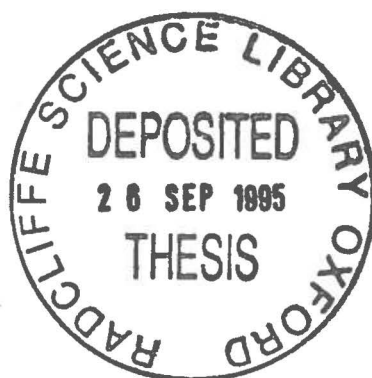


THE EFFECTS OF PROLONGED HYPOXIA ON RESPIRATION IN MAN

Thesis submitted for the degree of Doctor of Philosophy at the
University of Oxford



Luke Sebastian Geoffrey Eliot Howard
Christ Church

Hilary Term 1995

To My Parents
and my Darling Elizabeth

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ACKNOWLEDGEMENTS

I would like to express my thanks and appreciation to the following people who supported me, both with wisdom and friendship, throughout the time it took to complete this thesis.

I would like to thank first and foremost my supervisor, Dr Peter Robbins, for his patient guidance and dedicated supervision.

I owe much thanks to Dr Marc Poulin, a colleague and friend, who helped so much with the 96-hour study. Without him, it could not have happened.

I would also like to thank Dr Ian Clement for introducing me to the laboratory apparatus and for many interesting and amusing discussions.

I thank also Professor Don Paterson, Dr Keith Dorrington, Dr Mohammed Qayyum, Dr Clifford Barlow, Dr Jaideep Pandit, Dr Beatrix Nagyova and Mrs Pei-Ji Liang for their companionship throughout my time in this laboratory. I also thank Mr Mark O'Neill for his 'humour'.

I am deeply indebted to Mr David O'Connor for his technical assistance in constructing the chamber and for his willingness to help at all hours. I also thank him for his friendship and English sense of humour which always brought things into perspective.

I also thank Dr James Seymour Huntley for setting such a fine example of academic excellence and dedication to detail. I also thank him for the many stimulating discussions we shared over late-night port and Vincents' lunches.

I would like to thank the S.E.R.C. for providing the funds for my maintenance and fees and The Wellcome Trust for providing the funds for most of my research.

I thank Professor Colin Blakemore, for allowing me laboratory space.

Without the help of my moral tutor, Dr Ian Thompson, this would never have started and I am grateful to him.

I am eternally grateful for the support of my fiancée, Elizabeth, which has been loving, understanding and forgiving. I am also indebted to her for her direct assistance with this thesis.

I would like to thank Father David Albert Jones O.P. for his support and for his spiritual guidance. I would also like to thank Blackfriars, Oxford for their hospitality in these last days.

Finally, I would like to thank all my subjects who endured many hours of uninterrupted breathing and social deprivation for the sake of this research.

ABSTRACT

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D.Phil. Thesis, Hilary Term 1995

The Effects of Prolonged Hypoxia on Respiration in Man

This thesis is concerned with the effects of prolonged hypoxia on ventilatory control in humans.

Chapter 1 reviews some of the relevant studies in animals and humans relating to this thesis.

Chapter 2 describes the experimental apparatus and technique employed in dynamic end-tidal forcing using a computer-controlled gas-mixing system. It also details some of the basic analytical procedures used.

Chapter 3 describes the construction of a chamber for the long-term control of end-tidal gases. Preliminary testing showed this technique to be successful.

Chapter 4 documents the ventilatory response to 8 hours of isocapnic and poikilocapnic hypoxia. Ventilation rose dramatically during isocapnic hypoxia, but not during poikilocapnic exposure. The next chapter investigated the mechanisms responsible for these changes.

Chapter 5 assessed the changes in the ventilatory response to acute changes in hypoxia during the isocapnic and poikilocapnic hypoxic exposures of Chapter 4. Hypoxic sensitivity and hypoxia-independent ventilation increased during both hypoxic exposures and the changes were not significantly different between protocols. It was, therefore, proposed that hypoxia *per se* was responsible for the changes in ventilation during isocapnic hypoxia. Hypoxic sensitivity was thought to rise as a result of increased carotid body function. However, the mechanism responsible for the rise in hypoxia-independent ventilation was not clear.

Chapter 6 repeated the experiments of Chapters 4 and 5, but over a 48-hour period of hypoxic exposure and over the 48 hours of deacclimatization subsequent to the exposure. In addition, measurements of the eupnoeic ventilation and hypercapnic response were made. Ventilation continued to rise in the isocapnic exposure after 8 hours and also showed a gradual, but smaller, increase during the poikilocapnic exposure. Eupnoeic ventilation rose during both exposures and fell during deacclimatization towards the pre-exposure levels. These changes were similar in both protocols. The changes in hypoxic sensitivity and hypoxia-independent ventilation suggested that hypoxia was the major factor in determining the changes in respiratory control during prolonged hypoxia, in both poikilocapnia and isocapnia. Hypoxia was proposed to be the principal factor in the early stages of altitude acclimatization, and not the associated acid-base changes.

Chapter 7 describes the attempt to find a protocol to measure the hypoxic response (ventilation vs. P_{O_2}). This initial attempt was not successful, but, with modifications, the basic form of this protocol may provide a suitable test of the hypoxic response.

Chapter 8 presents a brief summary of the findings of this thesis and suggestions for future work.

CHAPTER 1

INTRODUCTION

*And the LORD God formed man of the dust of the ground,
and breathed into his nostrils the breath of life;
and man became a living soul.*

Genesis 2 v. 7

EARLY OBSERVATIONS

That pulmonary ventilation is required to maintain life has doubtless been known for millennia through observations of strangulation and choking. In 1543, Vesalius proved empirically that the opening of the thorax, and thus the collapse of the lungs, did not result in death when air flow was maintained by a pump. In 1667, Hooke repeated Vesalius's experiments, but punctured the lungs and forced air through the trachea which escaped through the holes on the surface of the lung. He established that life could be maintained in this way and concluded that thoracic movements themselves are not necessary for life, except to ensure air flow to the lungs. In 1789, Seguin and Lavoisier proposed that oxygen, "the respirable portion of air", was essential for respiration and a year later, Lavoisier suggested in a letter to Black, published in 1887, that hypoxia, a lack of oxygen, could cause a stimulation of ventilation. This was later confirmed in dogs by Pflüger (1868). In 1878, Paul Bert published La Pression Barométrique detailing his pioneering work using decompression chambers which, for some, earned him the title of "the father of altitude physiology" (Dejours, 1966). He showed that breathing air at reduced barometric pressure, as at altitude, was dangerous and he attributed this danger to hypoxia, since breathing oxygen at this same pressure restored function.

At about the same time, Miescher-Rüsch (1885) noted that carbon dioxide acted as a stimulus to pulmonary ventilation and was indeed more potent than hypoxia. Haldane and Priestley (1905) posited the involvement of carbon dioxide in a chemical feedback loop on ventilation because of the dependence of the partial pressure of carbon dioxide (P_{CO_2}) in the alveolar air on ventilation and the control of P_{CO_2} during exercise and hypoxia. They suggested that CO_2 might be the controlling link between ventilation and metabolism.

In 1913, Douglas, in collaboration with Haldane and others, produced the first comprehensive account of the time courses of the changes occurring during exposure to altitude on Pike's Peak, Colorado (Douglas *et al.*, 1913). They showed that the immediate

fall in alveolar P_{O_2} on arrival at altitude, caused by the drop in barometric pressure, was partially alleviated over a period of days to weeks by a gradual increase in ventilation. In association with the rise in ventilation and alveolar P_{O_2} , there was a steady fall in alveolar P_{CO_2} . The progressive increase in ventilation has become known as acclimatization.

STRUCTURES INVOLVED IN THE CONTROL OF RESPIRATION

Respiratory control is thought to involve a number of feedback and feedforward mechanisms (Figure 1.1). During steady state, the feedforward variables are not affected by changes in ventilation, unlike those of the feedback system. These feedback variables (arterial P_{O_2} , P_{CO_2} and H^+) are detected by central and peripheral chemosensitive structures and relayed neurally to the respiratory control centre lying deep in the medulla. Afferent neurones leading from this respiratory controller carry an output, which results in co-ordinated movements of the chest and diaphragm, giving rise to a level of ventilation appropriate for the current chemical status of the arterial blood and brain interstitial fluid.

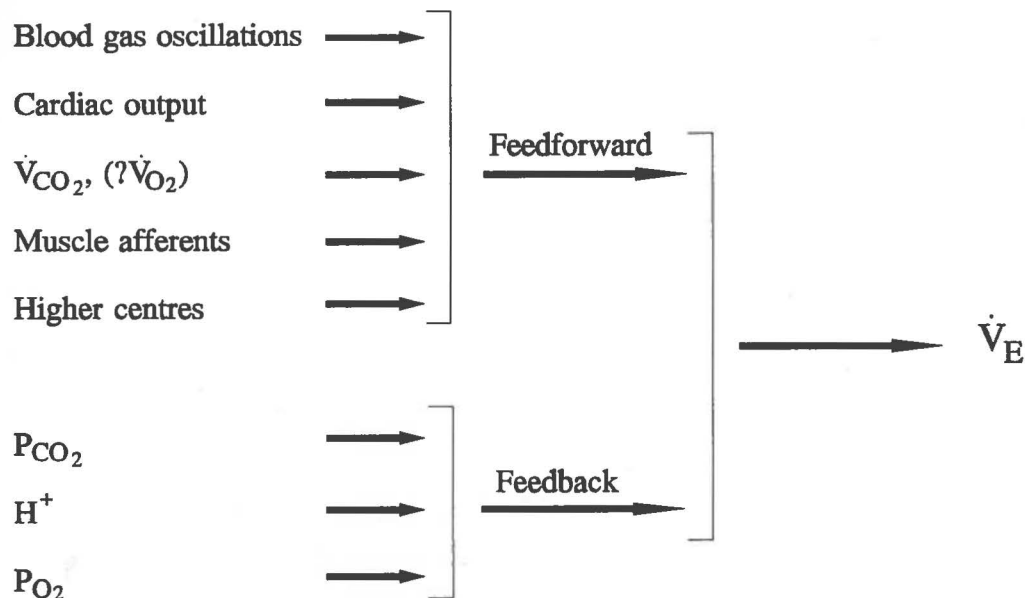


Figure 1.1: Feedforward and feedback contributions to the control of ventilation. (Adapted from Cunningham *et al.*, 1986).

Peripheral chemosensitive structures have been located in the carotid bodies, situated at the bifurcation of the carotid arteries, and in the aortic body in the arch of the aorta. They are richly perfused with arterial blood and can rapidly detect changes in P_{O_2} , P_{CO_2} and H^+ , although CO_2 is proposed to act indirectly on the chemoreceptors through H^+ (reviewed by Fidone and Gonzalez, 1986). In addition, there is much evidence, mainly in the cat, to suggest that multiplicative interaction between P_{O_2} and (P_{CO_2}/H^+) takes place in the carotid

and aortic bodies in determining the peripheral chemoreflex drive (reviewed by Cunningham *et al.*, 1986). Of these two peripheral chemosensitive bodies, the carotid body is more important for respiratory control and provides almost all the information concerning the blood gases and pH (reviewed by Honda, 1992).

Although alterations in arterial P_{CO_2} (P_{aCO_2}) can control ventilation via the peripheral chemoreflex, the dominant chemosensitive mechanism responding to changes in P_{aCO_2} is that of the intracranial or central chemoreceptors, located just below the surface of the ventral medulla (Mitchell *et al.*, 1963). Like the peripheral chemoreceptors, the unique stimulus of the central chemoreceptors is thought to be H^+ and more specifically, it is believed to be the transmembrane H^+ gradient of the chemosensitive cell (Xu *et al.* 1991,1992). However, as H^+ cannot cross the blood-brain barrier, only P_{aCO_2} stimulates these receptors [except during a state of a chronic arterial pH imbalance (Mitchell and Singer, 1965)]. Because of the relatively greater spatial separation of the central chemoreceptors from the arterial blood compared with the peripheral chemosensitive cells, the dynamics of the ventilatory response to step changes in P_{aCO_2} via the central chemoreflex (pure delay = 13.7 sec; time constant = 180 sec) are slower than those of the peripheral chemoreflex to changes in P_{aCO_2} and arterial P_{O_2} (P_{aO_2}) (pure delay = 9.7 sec; time constant = 14.8 sec) (Bellville *et al.*, 1979). The contribution of the peripheral and central chemoreflexes to the total ventilatory drive is thought to be independent and additive at the respiratory controller (van Beek *et al.*, 1984, Clement *et al.*, 1992), although there may be some small interaction (Bellville *et al.*, 1979; Robbins *et al.*, 1988). Figure 1.2 shows a scheme of the peripheral and central chemoreflexes and their contribution to ventilatory drive.

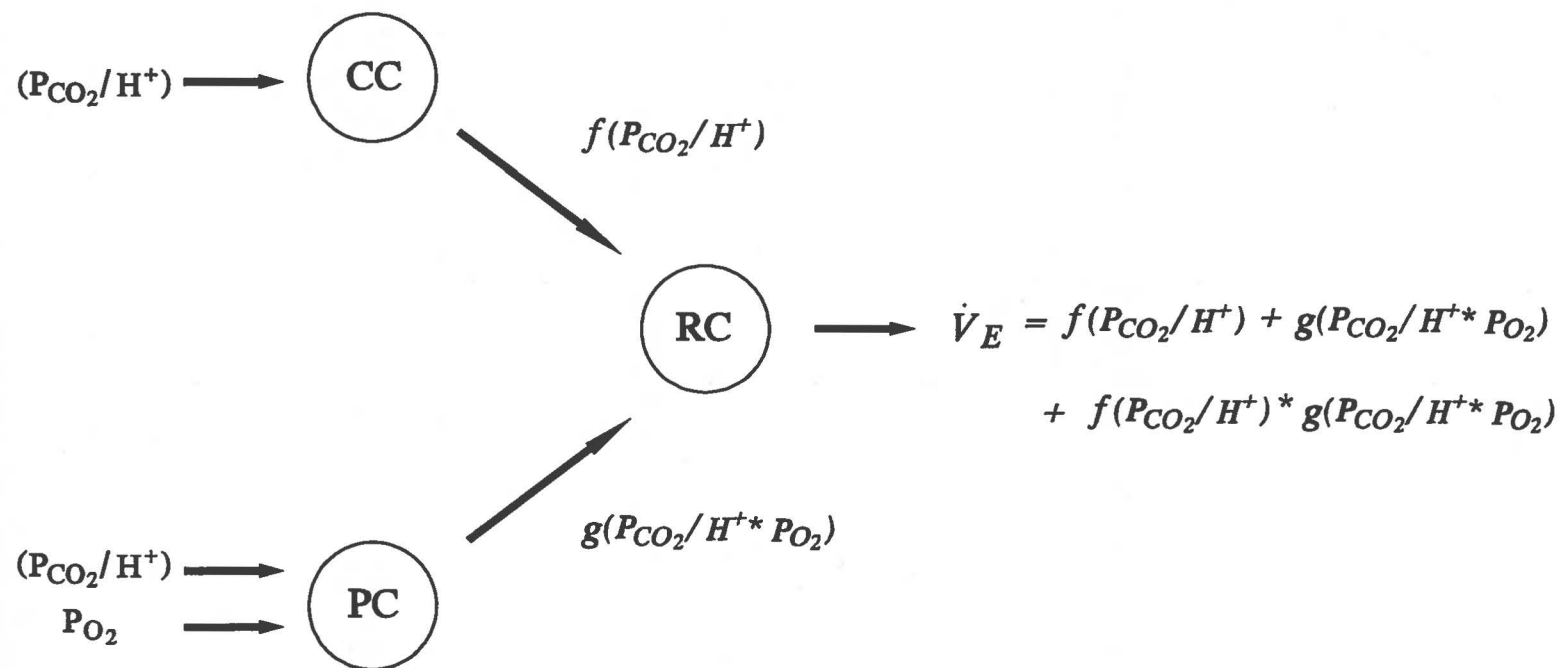


Figure 1.2: A schematic diagram illustrating the interactions between feedback stimuli. CC - central chemoreceptors; PC - peripheral chemoreceptors; RC - respiratory controller. $f(P_{CO_2}/H^+)$ - output of the central chemoreceptors as a function of P_{CO_2}/H^+ ; $g(P_{CO_2}/H^+ * P_{O_2})$ - output of the peripheral chemoreceptors as a function of P_{CO_2}/H^+ and P_{O_2} .

VENTILATORY RESPONSES TO HYPOXIA

In healthy subjects, hypoxia can be caused either by a decrease in the fractional concentration of O_2 in the air or by a reduction in the barometric pressure, as at altitude. The time course of the ventilatory response to hypoxia can be divided into four consecutive categories:

- 1) Acute hypoxia - from seconds to a few minutes;
- 2) Sustained hypoxia - from a few minutes to half-an-hour;
- 3) Prolonged/chronic hypoxia - from hours to days, weeks and years;
- 4) Life-long hypoxia - in life-long residents and natives at high altitude.

Although this is not true of all other species, each time course has elicited a different response in man. These responses are summarized in Figure 1.3.

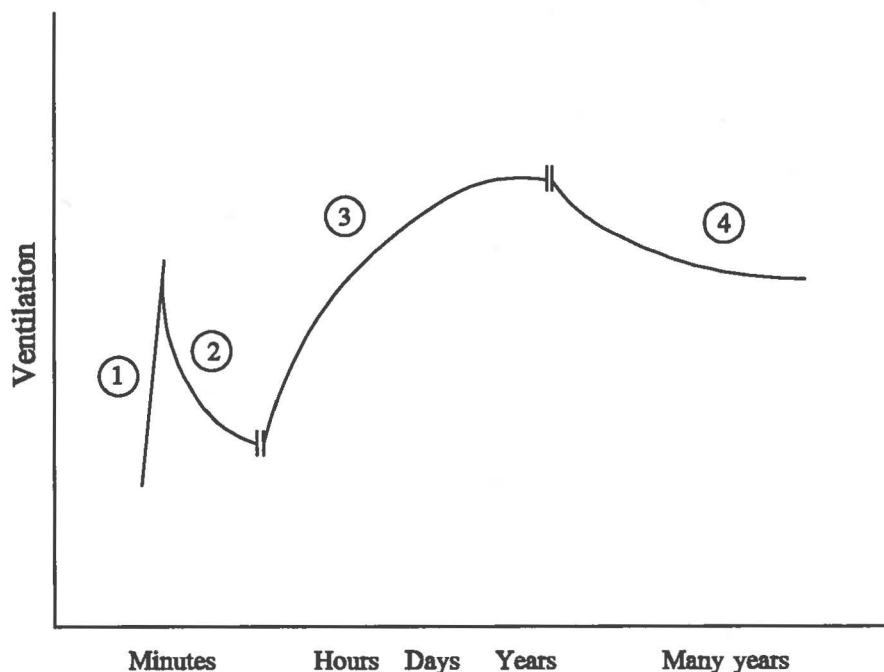


Figure 1.3: The ventilatory response to four categories of hypoxia. (1 - Acute hypoxia; 2 - sustained hypoxia; 3 - prolonged/chronic hypoxia; 4 - life-long hypoxia).

Acute hypoxia

On initial exposure to hypoxia, ventilation rises sharply and reaches a maximum within two minutes. The change in ventilation is termed the acute hypoxic ventilatory response (AHVR). Several functions have been formulated to describe the total ventilation as a function of P_{aO_2} . Cunningham *et al.* (1964) used a hyperbolic relationship; Kronenberg *et al.* (1972) employed an exponential function and Rebuck and Campbell (1974) suggested that ventilation was linearly related to the oxygen desaturation of the arterial blood. Carotid bodies are said to be primarily responsible for detecting changes in arterial P_{O_2} and triggering the ventilatory response. Studies in which the carotid bodies are non-functional have demonstrated no AHVR (Honda *et al.*, 1979).

The magnitude of the acute ventilatory response also depends upon P_{aCO_2} . When hypoxia is non-isocapnic - *i.e.*, poikilocapnic (from the Greek ΠΟΙΚΙΛΟΣ, meaning varied) - the ventilatory response is smaller than that of isocapnic hypoxia, as the initial hypoxia-induced hyperventilation causes a hypocapnic alkalosis which blunts the AHVR.

Sustained hypoxia

Following the initial rise, there is a drop in ventilation over 10 to 20 minutes in poikilocapnic hypoxia to a level above that of the pre-hypoxic control ventilation. This decline has been attributed to a fall in arterial P_{CO_2} resulting from the "blowing off" of CO_2 from the CO_2 stores of the body. However, a decline in ventilation over the same time course is seen in isocapnic hypoxia although ventilation settles to a level slightly higher than that observed during poikilocapnic hypoxia (Easton *et al.*, 1986; Edelman *et al.*, 1973; Weil and Zwillich, 1976; Weiskopf and Gabel, 1975). This decline has been termed hypoxic ventilatory decline (HVD). The mechanisms underlying HVD are still unclear for two reasons. First, there may be cross-species differences; secondly, studies carried out under conditions of anaesthesia produce dramatically different results from similar protocols in conscious animals (van Beek *et al.*, 1984).

Prolonged/chronic hypoxia

Most studies over this time course have been carried out under poikilocapnic conditions, either at altitude or in hypobaric chambers. Researchers have been able to study the early stages of the ventilatory response to prolonged poikilocapnic hypoxia by rapid reduction of the barometric pressure inside a chamber. Moore *et al.* (1986) demonstrated a rise in ventilation of the order of 2 to 3 l.min⁻¹ and a fall in PaCO₂ of approximately 4 mmHg during the first 4.5 hours of exposure to hypobaric hypoxia, simulating an altitude of 4,800 m. This rise in ventilation continues over days to weeks both at altitude (Douglas *et al.*, 1913) and simulated altitude (Åstrand, 1953) and is associated with an increased ventilatory response to acute hypoxia (Forster *et al.*, 1971, 1974; Goldberg *et al.*, 1992; Sato *et al.*, 1992, 1994; White *et al.*, 1987) and a leftward shift of the curve describing ventilation as a function of arterial Pco₂ (CO₂ response) (Åstrand, 1953; Eger *et al.*, 1968; Forster *et al.*, 1971; Kellogg *et al.*, 1957; Michel and Milledge, 1963; Sato *et al.*, 1992, 1994; White *et al.*, 1987). The question of whether the slope of the CO₂ response increases over time at altitude or stays the same remains a controversial one (Cruz *et al.*, 1980; Eger *et al.*, 1968; Forster *et al.*, 1971; Michel and Milledge, 1963; Sato *et al.*, 1992, 1994; White *et al.*, 1987). The rise in ventilation is also associated with a metabolic compensation of the respiratory alkalosis manifested by a progressive fall in arterial and cerebrospinal fluid (CSF) [HCO₃⁻] (reviewed by Dempsey and Forster, 1982).

Huang *et al.* (1984) suggested that HVD may extend into the first day of ventilatory acclimatization to hypoxia (VAH) and may even continue to blunt the long-term ventilatory acclimatization response. Indeed, a recent study over a 12-day sojourn at altitude by Sato *et al.* (1994) has shown the persistence of a steady level of HVD that underlies the acclimatization process. As HVD can be selectively observed only under isocapnic conditions, prolonged exposure to isocapnic hypoxia might help determine the role of HVD during the first hours and days of VAH. Only two experiments have attempted to do this in human subjects (Cruz *et al.*, 1980; Eger *et al.*, 1968): however, there were no ventilatory

measurements adequate for any conclusions concerning the nature of the ventilatory changes following HVD.

It is during the first few days of acclimatization that some human subjects suffer from acute mountain sickness (AMS), of which the principal symptoms are headache and nausea. Moore *et al.* (1986) established a correlation between the degree of ventilatory acclimatization and severity of symptoms of AMS, where asymptomatic subjects tended to show a greater degree of ventilatory acclimatization. More recently, it has been speculated that AMS is related to cerebral oedema secondary in part to hypoxic cerebral vasodilation and elevated cerebral capillary hydrostatic pressure. This results in reduced brain compliance with compression of intracranial structures (Krasney, 1994; Yang *et al.*, 1994). Therefore, subjects showing a greater degree of VAH are likely to suffer less from symptoms of AMS as a result of reduced cerebral vasodilation and capillary pressure.

Life-long hypoxia

After many years spent at high altitude, the ventilatory response to hypoxia diminishes (hypoxic desensitization). Weil *et al.* (1971) showed that the hypoxic responsiveness of high-altitude natives (born and living at high altitude) was lower than that of high-altitude residents (born at sea level, living at altitude), which in turn was less than that of lowlanders. It is thought that hypoxic desensitization involves a central depressive mechanism (Weil, 1986).

Much attention has been devoted to the ventilatory response to both acute and sustained hypoxia. More recently, research, in this laboratory and others, has focused on sustained isocapnic hypoxia to characterize HVD. Most studies of ventilatory acclimatization to hypoxia have documented the response under poikilocapnic conditions. The effect of long-term exposure to isocapnic hypoxia on the respiratory control system has been less thoroughly examined. The purpose of this thesis is to address this area of research. It is,

therefore, worthwhile briefly to review what is known about the mechanisms involved in HVD and, in more detail, VAH.

HYPOXIC VENTILATORY DECLINE

There is no doubt that the carotid bodies are responsible for the initial ventilatory response to acute hypoxia (Honda, 1992). However, the mechanisms responsible for the subsequent ventilatory decline in sustained hypoxia, and their sites of origin, remain unclear. Some animal studies have produced evidence for the involvement of a central mechanism, while others have suggested a role for the peripheral chemoreflex.

Investigations in anaesthetized animals have reported good evidence for the central origin of HVD. For example, carotid body-denervated cats demonstrate a depression of ventilation on exposure to hypoxia (Gautier and Bonora, 1980; Lahiri, 1976). In addition, van Beek *et al.* (1984) established that perfusion of the brain stem with hypoxic blood and systemic circulation with euoxic blood resulted in HVD, implying a central origin for HVD. A cross-perfusion technique used by Lee and Millhorn (1975) illustrated similar findings in anaesthetized, carotid body-denervated dogs, provided that the carotid bodies were perfused with euoxic or hyperoxic blood (which effectively renders them inactive). Lahiri (1976) and Lee and Millhorn (1975) both reported a decrease in the ventilatory sensitivity to CO_2 , but van Beek *et al.* attributed this to vagotomy. Their study, carried out on intact cats, showed no change in CO_2 sensitivity.

Investigations in conscious animals produce strikingly different results. Carotid body-denervated cats in the conscious state demonstrate an increase in ventilation during severe hypoxia which is completely suppressed by anaesthesia (Gautier and Bonora, 1980), and virtually no change in ventilation during moderate hypoxia (Long *et al.*, 1993). This provides strong conflicting evidence to the proposals of van Beek *et al.* (1984) that HVD is independent of the peripheral chemoreflex. Studies similar to that of van Beek *et al.* have been performed in the goat (Smith *et al.*, 1993; Weizhen *et al.*, 1992). Two principal differences were that the animals were conscious and that carotid body, rather than brainstem, perfusion was isolated from the systemic circulation. These experiments have

shown that central hypoxia results in a small rise in ventilation and no change in the CO₂ sensitivity. In contrast, CO inhalation sufficient to reduce brain oxygenation, but insufficient to stimulate the carotid bodies was shown to induce a depression of ventilation in awake ponies over 15 min (Lowry *et al.*, 1994). It is thus apparent that cross-species differences may be an important factor in explaining the disparities in these results, but in addition the state of consciousness has also been put forward. All of the above results may have some degree of validity and in fact some combination of facilitation and disfacilitation occurring as a result of central nervous system (CNS) hypoxia may be present, the balance of which may depend upon species and upon state of consciousness.

Recently, Long *et al.* (1994) have illustrated that intact, awake cats exhibit very similar ventilatory responses in sustained isocapnic hypoxia to humans. They suggest that HVD is dependent upon peripheral chemoreceptor input and may result from changes in the structures involved in the acute ventilatory response to hypoxia. They postulate that these structures may be a central pool of neurons involved in the hypoxic, and not the hypercapnic, ventilatory response (St. John, 1981) in order to comply with the observations of van Beek *et al.* (1984). In addition, they use the results of Tatsumi *et al.* (1992a) to propose that increased central dopamine may be a contributing factor in the ventilatory depression.

Studies in the rabbit and piglet have suggested a role for the peripheral chemoreflex loop in HVD. Observations of the discharge of single afferent fibres from the carotid body show a decline in activity during sustained isocapnic hypoxia (Li *et al.*, 1990; Mulligan and Bhide, 1989; Ponte and Sadler, 1989). This phenomenon is not present in the cat, which shows no time-dependent changes in carotid body activity (Barnard *et al.*, 1987), nor in the goat, in which nerve fibre activity was found to increase during 4 hours of isocapnic hypoxia (Nielsen *et al.*, 1988). Later, ventilation in goats was reported to increase over the same time course (Engwall and Bisgard, 1990), suggesting the absence of HVD. However, a recent study by Gershan *et al.* (1994) has demonstrated ventilatory "roll-off" during hypoxia, but

their experiments were carried out under conditions of poikilocapnia and so it is hard to interpret their results.

Three hypotheses have been proposed as mechanisms involved in the development of HVD in the conscious, adult human: 1) changes in the set point of the central chemoreceptors; 2) changes in the release, accumulation and/or degradation of chemicals in the brain; and/or 3) changes in the peripheral chemoreflex sensitivity. I shall review these hypotheses with their respective studies.

Changes in the set point of the central chemoreceptors

Systemic hypoxia results in a general vasodilation (except in the lung where alveolar hypoxia produces vasoconstriction) and therefore also an increased blood flow through the brain (Ellingsen *et al.*, 1987). In the unanaesthetized cat, increased blood flow is particularly marked in the brain stem (*i.e.*, the area of the brain in which the central chemoreceptors are situated) (Neubauer and Edelman, 1984). It has been suggested that increased blood flow causes a washout of CO₂ from the brain tissue, thus decreasing the stimulus at the central chemoreceptors. However, measurements of jugular venous Pco₂ do not follow a time course that is compatible with the decline in ventilation in sustained hypoxia (Suzuki *et al.*, 1989).

Sato *et al.* (1994) believe they have shown a steady level of HVD after 12 days of altitude acclimatization. They performed tests of ventilatory sensitivity to acute hypoxia and hypercapnia with and without a period of pre-oxygenation. The idea behind pre-oxygenation was to abolish HVD. They reported that pre-oxygenation had no effect on the gain of the ventilatory response to acute hypoxia or hypercapnia, except to lower the whole response. Thus, they propose that a resetting of the central chemoreceptor set point occurs, without changing the CO₂ sensitivity. They attributed this to hypoxic acidification of the chemoreceptor area by the chemosensitive cells (Xu *et al.*, 1991).

Changes in the neurochemical balance

Studies in the rat have shown that during sustained hypoxia there is an increase in the concentration of adenosine, an inhibitor of spontaneous and evoked discharge of neurons in the brain (Winn *et al.*, 1981). Investigators have reported that aminophylline, an adenosine blocker, attenuates HVD in humans (Easton *et al.*, 1988; Eldridge *et al.*, 1985; Georgopoulos *et al.*, 1989b) and thus it is plausible that an augmented level of adenosine in the brain may be in part responsible for HVD. Gamma-aminobutyric acid (GABA), a well-known depressor of nerve transmission and a primary inhibitor in the CNS, is also thought to be a possible mediator of HVD. Iversen *et al.* (1983) established that the concentration of GABA increased in the whole brain of neonatal rats in proportion to the severity of hypoxia, the highest concentration being found in the brain stem and midbrain. Midazolam, an anaesthetic drug known to potentiate the action of GABA, has been used in low doses in two studies of HVD in humans, which have reported contradictory results. Dahan and Ward (1991) showed an increase in the magnitude of HVD with midazolam, whereas Nagyova *et al.* (1993) did not. Although these changes in the neurochemical balance do not prove the central origin of HVD, they may act as factors in its development.

Changes in the peripheral chemoreflex

Honda and Sakakibara (1991) showed that two patients with bilateral carotid body resection maintained an almost constant ventilation during isocapnic hypoxia, but that one patient with unilateral carotid body resection exhibited HVD, which suggested a role for the peripheral chemoreflex. However, the CO₂ response appeared to be depressed during sustained hypoxia in carotid body-resected humans compared with control subjects (Honda and Hashizume, 1991), but the investigators attributed this depression to decreased Pco₂ in the central chemosensitive area as a result of increased cerebral blood flow rather than to HVD (Honda, 1992). More evidence in support of a decrease in the peripheral chemoreflex sensitivity to hypoxia during HVD was produced by Khamnei and Robbins (1990), who showed that the ventilatory on-response to isocapnic hypoxia was greater than the off-response to the relief of sustained hypoxia, indicating a drop in the peripheral chemoreflex

sensitivity. During a period of sustained hypoxia, Bascom *et al.* (1990) administered extra pulses of either hypoxia or hypercapnia, which were short enough not to effect changes in the central chemoreceptor drive, and demonstrated a significant decrease in the ventilatory response to the hypoxic pulses, but not to the hypercapnic pulses. Since single carotid body afferent fibres are non-specific to the type of stimulus (*i.e.*, hypoxia and/or hypercapnia) (Lahiri and DeLaney, 1975), Bascom *et al.* proposed that a specific decrease in the hypoxic sensitivity was occurring at the carotid body.

Easton *et al.* (1986) reported a correlation between the AHVR and HVD and therefore suggested that the site of origin of HVD was the same for that of AHVR (*i.e.*, the carotid body). This study was supported by a series of further studies in which AHVR was increased by almitrine, a carotid body stimulant (Georgopoulos *et al.*, 1989c), and hypercapnia (Georgopoulos *et al.*, 1989a; Khamnei and Robbins, 1990) and varied using different levels of hypoxia (Bascom *et al.*, 1992). All showed a positive correlation between AHVR and ensuing HVD. In an experiment by Bascom *et al.* (1991), dopamine (a naturally occurring inhibitory substance in the carotid body), infused intravenously, and domperidone (a peripheral dopamine blocker), taken orally, were both found to affect the magnitude of AHVR, but leave HVD unchanged. It appears, therefore, that AHVR and HVD may be linked at the carotid body, but may be modulated distinctly.

VENTILATORY ACCLIMATIZATION TO HYPOXIA

Ventilatory acclimatization to hypoxia (VAH) is characterized by a progressive, time-dependent rise in ventilation and an associated drop in alveolar and arterial Po_2 . The time course of the changes in these parameters depends not only upon the species, but also upon the severity of hypoxia. In humans exposed to an altitude of approximately 4,300 m (Mt. Evans, Colorado), maximal hyperventilation is achieved after 10 days (Forster *et al.*, 1975), whereas above 8,000 m (Mt. Everest), ventilation is estimated to take at least 30 days to reach maximum (West, 1991). In association with these rises in ventilation, PaCO_2 falls from an average of 40 mmHg to 25 mmHg at 4,300 m (Forster *et al.*, 1975) and alveolar Pco_2 to 7.5 mmHg at the summit of Everest, 8,848 m (West *et al.*, 1983). Generally, animals tend to acclimatize to altitude more rapidly than humans, except for the rat which shows a similar time course (Olson and Dempsey, 1978; Dempsey and Forster, 1982). Goats are almost completely acclimatized after 4 to 8 hours (Busch *et al.*, 1983; Engwall and Bisgard, 1990; Forster *et al.*, 1981; Smith *et al.*, 1986) and cats show significant acclimatization after 48 hours at 15,000 ft (Vizek *et al.*, 1987b).

A number of mechanisms responsible for VAH has been proposed, which I shall divide into 3 sections: 1) acid-base changes in the blood and in the brain; 2) increased CNS excitability and 3) peripheral chemoreceptor mechanisms.

Acid-base changes

Haldane *et al.* (1919) demonstrated that the kidneys excrete HCO_3^- from the blood during acclimatization to altitude and it was presumed that this metabolic acidosis was responsible for the increased ventilation. However, it became evident that arterial blood becomes alkaline at altitude despite the excretion of HCO_3^- . With the discovery of the carotid and aortic chemoreceptors, the alkalinity was accounted for by a respiratory alkalosis resulting from the acute ventilatory response to hypoxia. Further studies have shown that arterial pH is only slightly above normal despite a lower PaCO_2 in the acclimatized state,

confirming the notion of increased renal HCO_3^- excretion at altitude (Dempsey *et al.*, 1972, 1974; Forster *et al.*, 1975). However, a previous investigation has suggested that the level of HCO_3^- excretion is not sufficient to be responsible for the rise in ventilation during the first few days of acclimatization (Kellogg, 1963) and that ventilation might be driven by a more acidic pH in a compartment from which HCO_3^- is expelled faster.

At the same time as Kellogg proposed his theory, Severinghaus *et al.* (1963) showed that HCO_3^- was eliminated faster from the lumbar CSF than from the arterial blood at 3,800 m. They proposed that active transport of HCO_3^- or H^+ across the blood-brain barrier might be responsible for the selective restoration of CSF pH. Others postulated that increased lactic acid production was an alternative mechanism for part or all of CSF pH compensation (Sørensen, 1970; Sørensen and Mines, 1970). This led investigators to suggest that selective normalization of CSF pH reduced the damping effect of the decreased CSF Pco_2 on the central chemoreceptors, thereby allowing hypoxia to drive ventilation via the peripheral chemoreflex. In addition, this theory was a possible explanation for the residual hyperventilation noted during deacclimatization to altitude, since the increase in Pco_2 on return to sea level, combined with the lower CSF $[\text{HCO}_3^-]$, would produce an acid CSF pH when compared with normal. Moreover, Severinghaus *et al.* suggested that lower CSF $[\text{HCO}_3^-]$ would effect a greater change in pH, and hence ventilation, for a given change in Pco_2 on the basis of the Henderson-Hasselbalch equation:

$$\text{pH} = \text{pK}'_1 + \log \frac{[\text{HCO}_3^-]}{S \cdot \text{Pco}_2}$$

where pK'_1 is the log of the first dissociation constant for the reaction of CO_2 and water into HCO_3^- and H^+ in the CSF and S is the solubility constant of CO_2 , also in the CSF. This would account for the increased slope of the hyperoxic ventilation- Pco_2 relationship at altitude (Eger *et al.*, 1968; Forster *et al.*, 1971; Michel and Milledge, 1963; Sato *et al.*,

1992,1994). However, all the proposals of Severinghaus *et al.* assumed that CSF pH was a valid index for the pH at the central chemoreceptors and that the composition of the lumbar CSF was equal to the cisternal CSF.

The findings of Severinghaus *et al.* concerning the pH changes in the CSF have not been repeated and, indeed, much evidence has accumulated to contradict them. More recent studies have shown lumbar CSF to be alkalotic during VAH in man (Dempsey *et al.*, 1974; Forster *et al.*, 1975). What is more, they calculated that CSF pH increases or remains constant as ventilation continues to rise. As a result of uncertainty about possible cisternal-to-lumbar CSF gradients in acid-base variables during such changing conditions as acclimatization to altitude, cisternal CSF pH was measured in ponies at 3,400 m (Orr *et al.*, 1975) and at 4,300 m (Forster *et al.*, 1976; Orr *et al.*, 1975) and at both altitudes, it was found to be more alkaline than at sea level and still increasing during the first two days of exposure. Bureau and Bouverot (1975) repeated these observations in unanaesthetized dogs acclimatizing to 3,550 m for 4 weeks. Weiskopf *et al.* (1976) measured jugular venous P_{CO_2} in man during 5 days in a hypobaric chamber at 4,300 m in order to estimate cisternal CSF pH from lumbar CSF $[HCO_3^-]$ and they also found an alkaline shift in cisternal CSF pH.

In order further to characterize the changes in CSF pH at altitude, Dempsey *et al.* (1979) investigated CSF pH in men and in ponies during deacclimatization from altitude. Following one hour of deacclimatization after 3 to 5 days at 4,300 m, they found lumbar CSF to be normal in men and cisternal CSF pH to be 0.02 alkaline in ponies, whereas ventilation was only fractionally below the level reached at altitude. During the ensuing 12 hours ventilation and CSF pH fell, which is the direct opposite of acclimatization where both rise. This confirmed the notion that ventilation is not positively correlated to CSF $[H^+]$ and therefore perhaps not to the central chemoreceptor stimulus.

It remains possible that the bulk CSF $[H^+]$ does not reflect the $[H^+]$ at the chemoreceptor site. Many animal studies have shown that hypoxia acidifies the extracellular fluid over the

medullary ventral surface above the postulated sites of the central chemoreceptors, without acidifying the bulk CSF (Brown and Lawson, 1988; Davies *et al.*, 1986; Fencel *et al.*, 1979; Kiwull-Schöne and Kiwull, 1983; Millhorn *et al.*, 1984; Neubauer *et al.*, 1988; Nolan *et al.*, 1982; Xu *et al.*, 1991). This probably occurs through lactic acidosis (Dempsey and Forster, 1982; Musch *et al.*, 1981; Sørensen, 1970; Sørensen and Mines, 1970; Weyne *et al.*, 1977). However, Musch *et al.* (1983) could not establish any correlation between the time courses of lactic acid production and ventilation at altitude in rats. Xu *et al.* (1991,1992) used chronically implanted pH electrodes, lying in the subarachnoid space over areas believed to stimulate ventilation following topical application of H^+ , to detect rapid acidification during exposure to hypoxia in awake goats and anaesthetized cats. However, they showed no change in ventilatory drive, despite hypoxic acidification of these areas, with the peripheral chemoreceptors denervated, and thus, they speculated that the unique stimulus of the central chemoreceptors was their transmembrane H^+ gradient. However, Weizhen *et al.* (1992) demonstrated a small rise in ventilation maximal within 30 minutes in response to 4 hours of brain hypoxia with carotid body euoxia in the awake goat, but no rise in ventilation akin to acclimatization. There were no apparent changes in the CO_2 response, thus suggesting no role for any hypoxia-induced acid-base changes at the intracranial chemoreceptor site. These studies suggest that changes in $[H^+]$ at the central chemoreceptors elicited by hypoxia, in goats and cats, are not reflected by changes in the CSF $[H^+]$, and in addition that they do not produce any changes in respiratory control, although their short duration compared with the acclimatization time course in humans should be noted.

The Severinghaus data suggested that acclimatization depended upon the metabolic compensation of CSF pH, resulting from the associated hypocapnia of initial exposure to altitude. Thus, it was postulated that the prevention of hypocapnia would attenuate the progressive rise in ventilation during VAH. This theory was tested in two human studies (Eger *et al.*, 1968; Cruz *et al.*, 1980). They attempted to hold P_{CO_2} constant and so prevent any acid-base disturbances. In the study by Eger *et al.* (1968), no direct ventilatory measurements could be taken during the 8-hour hypoxic exposure, but they reported an

increase in the slope of the ventilation- P_{CO_2} response curve and a shift to a lower level of alveolar CO_2 , suggesting an increase in ventilation, contradicting the above postulate. Unfortunately, inadequate ventilatory measurements and poor control of CO_2 in the study by Cruz *et al.* (1980) mean that their results are hard to interpret.

However, successful exposure to prolonged periods of isocapnic hypoxia with good measurements of ventilation have been achieved in the awake goat (Engwall and Bisgard, 1990; Ryan *et al.*, 1993a). Engwall and Bisgard compared the effects of 4 hours of isocapnic hypoxia with those of poikilocapnic hypoxia and a summary of their results is presented in Figure 1.4. They found that VAH was not attenuated when CO_2 was controlled and, indeed, there was a striking progressive increase and subsequent stabilization of ventilation over the 4-hour exposure, suggesting almost complete acclimatization. In the parallel poikilocapnic study, they reported a significant development of VAH, despite the absence of any noticeable change in measured ventilation, as P_{aCO_2} fell significantly and stabilized at 4 hours. They took measurements of the CO_2 response before and after the hypoxic exposures and noted an increase in the slope (with ventilation plotted against either P_{aCO_2} or $[\text{H}^+]_{\text{a}}$) following the isocapnic protocol. In the poikilocapnic group, they observed a parallel leftward shift of the curve (*i.e.*, to a lower CO_2), indicative of a metabolic compensation of the respiratory alkalosis, but no change in slope. The authors offered no explanation for the lack of increase in slope following the poikilocapnic exposure, but remarked that this observation had been produced in two previous studies in the goat (Lahiri *et al.*, 1971; Mines and Sørensen, 1970). On return to euoxic conditions, ventilation returned to pre-exposure levels in the isocapnic group, but remained elevated in the poikilocapnic group: it was thought that the persistent hyperventilation of deacclimatization was associated with the hypocapnia of the poikilocapnic group, rather than with the mechanism producing the rise in ventilation during acclimatization. This further diminished the evidence for acid-base changes at the central chemoreceptors being of primary importance in producing VAH, but rather confirmed the postulate of Dempsey and Forster (1982) that they were consequences of it.

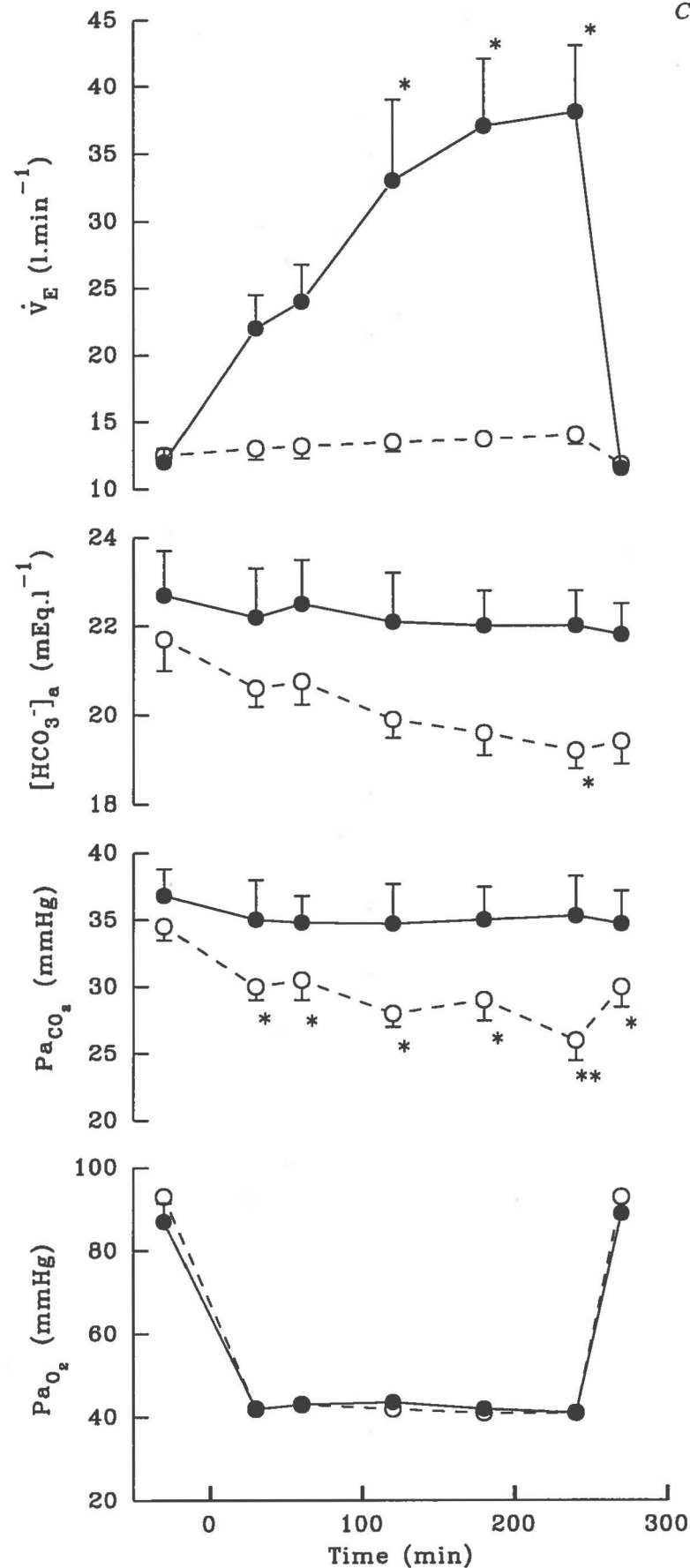


Figure 1.4: From top to bottom - the ventilatory response, arterial bicarbonate, arterial PCO_2 and arterial PO_2 in awake goats exposed to 4 hours of isocapnic hypoxia (closed circles) and poikilocapnic hypoxia (open circles). * Significantly different from control ($p < 0.05$); ** significantly different from $t = 0.5$ hours ($p < 0.05$). Adapted from Engvall and Bisgard (1990).

Increased CNS excitability

It has been speculated that increased excitability of the central nervous system may be involved in the ventilatory adaptations associated with VAH. Smith *et al.* (1962a,b) demonstrated that hyperventilation persists in paretic patients following the relief of 24 hours of artificial ventilation with a respirator. They also showed that hyperventilation persisted when the subject was maintained isocapnic during artificial ventilation. This is generally known as hyperventilation-induced hyperpnoea and may be due to the facilitation of CNS activity, which in this case was independent of hypoxia. In addition, Forster *et al.* (1975) and Schmeling (1977) have reported increases in CNS activity in EEG studies in humans at altitude, suggesting a role for CNS mechanisms in VAH.

Ou *et al.* (1983) compared intact and decerebrate cats and showed that suprapontine structures could contribute to VAH at 5,500 m, but that VAH was observed even in their absence. Gallman and Millhorn (1988) demonstrated a long-term facilitation of ventilation following only 10 minutes of hypoxia in peripheral chemoreceptor denervated cats. Sato (1990) also showed the development of a facilitative component of ventilatory drive, in humans, present at 15 min after the relief of short-term (30 min) isocapnic hypoxia. He suggested that this potentiation of ventilation was not related to the central chemoreceptors, but to an unspecified element elsewhere in the CNS. In addition, he proposed that this potentiation may be partly responsible for the rise in ventilation at altitude. These observation confirms the hypothesis of Forster and Dempsey (1981) that CNS hyperexcitability is an important source of increased ventilatory drive.

Researchers have found that repeated stimulation of the carotid sinus nerve results in long-term facilitation of ventilation in both anaesthetized and decerebrate cats (Millhorn *et al.*, 1980a,b). Tenney *et al.* (1971) showed that structures rostral to the pons, principally stimulated by peripheral chemoreceptor afferents, facilitated ventilation, largely through frequency, when activated by acute hypoxia. These studies support the view that repeated peripheral chemoreflex stimulation may result in persistent hyperventilation in euoxic

conditions. Some have attributed this mechanism to the persistent hyperventilation seen in deacclimatization from altitude.

Weizhen *et al.* (1992) and Lowry *et al.* (1994) have studied the effects of CNS hypoxia on ventilation in the goat and pony, respectively, both in the awake state. As discussed in the previous section, Weizhen *et al.* showed that brain hypoxia with carotid body euoxia produced an elevation of ventilation without any associated changes in the central chemoreceptor gain or offset, but no progressive rise in ventilation. However, Lowry *et al.* did note some acclimatization in the pony with carbon monoxide (CO) inhalation sufficient to reduce CNS oxygenation, but insufficient to increase carotid body activity, although the degree of acclimatization (quantified by the drop in PaCO_2) was small when compared with the same degree of deoxygenation produced with hypoxic hypoxia. The investigators suggest that a CNS mechanism may contribute to the early stages of VAH in the pony. However, they propose that this mechanism is independent of the intracranial chemoreceptors because of the transient nature of the hyperventilation and its return to control levels on relief of CO hypoxia. Rather than highlighting differences in experimental techniques, these studies serve to emphasize cross-species differences: previous carotid body denervation studies have established that goats do not exhibit any degree of acclimatization to hypoxia after section of the carotid sinus nerve (Forster *et al.*, 1981) unlike the pony, which shows some degree of acclimatization (Brown *et al.*, 1992; Forster *et al.*, 1976).

Peripheral chemoreceptor mechanisms

Attenuation, and in some cases abolition, of VAH in carotid body resected animals has established a primary role for the peripheral chemoreflex in acclimatization (Bouverot and Bureau, 1975; Fordyce and Tenney, 1984; Forster *et al.*, 1976, 1981; Olson *et al.*, 1988; Smith *et al.*, 1986). The role of the carotid bodies in VAH has been characterized further by a group of investigators in Wisconsin using an elegantly designed perfusion model in the awake goat which allows one carotid body to be perfused separately from the systemic arterial circulation (including the CNS) with the opposite carotid body denervated (Busch *et*

al., 1985): previously, it had been established that the ventilatory responses to acute and chronic hypoxia remain unaffected following unilateral carotid body attenuation in the goat (Busch *et al.*, 1983).

The first study to be carried out using this model addressed the question of whether brain hypoxia was required for VAH (Busch *et al.*, 1985). The investigators perfused the carotid body with isocapnic hypoxic blood for 6 hours while allowing the animal to breathe freely from the atmosphere. Assuming no change in the metabolic rate and using P_{aCO_2} as an index for ventilation (Forster *et al.*, 1981), it was shown that ventilation rose steadily over the 6-hour exposure, illustrating that brain hypoxia was not required to induce VAH. With the same model, the group performed a similar study, but over 4 hours, in which the carotid body was again perfused with hypoxic blood, but the systemic blood was also maintained isocapnic by controlling the inspired gases (Bisgard *et al.*, 1986b). VAH was still produced under these circumstances indicating that the respiratory systemic alkalosis typical of VAH was not a required element. Interestingly, on relief of carotid body hypoxia in the former study where acid-base changes were allowed to take place, there was a persistent hyperventilation as seen during deacclimatization, but none was seen in the latter study. This suggests that acclimatization and deacclimatization may be produced by different mechanisms; acclimatization may occur via changes in the peripheral chemoreflex and deacclimatization as a result of metabolic compensation of the respiratory alkalosis of acclimatization, principally affecting the central chemoreflex.

In a further study using this model, the mode of stimulation of the carotid body was changed from hypoxia to euoxic hypercapnia (Bisgard *et al.*, 1986a). This produced a steady hyperventilation, but no progressive rise in ventilation as with hypoxia. Considered in parallel with the previous observations of the effects of carotid body hypoxia, this result has two important implications. First, it suggests that hypoxia is acting within the carotid body to produce the progressive rise in ventilation and secondly, it refutes the idea that VAH is caused by the conditioning of the CNS by increased carotid body afferent activity, as

individual carotid body afferents are known to respond to both hypercapnia and hypoxia (Lahiri and DeLaney, 1975). In addition, Ryan *et al.* (1993b) sectioned the efferent sympathetic innervation of the carotid body, and showed no change in VAH, thus denying any conditioning of the carotid body by the CNS.

The same group of investigators also measured the carotid body discharge from single fibre afferents in anaesthetized goats during 4 hours of isocapnic hypoxia (Nielsen *et al.*, 1988) and euoxic hypercapnia (Engwall *et al.*, 1988). The results of these studies showed a pattern in carotid body discharge similar to that of ventilation, with discharge increasing over time during hypoxia and remaining constant, but elevated, during hypercapnia. These findings are compatible with the notion that VAH may primarily result from an increased carotid body sensitivity to hypoxia.

They extended their studies to compare 4 hours of whole body isocapnic hypoxia with poikilocapnic hypoxia (Engwall and Bisgard, 1990). The bulk of their results is presented in the section discussing the role of acid-base changes in VAH. Briefly, they demonstrated that VAH can develop when CO_2 , and hence pH, is controlled. What is more, they found that both protocols produced similar increases in the ventilatory sensitivity to acute changes in hypoxia and they attributed this to a hypoxia-induced increase in carotid body hypoxic sensitivity. Not only did this divorce VAH from acid-base changes, but it also established an augmented carotid body sensitivity to hypoxia as a primary factor in VAH. In the anaesthetized cat, Vizek *et al.* (1987b) produced similar results relating to the acute hypoxic response following 48 hours of acclimatization to hypobaric hypoxia simulating an altitude of 15,000 ft. They showed that both the ventilatory response and the peripheral chemoreceptor responsiveness to acute hypoxia increased in parallel during acclimatization. Moreover, there was a greater dependence of ventilation on carotid body function following acclimatization, demonstrated by an increase in PETCO_2 following unilateral section of the carotid sinus nerve in the acclimatized cat. These observations combined suggest that

enhanced central translation of the peripheral chemoreflex is not a major factor in the increased response to hypoxia, but that augmented carotid body function is.

Although it had been established that brain hypoxia is not a required element of VAH in the goat, the Wisconsin group decided further to characterize the effects of brain hypoxia on respiratory control using the carotid body perfusion model to maintain systemic hypoxia whilst perfusing the carotid body with isocapnic euoxic blood. Weizhen *et al.* (1992) showed that 4 hours of brain hypoxia, while producing a small elevation of ventilation, had no significant effect on the sensitivity of the CNS respiratory controller to carotid body input. Using the same protocol, but over 1.5 hours, brain hypoxia was found not to affect the respiratory pattern during acute hypoxic stimulation of the carotid bodies (Smith *et al.*, 1993). These studies provided further evidence for changes occurring locally within the carotid body in response to sustained hypoxia.

Elucidation of the role of the peripheral chemoreflex in VAH in humans has been restricted to studies at altitude. A group of researchers at Wisconsin, who were early sceptics of the Severinghaus proposals, showed that the ventilatory sensitivity to doxapram hydrochloride, a carotid body stimulant, was significantly increased after a period of 2 to 3 weeks at altitude (3,100 m), but not following 5 days of metabolic acidosis attempting to mimic the augmented medullary H^+ stimulation of altitude (Forster *et al.*, 1974). This led them to believe that a mechanism could be responsible for VAH other than acid-base changes in the CSF. In the light of the above animal studies, this result could be construed as an increase in the sensitivity of the carotid body to hypoxia. Although it has been shown that doxapram principally acts at the carotid body (Bairam *et al.*, 1991), care should be taken in relating the above observations to an increased carotid body function as other sites of action of doxapram cannot be conclusively ruled out.

A number of recent studies have reported progressive increases in the ventilatory sensitivity to acute hypoxia during prolonged exposure to altitude/hypobaric hypoxia (Goldberg *et al.*,

1992; Sato *et al.*, 1992,1994; White *et al.*, 1987). In one study, the hypoxic sensitivity was still rising at 12 days (Sato *et al.*, 1994), which would indicate that the mechanism responsible for the change in hypoxic sensitivity could also be a primary factor in the continuing rise in ventilation; although the rise in ventilation appeared to tail off at 48 hours. Such findings are compatible with the idea that there may be an increased carotid body sensitivity to hypoxia during acclimatization; however, they tell us relatively little in detail. Although they bear a resemblance with the results of Engwall and Bisgard (1990) in the goat and Vizek *et al.* (1987b) in the cat, the same conclusions cannot necessarily be drawn for the human. For example, the goat acclimatizes over a far shorter time course than humans and may not manifest HVD, unlike humans, illustrating differences in respiratory control. In order further to examine the role of a possible hypoxia-induced increase in carotid body sensitivity in man during the early stages of acclimatization, changes in central and peripheral CO_2/H^+ sensitivity need to be minimized: this would require the control of arterial CO_2 .

OBJECTIVES

The main objectives of the studies presented in this thesis are:

- 1). to construct a chamber to control the end-tidal gases in humans over long periods of time (Chapter 3);
- 2). to investigate the ventilatory response to 8 hours of isocapnic and poikilocapnic hypoxia (Chapter 4);
- 3). to compare the changes in the respiratory control system between 8 hours of isocapnic and poikilocapnic hypoxia (Chapter 5);
- 4). to examine the changes in ventilation, respiratory control and acid-base status of the blood during acclimatization to and deacclimatization from 48 hours of isocapnic and poikilocapnic hypoxia (Chapter 6) and
- 5). to investigate a suitable test for determining the shape and magnitude of the hypoxic ventilatory response (Chapter 7).

CHAPTER 2

METHODS

*Elaborate apparatus plays an important part in the science of to-day,
but I sometimes wonder if we are not inclined
to forget the most important instrument in research
must always be the mind of man.*

W.I.B. Beveridge

INTRODUCTION

This chapter describes the experimental techniques and apparatus associated with some of the studies presented in subsequent chapters. The bulk of the chapter is divided into two sections to describe the measurement of respiratory variables and the technique of dynamic end-tidal forcing (D.E.F.). First, each section presents the apparatus involved in the technique and secondly, an overview of the operation of the system is given. A section at the end of the chapter deals with the initial stages involved in data analysis and statistics and another with analysis of blood samples.

The apparatus and methods described in this chapter have been documented in detail by Khamnei (1990) and have been the subject of previous laboratory publications (Robbins *et al.*, 1982a,b; Howson *et al.*, 1986; Howson *et al.*, 1987). The methods described here are essentially the same.

SUBJECTS

Experiments were performed on healthy male and female volunteers, aged 18-24. Detailed descriptions of the subjects used in each study are to be found in the appropriate chapters. All subjects gave informed, written consent before each experiment, but were naïve as to the specific objectives of the experiments. In addition, their general practitioner was informed of their involvement in the study. All measurements described in this chapter were performed at rest with the subjects seated comfortably in a chair breathing through a mouthpiece with the nose occluded. The subjects were distracted with reading material, radio or television. All the studies presented in this thesis had received prior approval by the Central Oxford Research Ethics Committee.

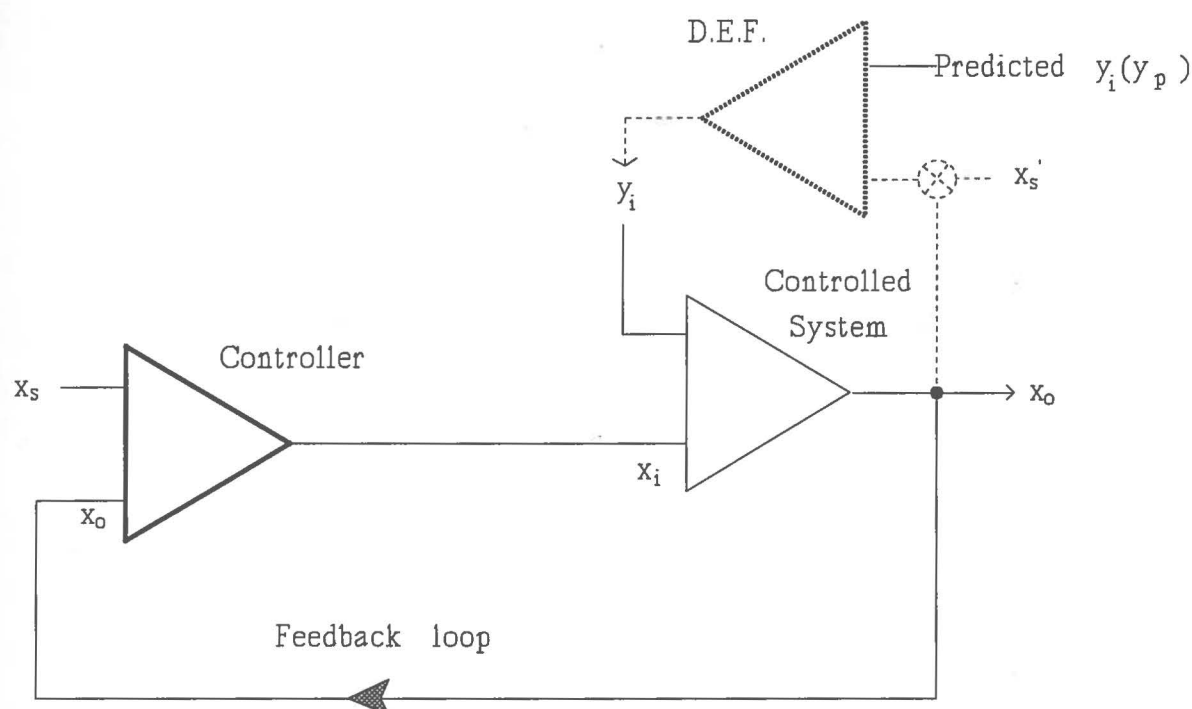


Figure 2.1: Schematic flow diagram of a respiratory chemoreflex loop (solid lines) and a flow diagram representation of the action of the dynamic end-tidal forcing system (D.E.F.) (broken lines).

OVERVIEW OF DYNAMIC END-TIDAL FORCING

The solid lines in the above schematic diagram (Fig. 2.1) represent a simple system consisting of a controller and a controlled system which aims to maintain an output, x_o , as close as possible to a set point, x_s , via a feedback loop. Thus the controller has two inputs, x_o and x_s . The controlled system also has two inputs, one from the controller, x_i (which is a function of x_o and x_s), and another, a disturbance element, y_i . Thus the output of the controlled system becomes a function of both inputs. An analogy can be drawn between the chemoreflex control loop of ventilation (described in Chapter 1) and this theoretical control system, where x_o is the arterial blood gas composition, x_i the ventilation, y_i a change in the inspired gas composition and x_s would remain as the set point of the system. Thus the chemoreflexes and respiratory centre (controller) evoke changes in ventilation via the

pulmonary apparatus (controlled system) in such a way as to minimize the deviation of the arterial gas composition from the set point, this deviation having served to act as the initial stimulus for the change in ventilation: this completes the feedback loop.

In order to study the properties of this type of controller, it is necessary to open the feedback loop. The aim of dynamic end-tidal forcing (represented by the broken lines in Fig. 2.1) is to do just this by imposing a new set point on the system (x_s') and monitoring the ensuing ventilatory changes, which now cease to affect the arterial blood gases.

In healthy subjects, the end-tidal gas composition is considered to be an accurate measure of the arterial blood gas composition. Thus, by monitoring the end-tidal gas partial pressures and comparing them with the desired values, the forcing system is able to generate the desired arterial gas profile by performing appropriate adjustments to the inspired gases. The feedback loop of the forcing system is combined with a feedforward element (predicted inspired composition, y_p) in order to prevent large swings in the inspire. The predicted inspired gas mixture is based on informed guesses of the ventilatory changes and is input to the system prior to experimentation. A fast system, as described in this chapter, can be used to generate sine wave, sawtooth and square wave changes in the end-tidal gases, thus enabling the study of the dynamic properties of the respiratory control system.

DATA ACQUISITION SYSTEM

This section describes the components, calibration and operation of the data acquisition system. A schematic representation of the whole dynamic end-tidal forcing system is given in Fig. 2.2.

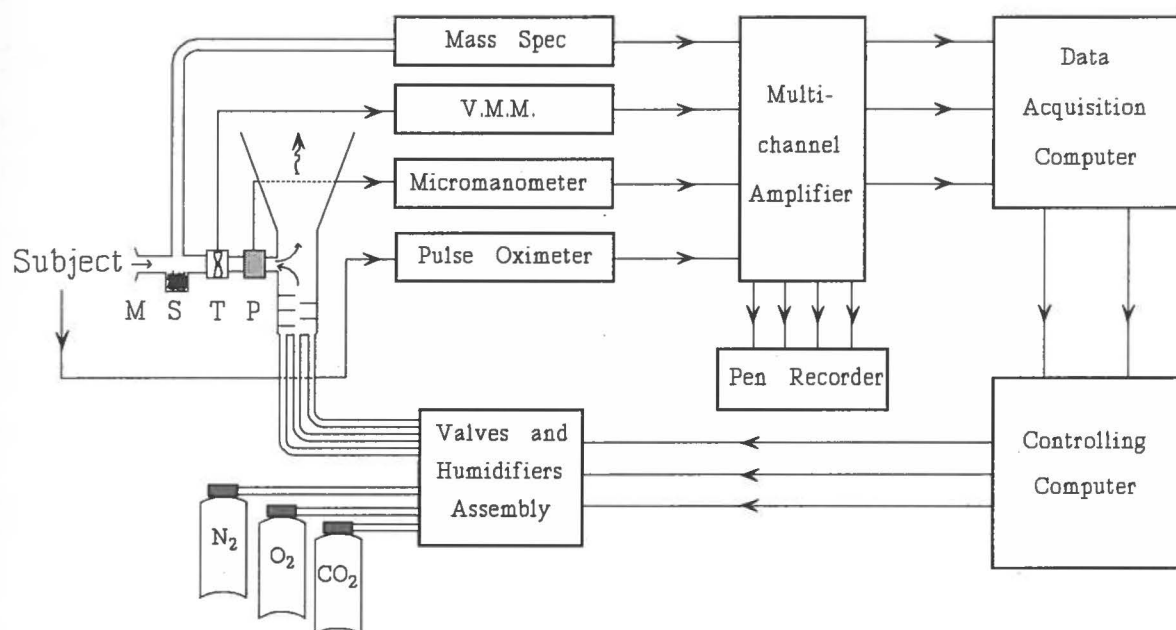


Figure 2.2: Schematic representation of the data acquisition and dynamic end-tidal forcing system. The upper part of the figure shows the elements of the data acquisition system and the lower part illustrates the computer-controlled gas-mixing system. (V.M.M. - ventilation measurement module; M - mouthpiece; S - saliva trap; T - turbine device; P - pneumotachograph.)

Apparatus

The principal elements of the data acquisition system are a mouthpiece assembly, a turbine device and associated ventilation measurement module, a pneumotachograph and associated micromanometer, a mass spectrometer, a multi-channel amplifier, a pen recorder and a data acquisition computer. A pulse oximeter is also used, although principally as a safety device.

Mouthpiece assembly. The mouthpiece assembly is the apparatus through which the subject breathes, and it forms a T-junction with the chamber through which newly mixed gas flows continuously. The assembly consists of a flexible plastic mouthpiece, a perspex tube with a saliva trap and a port for the sampling catheter from the mass spectrometer, a turbine flow cartridge, and a pneumotachograph. The perspex tube has a 60° bend in it so that the bulk of the assembly is moved from the subject's line of sight. During experiments the saliva trap is filled with cotton wool to reduce dead space and absorb saliva. The total dead space of the

mouthpiece assembly was measured at 90 ml by Khamnei (1990). Khamnei also showed that over a flow range of 5 l.min⁻¹ to 100 l.min⁻¹ the mouthpiece assembly had a small, flow-independent resistance of 0.1 mmH₂O.min.l⁻¹.

Turbine volume device. Volume flows through the mouthpiece assembly are measured by a turbine device (Cardiokinetics Ltd., U.K.) which consists of a volume cartridge, a photodetector pick-up assembly and an electronic processing module (Howson *et al.*, 1986). The volume cartridge is a transparent plastic tube incorporated into the mouthpiece, in the centre of which is a lightweight plastic impeller mounted on virtually frictionless sapphire bearings. The impeller makes approximately one revolution per 2 ml of gas flow. The pick-up assembly surrounds the impeller and consists of 4 infra red light-emitting diodes and 4 photodetectors. As the impeller rotates, the optical signal to each of the detectors is broken in sequence. The electronic processing module receives the output from the pick-up assembly and from this is able to derive both the direction and speed of rotation of the impeller. The output of the electronic processing module is an analogue signal for inspiratory and expiratory volume which is reset at each phase of the respiratory cycle. The volume signal is corrected for the effect of friction at low flows.

Khamnei (1990) studied the behaviour of the turbine device using a mechanical pump which could be made to "breathe" sinusoidally with different tidal volumes (range 0.5 to 3.0 l), frequencies (10 to 30 cycles.min⁻¹) and also with various gas compositions. The response of the turbine to different volumes was highly linear. When the frequency was changed from 10 to 30 cycles.min⁻¹, there was a mean drop in the measured volume of 1.7%, suggesting a small degree of frequency dependence. The measured volumes were virtually independent of gas composition, showing a change of less than 0.5% from pure oxygen to pure nitrogen.

Khamnei (1990) also compared the timings for respiratory phase switches measured by the turbine device with those measured by the pneumotachograph. The turbine reversal times lagged those of the pneumotachograph by up to 360 msec, presumably because of the inertia

of the impeller. Phase lags such as these make the turbine device unsuitable for determining phase switching times and instantaneous flow.

Pneumotachograph. The pneumotachograph (Fleisch, Switzerland; U.K. distributor, P.K. Morgan, Chatham, Kent) used is 27 mm in length by 27 mm in diameter and provides a measurement of flow. It also serves to sense respiratory timings from the reversal of airflow. It consists of a cylinder filled with a fine honeycomb of small tubes which provides a small, but measurable, resistance to flow (of the order of $3 \text{ mmH}_2\text{O} \cdot \text{sec} \cdot \text{l}^{-1}$), such that a small pressure drop develops across the resistance. For a given gas, this pressure drop is linear against flow, provided that flow is laminar. (Macfarlane (1985) reported that flow remained linear below $130 \text{ l} \cdot \text{min}^{-1}$.) The pressure drop across the pneumotachograph, and thus the flow through it, is transduced into an analogue voltage by a fast-responding micromanometer (Validyne Pressure Transducer, Model No. MP45-14-871); Validyne Engineering Corp., California, U.S.A.), set to $\pm 20 \text{ mmHg}$ full deflection.

Mass spectrometer. Gas close to the subject's mouth is sampled continuously by a fast-responding quadrupole mass spectrometer (Airspec MGA3000, C.A.S.E. Scientific, Biggin Hill, Kent, U.K.), at a rate of $80 \text{ ml} \cdot \text{min}^{-1}$ via a capillary tube seated in a port in the mouthpiece assembly. The mass spectrometer operates by measuring the mass to charge ratio of molecules and atoms in the gas sample. The molecules and atoms are ionized by electron bombardment and then are accelerated and passed through a fluctuating magnetic field in an almost perfect vacuum. The deflections in the trajectory of the ions induced by the magnetic field depend upon the ratio of the mass and charge of the ion. The mass spectrometer channels are tuned to respond to 4 mass/charge ratios: 32 for O_2 ; 14 for N; 44 for CO_2 and 40 for Ar. (The mass/charge ratio of 28 for N_2 was avoided since this coincides with CO, one of the products of CO_2 that can arise due to fragmentation during electron bombardment.) The mass spectrometer output is updated every 20 msec and returns values for O_2 , N, CO_2 and Ar. The total output of the 4 channels of the mass spectrometer is constrained to give a total gas composition of 100%, to prevent drift arising from factors

such as partial blockade of the sampling catheter. Zeroing, tuning and calibration of the mass spectrometer are described below in the calibration procedure.

Pulse oximeter. Arterial oxygen saturations were monitored using a finger probe connected to a pulse oximeter (Ohmeda Biox 3740, Hatfield, Herts., U.K.). This works by measuring the absorbence of the finger at two wavelengths, relating to oxygenated and deoxygenated haemoglobin. The data are processed to remove all the non-pulsatile elements of the signal so that the absorbence of the remaining signal is related to the arterial blood, and the arterial oxygen saturation can then be estimated. In practice, the machine was suitable only for measuring oxygen saturation in the steady state, because of the lag in the response following sudden changes in alveolar oxygen tension. It was principally used as a safety device.

Eight-channel amplifier. The analogue voltage outputs from the equipment described above are passed through a laboratory-built 8-channel amplifier. This serves to condition each of the analogue signals to a range of ± 5 Volts which is the input range required by the analogue-to-digital converters (Dash-8, Metrabyte; Keithley Instruments Ltd., U.K.) used by the data acquisition computer (IBM PC AT).

Pen recorder. A second set of outputs from the 8-channel amplifier is passed to a hot stylus chart recorder (Lectromed Devices M19, U.K.). The chart recorder displays the outputs of the CO₂ and O₂ channels of the mass spectrometer, the inspired and expired volumes from the ventilation measurement module and the output of the pulse oximeter.

Calibration

Prior to each experimental period, helium was introduced into the mass spectrometer in order to ascertain background levels of current for O₂, N, CO₂ and Ar mass numbers in the complete absence of these gases (the zeroing procedure). At this time, prevailing ion currents for O₂, N, CO₂ and Ar channels were stored and thereafter used as background signals to be subtracted from the total signal for each gas. The mass spectrometer was also

tuned and calibrated with air and a standard gas, whose composition had been verified by the Lloyd-Haldane procedure (composition approx. 6.5% CO₂ and 7.5% O₂ in N₂). Tuning involved finding the appropriate peak in the mass/charge ratio spectrum. The delay time from a change in gas composition at the tip of the sampling catheter to a 50% response in the mass spectrometer output was measured at the beginning of each experimental day and was typically about 160 msec, of which about 120 msec was transit delay arising largely from the catheter dead space. A fast-responding solenoid valve, connected to the standard gas supply, was used to provide rapid changes in CO₂ and O₂ composition at the tip of the catheter. The switch operating the valve provided the data acquisition computer with a time marker for the change in gas composition at the catheter tip, which allowed the computer to calculate the time lag before receiving the change in signal from the mass spectrometer. Delay times to 50% response of greater than 200 msec suggested an increased resistance in the sampling catheter, possibly due to partial obstruction, in which case the catheter was replaced.

Before each experimental trial, the complete data acquisition system was calibrated by performing a dummy experiment using a mechanical pump to "breathe" on the apparatus. The calibration pump was set to breathe at a tidal volume of 1 l and at a frequency of 20 cycles.min⁻¹. Data were collected for 2 min with the mass spectrometer first sensing air and then standard gas. After the end of the data collection period, the calibration program, CLDATA, was run. This calculates the appropriate calibration functions for each of the measured variables using the data collected together with user specified variables for standard gas composition, pump volume and the room temperature and barometric pressure. The CO₂ and O₂ calibration functions are unconstrained straight lines (two-point calibration), whereas the inspired and expired tidal volume calibration functions are straight lines constrained to pass through the origin. The conversion factors are stored in a DOS file (*.CAL). This file is used by the gas-mixing system controlling computer during the experiment and also in the analysis of the data after the experiment (see below).

Operation

Data acquisition during an experiment (both dummy and real) is performed by the program, RTDATA, written by Drs P.A. Robbins and K.J. Stratford which runs continuously on the data acquisition computer while an experiment is in progress. The program performs a number of functions which include collecting and storing data from the apparatus described above, detecting the end-expiratory values of PO_2 and Pco_2 and passing them to the computer controlling the gas-mixing apparatus associated with the D.E.F. system.

Data concerning the experimental time, gas flow and gas composition are stored every 20 msec by the RTDATA program in a *.RAW file, where * is a user defined file root name. The speed of the data acquisition makes it necessary for the *.RAW file to be written to a virtual disk. RTDATA receives information concerning the experimental time from an internal crystal running at 1 MHz, which in turn drives two internal counters in series to provide clocks running at 1 kHz and 1 Hz.

In order that the dynamic end-tidal forcing system works efficiently, and that the appropriate adjustments are made to the inspiratory gas composition, the RTDATA program needs to pass on accurate values of the end-tidal gases to the controlling computer. This is highly dependent upon the determination of the time of end of expiration. First, the program detects the current respiratory phase from a flow direction signal from the pneumotachograph. During the expiratory phase, the cue for the end of expiration comes when the turbine device indicates a phase switch. RTDATA then allows for the inertia of the impeller of the turbine (see above) by looking back in time at the pneumotachograph record for the last reversal of flow. The time associated with this is defined as the time at which the end of expiration occurred. The advantage of this procedure is that the inertia of the impeller allows for a genuine phase switch to be identified, whereas the pneumotachograph may show spurious, multiple flow reversals during a phase switch. Having determined the end-expiratory time, RTDATA then makes some allowance for the mass spectrometer delay by looking forward in time through the CO_2 and O_2 data record by 140 msec [this value will always be slightly less

than the pure delay of the mass spectrometer (see above)]. The CO_2 and O_2 values at this time are recorded as the end-expiratory values and can be passed on to the computer controlling the gas-mixing system. Inspiratory information, not passed on to the controlling computer, is determined in the same fashion.

RTDATA also receives input from a manual event marker through a digital input/output channel and records the experimental time of any event marks. At the end of the experiment RTDATA writes a *.PK file on the computer's hard disk. This file contains time, volume and gas data associated with the end-inspiratory and end-expiratory point of each breath.

Stability of Gas Analysis

In order to exclude a drift in the mass spectrometer during an experiment, the composition of a standard gas has been measured on a number of occasions both before and after repeated experimental periods. There was found to be no significant change in the mass spectrometer estimates of Pco_2 before and after an experimental period (mean change $\pm$ S.E. = 0.01 ± 0.006 mmHg, $n=18$) (Pandit, 1993).

GAS-MIXING SYSTEM

Accurate breath-by-breath control of end-tidal Pco_2 and Po_2 is effected by means of a computer-controlled gas-mixing system. The layout of the system is shown schematically in Fig. 2.3. The hardware of the system comprises two main elements, the controlling computer and the gas mixing unit.

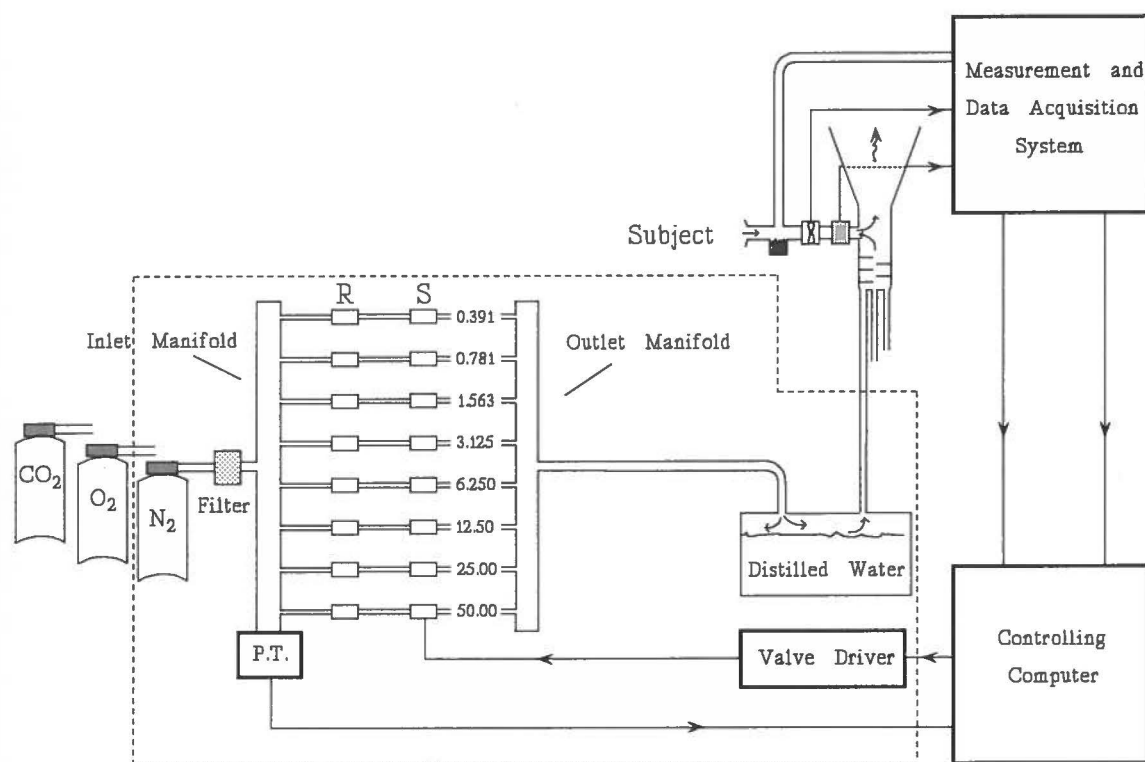


Figure 2.3: Schematic illustration of the layout of the gas-mixing system shown completely for one gas species (N₂). The flows through each channel (l.min⁻¹) at the standard supply pressure of 20 p.s.i. are shown. The resistance of each successive channel halves going from top to bottom in the illustration. (P.T. - pressure transducer; R - flow resistor (needle valve); S - solenoid valve.)

Gas Mixing Unit

Gas supply. O₂ and CO₂ are supplied from gas cylinders (British Oxygen Company) located in the laboratory, O₂ as compressed gas and CO₂ as liquid. A heating element is included in the CO₂ supply to vaporize the gas. The supply pressure from each gas-specific bank of cylinders is controlled by a two-stage regulator (M150, British Industrial Gases) and further downstream, a Linemaster single-stage regulator (M30, British Industrial Gases). The supply pressures are set to 20 p.s.i. for O₂ and 10 p.s.i. for CO₂. Liquid N₂ is stored in two 240 l tanks (Taylor Wharton, A.S. Scientific, Abingdon) outside the building. Pressure raising circuits in each of the tanks are set between 220 and 230 p.s.i. in order to drive liquid N₂ from each tank. (Only one tank is used at a time.) Downstream of the tank in use are a

Norgren Olympian single-stage regulator set at 100 p.s.i., a vaporizer constructed from 50 m of aluminium pipe and a further Norgren Olympian single-stage regulator set at 20 p.s.i..

Valve assembly. The valve assembly consists of 3 similar units, one for each of the gases. Each unit consists of a gas filter (LF-3/8-5, Festo Ltd., U.K.) to remove any particles, an inlet manifold incorporating a pressure transducer, 8 channels for gas flow running in parallel and an outlet manifold.

The pressure transducer serves to provide the controlling computer with information on the supply pressure, since the pressure regulators are unable to maintain a perfectly constant supply pressure.

Each of the 8 channels connecting the inlet and outlet manifolds consists of a needle valve flow resistor (GRO-1/80 and GRO-M5, Festo Ltd., U.K.) and a solenoid valve connected in series by 4 mm polythene tubing. The 8 parallel resistors are arranged so that the resistance of successively adjacent channels is halved (Fig. 2.3). The actual values of the resistances were set when the apparatus was built, such that the maximum flow through the valve assembly would be 100 l.min⁻¹ for N₂, 50 l.min⁻¹ for O₂ and 12.5 l.min⁻¹ for CO₂, at the respective supply pressures of 20, 20 and 10 p.s.i..

Downstream of the flow resistor in each of the 8 gas channels is a solenoid valve (MFH-2-M5, Festo Ltd. for CO₂; MC-2-1/8, Festo Ltd. for O₂ and N₂). Each valve is either open or closed and the combination of valves open and closed is under the control of the gas-mixing system controlling computer. The number of combinations possible therefore is 2⁸ or 256, giving the controlling computer the ability to select, for each gas species, any one of 256 possible gas flows ranging from zero to maximum. In order to open, each of the solenoids requires a 12 Volt d.c. input, provided by a laboratory-built power supply (valve driver). In the absence of this input, the solenoid valve is kept closed by a spring.

Expansion bags. One of the problems with the design of the valve assembly is that when any of the solenoid valves closes, the gas pressure immediately upstream of it builds up towards that of the supply pressure in the inlet manifold. When the valve opens again a burst of pressure travels downstream. In an attempt to dampen this out, anaesthetic bags (M289M, Leymed Ltd., U.K.) are incorporated into the gas circuits downstream of the outlet manifold, to act as compliant expansion chambers.

Humidifiers. In order to supply the subject with a warm, moist inspirate, heated humidifiers are incorporated into the O₂ and N₂ circuits. The humidifiers are laboratory-adapted perspex boxes (10 l volume) with gas inlet and outlet holes in the top. The boxes are kept about 90% filled with distilled water which is heated to ~30 °C by a thermostatically controlled 1 kW element (Elmatic Ltd., U.K.).

Mixing chamber. The inspirate is finally mixed when the O₂, N₂ and CO₂ gas circuits converge in the mixing chamber. The mixing chamber is a perspex cylinder with a volume of 0.12 l upstream of the T-junction with the mouthpiece assembly, from which the subject draws the inspiratory gas. The gas is thoroughly mixed by a number of perspex fins. By not mixing the inspirate until so close to the subject's mouth, the functional dead space of the system is kept very small, giving the gas-mixing system a rapid response time. During experiments, the total gas flow was designed to exceed the requirements of the subject so that all of the inspirate was supplied and the expirate washed out. If the subject's inspiratory flow happened to exceed that of the gas-mixing system at any moment, the conical outlet passage (volume approx. 2 l) downstream of the T-junction, with the mouthpiece assembly, acted as a reserve of gas to prevent inspiration of outside air.

Controlling Computer

The gas flow through each of the circuits of the gas-mixing unit is controlled by a computer (IBM PC AT) running the DEF program written by Dr P.A. Robbins. (This program was further adapted to meet the specific needs of particular protocols.) The

computer controls the combination of solenoid valves opened and closed for each gas circuit by means of an 8-bit port (DDA-06, Metrabyte; Keithley Instruments Ltd., U.K.) which communicates with the valve driver box for each gas circuit (see below).

Dynamic End-Tidal Forcing in Action

When an experiment is in progress, the process by which the gas-mixing system controlling computer successfully manipulates the inspiratory gas composition so as to force the alveolar gas composition to follow the desired time-course can be considered in two stages. First, the controlling computer decides what the inspired gas tensions should be, based on information received from the data acquisition computer (feedback stage). From this it decides what the flow for each gas should be and what combination of solenoid valve openings and closings will give these flows (output stage) and effects the necessary changes. A flow diagram representing this procedure is given in Fig. 2.4.

Feedback Stage. Following the end of expiration, the data acquisition computer identifies the measured end-tidal P_{CO_2} and P_{O_2} values in the way described above. These uncalibrated values are passed on to the gas-mixing system controlling computer (running the required version of the DEF program) which converts them to real values using the information in the *.CAL file. In order to calculate the appropriate inspiratory P_{CO_2} and P_{O_2} a feedforward/feedback procedure is used which takes 3 factors into account: 1) a predicted inspiratory partial pressure based on an estimate of the ventilatory response to the forcing function (feedforward); 2) the difference between the actual and the desired end-tidal gas value for the previous breath (proportional feedback) and 3) the sum of all previous differences (integral feedback). The calculation of the inspiratory gas partial pressure (P_I) for one gas species for breath number n can be represented as follows:

$$P_{I(n)} = P_{I_p(n)} + g_p \cdot (P_{ET_d(n-1)} - P_{ET_m(n-1)}) + g_i \cdot \left(\sum_{j=1}^{n-1} (P_{ET_d(j)} - P_{ET_m(j)}) \right)$$

where P_{I_p} is the predicted inspiratory gas tension, P_{ET_d} and P_{ET_m} are the desired and measured end-tidal gas tensions, and g_p and g_i are the proportional and integral feedback gains. The DEF program allows the values of these gains to be varied around a default value during the experiment by means of a potentiometer box. The default value of g_p is set to be 10 times that of the default g_i . The accuracy with which the desired forcing function is followed depends on the accuracy of the initial prediction and the values of g_p and g_i . If the gains are too low, the control is slack and if the gains are too high then the system can become unstable and oscillate. The integral control element serves to remove steady-state errors in the prediction, but is less effective for rapidly changing dynamic forcing functions (see Chapters 5 and 6).

Output stage. Having decided on the appropriate inspiratory gas partial pressures, the gas-mixing system controlling computer calculates the fractions of the total inspirate required to give these gas pressures (which depend on the barometric pressure). It then calculates the flow of each gas required (which depends on the total gas flow specified by the experimenter). After calculation of the O_2 and CO_2 gas flows, the balance is made up by N_2 . The final stage is to open the correct sequence of solenoid valves in the valve assembly to give the desired flow for each gas. The computer receives information about the supply pressure in the inlet manifold of the valve assembly from the pressure transducers. The DEF program then selects the appropriate combination of openings/closings from a previously determined pressure-flow table for each gas. The open/close instruction is then sent to the valve driver box for each gas as an 8 bit binary word. The valve driver then opens and closes the solenoid valves as instructed.

Speed of System. The inspiratory gas composition is updated (*i.e.*, one complete circuit of the loop in Fig. 2.4 is completed) every second and every time a new end-tidal gas value is detected. The speed of the response of the system is limited by dead space, which is minimal (see above), and by the delay in detecting the end-tidal gas composition after the end of

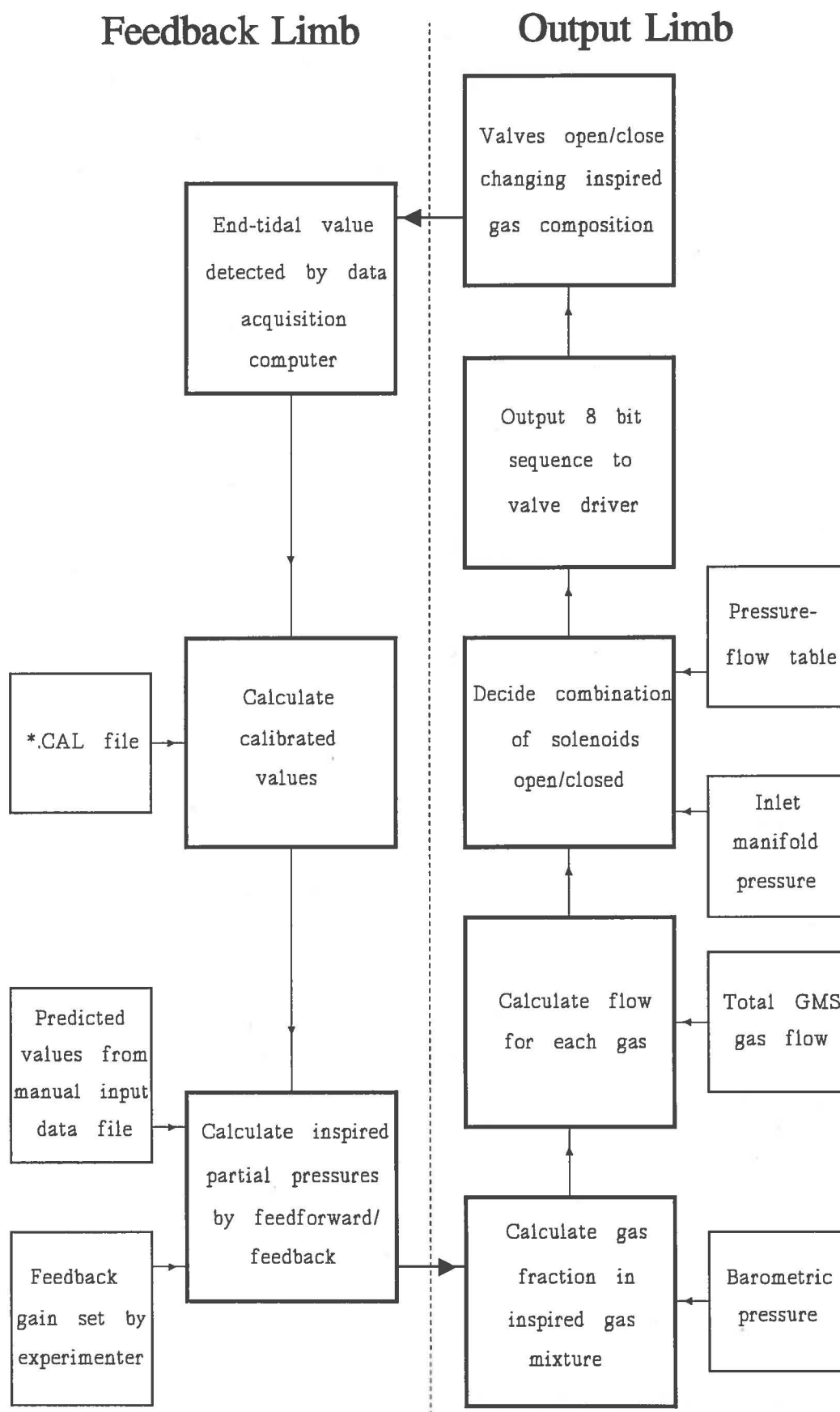


Figure 2.4: A schematic flow diagram illustrating the operation of the feedback and output limbs of the dynamic end-tidal forcing system.

expiration. This will be the longer of either the mass spectrometer delay or the turbine impeller reversal delay. The system is usually sufficiently fast to modify the gas composition of some of the subsequent inspiration following an expiration.

INITIAL DATA ANALYSIS

After the end of each experiment, the uncalibrated data in the *.RAW and *.PK files are converted into meaningful respiratory data using the calibration factors in the *.CAL file.

BRDATA program. The BRDATA program written by Drs P.A. Robbins and K.J. Stratford reads the *.RAW, *.PK and *.CAL files and writes to 4 files named *.D1, *.D2, *.D3 and *.D4. These files contain a variety of respiratory parameters described breath by breath. The *.D1 contains inspiratory and expiratory volumes, times and gas tensions, together with breath-by-breath expired ventilation calculated as expired volume divided by breath duration measured from the start of successive inspirations. The *.D2 and *.D3 files contain information regarding oscillations in alveolar P_{CO_2} and P_{O_2} , and metabolic production and consumption of CO_2 and O_2 respectively. The *.D4 file contains a record of the times of any marked events. For experiments presented in this thesis, only the *.D1 and *.D4 files were required (for direct analysis).

AVDATA program. The AVDATA program is used to bin-average the breath-by-breath data recorded in the *.D? files, over user-defined intervals. The program reads in a *.TMP file which gives the start and end periods for each averaging period. The results of the averaging are written into 3 files of the form *.M1, *.M2 and *.M3. For experiments presented in this thesis, only the *.M1 file was required for direct analysis.

CYCLE program. The CYCLE program (adapted from a program written by Dr I.D. Clement) is used to sort, into one-second time bins, the breath-by-breath data recorded in the *.D1 file collected from protocols employing dynamic oscillatory forcing functions of pre-defined period (e.g. square waves, sawtooth functions, etc.). The data are divided into intervals defined as the period of the input forcing function and these are superimposed to give a mean of all periods. The results are written into a *.AVG file.

BLOOD GAS ANALYSIS

Venous blood was taken from subjects participating in the study described in Chapter 6, and was analyzed for P_{CO_2} and pH using a blood gas analyzer (IL1306, Instrumentation Laboratory, Italy) and for O_2 saturation and haemoglobin content ($g\cdot dl^{-1}$) using a CO-oximeter (IL482, Instrumentation Laboratory, U.S.A.).

Blood gas analyzer. pH is sensed by a pH electrode which consists of a pH-sensitive glass membrane separating two solutions, one being the control solution of known pH and the other being the test solution. A potential difference, which is proportional to the pH difference between the two solutions, develops across the glass membrane. P_{CO_2} is measured using a modified form of pH electrode. The P_{CO_2} electrode consists of a pH electrode in contact with a solution contained within a CO_2 gas permeable membrane. When the electrode is in contact with a test solution, CO_2 diffuses across the gas permeable membrane until in partial pressure equilibrium with the test solution. A CO_2/H^+ equilibrium is established in the solution contained within the gas permeable membrane so that the pH of the solution is related to the P_{CO_2} of the test solution. The pH of the solution contained within the gas permeable membrane is then measured by the pH electrode.

The pH electrode is calibrated using two solutions of pH 6.838 and 7.382 and the P_{CO_2} electrode is calibrated using two humidified gases containing 5% and 10% CO_2 .

CO-oximeter. This instrument aspirates a sample of blood and haemolyzes it. A photodetector measures the absorbance of the sample at 4 different wavelengths and haemoglobin content and saturation are calculated from the known absorbance spectra of the different states of haemoglobin.

The CO-oximeter is calibrated by first, flushing through zeroing solution, and second, by flushing through a calibration dye of known absorbance at the specified wavelengths.

ANALYSIS OF VARIANCE

The predominant statistical test used in this thesis is analysis of variance (ANOVA). It provides a powerful tool for detecting the way in which the mean of a variable is affected by its division into different groups classified by levels of fixed-effect and/or random-effect factors. A fixed-effect factor may represent different levels of hypoxia used in a measurement of ventilation and a random-effect factor may represent different individuals used in an experiment. In order best to explain how ANOVA works, it is worth deriving the basic underlying algebraic principles.

Let us consider in general terms a variable, y , which may or may not depend on 3 factors, A, B and C. For the i th level of Factor A, j th level of Factor B and k th level of Factor C,

$$E(y_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k \quad (1)$$

where $E(y_{ijk})$ is the expected value for y at the levels of i, j and k for Factors A, B and C and α_i, β_j and γ_k are constants characterizing the factors. For a suitable value of μ ,

$$\sum_i \alpha_i = 0, \quad \sum_j \beta_j = 0 \quad \text{and} \quad \sum_k \gamma_k = 0.$$

However, the observed value of y_{ijk} will differ from the expected value and so an error term, e_{ijk} is introduced into the model, thus,

$$E(y_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk} \quad (2)$$

[Note that the factors in this model are additive in their effects.]

It is assumed that e_{ijk} is distributed normally with a mean of zero and a constant variance, σ^2 (which is the variance of the measured data). This assumption is not necessarily true if there is an interaction between the factors. This will be considered later.

If the overall mean of the data is $\bar{y}$, then any deviation of y_{ijk} from the overall mean can be defined by the identity,

$$y_{ijk} - \bar{y} = (\bar{y}_{i..} - \bar{y}) + (\bar{y}_{.j.} - \bar{y}) + (\bar{y}_{..k} - \bar{y}) + (y_{ijk} - \bar{y}_{i..} - \bar{y}_{.j.} - \bar{y}_{..k} + 2\bar{y}) \quad (3)$$

where $\bar{y}_{i..}$ is the average of all the data summed over the i th level of Factor A. The 4 quantities on the right-hand side of the equation reflect the fact that y_{ijk} deviates from $\bar{y}$ due to the effects of A, B and C and by an amount that is inexplicable. If equation (3) is squared and summed over all levels of A, B and C, then,

$$\begin{aligned} \sum_{i,j,k} (y_{ijk} - \bar{y})^2 &= \sum_i (\bar{y}_{i..} - \bar{y})^2 + \sum_j (\bar{y}_{.j.} - \bar{y})^2 + \sum_k (\bar{y}_{..k} - \bar{y})^2 \\ &\quad + \sum_{i,j,k} (y_{ijk} - \bar{y}_{i..} - \bar{y}_{.j.} - \bar{y}_{..k} + 2\bar{y})^2 \end{aligned} \quad (4)$$

with all the cross terms from squaring the right-hand side of equation (3):

$$2(\bar{y}_{i..} - \bar{y})(\bar{y}_{.j.} - \bar{y}) + 2(\bar{y}_{i..} - \bar{y})(\bar{y}_{..k} - \bar{y}) + 2(\bar{y}_{.j.} - \bar{y})(\bar{y}_{..k} - \bar{y})$$

and

$$2(\bar{y}_{ijk} - \bar{y}_{i..} - \bar{y}_{.j.} - \bar{y}_{..k} + 2\bar{y})(\bar{y}_{i..} + \bar{y}_{.j.} + \bar{y}_{..k} - 3\bar{y})$$

coming out as zero, since:

$$\sum_{i,j} \bar{y}_{i..} \bar{y}_{.j.} = \sum \bar{y}^2, \quad \sum_{i,j,k} \bar{y}_{i..} y_{ijk} = \sum_i \bar{y}_{i..}^2, \quad \sum_{i,j,k} \bar{y} y_{ijk} = \sum \bar{y}^2 \quad \text{and} \quad \sum_i \bar{y} \bar{y}_{i..} = \sum \bar{y}^2.$$

Thus, the 4 terms of the right-hand side of equation (4) are the sums of squared errors (SSq) between levels classified under Factors A, B and C (between-factor-levels SSq) and the residual SSq (within-factor-levels SSq). The left-hand side is the total SSq. This division of the total SSq into between-factor-levels SSq and within-factor-levels SSq is central to ANOVA. Thus, data can be divided up into pockets according to the specified factors and, by comparing the between-factor-levels SSq with the within-factor-levels SSq, the significance of the factors in the model (1) can be assessed.

For example, suppose ventilation is the variable being measured and that a model is proposed to define the effects that may influence its magnitude. Let A be the level of hypoxia; B, the colour of the experimenter's shirt and C, the flavour of jam on the subject's breakfast toast. Having described the model as that in equation (2), the data are averaged across the different levels of each factor and the between-factor-levels SSq are calculated from the deviation of the means of each level of hypoxia from the overall mean, as in (4). If the within-factor-levels SSq is much smaller than the between-factor-levels SSq for hypoxia, this indicates that hypoxia is a major effect in creating the total SSq. On the other hand, we would not expect the colour of the experimenter's shirt or the flavour of jam to affect ventilation and so would expect the between-factor SSq levels for Factors B and C (*i.e.*, the variance in the data caused by different colours or flavours) not to make any large contribution to the total SSq and therefore, not to have any large effect in the model. The contribution of the factors to the total variance can be tested statistically by dividing the mean squared error, MSq, (SSq/degrees of freedom) resulting from the between-factor-levels of the factor concerned by the residual MSq to produce an F-ratio. This, in effect, gives a ratio that compares the contribution of a factor to the total variance against the contribution from the error made in each ventilatory measurement, e_{ijk} . The significance of this ratio can be tested by comparing

it with the critical values for F at the specified significance level in the appropriate F distribution, which depends on the degrees of freedom of the two variances.

When there is an interaction between the factors in the model, for example if A is the level of hypoxia, B is the level of hypercapnia and C is the level of anaesthesia, then the residual SS_q will be inflated by discrepancies between $E(y_{ijk})$ and approximations given by the additive model (1). Thus it is necessary to expand the model with a factorial design:

$$E(y_{ijk}) = \mu + \alpha_i + \beta_j + \gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} \quad (5)$$

New terminology arises with this model. For example, α_i is described as the main effect of A (hypoxia), which is the effect of different levels of hypoxia averaged over all levels of the other factors, B (hypercapnia) and C (anaesthesia). $(\alpha\beta)_{ij}$ describes the effect of the interaction of hypoxia and hypercapnia averaged over all levels of anaesthesia which is not determined by the main effects of hypoxia and hypercapnia alone. This is called a first-order interaction term or a two-factor interaction term. Similarly, $(\alpha\beta\gamma)_{ijk}$ is a constant which describes how the mean response of all combinations of hypoxia, hypercapnia and anaesthesia is not affected purely by the main and first-order interaction effects. This constant is defined as a second-order interaction term or a three-factor interaction term. As with the additive model, y_{ijk} is considered to be normally distributed about $E(y_{ijk})$.

As hypoxia, hypercapnia and anaesthesia are all fixed-effect factors, their significance in the model can be determined using the analysis as described above for the additive model, by comparing the between-factor-levels MS_q with the within-factor-levels MS_q . However, suppose one of the factors, say anaesthesia, is instead a random-effect factor, such as different individuals; then an interaction between hypoxia and individuals would not preclude any interest in the main effect of hypoxia. All this would suggest is that different individuals have different ventilatory responses. More interesting would be the main effects of hypoxia within individuals. Thus, in order to test the main effects of hypoxia, the MS_q error for

hypoxia should be compared against the MSq for the first-order interaction of hypoxia and individuals. Similarly, to test for an interaction between hypoxia and hypercapnia, the first-order interaction term should be compared against the second-order interaction term, which includes the effects of subjects.

An alternative procedure to calculating all the SSq first and comparing the effects against the within-factor-levels SSq or other relevant SSq (see above) is to remove the SSq due to each term in the model sequentially from the total SSq and examine the effect of removing each term on the total SSq, using a variance ratio (F-ratio). If random-effect factors are present in the model then they should be removed first from the total SSq. For example, if model (5) is appropriate, the random-effect factor should be assigned to Factor A and the effects should be removed from the total SSq as they are expressed reading from left to right in the model. Examples of both these types of analysis are given in Chapters 4 and 5.

APPENDIX 2.1.

This appendix shows a partial listing of a .D1 file. The first 10 lines are headers which provide information concerning the file name and conditions of the experiment/data collection. The eleventh line consists of the following variables: BRNO, breath number; V_{TI} , inspired volume; V_{TE} , expired volume; T_I , inspiratory duration; T_E , expiratory duration; P_{ICO_2} and P_{IO_2} , inspired P_{CO_2} and P_{O_2} ; P_{ACO_2} and P_{AO_2} , alveolar P_{CO_2} and P_{O_2} ; WRK, work rate; and HRT, heart rate. The third column (E12345678) marks any events. Below the eleventh line are the calibrated data values. Minute ventilation for breath n is calculated as:

$$\dot{V}_{E(n)} = 60,000 \times \frac{V_{TE(n)}}{(T_{I(n)} + T_{E(n)})}$$

Data File written as 18761003.d1

DATE, 22: 5:1992

EXPERIMENTER, lsh

SUBJECT NO, 876

EXPERIMENT NO, 10

RUN NO, 3

NO.OF BREATHS, 596

MODE OF THIS EXPERIMENT, 0 1 0 1

INSPIRED GAS TEMPERATURE USED, 25.0

EXPIRED GAS TEMPERATURE USED, 38.6

BRNO	TIME	E12345678	VTI	TI	PIC2	PIO2	VTE	TE	PAC2	PAO2	VENT	WRK	HRT
2	5447	00000000	1.058	2940	0.2	148.8	1.048	3540	43.3	102.2	9.7	0	0
3	11927	00000000	1.009	2520	0.1	121.2	0.929	2960	39.7	103.0	10.2	0	0
4	17407	00000000	1.071	2640	32.0	127.7	1.442	3560	43.6	99.4	14.0	0	0
5	23607	00000000	1.234	2860	40.0	115.4	1.104	3120	47.7	94.4	11.1	0	0
6	29587	00000000	1.141	2800	33.2	126.4	1.186	3040	46.8	92.2	12.2	0	0
7	35427	10000000	1.207	2320	38.1	133.3	1.316	3180	47.2	97.2	14.4	0	0
8	40927	00000000	1.267	2220	39.8	0.5	1.357	3200	47.6	64.3	15.0	0	0
9	46347	00000000	1.376	2080	44.4	37.6	1.524	2460	47.5	52.2	20.1	0	0
10	50887	00000000	1.549	2200	46.9	51.1	1.716	2320	48.9	48.6	22.8	0	0
11	55407	00000000	2.197	2140	41.4	50.1	2.346	3080	47.3	49.1	27.0	0	0
12	60627	00000000	2.236	2460	46.5	49.8	2.356	3120	48.7	48.8	25.3	0	0
13	66207	00000000	2.265	2140	43.5	48.7	2.404	2940	49.8	47.7	28.4	0	0
14	71287	00000000	2.290	1940	40.2	50.9	2.393	2500	49.0	46.5	32.3	0	0
15	75727	00000000	2.352	1960	42.0	51.7	2.449	2580	47.4	47.1	32.4	0	0
16	80267	00000000	2.319	2040	43.8	51.5	2.500	2640	49.7	46.3	32.1	0	0
17	84947	00000000	2.166	1900	36.8	129.6	2.241	2600	49.4	83.8	29.9	0	0
18	89447	00000000	2.420	2000	36.6	76.5	2.373	3060	48.9	78.7	28.1	0	0
19	94507	00000000	1.826	2120	35.9	82.7	1.920	3000	49.9	71.1	22.5	0	0
20	99627	00000000	1.696	2580	32.9	97.7	1.747	2640	49.3	72.8	20.1	0	0
21	104847	00000000	1.650	2000	32.5	96.7	1.792	2540	47.9	74.0	23.7	0	0
22	109387	00000000	1.900	1960	36.2	86.6	2.008	3160	48.3	73.0	23.5	0	0

CHAPTER 3

A CHAMBER FOR CONTROLLING THE END-TIDAL GAS TENSIONS OVER SUSTAINED PERIODS

*'...Tis her breathing that
Perfumes the chamber thus...'
Cymbeline, II.ii.18*

INTRODUCTION

In Chapters 1 and 2 the negative feedback of the respiratory control system was described. Briefly, this system adjusts the ventilatory drive in order to minimize deviations of arterial Po_2 , Pco_2 and H^+ from the set point of the system (these deviations having acted as the initial stimulus for the adjustment, via the chemoreflexes). Many short-term experiments (<1 hour) have been performed to study this control system. For example, Padgett (1928) measured the ventilatory response to step changes in inspired gas tensions using a mouthpiece. Problems associated with this approach are that the respiratory control system responds to changes in arterial gas tensions, not inspired partial pressures, and also that the feedback loop remains closed. Therefore, the arterial blood gases will be altered by the ensuing ventilatory responses. To overcome this, Swanson and Bellville (1975) introduced a prediction-correction technique to control the end-tidal gas tensions and consequently the arterial gas composition (in healthy subjects), thus opening up the feedback loop. This was achieved using a computer controlled gas-mixing system and it enabled the response of the respiratory control system to be examined with the arterial gas tensions fully controlled in a pre-defined manner. This technique has been further developed in our laboratory to study the dynamics of the short-term responses to hypoxia and hypercapnia and is described in the previous chapter.

Longer-term studies exceeding one hour in duration cannot be performed using this apparatus as the subject becomes uncomfortable through wearing the mouthpiece and nose-clip, hence hypobaric chambers have been employed to study the effects of longer-term hypoxia. However, the hypobaric chamber suffers from the same inadequacies as the original mouthpiece apparatus in that the arterial gases are not being controlled.

In order to perform experiments of longer duration than one hour with adequate control of the end-tidal gases, a chamber was constructed in our laboratory based on the Swanson and Bellville principle, so that the inspire in the chamber is adjusted to control the end-tidal,

and hence arterial, partial pressures, at their desired experimental values. To overcome the problem of subject discomfort experienced with the mouthpiece apparatus, it was decided to sample gas at the opening of the nostrils.

METHODS

Equipment

Figure 3.1 shows a schematic diagram of the chamber and associated equipment. The clear-sided chamber (Bassaire, Southampton) is 2.3 m long, 1.7 m wide and 2.2 m high giving a chamber volume of 8.6 m³. The principal elements of the system are a nasal cannula apparatus; gas supplies, a soda lime CO₂ absorber and associated valve driver; a mass spectrometer; a multi-channel amplifier; a pen recorder; a pulse oximeter and a data acquisition/controlling computer. A photograph of the chamber and associated equipment, except the gas supplies is presented in Fig. 3.2.

Nasal catheter apparatus. The technique of monitoring gas at the nostril has previously been shown to give satisfactory measurements of end-tidal and inspiratory gas (Derrick *et al.*, 1993; Gothe *et al.*, 1982; Marsh, 1982). This technique was employed in the current setup. In the study described in Chapter 4, a single catheter, sampling at 80 ml.min⁻¹ and leading to the mass spectrometer, was taped in place at the opening of one of the nostrils. However, this technique was adjusted to suit experiments involving sleep (Chapter 6) where the subject may have either pulled out the catheter or wrapped it around his/her neck. Figure 3.3 shows a subject seated inside the chamber wearing the advanced apparatus comprising a nasal cannula (Hudson, Temecula, CA, U.S.A.) adapted to measure gas at the opening of the nostrils. Figure 3.4 illustrates a close-up of the prongs of the nasal cannula and associated components. A fine catheter is threaded through each of the prongs and along the ipsilateral flexible tubing leading from the prong. The two arms of the cannula run over the ears and are secured together at the back of the head by a toggle before converging into one tube that leads out of the chamber. Both catheters lead to separate inlets of the mass spectrometer, where one is selected to sample gas from its tip at 80 ml.min⁻¹.

Gas supplies and CO₂ absorber. The gas supplies and soda lime CO₂ absorber were

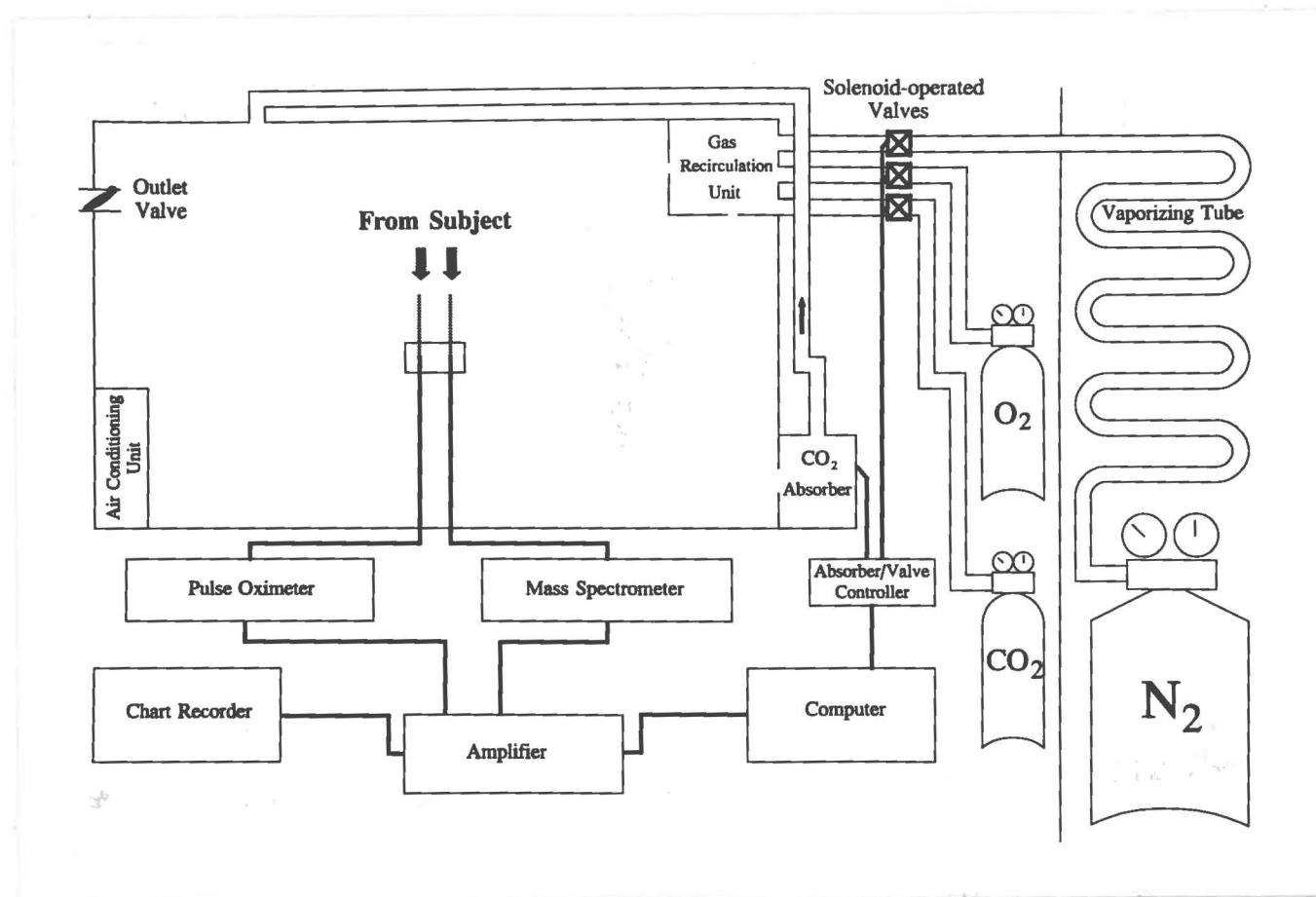


Figure 3.1: Schematic diagram illustrating the chamber and associated apparatus.



Figure 3.2: The chamber and associated equipment. The gas supplies are out of view.



Figure 3.3: Subject 910 seated inside the chamber seen wearing the adapted nasal cannula apparatus (used in Chapter 6) and a pulse oximeter probe on one of the fingers.

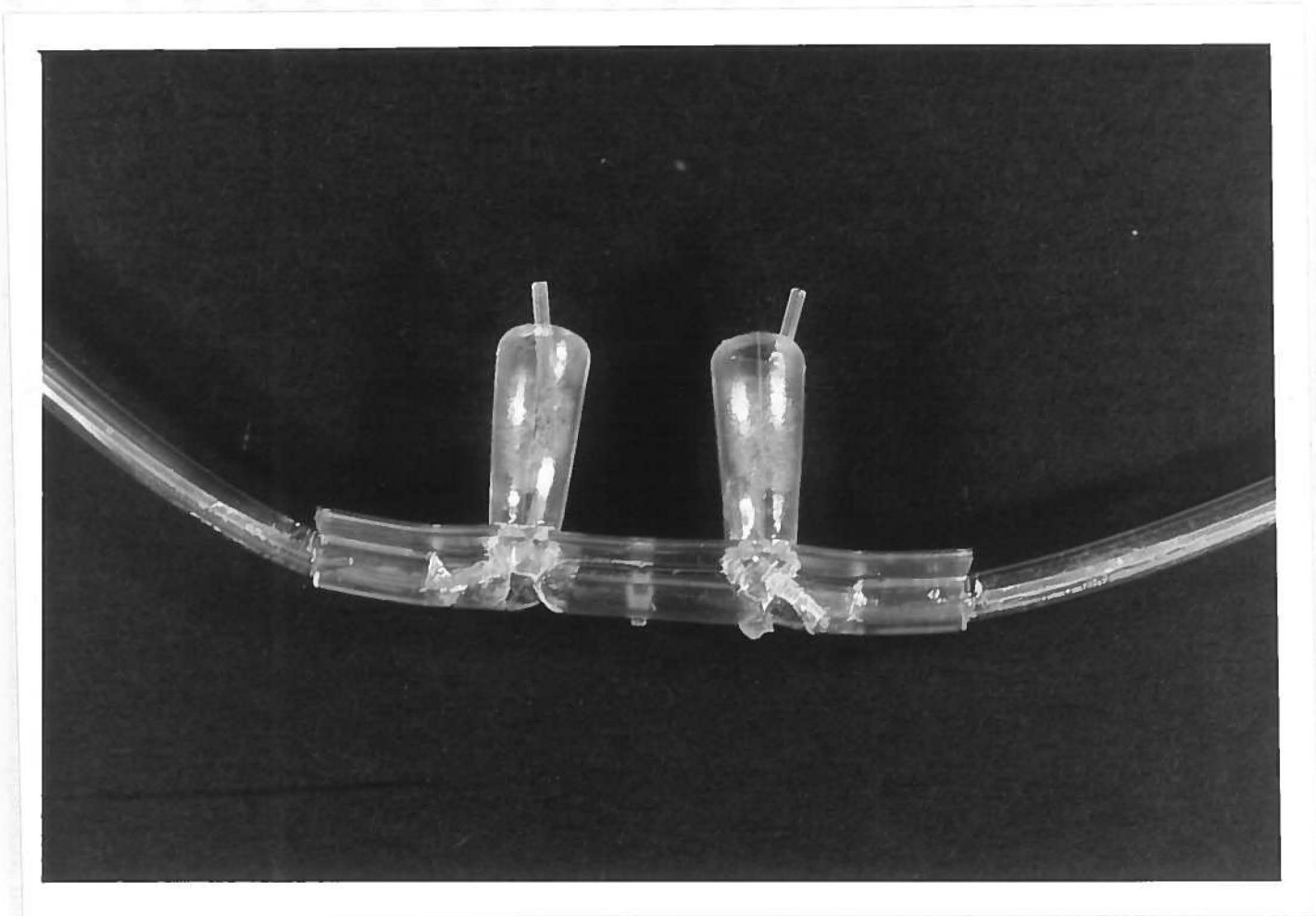


Figure 3.4: A close-up of the prongs of the nasal cannula with associated catheters leading to the mass spectrometer. The catheters are fixed in place with foam and glue.

designed to be able to change the CO_2 and O_2 concentrations by *ca.* 1% per min when at a chamber composition of 5% CO_2 and 10% O_2 . When required, CO_2 and O_2 can be injected into the chamber at a rate of 100 l.min⁻¹ and O_2 can be removed from the chamber by injecting N_2 at a rate of 1000 l.min⁻¹.

The gases are introduced into the chamber via multi-holed pipes in a gas recirculation unit to effect better mixing within the chamber. The oxygen is supplied from cylinders (British Oxygen Company) in a standard manifold. In order to obtain CO_2 flows of 100 l.min⁻¹, liquid withdrawal CO_2 cylinders (British Oxygen Company) in a manifold are used together with an external heater to vaporize the liquid. To supply the high volumes of N_2 required, a system was constructed outside the building consisting of two 240 l liquid N_2 tanks (Taylor Wharton, A.S. Scientific, Abingdon) and a vaporizer (50 m of aluminium pipe) to supply the laboratory (Figs 3.5 and 3.6). CO_2 is removed from the chamber by recirculating the inspire through a soda lime absorber (Bassaire) at 2000 l.min⁻¹. The regulation of pressure downstream of the gas supplies is as described in Chapter 2 for the fast gas-mixing system. The gas supplies are opened and closed by means of on-off solenoid valves (Platon Flowbits, Basingstoke) driven by the computer-controlled valve-driver box. Flows are regulated through the valves by laboratory-built taps and are registered by flowmeters (Platon Flowbits, Basingstoke). The CO_2 absorber is also controlled by a switch in the valve-driver box and the flow is monitored by an anemometer (Airflow EDRA 6, Airflow Developments, High Wycombe). The heat and water vapour produced by the soda lime absorber are counteracted by an air conditioning unit (Andrews Sykes, Swindon, Wilts.) installed inside the chamber. Gas leaves the chamber through a non-return flap valve.

Other equipment. The other equipment associated with the chamber apparatus, listed above, is the same as that described in the previous chapter relating to the data acquisition system. The mass spectrometer (Airspec QP9000, C.A.S.E. Scientific, Biggin Hill, Kent) is a different model to the one described in the previous chapter, but functions in the same manner. The computer used in this setup was an Elonex PC 2865-12.



Figure 3.5: The two 240 l nitrogen tanks.



Figure 3.6: The outdoor vaporizer consisting of 50 m of aluminium pipe.

Calibration

Before each experimental trial, the mass spectrometer is zeroed and calibrated as in Chapter 2. A calibration procedure is then performed on the data acquisition system using the program CLCHAMB. Data are collected for 10 sec with the mass spectrometer sensing air and the pulse oximeter set to zero scale deflection [*i.e.*, 0% arterial O_2 saturation (Sa_{O_2})]. Following this, the gas being sampled is switched to standard gas and the pulse oximeter is switched to full scale deflection (*i.e.*, 100% Sa_{O_2}). The program calculates the appropriate calibration functions for O_2 , CO_2 and Sa_{O_2} using the data collected and user specified values for standard gas composition, room temperature and barometric pressure. These functions are written to an ASCII file (*.CAL). This procedure is performed separately for both catheters and is repeated every 4 hours.

Data acquisition

Data acquisition and end-tidal gas control during an experiment are performed by the programs: CHCONTI (for protocols involving control of both Pet_{O_2} and Pet_{CO_2}); CHCONTP (for protocols involving the control of only Pet_{O_2}) and CHCONTC (a dummy program which monitors the end-tidal gases during an air-breathing control and periodically runs the CO_2 absorber and adds O_2 to the chamber to maintain euoxic and eucapnic conditions). Program CHCONTI is presented complete with subroutines in Appendix 3.1. The programs perform a number of functions which include collecting data from the apparatus described above, detecting and storing the Sa_{O_2} and end-inspiratory and end-expiratory values of Po_2 and Pco_2 and adjusting the inspirate in the chamber to correct deviations in the end-tidal gas averages from the desired values.

The amplified analogue signals from the CO_2 and O_2 channels of the mass spectrometer and pulse oximeter are input to the computer via analogue-to-digital converters (DAS-8PGA, Metrabyte; Keithley Instruments Ltd., U.K.), sampled every 20 msec by the CHCONTX program and stored in a ring buffer which has an array dimension of 50, therefore holding

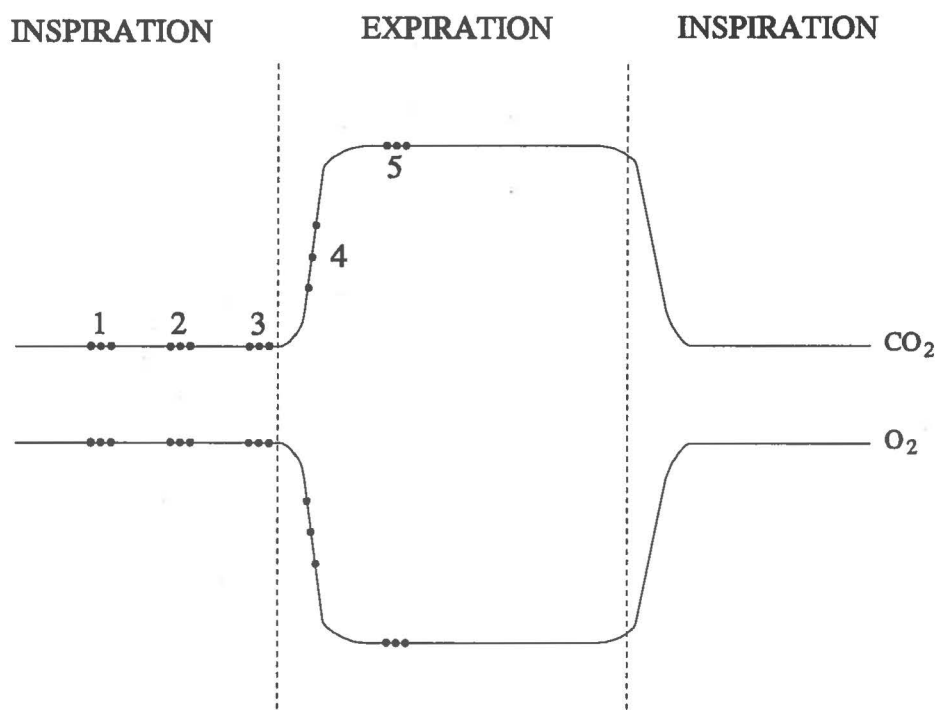


Figure 3.7: Diagram representing the time zones (1 to 5) used to detect a possible end of inspiration. The continuous line represents the amplified analogue signal from the mass spectrometer and the symbols represent digital data converted from the analogue signal every 20 msec relating to these time zones. (5, -40 to -80 msec; 4, -240 to -280 msec; 3, -440 to -480 msec; 2, -640 to -680 msec; 1, -840 to -880 msec.)

data for one second. CHCONTX receives information concerning experimental time from an internal crystal running at 1 MHz, which in turn drives two internal counters in series to provide clocks running at 1 kHz and 1 Hz.

A peak-detection algorithm is used to determine end-tidal Po_2 (PET_{O_2}) and end-tidal Pco_2 (PET_{CO_2}) from the mass spectrometer together with the inspired Po_2 (PI_{O_2}) and inspired Pco_2 (PI_{CO_2}). First, the program looks back over the last second of CO_2 and O_2 data (still in digital form) at various time points as shown in Fig 3.7 [from -40 to -80 msec (5); -240 to -280 msec (4); -440 to -480 msec (3); -640 to -680 msec (2); -840 to -880 msec (1)] and calculates the mean of the 3 points spanning each time zone (1 to 5). Second, when more than a program-specified difference between the values at time zones 3, 4 and 5 in the CO_2 data is detected, then the program registers a possible end of respiratory phase. In the case of a

possible end of inspiration (as in Fig. 3.7), the greatest value of O_2 and the lowest value of CO_2 from time zones 1, 2 and 3 are stored as possible inspired values. The reverse is true for end-of-expiration detection. Finally, in order to avoid attributing real phase switches to jumps in the trace during mid-phase, the program only verifies a true end of inspiration if the CO_2 is a program-specified value greater than the possible inspired value at a time point 300 msec after the possible phase switch was detected, provided no new possible ends of inspiration have been detected. This procedure is reversed for the detection of end of expiration. If a rogue end of inspiration is noted, then the program continues to search for an end of inspiration. Alternatively, the program begins the search for the next end of expiration.

Averages for P_{IO_2} , P_{ICO_2} , P_{ETO_2} and P_{ETCO_2} (in mmHg) and SaO_2 (in %) are calculated each minute. Values for individual breaths in which the end-tidal values are more than a program-specified deviation from the desired values are excluded to remove abnormal breaths (e.g. yawning) from the average. At the end of each expiration the values for P_{IO_2} , P_{ICO_2} , P_{ETO_2} , P_{ETCO_2} , SaO_2 , and the last minute average for P_{ETO_2} , P_{ETCO_2} and SaO_2 are relayed to the screen. This information, together with the breath number and time, is stored in a *.BBB file, where * is a user-defined file root name. The chart recorder plots the continuous amplified analogue signals from the CO_2 and O_2 channels of the mass spectrometer and SaO_2 from the pulse oximeter.

End-tidal gas control in action

At the start of the program, the experimenter is required to enter the desired values of P_{ETO_2} and P_{ETCO_2} into the computer. Subsequently, at 5-min intervals the computer compares the average end-tidal values from the 3 previous minute averages with the desired values. If both the P_{ETO_2} and P_{ETCO_2} are within 1 and 0.3 mmHg of the pre-defined, desired end-tidal pressures, respectively, then the inspire is left unchanged for the next 5 minutes. However, if the actual P_{ETO_2} and/or P_{ETCO_2} differ from the desired values by more than the specified amounts, a new inspired composition is calculated, taking into account the respiratory

changes that will result from the altered end-tidal gases. First, the ventilations associated with the current end-tidal gas partial pressures ($\dot{V}_{Eold}$) and with the desired end-tidal gas tensions ($\dot{V}_{Enew}$) are estimated using the Lloyd-Cunningham equation (Cunningham *et al.*, 1964):

$$\dot{V}_E = D.(P_{ETCO_2} - B) + \frac{DA.(P_{ETCO_2} - B)}{(P_{ETO_2} - C)}.$$

Then, assuming the metabolic rate remains constant and neglecting any differences between the alveolar ventilation ratio, $\dot{V}_{Aold}/\dot{V}_{Anew}$, and the expired ventilation ratio, $\dot{V}_{Eold}/\dot{V}_{Enew}$, the new required gas compositions can be calculated as follows:

$$P_{IO_2new} = P_{AO_2new} + \frac{(P_{IO_2old} - P_{AO_2old}).\dot{V}_{Eold}}{\dot{V}_{Enew}}$$

and

$$P_{ICO_2new} = P_{ACO_2new} - \frac{(P_{ACO_2old} - P_{ICO_2old}).\dot{V}_{Eold}}{\dot{V}_{Enew}}.$$

Considering CO_2 and O_2 separately, the volume of gas (Q) needed to raise the partial pressures of CO_2 and O_2 in the chamber by the appropriate amount may be calculated as follows:

$$Q_{O_2} = V.\log_e \frac{(1. - F_{O_2old})}{(1. - F_{O_2new})}$$

and

$$Q_{CO_2} = V.\log_e \frac{(1. - F_{CO_2old})}{(1. - F_{CO_2new})}$$

where V is the volume of the chamber and $F_{(CO_2/O_2)old}$ and $F_{(CO_2/O_2)new}$ are the starting and finishing dry fractions of CO_2 and O_2 of the adjustment, obtained from the inspiratory pressures, $P_{(CO_2/O_2)old}$ and $P_{(CO_2/O_2)new}$ respectively. If the CO_2 or O_2 need to be lowered, the appropriate flow of gas through the absorber or amount of nitrogen injected, respectively, is given by:

$$Q_{absorber} = \frac{V}{0.9997} \cdot \log_e \frac{F_{CO_2old}}{F_{CO_2new}}$$

and

$$Q_{N_2} = V \cdot \log_e \frac{F_{O_2old}}{F_{O_2new}},$$

where the constant 0.9997 derives from the amount of CO_2 in air. However, one complication of this procedure is that adjusting the O_2 concentration will affect the CO_2 concentration and *vice versa*. An allowance is made for this by planning to adjust the CO_2 and O_2 in alternate stages, and at the end of each stage calculating the effect of the one adjustment on the inspired partial pressure of the other gas species. The duration for which each valve remains open is then calculated using the precise flow for each gas. Information regarding the flows through the valves for each gas, the volume of the chamber and the values of A, B, C and D (Lloyd-Cunningham equation) are contained in a parameters file (PARS.LST) which is read at the start of the program.

The computer controls these gas flows using on-off solenoid valves, or in the case of the CO_2 absorber, by turning on or off the CO_2 absorber recirculation system, by means of an 8-bit port (DDA-06, Metrabyte; Keithley Instruments Ltd., U.K.) which communicates with the valve-driver/ CO_2 absorber switch-box.

CHCONTX receives an input from two switch-boxes through two digital input/output channels on the DDA-06 and DAS-8PGA boards. A number of functions can be performed using these switches. One box allows the experimenter to terminate the program, to stop

adjustments being made automatically every 5 minutes and to induce a change in the chamber inspire when automatic adjustments are deselected. The experimenter uses the other box to classify which catheter is active in sampling gas. Another switch was introduced on the second box for experiments in which the subjects slept inside the chamber to enable the experimenter to select a mode of end-tidal gas control used during periodic breathing.

Gas control during periodic breathing. Periodic breathing during sleep is manifested by an altered pattern of breathing involving alternating periods of apnoea of up to 23 sec and periods of hyperventilation of up to 37 sec, which has profound effects on the arterial blood gases (Gotoh *et al.*, 1969). During periodic breathing the average end-tidal gases as measured by CHCONTX are weighted towards the phase of hyperventilation as changes in the arterial blood gases go undetected during the periods of apnoea. However, Berssenbrugge *et al.* (1984) demonstrated that the average arterial gases and saturation did not change during periodic breathing and thus it was decided that, during periodic breathing, the experimenter should fix the inspired gases at the same levels that the program had used during normal breathing. When a period of apnoea lasting more than 8 sec is detected by the program, a prompt is printed to the screen to alert the experimenter and then he/she may decide whether to select the periodic breathing mode. When this mode is selected, the experimenter is requested to enter the desired inspired gas composition and the computer adjusts the inspire accordingly at 5-min intervals. With each breath, a flag is written to the screen and to the *.BBB file to confirm the mode of control selected.

Quality of the system

Assessment of accuracy of gas adjustment. In order to assess the accuracy with which the system alters the gas inside the chamber to meet a desired gas composition, a program incorporating subroutines from the main controlling program to calculate and implement adjustments to the inspire was written. The computer displayed a current 10 sec average of the gas composition inside the chamber to the screen and requested a new target composition.

The stages of adjustment to the CO_2 and O_2 were calculated and performed. Once the adjustments were complete the new composition of the chamber was displayed on the screen and compared with the desired composition. This was repeated 6 times for requested increases and decreases in Po_2 and Pco_2 .

Assessment of chamber performance. To calculate the amount of gas that might be leaking between the interior and the exterior of the chamber, the level of O_2 within the chamber was decreased to *ca.* 5%, with the addition of N_2 , and the subsequent rate of increase of Po_2 was examined. Assessment of the performance of the chamber in holding subjects' end-tidal gas compositions constant was undertaken with a series of trial experiments in 4 healthy subjects, whose characteristics are shown in Table 3.1. Each subject was studied over a *ca.* 7-hour period in the chamber. The desired end-tidal Pco_2 was set as the subjects' normal resting level, and the desired end-tidal Po_2 was set at 55 mmHg.

Subject	Sex	Height (m)	Weight (kg)	Age (years)
910	F	1.70	62	21
927	F	1.68	64	21
930	F	1.80	70	22
931	F	1.80	75	18

Table 3.1: Physical Characteristics of Subjects.

RESULTS

Accuracy of control of inspiratory partial pressures. The degree of accuracy with which the system adjusts the partial pressures of inspired gas to meet the desired inspiratory values can be expressed as the absolute value of the actual error in the adjustment (mmHg) divided by the magnitude of the total adjustment (mmHg) requested by the computer. For a requested increase in P_{O_2} and P_{CO_2} this error was 0.065 ± 0.077 (mean $\pm$ S.D.) and 0.072 ± 0.049 , respectively, and for a requested reduction in P_{O_2} and P_{CO_2} this error was 0.045 ± 0.041 and 0.055 ± 0.062 , respectively.

Accuracy of control of end-tidal gases. Immediately following construction, a leak of 16 l.min^{-1} was detected, although this may have increased subsequently. Figure 3.8 shows the rise in P_{O_2} resulting from the leak, when the level of O_2 was reduced by injection of N_2 . The leak was considered too small to have any major effect on end-tidal gas control.

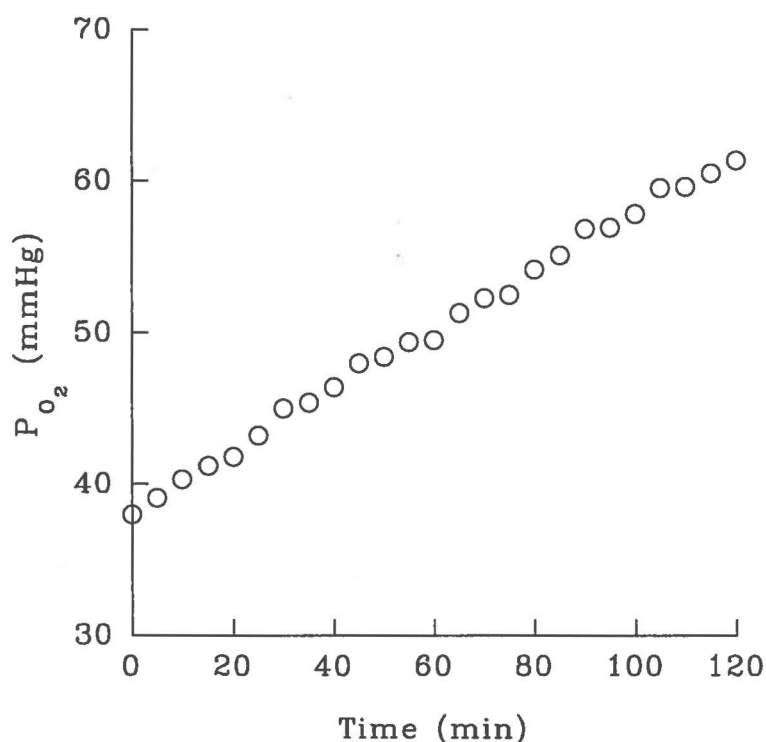


Figure 3.8: PO_2 (mmHg) vs. time (min) following the injection of N_2 into the chamber in order to estimate the leak from the rate of increase of PO_2 .

During the experiments, subjects spent most of the time seated, either reading, watching television, listening to music or playing video games. Satisfactory measurements of end-tidal and inspired gas composition could be obtained by mass spectrometer via the nasal catheter. None of the subjects reported problems with wearing the nasal catheter apparatus and pulse oximeter.

A section of a typical trace from an experiment where end-tidal P_{O_2} and P_{CO_2} were held at 55 mmHg and 46 mmHg respectively is shown in Fig. 3.9. The times at which the $P_{I_{O_2}}$ and $P_{I_{CO_2}}$ are adjusted are marked by the arrows. The drift seen in the inspired gas tensions ($0.25 \text{ mmHg} \cdot \text{min}^{-1}$ for P_{CO_2} and $0.5 \text{ mmHg} \cdot \text{min}^{-1}$ for P_{O_2}) results from the leak in the chamber, which appeared to have increased from the time of construction.

Figure 3.10 shows the end-tidal values for CO_2 and O_2 averaged every 5 minutes in 4 subjects over 7 hours, where the target $P_{ET_{O_2}}$ was 55 mmHg and the target $P_{ET_{CO_2}}$ was the subject's resting value while breathing air. This figure illustrates the quality of the control of the end-tidal gases attained by the system. The upper panel shows the deviation of the average $P_{ET_{CO_2}}$ from the target (in mmHg) and the lower panel shows the actual value of average $P_{ET_{O_2}}$. The means and standard deviations for the end-tidal CO_2 and O_2 (5 min averages) for each subject over the total 7 hour period are given in Table 3.2.

Subject	$P_{ET_{O_2}}$		$P_{ET_{CO_2}}$ error	
	Mean (mmHg)	S.D. (mmHg)	Mean (mmHg)	S.D. (mmHg)
910	56.67	2.01	0.32	0.81
927	56.76	2.34	0.35	0.48
930	56.86	1.89	0.14	0.46
931	56.33	2.37	0.22	0.51

Table 3.2: Mean values and standard deviations (S.D.) for 5-min averages of $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ error.

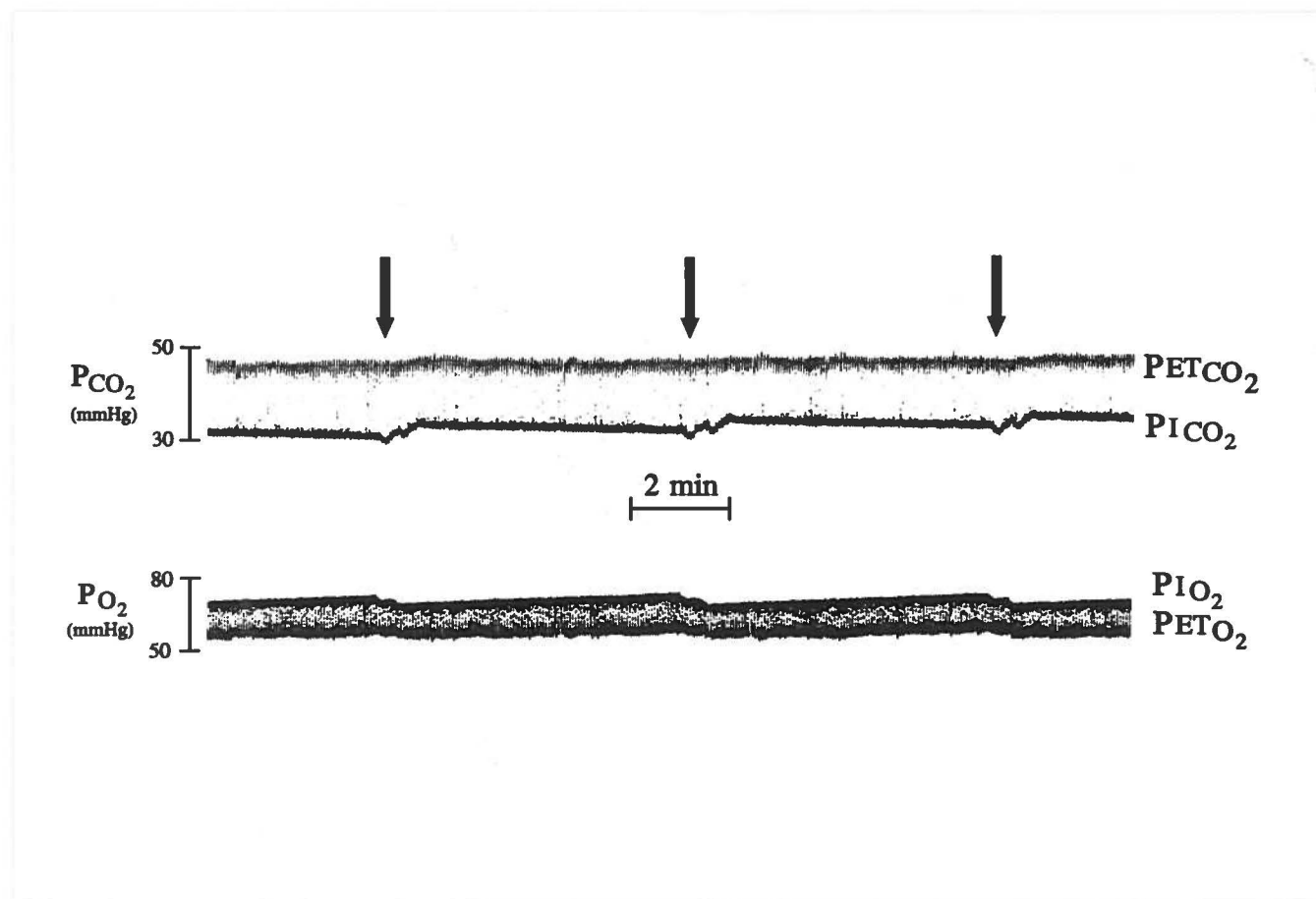


Figure 3.9: A section of a typical trace from an experiment in which $PETCO_2$ (upper trace) was held at 46 mmHg and $PETO_2$ (lower trace) was held at 55 mmHg. The arrows indicate the times at which the chamber compositions were altered.

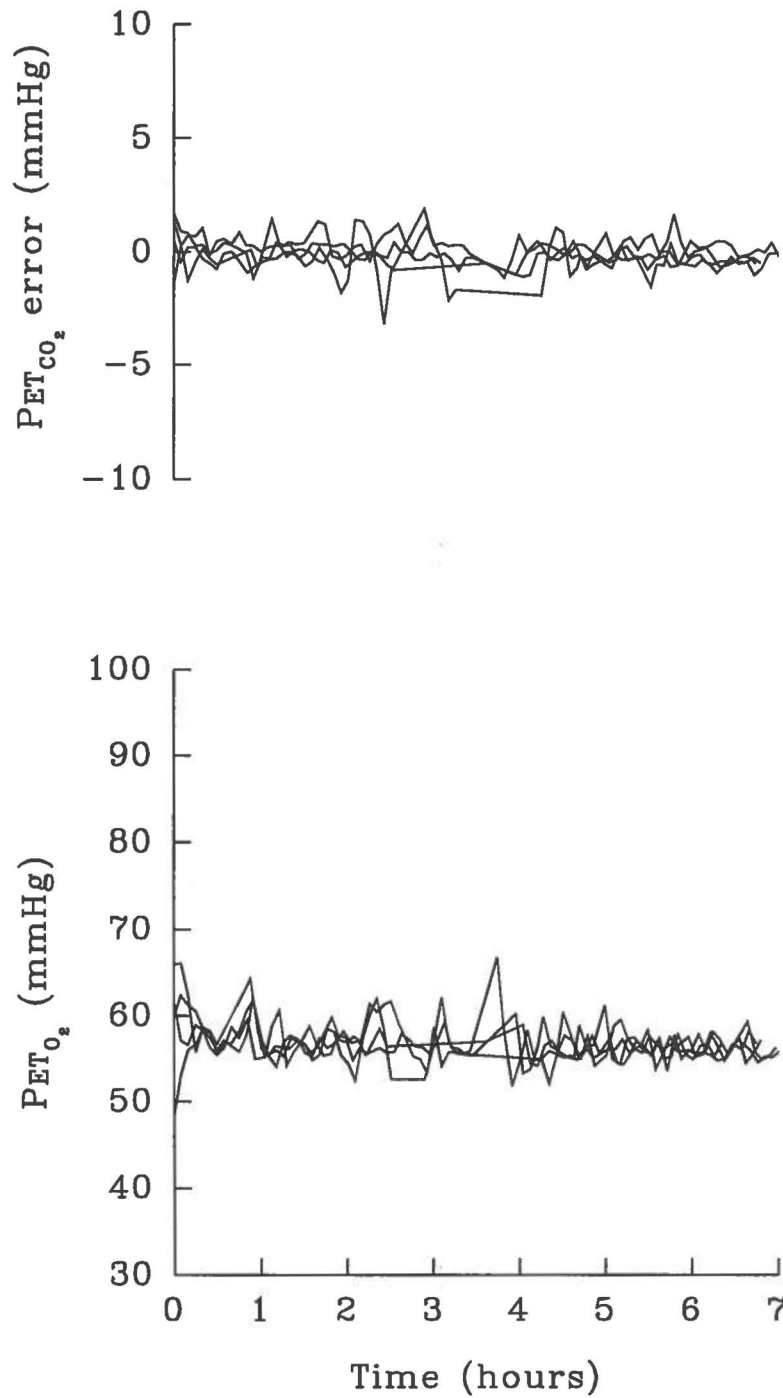


Figure 3.10: Five-minute averages for the deviation of the PET_{CO₂} from the subject's resting value (upper panel) and for the absolute value of PET_{O₂} (lower panel) in 4 subjects.

End-tidal gas control during periodic breathing. Figure 3.11 presents 5-min averages for the end-tidal gases and S_{aO_2} collected during a 48-hour exposure to poikilocapnic hypoxia (from Chapter 6). Periods when the subject was sleeping and also when the breathing pattern was periodic are indicated by boxes. During sleep, the subject's S_{aO_2} fell by *ca.* 2 to 3% despite a similar $P_{ET}O_2$. However, during periodic breathing, average $P_{ET}O_2$ rose and $P_{ET}CO_2$ fell, but with the CHCONT program in periodic breathing mode, S_{aO_2} was maintained at the previous level during normal breathing. Thus, the average arterial gases were assumed also to be similar to pre-periodic breathing levels.

Figure 3.12 shows a trace recorded at 1 mm.sec⁻¹ while subject 884 was demonstrating periodic breathing. The alternating periods of apnoea and hyperventilation are clearly illustrated.

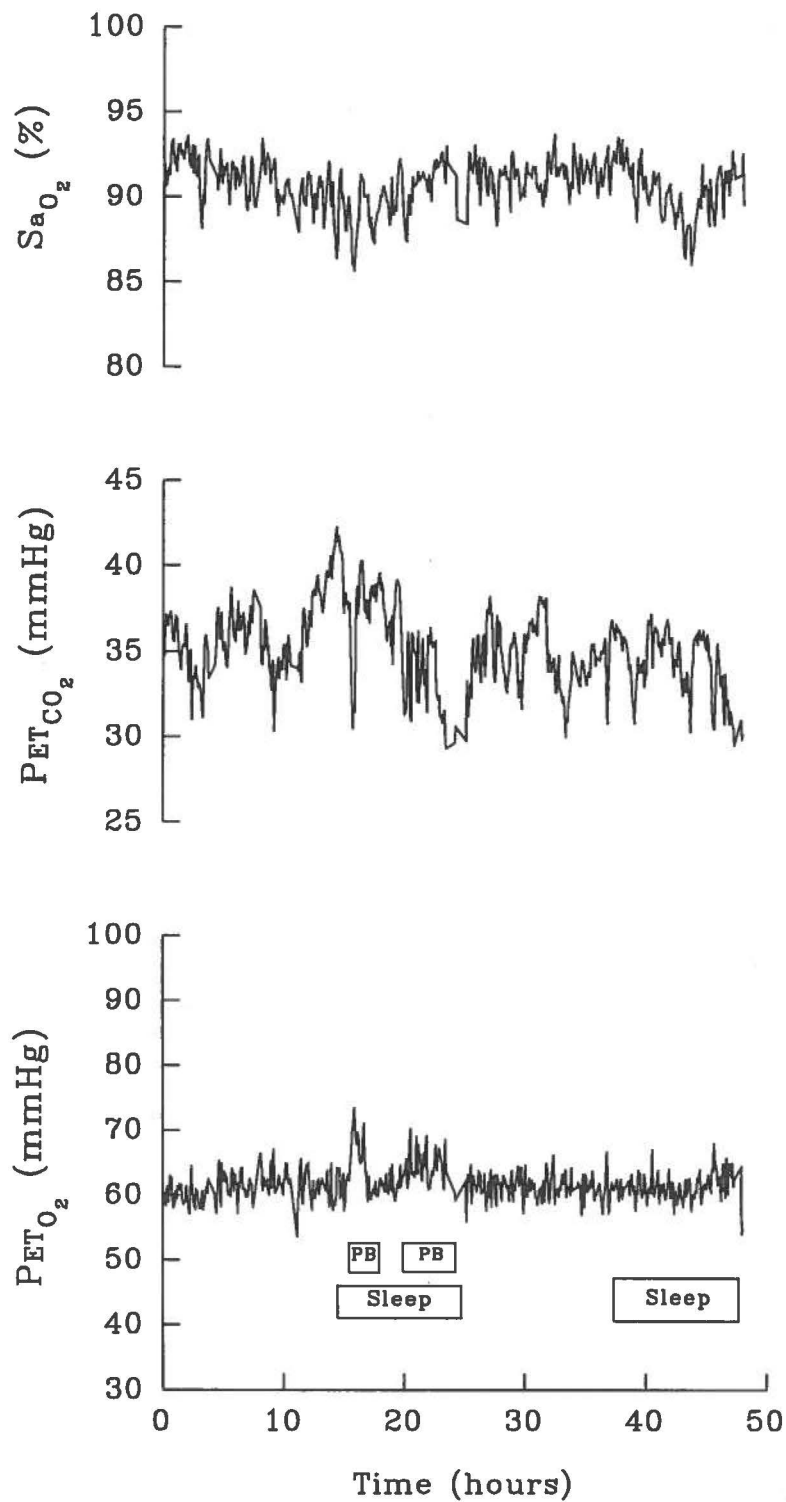


Figure 3.11: End-tidal PO_2 (P_{ETO_2}), PCO_2 (P_{ETCO_2}) and arterial saturation (S_{aO_2}) during 48 hours of poikilocapnic hypoxia in subject 884 (from Chapter 6). PB - periodic breathing.

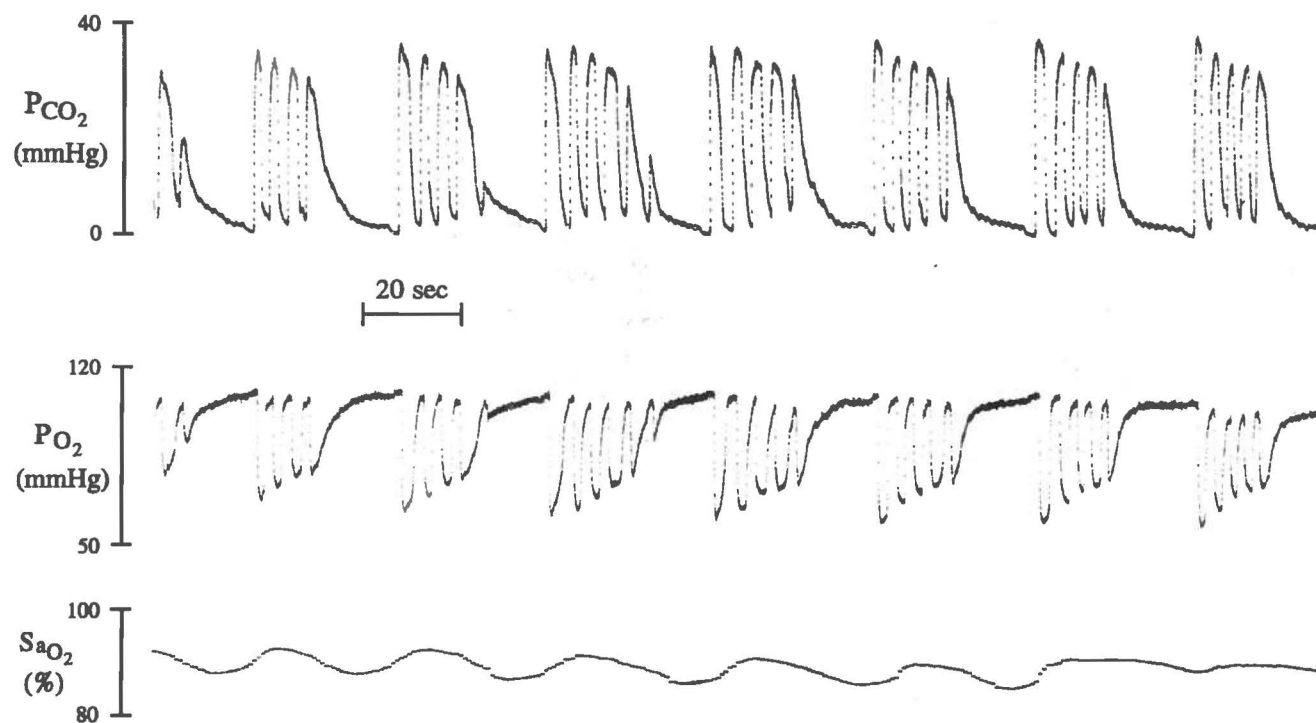


Figure 3.12: A section of a trace recorded at $1 \text{ mm} \cdot \text{sec}^{-1}$ during exposure to poikilocapnic hypoxia, with the subject (884) exhibiting periodic breathing. Upper trace, PCO_2 (mmHg); middle trace, PO_2 (mmHg); lower trace, SaO_2 (%).

DISCUSSION

This chamber provides a new technique for controlling the end-tidal gases of a subject for periods longer than previously practical. Eger *et al.* (1968) used an apparatus involving a face-mask to keep subjects hypoxic for 8 hours. The face-mask was attached to a deadspace to which CO_2 could be added in order to maintain isocapnia. In the 4 subjects studied, alveolar gas was sampled regularly and the investigators were successful in maintaining alveolar Po_2 between 46 and 51 mmHg and alveolar Pco_2 within 1.2 mmHg of the resting value. However, although methods used by Eger *et al.* held the alveolar gases reasonably constant, Kellogg (a co-author and subject in the study) reported that the protocol proved somewhat strenuous for subjects even with rests of 10 to 30 min every two hours (personal communication to Dr P.A. Robbins).

Cruz *et al.* (1980) used a hypobaric chamber to study the response of 4 subjects to 100 hours of hypoxia during which they attempted to maintain PETCO_2 constant by adding a fixed percentage of CO_2 to the inspire. End-tidal Po_2 could be manipulated by varying the pressure within the chamber. To a certain degree they were successful in preventing the development of the hypocapnic alkalosis seen during pure hypoxia, but this arrangement did not properly open the feedback loop since the effect of $\dot{V}_E$ on PETO_2 and PETCO_2 was not removed. In their experiments, they attempted to hold mean PETCO_2 at 39 mmHg and mean PETO_2 at 55 mmHg. After 27 hours, mean PETCO_2 had drifted up to 43 mmHg and mean PETO_2 to 62 mmHg.

Clearly, the chamber in our laboratory has advantages over both methods described above, in that it is possible to keep the subjects comfortable and to control his/her end-tidal gases both continuously and accurately through opening up the feedback loop between ventilation and the end-tidal gases. However, end-tidal Po_2 was consistently *ca.* 1.5 mmHg higher than the desired value: this is probably a function of the leak in the chamber. The immediate solution to this problem would be to define the desired PETO_2 1.5 mmHg lower than the actual desired

value. A longer-term proposal might be the installation of a servo system to introduce a continuous flow of N_2/CO_2 into the chamber, proportional to the actual chamber composition. There remains the problem of controlling the end-tidal gases during periodic breathing and no solution seems readily available; however, closing the feedback loop temporarily was thought to be sufficient, primarily due to minimal changes in ventilation between the different stages of sleep.

APPENDIX 3.1.

This appendix presents the program CHCONTI (written in Fortran 77, Microsoft Fortran v. 5.1) together with subroutines and functions listed at the end. Variables used and subroutines and functions called are all defined in the main program unit CHCONT, unless they are local only to subroutines or functions.

*No amount of brilliant programming will make fruit salad
out of garbage when data is being processed.*

William R. Best


```

C      PROGRAM CHCONT
C      THIS PROGRAM COLLECTS BREATH-BY-BREATH DATA FROM THE
C      SUBJECT
C      IN THE CHAMBER, WHILST CONTROLLING THE INSPIRED GASES TO
C      MAINTAIN THE DESIRED END-TIDAL VALUES.

C      ***** SUBROUTINES CALLED *****
C
C      RDCAL.....READ CALIBRATION FILE.
C      GETTIM....GET SYSTEM TIME.
C      SETCLK....SET CLOCK (ASSEMBLER CODE).
C      RDCLKI....READ CLOCK (ASSEMBLER CODE).
C      VLVCHG....CHANGE VALVE STATUS.
C      EXPSCH....SEARCH FOR END OF EXPIRATION.
C      INSSCH....SEARCH FOR END OF INSPIRATION.
C      CHANGE....CALCULATES NEW INSPIRED VALUES FOR O2 AND CO2 AND
C      TIMES FOR VALVES TO REMAIN OPEN.
C      ENDEXP....END OF EXPIRATION ROUTINE.
C      ENDINS....END OF INSPIRATION ROUTINE.
C      OUTDIG....WRITES INFORMATION TO A PORT (ASSEMBLER CODE).
C      INDIG.....READS INFORMATION FROM A PORT (ASSEMBLER CODE).
C      ADCONV....A/D CONVERSION FROM DASH-8 (ASSEMBLER CODE).
C      RDPARS....READS PARAMETERS FROM LISTING.
C      SHPARS....PRESENTS PARAMETERS TO THE SCREEN.
C      CHPARS....ALLOWS EXPERIMENTER TO CHANGE PARAMETERS FOR
C      THAT RUN.
C
C      PARS.LST..PARAMETER LISTING.

C      ***** FUNCTIONS CALLED *****
C
C      AVRAGE....CALCULATES MINUTE AVERAGE FOR O2, CO2 AND
C      SATURATION.
C      FFPP.....RETURNS A DRY FRACTION OF A GAS FROM ITS WET PARTIAL
C      PRESSURE.
C      PPFF.....RETURNS A WET PARTIAL PRESSURE OF A GAS FROM ITS DRY
C      FRACTION.
C      ITO2.....RETURNS THE TIME FOR WHICH THE O2 VALVE SHOULD
C      REMAIN OPEN.

C      ITC2.....RETURNS THE TIME FOR WHICH THE CO2 VALVE SHOULD
C      REMAIN OPEN.
C      ITN2.....RETURNS THE TIME FOR WHICH THE N2 VALVE SHOULD
C      REMAIN OPEN.
C      ITAC2.....RETURNS THE TIME FOR WHICH THE CO2 ABSORBER SHOULD
C      REMAIN ON.
C      FOONC.....RETURNS THE NEW VALUE FOR CO2 ONCE THE O2 HAS BEEN
C      ALTERED.
C      FNONC.....RETURNS THE NEW VALUE FOR CO2 ONCE THE N2 HAS BEEN
C      ALTERED.
C      FCONO.....RETURNS THE NEW VALUE FOR O2 ONCE THE CO2 HAS BEEN
C      ALTERED.
C      FACONO....RETURNS THE NEW VALUE FOR O2 ONCE THE CO2 HAS BEEN
C      ABSORBED.

C      ***** VARIABLES USED *****
C
C      ** INTEGERS **
C
C      ** ALL INTEGERS ARE 2-BYTE INTEGERS UNLESS STATED OTHERWISE.
C
C      IRNN.....RUN NO. FOR BREATH-BY-BREATH FILE.
C      IDELAY....MASS SPEC. DELAY.
C      IFLOK.....FLAG INDICATING SUCCESSFUL FILE HANDLING.
C
C      IOSECS....STARTING VALUE FOR CLOCK IN SECONDS (30000).
C      MOSECS....STARTING VALUE FOR CLOCK IN MILLISECONDS (1000).
C      ISECS.....SECONDS.
C      MSECS.....MILLISECONDS.
C      ISAMPL....SAMPLING INTERVAL FOR CYCLE (20 MSEC).
C      ITIME.....TIME.
C      ITIMES....TIME AT LAST EXIT FROM CYCLE.
C      ITIMED....TIME OF EXPERIMENT START.
C      ITIMEX....TIME AT WHICH LAST VALVE CHANGE WAS MADE.
C      ITIMEV....TIME AT WHICH VALVE PREDICTIONS WERE MADE.
C      ITIMEE....TIME OF END-EXPIRATION.
C      ITIMEA....TIME OF START OF MINUTE AVERAGE.
C      ITIMEP....300 MSEC AFTER POSS. END-EXPIRATION.
C      ITIMEI....300 MSEC AFTER POSS. END-INSPIRATION.

```

C MINGAP.....MINIMUM GAP (IN MINS) ALLOWED BETWEEN LAST VALVE
 C CHANGE AND NEXT.
 C ISTLTI.....SETTLING TIME ALLOWED IN MINS BEFORE CHAMBER CONC.
 C CAN CHANGE.
 C ISTAT.....ADJUSTING TIME LEFT.
 C IHR.....HOUR ON SYSTEM CLOCK.
 C IMIN.....MINUTES ON SYSTEM CLOCK.
 C ISEC.....SECONDS ON SYSTEM CLOCK.
 C I100TH.....MILLISECONDS ON SYSTEM CLOCK.
 C
 C IPHASE.....FLAG FOR INSPIRATION OR EXPIRATION (1=EXP., 2=INSP.).
 C IPO SSE.....FLAG INDICATING 1 IF POSSIBLE END-EXPIRATION WAS
 C FOUND.
 C IPOSSI.....FLAG INDICATING 1 IF POSSIBLE END-INSPIRATION WAS
 C FOUND.
 C IENDE.....FLAG INDICATING 1 IF END-EXPIRATION WAS FOUND.
 C IENDI.....FLAG INDICATING 1 IF END-INSPIRATION WAS FOUND.
 C IBRNO.....BREATH NUMBER IN RECORD.
 C JBRNO.....BREATH NO. AS INDEX FOR ARRAY FOR MINUTE AVERAGE.
 C
 C IADDCA.....PORT ADDRESS FOR SWITCHES FOR CHAMBER CONTROL.
 C ISWTCA.....8 BIT DATA FOR SWITCHES FOR CHAMBER CONTROL.
 C IADDCB.....PORT ADDRESS FOR SWITCHES FOR CATHETER NO.
 C AND PERIODIC BREATHING
 C ISWTCB.....8 BIT DATA FOR SWITCHES FOR CATHETER NO. AND PERIODIC
 C BREATHING
 C MANAUT.....BIT FOR MANUAL/AUTO SWITCH.
 C IONOFF.....BIT FOR ON/OFF SWITCH (MANUAL CONTROL).
 C ITERM.....BIT FOR TERMINATION SWITCH.
 C ICATH.....BIT FOR CATHETER SWITCH.
 C ICATHX.....CATHETER IN USE.
 C IPER.....BIT FOR PERIODIC BREATHING SWITCH.
 C IPERD.....FLAG FOR WHETHER EXPERIMENTER THINKS SUBJECT IN
 C PERIODIC BREATHING OR NOT. (1=PERIODIC, 0=NORMAL).
 C
 C ILIMIT.....VALUE IN UNITS (SAME MAGNITUDE AS A/D CONVERSION)
 C USED TO DEFINE A CHANGE BETWEEN PHASES OF INSPIRATION
 C AND EXPIRATION WHEN EXAMINING IPO2 ARRAY.
 C JPO2.....O2 VALUE FROM A/D CONVERSION.
 C JPCO.....CO2 VALUE FROM A/D CONVERSION.

C JSAT.....SATURATION VALUE FROM A/D CONVERSION.
 C IPO2().....O2 VALUE FROM A/D CONVERSION IN ARRAY.
 C IPCO2().....CO2 VALUE FROM A/D CONVERSION IN ARRAY.
 C ISAT().....SATURATION VALUE FROM A/D CONVERSION IN ARRAY.
 C IARRID.....ARRAY INDEX FOR RING BUFFER.
 C IARREF.....REFERENCE VALUE FOR ARRAY INDEX.
 C IAO2.....POSSIBLE END-EXPIRATION VALUE FOR O2.
 C IACO2.....POSSIBLE END-EXPIRATION VALUE FOR CO2.
 C IASAT.....POSSIBLE SATURATION VALUE.
 C IIO2.....POSSIBLE END-INSPIRATION VALUE FOR O2.
 C IICO2.....POSSIBLE END-INSPIRATION VALUE FOR CO2.
 C NOW??A.....RETURNED ALVEOLAR VALUE OF O2 OR CO2.
 C NOW??I.....RETURNED INSPIRED VALUE OF O2 OR CO2.
 C IAVCO().....AVERAGE OF 5 CONSECUTIVE CO2 VALUES FOR END CYCLE
 C SEARCH.
 C IAVO().....AVERAGE OF 3 CONSECUTIVE O2 VALUES FOR END CYCLE
 C SEARCH.
 C ICHPO2.....O2 CHANNEL ON DASH-8.
 C ICHPCO.....CO2 CHANNEL ON DASH-8.
 C ICHSAT.....SATURATION CHANNEL ON DASH-8.
 C
 C IP().....ARRAY DEFINING VALVE STATUS.
 C IVLVST.....CURRENT VALVE STATUS.
 C ITP().....ARRAY DEFINING DURATION OF VALVE STATUS.
 C
 C ** REALS **
 C
 C SFO2().....SCALE FACTOR FOR O2 CALIBRATION (CATHETER NO.).
 C SFCO2().....SCALE FACTOR FOR CO2 CALIBRATION ().
 C SFSAT().....SCALE FACTOR FOR SATURATION CALIBRATION ().
 C XINTO2().....O2 INTERCEPT FOR CALIBRATION ().
 C XINTCO().....CO2 INTERCEPT FOR CALIBRATION ().
 C XINTSA().....SATURATION INTERCEPT FOR CALIBRATION ().
 C
 C SAMFLO.....MASS SPEC. FLOW RATE.
 C BP.....BAROMETRIC PRESSURE.
 C RT.....ROOM TEMPERATURE IN KELVIN.
 C RMSAT.....ROOM WATER SATURATION.

C O2DIFF....DIFFERENCE (TORR) FOR O2 WHICH WHEN EXCEEDED
 C TRIGGERS VLVCAL.
 C CODIFF....DIFFERENCE (TORR) FOR CO2 WHICH WHEN EXCEEDED
 C TRIGGERS VLVCAL.
 C DESO2....DESIRED ARTERIAL O2 (TORR).
 C DESCO2....DESIRED ARTERIAL CO2 (TORR).
 C RPIO2.....INSPIRED PO2 (TORR).
 C RPICO2.....INSPIRED PCO2 (TORR).
 C RPAO2.....EXPIRED PO2 (TORR).
 C RPACO2.....EXPIRED PCO2 (TORR).
 C RSAT.....SATURATION (%).
 C
 C AVAO2()...ARRAY FOR HOLDING EXPIRED PO2S FOR MINUTE AVERAGE
 C (TORR).
 C AVACO2()..ARRAY FOR HOLDING EXPIRED PCO2S FOR MINUTE
 C AVERAGE (TORR).
 C AVASAT()..ARRAY FOR HOLDING SATURATIONS FOR MINUTE AVERAGE
 C (TORR).
 C AVIO2()...ARRAY FOR HOLDING INSPIRED PO2S FOR MINUTE AVERAGE
 C (TORR).
 C AVICO2()..ARRAY FOR HOLDING INSPIRED PCO2S FOR MINUTE
 C AVERAGE (TORR).
 C AVO2A(4)..LAST MINUTE AVERAGE FOR EXPIRED PO2 (TORR).
 C AVCO2A(4).LAST MINUTE AVERAGE FOR EXPIRED PCO2 (TORR).
 C AVSAT(4)..LAST MINUTE AVERAGE FOR SATURATION (%).
 C AVO2I(4)..LAST MINUTE AVERAGE FOR INSPIRED PO2 (TORR).
 C AVCO2I(4).LAST MINUTE AVERAGE FOR INSPIRED PCO2 (TORR).
 C
 C RNEWO2....NEW CALCULATED INSPIRED PO2.
 C RNEWCO....NEW CALCULATED INSPIRED PCO2.
 C PERDO2....INSPIRED O2 IN PERIODIC BREATHING.
 C PERDCO....INSPIRED CO2 IN PERIODIC BREATHING.
 C
 C V.....VOLUME OF CHAMBER.
 C QO2.....FLOW OF O2 THROUGH VALVE.
 C QC2.....FLOW OF CO2 THROUGH VALVE.
 C QN2.....FLOW OF N2 THROUGH VALVE.
 C QAC2.....FLOW RATE OF CO2 ABSORBER.
 C
 C A,B,C,D...LLOYD-CUNNINGHAM EQUATION CONSTANTS.

C ** CHARACTERS **

C FLCAL1....CALIBRATION FILE NAME WITH EXTENSION FOR CATHETER 1.
 C FLCAL2....CALIBRATION FILE NAME WITH EXTENSION FOR CATHETER 2.
 C FLCH.....BREATH-BY-BREATH FILE NAME WITH EXTENSION.
 C EXPR.....EXPERIMENTER.
 C DATE.....DATE.
 C STAT1.....STATUS OF VALVES (IE: CHAMBER ADJUSTING OR NOT).
 C PAR1.....FLAG FOR VIEWING OF PARAMETERS.
 C PAR2.....FLAG FOR CHANGING OF PARAMETERS.
 C SUBNO.....SUBJECT NO..
 C EXPNO.....EXPERIMENT NO..

INTEGER*2 IRNN, IDELAY, IFLOK, O, IARRID
 INTEGER*2 IOSECS, MOSECS, ISECS, MSECS, ISAMPL, MINGAP, ISTLTI
 INTEGER*4 ITIME, ITIMED, ITIMEX, ITIMEV, ITIMEA, ITIMES, ITIMEP, ITIMEI
 INTEGER*4 ISTAT, ITIMEY
 INTEGER*2 IPHASE, ILIMIT, IENDE, IENDI, IPOSSE, IPOSSI, IBRNO, JBRNO
 INTEGER*2 IADDCA, ISWTCA, IADDCB, ISWTCB, MANAUT, IONOFF, ITERM
 INTEGER*2 JPO2, JPCO, JSAT, IAO2, IACO2, IASAT, IPO2, IPCO2, ISAT
 INTEGER*2 ICHPO2, ICHPCO, ICHSAT
 INTEGER*2 IP, IVLVST, ICATHX, ICATH, IPERD
 INTEGER*4 ITP
 INTEGER*2 IHR, IMIN, ISEC, I100TH
 INTEGER*2 NOWCOI, NOWO2I, NOWCOA, NOWO2A, NOWSAT, IIO2, IICO2

REAL*8 SFO2, SFCO2, SFSAT, XINTO2, XINTCO, XINTSA
 REAL*8 SAMFLO, BP, RT, RMSAT
 REAL*8 DESO2, DESCO2, RPIO2, RPICO2, PERDO2, PERDCO
 REAL*8 AVAO2, AVACO2, AVASAT, AVIO2, AVICO2
 REAL*8 AVO2A, AVCO2A, AVSAT, AVO2I, AVCO2I
 REAL*8 V, QO2, QC2, QN2, QAC2
 REAL*8 O2DIFF, CODIFF, A, B, C, D

CHARACTER*12 FLCAL1, FLCAL2, FLCH
 CHARACTER*17 EXPR
 CHARACTER*14 STAT1
 CHARACTER*1 PAR1, PAR2

CHARACTER*16 DATE
 CHARACTER*16 SUBNO
 CHARACTER*17 EXPNO

COMMON /GASARR/IPO2(0:49),IPCO2(0:49),ISAT(0:49),ILIMIT
 COMMON /CALDAT/SFO2(2),SFCO2(2),SFSAT(2),XINTO2(2),XINTCO(2)
 ,XINTSA(2)
 COMMON /AVDATA/AVAO2(50),AVACO2(50),AVASAT(50),AVIO2(50)
 ,AVICO2(50),DESO2,DESCO2,JBRNO
 COMMON /AV/AVO2A(4),AVCO2A(4),AVSAT(4),AVO2I(4),AVCO2I(4)
 COMMON /VLV/IP(0:100),ITP(0:100)
 COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D
 COMMON /CHEYNE/PERDO2,PERDCO,IPERD,ITIMEY

DATA J,IP(0),IP(1),IP(2)/1,0,0,0/
 DATA ITP(0),ITP(1),ITP(2)/0,1800000,1800000/
 DATA IBRNO,JBRNO/1,1/
 DATA (IPO2(O),O=0,49)/50*0/
 DATA (IPCO2(O),O=0,49)/50*0/
 DATA (ISAT(O),O=0,49)/50*0/
 DATA O2DIFF,CODIFF/1.0,0.3/
 DATA ILIMIT/60/
 DATA ICHPCO,ICHPO2,ICHSAT/2,3,6/
 DATA IADDCA,IADDCB/778,770/
 DATA MANAUT,IONOFF,ITERM,ICATH/6,5,4,7/
 DATA IAO2,IACO2,IASAT/3000,0,0/
 DATA IIO2,IICO2/0,3000/
 DATA PERDO2,PERDCO/100.,15./
 DATA IPERD/0/

ISTAT=0

C GETTING FILE INFORMATION.

1 FORMAT (1X,'ENTER NAME OF CALIBRATION FILE FOR CATHETER 1 ',
 : TO BE READ (WITH EXTENSION)')
 PRINT 1
 2 FORMAT (A12)
 READ 2,FLCAL1

13 FORMAT (1X,'ENTER NAME OF CALIBRATION FILE FOR CATHETER 2 ',
 : TO BE READ (WITH EXTENSION)')
 PRINT 13
 READ 2,FLCAL2

C READ IN DATA FROM CALIBRATION FILES

CALL RDCAL(FLCAL1,FLCAL2,DATE,EXPR,SUBNO,EXPNO,DELAY,
 : SAMFLO,BP,RT,RMSAT,IFLOK)

IF (IFLOK.EQ.0) THEN
 3 FORMAT(1X,'ERROR IN ACCESS OF CALIBRATION FILES.')

PRINT 3
 GOTO 999

ENDIF

4 FORMAT (1X,'ENTER RUN NUMBER.')

PRINT 4
 5 FORMAT (I2)
 READ 5,IRNN

6 FORMAT (1X,'ENTER FILE NAME FOR DATA FILE, WITH EXTENSION.')

PRINT 6
 7 FORMAT (A12)
 READ 7,FLCH

C OPEN UNFORMATTED END OF BREATH FILE.

OPEN (UNIT=2,FILE=FLCH,STATUS='NEW')

C ACQUIRE PO2 AND PCO2 VALUES.

8 FORMAT (1X,'ENTER DESIRED VALUE FOR ARTERIAL PO2 (TORR)')

PRINT 8
 9 FORMAT (F5.1)
 READ 9,DESO2

10 FORMAT (1X,'ENTER DESIRED VALUE FOR ARTERIAL PCO2 (TORR)')

PRINT 10
 11 FORMAT (F5.1)
 READ 11,DESCO2

C	READ IN PARAMETERS FROM PARAMETER LISTING.	:	' AVO2, AVCO2, AVSAT, P/N')
	CALL RDPARS(MINGAP,ISTLTI,IFLOK)		WRITE (2,26)
	IF (IFLOK.EQ.0) THEN	C	CONVERT VALVE FLOWS TO LITRES PER MSEC.
12	FORMAT (1X,'ERROR IN ACCESS OF PARAMETER LISTING.')		QO2=QO2/60000.
	PRINT 12		QC2=QC2/60000.
	GOTO 999		QN2=QN2/60000.
	ENDIF		QAC2=QAC2/60000.
C	CHANGE PARAMETERS ?	C	
16	FORMAT (1X,'WOULD YOU LIKE TO SEE THE PARAMETERS (Y/N) ?')	C	SET UP PORTS A AND B AS INPUT AND PORT C AS OUTPUT PORTS.
	PRINT 16	C	
17	FORMAT (A1)		CALL OUTDIG (799,146)
	READ 17,PAR1		
	IF(PAR1.EQ.'Y'.OR.PAR1.EQ.'Y') CALL SHPARS(MINGAP,ISTLTI)	C	
18	FORMAT (1X,'WOULD YOU LIKE TO CHANGE THE PARAMETERS (Y/N) ?')	C	SET UP CLOCK.
	PRINT 18	C	
19	FORMAT (A1)		IOSECS=30000
	READ 19,PAR2		MOSECS=1000
	IF(PAR2.EQ.'Y'.OR.PAR2.EQ.'Y') CALL CHPARS(MINGAP,ISTLTI)		ISAMPL=20
C	CREATE HEADERS FOR BREATH-BY-BREATH FILE.	C	
20	FORMAT ('BREATH-BY-BREATH FILE WRITTEN AS ',A12)	C	INITIALISE ARRAY INDEX FOR RING BUFFERS.
	WRITE (2,20) FLCH	C	
21	FORMAT (A16)		IARRID=-1
	WRITE (2,21) DATE		
22	FORMAT (A17)		CALL SETCLK
	WRITE (2,22) EXPR		CALL RDCLKI(ISECS,MSECS)
	WRITE (2,21) SUBNO	27	IF ((IOSECS-ISECS).NE.0) GOTO 27
	WRITE (2,22) EXPNO		
23	FORMAT ('RUN NO, ',I2)	C	NOW COUNTING
	WRITE (2,23) IRNN		
24	FORMAT ('DESIRED O2, ',F5.1)	C	
	WRITE (2,24) DESO2	C	READY TO START.
25	FORMAT ('DESIRED CO2, ',F5.1)	C	
	WRITE (2,25) DESCO2	C	
26	FORMAT ('BR.NO.,EXP. TIME, PIO2, PICO2, PAO2, PACO2, SAT., ',		

PAUSE 'HIT RETURN TO START DATA ACQUISITION AND CONTROL.'

28 FORMAT (1X,'DATA ACQUISITION STARTED.')

PRINT 28

C
C
C
C
C
C

LOOK FOR END OF EXPIRATION BEFORE STARTING RECORDS. START
BY READING TIME FROM INTERNAL COUNTER (IE: NOT TIME OF DAY)
SO THAT A GAP OF 1040 MSEC MAY BE LEFT BEFORE EXPSCH IS CALLED
AS NO CO2 DATA EXISTS IN THE ARRAY, IPCO2().

CALL RDCLKI(ISECS,MSECS)
ITIMED=1000*(IOSECS-ISECS)+(MOSECS-MSECS)

29 CALL RDCLKI (ISECS,MSECS)
ITIME=1000*(IOSECS-ISECS)+(MOSECS-MSECS)
IF (ITIME.LT.(ITIMES+ISAMPL)) GOTO 29
ITIMES=ITIME
IARRID=IARRID+1
IARRID=MOD(IARRID,50)

CALL ADCONV (JPO2,ICHPO2)
CALL ADCONV (JPO2,ICHPO2)
IPO2(IARRID)=JPO2

CALL ADCONV (JPCO,ICHPCO)
CALL ADCONV (JPCO,ICHPCO)
IPCO2(IARRID)=JPCO

CALL ADCONV (JSAT,ICHSAT)
CALL ADCONV (JSAT,ICHSAT)
ISAT(IARRID)=JSAT

C CHECKING FOR 1040 MSEC INTERVAL BEFORE LOOKING FOR END OF
C FIRST EXP..

30 IF (ITIMES.LT.(ITIMED+1040)) GOTO 29
CALL EXPSCH(IARRID,IPOSSE,NOWCOA,NOWO2A,NOWSAT)
IF(IPOSSE.EQ.0) GOTO 29

CALL GETTIM(IHR,IMIN,ISEC,I100TH)

NOW START 20 MSEC LOOP FOR MAIN PROGRAM.

C SET TO INSPIRATION.

IPHASE=0
IPOSSE=0
IPOSSI=0
ITIMEP=29999999
ITIMEI=29999999
ITIMEV=ITIMES
ITIMEX=ITIMES
ITIMEA=ITIMES
ITIMEE=ITIMES

40 CALL RDCLKI (ISECS,MSECS)
ITIME=1000*(IOSECS-ISECS)+(MOSECS-MSECS)
IF (ITIME.LT.(ITIMES+ISAMPL)) GOTO 40
ITIMES=ITIME
IARRID=IARRID+1
IARRID=MOD(IARRID,50)

CALL ADCONV (JPO2,ICHPO2)
CALL ADCONV (JPO2,ICHPO2)
IPO2(IARRID)=JPO2

CALL ADCONV (JPCO,ICHPCO)
CALL ADCONV (JPCO,ICHPCO)
IPCO2(IARRID)=JPCO

CALL ADCONV (JSAT,ICHSAT)
CALL ADCONV (JSAT,ICHSAT)
ISAT(IARRID)=JSAT

IENDE=0
IENDI=0

IF(IPHASE.EQ.1) THEN

***** END OF EXPIRATION VERIFICATION *****

WHEN 300 MSEC AFTER LAST POSSIBLE END-OF-EXP. HAS BEEN PASSED
CHECK TO SEE IF CO2 VALUES ARE NEAR INSP. VALUES (200 UNITS LESS
THAN EXP. VALUES). IF YES, THEN EXP. HAS BEEN FOUND AND SET
IENDE TO 1.

IF(ITIMES.GT.ITIMEP) THEN
IF(JPCO.LT.(IACO2-200)) IENDE=1
ENDIF

ELSEIF(IPHASE.EQ.0) THEN

***** END OF INSPIRATION VERIFICATION *****

SAME AS EXP. VERIFICATION BUT CO2 VALUES MUST BE AT LEAST 200
UNITS MORE THAN INSP. VALUES.

IF(ITIMES.GT.ITIMEI) THEN
IF(JPCO.GT.(IICO2+200)) IENDI=1
ENDIF

ENDIF

LOOK TO CHANGE VALVE STATUS IN CHAMBER. THE PROGRAM WILL
ONLY
ENTER THE VALVE CHANGING SUBROUTINE IF J HAS BEEN MOVED ON
ONE.

IF (IP(J).EQ.0.AND.IP(J+1).EQ.0) THEN

STAT1='NOT ADJUSTING'

ELSE

STAT1='ADJUSTING'

ENDIF

A=J

IF (ITIMES.GE.(ITIMEV+ITP(J))) J=J+1

IVLVST=IP(J)

IF (A.NE.J) CALL VLVCHG(IVLVST)

TESTING FOR END OF INSPIRATION OR EXPIRATION.

IF (IPHASE.EQ.0) THEN

CALL INSSCH(IARRID,IPOSSI,NOWCOI,NOWO2I)

ELSEIF (IPHASE.EQ.1) THEN

CALL EXPSCH(IARRID,IPOSSE,NOWCOA,NOWO2A,NOWSAT)

ELSE

FORMAT(1X,'ERROR. NEITHER IN EXPIRATION NOR
INSPIRATION.')

PRINT 50

GOTO 999

ENDIF

RETURN TO START OF CYCLE IF NO POSSIBLE OR DEFINITE ENDS OF
CYCLE HAVE BEEN FOUND.

IF ((IPOSSE+IPOSSI+IENDI+IENDE).EQ.0) GOTO 40

ROOT OUT FLUCTUATIONS IN END-TIDAL GAS. IF AN END-OF-
INSPIRATION IS FOUND LESS THAN 800 MSEC AFTER A CONFIRMED
END-OF-EXPIRATION, THEN DISCOUNT IT. RESET ITIMEE TO THE
PRESENT TIME TO ACCOUNT FOR ANY OTHER FLUCTUATIONS.

IF(IPOSSI.EQ.1) THEN

IF(ITIMES.LE.(ITIMEE+800)) THEN

ITIMEE=ITIMES

GOTO 40

ENDIF

ENDIF

***** POSSIBLE END OF EXP. FOUND *****

SET UP A TIME 300 MSEC LATER TO CHECK WHETHER CO2 VALUES ARE
CLOSE

C TO INSP. OR EXP. VALUES. MEANWHILE STORE MAX CO2 AND MIN O2
 C FOR BEST
 C EXP. VALUES.

IF (IPOSSE.EQ.1) THEN
 ITIMEP=ITIMES+300
 IACO2=MAX0(NOWCOA,IACO2)
 IAO2=MIN0(NOWO2A,IAO2)
 IASAT=NOWSAT
 ENDIF

C ***** POSSIBLE END OF INSP. FOUND *****
 C
 C DITTO.
 C STORE MIN CO2 AND MAX O2 FOR BEST INSP. VALUES.

IF (IPOSSI.EQ.1) THEN
 ITIMEI=ITIMES+300
 IICO2=MIN0(NOWCOI,IICO2)
 IIO2=MAX0(NOWO2I,IIO2)
 ENDIF

C IF NO DEFINITE ENDS OF CYCLE FOUND RETURN TO START.

IF (IENDI.EQ.0.AND.IENDE.EQ.0) GOTO 40

C
 C CHECK WHICH CATHETER IN USE.
 C

CALL INDIG(IADDCB,ISWTCB)
 IF(BTEST(ISWTCB,ICATH)) THEN
 ICATHX=2
 ELSE
 ICATHX=1
 ENDIF

C
 C RUN THROUGH END OF EXPIRATION OR INSPIRATION ROUTINES.
 C

IF (IENDE.EQ.1) THEN
 ISTAT=ITIMEX-ITIMES
 IF(ISTAT.LE.0) ISTAT=0
 CALL ENDEXP(RPIO2,RPICO2,IAO2,IACO2,IASAT,IPHASE,
 : ITIMEP,ITIMEA,ITIMEE,IBRNO,STAT1,ISTAT,ICATHX,IADDCB,IFLOK)
 IPOSSE=0
 IF (IFLOK.EQ.0) GOTO 1015
 ELSEIF (IENDI.EQ.1) THEN
 CALL ENDINS(IIO2,IICO2,RPIO2,RPICO2,IPHASE,ITIMEI,ICATHX)
 IPOSSI=0
 ELSE
 60 FORMAT(1X,'ERROR. VARIABLES RETURNED FROM'
 : 'SUBROUTINE INVALID.')

PRINT 60
 GOTO 999
 ENDIF
 C
 C IF AT END OF EXPIRATION, THEN SEE IF WE NEED TO INITIATE A NEW
 C SET OF CHANGES CHAMBER TOCONCENTRATION OR WHETHER WE
 C WANT TO TO TERMINATE THE PROGRAM.
 C

IF (IENDE.NE.1) GOTO 40

C
 C ONLY TEST WHETHER CHAMBER NEEDS CHANGING IF ISTLTI MINS HAS
 C ELAPSED SINCE START OF PROGRAM. TEST FOR WHETHER CHANGING
 C CHAMBER CONCENTRATION IS ON MANUAL(0) OR AUTO(1). IF MANUAL
 C THEN TEST FOR ON(1) OR OFF(0). IF ON MANUAL AND ON THEN MUST
 C CHECK THAT MINGAP MINUTES HAVE ELAPSED SINCE LAST CHANGE
 C (THEREFORE ALLOWING FOR THE SWITCH BEING LEFT ON).
 C

IF (ITIMES.LT.(ITIMEV+(ISTLTI*60000))) GOTO 200

C CALL INDIG(IADDCA,ISWTCA)
 C IF (BTEST(ISWTCA,MANAUT)) THEN
 C


```

C   ON AUTO. THEREFORE CHECK THAT EITHER MINGAP MINS HAVE
C   PASSED BEFORE CHANGING CONCENTRATION AND THAT CONCS. HAVE C
C   DRIFTED BY MORE THAN EITHER O2DIFF OR CODIFF (TORR).
C
      IF (ITIMES.GT.(ITIMEX+100+(MINGAP*60000))) THEN
        IF(IPERD.EQ.1) THEN
          CALL CHANGE(DESO2,DESCO2,ITIMES,ITIMEX,
            BP)
          ISTAT=ITIMEX-ITIMES
C
C   RESET STARTING TIME FOR VALVE CHANNGES AND INITIALISE J.
      ITIMEV=ITIMES
      J=0
      ELSEIF ((ABS(AVO2A(4)-DESO2).LT.O2DIFF).AND.(ABS
        (AVCO2A(4)-DESCO2).LT.CODIFF)) THEN
        ITIMEX=ITIMEX+(MINGAP*60000)
        ISTAT=0
      ELSE
        CALL CHANGE(DESO2,DESCO2,ITIMES,ITIMEX,
          BP)
        ISTAT=ITIMEX-ITIMES
C
C   RESET STARTING TIME FOR VALVE CHANGES AND INITIALISE J.
      ITIMEV=ITIMES
      J=0
    ENDIF
  ENDIF
ELSEIF (BTEST(ISWTCA,IONOFF).NAND.BTEST(ISWTCA,MANAUT)) THEN
C
C   IN MANUAL + ON. TEST FOR WHETHER MINGAP HAS ELAPSED SINCE
C   LAST CHANGE.
      IF (ITIMES.GT.(ITIMEX+(MINGAP*60000))) THEN
        CALL CHANGE(DESO2,DESCO2,ITIMES,ITIMEX,BP)
        ISTAT=ITIMEX-ITIMES

```

RESET STARTING TIME FOR VALVE CHANGES AND INITIALISE J.

```

      ITIMEV=ITIMES
      J=0
    ELSE
      ISTAT=0
    ENDIF
  ELSE
    ISTAT=0
  ENDIF

```

TEST FOR REQUEST FOR TERMINATION.

```

CALL INDIG (IADDCA,ISWTCA)
IF (BTEST(ISWTCA,ITERM)) GOTO 999

```

IF TERMINATION HAS NOT BEEN REQUESTED THEN RETURN TO BEGINNING OF LOOP.

GOTO 40

END OF PROGRAM ROUTINE.

```

999 CONTINUE
IF (IBRNO.EQ.1) THEN
  STOP

```

```

ELSE
  IBRNO=IBRNO-1
  FORMAT ('NO. OF BREATHS, ',I4)
  WRITE (2,1001) IBRNO
  FORMAT ('TIME OF START : ',I2,',',I2,',',I2,',',I2)
  WRITE (2,1002) IHR,IMIN,ISEC,I100TH
  IVLVST=0
  CALL VLVCHG(IVLVST)
  CLOSE (2,ERR=1025)

```

```

ENDIF

1010  FORMAT(1X,'FILES WRITTEN SUCCESSFULLY AND PROGRAM
TERMINATED')
      PRINT 1010
      STOP

1015  CONTINUE
1020  FORMAT(1X,'ERROR IN WRITING FILE. PROGRAM TERMINATED.')
      PRINT 1020
      CLOSE (2,ERR=1025)
      STOP

1025  CONTINUE
1030  FORMAT(1X,'ERROR IN CLOSING FILE')
      PRINT 1030
      STOP
      END@

```

```

SUBROUTINE RDCAL(FLCAL1,FLCAL2,DATE,EXPR,SUBNO,EXPNO,
: IDELAY,SAMFLO,BP,RT,RMSAT,IFLOK)

```

```

C THIS SUBROUTINE READS INFORMATION FROM THE CALIBRATION FILE.

```

```

C
C IFLOK - INDICATOR FOR FILE OK (1=OK,0=FAIL)
C

```

```

CHARACTER*16 DUMMY
CHARACTER*12 FLCAL1,FLCAL2
CHARACTER*17 EXPR
CHARACTER*16 DATE
CHARACTER*16 SUBNO
CHARACTER*20 EXPNO

```

```

INTEGER*2 IFLOK,IDELAY,I

```

```

REAL*8 SFCO2,SFO2,SFSAT,XINTCO,XINTO2,XINTSA
REAL*8 SAMFLO,BP,RT,RMSAT

```

```

COMMON /CALDAT/SFO2(2),SFCO2(2),SFSAT(2),XINTO2(2),XINTCO(2)
,XINTSA(2)

```

```

: IFLOK IS SET TO 1 IF THE READ IS SUCCESSFUL, OTHERWISE A CODE
C OF 0 INDICATES TO THE CALLING ROUTINE THAT SOMETHING FAILED
C

```

```

C PRESUME THAT THIS WILL WORK
C

```

```

IFLOK=1

```

```

C
C OPEN CALIBRATION FILE 1 AND CHECK FOR ERROR
C

```

```

OPEN(UNIT=8,FILE=FLCAL1,STATUS='OLD',ERR=9000)

```

```

C MUST HAVE OPENED OK, SO READ THE DATA
C

```

```

C
C HEADER INFORMATION
C

```

```

1000  FORMAT(A16)
1010  FORMAT(A17)
1015  FORMAT(A15)
1020  FORMAT(A14)
1040  FORMAT(A10)

```

```

READ(8,1000,ERR=9000) DUMMY

```


C THIS SUBROUTINE READS IN THE PARAMETERS FROM A LISTING
C (PARS.LST)

INTEGER*2 MINGAP,ISTLTI,IFLOK

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 A,B,C,D

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

OPEN (UNIT=3,FILE='PARS.LST',STATUS='OLD',ERR=200)

100 FORMAT (20X,F6.1)
110 FORMAT (20X,F5.1)
120 FORMAT (27X,I2)
130 FORMAT (28X,I2)
140 FORMAT (/ ,1X,F4.1 / ,1X,F4.1 / ,1X,F4.1 / ,1X,F4.1)

READ (3,100,ERR=200) V
READ (3,110,ERR=200) QO2
READ (3,110,ERR=200) QC2
READ (3,100,ERR=200) QN2
READ (3,100,ERR=200) QAC2
READ (3,120,ERR=200) MINGAP
READ (3,130,ERR=200) ISTLTI
READ (3,140,ERR=200) A,B,C,D
PRINT *,A,B,C,D

IFLOK=1
CLOSE (3)

RETURN

200 IFLOK=0
CLOSE (3)

RETURN
END

SUBROUTINE SHPARS(MINGAP,ISTLTI)

C THIS SUBROUTINE PRESENTS THE PARAMETERS TO THE SCREEN.

INTEGER*2 MINGAP,ISTLTI

REAL*8 V,QO2,QC2,QN2,QAC2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2

300 FORMAT (1X,'VOLUME OF CHAMBER (LITRES) : ',F6.1)
310 FORMAT (1X,'FLOW THROUGH O2 VALVE (LITRES/MIN) : ',F5.1)
320 FORMAT (1X,'FLOW THROUGH CO2 VALVE (LITRES/MIN) : ',F5.1)
330 FORMAT (1X,'FLOW THROUGH N2 VALVE (LITRES/MIN) : ',F6.1)
340 FORMAT (1X,'FLOW THROUGH CO2 ABSORBER (LITRES/MIN) : ',F6.1)
350 FORMAT (1X,'MINIMUM GAP BETWEEN CHAMBER CONCENTRATION '
: 'CHANGES (MINUTES) : ',I2)
360 FORMAT (1X,'SETTLING TIME FOR CHAMBER (MINUTES) : ',I2)

PRINT 300,V
PRINT 310,QO2
PRINT 320,QC2
PRINT 330,QN2
PRINT 340,QAC2
PRINT 350,MINGAP
PRINT 360,ISTLTI

RETURN
END

SUBROUTINE CHPARS(MINGAP,ISTLTI)

C THIS SUBROUTINE ENABLES THE EXPERIMENTER TO CHANGE THE
C VOLUME OF THE CHAMBER AND THE FLOWS.

INTEGER*2 MINGAP,ISTLTI

REAL*8 V,QO2,QC2,QN2,QAC2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2

400 FORMAT (1X,'CURRENT VOLUME IS, 'F6.1,' (LITRES). ENTER NEW '
: 'VOLUME :')
410 FORMAT (1X,'CURRENT FLOW THROUGH O2 VALVE IS, 'F5.1,' (LI,
: 'TRES/MIN). ENTER NEW FLOW :')
420 FORMAT (1X,'CURRENT FLOW THROUGH CO2 VALVE IS, 'F5.1,' (LI,
: 'TRES/MIN). ENTER NEW FLOW :')
430 FORMAT (1X,'CURRENT FLOW THROUGH N2 VALVE IS, 'F6.1,' (LI,
: 'TRES/MIN). ENTER NEW FLOW :')
440 FORMAT (1X,'CURRENT FLOW THROUGH CO2 ABSORBER IS 'F6.1,' (LI,
: 'TRES/MIN). ENTER NEW FLOW :')
450 FORMAT (1X,'CURRENT MINIMUM GAP ALLOWED BETWEEN CHANGES'
: 'TO CHAMBER CONCENTRATION IS 'I2,' (MINS). ENTER NEW T',
: 'IME :')
460 FORMAT (1X,'CURRENT SETTLING TIME FOR CHAMBER IS,'I2,' (MINS)'
:, '. ENTER NEW TIME :')
470 FORMAT (F6.1)
480 FORMAT (F5.1)
490 FORMAT (I2)

PRINT 400,V
READ 470,V
PRINT 410,QO2
READ 480,QO2
PRINT 420,QC2
READ 480,QC2
PRINT 430,QN2
READ 470,QN2
PRINT 440,QAC2
READ 470,QAC2

PRINT 450,MINGAP
READ 490,MINGAP
PRINT 460,ISTLTI
READ 490,ISTLTI

RETURN
END@

SUBROUTINE SETCLK

C
C THIS ROUTINE INITIALISES THE COUNTER/TIMER ON THE PIO12 BOARD
C TO MODE 2. COUNTER 0 IS LOADED TO COUNT DOWN FROM 30000 AND
C COUNTER 1 IS LOADED TO COUNT DOWN FROM 1000

C
C THE PROGRAM CALLS:
C OUTDIG.....ASSEMBLER ROUTINE TO OUTPUT DATA TO A PORT
C
C PROGRAM VARIABLES
C PORT.....PORT ADDRESS
C CONT.....CONTROL VARIABLE
C CONTL.....COUNTS LOADED INTO COUNTER/TIMER

C
C INTEGER*2 PORT,CONT,CONTL

C
C LOAD CONTROL

C
C PORT=783
C CONT=52
C CALL OUTDIG(PORT,CONT)
C CONT=116
C CALL OUTDIG(PORT,CONT)
C CONT=180
C CALL OUTDIG(PORT,CONT)

C
C LOAD COUNTER 0
C

```

PORT=780
CONTL=48
CALL OUTDIG(PORT,CONTL)
CONTL=117
CALL OUTDIG(PORT,CONTL)

```

```

C
C   LOAD COUNTER 1
C

```

```

PORT=781
CONTL=232
CALL OUTDIG(PORT,CONTL)
CONTL=3
CALL OUTDIG(PORT,CONTL)

```

```

C
C   LOAD COUNTER 2
C

```

```

PORT=782
CONTL=232
CALL OUTDIG(PORT,CONTL)
CONTL=3
CALL OUTDIG(PORT,CONTL)
RETURN

```

```

END@

```

```

SUBROUTINE EXPSCH(IARRID,IPOSSE,NOWCOA,NOWO2A,NOWSAT)
C   THIS SUBROUTINE SEARCHES FOR A POSSIBLE END OF EXPIRATION
C   AND IPOSSE WILL BE RETURNED TO THE MAIN PROGRAM AS EITHER 0
C   OR 1. (0=END-EXPIRATION NOT FOUND, 1=END-EXPIRATION FOUND).
C

```

```

INTEGER*2 IPOSSE,IPO2,IPCO2,ISAT,ILIMIT
INTEGER*2 L,IAVCO(5),IAVO(3),NOWCOA,NOWO2A,NOWSAT
INTEGER*2 IARRID,IARREF

```

```

C   IAVO2(?)...AVERAGE FOR CO2 VALUES 4-6,14-16,...,44-46 : ?=1-5
C   IAVO(?)....AVERAGE FOR O2 VALUES 4-6,14-16,24-26 : ?=1-3

```

```

COMMON /GASARR/IPO2(0:49),IPCO2(0:49),ISAT(0:49),ILIMIT

```

```

IAVCO(1)=0
IAVCO(2)=0
IAVCO(3)=0
IAVCO(4)=0
IAVCO(5)=0

```

```

IAVO(1)=0
IAVO(2)=0
IAVO(3)=0

```

```

IARREF=MOD(IARRID+4,50)

```

```

DO L=IARREF,(IARREF+2)
    IAVCO(1)=IAVCO(1)+IPCO2(MOD(L,50))
    IAVO(1)=IAVO(1)+IPO2(MOD(L,50))

```

```

ENDDO
IAVCO(1)=IAVCO(1)/3
IAVO(1)=IAVO(1)/3

```

```

DO L=(IARREF+10),(IARREF+12)
    IAVCO(2)=IAVCO(2)+IPCO2(MOD(L,50))
    IAVO(2)=IAVO(2)+IPO2(MOD(L,50))

```

```

ENDDO
IAVCO(2)=IAVCO(2)/3
IAVO(2)=IAVO(2)/3

```

```

DO L=(IARREF+20),(IARREF+22)
    IAVCO(3)=IAVCO(3)+IPCO2(MOD(L,50))
    IAVO(3)=IAVO(3)+IPO2(MOD(L,50))

```

```

ENDDO
IAVCO(3)=IAVCO(3)/3
IAVO(3)=IAVO(3)/3

```

```

DO L=(IARREF+30),(IARREF+32)

```

```
IAVCO(4)=IAVCO(4)+IPCO2(MOD(L,50))
```

```
ENDDO
```

```
IAVCO(4)=IAVCO(4)/3
```

```
DO L=(IARREF+40),(IARREF+42)
```

```
IAVCO(5)=IAVCO(5)+IPCO2(MOD(L,50))
```

```
ENDDO
```

```
IAVCO(5)=IAVCO(5)/3
```

```
IF(IAVCO(3).GT.(IAVCO(4)+ILIMIT).AND.IAVCO(4).GT.(IAVCO(5)+ILIMIT)) THEN
```

```
    NOWCOA=MAX0(IAVCO(1),IAVCO(2))
```

```
    NOWCOA=MAX0(NOWCOA,IAVCO(3))
```

```
    NOWO2A=MIN0(IAVO(1),IAVO(2))
```

```
    NOWO2A=MIN0(NOWO2A,IAVO(3))
```

```
    NOWSAT=(ISAT(IARREF)+ISAT(MOD(IARREF+1,50)))+ISAT(MOD(IARREF+2,50))/3
```

```
    IPOSSE=1
```

```
ELSE
```

```
    IPOSSE=0
```

```
ENDIF
```

```
RETURN
```

```
END@
```

```
SUBROUTINE INSSCH(IARRID,IPOSSI,NOWCOI,NOWO2I)
```

```
THIS SUBROUTINE SEARCHES FOR A POSSIBLE END OF INSPIRATION  
AND IPOSSI WILL BE RETURNED TO THE MAIN PROGRAM AS EITHER 0  
OR 1. (0=END-INSPIRATION NOT FOUND, 1=END-INSPIRATION FOUND).
```

```
INTEGER*2 IPOSSI,IPO2,IPCO2,ISAT,ILIMIT
```

```
INTEGER*2 L,IAVCO(5),IAVO(3),NOWCOI,NOWO2I
```

```
INTEGER*2 IARRID,IARREF
```

```
C IAVCO(?)...AVERAGE FOR CO2 VALUES 4-6,14-16,...,44-46 : ?=1-5.
```

```
C IAVO(?)....AVERAGE FOR O2 VALUES 4-6,14-16,24-26 : ?=1-3
```

```
COMMON /GASARR/IPO2(0:49),IPCO2(0:49),ISAT(0:49),ILIMIT
```

```
IAVCO(1)=0
```

```
IAVCO(2)=0
```

```
IAVCO(3)=0
```

```
IAVCO(4)=0
```

```
IAVCO(5)=0
```

```
IAVO(1)=0
```

```
IAVO(2)=0
```

```
IAVO(3)=0
```

```
IARREF=MOD(IARRID+4,50)
```

```
DO L=IARREF,(IARREF+2)
```

```
IAVCO(1)=IAVCO(1)+IPCO2(MOD(L,50))
```

```
IAVO(1)=IAVO(1)+IPO2(MOD(L,50))
```

```
ENDDO
```

```
IAVCO(1)=IAVCO(1)/3
```

```
IAVO(1)=IAVO(1)/3
```

```
DO L=(IARREF+10),(IARREF+12)
```

```
IAVCO(2)=IAVCO(2)+IPCO2(MOD(L,50))
```

```
IAVO(2)=IAVO(2)+IPO2(MOD(L,50))
```

```
ENDDO
```

```
IAVCO(2)=IAVCO(2)/3
```

```
IAVO(2)=IAVO(2)/3
```

```
DO L=(IARREF+20),(IARREF+22)
```

```
IAVCO(3)=IAVCO(3)+IPCO2(MOD(L,50))
```

```
IAVO(3)=IAVO(3)+IPO2(MOD(L,50))
```

```
ENDDO
```

```
IAVCO(3)=IAVCO(3)/3
```

```
IAVO(3)=IAVO(3)/3
```

```
DO L=(IARREF+30),(IARREF+32)
  IAVCO(4)=IAVCO(4)+IPCO2(MOD(L,50))
ENDDO
IAVCO(4)=IAVCO(4)/3
```

```
DO L=(IARREF+40),(IARREF+42)
  IAVCO(5)=IAVCO(5)+IPCO2(MOD(L,50))
ENDDO
IAVCO(5)=IAVCO(5)/3
```

```
IF(IAVCO(3).LT.(IAVCO(4)-ILIMIT).AND.IAVCO(4).LT.(IAVCO(5)
:- ILIMIT)) THEN
  NOWCOI=MIN0(IAVCO(1),IAVCO(2))
  NOWCOI=MIN0(NOWCOI,IAVCO(3))
  NOWO2I=MAX0(IAVO(1),IAVO(2))
  NOWO2I=MAX0(NOWO2I,IAVO(3))
  IPOSSI=1
ELSE
  IPOSSI=0
ENDIF

RETURN
END@
```

```
SUBROUTINE ENDEXP(RPIO2,RPICO2,IAO2,IACO2,IASAT,IPHASE,
: ITIMEP,ITIMEA,ITIMEE,IBRNO,STAT1,ISTAT,ICATHX,IADDCB,IFLOK)
```

```
THIS SUBROUTINE COLLECTS THE END-EXPIRATORY VALUES (IN TORR)
AND CHANGES THE FLAG, IPHASE, TO INSPIRATION (0). IT ALSO
WRITES THE DATA TO THE SCREEN AND DISK.
```

```
C IGAS.....INDICATOR OF GAS TO BE AVERAGED.
C IAV.....BIT FOR 1 OR 2 MINUTE AVERAGING SWITCH.
C ITIMAV...TAKES VALUE OF 60000 OR 120000 MSEC FOR AVERGING
C PROCESS.
```

```
INTEGER*2 M,IFLOK,IGAS,IPERD
INTEGER*2 IPHASE,IBRNO,ICATHX,IADDCB,ISWTCB
INTEGER*2 IAO2,IACO2,IASAT,JBRNO
INTEGER*4 ITIMEP,ITIMEA,ITIMEE,ISTAT,ITIMEY
```

```
REAL*8 SFO2,SFCO2,SFSAT,XINTO2,XINTCO,XINTSA
REAL*8 AVAO2,AVACO2,AVASAT,AVIO2,AVICO2,AVRAGE
REAL*8 AVO2A,AVCO2A,AVSAT,AVO2I,AVCO2I,DESO2,DESCO2
REAL*8 RPIO2,RPICO2,RPAO2,RPACO2,RSAT,PERDO2,PERDCO
```

```
CHARACTER*14 STAT1
CHARACTER*3 STAT2
```

```
COMMON /CALDAT/SFO2(2),SFCO2(2),SFSAT(2),XINTO2(2),XINTCO(2)
: ,XINTSA(2)
COMMON /AVDATA/AVAO2(50),AVACO2(50),AVASAT(50),AVIO2(50)
: ,AVICO2(50),DESO2,DESCO2,JBRNO
COMMON /AV/AVO2A(4),AVCO2A(4),AVSAT(4),AVO2I(4),AVCO2I(4)
COMMON /CHEYNE/PERDO2,PERDCO,IPERD,ITIMEY
```

```
DATA IPER/6/
DATA IADDCB/770/
```

```
RPAO2=SFO2(ICATHX)*IAO2+XINTO2(ICATHX)
RPACO2=SFCO2(ICATHX)*IACO2+XINTCO(ICATHX)
RSAT=SFSAT(ICATHX)*IASAT+XINTSA(ICATHX)
```

```
ITIMEE=ITIMEP
IF(ITIMEE.GT.(ITIMEY+8000)) THEN
  PRINT *, 'CHECK FOR PERIODIC BREATHING'
  ITIMEY=ITIMEP
ELSE
  ITIMEY=ITIMEP
ENDIF
```


C ADD VALUES TO CURRENT MINUTE AVERAGE DATA ARRAY.

```

AVAO2(JBRNO)=RPAO2
AVACO2(JBRNO)=RPACO2
AVIO2(JBRNO)=RPIO2
AVICO2(JBRNO)=RPICO2
AVASAT(JBRNO)=RSAT

```

C CHECK WHETHER IN PERIODIC BREATHING MODE OR NOT.

IF(IPERD.EQ.0) THEN

CALL INDIG(IADDCB,ISWTCB)

IF(BTEST(ISWTCB,IPER)) THEN

10 FORMAT(1X,'ENTER DESIRED INSPIRED O2 (???.?)')

PRINT 10

20 FORMAT(F5.2)

READ 20,PERDO2

30 FORMAT(1X,'ENTER DESIRED INSPIRED CO2 (???.?)')

PRINT 30

40 FORMAT(F4.2)

READ 40,PERDCO

IPERD=1

STAT2='PER'

ELSE

IPERD=0

STAT2='NOR'

ENDIF

ELSE

CALL INDIG(IADDCB,ISWTCB)

IF(BTEST(ISWTCB,IPER)) THEN

IPERD=1

STAT2='PER'

ELSE

IPERD=0

STAT2='NOR'

ENDIF

ENDIF

C IS IT TIME TO UPDATE 1 MINUTE AVERAGE ? IF SO, DO IT.

IF (ITIMEE.GE.(ITIMEA+60000)) THEN

AVO2A(1)=AVO2A(2)

AVO2A(2)=AVO2A(3)

AVCO2A(1)=AVCO2A(2)

AVCO2A(2)=AVCO2A(3)

AVO2I(1)=AVO2I(2)

AVO2I(2)=AVO2I(3)

AVCO2I(1)=AVCO2I(2)

AVCO2I(2)=AVCO2I(3)

AVSAT(1)=AVSAT(2)

AVSAT(2)=AVSAT(3)

IGAS=1

AVO2A(3)=AVRAGE(IGAS)

IGAS=2

AVCO2A(3)=AVRAGE(IGAS)

IGAS=3

AVSAT(3)=AVRAGE(IGAS)

IGAS=4

AVO2I(3)=AVRAGE(IGAS)

IGAS=5

AVCO2I(3)=AVRAGE(IGAS)

AVO2A(4)=(AVO2A(1)+AVO2A(2)+AVO2A(3))/3.

AVCO2A(4)=(AVCO2A(1)+AVCO2A(2)+AVCO2A(3))/3.

AVO2I(4)=(AVO2I(1)+AVO2I(2)+AVO2I(3))/3.

AVCO2I(4)=(AVCO2I(1)+AVCO2I(2)+AVCO2I(3))/3.

AVSAT(4)=(AVSAT(1)+AVSAT(2)+AVSAT(3))/3.

DO M=1,JBRNO

AVAO2(JBRNO)=0.

AVACO2(JBRNO)=0.

AVASAT(JBRNO)=0.

AVIO2(JBRNO)=0.

AVICO2(JBRNO)=0.

ENDDO

JBRNO=1

ITIMEA=ITIMEE

ELSE

JBRNO=JBRNO+1

ENDIF

```

C      WRITE VALUES TO SCREEN.

600    FORMAT (1X,8(F5.1,1H,,),A14,1H,A3,1H,I8)
       WRITE (*,600) RPIO2,RPICO2,RPAO2,RPACO2,RSAT,AVO2A(3),AVCO2A(3),
       :    AVSAT(3),STAT1,STAT2,ISTAT

C      WRITE VALUES TO DISK.
610    FORMAT(2X,I4,1H,,1X,I8,8(1H,,1X,F5.1),1H,A1)
       WRITE (2,610,ERR=700) IBRNO,ITIMEE,RPIO2,RPICO2,RPAO2,RPACO2
       :    ,RSAT,AVO2A(3),AVCO2A(3),AVSAT(3),STAT2

       IBRNO=IBRNO+1

       IFLOK=1
       IPHASE=0
       ITIMEP=29999999
       IAO2=3000
       IACO2=0
       IASAT=0

       RETURN

700    IFLOK=0
       RETURN
       END@

SUBROUTINE ENDINS(IIO2,IICO2,RPIO2,RPICO2,IPHASE,ITIMEI,ICATHX)
C
C      THIS SUBROUTINE COLLECTS THE END-INPSIRATORY VALUES (IN
C      TORR) AND CHANGES THE FLAG, IPHASE, TO EXPIRATION (1).
C

       INTEGER*2 IPHASE,ICATHX
       INTEGER*2 IIO2,IICO2
       INTEGER*4 ITIMEI

```

```

REAL*8 RPIO2,RPICO2
REAL*8 SFO2,SFCO2,SFSAT,XINTO2,XINTCO,XINTSA

COMMON /CALDAT/SFO2(2),SFCO2(2),SFSAT(2),XINTO2(2),XINTCO(2)
:    ,XINTSA(2)

RPIO2=SFO2(ICATHX)*IIO2+XINTO2(ICATHX)
RPICO2=SFCO2(ICATHX)*IICO2+XINTCO(ICATHX)

IPHASE=1
ITIMEI=29999999
IIO2=0
IICO2=3000

RETURN
END@

```

```

SUBROUTINE CHANGE(DESO2,DESCO2,ITIMES,ITIMEX,BP)

```

```

C
C      THIS SUBROUTINE CALCULATES THE NEW INSPIRED VALUES FOR O2
C      AND CO2. IT ALSO CALCULATES CHANGES IN VALVE STATUS TO REACH
C      RNEWO2 AND RNEWCO FROM AVO2I AND AVCO2I. IT RETURNS THE
C      PROCEDURES REQUIRED AND THE TIMES AT WHICH THEY START AND
C      FINISH.
C      PROCEDURE #0 - IP()=0 - DO NOTHING.
C      PROCEDURE #1 - IP()=1 - ADD O2.
C      PROCEDURE #2 - IP()=2 - ADD N2.
C      PROCEDURE #3 - IP()=3 - ADD CO2.
C      PROCEDURE #4 - IP()=4 - ABSORB CO2.
C      O2 AND CO2 WILL BE CONTROLLED SEQUENTIALLY IN A NUMBER OF
C      STAGES, NSTAGE, IF THE OVERALL CHANGE NEEDED IS MORE THAN
C      0.5%.
C

```

```

C      VTOLD.....PREDICTED CURRENT VENTILATION FROM MODEL.
C      VTNEW.....PREDICTED NEW VENTILATION FROM MODEL.

```

```

C      A,B,C,D...CONSTANTS FOR LLOYD-CUNNINGHAM EQUATION.

C      FSO2.....STARTING FRACTION FOR O2.
C      FEO2.....FINISHING FRACTION FOR O2.
C      FSCO2.....STARTING FRACTION FOR CO2.
C      FECO2.....FINISHING FRACTION FOR CO2.
C      F0O2,F0CO2...STARTING FUNCTION FOR EACH STAGE.
C      F1O2,F1CO2...INTERMEDIATE FUNCTION FOR EACH STAGE.
C      F2O2,F2CO2...FINISHING FUNCTION FOR EACH STAGE.
C      F2TO2.....ACTUAL FINISHING FUNCTION FOR O2 AFTER CO2
C              ADJUSTMENTS.
C      ITPI(K).....TIME FOR INDIVIDUAL PROCEDURE.

C      DECLARE LOCAL VARIABLES.

      REAL*8 VTOLD,VTNEW
      REAL*8 AVO2A,AVCO2A,AVSAT,AVO2I,AVCO2I,DESO2,DESCO2
      REAL*8 RNEWO2,RNEWCO,PERDO2,PERDCO
      REAL*8 F0O2(50),F1O2(50),F2O2(50),F2TO2
      REAL*8 F0CO2(50),F1CO2(50),F2CO2(50)
      REAL*8 FSO2,FEO2,FSCO2,FECO2
      REAL*8 FFPP,PPFF
      REAL*8 FNONC,FOONC,FCONO,FAONO
      REAL*8 BP,A,B,C,D

      INTEGER*2 NSTAGE,N,K,IP,IPERD
      INTEGER*4 ITIMES,ITIMEX,ITPI(100),ITP,ITIMEY
      INTEGER*4 ITO2,ITN2,ITC2,ITAC2

      COMMON /VLV/IP(0:100),ITP(0:100)
      COMMON /AV/AVO2A(4),AVCO2A(4),AVSAT(4),AVO2I(4),AVCO2I(4)
      COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D
      COMMON /CHEYNE/PERDO2,PERDCO,IPERD,ITIMEY

      VTOLD=D*(AVCO2A(4)-B)*(1+(A/(AVO2A(4)-C)))
      VTNEW=D*(DESCO2-B)*(1+(A/(DESO2-C)))

C      IF IN PERIODIC BREATHING THEN ADJUST GASES TO THOSE PREDICTED
C      BY USER.

```

```

      IF(IPERD.EQ.1) THEN
          RNEWO2=PERDO2
          RNEWCO=PERDCO
      ELSE
          RNEWO2=PPFF(FFPP(DESO2,BP)+((FFPP(AVO2I(4),BP)-FFPP(AVO2A(4),BP))
:      *VTOLD/VTNEW),BP)
          RNEWCO=PPFF(FFPP(DESCO2,BP)-((FFPP(AVCO2A(4),BP)-FFPP(AVCO2I(4),
:      BP))*VTOLD/VTNEW),BP)
      ENDIF

      PRINT *,RNEWO2
      PRINT *,RNEWCO

C
C      MINIMUM CO2 LEVEL SET TO 3 TORR.
C

      IF(RNEWCO.LT.3.) RNEWCO=3.

      FSO2=FFPP(AVO2I(4),BP)
      FEO2=FFPP(RNEWO2,BP)
      FSCO2=FFPP(AVCO2I(4),BP)
      FECO2=FFPP(RNEWCO,BP)

      NSTAGE=INT(4*MAX(ABS((FSO2-FEO2)*100.),ABS((FSCO2-FECO2)*100.)
:      ))+1

      DO 800 N=1,NSTAGE
          F0O2(N)=FSO2+((N-1)*(FEO2-FSO2)/NSTAGE)
          F0CO2(N)=FSCO2+((N-1)*(FECO2-FSCO2)/NSTAGE)
          F1O2(N)=FSO2+((N)*(FEO2-FSO2)/NSTAGE)
          F1CO2(N)=FSCO2+((N-1)*(FECO2-FSCO2)/NSTAGE)
          F2O2(N)=FSO2+((N)*(FEO2-FSO2)/NSTAGE)
          F2CO2(N)=FSCO2+((N)*(FECO2-FSCO2)/NSTAGE)
800      CONTINUE

      ITP(0)=0

      DO 820 N=1,NSTAGE
          K=(2*N)-1

```

```

C      O2 STAGE.

810    IF(F0O2(N).EQ.F1O2(N)) THEN
        IP(K)=0
        ITP(K)=ITP(K-1)+0
    ELSEIF(F0O2(N).LT.F1O2(N)) THEN
        IP(K)=1
        ITPI(K)=ITO2(F0O2(N),F1O2(N))
        ITP(K)=ITPI(K)+ITP(K-1)
        F1CO2(N)=FOONC(F0CO2(N),ITPI(K))
    ELSEIF(F0O2(N).GT.F1O2(N)) THEN
        IP(K)=2
        ITPI(K)=ITN2(F0O2(N),F1O2(N))
        ITP(K)=ITPI(K)+ITP(K-1)
        F1CO2(N)=FNONC(F0CO2(N),ITPI(K))
    ENDIF

C      CO2 STAGE.

    IF(F1CO2(N).EQ.F2CO2(N)) THEN
        IP(K+1)=0
        ITP(K+1)=ITP(K)+0
    ELSEIF(F1CO2(N).LT.F2CO2(N)) THEN
        IP(K+1)=3
        ITPI(K+1)=ITC2(F1CO2(N),F2CO2(N))
        ITP(K+1)=ITPI(K+1)+ITP(K)
        F2TO2=FCONO(F1O2(N),ITPI(K+1))
    ELSEIF(F1CO2(N).GT.F2CO2(N)) THEN
        IP(K+1)=4
        ITPI(K+1)=ITAC2(F1CO2(N),F2CO2(N))
        ITP(K+1)=ITPI(K+1)+ITP(K)
        F2TO2=FACONO(F1O2(N),F1CO2(N),F2CO2(N))
    ENDIF

C      COMPARE ACTUAL O2, F2TO2, WITH DESIRED O2, F2O2 FOR THAT
C      STAGE.
C      CORRECT TARGET F1O2 IN CASE DIFFERENCE >0.05% AND RESET NEXT
C      START VALUE OF F0O2.

F0O2(N+1)=F2TO2

    IF(ABS(F2TO2-F2O2(N)).GT.0.0005) THEN
        F1O2(N)=F1O2(N)-(F2TO2-F2O2(N))
        GOTO 810
    ENDIF

820    CONTINUE

C      RESET ITIMEX SO THAT TIME OF END OF CHANGES IS KNOWN. THIS
C      ENABLES THE COMPUTER TO IGNORE A MANUAL REQUEST FOR A
C      CHAMBER CONCENTRATION CHANGE FOR A CERTAIN TIME AFTER THE
C      LAST VALVE CHANGE WAS MADE. IT ALSO SETS THE TIME AT WHICH
C      AN AUTOMATIC CHANGE SHOULD BE MADE WHEN ADDED TO A
C      DEFINED INTERVAL.

    ITIMEX=ITIMES+ITP(NSTAGE*2)

C      SET UP IP(NSTAGE*2+1) AND IP(NSTAGE*2+2) AS 0 WITH RESPECTIVE
C      ITP AS, SAY, 60 MINS, SO THAT ONCE THE CHANGES HAVE BEEN MADE
C      NOTHING OCCURS UNTIL NEXT CHANGE IS REQUESTED.

    IP(NSTAGE*2+1)=0
    IP(NSTAGE*2+2)=0
    ITP(NSTAGE*2+1)=60*60000
    ITP(NSTAGE*2+2)=60*60000

    RETURN
    END@

SUBROUTINE VLVCHG(IVLVST)

C      THIS SUBROUTINE CHANGES THE VALVE STATUS BY WRITING TO PORT
C      BIT 1 - O2 VALVE.
C      BIT 2 - N2 VALVE.
C      BIT 3 - CO2 VALVE.

```

C BIT 4 - CO2 ABSORBER.
 C IVLVST.....IP(J) FED DOWN FROM MAIN CONTROL PROGRAM GIVING
 C VALVE STATUS.
 C IVLVDT.....DATA FED TO PORT CONCERNING VALVE CHANGE.

INTEGER*2 IVLVST,IVLVDT

IF(IVLVST.EQ.0) THEN

IVLVDT=0

ELSE

IVLVDT=2** (IVLVST-1)

ENDIF

CALL OUTDIG (798,IVLVDT)

RETURN

END

FUNCTION AVRAGE(IGAS)

C THIS FUNCTION CALCULATES THE MINUTE AVERAGE EXCLUDING THE
 C VALUES
 C OF CO2 THAT ARE MORE THAN 5 MMHG FROM DESCO2 AND OF O2 THAT 10
 C ARE MORE
 C THAN 10 MMHG FROM DESO2. IT ALSO EXCLUDES VALUES OF INSPIRED
 C GASES
 C THAT ARE MORE THAN 2* STANDARD DEVIATION FROM THE AVERAGE.

C IAVBNO.....BREATH NO. IN AVERAGE.

C L.....LOOP VARIABLE.

C SUM.....TOTAL SUM OF ARRAY VARIABLES.

C SUMSQ.....SUM OF DIFFERENCE BETWEEN VALUE AND AVERAGE ALL
 C SQUARED.

C AVTEMP....TEMPORARY AVERAGE FOR CALCULATING SD.

C SD.....STANDARD DEVIATION.

C GAS().....ARRAY HOLDING GAS VALUES FOR MINUTE AVERAGE.

C ??EXCL....LIMIT OF EXCLUSION FROM AVERAGE FOR O2 AND CO2.

C DECLARE LOCAL VARIABLES.

INTEGER*2 L,IAVBNO,JBRNO,IGAS

REAL*8 SUM,SUMSQ,AVTEMP,GAS(50),SD,AVRAGE,O2EXCL,COEXCL

REAL*8 AVAO2,AVACO2,AVASAT,AVIO2,AVICO2,DESO2,DESCO2

COMMON /AVDATA/AVAO2(50),AVACO2(50),AVASAT(50),AVIO2(50)

: ,AVICO2(50),DESO2,DESCO2,JBRNO

O2EXCL=10.

COEXCL=5.

500 CONTINUE

SUM=0.

SUMSQ=0.

AVTEMP=0.

SD=0.

IAVBNO=JBRNO

GOTO (10,20,30,40,50),IGAS

DO L=1,JBRNO

GAS(L)=AVAO2(L)

ENDDO

GOTO 600

20 DO L=1,JBRNO

GAS(L)=AVACO2(L)

ENDDO

GOTO 600

```

30  DO L=1,JBRNO
      GAS(L)=AVASAT(L)
    ENDDO
    GOTO 600

40  DO L=1,JBRNO
      GAS(L)=AVIO2(L)
    ENDDO
    GOTO 600

50  DO L=1,JBRNO
      GAS(L)=AVICO2(L)
    ENDDO

600  CONTINUE

      DO 700 L=1,JBRNO
        SUM=SUM+GAS(L)
700  CONTINUE
      AVTEMP=SUM/JBRNO

C    EXCLUDE ROGUE MEASURED INSPIRED VALUES

      DO 710 L=1,JBRNO
        SUMSQ=SUMSQ+(GAS(L)-AVTEMP)**2
710  CONTINUE
      SD=(SUMSQ/(JBRNO-1))*0.5

      IF(IGAS.LE.5.AND.GE.4) THEN
        DO 720 L=1,JBRNO
          IF(ABS(GAS(L)-AVTEMP).GT.2.*SD) THEN
            SUM=SUM-GAS(L)
            IAVBNO=IAVBNO-1
          ENDIF
        ENDDO
        AVRAGE=SUM/IAVBNO
      ENDIF

720  CONTINUE

C    EXCLUDE ROGUE VALUES FOR MEASURED END-TIDAL GASES

```

```

      IF(IGAS.LE.2) THEN

        IF(IGAS.EQ.1) THEN
          DO L=1,JBRNO
            IF(ABS(GAS(L)-DESO2).GT.O2EXCL) THEN
              SUM=SUM-GAS(L)
              IAVBNO=IAVBNO-1
            ENDIF
          ENDDO
        ELSEIF(IGAS.EQ.2) THEN
          DO L=1,JBRNO
            IF(ABS(GAS(L)-DESCO2).GT.COEXCL) THEN
              SUM=SUM-GAS(L)
              IAVBNO=IAVBNO-1
            ENDIF
          ENDDO
        ENDIF

      ENDIF

C    REDO CALCULATION WITHOUT EXCLUDING BREATHS MORE THAN 10
C    AND 5 MMHG AWAY FROM DESO2 AND DESCO2 IF THERE ARE ZERO
C    BREATHS TO BE AVERAGED AFTER EXCLUSION

      IF(IAVBNO.EQ.0) THEN
        O2EXCL = O2EXCL+10.
        COEXCL = COEXCL+5.
        GOTO 500
      ENDIF

      AVRAGE=SUM/IAVBNO

    ENDIF

    RETURN
  END@

C    GASFUN.FOR HOLDS THE FUNCTIONS THAT :-

```

C 1. CALCULATE THE TIMES FOR VALVES TO BE OPEN.
 C 2. CALCULATE THE EFFECT OF CHANGING ONE GAS ON ANOTHER.
 C 3. CONVERT GASES FROM PARTIAL PRESSURES TO DRY FRACTIONS
 C AND VICE VERSA.

C THE IT??? FUNCTIONS ARE CALCULATED WITH THE GASES AS WET
 C FRACTIONS.
 C

FUNCTION ITO2(STRTO2,FNSHO2)

C THIS FUNTION CALCULATES THE TIME FOR WHICH THE O2 VALVE
 C SHOULD BE OPEN TO REACH FNSHO2 FROM STRTO2.

REAL*8 V,QO2,QC2,QN2,QAC2

C STRTO2.....O2 STARTING VALUE AS A DRY FRACTION.
 C FNSHO2.....O2 FINISHING VALUE AS A DRY FRACTION.

REAL*8 STRTO2,FNSHO2

INTEGER*4 ITO2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

ITO2=(V/QO2)*(DLOG((1.-STRTO2)/(1.-FNSHO2)))

RETURN
 END

FUNCTION ITC2(STRTC2,FNSHC2)

C THIS FUNTION CALCULATES THE TIME FOR WHICH THE CO2 VALVE
 C SHOULD BE OPEN TO REACH FNSHC2 FROM STRTC2.

C STRTC2.....CO2 STARTING VALUE AS A DRY FRACTION.
 C FNSHC2.....CO2 FINISHING VALUE AS A DRY FRACTION

REAL*8 V,QO2,QC2,QN2,QAC2
 REAL*8 STRTC2,FNSHC2

INTEGER*4 ITC2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

ITC2=(V/QC2)*(DLOG((1.-STRTC2)/(1.-FNSHC2)))

RETURN
 END

FUNCTION ITN2(STRTO2,FNSHO2)

C THIS FUNTION CALCULATES THE TIME FOR WHICH THE N2 VALVE C
 SHOULD BE OPEN TO REACH FNSHO2 FROM STRTO2.

C STRTO2.....O2 STARTING VALUE AS A DRY FRACTION.
 C FNSHO2.....O2 FINISHING VALUE AS A DRY FRACTION.

REAL*8 V,QO2,QC2,QN2,QAC2
 REAL*8 STRTO2,FNSHO2

INTEGER*4 ITN2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

ITN2=(V/QN2)*(DLOG(STRTO2/FNSHO2))

RETURN
 END

FUNCTION ITAC2(STRTC2,FNSHC2)

C THIS FUNTION CALCULATES THE TIME FOR WHICH THE CO2 ABSORBER
C SHOULD BE OPEN TO REACH FNSHC2 FROM STRTC2.

C STRTC2.....CO2 STARTING VALUE AS A DRY FRACTION.
C FNSHC2.....CO2 FINISHING VALUE AS A DRY FRACTION.

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 STRTC2,FNSHC2

INTEGER*4 ITAC2

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

ITAC2=(V/(QAC2*(-0.9997)))*(DLOG(FNSHC2/STRTC2))

RETURN
END

FUNCTION FOONC(STRTC2,ITCHG)

C THIS FUNCTION RETURNS THE DRY FRACTION OF CO2 IN THE CHAMBER
C ONCE THE O2 HAS BEEN CHANGED.

C STRTC2.....STARTING FRACTION FOR CO2.
C ITCHG.....TIME FOR WHICH O2 VALVE IS OPEN.

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 STRTC2,FOONC
INTEGER*4 ITCHG

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

C NO DRY TO WET CONVERSION IS NEEDED AS THE INPUT AND OUTPUT
C FRACTIONS ARE BOTH DRY FRACTIONS.

FOONC=STRTC2*(2.7182818**(-1.*QO2*ITCHG/V))

RETURN
END

FUNCTION FNONC(STRTC2,ITCHG)

C THIS FUNCTION RETURNS THE DRY FRACTION OF CO2 IN THE CHAMBER
C ONCE THE N2 HAS BEEN CHANGED.

C STRTC2.....STARTING FRACTION FOR CO2.
C ITCHG.....TIME FOR WHICH N2 VALVE IS OPEN.

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 STRTC2,FNONC
INTEGER*4 ITCHG

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

C NO DRY TO WET CONVERSION IS NEEDED AS THE INPUT AND OUTPUT
C FRACTIONS ARE BOTH DRY FRACTIONS.

FNONC=STRTC2*(2.7182818**(-1.*QN2*ITCHG/V))

RETURN
END

FUNCTION FCONO(STRTO2,ITCHG)

C THIS FUNCTION RETURNS THE DRY FRACTION OF O2 IN THE CHAMBER
C ONCE THE CO2 HAS BEEN CHANGED.

C STRTO2.....STARTING FRACTION FOR O2.

C ITCHG.....TIME FOR WHICH CO2 VALVE IS OPEN.

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 STRTO2,FCONO
INTEGER*4 ITCHG

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

C NO DRY TO WET CONVERSION IS NEEDED AS THE INPUT AND OUTPUT
C FRACTIONS ARE BOTH DRY FRACTIONS.

FCONO=STRTO2*(2.7182818**(-1.*QC2*ITCHG/V))

RETURN
END

FUNCTION FACONO(STRTO2,STRTC2,FNSHC2)

C THIS FUNCTION RETURNS THE DRY FRACTION OF O2 IN THE CHAMBER
C ONCE THE CO2 HAS BEEN ABSORBED.

C STRTO2.....STARTING FRACTION FOR O2.
C STRTC2.....STARTING FRACTION FOR CO2.
C FNSHC2.....FINISHING FRACTION FOR CO2.

REAL*8 V,QO2,QC2,QN2,QAC2
REAL*8 STRTO2,STRTC2,FNSHC2,FACONO

COMMON /FLOW/V,QO2,QC2,QN2,QAC2,A,B,C,D

C NO DRY TO WET CONVERSION IS NEEDED AS THE INPUT AND OUTPUT
C FRACTIONS
C ARE BOTH DRY FRACTIONS.

FACONO=STRTO2+(STRTC2-FNSHC2)*0.2094

RETURN
END

FUNCTION FFPP(PARTP,BP)

C THIS FUNCTION RETURNS THE DRY FRACTION FROM THE WET PARTIAL
C PRESSURE.

C PARTP.....PARTIAL PRESSURE.

REAL*8 FFPP,PARTP
REAL*8 BP

FFPP=PARTP/(BP-47.)

RETURN
END

FUNCTION PPFF(FRAC,BP)

C THIS FUNCTION RETURNS THE WET PARTIAL PRESSURE FROM A DRY
C FRACTION.

C FRAC.....FRACTION.

REAL*8 PPFF,FRAC
REAL*8 BP

PPFF=FRAC*(BP-47.)

RETURN
END@

*****ASSEMBLER CODE SUBROUTINES*****

***** RDCLKI.ASM TO READ FROM COUNTERS *****

```

;
; RDCLKI
;
; FROM RDCLK, KJS 23/2/88
;
; NOW WITH CLI AND STI TO SUSPEND
; INTERRUPTS. THIS IS REQUIRED IF
; THE ROUTINE IS TO RUN WITH CHCONT
; WHEN THE DASHINT MODULE IS IN USE
;

```

```

FRAME STRUC
SAVEDS DW ?      ; SAVED COPY OF DS REG.
SAVEBP DW ?      ; SAVED COPY OF BP REG.
RETADD DD ?      ; 4 BYTE RETURN ADDRESS
CLKRD1 DD ?      ; 4 BYTE ADDRESS OF MSEC.
CLKRD0 DD ?      ; 4 BYTE ADDRESS OF SEC.
FRAME ENDS

```

```

DATA SEGMENT PUBLIC 'DATA'
PORT_SEC DW 030CH ; PORT ADDR.FOR COUNTER 0
PORT_MSEC DW 030DH ; PORT ADDR.FOR COUNTER 1
LATCH_SEC DB 0000000B ; LATCH FOR READ OPERATION
LATCH_MSEC DB 01000000B ; DITTO
PORT_CONT DW 030FH ; PORT ADDR. FOR CONTROL
COMP_SEC1 DW ? ; COMPARE PREVIOUS SECS.
COMP_SEC2 DW ? ;
DATA ENDS
DGROUP GROUP DATA

```

```

TIME_SEG SEGMENT 'CODE'
ASSUME CS:TIME_SEG,DS:DGROUP,SS:DGROUP
PUBLIC RDCLKI
RDCLKI PROC FAR

```

CLI

```

PUSH BP      ;SAVE BP REGISTER
PUSH DS      ;SAVE DS REGISTER
MOV BP,SP    ;POINT BP REG.AT FRAME

```

SEC:

```

MOV DX,PORT_CONT ;LOAD ADD. OF CONT.PORT
MOV AL,LATCH_SEC ;LATCH CONTENTS IN REG.AX
OUT DX,AL        ;OUT LATCH INFO.FOR SEC

```

```

MOV DX,PORT_SEC ;LOAD PORT INTO REG.DX
LES BX,[BP].CLKRD0 ;LOAD SEC ADDRESS
IN AL,DX        ;READ LO BYTE SEC
MOV CL,AL       ;MOV LO INTO REG.CL
JMP SHORT $+2   ;I/O OPERATION DELAY
IN AL,DX        ;READ HI BYTE MSEC
MOV CH,AL       ;MOV HI INTO REG.CH
MOV ES:[BX],CX  ;MOV HI AND LO INTO CLKRD0
MOV COMP_SEC1,CX

```

MSEC:

```

MOV DX,PORT_CONT ;LOAD ADD.OF PORT CONTROL
MOV AL,LATCH_MSEC ;LATCH CONTENTS
OUT DX,AL        ;OUT LATCH INFO.FOR MSEC
JMP SHORT $+2    ;I/O OPERATION DELAY

```

```

MOV DX,PORT_MSEC ;LOAD PORT INTO REG.DX
LES BX,[BP].CLKRD1 ;LOAD MSEC ADDRESS
IN AL,DX        ;READ LO BYTE MSEC
MOV CL,AL       ;MOV LO INTO REG.CL
JMP SHORT $+2   ;I/O OPERATION DELAY
IN AL,DX        ;READ HI BYTE MSEC
MOV CH,AL       ;MOVE HI BYTE INTO REG.CH
MOV ES:[BX],CX  ;MOVE BOTH INTO CLKRD1

```

```

MOV DX,PORT_CONT ;LATCH FOR SECS
MOV AL,LATCH_SEC ;
JMP SHORT $+2    ;I/O OPERATION DELAY
OUT DX,AL        ;

```

```

MOV DX,PORT_SEC ;READ SECS AGAIN

```

```

LES BX,[BP].CLKRD0
IN AL,DX
MOV CL,AL
JMP SHORT $+2
IN AL,DX
MOV CH,AL
MOV ES:[BX],CX
CMP COMP_SEC1,CX ;COMPARE WITH PREV.SECs
JE OUT ;IF EQUAL THEN END
JMP SEC ;IF NOT READ SEC AGAIN
OUT: LES BX,[BP].CLKRD1 ;LOAD MSEC ADDRESS
MOV AX,ES:[BX]
CMP AX,0001H ;IS MSEC=1?
JNE FINISH ;NO GOTO FINISH
LES BX,[BP].CLKRD0 ;YES LOAD SEC ADDRESS
MOV AX,ES:[BX]
INC AX ;INC SECONDS
MOV ES:[BX],AX
FINISH:
NOP
POP DS
POP BP ;RESTORE BP AND DS REGS.

STI

RET 8
RDCLKI ENDP
TIME_SEG ENDS
END

```

***** OUTDIG.ASM - TO SET UP PORT CONFIGURATION *****

```

FRAME STRUC

SAVEDS DW ? ;SAVE COPY DS REG.
SAVEBP DW ? ;SAVE COPY BP REG.
RETADD DD ? ;4 BYTE RET. ADDR.

```

```

DATAADD DD ? ;4 BYTE ADDR. OF DATA
PORTADD DD ? ;4 BYTE ADDR. OF PORT
FRAME ENDS

```

```

DATA SEGMENT PUBLIC 'DATA'
DATA ENDS

```

```

DGROUP GROUP DATA
ASSUME CS:OUT_READ,DS:DGROUP,SS:DGROUP
OUT_READ SEGMENT 'CODE'
PUBLIC OUTDIG
OUTDIG PROC FAR
PUSH BP ;SAVE BP
PUSH DS ;SAVE DS
MOV BP,SP ;POINT BP AT FRAME
LES BX,[BP].PORTADD ;
MOV DX,ES:[BX] ;LOAD PORT NO.
LES BX,[BP].DATAADD ;LOAD DATA ADDR.
MOV AX,ES:[BX] ;LOAD DATA
OUT DX,AL ;OUTPUT LOW BYTE

POP DS
POP BP
RET 8

```

```

OUTDIG ENDP
OUT_READ ENDS
END

```

***** INDIG.ASM - TO READ DATA FROM PORT *****

```

FRAME STRUC
SAVEDS DW ? ;SAVED COPY DS REG.
SAVEBP DW ? ;SAVED COPY BP REG.
RETADD DD ? ;4 BYTE RETURN ADDR.
DATAADD DD ? ;4 BYTE ADDR.OF DATA
PORTADD DD ? ;4 BYTE ADDR. OF PORT

```

FRAME ENDS

DATA SEGMENT PUBLIC 'DATA'
DATA ENDS
DGROUP GROUP DATA

IN_READ SEGMENT 'CODE'

ASSUME CS:IN_READ,DS:DGROUP,SS:DGROUP

PUBLIC INDIG

INDIG PROC FAR

PUSH BP ;SAVE BP
PUSH DS ;SAVE DS
MOV BP,SP ;POINT BP AT FRAME

LES BX,[BP].PORTADD ;PORT ADDR.INTO BX
MOV DX,ES:[BX] ;PORT NO.INTO DX
LES BX,[BP].DATAADD ;DATA ADDR.INTO BX
MOV AX,0000H ;CLEAR AX
IN AL,DX ;INPUT DIG.DATA
MOV ES:[BX],AX ;MOVE INTO ADDR.

POP DS ;RESTORE BP/DS REGS.
POP BP
RET 8

INDIG ENDP
IN_READ ENDS
END

* ADCON2.ASM - TO CONVERT ANALOGUE DATA TO DIGITAL *

FRAME STRUC

SAVEDS DW ? ;SAVE COPY DS REGISTER
SAVEBP DW ? ;SAVE COPY BP REG.
RETADD DD ? ;4 BYTE RETURN ADDRESS
CHNOADD DD ? ;4 BYTE ADDRESS OF CHAN.NO.
DATAADD DD ? ;4 BYTE ADDRESS OF A/D NO.

FRAME ENDS

DATA SEGMENT PUBLIC 'DATA'

PORT_CONT DW 030AH ;PORT ADDR.FOR CONTROL
PORT_AD12 DW 0309H ;PORT ADDR.FOR 12 BIT A/D
PORT_AD8 DW 0308H ;PORT ADDR.FOR 8 BIT A/D
INIT DB 00H ;NO.FOR INITIALISING
END_CONV DB 10000000B ;END OF CONV.NO.
DATA ENDS

DGROUP GROUP DATA

CONV SEGMENT 'CODE'

ASSUME CS:CONV,DS:DGROUP,SS:DGROUP

PUBLIC ADCONV

ADCONV PROC FAR

PUSH BP ;SAVE BP REG.
PUSH DS ;SAVE DS REG.
MOV BP,SP ;POINT BP AT FRAME

CLI

LES BX,[BP].CHNOADD ;LOAD CHANN.NO.ADDR.
MOV AX,ES:[BX] ;LOAD CHANN.NO.

MOV DX,PORT_CONT ;LOAD ADDR.OF CONT.PORT
ADD AX,00001000B
OUT DX,AL ;OUT CHANN.NO.
JMP DELAY1 ;I/O OPERATION DELAY

DELAY1: MOV DX,PORT_AD12 ;LOAD A/D 12 BIT PORT NO.
MOV AL,INIT ;LOAD INIT.FOR 12 BIT A/D
OUT DX,AL ;OUT INIT.NO.
JMP DELAY2 ;I/O OPERATION DELAY

DELAY2: MOV DX,PORT_CONT ;LOAD CONTROL PORT
EOC: IN AL,DX ;READ STATUS BYTE
JMP SHORT \$+2 ;I/O OPERATION DELAY
AND AL,END_CONV ;CHECK FOR EOC
JNE EOC ;LOOP IF BUSY

```

MOV DX,PORT_AD12      ;LOAD A/D 12 PORT NO.
JMP SHORT $+2          ;I/O OPERATION DELAY
IN AL,DX               ;READ HI BYTE
MOV AH,AL              ;
MOV DX,PORT_AD8        ;LOAD A/D 8 PORT.NO.
JMP SHORT $+2          ;I/O OPERATION DELAY
IN AL,DX               ;READ LO BYTE
SAR AX,1               ;MOVE RIGHT 4 PLACES
SAR AX,1
SAR AX,1
SAR AX,1
AND AH,00001111B      ;CLEAN OFF HIGH ORDER BITS
LES BX,[BP].DATAADD    ;LOAD A/D NO.ADDRESS
MOV ES:[BX],AX         ;PUT NO.INTO LOCATION

```

```

STI

```

```

POP DS                ;RESTORE BP AND DS REGS.
POP BP
RET 8

```

```

ADCONV ENDP
CONV ENDS
END

```

CHAPTER 4

THE VENTILATORY RESPONSE TO EIGHT HOURS OF ISOCAPNIC AND POIKILOCAPNIC HYPOXIA

'How art thou out of breath, when thou hast breath

To say to me that thou art out of breath?'

Romeo and Juliet, II.v.31

INTRODUCTION

The ventilatory response in adult humans to sustained isocapnic hypoxia is biphasic (Weil and Zwillich, 1976). Ventilation rises initially and then tails off over a period of 15 to 20 minutes to a level above the euoxic base line. This phenomenon has been termed hypoxic ventilatory decline (HVD). On exposure to longer-term hypoxia at altitude (Douglas *et al.*, 1913) and at simulated altitude, using hypobaric chambers (Åstrand, 1953), there is a progressive increase in ventilation over a period of days to weeks which is known as ventilatory acclimatization to hypoxia (VAH). Whereas HVD is seen under isocapnic conditions, and so can be said to occur as a result of pure hypoxia, the process of acclimatization takes place with an associated hypocapnic alkalosis which develops due to an initial hyperventilation caused by hypoxic stimulation of the peripheral chemoreceptors. Two attempts to study the effects of prolonged isocapnic hypoxia have proved unsatisfactory, principally through limitations of the techniques available (Cruz *et al.*, 1980; Eger *et al.*, 1968). However, with the development of the chamber described in the previous chapter, it has become possible to impose good continuous control over the end-tidal gases for prolonged periods.

The aim of the study presented in this chapter was two-fold. The first objective was to examine the ventilatory response to hypoxia over the little-studied time course between HVD and VAH in both isocapnic and hypocapnic conditions: the first 8 hours of the response was monitored. The second objective was to perform a pilot study of relatively short duration for later longer-term studies lasting two days.

METHODS

Subjects. Eleven subjects (6 females, 5 males) aged between 18 and 24 were studied. Their individual characteristics are listed in Table 4.1. All were healthy, and none gave a history of respiratory symptoms or illnesses. All were non-smokers except for one subject (939) who smoked *ca.* 5 cigarettes per day.

Subject	Sex	Height (m)	Weight (kg)	Age (years)
884	M	1.87	75	24
894	F	1.58	51	20
910	F	1.70	62	21
927	F	1.68	64	21
930	F	1.80	70	22
932	F	1.73	62	21
934	M	1.83	75	19
935	M	1.82	75	21
939	F	1.68	62	21
945	M	1.78	69	23
947*	M	1.85	70	18

*Subject did not complete the study.

Table 4.1: Physical Characteristics of Subjects.

Equipment and experimental technique. The first 20 min of exposure to hypoxia were imposed using the fast gas-mixing system (described in Chapter 2). This was performed in order to record ventilation during a period in which it is known to vary (Weil and Zwillich, 1976) and it also enabled the chamber composition to be set to an initial correct level for

each individual subject. The subjects were seated comfortably inside the purpose-built chamber for controlling their end-tidal gases (see Chapter 3) for the remainder of the hypoxic exposure.

Measurements of ventilation made inside the chamber were collected with the subject breathing through a mouthpiece assembly fixed in series with a turbine volume measuring device and a pneumotachograph, with the nose occluded. The equipment listed above is identical to the data acquisition apparatus described in Chapter 2 with two exceptions. First, the whole assembly is open to the gas in the chamber and secondly, no gas is sampled. Thus, the calibration procedure of Chapter 2 did not involve the mass spectrometer in this case. Respiratory volumes, flows and durations were obtained as with the data acquisition system.

Although it is thought that using invasive techniques to monitor ventilation, such as a mouthpiece or facemask, may alter respiratory measurements in some subjects as a result of increased cortical stimulation (Shea *et al.*, 1989), a system involving respiratory inductance plethysmography (Shea *et al.*, 1987) was not employed to monitor ventilation inside the chamber despite the fact that the dynamic end-tidal forcing system was not being used. This was done in order to minimize any differences in cortical stimulation between the ventilatory measurements taken inside the chamber and those taken outside the chamber, when the dynamic end-tidal forcing system was in use: thus, reasonable comparisons could be made between the measurements (Chapters 5 & 6).

Protocols. Subjects were exposed to three different protocols, each lasting 8 hours, on three different days (in random order with each protocol separated by at least one week from the others). These were: isocapnic hypoxia (protocol I), where P_{ETO_2} was held at 55 mmHg and P_{ETCO_2} was held at the subject's resting value; poikilocapnic hypoxia (protocol P), where P_{ETO_2} was held at 55 mmHg and P_{ETCO_2} was uncontrolled; and control (protocol C), where the inspired gas was air. Subjects spent the first 20 min of exposure breathing from the fast gas-mixing system and were placed in the chamber following a 15-min test of the ventilatory

response to acute hypoxia, imposed by the fast gas-mixing system. The results of these tests are presented in Chapter 5.

Ventilation was measured over a 3-min period at the beginning of the exposure, after 20 min of exposure and then at hourly intervals from 1 ½ to 7 ½ hours. The subjects remained inside the chamber for the entire period, apart from 15 min at 4 hours when they were again assessed for their ventilatory response to acute hypoxia outside the chamber using the fast gas-mixing system.

Subjects were not allowed caffeinated drinks such as coffee, tea and certain canned drinks prior to and during the experiment and all ate lunch after 4 ½ hours.

Analysis. The last 15 breaths of each 3-min period of ventilatory measurement were used to calculate the subjects' ventilation at each point of measurement.

An analysis of variance was used to test for possible differences in the ventilatory response to the three protocols. Using the notation defined by Armitage and Berry (1987), the linear model for ventilation ($\dot{V}_E$) for the i th subject (S_i), at the j th time of measurement (T_j) and for the k th protocol (P_k) together with interaction terms used was:

$$\dot{V}_{Eijk} = \mu + S_i + T_j + P_k + (TP)_{jk} + (ST)_{ij} + (SP)_{ik} + (STP)_{ijk} + e_{ijk}$$

where e_{ijk} is an error term. In order to compare the isocapnic protocol (I) with the other two, and then the poikilocapnic protocol (P) with the air-breathing control (C), the protocol term was split into I versus other (I-PC) and P versus C (P-C). Thus the new model used was:

$$\begin{aligned} \dot{V}_{Eijkl} = & \mu + S_i + T_j + (I-PC)_k + (P-C)_l + (T(I-PC))_{jk} + (T(P-C))_{jl} + (ST)_{ij} + \\ & (S(I-PC))_{ik} + (S(P-C))_{il} + (ST(I-PC))_{ijk} + (ST(P-C))_{ijl} + e_{ijkl} \end{aligned}$$

The terms in this model act as individual sources of variance, contributing to the total error. Their significance as sources of variance was examined using two types of variance ratio test. In the first test (giving F-ratio (1)), sources of variance were removed from the error sequentially from left to right in the model, and each source of variance was tested for its significance by dividing it by the remaining mean square error to give an F-ratio. In the second test (F-ratio (2)), the mean square error from the source of variance being tested was divided by the mean square error from the interaction between the subject term and the source of variance to give a second F-ratio. The analysis was performed using GLIM statistical software.

RESULTS

The subjects generally spent their time in the chamber either reading, watching television or videos, playing computer games or listening to the radio. Some subjects reported mild headaches and nausea during each of the three protocols, but more so during the protocols involving hypoxia. One subject (947) had to be withdrawn from the study following more severe sensations of light-headedness. Two subjects did not remain in the chamber for the full 8-hour duration of every protocol because of time constraints, but none left the chamber before at least 7 hours had been completed.

End-tidal gases. Five-min averages of the end-tidal gases recorded in each subject while in the chamber are presented in Fig. 4.1 (isocapnic hypoxia), Fig. 4.2 (poikilocapnic hypoxia) and Fig. 4.3 (control). These plots illustrate the quality of control of both $P_{ET}O_2$ (held at 56.87 ± 0.08 mmHg throughout; mean $\pm$ S.E.) and $P_{ET}CO_2$ (held at 38.36 ± 0.09 mmHg throughout) during isocapnic hypoxia and of $P_{ET}O_2$ (held at 57.47 ± 0.08 mmHg throughout) during poikilocapnic hypoxia. Within the first minute of exposure to poikilocapnic hypoxia, $P_{ET}CO_2$ dropped from the control value of 38.85 ± 0.73 mmHg to 35.65 ± 0.78 mmHg and as ventilation tailed off over the first 20 min, $P_{ET}CO_2$ rose to 36.38 ± 0.73 mmHg. Over the course of the remaining exposure $P_{ET}CO_2$ dropped to 34.1 ± 0.71 mmHg, dipping to 33.51 ± 0.91 mmHg in the fourth hour. These values of $P_{ET}CO_2$ were both significantly lower than the value at 20 min ($p < 0.05$; paired t-test). A reduction in $P_{ET}CO_2$ from the initial control reading (38.85 ± 0.73 mmHg) was also detected in the control condition when the subject was breathing air. The lowest reading detected was 35.75 ± 0.24 mmHg at 1 ½ hours and at the end of 8 hours the $P_{ET}CO_2$ was 36.01 ± 0.34 mmHg. The mean value of measured $P_{ET}O_2$ during the control was 110.61 ± 0.22 mmHg. The average end-tidal P_{CO_2} (in one-hour time-bins) for all subjects in each protocol is plotted in Fig. 4.4 (lower panel).

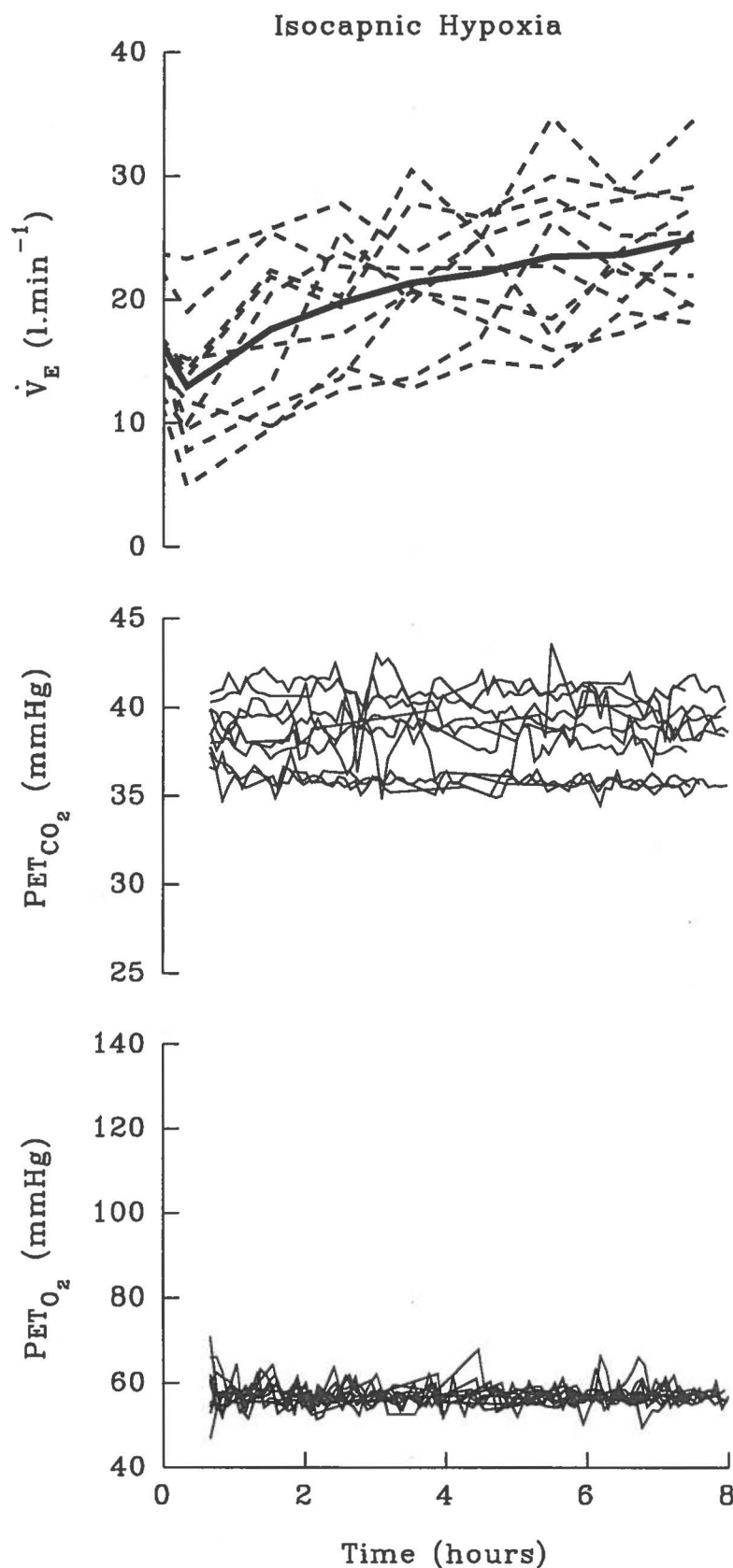


Figure 4.1: The upper panel shows the individual (dashed lines) and mean (solid line) ventilatory responses ($\dot{V}_E$) to isocapnic hypoxia. The lower two panels show the end-tidal gases (PET_{CO_2} and PET_{O_2}) averaged every 5 min, for each subject while in the chamber.

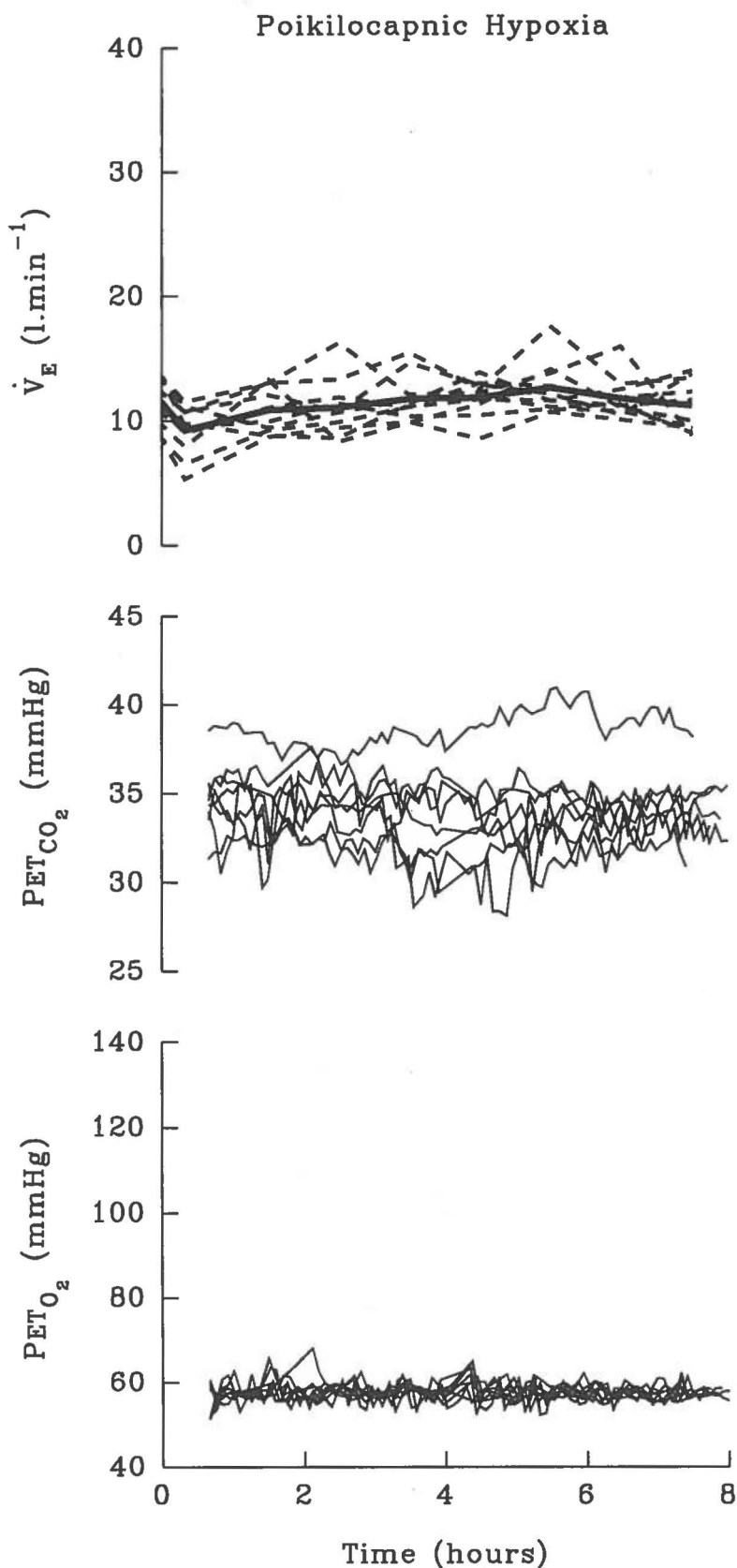


Figure 4.2: The upper panel shows the individual (dashed lines) and mean (solid line) ventilatory responses ($\dot{V}_E$) to poikilocapnic hypoxia. The lower two panels show the end-tidal gases (PET_{CO_2} and PET_{O_2}) averaged every 5 min, for each subject while in the chamber.

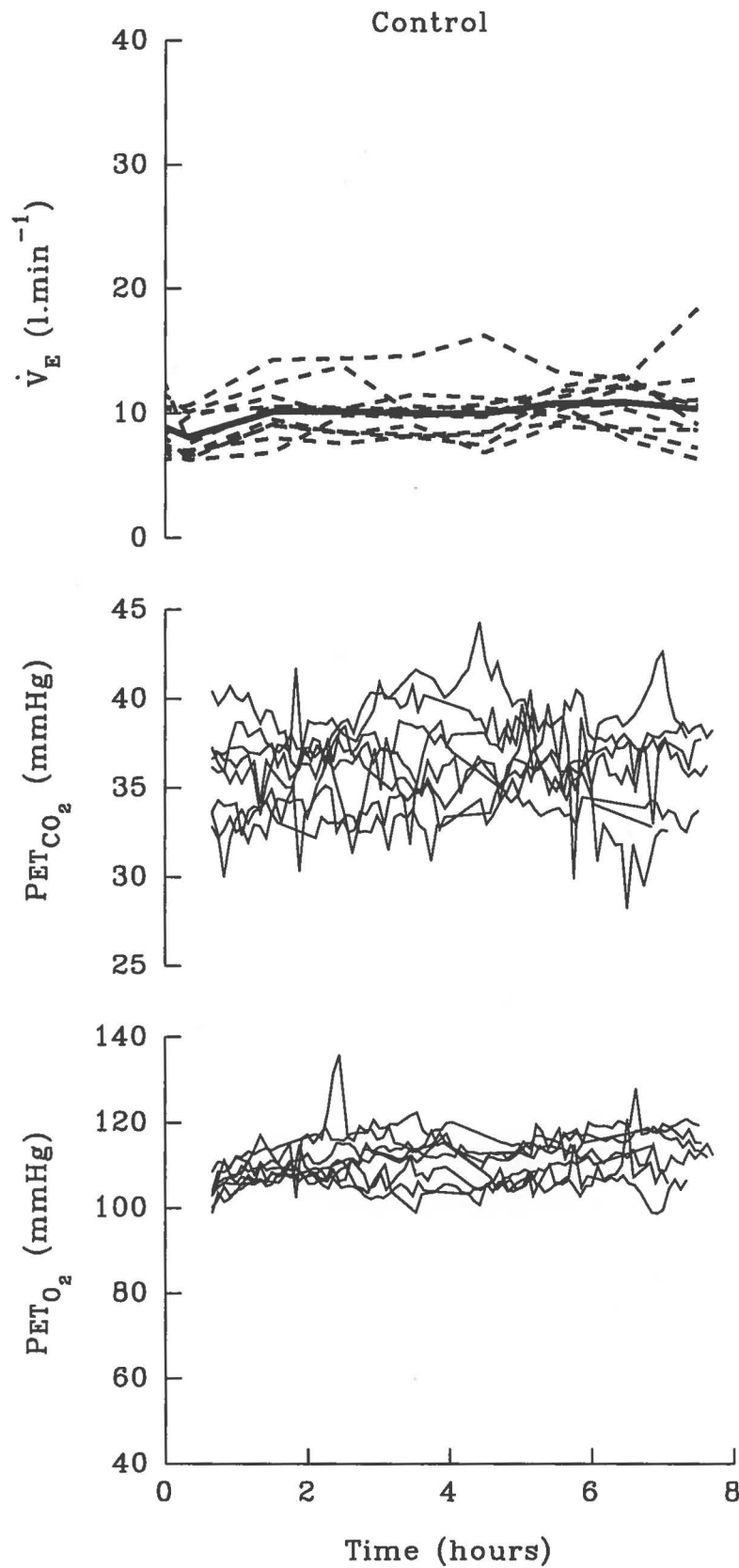


Figure 4.3: The upper panel shows the individual (dashed lines) and mean (solid line) ventilatory responses ($\dot{V}_E$) to the control protocol. The lower two panels show the end-tidal gases (PETCO₂ and PETO₂) averaged every 5 min, for each subject while in the chamber.

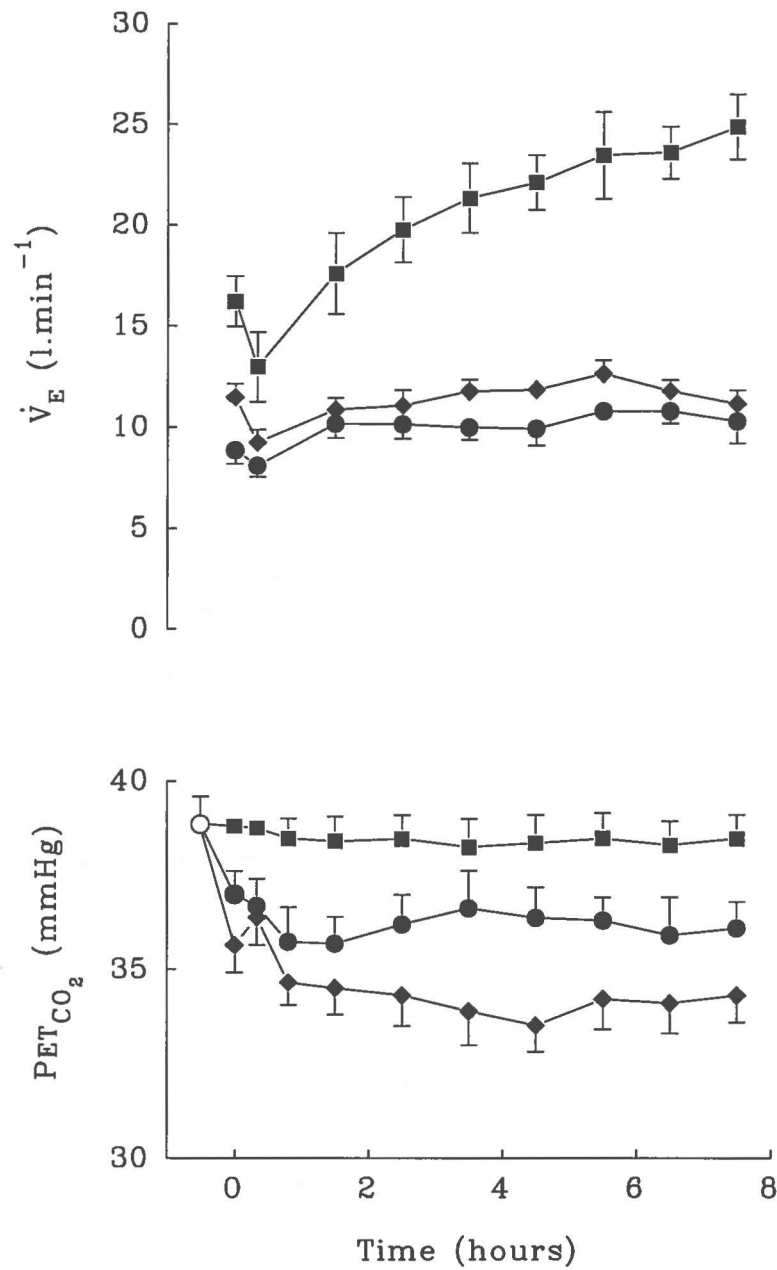


Figure 4.4: Upper panel: Mean $\pm$ S.E. of ventilatory responses ($\dot{V}_E$) of all subjects for each protocol. Lower panel: Mean $\pm$ S.E. of PETCO₂ of all subjects for each protocol, averaged over one-hour time-bins with data from the end-tidal forcing system measuring via the nasal catheter. (■ = isocapnic hypoxia; ◆ = poikilocapnic hypoxia; ● = control; ○ = mean resting value of PETCO₂).

In two subjects, we failed to get adequate signals for arterial oxygen saturation from the pulse oximeter throughout the whole 8 hour period. The reasons for this are unclear. In the subjects in which satisfactory records were obtained there were no significant changes in saturation during isocapnic hypoxia ($90.79 \pm 0.16\%$, 1st hour; $89.86 \pm 0.20\%$, 8th hour; $n=8$ (mean $\pm$ S.E.); $p=NS$, paired t-test), poikilocapnic hypoxia ($90.42 \pm 0.24\%$, 1st hour; $89.72 \pm 0.14\%$, 8th hour; $p=NS$, paired t-test) or air-breathing control ($98.40 \pm 0.33\%$, 1st hour; $98.60 \pm 0.15\%$, 8th hour; $p=NS$, paired t-test).

Ventilation. The ventilatory responses of each subject (dashed lines) and the mean ventilatory response of all the subjects combined (solid lines) for the 8-hour duration of the exposure are presented in Fig. 4.1 (isocapnic hypoxia), Fig. 4.2 (poikilocapnic hypoxia) and Fig. 4.3 (control). Figure 4.4 (upper panel) shows the mean responses superimposed on the same plot. Ventilation fell significantly during the first 20 min in all subjects in both isocapnic hypoxia (from 16.20 ± 1.3 l.min⁻¹ to 12.96 ± 1.7 l.min⁻¹ (mean $\pm$ S.E.); $p<0.005$, paired t-test) and poikilocapnic hypoxia (from 11.46 ± 0.7 l.min⁻¹ to 9.22 ± 0.6 l.min⁻¹; $p<0.001$, paired t-test), but not during the control. In all subjects, ventilation rose steadily during exposure to isocapnic hypoxia (from 16.2 ± 1.3 l.min⁻¹ to 24.8 ± 1.6 l.min⁻¹; mean $\pm$ S.E.) versus exposure to poikilocapnic hypoxia and control conditions ($p<0.001$, ANOVA), but exposure to poikilocapnic hypoxia did not elicit a progressive rise in ventilation when compared with control. However, in poikilocapnic hypoxia, ventilation was greater than during the control ($p<0.01$, ANOVA(F-ratio(1)); $p<0.025$, ANOVA (F-ratio(2))) and ventilation during isocapnic hypoxia was also greater than during the two other protocols, poikilocapnic hypoxia and control ($p<0.001$, ANOVA). Table 4.2 summarizes the results of the analysis of variances for the model of ventilation. Table 4.3 in the appendix to this chapter presents the ventilatory data collected from each experiment.

During both poikilocapnic hypoxia and control, there is a noticeable bulge in ventilation at 5 ½ hours. However, this measurement was consistently made 30 min after lunch and a rise in ventilation would be expected, due to an increased metabolic rate.

Source of Variance	Degrees of Freedom	Sum of squares (l.min ⁻¹) ²	Degrees of Freedom of Error	Error (l.min ⁻¹) ²	F-ratio (1)	F-ratio (2)
No Factor	---	---	269	9978.7	---	---
S _i	9	858.2	260	9120.4	---	---
T _j	8	793.8	252	8326.6	---	---
(I-PC) _k	1	5547.8	251	2778.8	501.1***	53.7***
(P-C) _l	1	89.9	250	2688.9	8.4**	8.9*
T _j *(I-PC) _k	8	555.5	242	2133.4	7.9**	9.5***
T _j *(P-C) _l	8	16.5	234	2116.9	0.2	0.8
S _i *T _j	72	387.1	162	1729.8	---	---
S _i *(I-PC) _k	9	929.2	153	800.7	---	---
S _i *(P-C) _l	9	90.5	144	710.2	---	---
S _i *T _j *(I-PC) _k	72	523.9	72	186.3	---	---
S _i *T _j *(P-C) _l	72	186.3	0	0.00	---	---
Total	269	9978.7	---	---		

Table 4.2: Statistical analysis of the linear model of ventilation using analysis of variance. F-ratio (1) is determined from the ratio of the error from the source of variance being considered (column 3) to the remaining error (column 5). F-ratio (2) is determined from the ratio of the error from the source of variance being considered to the error from the interaction term between the source of variance and subjects. (*, $p < 0.025$; **, $p < 0.01$; ***, $p < 0.001$.)

DISCUSSION

The major finding of this study is that ventilation rose steadily over 8 hours of isocapnic hypoxia once the phenomenon of HVD had been completed. Although P_{ETCO_2} in the isocapnic study was 1.8 mmHg higher than actually determined over the 8 hour control period, it is likely that P_{ETCO_2} during isocapnic hypoxia was held close to the subject's natural value, as the ventilation and P_{ETO_2} data for the air-breathing control would suggest a mild hyperventilation. The cause of this hyperventilation is not known, but may be due to the unfamiliar environment of the chamber. Despite no significant time-dependent change in the ventilation during poikilocapnic hypoxia, there was a significant drop in P_{ETCO_2} throughout the 8-hour exposure to hypoxia. Assuming no change in metabolic rate or dead space, this would suggest an increase in ventilation (Forster *et al.*, 1981).

Studies of sustained isocapnic hypoxia in humans. There have been previous attempts to look at sustained isocapnic hypoxia in humans (Cruz *et al.*, 1980; Eger *et al.*, 1968), but the authors did not take any direct measurements of ventilation over the time course employed in the current study. Eger *et al.* (1968) used a face-mask and rebreathing arrangement to expose subjects to 8 hours of hypoxia with and without hypocapnia. They did not report on changes in ventilation since in some cases a respirator was used to lower alveolar P_{CO_2} (P_{ACO_2}), but instead examined changes in the relationship between ventilation and P_{CO_2} in hyperoxia (the CO_2 response). They showed that there was an increase in the slope of the CO_2 response following exposure to isocapnic, hypercapnic and hypocapnic hypoxia, but not following euoxic exposure with any condition of P_{ACO_2} . They also demonstrated that a leftward shift of the CO_2 response curve occurred during exposure to hypocapnic hypoxia, which was reduced, but not eliminated, when the subject was kept either isocapnic or hypercapnic during the hypoxic exposure. These results would seem consistent with the observation from this study that isocapnic hypoxia causes a rise in ventilation over 8 hours, and are not inconsistent with the notion that this rise would be masked by the associated alkalosis in poikilocapnic hypoxia. However, Eger *et al.* did point out that the observed increase in slope

of the CO_2 response in hypoxia was questionable, since the slope of the CO_2 response preceding the hypoxic studies was significantly less steep than preceding the euoxic studies. Furthermore, the method of controlling gas tensions suffered drawbacks: in particular, the subjects found it stressful (Kellogg, personal communication to Robbins) and had taken breaks of 10 to 30 min every two hours to eat, drink and attend to other necessities. For these reasons, the changes in the CO_2 response may have been spurious. It is, therefore, difficult to account for the findings of this study using the observations of Eger *et al.*.

Cruz *et al.* (1980) used a hypobaric chamber to study ventilation over 100 hours. Subjects were studied using both an isocapnic and poikilocapnic protocol at the same PO_2 as in the current study. In order to try to attain isocapnia, a fixed proportion of 3.77% CO_2 was added to the chamber inspire and a lower pressure was used in order to try to match PET_{O_2} between the two hypoxic protocols. They observed a sharp rise in ventilation after 3 hours of exposure to isocapnic hypoxia and then a steady level of ventilation over the remaining period. In subjects exposed to poikilocapnic hypoxia they saw a gradual rise in ventilation towards levels of ventilation produced in the isocapnic protocol. However, during the first 24 hours of exposure, they only measured ventilation once (at 3 hours) and may have missed the progressive increase in ventilation occurring over 8 hours of isocapnic hypoxia reported in the current study. Furthermore, their control of the alveolar gases was relatively poor, as after 27 hours of exposure PET_{O_2} had drifted from 55 mmHg to 62 mmHg and PET_{CO_2} had drifted from 39 mmHg to 43 mmHg. This may have contributed to the discrepancy between the ventilations that they recorded in 'isocapnic' hypoxia and those recorded in this study. The maximum average ventilation that was elicited in their experiments was approximately half the maximum value reported in the current study.

Studies of sustained isocapnic hypoxia in animals. The ventilatory response to prolonged hypoxia has been studied in some detail using a carotid body perfusion model in awake goats, in which one of the carotid bodies is perfused separately from the systemic arterial circulation (and central nervous system, CNS) with the opposite carotid body denervated.

The investigators showed that perfusion of the carotid body with isocapnic hypoxic blood while the animal was breathing air produced a progressive increase in ventilation (Busch *et al.*, 1985). When the systemic circulation was maintained euoxic and eucapnic, by addition of CO₂ to the inspired gas, a rise in ventilation was still seen (Bisgard *et al.*, 1986b). It was thus concluded that neither brain hypoxia nor brain hypocapnia was required for an increase in ventilation to occur. This model was used further to demonstrate that when the carotid body stimulus is changed from hypoxia to hypercapnia, there is a steady hyperventilation but no time-dependent rise as with hypoxia (Bisgard *et al.*, 1986a). This established carotid body hypoxia as the essential stimulus in producing this phenomenon, and that the changes in ventilation were not the result of CNS conditioning by carotid body afferent activity.

A progressive rise in ventilation over 4 hours of whole body isocapnic hypoxia has also been demonstrated in the conscious, intact goat (Engwall and Bisgard, 1990; Ryan *et al.*, 1993a). In both these studies and in the study of systemic eucapnia with carotid body hypoxia (Bisgard *et al.*, 1986b), ventilation returned to baseline on relief of hypoxia. Furthermore, with the observation that no time-dependent rise in ventilation was seen during carotid body hypercapnia (Bisgard *et al.*, 1986a), the progressive rise in ventilation can be attributed to a time-dependent increase in ventilatory sensitivity to hypoxia. The carotid body perfusion and other related experiments by the Wisconsin group of researchers have been presented in greater detail in Chapter 1, *Ventilatory Acclimatization to Hypoxia*.

However, there are some substantial differences between the results from goats and these and other observations in conscious humans. There is a significant progressive fall and subsequent stabilization in P_{ETCO_2} in goats after 4 to 8 hours of poikilocapnic hypoxia (Busch *et al.*, 1983; Engwall and Bisgard, 1990; Forster *et al.*, 1981; Smith *et al.*, 1986). This pattern of change in P_{ETCO_2} is characteristic of near complete acclimatization to high altitude in the goat (Busch *et al.*, 1983; Engwall and Bisgard, 1990; Forster *et al.*, 1981; Smith *et al.*, 1986), and so it is reasonable to consider that the concomitant increased carotid body response to sustained hypoxia seen in the above experiments may be the primary mechanism

in ventilatory acclimatization in these animals. In the current study, there was a significant drop in P_{ETCO_2} , but no progressive decline and subsequent stabilization. This was as expected since the acclimatization process is known to take days to weeks in humans (Douglas *et al.*, 1913; Åstrand, 1953). Thus the mechanisms proposed to account for acclimatization in the goat cannot simply be extended to humans without due caution.

Possible mechanisms for the rise in ventilation during isocapnic hypoxia. Chapter 1 describes the mechanisms proposed to account for ventilatory acclimatization to hypoxia: changes in the acid/base status of the blood, cerebro-spinal fluid or extracellular fluid bathing the intracranial chemoreceptors; increased excitability of the CNS and peripheral chemoreceptor mechanisms. Unlike the goat, it is not clear, from the reasons given earlier, that the processes thought to underlie VAH are appropriate in humans for the changes in ventilation over the time course investigated here. However, the above processes can still be considered when discussing the results of the current study. Clearly, one possible mechanism for the rise in ventilation during isocapnic hypoxia is that it is a progressive effect of hypoxia on the carotid body, analogous to that described in the goat. If so, then the failure to observe any rise in ventilation during poikilocapnic hypoxia (despite observing a drop in P_{ETCO_2}) could be attributed to the concurrent hypocapnic alkalosis masking the change in carotid body sensitivity. However, mechanisms involving enhanced CNS activity may be responsible (Forster *et al.*, 1975; Sato, 1990; Schmeling *et al.*, 1977). For example, the progressive rise in ventilation during isocapnic hypoxia may arise from the higher initial level of ventilation as compared with poikilocapnic hypoxia, as a consequence of hyperventilation-induced hyperpnoea (Smith *et al.*, 1962a,b). Alternatively, CNS hyperexcitability may result from an effect of augmented peripheral chemoreceptor activity (Millhorn *et al.*, 1980a,b; Tenney *et al.*, 1971) or a direct effect of CNS hypoxia (Lowry *et al.*, 1994), which would also have been masked by the associated alkalosis of the poikilocapnic exposure. The latter phenomenon, although causing an increase in ventilation in the pony during exposure to CNS hypoxia, is still too small, however, to explain the rise in ventilation seen in this study (Lowry *et al.*, 1994).

Despite attempting to maintain arterial isocapnia, and so pH, in this study, the possibility remains that central hypoxia may cause changes in the pH at the central chemoreceptors. It has been established that hypoxia *per se* causes an acidification of the ventral medullary surface above the postulated sites of the central chemoreceptors in both anaesthetized and conscious animals (Brown and Lawson, 1988; Davies *et al.*, 1986; Neubauer *et al.*, 1988; Xu *et al.*, 1991,1992). However, as discussed in Chapter 1, it is thought that hypoxic acidification of the central chemoreceptor site does not result in any alterations in respiratory control that might lead to VAH (Dempsey and Forster, 1982; Fencel *et al.*, 1979; Musch *et al.*, 1983; Weizhen *et al.*, 1992; Xu *et al.*, 1991,1992). It remains just possible, however, that in the current study, hypoxia is causing an increase in ventilation through lacticacidosis, which is masked during poikilocapnic hypoxia because of the associated hypocapnic alkalosis.

Conclusion. A progressive time-dependent rise in ventilation was observed during exposure to 8 hours of isocapnic, but not poikilocapnic, hypoxia. A possible explanation for this phenomenon is a progressive effect of hypoxia *per se*, probably on the carotid body, but other theories remain possible.

APPENDIX 4.1.

This appendix presents the ventilatory data collected during each individual experiment from every subject.

Protocol	Time	Ventilation (l.min ⁻¹)									
		884	894	910	927	Subject No. 930	932	934	935	939	945
Isocapnic Hypoxia	0	15.00	22.00	12.00	14.00	11.02	16.12	16.33	15.19	23.65	16.73
	0.3*	9.95	19.07	9.50	11.78	4.91	15.21	13.80	7.75	23.28	14.34
	1.5	20.46	25.39	13.17	9.73	9.51	16.32	21.89	11.26	25.70	22.36
	2.5	23.88	22.64	25.50	12.63	14.69	17.17	19.40	13.63	27.75	20.23
	3.5	21.11	22.50	19.76	13.68	12.79	20.63	27.76	20.80	23.62	30.41
	4.5	24.87	22.50	25.00	16.96	15.02	19.87	26.59	18.31	26.80	25.00
	5.5	16.92	22.70	34.65	26.21	14.44	18.43	28.21	15.91	29.88	27.00
	6.5	23.97	19.83	28.71	22.07	19.01	22.87	25.10	17.26	28.77	28.00
	7.5	27.29	25.37	27.91	21.89	18.03	19.44	25.30	19.77	34.38	29.00
Poikilocapnic Hypoxia	0	13.50	10.50	11.01	8.60	8.56	12.28	13.63	13.48	13.33	9.71
	0.3*	11.50	9.76	9.00	6.57	5.33	10.70	10.70	9.53	11.11	7.95
	1.5	13.01	8.78	11.01	9.24	8.71	12.07	13.01	9.90	9.45	13.26
	2.5	16.23	8.58	11.88	9.87	9.43	10.70	13.34	11.38	10.63	8.32
	3.5	12.08	11.32	11.02	11.07	9.89	14.62	15.44	10.32	11.67	9.82
	4.5	12.21	13.86	12.99	11.69	8.51	12.81	12.48	10.42	11.90	11.26
	5.5	13.76	11.50	12.72	11.59	10.62	12.09	17.56	10.88	11.04	14.10
	6.5	15.87	12.45	11.79	11.13	10.00	10.41	12.89	10.68	11.22	10.97
	7.5	8.75	13.95	12.24	11.59	9.35	13.64	13.39	9.41	8.96	9.94
Control	0	10.50	10.36	7.46	6.27	8.01	11.45	8.31	12.35	6.76	7.00
	0.3*	10.61	10.14	6.75	6.53	6.33	10.08	9.74	7.52	6.21	7.00
	1.5	14.30	12.39	9.48	8.99	9.08	10.50	11.31	10.53	6.80	8.00
	2.5	14.40	13.77	8.33	8.37	8.45	10.44	9.78	10.36	9.82	7.55
	3.5	14.57	9.84	8.97	8.05	8.21	10.21	10.51	11.49	9.69	8.12
	4.5	16.17	9.63	6.75	7.32	8.40	10.64	10.38	11.20	10.28	8.21
	5.5	13.34	12.03	8.95	10.75	9.15	11.72	10.12	10.50	9.77	11.17
	6.5	12.63	12.99	8.44	7.78	10.27	12.00	12.06	10.60	8.62	12.30
	7.5	18.30	9.00	7.09	6.21	8.53	12.64	10.54	11.00	8.55	10.50

Table 4.3: Ventilatory data (l.min⁻¹) calculated from the last 15 breaths of a 3-min measurement period at specified times of 0, 20 min, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 hours in each of the three protocols.

CHAPTER 5

ALTERATIONS IN RESPIRATORY CONTROL DURING EIGHT HOURS OF ISOCAPNIC AND POIKILOCAPNIC HYPOXIA

*L'homme vit et se meurt dans ce qu'il voit,
mais il ne voit ce qu'il songe.*

Paul Valéry

INTRODUCTION

In the previous chapter, it was reported that a large increase in ventilation occurs during an 8-hour exposure to isocapnic hypoxia, but not during an 8-hour exposure to either poikilocapnic hypoxia or control (air breathing) conditions. This rise in ventilation could be due to an increase in the component of ventilation that is acutely sensitive to rapid changes in hypoxia, or to an increase in the component of ventilation that is insensitive to rapid changes in hypoxia or both. Further, the increase in ventilation could be a primary effect of the sustained hypoxia, which is present in both the isocapnic and poikilocapnic exposures, but masked by the associated hypocapnic alkalosis of poikilocapnic hypoxia, or, alternatively, it could be the higher initial level of ventilation in isocapnic hypoxia that causes the progressive rise in ventilation as a result of hyperventilation-induced hyperpnoea (Smith *et al.*, 1962a,b).

During the experiments described in Chapter 4, the acute ventilatory response to hypoxia (AHVR) was measured before, during and after exposure to each of the protocols (isocapnic hypoxia, poikilocapnic hypoxia and control). These measurements enable the above hypotheses to be tested. First, the AHVR is a direct measure of whether the component of ventilation that is sensitive to rapid fluctuations in hypoxia has increased. Secondly, measurements of AHVR provide a test of the alternative hypotheses for the increase in ventilation observed during isocapnic hypoxia. In particular, if the increase in ventilation was due to a direct effect of hypoxia, then similar changes in AHVR would be expected in both the isocapnic and poikilocapnic hypoxic exposures. Alternatively, if the observed rise was due to hyperventilation-induced hyperpnoea, then one would expect differences between the two hypoxic protocols.

METHODS

Subjects. The study in this chapter was performed in parallel with the study in Chapter 4, therefore the subjects who undertook these experiments are the same as those listed in Table 4.1 of the previous chapter.

Equipment and experimental technique. During the bulk of the experiment, individual subjects were seated inside the purpose-built chamber (described in Chapter 3). Measurements of the ventilatory response to rapid changes in hypoxia were made outside the chamber using the fast gas-mixing system. This system is described in Chapter 2.

Chapter 2 also described how the dynamic end-tidal forcing system predicts the inspired gases in order to generate the desired end-tidal gas composition using an estimate of the inspired composition entered prior to experimentation, the difference between the actual and the desired end-tidal gas values for the previous breath (proportional feedback) and the sum of all the previous differences (integral feedback). During steady-state inputs of hypoxia and/or hypercapnia this system can function well with one estimate of the inspired composition, since the end-tidal gases soon reach the desired levels as a result of the two feedback mechanisms. However, during on-going dynamic changes in the level of hypoxia, as in this study, good second-by-second predictions of the inspiratory gases are needed. Prior to each test of AHVR, a set of cardio-respiratory simulation programs were run in order to calculate these predictions. This set of programs was originally written by Dr P.A. Robbins and then modified by Dr I.D. Clement to run under UNIX on a Sun Sparc Station computer and to allow square wave and sawtooth functions of hypoxia to be defined. They were further adapted for this study to impose sawtooth functions in terms of saturation (see below).

The first of the programs predicts the ventilatory response to the alveolar gas forcing function specified by the experimenter, using a steady-state controller equation [the Lloyd-

Cunningham equation, Cunningham *et al.* (1964)] and a two-compartment model relating to the dynamic properties of the respiratory control system. The steady-state parameters for the Lloyd-Cunningham equation, A, B, C, and D, the time constant and pure delay of the predicted ventilatory response to hypoxia, the resting gas composition and the resting $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ are defined by the user. The second program runs a five-tissue model of the circulatory system in order to predict the changes in the venous return of the gases to the lung. The results of these two programs are combined to give a second-by-second prediction of the inspired gases to generate the desired end-tidal gas profile, which are also given in the prediction file.

Protocols. As described in Chapter 4, subjects were exposed to 8 hours of either isocapnic hypoxia (protocol I), where $P_{ET_{O_2}}$ was held at 55 mmHg and $P_{ET_{CO_2}}$ was held at the subject's resting value; poikilocapnic hypoxia (protocol P), where $P_{ET_{O_2}}$ was held at 55 mmHg and $P_{ET_{CO_2}}$ was left to vary; or air as a control (protocol C). The first test of AHVR was carried out before the start of the 8-hour period. The first 20 minutes of the exposure were performed with the subject attached to the fast gas-mixing system and this was immediately followed by a second test of AHVR. Subjects entered the chamber following this test and were removed for 15 min at 4 hours and 8 hours for a third and fourth measurement of AHVR.

The protocol for measuring AHVR consisted of 3 ½ min of steady $P_{ET_{O_2}}$ (100 mmHg for the first test and 55 mmHg for the second, third and fourth tests, apart from the control protocol where 100 mmHg was used throughout) followed by 3 ½ square waves, stepping between 45 and 100 mmHg and 4 sawtooth inputs of hypoxia between 45 and 100 mmHg. Both the square wave and sawtooth inputs had periods of 90 sec giving a total duration of 15 min. The sawtooth P_{O_2} was calculated using an equation derived by Ellis (1989),

$$P_{O_2} = \sqrt[3]{\frac{11,700}{(1/S - 1)} + \sqrt[2]{50^3 + \frac{11,700^2}{(1/S - 1)^2}}} + \sqrt[3]{\frac{11,700}{(1/S - 1)} - \sqrt[2]{50^3 + \frac{11,700^2}{(1/S - 1)^2}}} \quad (1)$$

from the relationship between saturation and P_{O_2} defined by Severinghaus (1979),

$$S_{aO_2} = \{23,400 * (P_{O_2}^3 + 150 * P_{O_2})^{-1} + 1\}^{-1} \quad (2)$$

to impose a linear rise and fall against time in the haemoglobin saturation (S_{aO_2}). This input provided some stimulation at P_{ETO_2} s other than the two values used for the lower and upper levels of the square wave input. The P_{ETCO_2} was held constant throughout the test at 1-1.5 mmHg above the subject's initial air-breathing value before the start of the experiment. The changes in end-tidal P_{O_2} employed to measure AHVR are summarized in Fig. 5.1.

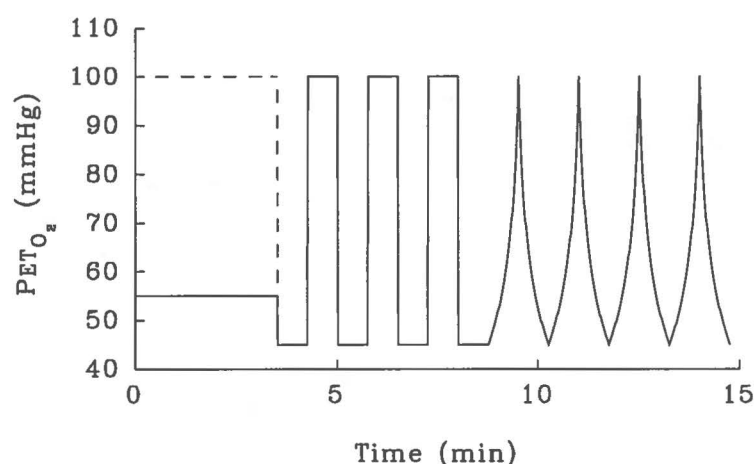


Figure 5.1: Desired profile for P_{ETO_2} during measurement of AHVR. (Initial dashed line - initial euoxic period used at $t=0$ in the hypoxic protocols (I and P) and all tests in the control (C); initial solid line - initial hypoxic period used at $t=20$ min, 4 hours and 8 hours in protocols I and P).

Model of hypoxic response. In order to characterize any changes in AHVR, the data from each measurement were fit by a first-order model as defined by Clement and Robbins (1993). In this model, total ventilation, $\dot{V}_E$, is divided up into a component which is sensitive to rapid

changes in hypoxia, $\dot{V}_P$ (which is constrained to have both a time constant and delay less than 30 sec), and a component which is insensitive to such changes, $\dot{V}_C$. Thus,

$$\dot{V}_E = \dot{V}_P + \dot{V}_C. \quad (3)$$

In the current tests of hypoxic sensitivity, where rapid changes in hypoxia were employed, isocapnia was maintained and so it is assumed that the hypoxia-independent ventilation as per the model (*i.e.*, $\dot{V}_C$) is constant.

During steady-state, the hypoxia-dependent component can be linearly related to the hypoxic stimulus as follows:

$$\dot{V}_P = G_P \cdot Hyp. \quad (4)$$

where G_P is the hypoxic sensitivity or gain and Hyp is the hypoxic stimulus, a non-linear function of P_{O_2} , in this case derived from the haemoglobin saturation function described by Severinghaus (1979), shown in equation (2), to give desaturation. If the hypoxic stimulus varies with time, then the dynamic response of $\dot{V}_P$ can be represented by a first-order model as follows:

$$\tau \frac{d\dot{V}_P}{dt} + \dot{V}_P = G_P \cdot Hyp(t - T_d) \quad (5)$$

where τ is the time constant and T_d is the time delay of the ventilatory response. In fitting this model to the data, the non-linear conversion of P_{O_2} to desaturation is performed before the dynamics as shown in equation (5).

The fitting technique used was as described by Clement and Robbins (1993). First, P_{O_2} was converted into a continuous variable from a discrete variable by first-order interpolation between breaths. Second, rather than adopt a similar procedure for ventilation, equation (5)

was solved by integration, assuming a constant hypoxic input over a single breath i ending at time t , to give a difference equation describing the hypoxia-dependent model ventilation in terms of desaturation, Hyp , breath duration, $T_{B(i)}$, and the hypoxia-dependent model ventilation for the previous breath ($i-1$):

$$\dot{V}_{P(i)} = G_P \cdot Hyp(t - T_d) - (G_P \cdot Hyp(t - T_d) - \dot{V}_{P(i-1)}) \cdot e^{-\frac{T_{B(i)}}{\tau}}. \quad (6)$$

Model fitting. Preliminary data analysis on each test of AHVR was performed using the program BRDATA, described in the initial data analysis section, Chapter 2. The first 5 min of the *.D1 and *.D4 files written by BRDATA were omitted from the analysis, to allow for the time during which the respiratory control system adapts to the level of PET_{CO_2} used in the test. The remaining 10 minutes of the test were used in the model-fitting.

The breath-by-breath model ventilation ($\dot{V}_{E(i)}$) can be calculated from the solution for $\dot{V}_{P(i)}$ as follows:

$$\dot{V}_{E(i)} = \dot{V}_{P(i)} + \dot{V}_C \quad (7)$$

Provided a value of $\dot{V}_{P(i)}$ is known for the first breath, $\dot{V}_{P(1)}$, of the period to be fit by the model, $\dot{V}_{E(1)}$ can be calculated. An initial value for $\dot{V}_{P(initial)}$ is chosen from the existing breath two min before the start of the model-fitting period; then the model is run on the intervening period before the start of the fitting procedure to allow $\dot{V}_{P(i)}$ to settle down to a value appropriate to the on-going dynamic stimulation.

The parameters to be estimated for the model were τ , T_d , G_P and $\dot{V}_C$. All the parameters were constrained to be greater than zero and the dynamic parameters to be less than 30 sec. The best fit parameters are estimated by a non-linear regression using the NAG (Numerical Algorithms Group, Oxford, UK) Fortran library routine E04FDF to minimise the function E , where:

$$E = \sum_{i=1}^n (e_{(i)})^2 \quad (8)$$

and

$$e_{(i)} = (\dot{V}_{E(i)} - \dot{V}_{E(i)}^*) \cdot \sqrt{T_{B(i)}} \quad (9)$$

where $\dot{V}_{E(i)}^*$ is the measured ventilation and n is the total number of breaths. The squared residual was weighted by the breath duration $T_{B(i)}$ to avoid the fit being weighted towards periods of higher frequency ventilation.

The model was fit to the individual tests of hypoxic sensitivity from 0 hours, 20 min and 4 and 8 hours and parameters τ , T_d , G_p and $\dot{V}_C$ were estimated for each test (fitting procedure A). In fitting procedure B, the model was fit to all 12 tests from each subject simultaneously. G_p and $\dot{V}_C$ were allowed to vary for each test, but τ and T_d were constrained to take the same value for all 12 data sets. The test carried out at 20 min was excluded from further analysis as the study of HVD was not one of the objectives of this chapter.

Statistical analysis. An analysis of variance, similar to that in the previous chapter, was used to test for possible differences in the hypoxic sensitivity, G_p , and hypoxia-independent ventilation, $\dot{V}_C$, between the three protocols. Using the notation defined by Armitage and Berry (1987), the linear model for G_p and $\dot{V}_C$ for the i th subject (S_i), for the j th time of measurement (T_j) and for the k th protocol (P_k) together with interaction terms used was:

$$G_{Pijk} \text{ or } \dot{V}_{Cijk} = \mu + S_i + T_j + P_k + (TP)_{jk} + (ST)_{ij} + (SP)_{ik} + (STP)_{ijk} + e_{ijk}$$

where e_{ijk} is an error term. The protocol term was split into control, C, versus other (C-IP) and isocapnic hypoxia, I, versus poikilocapnic hypoxia, P, (I-P) and the times were

split into quadratic and linear effects, T_Q and T_L , respectively. Thus the new model used was:

$$\begin{aligned} G_{Pijklm} \text{ or } \dot{V}_{Eijklm} = & \mu + S_i + T_{Qj} + T_{Lk} + (C-IP)_l + (I-P)_m + (T_Q(C-IP))_{jl} + (T_L(C-IP))_{kl} \\ & + (T_Q(I-P))_{jm} + (T_L(I-P))_{km} + (ST_Q)_{ij} + (ST_L)_{ik} + (S(C-IP))_{il} + (S(I-P))_{im} \\ & + (ST_Q(C-IP))_{ijl} + (ST_L(C-IP))_{ikl} + (ST_Q(I-P))_{ijm} + (ST_L(I-P))_{ikm} + e_{ijklm} \end{aligned}$$

The effects in the model were analyzed, as in Chapter 4, for their contribution to the total variance of the data by two variance ratio tests. The first test involved removing the error contributed by each effect from the total error in a sequential, hierarchical order as defined in the model from left to right. The mean squared error from each effect in the model was divided by the remaining mean squared error to give F-ratio (1). In the second test the mean squared error from each effect was divided by the mean squared error from the interaction between the effect and the subject term to give F-ratio (2). The analysis was performed using GLIM statistical software.

RESULTS

Ten subjects completed the study successfully, although some reported discomfort from high levels of ventilation during the final measurement of AHVR at 8 hours. Two subjects were removed from the chamber before the 8-hour duration had been completed due to time constraints, but none was withdrawn before at least 7 hours had been completed.

Figure 5.2 shows the breath-by-breath ventilatory response and the end-tidal gases of subject 910 during a sensitivity test carried out after 4 hours of poikilocapnic hypoxia. This figure demonstrates the quality of control of the end-tidal gases attained by the dynamic end-tidal forcing system. The average end-tidal gas tensions and ventilatory responses of the last 3 superimposed hypoxic square waves averaged over all subjects are shown in Fig. 5.3 (isocapnic hypoxia), Fig. 5.4 (poikilocapnic hypoxia) and Fig. 5.5 (control). (Separate averages were calculated for data from each protocol and from each measurement period within a protocol.) The average end-tidal gases and ventilatory responses for all 4 superimposed sawtooth inputs averaged over all subjects [presented in Fig. 5.6 (isocapnic hypoxia), Fig. 5.7 (poikilocapnic hypoxia) and Fig 5.8 (control)] were calculated in a manner analogous to that used in Figs 5.3, 5.4 and 5.5. The impressions gained from these figures suggest the presence of a progressive increase in the hypoxic sensitivity (magnitude of the ventilatory response) during the 8-hour exposure to both poikilocapnic and isocapnic hypoxia, when the 20 min measurement is excluded. The changes in the hypoxia-independent offset of ventilation (baseline) are less clear cut. There is a strong suggestion that the offset increases during both types of hypoxic exposure; however, the dynamics appear to have altered and thus it is hard to tell precisely the true hypoxia-independent ventilation. The figures also illustrate the lack of any change in the ventilatory response during the exposure to control conditions.

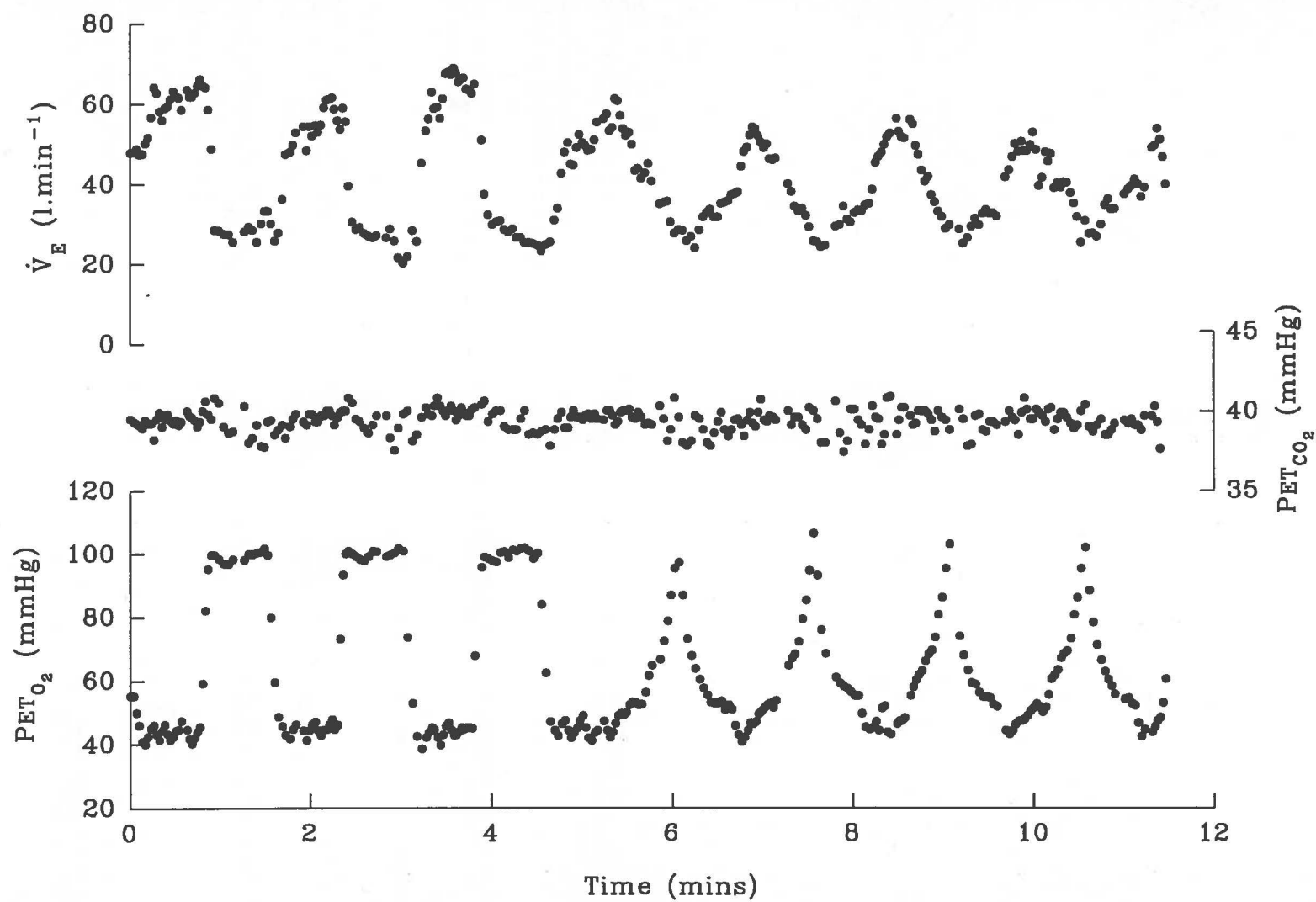


Figure 5.2: Breath-by-breath ventilation ($\dot{V}_E$), end-tidal PCO_2 and PO_2 (PET_{CO_2} and PET_{O_2}) during a measurement of AHVR. (Subject 910 at $t=4$ hours during poikilocapnic hypoxia.)

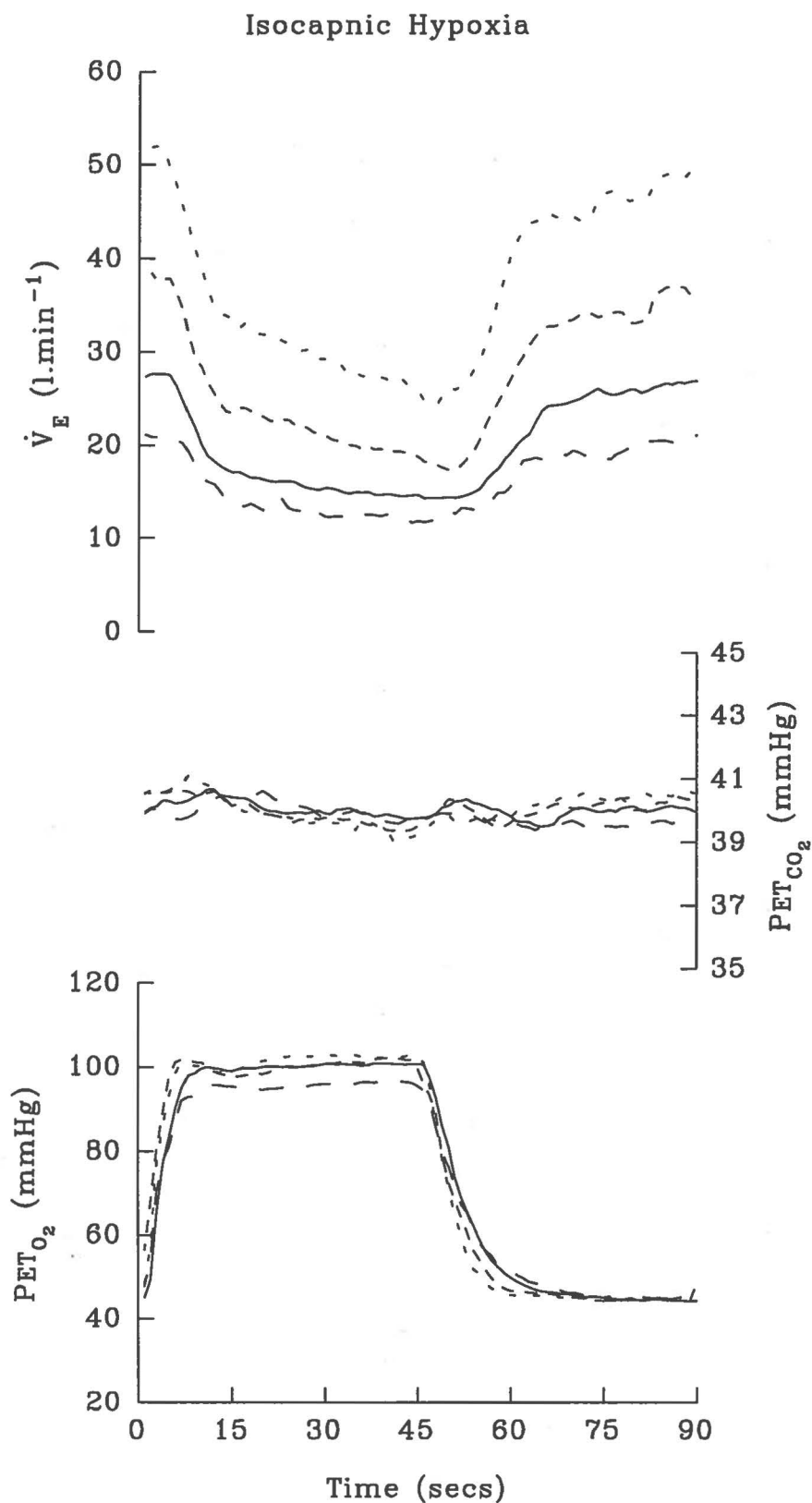


Figure 5.3: PET_{CO_2} , PET_{O_2} and $\dot{V}_E$ for average of last 3 square waves during tests of AHVR averaged for all 10 subjects during the isocapnic protocol. (Solid line, $t=0$; long dashes, $t=20$ min; medium dashes, $t=4$ hours; short dashes, $t=8$ hours.)

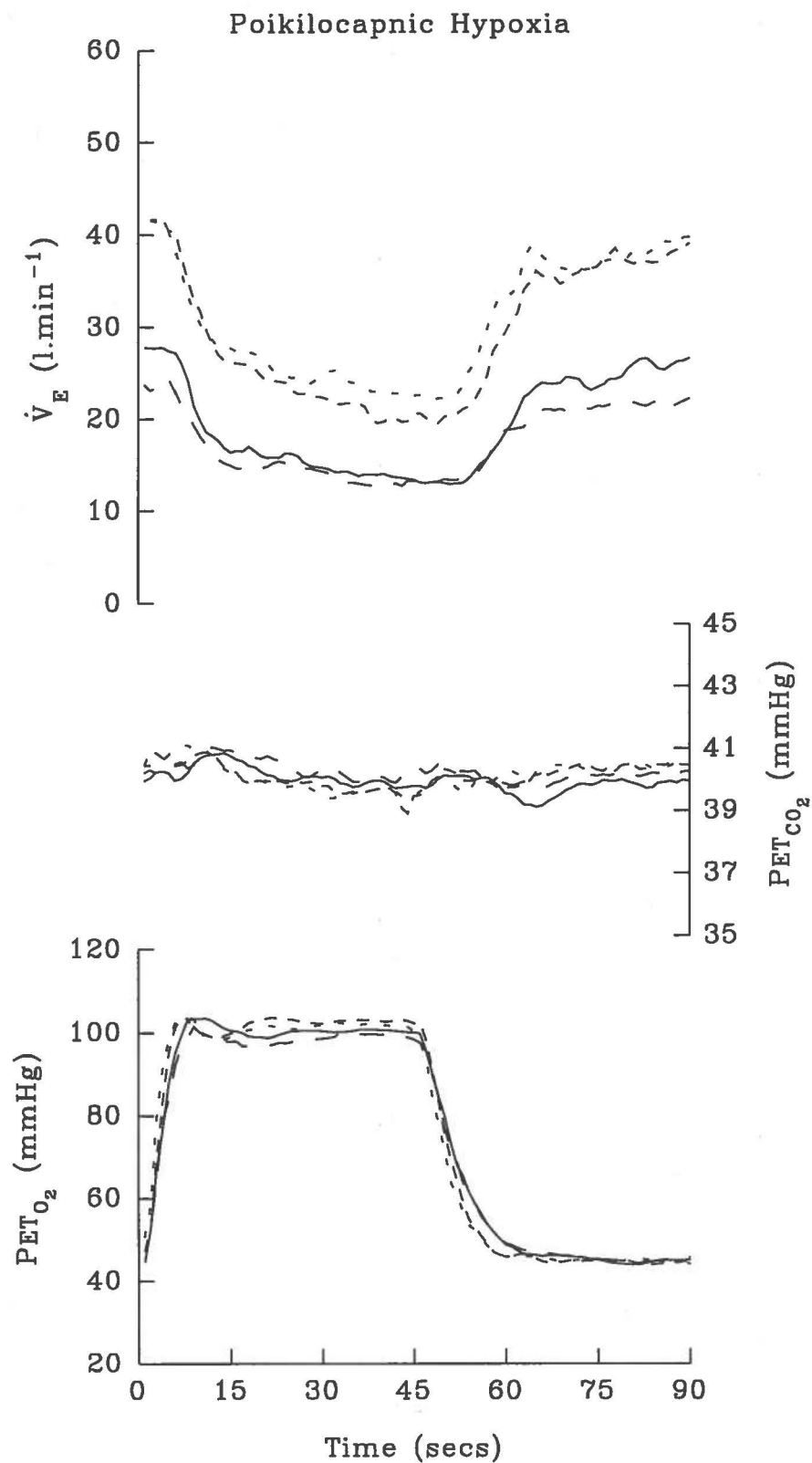


Figure 5.4: PET_{CO_2} , PET_{O_2} and $\dot{V}_E$ for average of last 3 square waves during tests of AHVR averaged for all 10 subjects during the poikilocapnic protocol. (Solid line, $t=0$; long dashes, $t=20$ min; medium dashes, $t=4$ hours; short dashes, $t=8$ hours.)

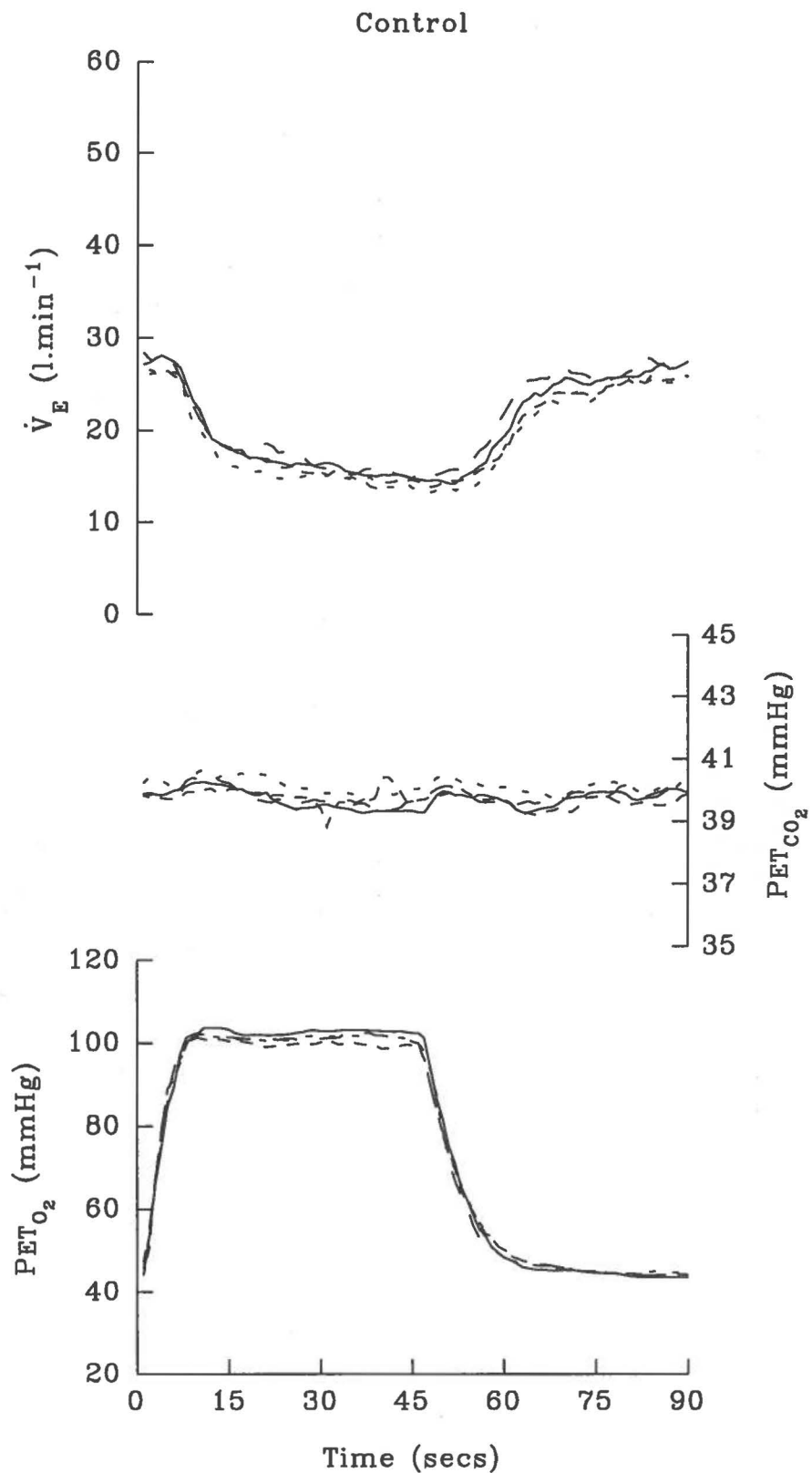


Figure 5.5: PET_{CO_2} , PET_{O_2} and $\dot{V}_E$ for average of last 3 square waves during tests of AHVR averaged for all 10 subjects during the control protocol. (Solid line, $t=0$; long dashes, $t=20$ min; medium dashes, $t=4$ hours; short dashes, $t=8$ hours.)

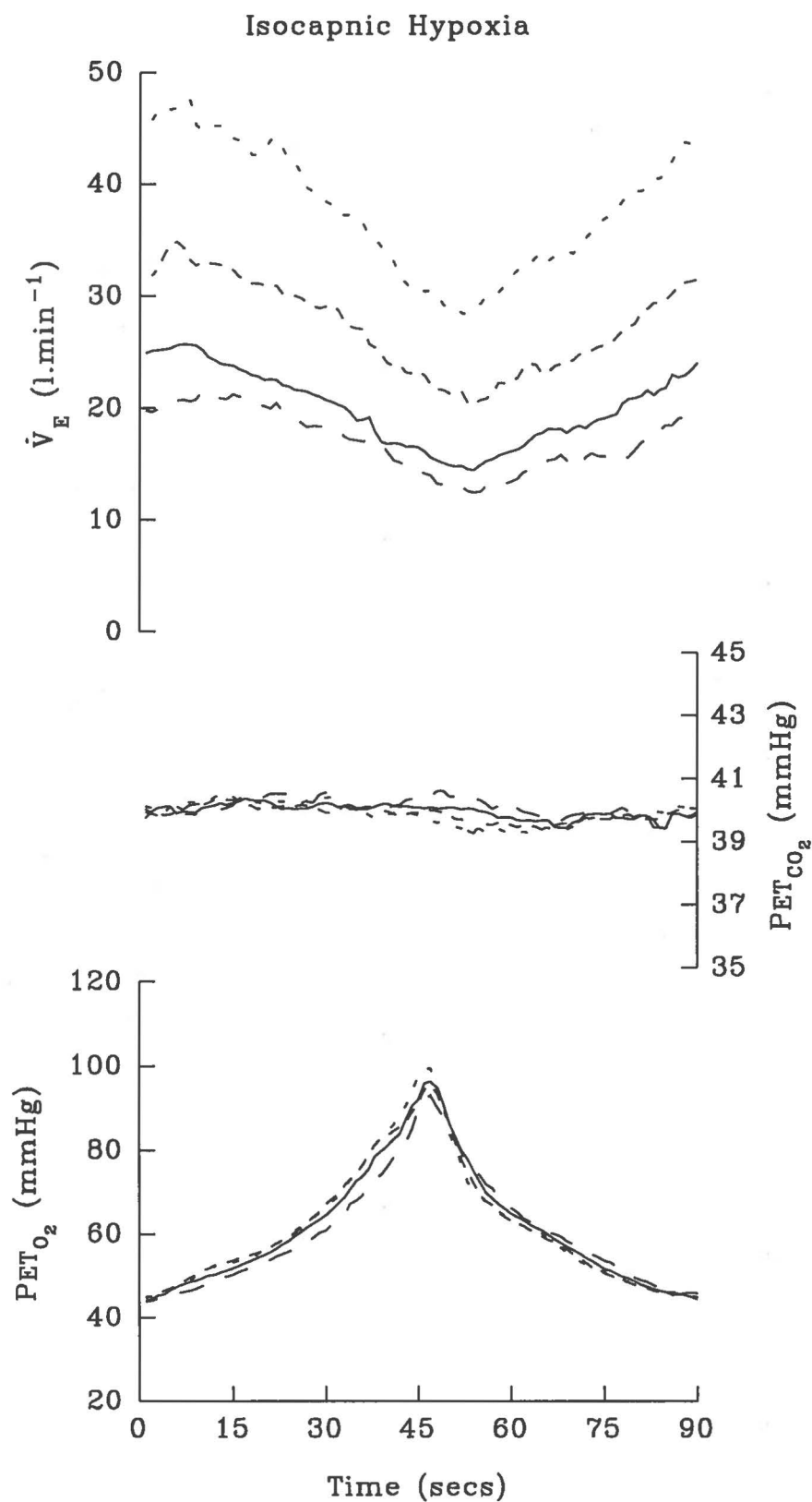


Figure 5.6: PET_{CO_2} , PET_{O_2} and $\dot{V}_E$ for average of all sawtooth inputs during tests of AHVR averaged for all 10 subjects during the isocapnic protocol. (Solid line, $t=0$; long dashes, $t=20$ min; medium dashes, $t=4$ hours; short dashes, $t=8$ hours.)

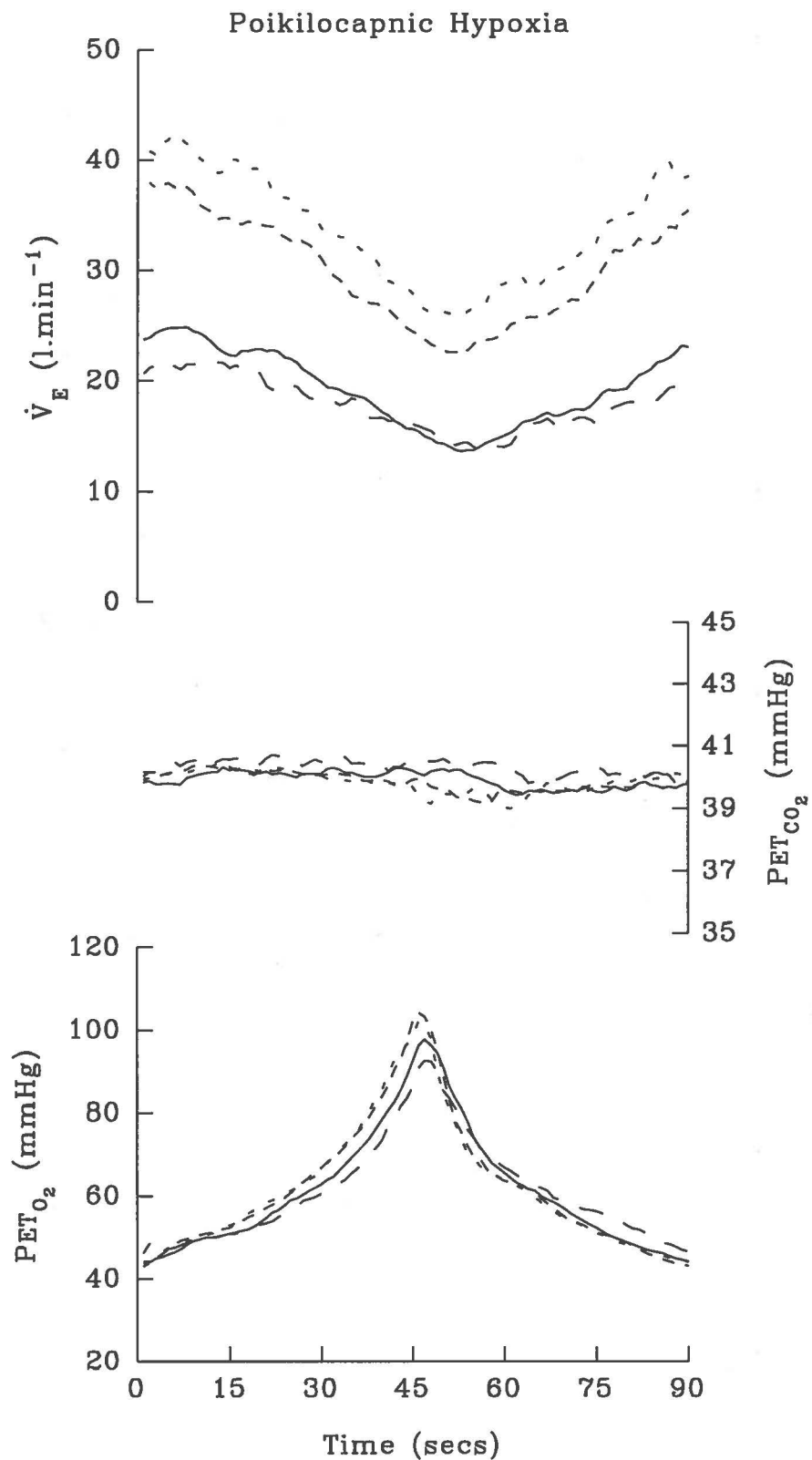


Figure 5.7: PET_{CO_2} , PET_{O_2} and $\dot{V}_E$ for average of all sawtooth inputs during tests of AHVR averaged for all 10 subjects during the poikilocapnic protocol. (Solid line, $t=0$; long dashes, $t=20$ min; medium dashes, $t=4$ hours; short dashes, $t=8$ hours.)

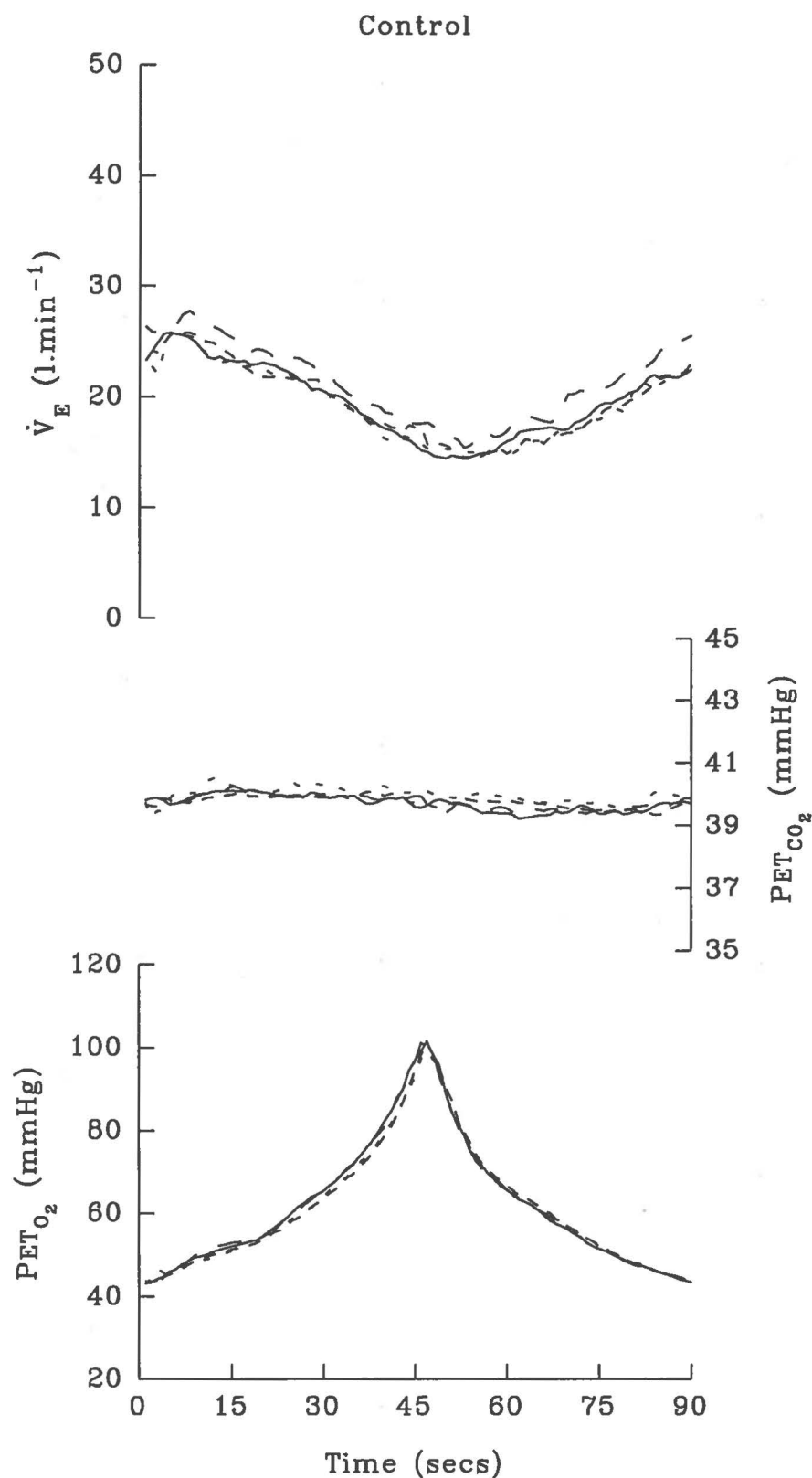


Figure 5.8: PETCO₂, PETO₂ and $\dot{V}_E$ for average of all sawtooth inputs during tests of AHVR averaged for all 10 subjects during the control protocol. (Solid line, t=0; long dashes, t=20 min; medium dashes, t=4 hours; short dashes, t=8 hours.)

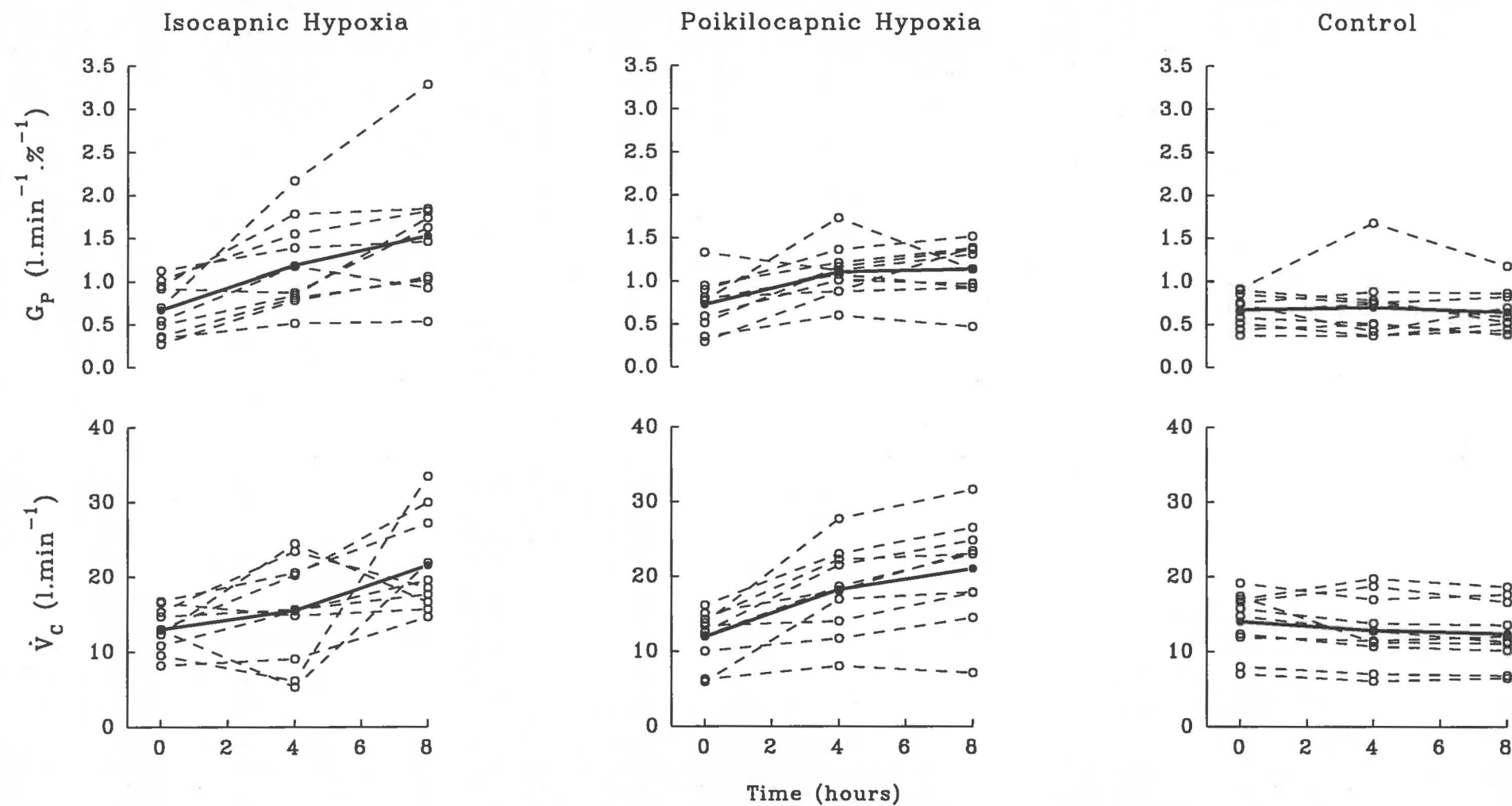


Figure 5.9: Estimates using fitting procedure A for G_p and $\dot{V}_C$ at 0, 4 and 8 hours. Left, isocapnic hypoxia; middle, poikilocapnic hypoxia; right, control. (Dashed lines, individual subjects; solid lines, mean values.)

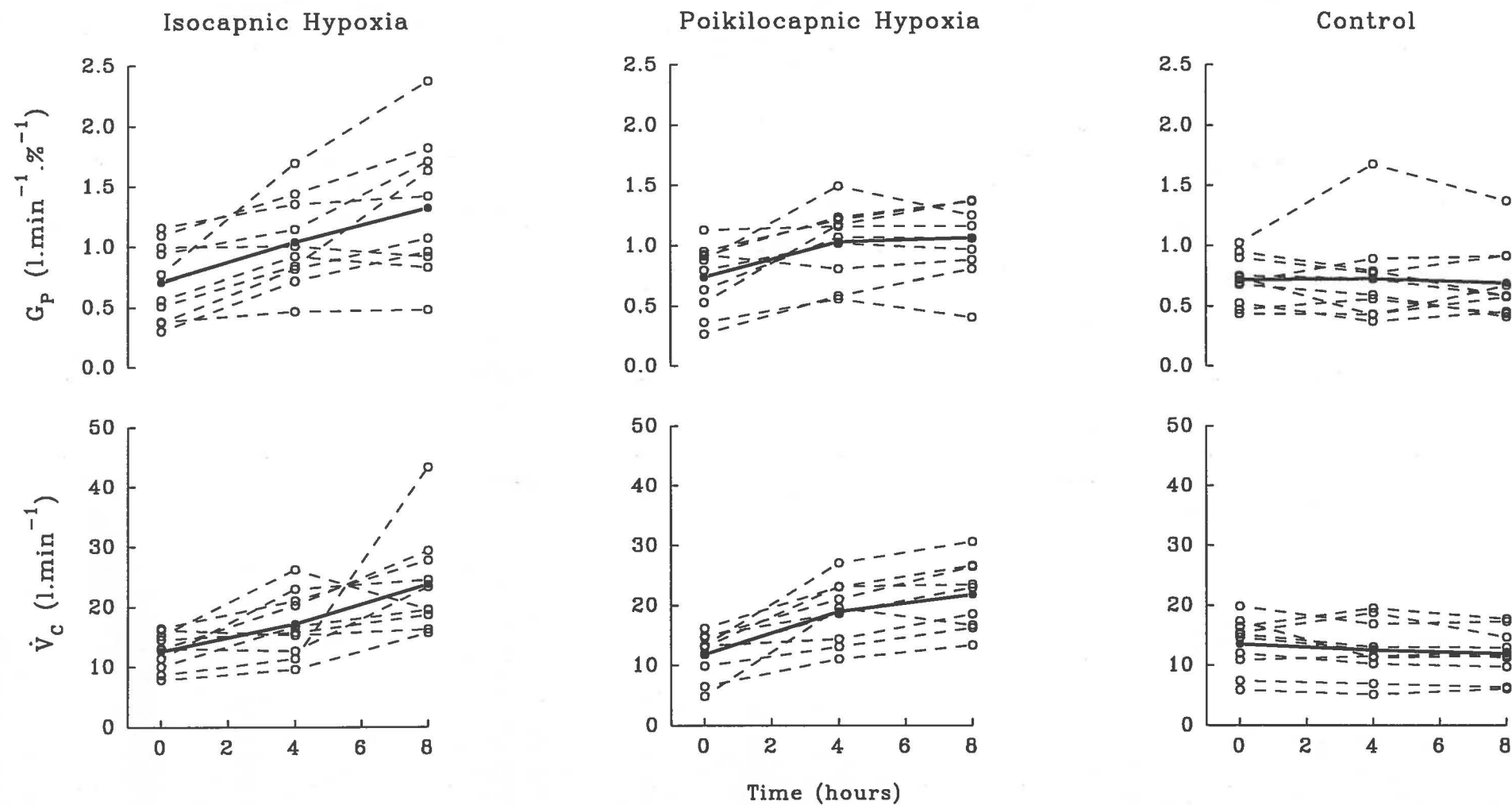


Figure 5.10: Estimates using fitting procedure B for G_p and $\dot{V}_c$ at 0, 4 and 8 hours. Left, isocapnic hypoxia; middle, poikilocapnic hypoxia; right, control. (Dashed lines, individual subjects; solid lines, mean values.)

The dynamic model was fit to individual AHVR tests (procedure A) and estimates for G_P , $\dot{V}_C$, T_d and τ obtained. Figure 5.9 shows the mean and individual estimates for the sensitivity and offset terms (G_P and $\dot{V}_C$) using fitting procedure A. As the fitted values for the sensitivity and offset terms are dependent upon the fitted values T_d and τ , it was decided that, for each subject, a separate fitting procedure (procedure B) should be employed, where T_d and τ were constrained to take just one value for all 12 data sets. Figure 5.10 shows the mean and individual estimates for the sensitivity and offset terms using fitting procedure B. The data obtained from both fitting procedures are represented in Appendix 5.1 and the mean estimates (using procedure B) are summarized in Fig. 5.11.

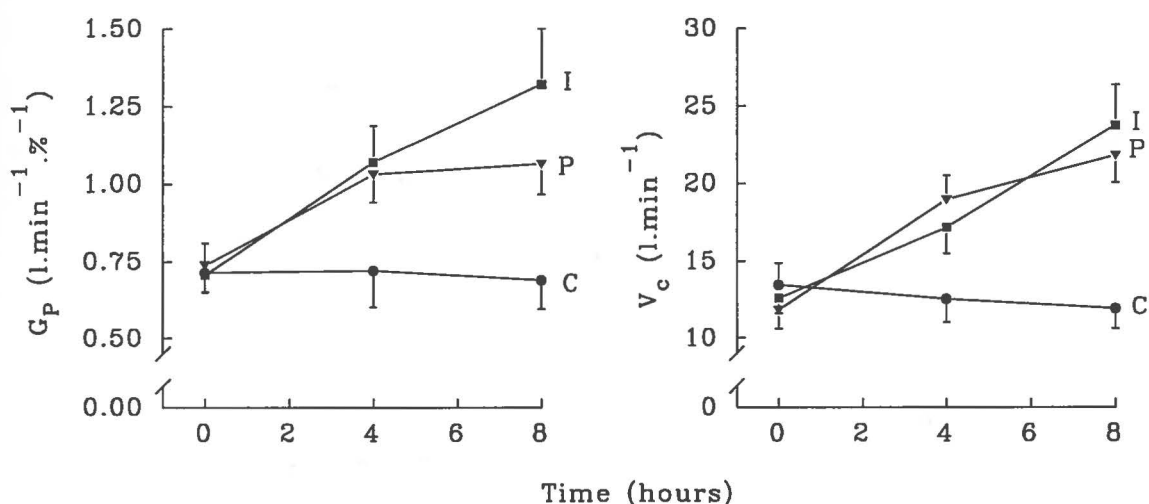


Figure 5.11: Mean estimates ($\pm$ S.E.) across all subjects for hypoxic sensitivity, G_P (l.min⁻¹.%⁻¹) and offset, V_C (l.min⁻¹) during isocapnic hypoxia (I), poikilocapnic hypoxia (P) and control (C) (using fitting procedure B).

The quality of the model fit is illustrated in Figs 5.12 to 5.14 in Appendix 5.2. These figures present examples of the average calculated arterial saturation profile (Severinghaus, 1979) and ventilatory responses, with the model fit, for the last 3 superimposed hypoxic square waves and the four superimposed sawtooth inputs from one subject (930) in each protocol.

The overall impressions gained are very similar between Figs 5.9 and 5.10, of increasing values for both G_P and $\dot{V}_C$ during both hypoxic protocols, but not during the control

protocol. The impressions obtained from these figures were tested statistically using analysis of variance on both the sensitivity and offset terms. The results of the analysis are shown for parameters from fitting procedure A in Tables 5.1 and 5.2 and for parameters from procedure B in Tables 5.3 and 5.4. For both G_p and $\dot{V}_C$, the linear component of the time factor (T_L), the difference between the control and hypoxic protocols (C-IP) and the interaction between these two factors, were all shown to be major factors in the analysis of variance. This outcome was significant whichever fitting procedure was used and whichever form of F-ratio test was used, and demonstrates that there is a general trend for G_p and $\dot{V}_C$ to increase linearly during 8 hours of hypoxia; both poikilocapnic and isocapnic. In no case did the quadratic time factor or its interaction with (C-IP) or (I-P) have a significant effect.

For the interaction between T_L and the comparison between the two hypoxic protocols (I-P), there were no significant effects on $\dot{V}_C$. However, for G_p the results were less clear cut, in that a significant difference was obtained using the F-ratio (1) test on parameters obtained using fitting procedure A ($p < 0.025$), but use of either the F-ratio (2) test or parameters obtained using fitting procedure B (both F-ratio tests) just failed to reach significance ($0.05 < p < 0.1$). In general, no certain differences between the two hypoxic protocols can be inferred from these results.

Source of Variance	Degrees of Freedom	Sum of squares (l.min ⁻¹) ²	Degrees of Freedom of Error	Error (l.min ⁻¹ .% ⁻¹) ²	F-ratio (1)	F-ratio (2)
No Factor	---	---	89	21.4966	---	---
S _i	9	6.5896	80	14.9070	---	---
(T _Q) _j	1	0.1977	79	14.7094	1.06	3.99
(T _L) _k	1	2.5777	78	12.1316	16.57***	28.71***
(C-IP) _l	1	3.0175	77	9.1142	25.49***	15.47*
(I-P) _m	1	0.2833	76	8.8309	2.44	2.20
(T _Q) _j *(C-IP) _l	1	0.0335	75	8.7974	0.29	1.00
(T _L) _k *(C-IP) _l	1	1.4522	74	7.3452	14.30***	16.15***
(T _Q) _j *(I-P) _m	1	0.0205	73	7.3246	0.20	0.49
(T _L) _k *(I-P) _m	1	0.4992	72	6.8255	5.27*	3.84
S _i *(T _Q) _j	9	0.4460	63	6.3794	---	---
S _i *(T _L) _k	9	0.8081	54	5.5713	---	---
S _i *(C-IP) _l	9	1.7556	45	3.8157	---	---
S _i *(I-P) _m	9	1.1600	36	2.6557	---	---
S _i *(T _Q) _j *(C-IP) _l	9	0.3023	27	2.3534	---	---
S _i *(T _L) _k *(C-IP) _l	9	0.8095	18	1.5439	---	---
S _i *(T _Q) _j *(I-P) _m	9	0.3751	9	1.1688	---	---
S _i *(T _L) _k *(I-P) _m	9	1.1688	0	0	---	---
Total	89	21.4966	---	---		

Table 5.1: Analysis of variance for hypoxic sensitivity term, G_p (estimated via procedure A). F-ratio (1) is determined from the ratio of the mean squared error from the source of variance being considered (column 3/column 2) to the remaining mean squared error (column 5/column 4). F-ratio (2) is determined from the ratio of the mean squared error from the source of variance being considered to the mean squared error from the interaction term between the source of variance and subjects. (*, $p < 0.025$; **, $p < 0.01$; ***, $p < 0.001$.)

Source of Variance	Degrees of Freedom	Sum of squares (l.min ⁻¹) ²	Degrees of Freedom of Error	Error (l.min ⁻¹) ²	F-ratio (1)	F-ratio (2)
No Factor	---	---	89	3223.4	---	---
S _i	9	996.3	80	2327.1	---	---
(T _Q) _j	1	0.3	79	2326.7	0.01	0.03
(T _L) _k	1	425.5	78	1901.2	17.46***	25.75***
(C-IP) _l	1	285.5	77	1615.8	13.61***	8.34*
(I-P) _m	1	2.0	76	1613.7	0.10	0.11
(T _Q) _j *(C-IP) _l	1	0.57	75	1613.2	0.03	0.08
(T _L) _k *(C-IP) _l	1	356.3	74	1256.8	20.98***	46.68***
(T _Q) _j *(I-P) _m	1	40.4	73	1216.4	2.43	1.88
(T _L) _k *(I-P) _m	1	0.8	72	1215.6	0.05	0.05
S _i *(T _Q) _j	9	124.5	63	1091.0	---	---
S _i *(T _L) _k	9	148.7	54	942.3	---	---
S _i *(C-IP) _l	9	308.2	45	634.1	---	---
S _i *(I-P) _m	9	161.6	36	472.5	---	---
S _i *(T _Q) _j *(C-IP) _l	9	62.4	27	410.1	---	---
S _i *(T _L) _k *(C-IP) _l	9	68.7	18	341.4	---	---
S _i *(T _Q) _j *(I-P) _m	9	193.6	9	147.8	---	---
S _i *(T _L) _k *(I-P) _m	9	147.8	0	0.0	---	---
Total	89	3223.4	---	---		

Table 5.2: Analysis of variance for offset term, $\dot{V}_C$ (estimated via procedure A). F-ratio (1) is determined from the ratio of the mean squared error from the source of variance being considered (column 3/column 2) to the remaining mean squared error (column 5/column 4). F-ratio (2) is determined from the ratio of the mean squared error from the source of variance being considered to the mean squared error from the interaction term between the source of variance and subjects. (*, $p < 0.025$; **, $p < 0.01$; ***, $p < 0.001$.)

Source of Variance	Degrees of Freedom	Sum of squares (l.min ⁻¹) ²	Degrees of Freedom of Error	Error (l.min ⁻¹ .% ⁻¹) ²	F-ratio (1)	F-ratio (2)
No Factor	---	---	89	14.0312	---	---
S_i	9	5.7028	80	8.3284	---	---
$(TQ)_j$	1	0.0668	79	8.2615	0.64	4.06
$(T_L)_k$	1	1.3887	78	6.8729	15.76***	22.50**
$(C-IP)_l$	1	1.4910	77	5.3818	21.33***	8.27*
$(I-P)_m$	1	0.0895	76	5.2923	1.29	1.87
$(TQ)_j*(C-IP)_l$	1	0.0144	75	5.2779	0.20	0.54
$(T_L)_k*(C-IP)_l$	1	0.8148	74	4.4631	13.51***	10.48*
$(TQ)_j*(I-P)_m$	1	0.0395	73	4.4272	0.65	3.79
$(T_L)_k*(I-P)_m$	1	0.2031	72	4.2241	3.46	4.25
$S_i*(TQ)_j$	9	0.1483	63	4.0758	---	---
$S_i*(T_L)_k$	9	0.5556	54	3.5202	---	---
$S_i*(C-IP)_l$	9	1.6232	45	1.8970	---	---
$S_i*(I-P)_m$	9	0.4312	36	1.4658	---	---
$S_i*(TQ)_j*(C-IP)_l$	9	0.2417	27	1.2241	---	---
$S_i*(T_L)_k*(C-IP)_l$	9	0.6996	18	0.5245	---	---
$S_i*(TQ)_j*(I-P)_m$	9	0.0940	9	0.4305	---	---
$S_i*(T_L)_k*(I-P)_m$	9	0.4305	0	0	---	---
Total	89	140312	---	---		

Table 5.3: Analysis of variance for hypoxic sensitivity term, G_P (estimated via procedure B). F-ratio (1) is determined from the ratio of the mean squared error from the source of variance being considered (column 3/column 2) to the remaining mean squared error (column 5/column 4). F-ratio (2) is determined from the ratio of the mean squared error from the source of variance being considered to the mean squared error from the interaction term between the source of variance and subjects. (*, $p < 0.025$; **, $p < 0.01$; ***, $p < 0.001$.)

Source of Variance	Degrees of Freedom	Sum of squares (l.min ⁻¹) ²	Degrees of Freedom of Error	Error (l.min ⁻¹) ²	F-ratio (1)	F-ratio (2)
No Factor	---	---	89	3764.8	---	---
S_i	9	861.6	80	2903.2	---	---
$(T_Q)_j$	1	2.3	79	2900.9	0.06	0.22
$(T_L)_k$	1	638.8	78	2262.2	22.02***	28.03***
$(C-IP)_l$	1	507.6	77	1754.6	22.28***	16.42**
$(I-P)_m$	1	1.6	76	1753.0	0.07	0.10
$(T_Q)_j*(C-IP)_l$	1	2.3	75	1750.6	0.10	0.44
$(T_L)_k*(C-IP)_l$	1	490.1	74	1260.6	28.77***	62.20***
$(T_Q)_j*(I-P)_m$	1	33.0	73	1227.6	1.96	1.71
$(T_L)_k*(I-P)_m$	1	3.6	72	1223.9	0.21	0.16
$S_i*(T_Q)_j$	9	91.8	63	1132.1	---	---
$S_i*(T_L)_k$	9	205.1	54	927.0	---	---
$S_i*(C-IP)_l$	9	278.2	45	648.8	---	---
$S_i*(I-P)_m$	9	145.2	36	503.7	---	---
$S_i*(T_Q)_j*(C-IP)_l$	9	48.2	27	455.4	---	---
$S_i*(T_L)_k*(C-IP)_l$	9	70.9	18	384.6	---	---
$S_i*(T_Q)_j*(I-P)_m$	9	174.2	9	210.3	---	---
$S_i*(T_L)_k*(I-P)_m$	9	210.3	0	0.0	---	---
Total	89	3764.8	---	---		

Table 5.4: Analysis of variance for offset term, $\dot{V}_C$ (estimated via procedure B). F-ratio (1) is determined from the ratio of the mean squared error from the source of variance being considered (column 3/column 2) to the remaining mean squared error (column 5/column 4). F-ratio (2) is determined from the ratio of the mean squared error from the source of variance being considered to the mean squared error from the interaction term between the source of variance and subjects. (*, $p < 0.025$; **, $p < 0.01$; ***, $p < 0.001$.)

DISCUSSION

The main findings of this study are that the component of ventilation sensitive to acute changes in hypoxia, G_P , and the component of ventilation insensitive to acute changes in hypoxia, $\dot{V}_C$, both rise during exposure to 8 hours of hypoxia. The changes in G_P and $\dot{V}_C$ over the 8-hour period were not shown to be significantly different between isocapnic and poikilocapnic hypoxia, suggesting that these changes do not depend principally on alterations in the acid-base status of the blood, and are caused by hypoxia *per se*.

Modelling of hypoxic responses. The first-order model employed in this study has been used in previous studies to model the ventilatory sensitivity to acute hypoxia (Clement and Robbins, 1993; Paterson *et al.*, 1993). It has been used to describe the responses to isocapnic sawtooth inputs of hypoxia of periods 120, 90, 60, 45 and 30 sec, and square wave inputs of 120, 90, 60 and 30 sec. For each given input, the model could describe the data well. Furthermore, for the square wave inputs of periods 120, 90 and 60 sec, a reasonable plateau phase in ventilation after the initial transient could be observed, suggesting that the rapid component of the response had evolved fully. For these reasons, a period of 90 sec for the acute hypoxic stimuli was chosen in the current study.

Inspection of the ventilatory responses to acute hypoxia measured before the exposures, and of the ventilatory responses to acute hypoxia measured during the control (air breathing) exposure (Figs 5.3, 5.4 and 5.5), suggests that a reasonable, if not totally complete, plateau phase in ventilation had been achieved in these tests of hypoxic sensitivity. However, the ventilatory responses to acute hypoxia after 4 and 8 hours exposure to hypoxia in the chamber, both in the isocapnic and poikilocapnic states, do not show this clear plateau phase. Instead there appears to be a slower component of the response present. This could arise in two ways. The first is that there is a general slowing of the response in the subjects, but that the single compartment model used is still appropriate. In this case, the biphasic appearance of the mean responses would result from the process of averaging across subjects who show a

range of response speeds, rather than from any additional component. The second possibility is that the exposure to 4 or 8 hours of hypoxia has resulted in the development of a second phase of the acute ventilatory response to hypoxia, which is slower than the first phase. In the modelling process, part of this intermediate component would be attributed to G_P and part of it would be attributed to $\dot{V}_C$. In order to distinguish between the two possibilities, further experimentation is required. In addition, a longer period hypoxic square wave is needed in order to allow the plateau phase to develop.

Two fitting procedures were used, one where each individual test of AHVR was fit to produce estimates for G_P , $\dot{V}_C$, T_d and τ , and one where T_d and τ were constrained to take just one value for all 12 data sets from one subject. This was done since the fitted values for G_P and $\dot{V}_C$ were dependent on the fitted values for T_d and τ , and therefore, rogue estimates for G_P and $\dot{V}_C$ were occasionally obtained when T_d and τ were poorly estimated as a result of only 12 min of available data.

Changes in hypoxic sensitivity: comparison with other studies. There are no previous studies in humans of the sensitivity to acute changes in hypoxia during exposure to prolonged hypoxia, isocapnic or poikilocapnic, over the time course employed in this study. Shorter duration (tens of minutes) exposures to isocapnic hypoxia are associated with a decrease in hypoxic sensitivity (Bascom *et al.*, 1990). Cruz *et al.* (1980) examined the ventilatory response to acute hypoxia at 24 hour intervals over 100 hours of both isocapnic and poikilocapnic hypoxia. Although they only detected an increase in hypoxic sensitivity in the poikilocapnic state, their data suggest that hypoxic sensitivity almost doubled in isocapnic hypoxia, indicating that a possible type 2 statistical error was made. Apart from this study, longer-duration studies of hypoxic sensitivity during hypoxic exposure have only been accomplished in the poikilocapnic state (Goldberg *et al.*, 1992; Sato *et al.*, 1992, 1994; White *et al.*, 1987). These studies report a progressive time-dependent increase in AHVR during exposure to hypoxia at altitude or at simulated altitude (hypobaria), over a periods of up to 12 days.

However, one problem in measuring changes in AHVR during the poikilocapnic state is the determination of a suitable P_{CO_2} at which to conduct the test, when acid-base changes following different time courses occur in the fluids bathing the central and peripheral chemoreceptors. Initial exposure to hypoxia causes a respiratory systemic hypocapnic alkalosis to develop. Subsequently, over a period of hours to days, cerebrospinal fluid (CSF) and medullary interstitial fluid $[HCO_3^-]$ fall through metabolic compensation, although CSF pH stays alkaline (Forster *et al.*, 1974) because of a continuing rise in ventilation. However, renal compensation of the arterial alkalosis by excretion of HCO_3^- does not follow the same time course and occurs over a period of days to weeks (Forster *et al.*, 1975). Thus, the central and peripheral chemoreceptors may be operating at different "set points" of pH during the first few days of acclimatization.

Three different levels of P_{ETCO_2} were chosen at which to assess AHVR in the studies by Goldberg *et al.*, (1992), Sato *et al.*, (1992,1994) and White *et al.*, (1987). Goldberg *et al.* (1992) used the resting P_{ETCO_2} during the hypoxic exposure; Sato *et al.* (1992,1994) used the level of P_{ETCO_2} that forced a hyperoxic inspired ventilation of $140 \text{ ml.kg}^{-1}.\text{min}^{-1}$ in order to standardize the central component of the ventilatory drive and White *et al.* (1987) performed the tests at the hyperoxic P_{ETCO_2} . Despite these methods which attempted to nullify the effects of changes in the pH/ CO_2 "set point" of the central chemoreceptors, it is not clear that any of these P_{ETCO_2} levels is necessarily appropriate, given the interaction between H^+ and PO_2 that occurs at the carotid body. Indeed, it is likely that in these studies the arterial pH was alkaline and therefore the change in AHVR was underestimated; moreover, this would have affected the time course of the observed rise in AHVR. Cruz *et al.* (1980) used the same level of alveolar P_{CO_2} for all tests of AHVR. This probably would have led to an overestimation of AHVR in the latter stages of the exposure to poikilocapnic hypoxia. Over the time course of the present study, however, minimal metabolic compensation of arterial $[HCO_3^-]$ is expected, and therefore, the level of P_{ETCO_2} was the same for all measurements of AHVR.

A study similar to this one has been undertaken in the conscious goat (Engwall and Bisgard, 1990). The findings from this study closely reflect those presented here, in that there is a rise in AHVR with 4 hours of hypoxia, and that this rise does not depend upon whether the hypoxia is isocapnic or poikilocapnic.

Changes in hypoxic sensitivity: mechanisms. The observation of the rise in AHVR associated with the longer-term poikilocapnic exposures in humans (Goldberg *et al.*, 1992; Sato *et al.*, 1992,1994; White *et al.*, 1987) provides relatively little information about the mechanism by which it occurs. The change in AHVR could result from a direct effect of hypoxia *per se*, or it could evolve from the alkalosis associated with hypocapnia. The isocapnic hypoxia protocol of the current study helps to distinguish between the two possibilities, since the hypocapnic alkalosis is prevented in this protocol. The similarity of the changes in AHVR between the isocapnic and poikilocapnic protocols suggests that the hypocapnic alkalosis is not important in the rise in AHVR, although care should be taken not to extend this conclusion uncritically to the effects seen with several days of hypoxia.

Researchers using experimental animals have been able to investigate the mechanisms underlying the rise in AHVR during sustained hypoxic exposures to a greater degree. Studies involving carotid body denervation have reported that the rise in ventilation seen during prolonged exposure to hypoxia is significantly attenuated in the denervated animal (Fordyce and Tenney, 1984; Forster *et al.*, 1976; Forster *et al.*, 1981; Smith *et al.*, 1986) and Vizek *et al.* (1987b) found an increased dependency of ventilation on carotid body function in cats after exposure to 48 hours of hypobaric hypoxia. These studies illustrate the important, if not primary, role of the carotid body in the ventilatory adaptations to prolonged hypoxia.

Detailed investigations in the goat by a research group in Wisconsin (discussed in greater detail in Chapters 1 and 4) have demonstrated that carotid body hypoxia appears to be the primary stimulus in producing the rise in ventilation during prolonged hypoxia (Bisgard *et al.*, 1986a,b; Busch *et al.*, 1985; Engwall and Bisgard, 1990; Engwall *et al.*, 1988; Nielsen *et*

al., 1988; Ryan *et al.*, 1993a; Weizhen *et al.*, 1992). As AHVR and ventilation rise contemporaneously during 4 hours of whole body isocapnic hypoxia in the goat (Engwall and Bisgard, 1990), it is reasonable to link an augmented carotid body function with elevated AHVR, as well as ventilation. It thus seems likely that increased carotid body function could be responsible for the rise in AHVR in prolonged hypoxia observed in the present study, although it should be noted that the time course may be different from that in the goat, since, in the goat, most of the acclimatization to hypoxia takes place within 4 to 8 hours (Busch *et al.*, 1983; Engwall and Bisgard, 1990; Forster *et al.*, 1981; Smith *et al.*, 1986).

Figure 5.11 indicates that there may be a slight difference in G_P between the two hypoxic protocols, where G_P continues to rise more so than in the poikilocapnic state. One of the 4 statistical tests showed this effect to be significant, whereas the others just failed. It is possible that facilitation of CNS activity resulting from the higher level of ventilation in the isocapnic exposure [hyperventilation-induced hyperpnoea (Smith *et al.* (1962a,b))] may be adding to this further rise in G_P alongside any postulated changes in the carotid body sensitivity.

Changes in hypoxia-independent component of $\dot{V}_E$: comparison with other studies. Eger *et al.* (1968) found that the ventilatory response curve to CO_2 had been steepened and shifted to the left following 8 hours of either poikilocapnic, isocapnic or hypercapnic hypoxia. This is consistent with the rise in $\dot{V}_C$ in the current study. However, in the case of Eger *et al.*, this finding was greatest with poikilocapnic conditioning and least with hypercapnic conditioning, a feature which was not observed in the current study. One possible explanation for this is that Eger *et al.* measured the CO_2 response under hyperoxic conditions, whereas in the present study, $\dot{V}_C$ was calculated in the process of modelling the response to hypoxia. In addition, for reasons given in the previous chapter, the leftward shift may have been spurious. However, an increased slope of the CO_2 response would be compatible with an increase in $\dot{V}_C$ in the present study, as the level of P_{ETCO_2} used was 1 to 1.5 mmHg above the resting value before the start of the experiment. Longer duration

studies, following the prolonged poikilocapnic hypoxia of altitude, do report an increase in ventilation that is not totally relieved by either return to sea level or exposure to hyperoxia (reviewed by Dempsey and Forster (1982)). Again, this is consistent with an increase in a component of ventilation which is insensitive to rapid changes in P_{O_2} .

In the awake goat, there is a difference between 4 hours of poikilocapnic and isocapnic hypoxia (Engwall and Bisgard, 1990). With the latter condition, no change in baseline ventilation is seen on return to air breathing, whereas with the former condition there is a persistent elevation of ventilation above the pre-hypoxic baseline following return to air. However, one should again note the differences in the technique with which $\dot{V}_C$ was assessed between the two studies, in particular the level of P_{ETCO_2} employed to assess this parameter. Moreover, there may be differences between humans and goats concerning this aspect of respiratory control during prolonged hypoxia.

Changes in hypoxia-independent component of ventilation: mechanisms. The mechanisms involved in the increase in the component of ventilation insensitive to acute changes in hypoxia remain difficult to determine. It should again be noted that in the current study, the increase in this component of ventilation has been detected using rapidly-varying hypoxia. Thus it may be the case that, with slightly lower-frequency hypoxic stimulation and a different model of the dynamic ventilatory response to hypoxia, no increase in this component would have been detected. Alternatively, it is possible that separate mechanisms may be responsible for the rise in $\dot{V}_C$ in the poikilocapnic and isocapnic exposures. It may be that the elevation of $\dot{V}_C$ during poikilocapnic hypoxia is related to the persistent hyperventilation during deacclimatization from altitude. A popular theory is that this is a consequence of the metabolic compensation of blood and CSF $[HCO_3^-]$ in response to the hypocapnic alkalosis. Indeed, Bisgard *et al.* (1986b) established in the goat that on relief of carotid body hypoxia, hyperventilation only persisted when the systemic circulation had previously been allowed to become alkalotic through hypocapnia. However, CSF pH has been shown to be alkaline on relief of prolonged hypoxia and, as ventilation drops towards

control levels, to become progressively more acidotic in both humans and ponies (Dempsey *et al.*, 1979), implying that CSF pH cannot be directly linked to central ventilatory drive. Nevertheless, this phenomenon of metabolic compensation may account for the rise in $\dot{V}_C$ during the poikilocapnic exposure, although, the isocapnic conditioning should have blunted any changes in the acid-base state of the systemic circulation. However, it cannot be certain that there were no pH changes at the central chemoreceptor site, as hypoxia has been reported to acidify the ventral medullary surface above the postulated sites of the central chemoreceptors without affecting the bulk CSF pH (Xu *et al.*, 1991,1992), but, as discussed in Chapter 1, this appears to have little or no effect on ventilation (Dempsey and Forster, 1982; Fencel *et al.*, 1979; Musch *et al.*, 1983; Weizhen *et al.*, 1992; Xu *et al.*, 1991,1992). It could be, however, that isocapnic hypoxic acidification is causing an increase in hypoxia-independent ventilation in humans, where, in the goat, it is not. Conscious goats do not exhibit persistent hyperventilation on relief of isocapnic hypoxia (Engwall and Bisgard, 1990), whereas the findings of the current study, and those of Eger *et al.* (1968), would suggest that humans do. There remains the possibility that isocapnic hypoxia, via an increased cerebral blood flow (Ellingsen *et al.*, 1987) may lower the P_{CO_2} of the brain interstitial fluid. This in turn may induce some metabolic regulation of pH, via alterations in $[HCO_3^-]$, which could then be responsible, in part, for the increase in $\dot{V}_C$. The current study provides no information concerning the CO_2 response and, therefore, conclusions regarding its contribution to the changes in $\dot{V}_C$ cannot be drawn.

As an alternative to the above theories, the rise in $\dot{V}_C$ may be due to a facilitation of central nervous system (CNS) activity. In the previous chapter, it was suggested that increased CNS excitability may be responsible for the observed increase in ventilation during isocapnic hypoxia (Forster *et al.*, 1975; Lowry *et al.*, 1994; Millhorn *et al.*, 1980a,b; Schmeling *et al.*, 1977; Smith *et al.*, 1962a,b; Tenney *et al.*, 1971). It is possible that these phenomena are also responsible for the rise in $\dot{V}_C$, with the exception of hyperventilation-induced hyperpnoea in the poikilocapnic state, where ventilation demonstrated no time-dependent increase. In a previous section of this discussion, *Modelling of the hypoxic responses*, two

theories were presented to explain the slowing of the response to acute hypoxia. First, the dynamics of the acute hypoxic response may have changed and this would have contributed to an increase in $\dot{V}_C$ as determined by the present analytical procedure. Secondly, an additional slower component of the hypoxic response may have developed: this could be attributable to an increase in CNS excitability.

Ventilatory afterdischarge is a phenomenon that occurs following sudden relief of hypoxic stimuli and is thought to serve as a smoothing influence on respiratory drive (Engwall *et al.*, 1991,1994; Georgopoulos *et al.*, 1990). It is manifested as an elevation of ventilation, following cessation of excitatory input from the peripheral chemoreflex, which decays in a monoexponential fashion with a time constant in the range of 45 sec in the human (Georgopoulos *et al.*, 1990). Recently, Engwall *et al.* (1994) have established that ventilatory afterdischarge following 5 to 10 min of selective peripheral chemoreceptor stimulation in awake goats is prolonged with central hypercapnia, from having a time constant of 16.8 sec to a time constant of 39.9 sec. It may also be the case that the increased hypoxic sensitivity in the current study, acting through increased peripheral chemoreceptor output, may prolong ventilatory afterdischarge and thus be responsible for the slowing of the dynamics of the off-response from hypoxia. A facilitation of CNS activity, with similar dynamic characteristics, may also be present in the on-response.

As postulated for the case of G_P , it may be that different mechanisms are responsible in differing degrees for the rise in $\dot{V}_C$ in the isocapnic and poikilocapnic states, those CNS-excitability mechanisms contributing to most of the changes in $\dot{V}_C$ during isocapnic hypoxia and those acid-base-related mechanisms in the bulk CSF contributing to most of the rise in $\dot{V}_C$ during poikilocapnic hypoxia, although the dynamics of the AHVR would suggest similar processes in both states. Square waves of longer duration, a measure of the CO_2 response and periods of air breathing are needed in order to characterize the mechanisms underlying the rise in $\dot{V}_C$ in both hypoxic exposures. These are presented in Chapter 6.

The role of AHVR in acclimatization. The time course of the rise in AHVR during exposure to isocapnic and poikilocapnic hypoxia is similar to that of the progressive time-dependent rise in ventilation in isocapnic hypoxia reported in Chapter 4, in that both AHVR and ventilation are elevated at 4 hours and further augmented at 8 hours. This observation suggests that increased hypoxic sensitivity is in part responsible for the rise in ventilation in isocapnic hypoxia, but is masked by the associated hypocapnic alkalosis of poikilocapnic hypoxia. In the goat, Engwall and Bisgard (1990) proposed that increased hypoxic sensitivity was the primary mechanism responsible for the rise in ventilation during isocapnic hypoxia and they extended their proposal to suggest that increased hypoxic sensitivity was responsible for the ventilatory acclimatization to the hypoxia of altitude. They justified this theory by establishing, in the same study, that AHVR rose during exposure to 4 hours of poikilocapnic hypoxia over the same time course during which there was a progressive decline and subsequent stabilization of arterial P_{CO_2} , characteristic of almost complete ventilatory acclimatization [using arterial P_{CO_2} as an index of alveolar ventilation-to- CO_2 production ratio (Forster *et al.*, 1981)]. Ventilatory acclimatization to altitude in man is known to occur over a period of days and thus the current study cannot necessarily draw the same conclusion. However, 4 recent studies in man at altitude (Goldberg *et al.*, 1992; Sato *et al.*, 1992,1994; White *et al.*, 1987) show that AHVR continues to rise steadily over a period of days to almost a fortnight following the initial exposure, thus supporting the view that an enhanced hypoxic ventilatory drive is important, if not primarily responsible, for VAH. This would account for the observation that arterial and CSF pH become increasingly alkaline during the first week of VAH (Dempsey *et al.*, 1974; Forster *et al.*, 1974,1975), implying an increase in ventilatory drive other than from acid-base changes; although CSF pH is probably not an appropriate indicator of the pH at the site of the central chemoreceptors (Dempsey and Forster, 1982; Xu *et al.*, 1991,1992).

The increases in hypoxia-independent ventilation in both hypoxic exposures are indeed suggestive of changes occurring within the CNS, related either to local acid-base mechanisms or to excitability. In the isocapnic protocol, such changes may be partly responsible, in

association with changes in hypoxic sensitivity, for the rise in ventilation described in the previous chapter. However, the nature of these changes is not clear from the current study and, thus, whether they are important in the early stages of VAH is also hard to determine.

Conclusions. The results from this study suggest that prolonged hypoxia *per se* causes an increase in AHVR in man and that this is, at least in part, responsible for the increase in ventilation reported during prolonged isocapnic hypoxia in Chapter 4. Although ventilatory acclimatization to altitude takes place over a period of days in humans, it seems possible that the increased AHVR observed in this study may be characteristic of the very early stages of acclimatization. Longer studies are clearly required.

APPENDIX 5.1.

This appendix gives the estimates for the hypoxic sensitivity term, G_p ($\text{l}\cdot\text{min}^{-1}\cdot\%$), and offset term, $\dot{V}_C$ ($\text{l}\cdot\text{min}^{-1}$), time constant, τ (sec), and pure delay T_d (sec) at 0, 4 and 8 hours from each protocol using both fitting procedures, A and B. Fitting procedure A allowed the dynamic parameters to vary for each test of AVHR and fitting procedure B constrained these parameters to be the same for all the tests from one subject.

Subject	Protocol	Time (hours)	G_P ($\text{l}\cdot\text{min}^{-1}\cdot\%^{-1}$)	$\dot{V}_C$ ($\text{l}\cdot\text{min}^{-1}$)	τ (sec)	T_d (sec)
884	I	0	0.9106	12.257	5.247	3.801
		4	0.8665	24.401	1.502	8.100
		8	1.6180	16.623	24.106	.180
	P	0	0.8125	14.214	.081	6.673
		4	0.8799	22.205	11.363	2.280
		8	0.9223	22.993	9.895	3.540
	C	0	0.8477	16.914	6.781	5.401
		4	0.7480	19.689	7.473	3.725
		8	0.8294	18.566	5.128	6.180
894	I	0	0.9549	12.869	13.974	2.138
		4	1.7840	5.330	30.000	.467
		8	1.8450	21.805	15.735	.840
	P	0	1.3326	12.464	18.255	.000
		4	1.1178	21.453	12.876	2.180
		8	1.3107	24.780	16.690	3.290
	C	0	0.9088	16.590	9.830	3.040
		4	1.6781	18.715	13.435	2.680
		8	1.1820	16.632	9.229	4.036
910	I	0	1.0104	10.821	3.525	6.277
		4	1.5523	15.396	9.222	4.582
		8	1.8126	19.539	6.584	3.880
	P	0	0.8984	9.988	6.216	4.361
		4	1.3575	11.702	9.810	3.255
		8	1.5248	14.525	10.211	3.040
	C	0	0.9003	7.946	4.492	4.933
		4	0.7753	6.955	6.484	4.668
		8	0.5342	6.764	2.184	7.660
927	I	0	0.6953	9.509	8.077	2.581
		4	2.1668	6.124	21.596	.000
		8	3.2817	33.364	23.093	.000
	P	0	0.7748	5.946	8.148	3.901
		4	1.7275	16.926	17.441	.000
		8	1.1398	17.938	9.903	3.701
	C	0	0.5755	6.985	4.917	5.287
		4	0.5102	6.015	3.237	5.540
		8	0.3813	6.370	4.291	5.560
930	I	0	0.2662	8.094	5.132	4.720
		4	0.7774	8.952	13.871	3.020
		8	1.0539	14.590	14.530	2.420
	P	0	0.2918	6.160	12.597	9.114
		4	0.8825	8.032	24.386	.000
		8	1.3861	7.149	25.800	3.800
	C	0	0.4353	12.322	8.787	6.340
		4	0.5027	10.736	7.499	6.194
		8	0.3879	10.093	6.623	6.820

Table 5.5: Estimates for the hypoxic sensitivity term, G_P ($\text{l}\cdot\text{min}^{-1}\cdot\%^{-1}$), offset term, $\dot{V}_C$ ($\text{l}\cdot\text{min}^{-1}$), time constant, τ (sec), and pure delay, T_d (sec) obtained using fitting procedure A for subjects 884 to 930. (I, isocapnic hypoxia; P, poikilocapnic hypoxia; C, control.)

Subject	Protocol	Time (hours)	G_P (l.min ⁻¹ .% ⁻¹)	$\dot{V}_C$ (l.min ⁻¹)	τ (sec)	T_d (sec)
932	I	0	0.3396	16.548	7.240	2.800
		4	0.5137	14.807	14.104	1.580
		8	0.5346	15.584	14.318	1.820
	P	0	0.3471	13.414	9.527	2.500
		4	0.5981	13.963	13.136	.741
		8	0.4718	17.750	15.700	.000
	C	0	0.3734	15.726	6.141	4.533
		4	0.3650	13.687	5.541	4.580
		8	0.5184	13.479	7.495	2.340
934	I	0	0.4921	14.662	1.676	5.800
		4	0.8387	15.636	4.647	5.500
		8	1.7251	17.647	6.151	4.600
	P	0	0.5078	12.020	.183	6.160
		4	1.1643	18.608	4.113	4.880
		8	1.3648	22.982	3.965	5.023
	C	0	0.7318	17.306	3.669	3.561
		4	0.4191	11.161	.396	7.401
		8	0.7047	11.058	6.487	4.020
935	I	0	0.3553	13.032	.966	6.141
		4	0.8082	20.204	2.741	6.820
		8	1.0177	29.854	.104	5.566
	P	0	0.7814	14.960	3.125	3.461
		4	1.0694	18.074	6.168	3.420
		8	0.9253	23.424	.099	5.292
	C	0	0.5148	14.692	2.966	3.880
		4	0.3677	12.670	3.171	5.040
		8	0.4403	11.338	.102	7.330
939	I	0	1.1232	16.706	2.453	4.701
		4	1.3927	20.501	4.804	2.080
		8	1.4627	27.128	5.369	3.060
	P	0	0.9500	16.102	5.084	2.976
		4	1.2117	23.001	3.461	3.826
		8	1.3807	26.473	4.096	3.500
	C	0	0.7538	19.114	7.214	2.655
		4	0.8762	16.928	3.206	4.881
		8	0.8709	17.565	.075	5.764
945	I	0	0.5389	15.296	8.932	4.740
		4	1.1674	23.350	17.195	3.261
		8	0.9151	18.524	13.004	3.200
	P	0	0.5883	13.748	5.417	10.560
		4	1.0077	27.651	8.120	4.460
		8	0.9699	31.584	6.829	5.220
	C	0	0.6498	11.921	3.599	8.760
		4	0.7366	11.431	10.887	4.200
		8	0.5870	11.983	10.264	4.968

Table 5.6: Estimates for the hypoxic sensitivity term, G_P (l.min⁻¹.%⁻¹), offset term, $\dot{V}_C$ (l.min⁻¹), time constant, τ (sec), and pure delay, T_d (sec) obtained using fitting procedure A for subjects 932 to 945. (I, isocapnic hypoxia; P, poikilocapnic hypoxia; C, control.)

Subject	Protocol	Time (hours)	G_p ($l \cdot min^{-1} \cdot \%^{-1}$)	$\dot{V}_C$ ($l \cdot min^{-1}$)	τ (sec)	T_d (sec)
884	I	0	0.9944	11.320	8.540	3.640
		4	1.0018	22.921	8.540	3.640
		8	0.9124	24.512	8.540	3.640
	P	0	0.9215	12.977	8.540	3.640
		4	0.8033	23.089	8.540	3.640
		8	0.8816	23.436	8.540	3.640
	C	0	0.8983	16.386	8.540	3.640
		4	0.7691	19.464	8.540	3.640
		8	0.9126	17.632	8.540	3.640
894	I	0	0.9380	13.039	13.456	2.168
		4	1.1453	12.658	13.456	2.168
		8	1.7074	23.252	13.456	2.168
	P	0	1.1276	14.613	13.456	2.168
		4	1.1543	21.010	13.456	2.168
		8	1.1600	26.440	13.456	2.168
	C	0	1.0199	15.370	13.456	2.168
		4	1.6781	18.696	13.456	2.168
		8	1.3678	14.611	13.456	2.168
910	I	0	1.0947	9.960	6.855	4.600
		4	1.4352	16.725	6.855	4.600
		8	1.8170	19.470	6.855	4.600
	P	0	0.9111	9.845	6.855	4.600
		4	1.2333	13.055	6.855	4.600
		8	1.3656	16.187	6.855	4.600
	C	0	0.9480	7.369	6.855	4.600
		4	0.7869	6.816	6.855	4.600
		8	0.5844	6.255	6.855	4.600
927	I	0	0.7704	8.590	13.497	1.441
		4	1.6922	11.364	13.497	1.441
		8	2.3682	43.237	13.497	1.441
	P	0	0.8779	4.797	13.497	1.441
		4	1.4905	19.506	13.497	1.441
		8	1.2526	16.652	13.497	1.441
	C	0	0.6776	5.811	13.497	1.441
		4	0.5901	5.050	13.497	1.441
		8	0.4075	5.988	13.497	1.441
930	I	0	0.2954	7.796	11.598	4.088
		4	0.7130	9.577	11.598	4.088
		8	0.9569	15.624	11.598	4.088
	P	0	0.2624	6.463	11.598	4.088
		4	0.5831	11.034	11.598	4.088
		8	0.8027	13.298	11.598	4.088
	C	0	0.4692	11.947	11.598	4.088
		4	0.5574	10.144	11.598	4.088
		8	0.4359	9.625	11.598	4.088

Table 5.7: Estimates for the hypoxic sensitivity term, G_p ($l \cdot min^{-1} \cdot \%^{-1}$), offset term, $\dot{V}_C$ ($l \cdot min^{-1}$), time constant, τ (sec), and pure delay, T_d (sec) obtained using fitting procedure B for subjects 884 to 930. (I, isocapnic hypoxia; P, poikilocapnic hypoxia; C, control.)

Subject	Protocol	Time (hours)	G_P ($l \cdot min^{-1} \cdot \%^{-1}$)	$\dot{V}_C$ ($l \cdot min^{-1}$)	τ (sec)	T_d (sec)
932	I	0	0.3793	16.143	11.078	1.745
		4	0.4631	15.325	11.078	1.745
		8	0.4756	16.193	11.078	1.745
	P	0	0.3634	13.260	11.078	1.745
		4	0.5583	14.380	11.078	1.745
		8	0.4017	18.500	11.078	1.745
	C	0	0.4340	15.108	11.078	1.745
		4	0.4307	13.009	11.078	1.745
		8	0.5756	12.881	11.078	1.745
934	I	0	0.5069	14.505	4.113	4.880
		4	0.8359	15.704	4.113	4.880
		8	1.6292	18.634	4.113	4.880
	P	0	0.5287	11.810	4.113	4.880
		4	1.1709	18.580	4.113	4.880
		8	1.3717	22.942	4.113	4.880
	C	0	0.7338	17.297	4.113	4.880
		4	0.4235	11.162	4.113	4.880
		8	0.6671	11.507	4.113	4.880
935	I	0	0.3690	12.871	4.152	3.960
		4	0.8104	20.201	4.152	3.960
		8	1.0669	29.352	4.152	3.960
	P	0	0.7907	14.859	4.152	3.960
		4	1.0142	18.670	4.152	3.960
		8	0.9643	22.973	4.152	3.960
	C	0	0.5252	14.579	4.152	3.960
		4	0.3716	12.613	4.152	3.960
		8	0.4516	11.193	4.152	3.960
939	I	0	1.1566	16.369	3.901	3.919
		4	1.3522	20.969	3.901	3.919
		8	1.4159	27.652	3.901	3.919
	P	0	0.9500	16.102	3.901	3.919
		4	1.2117	23.001	3.901	3.919
		8	1.3728	26.561	3.901	3.919
	C	0	0.6898	19.796	3.901	3.919
		4	0.8883	16.818	3.901	3.919
		8	0.9078	17.200	3.901	3.919
945	I	0	0.5550	15.121	10.069	4.397
		4	0.9171	26.194	10.069	4.397
		8	0.8260	19.503	10.069	4.397
	P	0	0.6328	13.247	10.069	4.397
		4	1.0664	27.004	10.069	4.397
		8	1.0589	30.617	10.069	4.397
	C	0	0.7486	10.852	10.069	4.397
		4	0.7205	11.493	10.069	4.397
		8	0.5806	12.044	10.069	4.397

Table 5.8: Estimates for the hypoxic sensitivity term, G_P ($l \cdot min^{-1} \cdot \%^{-1}$), offset term, $\dot{V}_C$ ($l \cdot min^{-1}$), time constant, τ (sec), and pure delay, T_d (sec) obtained using fitting procedure B for subjects 932 to 945. (I, isocapnic hypoxia; P, poikilocapnic hypoxia; C, control.)

APPENDIX 5.2.

This appendix presents examples of the average calculated arterial saturation profile (Severinghaus, 1979) and ventilatory responses, with the model fit, for the last 3 superimposed hypoxic square waves and the 4 superimposed sawtooth inputs from one subject (930). (Fig. 5.12, isocapnic hypoxia; Fig. 5.13, poikilocapnic hypoxia; Fig. 5.14, control.)

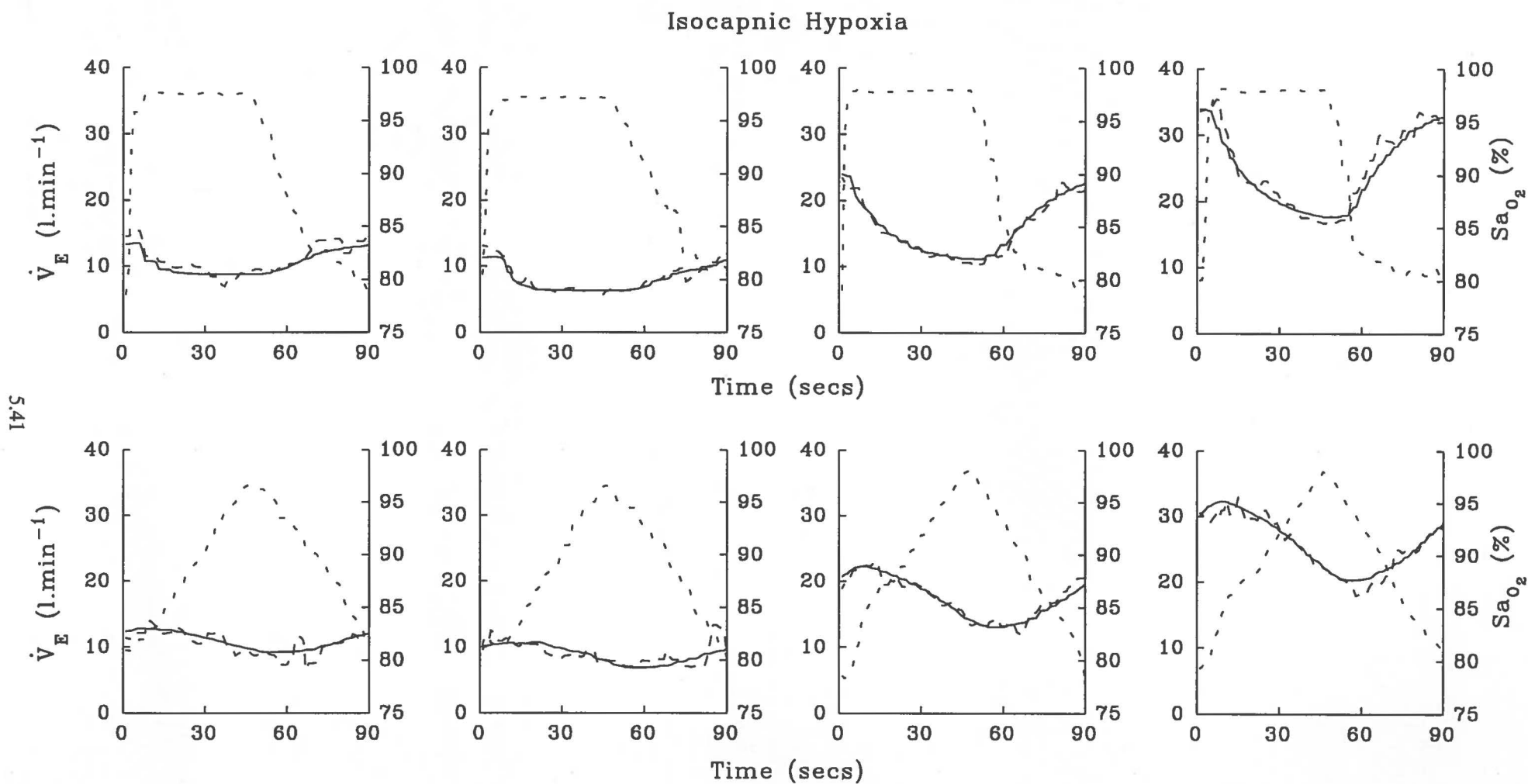
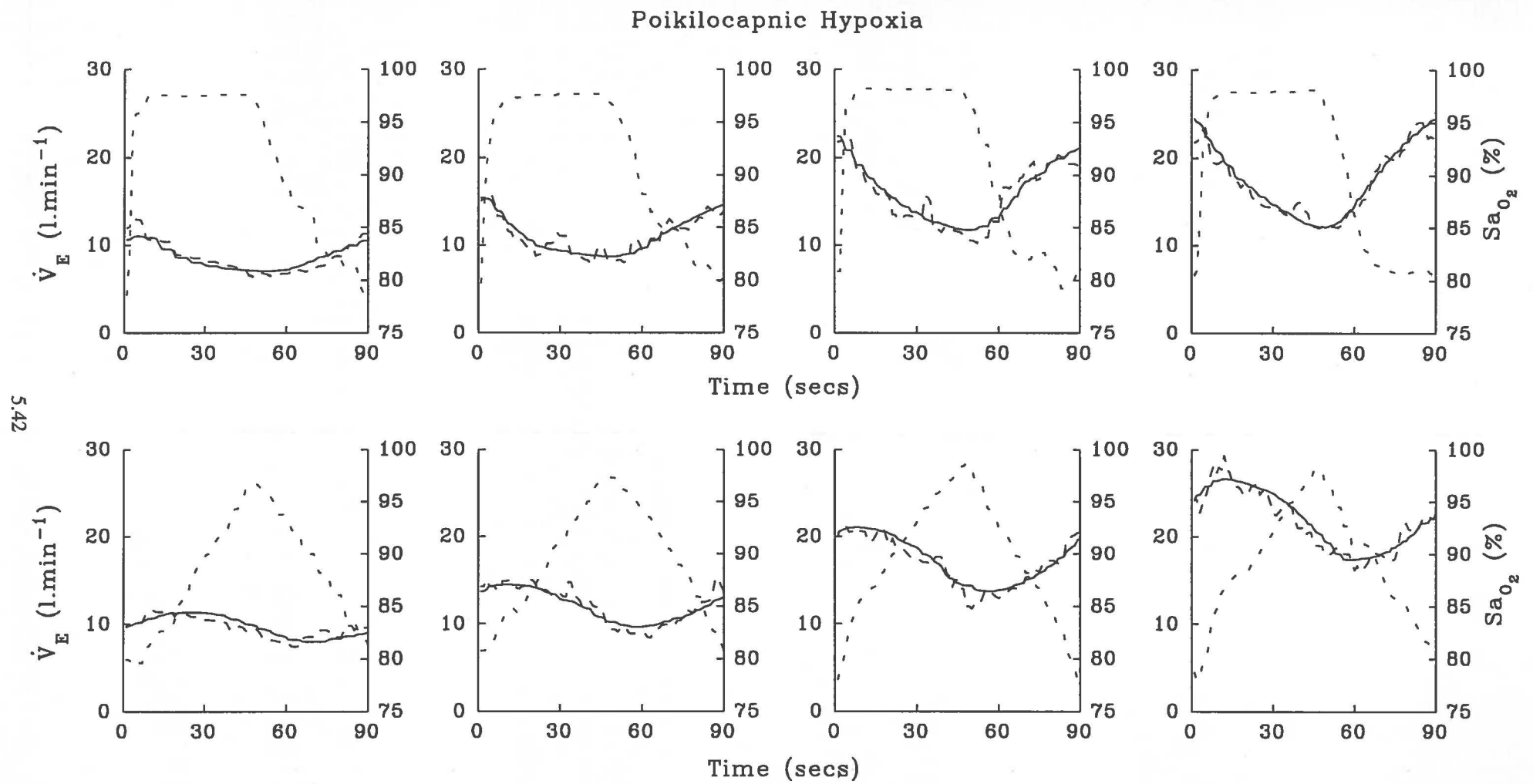


Figure 5.12: Calculated saturation (%), short-dashed line, actual ventilation, $\dot{V}_E$ ($\text{l}\cdot\text{min}^{-1}$), long-dashed line, and model ventilation, solid line, for average of last 3 square waves (top 4 panels) and all 4 sawtooth inputs (lower 4 panels) during a test of AHVR in protocol I, isocapnic hypoxia. From left to right are represented measurements at $t=0$, 20 min, 4 hours and 8 hours. (Subject 930.)



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Figure 5.12: Calculated saturation (%), short-dashed line, actual ventilation, $\dot{V}_E$, ($\text{l}\cdot\text{min}^{-1}$), long-dashed line, and model ventilation, solid line, for average of last 3 square waves (top 4 panels) and all 4 sawtooth inputs (lower 4 panels) during a test of AHVR in protocol I, isocapnic hypoxia. From left to right are represented measurements at t=0, 20 min, 4 hours and 8 hours. (Subject 930.)

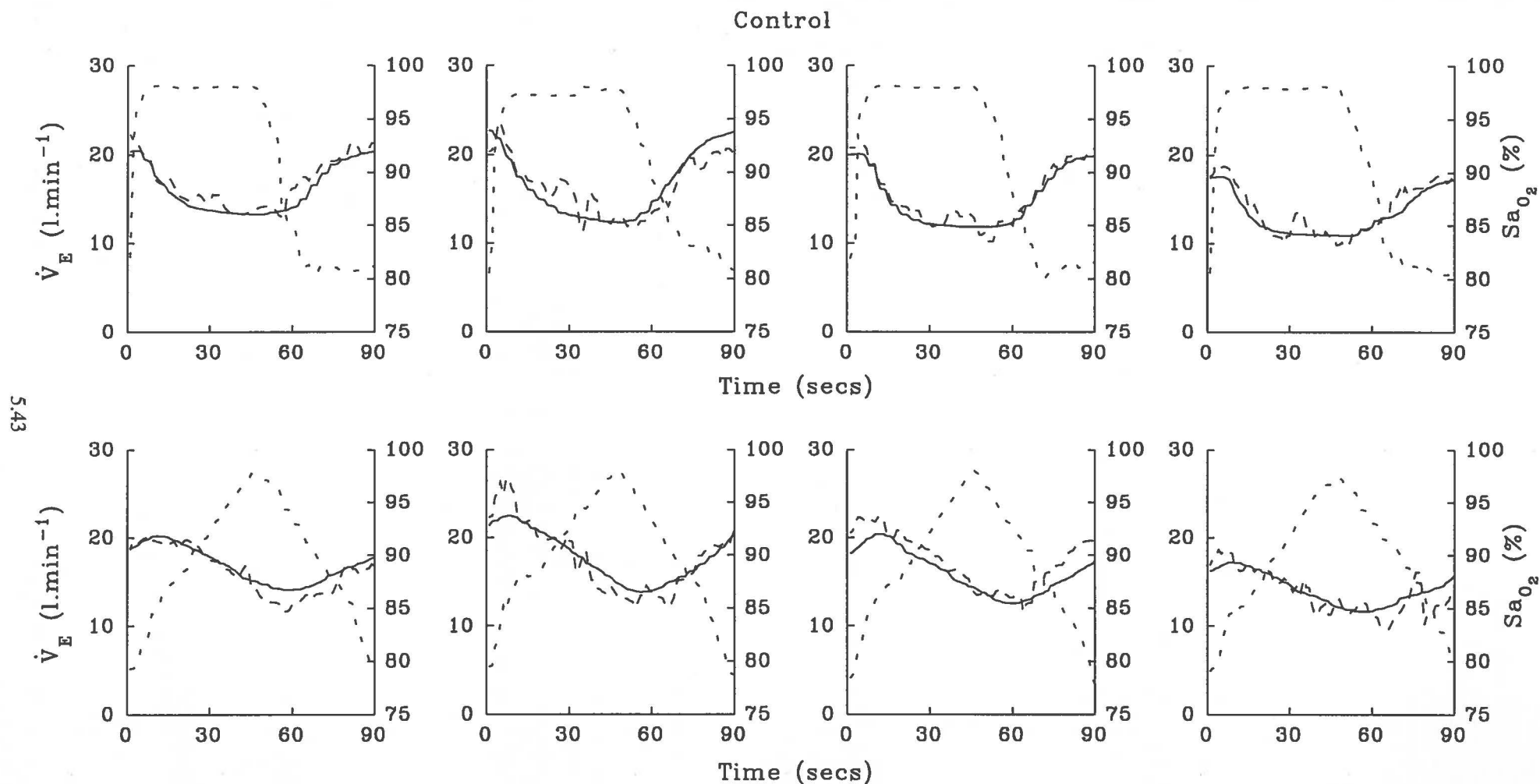


Figure 5.14: Calculated saturation (%), short-dashed line, actual ventilation, $\dot{V}_E$, ($\text{l}\cdot\text{min}^{-1}$), long-dashed line, and model ventilation, solid line, for average of last 3 square waves (top 4 panels) and all 4 sawtooth inputs (lower 4 panels) during a test of AHVR in protocol C, control. From left to right are represented measurements at $t=0$, 20 min, 4 hours and 8 hours. (Subject 930.)

CHAPTER 6

THE EFFECTS OF 48 HOURS OF HYPOXIA

Is it finished yet?

Subjects 884, 910, 964, 969, 970.

INTRODUCTION

In the studies described in Chapters 4 and 5, the changes in ventilation and respiratory control were examined during 8 hours of isocapnic and poikilocapnic hypoxia. An increase in ventilatory sensitivity to hypoxia was thought to be partly responsible for a progressive increase in ventilation observed during isocapnic hypoxia, in addition to a possible rise in hypoxia-independent ventilation. It was further proposed that this change in respiratory control may be involved in the early stages of ventilatory acclimatization to the hypoxia of altitude. However, since no real change in ventilation was seen in poikilocapnic exposure (pseudoaltitude) and, further, acclimatization in man is known to take place over days to weeks, it is hard to justify this statement with 8 hours of data. It was therefore decided to extend the protocol to 48 hours. In addition, since many researchers have studied the process of deacclimatization from altitude in order to elucidate the mechanisms responsible for the acclimatization process, the 48 hours following exposure were also investigated.

Unlike the previous study, progressive changes in arterial pH and P_{CO_2} would be expected in the poikilocapnic state over the 48-hour exposure as a result of renal excretion of HCO_3^- (Forster *et al.*, 1975) and thus the changes in the acid-base variables of the blood and the ventilatory response to CO_2 were also monitored. The previous study highlighted two aspects of the protocol which could be improved. These were: 1) the use of a longer period hypoxic square wave in the measurement of the acute hypoxic ventilatory response and 2) the measurement of ventilation in air at various time points during the exposure. These changes were incorporated into the current protocol and employed in both the acclimatization and deacclimatization phases.

Because of the length of the protocol, it was decided that only a pilot study could be undertaken as part of this thesis. The first subject (969) studied was used purely to sort out details of the protocol. This was invaluable, although it does mean that the results obtained from this subject cannot be compared with the later subjects and thus will not be considered

further. Four further subjects were studied. Although this limits the statistical analysis and significance that can be attributed to the results, responses were in general consistent and I feel the data are worth presenting. This study provides valuable pilot data for a more definite investigation to be undertaken.

METHODS

Subjects. Five subjects (4 males, 1 female) aged between 20 and 25 were studied. Their individual characteristics are listed in Table 6.1. All were healthy, and none gave a history of respiratory symptoms or illnesses. All were non-smokers except for one subject (970) who smoked *ca.* 10 cigarettes per day. He stopped smoking one week prior to experimentation.

Subject	Sex	Height (m)	Weight (kg)	Age (years)
884	M	1.87	75	25
910	F	1.70	64	22
964	M	1.80	72	23
969	M	1.80	73	21
970	M	1.82	80	20

Table 6.1: Physical Characteristics of Subjects.

Hypoxic exposure. The hypoxic exposure was imposed using the purpose-built chamber described in Chapter 3. Subjects were exposed to two protocols, the hypoxic exposure associated with each lasting 48 hours, in random order with one protocol separated by at least 5 days from the other. The exposures were: isocapnic hypoxia, where end-tidal PO_2 (PET_{O_2}) was held at 60 mmHg and end-tidal PCO_2 (PET_{CO_2}) was held at the subject's resting value; and poikilocapnic hypoxia, where PET_{O_2} was held at 60 mmHg and PET_{CO_2} was uncontrolled. However, in subject 964, PET_{O_2} was held at 55 mmHg in both protocols. Since progesterone is known to affect resting PET_{CO_2} (Lyons and Huang, 1968; Skatrud *et al.*, 1978), in the one female subject studied (910), both experiments were performed during the first two weeks of the menstrual cycle so that levels of progesterone would be similar between protocols. In addition, she was not using the contraceptive pill.

Post-hypoxic "exposure". Subjects were removed from the chamber after 48 hours and then allowed to carry out their normal daily procedures. However, they were requested to refrain from excessive alcohol consumption and late nights for the following 48 hours while still under observation.

Acute mountain sickness. The subjects were given a questionnaire to answer every 4 hours while awake to record the absence or severity of 5 symptoms: anorexia, nausea, headache, lethargy and subcutaneous oedema. The subjects could grade the degree of severity of each symptom from zero to two (0, absent; 1, mild; 2, severe). An example of an acute mountain sickness score sheet is given in Appendix 6.1.

Blood measurements. Blood was taken from each subject at 0, 4, 8, 12, 24, 36 and 48 hours during both the hypoxic exposure and the deacclimatization period. Venous samples were taken either from the cubital fossa or from the dorsum of the hand. During the hypoxic exposure, samples were taken while the subject was seated inside the chamber and during the deacclimatization period, samples were taken following 20 min rest with the subject seated in the laboratory. Values for venous pH, O₂ saturation, haemoglobin concentration, [HCO₃⁻] and standard [HCO₃⁻] were obtained from the blood-gas analyzer and CO-oximeter (described in Chapter 2). [Standard [HCO₃⁻] is the concentration of bicarbonate that would exist in a sample of blood if it were equilibrated to 40 mmHg Pco₂ at 37°C.] Simultaneous readings of the end-tidal gases were recorded during the drawing of venous blood. Thus arterial pH and [HCO₃⁻] could be calculated (Appendix 6.2).

Ventilation. Ventilation during the hypoxic exposure was measured over a 3-min period half an hour into the exposure and then at two-hourly intervals for the remainder of the 48-hour protocol (except during sleep). The last 30 sec of this period was used to calculate the ventilation for that time point. Measurements of ventilation made inside the chamber were collected with the subject breathing through a mouthpiece assembly fixed in series with a turbine volume measuring device and a pneumotachograph, with the nose occluded. The

equipment listed above is identical to the data acquisition apparatus described in Chapter 2 with one exception: the whole assembly was open to the gas in the chamber. Respiratory volumes, flows and durations were obtained as with the data acquisition system.

Assessment of respiratory control. In order to assess changes in the respiratory chemoreflex sensitivities, it was decided to obtain measures of the acute hypoxic ventilatory response (AHVR), the acute hypercapnic ventilatory response (AHCVR) and the eupnoeic ventilation (i.e., while breathing air) ($\dot{V}_{E100}$). AHVR and $\dot{V}_{E100}$ were measured following each venous blood sample (i.e., 0, 4, 8, 12, 24, 36, 48, 52, 56, 60, 72, 84 and 96 hours after the start of experimentation). AHCVR was measured at the start of each day (i.e., 0, 24, 48, 72 and 96 hours). These measurements were all made using the fast gas-mixing system described in Chapter 2.

Chapter 5 described how the fast gas-mixing system could be used to generate square waves of end-tidal P_{O_2} with the end-tidal P_{CO_2} held constant. The same procedure was used to test AHVR in this study; however, the pattern of end-tidal gases involved in the test was different. The protocol began with 3 min of breathing air in order to incorporate a measure of $\dot{V}_{E100}$. Subsequently, P_{ETO_2} was held constant at 100 mmHg for 3 min at an appropriate P_{ETCO_2} (see below) followed by 6 square waves of 2-min period from 50 to 100 mmHg at the same P_{ETCO_2} .

At each time point defined above, the measurement of AHVR was performed with P_{ETCO_2} held at 2 mmHg above the subject's resting value before the start of experimentation (' P_{CO_2} -matched' measurements of AHVR). In addition to these, tests were also carried out at an appropriate P_{ETCO_2} that would elicit the same arterial pH as the first test of AHVR ($t=0$ hours), calculated as in Appendix 6.3. These tests were termed 'pH-matched' and were performed at 24, 48, 72 and 96 hours. The level of P_{ETCO_2} was calculated using data from the simultaneous venous blood samples and end-tidal gases (Appendix 6.4). The changes in end-tidal P_{O_2} employed to measure AHVR are summarized in Fig. 6.1.

In order to characterize any changes in AHVR, the data from each measurement were fit by a first-order model as defined by Clement and Robbins (1993). Values for the ventilatory sensitivity to rapid changes in hypoxia [G_p ($\text{l}\cdot\text{min}^{-1}\cdot\%^{-1}$)], the ventilation insensitive to rapid changes in hypoxia, [$\dot{V}_C$ ($\text{l}\cdot\text{min}^{-1}$)] and the dynamic parameters of the ventilatory response [pure delay, T_d , and time constant, τ] were estimated using this model. This procedure is described in Chapter 5.

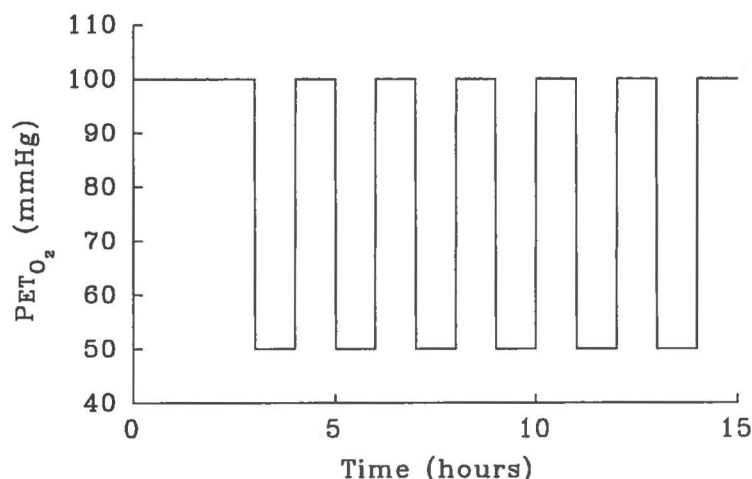


Figure 6.1: Desired profile for PET_{O₂} during measurement of AHVR.

The ventilatory response to hypercapnia was assessed by measuring steady-state ventilation at two levels of PET_{CO₂}. The two levels of PET_{CO₂} were estimated by the experimenter in order to elicit a ventilatory response in the same range as the previous recording and were always carried out as a continuation of a test of pH-matched AHVR. The lower level of PET_{CO₂} was given 5 min and the higher level was held for 8 min in order to allow time to reach a steady state in ventilation. The final minute at each level was taken as the resultant ventilation at that P_{CO₂}.

Statistical analysis. An analysis of variance similar to that described in Chapters 4 and 5 was used to investigate changes in the parameters measured. Each analysis was based on the same linear model for variance:

$$X_{ijk} = \mu + S_i + T_j + P_k + (ST)_{ij} + (SP)_{ik} + (TP)_{jk} + (STP)_{ijk} + e_{ijk}$$

where X is the parameter being analyzed (e.g., $\dot{V}_E$, P_{CO_2} , pH etc.) for the i th subject (S_i), at the j th time of measurement (T_j) and for the k th protocol (P_k) and e_{ijk} is an error term. Each term was tested for its contribution to the total variance of X with a variance-ratio (F-ratio) test. The F-ratio for a term was calculated by dividing the MSq for that term by the MSq for the interaction between that term and the subject term. This analysis was performed using Minitab statistical software.

RESULTS

Apart from periods spent sleeping, subjects spent most of their time in the chamber either reading, watching television or videos, playing computer games or listening to the radio. Subjects expressed some discomfort towards the end of the 48-hour isocapnic hypoxic exposure as a result of the high levels of resting ventilation (up to 30 l.min⁻¹). In particular, discomfort was expressed during the tests of the acute hypoxic and hypercapnic ventilatory responses during the middle 48 hours of the total experiment as levels of maximum ventilation reached *ca.* 80 l.min⁻¹. During sleep, periodic breathing was only observed in one subject (884) during the poikilocapnic exposure: this is illustrated in Fig. 3.9 (p. 3.21).

End-tidal gases. Five-min averages of the end-tidal gases and arterial oxygen saturation recorded in each subject were calculated and then averaged across subjects in two-hourly sectors: these are presented in Fig. 6.2. This plot illustrates the quality of the control of the $P_{ET}O_2$ (held at 60.07 ± 0.13 mmHg during the isocapnic exposure and 60.43 ± 0.16 mmHg during the poikilocapnic exposure; mean $\pm$ S.E.). End-tidal P_{CO_2} appears to be less well-controlled in the early isocapnic exposure, in particular when compared with the previous study: this may be a result of a lower hypoxic drive. During the isocapnic exposure, $P_{ET}CO_2$ was held at 38.72 ± 0.14 mmHg throughout. In the poikilocapnic state, $P_{ET}CO_2$ averaged 36.72 ± 0.25 mmHg during the first day and rose to 39.00 mmHg in the night. The following day, $P_{ET}CO_2$ averaged 34.07 ± 0.43 mmHg and peaked at 36.91 mmHg in the second night. The mean $P_{ET}CO_2$ after waking on the third morning was 31.89 mmHg. The arterial oxygen saturation was held at 91.12 ± 0.13 % during the isocapnic exposure and at 90.84 ± 0.13 % during the poikilocapnic state outside a 6-hour period during the latter half of the first night, where it fell by *ca.* 2% despite maintained $P_{ET}O_2$.

Acid-base parameters. Figure 6.3 presents the calculated arterial acid-base data recorded over the 96-hour protocol from the venous blood samples. The data from each subject are

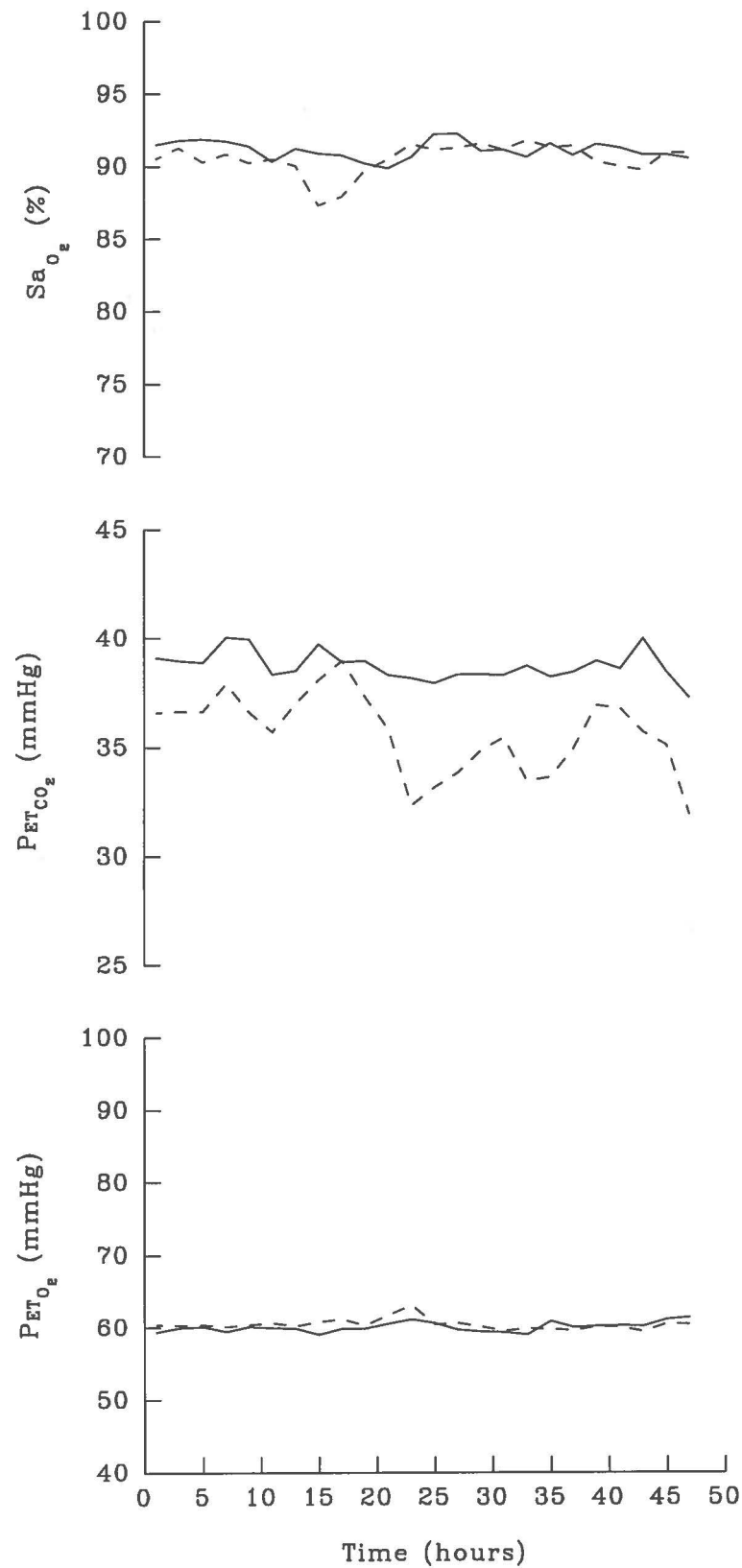


Figure 6.2: Two-hour averages of the 5-min averages of the end-tidal gases (P_{ETO_2} and P_{ETCO_2}) and O_2 saturation (Sa_{O_2}) averaged over all subjects. Solid line, isocapnic hypoxia; broken line, poikilocapnic hypoxia. Note the difference in P_{ETCO_2} between the two protocols.

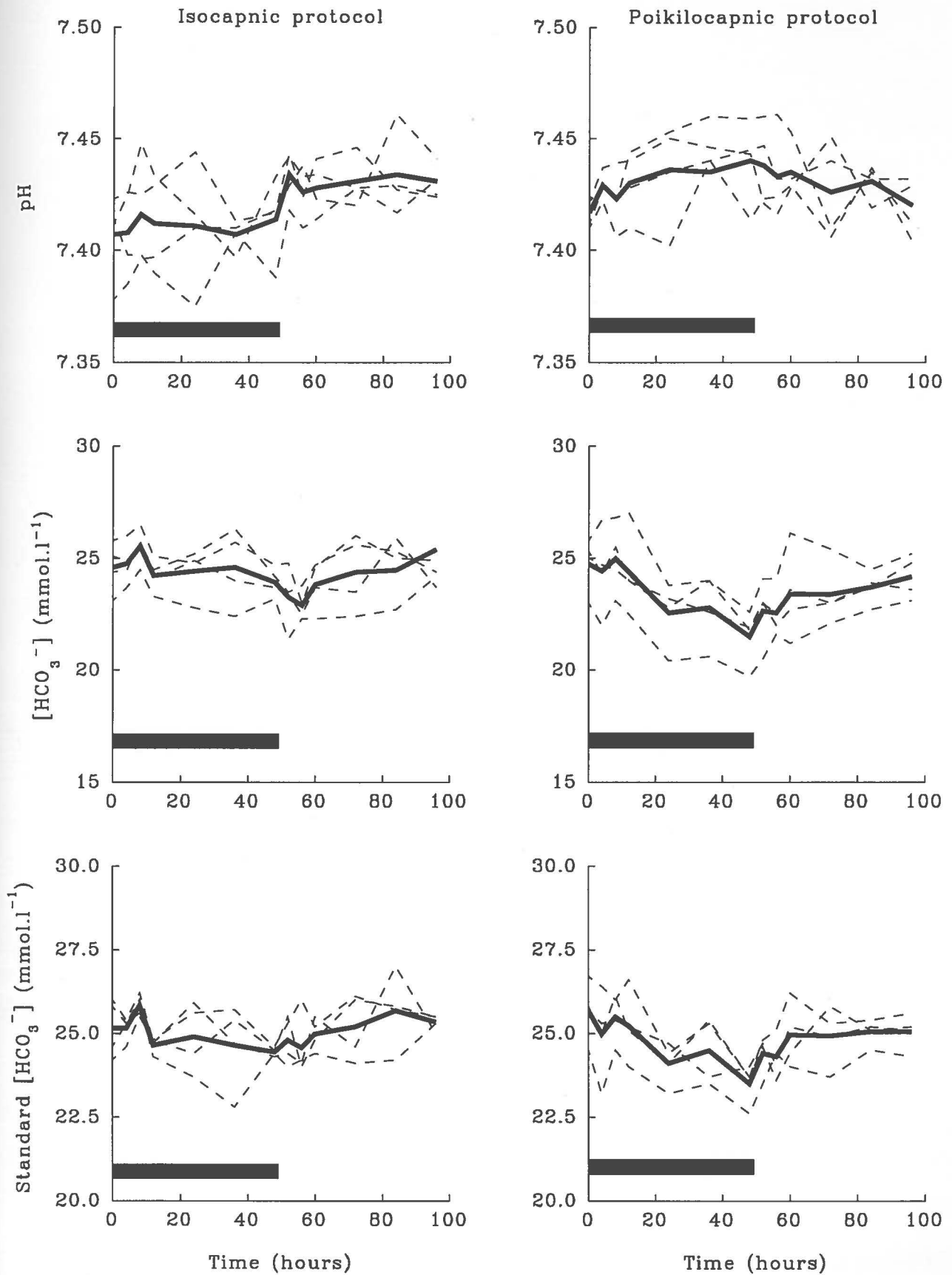


Figure 6.3: Calculated arterial pH, $[\text{HCO}_3^-]$ and standard $[\text{HCO}_3^-]$. (Individual subject data, dashed lines; mean data, solid lines.) Solid bar indicates hypoxic exposure.

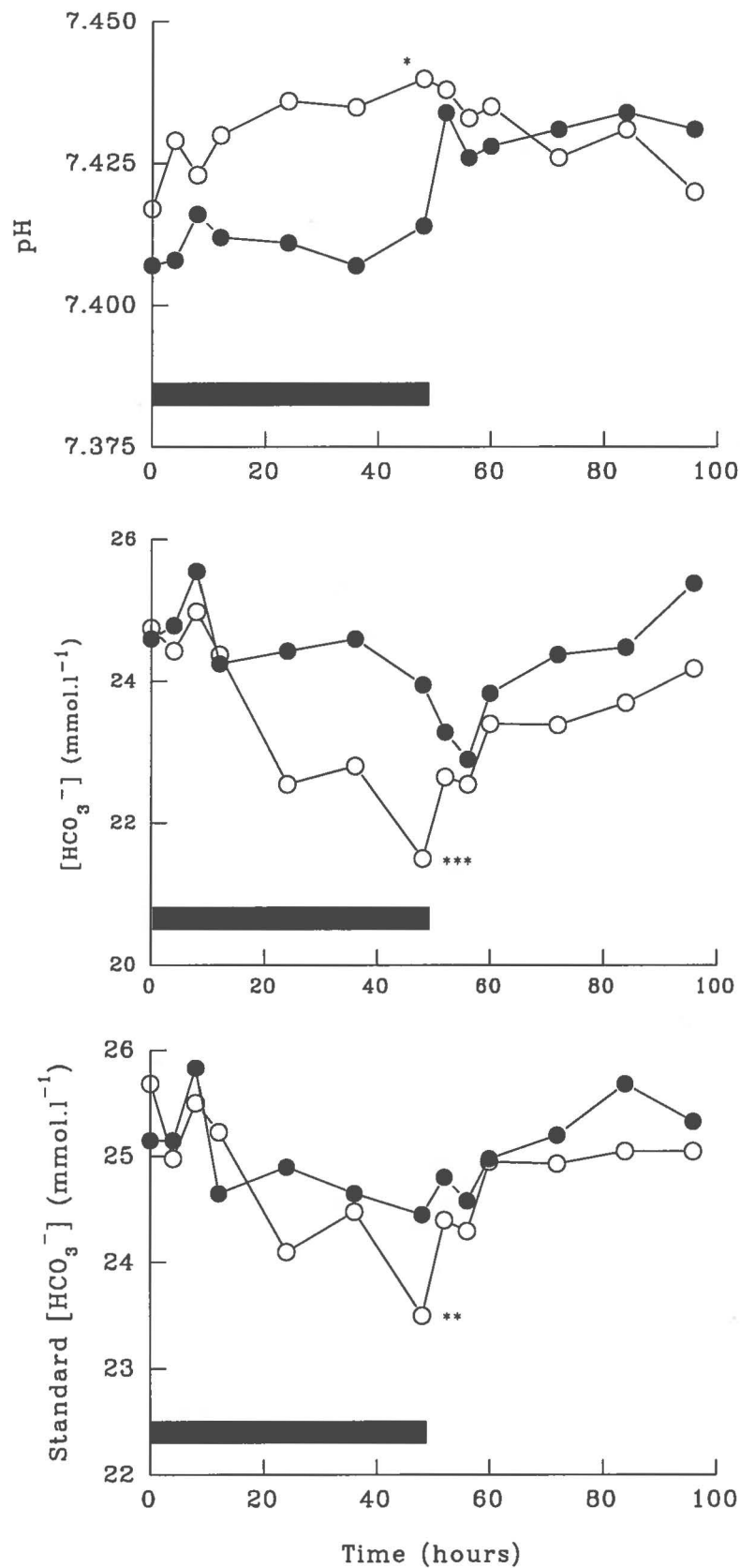


Figure 6.4: Mean calculated arterial pH, $[HCO_3^-]$ and standard $[HCO_3^-]$. (Isocapnic protocol, filled circles; poikilocapnic protocol; hollow circles.) *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.001$ that value is different compared with value at $t = 0$. Solid bar indicates hypoxic exposure.

represented by the dashed lines and the mean data are represented by the solid lines. Figure 6.4 shows the mean data for both protocols superimposed on the same plots (pH, upper panel; bicarbonate concentration, middle panel; standard bicarbonate concentration, lower panel). After 48 hours of isocapnic hypoxia, pH remained unchanged (7.407 ± 0.010 at $t=0$ hours (mean $\pm$ S.E.); 7.414 ± 0.010 at $t=48$ hours; $p=\text{NS}$, paired t -test); however, pH was elevated following the poikilocapnic exposure (7.417 ± 0.003 , $t=0$ hours; 7.440 ± 0.009 , $t=48$ hours; $p<0.05$). The arterial bicarbonate concentration fell significantly during poikilocapnic hypoxia (from 24.78 ± 0.61 mM, $t=0$ hours, to 21.50 ± 0.63 mM, $t=48$ hours; $p<0.001$), but not during isocapnic hypoxia (24.60 ± 0.58 mM at $t=0$ hours; 23.95 ± 0.32 mM at $t=48$ hours). The standard bicarbonate concentration demonstrated a similar trend, falling significantly from 25.65 ± 0.45 mM to 23.50 ± 0.31 mM ($p<0.005$) during poikilocapnic hypoxia, but not during isocapnic hypoxia (25.15 ± 0.44 mM, $t=0$ hours; 24.45 ± 0.06 mM, $t=48$ hours).

Acute mountain sickness. Subjects reported symptoms of illness in both protocols, but these were more severe during isocapnic hypoxia. The maximum symptom score was 45 per person, thus total scores are out of 180. Isocapnic hypoxia produced a total sickness rating of 36 and poikilocapnic hypoxia, 22. These differences were not significant when analysed with a paired t -test. In addition, there were no differences in presenting symptoms between protocol types.

Ventilation. The ventilatory responses of each subject (dashed lines) and the mean ventilatory response of all the subjects combined (solid lines) for the 48-hour duration of the exposure are presented in Fig. 6.5. Figure 6.6 shows the mean responses superimposed on the same plot. Ventilation rose steadily from 11.77 ± 1.10 l.min⁻¹ to 29.08 ± 3.26 l.min⁻¹ over the course of the isocapnic exposure and from 9.88 ± 0.68 l.min⁻¹ to 13.36 ± 1.22 l.min⁻¹ in the poikilocapnic exposure. Moreover, ventilation rose significantly over time in the isocapnic exposure when compared with the poikilocapnic exposure ($p<0.001$, ANOVA).

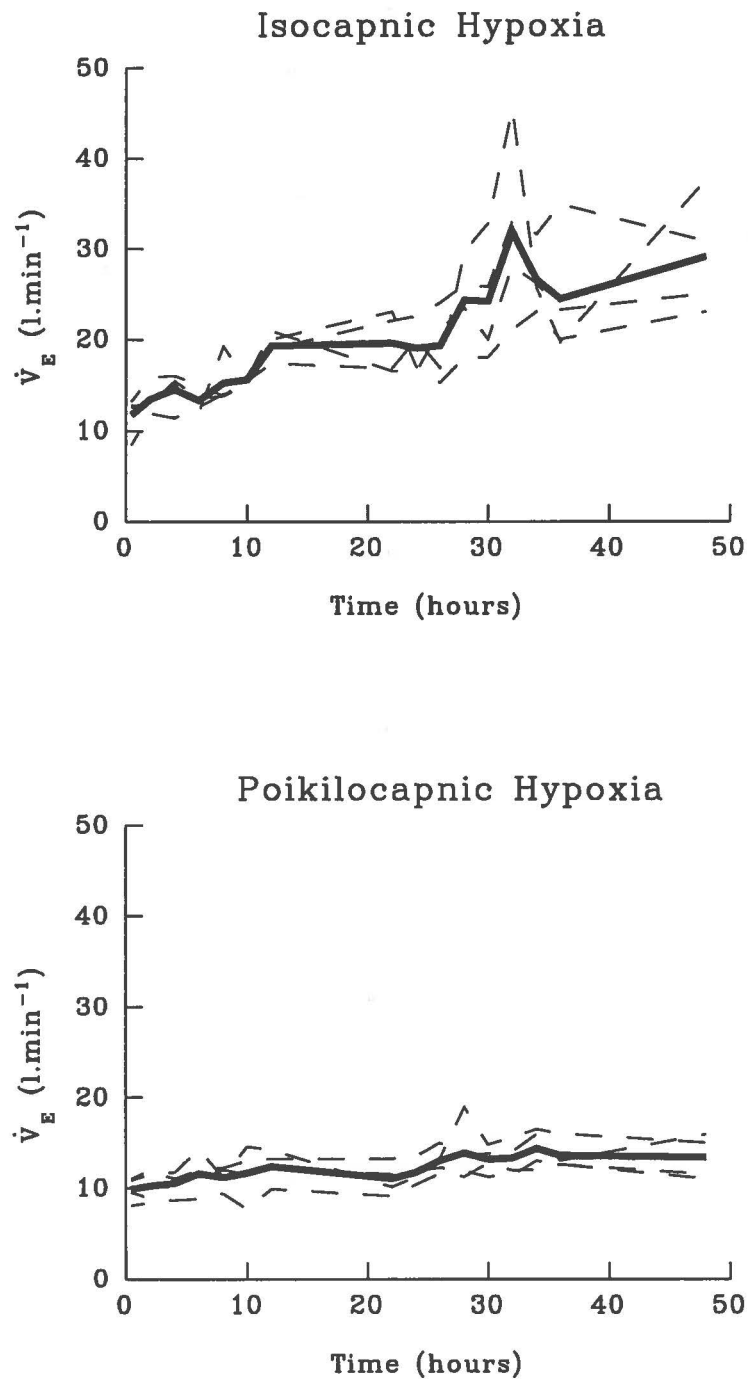


Figure 6.5: Ventilatory responses of each subject (dashed lines) and the mean response of all the subjects combined (solid lines). (Upper panel, isocapnic hypoxia; lower panel, poikilocapnic hypoxia.)

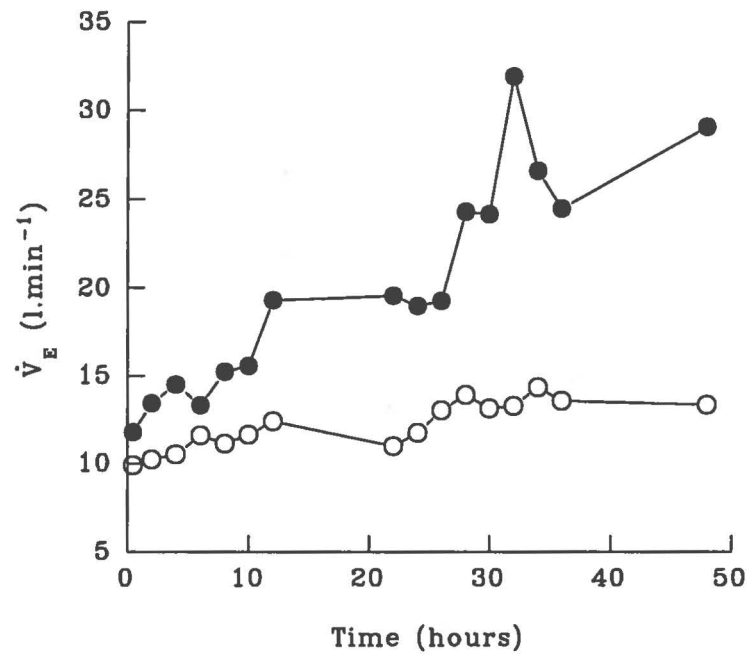


Figure 6.6: The mean ventilatory responses of all subjects during isocapnic hypoxia (filled circles) and poikilocapnic hypoxia (hollow circles).

Source of Variance	Degrees of Freedom	Sum of squares ($\text{l}\cdot\text{min}^{-1}$) ²	Mean Squared Error ($\text{l}\cdot\text{min}^{-1}$) ²	F-ratio	P value
S_i	3	297.6	99.2	---	---
T_j	15	1594.2	106.3	11.84	<0.001
P_k	1	1997.8	1997.8	62.92	<0.005
ST_{ij}	45	403.8	9.0	---	---
SP_{ik}	3	95.3	31.8	---	---
TP_{jk}	15	738.4	49.2	5.30	<0.001
STP_{ijk}	45	417.7	9.3	---	---
Total	127	5544.7	---		

Table 6.2: Statistical analysis of the linear model of ventilation using analysis of variance.

Table 6.2 summarizes the results of the analysis of variance for the model of ventilation. Table 6.5 in Appendix 6.5 presents the ventilatory data collected from each experiment.

Eupnoeic ventilation and P_{CO_2} . The eupnoeic ventilation ($\dot{V}_{E100}$) and P_{CO_2} ($P_{CO_{2100}}$) are shown in Fig. 6.7 (individual subjects, dashed lines; mean data, solid lines). The mean responses for both protocols are shown on the same plot in Fig. 6.8. $\dot{V}_{E100}$ appears to rise during both types of hypoxic exposure and tail off following the exposure. The plots are hard to interpret because of the large oscillations; however, the P_{CO_2} data are less affected. During isocapnic hypoxia, $P_{CO_{2100}}$ fell significantly from 37.89 ± 1.04 mmHg (mean $\pm$ S.E.) to 32.94 ± 0.60 mmHg ($p < 0.01$; paired t-test) and during poikilocapnic hypoxia, $P_{CO_{2100}}$ fell from 36.03 ± 0.59 mmHg to 32.40 ± 1.24 mmHg ($p < 0.025$). Since end-tidal P_{CO_2} can be used as an index of ventilation, assuming that the metabolic rate is constant and that there is no change in dead space (Forster *et al.*, 1981), the changes in $P_{CO_{2100}}$ can be related to an increase in eupnoeic ventilation during both exposures and a subsequent fall on relief of hypoxia. The changes in $P_{CO_{2100}}$ would suggest similar changes in ventilation between both protocols. ANOVA showed no significant differences between the two protocols for either $\dot{V}_{E100}$ or $P_{CO_{2100}}$. Figure 6.9 (lower panel) shows the differences between the isocapnic and poikilocapnic exposures for $\dot{V}_{E100}$ and $P_{CO_{2100}}$ at each time point and for each subject. The upper panel of the figure presents the mean paired difference for each time point and the standard error, multiplied by a factor of two (dashed lines), for each mean difference. Most of the points lie within the limits set up by the double standard error: this suggests that the isocapnic and poikilocapnic exposures produce similar data distributions. Table 6.6 in Appendix 6.6 presents the ventilatory and P_{CO_2} data collected for each measure of eupnoeic ventilation.

Acute hypoxic ventilatory response. The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of P_{CO_2} -matched AHVR are presented in Fig. 6.10 (isocapnic hypoxia, 0-48 hours), Fig. 6.11

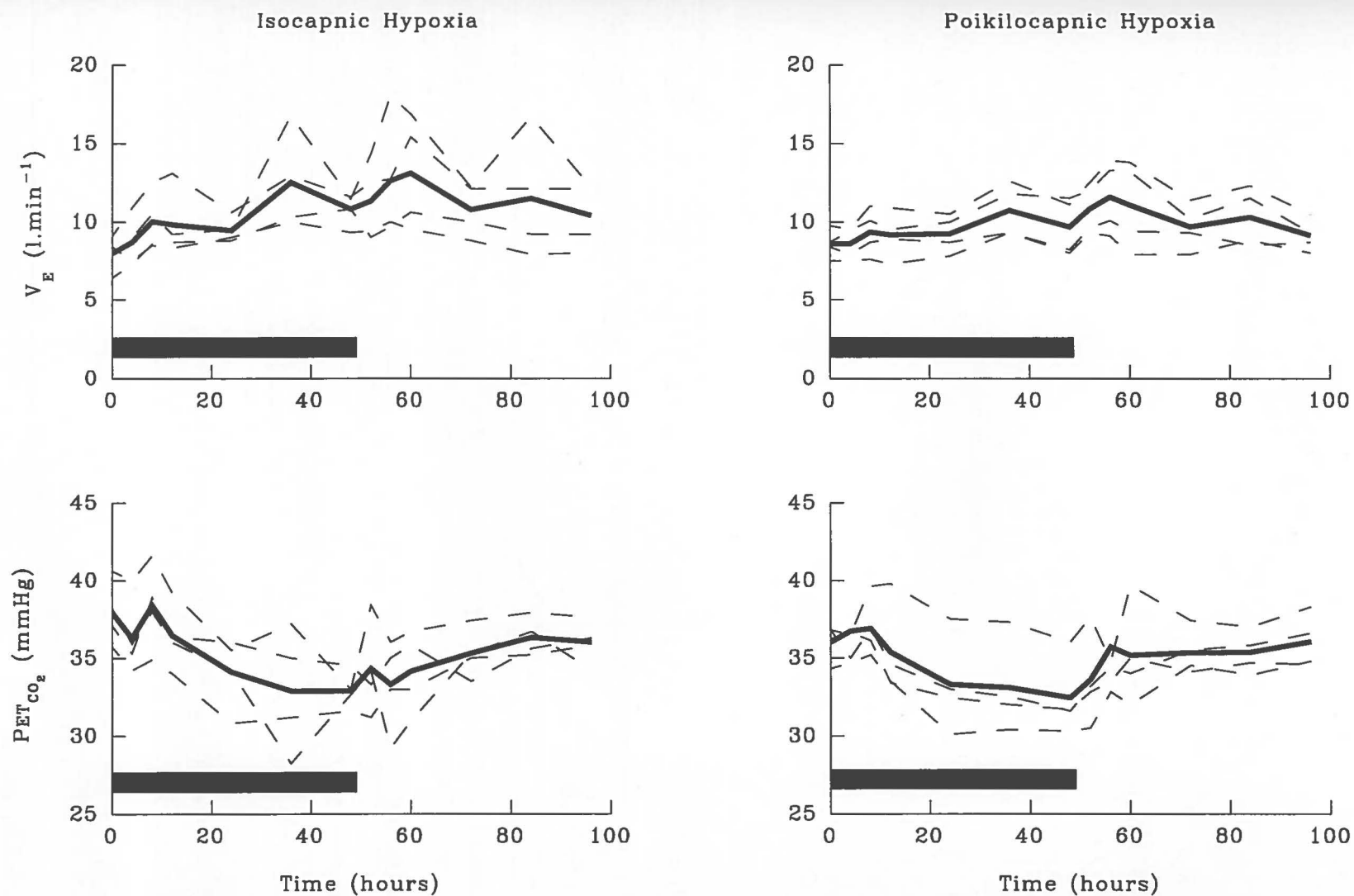


Figure 6.7: Eupnoeic ventilation (V_E) and PCO_2 measured in both protocols - left panels, isocapnic protocol; right panels, poikilocapnic protocol. (Dashed lines, individual subjects; solid lines, mean response.) Solid bar indicates period of hypoxia.

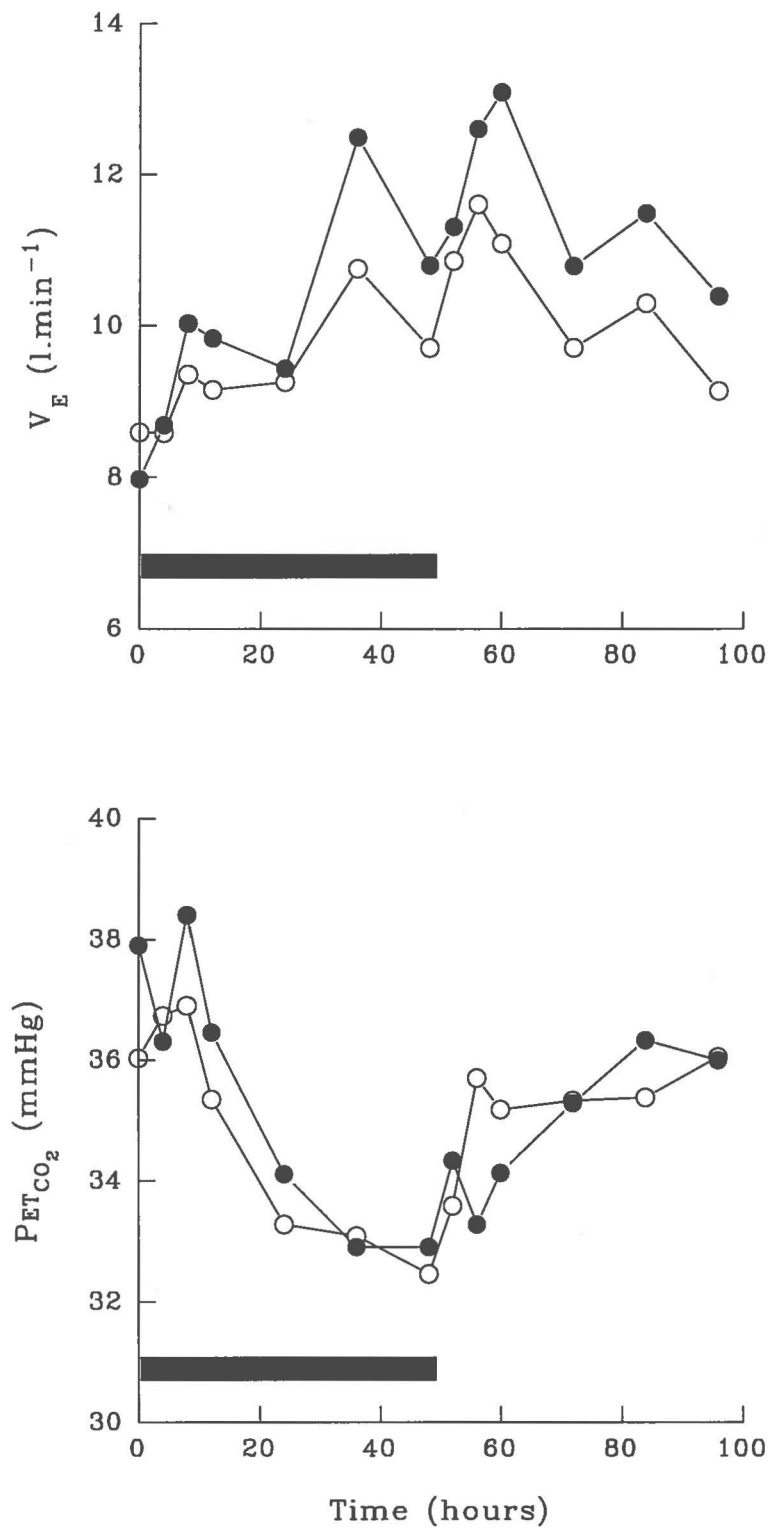


Figure 6.8: Means of the eupnoeic ventilation (V_E) and associated PCO_2 . (Filled circles, isocapnic protocol; hollow circles, poikilocapnic protocol.) Solid bar indicates hypoxic exposure.

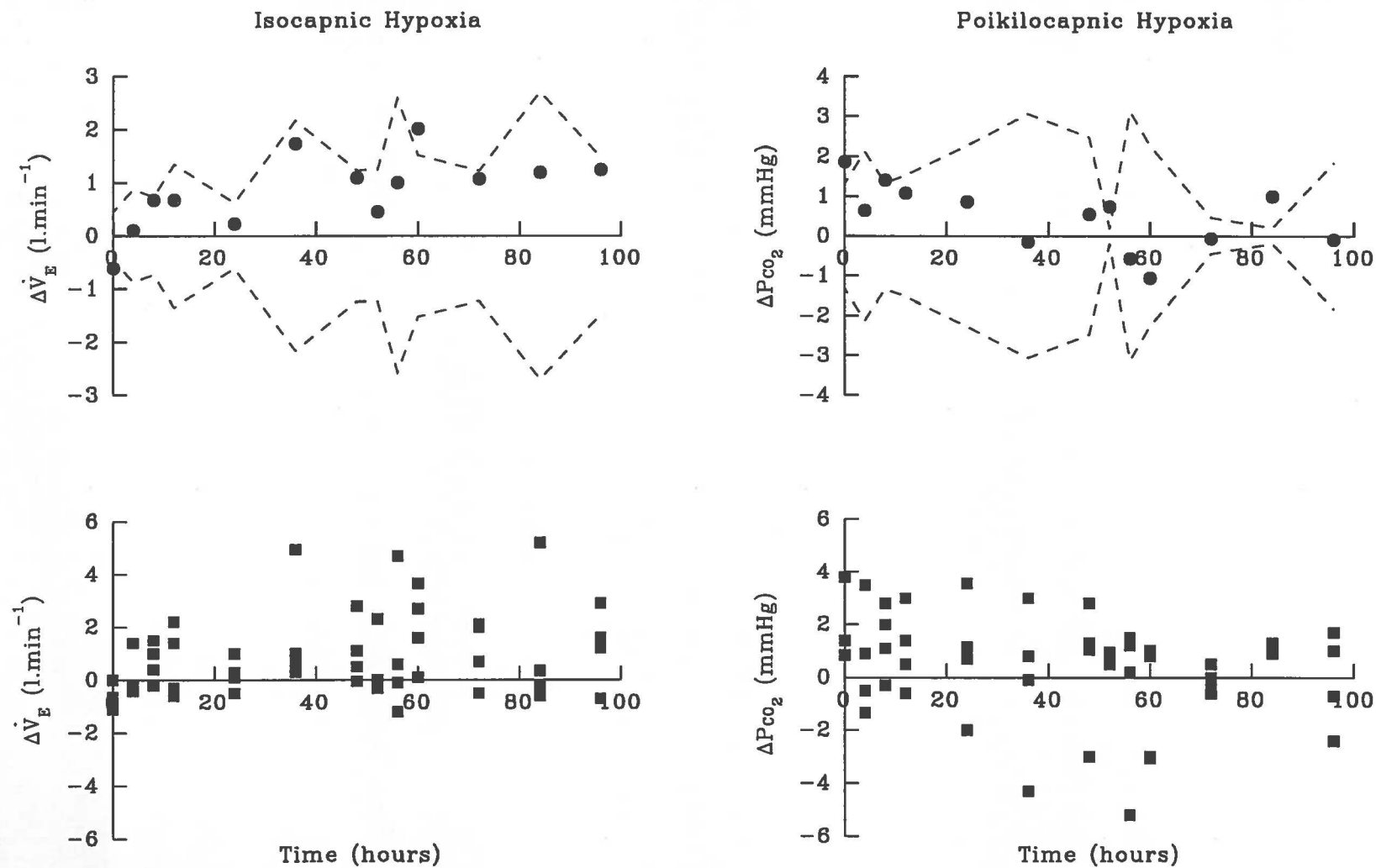


Figure 6.9: Lower panels - differences between isocapnic and poikilocapnic protocols for eupnoeic ventilation, $\dot{V}_E$ (left) and PCO_2 (right) for each subject. Upper panels - mean paired difference for each time point and the standard error multiplied by a factor of two, for each mean difference (dashed lines).

(post-isocapnic hypoxia, 48-96 hours), Fig. 6.12 (poikilocapnic hypoxia, 0-48 hours), Fig. 6.13 (post-poikilocapnic hypoxia, 48-96 hours). The impressions gained from these figures suggest an increase in both the hypoxic sensitivity of the ventilatory response and the ventilation insensitive to rapid changes in hypoxia (hypoxia-independent ventilation or baseline), during the both types of hypoxic exposure. In addition, these increases appear to be almost complete by 36 hours. However, since ventilation fails to settle during the euoxic step of the square wave, changes in the baseline are difficult to interpret. During the period of recovery from hypoxia, the changes observed during hypoxia are reversed (i.e., hypoxic sensitivity and ventilation baseline fall). However, these changes appear to take place more slowly than during the exposure and do not return to pre-exposure levels.

The pH-matched AHVR measurements were manipulated in a manner analogous to the P_{CO_2} -matched tests and are presented in Fig. 6.14 (isocapnic hypoxia, 0-48 hours), Fig. 6.15 (isocapnic hypoxia, 48-96 hours), Fig. 6.16 (poikilocapnic hypoxia, 0-48 hours), Fig. 6.17 (poikilocapnic hypoxia, 48-96 hours). The impressions gained from these figures are similar to those expressed above. However, on observation, there appear to be some differences in the changes in the ventilation baseline between the two types of exposure, despite pH being matched. In the isocapnic exposure, the ventilatory baseline, despite rising less than in the P_{CO_2} -matched tests, increased considerably more than in the poikilocapnic exposure.

The dynamic model was fit to individual AHVR tests and estimates for hypoxic sensitivity, G_p , ventilation baseline, $\dot{V}_C$, pure delay, T_d , and time constant, τ , obtained (procedure A). Since the fitted values for the sensitivity and baseline terms are dependent upon the fitted values of the dynamic parameters, it was decided, as in the previous chapter, to employ a separate fitting procedure for each subject whereby T_d and τ were constrained to take just one value for all 17 data sets from one 96-hour experiment (procedure B). The estimates for all 4 parameters using fitting procedure A is given in Tables 6.7 and 6.8 in Appendix 6.7.

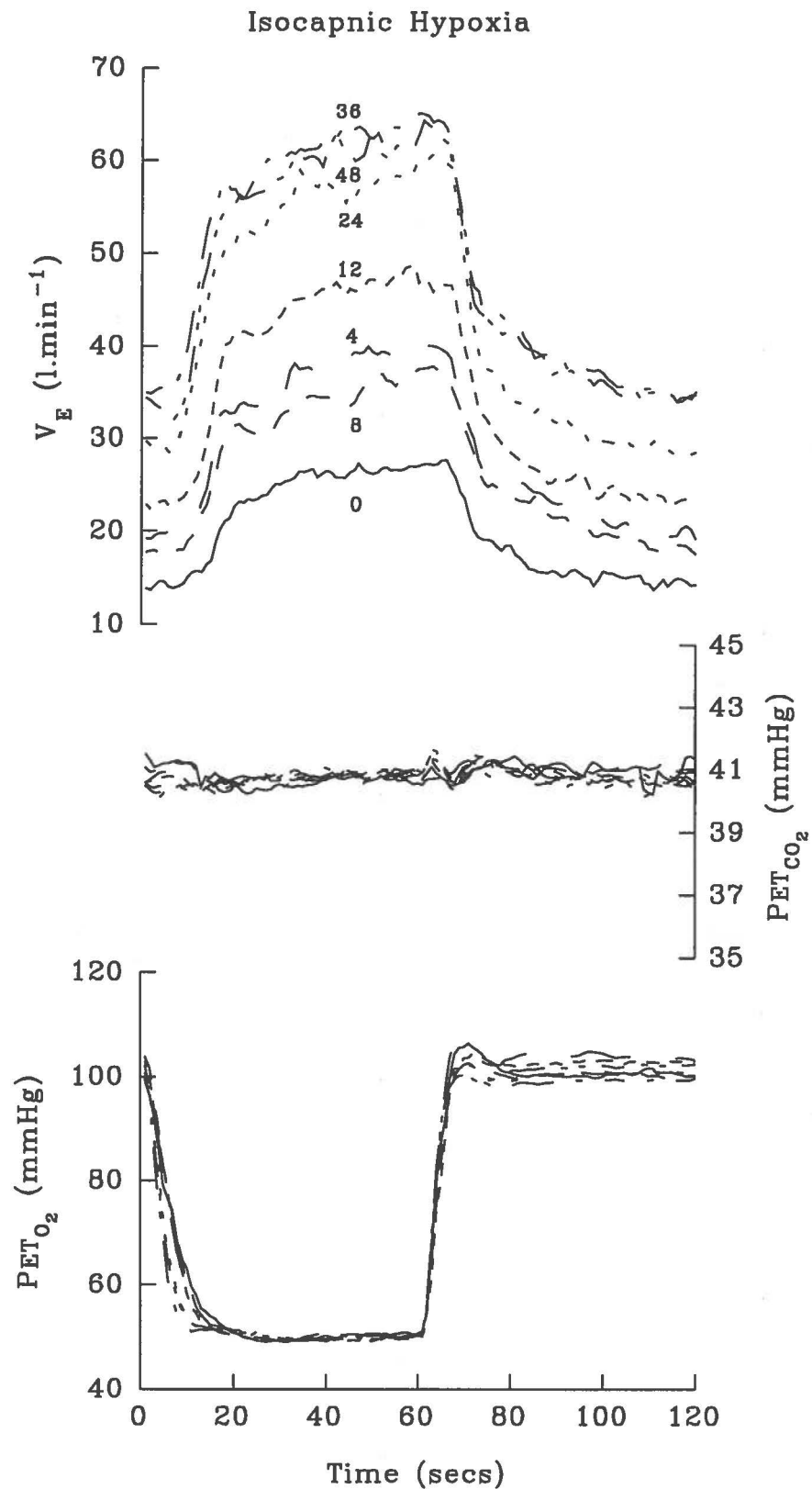


Figure 6.10: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of PCO_2 -matched AHVR between 0 and 48 hours of isocapnic hypoxia.

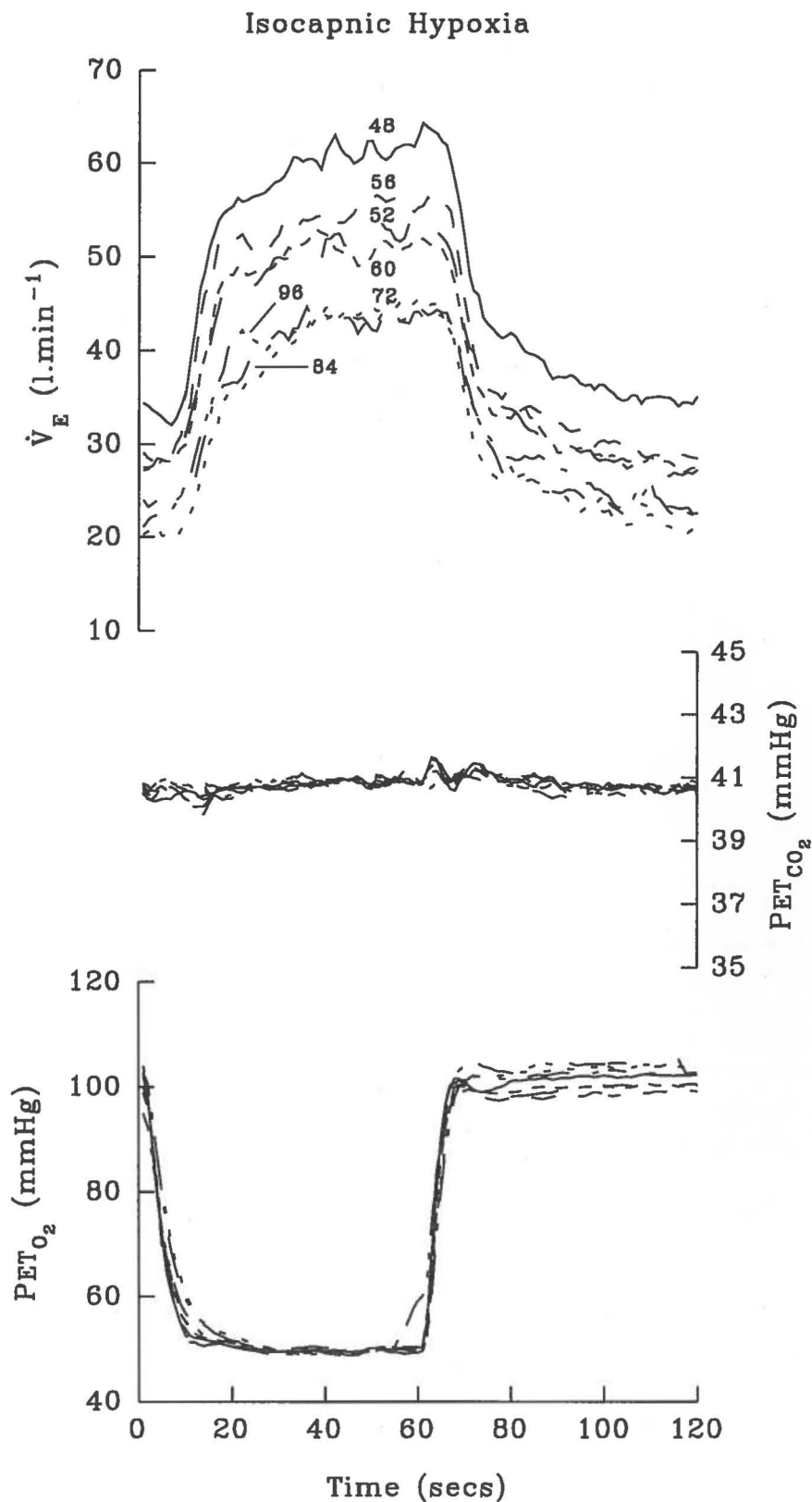


Figure 6.11: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of PCO_2 -matched AHVR between 48 and 96 hours of isocapnic protocol.

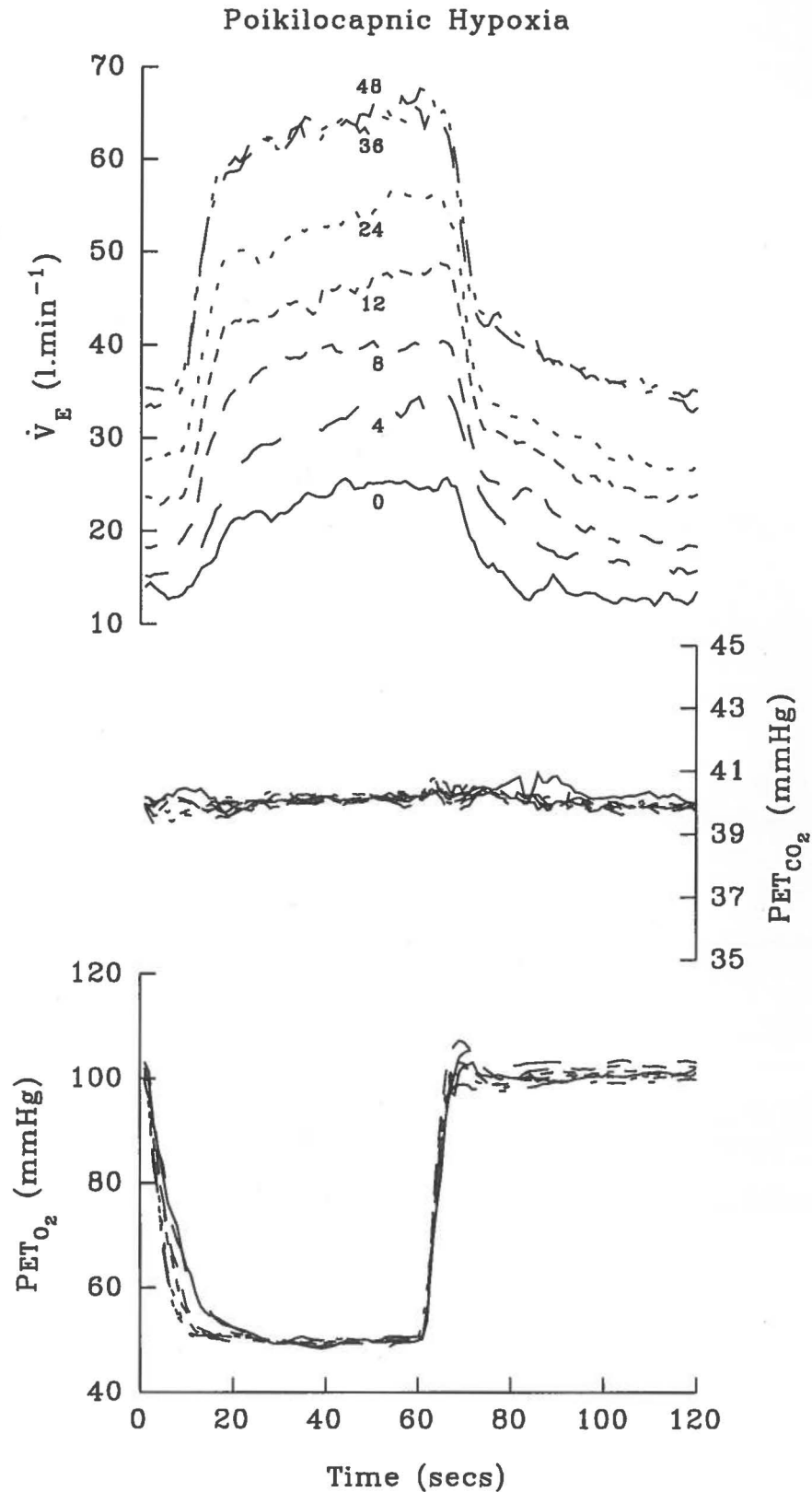


Figure 6.12: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of PCO_2 -matched AHVR between 0 and 48 hours of poikilocapnic hypoxia.

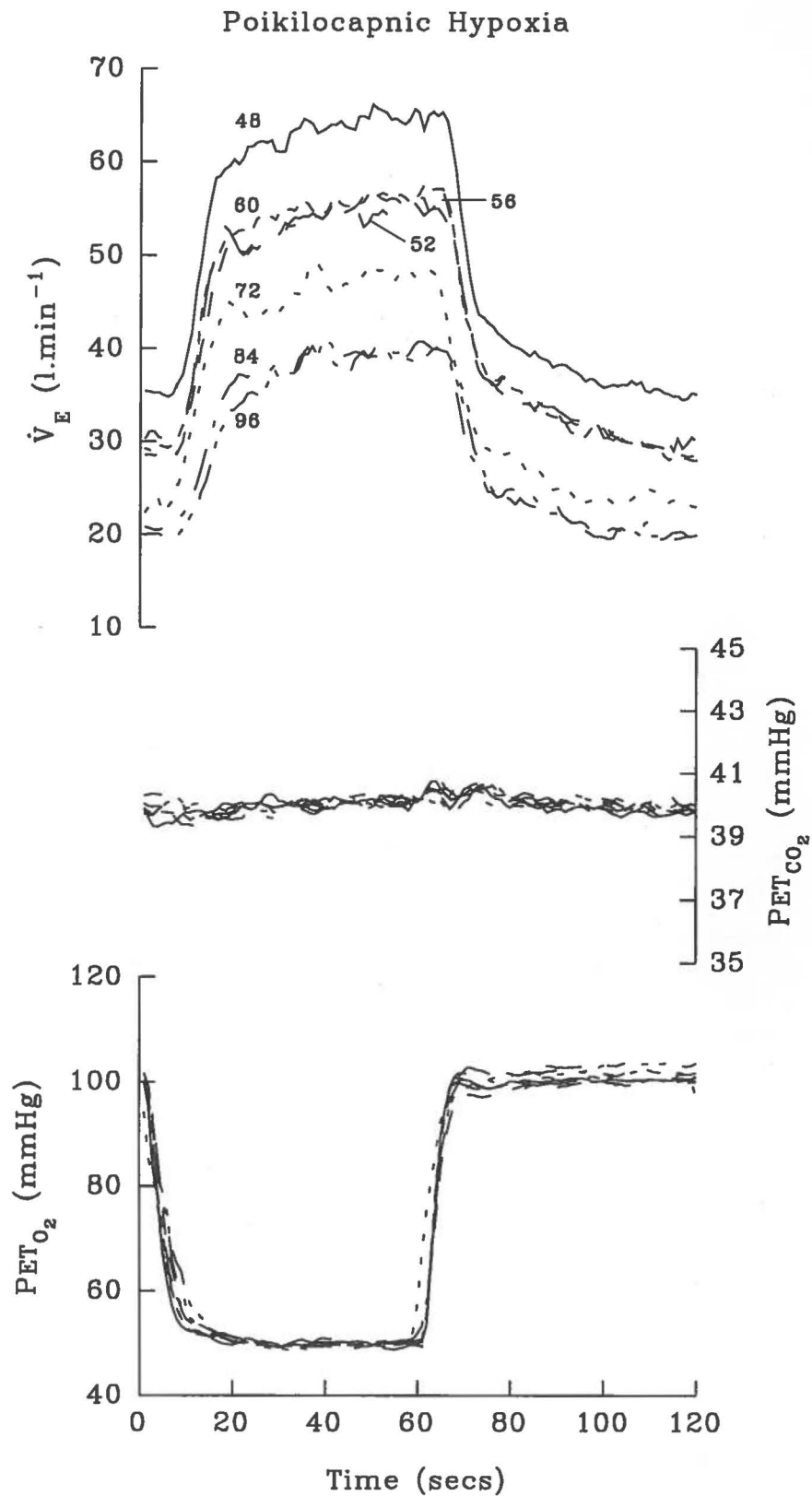


Figure 6.13: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of PCO_2 -matched AHVR between 48 and 96 hours of poikilocapnic protocol.

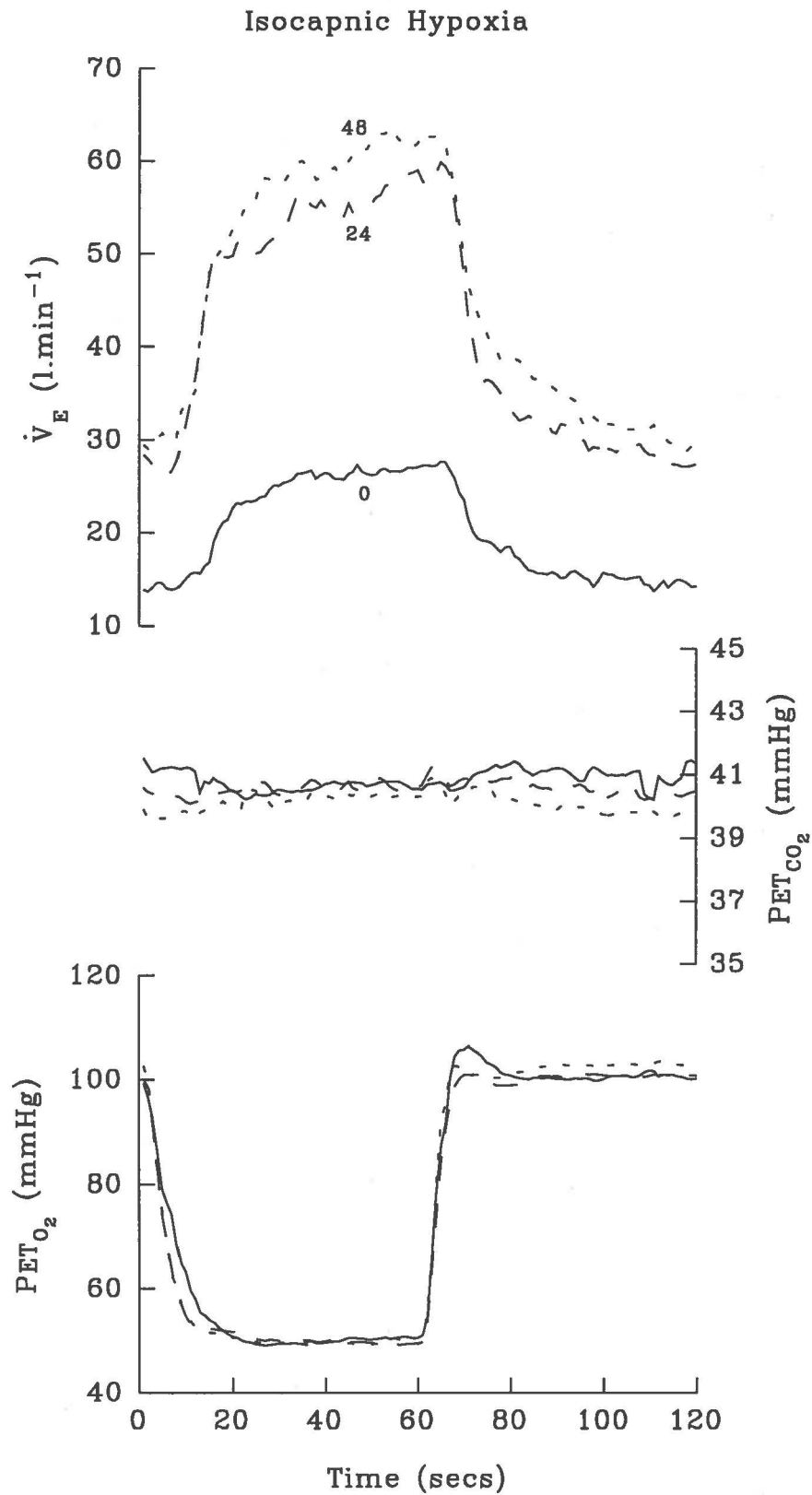


Figure 6.14: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of pH-matched AHVR between 0 and 48 hours of isocapnic hypoxia.

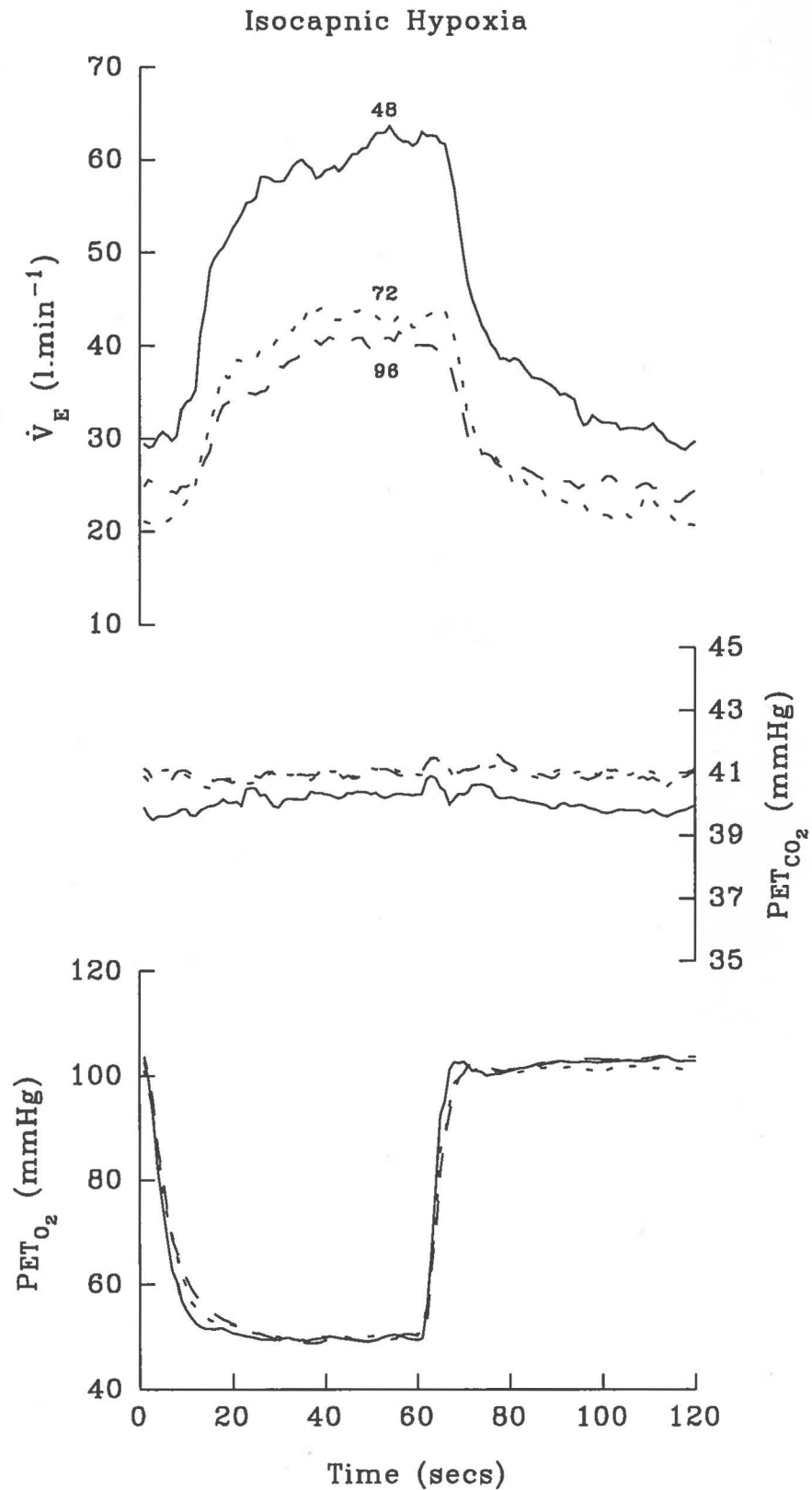


Figure 6.15: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of pH-matched AHVR between 48 and 96 hours of isocapnic protocol.

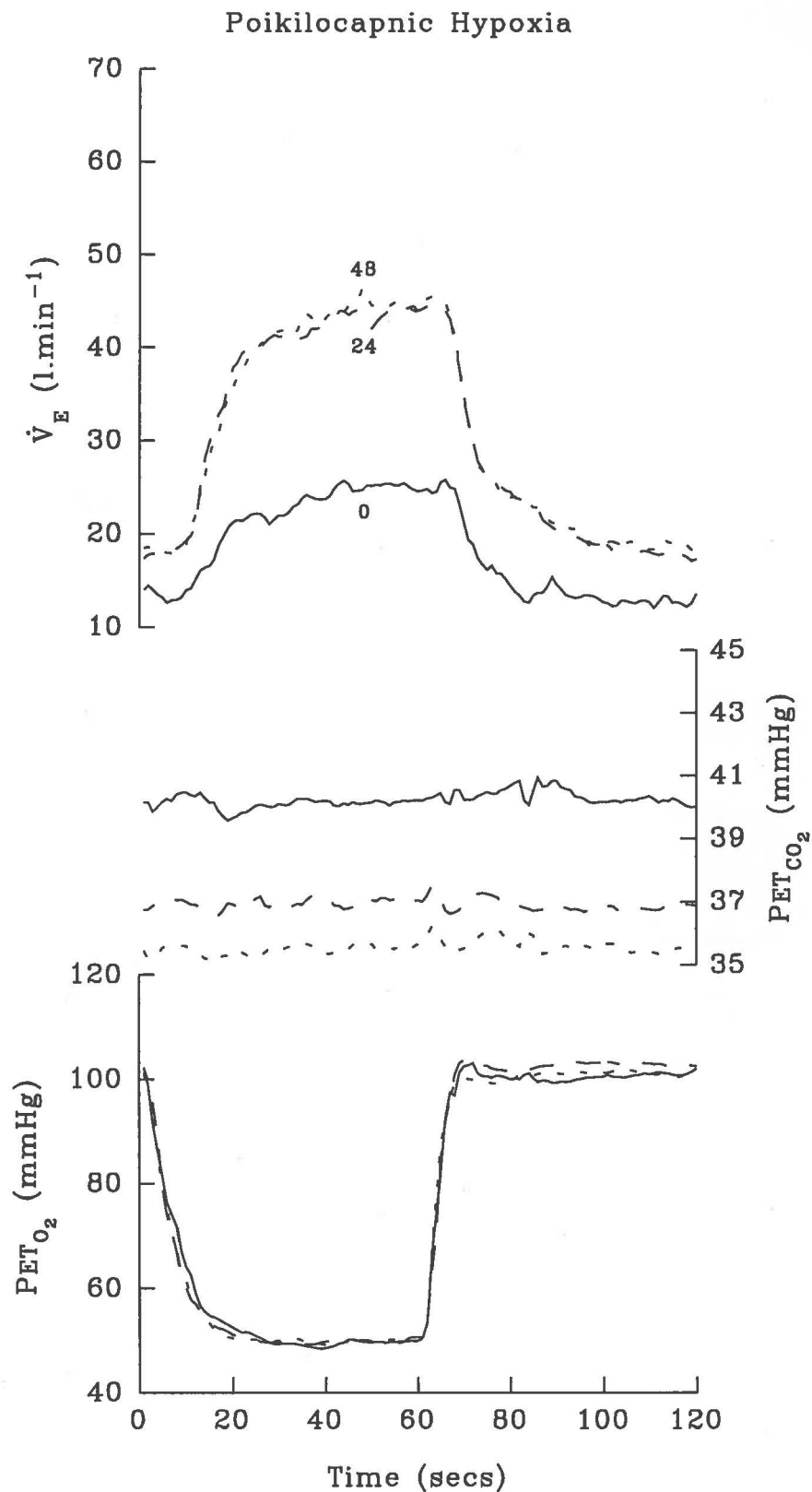


Figure 6.16: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of pH-matched AHVR between 0 and 48 hours of poikilocapnic hypoxia.

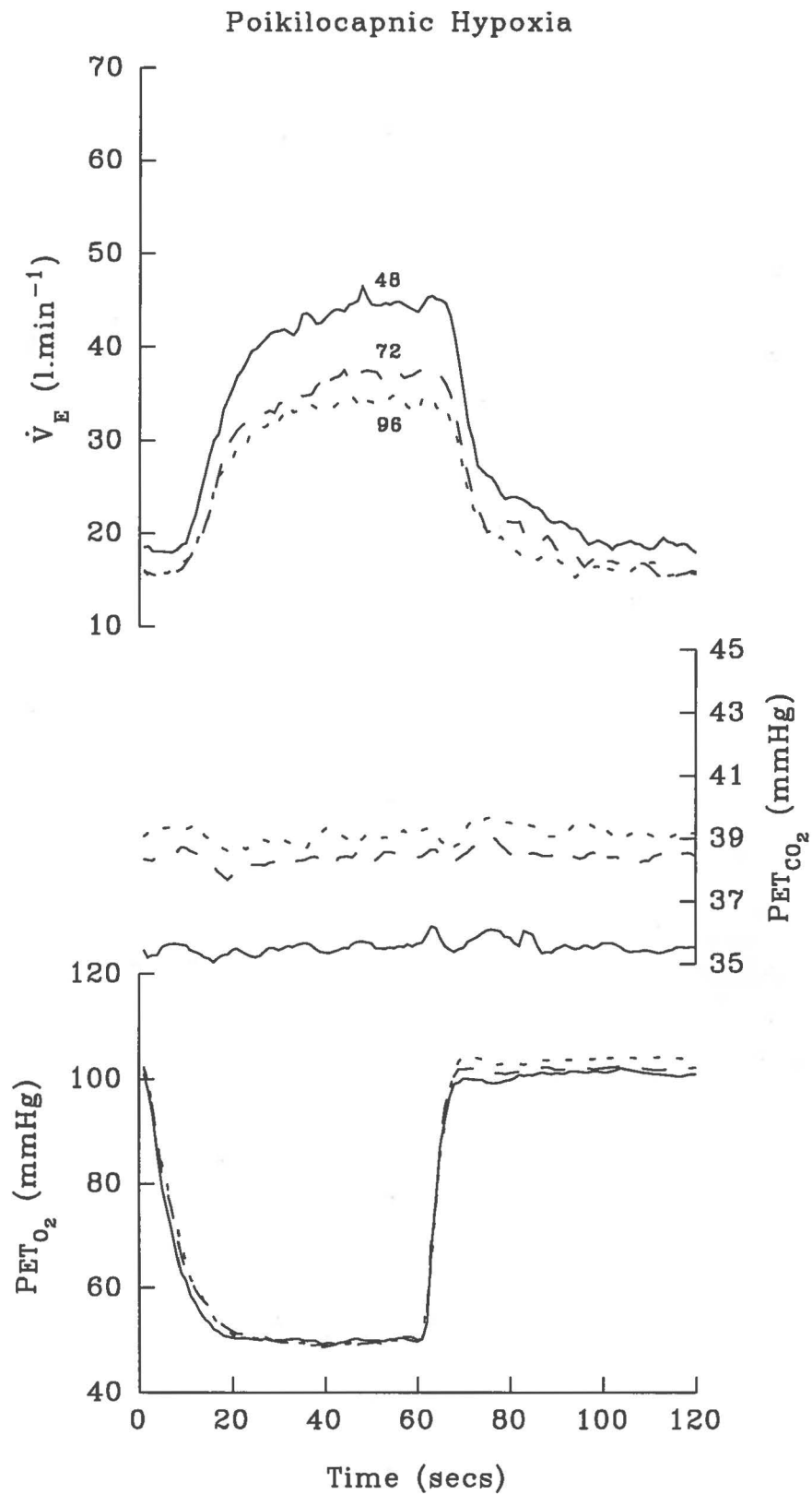


Figure 6.17: The average end-tidal gas tensions and ventilatory responses of the last 5 square waves superimposed and averaged across all subjects for tests of pH-matched AHVR between 48 and 96 hours of poikilocapnic protocol.

The mean and individual estimates for the sensitivity and baseline terms from the P_{CO_2} -matched tests of AHVR are shown in Fig. 6.18 (procedure A) and Fig. 6.19 (procedure B). The mean estimates using both fitting procedures for both protocols are superimposed in Fig. 6.20. These plots confirm the observations made above that both the hypoxic sensitivity and the ventilation baseline increase, and by similar amounts, during both types hypoxic exposure. In addition, these changes are slower to decline during the recovery. The results are presented in Figs 6.21 and 6.22 in an alternative manner, as used for analysis of eupnoeic ventilation above, whereby the data from the poikilocapnic exposure is subtracted from the isocapnic data for each subject and averaged, resulting in a mean difference and standard error. This technique was employed for both fitting procedures (Fig. 6.21, procedure A; Fig. 6.22, procedure B) and for both G_P and $\dot{V}_C$. These plots show that almost all of the mean paired differences between the protocols lie within the $2 \times S.E.$ confidence intervals, indicating that the isocapnic and poikilocapnic protocols result in similar distributions of data. ANOVA showed no differences between the two protocols.

The same data for the pH-matched measurements of AHVR are presented in Fig. 6.23 (mean and individual estimates; procedure A), Fig. 6.24 (mean and individual estimates; procedure B) and Fig. 6.25 (mean estimates) and Table 6.9 in Appendix 6.7. Figures 6.26 and 6.27 show the paired differences between the two protocols (lower panels) and the mean paired differences and $2 \times S.E.$ confidence intervals (upper panels). The plots of the estimates of hypoxic sensitivity are made hard to interpret qualitatively by the anomalous result recorded in one subject (970) at 72 hours. However, most of the mean paired differences lie within the confidence limits (Figs 6.26 and 6.27) indicating that the two distributions of data are the same, although ANOVA narrowly failed to demonstrate that there was significant interaction between the time (T_j) and protocol (P_k) factors in the model of G_P ($0.05 < p < 0.1$). The hypoxia-independent ventilation shows some, but very little, change during and after exposure to poikilocapnic hypoxia, whereas it rises during isocapnic hypoxia and subsequently falls off. This is also illustrated in Figs 6.26 and 6.27 where all mean paired

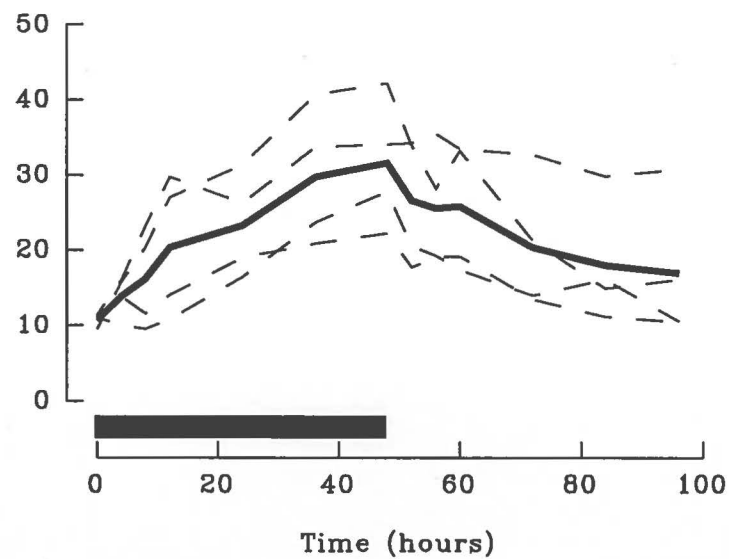
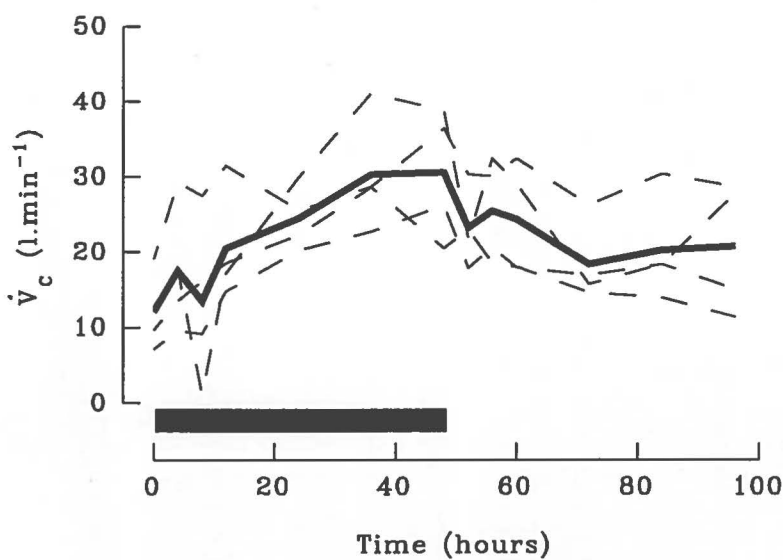
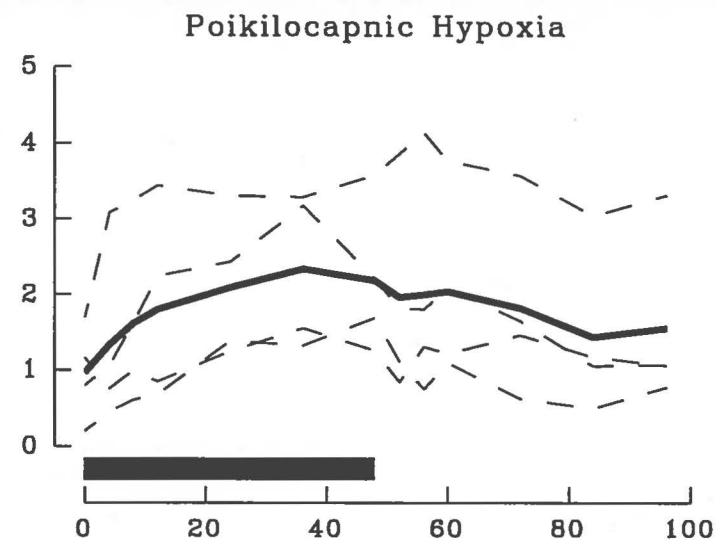
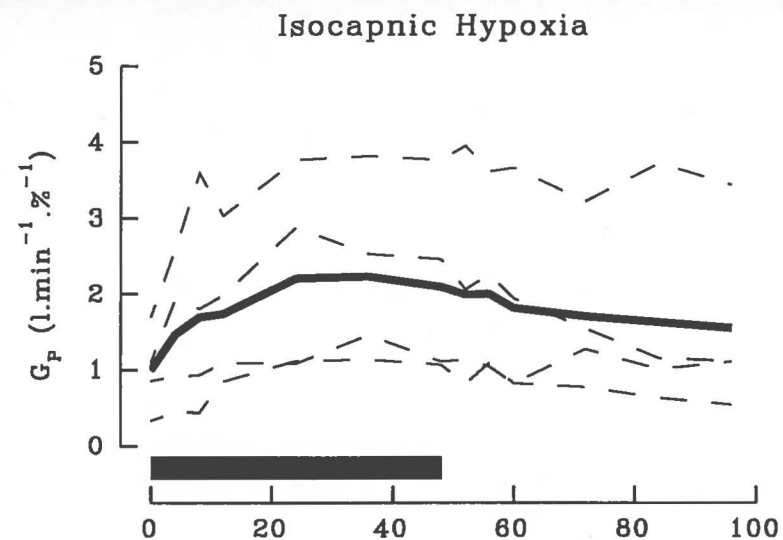


Figure 6.18: Mean (solid lines) and individual (dashed lines) estimates for the hypoxic sensitivity (G_p) and hypoxia-independent ventilation ($\dot{V}_C$) from the PCO_2 -matched tests of AHVR using fitting procedure A. (Isocapnic hypoxia, left; poikilocapnic hypoxia, right.) Solid bar indicates period of hypoxic exposure.

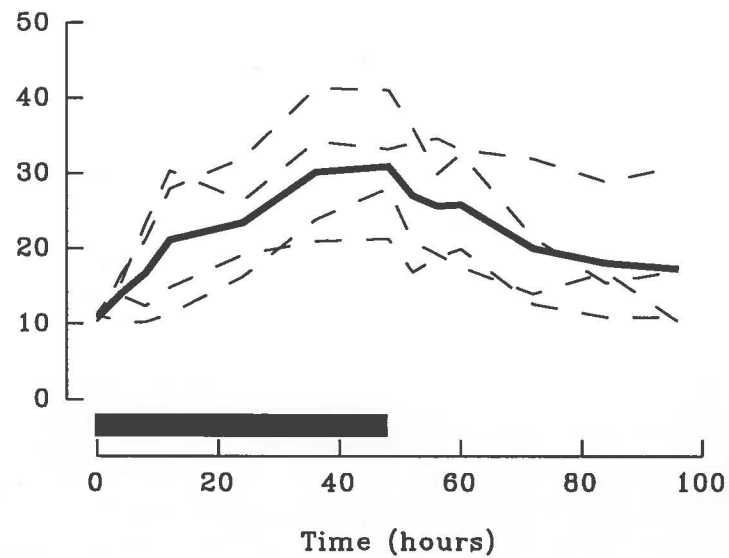
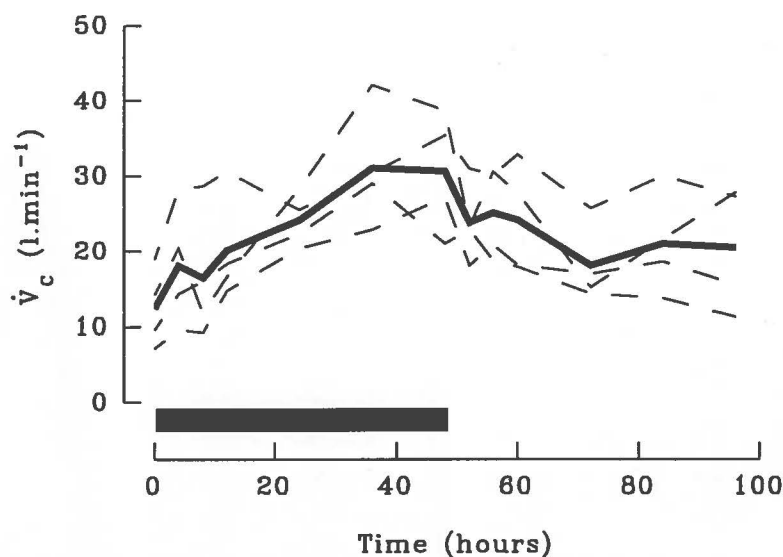
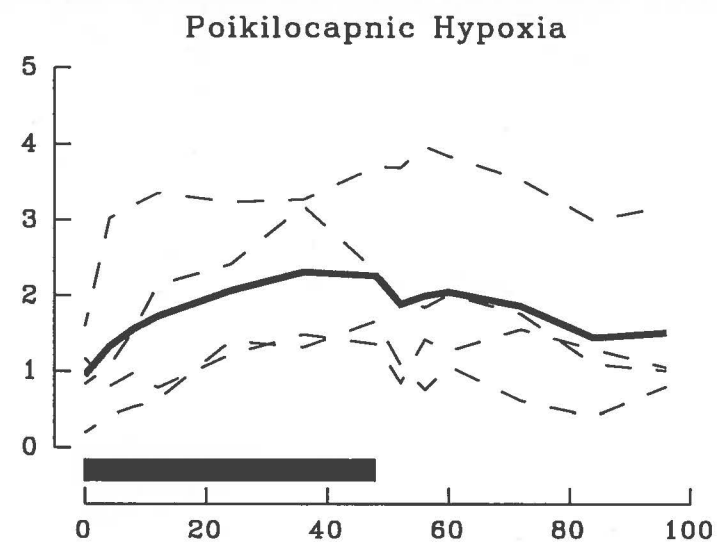
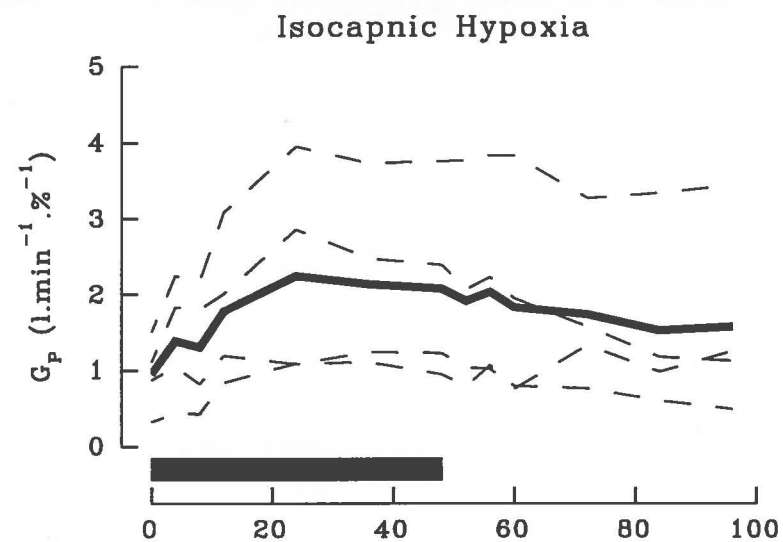


Figure 6.19: Mean (solid lines) and individual (dashed lines) estimates for the hypoxic sensitivity (G_P) and hypoxia-independent ventilation ($\dot{V}_C$) from the PCO_2 -matched tests of AHVR using fitting procedure B. (Isocapnic hypoxia, left; poikilocapnic hypoxia, right.) Solid bar indicates period of hypoxic exposure.

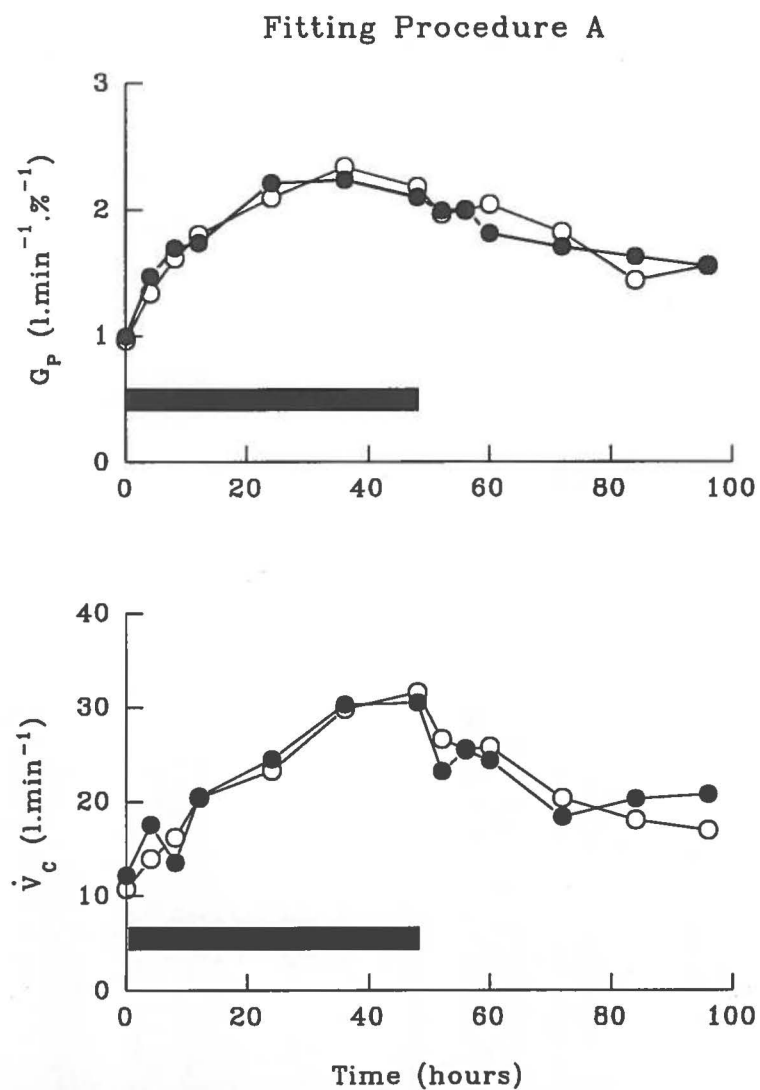


Figure 6.20: Mean estimates of hypoxic sensitivity (G_P) and hypoxia-independent ventilation ($\dot{V}_C$) from the PCO_2 -matched tests of AHVR. (Fitting procedure A, left; fitting procedure B, right.) Solid bar indicates period of hypoxic exposure.

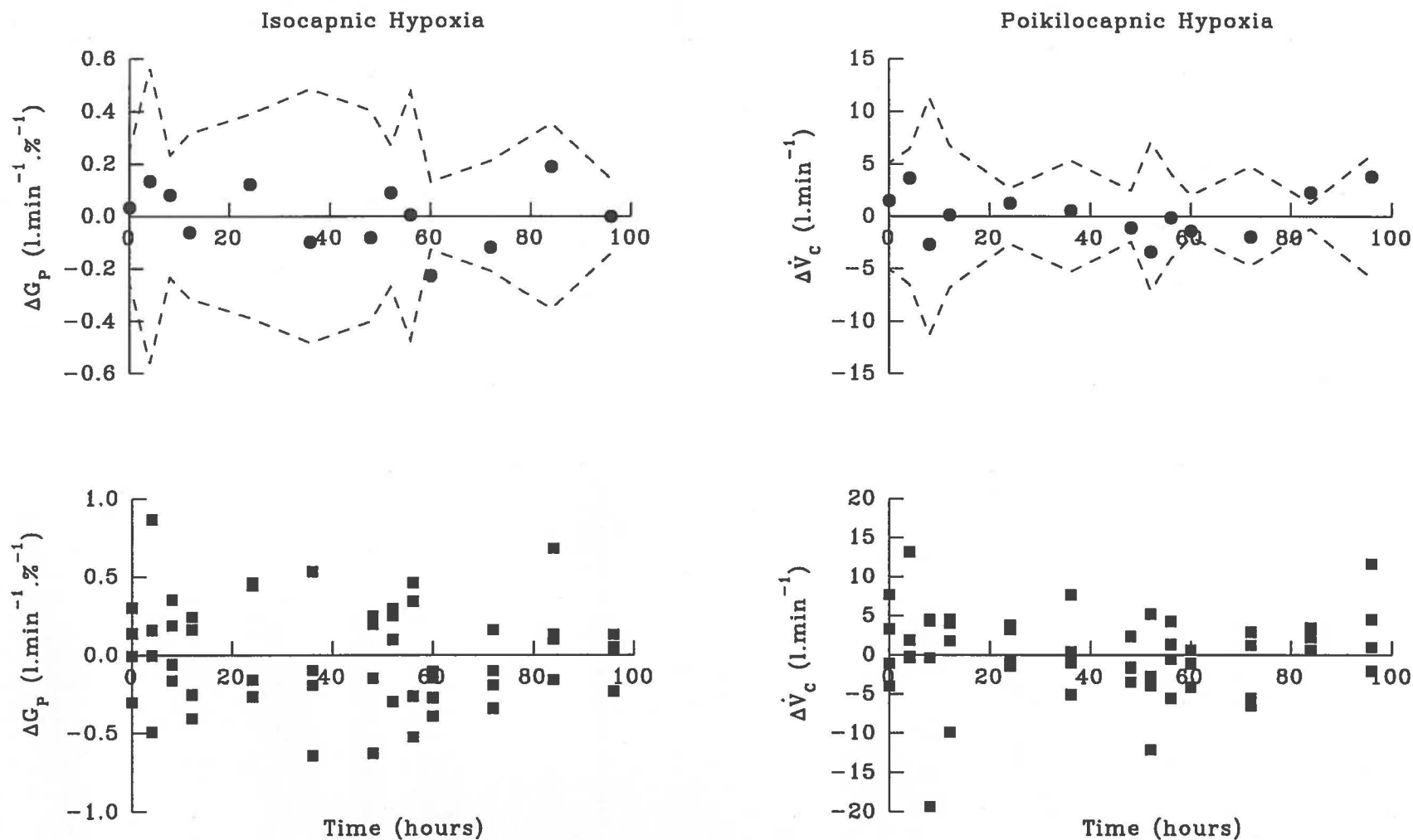


Figure 6.21: Lower panels - differences between isocapnic and poikilocapnic protocols for hypoxic sensitivity, G_p (left), and hypoxia-independent ventilation, $\dot{V}_C$ (right) from PCO_2 -matched tests of AHVR for each subject (fitting procedure A). Upper panels - mean paired difference for each time point and the standard error multiplied by a factor of two, for each mean difference (dashed lines).

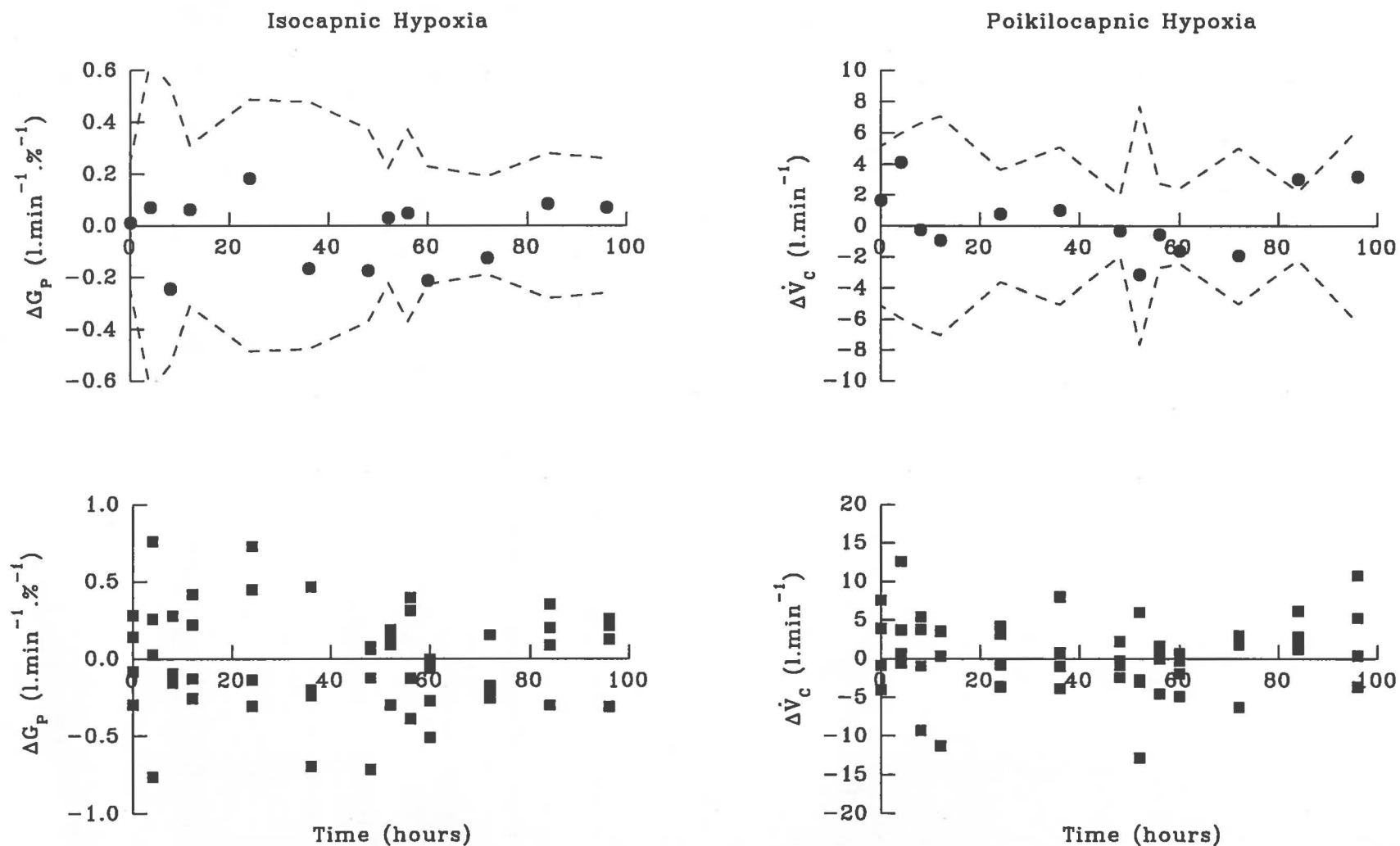


Figure 6.22: Lower panels - differences between isocapnic and poikilocapnic protocols for hypoxic sensitivity, G_p (left), and hypoxia-independent ventilation, $\dot{V}_C$ (right) from PCO_2 -matched tests of AHVR for each subject (fitting procedure B). Upper panels - mean paired difference for each time point and the standard error multiplied by a factor of two, for each mean difference (dashed lines).

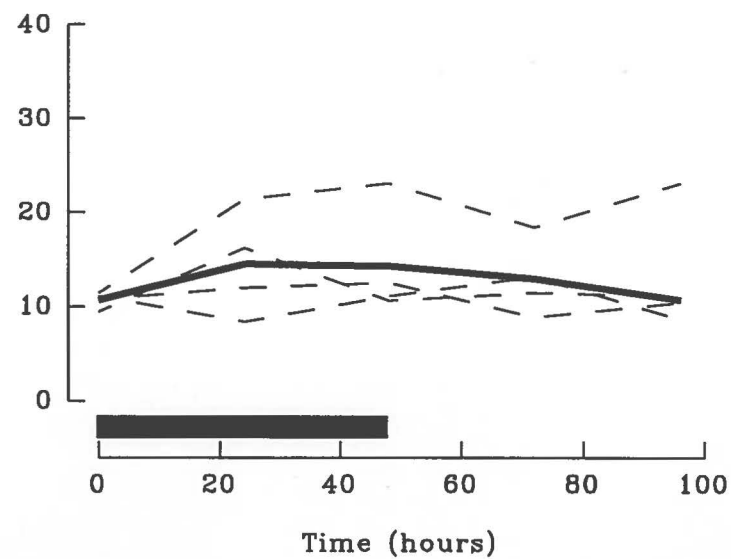
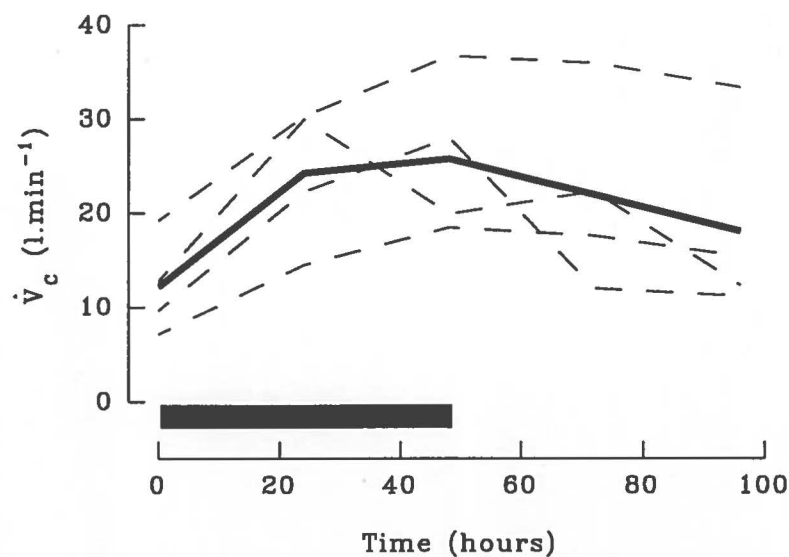
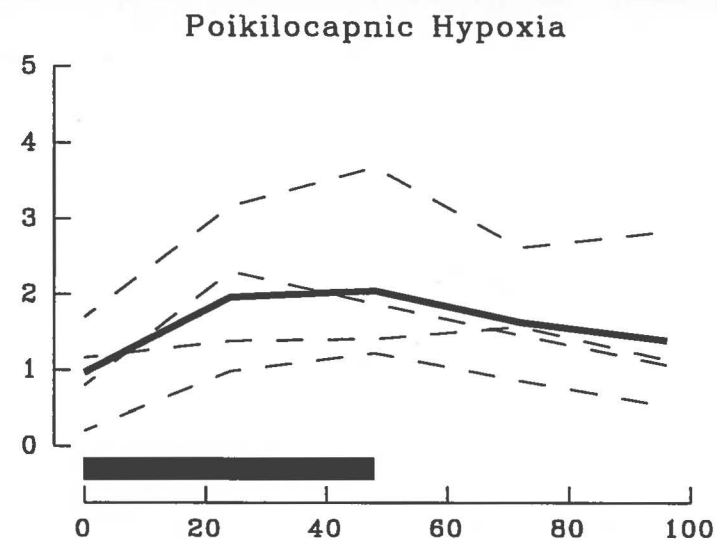
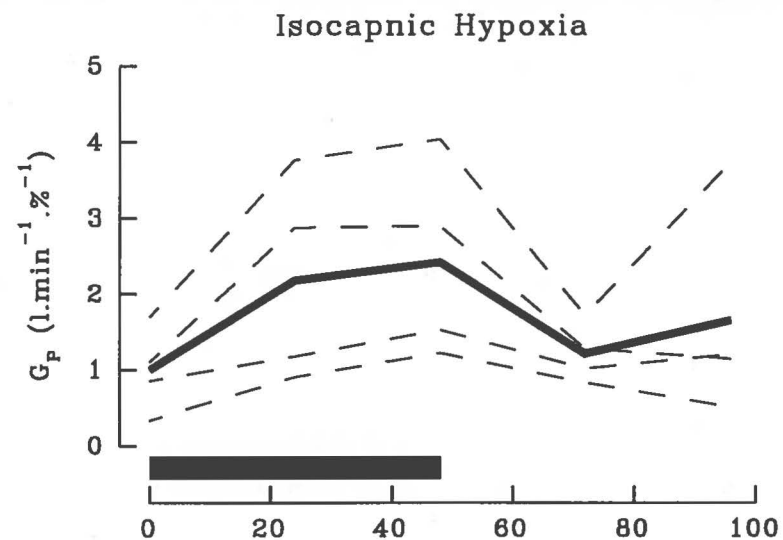


Figure 6.23: Mean (solid lines) and individual (dashed lines) estimates for the hypoxic sensitivity (G_p) and hypoxia-independent ventilation ($\dot{V}_c$) from the pH-matched tests of AHVR using fitting procedure A. (Isocapnic hypoxia, left; poikilocapnic hypoxia, right.) Solid bar indicates period of hypoxic exposure.

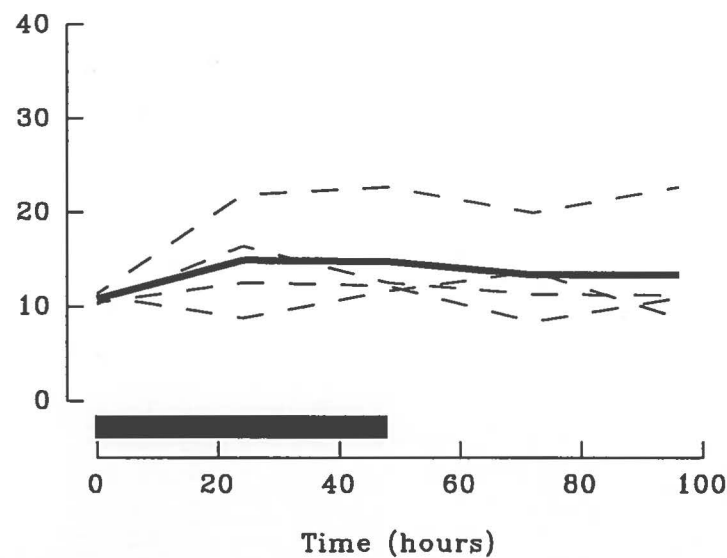
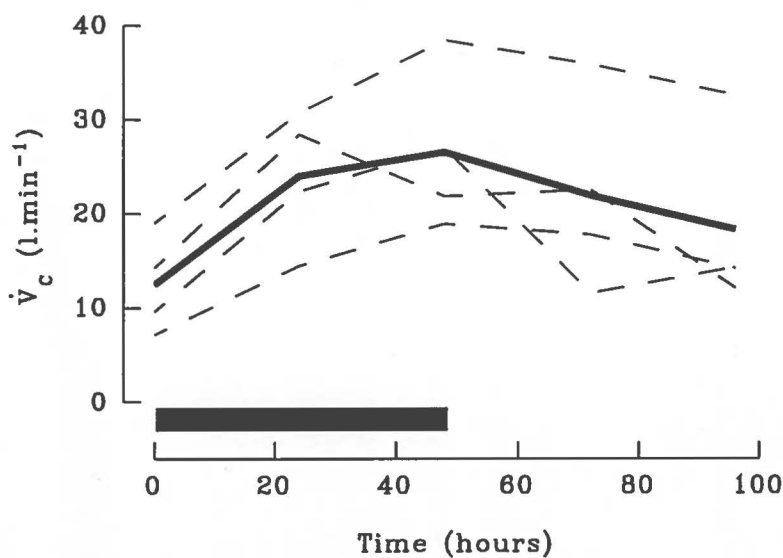
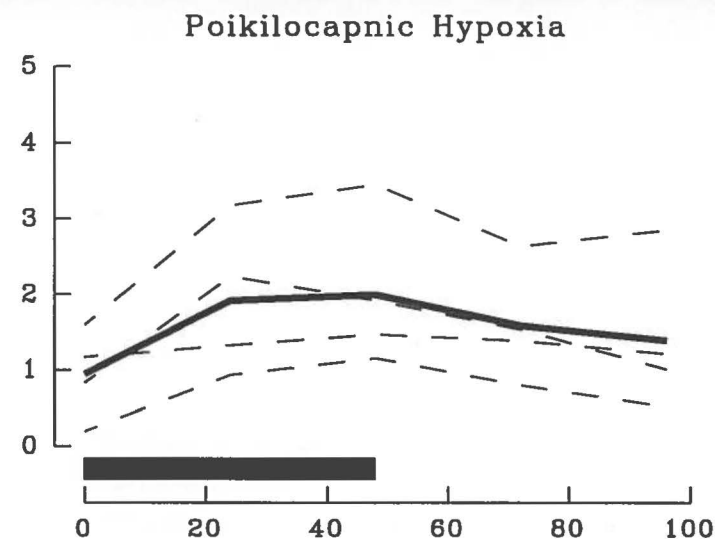
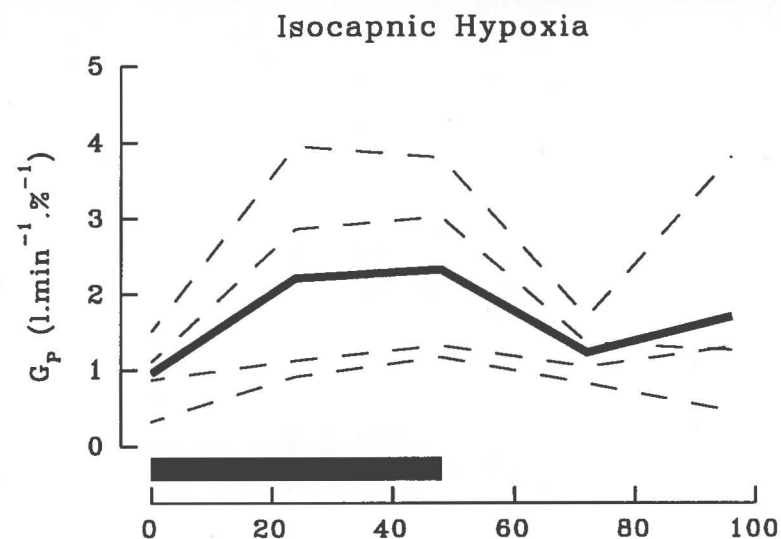


Figure 6.24: Mean (solid lines) and individual (dashed lines) estimates for the hypoxic sensitivity (G_p) and hypoxia-independent ventilation ($\dot{V}_C$) from the pH-matched tests of AHVR using fitting procedure B. (Isocapnic hypoxia, left; poikilocapnic hypoxia, right.) Solid bar indicates period of hypoxic exposure. Solid bar indicates period of hypoxic exposure.

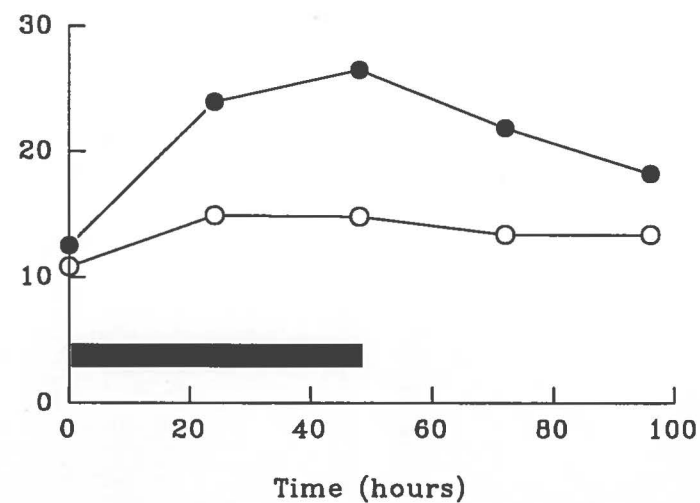
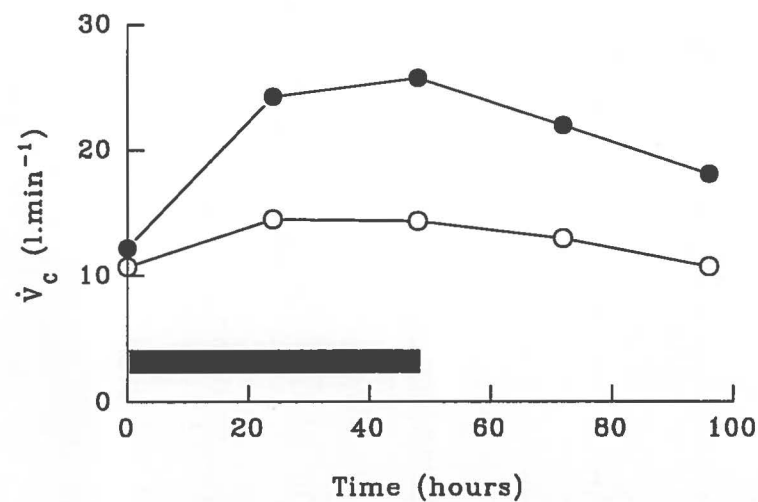
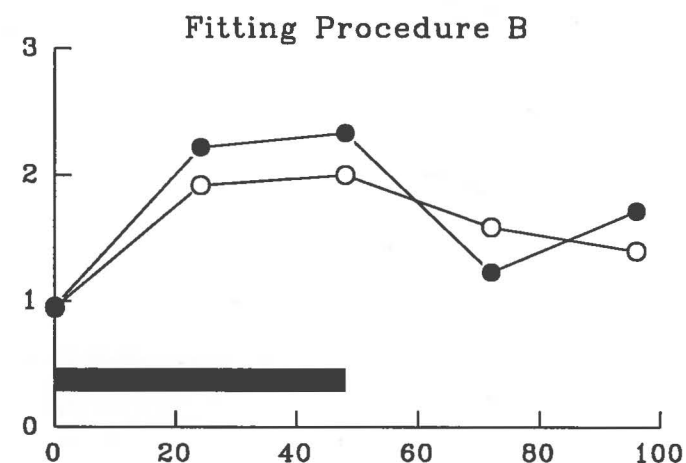
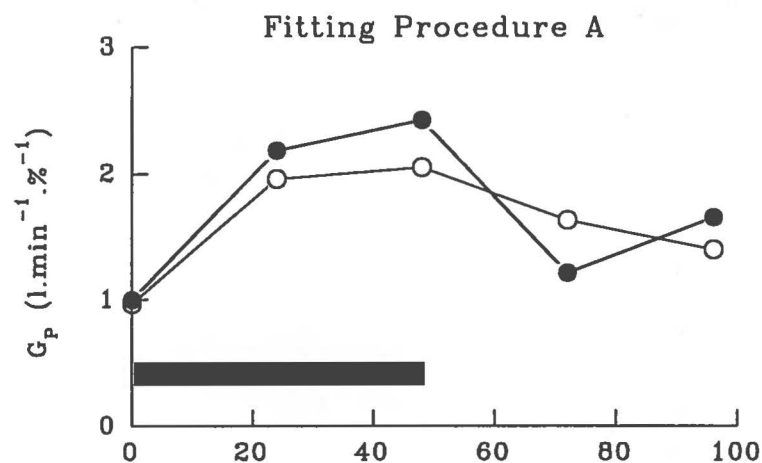


Figure 6.25: Mean estimates of hypoxic sensitivity (G_p) and hypoxia-independent ventilation ($\dot{V}_c$) from the pH-matched tests of AHVR. (Fitting procedure A, left; fitting procedure B, right.) Solid bar indicates period of hypoxic exposure.

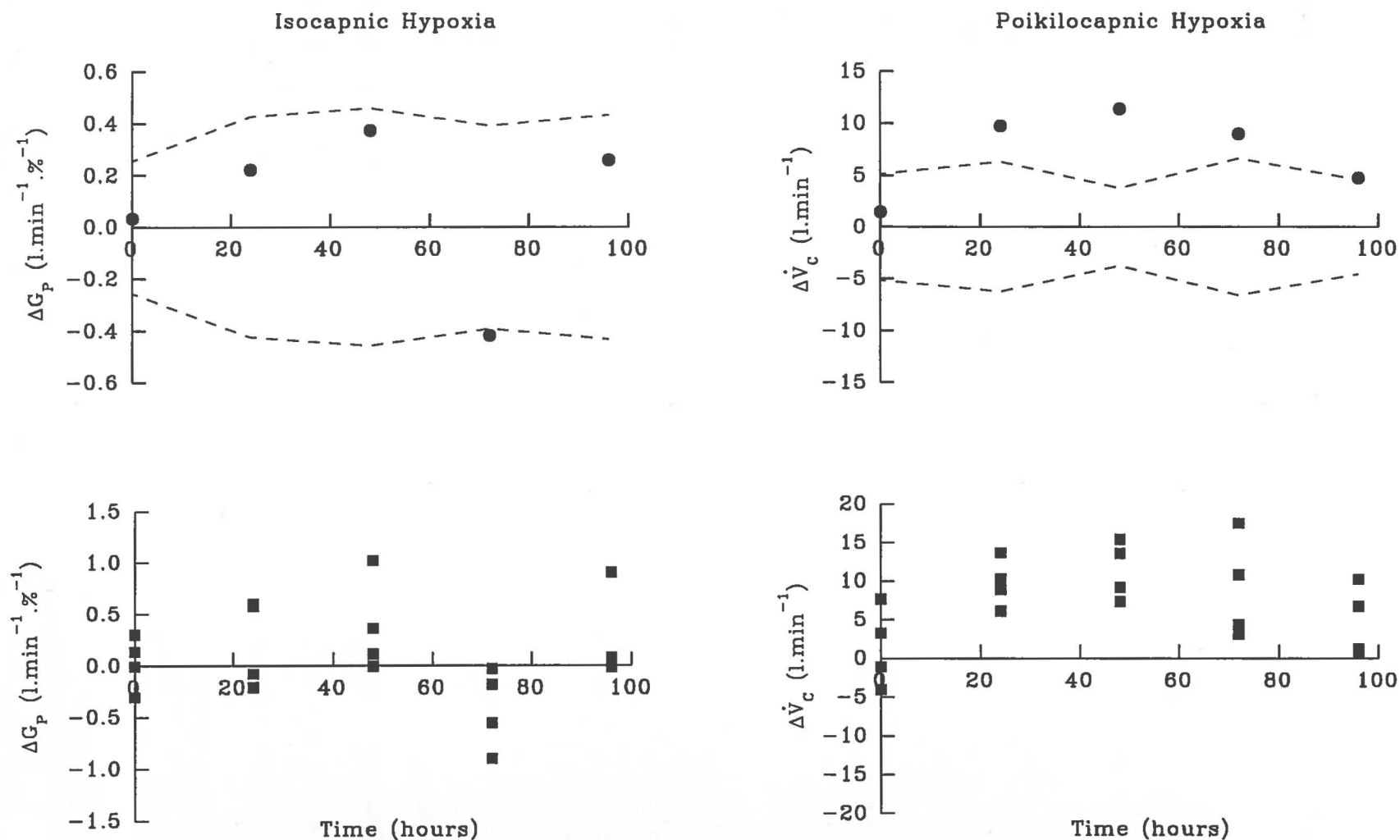


Figure 6.26: Lower panels - differences between isocapnic and poikilocapnic protocols for hypoxic sensitivity, G_p (left), and hypoxia-independent ventilation, $\dot{V}_C$ (right) from pH-matched tests of AHVR for each subject (fitting procedure A). Upper panels - mean paired difference for each time point and the standard error multiplied by a factor of two, for each mean difference (dashed lines).

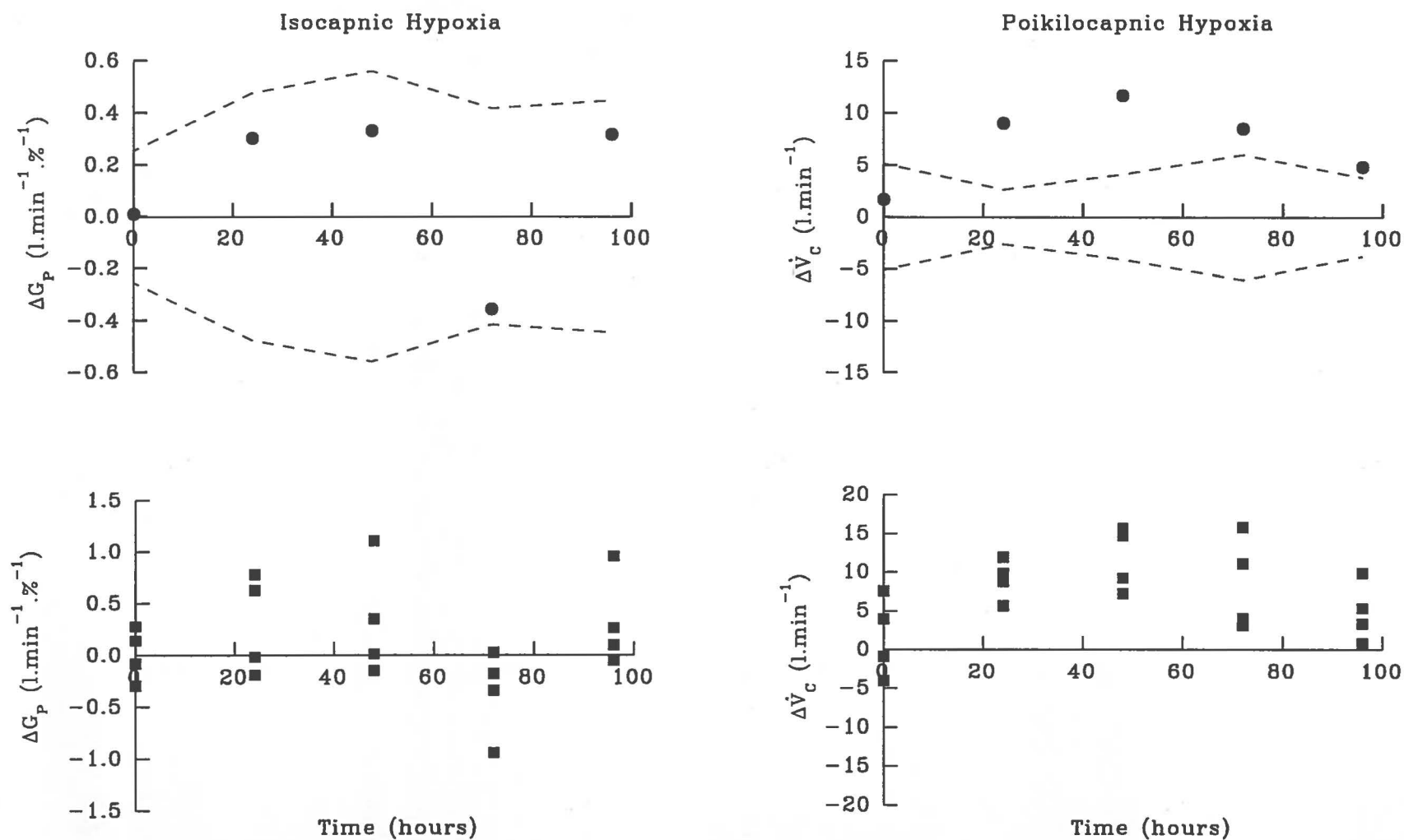


Figure 6.27: Lower panels - differences between isocapnic and poikilocapnic protocols for hypoxic sensitivity, G_p (left), and hypoxia-independent ventilation, $\dot{V}_c$ (right) from pH-matched tests of AHVR for each subject (fitting procedure B). Upper panels - mean paired difference for each time point and the standard error multiplied by a factor of two, for each mean difference (dashed lines).

differences during and after exposure lie outside the confidence intervals. In addition, there was a significant interaction between the protocol and time effects in the model of this parameter ($p < 0.025$; ANOVA). The summary of the analysis of variance for the hypoxia-independent ventilation is given in Table 6.3.

Source of Variance	Degrees of Freedom	Sum of squares (l.min-1) ²	Mean Squared Error (l.min-1) ²	F-ratio	P value
S _i	3	1008.4	336.1	---	---
T _j	4	379.1	94.8	3.84	<0.05
P _k	1	525.3	525.3	20.16	<0.025
ST _{ij}	12	296.1	24.7	---	---
SP _{ik}	3	78.2	26.1	---	---
TP _{jk}	4	132.0	33.0	4.18	<0.025
STP _{ijk}	12	94.6	7.9	---	---
Total	39	2513.6	---		

Table 6.3: Statistical analysis of the linear model of hypoxia-independent ventilation, calculated using fitting procedure A, using analysis of variance.

Individual hypoxic sensitivities before both hypoxic exposures are plotted against the hypoxic sensitivities at 48 hours, measured by the pH-matched method and calculated by fitting procedure A, in Fig. 6.28. An interpretation of this plot is that the magnitude of the rise in hypoxic sensitivity during long-term hypoxia is dependent on the subject's resting hypoxic sensitivity. The Pearson correlation coefficient was calculated as 0.87, which was significantly different from zero ($p < 0.005$) when the function

$$\frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

was assumed to have a t -distribution with $n-2$ degrees of freedom, where r is the Pearson correlation coefficient and an estimate for the correlation in the population (ρ).

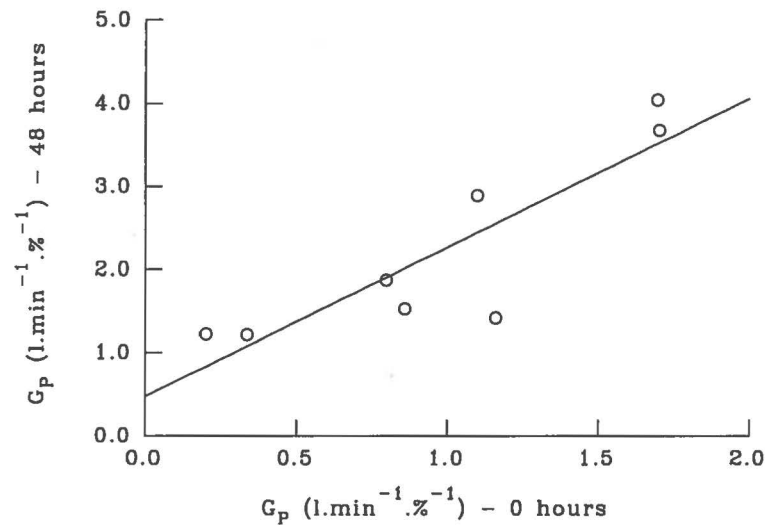


Figure 6.28: Hypoxic sensitivity (G_p , l.min⁻¹.%⁻¹) at 0 hours vs. hypoxic sensitivity at 48 hours, in both protocols measured by the pH-matched method and calculated by fitting procedure A.

Figure 6.29 shows the relationship between hypoxic sensitivity at 0 hours and ventilation at 48 hours and also between hypoxic sensitivity at 48 hours and ventilation at 48 hours for both isocapnic and poikilocapnic states. The Pearson correlation coefficient failed to reach significance in the poikilocapnic exposure; however, ventilation at 48 hours was shown to be significantly correlated with hypoxic sensitivity at 0 hours ($r=0.965$, $p<0.025$) and hypoxic sensitivity at 48 hours ($r=0.997$, $p<0.005$) in the isocapnic study.

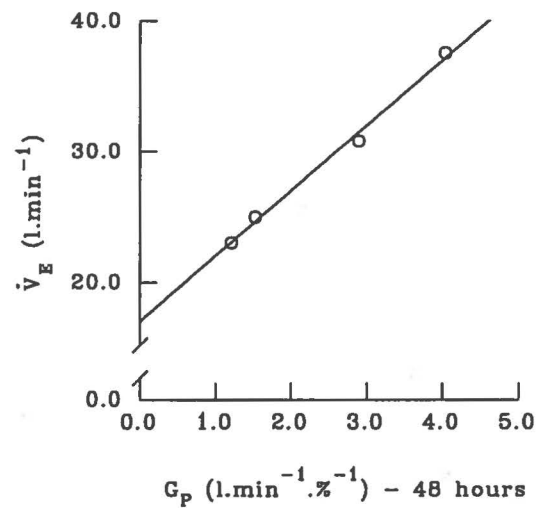
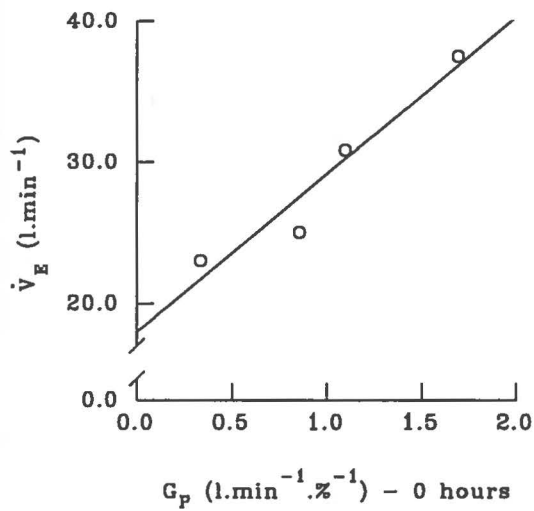
Acute hypercapnic ventilatory response. The data describing the CO_2 response (i.e., the slope and x -axis intercept of the CO_2 response curve, B) are presented in Table 6.4. These data are also displayed in Fig. 6.30.

Time (hours)	Isocapnic Hypoxia				Poikilocapnic Hypoxia			
	Slope (l.min ⁻¹ .mmHg ⁻¹)		B (mmHg)		Slope (l.min ⁻¹ .mmHg ⁻¹)		B (mmHg)	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
0	2.71	0.81	35.13	1.35	2.19	0.24	34.57	2.04
24	3.14	0.89	31.84	1.56	3.45	1.25	32.04	1.62
48	3.66	0.60	28.70	3.47	4.29	1.43	31.29	1.73
72	3.37	1.13	32.91	1.48	3.51	0.81	33.23	1.66
96	3.66	1.52	32.60	1.09	3.32	1.00	32.72	1.77

Table 6.4: Slope (l.min⁻¹.mmHg⁻¹) and x-axis intercept, B, (mmHg) of the CO₂ response during exposure to and recovery from isocapnic and poikilocapnic hypoxia.

In the isocapnic protocol, there appears to be a small change in the slope of the response; however, there is a marked left-ward shift of the response during the hypoxic exposure (from 35.13 ± 1.35 mmHg to 28.70 ± 3.47 mmHg; mean $\pm$ S.E.). During the recovery from isocapnic hypoxia, the CO₂ response shifts back towards pre-exposure levels, although not completely (to 32.60 ± 1.09 mmHg). The poikilocapnic exposure elicits an increase in slope during the exposure (from 2.19 ± 0.24 l.min⁻¹.mmHg⁻¹ to 4.29 ± 1.43 l.min⁻¹.mmHg⁻¹), which decreases during recovery towards pre-exposure levels (to 3.32 ± 1.00 l.min⁻¹.mmHg⁻¹). There is also a leftward shift in the curve during the poikilocapnic exposure (from 34.57 ± 2.04 mmHg to 31.29 ± 1.73 mmHg), with a subsequent incomplete return during recovery (to 32.72 ± 1.77 mmHg). However, this is not as marked as in the isocapnic protocol. None of these findings proved statistically significant.

Isocapnic Hypoxia



Poikilocapnic Hypoxia

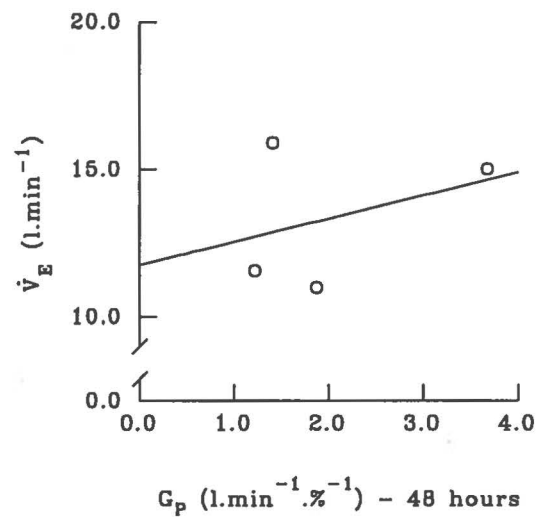
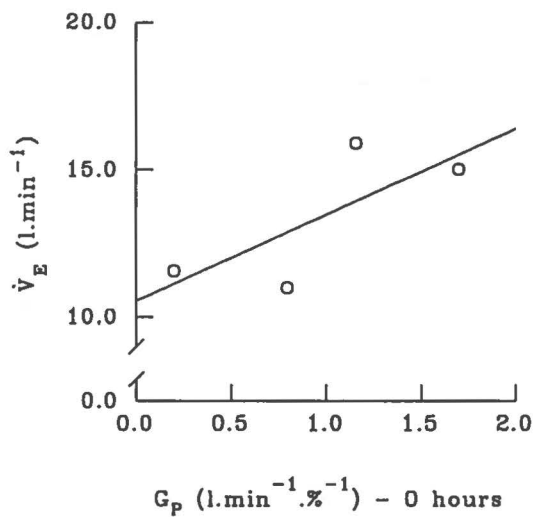


Figure 6.29: Hypoxic sensitivity (G_P , $\text{l} \cdot \text{min}^{-1} \cdot \text{\%}^{-1}$) at 0 hours vs. ventilation at 48 hours (left panels) and hypoxic sensitivity at 48 hours vs. ventilation at 48 hours (right panels). (Upper panels, isocapnic hypoxia; lower panels, poikilocapnic hypoxia.)

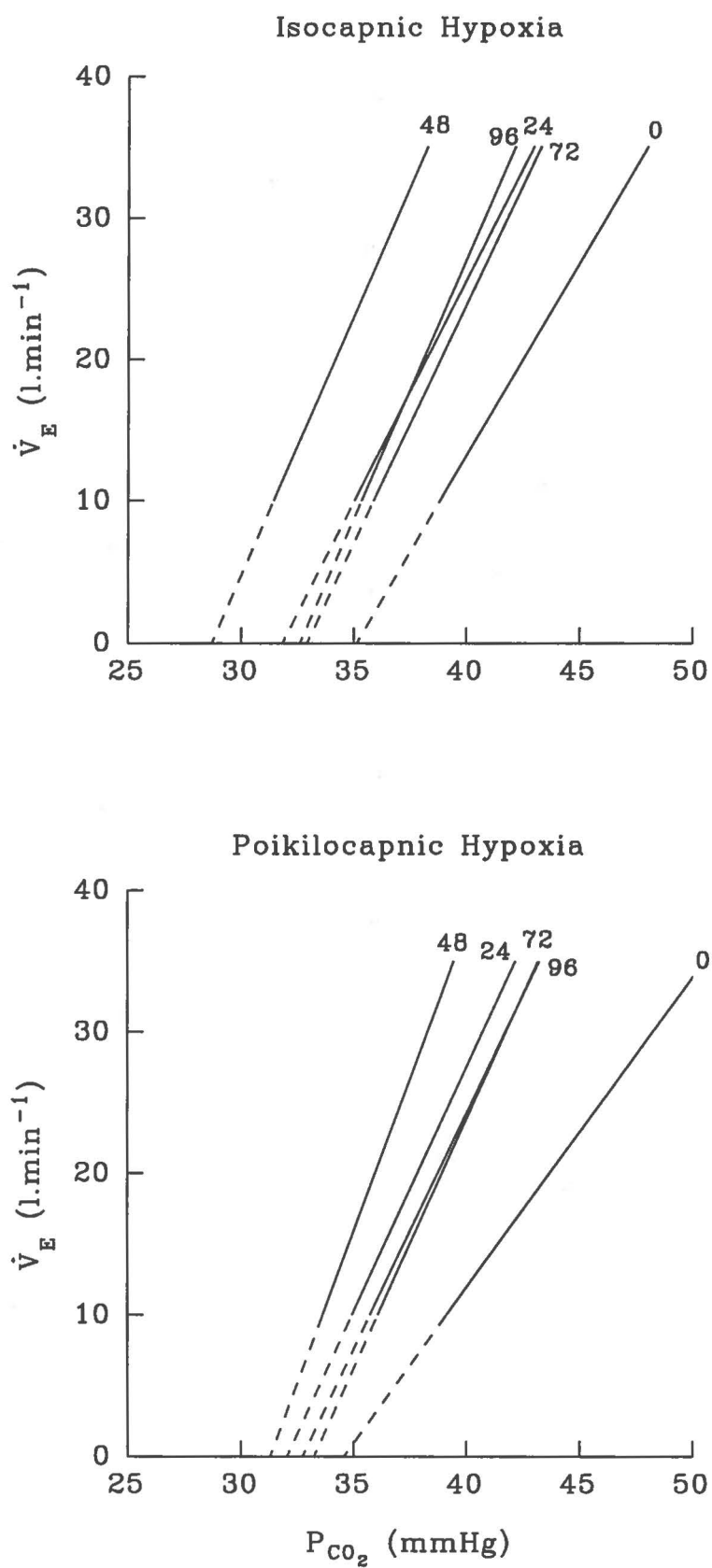


Figure 6.30: AHCVR calculated from the mean slope and x-axis intercept throughout both protocols. (Upper panel, isocapnic hypoxia; lower panel, poikilocapnic hypoxia.)

DISCUSSION

The purpose of this study was to examine the changes in ventilation and respiratory control during exposure to 48 hours of hypoxia, in both the isocapnic and poikilcapnic states, and during a further 48 hours following exposure. A 48-hour exposure was chosen in order to monitor the changes over a time course during which a reasonable degree of ventilatory acclimatization to the hypoxia of altitude (VAH) becomes apparent. Generally accepted signs of VAH are a gradual increase in ventilation and gradual decreases in P_{aCO_2} and arterial and standard $[HCO_3^-]$. Indeed, the poikilcapnic exposure of this study (akin to altitude) elicited these indicators of VAH. However, the isocapnic exposure, in which there were no changes in P_{ETCO_2} , pH or $[HCO_3^-]$, produced a striking increase in ventilation which, like the poikilcapnic exposure, showed no evidence of a clear plateau. Furthermore, during both exposures, eupnoeic ventilation gradually increased and then fell off throughout the recovery period.

In brief, further to these changes, hypoxic sensitivity and ventilation baseline, when measured by rapid square wave inputs of hypoxia, increased during both hypoxic exposures and tailed off during recovery at a slower rate. These changes were similar in both protocols when measured at the same level of CO_2 (P_{CO_2} -matched). However, when measured at the same level of arterial pH (pH-matched), the ventilation baseline remained almost unchanged during the poikilcapnic exposure and associated recovery, but increased nevertheless in the isocapnic exposure with a subsequent decline during the recovery. Hypoxic sensitivity increased during both types of exposure and decreased subsequently when measured by the pH-matched method. The changes were similar in both protocols, although the isocapnic protocol may have produced slightly larger increases: ANOVA showed that the isocapnic and poikilcapnic protocols almost resulted in different distributions of G_p . However, the anomalous result detected in subject 970 may have interfered with the analysis, particularly as the number of cases was so small. The magnitude of the increase in hypoxic sensitivity during both exposures in each individual was thought to be positively related to the measure

of hypoxic sensitivity before the exposures and, interestingly, the ventilation at 48 hours was also shown to be correlated with the hypoxic sensitivity (both at 0 and 48 hours) in the isocapnic protocol.

No significant changes were detected in the hypercapnic response, although the plots suggest that with more trials, some may become apparent. Interestingly, despite any changes in arterial acid-base parameters, the CO_2 response was shifted leftward/upward in the isocapnic exposure with a small change in the slope. This reversed, although more slowly, during the recovery from hypoxia. The poikilocapnic exposure produced a smaller shift in the curve but did result in a larger increase in slope. Again, this was reversed during recovery, but at a slower rate.

No significant difference in the level of reported sickness was detected between the protocols. However, the sickness scores were 50% greater during the isocapnic exposure, which would indicate that the addition of CO_2 to prevent the respiratory alkalosis does not alleviate the symptoms, but may in fact aggravate them. This is the only known study to show this in humans and it helps confirm the notion based on behavioural studies in goats, that acute mountain sickness may be related to cerebral blood flow and oedema (Krasney, 1994; Yang *et al.*, 1994). These factors would be potentiated by isocapnia (Ellingsen *et al.*, 1987).

Periodic breathing was only observed in one subject during the first night of poikilocapnic hypoxia. However, this breathing pattern was prevented in the isocapnic exposure, illustrating the importance of chemical control in the genesis of periodic breathing. Similar to the findings relating to mountain sickness, this study is again a novel one in that it is able to produce such data. These two areas of research clearly merit further investigation.

Control of arterial parameters. End-tidal Po_2 throughout both exposures and end-tidal Pco_2 during the isocapnic exposure were well maintained. A Po_2 of 55 mmHg was chosen for

subject 964, since he was found to be relatively insensitive to acute changes in hypoxia and a report by Reeves *et al.* (1993) suggested that AHVR at sea level is inversely related to VAH. The others were studied at 60 mmHg. Arterial O₂ saturation (SaO₂), which provides a good index for PaO₂, was well controlled, except for a period of 6 hours during the first night of poikilocapnic exposure. Since a routine had been incorporated into the controlling program to maintain arterial saturation during periodic breathing (p. 3.16) and moreover, this phenomenon of altered breathing pattern was only detected in one subject, it is unlikely that this mode of respiration was responsible for the difference between alveolar Po₂ and arterial Po₂ (measured as saturation). It is also unlikely that the Bohr Shift is responsible since this fall does not occur during the second night. It is probable that this decrease in SaO₂ resulted from an increase in ventilation-perfusion mismatch during the first night of exposure. Arterial pH and [HCO₃⁻] (and standard [HCO₃⁻]) were well maintained during isocapnic hypoxia, indicating that overall control of the P_{ET}CO₂ was appropriate.

Ventilation. Chapter 4 described how ventilation was seen to increase dramatically over 8 hours of isocapnic hypoxia, when ventilation remained unchanged over the same duration in poikilocapnic hypoxia. In this study, one aim was to examine whether this rise in ventilation during isocapnic hypoxia continued over 48 hours and thus into the period when VAH is known to occur at altitude in order to determine whether this rise was involved in the acclimatization process.

The current study did demonstrate a continuation of the rise in ventilation subsequent to 8 hours. In Chapter 4, two hypotheses were presented to account for the rise in ventilation during isocapnic hypoxia. The first hypothesis proposed that hypoxia was having a direct effect on the respiratory control system and the second was that elevated central nervous system (CNS) excitability resulting from an initial higher level of ventilation was responsible for the increase in ventilation.

Chapter 5 provided results to favour the first hypothesis over the 8-hour time course by showing that the ventilatory responses to rapid square wave and sawtooth changes in hypoxia were similar between the isocapnic and poikilocapnic hypoxic exposures. In particular, it was thought that an augmented hypoxic sensitivity was responsible for the rise in ventilation during isocapnic hypoxia. Other investigations, principally in the goat (described in Chapters 1, 4 and 5), suggest that an increase in the ventilatory sensitivity to acute changes in hypoxia and, in particular, an increase in carotid body function are responsible for the rise in ventilation during isocapnic hypoxia. The fact that the ventilation continues to rise in the isocapnic protocol over 48 hours suggests that an increase in hypoxic sensitivity of the peripheral chemoreflex may play a part in VAH at altitude/poikilocapnic hypoxia. However, it is necessary to test whether the first hypothesis of an increased AHVR still applies during this prolonged period. For this reason, the ventilatory responses to acute changes in hypoxia and hypercapnia were measured, together with the eupnoeic ventilation.

Modelling of the hypoxic responses. In Chapter 5, a period of 90 sec was chosen for the hypoxic square wave and sawtooth inputs in the tests of AHVR. However, a complete plateau phase in the ventilatory response was not realized in the measurements made during and after the hypoxic exposure, therefore a period of 120 sec was selected for this study. The sawtooth input was omitted in order not to lengthen the overall duration on the mouthpiece.

Inspection of the ventilatory responses to acute hypoxia measured before the exposures suggests an almost complete plateau phase in ventilation. However, the remaining ventilatory responses (up to 96 hours) do not show this clear plateau. Chapter 5 suggested two explanations for this. The first was a change in the dynamics of the response and the second was the development of an additional phase of the ventilatory response to hypoxia. It is not clear from the longer duration square waves of this study which of these apply and further experimentation is still required.

Results obtained from data calculated using fitting procedure A were not different from those using data acquired via procedure B, indicating that appropriate estimates of time delay and time constant were fit to the ventilatory response profiles using procedure A, where individual measurements of AHVR were fit.

Changes in respiratory control. Measurements of AHVR were made under two conditions of P_{CO_2} , P_{CO_2} -matched and pH-matched, where in the latter, P_{CO_2} was adjusted in order to elicit the same arterial pH as in the control experiment at $t=0$. The P_{CO_2} -matched measurements were made to monitor any raw changes in the acute hypoxic response and the pH-matched tests were performed in order to minimize any differences in the interaction of H^+ and PO_2 at the carotid body between measurements.

HYPOXIC SENSITIVITY IN AHVR. As in the study presented in Chapter 5, the hypoxic sensitivity, G_p , increased in both protocols during hypoxic exposure. Furthermore, the changes were similar in both protocols, implying that they were caused by a direct effect of hypoxia. In addition, the increase in hypoxic sensitivity appeared to be almost complete by 24 to 48 hours. The recovery from hypoxic exposure elicited similar declines in hypoxic sensitivity in both protocols, further establishing the proposition that hypoxia was directly effecting changes in the acute hypoxic response mechanism, which, in turn, were acting as a principal cause of the rise in ventilation during the isocapnic protocol. This hypothesis is further supported by the observation that there is a correlation within individuals between the ventilation measured after 48 hours of isocapnic hypoxia and the hypoxic sensitivity measured at 0 and 48 hours. That the rise in hypoxic sensitivity was proportional to the control hypoxic sensitivity suggests that the mechanism responsible for this rise is closely related to the mechanism involved in the hypoxic response *per se* (i.e., the peripheral chemoreflex).

P_{CO_2} -matched and pH-matched measurements of AHVR showed the same pattern of change, confirming the hypothesis that hypoxia is directly affecting hypoxic sensitivity. However,

there is the possibility that hypoxic sensitivity was slightly higher in the isocapnic protocol when the arterial blood was pH-matched. This suggests the involvement of another factor in the rise in G_p . This should be discussed in conjunction with $\dot{V}_C$ and the CO_2 response.

VENTILATION BASELINE IN AHVR. When measured by the P_{CO_2} -matched method, the hypoxia-independent ventilation or ventilation baseline increased by similar amounts during hypoxia and decreased by similar amounts during recovery. Moreover, these changes followed comparable time courses. pH-matched measurements of AHVR resulted in lower values of ventilation baseline being detected in both isocapnic and poikilocapnic protocols. Poikilocapnic hypoxia produced minimal changes in $\dot{V}_C$, whereas isocapnic hypoxia still elicited a large increase in this parameter. This suggests that $\dot{V}_C$ seems to be very closely related to pH and yet, the P_{CO_2} -matched measurements of AHVR provide better matching of pH at the site of the central chemoreceptors. The pH-matched protocols resulted in poor matching of the central stimulus (*i.e.*, lower in the poikilocapnic protocol), which would account for the difference in $\dot{V}_C$. The simplest conclusion to draw from these observations is that hypoxia *per se* is producing these changes in $\dot{V}_C$ at the site of the central chemoreceptors. Previously, it has been suggested that cerebrospinal fluid (CSF) $[HCO_3^-]$ falls during acclimatization to hypoxia as a result of metabolic compensation of the respiratory alkalosis (Dempsey and Forster, 1982). However, since there was no such alkalosis in the isocapnic protocol as the feedback loop of ventilation on arterial P_{CO_2} had been opened, then these postulated changes in acid-base status of the CSF are not appropriate. It appears that any cause of changes at the site of the central chemoreceptors would be related more directly to hypoxia than to systemic acid-base disturbances. There are 4 possible mechanisms that may account for this phenomenon. First, there may be a change involving the transduction mechanism of the central chemoreceptors, which would result in a reduction of the CO_2 "set point" of the central chemoreflex or a decrease in B (as defined by the Lloyd-Cunningham equation (Cunningham *et al.*, 1964)). Secondly, the excitability of the respiratory controller, and not the central chemoreceptors, may be augmented. These first two mechanisms come under the general title of hypoxia-induced increased CNS

excitability. Thirdly, hypoxic lacticacidosis may be causing an increase in $[H^+]$ in the local environment of the central chemoreceptors (Dempsey and Forster, 1982; Fencel *et al.*, 1979; Musch *et al.*, 1981). Moreover, this may be effecting the alterations in the set point of the central chemoreflex discussed above. Finally, changes in the dynamics of the ventilatory response to the hypoxic square waves may be producing erroneous measurements of $\dot{V}_C$. This latter mechanism is unlikely, however, since the CO_2 response, measured under steady-state conditions of PO_2 , was shifted leftward/upward.

An argument could exist that interaction between the central chemoreceptors and the peripheral chemoreflex may produce a spurious increase in hypoxic sensitivity as a result of the elevated $\dot{V}_C$. However, since the difference in $\dot{V}_C$ between the P_{CO_2} -matched and pH-matched tests of AHVR did not result in a large change in G_p , then an interaction effect would be too small to be responsible for the rise in G_p in isocapnic hypoxia. Assuming, therefore, that the increase in G_p during isocapnic hypoxia is a genuine increase in peripheral chemoreflex sensitivity to hypoxia as in the poikilcapnic exposure, then an interaction between $\dot{V}_C$ and the peripheral chemoreflex may be causing the small difference in G_p between the two types of exposure, as measured by the pH-matched protocol. More importantly, the rise in $\dot{V}_C$ may be partly responsible for the rise in ventilation during the isocapnic exposure in addition to the increase in hypoxic sensitivity.

HYPERCAPNIC RESPONSE. The changes in the ventilation baseline can be further examined with the hypercapnic response. During isocapnic hypoxia, there was a leftward/upward shift in this response with a small change in slope despite no change in arterial pH or $[HCO_3^-]$. This corresponds with the rise in $\dot{V}_C$ during this protocol and suggests a change at the central chemoreceptor/respiratory controller level. However, an increase in the excitability of the respiratory controller might be expected to be associated with an increase in the slope of AHCVR. In the poikilcapnic exposure, the CO_2 response was also displaced to the left with an additional increase in slope. This is commensurate with a decrease in the CO_2 set point at the intracranial chemoreceptors, either as a result of a change within the cells relating to the

transduction mechanism or outside the cells relating to an increase in $[H^+]$ via hypoxic lacticacidosis or a decrease in $[HCO_3^-]$ due to metabolic compensation of the respiratory alkalosis.

EUPNOEIC VENTILATION. The eupnoeic ventilation and P_{CO_2} confirm all of the above observations. On relief of isocapnic hypoxia, the increased central ventilatory drive results in an increase in ventilation and a decrease in P_{CO_2} relative to control. On relief of poikilocapnic hypoxia, the loss of hypoxic drive tends to result in a slight increase in P_{CO_2} ; however, ventilation remains elevated and P_{CO_2} depressed relative to control since the CO_2 response curve has been shifted to lower levels of P_{CO_2} .

Comparison with other studies in humans. The time duration examined in the current study during isocapnic hypoxia has only been studied once previously in humans by Cruz *et al.* (1980). They examined 100 hours of both isocapnic and poikilocapnic hypoxia. They observed no such trend in ventilation after 75 hours of isocapnic hypoxia as shown in the present report. However, in the poikilocapnic exposure, where CO_2 was left uncontrolled, they demonstrated a progressive rise in ventilation from 8.5 l.min^{-1} to 12.0 l.min^{-1} at 75 hours at an alveolar PO_2 of *ca.* 60 mmHg. They reported no change in AHVR during 75 hours of isocapnic hypoxia, but a marked increase during poikilocapnic hypoxia. However, there are two reasons why their results should be treated with caution. First, the gas control was poor throughout the exposure as discussed in Chapters 4 and 5. Secondly, AHVR did double during the isocapnic protocol on examination of their data, but the statistical test was not suitable for detecting this change. Their measurements of AHCVR showed no significant increase in slope in either protocol but showed instead a drop in the x -axis intercept of the CO_2 response curve in both exposures; however, this was only significant in the poikilocapnic group. This latter phenomenon conflicts with the current study, but their poor gas control may have contributed largely to this. In addition, their results were not paired since they divided 8 subjects into two groups and made comparisons between the groups.

Eger *et al.* (1968) also studied exposure to both isocapnic and poikilocapnic hypoxia in humans, but only over an 8-hour period. They did not measure AHVR, but did report on AHCVR and eupnoeic ventilation. They found a leftward shift in both types of hypoxia and even in hypercapnic hypoxia, although the shift was greatest in poikilocapnic hypoxia and least in hypercapnic hypoxia. Furthermore, they demonstrated an increase in eupnoeic ventilation in all modes of hypoxia compared with euoxia, under the same conditions of P_{CO_2} . These results are consistent with the observations of the current study, and further that long-term hypoxia can affect hypoxia-independent ventilation. However, they also illustrate the role of long-term P_{CO_2} in determining AHCVR and therefore, in the control of the central chemoreflex. Interestingly, they showed, in two subjects tested, an increase in eupnoeic ventilation after 8 hours of voluntary isocapnic hyperventilation at $30 \text{ l}\cdot\text{min}^{-1}$. This is consistent with the phenomenon of hyperventilation-induced hyperpnoea (Smith *et al.*, 1962a,b).

No studies have been performed on the recovery from isocapnic hypoxia.

In contrast with isocapnic hypoxia, many studies have focused on changes in ventilation and respiratory control during poikilocapnic hypoxia/altitude. Ventilation has been reported to rise over a period of weeks at altitude (Douglas *et al.*, 1913) and at simulated altitude (Åstrand, 1953) with a concurrent drop in P_{aCO_2} . In addition, these phenomena have been shown to take place while CSF pH remains alkaline or even increases (Dempsey *et al.*, 1974; Forster *et al.*, 1975; Weiskopf *et al.*, 1976), suggesting that an alternative increased ventilatory drive other than the central chemoreceptors has a role in VAH. However, it has become accepted that local acid-base changes can take place at the intracranial chemoreceptors and not be reflected in the bulk CSF, for example, as a result of hypoxic lacticacidosis (Dempsey and Forster, 1982).

Four studies at altitude or in a hypobaric chamber have investigated respiratory control and shown that AHVR increases progressively with time (Goldberg *et al.*, 1992; Sato *et al.*,

1992,1994; White *et al.*, 1987). Goldberg *et al.* (1992) only took measurements before and after exposure, but the other 3 studies reported on measurements at regular intervals at altitude and all showed AHVR to be still increasing at the end of the sojourn: the longest of these 3 studies was 12 days (Sato *et al.*, 1994). This conflicts with the evidence from the present investigation that the rise in AHVR starts to reach a plateau by 48 hours. Naturally, the present report cannot confirm that the rise in hypoxic sensitivity does not continue to rise over 12 days and it is possible that the current study does not witness complete VAH, although Sato *et al.* claimed that VAH was almost complete in their study by 48 hours (Sato *et al.*, 1994). However, it is thought that HCO_3^- continues to be excreted by the kidneys over this time course (Forster *et al.*, 1975). It is possible that the results of Sato *et al.* (1992,1994) and White *et al.* (1987) can be linked with the effect of metabolic compensation of $[\text{HCO}_3^-]$. By employing an inappropriate level of P_{ETCO_2} at which to conduct the measurements of AHVR, the investigators may not have elicited the correct time course of the changes in hypoxic sensitivity. Sato *et al.* used a level of P_{ETCO_2} that induced an inspired ventilation of $140 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ at a $\text{P}_{\text{ETO}_2} > 150 \text{ mmHg}$ ($\text{P}^*_{\text{CO}_2}$). This would have corrected for any central changes in respiratory drive; however, arterial $[\text{HCO}_3^-]$ is thought to fall over days to weeks, whereas central respiratory drive alters more rapidly (Dempsey and Forster, 1982). In the early stages of the altitude sojourn, therefore, $\text{P}^*_{\text{CO}_2}$ would have produced an arterial alkalosis and as a consequence an underestimation of the hypoxic sensitivity due to interaction between H^+ and Po_2 at the carotid body. In the latter stages of the exposure, arterial $[\text{HCO}_3^-]$ would still be falling and thus producing further increases in hypoxic sensitivity. White *et al.* used hyperoxic P_{ETCO_2} , but it is not clear how this would affect arterial pH. Nonetheless, neither protocol attempted to normalize arterial pH for the tests of AHVR and this would have interfered with the time course of the observed rise. The current study did produce arterial pH-matched data which showed that the rise in hypoxic sensitivity slowed dramatically after 24 hours in both hypoxic exposures.

Only the two studies by Sato *et al.* followed AHVR during deacclimatization. Both found that the fall in hypoxic sensitivity immediately followed descent to sea level, which is

compatible with the present study. The speed of the decline varied between the two studies, being faster in the 12-day study (Sato *et al.*, 1994) compared with the 3-day sojourn (Sato *et al.*, 1992) and reaching control levels within 4 days.

In a study by Moore *et al.* (1986), ventilatory acclimatization to 4.5 hours of hypobaric hypoxia was found to be positively related to ventilatory responsiveness to acute isocapnic changes in hypoxia measured before the exposure. This observation was confirmed over 19 days at altitude (Reeves *et al.*, 1993). As discussed earlier, this phenomenon was noted in the isocapnic protocol of the current study and not the poikilocapnic protocol. However, Fig. 6.29 does suggest a correlation in the poikilocapnic protocol between AHVR at $t=0$ and ventilation at the end of the exposure, despite the correlation coefficient not reaching significance. The small number of trials and/or the lower drive to breathe in the poikilocapnic state, producing greater errors in measurement of true ventilation caused by differing levels of consciousness of subjects, may have rendered the correlation insignificant. The above studies would suggest that there is indeed a positive correlation and imply that VAH may be related to the peripheral chemoreflex. Compatible with this proposition is that the current study also demonstrates a correlation between AHVR at $t=0$ and $t=48$ hours in both protocols.

AHCVR has also been widely studied at altitude/poikilocapnic hypoxia. That the CO_2 response curve shifts leftward during hypoxia remains unchallenged (Åstrand, 1953; Eger *et al.*, 1968; Forster *et al.*, 1971; Kellogg *et al.*, 1957; Michel and Milledge, 1963; Sato *et al.*, 1992,1994; White *et al.*, 1987). The current study did not show a significant shift; however, a longer duration study and/or more cases may have produced a significant result. Sato *et al.* (1992,1994) indirectly showed a shift of the hypercapnic response towards control levels on descent from altitude, consistent with this study.

Severinghaus *et al.* (1963) proposed that a leftward shift of the CO_2 response curve caused by a fall in CSF $[\text{HCO}_3^-]$, and thus a change in $[\text{H}^+]$ at the intracranial chemoreceptors, would

be associated with an increase in the slope of the curve on the basis of the Henderson-Hasselbalch equation (p. 1.17). An increase in the slope has been demonstrated in a number of studies (Forster *et al.*, 1971; Michel and Milledge, 1963; Sato *et al.*, 1992,1994); some reports do not confirm this (Cruz *et al.*, 1980; White *et al.*, 1987) and one remains equivocal (Eger *et al.*, 1968). However, all the studies did show some increase in slope, but not all reached significance. Sato *et al.* (1992,1994) chose to correct the slope of the response curve by the fall in ambient P_{ETCO_2} to account for the use of P_{CO_2} instead of $\log_{10}(P_{CO_2})$ or CSF pH. After correction, both studies showed a rise in slope, however, only the first study showed any significant change, which occurred in one measurement (Sato *et al.*, 1992). It is thus possible that there is some increase in CNS excitability or a local additional acidic stimulus due to the presence of lactic acid, but the data are not convincing enough to confirm this. However, since the fall in CSF $[HCO_3^-]$ is thought not to reflect the stimulus at the chemoreceptor site, it may be that CNS excitability and/or lacticacidosis are the relevant parameters in this change in AHCVR (Dempsey and Forster, 1982). The fact that hypoxia has been shown not to alter bulk CSF $[HCO_3^-]$ (Dempsey *et al.*, 1975), but rather brain P_{CO_2} has (Dempsey *et al.*, 1978), yet prolonged hypoxia has been shown to alter AHCVR (Eger *et al.*, 1968) might suggest that hypoxia is having a local effect at the site of the central chemoreceptors/respiratory controller. As discussed earlier, that the slope of AHCVR does not appear to rise significantly, yet undergoes a leftward shift, suggests that the respiratory controller is not involved.

Comparison with other studies in animals. Studies of this time course have been conducted in conscious sheep (Krasney *et al.*, 1984,1985), although acclimatization in sheep is known to take much less time. Ventilation was found to be maximal after 24 hours in the isocapnic study (Krasney *et al.*, 1985) and after 24 to 48 hours in the poikilocapnic study (Krasney *et al.*, 1984). However, since only 3 measurements of ventilation were made during the first 48 hours it is hard to decipher the time course of changes. Engwall and Bisgard (1990) have reported on the effects of both isocapnic and poikilocapnic hypoxia on ventilation measured at regular short intervals, AHVR and AHCVR over an almost complete time course of

ventilatory acclimatization to hypoxia in the conscious goat (4 hours). As in the current study, they demonstrated that ventilation rose dramatically and stabilized during isocapnic hypoxia. In addition, the poikilocapnic exposure produced a significant drop and subsequent stabilization of P_{aCO_2} indicative of almost complete acclimatization. Moreover, this report described similar increases in the hypoxic sensitivity at the end of both hypoxic exposures, as in the present investigation. They suggested that the rise in hypoxic sensitivity was responsible for the change in ventilation during isocapnic hypoxia and the primary cause of VAH in the poikilocapnic exposure. Furthermore, studies in the goat in which the carotid bodies have been isolated from the systemic circulation have found an increase in hypoxic sensitivity of the carotid bodies as a result of perfusion with isocapnic hypoxic blood separate from the systemic circulation (Bisgard *et al.*, 1986b; Busch *et al.*, 1985).

A study of isolated CNS isocapnic hypoxia in the conscious goat (Weizhen *et al.*, 1992) elicited no change in respiratory control (AHVR, AHCVR and eupnoeic ventilation) indicating no alteration in CNS sensitivity or acid-base status as a result of direct central hypoxia, although ventilation did rise over 30 min by a small, yet significant, degree. Furthermore, Xu *et al.* (1991, 1992) could not demonstrate any stimulation of ventilation by isocapnic hypoxic acidification of the central chemoreceptor site in awake goats or anaesthetized cats. However, goats do not exhibit persistent hyperventilation following whole body isocapnic hypoxia (Engwall and Bisgard, 1990), unlike man in the current study and that of Eger *et al.*, (1968), and thus, central hypoxic lacticacidosis remains a possible explanation for the changes in $\dot{V}_C$ in this study.

Investigations in ponies (Forster *et al.*, 1976; Orr *et al.*, 1975) and dogs (Bureau and Bouverot, 1975) at altitude have shown that ventilation rises over days to weeks with an alkaline shift in CSF pH. This, as in the human studies (Dempsey *et al.*, 1974; Forster *et al.*, 1975; Weiskopf *et al.*, 1976), suggests changes in respiratory control that are not dependent on metabolic compensation of CSF pH. Studies in the goat and cat have demonstrated increases in AHVR during poikilocapnic hypoxia that appear to be dependent upon elevated

carotid body function (Barnard *et al.*, 1987; Engwall and Bisgard, 1990; Vizek *et al.*, 1987b). Moreover, these studies suggest that increased AHVR is of primary importance in the rise in ventilation in VAH when combined carotid body studies (Bisgard *et al.*, 1986a,b; Busch *et al.*, 1985; Weizhen *et al.*, 1992) and carotid body afferent discharge investigations (Engwall *et al.*, 1988; Nielsen *et al.*, 1988).

Bisgard *et al.* (1986b) demonstrated that the persistent hyperventilation of deacclimatization from hypoxia was dependent upon a systemic respiratory alkalosis during the acclimatization period, which they presumed was acting through a decrease in $[\text{HCO}_3^-]$ at the intracranial chemoreceptors. However, some experiments have provided evidence to suggest that a reduction in Pco_2 can produce lacticacidosis in rat whole brain or cortex and brainstem (Musch *et al.*, 1981; Weyne *et al.*, 1977) and it may be that CSF $[\text{HCO}_3^-]$ is not the relevant parameter, but Pco_2 producing lacticacidosis, in creating the observations of Bisgard *et al.* (1986b). Experiments by Fencil *et al.* (1979) estimated in the acclimatizing goat that there was an acidotic shift (-0.07 U) in cerebral interstitial fluid pH, and thus intracranial chemoreceptor extracellular fluid pH, in the face of an alkaline bulk CSF pH. However, doubts have been raised as to the contribution of this phenomenon to VAH as a result of reports in the acclimatizing rat showing that the time courses of cerebral lactic acid production and the rise in ventilation at altitude do not correlate (Musch *et al.*, 1983). However, the current study is not claiming that hypoxic lacticacidosis is involved in VAH, but that it may be a mechanism responsible for the rise in hypoxia-independent ventilation observed during tests of AHVR and in eupnoeic ventilation. However, Musch *et al.* (1981) and Weyne *et al.* (1977) both demonstrated that lactate reaches its maximum value at 2 hours and this also does not correlate with the changes in $\dot{V}_C$ in the current study.

Low level carbon monoxide, resulting in hypoxia insufficient to stimulate the peripheral chemoreceptors, has been shown to produce a slight rise in ventilation in the awake pony (Lowry *et al.*, 1994) indicating that central hypoxia may increase ventilatory drive and thus elicit a change in ventilation baseline and the CO_2 response as in the present study. The

authors suspected that this was caused by a CNS mechanism, independent of changes in the acid-base environment of the intracranial receptors. Interestingly, this hyperventilation was more marked than the rise in ventilation produced by more severe hypoxic hypoxia in a previous study on carotid body-denervated ponies (Brown *et al.*, 1992). This suggests that carotid body input may be important in alterations of CNS excitability. This is compatible with other studies which support the notion that peripheral chemoreflex stimulation may result in persistent hyperventilation under euoxic conditions (Millhorn *et al.*, 1980a,b; Tenney *et al.*, 1971). However, hypoxia may also be having a direct effect on CNS excitability (Gallman and Millhorn, 1988; Ou *et al.*, 1983; Sato, 1990).

Conclusions. The results of this study suggest that hypoxia *per se* causes a progressive time-dependent rise in acute hypoxic sensitivity which appears to be almost complete by 24 to 48 hours. The findings also suggest that prolonged hypoxia leads to an increase in hypoxia-independent ventilation. Changes in both hypoxic sensitivity and hypoxia-independent ventilation are postulated to be responsible for the progressive rise in ventilation during the isocapnic hypoxia. In addition, the hypoxia-induced changes in acute hypoxic sensitivity probably play a major role in the rise in ventilation during poikilocapnic hypoxia over the observed time course. However, changes in hypoxia-independent ventilation cannot be ruled out of taking part in these ventilatory changes. Although both the changes in $\dot{V}_C$, and thus central respiratory drive, in the pH-matched measurements of AHVR and the changes in eupnoeic ventilation are small, they may represent a reasonable proportion of the increased ventilatory drive leading to the relatively larger changes in ventilation during the poikilocapnic exposure. Nevertheless, this study demonstrates that hypoxia *per se* is a significant factor during the first two days of ventilatory acclimatization to hypoxia at altitude and not the acid-base changes associated with hypoxic exposure.

It is thought that the increase in hypoxic sensitivity is due to an increase in peripheral chemoreflex sensitivity, and more specifically, an elevation of carotid body function. The mechanism responsible for the rise in hypoxia-independent ventilation is harder to elucidate.

Hypoxic lacticacidosis is a possible phenomenon that could account for this observation, however, the expected time course of the rise and fall of lactate at the intracranial chemoreceptors does not correlate with the changes in this parameter. Furthermore, hypoxic lacticacidosis has been shown not to produce any stimulation of ventilation in animals (Xu *et al.*, 1991,1992). However, in man, hypoxia has been shown to act synergistically with hypocapnia (induced either by voluntary hyperventilation or with a respirator) in raising eupnoeic ventilation and lowering the set point of the central chemoreflex, as in the current study (Dempsey *et al.*, 1975; Eger *et al.*, 1968): it may be that hypoxia is producing changes in the acid-base environment of the central chemoreceptors. However, this study cannot reconcile the fact that the difference in P_{CO_2} between the two protocols does not affect the measured parameters with the fact that the above studies do, if indeed the changes in the central chemoreflex are occurring through acid-base mechanisms.

It, therefore, seems likely that hypoxia may be causing an increase in CNS excitability either directly by central hypoxia (Gallman and Millhorn, 1988; Ou *et al.*, 1983; Sato, 1990) or indirectly through a persistent increase in peripheral chemoreflex stimulation (Millhorn *et al.*, 1980a,b; Tenney *et al.*, 1971). (This latter theory relating to increased peripheral chemoreflex activity might account for the similarity in G_p and $\dot{V}_C$ during both acclimatization and deacclimatization.) It is unlikely that the excitability of the respiratory controller is being altered, since the slope of the CO_2 response appears not to be elevated in the current study and in others, when corrected using $\log_{10}P_{CO_2}$ instead of P_{CO_2} (Sato *et al.*, 1992,1994). The central chemoreceptors are therefore probably the site of the increased excitability, and this may be manifested as a decrease in their set point or B . This is proposed to account for the changes in $\dot{V}_C$, AHCVR and eupnoeic ventilation during acclimatization and deacclimatization, in both protocols.

There is very little doubt that CSF and arterial $[HCO_3^-]$ does fall in time at altitude (Dempsey and Forster, 1982) and this may have become apparent over a longer time course, manifested as a further increase in ventilation beyond any changes in hypoxic sensitivity. Clearly,

longer experimentation is still further required in order to clarify the role of acid-base changes, although this study does suggest that hypoxia is the major factor in VAH during the first 48 hours and during deacclimatization from this period.

APPENDIX 6.1.

This appendix presents the acute mountain sickness score sheet to record the absence or severity of symptoms of sickness throughout both hypoxic exposures.

Acute Mountain Sickness Score SheetName:Date:Protocol:

Time/hrs	Anorexia	Nausea	Headache	Lethargy	Oedema
0					
4					
8					
12					
24					
28					
32					
36					
48					

Scoring Scheme :-

<i>Anorexia</i>	<i>None</i>	<i>0</i>
	<i>Not v. hungry</i>	<i>1</i>
	<i>Manage a few mouthfuls</i>	<i>2</i>
<i>Nausea</i>	<i>None</i>	<i>0</i>
	<i>Nausea</i>	<i>1</i>
	<i>Vomiting</i>	<i>2</i>
<i>Headache</i>	<i>None</i>	<i>0</i>
	<i>Mild</i>	<i>1</i>
	<i>Severe</i>	<i>2</i>
<i>Lethargy</i>	<i>None</i>	<i>0</i>
	<i>Hard to work</i>	<i>1</i>
	<i>Cannot work</i>	<i>2</i>
<i>Oedema</i>	<i>None</i>	<i>0</i>
	<i>Mild (fingers)</i>	<i>1</i>
	<i>Severe (facial)</i>	<i>2</i>

APPENDIX 6.2.

This appendix describes the calculation of values for arterial pH and $[\text{HCO}_3^-]$ from the measured values of venous pH, $[\text{HCO}_3^-]$, oxygen saturation and [haemoglobin], and the P_{ETCO_2} . The calculations are based upon the observation that there is a linear relationship between pH and $[\text{HCO}_3^-]$ (Davenport diagram) in the blood in response to changes in P_{CO_2} (Michel *et al.*, 1966):

$$\text{pH} = A + B.[\text{HCO}_3^-]. \quad (1)$$

The term buffer line is used to describe this relationship. The strategy is first to quantify the venous buffer line equation, which passes through the measured pH- $[\text{HCO}_3^-]$ co-ordinate. Then, from this, an equation for the arterial buffer line can be derived, by calculating the effect of changes of oxygen saturation on the values for A and B. The arterial pH can be calculated using the arterial buffer line together with the Henderson-Hasselbalch equation from the arterial P_{CO_2} (assumed to be equal to P_{ETCO_2}).

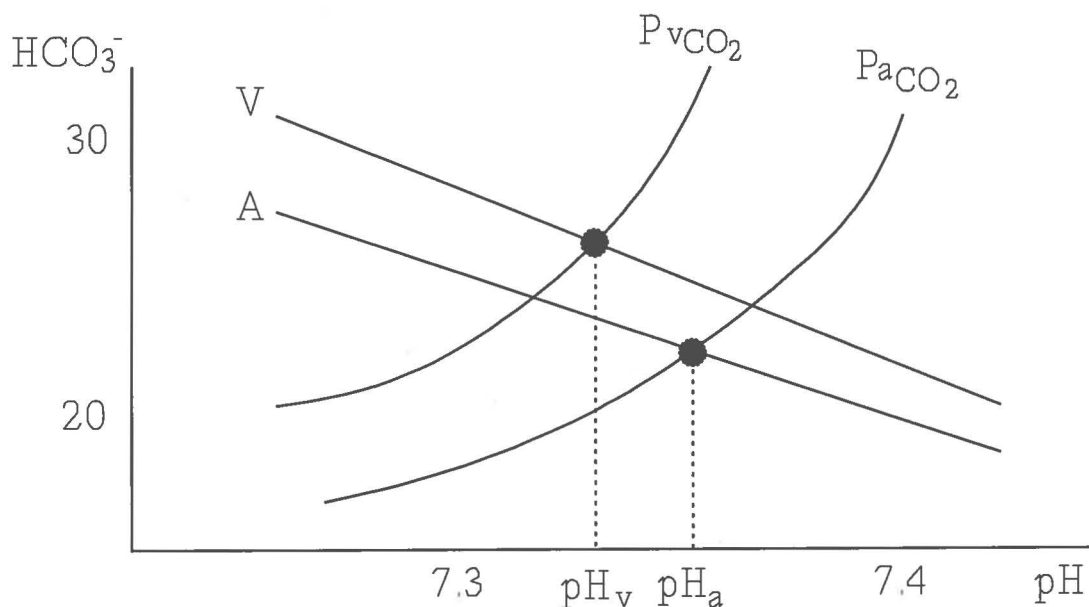


Figure 6.31: Schematic Davenport diagram showing the calculation of arterial pH. (V - venous buffer line; A - arterial buffer line.)

Venous buffer line. Differentiation of equation (1) yields:

$$B = \frac{d(pH)}{d([HCO_3^-])} \quad (2)$$

and Lloyd and Michel (1966) give an equation for the *in vitro* buffer line as follows:

$$\left(\frac{d(pH)}{d([HCO_3^-])} \right)_{in\ vitro} = -0.005 - (0.273 - 0.01 \cdot D)/[Hb], \quad (3)$$

where D is the fractional oxygen desaturation and $[Hb]$ is the concentration of haemoglobin in the blood. The intercept of the venous buffer line, A_v , can be calculated from equation (1) with the experimentally-measured values for venous pH (pH_v) and $[HCO_3^-]$ ($[HCO_3^-]_v$) as follows:

$$A_v = pH - B_v \cdot [HCO_3^-]. \quad (4)$$

Arterial buffer line. A can be described for the *in vitro* buffer line as being linearly dependent upon D where (Lloyd and Michel, 1966),

$$\frac{dA}{dD} = 0.034 + 0.004 \cdot [Hb] \quad (5)$$

and after integration this becomes

$$A_a = A_v + (0.034 + 0.004 \cdot [Hb]) \cdot \Delta D \quad (6)$$

where

$$\Delta D = D_a - D_v. \quad (7)$$

Arterial blood saturation was calculated as per Kelman (1966):

$$S = 100. \cdot \frac{(P_{O_2}' \cdot (P_{O_2}' \cdot (P_{O_2}' \cdot (P_{O_2}' + C_1) + C_2) + C_3))}{(P_{O_2}' \cdot (P_{O_2}' \cdot (P_{O_2}' \cdot (P_{O_2}' + C_4) + C_5) + C_6) + C_7)} \quad (8)$$

where,

$$P_{O_2}' = P_{aO_2} * 10^{(0.4 \cdot (pH - 7.4) + 0.06 \cdot (\log_{10} 40. - \log_{10} P_{aCO_2}))} \quad (9)$$

and

$C_1 = -67.07399$, $C_2 = 2121.401$, $C_3 = -8532.229$, $C_4 = -67.10441$, $C_5 = 2396.167$, $C_6 = -31346.26$ and $C_7 = 935960.9$.

The slope of the arterial buffer line was calculated as in equation (3) and thus the equation for the arterial buffer line is as follows:

$$pH_a = A_a + B_a \cdot [HCO_3^-]. \quad (10)$$

Calculation of arterial pH. The Henderson-Hasselbalch equation relates pH, P_{CO_2} and $[HCO_3^-]$ as:

$$pH = pK_1' + \log_{10} \frac{[HCO_3^-]}{0.03 \cdot P_{CO_2}}, \quad (11)$$

where the dissociation co-efficient $pK_1' = 6.1$. The $[\text{HCO}_3^-]$ term can be eliminated using equation (9) to give an equation only in terms of pH and arterial Pco_2 :

$$pH_a = pK_1' + \log_{10} \frac{pH_a - A_a}{B_a \cdot 0.03 \cdot Pa_{CO_2}}. \quad (12)$$

This equation was solved iteratively using a Fortran 77 program by Newton-Raphson approximation, as shown in equation (13).

$$pH_{a(n+1)} = pH_{a(n)} - \frac{f(pH_{a(n)})}{f'(pH_{a(n)})} \quad (13)$$

where,

$$f(pH_a) = 0.03 \cdot Pa_{CO_2} \cdot 10^{pH - pK_1'} - \frac{(pH_a - A_a)}{B_a}. \quad (14)$$

With a value for arterial pH, it is possible to calculate arterial $[\text{HCO}_3^-]$ by rearranging the Henderson-Hasselbalch equation (11).

The slope of the *in vivo* buffer line differs from that of the *in vitro* buffer line since the buffering capacity in the *in vivo* blood is diluted to some extent by the extracellular fluid (Michel *et al.*, 1966). However, in circulating through the lungs from the venous to the arterial side, the blood will not be in exchange with the bulk of the extracellular space and therefore, the *in vitro* buffer line is most appropriate on this occasion.

APPENDIX 6.3.

This appendix describes the calculation of an effective arterial pH (pH_a) for fully saturated blood from any arterial Pco_2 (P_{aCO_2}), using known values for pH_a and arterial $[\text{HCO}_3^-]$ ($[\text{HCO}_3^-]_a$) at a measured P_{aCO_2} (Appendix 6.2). The effective pH_a is defined as the value of pH_a that would coincide with the value of P_{aCO_2} during normal acid/base balance. These calculations are founded on the observation that there is a linear relationship between pH and $[\text{HCO}_3^-]$ in the blood in response to changes in Pco_2 (Davenport diagram) (Michel *et al.*, 1966). This relationship is termed the buffer line:

$$\text{pH} = A + B.[\text{HCO}_3^-]. \quad (1)$$

The basis of the calculation is first, to find values for A and B, and secondly, to calculate the effective pH_a using the Henderson-Hasselbalch equation.

Buffer line for 100% O_2 saturation of haemoglobin. Differentiation of equation (1) gives

$$B = \frac{d(\text{pH})}{d([\text{HCO}_3^-])}. \quad (2)$$

The *in vitro* buffer line is defined by Lloyd and Michel (1966) as

$$\left(\frac{d(\text{pH})}{d([\text{HCO}_3^-])} \right)_{\text{in vitro}} = -0.005 - (0.273 - 0.01 \cdot D)/[\text{Hb}], \quad (3)$$

where D is the fractional haemoglobin O_2 desaturation of the blood and $[\text{Hb}]$ is the haemoglobin concentration in the blood. However, the slope of the *in vivo* buffer line differs from the slope of the *in vitro* line since the extracellular fluid reduces the buffering capacity

of the blood as it circulates through the vascular system. For the case $D=0$, Michel *et al.* (1966) give

$$B_{100} = \left(\frac{d(pH)}{d([HCO_3^-])} \right)_{in\ vivo} = 1.7 \cdot \left(\frac{d(pH)}{d([HCO_3^-])} \right)_{in\ vitro}. \quad (4)$$

Thus, B_{100} can be calculated for the 100% saturated haemoglobin buffer line from equations (3) and (4). The constant A , in equation (1), can be calculated from the calculated values of pH_a and $[HCO_3^-]_a$ (pH'_a and $[HCO_3^-]'_a$) from the venous measurement (Appendix 6.2) assuming that there is a negligible difference between the arterial blood and fully saturated blood. Thus,

$$A_{100} \approx pH'_a - B_{100} \cdot [HCO_3^-]'_a, \quad (5)$$

where, A_{100} is the intercept of the buffer line on the pH_a axis and B_{100} is the buffer line for the case $D=0$.

The effective pH_a can be calculated for any P_{aCO_2} (for fully saturated blood) from the following equation (derived in Appendix 6.2; equation (12)) by iteration using a Newton-Raphson approximation:

$$pH_a = pK'_1 + \log_{10} \frac{pH_a - A_{100}}{B_{100} \cdot 0.03 \cdot P_{aCO_2}}. \quad (6)$$

APPENDIX 6.4.

In Appendix 6.2 it was described how arterial pH (pH_a) and arterial $[HCO_3^-]$ ($[HCO_3^-]_a$) were calculated from a venous sample of blood. Appendix 6.3 outlines the calculation of how a new level of P_{aCO_2} would quantitatively affect the effective pH_a of the blood. (The effective pH_a is defined as the value of pH_a that would coincide with the value of P_{aCO_2} during normal acid/base balance.) For a separate sample of venous blood, this appendix describes the calculation of a value of arterial P_{CO_2} (P_{aCO_2}') required to produce the same effective pH_a as calculated in Appendix 6.3 (pH_a'), for blood with 100% haemoglobin saturation. The procedure outlined in Appendix 6.2 is first employed to calculate the arterial parameters (at arterial $P_{O_2}=100$ mmHg), pH_a and $[HCO_3^-]_a$, pertinent to the new venous sample. The second calculation is based on two relationships, the buffer line,

$$pH = A + B.[HCO_3^-], \quad (1)$$

and the Henderson-Hasselbalch equation,

$$pH = pK_1' + \log_{10} \frac{[HCO_3^-]}{0.03 \cdot P_{CO_2}}. \quad (2)$$

The $[HCO_3^-]_a$ at pH_a' , $[HCO_3^-]_a'$, can be calculated from equation (1) thus:

$$[HCO_3^-]_a' = [HCO_3^-]_a - \frac{pH_a - pH_a'}{B_{in\ vivo}} \quad (3)$$

where $B_{in\ vivo}$ is the buffer line slope *in vivo*. Thus, P_{aCO_2}' can be calculated from equation (2):

$$P_{aCO_2}' = \frac{[HCO_3^-]_a'}{0.03} \cdot 10^{pK_1' - pH_a'}. \quad (4)$$

As in Appendix 6.3, it is assumed that there is a negligible difference in acid/base status between arterial blood at a P_{O_2} of 100 mmHg and fully saturated blood.

APPENDIX 6.5.

This appendix presents the ventilatory data collected during each individual experiment from every subject.

Protocol	Time	Ventilation (l.min ⁻¹)			
		Subject Number			
		884	910	964	970
Isocapnic Hypoxia	0.5	13.2	12.8	8.5	12.5
	2	15.8	12.8	11.9	13.1
	4	15.9	15.4	11.4	15.1
	6	15.0	13.5	12.6	12.1
	8	13.8	13.8	14.0	19.1
	10	15.7	15.0	16.0	15.4
	12	20.9	18.9	20.0	17.3
	22	16.5	22.0	23.0	16.7
	24	16.4	22.5	17.0	20.0
	26	21.0	24.0	15.2	16.8
	28	23.4	26.0	18.0	29.6
	30	20.0	25.8	18.0	32.8
	32	28.1	33.0	21.0	45.5
	34	26.1	31.6	23.0	25.6
	36	23.2	34.9	20.0	19.7
	48	25.0	30.8	23.0	37.5
Poikilocapnic Hypoxia	0.5	11.0	9.5	8.1	10.9
	2	12.0	8.9	8.4	11.5
	4	11.0	10.6	8.7	11.7
	6	12.0	11.3	8.8	14.3
	8	12.2	12.0	9.4	11.1
	10	13.0	11.5	7.7	14.6
	12	13.2	12.2	9.9	14.2
	22	13.2	11.5	9.1	10.1
	24	13.5	11.9	10.4	11.2
	26	15.0	12.2	11.6	13.3
	28	13.5	11.9	11.2	18.9
	30	13.8	11.2	12.7	14.8
	32	14.0	11.4	11.9	15.6
	34	15.9	13.0	12.0	16.5
	36	13.0	12.6	12.6	16.0
	48	15.9	11.0	11.5	15.0

Table 6.5: Ventilatory data (l.min⁻¹) calculated from the last 30 sec of a 3-min measurement period at specified times of 0.5, 2, 4, 6, 8, 10, 12, 22, 24, 26, 28, 30, 32, 34, 36 and 48 hours in both protocols.

APPENDIX 6.6.

This appendix presents the ventilatory and Pco_2 data collected during measurements of eupnoeic ventilation from every subject.

Protocol	Time	884		910		964		970	
		V_E	PCO_2	V_E	PCO_2	V_E	PCO_2	V_E	PCO_2
Isocapnic Hypoxia	0	9.1	37.1	8.4	35.7	6.4	40.6	8.0	38.2
	4	10.9	35.3	7.5	34.2	7.3	40.0	9.0	35.9
	8	12.5	38.9	8.5	34.9	8.6	41.6	10.5	38.0
	12	13.1	36.4	8.3	34.0	8.7	39.2	9.2	36.0
	24	10.6	36.0	9.0	30.8	8.8	35.5	9.5	34.2
	36	12.9	35.0	10.0	31.2	10.3	37.2	16.8	28.2
	48	11.6	34.5	9.3	31.6	10.8	33.0	11.5	32.7
	52	12.5	33.3	9.4	31.2	9.0	38.4	14.3	34.2
	56	12.7	35.0	10.0	33.0	9.7	36.0	18.0	29.2
	60	15.4	35.8	9.5	33.0	10.6	36.7	16.9	31.0
	72	12.1	33.5	8.8	35.0	10.0	37.4	12.2	35.3
	84	12.1	35.6	7.9	35.2	9.2	37.9	16.7	36.7
	96	12.1	36.3	8.0	35.8	9.2	37.6	12.2	34.2
Poikilocapnic Hypoxia	0	9.8	36.2	8.4	34.3	7.5	36.8	8.7	36.8
	4	9.5	36.7	7.9	34.7	7.5	36.5	9.4	35.0
	8	11.0	36.1	8.7	35.2	7.6	39.6	10.1	36.9
	12	10.9	33.4	8.9	33.5	7.3	39.8	9.5	34.6
	24	10.5	32.5	8.7	30.1	7.8	37.5	10.0	33.0
	36	12.6	32.0	9.3	30.4	9.3	37.3	11.8	32.5
	48	11.1	31.7	8.2	30.3	8.0	36.0	11.5	31.6
	52	12.5	32.8	9.6	30.5	9.3	37.6	12.0	33.2
	56	13.9	33.5	10.1	32.8	9.1	34.8	13.3	34.4
	60	13.8	35.0	9.4	32.0	7.9	39.7	13.2	34.0
	72	11.4	34.1	9.3	34.5	7.9	37.4	10.2	35.5
	84	12.3	34.7	8.5	33.9	8.9	37.0	11.5	35.8
	96	10.5	34.6	8.7	34.8	8.0	38.3	9.3	36.6

Table 6.6: Ventilatory data (V_E , $\text{l}\cdot\text{min}^{-1}$) and PCO_2 (mmHg) calculated from the last 30 sec of a 3-min measurement period prior to measurement of AHVR.

APPENDIX 6.7.

This appendix gives the estimates for the hypoxic sensitivity term, G_p ($\text{l}\cdot\text{min}^{-1}\cdot\%$), and offset term, $\dot{V}_C$ ($\text{l}\cdot\text{min}^{-1}$), time constant, τ (sec), and pure delay T_d (sec) at all measurement points during both 96-hour protocols using fitting procedure A. (Fitting procedure A allowed the dynamic parameters to vary for each test of AVHR.) Data from both Pco_2 -matched and pH-matched methods of measurement of AHVR are included.

Subject	Time (hours)	ISOCAPNIC PROTOCOL				POIKILOCAPNIC PROTOCOL			
		G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)	G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)
884	0	0.858	19.138	8.203	6.421	1.161	11.452	7.890	3.480
	4	0.917	29.234	0.094	6.500	0.758	16.061	6.825	6.701
	8	0.935	27.493	12.640	3.620	0.995	23.161	9.362	3.560
	12	1.090	31.474	5.174	5.321	0.847	29.717	11.711	3.726
	24	1.084	25.514	9.089	3.700	1.244	26.227	8.595	4.941
	36	1.459	28.633	14.692	2.426	1.557	33.722	10.578	2.421
	48	1.105	36.401	2.761	6.598	1.253	34.071	3.980	5.397
	52	1.134	30.278	11.979	2.440	0.838	34.240	8.739	3.744
	56	1.041	29.967	8.590	4.780	1.304	35.505	2.656	5.065
	60	0.824	32.389	14.154	2.560	1.215	33.429	6.285	5.062
	72	1.269	26.166	7.358	7.640	1.464	32.687	5.317	4.640
	84	1.006	30.384	8.353	2.860	1.167	29.771	2.853	6.264
	96	1.105	28.666	2.888	6.240	1.062	30.729	8.964	2.644
910	0	1.099	9.649	6.287	4.480	0.797	10.730	4.652	4.718
	4	1.929	13.517	8.813	3.986	1.063	13.741	6.713	4.961
	8	1.804	16.126	6.947	3.684	1.616	11.595	9.502	3.021
	12	1.986	18.541	5.976	4.882	2.241	14.007	8.776	4.402
	24	2.875	22.228	7.199	3.141	2.431	19.020	7.646	3.250
	36	2.533	28.515	7.630	3.252	3.174	20.847	6.887	2.540
	48	2.460	20.484	8.261	2.820	2.212	22.183	4.979	4.323
	52	2.068	22.925	6.276	2.921	1.817	17.705	3.694	5.361
	56	2.259	18.549	7.335	3.404	1.798	19.089	5.902	3.920
	60	1.942	18.002	6.672	3.901	2.089	19.112	8.258	3.424
	72	1.545	14.642	6.017	3.781	1.647	13.420	4.056	4.900
	84	1.151	13.924	5.691	4.465	1.051	11.184	4.867	5.642
	96	1.118	11.373	7.386	2.816	1.067	10.415	9.809	3.123

Table 6.7: Estimates for the hypoxic sensitivity term, G_P (l.min⁻¹.%⁻¹), offset term, V_C (l.min⁻¹), time constant, τ (sec), and pure delay, T_d (sec), from PCO₂-matched tests of AHVR, obtained using fitting procedure A for subjects 884 and 910.

Subject	Time (hours)	ISOCAPNIC PROTOCOL				POIKILOCAPNIC PROTOCOL			
		G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)	G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)
964	0	0.336	7.074	3.180	5.850	0.200	11.095	4.083	1.849
	4	0.443	9.572	2.613	8.350	0.447	9.956	9.334	3.660
	8	0.437	9.089	2.843	5.560	0.600	9.491	9.508	3.300
	12	0.842	14.790	3.124	7.260	0.679	10.764	9.194	3.142
	24	1.117	20.216	4.229	4.871	1.384	16.421	4.004	6.915
	36	1.134	22.659	3.102	6.520	1.328	23.690	4.880	5.210
	48	1.065	26.182	8.490	4.438	1.692	27.759	5.823	5.921
	52	0.811	17.803	4.493	5.181	1.110	20.549	5.280	4.941
	56	1.091	20.546	4.210	5.241	0.749	19.234	3.776	6.640
	60	0.820	17.937	4.538	6.443	1.094	17.336	5.963	5.742
	72	0.773	16.861	3.724	4.348	0.614	13.964	4.744	4.129
	84	0.628	18.321	3.941	5.081	0.495	16.101	13.459	0.060
	96	0.543	15.033	7.881	2.463	0.775	10.507	1.676	6.366
970	0	1.695	12.762	12.417	4.672	1.702	9.476	9.683	3.465
	4	2.588	17.696	13.291	3.080	3.080	15.801	9.015	4.480
	8	3.597	1.037	25.799	0.000	3.244	20.369	7.197	5.220
	12	3.029	17.123	7.200	5.760	3.435	26.994	7.709	4.180
	24	3.766	29.914	5.689	4.340	3.303	31.333	8.054	3.180
	36	3.818	41.080	8.746	3.388	3.285	40.687	7.681	3.866
	48	3.766	38.760	8.670	5.540	3.572	42.241	5.074	4.432
	52	3.949	21.704	10.099	3.541	3.851	33.822	8.295	2.828
	56	3.611	32.422	5.363	5.132	4.135	28.183	8.639	3.146
	60	3.658	29.009	5.114	4.300	3.764	33.144	5.551	3.660
	72	3.219	15.724	7.621	4.390	3.561	21.238	7.346	3.996
	84	3.718	18.360	12.066	2.203	3.037	14.908	7.140	2.723
	96	3.439	27.709	8.363	3.820	3.310	16.058	8.687	4.340

Table 6.8: Estimates for the hypoxic sensitivity term, G_P (l.min⁻¹.%⁻¹), offset term, V_C (l.min⁻¹), time constant, τ (sec), and pure delay, T_d (sec), from PCO₂-matched tests of AHVR, obtained using fitting procedure A for subjects 964 and 970.

Subject	Time (hours)	ISOCAPNIC PROTOCOL				POIKILOCAPNIC PROTOCOL			
		G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)	G_P (l.min ⁻¹ .% ⁻¹)	V_C (l.min ⁻¹)	τ (sec)	T_d (sec)
884	0	0.858	19.138	8.203	6.421	1.161	11.452	7.890	3.480
	24	1.176	30.262	10.886	3.220	1.385	21.378	9.497	2.861
	48	1.525	36.663	14.582	1.950	1.412	23.073	6.431	5.463
	72	1.013	35.883	7.366	3.541	1.569	18.372	12.383	2.500
	96	1.206	33.331	5.455	5.380	1.140	23.100	4.220	4.891
910	0	1.099	9.649	6.287	4.480	0.797	10.730	4.652	4.718
	24	2.875	22.228	7.199	3.141	2.300	11.980	8.700	1.822
	48	2.896	27.922	4.461	3.271	1.875	12.507	6.070	4.140
	72	1.277	11.951	4.320	4.361	1.461	8.861	4.592	4.213
	96	1.145	11.109	7.852	2.571	1.067	10.415	9.809	3.123
964	0	0.336	7.074	3.180	5.850	0.200	11.095	4.083	1.849
	24	0.906	14.489	0.158	7.480	0.984	8.354	6.802	6.341
	48	1.218	18.385	5.063	5.120	1.221	11.061	7.023	5.033
	72	0.828	17.533	2.772	4.981	0.858	13.157	8.907	2.441
	96	0.508	15.382	5.225	4.923	0.525	8.662	5.799	3.076
970	0	1.695	12.762	12.417	4.672	1.702	9.476	9.683	3.465
	24	3.766	29.913	5.695	4.340	3.167	16.218	6.187	4.909
	48	4.040	19.822	10.473	5.161	3.678	10.641	9.612	3.340
	72	1.721	22.229	8.941	4.601	2.622	11.440	6.724	4.284
	96	3.737	12.310	6.973	4.727	2.834	11.105	6.267	3.600

Table 6.9: Estimates for the hypoxic sensitivity term, G_P (l.min⁻¹.%⁻¹), offset term, V_C (l.min⁻¹), time constant, τ (sec), and pure delay, T_d (sec), from pH-matched tests of AHVR, obtained using fitting procedure A for all subjects.

CHAPTER 7

PROBLEMS WITH DETERMINING THE HYPOXIC RESPONSE USING STEPWISE CHANGES IN END-TIDAL P_{O_2}

*Not the possession of truth
but the effort in struggling to attain to it
brings joy to the researcher.
Gotthold Lessing*

INTRODUCTION

The relationship between ventilation ($\dot{V}_E$) and end-tidal P_{O_2} (P_{ETCO_2}), the hypoxic response, is curvilinear and a number of functions has been formulated to describe its shape. Cunningham *et al.* (1964) employed a hyperbolic relationship of the form:

$$\dot{V}_E = D.(P_{ETCO_2} - B) + \frac{DA.(P_{ETCO_2} - B)}{(P_{ETO_2} - C)}$$

where A, B, C and D are constants and P_{ETCO_2} , end-tidal P_{CO_2} . The first term represents the oxygen-insensitive component and the second term the oxygen sensitive component of the ventilatory drive. They found considerable variation between individuals in the magnitude of the response, but interestingly also found that the value of C was different for individuals, suggesting that the shape of the hypoxic response varied. Kronenberg *et al.* (1972) used an exponential function to describe the hypoxic response:

$$\dot{V}_E = \dot{V}_{100} + \Delta \dot{V}_0 e^{-P_{ETO_2}/k}$$

where $\dot{V}_{100}$ is the non-hypoxic component of ventilation and $\dot{V}_{100} + \Delta \dot{V}_0$ is the ventilation intercept at $P_{ETO_2}=0$. Rebuck and Campbell (1974) suggested that ventilation was linearly related to the oxygen saturation of the arterial blood, when measured by oximetry. In the latter case, or in the case of the exponential formulation where the value of the constant, k, is considered fixed, the shape of the hypoxic response is regarded as the same between subjects, although the overall magnitude of the response may vary.

One major problem in measuring the form of the hypoxic response relates to the method by which it is measured. Lloyd *et al.* (1958) exposed subjects to constant levels of inspired CO_2 and O_2 for at least 8 min before taking ventilatory readings to allow time for ventilation to

settle if the end-tidal CO_2 changed. A subsequent workshop chaired by Cherniack (Cherniack *et al.*, 1977) recommended that differing gas mixtures containing diminished concentrations of O_2 should be administered for at least 5 to 10 min before ventilation was measured. It has become accepted, however, that the ventilatory response to a step input of isocapnic hypoxia is biphasic, involving an initial rise and a subsequent decline over 20 to 30 min, termed hypoxic ventilatory depression, HVD (Weil and Zwillich, 1976; Easton *et al.*, 1986). It is clear from this work that the long exposure to hypoxia in the study by Lloyd *et al.* would not result in a measurement of the initial acute ventilatory sensitivity to hypoxia, but a measurement only after a certain decline in ventilation had occurred. Faster measurements of the hypoxic response using a rebreathing technique, where there is a progressive decrease in P_{O_2} over about 15 min had been employed even before the importance of HVD had been realized (Weil *et al.*, 1970). This test was shortened by others to 6 to 12 min for use in measurements of the hypoxic response at altitude (Cruz *et al.*, 1980; Goldberg *et al.*, 1992; White *et al.*, 1987). However, objections that may be raised to this approach are first, that the $\text{P}_{\text{ET}\text{O}_2}$ is constantly changing and so a ventilation cannot be related to a steady level of $\text{P}_{\text{ET}\text{O}_2}$, and secondly, that it is difficult to maintain a constant level of $\text{P}_{\text{ET}\text{CO}_2}$.

In addition to the methods described above, a technique for varying the end-tidal gases exists in the form of end-tidal forcing (presented in Chapter 2) (Swanson and Bellville, 1975; Robbins *et al.*, 1982a). With this technique it is possible to obtain good control of many pre-defined levels of end-tidal P_{CO_2} and P_{O_2} , thus the steady decline in $\text{P}_{\text{ET}\text{O}_2}$ of the rebreathing technique can be modified to a reduction of $\text{P}_{\text{ET}\text{O}_2}$ in a series of steps, thereby overcoming the two major objections to the rebreathing technique. The aim of the study presented in this chapter was to examine whether such a protocol could be used for determining the shape of the hypoxic response. Severinghaus (1976) proposed that two minutes was sufficient time for the acute responses to hypoxia to develop, and consequently it was decided to vary $\text{P}_{\text{ET}\text{O}_2}$ in two-minute periods and take a measurement of ventilation during the last 30 sec of each step. Seven levels of $\text{P}_{\text{ET}\text{O}_2}$ were chosen, giving a protocol

duration of 14 min in total, but only part of which involves the more severe levels of hypoxia.

If this protocol was to determine the acute hypoxic response without itself altering the hypoxic response through the development of HVD, then the order in which the steps were imposed should not matter. To test this, the responses to separate protocols with ascending and descending steps of P_{ETO_2} were compared.

One motivation behind finding a test for the hypoxic response was to have a tool to examine the nature of the changes in respiratory control during exposure to hypoxia, both sustained and prolonged. Many studies have investigated the decrease in the ventilatory sensitivity to hypoxia associated with HVD (Bascom *et al.*, 1990; Bascom *et al.*, 1992; Khamnei and Robbins, 1990) and increase in hypoxic sensitivity associated with prolonged exposure to hypoxia over a period of hours to days (Chapters 5 and 6; Cruz *et al.*, 1980; Goldberg *et al.*, 1992; Sato *et al.*, 1992,1994; White *et al.*, 1987); however, none has successfully examined the change in the shape of the hypoxic response itself. Consequently, it was decided that the test proposed in this chapter be performed following a 20-min period of isocapnic hypoxia (50 mmHg) allowing HVD time to develop, as well as following a control period of 20 min of isocapnic euoxia.

METHODS

Subjects. Six subjects (5 males, 1 female) took part in the study. Their individual characteristics are listed in Table 7.1. All were healthy non-smokers, and none gave any history of respiratory symptoms or illnesses.

Subject	Sex	Height (m)	Weight (kg)	Age (years)
875	M	1.84	74	18
879	M	1.77	72	23
884	M	1.86	75	22
886	M	1.82	70	22
889	M	1.88	91	22
910	F	1.70	60	20

Table 7.1: Physical characteristics of subjects.

Equipment and experimental technique. The equipment and experimental technique used in this study were those of the dynamic end-tidal forcing system, described in Chapter 2.

Protocols. In order to describe the hypoxic response, the hypoxic stimulus to the peripheral chemoreceptors was varied by holding the end-tidal P_{O_2} at 7 different pre-determined levels each for 2 min over the range of 45 to 100 mmHg with the end-tidal P_{CO_2} held constant 2 mmHg above resting. These levels were calculated to provide equal steps in oxygen saturation of the arterial blood and were imposed either in ascending or descending order. Both protocols were preceded by either a 20-min isocapnic exposure to an end-tidal P_{O_2} of 100 mmHg (euoxia) or 50 mmHg (hypoxia) in order to describe any possible change in the

hypoxic response following the development of HVD. Each protocol was repeated 6 times on each subject, resulting in 24 data sets per subject and a total of 144 data sets for the study. Figure 7.1 displays the procedure for each protocol.

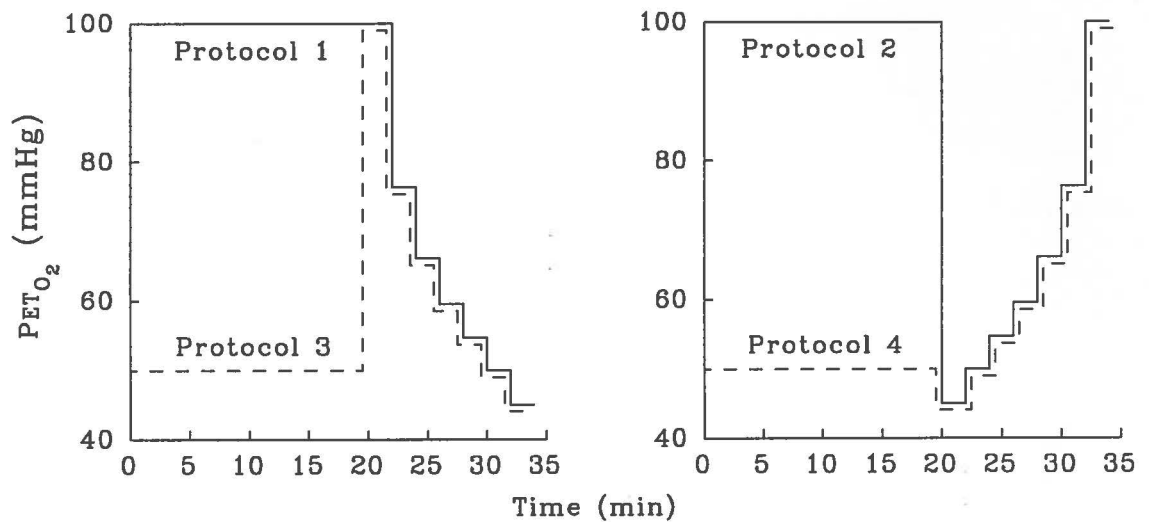


Figure 7.1: End-tidal P_{O_2} time course employed for each of the four protocols.

Data analysis. Data were analyzed using the programs BRDATA and AVDATA, described in the preliminary data analysis section, Chapter 2. The average ventilation from the last 30 sec of exposure to each level of $P_{ET}O_2$ was taken as the resulting ventilation for that level of $P_{ET}O_2$ for that data set.

An analysis of variance was used to examine the possible interaction between the protocol employed and the level of $P_{ET}O_2$. Using the notation defined by Armitage and Berry (1987), the linear model for ventilation ($\dot{V}_{Eijk}$) at the i th protocol (P_i), j th level of hypoxia or $P_{ET}O_2$ (H_j) and k th subject (S_k), together with interaction terms used was:

$$\dot{V}_{Eijk} = \mu + P_i + H_j + S_k + (PH)_{ij} + (PS)_{ik} + (HS)_{jk} + (PHS)_{ijk} + e_{ijk}$$

where e_{ijk} is an error term. In this model the hypoxia and protocol factors are fixed effects and the subject factor is a random effect. The sum of squared errors from the two-factor interaction term $(PH)_{ij}$ was compared with the three-factor interaction error term $(PHS)_{ijk}$ in an F-ratio test to determine the significance of the interaction between the protocol and hypoxia effects on ventilation. Data from protocols 1 and 2 (protocols with prior euoxia) were compared, as were data from protocols 3 and 4 (protocols with prior hypoxia). The analysis was performed using Minitab statistical software.

RESULTS

Figure 7.2 (upper and middle panels) shows breath-by-breath plots of P_{ETCO_2} and P_{ETO_2} against time during an experiment (protocol 4) on subject 910. This figure illustrates the quality of input attained by the system. The resulting ventilatory response for each breath is also plotted in Fig. 7.2 (lower panel). All subjects, except 889, tolerated the hypoxia well. Subject 889 expressed some discomfort, in particular in association with the increasing hypoxia of protocol 1.

Figure 7.3(a) presents the hypoxic responses to all 4 protocols averaged across the subjects ($\dot{V}_E$ vs. calculated arterial saturation). First, comparing the two panels, the expected decrease in the hypoxic sensitivity with prior exposure to hypoxia (panel B) is indeed evident when compared with the data without prior exposure to hypoxia (panel A). Secondly, comparing the responses within the panels suggests that the order in which the steps of P_{ETO_2} are performed does affect the outcome of the test. In panel A, it appears that the initial exposure to intense hypoxia associated with protocol 2 depresses the ventilatory response to the remainder of the test. In panel B, there is a suggestion that ventilation falls to a lower level with the sudden relief of hypoxia in protocol 3 at the end of the 20-min hypoxic exposure than with the gradual relief of hypoxia in protocol 4.

The analysis of variance to test for a significant interaction between the protocol and P_{ETO_2} effects revealed a non-significant interaction ($p < 0.056$) when comparing protocols 1 and 2. It was decided to re-analyze this interaction, with the exclusion of subject 889 should the subject's discomfort have distorted the mean response and caused a type 2 statistical error. Indeed, exclusion of this subject from the analysis results in a highly significant interaction ($p < 0.001$). The comparison of protocols 3 and 4 showed a highly significant interaction ($p < 0.005$), which remained significant after removal of subject 889 ($p < 0.0005$). The details of the analysis, both with and without the exclusion of subject 889, are displayed in Table

7.2. In addition, Fig. 7.3(b) presents the hypoxic responses for all 4 protocols averaged across all subjects, except subject 889.

The data for each experimental run are presented in Tables 7.3 to 7.8 in Appendix 7.1 of this chapter. Appendix 7.2 contains Figs 7.4 to 7.9 which show the mean hypoxic responses ($\dot{V}_E$ vs. P_{O_2}) to each of the protocols 1 to 4 for each subject.

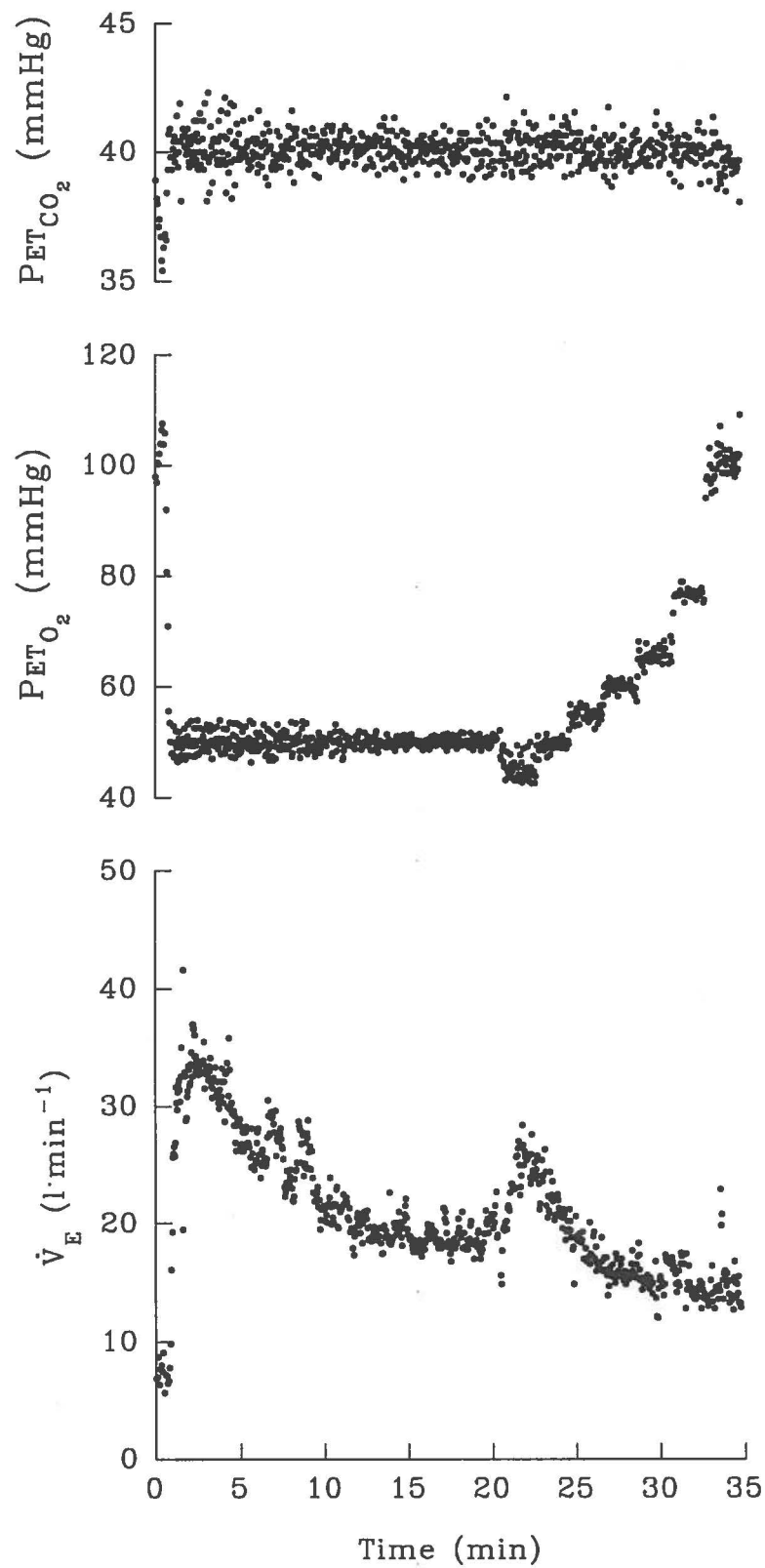


Figure 7.2: Record of breath-by-breath end-tidal PCO₂ (PETCO₂), end-tidal PO₂ (PETO₂) and ventilation ($\dot{V}_E$) against time for one experimental run (protocol 4; subject 910).

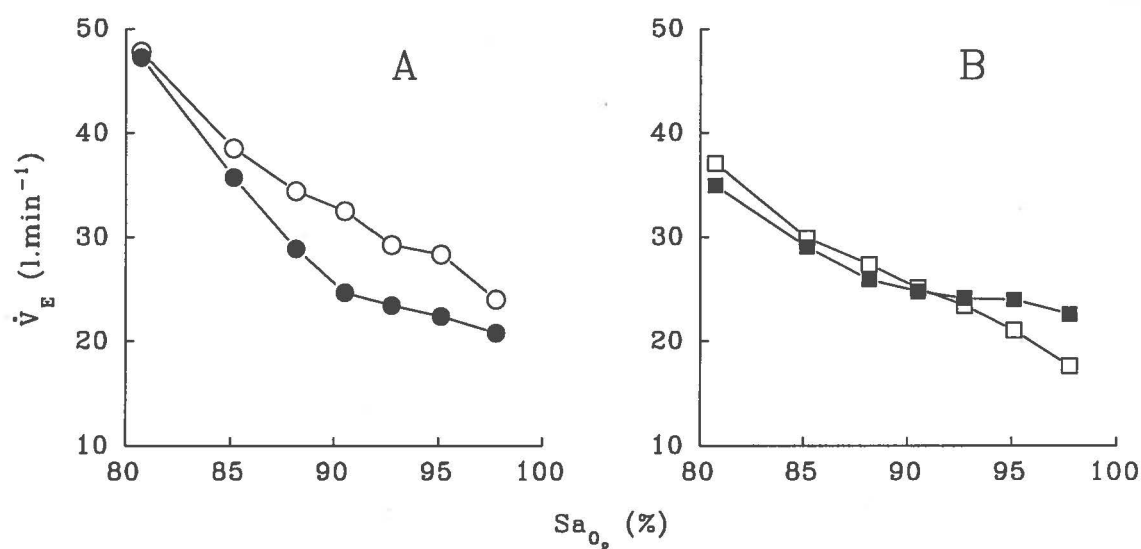


Figure 7.3(a): Relationship between $\dot{V}_E$ and calculated arterial saturation (Sa_{O_2}), averaged across all subjects. ($\circ$ = protocol 1; $\bullet$ = protocol 2; $\square$ = protocol 3; $\blacksquare$ = protocol 4.)

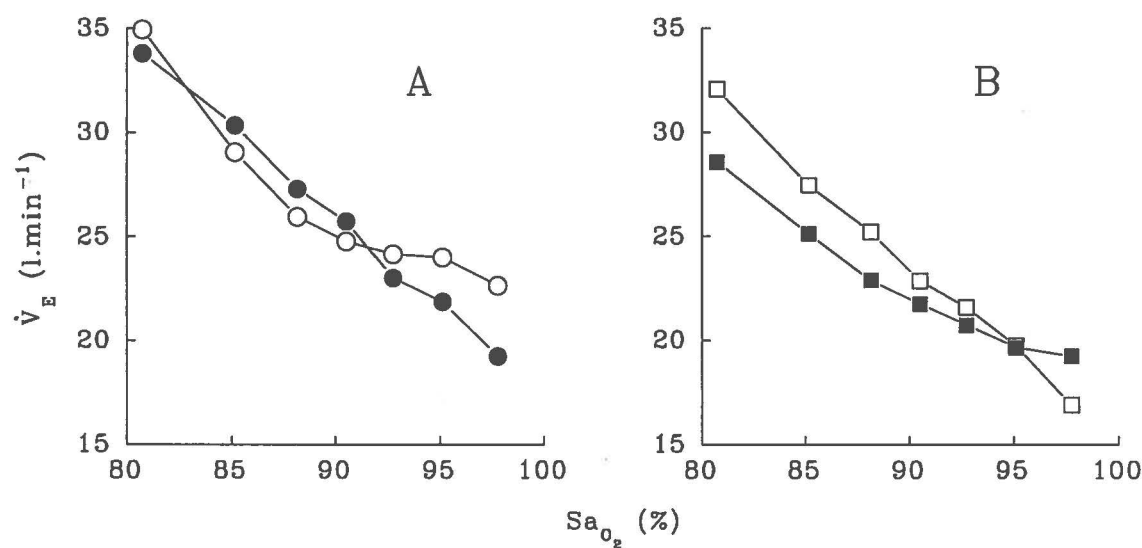


Figure 7.3(b): Relationship between $\dot{V}_E$ and calculated arterial saturation (Sa_{O_2}), averaged across all subjects, except subject 889. ($\circ$ = protocol 1; $\bullet$ = protocol 2; $\square$ = protocol 3; $\blacksquare$ = protocol 4.)

Protocols under comparison	Source of Variance	Degrees of Freedom	Sum of Squared Errors (l·min ⁻¹) ²	Mean Squared Error (l·min ⁻¹) ²	F-ratio	p value
1 vs. 2	Protocol*P _{O₂} (PH) _{ij}	6 {6}	460.5 {449.3}	76.8 {74.9}	2.35 {6.05}	<0.056 {<0.001}
	Protocol*P _{O₂} *Subject (PHS) _{ijk}	30 {24}	981.7 {296.9}	32.7 {12.4}	-	-
3 vs. 4	Protocol*P _{O₂} (PH) _{ij}	6 {6}	765.4 {313.1}	127.6 {52.9}	3.98 {7.83}	<0.005 {<0.0005}
	Protocol*P _{O₂} *Subject (PHS) _{ijk}	30 {24}	962.2 {159.9}	32.1 {6.66}	-	-

Table 7.2: Summary of statistical analysis (ANOVA) for comparison of hypoxic response between protocols. Values in brackets relate to statistical analysis which excluded subject 889.

DISCUSSION

In this study, it is considered that the normal acute ventilatory response to a level of hypoxia is the expected value at the maximum of the rise in ventilation reached in the first few minutes following an isocapnic step from a period of sustained euoxia into hypoxia. Consequently, the form of the hypoxic response is defined as the relation between ventilation and $P_{ET}O_2$ generated from the acute ventilatory responses to every level of hypoxia. The hypoxic response is only said to be normal when the response is measured following a period of sustained euoxia, as periods of sustained hypoxia are known to elicit HVD. Different hypoxic responses exist for different levels of $P_{ET}CO_2$. Such a definition of the hypoxic response cannot be used practically as a method to determine the ventilatory response to hypoxia. However, as outlined in the introduction, the methods that have been employed to determine the hypoxic response all suffer significant drawbacks either as a result of the development of an indeterminate degree of HVD during the test, or because $P_{ET}O_2$ is never steady, or because the control of $P_{ET}CO_2$ is poor. The aim of the current study was to develop a protocol using the end-tidal forcing technique to maintain good $P_{ET}CO_2$ control and to generate a sequence of steps into different levels of hypoxia, each step being long enough to allow time for a maximal ventilation to develop and short enough to prevent a significant change in the degree of HVD.

The results of this study indicate that the order in which the steps into the various levels of hypoxia were performed affected the variable being measured. Consequently, it is not possible for both protocols to reflect the hypoxic response as defined above, although it remains theoretically possible that either one on its own may do so. It can be seen from Fig. 7.3(a) that the relation between ventilation and arterial oxygen saturation appears much more curvilinear when measured using protocols 2 and 4 than with protocols 1 and 3. One possible explanation for this observation is that the degree of HVD changed during the test procedure, although other explanations may exist.

Two features of the protocol chosen which may have affected the degree of the change in HVD during the protocol were the number of steps into the different levels of hypoxia and the duration of each step. In order to limit this change in HVD, the duration of the protocol could be reduced by decreasing the number of steps from 7 to 6 or even 5. However, such a change would increase the degree of interpolation between points and consequently might reduce the ability to distinguish possible changes in the shape of the relation between ventilation and $P_{ET}O_2$. Secondly, it would be possible to reduce the duration for which each level of $P_{ET}O_2$ is held constant. The choice of two min for the protocols employed in this study was based on a proposal by Severinghaus (1976) and findings from our own laboratory showing that, following an isocapnic step into hypoxia, ventilation is usually maximal in the second minute of the response, when averaged over one-minute periods (Khamnei and Robbins, 1990; Bascom *et al.*, 1992). Recent modelling studies of the acute response to rapid changes in $P_{ET}O_2$ from our laboratory (Clement and Robbins, 1993) provide estimates for a pure delay (4.85 ± 1.95 sec, mean $\pm$ S.D.) and a time constant (6.11 ± 4.21 sec) for a first order model describing the ventilatory response to acute hypoxia. These estimates suggest that it should be possible to shorten the two-min period employed in this protocol substantially and still obtain a steady level of ventilation in the majority of cases.

It may transpire that reducing the number of steps, and so data points, and reducing the time spent at each level of hypoxia still might prove insufficient to ensure that the response to the protocol is independent of the order in which the hypoxic stimuli are presented. If this is the case then the idea of using steady levels of $P_{ET}O_2$ may have to be abandoned, and an approach using rapidly varying $P_{ET}O_2$ together with model fitting to allow for system dynamics be explored in its place.

APPENDIX 7.1.

This appendix shows the data from each experimental run for every subject. In each cell are displayed the average values for ventilation, $\dot{V}_E$ ($\text{l}\cdot\text{min}^{-1}$), end-tidal Po_2 and Pco_2 (mmHg) from the last 30 sec of exposure to each level of Po_2 .

Subject 875

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	16.4	8.4	13.9	11.5	22.1	16.9	19.1	17.1	15.0	12.2	26.4	20.4
PO ₂ (mmHg)	99.1	100.3	99.2	100.9	101.1	99.0	98.7	101.7	101.3	100.6	100.1	99.8
PCO ₂ (mmHg)	42.3	42.1	42.2	41.9	42.2	42.1	41.8	41.5	41.6	41.6	42.1	41.7
$\dot{V}_E$	19.5	8.7	12.9	14.0	26.8	23.0	19.6	17.2	15.0	11.2	23.8	19.8
PO ₂	75.8	76.4	76.1	76.5	76.4	76.4	76.1	78.9	78.7	77.8	78.6	76.8
PCO ₂	41.5	42.6	41.3	41.4	42.0	41.5	42.0	41.1	40.6	41.8	41.8	41.8
$\dot{V}_E$	18.4	9.0	11.7	10.3	27.6	23.6	23.5	16.2	14.0	11.9	23.8	21.0
PO ₂	65.9	64.5	65.8	64.4	66.5	65.5	65.4	67.4	66.8	66.2	65.9	66.3
PCO ₂	41.8	42.3	42.1	42.1	42.1	42.4	41.8	41.5	41.3	42.0	42.4	42.2
$\dot{V}_E$	14.7	8.1	11.5	11.7	34.8	23.4	25.7	15.4	16.5	12.7	23.1	25.8
PO ₂	58.2	57.1	59.3	57.8	58.7	59.3	60.2	61.0	61.3	60.1	60.5	59.9
PCO ₂	42.3	42.7	42.2	42.4	42.8	42.2	41.7	41.7	41.3	41.3	41.8	41.7
$\dot{V}_E$	16.0	9.4	10.1	10.4	27.8	20.0	20.9	18.3	17.2	12.6	23.8	23.3
PO ₂	54.9	53.8	54.2	51.4	54.7	54.7	55.4	55.9	54.8	55.0	54.4	55.0
PCO ₂	41.8	42.3	42.4	42.6	41.9	42.5	42.1	41.6	41.6	41.8	41.6	41.5
$\dot{V}_E$	15.9	8.1	11.2	17.1	28.8	22.9	26.5	18.6	19.8	13.2	27.5	27.4
PO ₂	49.9	48.8	49.2	49.1	50.2	49.4	49.7	51.1	49.6	50.0	50.5	49.8
PCO ₂	41.7	42.7	42.0	41.1	41.8	42.0	42.0	42.0	41.7	41.7	42.1	42.2
$\dot{V}_E$	18.0	8.7	13.5	13.6	27.8	26.1	36.3	23.5	25.2	16.3	32.8	35.5
PO ₂	45.7	43.9	46.1	43.8	44.7	45.0	44.6	46.0	45.0	44.9	45.4	44.7
PCO ₂	42.1	42.2	42.2	41.6	42.1	41.8	42.3	41.4	41.3	41.8	42.0	42.1
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	10.9	7.5	17.6	14.9	18.3	10.4	27.8	16.3	11.1	11.8	20.6	19.4
PO ₂ (mmHg)	99.3	102.5	102.5	102.3	102.9	100.0	100.2	102.1	101.5	101.3	100.9	102.3
PCO ₂ (mmHg)	41.8	43.5	41.2	41.6	41.4	42.0	41.4	41.0	41.2	41.4	41.4	41.9
$\dot{V}_E$	16.7	9.5	22.5	18.6	20.0	10.9	23.9	15.9	12.2	8.3	18.1	19.5
PO ₂	77.0	77.4	76.8	75.6	76.4	76.3	77.1	77.0	77.3	75.4	77.1	77.0
PCO ₂	41.4	41.8	42.0	41.2	41.9	42.0	41.8	41.3	40.8	42.3	42.0	41.9
$\dot{V}_E$	13.9	7.6	27.7	23.4	20.2	11.6	24.4	23.4	9.8	8.5	19.0	20.4
PO ₂	66.4	64.9	63.8	66.0	65.5	66.1	66.0	66.6	66.8	65.7	66.7	67.0
PCO ₂	41.6	42.4	42.4	42.4	42.2	42.0	41.9	41.7	41.2	42.5	41.5	41.5
$\dot{V}_E$	14.1	10.0	23.7	22.3	22.2	15.9	23.8	15.8	10.2	10.1	18.6	17.6
PO ₂	59.9	59.7	59.2	59.5	59.6	59.5	61.3	59.7	60.8	59.2	59.7	57.9
PCO ₂	41.1	41.9	41.9	42.2	42.1	42.0	41.9	41.9	41.0	42.3	41.9	42.2
$\dot{V}_E$	15.0	9.6	32.1	26.0	24.9	13.7	21.8	17.5	9.9	8.4	17.5	19.5
PO ₂	56.0	54.0	53.6	54.9	54.9	54.7	54.4	55.4	54.5	53.3	55.3	54.8
PCO ₂	41.1	41.9	42.3	41.7	41.9	42.0	41.6	41.7	41.9	42.6	42.3	42.3
$\dot{V}_E$	15.2	11.2	27.4	24.5	24.2	14.1	24.1	16.8	10.5	11.4	20.7	24.2
PO ₂	50.1	50.0	49.9	49.4	49.8	50.0	49.1	50.3	49.5	48.0	49.6	49.4
PCO ₂	41.8	41.6	42.0	42.3	42.3	42.0	41.6	42.3	42.6	42.7	42.0	42.3
$\dot{V}_E$	13.2	10.3	34.2	25.9	26.4	15.9	22.6	18.1	10.3	10.4	20.5	25.7
PO ₂	44.8	45.2	44.7	45.1	44.9	45.0	44.9	44.2	45.3	43.6	43.7	44.5
PCO ₂	42.3	41.8	42.2	41.8	42.0	42.0	41.7	42.4	42.2	42.8	42.3	41.9

Table 7.3: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 875, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

Subject 879

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	15.3	12.7	14.1	14.6	12.8	15.6	9.7	8.4	13.1	12.0	12.3	10.2
PO ₂ (mmHg)	99.5	99.7	100.0	100.0	98.3	100.6	100.7	98.5	99.7	101.9	101.9	97.6
PCO ₂ (mmHg)	42.3	42.2	42.2	42.1	42.9	41.8	42.3	42.4	42.8	42.0	42.1	43.3
$\dot{V}_E$	14.8	12.8	17.1	17.9	15.0	16.4	10.4	13.1	16.5	13.4	15.9	13.1
PO ₂	75.9	75.8	74.4	76.2	76.7	75.8	78.4	78.2	76.8	77.0	77.9	77.7
PCO ₂	43.3	42.2	42.2	41.7	41.3	41.5	42.2	42.0	42.6	42.2	41.9	42.7
$\dot{V}_E$	16.2	13.1	17.4	16.1	15.8	16.1	9.1	10.2	16.6	15.7	13.0	11.2
PO ₂	65.3	63.8	65.6	66.0	65.4	66.2	63.6	65.1	65.1	66.0	66.1	66.0
PCO ₂	42.0	42.3	42.5	41.9	42.1	41.8	42.4	42.8	42.4	42.6	42.5	43.1
$\dot{V}_E$	18.7	12.4	21.8	18.6	16.3	22.9	10.7	12.2	19.1	16.5	17.0	12.6
PO ₂	59.0	57.9	58.9	58.6	59.1	59.3	60.2	59.4	59.6	59.5	61.0	59.0
PCO ₂	41.9	42.4	42.5	41.7	41.9	41.9	42.4	42.4	41.8	41.7	42.1	43.0
$\dot{V}_E$	17.0	12.8	21.4	19.7	22.8	21.4	13.6	13.8	22.0	20.5	17.6	13.5
PO ₂	54.3	53.7	53.8	53.3	54.6	55.0	52.8	53.0	54.7	55.1	55.6	52.4
PCO ₂	42.2	42.7	42.5	41.6	41.8	42.1	42.1	42.2	42.1	41.5	42.5	43.3
$\dot{V}_E$	18.3	16.1	25.9	18.6	17.9	22.8	18.0	18.6	26.2	22.3	21.0	17.9
PO ₂	50.5	49.1	48.2	48.8	48.3	49.3	49.3	49.5	49.4	50.8	48.6	48.6
PCO ₂	41.6	42.0	42.6	42.0	42.3	41.8	42.3	42.0	41.5	41.8	42.1	43.3
$\dot{V}_E$	21.7	17.4	28.7	23.9	20.3	26.5	21.6	31.1	39.6	30.7	29.5	25.4
PO ₂	46.1	44.4	44.7	44.0	44.1	45.6	44.8	45.9	45.5	45.6	44.3	43.9
PCO ₂	42.0	42.4	42.1	41.8	42.4	41.8	41.9	41.5	41.5	41.3	41.5	42.5
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	14.2	9.8	14.6	13.0	12.4	9.8	9.3	10.8	14.0	14.3	9.5	12.2
PO ₂ (mmHg)	102.2	100.2	101.1	101.0	110.1	100.9	97.7	100.0	101.0	100.7	104.6	103.6
PCO ₂ (mmHg)	41.6	42.3	41.3	41.8	40.7	42.3	43.1	42.2	42.3	41.4	41.4	41.8
$\dot{V}_E$	15.7	13.2	15.3	13.8	10.6	14.0	9.9	13.5	14.5	17.3	9.5	15.9
PO ₂	74.0	76.9	76.9	76.1	74.4	78.3	78.3	77.2	77.0	78.0	78.2	76.4
PCO ₂	43.4	41.5	41.5	41.5	42.1	41.4	41.7	42.2	41.9	41.6	41.3	41.8
$\dot{V}_E$	17.6	17.9	17.6	16.1	12.1	14.1	10.8	15.6	15.5	17.8	11.8	16.7
PO ₂	66.5	65.9	66.1	67.2	65.7	66.8	66.3	67.7	68.0	66.5	66.7	68.0
PCO ₂	41.7	42.3	41.6	41.8	42.3	41.8	42.0	42.0	41.7	41.7	42.1	41.1
$\dot{V}_E$	18.9	15.2	18.2	17.8	11.1	14.9	9.5	18.0	15.3	17.6	10.9	17.2
PO ₂	58.4	58.5	59.5	59.8	57.1	59.8	58.6	60.6	60.5	61.2	60.3	59.1
PCO ₂	42.3	41.8	41.8	41.7	42.7	42.0	42.0	41.9	42.3	42.0	42.0	41.1
$\dot{V}_E$	19.1	15.8	17.9	17.4	12.6	15.9	12.9	17.5	17.2	19.6	11.2	17.3
PO ₂	54.2	54.6	54.5	53.7	53.2	55.2	56.2	55.3	55.1	55.7	54.9	54.7
PCO ₂	42.2	41.5	41.9	42.3	42.6	42.8	41.9	42.2	42.4	41.9	42.2	41.7
$\dot{V}_E$	22.6	20.3	20.4	20.5	13.8	16.2	11.0	18.8	15.3	22.2	12.5	19.6
PO ₂	48.6	48.5	50.2	49.3	48.0	50.9	50.0	49.4	48.8	50.3	47.2	50.0
PCO ₂	42.0	42.2	41.6	42.0	42.8	42.8	42.2	42.8	42.8	42.3	42.9	42.1
$\dot{V}_E$	22.2	21.0	23.1	18.5	16.1	25.6	17.8	20.8	18.2	20.8	17.6	22.9
PO ₂	45.1	44.7	44.8	43.9	43.6	43.9	45.9	44.0	46.0	45.1	44.5	45.8
PCO ₂	41.9	41.9	41.7	42.5	42.5	41.5	41.0	42.6	42.0	42.4	42.0	41.3

Table 7.4: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 879, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

Subject 884

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	37.0	17.6	24.6	27.3	26.5	27.6	22.7	28.4	25.6	29.8	19.6	30.9
PO ₂ (mmHg)	99.2	99.6	100.8	100.6	100.5	100.3	99.8	100.5	101.6	100.1	99.7	101.1
PCO ₂ (mmHg)	42.1	42.0	41.8	41.7	42.1	41.8	42.2	42.1	41.1	41.5	41.9	41.7
$\dot{V}_E$	36.6	26.0	30.7	29.3	31.0	30.4	25.9	33.7	26.6	33.1	20.2	29.4
PO ₂	76.1	75.5	76.3	76.1	75.7	76.2	77.3	76.5	78.4	77.1	76.1	76.6
PCO ₂	42.3	42.5	42.2	42.0	41.7	42.1	41.7	41.7	41.7	41.7	42.4	42.0
$\dot{V}_E$	38.6	25.4	28.8	31.9	33.2	31.2	29.9	33.1	28.6	29.7	24.3	32.4
PO ₂	66.6	66.7	66.3	66.3	66.2	65.6	65.4	66.2	65.7	66.5	65.2	65.2
PCO ₂	41.7	41.6	41.8	42.0	41.6	42.1	41.7	41.7	42.0	41.9	42.0	42.5
$\dot{V}_E$	48.6	28.1	30.8	35.3	29.2	33.9	30.2	33.3	29.2	35.7	23.6	33.0
PO ₂	59.5	59.8	59.6	60.3	59.4	59.5	59.0	59.1	59.3	59.4	59.6	59.4
PCO ₂	41.8	41.7	41.8	41.9	42.1	41.9	42.5	42.1	41.5	41.9	42.0	42.1
$\dot{V}_E$	46.5	29.3	31.5	32.0	36.6	35.2	32.4	41.3	28.9	35.1	25.0	34.8
PO ₂	54.3	53.9	54.5	55.1	54.9	55.1	55.0	55.7	54.8	54.1	54.7	54.8
PCO ₂	42.1	42.1	42.0	42.2	42.2	41.8	42.0	41.6	42.2	42.2	41.8	41.8
$\dot{V}_E$	43.3	33.5	38.9	37.5	33.0	40.2	39.7	43.6	35.6	40.4	31.5	34.8
PO ₂	50.3	49.3	49.2	50.1	50.3	49.4	51.2	49.9	48.7	50.5	50.1	49.8
PCO ₂	42.3	42.7	41.7	42.4	42.0	42.4	42.2	41.9	42.1	42.1	42.1	41.9
$\dot{V}_E$	46.4	35.6	38.1	43.0	39.3	39.3	45.9	44.6	34.1	38.5	30.0	39.0
PO ₂	44.7	44.7	45.6	45.4	45.6	44.5	44.4	45.9	44.6	44.6	45.4	45.1
PCO ₂	42.3	42.2	42.1	42.2	42.1	42.2	41.9	42.3	42.6	42.3	41.9	42.2
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	21.7	28.7	32.3	24.3	21.8	21.9	28.4	30.9	25.8	25.1	19.4	21.7
PO ₂ (mmHg)	100.3	101.5	100.4	98.7	99.3	99.2	101.2	101.2	100.4	98.2	100.8	101.0
PCO ₂ (mmHg)	41.7	41.2	42.0	42.3	41.8	41.9	41.9	41.7	41.4	42.4	42.2	41.8
$\dot{V}_E$	25.9	36.1	32.2	27.7	25.4	25.6	27.3	29.7	27.1	27.4	20.2	20.8
PO ₂	76.5	76.3	76.9	75.7	75.5	76.1	77.6	76.3	75.9	76.7	76.4	76.2
PCO ₂	42.1	42.1	41.8	41.8	42.0	42.1	41.9	41.8	42.1	41.9	42.1	42.1
$\dot{V}_E$	29.7	33.6	33.8	33.5	28.0	30.0	29.9	28.1	26.7	24.1	20.8	23.4
PO ₂	65.8	66.5	66.0	66.3	65.6	65.7	67.1	66.7	66.9	66.4	66.9	66.2
PCO ₂	41.8	41.6	41.9	42.0	42.3	41.9	41.8	41.8	41.4	41.9	41.9	42.0
$\dot{V}_E$	30.1	39.2	33.3	33.2	24.1	31.3	31.8	33.0	29.1	38.9	21.7	25.2
PO ₂	59.1	60.0	59.8	59.8	60.1	59.4	60.0	59.9	59.4	59.9	59.9	59.8
PCO ₂	42.1	42.0	42.0	42.3	42.0	41.9	42.1	41.8	41.6	42.2	41.9	42.1
$\dot{V}_E$	31.0	43.1	36.2	36.6	30.2	33.6	34.6	32.1	33.0	38.3	23.7	25.4
PO ₂	54.8	54.8	55.3	55.7	53.4	55.2	54.3	54.1	54.7	55.6	54.7	54.8
PCO ₂	41.7	42.1	41.8	41.9	42.0	42.1	41.9	42.1	41.8	41.5	42.3	41.6
$\dot{V}_E$	37.7	40.5	39.5	40.4	30.4	36.7	34.2	32.0	33.1	40.8	25.9	29.0
PO ₂	49.3	49.8	49.9	49.6	50.3	50.4	50.7	49.4	49.5	50.2	49.9	50.1
PCO ₂	42.0	41.9	42.3	42.0	42.0	41.9	41.7	41.8	42.0	41.6	41.8	42.1
$\dot{V}_E$	40.2	46.6	43.1	40.3	36.4	35.1	40.1	36.1	36.3	38.7	32.5	33.9
PO ₂	44.8	44.5	44.5	45.0	44.5	48.1	44.8	44.8	45.1	44.9	45.5	45.2
PCO ₂	42.3	42.2	41.0	42.1	41.8	42.1	42.2	42.1	42.0	42.2	41.6	41.9

Table 7.5: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 884, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

Subject 886

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	26.1	15.7	15.7	27.4	31.3	36.0	20.4	32.3	17.3	13.9	25.3	29.3
PO ₂ (mmHg)	99.9	100.5	100.3	100.2	100.3	100.1	99.2	101.3	100.8	100.3	100.5	100.2
PCO ₂ (mmHg)	46.0	45.9	45.8	45.9	46.2	45.9	46.3	45.6	45.5	45.1	45.7	46.1
$\dot{V}_E$	21.1	20.5	16.4	28.1	39.5	36.8	22.2	33.2	21.8	17.0	28.1	33.6
PO ₂	77.3	76.6	76.8	76.9	76.0	76.4	75.5	78.0	76.7	75.7	76.8	76.6
PCO ₂	44.9	45.9	45.4	46.0	46.0	45.5	46.2	45.2	45.9	45.6	45.8	46.0
$\dot{V}_E$	27.4	22.2	17.7	31.8	43.3	37.3	25.6	37.4	21.8	17.0	30.9	35.0
PO ₂	67.0	65.9	66.2	66.3	66.2	65.4	65.9	65.8	65.6	65.4	66.6	66.5
PCO ₂	44.9	45.6	45.3	46.1	45.8	46.1	45.9	45.7	45.9	45.8	46.2	45.8
$\dot{V}_E$	26.8	22.6	18.1	38.0	48.7	43.3	25.6	39.9	25.2	19.3	32.8	32.3
PO ₂	60.8	59.8	59.4	59.8	59.9	58.9	59.5	60.3	59.6	60.5	58.8	59.4
PCO ₂	44.8	45.8	45.4	45.7	46.0	46.5	45.9	45.4	46.6	46.2	45.3	45.6
$\dot{V}_E$	27.9	25.1	21.6	39.6	54.4	46.9	31.0	36.8	21.6	21.6	35.8	38.6
PO ₂	55.6	54.4	55.3	55.7	54.5	54.9	54.4	54.9	55.0	54.9	54.7	54.9
PCO ₂	45.3	45.5	45.1	45.9	46.2	46.3	45.7	45.4	46.3	45.8	46.1	45.9
$\dot{V}_E$	32.3	26.5	25.5	40.8	57.4	47.5	33.4	42.5	25.4	26.7	39.7	45.9
PO ₂	50.5	50.8	49.3	50.5	49.8	49.9	50.7	50.4	50.7	50.2	50.6	49.8
PCO ₂	45.5	45.8	45.3	46.0	46.2	46.1	45.8	45.7	46.1	45.6	45.9	46.0
$\dot{V}_E$	38.7	30.2	30.7	45.9	63.4	49.9	43.1	56.4	42.3	37.1	54.3	64.3
PO ₂	45.5	43.8	45.1	45.3	44.7	44.9	45.6	44.7	45.0	44.8	45.2	44.7
PCO ₂	45.7	46.0	45.1	46.2	46.2	46.3	45.8	45.7	45.7	45.6	46.0	46.4
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	20.3	18.8	16.2	15.2	21.2	29.4	17.9	16.8	36.0	34.6	29.9	22.1
PO ₂ (mmHg)	99.9	100.0	100.1	100.2	101.0	99.9	99.6	101.4	101.5	100.0	100.8	100.4
PCO ₂ (mmHg)	45.8	45.6	45.2	45.4	45.7	45.2	45.4	45.8	45.7	45.9	45.7	45.7
$\dot{V}_E$	29.1	18.4	20.0	15.4	22.7	34.4	23.6	19.2	28.5	35.9	33.0	24.4
PO ₂	76.5	74.3	76.3	76.0	77.8	75.8	76.8	76.5	77.4	75.7	75.9	77.3
PCO ₂	45.5	45.9	44.9	45.4	45.4	46.2	45.2	45.6	46.2	46.1	46.0	45.5
$\dot{V}_E$	32.4	21.4	21.8	15.6	21.4	34.9	23.2	19.1	33.5	43.2	31.9	25.3
PO ₂	67.0	65.7	66.6	65.2	65.0	66.7	66.2	67.1	66.2	65.7	66.1	66.3
PCO ₂	45.9	45.4	45.7	46.3	46.4	45.5	45.6	45.4	45.7	45.8	45.6	45.5
$\dot{V}_E$	32.4	24.6	24.9	18.5	28.5	39.6	22.0	22.5	35.1	45.5	33.4	23.2
PO ₂	60.3	60.9	59.8	58.9	60.1	58.5	60.6	60.5	59.5	59.7	59.4	59.8
PCO ₂	45.3	45.1	45.3	45.8	45.2	46.3	45.7	45.5	46.2	45.8	45.8	44.8
$\dot{V}_E$	37.1	29.6	25.2	16.8	31.1	37.9	27.7	22.0	36.4	45.7	33.8	25.2
PO ₂	55.2	55.8	56.3	54.3	55.1	55.2	53.5	54.6	54.9	55.4	54.7	54.5
PCO ₂	45.5	45.3	45.4	46.0	46.2	45.7	46.2	45.8	46.1	45.7	45.9	46.5
$\dot{V}_E$	39.5	31.8	23.3	21.5	32.7	39.6	25.6	26.7	41.2	49.3	38.0	27.0
PO ₂	50.3	50.0	50.7	50.5	50.0	49.5	50.2	50.5	50.5	50.1	50.2	50.3
PCO ₂	45.7	45.8	45.8	45.9	45.8	46.0	45.6	46.0	45.8	45.9	45.9	46.2
$\dot{V}_E$	46.1	32.8	32.7	29.8	39.0	49.4	28.1	29.4	45.1	47.1	44.7	30.4
PO ₂	44.9	45.3	45.9	44.8	45.6	44.9	44.5	45.1	44.6	44.6	44.8	45.5
PCO ₂	46.2	45.8	45.8	45.7	45.9	45.9	45.6	46.1	45.8	45.8	46.0	45.9

Table 7.6: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 886, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

Subject 889

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	48.3	49.6	37.4	25.8	60.1	65.1	34.8	40.0	11.5	13.8	25.9	53.4
PO ₂ (mmHg)	100.2	99.6	100.4	100.4	100.1	100.2	100.4	100.7	97.4	99.4	99.8	100.1
PCO ₂ (mmHg)	42.2	42.2	42.0	42.1	42.0	42.0	42.1	41.8	43.0	42.2	41.8	41.9
$\dot{V}_E$	57.5	56.0	69.6	37.8	79.2	63.4	40.7	32.9	24.7	14.9	22.3	50.2
PO ₂	76.6	76.3	76.7	77.6	75.4	76.1	76.3	76.6	74.1	76.9	76.8	76.9
PCO ₂	42.1	42.4	41.9	42.3	41.9	41.6	41.9	42.0	42.8	42.4	41.7	41.8
$\dot{V}_E$	55.0	62.9	80.9	36.0	70.9	55.9	39.8	41.8	21.5	15.2	29.9	44.4
PO ₂	66.0	66.0	67.4	66.0	65.9	66.0	67.2	66.9	66.0	66.7	67.0	66.6
PCO ₂	42.2	42.1	41.6	42.1	41.9	42.1	41.5	42.2	42.0	42.2	41.2	41.9
$\dot{V}_E$	60.1	60.5	59.1	49.4	75.0	94.8	33.9	35.1	26.7	15.9	35.8	44.2
PO ₂	59.4	58.9	57.3	59.5	65.9	59.8	59.0	58.7	60.6	59.5	61.0	59.4
PCO ₂	41.8	42.4	42.7	41.7	41.9	41.9	42.1	42.3	42.4	42.5	41.3	42.0
$\dot{V}_E$	66.2	78.9	49.2	39.0	80.0	109.6	59.7	63.9	37.5	12.2	37.9	57.1
PO ₂	54.5	54.0	54.8	54.2	56.0	54.8	54.5	54.7	54.4	53.4	55.4	54.5
PCO ₂	41.9	42.2	41.3	42.3	42.3	41.9	42.0	41.4	42.3	42.9	41.7	42.0
$\dot{V}_E$	70.2	93.1	54.9	54.9	89.7	113.8	85.7	67.3	58.0	22.2	58.6	70.9
PO ₂	50.4	50.5	49.8	50.0	49.8	50.2	48.0	49.9	49.7	48.1	50.0	50.2
PCO ₂	42.0	42.4	42.1	42.1	42.0	42.0	42.8	42.1	41.9	42.2	41.9	42.0
$\dot{V}_E$	94.3	74.0	72.5	178.1	157.3	99.4	103.4	89.1	30.2	84.6	112.5	130.3
PO ₂	48.0	46.9	44.7	45.9	45.0	48.8	43.7	45.0	45.2	45.3	46.4	44.7
PCO ₂	41.3	42.0	42.3	42.0	41.7	41.4	42.1	42.1	42.0	42.0	41.4	42.5
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	20.9	31.0	15.5	17.1	15.6	28.9	54.3	47.2	54.6	21.6	21.0	38.2
PO ₂ (mmHg)	99.9	104.3	102.6	100.5	101.4	101.8	100.1	100.3	100.4	100.9	100.9	100.2
PCO ₂ (mmHg)	42.3	40.8	42.1	41.5	42.8	42.0	41.8	41.6	41.8	41.6	41.4	42.1
$\dot{V}_E$	24.1	42.2	17.1	29.0	18.0	35.2	42.2	47.2	70.6	22.3	24.1	67.7
PO ₂	79.2	76.2	77.3	77.6	75.7	77.4	77.3	76.9	77.2	76.9	77.1	74.1
PCO ₂	40.5	41.8	42.4	40.9	41.6	41.7	41.9	41.7	41.9	41.9	41.0	42.1
$\dot{V}_E$	34.3	42.5	14.2	30.3	23.9	50.7	32.9	44.7	45.8	24.5	23.2	76.0
PO ₂	66.3	66.4	65.3	65.7	66.2	65.9	66.0	66.0	66.3	65.7	66.2	66.4
PCO ₂	41.5	42.2	42.5	42.0	42.1	42.1	42.0	41.4	41.9	41.9	41.5	41.8
$\dot{V}_E$	51.3	42.0	19.6	34.6	24.5	46.6	53.7	39.4	58.6	23.1	20.1	44.0
PO ₂	59.5	59.5	58.4	60.4	59.8	58.7	59.8	60.2	59.6	60.4	60.5	60.3
PCO ₂	42.3	40.6	42.8	41.5	41.7	42.1	41.9	41.9	41.7	42.2	41.6	41.9
$\dot{V}_E$	57.3	41.8	16.1	31.2	23.4	58.9	66.9	37.5	47.8	22.8	17.2	54.9
PO ₂	54.6	50.1	53.5	53.9	54.8	54.4	55.0	53.3	54.6	53.9	54.3	54.6
PCO ₂	42.1	43.0	42.8	42.0	42.2	42.2	42.1	42.1	41.8	42.3	42.3	41.9
$\dot{V}_E$	84.3	37.4	15.5	35.4	29.9	50.7	74.7	61.9	61.3	25.1	19.4	49.1
PO ₂	50.3	50.2	50.4	50.4	49.9	50.2	50.5	50.7	50.4	50.2	48.6	48.7
PCO ₂	42.0	41.9	42.7	42.0	41.7	42.1	41.9	42.1	41.9	41.6	42.0	42.3
$\dot{V}_E$	98.0	69.2	17.3	43.6	38.5	110.7	66.2	60.3	86.8	46.6	25.7	116.1
PO ₂	44.7	45.4	45.2	45.2	45.1	46.2	44.4	44.7	45.4	44.7	44.3	45.5
PCO ₂	42.4	42.0	42.2	41.9	41.8	42.0	42.1	42.0	42.1	42.1	41.6	42.0

Table 7.7: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 889, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

Subject 910

	Protocol 1						Protocol 2					
$\dot{V}_E$ (l.min ⁻¹)	18.6	12.3	12.7	13.0	15.9	16.9	18.3	14.5	12.8	14.5	18.8	17.3
PO ₂ (mmHg)	97.7	99.5	99.7	99.8	100.1	100.4	100.3	101.1	102.8	100.3	99.6	101.0
PCO ₂ (mmHg)	40.3	39.9	40.1	39.8	39.5	40.0	40.3	39.0	40.2	39.7	40.1	39.7
$\dot{V}_E$	23.5	16.6	14.7	15.9	17.2	23.0	23.1	16.6	14.5	15.4	19.5	17.7
PO ₂	76.8	74.9	76.4	75.9	76.5	75.7	77.1	76.2	75.0	76.9	78.4	75.7
PCO ₂	40.2	40.9	40.0	40.3	40.1	39.8	40.1	40.1	39.9	40.2	39.9	40.0
$\dot{V}_E$	28.6	16.1	18.1	18.6	21.6	23.0	23.3	20.7	15.0	18.5	21.4	20.6
PO ₂	65.8	67.4	66.6	66.0	64.4	66.0	66.2	64.7	65.6	66.2	66.5	66.5
PCO ₂	40.1	39.8	39.6	39.9	40.1	39.8	40.3	40.2	40.3	40.1	39.9	40.1
$\dot{V}_E$	32.8	21.9	22.4	22.0	22.8	32.0	26.3	23.0	16.9	20.4	21.2	21.0
PO ₂	59.3	60.5	59.4	58.9	59.3	59.7	58.6	59.7	59.2	59.5	60.6	59.0
PCO ₂	39.9	39.6	39.8	40.1	40.1	40.2	40.3	40.0	40.2	39.9	40.0	40.0
$\dot{V}_E$	39.6	24.4	32.9	24.5	27.5	33.5	34.4	30.5	22.1	25.9	29.1	28.5
PO ₂	55.1	54.3	54.9	54.5	54.6	54.7	54.3	54.6	54.3	54.5	55.6	55.0
PCO ₂	40.0	39.9	39.8	40.1	40.0	40.1	40.2	40.0	40.4	40.3	40.0	40.0
$\dot{V}_E$	50.0	39.1	37.8	34.9	30.9	37.9	46.3	41.2	29.8	36.4	39.2	35.5
PO ₂	50.1	49.2	50.5	50.1	49.9	49.4	49.8	49.7	50.4	49.9	50.9	50.1
PCO ₂	40.1	39.9	39.9	39.9	39.9	40.0	40.1	40.4	40.0	39.9	39.9	39.8
$\dot{V}_E$	50.3	31.9	47.1	46.4	39.7	52.1	60.0	49.8	43.1	48.2	52.3	51.0
PO ₂	47.2	45.4	45.9	45.4	45.3	44.7	44.4	44.6	44.7	45.4	45.8	44.7
PCO ₂	40.0	39.8	40.1	40.0	40.0	40.1	40.2	40.0	39.9	40.0	39.9	40.0
	Protocol 3						Protocol 4					
$\dot{V}_E$ (l.min ⁻¹)	14.3	14.1	9.6	11.7	15.5	12.8	21.1	14.7	14.2	13.5	14.4	13.9
PO ₂ (mmHg)	98.5	99.9	100.7	100.1	100.3	99.0	99.5	99.8	100.1	99.8	101.1	100.0
PCO ₂ (mmHg)	40.0	40.6	40.2	40.2	39.8	40.1	39.7	39.4	40.0	39.9	40.3	40.0
$\dot{V}_E$	15.0	15.5	11.2	13.2	21.7	15.0	21.1	14.2	16.9	14.7	12.8	14.1
PO ₂	74.3	76.5	76.6	74.8	76.5	76.6	76.5	76.4	76.8	76.4	77.9	76.3
PCO ₂	40.2	40.0	40.1	40.0	39.9	40.5	40.0	40.1	39.9	40.0	39.6	40.0
$\dot{V}_E$	17.4	18.3	12.5	15.0	22.5	19.1	20.4	16.7	16.6	15.5	13.9	16.1
PO ₂	65.4	66.3	65.6	65.2	66.6	66.2	66.1	66.1	66.0	66.2	65.9	66.1
PCO ₂	39.8	39.8	39.7	39.7	39.9	39.8	40.0	40.3	40.1	39.9	40.1	40.0
$\dot{V}_E$	24.6	17.4	13.6	19.3	23.1	21.6	23.5	16.4	17.5	17.1	15.3	17.1
PO ₂	59.1	58.8	59.3	58.9	59.3	59.5	59.6	59.5	59.8	59.9	60.4	59.5
PCO ₂	39.8	39.8	40.0	40.0	39.8	39.9	39.9	40.1	39.9	39.9	39.8	40.0
$\dot{V}_E$	26.9	25.5	16.5	21.0	28.0	22.4	24.7	16.8	19.9	18.4	18.1	20.2
PO ₂	53.8	55.2	54.9	54.0	55.0	54.5	54.8	55.3	54.3	54.8	54.9	54.7
PCO ₂	40.1	40.0	40.0	40.1	40.1	39.8	40.3	40.0	40.2	40.2	39.8	40.0
$\dot{V}_E$	35.9	27.4	18.4	30.3	36.6	24.5	30.3	20.0	24.0	24.0	19.7	26.2
PO ₂	49.3	49.7	50.4	49.2	50.7	50.0	49.9	50.4	50.0	49.2	50.6	50.0
PCO ₂	40.1	40.2	40.1	40.3	40.0	40.1	40.1	40.4	40.2	40.3	40.5	40.0
$\dot{V}_E$	44.0	35.7	26.5	39.3	51.6	30.4	40.6	24.8	28.8	27.9	30.0	36.7
PO ₂	45.8	44.4	45.1	44.8	44.6	43.8	45.5	44.9	44.8	45.6	45.2	45.0
PCO ₂	40.0	40.0	39.9	40.0	40.2	39.8	39.9	40.1	40.0	40.1	40.1	40.0

Table 7.8: Average values of ventilation ($\dot{V}_E$), end-tidal PO₂ and PCO₂ for subject 910, from each experimental run. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps.)

APPENDIX 7.2.

This appendix shows the mean hypoxic responses (ventilation ($\dot{V}_E$) vs. P_{O_2}) to each of the protocols 1 to 4 for each subject.

Subject 875

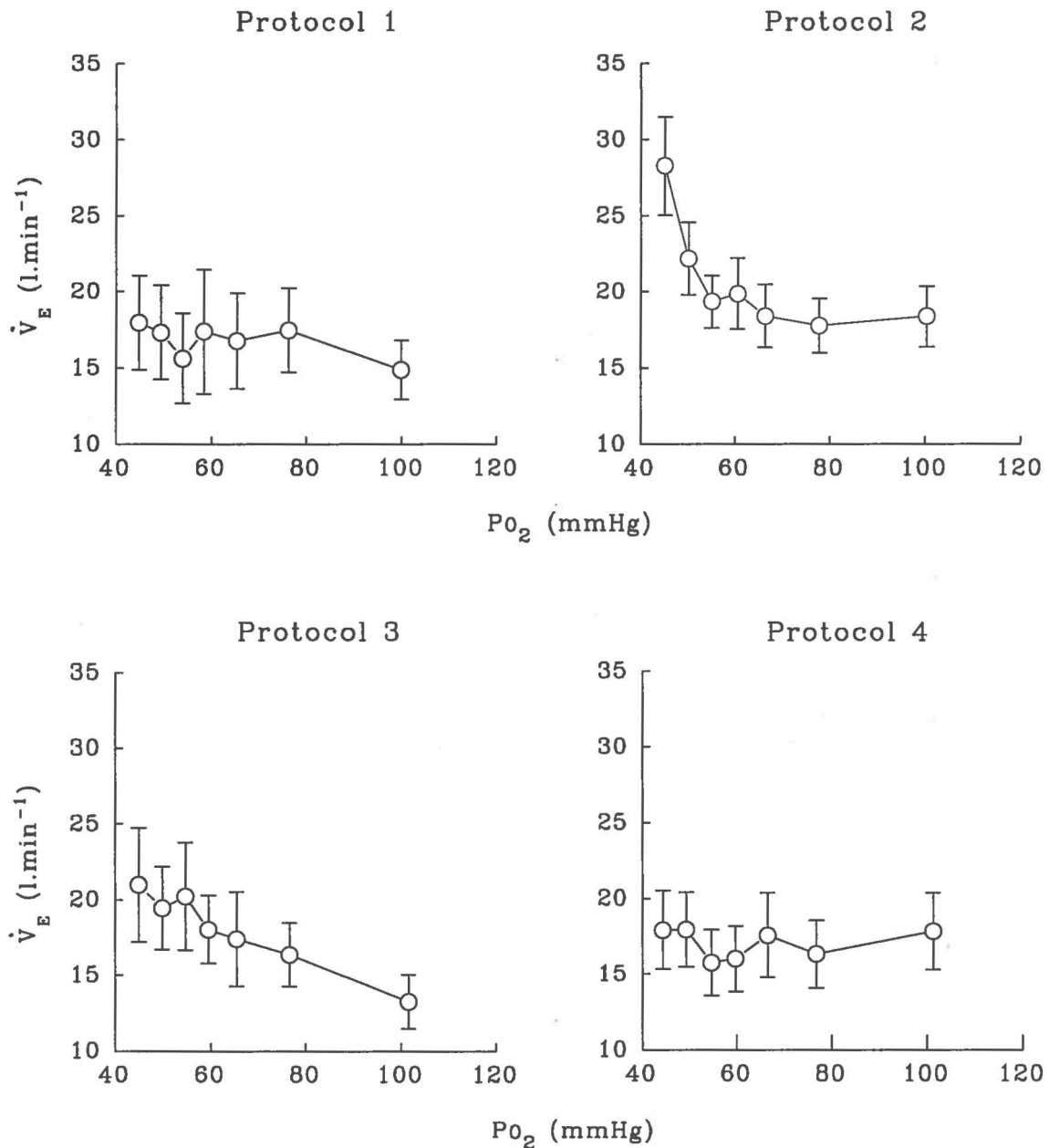


Figure 7.4: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 875. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

Subject 879

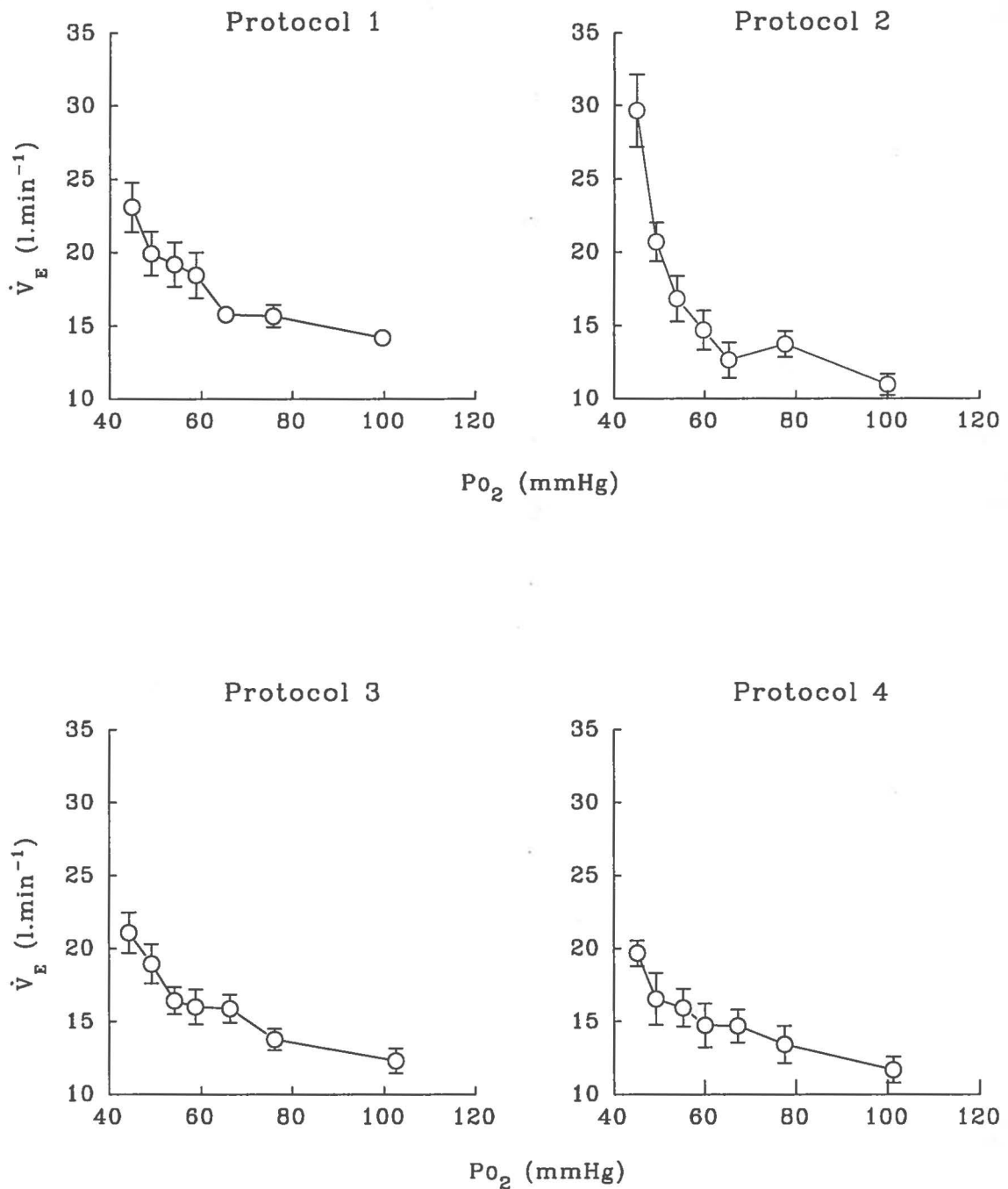


Figure 7.5: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 879. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

Subject 884

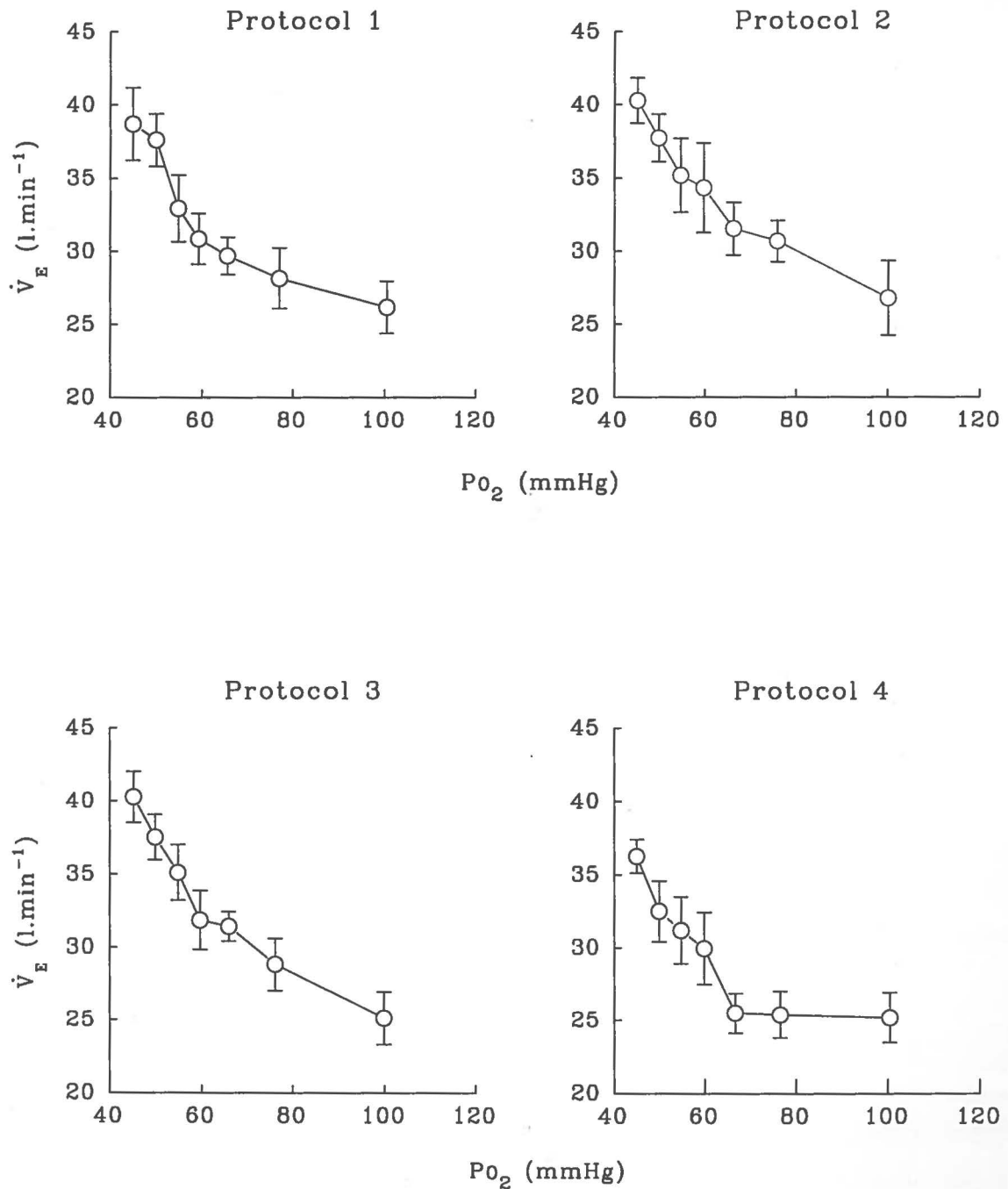


Figure 7.6: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 884. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

Subject 886

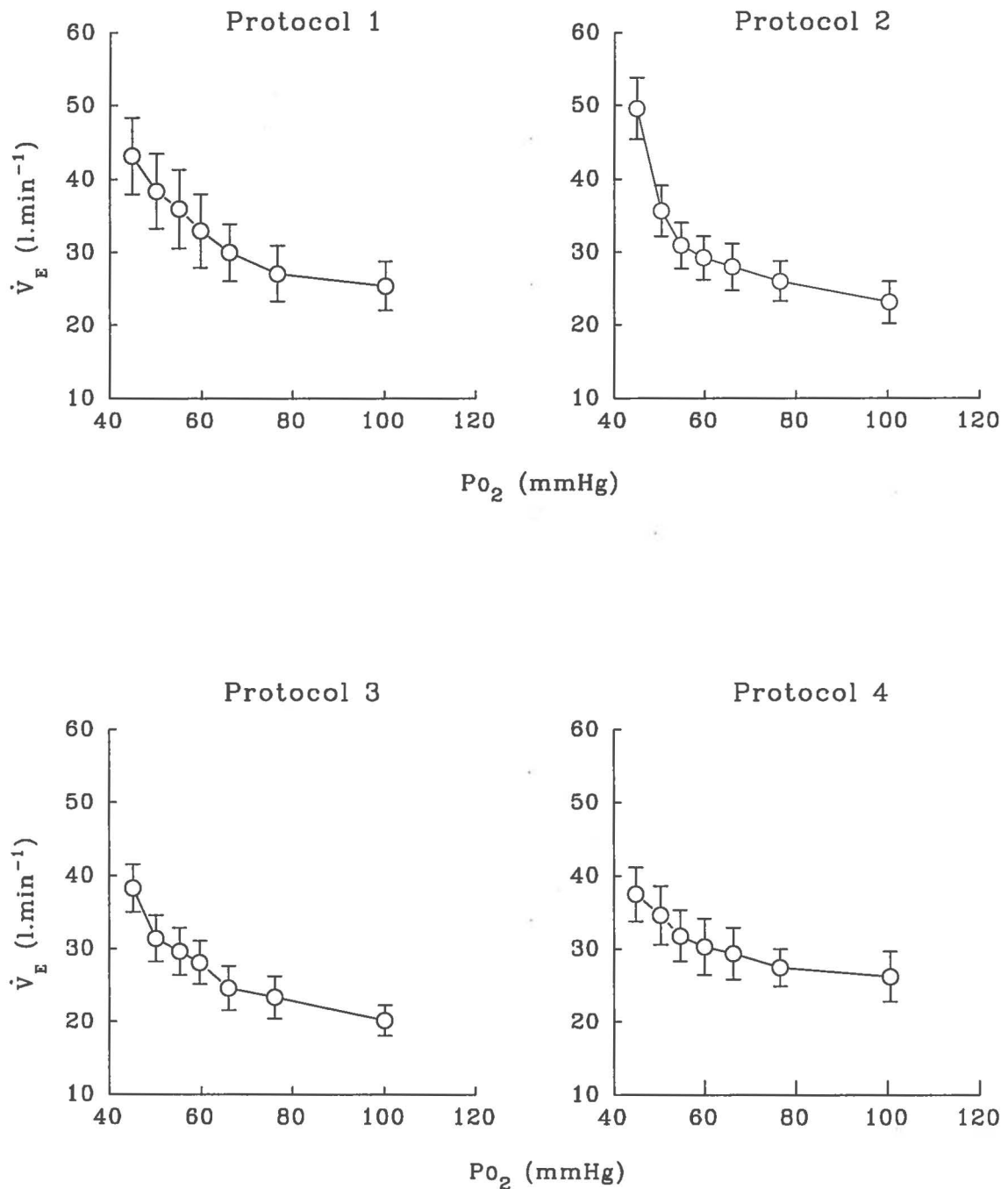


Figure 7.7: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 886. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

Subject 889

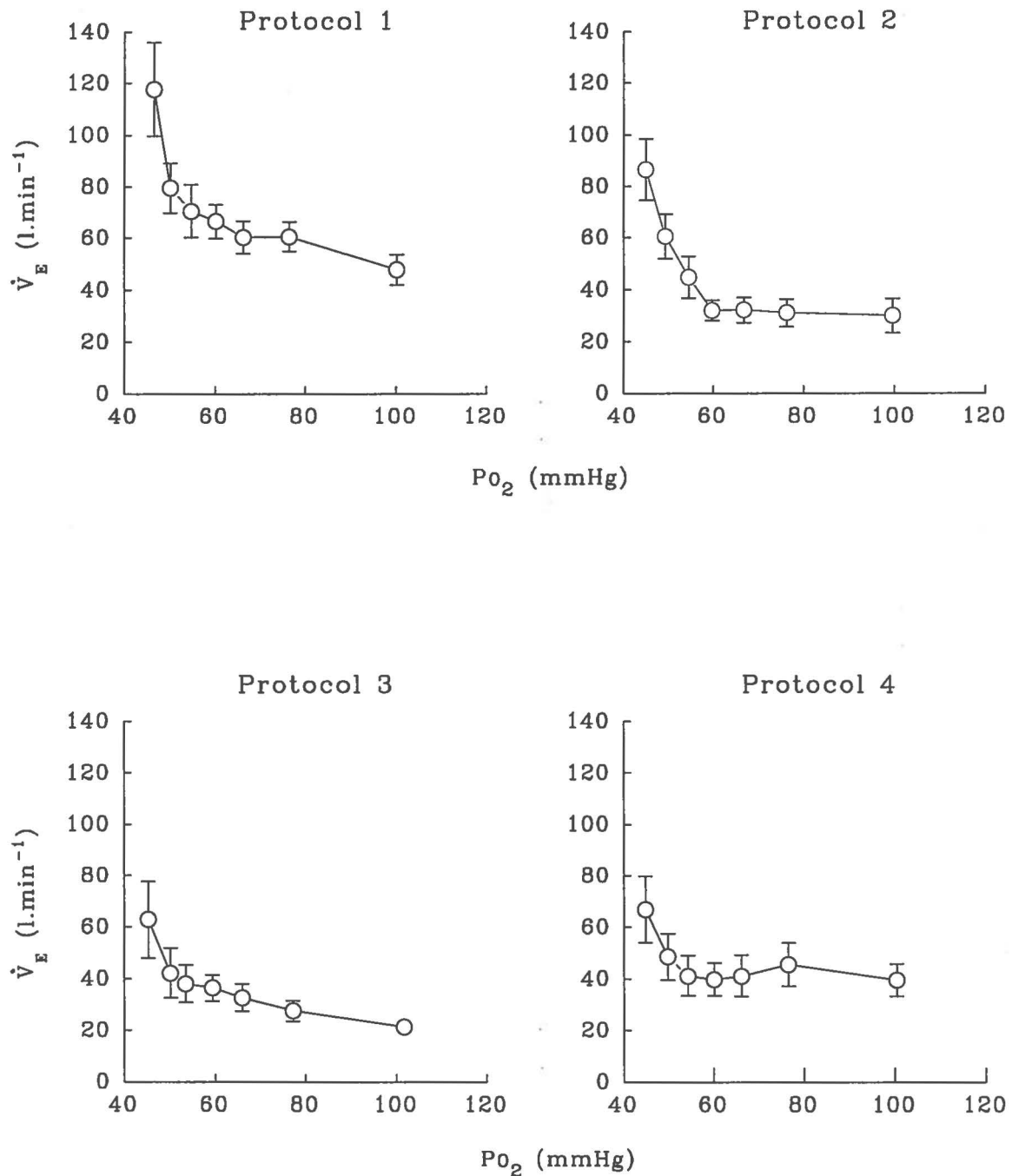


Figure 7.8: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 889. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

Subject 910

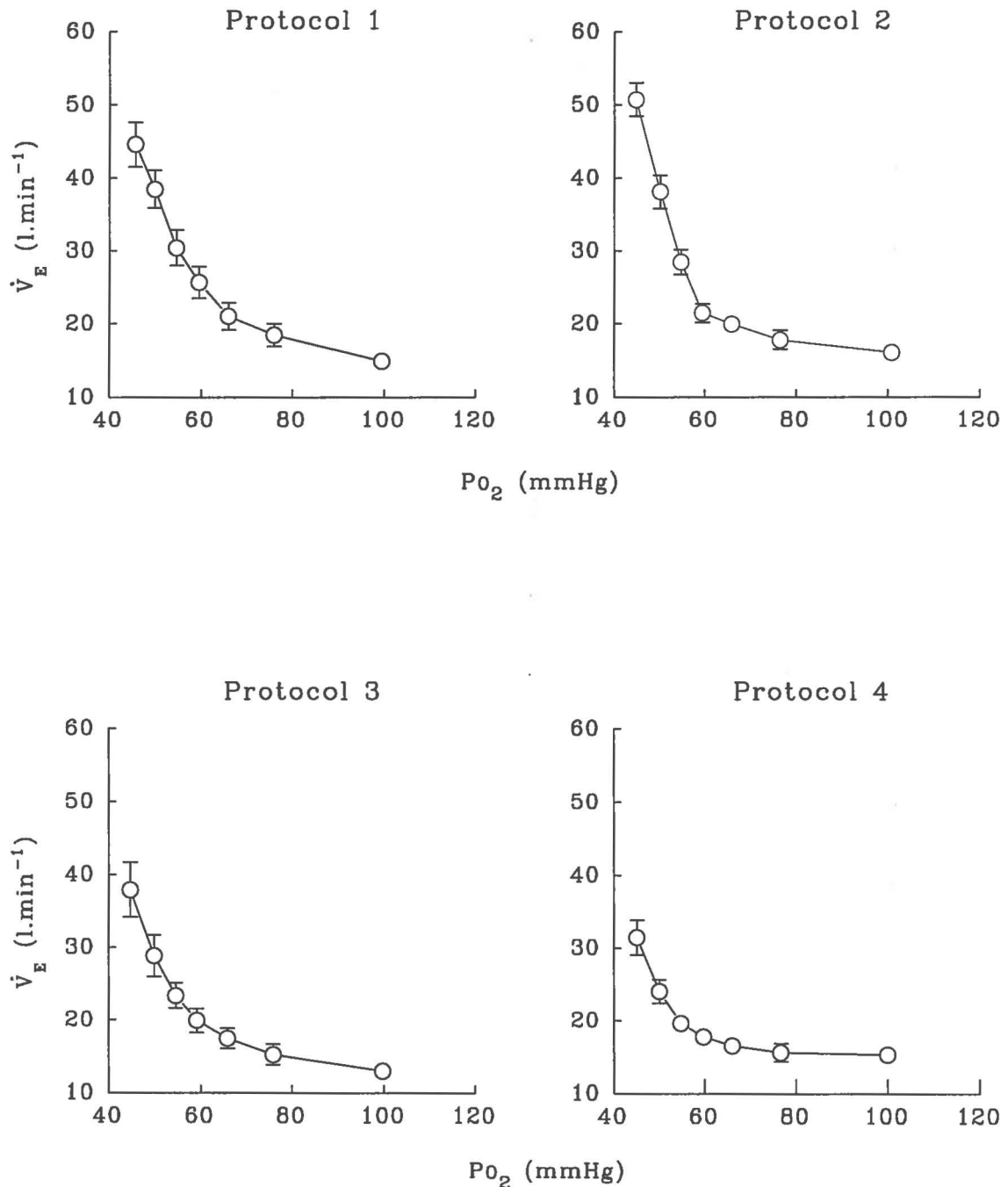


Figure 7.9: Mean ($\pm$ S.E.) hypoxic responses to protocols 1 to 4 for subject 910. (Protocol 1 - euoxic pre-conditioning, descending steps; protocol 2 - euoxic pre-conditioning, ascending steps; protocol 3 - hypoxic pre-conditioning, descending steps; protocol 4 - hypoxic pre-conditioning, ascending steps).

CHAPTER 8

CONCLUDING REMARKS

I trust I have not wasted breath.

Tennyson.

In this chapter I should like to summarize the findings of this thesis and propose possible future work that may help in clarifying and extending conclusions drawn here.

Chapter 3 presented the construction of a chamber within our laboratory designed to control end-tidal gases in man for longer periods of time than previously feasible. Initial testing proved successful over an 8-hour period with good quality control of end-tidal gas composition. Although there was a small leak through the chamber walls at construction, it appeared to have increased by the time of experimentation. In order to counter this effect, it should be possible in the future to install a servo system to introduce a continuous flow of N_2/CO_2 into the chamber, proportional to the actual chamber composition.

Chapters 4 and 5 were concerned with the comparison between isocapnic and poikilocapnic conditioning during 8 hours of hypoxia. The first of these two chapters documented the ventilatory changes and described a striking, progressive rise in ventilation during isocapnic hypoxia, but no change when P_{CO_2} was allowed to fall. The following chapter presented data from tests of the ventilatory response to acute changes in hypoxia (AHVR). These measurements suggested that hypoxia *per se* was principally responsible for the rise in ventilation observed in isocapnic hypoxia and that one underlying mechanism was probably an increase in hypoxic sensitivity. In line with animal studies, it is probable that this change in hypoxic sensitivity is a result of an elevated carotid body function. An increase was also noted in the hypoxia-independent ventilation during both hypoxic exposures, but the degree of this change was not clear using the current protocol. However, its role in the rise in ventilation during isocapnic hypoxia was not ruled out.

The length of the study meant that the importance of these changes in respiratory control was not easily definable in relation to the progressive rise in ventilation at altitude over days to weeks (ventilatory acclimatization to hypoxia (VAH)). Chapter 6, therefore, attempted to extend the period of observation to 48 hours of hypoxic exposure, again in both

poikilocapnia and isocapnia, and the 48 hours of deacclimatization following relief of hypoxia.

Ventilation continued to rise during 48 hours of isocapnic hypoxia, with no real evidence of a plateau. In addition, ventilation rose during the poikilocapnic exposure, suggestive of VAH. Increases in both hypoxic sensitivity and hypoxia-independent ventilation, were thought to be responsible for the ventilatory changes in isocapnic hypoxia. In addition, they were both thought to account for the rise in ventilation during poikilocapnic hypoxia. This is an interesting observation, since researchers, particularly experimenting in the goat and in the cat, have suggested that increased carotid body function is the primary cause of VAH, and that central changes play only a small role. Indeed, the well-documented dependence of VAH on the presence of carotid bodies (in carotid body denervation studies) and the correlation between hypoxic sensitivity at $t=0$ and the degree of VAH noted in this study and another recent investigation by Reeves *et al.* (1993) supports the important role of the intact peripheral chemoreflex.

However, the protocol employed in this chapter provides more information regarding the changes in the central component of ventilation and its genesis. It was proposed that hypoxia was causing an increase in the central chemoreflex excitability, represented by a decrease in its set point. Hypoxia may be acting directly on the CNS or via an increased peripheral chemoreflex stimulation. The fact that G_p and $\dot{V}_C$ appear to rise with similar time courses implies that $\dot{V}_C$ may be dependent on the peripheral chemoreflex, thus agreeing with data from this and many other investigations that neither VAH nor large central changes occur in the absence of the carotid bodies during prolonged hypoxia. Changes in the central chemoreflex, elicited by an increased peripheral chemoreflex, may therefore be important in the changes in ventilation at high altitude.

In the discussion of Chapter 6, it was noted that other studies have shown that prolonged hypocapnia, in the absence of hypoxia, can alter the CO_2 response and eupnoeic ventilation

(Dempsey *et al.*, 1975; Eger *et al.*, 1968): this was termed hyperventilation-induced hyperpnoea. The hypocapnia of these studies was elicited by either voluntary hyperventilation (Dempsey *et al.*, 1975; Eger *et al.* 1968, in one subject) or using a respirator (Eger *et al.*, 1968). It was thought that acid-base changes due to the respiratory alkalosis were responsible for the changes in respiratory control, however, it may have been that suprapontine structures were generating these changes by a similar mechanism as the peripheral chemoreflex. Indeed, animal studies have suggested that both the peripheral chemoreflex (Millhorn *et al.*, 1980a,b; Tenney *et al.*, 1971) and suprapontine structures (Gallman and Millhorn, 1988; Ou *et al.*, 1983) can modulate CNS excitability. Since the poikilocapnic and isocapnic exposures result in similar peripheral chemoreflex and different central chemoreflex activities, then the site of the increased excitability is unlikely to be the respiratory controller, which would receive the different levels of stimuli, but rather the central chemoreceptors since the changes in CNS excitability appear to be the same in both protocols.

If suprapontine structures are important in the changes during voluntary and imposed hyperventilation, then a further increase in CNS excitability might be expected during the isocapnic exposure of the current study. This is not seen, however, and thus it is hard to integrate fully all of the observations of this and other studies.

The results of this thesis suggest that hypoxic sensitivity shows a plateau after 48 hours of hypoxic exposure, although a plateau in ventilation appears less obvious. There are two possible conclusions that can be drawn from this. The first is that there is a further mechanism causing a prolonged slow rise in ventilation: this may be related to HCO_3^- continuing to be excreted from the plasma (in response to the alkalosis of the poikilocapnic protocol) or an increase in central nervous system (CNS) excitability in both protocols. The second is that almost complete ventilatory acclimatization is being witnessed in both protocols, but is less clear-cut. Sato *et al.* (1994) elicited almost complete VAH after 48 hours by demonstrating a stabilization of end-tidal Pco_2 . Since the duration of

acclimatization is known to depend upon the level of hypoxaemia, it would seem reasonable that the relatively low level of hypoxaemia in the current study and that of Sato *et al.* could elicit almost complete acclimatization over this time course. This suggests a strong link between the changes in hypoxic sensitivity and VAH. Further evidence in favour of this notion is that this thesis and others have noted a correlation between hypoxic sensitivity before hypoxic exposure and the degree of ventilatory acclimatization and also between hypoxic sensitivities at $t=0$ and $t=48$ hours, the latter correlation only being investigated in this thesis. These correlations suggest that the mechanism responsible for the acute hypoxic response (*i.e.*, the peripheral chemoreflex) is also connected with the mechanism responsible for the rise in ventilation during long-term hypoxia. The studies presented in this thesis are the first of their kind in man to show definitively that the acid-base changes at altitude are not important in eliciting the *early* stages of VAH and that hypoxia *per se* is the principal factor. However, what this thesis cannot confirm is that acid-base changes are not important at a later stage, *i.e.*, after several days.

With a view to future investigations, it is clear that even further protracted exposures would be desirable in order to observe whether ventilation continues to rise after 48 hours of isocapnic hypoxia and whether a true plateau in hypoxic sensitivity has been elicited at 48 hours as thought in Chapter 6 of this thesis. In addition, this would need to be compared with an equivalent poikilocapnic exposure in order to investigate the presence of a second mechanism, with a slower time course, related to acid-base changes, in VAH. Indeed, if a true plateau is being seen in hypoxic sensitivity at 48 hours and ventilation continues to rise (as suspected from the majority of the literature), then acid-base changes must be a strong candidate for the cause of the continuing acclimatization.

Clearly, it is important that more experimentation is applied to the understanding of central changes in respiratory control during prolonged hypoxia. Exposure to prolonged hypercapnia would provide information on this issue. For example, if ventilation were to increase progressively in response to this stimulus, then there is an implied role for

suprapontine structures and/or increased peripheral chemoreflex activity causing hyperventilation-induced hyperpnoea during isocapnic hypoxia. If ventilation were to remain constant, then the postulates of this thesis may be true and hypoxia may in fact be the important stimulus in mediating changes in central respiratory control. Alternatively, if ventilation was to fall after the acute hyperventilatory response, the elevated P_{CO_2} could be shifting the CO_2 response towards higher levels of P_{CO_2} via changes in CSF $[HCO_3^-]$, which, in this thesis, are thought to be playing virtually no part in effecting changes in ventilation. Experiments have been performed on the ventilatory response to prolonged inspired P_{CO_2} , and these suggest that central respiratory drive may not alter significantly (Dempsey and Forster, 1982). However, a long-term study using the chamber described in this thesis is needed in order to monitor the response to arterial hypercapnia.

One of the problems in assessing the contribution of CNS changes to VAH, by measuring the eupnoeic ventilation or hypoxia-independent ventilation, is that the subjects are required to come out of the chamber. This results in an increased state of arousal; thus it is difficult to compare the hypoxic ventilation in the chamber with the eupnoeic ventilation. In order to overcome this problem, the eupnoeic ventilation could be measured immediately after the "chamber" ventilation, by allowing air to pass into a mouthpiece containing one-way valves, via a tube from outside the chamber. Expired air could then be removed via another tube leading out of the chamber.

One of the initial questions raised was whether hypoxic ventilatory depression (HVD), present at 20 min of isocapnic hypoxia, develops into VAH or whether it is still present during VAH. A recent study by Sato *et al.* (1994) documents the persistence of HVD throughout acclimatization and they used this observation to propose that HVD was occurring within the central chemoreceptor mechanism. Results from our laboratory and recent reports from others indicate that the peripheral chemoreflex and more specifically, the carotid body, is responsible for HVD in man and awake animals. It would be interesting, therefore, to examine a 20-min period of isocapnic hypoxia following a pre-conditioning

period of hyperoxia at various time points during exposure to prolonged isocapnic hypoxia. Should an initial rise in ventilation with a subsequent progressive fall, akin to HVD, be present, then a reversal of the mechanisms involved in HVD to give VAH could be ruled out and *vice versa*.

Further experimentation in our laboratory has indicated that HVD is not related to an endogenous dopaminergic inhibitory mechanism within the carotid bodies. Conversely, Tatsumi *et al.* (1992b) and Bisgard *et al.* (1987) have suggested that diminished dopaminergic inhibition within the carotid body may be involved in VAH, in cats and goats, respectively. These data suggest that HVD and VAH may be independent; thus, it would be informative to examine the ventilatory response to prolonged isocapnic hypoxia with oral domperidone (a peripheral dopamine blocker) or to compare the sensitivity of AHVR to domperidone before and after prolonged isocapnic exposure. This could further establish the dissociation of HVD and VAH and also aid in understanding the mechanisms involved in VAH.

The novelty of the protocol in Chapter 6 elicited two other interesting findings: first, that isocapnia during hypoxia may worsen the symptoms of acute mountain sickness (possibly by increased cerebral blood flow) and second that isocapnia may prevent periodic breathing during sleep. These findings are the first of their kind in humans and indeed merit further investigation.

Chapter 7 described the attempt to design a protocol to measure the hypoxic response, defined as the relation between ventilation and end-tidal P_{O_2} generated from the acute ventilatory responses to every level of hypoxia; where the acute ventilatory response is the expected value at the maximum of the rise in ventilation reached in the first few minutes in stepping from a period of sustained euoxia into hypoxia under isocapnic conditions. The protocol consisted of a series of 7 either ascending or descending 2-min steps of hypoxia. This protocol was found to be unsatisfactory, however, and it was thought that HVD

associated with the hypoxic steps was altering the hypoxic response itself. Because the development of a suitable test for measuring the shape of the hypoxic response would be useful in studies such as those described in this thesis, it would be beneficial to re-attempt to design a successful protocol by reducing the duration of each hypoxic step and/or reducing the number of steps.

In conclusion, therefore, the development of the chamber as described here has clearly opened up many avenues for research into prolonged alterations of certain physiological stimuli and their effects upon specific homeostatic control mechanisms in man. The results presented in this thesis, from the preliminary experiments using this technique, suggest that hypoxia is the important factor in creating the changes in respiratory control during the first two days at altitude, *i.e.*, increases in hypoxic sensitivity, CO₂ response and eupnoeic ventilation, and that the associated acid-base changes of the respiratory alkalosis are not. This is indeed a surprising finding and merits further investigation.

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*If you steal from one author, it's plagiarism;
if you steal from many, it's research.*

Wilson Mizner.

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