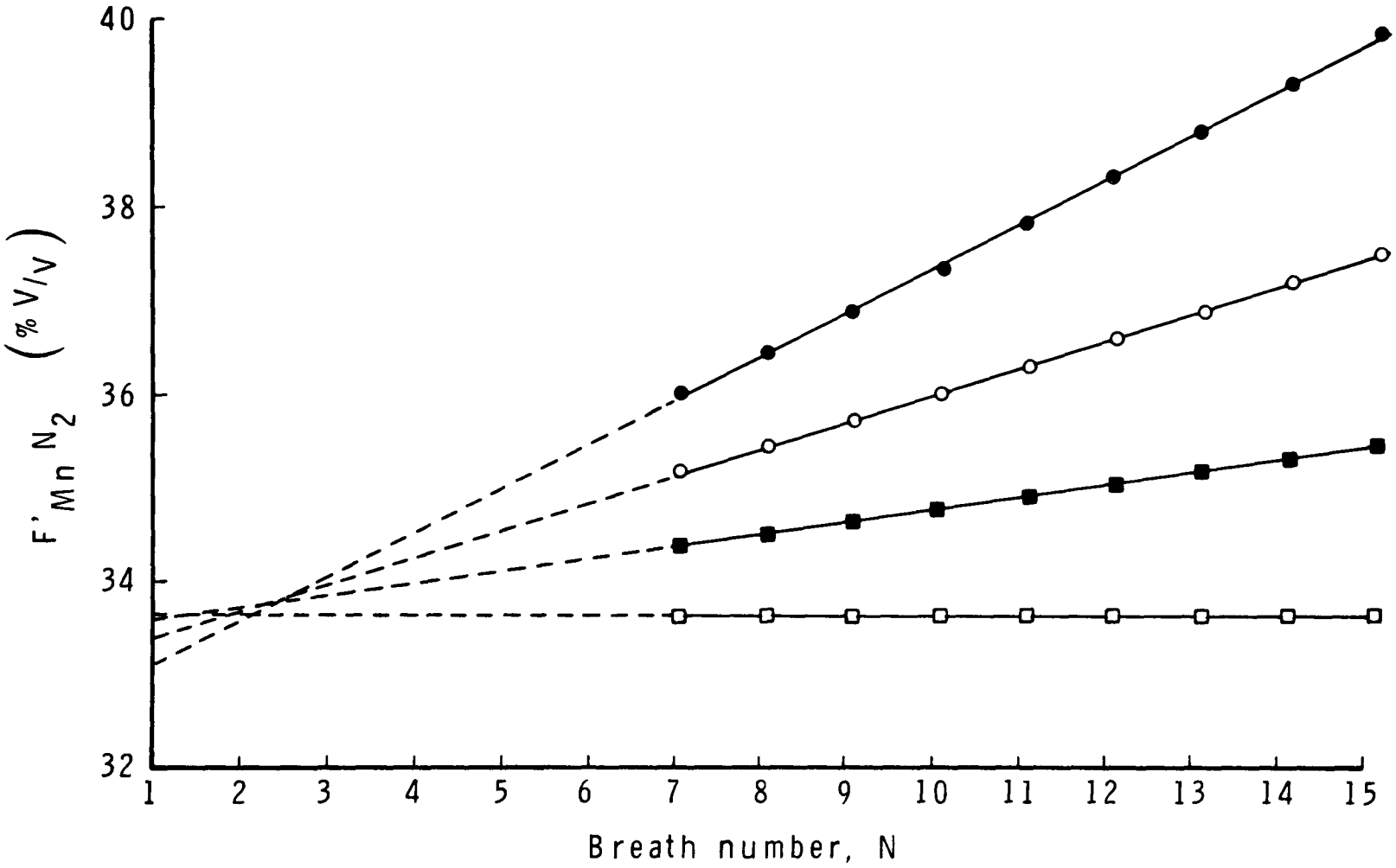


Figure 4.10 The same plot as Figure 4.9 but for nitrogen. Once again the error increases with increasing O_2 uptake.

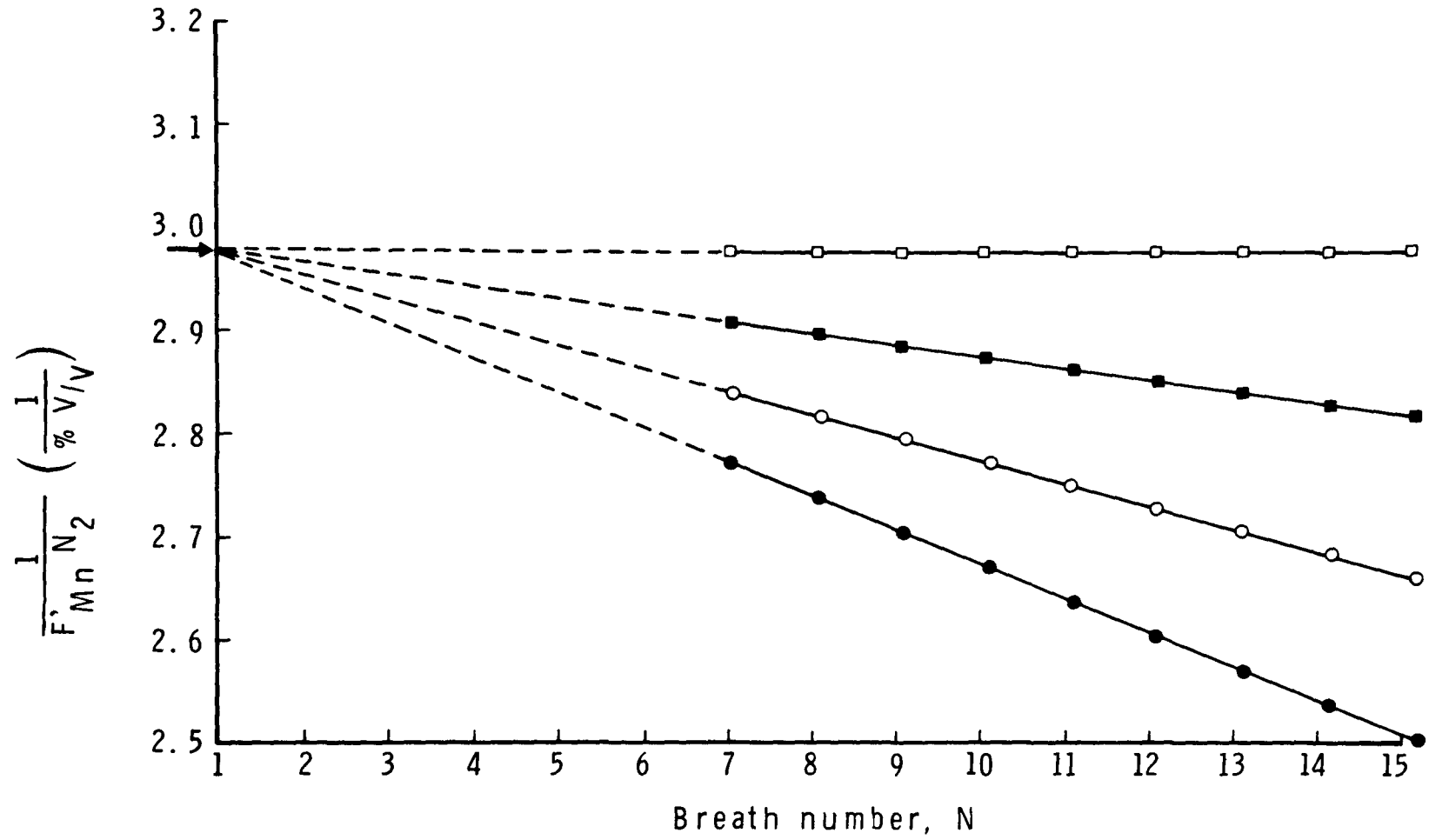


Figure 4.11 A similar plot to Figure 4.9, except that the reciprocal of the transformed nitrogen concentration is plotted. This yields the same (correct) initial mixing concentration at all levels of oxygen uptake.

4.6.3 Limitations of the Simulation of Apparent Lung Volumes

The assumption of zero alveolar-capillary flux for N_2 and Ar is strictly inaccurate. However, because of the insolubility of these gases, the effects of these exchanges will be small in comparison to the primary cause of the discrepancies in apparent lung volumes, namely the CO_2 output.

The profiles assumed for the breath-by-breath output of CO_2, or for the uptake or output of N_2O, are extremely influential in determining the pattern of discrepancy. Appreciable changes in the pattern of simulated results could be obtained by appropriate choice of the values for the first breath uptakes and outputs, and by choosing the decrements for subsequent breaths, even though these choices were made within ranges which would be considered quite reasonable. A preliminary attempt to measure the breath-by-breath changes in CO_2 occuring during a rebreathing manoeuvre was made whilst the author of this thesis was visiting Professor G.Swanson at the University of Colorado. By assuming zero net capillary exchange of N_2 during rebreathing, it is possible to extend the approach of Swanson (1980) to estimate capillary gas exchange during rebreathing. These estimates suggest that the value of 60 ml of CO_2 in the first breath may be an underestimate, and the true output may be closer to 80 ml. The decline in CO_2 output, over the first 20 seconds at least, appeared to conform to the geometrical decline used in the simulations.

It is likely that a major improvement in the accuracy of the simulation would result from choosing the correct gas exchange profiles for the particular rebreathing manoeuvre which was being simulated. However, the effort devoted to more accurate simulation was deliberately limited, since even the most accurate simulation of results is no proof that the mechanism proposed in the simulation is the one operating in real life.

4.6.4 Limitations of the Correction

The assumptions of a constant oxygen uptake and negligible alveolar-capillary exchange of nitrogen and argon, are crucial in the theoretical formulation of the correction procedure. Thus, it is reasonable to test, in the model, the effect that deviations from these assumptions has upon the correction procedure.

By taking the simulated breath-by-breath alveolar concentrations for the maneouvre featured in Table 4.5, the gradient of inert gas from F_{L1} to F_{Ln} can be assessed for each breath. These gradients are inaccurate, in so far as they are calculated for each breath in isolation from the output in the previous breath. With the Ostwald solubilities shown in Chapter 3 (Figure 3.1) and an estimated cardiac output, a profile of inert gas exchange can be produced. It is assumed that, at the start of rebreathing, the cardiac output is doubled to 10 L/min and remains at this level for 3 breaths, falling linearly to 5 L/min by breath 8. This assumption produces an inert gas exchange which increases initially and then decreases to a stable plateau. For nitrogen, the plateau rate is 3.5 ml/breath, the maximum rate is 7.1 ml/breath and the total output is 66 ml over 15 breaths. For argon, the plateau rate is 5.6 ml/breath, the maximum rate is 10.9 ml/breath and the total uptake is 103 ml. The values seem reasonable when compared to the expectations of other authors, as discussed by Kety (1951). When introduced into the computer model, the above inert gas exchanges cause the corrected lung volumes (by all three indicators) to overestimate the lung volume which was calculated with no inert gas exchanges. However, this overestimation is only 20 ml for O_2 and 30 ml for N_2 and Ar.

Figure 4.12 illustrates a real rebreathing manoeuvre in which an argon output and a nitrogen uptake would be expected. In the period between the 30^{th} and 90^{th} second of the manoeuvre the rebreathing volume is

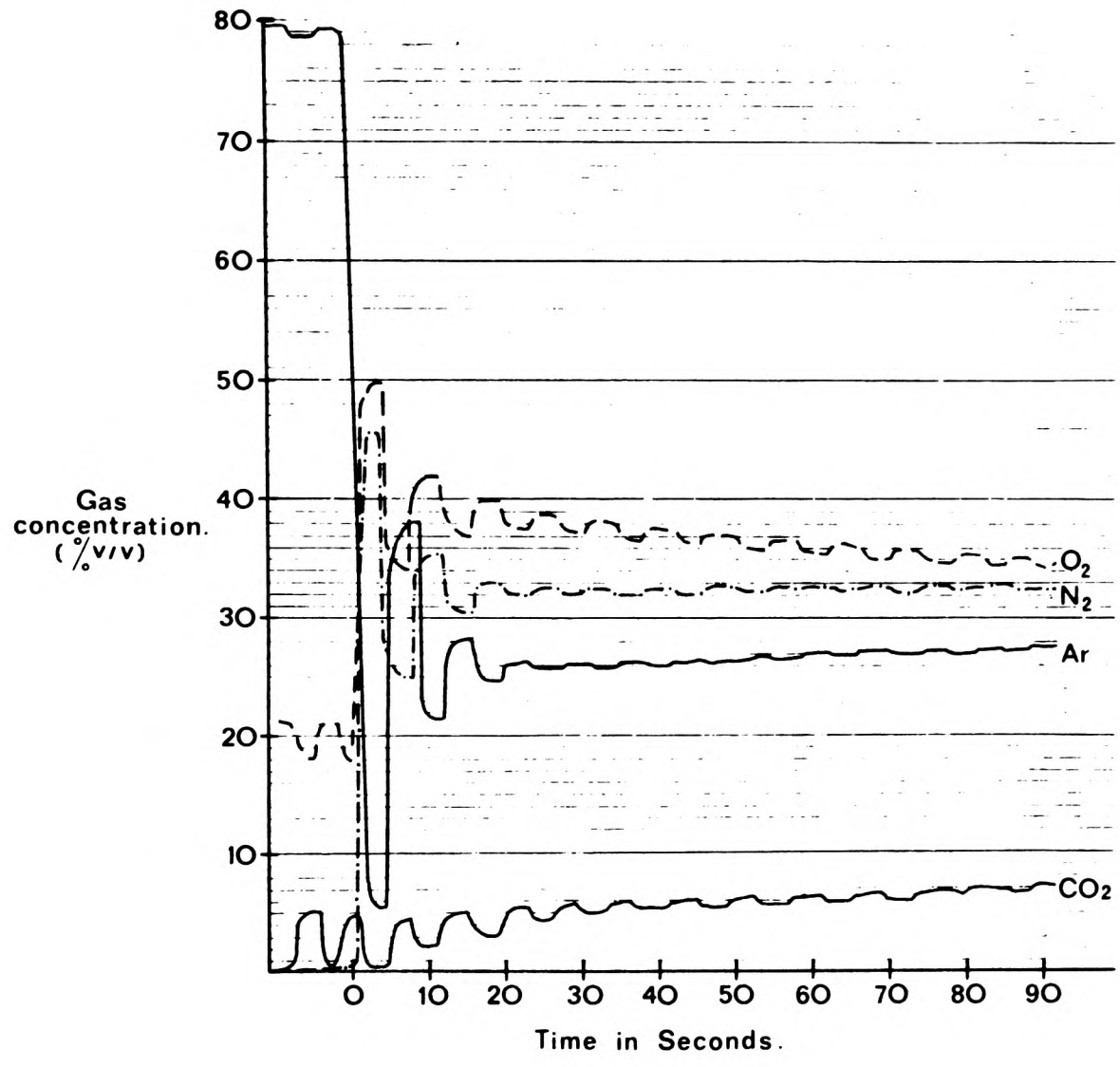


Figure 4.12 Example of a real manoeuvre illustrating slope of the later mixed portions. See text for details. Redrawn for monochrome presentation.

equilibrated. During this time, both gases increase in concentration. If there was no overall volume change between these times, the increase in concentration would be consistant with the excretion of 100-120 mls of argon in 60 seconds, and the excretion of 25-30 ml of nitrogen. The reason why both gases appear to be excreted is that the system volume is decreasing (due to the oxygen uptake). Since both gases are present at almost the same mixed concentration the concentrating effect, due to the net volume shrinkage, will be nearly the same for both gases. Therefore, Figure 4.12 may be interpreted as indicating that between 65 and 85 ml more argon is excreted than nitrogen. When the expected inert gas exchanges are calculated, as described previously (on the basis of a 5 L/min pulmonary blood flow), the argon output is predicted as 40 ml/min and the nitrogen uptake 20 ml/min. This results in a calculated excess of argon excretion over that of nitrogen of 60 ml/min, very close to that observed. This suggests that the inert gas exchanges previously calculated (for the later breaths of the manoeuvre) are within the range that occurs in real manoeuvres. Conversely, this suggests that in real manoeuvres the calculated lung volume will be only slightly effected by inert gas exchanges.

If the oxygen uptake is assumed to vary in proportion to the cardiac output (25 ml/breath equivalent to 5 L/min) then in the previous manoeuvre (where the cardiac output is initially doubled but declines to normal by breath 8) the large errors are simulated. The corrected lung volume calculated with nitrogen underestimates the true lung volume by 80 ml, and the argon overestimates by 120 ml. Most dramatic of all is the estimate of lung volume by oxygen which overestimates the true lung volume by 870 ml!

Therefore, the assumption concerning the stability of the oxygen uptake seems to be the most critical. The suceptibility of the oxygen estimate to this error will depend on size of the bag-lung oxygen

concentration gradient. The larger this gradient, the larger the numerator and denominator of equation 4.2 will be, and thus the scope for error is less. Increasing the $F_{Bo}O_2$ to 50% v/v, in the above situation, reduces the the O_2 overestimate to 470 ml.

4.6.5 The Benefits of Modelling

The computer model provides a numerical simulation of rebreathing based on the theoretical constraints which will be discussed in Chapter 6. The benefits of this model were threefold. Firstly, it allowed a comparison between real and idealised data to be made. This provided an insight into the mechanisms operating in the former. Secondly, the model provided predictions on the effects of a number of factors in real manoeuvres. Specifically, the model has allowed the correction procedure to be tested in an error free system, and the sensitivity of the correction to deviations from the fundamental assumptions has been gauged. Thirdly, through providing the facility to investigate physiologically impossible situations, the model has allowed insights into the general theory which would not have been obvious analytically.

4.7 CONCLUSIONS

Sections 4.2 to 4.4 show that the pattern of apparent lung volumes, observed with the different gas composition changes, can be explained economically and simulated by the model. This supports the hypothesis as to the underlying cause of these discrepancies. The correction technique was tested in the model, where its assumptions were valid. Even under these conditions, the correction was not perfect. Two errors of extrapolation were identified. The first concerned identifing the true mixed concentration of each breath. The other, identified in sections 4.5 to 4.6, concerned the inappropriateness of a linear extrapolation. Removal

of these errors allowed a perfect correction under the ideal conditions of the model.

In a real situation, some of the assumptions, implicit in the correction, will not be valid. The ultimate test will be in real subjects. If a unique volume of distribution is obtained from three indicators (O_2, N_2, and Ar) for a given composition change in a real rebreathing manoeuvre, the crucial assumptions of constant oxygen uptake and zero alveolar-capillary exchanges of nitrogen and argon would be less suspect. If the same unique volume of distribution is obtained, irrespective of the composition change imposed, one would be even more reassured.

The most efficient way to apply this technique in real subjects would be to develop the means of automatically collecting data points analogous to those generated by the model. The extrapolation and correction can then be performed numerically on these data.

CHAPTER 5

On-Line Computation of Lung Volume

5.1 INTRODUCTION

In Chapter 3, it was shown that the linear extrapolation, which was employed to allow for oxygen uptake from the bag-plus-lung system, failed to allow adequately for the output of CO_2. As a result, the imposition of different gas composition changes between "control" and rebreathing yielded consistently different patterns of discrepancy between the apparent volumes of distribution for the different indicators. In Chapter 4, these patterns were simulated in a simplified numerical model of rebreathing. An essential requirement was an output of CO_2 which was initially high but which then fell progressively in the first few breaths of the rebreathing manoeuvre. Simulations were also produced for situations where N_2O, as well as CO_2, contributed to the early volume changes in the bag/lung system. The model was unrealistically simple but apparently contained the essential features needed to explain the observations which had been made. A correction procedure was devised and tested in the model. The correction enabled the "correct" lung volume to be estimated, largely independent of the concentration gradients of the indicator gases.

In the model, the quality of the correction depended heavily on the assumptions that the O_2 uptake was constant, or nearly so, and that the alveolar-capillary exchanges of nitrogen and argon were negligible. If such a correction is effective for data from real rebreathing manoeuvres, these assumptions would appear to be justified for the purposes of lung volume measurement.

The correction procedure added considerably to the number of calculations required for each manoeuvre. In order to cope with this and automate the analysis, a microcomputer (Research Machines 380Z, Oxford) was used.

5.2 METHODS

As expertise in the use of microprocessor technology developed, so the methodology improved. Three phases of increasing sophistication are identified below. The methods and results are discussed with respect to these phases.

5.2.1 The Phases of the Study

PHASE I

(i) Nitrous oxide was not used. The concentration changes of O_2, N_2 and Ar between control and rebreathing, exposed the subject and the mass spectrometer to 100% v/v O_2. The mass spectrometer was calibrated on zero and 100% v/v gas concentrations.

(ii) The rebreathing switch was as previously described in Chapter 3.

(iii) The mass spectrometer output, during a manoeuvre, was recorded on magnetic tape. Computer data collection and analysis were performed "off-line" from these recorded signals.

(iv) The selection of the requisite data points, for the calculations of lung volume, was based entirely on the information contained in the O_2, N_2, Ar and CO_2 concentration traces.

PHASE II

(i) Nitrous oxide was not involved. In the concentration changes between control and rebreathing, the subject and mass spectrometer were never exposed to an O_2 concentration greater than 75% v/v or an inert gas concentration greater than 80% v/v. The mass spectrometer was calibrated without exposure to 100% v/v of any gas.

(ii) The rebreathing switch was as used in Phase I.

(iii) The data collection and analysis was performed "on-line" as each rebreathing manoeuvre was performed.

(iv) The selection of data points was as described in Phase I.

PHASE III

(i) In this phase, N_2O was incorporated. All concentration changes avoided exposure to 100% v/v of any gas. The calibration of the mass spectrometer was as in Phase II.

(ii) The rebreathing switch was improved (as will be described in section 5.2.4) and was operated automatically by the computer.

(iii) Computation was "on-line" as in Phase II.

(iv) The selection of the requisite data points was prompted by a flow sensing device, located within the improved rebreathing switch, and rebreathing was initiated by the computer.

5.2.2 Calibration of the Mass Spectrometer

In Phase I, the mass spectrometer (Centronic 200 MGA), was calibrated on the zero and 100% v/v points for O_2, N_2 and Ar and the zero and 10% v/v points for CO_2. By the completion of Phase I, it was clear that exposure of the mass spectrometer to 100% v/v O_2, N_2 or Ar could introduce serious errors, (Gillbe et al, 1981). Indeed, the results from Phase I suggested that this was the case (see also Chapter 3, section 3.3.4a).

For Phase II, three gas mixtures were made up into "calibration cylinders". These cylinders were first evacuated and then medically pure gases were decanted from larger cylinders into the "calibration cylinders". By choosing a final cylinder pressure of 1000 psi, the final volume percentage concentration of each gas was equivalent to 1% v/v for every 10 psi added during decanting (From Dalton's law of partial pressures). Each cylinder, when full, contained about 250 litres of calibration gas, at ambient temperture and pressure. Including "air", this

provided 4 mixtures available for mass spectrometer calibration. For Phase III, two further "calibration cylinders" were acquired, both containing N_2O. One mixture was decanted from pure gases as described above, the other was an Entonox cylinder (BOC size 3). The compositions of these six mixtures are shown in Table 5.1.

The O_2 concentrations in the "calibration cylinders" were determined with a paramagnetic oxygen analyser (Servomex Control Ltd., Model DCL 0A10). The CO_2 concentrations were determined by Haldane analysis (Lloyd Gas Analyser, GAS-560-A, Gallenkamp) and ~~confirmed by~~ an infra-red analyser (Gould/Godart Capnograph Mk III). Mixtures numbers 4 and 6 contained a single inert gas (Ar and N_2O respectively). The concentration was, therefore, determined by subtraction of the CO_2 plus O_2 fraction from unity. Mixtures 2, 3 and 4 contained two inert gases. These concentrations were determined by mass spectrometry, after a careful initial calibration of the mass spectrometer using binary gas mixtures produced by a gas mixing pump (H. Wosthoff. o.H.G., Bochum).

The use of these calibrated gas mixtures avoided exposing the mass spectrometer to concentrations of greater than 80% v/v during calibration. In addition, each channel was provided with a "three point" calibration. Having these mixtures stored in cylinders greatly increased the speed with which calibration and linearity checks could be performed.

5.2.3 The Protocols of Concentration Change

For each rebreathing manoeuvre, the subject sat in a standardised position. After an initial period of breathing a "control" gas mixture, he or she rebreathed from a 6 litre anaesthetic bag containing one of a number of rebreathing gas mixtures. The change from control to rebreathing was effected in Phases I and II, by the remotely operated rebreathing switch described in Chapter 3. In Phase III, this was replaced by an improved switch incorporating a flow sensor (described in section 5.2.4a).

GAS MIXTURES FOR CALIBRATING MASS SPECTROMETRY

	1 (AIR)	2	3	4	5	6 (ENTONOX)
N_2	78.1	27.8	9.4	0	0	0
O_2	20.9	0	37.4	57.7	0	48.4
CO_2	0	0	7.8	11.8	0	0
N_2O	0	0	0	0	25.6	51.6
AR	0.9	72.2	45.4	30.5	74.4	0

Table 5.1 Composition of the calibration gases in volumes percent.

The protocol used in Phase I consisted of 8 types of composition change, which were imposed on only one subject. The composition changes will be discussed in relation to the results. Both the subject and mass spectrometer were exposed to 100% v/v O_2. Such exposure was avoided in Phases II and III, which were carried out on 7 subjects. The protocols in Phases II and III each consisted of 4 composition changes, shown in Table 5.2. Each type of concentration change was repeated 5 times. The repetetions were in random sequence in Phase II, but Phase III was undertaken in two halves. The first half resulted in a nitrous oxide uptake and the second half a nitrous oxide output. It took too long to saturate and desaturate the body stores of nitrous oxide when changing randomly between stable control states breathing air, on the one hand, and 40 - 50% v/v N_2O on the other. The choice of 40 or 50% v/v N_2O depended on the ability of individual subjects to remain co-operative.

5.2.4 The Rebreathing Switch

Phases I and II employed the rebreathing switch described in Chapter 3. The switch used in Phase III was based upon a design by Ellis and Lampman (1980) and is illustrated in Figure 5.1. The switch allowed the mouthpiece port to communicate either with the "control" breathing port or the rebreathing port, depending on the position of two mushroom shaped seals. These seals were mounted at either end of a hollow cylindrical shuttle. This "double mushroom" arrangement moved between the orifices of the "control" and rebreathing ports in such a way that when one "mushroom seal" occluded one orifice, whilst the other seal was 5 mm clear of the other orifice. The cylindrical shuttle carrying the "mushrooms" moved on a hollow projecting rod, the base of which was mounted within the lumen of the rebreathing port. The cylindrical shuttle was closed at one end. The other end, where it engaged the projecting rod, was sealed with an "O" ring. A capillary lumen was drilled, from the outside of the rebreathing

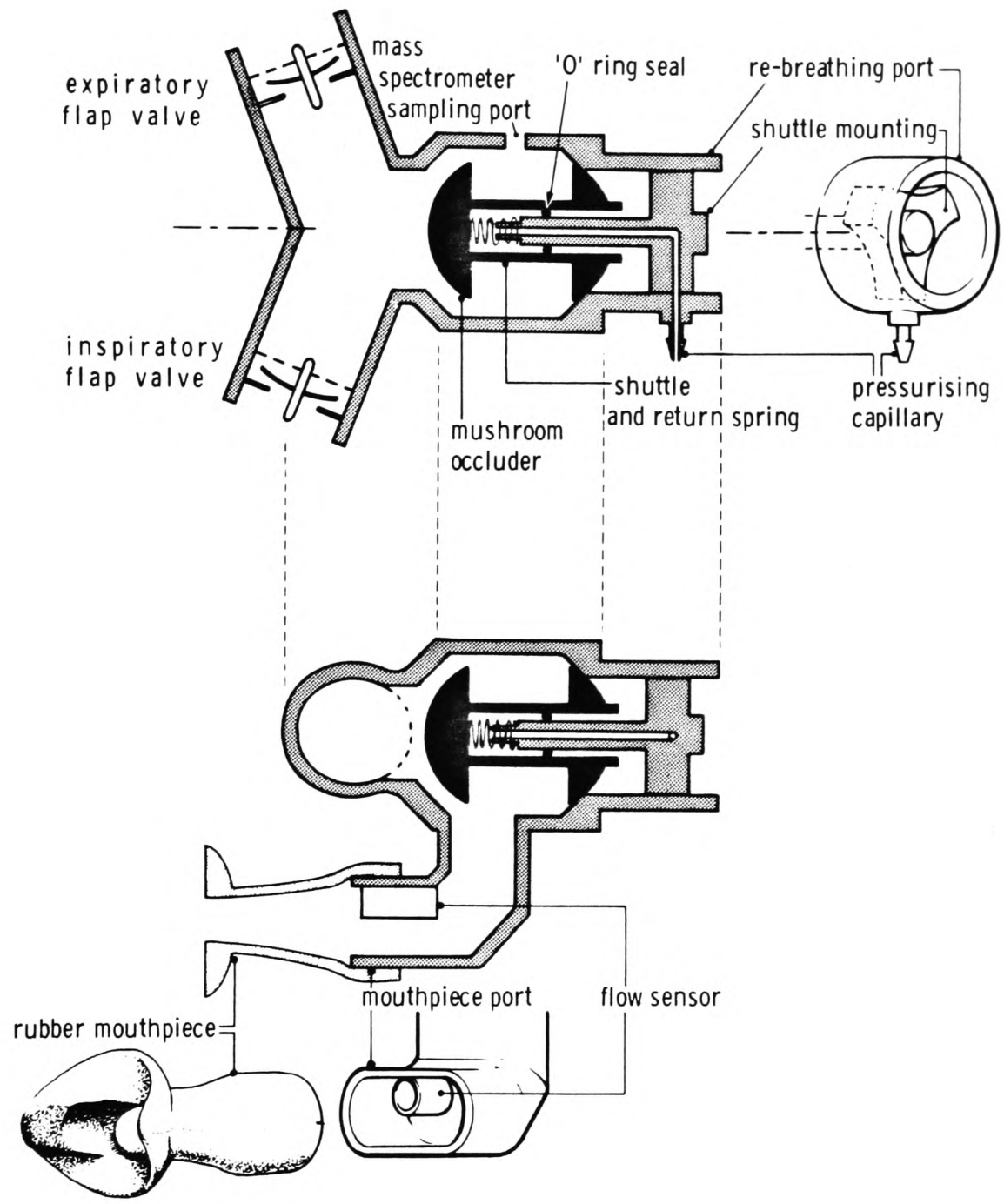


Figure 5.1 The rebreathing switch used in Phase III, as viewed from above (top) and from the side (bottom).

port, through the shuttle mounting and into the projecting rod. This allowed the lumen of the cylindrical shuttle to be pressurised. During the "control" period, a small spring held the shuttle so that a "mushroom seal" occluded the rebreathing port. Application of a pressure of 20 psi, via the capillary, to the lumen of the cylindrical shuttle moved the shuttle so that the other "mushroom" seal occluded the "control" breathing port. This movement was reversed by the spring when the pressure was removed. The 20 psi pressure was supplied from a reducing valve and directed by a solenoid valve (Sirai, H350 B), triggered electronically by the microcomputer.

The cross-sectional area of the mouthpiece was 330 mm^2, 24% of which was occupied by the flow sensor assembly. This did not appreciably increase the resistance. The dead space of the valve was 60 ml before, and 70 ml during, rebreathing (see Figure 5.2). The rebreathing bag (in a bottle), mounted on the rebreathing port, could be emptied and refilled in situ, through a tap in its distal end (Figure 5.2). The "control" gas mixture was supplied to the "control" port via an inspiratory one way valve and the subject's expirate left via an expiratory one way valve.

5.2.4a The Flow-Direction Sensor, (Phase III)

The flow-direction sensor (Micco, 1973) employed the principle of hot wire anemometry, made direction sensitive by the thermal coupling of two electrically heated platinum wires. The wires were 1.5 mm long and 25 μm thick and arranged in parallel 0.5 mm apart. The wires were heated by a constant current, one wire was upstream of the other and heated the gas steam impinging on the downstream wire. The wires constituted two resistor arms of a "Wheatstone bridge" circuit. Thus, differences in temperature between the two wires could be detected with great sensitivity from the ensuing resistances changes. The electronics were modified (Micco, personal communication, see Appendix 3) so that the output of the

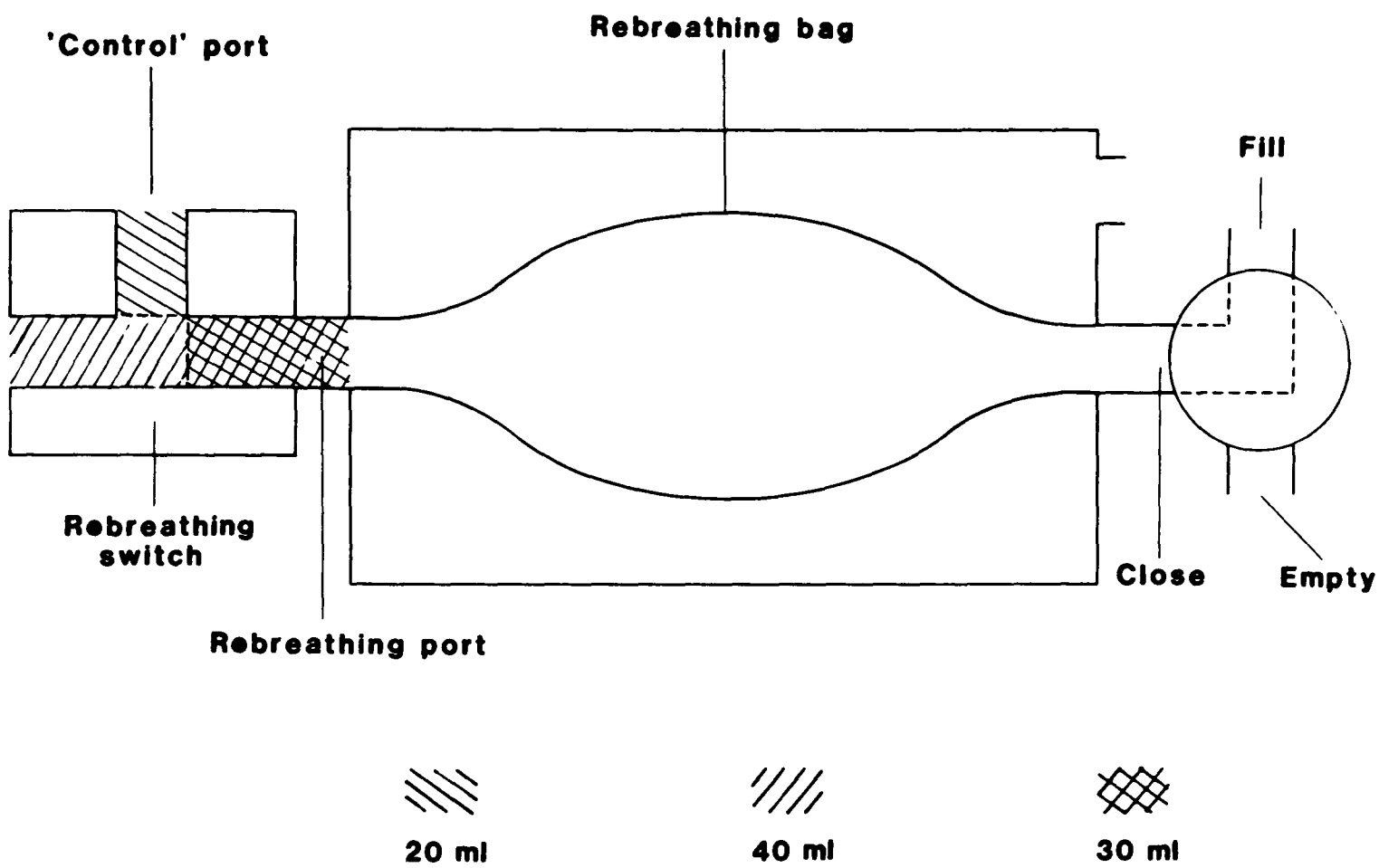


Figure 5.2 Schematic diagram of the rebreathing switch and the "bag in bottle" arrangement. The shaded areas illustrate the dead space of the rebreathing switch, 60 ml (20+40 ml) during "control" breathing and 70 ml (40+30 ml) during rebreathing. The rebreathing bag can be emptied and filled in situ, as indicated.

"bridge amplifier" formed one input to a comparator circuit. The comparator incorporated an adjustable "dead-band". The dead-band set a threshold for the "bridge" output, below which the comparator did not respond. This prevented instability of the comparator's output around the point of zero flow. It had the added advantage of providing a third stable comparator output which indicated zero or low flows. This will henceforth be termed the "zero flow state". The effect of this is seen schematically in Figure 5.3. A gradually changing flow signal is transformed into a three state output:- an inspiratory flow state, an expiratory flow state and a zero flow state. The three states of the comparator were passed to the microcomputer by two transistor-transistor logic (TTL) compatible outputs. Each output gave a signal of zero volts, (binary logic "0"), or 5 volts, (binary logic "1"). Inspiratory flow was signalled by one output being "1" with the other "0". Expiratory flow was signalled by the converse situation. The zero flow state was signalled by both inputs being "1".

The sensor was mounted within a perspex tube. This served to protect the transducer and to minimise turbulent flow around it. This tube was 30 mm long, with an internal diameter of 7 mm. One end of the tube faced the inspiratory flow and the other faced the expiratory flow. The whole assembly was mounted within the mouthpiece of the Phase III rebreathing switch (Figure 5.1).

The protective perspex tube somewhat reduced the sensitivity of the flow-direction detector. By itself, the bridge amplifier will provide an adequate signal in response to gas flow velocities as low as 1 m/min, with a response time of only 2 msec (Micco, 1973). When installed in the perspex tube, a 2.6 m/min expiratory flow velocity and 1.6 m/min inspiratory flow velocity was required to initiate the TTL compatible outputs. This was equivalent to a flow through the perspex tube of 100 and

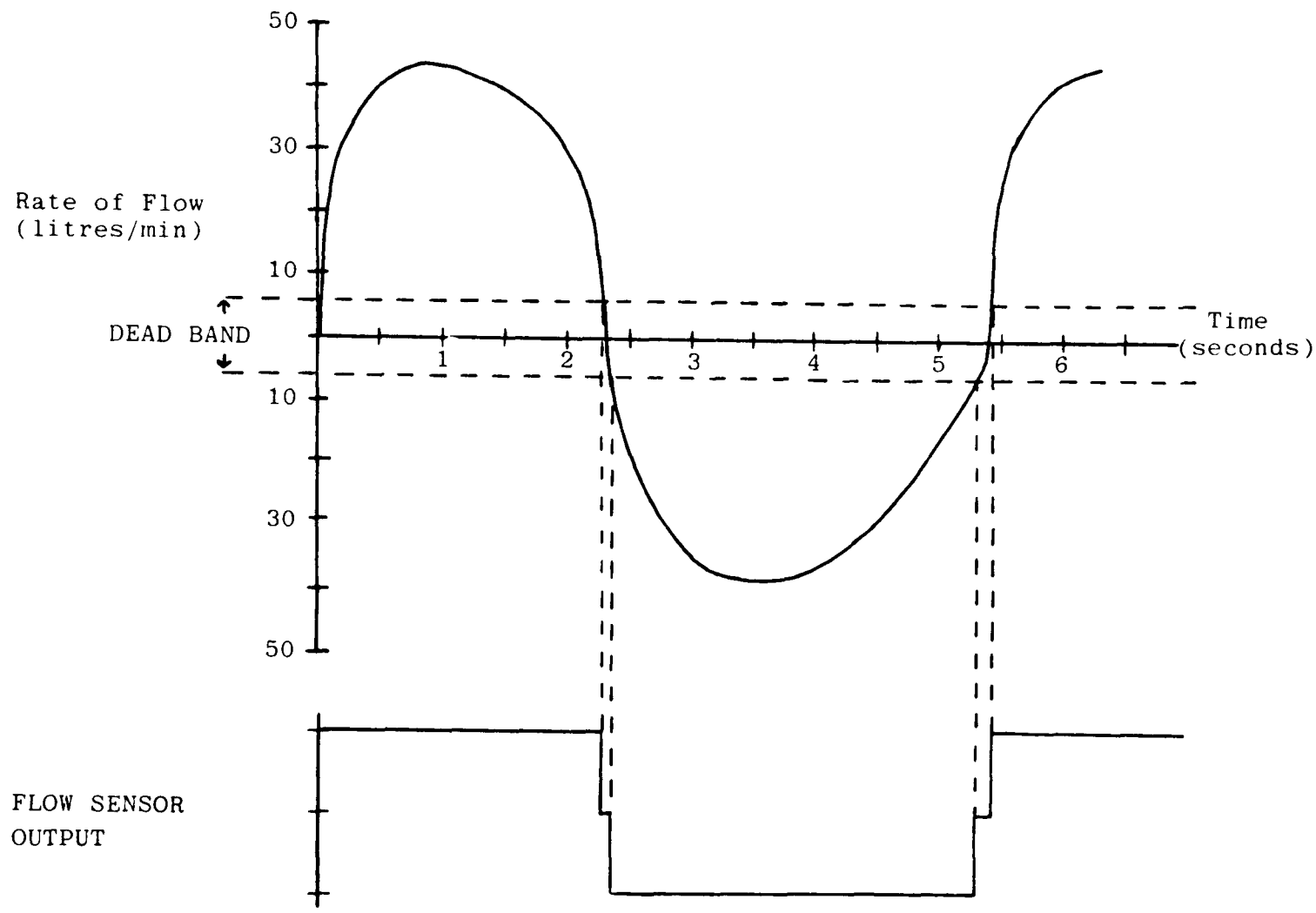


Figure 5.3 Normal respiratory flow pattern (Cain and Otis, 1949), and the three "flow states" which would result from the flow direction sensor.

60 ml/min respectively. Smaller flows than this fall within the dead band. Inside the mouthpiece port, the high flows associated with hyperventilation caused the sensor's output to "chatter" between it's three states, presumably due to turbulence. As an empirical solution to this problem, the inspiratory end was partially occluded so that access to the sensor comprised a lumen 1 mm in diameter and 5 mm long. The access at the expiratory end was a lumen 1 mm in diameter and 22 mm long. With the above arrangement, the minimum detectable flow through the mouthpiece was 6 L/min in either direction. During spontaneous respiration with a minute ventilation of 11 L/min, the peak inspiratory and expiratory flows are of the order of 40 L/min (Cain and Otis, 1949). Cain and Otis's data also show that flow exceeds 6 L/min within 100 msec of the start of expiration and 50 msec from the start of inspiration. Under the conditions of these experiments, these latencies were even shorter for a number of reasons. During the "control" breathing period, a major factor was the opening pressure of the valves. The initial resistance of the valves, followed by the sudden opening, caused a larger than normal initial flow. Even if a flow of 6 L/min was sustained for 100 msec, this would only amount to the movement of 10 ml of gas, considerably less than the valve dead space. During rebreathing, there were no valves to direct the gas flow. However, the forced ventilation, which was employed, generated very large flows so that the initial inspiratory and expiratory flow thresholds were again very rapidly attained.

5.2.5 Computing Objectives

For calculating the lung volumes and correcting them for initial volume changes in the rebreathing system, the microcomputer was required to select time related data from a minimum of three and a maximum of five concentration channels. On each channel, the initial bag and lung concentrations, along with bag and lung data for the mixing portion, were

required. With this information and the algorithms developed in the model (Chapter 4), lung volume was estimated. Thus, the following specific objectives guided the acquisition of hardware (electronic devices) and the development of software (computer programme and routines).

Hardware objectives:

1. Convert analogue input into a digital form and interface this with the computer.
2. Digitise more than one channel of input.
3. Provide a measurement of time.

Software objectives:

1. Select a channel and take the concentration-time data into the computer.
2. Identify the end-tidal lung concentration prior to the start of rebreathing.
3. Identify the initial bag concentration.
4. Identify the mixing phase and, subsequently, accumulate sufficient information on concentrations to enable a mixing line to be drawn.
5. Using the above data, perform the calculations previously tested in the model.

In order to test the electronics (hardware) and computer programmes (software) on real data, the O_2, N_2, Ar and CO_2 concentration-time signals (for Phase I) were recorded on magnetic tape using an "FM" tape recorder (Store 7DS, Racal Recorders Ltd., Southampton). This aided the development, "debugging" and validation of both the software and the hardware, since a standard set of recorded signals could be conveniently replayed to an evolving set of hardware and software.

5.2.5a Hardware

PHASES I and II

In Phases I and II, the hardware consisted of a purpose built input-output board. This carried a parallel input-output controller ("PIO chip", Mostek MK 3881), an 8 bit analogue to digital converter ("ADC", type 8703), a counter-timer circuit ("CTC", Mostek MK 3882), a four way analogue switch (type CD 4066) and a number of binary logic gates.

The PIO chip provided a programmable interface between the central processing unit (CPU) of the microcomputer and two peripheral devices. In this case, these "peripherals" were the ADC and the analogue gate. The ADC was "free-running". That is to say, it was continually converting at its maximal rate of 800 conversions per second. Thus, each conversion took 1.25 msec, 5 μsec of which was taken up in changing the value on the ADC output from the previous value to the one which had just been converted. During this period of updating, the value on the output of the ADC was incorrect- that is for 1/250 of the time. The digital output of the ADC formed the input to the PIO. The analogue gate was controlled by the programme in the microcomputer to select, in turn, the outputs of each of the channels of the mass spectrometer. Thus, up to four channels of analogue input were "multiplexed" into the ADC, and the digital output was fed into the computer. After opening the gate for each channel, a "software delay" of about 4 msec was interposed to allow time for conversion. The digital output was then read, and the next channel was selected. In this way, four channels were read in sequence, 45 times a second.

The CTC was similarly connected to the CPU. The microcomputer started the counter and the output was read in the same way as that of the PIO.

Since the ADC, used in Phases I and II, could not register voltages

greater than 2.5 volts and since full scale deflection of the mass spectrometer corresponded to a voltage range of 0-10 volts, a scaling unit was interposed. Such that 0% v/v of each gas gave zero from the ADC and 100% v/v gave 200. Thus, the resolution was $\pm$ 0.25% of full scale deflection.

PHASE III

Phase III used a commercially made 16 channel PIO board, with a 10 bit analogue to digital converter (Research Machines, Oxford). Although the components were basically the same as on the purpose built board, the operational characteristics were significantly better and more sophisticated data collection was possible. The commercial board allowed a selection of operating voltages. It was set up so that a mass spectrometer output of 0% v/v gave zero and 100% v/v gave 1000. The 10 bit ADC improved the resolution from $\pm$ 0.25% of full scale to $\pm$0.05%. The multiplexer also provided up to 16 channels for analogue input, as oppossed to only 4 (in Phases I and II). This allowed N_2O data to be collected. The output of the flow sensor, being TTL compatible, was fed directly into a PIO port. The ADC was no longer "free running" but controlled through the PIO in such a way that the ADC only converted when instructed to do so. When conversion was completed, this was signalled to the PIO. This eliminated the need for a software time delay, and prevented the collection of invalid data during updating of the ADC output, which was described for the purpose built board. With the 10 bit ADC, the time taken for a conversion was dramatically improved to 50 μsec, giving a maximum conversion rate of 20 KHz.

5.2.5b Software

The software was designed in a three tier architecture:-

1. Channel selection and data collection were controlled by routines

written in "machine code".

2. Subroutines for individual functions were written in "Basic".
3. A "master programme" for coordinating the subroutines was written in Basic.

The machine code routines were incorporated into the Basic language and could be called from the subroutines or the master programme.

PHASES I and II

The channel to be read was selected by sending a signal to the analogue gate. After a 2 msec delay, the data were read. The data were temporarily stored in a "first in first out" (FIFO) buffer. This was created in software and consisted of 11 data items in a sequence of addresses. As each new datum item was added, the previous 11 data items were moved along one address. The least recent datum item was ejected entirely from the buffer. During the "control" period, the gas compositions at the end of the inspiratory and expiratory plateaux were recognised by an algorithm based on the abrupt changes in CO_2 concentration at these times. This approach has also been used by Gothard et al (1980) and Ozanne et al (1981). A basic subroutine collected CO_2 data points 45 times a second. The most recent value was compared with the immediately previous one. At the beginning of inspiration, the sharp fall in CO_2 concentration was recognised when the new CO_2 value differed from the immediately preceding value by more than a preset amount, which was termed the "CO_2 sensitivity". When the change was recognised, the average of 10 previous values in the FIFO buffer was taken to be the end-expiratory CO_2 concentration. The value of the "CO_2 sensitivity" was adjusted empirically to prevent noise on the CO_2 signal from being misinterpreted. When the end-expiratory CO_2 concentration was registered, the corresponding end-expiratory concentrations for O_2, N_2 and Ar were simultaneously

registered from the FIFO buffers on these channels. The programme was then set to detect a sharp rise of CO_2 concentration. In similar fashion, the sharp rise in CO_2 concentration caused the end-inspiratory concentrations on all four channels to be registered, and a sharp fall in CO_2 was again looked for.

Thus, during the "control" breathing period, end-expiratory and end-inspiratory data points were collected in sequence. After each end-inspiratory point had been detected, a further Basic subroutine compared the inspired O_2 and N_2 concentrations with those of the previous expiration.

This subroutine was designed to detect the large concentration changes which marked the first inspiration from the rebreathing bag. Detection occurred either when the difference between a pair of end-expiratory and end-inspiratory O_2 concentrations exceeded twice that between the preceding pair or when the difference for N_2 varied by more than 10% v/v from the preceding difference. Once a sufficiently large concentration difference was detected, the immediately preceding end-expiratory value on each channel was registered as the initial lung concentration and the current end-inspiratory value was registered as the initial bag concentration.

Having registered and stored these initial values, the microcomputer waited for a preset period before collecting any further values. The period was adjusted empirically to about 15 seconds. With a normal subject rebreathing from a 4 litre bag, this was sufficient for the bag and lung O_2 concentrations to approach each other to within 2% v/v. By this time, inspired-to-expired concentration difference for CO_2 had become too small to be used to detect end-expiratory and end-inspiratory values. Instead, values on all four channels were collected indiscriminately at between 5 and 6 points per second. These were stored either until 400 values for each channel had been accumulated or until the rebreathing manoeuvre was

terminated. Termination of the manoeuvre was signalled by a hand held switch which produced a TTL compatible signal on the PIO.

Although this procedure allowed for lung volume to be calculated on-line, it was too inflexible and wasteful of computer time and memory. The programme depended on pattern recognition based on the concentration traces, and required a number of assumptions to be made as to the format of these traces. In addition, because of the difficulty in detecting end-expiratory and end-inspiratory concentrations during rebreathing, excessive amounts of data were collected. This made disc storage impractical.

PHASE III

As described earlier the flow sensor output provided an unequivocal signal of inspiratory flow, expiratory flow and zero or low flow. The state of the flow sensor was inspected (or "polled") by the computer 120 times a second. The delay in passage of gas, through the 1.25 m mass spectrometer sampling capillary, was at least 160 msec. Thus it was 160 msec after the flow sensor registered a change in flow state before the mass spectrometer recorded the corresponding change in gas composition. At a sampling frequency of 66 per second on each channel, this gave time for 10 values to be sampled and averaged. Because of the dead space of the upper respiratory tract during expiration and because of the valve dead space during inspiration, the composition change was further retarded in relation to the flow. This decreased the chances of the flow sensor missing an end-expiratory or end-inspiratory concentration sample.

During the "control" breathing period, the microcomputer was programmed to wait until the end-expiratory and end-inspiratory concentrations of N_2 and Ar were within 0.5% v/v of each other. This

ensured that the subject was properly equilibrated with the "control" gas mixture. The microcomputer then initiated the rebreathing manoeuvre, 5 to 10 breaths later. This was accomplished by activating the solenoid (which operated the rebreathing switch) during an expiratory pause. An expiratory pause was detected by "polling" the flow sensor output. Any expiration followed by a zero flow state for at least a third of a second provided a suitable end-expiratory point. During a quiet expiration the flow may be below 6 L/min for up to 1 sec (Cain and Otis, 1949) so that (if anything) the rebreathing port will be opened a fraction of a second before the end of an expiration. Even assuming a mean flow of 3 L/min, after opening, it would take nearly half a second to wash out the valve dead space distal to the rebreathing port (30 ml) so that contamination of the bag with alveolar gas was most unlikely.

Using the flow sensor, and the computer actuated valve, meant that the computer was already prepared for the relevant events of the manoeuvre before their consequences appeared as concentration signals. In Phases I and II, a recent history of concentration events had to be stored and consulted and events were detected retrospectively.

The flow sensor also allowed end-expiratory and end-inspiratory concentrations to be read during rebreathing, when the differences between these two was small. This was a significant advance on Phases I and II. During rebreathing, end-expiratory and end-inspiratory concentrations were stored from each channel for each breath of the rebreathing manoeuvre, up to and beyond the time when the bag and lung contents had been thoroughly mixed. The effectiveness of mixing was quantitatively determined from the progressive approximations of the bag and lung concentrations during rebreathing. As the manoeuvres lasted for between 1 and 1.5 minutes, some 8 to 20 pairs of values were recorded on each concentration channel. Along with the time of their occurrence, this made a total of 130 values on average. With this number of raw data points, about 60 rebreathing

manoeuvres could be stored per side of a "mini-floppy disc". During the manoeuvre, the computer's visual display unit (VDU) exhibited a concentration versus time trace for oxygen, along with a digital readout of rebreathing time calibrated in seconds. The oxygen plot was initially drawn using "low resolution graphics", and later on, in "high resolution" graphics. The data stored on disc could be read by an "off-line" Basic programme and the raw data from any channel were plotted on the VDU or printed on paper for checking.

Throughout Phases I and II, and in the early experiments of Phase III, the concentration traces of each rebreathing manoeuvre were recorded on a four channel potentiometric pen recorder (Linseis, LS 4). An example is shown in Figure 5.4 of a rebreathing manoeuvre in which a subject rebreathed 50% v/v oxygen in nitrogen after a control period breathing 20% v/v oxygen in argon. Traces of this sort were used in checks of the accuracy of the analogue-to-digital conversion and of the on-line computation.

5.2.5c Summary of the Phase III Procedures

The software and hardware of Phase III is shown schematically in Figure 5.5. On the right of the dotted line the hardware and apparatus are shown and on the left the software is summarised. (The computer programmes are printed out in Appendix 4). The subject is seated and breathing through the rebreathing switch. The concentrations at the mouth, which are recorded by the mass spectrometer, are fed into the PIO. The PIO is shown as a multiplexer (MUX) connected to an ADC. The flow-direction sensor is recording the gas flow at the mouth. The "flow box" converts this into the three recognised flow states, which are communicated to the microprocessor through two inputs.

The logical steps of the software are shown in the form of a flow

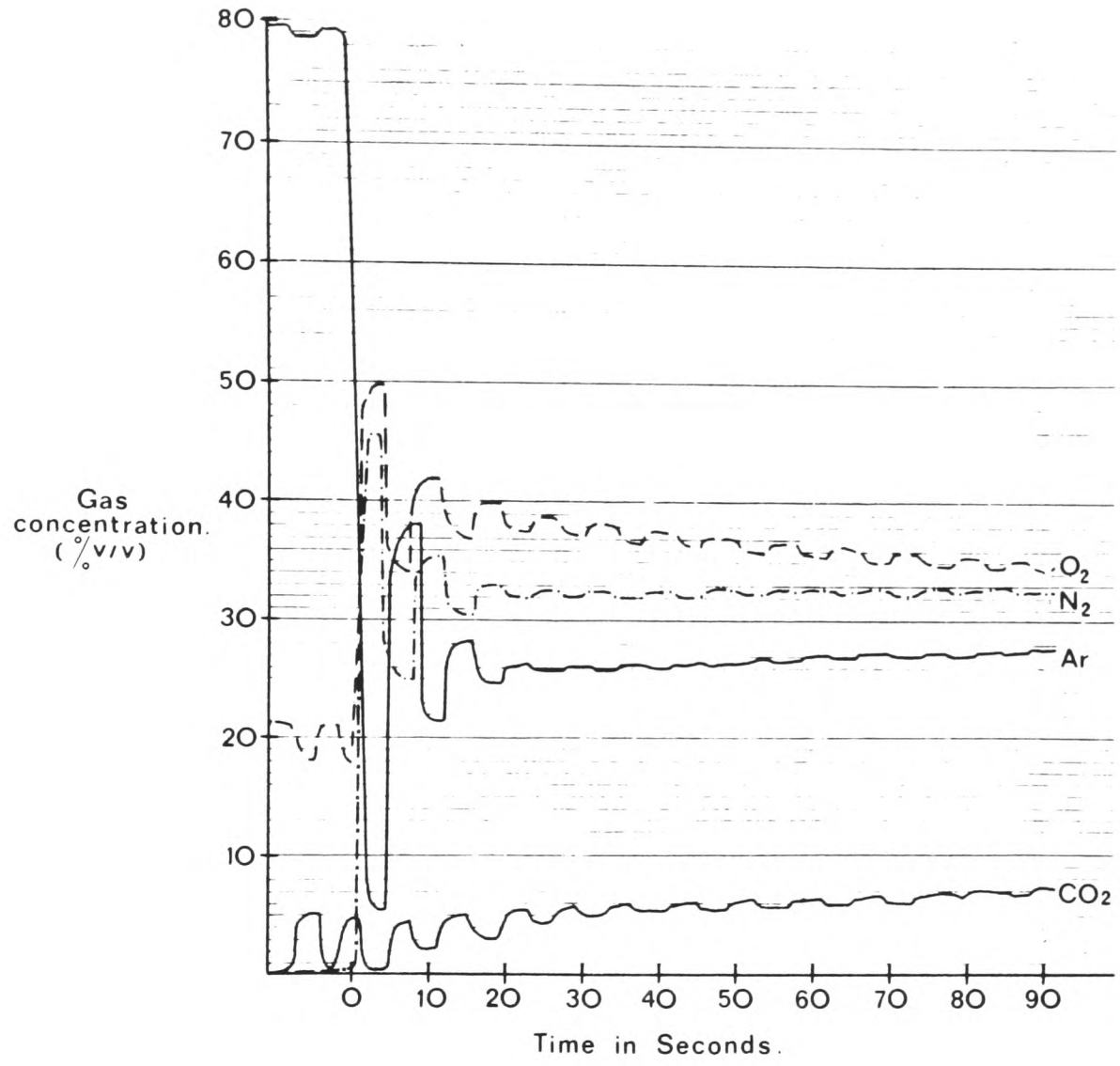


Figure 5.4 A concentration verses time trace of the oxygen, nitrogen, argon and carbon dioxide concentrations during a Phase III rebreathing manoeuvre. Redrawn for monochrome presentation.

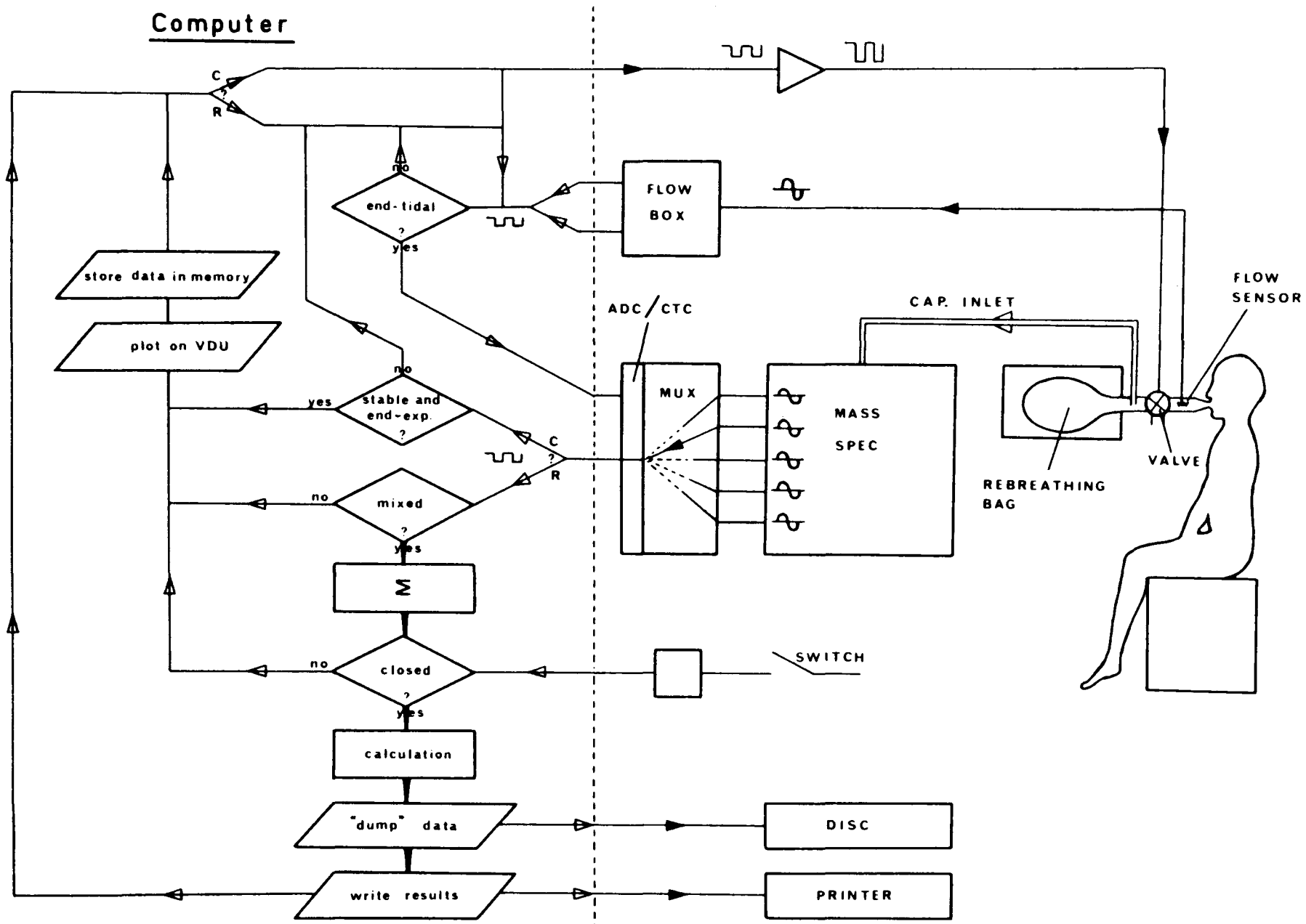


Figure 5.5 A schematic diagram of the hardware and software used in Phase III. See text for details.

diagram. Diamond shapes are used to represent decision processes (decision boxes, "DB"). Parallelograms illustrate input/output instructions and rectangles are operations not covered by the other two shapes.

The "end-tidal?" DB inspects the flow sensor's outputs and decides whether an end-expiratory or an end-inspiratory condition has occurred. If not "polling", these outputs continue, if so, then the computer requests five channels of input from the ADC along with "time" from the CTC. Initially, the software is in the "control" condition ("C") so that the "stable and end-exp?" DB is entered. This tests to see if the subject is in a stable "control" breathing state, if not then the programme returns to collecting end-expiratory and end-inspiratory values. Once a stable "control" state is attained at end-expiration, these data are displayed and stored. The solenoid valve is then activated to start rebreathing. The programme returns to the "end-tidal?" DB, but now in the rebreathing state ("R"). Upon detection of an end-expiratory or end-inspiratory condition, more data are collected but the "mixed?" DB is entered. If a sufficient degree of mixing has been achieved, the data are used to calculate a regression line ("Σ"). The status of the hand-held switch is then inspected ("closed?" DB). When this switch is open, the data are displayed and stored and the programme returns to collect still more data. When the switch is closed, the ~~the~~ software stops collecting data and performs the necessary steps to calculate lung volume. The raw data are saved on disc and the results printed. The programme is now ready to perform the next manoeuvre.

5.2.6 The Calculation and Correction Procedures

Calculation of the apparent initial mixing concentrations was based on the linear extrapolation of the "mixing line" to the initial instant of the rebreathing manoeuvre (Chapter 3, 3.3.2). The "mixing line" for each gas was the later portion of the concentration trace, after mixing of bag

and lung gas contents had occurred. In phases I and II, the "mixing lines" were represented by the 300 to 400 samples stored for each concentration channel from about the 15th second of the rebreathing manoeuvre. In Phase III, it was represented by by 6 to 12 pairs of values for end-expiratory and end-inspiratory concentration, occurring after those concentrations had come within 1.5% v/v of each other. These two methods are not equivalent. In Phases I and II, the extrapolation was from a time weighted average over the whole of the equilibrated portion of the trace. In Phase III, only end-expiratory and end-inspiratory points were selected and both concentrations were considered to have occurred at end-expiration. The latter was more in keeping with the theory developed from the model (Chapter 4) and the bag concentrations were transposed so that they occurred at the same time as the corresponding end-tidal lung concentrations. Following the procedure described for the model simulation, linear regressions were performed on the values representing the time courses of the mixing lines. These were numerically extrapolated to the initial instant of the manoeuvre.

The calculation of apparent volumes of distribution from the initial lung, initial bag and apparent initial mixing concentration was as described previously in Chapter 3 (equation 3.1). A linear mixing line was fitted despite the small errors which had been recognised (Chapter 4, section 4.6.2). The correction procedure was essentially similar, in all phases of the study, to that described for the simulations (Chapter 4, equation 4.6). The O_2, N_2 and Ar signals were transformed by dividing each by their sum. The transformed concentrations were stored in memory, and the regression, extrapolation and calculation from these values yielded the "corrected" pulmonary volumes of distribution of O_2, N_2 and Ar.

5.3 RESULTS

PHASE I

Table 5.2 shows the results obtained in Phase I. The composition changes were presented in a random sequence to a single subject. The table is divided into three sections: the first shows the composition changes and the second the apparent lung volumes obtained in the subject, as compared with the model. The third section compares the corrected lung volumes in the subject and the model. The values from the subject are the means from either 4 or 5 rebreathing manoeuvres. The standard deviations are shown in small print within parentheses.

Subject and model show a passable similarity regarding the patterns of apparent volumes of distribution, for different indicator gases, between the different composition changes. In the model simulation, discrepancies within a given manoeuvre are corrected very nearly to the true lung volume in all manoeuvres. The residual discrepancies are due to the inaccuracy of fitting a linear mixing line as discussed in Chapter 4 (section 4.6.2). In the subject, however, the correction procedure left appreciable discrepancies within manoeuvres and between manoeuvres. Because the results resisted physiological explanation and appeared to indicate a technical fault, they were not subjected to detailed statistical analysis, and no further subjects were studied in this phase.

The initial bag, lung and mixing gas compositions registered by the microcomputer, and the apparent volumes of distribution calculated from them, agreed with those from the simultaneously recorded paper records. Applying the correction procedure manually to the concentrations measured, breath by breath, from the paper records, yielded a similarly imperfect correction. Thus, the fault was evidently present in the mass spectrometer output.

It seems likely, in retrospect, that this fault arose because of the exposure of the mass spectrometer to 100% v/v O_2, N_2, and Ar during the

CONTROL			REBREATHING			UNCORRECTED Apparent Lung Volumes						CORRECTED Apparent Lung Volumes					
						SUBJECT			MODEL			SUBJECT			MODEL		
O_2%	N_2%	Ar%	O_2%	N_2%	Ar%	O_2	N_2	Ar	O_2	N_2	Ar	O_2	N_2	Ar	O_2	N_2	Ar
20	80	-	50	50	-	2.91 (0.16)	2.04 (0.14)	-	3.05	2.25	-	2.62 (0.15)	2.67 (0.16)	-	2.76	2.76	-
20	80	-	50	-	50	3.61 (0.37)	2.77 (0.20)	3.22 (0.27)	3.04	2.68	2.97	3.14 (0.50)	3.06 (0.43)	3.01 (0.42)	2.76	2.79	2.81
20	80	-	100	-	-	2.70 (0.13)	2.38 (0.13)	-	3.01	2.68	-	2.40 (0.11)	2.40 (0.11)	-	2.79	2.79	-
20	-	80	50	50	-	2.60 (0.31)	2.21 (0.36)	2.01 (0.24)	3.05	2.97	2.68	2.30 (0.59)	1.90 (0.37)	2.05 (0.28)	2.76	2.81	2.79
20	-	80	50	-	50	2.69 (0.20)	-	1.67 (0.16)	3.05	-	2.25	2.37 (0.19)	-	2.08 (0.16)	2.76	-	2.79
20	-	80	100	-	-	2.31 (0.05)	-	1.82 (0.11)	3.01	-	2.68	2.01 (0.06)	-	1.86 (0.07)	2.01	-	1.89
100	-	-	50	50	-	2.25 (0.26)	2.69 (0.10)	-	2.42	2.97	-	2.49 (0.05)	2.42 (0.05)	-	2.81	2.81	-
100	-	-	50	-	50	2.45 (0.56)	-	3.12 (0.32)	2.42	-	2.97	2.88 (0.28)	-	3.25 (0.11)	2.81	-	2.81

Table 5.2 Results for Phase I. On the left the composition changes are shown. In the middle, the uncorrected lung volumes in litres, for the subject and model. On the right the corrected results are shown. The results are the means of five obsevations, the standard deviations are shown in brackets.

calibration procedure and possibly because of the additional exposure to 100% v/v O_2 during some steps in Phase I, (Gillbe et al 1981). These errors would have been compounded by the Automatic Sensitivity Control (ASC) mode of the mass spectrometer, and the resulting errors could be very complicated (see Chapter 2). The Phase I procedures were abandoned in favour of Phase II procedures. Phase I had resulted in a thorough validation of the data collection and analysis to the extent that these could confidently be applied "on-line" in Phase II.

PHASE II

Table 5.4 shows mean values for the apparent volumes of distribution for O_2, N_2, and Ar, in 7 normal subjects with the 4 composition changes: A, B, C, and D, shown for Phase II in Table 5.3. Each composition change was repeated five times in random sequence. Because of technical difficulties seven of the manoeuvres gave unusable results. Thus, seven sets of means (indicated by asterisks) are of four, rather than five values. The first line of Table 5.4 shows the corresponding model simulations. The second line shows the results obtained for the equivalent composition changes from Phase I. The initial impression is that there is considerable variation in the lung volume between subjects, but the pattern of discrepancies in relation to composition change is reproduced from subject to subject.

The effect of the correction procedure in Phase II is shown in Table 5.5. The initial impression is that the inter-subject variation in lung volume remains, but the discrepancies within and between composition changes are very much reduced.

PHASE III

The concentration changes used in Phase III are shown in Table 5.3. Tables 5.6 and 5.7 show, respectively, the apparent and corrected volumes of distribution obtained for O_2, N_2 and Ar. The top line shows the

		Control period (Nominal concentrations)			Rebreathing period (Nominal concentrations)		
		O_2	N_2	Ar	O_2	N_2	Ar
PHASE II (Composition changes <u>without</u> N_2O)	A	20%	80%	-	50%	50%	-
	B	20%	80%	-	50%	-	50%
	C	20%	-	80%	50%	50%	-
	D	20%	-	80%	50%	-	50%

		O_2	N_2	Ar	N_2O	O_2	N_2	Ar	N_2O
PHASE III (Composition changes <u>with</u> N_2O)	A	20%	80%	-	-	50%	-	-	50%
	B	20%	80%	-	-	50%	-	25%	25%
	C	50%-60%	-	-	50%-40%	20%	40%	40	-
	D	50%-60%	-	-	50%-40%	75%	25%	-	-

Table 5.3 Key to the composition changes for Tables 5.4 to 5.7 (concentrations are in volumes percent).

COMPOSITION CHANGES WITHOUT N_2O:

UNCORRECTED VALUES.

	A		B			C			D	
	O_2	N_2	O_2	N_2	Ar	O_2	N_2	Ar	O_2	Ar
Model	3.05	2.25	3.04	2.68	2.97	3.05	2.97	2.61	3.05	2.25
Phase I	2.91 (0.16)	2.04 (0.14)	3.61 (0.37)	2.77 (0.20)	3.22 (0.27)	2.60 (0.31)	2.21 (0.36)	2.01 (0.24)	2.69 (0.20)	1.67 (0.16)
Subject 1	3.02 (0.30)	2.30 (0.19)	3.21 (0.27)	2.83 (0.20)	3.08 (0.24)	3.26* (0.31)	3.06* (0.20)	2.81* (0.23)	3.15 (0.27)	2.25 (0.30)
Subject 2	3.06 (0.03)	2.17 (0.06)	2.91 (0.13)	2.54 (0.11)	2.79 (0.14)	2.89 (0.27)	2.85 (0.16)	2.63 (0.17)	2.81 (0.27)	2.20 (0.21)
Subject 3	2.99 (0.19)	2.32 (0.18)	3.03 (0.26)	2.77 (0.27)	2.94 (0.27)	2.78 (0.38)	2.78 (0.35)	2.57 (0.32)	2.91 (0.23)	2.44 (0.16)
Subject 4	3.21 (0.16)	2.40 (0.35)	3.11 (0.16)	2.76 (0.10)	3.03 (0.12)	2.92* (0.14)	2.84* (0.11)	2.58* (0.13)	3.15 (0.25)	2.52 (0.29)
Subject 5	1.82 (0.15)	1.10 (0.08)	1.81* (0.14)	1.48* (0.11)	1.70* (0.11)	1.79* (0.18)	1.67* (0.13)	1.44* (0.14)	1.86 (0.14)	1.22 (0.14)
Subject 6	1.78 (0.04)	1.17 (0.11)	1.74 (0.12)	1.44 (0.12)	1.64 (0.13)	1.74 (0.08)	1.68 (0.07)	1.44 (0.06)	1.77 (0.11)	1.24 (0.10)
Subject 7	3.58* (0.34)	2.82* (0.19)	3.48* (0.19)	3.09* (0.17)	3.34* (0.22)	3.40* (0.31)	3.38* (0.33)	3.07* (0.27)	3.54 (0.21)	2.80 (0.09)
Means of 7 Subjects	2.78	2.04	2.76	2.41	2.69	2.68	2.61	2.36	2.74	2.09

Table 5.4 The Phase II results, before correction. The table is divided between the 4 composition changes (refer to Table 5.3). The apparent lung volumes are shown for oxygen, nitrogen and Argon, in litres. Each result is the mean of 5, standard deviations are in brackets. The first line shows the model simulation. The second line is borrowed from Phase I. The next lines show the results for the seven subjects. The last line shows the mean value, for the seven subjects, for each column.

COMPOSITION CHANGES WITHOUT N_2O:

(CORRECTED VALUES).

	A		B			C			D	
	O_2	N_2	O_2	N_2	Ar	O_2	N_2	Ar	O_2	Ar
Model	2.76	2.76	2.76	2.79	2.81	2.76	2.81	2.79	2.76	2.76
Phase I	2.62 (0.15)	2.67 (0.16)	3.14 (0.50)	3.06 (0.43)	3.01 (0.42)	2.30 (0.59)	1.90 (0.37)	2.05 (0.28)	2.37 (0.19)	2.08 (0.16)
Subject 1	2.77 (0.25)	2.76 (0.27)	2.94 (0.23)	2.94 (0.23)	2.94 (0.22)	2.94* (0.29)	2.91* (0.20)	2.91* (0.23)	2.87 (0.29)	2.68 (0.28)
Subject 2	2.73 (0.02)	2.71 (0.05)	2.63 (0.12)	2.64 (0.11)	2.65 (0.14)	2.67 (0.20)	2.74 (0.15)	2.71 (0.17)	2.53 (0.24)	2.60 (0.25)
Subject 3	2.76 (0.19)	2.74 (0.16)	2.82 (0.26)	2.84 (0.27)	2.85 (0.27)	2.55 (0.34)	2.68 (0.33)	2.64 (0.34)	2.71 (0.20)	2.74 (0.20)
Subject 4	2.88 (0.18)	2.87 (0.18)	2.82 (0.11)	2.87 (0.11)	2.91 (0.11)	2.63* (0.11)	2.70* (0.12)	2.67* (0.12)	2.85 (0.28)	2.84 (0.24)
Subject 5	1.55 (0.10)	1.52 (0.13)	1.56* (0.14)	1.55* (0.12)	1.55* (0.11)	1.49* (0.16)	1.51* (0.13)	1.50* (0.14)	1.59 (0.13)	1.54 (0.15)
Subject 6	1.53 (0.07)	1.54 (0.06)	1.49 (0.11)	1.50 (0.12)	1.50 (0.12)	1.46 (0.07)	1.53 (0.07)	1.50 (0.06)	1.51 (0.09)	1.56 (0.12)
Subject 7	3.33* (0.29)	3.28* (0.28)	3.22* (0.15)	3.21* (0.18)	3.20* (0.21)	3.13* (0.27)	3.24* (0.31)	3.19* (0.29)	3.25 (0.17)	3.29 (0.13)
Means of 7 Subjects	2.50	2.49	2.48	2.50	2.51	2.41	2.47	2.45	2.47	2.46

Table 5.5 The Phase II results, after correction. The format is the same as Table 5.4.

COMPOSITION CHANGES WITH N_2O:

(UNCORRECTED VALUES).

	A		B			C			D	
	O_2	N_2	O_2	N_2	AR	O_2	N_2	AR	O_2	AR
MODEL	2.94	2.73	3.00	2.69	2.96	2.40	3.00	3.00	3.68	3.04
SUBJECT 1	3.28 (0.38)	3.32 (0.29)	3.38 (0.07)	3.08 (0.06)	3.41 (0.23)	2.90 (0.26)	3.48 (0.27)	3.50 (0.28)	4.87 (0.41)	3.54 (0.23)
SUBJECT 2	2.60 (0.10)	2.40 (0.08)	2.69 (0.08)	2.36 (0.07)	2.65 (0.08)	1.96 (0.04)	2.64 (0.27)	2.64 (0.26)	3.65 (0.13)	2.80 (0.05)
SUBJECT 3	3.88 (0.33)	3.73 (0.29)	4.08 (0.31)	3.64 (0.27)	3.92 (0.30)	2.75 (0.31)	3.54 (0.38)	3.53 (0.40)	4.09 (0.33)	3.16 (0.41)
SUBJECT 4	2.76 (0.18)	2.65 (0.11)	2.92 (0.17)	2.59 (0.14)	2.85 (0.16)	2.24 (0.15)	2.90 (0.09)	2.89 (0.09)	3.92 (0.24)	3.11 (0.08)
SUBJECT 5	3.82 (0.14)	3.63 (0.15)	3.89 (0.07)	3.39 (0.43)	3.83 (0.07)	3.16 (0.05)	3.92 (0.20)	3.96 (0.20)	6.31 (1.25)	3.81 (0.15)
SUBJECT 6	2.76 (0.17)	2.83 (0.15)	3.08 (0.36)	2.87 (0.35)	3.07 (0.35)	2.98 (0.29)	3.48 (0.33)	3.54 (0.27)	4.83 (0.45)	3.47 (0.32)
SUBJECT 7	2.86 (0.13)	2.73 (0.16)	2.99 (0.11)	2.61 (0.09)	2.85 (0.10)	2.40 (0.06)	3.16 (0.08)	3.17 (0.07)	6.22 (0.87)	3.35 (0.14)
MEANS OF 7 SUBJECTS	3.14	3.04	3.29	2.93	3.23	2.62	3.30	3.30	4.84	3.32

Table 5.6 The Phase III results, before correction. The format is as for Table 5.4 except that the "Phase I" line is omitted.

COMPOSITION CHANGES WITH N_2O:

(CORRECTED VALUES).

	A		B			C			D	
	O_2	N_2	O_2	N_2	AR	O_2	N_2	AR	O_2	N_2
MODEL	2.78	2.78	2.76	2.78	2.82	2.80	2.80	2.80	2.83	2.83
SUBJECT 1	3.32 (0.31)	3.32 (0.31)	3.18 (0.07)	3.19 (0.05)	3.21 (0.03)	3.30 (0.27)	3.29 (0.27)	3.30 (0.28)	3.35 (0.24)	3.36 (0.22)
SUBJECT 2	2.47 (0.08)	2.46 (0.08)	2.44 (0.07)	2.47 (0.07)	2.51 (0.07)	2.47 (0.19)	2.48 (0.20)	2.47 (0.18)	2.58 (0.03)	2.59 (0.05)
SUBJECT 3	3.81 (0.31)	3.80 (0.31)	3.82 (0.29)	3.80 (0.29)	3.77 (0.29)	3.31 (0.38)	3.31 (0.39)	3.32 (0.38)	3.16 (0.33)	3.20 (0.35)
SUBJECT 4	2.69 (0.13)	2.70 (0.13)	2.67 (0.15)	2.69 (0.15)	2.72 (0.15)	2.70 (0.10)	2.70 (0.10)	2.70 (0.11)	2.90 (0.05)	2.92 (0.07)
SUBJECT 5	3.71 (0.15)	3.71 (0.14)	3.70 (0.07)	3.70 (0.07)	3.71 (0.07)	3.69 (0.14)	3.67 (0.14)	3.72 (0.13)	3.56 (0.14)	3.60 (0.15)
SUBJECT 6	2.81 (0.16)	2.82 (0.15)	2.91 (0.36)	2.94 (0.35)	2.98 (0.35)	3.36 (0.29)	3.34 (0.30)	3.38 (0.28)	3.21 (0.24)	3.30 (0.32)
SUBJECT 7	2.78 (0.15)	2.79 (0.16)	2.74 (0.10)	2.73 (0.10)	2.71 (0.10)	2.90 (0.06)	2.89 (0.07)	2.91 (0.04)	3.08 (0.15)	3.11 (0.13)
MEANS OF 7 SUBJECTS	3.08	3.09	3.06	3.07	3.09	3.10	3.10	3.11	3.12	3.15

Table 5.7 The Phase III results, after corrections in the same format in Table 5.6.

corresponding model simulation. The composition changes A and B resulted in an uptake of nitrous oxide during rebreathing, whereas changes C and D created an output. Corrected volumes of distribution are presented only for O_2, N_2 and Ar, since N_2O exchange was assumed to be neither constant nor negligible. Again the initial impressions are that the correction procedure has largely removed the variability dependent upon composition change and indicator gas.

5.4 ANALYSIS OF VARIANCE

The results of Phases II and III were subjected to a "Split plot" analysis of variance, adapted to the experimental designs of Phases II and III. The arithmetical details are presented in Appendix 1. The general background to inferential statistics is clearly outlined by Schefler (1979), including an introduction to the analysis of variance or "anova" for short. Both Sokal and Rholf (1969) and Zar (1974) provide more advanced yet readable accounts of the analysis of variance.

In the experimental protocol described in section 5.2.3, four sources of variability can be identified, attributable to subject, composition change, indicator gas and error. The term "error" does not imply a source of variability leading to incorrect results but refers to any uncontrolled variation (ie. that not accounted for by the other three groups). The estimates of lung volume vary between manoeuvres as a result of between-manoeuve treatments and between-maneouvre error. Estimates vary within manoeuvres as a result of within-manoeuvre treatments and within-manoeuvres error. In essence, the error structure is split. This conforms to a "split-plot anova" which is one of a number of possible designs for an analysis of variance. Such analyses are discussed at length by Steel and Torrie (1960) and Cochran and Cox (1957).

5.4.3 The Factors affecting the Scatter of Results

The 70 mean values in Tables 5.4 and 5.5 represent 338 individual estimates of lung volume from 133 manoeuvres. The 70 in Tables 5.6 and 5.7 represent 350 individual estimates from 140 rebreathing manoeuvres. The variation between these individual estimates consists of variation between rebreathing manoeuvres and a variation within manoeuvres. The variation between manoeuvres is made up of the factors which cause lung volume estimates to change from one manoeuvre to the next. This includes variation between subjects, real variations in a single subject's end-expiratory volume and variability between manoeuvres. The last has been shown in Chapter 3 to be dependent on the composition change imposed (at least for uncorrected values). It also includes the within-manoeuvre variation which incorporates measurement error. The variation within a manoeuvre, on the other hand, is contributed to by any effect which causes the volume estimated for a individual FRC to differ with different indicator gases (O_2, N_2 and Ar), including the within-group error.

Table 5.8 shows the results of the analysis of variance of Phase II results, and Table 5.9 the analysis of Phase III. The top half of each table shows the analysis of the sum of squares between-manoeuvres, into that owing to subjects (Subj.SS), composition changes (Comp.SS), and interactions between the two (Sub*Comp.SS). The bottom half is the analysis of the sum of squares within-manoeuvres, into that between indicator gases within a composition change (GwC.SS), and that owing to interactions of this with subject (S*GwC.SS). The ~~right~~ left hand of each table shows the results for the uncorrected apparent volumes of distribution (corresponding to Tables 5.4 and 5.6) and the right hand side the results for the corrected volumes of distribution (corresponding to Tables 5.5 and 5.7).

Tables 5.8 and 5.9 differ slightly. Table 5.8 contains fewer degrees of freedom than Table 5.9 because of the missing values in Phase

SPLIT-PLOT ANALYSIS OF VARIANCE, WITHOUT NITROUS OXIDE

SOURCE OF VARIATION	ABBREVIATIONS USED IN APPENDIX	DEGREES OF FREEDOM	UNCORRECTED SUM OF SQUARES	% OF TCSS	MEAN SUM OF SQUARES	F	CORRECTED SUM OF SQUARES	% OF TCSS	MEAN SUM OF SQUARES	F
Subjects	Subj.SS	6	132	78.6	22.1	312 ‡‡	137	92.3	22.9	368 ‡‡
Composition Changes	Comp.SS	3	2.44	1.4	0.81	11.5 ‡‡	0.25	0.2	0.082	1.3
Interaction of Subjects and Composition Changes	Sub*Comp.SS	18	1.27	0.8	0.071	0.8	1.12	0.8	0.062	0.7
Residual between Manoeuvres		105	9.76	5.8	0.087		9.64	6.5	0.086	
Total between Manoeuvres	Between.SS	132	146	86.6			148	99.7		
Gases within a Composition Change	GwC.SS	6	21.0	12.4	3.49	22 ‡‡	0.085	0.06	0.0142	2.7 ‡
Interaction of Subject and Gases within a Composition Change	S*GwC.SS	36	0.57	0.3	0.016	2.4 ‡‡	0.188	0.12	0.0053	4.1 ‡‡
Residual within Manoeuvres		155	1.11	0.7	0.007		0.225	0.15	0.0013	
Total within Manoeuvres	Within.SS	197	22.6	13.4			0.499	0.3		
Column Totals		329	169 (TCSS)	100			149 (TCSS)	100		

Table 5.8 Phase II. Partitioning of the total sum of square and the subsequent "split-plot" analysis of variance. Results are shown before (left) and after (right) correction.
"‡ " indicates a significant F ratio $p < 0.05$. "‡‡" indicates a significant F ratio $p < 0.01$.

SPLIT-PLOT ANALYSIS OF VARIANCE, WITH NITROUS OXIDE

SOURCE OF VARIATION	ABBREVIATIONS USED IN APPENDIX	DEGREES OF FREEDOM	UNCORRECTED SUM OF SQUARES	% OF TCSS	MEAN SUM OF SQUARES	F		CORRECTED SUM OF SQUARES	% OF TCSS	MEAN SUM OF SQUARES	F	
Subjects	Subj.SS	6	60.4	26.8	10.1	8.1	‡‡	55.3	73.3	9.21	21.9	‡‡
Between Halves	BH-Comp.SS	1	11.1	4.9	11.1	4.9		0.131	0.2	0.131	0.12	
Within Halves	WH-Comp. SS	2	42.0	18.7	22.0	29.7	‡‡	0.053	0.1	0.026	0.32	
Composition Changes	Comp.SS	3	53.1	23.6	17.7	14.3	‡‡	0.184	0.3	0.061	0.15	
Between Halves	BH-S*C.SS	6	13.5	6.0	2.25	13.6	‡‡	6.59	8.7	1.10	10.1	‡‡
Within Halves	WH-S*C. SS	12	8.9	3.9	0.74	4.4	‡‡	1.00	1.3	0.083	0.77	
Interaction of Subjects and Composition Changes	Sub*Comp.SS	18	22.3	9.9	1.24	7.5	‡‡	7.59	10.0	0.42	3.88	‡‡
Residual between Manoeuvres		112	18.6	8.3	0.17			12.2	16.1	0.11		
Total between Manoeuvres	Between.SS	139	154	68.6				75.2	99.8			
Gases within a Composition Change	GwC.SS	6	54.1	24.0	9.01	29.6	‡‡	0.035	0.05	0.0058	4.1	‡‡
Interaction of Subjects and Gases within a Composition Change	S*GwC.SS	36	11.0	4.9	0.304	9.2	‡‡	0.049	0.07	0.0014	2.5	‡‡
Residual within Manoeuvres		168	5.52	2.5	0.033			0.096	0.13	0.0006		
Total within Manoeuvres	Within.SS	210	70.6	31.4				0.18	0.2			
Column Totals		349	225 (TCSS)	100				75.4 (TCSS)	100			

Table 5.9 Phase III. Same format as Table 5.8. "‡‡" indicates a significant F ratio $P < 0.01$.

II. In Table 5.9, the composition changes were considered in two groups of two rather than one group of four. This is because the composition changes in Phase II were presented in a sequence randomly distributed between changes A, B, C and D. Whereas, in Phase III, the composition changes were presented in two halves. Randomly chosen presentations of A and B were followed by the randomly chosen presentations of C and D. This provided an "a priori" reason for a further subdivision of the TCSS, the Comp.SS being divided into that between the halves (BH-Comp.SS) and within the halves (WH-Comp.SS) of the protocol. This entailed a slight difference in the detail of the analysis, although the overall principle remained the same.

Considering for the moment only the columns containing the "sums of squares" and the "% of TCSS", these results confirm the initial impressions formed by examination of Tables 5.4 to 5.7.

Before correction:

1.(i) The major part of the variation between apparent volumes of distribution in Phase II is accounted for by the variation between manoeuvres, and most of this is between subjects (Subj.SS).

(ii) In Phase III most of the TCSS is again attributable to the between-manoeuvres sum of squares. Comp.SS and Subj.SS contribute almost equally the major components of this.

2. Differences between estimates by different indicator gases (GwC.SS) are the major component of the within-manoeuvres sum of squares. They also account for the second largest proportion of TCSS, behind Subj.SS.

After correction:

1. The correction procedure reduces the total variability of the estimates of lung volume (TCSS).

2. The variability between manoeuvres contributes over 99% of the TCSS, Subj.SS being by far the largest component.

5.4.4 Comparisons of Mean Squares

The mean squares are the sum of squares divided by the associated degrees of freedom and provide estimates of variance. The choice of which mean squares to compare in order to calculate a variance ratio of "F" statistic was made on the basis of a "Model II"anova (Sokal and Rholf, 1969). The mean squares due to both subjects and compositions were compared to the subject/composition interaction mean square. The subject/composition interaction represents that portion of the sum of squares between-manoeuvres and within a subject and composition change, which is not accounted for by subjects or composition changes alone. It is compared with the residual sum of squares between-manoeuvres. Within each manoeuvre, the variance due to the different indicator gases is compared to the interaction of subject and gases within a composition change. The F statistics, for identified sources of variation, are shown in Tables 5.8 and 5.9. Significant values are indicated by "£" signs. The significances are the probabilities (1% or 5%) of wrongly rejecting the relevant null hypothesis. By examining the F statistics in Tables 5.8 and 5.9, using the above logic, one can make a number of statements:

1. There is, in Phase II and III, a significant difference in lung volumes between subjects before and after correction.
2. Before correction, the effect of composition change is significant in determining the lung volume. However, following the correction the effect of composition change is not significant.
3.(i) Without N_2O (Phase II), there is no significant interaction of subject and composition change.

   (ii) With N_2O (Phase III), there is such an interaction before correction. After correction the subject/composition interaction is

only between halves but not within the two halves of Phase III.

4. The effects of the different indicator gases within a manoeuvre are significant in Phase II and III, both before and after correction.
5. The interaction of subjects with GwC.SS (above) is significant in both Phase II and III, before and after correction.

5.5 DISCUSSION

The aim of this study was to assess the performance of the correction technique in real subjects. There were two purposes:- firstly to validate, or otherwise, the conclusions made in the model (Chapter 4) and, secondly, to establish the precision of this technique for use as a practical method of measuring lung volume.

PHASE I

Phase I confirms that exposure of the Centronic MGA 200 to 100% v/v gases during the calibration procedure leads to measurement errors. These errors were not sufficient to alter the underlying pattern of the apparent volumes of distribution. However, the correction was ineffective in the presence of such errors. This can be seen by comparison of the Phase I and Phase II in Table 5.5.

PHASES II and III

5.5.1 Assumptions of the Analysis of Variance

When using a test such as the analysis of variance a number of implicit assumptions are made.

1. The uncontrolled variation of lung volume between manoeuvres and within manoeuvres was assumed to be randomly distributed between the composition changes. This was achieved by randomisation of the application of composition change. In Phase III, when complete randomisation was not

possible a further sub-division of the sum of squares was necessary.

2. The variances within the groups of the anova design should have been homogeneous. This requires that:-

(i) The variances of the estimates for a particular composition change were the same irrespective of the subject.

(ii) The variance between composition changes in the same subject was the same.

The square roots of the former variances are listed in the columns in Tables 5.4 to 5.7 and the latter are listed across the rows. Clearly, there is not a homogeneous variance between subjects (ie. down the columns) and one would not expect there to be. However, the significance of the difference between different subjects lung volumes is of no interest. Between composition changes (rows), the variances appear more similar. It is possible to test the homogeneity of the variances using Bartlett's test (Sokal and Rholf, 1969). However, there is some doubt as as to the usefulness of this test. Zar (1974) states that it is seldom necessary to use Bartlett's test with the analysis of variance and goes on to say:- "Fortunately, the analysis of variance is robust enough to operate well even with considerable heterogeneity of variances...". This is only true if the sample sizes are equal or nearly equal, as in this study. Most statistical texts concur with this opinion especially if the "F" values are not on the borderline of significance.

3. The variable considered (lung volume) must be normally distributed in the population (within and between subjects) and there is no reason to suppose otherwise. Even so, quoting from Zar (1974): "The analysis of variance is also robust enough with respect to the assumption of normality in the underlying populations. The validity of the analysis is affected only slightly by even great deviations from normality...".

Thus, although some of the assumptions of the analysis may have been

compromised it is likely that the test is robust enough to cope. Furthermore, the conclusions are drawn from comparisons of two analysis on similar data, one before and after correction.

5.5.2 The Correction

The success of the correction technique will be assessed in terms of its ability to eliminate the between-composition change and the between-indicator gas sources of variability, by comparing the results before and after correction.

5.5.2a Composition Changes

Tables 5.8 and 5.9 show that, before correction, composition changes contribute significantly to the between-manoeuvres variation in lung volumes. After correction, the effect is no longer evident. Although one is unable to reject the null hypothesis that, after correction, the Comp.SS is not a significant component of the between-manoeuvres sum of squares, one cannot prove this hypothesis. When accepting the null hypothesis, the risk that this is really false is incurred. By examining the power of the experimental design and associated statistical test, one can quantify the risks of falsely accepting the null hypothesis. The power of both the anovas can be calculated by standard methods (see Snedecor and Cochran, 1980; and Mood, Graybill and Boes, 1974). The subject/protocol interaction mean square is used as an estimate of the within-subject population variance. For a test on a given set of data, the power $(1-\beta)$ is related to the risk, α, which one is prepared to accept of falsely rejecting the null hypothesis. β is then the risk of falsely accepting the null hypothesis. For an "α" of 0.05 the power curves are shown in Figure 5.6. The probability of concluding that there was no effect of composition change and missing a real difference of 150 ml (ie β) appears to be 0.29 in Phase II and 0.41 in Phase III. A difference of 150 mls is probably of practical

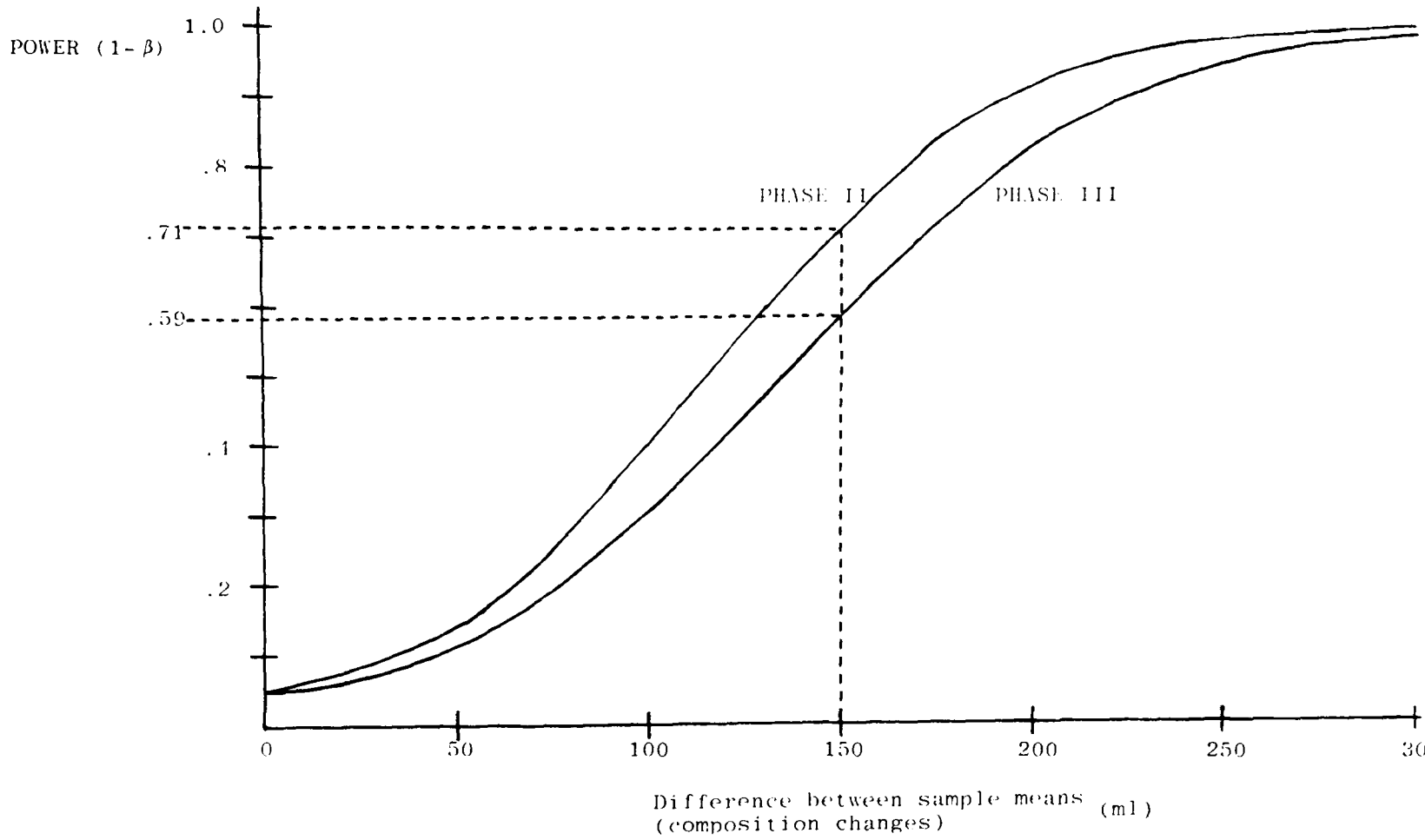


Figure 5.6 The "power curves" for the analysis of the variance due to composition changes, for Phases II and III. The dotted lines indicate the probability $(1-\beta)$ of detecting a true difference between means of 150 ml.

significance. One way of increasing the power (decreasing β), whilst keeping "α" the same, would be to increase the the number of repetitions. To reduce, to about 0.05, the chance of missing a real difference between composition changes of 150 ml would require about twice as many repetitions. To detect 50 ml differences 95% of the time would require twenty times as many repetitions. This highlights the difficulties of comparing different methods of lung volume measurement, in the face of the normally large variations of the end-expired volume between estimates. Many authors (Willmon and Behnke, 1948; Sloan and Bredell, 1973; Nunneley et al, 1974; Chiang and Yang, 1973; Wilmore, 1969) have attempted such a comparison and concluded that since the methods are not significantly different they must be the same.

In Phase III (Table 5.9), a significant subject with composition change interaction was evident (Sub*Comp.SS). After correction, this interaction persisted even though the effect of composition changes was eliminated. Partitioning of the sum of squares due to composition changes between the two halves of the protocol resulted in a significant interaction of subject and composition change between halves (BH-S*C.SS) but not within halves (WH-S*C.SS). This implies that the corrected lung volume varied between the two halves of the protocol, and that the direction and extent of the variation was subject dependent. Over the seven subjects, these effects tended to cancel out, as witnessed by the failure of BH-Comp.SS to achieve significance. There is quite adequate explanation for the between-halves subject with composition interaction. The two halves of the Phase III protocol were separated in time, with a break for relaxation interposed. In addition, breathing entonox may have influenced the maintainance of FRC differently in different subjects. The observation justifies the extra partitioning of the TCSS in Phase III.

5.5.2b Gases within a Composition Change

Discrepancies between the corrected lung volumes, estimated from different indicators, are still apparent after correction (Tables 5.8 and 5.9; GwC.SS). The column totals in Tables 5.4 and 5.6 are the mean lung volumes (over the seven subjects) for each particular gas in each composition change. The statistical significance of the GwS.SS refers to the differences between the two or three means under each composition change. Most subjects displayed the same order for the relative sizes of the lung volume estimates from the different indicator gases. These consistent patterns were specific to each composition change and it was this which was detected by the anova. "Split-plot" designs are rather sensitive to within-manoeuvres variability (Steel and Torrie, 1960). However, after correction, the total within-manoeuvres sum of squares is small in comparison to the differences which occur in a single subject between repetitions of a similar rebreathing manoeuvre. (This latter variability is estimated by the residual between-manoeuvres sum of squares). The statistical significance of the differences between gases within a composition change refers to their consistency. Their size (around 30-40 mls) is of little practical significance in the context of lung volume measurement. Discrepancies between the indicator gases after correction will be referred to as "residual discrepancies" (as they were in Chapter 4) and the factors which cause them will now be discussed.

5.5.3 Residual Within-Manoeuvre Variation

The presence of residual discrepancies within manoeuvres is predicted from the model simulations (Chapter 4). In the model these were mainly the result of a non-linear mixing line and were corrected for by the use of reciprocal extrapolation. In real subjects, the reciprocal extrapolation increased rather than decreased the residual discrepancies. This difference in performance between the subjects and the model may

reflect deviations between the model's assumptions and real life. Pulmonary gas exchange deviations were considered to be of minor practical significance (Chapter 4, section 4.6.4), although they may explain some of the differences between the subject and the model. Non-homogeneities of the gas mixing within the lung may also have contributed to the residual discrepancies. Two particular instances of this were identified.

The first instance was in Phase II. A subject dependent residual difference was observed as a significant S*GwC.SS. The source of this was traced to Subject 1, since repeating the anova whilst omitting the first subject produced a similar analysis except that the above interaction was no longer significant. The third and fourth composition change (C and D) of this Phase II entailed a "control" period breathing 20% v/v oxygen in argon. In the first subject, insufficient time was allowed for complete "wash-out" of nitrogen and "wash-in" of argon. This was evidenced, in retrospect, by a difference greater than 6% v/v, at the end of the "control" breathing period, between the inspiratory and expiratory concentrations of both nitrogen and argon. Under these circumstances, the end-expiratory argon and nitrogen concentrations may not have represented the true mean alveolar concentration. Correspondingly, the patterns of residual discrepancies for Subject 1 are different in "C" and larger in "D" than for all other subjects (Table 5.5). In subsequent subjects in Phase II, the on-line paper trace records were consulted before the computer was activated, to ensure that proper equilibration with control gas mixtures had occurred. In Phase III, initiation of the rebreathing manoeuvre was automatically prevented until the inspiratory to expiratory concentration differences had fallen to less than 1.5% v/v for the inert gases (as described in section 5.2.5b, Phase III).

The second instance was in Phase III, where despite the above

precautions, the S*GwC.SS was again significant. Examination of the raw data indicated a larger than expected discrepancy between the corrected estimates of lung volume given by O_2 and N_2 in composition change "D", of subject 6. A re-run of the analysis, omitting Subject 6, reduced the interaction, S*GwC.SS, but failed to remove its significance. The concentration data for this subject did not reveal any irregularities during the "control" period, as before, but these were evident during rebreathing.

Small, but measureable, argon concentrations later in rebreathing formed a mixing line which was extrapolated, in the usual way, to the start of the manoeuvre giving an "extrapolated" initial mixing concentration for argon. In theory, this concentration can also be calculated from the initial lung and bag concentrations of argon, both weighted by the respective volumes of the lung and bag. By assuming the true lung volume was a value midway between the corrected volumes as calculated from the O_2 and N_2 traces, an "expected" initial mixed argon concentration was calculated. When the "expected" mixing concentration was compared with the "extrapolated", a difference was found. This difference, multiplied by the estimated system volume, indicated the appearance of 10-20 ml of argon during rebreathing which could not be accounted for by the initial measured concentrations of argon in the bag and lung. The extrapolated-to-expected difference correlated with the discrepancy between the corrected volumes of distribution for O_2 and N_2. In Figure 5.7,the data from Subject 6 are compared to those from the other subjects. Three manoeuvres from Subject 6 stand out. These were the only manoeuvres which were preceded by a period of rebreathing with argon (composition change "C"). The above volume of argon could have come from two sources, the pulmonary circulation or from within the lungs. Since, during the control period the end-tidal argon concentration was less than 1.5% v/v, the concentration gradient for argon evolution from the blood was too small to account for the observed volume.

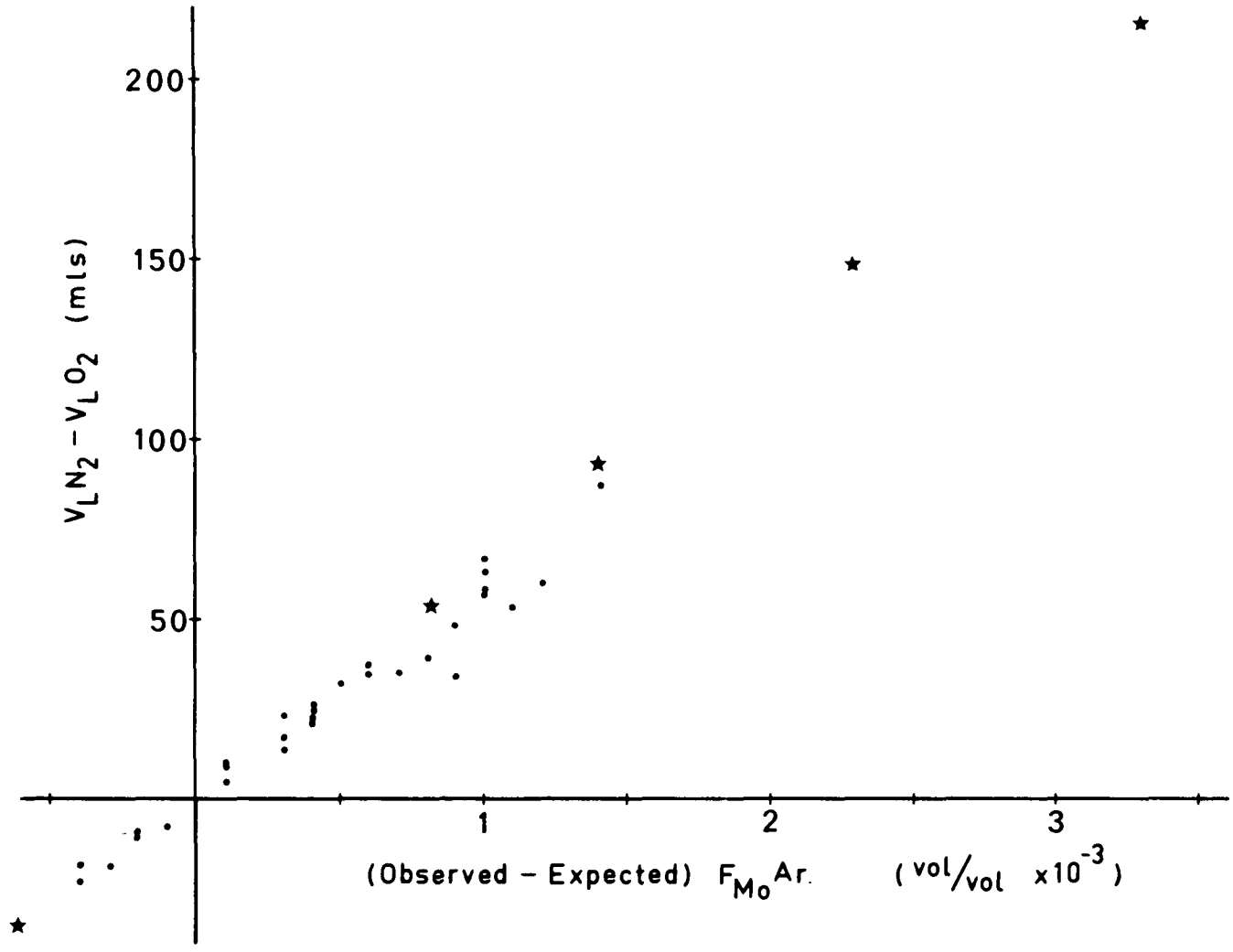


Figure 5.7 The relationship of the difference in corrected values of distribution, of O_2 and N_2, and the discrepancy between the observed and expected initial mixed concentration of argon. Results are from composition change "D" for all seven subjects (Subject 6 is indicated by the "★").

Another possible interpretation is the presence of very poorly ventilated gas spaces or trapped gas during the "control" breathing period. During rebreathing, the large tidal volumes equilibrate the entire lung volume with the contents of the rebreathing bag. During quiet breathing, poorly ventilated alveoli may not be fully equilibrated with the "control" mixture, although this disequilibrium may not be apparent in the expired gas. Thus, after rebreathing, the O_2, N_2 and Ar mixture, shown for composition change "C", the end-expired concentrations immediately before rebreathing may underestimate the mean alveolar argon concentrations. Gas from this previously poorly ventilated area becomes part of the bag/lung system during rebreathing and the argon in these underventilated areas may account for the difference between the extrapolated and observed argon. Indeed, the volume of nitrogen mobilised by forced rebreathing after a period of nitrogen washout has been used to measure the volume of trapped gas (Christensson et al, 1981). If a volume of gas containing 20% v/v argon was trapped and then reopened during rebreathing the observed to expected difference would indicate a trapped gas volume of 50-100 ml. The presence of trapped or underventilated gas would cause an underestimation of the initial nitrogen concentration of the lung and would result in an overestimation of lung volume as measured by nitrogen. The reason why such a feature is only seen for composition change "D" may be because this has the smallest concentration change for nitrogen and, therefore, is the most sensitive to measurement errors (Chapter 6).

There is some evidence which suggests that breathing high concentrations of nitrous oxide may impair gas exchange. Intrapulmonary shunt, measured in anaesthetised sheep, was found to double after the administration of 70% v/v N_2O (Dueck and Prutow, 1982). One explanation of this observation would be in terms of airway closure, since nitrous oxide has been observed to cause bronchoconstriction in humans (Douglas et al, 1974). Thus, the impaired gas mixing observed in Subject 6 may only have

been the result of breathing entonox during the "control" period.

These observations raised an interesting question which was how discrepancies can occur in a two gas correction system. In Chapter 4 (section 4.4.3), it was shown that discrepancies could not occur in such a system. However, in these experiments a true two gas system was not present since small quantities of argon were present. This was sufficient to "uncouple" the two gas system and allow discrepancies between the two indicator gases to be expressed.

5.5.4 In-Built Quality Control

This study confirms the "quality control" provided by the correction procedure. If only two gases are included in the correction procedure, a unique lung volume will inevitably be obtained. For a number of different manoeuvres, this unique lung volume will vary. However, for an individual manoeuvre, there is little to indicate whether or not this lung volume is correct. The use of a third gas within a manoeuvre (even in concentrations too small to be useful as an indicator gas) provides for a check of consistency, by allowing within-manoeuvre discrepancies to occur. These discrepancies have been found to be very sensitive to a number of errors, as described below.

Within-manoeuvre discrepancies led to the discovery of the calibration error in Phase I and the errors attributed to "inhomogeneity" in Phases II and III. There have been other errors of a more technical kind all of which were recognised from within-manoeuvres discrepancies. On one occasion, the rebreathing valve (Figure 5.1) was used upside down, and condensed moisture and saliva was intermittently aspirated into the sampling catheter of the mass spectrometer. Early in the Phase III study, the pressure of 20 psi which was used to operate the shuttle was supplied from a compressed air supply. Leaks in the "O-ring" seal of the shuttle

cylinder allowed the pressurising air to contaminate the gases under measurement. All of these errors, and a number of other possible errors were examined in the simulation, and were readily detected by the discrepancies which they induced between the corrected volumes of distribution of the three indicators. The only exception, in simulations, was the effect of gas sampling by the mass spectrometer. An identical lung volume was obtained for all three gases but this was in error. This error was very small:- only 6 ml for a simulated sampling rate of 100 ml per minute.

5.6 CONCLUSIONS

The specific objective attained in this study has been the validation of a method for measuring lung volume in normal subjects, without the procedural complexity of the conventional helium rebreathing technique. There are certainly arithmetical complexities but these are conveniently handled by the microcomputer. The method seems to be adequate for lung volume measurement in normal subjects. The various assumptions implicit within the method do not appear to significantly affect the results when considered in the context of normal lung volume variation. The method contains an internal check of consistency by the simultaneous use of three indicators. Large discrepancies between the indicator gases suggest the presence of errors without indicating what they are. The method is very flexible. It does not require any special gas mixtures, and will work the presence of nitrous oxide. The technique is being assessed for measuring FRC in patients with abnormal lungs. Some limitations of the method may become apparent in this context.

The primary disadvantage is the requirement for a mass spectrometer. This is expensive and requires a skilled operator. However, the clinical applications of the mass spectrometer have long been

appreciated (Fowler and Hugh-Jones, 1957). Many have reported the use of mass spectrometry for multi-patient monitoring (Severinghaus and Ozzane, 1978; Davies and Denison, 1979; Gothard et al, 1980; Gillbe et al, 1981; Ozzane et al, 1981a). When divided between several patients, the mass spectrometer can dispense with a number of single gas monitors. This study has shown that even in a single subject, there can be advantages of using mass spectrometery over conventional respiratory monitoring. Examination of the simultaneous concentration traces of several gases yields considerably more information than the sum of the information contained in the separate concentration traces. If the effect of CO_2 output early in rebreathing had been negligible, as Suwa and Bendixen (1971) had supposed, then the information from the O_2 trace would have sufficed for obtaining a value for lung volume. The lung volume yielded by the trace of a second gas (eg. N_2) would have been very similar and the information in that trace would have been redundant. Because the CO_2 output is not negligible, the information from the second gas is not redundant but allows a correction to be made for the CO_2 output. Similarly, if the system was error-free, the information supplied by a third gas would be redundant. As errors are inevitable, an "error monitor" is a very worthwhile feature. The advantages of an error monitor were appreciated by Ozzane et al (1981b) who used a simultaneous open circuit wash-in/wash-out technique for measuring lung volume and differences between the two estimates were taken to indicate a procedural error.

CHAPTER 6

Rebreathing Methods of Measuring Lung Volume

Theory and Comparison of Methods

6.1 INTRODUCTION

Chapter 1 described the historical background to the methods of lung volume measurement. Chapters 3, 4 and 5 described the empirical development of a new approach. In this Chapter, this new approach will be placed within the context of existing approaches, and an attempt will be made to compare results obtained by a number of different approaches.

6.2 MASS BALANCE AS APPLIED TO REBREATHING

The algebraical approach to lung volume measurement by rebreathing is based on accounting for the mass of gas present in the lung and rebreathing bag from the initial instant of the rebreathing manoeuvre to its termination.

It follows from the laws of Boyle, Gay-Lussac and Dalton that the mass of a species of gas in a mixture is proportional to the product of its fractional concentration and the total volume of the mixture. The constant of proportionality is $P_B/(R.T)$, where P_B is the barometric pressure, R is the gas constant and T is the absolute temperature. It is customary to deal with dry gas concentrations and therefore the dry barometric pressure. Where the algebra is applicable to any gas species, specific gas symbols will be omitted.

The amount of gas present initially in the rebreathing system (Bag: $V_{Bo}.F_{Bo}$, Lung: $V_{Lo}.F_{Lo}$) equals that present at the n^{th} breath (Bag: $V_{Bn}.F_{Bn}$, Lung: $V_{Ln}.F_{Ln}$) plus the total amount of gas removed by alveolar-capillary gas exchange (this quantity is negative if gas is added to the system). If V_{bi} is the uptake from the system in the i^{th} breath, $\sum_{n} V_{bi}$ will have been removed by breath n, at constant temperature and

barometric pressure:

$$\frac{P_B}{R.T}.\left(V_{Bo}.F_{Bo} + V_{Lo}.F_{Lo} = V_{Bn}.F_{Bn} + V_{Ln}.F_{Ln} + \sum^{n} V_{bi}\right) \qquad (6.1)$$

There are too many unknowns in this equation to attempt a solution to the general case. Simplifying assumptions must be made and restricting conditions must be imposed on the performance of the manoeuvre in order to solve for V_{Lo}.

6.2.1 In the absence of any Alveolar-Capillary Gas Exchange

A very great simplification is achieved by assuming no net alveolar capillary exchange for any gas. Thus, $\sum^{n} V_{bi}$ is zero and, therefore, equation 6.1 simplifies to:

$$V_{Bo}.F_{Bo} + V_{Lo}.F_{Lo} = V_{Bn}.F_{Bn} + V_{Ln}.F_{Ln} \qquad (6.1a)$$

and:

$$V_{Bn} + V_{Ln} = V_{Bo} + V_{Lo} \qquad (6.1b)$$

If also $V_{Ln} = V_{Lo}$ and $V_{Bn} = V_{Bo}$, then:

$$V_{Lo} = V_{Bo}.\frac{F_{Bo} - F_{Bn}}{F_{Ln} - F_{Lo}} \qquad (6.2)$$

Solving for V_{Lo} from equation 6.2 requires both F_{Bn} and F_{Ln} to be measured when V_{Ln} and V_{Lo} are equal. Such an approach could provide a solution for lung volume for any breath. However, in the first few breaths, inhomogeneities of alveolar mixing make F_{Ln} difficult or impossible to estimate from the end-tidal concentration. This is especially so if the inspired to expired concentration difference is large (Chapter 3, section 3.3.1). Similarly, F_{Bn} may not be a homogeneous concentration in certain spirometer circuits.

6.2.1a The advantages of attaining a Mixed Concentration

If measurement is delayed until $F_{Bn} = F_{Ln} = F_M$ equation 6.2 becomes:

$$V_{Bo}.F_{Bo} + V_{Lo}.F_{Lo} = (V_{Bn} + V_{Ln}).F_M \qquad (6.3)$$

Using equation 6.1b, equation 6.3 becomes:

$$V_{Lo} = V_{Bo} \cdot \frac{F_{Bo} - F_M}{F_M - F_{Lo}} \qquad (6.4)$$

A further simplification can be effected which eliminates the uncertainty as to the true F_{Lo}. This was first used by Davy (1800). If a "foreign" inert gas is used as the indicator, then $F_{Lo} = 0$ and, therefore, equation 6.4 becomes:

$$V_L = V_{Bo} \cdot (F_{Bo} - F_M)/F_M \qquad (6.5)$$

There are three advantages in using the mixed concentration, F_M, to calculate lung volume. Firstly, equation 6.3 is independent of the value of V_{Ln}, provided that: $(V_{Bn} + V_{Ln}) = (V_{Bo} + V_{Lo})$. Secondly, when F_M is attained there can be no inhomogeneities of mixing. Thirdly, only one concentration, F_M, need be measured after rebreathing and this may be determined from F_{Bn}. The conventional helium rebreathing technique with quiet breathing, CO_2 absorption and maintainance of a constant volume (see Meneeley and Kaltreider, 1949) is designed to fulfil the requirement of equation 6.5.

6.2.1b Effects of Errors of estimating F_M

Consider a constant volume technique in which volume is added to maintain $(V_{Bn} + V_{Ln})$ the same as $(V_{Bo} + V_{Lo})$. In practice, this volume adjustment must be made at end-expiration, with the assumption that the FRC remains constant at V_{Lo}. If however, FRC changes by an amount, V_{err}, there will be an error in the maintainance of a constant volume. This will result in the measurement of an apparent mixing concentration, aF_M, such that:

$$F_M \cdot (V_{Bo} + V_{Lo}) = aF_M \cdot (V_{Bo} + V_{Lo} + V_{err}) \qquad (6.6)$$

Substituting from equation 6.3 into 6.6 (using 6.1b):

$$V_{Bo} \cdot F_{Bo} + V_{Lo} \cdot F_{Lo} = aF_M \cdot (V_{Bo} + V_{Lo} + V_{err}) \qquad (6.7)$$

Solving for V_{Lo}:

$$V_{Lo} = \frac{V_{Bo} \cdot (F_{Bo} - aF_M) - V_{err} \cdot aF_M}{aF_M - F_{Lo}} \qquad (6.8)$$

Entering aF_M into equation 6.4, instead of F_M, would result in an apparent lung volume, aV_{Lo}, such that:

$$aV_{Lo} = V_{Bo} \cdot \frac{F_{Bo} - aF_M}{aF_M - F_{Lo}} \qquad (6.9)$$

Combining equation 6.8 and 6.9 and rearranging, shows that the error in lung volume measurement $(aV_{Lo} - V_{Lo})$ resulting from an apparent mixing concentration, aF_M, is (using equation 6.7)

$$aV_{Lo} - V_{Lo} = \frac{V_{err}}{1 - \frac{(1 + V_{Bo}/V_{Lo} + V_{err}/V_{Lo})}{(1 + F_{Bo}/F_{Lo} \cdot V_{Bo}/V_{Lo})}} \qquad (6.10)$$

This equation is formally identical to the equation in section 4.6.1 (Chapter 4), V_{err} equating to V_{miss} (See section 6.2.3). Equation 6.10 is a general equation such that any source of error in estimating F_M can be expressed as an effective V_{err} using equation 6.6. The effects of the ratios F_{Bo}/F_{Lo} and V_{Bo}/V_{Lo} on the expression of this error in terms of lung volume estimation will be as in equation 6.10.

6.2.2 Net Rebreathing Volume Change without Indicator Uptake or Output

These conditions are more realistic than the very simplified and restricted conditions considered above. The indicator gas is assumed to be insoluble and inert, however, the net uptake of O_2 in excess of CO_2 output will cause $(V_{Bn} + V_{Ln})$ to change with breath number. In all rebreathing methods, other than the "constant volume" techniques, there is a change in

system volume with breath number. In the quiet breathing techniques, a combination of prolonged rebreathing and CO_2 absorption from the spirometer cause an appreciable volume change. Consequently, a final mixed concentration (F_M) of the indicator gas is never attained. The inert gas concentration in the system will tend to change reciprocally with the change in volume. Nevertheless, for each breath, a mixed concentration F_{Mn} would be measurable. If the net volume change up to the n^{th} breath was measured, ($Vadd_n$) then F_{Mn} could be corrected to the true F_M in a way analogous to equation 6.6, $Vadd_n$ equates to the V_{err}.

$$F_M = F_{Mn} \cdot \frac{(V_{Bo} + V_{Lo} + Vadd_n)}{(V_{Bo} + V_{Lo})} \qquad (6.11)$$

Where $V_{Bo} + V_{Lo} + Vadd_n = V_{Bn} + V_{Ln}$

In forced breathing methods this volume change is either ignored (Davy, 1800; Lundsgaard and Van Slyke, 1918) or accounted for (Rauwerda, 1946 (cited by Rahn); and Rahn et al 1949). In quiet breathing techniques, the volume change must be measured and a correction, of the sort described by equation 6.11, performed (Anthony, 1930; Christie, 1932).

6.2.2a Measuring a mixed concentration

The above approach ignores a further error which arises from the fact that, in a system with volume changes in the lung ($R \neq 1$) and in the spirometer (CO_2 absorption), F_{Mn} does not remain equal to F_{Ln} or F_{Bn} in the steady state. Therefore, F_{Mn} cannot be measured as F_{Bn} as is assumed in all the techniques which measure a single "mixed" concentration.

Suppose that, at the end of the p^{th} expiration, the initial and subsequent conditions of the manoeuvre were such that $F_{Bp} = F_{Lp} = F_{Mp}$. The next inspiration would take in a volume of indicator gas, $V_{Ai} \cdot F_{Lp}$, into the alveolar space and subsequently expire a volume, $V_{Ae} \cdot F_{Lp+1}$ from the alveolar space.

Thus:

$$F_{Lp+1} = \frac{V_L + V_{Ai}}{V_L + V_{Ae}} \cdot F_{Lp} \qquad (6.12)$$

If $Vabs_p$ is the volume removed from the spirometer circuit in breath "p" (owing to CO_2 absorption plus the volume added (O_2 replenishment)), the resulting concentration of the indicator gas in the spirometer will be, F_{Bp+1}, where:

$$F_{Bp+1} = \frac{(V_{Bp} - V_{Ai}).F_{Bp} + V_{Ae}.F_{Lp+1}}{V_{Bp} - V_{Ai} + V_{Ae} - Vabs_p} \qquad (6.13)$$

Substituting for F_{Bp} from 6.12 (since $F_{Bp} = F_{Lp}$):

$$\frac{F_{Bp+1}}{F_{Lp+1}} = \frac{(V_{Bp} - V_{Ai}).\dfrac{V_L + V_{Ae}}{V_L + V_{Ai}} + V_{Ae}}{V_{Bp} - V_{Ai} + V_{Ae} - Vabs_p} \qquad (6.14)$$

Rearranging:

$$\frac{F_{Bp+1}}{F_{Lp+1}} = \frac{(V_L + V_{Ai}).V_{Bp} - (V_{Bp} + V_L).(V_{Ai} - V_{Ae})}{(V_L + V_{Ai}).V_{Bp} - (V_L + V_{Ai}).(V_{Ai} - V_{Ae} + Vabs_p)} \qquad (6.15)$$

Thus, even if $F_{Bp} = F_{Lp}$, F_{Bp+1} will not, in general, equal F_{Lp+1} except in the special conditions such that:

$$(V_{Bp} + V_L).(V_{Ai} - V_{Ae}) = (V_L + V_{Ai}).(V_{Ai} - V_{Ae} + Vabs_p) \qquad (6.16)$$

If the difference between the inspired and expired alveolar tidal ventilation ($V_{Ai} - V_{Ae}$) is due to O_2 uptake and CO_2 output ($V_{bp}O_2 - V_{bp}CO_2$), and $Vabs_p$ is equivalent to $V_{bp}CO_2$, then equation 6.16 can be written

$$(V_{Bp} + V_L).(1-R) = (V_L + V_{Ai})$$

Where R is the respiratory quotient, $V_{bp}CO_2/V_{bp}O_2$.

The size of the bag in relation to the lung at end-inspiration is clearly important in determining the size and direction of the inequality which develops between F_{Bp+1} and F_{Lp+1}. The implication of this was

discussed in Chapter 1 (section 1.4.3a(ii)) in relation to the manifestation of the "N_2 lag effect" (Lassen et al, 1937).

In the "constant volume" techniques the quantity: $V_{Ai} - V_{Ae} + Vabs_p$ (equation 6.13), is constrained to be zero so that V_B remains constant with breath number. Under these conditions it can be seen (equation 6.15) that if R<1 then F_{Lp+1} will always be greater than F_{Bp+1}. There is one situation where the the bag and lung concentrations would be equal, and remain so. If V_B were equal to V_{Ai} (ie. there were no dead space), the bag concentration (F_{Bp+1}) would be equal to F_{Lp+1} and remain so on subsequent breaths. This is best seen in equation 6.14. However, since the dead space is inevitably present such a situation can never arise as a stable state.

6.2.2b The double indicator approach

The methods, which involved measuring the volume change and using it to correct a measured mixing concentration as in equation 6.11, were preceded by a method involving two inert gases, initially H_2 and N_2 (Van Slyke and Binger, 1923). Equation 6.1 can be written for the two gases, with the uptake term $\sum^{n} V_{bi}$ assumed to be zero. By dividing one by the other:

$$\frac{V_{Bo}\cdot F_{Bo}N_2 + V_{Lo}\cdot F_{Lo}N_2}{V_{Bo}\cdot F_{Bo}H_2 + V_{Lo}\cdot F_{Lo}H_2} = \frac{V_{Bn}\cdot F_{Bn}N_2 + V_{Ln}\cdot F_{Ln}N_2}{V_{Bn}\cdot F_{Bn}H_2 + V_{Ln}\cdot F_{Ln}H_2} \qquad (6.17)$$

After a sufficient number of breaths a steady state will be produced. The initial difference between bag and lung concentrations, for N_2 and H_2, will have been abolished by convective mixing. This will have been replaced by a persistant difference due to the pulmonary gas exchange of O_2 and CO_2. For each gas, the bag concentration will be proportional to the lung concentration, the constant of proportionality for each breath being given

by the right hand side of equation 6.15. If this is designated k then:

$$\frac{V_{Bo}\cdot F_{Bo}N_2 + V_{Lo}\cdot F_{Lo}N_2}{V_{Bo}\cdot F_{Bo}H_2 + V_{Lo}\cdot F_{Lo}H_2} = \frac{(V_{Bn} + k.V_{Ln})\cdot F_{Bn}N_2}{(V_{Bn} + k.V_{Ln})\cdot F_{Bn}H_2} \qquad (6.18)$$

Solving for V_{Lo}:

$$V_{Lo} = V_{Bo}\cdot \frac{F_{Bo}H_2\cdot F_{Bn}N_2 - F_{Bn}H_2\cdot F_{Bo}N_2}{F_{Bn}H_2\cdot F_{Lo}N_2 - F_{Lo}H_2\cdot F_{Bn}N_2} \qquad (6.19)$$

The solution to equation 6.17 does <u>not</u> require the measurement of volume changes <u>nor</u> does it require the assumption that $F_{Bn} = F_{Mn}$, after sufficient mixing. This approach is, however, sensitive to the assumption that $\sum^{n} V_{bi}$ is zero for both H_2 and N_2 (Nunneley et al, 1974)

6.2.3 Solutions with Alveolar-Capillary Exchange of the Indicator

There are many applications of rebreathing techniques which are intended to study the exchange of the chosen indicator gas between the alveoli and the lung tissue or pulmonary capillary blood (eg. Cerretelli et al, 1970; Sackner et al, 1975; Peterson et al, 1978). For such approaches, the changing concentration of the indicator gas should be measured after mixing of the bag and lung contents has taken place or some allowance must be made for the mixing process (Adaro et al, 1973). The suggestion of Suwa and Bendixen (1971) for lung volume measurement by oxygen dilution was the obverse of these approaches. The uptake of oxygen, per se, does not provide any information concerning the lung volume, in fact it serves to obscure the quantity of real interest which is the mixed oxygen concentration before any pulmonary gas exchange has taken place. An extrapolation is used to account for the changes in the mixed concentration of oxygen and this allows a mixed concentration independent of pulmonary gas exchange to be calculated. If there were no carbon dioxide output and the oxygen uptake was constant from breath to breath (at a value of V_bO_2)

then equation 6.1 could be written:

$$V_{Bo}.F_{Bo}O_2 + V_{Lo}.F_{Lo}O_2 = V_{Bn}.F_{Bn}O_2 + V_{Ln}.F_{Ln}O_2 + N.V_bO_2 \qquad (6.19a)$$

Where "N" is the number of breaths. Dividing by $V_{Bn}+ V_{Ln}$, and substituting to give F_{Mn} from equation 6.3:

$$F_{Mn}O_2 = \frac{V_{Bo}.F_{Bo}O_2 + V_{Lo}.F_{Lo}O_2 - N.V_bO_2}{V_{Bn}+ V_{Ln}} \qquad (6.20)$$

(Substituting $V_{Bo}+ V_{Lo}- N.V_bO_2$ for $V_{Bn}+ V_{Ln}$:)

$$F_{Mn}O_2 = \frac{V_{Bo}.F_{Bo}O_2 + V_{Lo}.F_{Lo}O_2}{V_{Bo}+ V_{Lo}- N.V_bO_2} - \frac{N.V_bO_2}{V_{Bo}+ V_{Lo}- N.V_bO_2} \qquad (6.21)$$

If $N.V_bO_2$ is sufficiently small in relation to $V_{Bo}+ V_{Lo}$, this approximates to a linear function ($F_{Mo} = (V_{Bo}.F_{Bo} + V_{Lo}.F_{Lo})/(V_{Bo}+V_{Lo})$).

$$F_{Mn}O_2 \approx F_{Mo}O_2 - N.K \qquad (6.22)$$

F_{Mo} can be determined from linear extrapolation of the time course of F_{Mn}. Even in the situation with no CO_2 output, the defects in equation 6.22 were readily identified in an error free model (Chapter 4, section 4.6.2). They were not however identified in real life (Chapter 5, section 5.5.3). By contrast, the flawed assumption that the CO_2 output is negligible produced gross errors in the estimation of $F_{Mo}O_2$ by the extrapolation. This produced patterns of discrepancy observed in Chapter 3 which were as described by the general error equations 6.10 (Chapter 4, section 4.6.1). The V_{err} appropriate in this situation is shown in Figure 6.1. The figure plots the volume change in the bag/lung system against the breath number. The dotted line illustrates the situation where the change is due entirely to the constant O_2 uptake. An extrapolation of this time course of volume change intersects the time zero at the correct value for $V_{Bo}+ V_{Lo}$. The solid lines illustrate the effect of a superimposed CO_2 output which is initially appreciable but becomes negligible by breath 9. A line drawn from breath 15 to 9 and extrapolated to time zero gives an initial volume

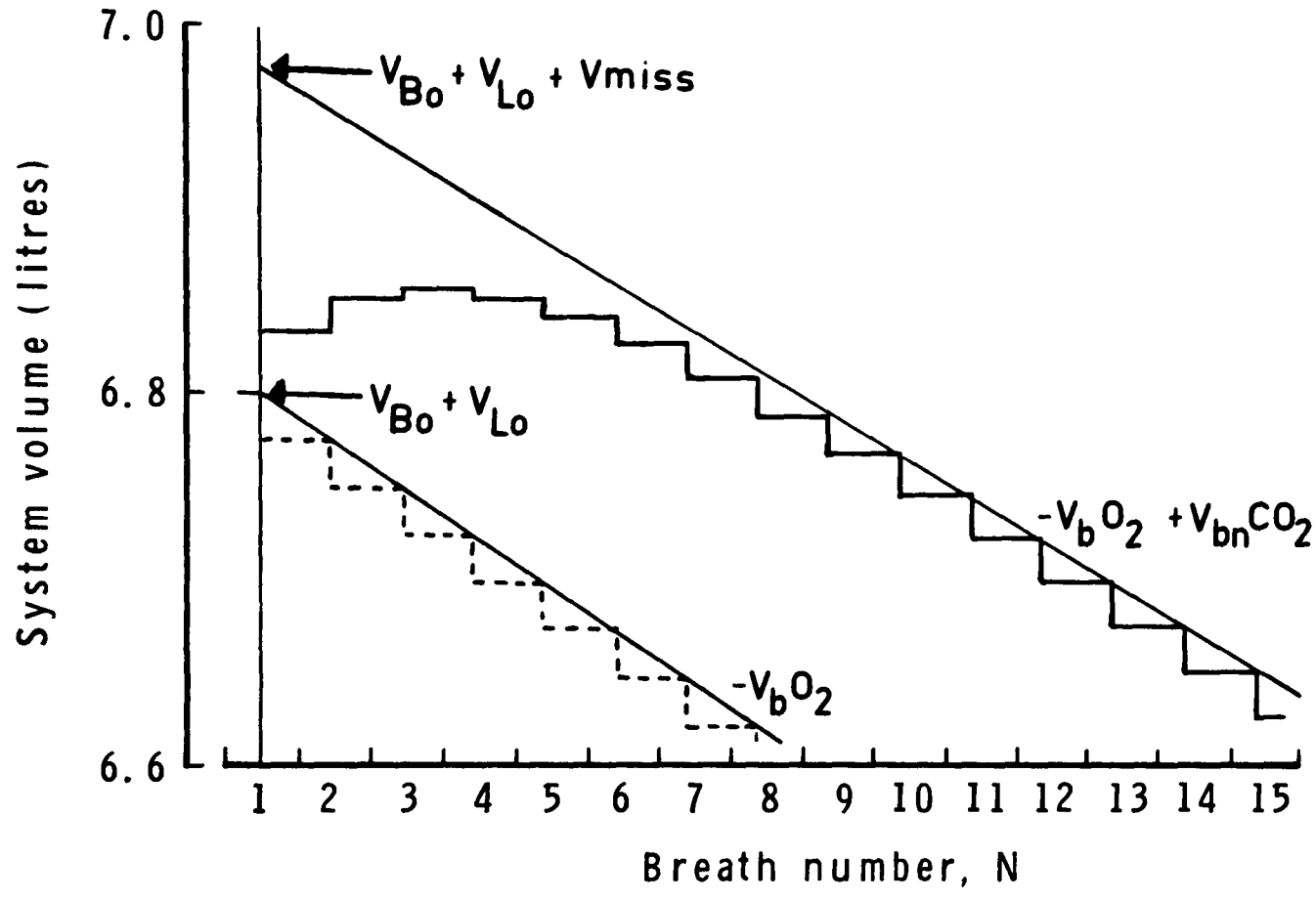


Figure 6.1 The volume change, per breath, in a bag/lung system. The dotted "stepped" line illustrates a situation where the volume change is due to oxygen uptake, while the continuous "stepped" line illustrates the case for an oxygen uptake and a non-sustained carbon dioxide output. See text for details.

which exceeds $V_{Bo} + V_{Lo}$ by an amount approximately equal to the total CO_2 output of breaths 1 to 9. This error, Vmiss, may be less than the CO_2 output to the extent that the extrapolation is influenced by the small CO_2 output later than breath 9 (Chapter 4, section 4.6.1).

A correction technique was developed in Chapter 4 (section 4.2.2b) which eliminated the effect of this CO_2 and still enabled an extrapolation to be used to account for the oxygen uptake. This correction technique is analogous, under certain circumstances, to that described in equation 6.19. Recall equation 4.6 (Chapter 4), which can be written in terms of real concentrations divided by the normalising factors (Bo, Mo and Lo). The factors are the sum of the concentrations of indicator gases in the bag:- Bo, mixed:- Mo and Lung:- Lo.

$$V_{Lo} = V_{Bo} \cdot \frac{Bo.(F_{Bo}H_2/Bo - F_{Mo}H_2/Mo)}{Lo.(F_{Mo}H_2/Mo - F_{Lo}H_2/Lo)} \qquad (6.23)$$

Rearranging:

$$V_{Lo} = V_{Bo} \cdot \frac{(F_{Bo}H_2.Mo - F_{Mo}H_2.Bo)}{(F_{Mo}H_2.Lo - F_{Lo}H_2.Mo)}$$

In a two gas system (eg $Mo = F_{Mo}H_2 + F_{Mo}N_2$) and with no alveolar-capillary gas exchange (ie. $F_{Mo} = F_{Mn}$) then equation 6.23 is identical to equation 6.19, given that $F_{Mn} = k.F_{Bn}$ (so that eg $Mo = k.(F_{Bn}H_2 + F_{Bn}N_2)$). This last equality can readily be proved. Since by definition:

$$F_{Mn} = \frac{V_{Bn}.F_{Bn} + V_{Ln}.F_{Ln}}{V_{Bn} + V_{Ln}} \qquad (6.24)$$

From equation 6.15, F_{Bn} is proportional to F_{Ln} so that from equation 6.24 it follows that F_{Mn} is proportional to F_{Bn} (after sufficient mixing). Therefore, when the two above conditions are satisfied (two gas system, and no gas exchange) equations 6.23 and 6.19 are the same. It is appropriate to examine the performance of the two approaches represented by equations

6.19 and 6.23 under conditions where they are not the same.

6.2.4 Equation 6.19 versus Equation 6.23

There are two circumstances which make using equation 6.23 more advantageous than equation 6.19. The first is in the presence of inert gas exchange. In the presence of inert gas exchange equation 6.19 becomes.

$$V_{Lo} = V_{Bo} \cdot \frac{F_{Bo}H_2 \cdot F_{Bn}N_2 - F_{Bn}H_2 \cdot F_{Bo}N_2 + X}{F_{Bn}H_2 \cdot F_{Lo}N_2 - F_{Lo}H_2 \cdot F_{Bn}N_2} \qquad (6.25)$$

$$\text{Where: } X = F_{Bn}Ar \cdot \sum^{n}(V_{bi}N_2) - F_{Bn}N_2 \cdot \sum^{n}(V_{bi}Ar)$$

Suppose that the concentration gradients for Ar and N_2, between the bag and the lung, are initially opposite (as is usually the case). The resulting pulmonary gas exchanges would both have an additive effect on X in equation 6.25. In equation 6.~~19~~23, however, F_{Mo} is calculated by extrapolation and this will take account of these gas exchanges to the extent that they cause linear changes in concentration.

The second advantage is that using an extrapolation affords information from a third gas bound always to be present, O_2. A two gas correction, using equation 6.23 or 6.19, will always produce a unique solution, which will compound error of measurement and assumption. Three gases provides a check on error of measurement or assumption (Chapter 5, section 5.5.4).

6.3 COMPARISON OF ALTERNATIVE APPROACHES TO LUNG VOLUME MEASUREMENT

6.3.1 Introduction

Working with a very early modification of the Suwa and Bendixen (1971) approach, ten FRC measurements were found to be indistinguishable from a further 10 using the conventional helium dilution technique of Meneeley and Kaltreider (1949). The experimental work presented in Chapter 5 subsequently highlighted the difficulty of demonstrating differences in

lung volume, between manoeuvres, in the face of large performance variations. The implication was that comparisons between measurement techniques would ideally be made within the same manoeuvre. In the previous two sections of this Chapter the theoretical background to lung volume measurement is discussed. A number of different approaches are described each containing its own set of assumptions. It is appropriate to attempt to compare the approaches (including that developed in the previous two Chapters) in real and simulated rebreathing manoeuvres. Such comparisons present a problem, since each technique imposes its own requirements on the performance of the rebreathing manoeuvre. The comparisons are, therefore, made during three types of rebreathing manoeuvre.

1. Forced breathing, no CO_2 absorption.
2. Quiet breathing, CO_2 absorption, no volume adjustment.
3. Quiet breathing, CO_2 absorption and constant volume.

The comparisons were first made in an error free computer simulation of the sort described in Chapter 5. They were then attempted in real manoeuvres performed on a single subject.

6.3.2 Equations used in the comparisons

For all real manoeuvres, attempts were made to ensure that the initial lung volume was maintained, at least for the moments at which the measurements were taken.

6.3.2a The Bag-Lung equation

With constant lung volume equation 6.1 becomes:

$$V_L = \frac{V_{Bo} \cdot F_{Bo} - V_{Bn} \cdot F_{Bn}}{F_{Ln} - F_{Lo}} \qquad \text{---"B-L"}$$

This was denoted the "B-L" equation, since it relied on bag and lung concentration data. The solution also requires the measurement of the

change in spirometer volume in order to calculate V_{Bn}, which assumes the lung volume does not change during the manoeuvre.

6.3.2b The Bag only equation

If mixing is assumed to be sufficient to make $F_{Bn} = F_{Ln}$, then the "B-L" equation becomes:

$$V_L = \frac{V_{Bo} \cdot F_{Bo} - V_{Bn} \cdot F_{Bn}}{F_{Bn} - F_{Lo}} \qquad \text{---"B"}$$

This was called the "B" equation, since it only requires the measurement of bag concentrations.

6.3.2c The double indicator equation

This equation was described earlier (equation 6.19). In this case Ar replaces He:

$$V_{Lo} = V_{Bo} \cdot \frac{F_{Bo}Ar \cdot F_{Bn}N_2 - F_{Bn}Ar \cdot F_{Bo}N_2}{F_{Bn}Ar \cdot F_{Lo}N_2 - F_{Bn}N_2 \cdot F_{Lo}Ar} \qquad \text{---"B/B"}$$

This was known as the "B/B" equation, since it relied on bag concentration data from two inert gases.

6.3.2d The transformation and extrapolation

From equation 6.23, where $F'_{Bo} = F_{Bo}/Bo$, and F'_{Mo} and F'_{Lo} are similarly defined:

$$V_{Lo} = V_{Bo} \cdot \frac{Bo.(F'_{Bo} - F'_{Mo})}{Lo.(F'_{Mo} - F'_{Lo})} \qquad \text{---"TE"}$$

This was known as the "TE" equation, since it relied on a transformation of the real concentrations followed by an extrapolation to calculate F'_{Mo}.

For the rebreathing manoeuvres with the maintenance of constant volume "B-L" and "B" were simplified, since $V_{Bo} = V_{Bn}$. The "B/B" and "TE"

equations did not require knowledge of volume change.

6.3.3 METHODS

6.3.3a Computer simulation

The computer simulation described in Chapter 5 was modified in order to imitate the conditions planned for the real rebreathing manoeuvres. A lung volume of 5.5 litres was chosen for all simulations. This appeared to be appropriate to the real subject's FRC. The initial bag composition, for all manoeuvres, was chosen to be 40% v/v O_2, 30% v/v N_2 and 30% v/v Ar and the lung concentration was 15% v/v O_2, 80% v/v N_2 and 5% v/v CO_2. These were in the range of the compositions used in the real manoeuvres. The respiratory rate was 10 per minute throughout.

The initial volume of the spirometer was 5.5 litres for forced breathing and 6.5 for the quiet breathing. The alveolar minute ventilation was 2 5 litres per minute for forced breathing simulations and 5 litres per minute for quiet breathing.

The oxygen uptake was constant at 300 ml per minute in all manoeuvres. For forced breathing, the carbon dioxide output was chosen to be 60 ml in the first breath declining by a factor of 0.8 in subsequent breaths. In quiet breathing, the CO_2 output was 240 ml per minute. The inert gases, N_2 and Ar, were assumed not to take part in alveolar-capillary gas exchange.

6.3.3b Experiments with a real subject

6.3.3b(i) Spirometer circuit

The spirometer circuit (P.K. Morgan resparameter MK 4, Chatham, Kent) incorporated a removable carbon dioxide absorber, a circulating fan and a spirometer volume writeout facility. The standard metal tap, normally used to start rebreathing, was replaced with the remotely operated rebreathing switch (Chapter 5, section 5.2.4).

6.3.3b(ii) Gas analysis

Although helium is part of the accepted standard for rebreathing lung volume determination (Meneeley and Kaltreider, 1949), its use was not compitable with the continuous and simultanoeus measurement of at least one other gas (as required by the "B/B" and "TE" approaches). Accurate detection of helium by the mass spectrometer proved difficult. Argon was used to replace helium without altering the principles of the above equations.

Helium was used for a preliminary determination of spirometer dead space, with and without a CO_2 absorber. This was accomplished using a Katharometer (Cambridge Instruments Co., Cambridge) and serial helium dilution, as described by Cotes (1965). The katharometer had the advantage, over the mass spectrometer, of not depleting the spirometer gas volume.

6.3.3b(iii) The effect of mass spectrometer sampling

The effect of the mass spectrometer sampling was to deplete the system volume. If the volume had been removed from the bag and lung before mixing, it would not have affected the final mixed concentration provided the volumes removed were in proportion to their respective volumes. Therefore, if the mass spectrometer was sampling from the junction of a homogeneous lung and homogeneous bag (at a constant rate), the above condition would be satisfied if the time taken for inspiration and expiration was in proportion to the bag and lung volumes respectively. Once the bag and lung contents are mixed further sampling does not affect the composition. Also, the sampling artifact becomes smaller as the rebreathing system becomes larger. Results obtained from the computer simulation, with the inclusion of a mass spectrometer sampling, suggest that the measurements of the change in spirometer volume must be corrected so as not to include the volume removed by the mass spectrometer. When this correction is made, a

mass spectrometer sampling rate of 100 ml/min does not materially affect the calculated lung volume by any of the methods used in this study. Any artifact was minimised by having the subject and spirometer volumes similar and employing a reasonably constant and equal inspiratory and expiratory period.

6.3.3(iv) Preparation for rebreathing

The spirometer dead space was first flushed several times with room air. During forced breathing, approximately 1.5 litres of dry oxygen and 1.5 litres of dry argon were added, the exact amount being read later from the kymograph tracing. The contents of the spirometer were then mixed using the fan. The initial composition was approximately 36% v/v O_2, 36% v/v N_2 and 28% v/v Ar. The exact compositions were measured as the F_{Bo} during the first breath.

For the quiet breathing manoeuvres, approximately 2 L of O_2 and 2 L of Ar were added. This gave an initial spirometer concentration of approximately 38% v/v O_2, 28% v/v N_2 and 34% v/v Ar. The larger spirometer volume during quiet breathing was to allow for oxygen uptake.

The actual dry spirometer dead space (V_D) for each manoeuvre was calculated from the dilution of O_2, N_2 and Ar in the initial spirometer volume.

$$V_D = \frac{Vgas - F_{Bo}.Vtotal}{-F_D + F_{Bo}}$$

Where: Vgas is the volume of the particular gas added to the spirometer and Vtotal is the total volume added. F_D is the initial dry gas composition of the spirometer dead space (assumed to be the same as air). F_{Bo} is the concentration of the spirometer recorded during the first breath.

6.3.3b(v) Performance of rebreathing manoeuvres

The subject was seated at the spirometer breathing through the mouthpiece, with a noseclip in place. Once the subject had settled, the computer programme was started. The initiation of rebreathing and the collection of end-expired and end-inspired data were computer controlled, as described in Chapter 5.

FORCED BREATHING:

When the valve was opened, the subject was encouraged to breathe deeply and regularly as in Chapters 3. The output of the mass spectrometer was displayed on a four channel potentiometric recorder (Linsies, series LS 4), which allowed the operator to assess when sufficient data had been collected. This took about 90 seconds. The subject then breathed quietly for about five to ten breaths in an attempt to reproduce his original FRC, while the volume change was recorded on the spirometer writeout. At the end of the manoeuvre the concentration data were stored on "floppy disc", for later analysis. This manoeuvre was successfully completed four times.

QUIET BREATHING- DIMINISHING VOLUME:

The subject attempted to maintain the same breathing pattern before and during rebreathing. A soda lime cannister was placed inside the spirometer, in order to absorb the expired CO_2. This was found to maintain the inspired CO_2 at less than 0.5% v/v during rebreathing. The reduction in the system volume was recored on the spirometer. This was an indication of the subject's O_2 uptake. Rebreathing continued for five minutes (with the circulating fan running). Four manoeuvres were sucessfully completed.

QUIET BREATHING:- CONSTANT VOLUME

The experimental procedure was the same as for diminishing volume except that the system volume was maintained by replacing the subjects oxygen uptake (calculated above) with an in-flow of O_2. This rate was adjusted, after the lung and spirometer had equilibrated, in an attempt to

maintain a constant inspired oxygen concentration. The volume of the spirometer system continued to decline because of the mass spectrometer sampling. Thus, maintainance of an end-expiratory spirometer volume was inapplicable. Two manoeuvres were successfully completed.

6.3.4 Results and Discussion of Simulations

These results are presented in Table 6.1 and will be discussed in terms of the four equations in subsections 6.3.4a to 6.3.4d.

6.3.4a The bag lung equation, "B/L"

Since the equation was supplied with the correct information for each breath, it calculated the correct lung volume for each breath irrespective of the indicator and rebreathing manoeuvre.

6.3.4b The bag only equation, "B"

This equation produced an incorrect estimate of lung volume. The estimates for N_2 and Ar straddled the correct volume. The discrepancy between N_2 and Ar determinations was smaller with forced breathing than with quiet breathing (with constant volume), although they were in the same direction (the Ar dilution volume was greater). With a constant volume technique, these discrepancies were reduced by doubling the rebreathing bag volume. When quiet breathing (with a diminishing volume) was used a discrepancy persisted but with reversed sign (the N_2 dilution volume was greater). This was again reduced with a larger bag volume. The above results discrepancies are due to the fact that $F_{Bn} \neq F_{Ln}$, and, therefore, $F_{Mn} \neq F_{Bn}$, as described in section 6.2.2a.

6.3.4c The double indicator equation, "B/B"

Incorrect values were obtained for the early breaths until a situation where $F_{Ln}N_2/F_{Bn}N_2$ was equal to $F_{Ln}Ar/F_{Bn}Ar$ (section 6.2.2b).

	Initial spirometer volume	Volume change	CALCULATED LUNG VOLUME (true lung volume 5.5 litres) "B-L N2"	"B-L Ar"	"B N2"	"B Ar"	"B/B"	"TE" O_2	"TE" N_2	"TE" Ar
FORCED BREATHING	5.5	$-O_2$ uptake $+CO_2$ output	5.50	5.50	5.46	5.52	5.50	5.47	5.49	5.51
					Rauwerda (1946)		Nunneley et al (1974)			
QUIET BREATHING	6.5	$-O_2$ uptake	5.50	5.50	5.90	5.38	5.50	5.50	5.50	5.50
	13.0		5.50	5.50	5.56	5.44	5.50	5.50	5.50	5.50
			Lassen al (1937)	Anthony (1938)	Christie (1932)	Anthony (1930)	Van Slyke and Binger (1923)			
QUIET BREATHING	6.5	nil	5.50	5.50	5.37	5.57	5.50	5.50	5.50	5.50
	13.0		5.50	5.50	5.40	5.55	5.50	5.50	5.50	5.50
					Herrald & McMichael (1939)	McMicheal (1939)	Lewis (1971)			

Table 6.1 Results of the model simulation. The simulation was performed under different rebreathing conditions. These conditions are summarised in the first three columns. The rest of the columns show the lung volume (litres) as calculated from 4 different equation ("B-L", "B", "B/B" and "TE"). Where possible the results of two or three indicator gases are given. The references indicate the authors who first measured lung volume using techniques which were, in principle, the same as those used here.

Thereafter, the correct values were obtained for every breath and these values are shown in the table.

6.3.4d The transformation and extrapolation, "TE"

The"TE" procedure for calculating lung volume was evaluated in relation to forced breathing in Chapter 5. There it was found, using a linear regression, that the lung volume as estimated by any of the three gases within a single manoeuvre was less than 50 ml from true lung volume. Quiet breathing methods impose a considerable delay before mixing, which increases the error in identifying the true mixing line and extrapolating correctly from it. This error is the result of two factors.

IDENTIFYING THE TRUE MIXING LINE:

For each breath, the F_{Mn} is given by equation 6.24, this is illustrated in Figure 6.2. F_{Mn} is the weighted average of F_{Bn} and F_{Ln} at end expiration, such that:

$$F_{Mn} = A.F_{Bn} + B.F_{Ln} \qquad (6.26)$$

Where: $A = V_{Bn}/(V_{Bn} + V_{Ln})$ and $B = V_{Ln}/(V_{Bn} + V_{Ln})$, and thus, $A + B = 1$. In practice the values of V_{Bn} and V_{Ln} are unknown and, therefore, the weighting factors "A" and "B" need to be determined empirically. For forced breathing, F_B and F_L are so close that there is not much scope for error in placing F_{Mn} between them.

CURVATURE OF THE MIXING LINE:

This was discussed in Chapter 4 (section 4.6.2) and in relation to equation 6.22 (section 6.2.3). Over the short period of a forced breathing manoeuvre the curvature of the mixing line produced only small errors in an otherwise error-free system. Over the longer period of quiet breathing, such errors are considerably magnified.

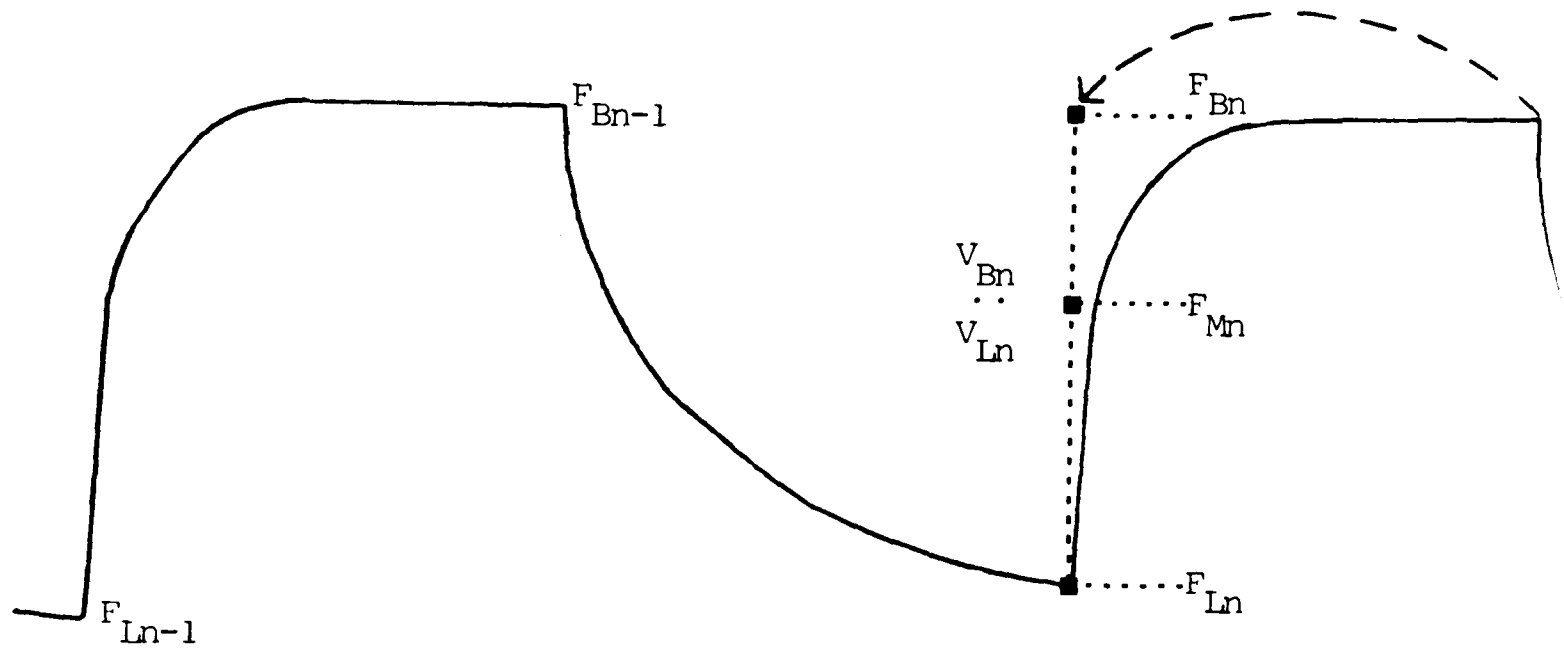


Figure 6.2 The position of the mixed concentration (F_{Mn}) in relation to disparate bag (F_{Bn}) and lung (F_{Ln}) concentrations.

The effects of the two errors described above were overcome by the following empirical procedure described in relation to Figure 6.3. For breaths 30 to 50 of a simulated quiet rebreathing manoeuvre (with diminishing volume) a line was fitted to the reciprocal of the bag concentrations for O_2, N_2 and Ar. These lines were extrapolated to zero time to give a value which was used to calculate lung volume for the three indicators. This was equivalent to using a weighting factor, in equation 6.26, of A=1 and, therefore, B=0. Figure 6.3 shows the value of A on the x-axis and the apparent lung volume produced by this weighting on the y-axis for the three gases.

The procedure was repeated for values of A decreasing from 1 to 0 in steps of 0.1. The points representing the apparent lung volume for O_2, N_2 and Ar at these values of a form three lines which intersect at a single point. If the value of A at that point is taken a unique lung volume can be calculated. This procedure works for the constant volume simulations.

6.3.4e Computer simulations with gas exchange

Subsiduary model simulations with constant alveolar-capillary inert gas exchanges confirm that these have a negligible effect on the "TE" procedure, whilst having a marked effect on the "B/B" calculation. In Chapter 4 (section 4.6.4), the effect of a more realistic pulmonary inert gas exchange profile was assessed in terms of producing errors in lung volume determination by the "TE" equation. The profile described produced a less than 50 ml error in all the indicators. By calculating the lung volume by the "B/B" equation in the same manoeuvre, a 200 ml overestimate of lung volume was produced. This was confirmed by using the total inert gas uptakes and outputs in equation 6.24. This illustrates the benefit of the extrapolation in reducing the sensitivity to inert gas exchanges.

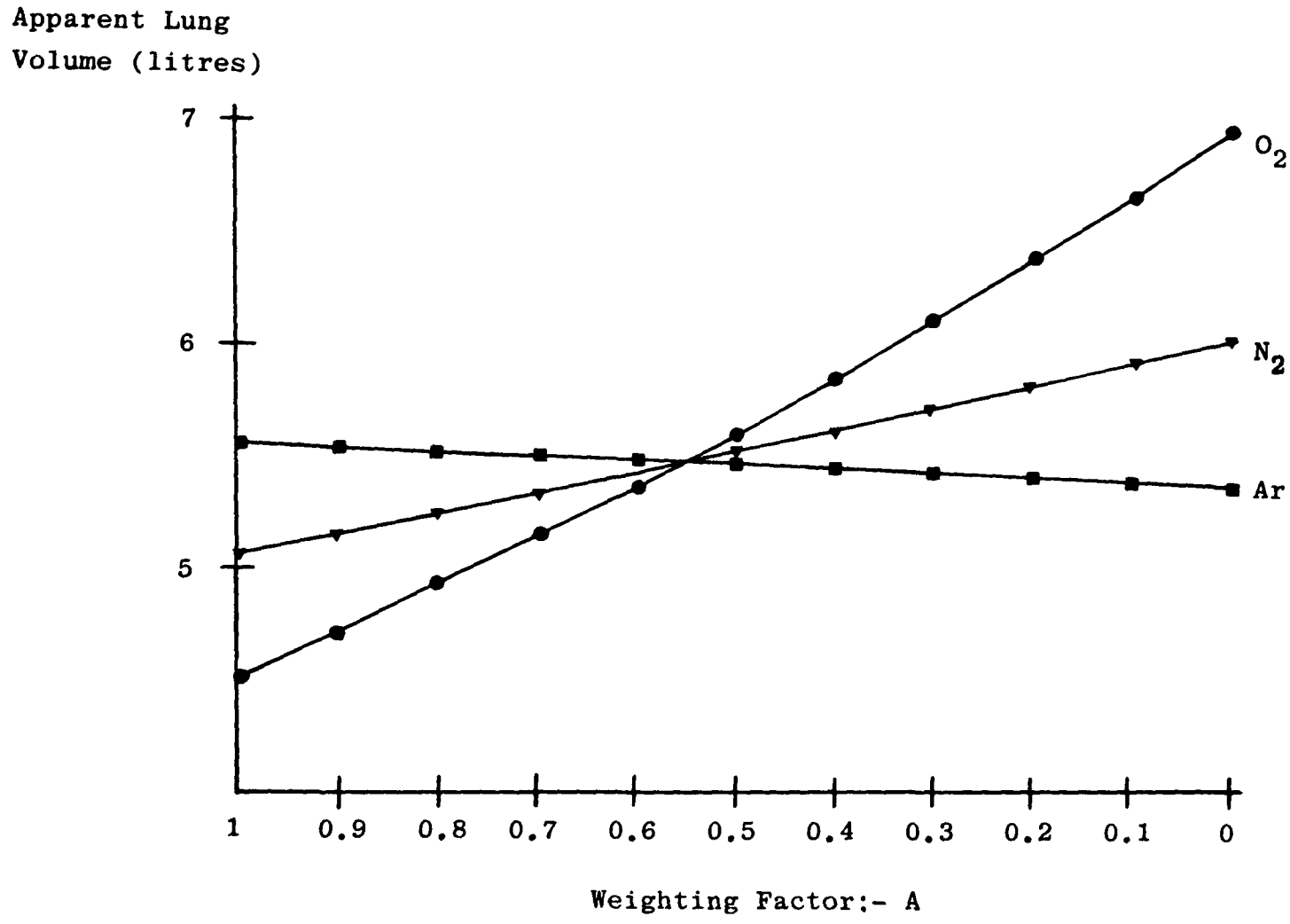


Figure 6.3 Results of a computer simulation, which shows the apparent lung volumes for oxygen, nitrogen and argon over a range of values of "A". "A" is a weighting factor (see text) which defines the position of the mixing concentration between the bag and the lung concentrations (see Figure 6.2). There is a value of "A" when all the apparent lung volume are equal.

6.3.5 Results of real manoeuvres

Data on volume changes were added to the stored concentration data and this allowed the computation of V_L using the same equations as were used for the simulations. These are shown in Table 6.2 and this will be described column by column.

FIRST COLUMN:

This shows average dead space as calculated from O_2, N_2 and Ar dilution. By serial helium dilution, this volume (dry) was 2.72 L $\pm$ 50 ml without a CO_2 absorber and 2.36 L $\pm$ 50 ml with a CO_2 absorber. The CO_2 absorber displaces a volume of gas within the spirometer.

SECOND COLUMN:

This indicates the calculated change in system volume during rebreathing. For forced breathing this is difficult to estimate, as is witnessed by the variability in this measurement between the four manoeuvres.

THIRD AND FOURTH COLUMNS:

These show the application of the "B-L" equation. For forced breathing there is 660 ml difference between N_2 and Ar determinations (the N_2 dilution volume was greater). For quiet breathing (diminishing volume), this is even larger, 790 ml (the Ar dilution volume was greater). With constant volume, this discrepancy is 350 ml (the Ar dilution volume was greater).

FIFTH AND SIXTH COLUMNS:

These show the results of "B" equation. During forced rebreathing, a 740 ml discrepancy is apparent between the N_2 and Ar dilution (N_2 greater). For quiet breathing (diminishing volume), the two estimates are identical. With constant volume, there is a 660 ml discrepancy (Ar greater).

SEVENTH COLUMN:

This shows the results of the "B/B" equation and, therefore, only a single estimate is produced.

	Spirometer dead space	Decrease in system volume	CALCULATED LUNG VOLUMES "B-L N_2"	"B-L AR"	"B N_2"	"B Ar"	"B/B"	"TE" O_2	"TE" N_2	"TE" Ar
FORCED BREATHING	2.55	0	6.30	5.54	6.61	5.58	5.85	5.63	5.49	5.40
	2.52	0.5	5.52	5.12	5.77	5.19	5.35	5.41	5.16	4.98
	2.51	0.4	5.18	5.06	5.66	5.19	5.32	5.25	5.09	4.98
	2.56	0.1	6.37	5.61	6.54	5.68	5.90	6.08	5.69	5.40
			$\bar{X}$ 5.84	5.33	6.15	5.41	5.61	5.59	5.36	5.19
			SD (0.59)	(0.28)	(0.50)	(0.26)	(0.29)	(0.36)	(0.28)	(0.24)
QUIET BREATHING volume shrinkage	2.23	1.88	5.31	6.27	5.85	6.01	5.98	5.27	5.29	5.25
	2.24	1.91	5.70	6.53	6.29	6.27	6.27	5.65	5.70	5.67
	2.23	1.75	5.51	6.05	6.08	5.77	5.84	5.27	5.26	5.25
	2.14	1.63	5.43	6.29	5.86	6.02	5.98	5.38	5.36	5.40
			$\bar{X}$ 5.49	6.28	6.02	6.02	6.02	5.39	5.40	5.59
			SD (0.16)	(0.20)	(0.21)	(0.20)	(0.18)	(0.18)	(0.20)	(0.20)
QUIET BREATHING constant volume	2.07	0.0	5.86	6.07	5.61	6.17	5.96	5.43	5.45	5.44
	1.93	0.0	5.90	6.39	5.66	6.42	6.18	5.20	5.19	5.19
			$\bar{X}$ 5.88	6.23	5.64	6.30	6.07	5.32	5.32	5.32

Table 6.2 Results of a single subject for three types of rebreathing manoeuvre, shown up the left hand side. All values are in litres. The calculated lung volumes are presented in the same format as in Table 6.1. The means for repeated observations are shown ($\bar{X}$), along with the standard deviation (SD), if there are more than 2 repetitions.

LAST THREE COLUMNS:

These are the results of the "TE" equation and, therefore, a third indicator gas is present. During forced breathing, the discrepancies between the three indicators are much larger than observed in Chapter 5. However, in these experiments the equipment is different. During both quiet breathing techniques the use of the optimal weighting factor reduces the "between-indicator" discrepancy to a minimum.

6.3.6 DISCUSSION

6.3.6a Alveolar-capillary inert gas exchange

The concentration gradient for inert gas exchange in all the above experiments was about 30% v/v for both N_2 and Ar. Hill and Cambell's (1931) results indicate that such a concentration gradient would cause a N_2 output of at least 65 ml in five minutes. Since Ar is 2.1 times as soluble as nitrogen, a similar, but opposite, concentration gradient for argon would cause an uptake of 130 ml. For forced breathing, the time of rebreathing is shorter but the cardiac output is probably raised as a result of the hyperventilation. Arbritary figures of 50 ml for N_2 and 100 ml for Ar were assumed.

Table 6.3 indicates the mean values from Table 6.2 after a correction for the above pulmonary gas exchanges. The effect of accounting for gas exchange is to reduce all estimates of lung volume. This does not remove discrepancies and in many cases makes them worse

6.3.6b Comparing V_{Lo} estimates in the same manoeuvre

This comparison is made along the rows of Table 6.2, for the forced and then quiet breathing manoeuvres.

6.3.6b(i) Forced breathing

The transformation and extrapolation technique ("TE") failed to

	CALCULATED LUNG VOLUME (corrected for pulmonary gas exchange)				
	"B-L N2"	"B-L Ar"	"B N2"	"B Ar"	"B/B"
FORCED BREATHING	5.57	4.60	5.88	4.67	5.00
QUIET BREATHING volume shrinkage	5.15	5.57	5.64	5.34	5.44
QUIET BREATHING constant volume	5.63	5.29	5.33	5.37	5.36

Table 6.3 This table shows the effect of allowing for pulmonary inert gas exchange on mean lung volumes calculated Table 6.2. The values choosen for these exchanges are discussed in the text (section 6.3.6a). Such allowances are only made in the "B-L", "B" and "B/B" equations, since for the "TE" approach the profile, and not just the total amount, of the gas exchange is important.

produce a common lung volume for the three gases, the residual difference being extremely large in some manoeuvres. These discrepancies in the past (Chapter 5, section 5.5.4) have suggested the presence of errors of measurement or assumption. The tidal volumes were large and it is possible that not enough time was available for all the gas throughout the spirometer to be mixed. The end-inspiratory spirometer concentration may not have been the mean inspired concentration. This would be analogous to a serial inhomogeniety of the lung (Chapter 4, Figure 4.11).

Discrepancies within the same manoeuvre are evident in the "B-L" and "B" techniques. These may be accounted for as above or there may be an additional source. Attempting to measure (by spirometry) the change in system volume during a forced breathing manoeuvre is likely to be unreliable. It relies upon being able to reproduce FRC at the end of a manoeuvre. Most subjects have difficulty in returning to spontaneous quiet respirations after a period of forced hyperventilation. This is particularly true when the level of CO_2 is high since this itself may cause changes in the end-expiratory position (Green, 1933). The variation between the estimated decrease in system volume (Table 6.2) probably reflects these uncertainties.

6.3.6b(ii) Quiet breathing

The "TE" discrepancies were minimal but they were the result of an empirical technique (weighting) which may compound real differences. In the "B-L" and "B" techniques, errors may be due to estimation of the volume change or the presence of gas exchange.

During rebreathing, heating and humidification of the spirometer takes place. Thus, the temperature and water vapour saturation of the spirometer must be established before and after rebreathing, or the rate of change of spirometer volume must be established during the manoeuvre when the volume changes due to heating and humidification, have stopped. The

latter method was employed in these experiments. A 100 ml under-estimation in the O_2 consumption would lead to a 400 ml difference between "B-L N_2" and "B-L Ar" estimates, in the direction observed. Similarly, an increase in lung volume at the start of rebreathing (of the same size) would have a similar effect. Therefore, techniques, which rely on volume measurement, are subject to more errors of measurement than techniques not requiring volume measurement.

The gas exchanges shown in Table 6.3 do not seem to help to explain the discrepancies.

6.4 CONCLUSIONS

The model simulations confirm the impressions gained from inspection of the theory:

1. Techniques, which only measure the bag concentrations, were found to be in error as a result of net volume changes in the lung and spirometer. This effect is modulated by many variables including the type of rebreathing manoeuvre and the system volume. However, the conventional constant volume techniques will suffer from this error.
2. The above error is overcome by using bag and lung data which effectively determines a mixed concentration.
3. The double indicator correction is shown to overcome this error, in determining a mixed concentration, by using two indicator gases. The need to measure volume change is also overcome.
4. The technique developed in this thesis is shown to be similar to the double indicator technique under certain ideal circumstances. In more realistic circumstances, it benefits by being less sensitive to pulmonary gas exchange of the indicator gases and also provides an internal check of consistency. When applied in quiet breathing techniques, a further empirical correction must be made in order to

determine the true mixing concentrations.

In terms of comparing techniques in real manoeuvres, this was not successful. In Chapter 5, the large inherent variability between lung volume estimates was shown to make between-manoeuvre comparisons difficult. This Chapter has shown one problem involved in making comparisons within a manoeuvre, which is that if in order to make the comparison the technique is modified, this comparison is no longer concerned with the original technique. The "TE" equation produced lower estimates of lung volume than the other techniques (Table 6.2). However, the effect of pulmonary inert gas exchange was to cause an overestimate of lung volume by these other techniques, whilst the "TE" technique would not be so greatly affected. Conclusions concerning the comparisons can only be made with extreme caution because of the paucity of data and large discrepancies between all techniques. The attempted comparison has served to shown the sensitivity of other techniques to a number of assumptions. The "TE" technique, at least in forced breathing, has been shown to provide an internal check of consistancy (Chapter 5, section 5.5.4). When similar checks were attempted in other the techniques, they failed to demonstrate internal consistancy. The use of two indicators for this purpose would seem to be a useful addition in other techniques.

The problems of adapting the "TE" technique to a quiet breathing approach have been explored. The practical and computational modifications, which were required to apply the "TE" technique during quiet breathing, may prove useful in further studies on patients, for whom forced breathing is not possible.

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Calculations for split-plot anova

PHASE III

The results from composition changes with N_2O were a complete set. Each of the 4 composition changes was successfully repeated 5 times in 7 subjects, -- 140 rebreathing manoeuvres in all. 70 of these manoeuvres yielded lung volume estimates from 2 indicator gases, and 70 yielded estimates from 3,-- 350 observations in all. The subscripts s,c,g and r, indicate summations over subjects, compositions, indicator gases and repetitions, respectively (except where specified). The individual observations would be more correctly characterised by:- $y_{s,c,g,r}$, but in the interests of simplicity this will be written:- y.

The sum of squares of the deviations of the 350 individual observations from their mean is calculated, in conventional manner as the Total Corrected Sum of Squares, (TCSS). This is the sum of squares of the observations "corrected" by subtracting a "Correction Factor", (CF), which consists of the square of the sum of the 350 observations divided by 350.

$$CF = \frac{\left[\sum_s \sum_c \sum_g \sum_r y\right]^2}{350}$$

Thus, $$TCSS = \sum_s \sum_c \sum_g \sum_r (y)^2 - CF$$

The TCSS arises from differences between rebreathing manoeuvres, (Between.SS), and within rebreathing manoeuvres, (Within.SS).

TCSS = Between.SS + Within.SS

The Between.SS is computed by squaring the sum of the 2 or 3 estimates of lung volume yielded by each manoeuvre, dividing appropriately by 2 or 3, totalling the results for the 140 rebreathing manoeuvres and "correcting" by CF.

$$\text{Between.SS} = \frac{\overset{1,4}{\sum_{c}}\sum_{s}\sum_{r}\left[\sum_{g} y\right]^2}{2} + \frac{\overset{2,3}{\sum_{c}}\sum_{s}\sum_{r}\left[\sum_{g} y\right]^2}{3} - CF$$

The Within.SS is obtained by subtracting the Between.SS from TCSS.

Analysis of "Between.SS"

The Between.SS contains sums of squares owing to differences between subjects, (Subj.SS), and between composition changes, (Comp.SS), and to the interactions between subjects and composition changes, (Sub*Comp.SS). The Subj.SS is the square of the sum of the 50 observations per subject, divided by 50, summed for the 7 subjects and "corrected" by CF.

$$\text{Subj.SS} = \frac{\sum_{s}\left[\sum_{c}\sum_{g}\sum_{r} y\right]^2}{50} - CF$$

The Comp.SS is the square of all the estimates within each composition change, divided by the number of estimates, summed for the 4 composition changes and "corrected" by CF.

$$\text{Comp.SS} = \frac{\overset{1,4}{\sum_{c}}\left[\sum_{s}\sum_{g}\sum_{r} y\right]^2}{70} + \frac{\overset{2,3}{\sum_{c}}\left[\sum_{s}\sum_{g}\sum_{r} y\right]^2}{105} - CF$$

The Sub*Comp.SS is the square of the 10 or 15 estimates per subject per composition change, divided appropriately by 10 or 15, summed by composition change and subject and "corrected" by Subj.SS, Comp.SS and CF. If Subj.SS + Comp.SS + CF is designated "SCCF":

$$\text{Sub*Comp.SS} = \frac{\overset{1,4}{\sum_{c}}\sum_{s}\left[\sum_{g}\sum_{r} y\right]^2}{10} + \frac{\overset{2,3}{\sum_{c}}\sum_{s}\left[\sum_{g}\sum_{r} y\right]^2}{15} - SSCF$$

For the composition changes with N_2O, there was a priori reason to expect different circumstances between the first two composition changes and the second two. Thus the Comp.SS and the Sub*Comp.SS were each subdivided into a "between-halves", (BH-) and a "within-halves", (WH-), sum

of squares.

For the BH-Comp.SS, the two totals of the 175 observations in each half of the protocol were squared, added together, divided by 175, and corrected by the correction factor.

$$\text{BH-Comp.SS} = \frac{\left[\sum_{c}^{1,4}\sum_{s}\sum_{g}\sum_{r} y\right]^2 + \left[\sum_{c}^{2,3}\sum_{s}\sum_{g}\sum_{r} y\right]^2}{175} - CF$$

The WH-Comp.SS is the difference between this and the Comp.SS.

For the BH-Sub*Comp.SS, the totals of the 25 observations per subject were squared, summed by subject, divided by 25, and corrected by a composite correction factor, (BHSCCF), equal to the sum of Subj.SS, BH-Comp.SS and CF.

$$\text{BH-Sub*Comp.SS} = \frac{\sum_{s}\left[\sum_{c}^{1,4}\sum_{g}\sum_{r} y\right]^2 + \sum_{s}\left[\sum_{c}^{2,3}\sum_{g}\sum_{r} y\right]^2}{25} - BHSCCF$$

The WH-Sub*Comp.SS is the difference between this and the Sub*Comp.SS.

The remainder of the Between.SS , after subtraction of Subj.SS, Comp.SS and Sub*Comp.SS, was the residual sum of squares for the variation between rebreathing manoeuvres.

Analysis of the Within.SS

The Within.SS contains the variation between gases within composition change, (GwC.SS), and the interaction of this with subject, (S*GwC.SS).

The GwC.SS is obtained by squaring the sums of the 35 estimates, (7 subjects times 5 repetitions), for each indicator gas within each

composition change, dividing by 35, summing over gases and composition changes and correcting by Comp.SS and CF

$$GwC.SS = \frac{\sum_{c}\sum_{g}\left[\sum_{s}\sum_{r} y\right]^2}{35} - \left[Comp.SS + CF\right]$$

The S*GwC.SS is obtained by squaring the sums of the 5 repetitions of each gas within composition change within subject, diving by 5, summing over subjects, compositions and gases, and correcting by a composite factor, (SCGCF) consisting of the sum of Subj.SS, Comp.SS , Sub*Comp.SS , GwC.SS and CF.

$$S*GwC.SS = \frac{\sum_{s}\sum_{c}\sum_{g}\left[\sum_{r} y\right]^2}{5} - SCGCF$$

The remainder of WithinSS after subtracting GwC.SS and S*GwC.SS is the residual sum of squares for variations of estimate within rebreathing manoeuvre.

If each of the above components of the TCSS is divided by its associated degrees of freedom, the mean sum of squares is obtained. For tests of significance, the variance ratios, (F values) for subjects and composition changes are the ratios of mean Subj.SS and mean Comp.SS with mean WH-Subj*Comp.SS. The variance ratio for the subject/composition interaction, (and its components), is that between mean Subj*comp.SS, (and its BH and WH components), and the mean between manoeuvre residual. The F value for gases within composition changes was the ratio GwC.SS : S*GwC.SS . That for the interaction of indicator gas within composition change with subject was the ratio of S:GwC.SS with the mean within-manoeuvre residual.

PHASE II

The analysis of results for composition changes without N_2O followed

the principles outlined above. It was slightly simpler as there was no a priori reason to consider composition changes 1 and 2 separately from changes 3 and 4. It was slightly more complex because some of the composition changes were repeated only 4 times. Appropriate allowance had to be made for the irregularly changing numbers of estimates in the summations.

PROGRAMMES FOR A NUMERICAL SIMULATION OF REBREATHING

```
0: dim B$[10];"NOBEL"→B$
1: dsp "FRC Model";wait 500;fxd 4;sfg 14;dim A$[2,40],F$[1,10],X$[10]
2: dim L[30],B[60],M[60],O[15],C[15],N[15],I[15],D[15],G[15],Q[15]
3: dim Y[4],T[45,2],V[45,2],W[45,2],E[45,2],H[45,2],X[45],A[45,2]
4: "REM":
5: fmt 0,/,5x,"Tidal Vol.",5x,"Resp. rate",9x,"F.R.C.",5x,"Dead Space",z
6: fmt 1,8f15.3
7: fmt 2,3f12.4,f18,6f12.4
8: fmt 3,5/;"BREATH NUMBER"→A$[1]
9: fmt 4,/,8x,"FB",10x,"FM",10x,"FL",15x,"VO2/F",6x,"VCO2/F",6x,"VN2O/F",z
10: fmt 7,9x,"CorrO2",8x,"CorrCO2",8x,"CorrN2O",6x,"CorrInert"
11: fmt 8,5x,"VInert/F",3x,"Net Uptk/F",3x,"Total Gas",8x,"VB"
12: 1→r30;if r50>=1 and r50<=3;gto 68
13: ent "Reciprical Regression? YES/NO",X$;cap(X$)→X$
14: ent "FB0",B[1],"FA0",L[1],"FBIn",Y[1],"FAIn",Y[3],"FBA",Y[2]
15: ent "FAA",Y[4],"Vt",T,"freq",F,"VB",B,"FRC",L,"Vd",D
16: dsp "UPTAKES +ve----OUTPUTS -ve";wait 2000
17: ent "VO2",O;if flg13;for I=1 to 15;ent O[I];next I;1→r22
18: ent "VCO2",C,"VInert",I,"VA",N
19: if r22#1;ent "CorrO2",r1
20: ent "CorrCO2",r2
21: ent "CorInert",r4,"CorAr",r3,"C02 &N20 out of ASC?-Y→1,C→2,N→3",r50
22: ent "Sample Rate(L/m)",S;S/(2*F)→S
23: ent "Shortened printout?  Y→0,N→1",r20
24: B[1]→r83;L[1]→r82;B→r40
25: if r22=1;O[1]*F→O
26: O/F→O[1];C/F→C[1];N/F→N[1];I/F→I[1];(O+N+C+I)/F→D[1];1→Z
27: T-D→A;(1-A/B)/(1+A/L)→Q;B*B[1]+L*L[1]→Q[1];B+L→G[1];Q[1]/G[1]→M[1]
28: for I=2 to 15;r2*C[I-1]→C[I];r3*N[I-1]→N[I];r4*I[I-1]→I[I]
29: if r22#1;r1*O[I-1]→O[I]
30: O[I]+N[I]+C[I]+I[I]→D[I];G[I-1]-D[I-1]-2S→G[I]
31: if Z=2;(L*L[I-1]+(A-S)*B[I-1]-I[I-1])/(L+A-S-D[I-1])→L[I]
32: if Z=2;Q[I-1]-I[I-1]-S*B[I-1]-S*L[I]→Q[I];Q[I]/G[I]→M[I]
33: if Z=3;(L*L[I-1]+(A-S)*B[I-1]-N[I-1])/(L+A-S-D[I-1])→L[I]
34: if Z=3;Q[I-1]-N[I-1]-S*B[I-1]-S*L[I]→Q[I];Q[I]/G[I]→M[I]
35: if Z=1;(L*L[I-1]+(A-S)*B[I-1]-O[I-1])/(L+A-S-D[I-1])→L[I]
36: if Z=1;Q[I-1]-O[I-1]-S*B[I-1]-S*L[I]→Q[I];Q[I]/G[I]→M[I]
37: ((G[I-1]-L-A)*B[I-1]+(A-2S-D[I-1])*L[I])/(G[I]-L)→E[I]
38: if r50>=1 and r50<=3;jmp 4
39: if Z=1;(B[I]-L[I])/(B[I-1]-L[I-1])→B[I+15]
40: if Z=2;(B[I]-L[I])/(B[I-1]-L[I-1])→M[I+15]
41: if Z=3;(B[I]-L[I])/(B[I-1]-L[I-1])→L[I+15]
42: next I
43: if Z#1;gto 48
44: wrt 6;wrt 6.7
45: wrt 6.1,T,F,L,D,r1,r2,r3,r4
46: wrt 6.3
47: wrt 6.4;wrt 6.8
48: if Z=2;gto 52
49: if Z=3;gto 54
50: for I=1 to 15;I→T[I,1];B[I]→T[I,2];I→T[15+I,1]
51: M[I]→T[15+I,2];I→T[30+I,1];L[I]→T[30+I,2];gto 60
52: for H=1 to 15;H→V[H,1];B[H]→V[H,2];H→V[15+H,1]
53: M[H]→V[15+H,2];H→V[30+H,1];L[H]→V[30+H,2];next H;gto 65
54: for K=1 to 15;K→W[K,1];B[K]→W[K,2];K→W[15+K,1]
55: M[K]→W[15+K,2];K→W[30+K,1];L[K]→W[30+K,2]
56: K→E[K,1];B[15+K]→E[K,2];K→E[15+K,1];M[15+K]→E[15+K,2]
57: K→E[30+K,1];L[15+K]→E[30+K,2];K→H[K,1]→H[K+15,1]→H[K+30,1]
58: 1-T[K,2]-V[K,2]-W[K,2]→H[K,2];1-T[15+K,2]-V[15+K,2]-W[15+K,2]→H[15+K,2]
59: 1-T[30+K,2]-V[30+K,2]-W[30+K,2]→H[30+K,2];next K;gto 65
60: if Z>1 or r21=2;gto "Next"
*29259
```

```
61: wrt 6.2,B[I],M[I],L[I],O[I],C[I],N[I],I[I],D[I],Q[I],G[I]-L
62: if r20=0;2→r21
63: "Next":next I
64: if r21#2;wrt 6.3
65: Z+1→Z;if Z>3;gto 67
66: Y[Z-1]→B[1];Y[Z+1]→L[1];gto 27
67: if r50>=1 and r50<=3;chain "WRTLIN"
68: gto +2;if r50>=1;gsb "ASC";if r50>1;gsb "CO2";if r50>2;gsb "N20"
69: if r50>3;gto +1
70: wrt 6.3
71: fmt 0,/,3x,"FBN2",7x,"FMN2",7x,"FLN2",6x,"FBArg",6x,"FMArg",6x,"FLArg",z
72: fmt 1,1f7.4,2f11.4,z;fmt 2,6f11.4,z;fmt 6,3f11.4
73: fmt 4,6x,"FBCO2",6x,"FMCO2",6x,"FLCO2",7x,"Q 02",7x,"Q N2",6x,"Q N20"
74: fmt 5,2/,"-----------------THEORETICAL Q [(1-VA/VB)/1-VA/VL)] =",1f6.4
75: wrt 6;wrt 6.4
76: for I=1 to 14*r20+1;wrt 6.1,V[I,2],V[I+15,2],V[I+30,2]
77: wrt 6.2,W[I,2],W[I+15,2],W[I+30,2],H[I,2],H[15+I,2],H[30+I,2]
78: wrt 6.6,E[I,2],E[I+15,2],E[30+I,2];next I
79: wrt 6.5,Q;if r21#2;wrt 6.3
80: if r50<1 or r50>3;jmp 5
81: for I=1 to 45;M[I+15]→V[I,2];B[I+15]→T[I,2]
82: if r50=2;X[I]→W[I,2]
83: if r50=3;X[I]→H[I,2]
84: next I
85: 2→r30
86: chain "WRTLIN"
87: "CO2":for I=1 to 45;W[I,2]→X[I];next I;gsb "ASC"
88: "*** CO2 OUT OF ASC***"→A$[2]
89: ret
90: "N20":for I=1 to 45;H[I,2]→X[I];next I;gsb "ASC"
91: "*** N2O OUT OF ASC***"→A$[2]
92: ret
93: "ASC":for I=1 to 45;T[I,2]→r51;V[I,2]→r52
94: if r50=1;0→X[I]
95: r52/(r51+r52+X[I])→M[I+15];r51/(r51+r52+X[I])→B[I+15]
96: if r50=3 or r50=2;X[I]/(r51+r52+X[I])→X[I]
97: if r50=1;"*** CO2 AND N2O OUT OF ASC***"→A$[2]
98: next I
99: for I=2 to 15;(B[I+15]-B[I+45])/(B[I+14]-B[I+44])→E[I,2]
100: (M[I+15]-M[I+45])/(M[I+14]-M[I+44])→E[I+15,2]
101: if r50=2;(X[I]-X[30+I])/(X[I-1]-X[29+I])→E[I+30,2]
102: next I;ret
*5163
```

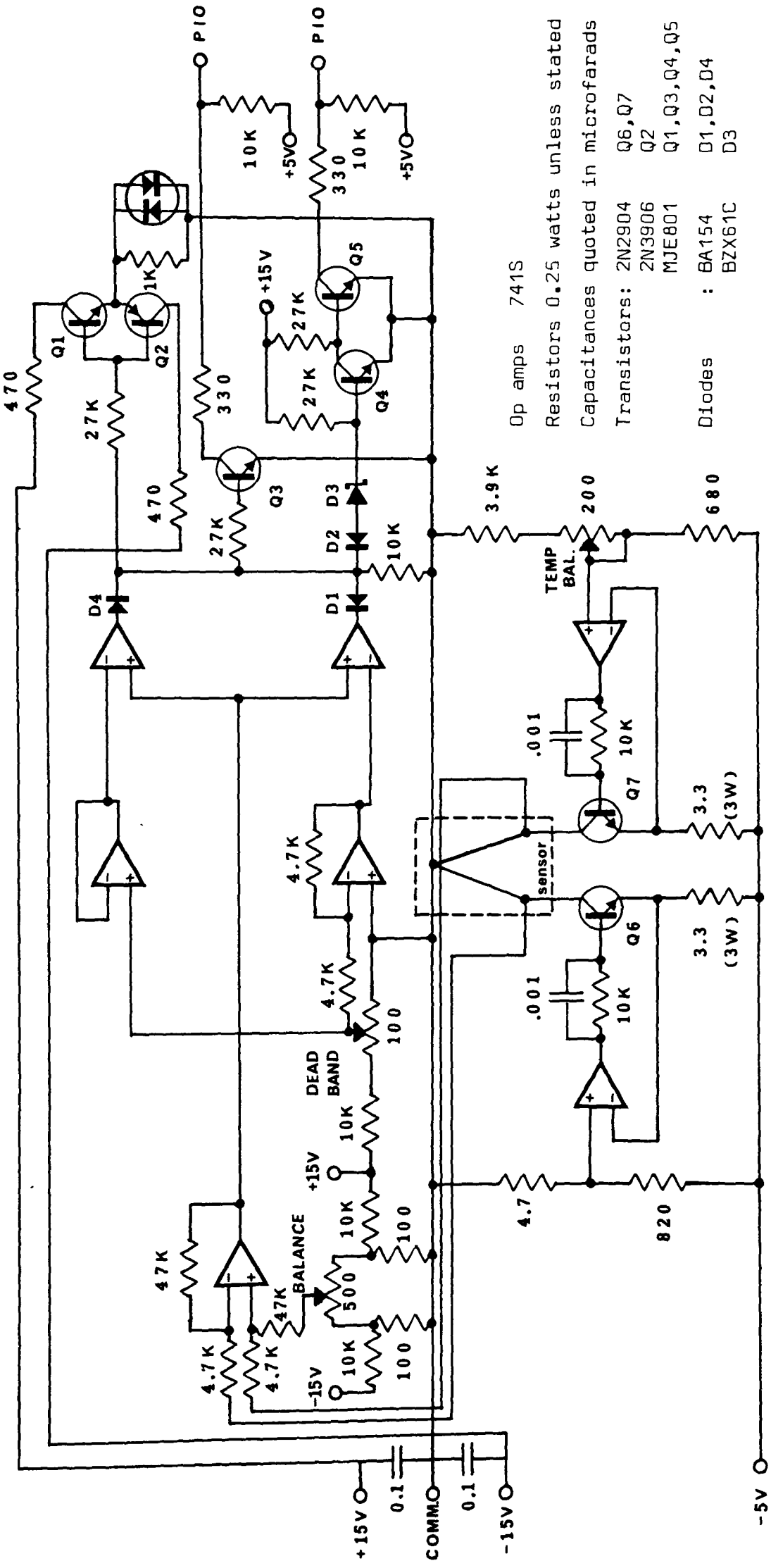
```
0: fmt 5,/,"======= Y=A+BX======="
1: fmt 6,"A =",f8.4,"    B =",f12.4
2: fmt 9,"R =",f10.4,"  ",f10.4
3: 1→r41;3→r98;if r30=2 and r50>=1 and r50<=3;wrt 6,A$[2]
4: "0":7→I;0→Z
5: "E":0→r9→r10→r18→r19→r22
6: "G":if I>15;Z→r0;0→Z;gto "B"
7: if r98=1;T[I+15,1]→X;T[1,2]→r88;"O2 FRC"→F$[1]
8: if r98=1;(T[I,2]*2+T[I+30,2])/3→Y;T[31,2]→r87
9: if r98=2;V[I+15,1]→X;V[1,2]→r88;"N2 FRC"→F$[1]
10: if r98=2;(V[I,2]*2+V[I+30,2])/3→Y;V[31,2]→r87;B+A→r85
11: if r98=3;W[I+15,1]→X;W[1,2]→r88;"N20 FRC"→F$[1]
12: if r98=3;(W[I,2]*2+W[I+30,2])/3→Y;W[31,2]→r87;B+A→r84
13: if X$="YES";1/Y→Y
14: Z+1→Z;r9+X→r9;r10+XX→r10;r18+Y→r18;r19+YY→r19;r22+XY→r22
15: I+1→I;gto "G"
16: "B":
17: if Y=0;gto "D"
18: r9→A;r10→B;r18→X;r19→Y;r22→r7
19: (BX-Ar7)/(Br0-AA→r8)→B
20: (r0r7-AX)/r8→A
21: if Yr0-XX=0;1→r8;jmp 2
22: if X$="YES";fmt 5,/,"=========1/Y=A+BX======="
23: AAr8/(Yr0-XX)→r8
24: fxd 4;wrt 6.5
25: wrt 6.6,B,A
26: if X$="YES";jmp 2
27: r40*(r88-B-A)/(B+A-r87)→r86;jmp 5
28: if r98=3;if r50=2;r41-1/(B+A)→r41
29: if r98=2;r41-1/(B+A)→r41
30: if r98=1;0→B;1/r41→A
31: r40*(r88-1/(B+A))/(1/(B+A)-r87)→r86
32: if r50=1 and r30=2;(1-H[1,2]-W[1,2])/(1-H[31,2]-W[31,2])→J;J*r86→r86
33: if r50=1 and r30=2 and r98=3;r86/J→r86
34: if r50=2 and r30=2;(1-H[1,2])/(1-H[31,2])→J;J*r86→r86
35: if r50=3 and r30=2;(1-W[1,2])/(1-W[31,2])→J;if r98=3;gto 36;J*r86→r86
36: wrt 6.9,r8,F$[1],r86
37: "D":if r98=3;B+A→r83
38: r98-1→r98;if r98<1;gto "END"
39: gto "0"
40: "END":chain "STUFED"
*23098
0: fmt 0,/,"PROP",f11.4;fmt 1,"FMO ^",f10.4
1: fmt 2,"FMN ^",f10.4;fmt 3,"FRC",2f10.4
2: T[1,2]→B;T[31,2]→A;r85→M;V[1,2]→N;V[31,2]→O;r84→P
3: r83→F;W[1,2]→E;W[31,2]→D
4: if r84=0;W[1,2]→N;W[31,2]→O;r83→P
5: M/(M+P+F)→C;P/(M+P+F)→G;wrt 6,C
6: N*A-B*O→K
7: N-O→X;A-B→Y
8: (K*C+(P*C-M*G)*Y)/(G*Y+X*C)→W
9: (K-W*X)/Y→V
10: 4*(B-W)/(W-A)→r1;4*(N-V)/(V-O)→r2
11: if F=0;gto +2
12: ((1-C-G)*(V-r84)+F*G)/G→F;4*(E-F)/(F-D)→r3
13: wrt 6.1,W-M;wrt 6.2,V-P;wrt 6.3,r1,r2,r3
14: if r50>=1 and r50<=3 and r30=1;chain B$,0,5
15: chain "WRTPLT"
*17686
```

```
0: if rds(6)=32;stp ;dsp "SWITCH PRINTER ON,CONTINUE"
1: 0→T→F→B→L→D→O→C→N→J→Z→I→H→K→r1→r2→r3→r4→r100→r19→r18
2: 0→r17;6→r0;wtb r0,13,10
3: cfg 13
4: "CHOOSE":
5: if flg13;jmp 2
6: for I=1 to 15;I→A[I,1]→A[I+15,1]→A[I+30,1];next I
7: ent "PLOTS?-O2→0,N2→1,N20→2,C02→3,Q→4",r17
8: if r17=1;for I=1 to 45;V[I,2]→A[I,2];next I;if flg13;gto 14
9: if r17=2;for I=1 to 45;W[I,2]→A[I,2];next I;if flg13;gto 14
10: if r17=4;for I=1 to 45;E[I,2]→A[I,2];next I;if flg13;gto 14
11: if r17=3;for I=1 to 45;H[I,2]→A[I,2];next I;if flg13;gto 14
12: if r17=5;gto "end"
13: if r17=0;for I=1 to 45;T[I,2]→A[I,2];next I
14: r17+1→r17;gto "paper size"
15: "move":
16: wtb r0,27,65,int((p1-X)U/64),int((p1-X)U),int((p2-Y)V/64),int((p2-Y)V)
17: ret
18: "plt":
19: wtb r0,27,65,int((p1-X)U/64),int((p1-X)U),int((p2-Y)V/64),int((p2-Y)V)
20: if p3=0;46→p3
21: if p3=46;wtb r0,27,82,0,0,0,6
22: wtb r0,p3;wtb r0,8
23: if p3=46;wtb r0,27,82,0,0,63,-6
24: ret
25: "fplt":
26: wtb r0,27,97,int((p1-X)U/64),int((p1-X)U),int((p2-Y)V/64),int((p2-Y)V)
27: if p3=0;46→p3
28: if p3=46;wtb r0,27,82,0,0,0,6
29: wtb r0,p3;wtb r0,8
30: if p3=46;wtb r0,27,82,0,0,63,-6
31: ret
32: "char":
33: if p2=0;5→p2;0→p3
34: wtb r0,27,46,p1,int(p2/64),p2,p3
35: ret
36: "psiz":
37: p1→H;p2→W
38: wtb r0,27,79,int(p4*120/64),p4*120,int(p3*96/64),p3*96
39: ret
40: "scl":
41: 120W/(p2-p1)→U
42: 96H/(p4-p3)→V
43: p1→X;p3→Y
44: ret
45: "xaxis":
46: wtb r0,27,46,95,0,5,9
47: if p3=0 and p4=0;X→p3;X+120W/U→p4
48: if p2=0;p4-p3→p2
49: wtb r0,27,65,int((p3-X)U/64),int((p3-X)U),int((p1-Y)V/64),int((p1-Y)V)
50: p3→p5;wtb r0,43;wtb r0,8
51: wtb r0,27,114,int(p2U/64),int(p2U),0,0;wtb r0,43,8;jmp (p5+p2→p5)>=p4
52: ret
53: "yaxis":
54: wtb r0,27,46,124,0,3,0
55: if p3=0 and p4=0;Y→p3;Y+96H/V→p4
56: if p2=0;p4-p3→p2
57: wtb r0,27,65,int((p1-X)U/64),int((p1-X)U),int((p3-Y)V/64),int((p3-Y)V)
58: p3→p5;wtb r0,43;wtb r0,8
59: wtb r0,27,114,0,0,int(p2V/64),int(p2V);wtb r0,43,8;jmp (p5+p2→p5)>=p4
60: ret
*29051
```

```
61: "space":
62: if p1<0;gto +2
63: wtb r0,32;jmp 2((p1-1→p1)=0)
64: wtb r0,8;jmp (p1+1→p1)=0
65: ret
66: "skip":
67: if p1<0;gto +2
68: wtb r0,10;jmp 2((p1-1→p1)=0)
69: wtb r0,27,10;jmp (p1+1→p1)=0
70: ret
71: "form":
72: wtb r0,27,77
73: wtb r0,27,84
74: if p1=0;13.2→p1;11→p2→p3
75: wtb r0,27,87,int(120*p1/64),120*p1
76: wtb r0,27,76,int(96*p2/64),96*p2
77: wtb r0,27,70,int(96*p3/64),96*p3
78: ret
79: "paper size":
80: wtb r0,13;12→W;8→F;0→r1;F-1→L
81: cll 'form'(W,L,F)
82: 3W/4→Q;W/6→S;1→r1;2→R
83: L-2→P
84: cll 'psiz'(P,Q,R,S)
85: 0→A;A→r10;15→B
86: 0→C;C→r11;1→D;if r17=5;1.6→D
87: if r17=4;.1→D
88: cll 'scl'(A,B,C,D)
89: 0→E;5→F;0→r15
90: A→G;B→J;cll 'xaxis'(E,F,G,J)
91: 0→K;.2→M;2→r16;if r17=4;.02→M
92: C→N;D→O;cll 'yaxis'(K,M,N,O)
93: G→I;fmt f5.0,z
94: cll 'move'(I,E)
95: cll 'space'(-4)
96: cll 'skip'(1)
97: wrt r0,I;if (I+F→I)<=J;gto -3
98: cll 'move'(A+(B-A)/3,E)
99: cll 'skip'(3)
100: if r19=0;1→r19
101: wrt r0,A$[1]
102: N→I
103: if r16=2;fmt f5.2,z
104: cll 'move'(K,I)
105: cll 'space'(-8)
106: wrt r0,I;if (I+M→I)<=O;gto -2
107: cll 'move'(K,D)
108: cll 'space'(5)
109: cll 'skip'(-1)
110: if r17=2;" INERT Concs"→A$[2]
111: if r17=3;"N2O Concs"→A$[2]
112: if r17=1;"O2 Concs"→A$[2]
113: if r17=5;"Q      #=O2  *=N2  o=N2O"→A$[2]
114: if r17=4;"CO2 Concs"→A$[2]
115: wrt r0,A$[2]
116: 1→Z;1→r5
117: "BOB":if r18=3;0→r18;wtb r0,13;cll 'skip'(30);gto "CHOOSE"
118: "SKP":if Z<45;111→F
119: if Z<30;42→F
120: if Z<15;35→F
*7676
```

```
121: "plot":0→r1→r2→r3→r22
122: cll 'move'(A[Z,1],A[Z,2])
123: 0→A→D→E→G→H→N→r12
124: for Z=r5 to 15+r5-1
125: cll 'plt'(A[Z,1],A[Z,2],F)
126: next Z
127: Z→r5;if Z>45;1→Z→r5
128: r18+1→r18
129: gto "BOB"
130: "end":if r30=2;0→r50;chain B$,0,5
131: chain "FRCWRT",0,3
*16453
```

CIRCUIT DIAGRAM FOR FLOW DIRECTION SENSOR



Reproduced from a diagram supplied by A. Micco, Department of Anesthesiology and Biometrics, University of Colorado, Health Sciences Center, Colorado.

MACHINE CODE ROUTINES FOR ON-LINE DATA COLLECTION

```
32D2                    ORG 32D2H
32D2 CD8533             CALL SWTCH
32D5 DD212434           LD IX,ADDR+3        ;LAST DATA
32D9 3E00               LD  A,00H
32DB 322134             LD  (ADDR),A
32DE 3A2134         START:LD  A,(ADDR)      ;CHANNEL No
32E1 D320               OUT (20H),A
32E3 D321               OUT (21H),A         ;START CONVERT
32E5 012200             LD  BC,0022H
32E8 ED78           WAIT:IN A,(C)           ;READ HIGH BYTE
32EA B7                 OR  A               ;SET FLAG
32EB F2E832             JP  P,WAIT          ;JP UNTIL BIT7=0
32EE E603               AND  3              ;MASK NON DATA BITS
32F0 C5                 PUSH BC
32F1 010200             LD  BC,0002H
32F4 DD222234           LD  (ADDR+1),IX     ;DE=IX
32F8 ED5B2234           LD  DE,(ADDR+1)
32FC DD09               ADD IX,BC           ;IX=IX+2
32FE DD222234           LD  (ADDR+1),IX
3302 2A2234             LD  HL,(ADDR+1)     ;HL=IX
3305 011400             LD  BC,0014H
3308 EDB0               LDIR                ;ROTATE DATA BLOCK
330A DD7713             LD   (IX+13H),A     ;STORE HIGH BYTE
330D C1                 POP BC
330E 0C                 INC C
330F ED78               IN  A,(C)           ;GET LOW BYTE
3311 C600               ADD A,00H           ;ADD OFFSET
3313 DD7712             LD  (IX+12H),A      ;STORE LOW BYTE
3316 D22033             JP NC,CARRY
3319 DD7E13             LD  A,(IX+13H)
331C 3C                 INC A               ;INC HIGH BYTE IF OFFSET GIVES CARRY
331D DD7713             LD  (IX+13H),A
3320 C5             CARRY:PUSH BC
3321 011400             LD  BC,0014H
3324 DD09               ADD IX,BC           ;IX=IX+13
3326 C1                 POP BC
3327 3A2134             LD  A,(ADDR)
332A 3C                 INC A
332B 322134             LD (ADDR),A         ;INC CHANNEL No
332E D600               SUB A,00H           ;n POKED IN FROM BASIC
3330 C2DE32             JP NZ,START
3333 C9                 RET
                    ;
                    ;
3334 DD212434           LD IX,ADDR+3        ;SUM LAST 10 DATA
3338 FD219234           LD IY,ADDR+113
333C CD6C33             CALL ZERO
333F 1E05               LD   E,05H
3341 160A           LOOP:LD  D,0AH
3343 FD7E00         AGAIN:LD  A,(IY+0)
3346 DD8600             ADD A,(IX+0)
3349 FD7700             LD (IY+0),A
334C FD7E01             LD  A,(IY+1)
334F DD8E01             ADC A,(IX+1)
3352 FD7701             LD (IY+1),A
3355 DD23               INC IX
3357 DD23               INC IX
3359 15                 DEC D
335A C24333             JP NZ,AGAIN
```

```
335D 010200          LD  BC,0002H
3360 DD09            ADD IX,BC
3362 010200          LD  BC,0002H
3365 FD09            ADD IY,BC
3367 1D              DEC E
3368 C24133          JP NZ,LOOP
336B C9              RET
                   ;
336C 1E0A          ZERO:LD  E,0AH        ;ZERO SUMS
336E 019234          LD  BC,ADDR+113
3371 3E00          REP:LD   A,00H
3373 02              LD  (BC),A
3374 03              INC  BC
3375 1D              DEC E
3376 C27133          JP NZ,REP
3379 C9              RET
                     ;
                     ;
337A 212234          LD HL,ADDR+1      ;DAC ROUTINE
337D 0E24            LD  C,24H
337F EDA3            OUTI
3381 0C              INC C
3382 EDA3            OUTI
3384 C9              RET
                   ;
                   ;
3385 3AFFFB        SWTCH:LD A,(0FBFFH)
3388 CB47            BIT 0,A
338A CA8533          JP Z,SWTCH
338D C9              RET               ;RET ONLY IF BIT 0 SET
                   ;
                   ;
338E 3AFFFB          LD  A,(0FBFFH)    ;TEST FLOW SENSOR
3391 4F              LD  C,A           ;00 ⅜ 0
3392 3E00            LD  A,00H         ;01 ⅜ 3
3394 0600            LD  B,00H         ;10 ⅜ 1
3396 CB49            BIT 1,C           ;11 ⅜ 2
3398 C29E33          JP NZ,LINEA
339B C3A033          JP LINEB
339E 0601          LINEA:LD  B,01H
33A0 CB51          LINEB:BIT 2,C
33A2 C2A933          JP NZ,LINEC
33A5 78              LD  A,B
33A6 C3AC33          JP LINED
33A9 3E03          LINEC:LD  A,03H
33AB 90              SUB A,B
33AC 47            LINED:LD  B,A
33AD 3E00            LD  A,00H
33AF CD0C01          CALL 010CH        ;RET VALUE TO BASIC
33B2 C9              RET
                   ;
                   ;
                   ;SET UP CTC
33B3 012C00          LD  BC,002CH
33B6 3E57            LD  A,57H
33B8 ED79            OUT (C),A         ;CHANNEL 0 CONTROL WORD
33BA 3E7A            LD  A,07AH
33BC ED79            OUT (C),A         ;TIME CONSTANT
33BE 0E2D            LD  C,2DH
33C0 3E57            LD  A,57H          ;CHANNEL 1 CONTROL WORD
```

```
33C2 ED79          OUT (C),A
33C4 3EFF          LD  A,0FFH
33C6 ED79          OUT (C),A        ;TIME CONSTANT
33C8 0E2F          LD  C,2FH
33CA 3E57          LD  A,57H
33CC ED79          OUT (C),A        ;CHANNEL 3 CONTROL WORD
33CE 3EFF          LD  A,0FFH
33D0 ED79          OUT (C),A
33D2 C9            RET
                   ;
                   ;
                   ;READ CTC
33D3 012C00        LD  BC,002CH
33D6 ED78          IN  A,(C)
33D8 322134        LD  (ADDR),A
33DB 0E2D          LD  C,2DH
33DD ED78          IN  A,(C)
33DF 322234        LD  (ADDR+1),A
33E2 0E2F          LD  C,2FH
33E4 ED78          IN  A,(C)
33E6 322334        LD  (ADDR+2),A
33E9 C9            RET
                   ;
                   ;
33EA 3AFFFB        LD  A,(0FBFFH)   ;READ PIO BIT 0
33ED CB47          BIT 0,A
33EF 3E00          LD  A,00H
33F1 CAF633        JP Z,ROT
33F4 3E01          LD  A,01H
33F6 CD0C01    ROT:CALL 010CH       ;RET VALUE TO BASIC
33F9 C9            RET
                   ;
                   ;
33FA CD0901        CALL 0109H       ;SET UP CTC 3
33FD 3E37          LD  A,37H        ;TIMER MODE
33FF D32E          OUT (2EH),A
3401 7B            LD  A,E
3402 321034        LD (3410H),A
3405 321834        LD (3418H),A
3408 D32E          OUT (2EH),A
340A 012E00        LD  BC,002EH     ;WAIT FOR CTC 3
340D ED58      MRK1:IN E,(C)
340F 3EFF          LD  A,0FFH
3411 93            SUB A,E
3412 CA0D34        JP Z,MRK1
3415 ED58      MRK2:IN E,(C)
3417 3EFF          LD  A,0FFH
3419 93            SUB A,E
341A C21534        JP NZ,MRK2
341D CDD232        CALL 32D2H
3420 C9            RET
3421 0000          ADDR:DEFW 00
0000               END
```

```
3421 ADDR     3343 AGAIN    3320 CARRY    339E LINEA    33A0 LINEB
33A9 LINEC    33AC LINED    3341 LOOP     340D MRK1     3415 MRK2
3371 REP      33F6 ROT      32DE START    3385 SWTCH    32E8 WAIT
336C ZERO
```

```
10 REM *********BASIC MASTER PROGRAM******
20 PRINTER 4,3
30 POKE&FBFF,0
40 PUT 17:PUT 12
50 CLOSE £10:CLEAR 200
60 WT=50:REM 1/4 SEC
70 INPUT "NUMBER  ",NUMBER
80 INPUT"DISC STORE  ? (YES/NO)  ",C$
90 IF C$<>"YES"THEN 230
100 ON ERROR GOTO 3110
110 INPUT"FILE NAME  ",A$
120 INPUT "WHICH DRIVE A:,B:,C:,D: ?. ",E$
130 A$=E$+A$
140 IF LOOKUP (A$)=0 THEN 220
150 RENAME E$+"TEMP",A$
160 OPEN £10,E$+"TEMP":CREATE £10,A$
170 ON EOF GOTO210
180 INPUT LINE £10,D$:PRINT £10,D$
190 INPUT LINE £10,B$:PRINT£10,B$
200 GOTO 190
210 ERASE E$+"TEMP":GOTO260
220 CREATE £10,A$
230 INPUT "SUBJECT ,DATE   ",D$
240 IF C$<>"YES" GOTO 260
250 PRINT£10,"%"+D$,NUMBER
260 DIM A(4,5,50),T(2,50),F(5,5),S(5,10)
270 DIM B$(5)
280 B$(1)="NITROGEN":B$(2)="OXYGEN":B$(3)="CO2":B$(4)="N20":B$(5)="ARGON"
290 DIM R(5,3),U(2,50),O(5)
300 GRAPHO:PUT12
310 INPUT "BAG VOLUME=",V
320 ON BREAK GOTO 3340
330 ON ERROR GOTO 3110
340 NUMBER=NUMBER+1
350 GOSUB 1480
360 N=0:Z=&3421:W1=1:W2=5:W3=1:W4=5
370 H=1:H1=1:G=.015:G1=.01:K=1
380 O(1)=1:O(2)=2:O(3)=0:O(4)=0:O(5)=5
390 C=0:D=2
400 POKE &341E,&D2:POKE&332F,5
410 REM
420 ****************START**************
430 REM
440 GOSUB 2370
450 RANDOMIZE
460 A=INT(RND(-1)*5)+5
470 POKE &107,&8E:POKE&108,&33:GOTO490
480 A=2
490 B=USR(X)
500 ON B GOTO 520,540,530
510 ?"ATTEND TO POWER SUPPLY":INPUT B:GOTO 450
520 C=1:GOTO490
530 C=0:GOTO490
540 A=A-C:C=0:IF A=0 GOTO560
550 GOTO 490
560 FOR J=1 TO WT:B=USR(X)
570 ON B GOTO 480,580,480
580 NEXT J
590 POKE&FBFF,1
600 A=200:POKE&107,&FA:POKE&108,&33:FOR J=1TO11:Y=USR(A)
```

```
610 NEXTJ
620 CALL &33B3
630 CALL &3334
640 I=0:B=3
650 GOSUB 1840
660 T(2,1)=0
670 GOSUB2140
680 D=3:GOSUB 1570
690 POKE&FBFF,0
700 U(2,1)=1:U(1,1)=1
710 GOSUB2590
720 N1=N:N=0:K=3:I1=I:GOSUB 1480
730 GOSUB 3420
740 GOSUB 2040
750 OX=1:FOR W=1TO 5
760 IF W=2 GOTO 820
770 IF S(W,1+(K-1)*2)=0GOTO 790
780 S(W,1+(K-1)*2)=1/S(W,1+(K-1)*2)
790 IFS(W,2+(K-1)*2)=0 GOTO 820
800 S(W,2+(K-1)*2)=1/S(W,2+(K-1)*2)
810 IF O(W)=W THEN OX=OX-S(W,2+(K-1)*2)
820 NEXT W
830 S(2,2+(K-1)*2)=OX
840 GOSUB 2590
850 S(1,9)=0:FOR W=1TO 5
860 IFO(W)<>WTHEN 880
870 S(1,9)=S(W,2)+S(1,9)
880 NEXT W
890 FOR W=1TO 5
900 IF O(W)<>W THEN S(W,10)=S(W,2):GOTO920
910 S(W,10)=S(W,2)/S(1,9)
920 NEXTW
930 K=5:GOSUB2730
940 AS=2:GOSUB 2590
950 K=2:N=0
960 GOSUB1480
970 FORI=I1-N1+1TOI1
980 GOSUB 1950
990 NEXT I:I=I1
1000 GOSUB 2040
1010 GOSUB 2590
1020 GOSUB 1480:N=0:K=4:GOSUB 3420
1030 GOSUB 2040
1040 OX=1:FOR W=1TO 5
1050 IF W=2 THEN 1110
1060 IF S(W,1+(K-1)*2)=0 THEN 1080
1070 S(W,1+(K-1)*2)=1/S(W,1+(K-1)*2)
1080 IF S(W,2+(K-1)*2)=0 THEN 1110
1090 S(W,2+(K-1)*2)=1/S(W,2+(K-1)*2)
1100 IF O(W)=W THENOX=OX-S(W,2+(K-1)*2)
1110 NEXT W
1120 S(2,2+(K-1)*2)=OX
1130 GOSUB 2590
1140 GOSUB 1180
1150 IF C$="YES"THEN GOSUB 2920
1160 GOTO 300
1170 REM ------------------------------
1180 REM  *****LINE PRINT ****
1190 REM
1200 REM
```

```
1210 LPRINT D$,NU
1220 LPRINT:LPRINT
1230 LPRINT "      ","BAG","MIXED","LUNG","L.V","REC"
1240 LPRINT:LPRINT "*ASC*"
1250 LPRINT
1260 LPRINT "Ch 1 (N2)",A(3,1,1),S(1,4),A(4,1,1),F(2,1),F(4,1)
1270 LPRINT "Ch 2 (O2)",A(3,2,1),S(2,4),A(4,2,1),F(2,2),F(4,2)
1280 LPRINT "Ch 3 (CO2)",A(3,3,1),S(3,4),A(4,3,1),F(2,3),F(4,3)
1290 LPRINT "Ch 4 (N2O)",A(3,4,1),S(4,4),A(4,4,1),F(2,4),F(4,4)
1300 LPRINT "Ch 5 (AR)",A(3,5,1),S(5,4),A(4,5,1),F(2,5),F(4,5)
1310 LPRINT
1320 LPRINT "Ch 1 (N2)",A(1,1,1),S(1,2),A(2,1,1),F(1,1),F(3,1),F(5,1)
1330 LPRINT "Ch 2 (O2)",A(1,2,1),S(2,2),A(2,2,1),F(1,2),F(3,2),F(5,2)
1340 LPRINT "Ch 3 (CO2)",A(1,3,1),S(3,2),A(2,3,1),F(1,3),F(3,3),F(5,3)
1350 LPRINT "Ch 4 (N2O)",A(1,4,1),S(4,2),A(2,4,1),F(1,4),F(3,4),F(5,4)
1360 LPRINT "Ch 5 (AR)", A(1,5,1),S(5,2),A(2,5,1),F(1,5),F(3,5),F(5,5)
1370 LPRINT:LPRINT
1380 LPRINT"TIME TAKEN=          ";INT(T(1,I1)/122);"sec"
1390 LPRINT"No OF DATA POINTS=   ";I1
1400 LPRINT"No OF EXTRAP POINTS=";N1
1410 LPRINT "BAG VOLUME=         ";V
1420 LPRINT:LPRINT:LPRINT:LPRINT
1430 RETURN
1440 REM ------------------------------
1450 REM  ****ZEROS REGRESSION ARRAY****
1460 REM
1470 REM
1480 R1=0:R2=0:R3=0
1490 FOR J1=1TO5:FOR J=1TO3
1500 R(J1,J)=0
1510 NEXTJ:NEXTJ1
1520 RETURN
1530 REM ------------------------------
1540 REM  ****SAMPLE MIXING LINE****
1550 REM
1560 REM
1570 POKE &341E,&D5
1580 I=1:F=1:AS=0
1590 POKE &107,&8E:POKE &108,&33
1600 B=USR(X):ON B GOTO 1620,1600,1620
1610 ?"ATTEND TO POWER SUPPLY":INPUT B:GOTO1600
1620 IF ABS(B-D)>1 GOTO 1640
1630 D=B:GOTO 1600
1640 POKE &107,&FA:POKE&108,&33:FOR J=1TO11:Y=USR(A)
1650 NEXTJ
1660 CALL &33D3
1670 CALL &3334
1680 POKE &107,&EA:POKE &108,&33:E=USR(X)
1690 E=INT(E/255)
1700 D=B:GOSUB 1840
1710 ON F GOTO 1720,1750
1720 GOSUB 2190:ON E*2+B GOTO 300,300,1730,1590
1730 IF ABS(A(1,W1,I)-A(2,W1,I))>G OR ABS(A(1,W2,I)-A(2,W2,I))>G GOTO 1590
1740 F=2:GOTO1710
1750 GOSUB 2290:ON B GOTO 1760,1590
1760 GOSUB 1950
1770 ON E+1 GOTO 1780,1590
1780 GOSUB 2040
1790 RETURN
1800 REM ------------------------------
```

```
1810 REM  ****READ ADC VALUES****
1820 REM
1830 REM
1840 B=(B+1)/2:I=I+B-1
1850 T(B,I)=122-PEEK(Z)+(255-PEEK(Z+1))*122
1860 FOR W=1TO5
1870 A(B,W,I)=PEEK(Z+W*2+111)+256*PEEK(Z+W*2+112)
1880 A(B,W,I)=A(B,W,I)/10000
1890 NEXT W
1900 RETURN
1910 REM ------------------------------
1920 REM  ****SUMS FOR REGRESSION****
1930 REM
1940 REM
1950 FOR W=1TO 5:R1=(H*A(2+AS,W,I)+H1*A(1+AS,W,I))/(H+H1)
1960 R(W,1)=R(W,1)+R1:R(W,2)=R(W,2)+R1*R1:R(W,3)=R(W,3)+R1*T(2,I)
1970 NEXTW
1980 R2=R2+T(2,I):R3=R3+T(2,I)*T(2,I)
1990 N=N+1:RETURN
2000 REM ------------------------------
2010 REM  ****PEFORMS REGRESSION****
2020 REM
2030 REM
2040 FOR W=1TO 5
2050 IF R3*N-R2*R2=0 THEN S(W,1+(K-1)*2)=0:GOTO2070
2060 S(W,1+(K-1)*2)=(R(W,3)*N-R2*R(W,1))/(R3*N-R2*R2)
2070 S(W,2+(K-1)*2)=(R(W,1)-S(W,1+(K-1)*2)*R2)/N
2080 NEXT W
2090 RETURN
2100 REM ------------------------------
2110 REM  ****PLOT CHANNEL 2****
2120 REM
2130 REM
2140 GRAPH 1:L=100
2150 FOR J=1TO 59:PLOTO,J,108:NEXTJ
2160 FOR J=1TO 79:PLOTJ,0,96:NEXTJ
2170 X=50:Y=58:S$="Time=    sec":GOSUB 3580
2180 GOTO2320
2190 IF I>1 GOTO2270
2200 M=A(2,2,1)
2210 IFA(1,2,1)>A(2,2,1) THEN M=A(1,2,1)
2220 M=M/55
2230 PLOT 2,A(2,2,1)/M,1
2240 X=0:Y=58:S$="02":GOSUB 3580
2250 PRINT "                    Time"
2260 ?:?
2270 PLOT 2+T(B,I)/L,A(B,2,I)/M,1
2280 GOTO2300
2290 PLOT 2+T(B,I)/L,A(B,2,I)/M,2
2300 X=60:Y=58:S$=STR$(INT(T(B,I)/122))
2310 GOSUB 3580
2320 RETURN
2330 REM ------------------------------
2340 REM  ****PREBREATH EQUILIBRATION****
2350 REM
2360 REM
2370 F=1
2380 POKE &107,&8E:POKE&108,&33
2390 B=USR(X):ON B GOTO 2410,2390,2410
2400 ? "SWITCH ON POWER SUPPLY":INPUT B:GOTO 2370
```

```
2410 IF ABS(B-D) 1⟩GOTO2430
2420 D=B:GOTO2390
2430 D=B:A=200:POKE&107,&FA:POKE&108,&33:FOR J=1TO11:Y=USR(A)
2440 NEXTJ
2450 CALL &3334
2460 B=(B+1)/2:I=1:GOSUB 1860
2470 A1=A(B,W3,1):A3=A(B,W4,1)
2480 IF F=1 GOTO2540
2490 IF ABS(A1-A2)⟩G1OR ABS(A3-A4) G1 GOTO 2540
2500 F=F+1:IF F⟩5 GOTO 2520
2510 A2=A1:A4=A3:GOTO 2380
2520 PRINT "OK PRE BREATHE EQUILIBRATION"
2530 RETURN
2540 A2=A1:A4=A3:F=2:GOTO 2380
2550 REM ------------------------------
2560 REM  ****CALCULATES LUNG VOLUMES****
2570 REM
2580 REM
2590 FOR W=1TO 5:IF ABS(A(2,W,1)-A(1,W,1))⟩.2 THEN GOTO2610
2600 F(K,W)=0:GOTO2680
2610 F(K,W)=(A(1+AS,W,1)-S(W,2+(K-1)*2))/(S(W,2+(K-1)*2)-A(2+AS,W,1))
2620 F(K,W)=F(K,W)*V
2630 IF O(W)<>W THEN GOTO 2650
2640 F(K,W)=F(K,W)*U(1,1)/U(2,1)
2650 FX=INT (F(K,W)*1000)
2660 IF F(K,W)*1000-FX⟩=.5 THEN FX=FX+1
2670 F(K,W)=FX/1000
2680 NEXTW:RETURN
2690 REM ------------------------------
2700 REM  ****ASC NORMALISATION****
2710 REM
2720 REM
2730 FOR J=1 TO I1
2740 FOR B=1TO2
2750 U(B,J)=0
2760 FOR W=1TO5
2770 IF O(W)<>W GOTO  2790
2780 U(B,J)=A(B,W,J)+U(B,J)
2790 NEXT W
2800 NEXT B:NEXT J
2810 FOR J=1TO I1:FOR B=1TO2
2820 FOR W=1TO5:IF O(W)<>W GOTO2840
2830 A(B+2,W,J)=A(B,W,J)/U(B,J):GOTO2850
2840 A(B+2,W,J)=A(B,W,J)
2850 NEXT W
2860 NEXTB:NEXT J
2870 RETURN
2880 REM ------------------------------
2890 REM  ****STORES VALUES ON DISC****
2900 REM
2910 REM
2920 FOR W=1TO5
2930 PRINT £10,"%"+B$(W),NU
2940 FOR J=1TO I1
2950 A1$=STR$(A(2,W,J)):A2$=STR$(A(1,W,J))
2960 A1$=A1$+","+A2$
2970 PRINT£10,A1$
2980 NEXTJ
2990 NEXTW
3000 PRINT£10,"%TIME"
```

```
3010 FOR J=1TO I1
3020 A1$=STR$(T(2,J)):A2$=STR$(T(1,J))
3030 A1$=A1$+","+A2$
3040 PRINT £10,A1$
3050 NEXTJ
3060 RETURN
3070 REM ------------------------------
3080 REM  ****CATCHES DISC FULL ERRORS****
3090 REM
3100 REM
3110 ER=ERR
3120 IF ER)38AND ER(41 THEN3150
3130 TEXT:PRINT "***ERROR*** AT"; ERL
3140 ERROR ER:STOP
3150 PRINT"DISC FULL"
3160 ON ERROR GOTO3110
3170 CLOSE £10
3180 INPUT "NEW DISC? YES/NO  ",A$
3190 IF A$="YES" THEN RESET:GOTO3210
3200 RESUME
3210 INPUT "NEW FILE NAME  ",A$
3220 INPUT"WHICH DRIVE A:,B:,C:,D:, ?. ",E$
3230 A$=E$+A$
3240 IF LOOKUP(A$)=0THEN 3260
3250 ?"FILE EXISTS":GOTO 3180
3260 CREATE £10,A$
3270 IF ERL(1000THEN GOTO 230
3280 PRINT£10,"%"+D$,NU
3290 GOTO 2920
3300 REM ------------------------------
3310 REM  ****CATCHES ½Z AND CLOSE FILES****
3320 REM
3330 REM
3340 CLOSE£10
3350 TEXT:POKE &FBFF,0
3360 PUT 19
3370 STOP
3380 REM ------------------------------
3390 REM ****RECIPRICAL EXTRAPOLATION****
3400 REM
3410 REM
3420 FOR I=I1-N1+1 TO I1
3430 FOR W=1TO 5
3440 IFA(2+AS,W,I)=0 GOTO 3460
3450 A(2+AS,W,I)=1/A(2+AS,W,I)
3460 IFA(1+AS,W,I)=0 GOTO 3480
3470 A(1+AS,W,I)=1/A(1+AS,W,I)
3480 NEXT W
3490 GOSUB 1950
3500 FOR W=1TO 5
3510 IF A(2+AS,W,I)=0 GOTO 3530
3520 A(2+AS,W,I)=1/A(2+AS,W,I)
3530 IF A(1+AS,W,I)=0 GOTO 3550
3540 A(1+AS,W,I)=1/A(1+AS,W,I)
3550 NEXT W
3560 NEXT I
3570 RETURN
3580 FOR J1=1TO LEN(S$)
3590 PLOT X,Y,ASC(MID$(S$,J1))
3600 X=X+2
3610 NEXTJ1:RETURN
```