PULMONARY RECEPTORS AND THEIR ROLE IN THE
CONTROL OF BREATHING IN TURTLES

by

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The normal breathing pattern recorded in unanaesthetized, lightly restrained turtles, Chrysemys picta, consisted of periods of continuous breathing interspersed with periods of breath holding. During each ventilatory period, respiratory frequency and tidal volume were controlled separately and independently of breath length, the total inspiratory interval, the active inspiratory interval and the expiratory interval. Increases in pulmonary minute ventilation during hypercapnia were caused by increases in respiratory frequency due solely to shortening of the intervals of breath holding. The frequency of breathing within each ventilatory period remained constant. There was a large variability in inspiratory and expiratory gas flow rates yet tidal volume was maintained within narrow limits by adjustment of the lengths of the active inspiratory and expiratory intervals. This mechanism was dependent upon lung volume information carried within the vagus nerve. Following vagotomy, changes in minute ventilation due to hypercapnia stemmed primarily from changes in tidal volume while changes in respiratory frequency were markedly reduced.

Lung volume information carried within the vagus nerve arose from slowly adapting pulmonary stretch receptors. Single fibre nerve activity from pulmonary receptors was recorded from vagal slips in single-pithed tidally ventilated turtles. The major stimulus of these receptors was the change in lung volume throughout each breathing cycle. The rate and degree of change in transpulmonary pressure were without direct effect on receptor discharge. The functional characteristics of these receptors differed only quantitatively from those recorded in pulmonary stretch receptors of mammals.
and these differences probably stem from the lower body temperature of the turtle and the location of the receptors in the turtle lung.

Most receptors were sensitive to $CO_2$, several sufficiently sensitive that both tonic and phasic receptor discharge were totally inhibited throughout the ventilatory cycle by 5 to 10% $CO_2$ in the inspired gas. Pulmonary mechanoreceptors in the frog were also shown to be sensitive to $CO_2$. The acute sensitivity to $CO_2$ of a few receptors in turtles and frogs parallels that of the intrapulmonary $CO_2$ receptors described in birds and suggests that a pulmonary receptor with distinct mechano- and chemosensitive properties may represent the functional precursor of the variety of pulmonary receptor types which appear in modern day vertebrates.

To examine the role of $CO_2$ sensitivity of pulmonary receptors in the overall response of turtles to inhaled $CO_2$, ventilatory responses of unanaesthetized turtles to changes in the intrapulmonary $CO_2$ content of a vascularly isolated lung (constant $Pa_{CO_2}$) and an intact lung were measured during spontaneous breathing. The isocapnic hyperpnea associated with inhalation of $CO_2$ by the vascularly isolated lung was small and abolished by vagotomy. It is concluded that both inhibition of pulmonary stretch receptor discharge with increasing levels of $F_{ICO_2}$ and a functional increase in central inspiratory volume threshold contributed significantly to tidal volume increases during hypercapnia. The primary ventilatory response of intact turtles to increasing levels of $F_{ICO_2}$ was an increase in respiratory frequency and this response was greatly reduced when $CO_2$ was inspired only by the vascularly isolated lung. Thus the ventilatory response of turtles to increasing levels of $F_{ICO_2}$ is
primarily dependent upon increased levels of arterial CO₂.

The effect of vagotomy in producing apneusis in turtles supports suggestions they lack a pneumotaxic centre. The arrhythmic breathing pattern in turtles with intact vagal nerves, however, bears no similarity to the pattern of breathing in mammals with only the pneumotaxic centre ablated. It is concluded that the vagal input from pulmonary receptors to the respiratory centres in turtles is qualitatively similar to that in mammals yet the differences in central integration of lung volume information in turtles and mammals are not due solely to the absence of a pneumotaxic centre in the turtle. Many of the remaining differences may arise from the lower metabolic demand of turtles but how this affects central integration and respiratory pattern generation is unknown.
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GENERAL INTRODUCTION

Although modern reptiles exhibit a vast diversity of respiratory mechanics, lung morphology and gas transport mechanisms, the breathing pattern of almost all reptiles is similar; an arrhythmic breathing pattern consisting of series of one to several breaths separated by a highly variable, respiratory pause (from a few seconds to several hours) commencing at end-inspiration (turtles, McCutcheon, '43; Gans & Hughes, '67; crocodiles, Naifeh et al., '70; Gans & Clark, '76; snakes, Glass & Johansen, '76). Although the respiratory pattern is very similar in these animals, the terminology used to describe the events during ventilation is not. A continuous series of breaths has been described as a ventilatory period (Naifeh et al., '70; Glass & Johansen, '76), a respiratory sequence (Gaunt & Gans, '70; Lucey & House, '77), a respiratory series (McCutcheon, '43; Shelton & Burggren, '76), a breathing period (Lenfant et al., '70), a breathing group (Huggins et al., '70), or an active period (Lucey & House, '77; Frankel et al., '69). The period between consecutive series of breaths has been labelled the nonventilatory period (Glass & Johansen, '76; Naifeh et al., '70), breath holding period (Lenfant et al., '70), interval between series (McCutcheon, '43; Shelton & Burggren, '76) or the passive or apneic period (Frankel et al., '69; Lucey & House, '77). To add further confusion, Frankel et al. ('69) use the term respiratory sequence to describe jointly the period during which there is a continuous series of breaths and the subsequent interval before the next such series. Many of these terms are confusing, misleading or incorrect. The simplest
yet adequately descriptive terms for these events, viz ventilatory period and nonventilatory period will be used throughout this study.

This arrhythmic breathing pattern bears many similarities to abnormal breathing patterns in mammals with defective central respiratory controls. As a consequence, the pattern has been variously termed apneic breathing (Boyer, '63; Frankel et al., '69; Jackson et al., '74; Shelton & Burggren, '76; Glass & Johansen, '76), apneustic breathing (Lumsden, '23a; Randall et al., '44; Lucey and House, '77), Cheyne-Stokes respiration (Hoff & Breckenridge, '54), and Biot's respiration (Hoff and Breckenridge, '54; Frankel et al., '69).

In keeping with this image of poor ventilatory control, reports of widescale variations in the ventilatory response to hypoxia, hypercapnia and temperature, across reptilian groups, are prevalent in the literature (cf. Wood & Lenfant, '76, for review). Many of these studies assess only the effects of the respiratory stimuli on metabolism, ventilation rate or dive length in aquatic species. This lack of complete analysis has made meaningful comparisons impossible. Many reptiles show large transient alveolar-arterial differences in $P_{CO_2}$ and $P_{O_2}$ when the levels of inspired gases are altered due to variable degrees of intracardiac or central vascular shunting (right to left) (Frankel et al., '69; Lenfant et al., '70). These transients may last over long periods (½-hr) but have not always been accounted for when the respiratory sensitivity to $CO_2$ and $O_2$ of these animals was measured. Further, comparisons of the minute ventilation ($V_E$) response to inspired $CO_2$ in ectotherms at various temperatures have now been shown to be valid only if oxygen consumption ($V_O_2$) differences are taken into account, i.e., it is more meaningful to measure $V_E/V_O_2$ as a function
of \( P_{CO_2} \) than simply measuring \( \dot{V}/P_{CO_2} \) (Dejours et al., '70). In view of all this, it is not surprising that there is no coherent concept of respiratory control in reptiles.

It has become clear, however, that the concepts of respiratory control in mammals emphasizing blood \( P_{CO_2} \) and pH homeostasis, do not strictly apply to ectotherms. In ectotherms, blood pH changes with temperature in such a way that the \( OH^-/H^+ \) ratio remains constant, and, therefore, the difference between blood pH and pN (neutrality of water) remains constant. Since the pH of the blood is always greater than pN, it follows that the blood is always alkaline with respect to water at the same temperature. As long as this relative alkalinity (pH-pN) remains constant, the \( OH^-/H^+ \) ratio of the animal is constant and acid-base balance is maintained (Howell & Rahn, '76).

The net consequence of this is that as temperature increases, changes in respiratory frequency, tidal volume and minute ventilation will be smaller than anticipated on the basis of measured increases in oxygen consumption. This leads to a relative "hypoventilation" (a decrease in \( \dot{V}_E/\dot{V}_O_2 \)), an increase in arterial \( P_{CO_2} \) and pH, and maintenance of a constant \( OH^-/H^+ \) ratio (Jackson, '71; Rahn, '73). These observations suggest that respiration is controlled to maintain a constant relative alkalinity of the blood (temperature dependent pH and \( P_{CO_2} \)) (Reeves, '72) and it is from this homeostatic state that ventilation is altered in response to metabolic load as has now been demonstrated for the turtle (Jackson, '71).

Thus, it begins to appear that reptiles accurately control ventilation, despite an arrhythmic breathing pattern. Further, as a consequence of the arrhythmic pattern; there are now three major variables in respiratory control; tidal volume, respiratory rate and the duration of the nonventila-
tery period. To date virtually nothing is known of the way the breathing pattern is changed, either to maintain relative alkalinity or in response to changing metabolic loads, or of the means by which this regulation of breathing is achieved.

A Hering-Breuer (vagal, inflation volume related, inspiratory inhibitory reflex) or similar reflex has now been demonstrated for fish (see Shelton, '70), frogs (Taglietti & Casella, '66,'68), reptiles (Huggins et al.,'71), birds (see Eaton et al.,'71; Osborne & Burger, '74) and mammals (see Guz et al.,'69) and several other ventilatory control mechanisms reflexly induced by pulmonary mechanoreceptors have been described. The function of pulmonary mechanoreceptors appears to be in maintenance of a balance between tidal volume ($V_t$) and respiratory frequency ($f_{res}$) in such a way that adequate ventilation is achieved with minimum work or minimum average force developed by the respiratory muscles (Otis et al.,'50; Mead, '60; Euler et al.,'70).

As lung volume is increased above functional residual capacity (FRC) in mammals, inhibition of inspiration and promotion of expiration are reflexly elicited by pulmonary stretch receptors.

The response of mammalian pulmonary mechanoreceptors to inflation shows both a phasic and a tonic component. The phasic component of this response is responsible for the true Hering-Breuer reflex. The inflation accompanying inspiration stimulates pulmonary mechanoreceptors increasing their discharge rate and reflexly inhibiting the ongoing inspiration. The rate of decay of the phasic activity (which is faster for smaller inflation volumes) and the level of control excitatory activity in the respiratory centres will determine the onset of the next breath. This phasic activity, then, affects the time course of inspiration ($T_I$) and the respiratory
frequency \( f_{\text{resp.}} \) (Guz et al., '69; Bystrzycka & Huszczuk, '73; Younes et al., '74). This particular reflex may further contribute to ventilatory stability by balancing vagal information on rate of inflation with central inspiratory drive and reflexly increase or decrease respiratory muscle recruitment during the time course of an inspiration depending on the load (resistance to air flow) encountered (McClelland et al., '72).

The widespread appearance of this response in amphibians to mammals (lunged fishes remain uninvestigated) is an indication of its importance. The convergent appearance of similar mechanoreceptors on the branchial processes of the gills of fish which when stimulated by displacement react to the mechanostimulation by reflexly decreasing \( f_{\text{resp.}} \) via prolonging \( T_I \), is not, then, surprising (Satchell & Way, '62). Adjusting and optimizing respiratory work according to load appears to have long been a function of peripheral (gill or pulmonary) mechanoreceptors.

The tonic component of the inflation response has only recently been the subject of much attention. Research indicates that a sensitive vagal control of central respiratory frequency by changes in tonic, non-modulated vagal activity exists which affects the duration of expiratory activity \( T_E \) (Knox, '73; Bystrzycka & Huszczuk, '73; Younes et al., '74; Miserocchi & Milic-Emili, '75; D'Angelo & Agostoni, '75). At end-inspiration, phasic respiratory activity decays. The tonic, vagal, inspiratory-inhibitory activity which remains may establish a level of central inhibition which inspiratory drive must overcome to initiate the next inspiration. It is this component of the response which determines the length of apnoea following forced inflation (Younes et al., '74). Tonic activity is a function of lung volume. When end-inspiratory volume is low and therefore tonic activity is small, or inspiratory drive is high, the rate of decay
of phasic vagal activity will determine $f_{\text{resp}}$. If, however, tonic vagal activity is high accompanying a large inflation volume, or inspiratory drive is low, the tonic vagal activity will determine $f_{\text{resp}}$ by affecting $T_E$.

The peripheral control of breathing in birds has also been the subject of much recent investigation. Since bird lungs are relatively inexpansible, there are shortcomings in the theory of using lung stretch as the sensitive component of a breath-by-breath control system. Inflation of the lung-air sac system is, however, accompanied by a massive vagal afferent discharge and debate has centered around the modality for this response: mechano-receptor stimulation; CO$_2$ receptor stimulation or stimulation of CO$_2$ sensitive mechanoreceptors.

Histological studies (King et al., '74) indicate the most likely candidates to act as pulmonary receptors are granular cells. These cells look like both neurite receptor cell complexes such as carotid body chief cells which are presumed to be chemosensitive, and like Merkel cells which are known to be mechanosensitive. Consequently the histological evidence contributes nothing conclusive to this controversy. The majority of investigators, however, agree with the classification system of Molony ('74) which distinguishes between: type I cells, CO$_2$ sensitive with some mechanosensitivity; and type II cells, sensitive to mechanical stimulation only (Molony, '74; Fedde et al., '74a; Scheid et al., '74; Osborne and Burger, '74; Burger et al., '74). By far the majority of receptors recorded from are of type I (60-80%) (Molony, '74; Fedde et al., '74a) and are located in the major airways (Scheid et al., '74). Type II receptors are thought to comprise only a small fraction of the total receptor population (18-20%) and have been tentatively isolated to the oblique septum (not in
the lung) (Scheid et al., '74). Their location tends to indicate that they are unlikely to function like stretch receptors in a Hering-Breuer type reflex.

The other major classification scheme for avian pulmonary receptors (Leitner and Roumy, '74) is based on discharge patterns of slowly and rapidly adapting receptors responding to inflation, deflation, or both inflation and deflation. It has been pointed out (Molony, '74; Fedde et al., '74b; Burger et al., '74) that the pattern and magnitude of CO₂ receptor discharge would depend on the CO₂ concentration at the receptor site, a function of the amount of gas exchange around the site and the magnitude of the gas flow at the site throughout the ventilatory cycle. It is possible that areas of the dorso-bronchi of the avian lung are exposed to high CO₂ levels during inflation or deflation alone or both inflation and deflation.

Although the majority of evidence supports the involvement of pulmonary CO₂ receptors in the reflex control of ventilation in birds, these studies pose a further question. Are these receptors which monitor pulmonary CO₂ levels acting as part of an inspiratory-inhibitory reflex similar to mammalian pulmonary mechanoreceptors, or are they part of a reflex control of blood or pulmonary gas CO₂?

Several investigators (Fedde et al., '74b; Scheid et al., '74) support the view that CO₂ receptors monitor metabolic CO₂ changes at different times in the respiratory cycle and may be involved in establishing local air flow patterns within the lung or ventilation-perfusion matching to regulate blood CO₂ partial pressures (P_{CO₂}). Burger et al. ('74) suggest that these receptors may have originally monitored tissue or blood P_{CO₂} but because ventilation removes pulmonary arterial CO₂, they also indirectly
monitor ventilation. Dynamic responses must have evolved to permit intrapulmonary CO₂ receptors to further monitor breath size and flow rate. Osborne and Burger ('74) have pointed out that by monitoring the disappearance of dead space CO₂ in inspiration, they may, in effect, monitor rate and depth of inspiration and may produce respiratory inhibition similar to that achieved by mammalian pulmonary mechanoreceptors. Further support for this argument comes from carotid body denervation studies in ducks (Jones & Purves, '70a, b) which indicate that the speed of the ventilatory response to transient CO₂ changes is reduced by carotid body denervation. This indicates that in the absence of the carotid bodies there is no rapid ventilatory response to CO₂ and afferent information from pulmonary CO₂ receptors must therefore be involved in a Hering-Breuer type reflex only.

Partially as a result of these avian studies, investigators have begun to re-examine the effects of changing gas concentrations (particularly CO₂) on the response of mammalian pulmonary stretch receptors. There have been many scattered reports in the literature of lung sensitivity to CO₂ but nothing conclusive (Adrian, '33; Pi-Suñer, '47; Hortolomei et al., '56; Petit, '60; Bishop & Bachofen, '73). The older literature suggests that there is no effect of inhaled CO₂ on the stretch receptor discharge of the cat and rabbit. Mustafa and Purves ('72), however, have recently shown that CO₂ does reduce stretch receptor discharge in rabbits. Increasing the P<sub>CO₂</sub> in the lung from 27 to 67 mm Hg resulted in a decreased average rate of discharge at peak inflation (≈25% reduced), an altered interval histogram, a decreased discharge rate at any given lung volume, a rise in the mechanical threshold and no effect on total lung resistance (TLR). These results have been confirmed by others for the dog (Sant' Ambrogio et
al., '74) and they are compatible with more recent studies of Bartoli et al. ('73, '74). Bartoli and co-workers have further shown that the changes in $f_{\text{resp}}$ resulting from the effects of $CO_2$ on pulmonary stretch receptors are due to changes in $T_E$ implying the effect is mediated primarily by the tonic component of the inflation response. This has been confirmed by Phillipson ('74).

Recently, it has been shown that lizards appear to possess both $CO_2$ receptors which have functional characteristics similar to those in birds and mechanoreceptors with functional characteristics similar to pulmonary stretch receptors in mammals (Fedde et al., '77; Scheid et al., '77).

Thus it appears that all lunged vertebrates show a ventilatory response to imposed changes in lung volume. It also appears that lizards, birds and mammals provide similar vagal inputs to the respiratory centres of the brain throughout the breathing cycle, regardless of the nature of the stimulus specificity of the receptors involved. This would seem to imply that the arrhythmic breathing pattern of turtles is primarily the product of the central respiratory generator.

It is known that turtles lack a pneumotaxic centre within the pons of the brainstem (Lumsden, '23a). It is suggested that the normal function of the pneumotaxic centre in mammals is to reduce the threshold of the respiratory centres which has to be exceeded by an inhibitory signal before inspiration is terminated (Bradley, '77). There is also some evidence that this centre may be important in terminating expiration (Cohen, '71). As a consequence, section of the brain stem in the mid-pontine region in mammals produces slow, deep breathing (Tang, '67) although this tends to revert to normal with time (St. John et al., '72). It is also well established that
afferent information from pulmonary receptors in mammals is important for
termination of inspiration and expiration (Breuer, 1868; Knox, '73). The
influences of both the pneumotaxic centre and the lung receptors must inter-
act and the consequences will be reflected in the rate and depth of breath-
ing. When destruction of the pneumotaxic centre is combined with vagotomy,
apneusis ensues (Stella, '38).

As mentioned earlier, many authors have noted a superficial similarity
between the breathing pattern in turtles and apneusis in mammals (Lumsden,
'23a; Randall et al., '44; Lucey & House, '77). Closer analysis reveals,
however, that this is not a true apneusis in turtles as the nonventilatory
period is not associated with tonic or tetanic contraction of inspiratory
muscles as occurs in mammals (Sears, '77; St. John, '77). Further, this
arrhythmic breathing pattern in turtles occurs with intact vagal nerves
yet bears no similarity to the pattern of breathing in mammals with only
the pneumotaxic centre ablated (Tang, '67). Obviously then, the vagal
nerves of turtles have a different influence on the breathing pattern than
in mammals and there is no evidence to suggest whether this is attributable
to the nature of the vagal input and/or to the central integration of this
information.

Very little information exists concerning vagal influences on respira-
tion in turtles. It has been shown that an inverse linear relation exists
between total lung volume and breathing frequency (Milsom & Johansen, '75)
and that vagotomy prolongs the breath length and decreases breathing
frequency (Frankel et al., '69). There is no doubt that lung receptors
play an essential role in regulating ventilation and this evidence suggests-
their responses are qualitatively similar to those of mammals. As no
recordings have been made from pulmonary receptors in turtles, however, their functional characteristics and stimulus specificity is unknown. Without this information, the nature of the vagal input cannot be assessed.

In the present study, the arrhythmic breathing pattern of turtles has been studied with particular regard to the changes which occur during respiratory stimulation. The nature of the vagal afferent input from the lungs has also been studied with special emphasis on its role in the control of the breathing pattern. In the first section, the functional characteristics of pulmonary receptors in the turtle are analyzed. The receptor specificity for volume, transpulmonary pressure and CO\textsubscript{2} is determined and from this analysis, the nature of the vagal afferent input to the respiratory centres throughout the breathing cycle is assessed. In the second section, changes in the breathing pattern during hypercapnic stimulation are studied. The role of information regarding rate and depth of lung inflation on the changes which occur in the breathing pattern is determined and an attempt is made to describe the control of the arrhythmic breathing pattern in terms of peripheral input to the respiratory centres and central respiratory pattern generation. In the third section, changes in the respiratory pattern are measured when levels of alveolar P\textsubscript{CO\textsubscript{2}} are changed at constant arterial P\textsubscript{CO\textsubscript{2}}. This information is used to appraise the suggestion that the CO\textsubscript{2} sensitivity of pulmonary receptors is important in the ventilatory response to inhaled CO\textsubscript{2}. The final section extends the analysis of pulmonary receptor characteristics to the amphibia to allow comparison of the diversity of responses to chemical (CO\textsubscript{2}) and mechanical stimuli which have arisen throughout the vertebrate groups.
SECTION I

An Analysis of Slowly Adapting Pulmonary Stretch Receptors in the Turtle

INTRODUCTION

Pulmonary receptors with varying degrees of CO$_2$-sensitivity have been demonstrated in a variety of animals. Birds possess intrapulmonary chemoreceptors having no apparent mechanosensitivity but responding solely to changes in airway CO$_2$ concentrations throughout the breathing cycle (Fedde & Petersen,'70; Osborne & Burger,'74; Fedde et al.,'74a,b). Similar receptors have been described in the lizard (Fedde et al.,'77). The discharge of slowly adapting pulmonary stretch receptors in cats, dogs and rabbits is partially modified by the level of alveolar CO$_2$ (Adrian,'33; Mustafa & Purves,'72; Schoener & Frankel,'72; Bartoli et al.,'74; Sant'Ambrogio et al.,'74) as is the discharge of bronchial stretch receptors in the extrapulmonary airways of the dog (Bartlett & Sant'Ambrogio,'76). The effect of CO$_2$ on pulmonary receptor discharge in the bird is a major, if not dominant factor in the regulation of avian respiration (Kunz & Miller,'74a,b). Although not a major factor in control of mammalian respiration, the effect of CO$_2$ on the discharge of mammalian pulmonary receptors does account for a vagally mediated tachypnoeic response to inhaled CO$_2$(Mustafa & Purves,'72; Bartoli et al.,'74; Bradley et al.,'76). Recently there have been preliminary observations of slowly adapting pulmonary stretch receptors in turtles (Milsom & Jones,'76) and lizards
(Fedde et al., '77) which are typically mechanosensitive but which exhibit a range of variation in their sensitivity to CO$_2$ which encompasses the different sensitivities to CO$_2$ found in the avian and mammalian receptor types. At the moment the functional significance of such diversity is unclear. Before the role of this diversity in the control of breathing can be studied, however, it is necessary to carefully characterize these receptors. As a first step, the following study was designed to provide a detailed analysis of the static and dynamic characteristics of pulmonary receptors in the turtle and the effects of changes in airway CO$_2$ concentrations on the receptor response.
Experiments were performed on thirty turtles (*Chrysemys picta*, 500-1500 g) single-pithed and restrained in a ventral position at room temperature (22-23°C). A pneumotachograph with a side arm for tracheal pressure measurement and gas sampling was attached to a tracheal cannula inserted as low in the neck as possible. The distal end of the tracheal cannula was attached to a constant volume positive pressure respiration pump for tidal ventilation (Fig. 1). A catheter inserted into the abdominal cavity through a hole drilled in the carapace was sealed in place with dental acrylic cement. Intratracheal pressure ($P_{it}$), taken as an index of intrapulmonary pressure and intra-abdominal pressure ($P_{ia}$) were measured with Statham P23V pressure transducers. Since turtles possess a pleuro-peritoneal cavity, the abdominal cavity pressure with respect to tracheal pressure measured by a Hewlett-Packard 267 BC differential pressure transducer was taken as the transpulmonary pressure ($P_{tp}$) (Fig. 1). The pressure across the pneumotachograph screen during tracheal air flow was measured with a Hewlett-Packard 268 BC differential pressure transducer and this air flow signal was fed into a Hewlett-Packard 350-3700 A integrating preamplifier to give tidal volume. All measurements; pressures, flow and volume were continuously recorded on magnetic tape and monitored on a Sanborn 4 channel chart recorder writing on rectilinear co-ordinates. The $O_2$ and $CO_2$ composition of inspired and expired gases was either determined on samples taken through the side arm of the pneumotachograph and measured on a Fisher-Hamilton gas partitioner or by continuous sampling with a Centronic 200 MGA clinical mass spectrometer (sample rate <10 ml/min).
Figure 1. Schematic diagram illustrating experimental arrangement during acute experiments. For description, see text.
Either the right or left vagosympathetic nerve was cut high in the neck, dissected free of surrounding tissue and placed on a dissection platform. Small filaments were dissected from the proximal cut end of the nerve and single unit action potentials from slowly adapting stretch receptors were recorded by conventional means using bipolar silver electrodes. This activity was amplified, monitored with an oscilloscope, audio-amplifier and instantaneous rate meter, and recorded on magnetic tape.

**Location of Pulmonary Receptors**

Slowly-adapting stretch receptor discharge was attributed to pulmonary receptors if the discharge was modulated by artificial ventilation and unaffected by pulmonary artery occlusion. For confirmation, the precise locations of seventeen slowly adapting stretch receptors were further determined in ten turtles. The chest of these animals was opened by removal of the carapace using a necropsy saw and the lungs were exposed by surgical removal of all obstructing viscera. While the activity of each receptor was being monitored, its longitudinal and circumferential position within the lung was determined by gentle probing with a fine bristle. This method always sufficed to locate the receptors to the major internal septa which subdivide the lung (Fig. 2). For nine of these units, conduction velocities were also measured from photographic records of evoked potentials during simultaneous stimulation with two pairs of stimulating electrodes placed 0.5 cm apart on the pulmonary vagus where it emerged from the lung (Fig. 1).

**Experimental Protocol**

While monitoring the discharge of each receptor, the turtles were ventilated with mixtures of humidified air containing 0.5 and 10% CO₂ at pump
Figure 2. (a) Dry mount of left lung showing internal septation.

(b) Schematic diagram of left lung showing approximate location of pulmonary receptors (●) located by punctate stimulation.

(c) Recording of discharge from a pulmonary receptor during punctate stimulation (arrow) with a fine bristle.
frequencies from 3 to 20/min and tidal volumes of 10 to 50 ml. The pump was briefly stopped to obtain maintained deflation of the lungs equilibrated to atmospheric pressure and inflation of the lungs at various volumes. The rate of inflation to, or deflation from these volumes was altered by changing the pump rate along with the pump inflation-deflation phase ratio.

In a further five animals, the inguinal and cervico-axillary pockets were tightly packed with plasticene and bound with reinforced tape which, in conjunction with the shell, rendered the body of the animal totally inflexible. An adjustable pressure reservoir was connected to the body cavity through the hole drilled in the carapace so that intra-abdominal pressure could be varied at will. The ventilation pump was arranged for sinusoidal pumping of a fixed volume (Fig. 1) and care was taken to always begin the first inflation from a constant functional residual capacity. By adjusting the starting pump volume and/or the intra-abdominal cavity pressure, receptor discharge could be monitored while the peak transpulmonary pressure was varied at any given inflation volume or while the same peak transpulmonary pressure was developed for a variety of inflation volumes.

**Measurements and Analysis**

All data stored on magnetic tape was analyzed on a Digital PDP Lab 8e mini-computer using conventional software. The primary parameters measured were peak inspiratory discharge, end-expiratory discharge and instantaneous discharge rate throughout each breathing cycle. In several instances both time interval histograms (T.I.H.) and post-stimulus time histograms (P.S.T.H.) were constructed from 5 to 10 successive pump cycles to analyze the effects of CO₂ on the relations between instantaneous discharge and inflation volume.
and pressure throughout the phases of the pump cycle. The appropriate signals were also fed into an XY plot on a Hewlett-Packard 1201A storage oscilloscope to provide pressure-volume curves for determination of dynamic lung compliance and to observe the degree of hysteresis in the pulmonary receptor discharge versus pressure and versus volume plots for any ventilation cycle.
RESULTS

Response of Pulmonary Stretch Receptors to Changes in Lung Volume

The discharge pattern in 62 fibres studied varied from a low threshold pattern (n = 54) lasting throughout the respiratory cycle to a high threshold pattern (n = 8) in which discharge occurred only during inspiration and the early part of expiration. All fibres exhibited some adaptation to a step change in pressure over a 2 kPa range but the rate of adaptation estimated from Index 1 of Davis et al. ('56) was always less than 30%. The conduction velocities of these fibres, at 23°C, ranged from 3 to 16 m/sec (mean = 7.4) suggesting fibres 3-11 μ in diameter (Erlanger & Gasser, '37).

The steady discharge of these fibres was measured after adaptation in response to maintained lung inflation to various volumes above end-expiratory volume. The relation between discharge rate and lung volume was linear over the range studied (0 to 1.5 kPa tracheal pressure) (Fig. 3).

A range of inflation and deflation rates corresponding roughly with the range found in spontaneously breathing turtles was used in these experiments (0 and 10% CO₂ in air resulted in average flow rates of 5 and 10 ml/min/kg respectively). At low inflation volumes there was no difference in the maximal discharge frequency attained whether the inflation was slow or rapid. For larger inflation volumes, however, maximal discharge rate was a function of both the rate and volume of inflation (Fig. 4). Despite this, discharge in any given fibre fell to the same level within 5 sec of maintained inflation regardless of inflation rate (Fig. 4, b-d). Discharge per unit volume or per unit pressure at mid-inflation showed very little rate dependency implying that the graded overshoot in maximal discharge rate seen at different
Figure 3. The effect of changing inspired CO₂ (FLCO₂) on the relationship between discharge frequency of pulmonary stretch receptors (n = 62) and intratracheal pressure at least 30 sec after completion of inflation to various volumes. Curves and equations are from linear regression analysis of values recorded at three levels of inspired CO₂ (0, 5 and 10%).
Figure 4. The effect of changing the rate of lung inflation on inflation volume, transpulmonary pressure, intratracheal pressure and the discharge rate and electroneurogram of a single pulmonary stretch receptor. a. and b. represent inflations of the same approximate rate but to different maximal lung volumes. b., c. and d. are the responses to lung inflations of equal volume at varying rates. The rates of change in volume, pressure and discharge are tabulated beneath with the relative sensitivity (discharge per unit volume or per unit pressure at mid-inflation) of the fibre.
<table>
<thead>
<tr>
<th></th>
<th>Rate of Volume Increase (ml/sec)</th>
<th>Rate of Pressure Increase (kPa/sec)</th>
<th>Rate of Discharge Increase (/sec)</th>
<th>Discharge / Unit Volume Mid-Inflation (Hz/ml)</th>
<th>Discharge / Unit Pressure Mid-Inflation (Hz/kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>11.3</td>
<td>1.2</td>
<td>10.3</td>
<td>0.76</td>
<td>9.2</td>
</tr>
<tr>
<td>b</td>
<td>12.5</td>
<td>1.5</td>
<td>14.1</td>
<td>0.81</td>
<td>10.5</td>
</tr>
<tr>
<td>c</td>
<td>6.3</td>
<td>0.9</td>
<td>8.0</td>
<td>0.96</td>
<td>11.0</td>
</tr>
<tr>
<td>d</td>
<td>3.3</td>
<td>0.4</td>
<td>4.2</td>
<td>0.83</td>
<td>10.3</td>
</tr>
</tbody>
</table>
inflation rates was a function of rate dependent changes in lung mechanics. Deflation rate had no effect on end-expiratory discharge rate.

Effect of Changing Inspired CO$_2$ Concentration on Pulmonary Stretch Receptor Discharge

The discharge rate of all fibres during maintained lung inflation to various volumes decreased as the level of inspired CO$_2$ increased (Fig. 3). The relation between rate of discharge and tracheal pressure remained linear, the greatest changes in receptor discharge occurring over the lower range of airway CO$_2$ concentrations.

When animals were given CO$_2$ during pump ventilation, a reduction in the rates of peak-inspiratory and end-expiratory discharge were noticeable after 2 to 4 ventilation cycles (Fig. 5). As alveolar CO$_2$ levels rose to a new steady level, there was a progressive reduction in the levels of peak inspiratory and end-expiratory discharge (reduced sensitivity) and in the onset of discharge during inspiration (increased threshold). The rates of decrease in peak-inspiratory and end-expiratory discharge were similar and dependent on the degree of change in inspired CO$_2$ levels (Fig. 5).

There was a marked depression of activity throughout the entire respiratory cycle in 59 of 63 stretch receptors studied. Figure 6 illustrates the typical effects of CO$_2$ on one fibre. The time interval histograms (T.I.H.) represent the discharge profiles of each of eight successive breaths during pump ventilation and the post stimulus time histograms (P.S.T.H.) represent the cumulative average of the discharge during these same eight ventilation cycles portrayed over the time course of a single cycle. It can be seen that CO$_2$ reduces discharge almost equally throughout
Figure 5. The time relationship between changes in the level of inspired CO$_2$ and pulmonary receptor discharge. The upper pair of traces illustrates the effects of introducing 10% CO$_2$ to the inspired gas and the second pair of traces illustrates the return to ventilation with air. In both pairs of recordings, the top trace is the intratracheal pressure and the lower trace the discharge of a single pulmonary receptor during pump ventilation. The time relationships portrayed below these traces represent the breath by breath end-inspiratory (solid symbols) and end-expiratory discharge (open symbols) of the same receptor following the introduction (arrow, left) and removal (arrow, right) of 5% (dashed lines) and 10% CO$_2$ (solid lines) to the ventilatory gas.
Figure 6. Effect of changing inspired CO$_2$ on pulmonary stretch receptor discharge throughout the ventilation cycle.
The time interval histograms (T.I.H.) and post stimulus time histograms (P.S.T.H.) of eight successive breaths are shown at two levels of inspired CO$_2$ and three levels of inflation volume ($V_T$). The bin lengths for the T.I.H. were 500 msec and 150 msec for the P.S.T.H.
Pulmonary stretch receptor discharge
all phases of the ventilation cycle regardless of ventilation volume. Figure 7 shows the relative effect of increasing the inspired CO$_2$ concentration on the peak-inspiratory and end-expiratory discharge of all fibres studied. There is a large range of variation in response between fibres. As under steady state conditions, the response was most sensitive over the lower range of airway CO$_2$ concentrations.

Recovery to previous discharge levels on return to breathing room air was much slower than the inhibition of discharge during CO$_2$ administration (Fig. 5). Recovery rates were inversely related to the CO$_2$ levels employed and peak-inspiratory discharge rate invariably recovered quicker than end-expiratory discharge rate (Fig. 5).

**Effect of Changing the Inspired CO$_2$ Concentration on Lung Mechanics**

There was little or no effect of low levels of CO$_2$ on lung compliance in these studies. At higher levels of CO$_2$, in eight animals examined, there was usually a slight decrease in dynamic compliance; average values falling from $0.96 \pm 0.09$ (S.E. of mean) to $0.82 \pm 0.10$ ml/kPa/kg in animals breathing air and 10% CO$_2$ in air respectively (Table 1 & Fig. 8).

**Effect of Changes in Transpulmonary Pressure and Lung Volume on Pulmonary Stretch Receptor Discharge**

The independent effects of transpulmonary pressure and lung volume on pulmonary stretch receptor discharge were analyzed on six fibres in five animals. Large scale changes in transpulmonary pressure achieved by altering intra-abdominal pressure during constant volume ventilation, had very little effect on pulmonary receptor discharge (Fig. 9). Only when transpulmonary pressure was reduced to very low values was there any response in
Figure 7. The effect of changing airway CO$_2$ concentrations on pulmonary receptor discharge during pump ventilation. Peak inspiratory and end-expiratory discharge frequency in 62 pulmonary stretch receptors at inspired CO$_2$ concentrations of 0, 5 and 10% are shown. The mean values for all fibres at each gas concentration are shown by the solid symbols (Φ).
Pulmonary stretch receptor discharge
 (% peak inspiratory discharge at $F_{ICO_2} = 0$)
Table 1. The effect of changing airway CO$_2$ concentrations on dynamic lung compliance.

<table>
<thead>
<tr>
<th>Turtle</th>
<th>Lung Compliance (ml/kPa/kg)</th>
<th>Air</th>
<th>Air + 10% CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.12</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.97</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.12</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.18</td>
<td>1.14</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.96</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.36</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.98</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>

$\bar{x}$ 0.96 ± 0.09* 0.82 ± 0.10*  

*±S.E.
Figure 8. The effect of changing airway CO₂ concentrations on pulmonary stretch receptor discharge and dynamic compliance. The top trace shows the discharge rate of a single pulmonary stretch receptor; the middle trace, inflation volume; and the bottom trace, transpulmonary pressure. 10% CO₂ was removed from the ventilating gas at the arrow (↓). The traces for two cycles (indicated), one before (left) and one after (right) removal of the CO₂ are shown on an extended time scale to the sides of the continuous recordings. The pressure-volume plots for these two breaths are shown below the extended trace of each respective breath along with their computed dynamic compliances. Both plots cycle in a clockwise direction.
Figure 9. The effect of changes in transpulmonary pressure at constant inflation volume on pulmonary receptor discharge. The upper trace shows the instantaneous discharge frequency of a single pulmonary receptor, the second trace shows transpulmonary pressure, the third trace shows inflation volume and the fourth trace shows the intratracheal pressure during two ventilation cycles to each of four peak transpulmonary pressures.
receptor discharge rate. Changes in ventilation volume during inflation to constant peak transpulmonary pressure, on the other hand, were always successful in altering receptor discharge rate (Fig. 10). Further, during normal breathing, for any given lung volume, transpulmonary pressure is always greater during inflation. This gives rise to a clockwise rotating hysteresis loop when transpulmonary pressure is plotted against volume through a complete pump cycle (Fig. 11). When the discharge rate of a pulmonary receptor is plotted against the associated transpulmonary pressure during a single ventilation cycle, a counter clockwise hysteresis loop results. At any given level of transpulmonary pressure, discharge is always less during inflation. When the same receptor discharge is plotted against the associated lung volume for a given pump cycle, there is no hysteresis; discharge is always the same at any given lung volume regardless of whether the lungs are inflating or deflating.
Figure 10. The effect of changes in inflation volume with inflation to constant peak transpulmonary pressure on pulmonary receptor discharge. The upper trace is the instantaneous discharge frequency of a single pulmonary stretch receptor, the middle trace is the transpulmonary pressure and the lower trace is the inflation volume of two ventilation cycles each with two inflation volumes.
discharge rate (Hz)

Transpulmonary Pressure (kPa)

Volume

5 sec

40

20

0
Figure 11. The relationships between changes in transpulmonary pressure, inflation volume and pulmonary stretch receptor discharge during a single breath. (a) Plot of transpulmonary pressure versus inflation volume during a single breath. (b) Plot of discharge frequency of a single pulmonary stretch receptor versus transpulmonary pressure during the same breath. (c) Plot of the same changes in discharge frequency versus inflation volume during the same breath. The time relation of each plot is indicated by the arrows.
DISCUSSION

The functional characteristics of slowly adapting pulmonary stretch receptors in turtles are very similar to those of mammals. Most receptors exhibited tonic activity at resting lung volume (zero transmural pressure) as do most intra- and extrapulmonary stretch receptors in mammals (Mustafa & Purves, '72; Paintal, '73; Miserocchi and Sant'Ambrogio, '74; Bartlett et al., '76). The receptors exhibited a linear response to increasing lung volume over the range studied. At low inflation volumes peak discharge was unaffected by the inflation rate, however, as the inflation volume increased, peak discharge increased with both the rate and degree of inflation. Regardless of the inflation rate, discharge always fell to a tonic level dependent only on lung volume. Similar results are well documented in the cat and dog (Adrian, '33; Davis et al., '56). The axons arising from pulmonary receptors are of similar diameter in turtles and mammals (Paintal, '73) yet the conduction velocities in turtles ($\bar{x} = 7.4$ m/sec) are much slower than those of mammals (36-39 m/sec) (Paintal, '73). Also, the discharge frequencies of the receptors in turtles were much lower and the sensitivity of the receptors (defined as the change in discharge rate for a given change in pulmonary pressure during inflation) was also less; 15.1 Hz/kPa in the turtle compared to 30 Hz/kPa in the dog (Miserocchi & Sant'Ambrogio, '74; Bradley et al., '76). These differences, however, are probably attributable to the much lower body temperature of the turtles (22-23°C).

In mammals, the discharge of pulmonary stretch receptors associated with any given lung volume during lung inflation appears to consist of a component associated with the actual volume and a component associated with air...
flow (Davis et al., '56). Since this same relation also exists between transmural pressure and volume and flow, many investigators have successfully correlated receptor discharge with the level and rate of change (dP/dt) of transpulmonary pressure, both \textit{in vivo} (Davis et al., '56; Sant'Ambrogio et al., '74; Bartlett et al., '76) and \textit{in vitro} (Bradley & Scheurmier, '77). Two separate lines of evidence presented in this study, however, show that in the turtle, pulmonary stretch receptor discharge is associated only with lung volume. Wide scale alterations in the transpulmonary pressure developed during lung ventilation failed to have much noticeable effect on peak receptor discharge frequency as long as the inflation volume was held constant (Fig. 9). When, on the other hand, a constant level of transpulmonary pressure was developed with varying inflation volumes, peak receptor discharge rate always reflected the changes in inflation volume (Fig. 10). Further, in the absence of such manipulations, but during normal pump ventilation, pulmonary stretch receptor discharge frequency always followed the changes in lung volume exactly while both discharge and volume lagged behind the changes in transmural pressure (Fig. 11).

There is no question that the response of a receptor to transpulmonary pressure changes requires that a net force be developed across the septa or wall containing the receptor. The extent to which this occurs decreases with each generation of airway division (Fung, '75). In turtles, the primary bronchi (first generation) do not divide but pass the length of the lung and end. Each bronchus remains patent but is perforated along its length providing access to the 8 to 10 major chambers of the lung (Fig. 2a, b) (Gans & Hughes, '67). Each chamber is further subdivided by
numerous septa and it is on these septa that the receptors are located (Fig. 2b). In view of the fact these septa must be exposed to the same pressure and flow changes on each side during inflation (Fung, '75), it is not surprising that the receptors were insensitive to changes in transpulmonary pressure. In light of this, however, the mechanism behind the response to rate of inflation recorded at high inflation volumes remains unclear. It is possible that the response arises from transient changes in the longitudinal tension of the septa which only appear during air flow once the lungs are distended beyond a certain point. For the moment, this must remain speculative.

The average response of pulmonary receptors in the turtle to CO\textsubscript{2} was also similar to that reported for mammals. The sensitivity of receptors to static volume inflations was reduced to 9.2 Hz/kPa and 6.8 Hz/kPa when animals were ventilated with 5 and 10\% CO\textsubscript{2} respectively. These values represent 39 and 55\% reductions, slightly greater than the 20-40\% reductions reported in rabbits, cats and dogs ventilated with 7-9\% CO\textsubscript{2} in O\textsubscript{2} (Mustafa & Purves, '72; Kunz et al., '76; Bradley et al., '76). As reported for the dog (Bradley et al., '76) the effects were not linear but were greater over the lower range of CO\textsubscript{2} concentrations.

The time course of the increase in mechanical threshold and decrease in sensitivity of receptors when CO\textsubscript{2} was administered during pump ventilation were similar in both turtles and mammals (Mustafa & Purves,'72; Schoener & Frankel,'72; Bradley et al.,'76) as was the time course of their recovery when CO\textsubscript{2} was removed (Adrian,'33; Mustafa & Purves,'72; Bradley et al.,'76; Bartlett & Sant'Ambrogio,'76). Interestingly, however, although the rate of response of peak inspiratory and end-expiratory discharge were similar when CO\textsubscript{2} was administered, peak inspiratory rate often recovered more quickly than the
end-expiratory discharge rate when $\text{CO}_2$ was removed. Hypercapnia has been found to inhibit stretch receptor discharge less during the inspiratory phase of artificial ventilation than during the expiratory phase in mammals (Mustafa & Purves, '72; Sant'Ambrogio et al., '74; Bradley et al., '76; Bartlett & Sant'Ambrogio, '76) but this only occurs during this recovery phase in the turtle.

During pump ventilation, as with static lung inflations, the sensitivity of receptor discharge to $\text{CO}_2$ was greater over the lower range of $\text{CO}_2$ concentrations. This too has been observed in mammals (Bradley et al., '76; Bartlett & Sant'Ambrogio, '76). Over a range of $\text{CO}_2$ concentrations from 0 to 5%, there was an average reduction of 10% of control in peak discharge rate per 10 mm Hg increase in $P_{CO_2}$ and 7% of control in end-expiratory discharge per 10 mm Hg increase in $P_{CO_2}$. These values tend to be slightly greater than those reported for mammals (Mustafa & Purves, '72; Bradley et al., '76).

It seems evident that $\text{CO}_2$ acts directly on the pulmonary receptors in mammals rather than on lung volume or transpulmonary pressure as $\text{CO}_2$ appears to have little direct or reflex effect on either the functional residual capacity (FRC) (Mustafa & Purves, '72) or the total lung resistance (TLR) (Mustafa & Purves, '72; Sant'Ambrogio et al., '74; Bradley et al., '76). This would certainly appear to be the case in turtles since any $\text{CO}_2$ induced decrease in lung resistance must appear as a decrease in transpulmonary pressure during constant volume ventilation and it has been shown that changes in transpulmonary pressure have very little effect on receptor discharge. Further, high levels of $\text{CO}_2$ decrease dynamic lung compliance in the turtle (Fig. 8) (Table 1), thus if the receptors were sensitive to transpulmonary pressure, this effect would tend to mask, not enhance, the
effects of the CO₂.

It is interesting to note that the quantification of the dP/dt sensitivity of stretch receptors in mammals has been focused primarily upon tracheal (Bartlett et al., '76; Bradley & Scheurmier, '77) or type I (Miserocchi et al., '73) receptors in the extrapulmonary airways and that these receptors are insensitive to CO₂. The pulmonary receptors which are sensitive to CO₂ appear to be the bronchial (Bartlett & Sant'Ambrogio, '76) or type II receptors (Miserocchi et al., '73) which are the exclusive receptor type in their primary location, the small bronchi (>1 mm diameter). This is a position where lung stretch (circumferential and longitudinal) and not transpulmonary pressure will be the primary stimulus. On the basis of functional characteristics, location and sensitivity to CO₂, it would appear that turtle pulmonary stretch receptors differ only quantitatively from these mammalian intrapulmonary (bronchial) stretch receptors.

The range of sensitivity to CO₂ exhibited by turtle pulmonary receptors was much greater than that found in mammals and although some (n = 3) were insensitive to CO₂ several were totally inhibited throughout all phases of the ventilation cycle at CO₂ levels of 5 to 10% (Fig. 5, 7). The degree of sensitivity to CO₂ exhibited by these few receptors is equal to that of the intrapulmonary CO₂ receptors which have been described in birds and lizards (Fedde et al., '74a; Fedde et al., '77). These pulmonary CO₂ receptors are extremely sensitive to CO₂ and are apparently insensitive to changes in lung volume and transmural pressure (Osborne & Burger, '74; Fedde et al., '74a). It is believed that these receptors monitor the rate and degree of lung ventilation by the fluctuations in intrapulmonary CO₂ concentration throughout the respiratory cycle (Molony, '74; Fedde et al., '74b) and are a major
factor in the regulation of avian respiration (Osborne & Burger, '74; Kunz & Miller, 74a,b). The finding that such levels of CO₂ sensitivity are not without equal in the pulmonary stretch receptors of other vertebrates places new emphasis on attempts to describe the physiological roles and functional characteristics of these receptor groups.
SECTION II

The Role of Pulmonary Afferent Information and Hypercapnia in Control of the Breathing Pattern in the Turtle

INTRODUCTION

A pattern of arrhythmic breathing consisting of a series of one to several breaths separated by a highly variable, respiratory pause (from a few seconds to several hours) commencing at end-inspiration has been described for most species of reptiles (turtles, McCutcheon, '43; Gans & Hughes, '67; crocodiles, Naifeh et al., '70; Gans & Clark, '76; snakes, Glass & Johansen, '76). Despite the similarities between this breathing pattern and various patterns of abnormal breathing in mammals with defective central respiratory control, evidence indicates that reptiles accurately adjust ventilation to maintain blood pH at a specific, temperature-dependent value (cf. Howell & Rahn, '76, for review). Although many studies have analyzed the effects of respiratory stimuli such as hypoxia, hypercapnia and temperature in reptiles, these studies primarily assess the effects upon metabolism, total pulmonary ventilation or dive length in aquatic species (cf. Wood & Lenfant, '76, for review). Very little is known about the factors and mechanisms controlling the arrhythmic breathing pattern.

Hypoxia appears to be the major factor controlling respiration in reptiles (Nielsen, '61a,b; Templeton & Dawson, '63; Glass & Johansen, '76; Wood & Lenfant, '76) including turtles (Lenfant et al., '70), yet many of these animals exhibit an incredible tolerance to anoxia (Belkin, '63a,b, '68).
Reports on the effects of hypercapnia on ventilation in turtles range from a slight or moderate increase (Millen et al., '63; Wood & Lenfant, '76) to a powerful stimulation (Jackson et al., '74). Many of these discrepancies arise from species differences, levels of anaesthesia and the presence or absence of avenues of cutaneous gas exchange. Furthermore, in many studies only ventilation rates and not volumes were measured which makes accurate comparison almost impossible.

The present study was undertaken to describe and analyze the breathing pattern in the turtle *Chrysemys picta*. The role of pulmonary afferent information carried in the vagus nerve, with and without concomitant hypercapnia (at normoxia and constant temperature), have been studied as a first step in assessing the mechanisms involved in the control of this breathing pattern.
METHODS

Surgical Procedures

Experiments were performed on unanaesthetized, lightly restrained specimens of the freshwater turtle, *Chrysemys picta* (600-1200 g) at room temperature (22-23°C). The optimum temperature range for this temperate species is 20-25°C (Cagle, '54). Using a combination of cold (1-4 hr at -20°C) and local anaesthesia (2% Lidocaine hydrochloride), a tracheal cannula was inserted and the vagi bilaterally exposed. A pneumotachograph with a side arm for tracheal pressure measurement and gas sampling was attached to the tracheal cannula and, in the majority of cases, the open end of the pneumotachograph was attached to a plastic T-piece. One arm of the T was open to atmosphere and the other was attached to a gas supply. Using a system of air flow meters, the composition of the gas flowing past the end of the tracheal cannula could be altered thus controlling the composition of the inspired air when the turtle breathed. Due to this procedure, the lung remained open to atmosphere throughout the respiratory pause and thus lung volume returned to a constant functional residual volume during this period. In the intact animal, the respiratory pause occurs at end-inspiration and the established lung volume is not only variable but influences the respiratory frequency (Milsom & Johansen, '75). To establish whether the maintenance of a constant functional residual capacity by our procedure influenced the breathing pattern or time course of the various respiratory phases, the distal end of the tracheal cannula was reattached to the cut peripheral end of the trachea on several occasions. These animals breathed through an intact glottis and showed no differences in the measured variables from the
experimental animals. However, as a consequence of holding the end-inspira-
tory (breath hold) volume constant, any effects of CO₂, vagotomy, tidal
volume or breath hold length on this volume will not have been observed.

Recording Techniques

Tracheal pressure was measured with a Statham P23V pressure transducer
and the pressure drop across the pneumotachograph screen during tracheal air
flow with a Hewlett-Packard 268 BC differential pressure transducer. The
air flow signal was fed through a Hewlett-Packard 350-3700 A integrating
preamplifier to give tidal volume and all measurements; pressure, flow and
volume, were continuously recorded on a Sanborn 4 channel chart recorder
writing on rectilinear co-ordinates. The O₂ and CO₂ composition of inspired
and expired gases was either determined on samples taken through the side
arm of the tracheal cannula and measured on a Fisher-Hamilton gas partition-
er or by continuous sampling with a Centronic 200 MGA clinical mass spectro-
meter (sample rate <10 ml/min).

Protocol

Animals were allowed to recover from anaesthesia for 4 to 6 hours
before experimentation began. The animals were surrounded by opaque
screens to shield them from all activities of the experimenters and when
resting quietly were presented with mixtures of 0, 5 or 10% CO₂ in air to
breathe for one-half to one hour periods in random order. All measurements
were recorded continuously but data were selected for analysis only after
the responses to each gas mixture had stabilized. It must be stressed that
due to the incompletely divided ventricle of the turtle heart there can be
right to left shunting of blood caused by pulmonary arterial vasoconstric-
tion (White, '76; Milsom et al., '77) and thus turtles may maintain a
considerable partial pressure difference between alveolar and arterial CO₂ (AaΔCO₂) for long periods of time following changes in the composition of inspired gases (Glass et al., '78). Consequently care was taken to assure that ample time was allowed for stable responses to develop before any measurements were made.

On the second day of experimentation, the vagi were sectioned under local anaesthetic and the above protocol repeated.

**Measurements and Analysis**

The intervals of the various respiratory phases were all measured from the air flow recordings. The slopes of all graphs were computed by simple linear regression analysis of the data or logarithmic transformations of the data on a PDP 12 lab mini-computer. Unless otherwise stated all values are means ± S.E.
RESULTS

The Pattern of Breathing

(a) Intact Animals

After a variable period of breath holding in the inspiratory position (non-ventilatory period, NVP) a series of one to several breaths would commence with an active expiration and terminate again in the end-inspiratory phase (Fig. 12a and 13). This constitutes the ventilatory period (VP) (Fig. 13). Each ventilatory period commenced with active expiration regardless of whether the animal had an intact or bypassed glottis (i.e. regardless of lung volume). The time interval of each breath (T_{tot}) consisted of the expiratory interval (T_E) and the subsequent inspiratory interval (T_I) which usually consisted of an active inspiratory phase (T_I') (this phase would be similar to the T_I in mammals), and a short interval during which this breath was held at end-inspiration (T_I-T_I') (Fig. 13).

Table 2 lists the mean values (±S.E.) of several of the variables measured for each trial (n) in sixteen animals. Increasing the percentage of CO_2 in the inspired gas (F_{CO_2}) resulted in an increased frequency of breathing (f, calculated on all breaths taken per unit time), tidal volume (V_t) and total pulmonary ventilation (V_E). The incidence of ventilatory periods (VP·min^{-1}) increased as a result of the large reduction in the duration of the non-ventilatory period (T_{NVP}) while the number of breaths within each ventilatory period (Breaths·VP^{-1}) also increased (Fig. 12a). Since the mean duration of each individual breath (T_{tot}) did not change noticeably, the frequency of breaths within each ventilatory period (f_{VP}) remained unchanged.
Figure 12. Representative records of the breathing pattern of a turtle and the effects of vagotomy and hypercapnia on this pattern. Values of $F_{I}^{O_2}$ and $F_{I}^{CO_2}$ apply to both the prevagotomy and postvagotomy records.
Figure 13. Schematic diagram of the breathing pattern in a turtle illustrating the various respiratory intervals. See text for the explanation of all abbreviations.
Ventilatory Period

Breathing cycle

Non-Ventilatory Period

\[ T_{VP} \]

\[ T_{NVP} \]

\[ T_{tot} \]

\[ T_{E} \]

\[ T_{i} \]

\[ T_{r} \]

5 sec
Table 2. Ventilatory variables of turtles breathing air and CO₂ gases.

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<tr>
<th></th>
<th>air</th>
<th>5% CO₂ + air</th>
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<td>pre vagotomy</td>
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<td><strong>f (min⁻¹)</strong></td>
<td>31</td>
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<td>1.8</td>
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<td><strong>f_VP (min⁻¹)</strong></td>
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<td><strong>V_T (ml BTPS·Kg⁻¹)</strong></td>
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<td><strong>V̇_E (ml BTPS·min⁻¹·Kg⁻¹)</strong></td>
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<td>44.9</td>
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<td>±11.4</td>
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<td><strong>T′_I (sec)</strong></td>
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<td>16.6</td>
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<td><strong>T_NVBP (sec)</strong></td>
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<td><strong>T_VBP+NVP (sec)</strong></td>
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<td>185.6</td>
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<td><strong>T TP/VP+NVP ·100</strong></td>
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while the duration of each ventilatory period ($T_{VP}$) increased as a result of the larger number of breaths in each. As a consequence of this increase in $T_{VP}$ and the decrease in the subsequent $T_{NVP}$, the percentage of time spent actively breathing ($\frac{T_{VP}}{VP + NVP} \times 100$) increased.

It is interesting to note that the observed increases in tidal volume when animals were breathing the CO$_2$ gas mixtures were due to an increase in both force (Fig. 12a) and rate (constant $T_{tot}$, Table 2) of expiration and inspiration. Despite the increased respiratory drive the breathing pattern remained arrhythmic. At the maximum ventilatory frequencies measured under severe hypercapnic stress ($F_{CO2} = 15\%$) breathing became virtually eupneic as $T_{NVP}$ was eliminated and $f$ approached $f_{VP}$, the duration of each breathing cycle still remaining unchanged.

(b) After Bilateral Vagotomy

The effect of vagotomy on the breathing pattern, studied in five animals (Fig. 12b, Table 2), was to decrease the respiratory frequency and increase the tidal volume and total pulmonary ventilation. The number of breaths per ventilatory period increased slightly but the frequency of ventilatory periods decreased. The average breath length was prolonged and thus the breathing frequency within each ventilatory period decreased. The net effect of these changes was a slight lengthening of $T_{VP}$ but since $T_{NVP}$ was greatly lengthened the actual time spent actively breathing decreased. Increasing the CO$_2$ concentration in the inspired air of these vagotomized animals produced similar trends to those observed in intact animals. Although the amount of time spent actively breathing was less after vagotomy, the presence of 5 and 10% CO$_2$ in the inspired air produced proportionately the same effect in normal
and vagotomized animals, a doubling and tripling respectively of the amount of time spent actively breathing compared to ventilation with room air.

The tidal volume in intact animals breathing room air was approximately 10% of the lung volume (Milsom, '75). Following vagotomy, tidal volume increased to 39% of the lung volume. Addition of 10% CO₂ to the inspired gas mixture raised these values to 25% and 87% for intact and vagotomized animals respectively.

The Relationships Between \( \dot{V}_E \), \( V_T \) and \( f \)

(a) Intact Animals

There is a linear relationship between \( \dot{V}_E \) plotted as a function of \( V_T \) ("Hey Plot") in agreement with results obtained at low levels of ventilation in humans (Milic-Emili & Cajani, '57; Hey et al., '66) and anaesthetized mammals (Euler et al., '70) (Fig. 14a, open circles). Higher levels of ventilation were obtained by hypercapnic stimulation which, consistent with mammalian studies (Hey et al., '66; Euler et al., '70) did not alter the \( \dot{V}_E, V_T \) relation. This relation has a very steep slope since in the unanaesthetized turtle, \( V_T \) varies little and increases in \( \dot{V}_E \) are primarily the result of increases in \( f \) (Fig. 14b). The inflexion or curvilinear bend found in the \( \dot{V}_E, V_T \) relation of man and other mammals at roughly half vital capacity (Hey et al., '66; Euler et al., '70) was not found in turtles. Although it is possible that CO₂ stimulation alone is inadequate to raise ventilation above the critical level at which such inflexions occur (Euler et al., '70), the primary dependence of increases in \( \dot{V}_E \) on increases in \( f \) above this critical level in mammals parallels the situation which normally exists at all levels of \( \dot{V}_E \) in turtles suggesting that such an inflexion
Figure 14. The relationships between (a) $V_E$ and $V_T$, (b) $V_E$ and $f$, and (c) $V_T$ and $1/f$ for turtles breathing air and CO$_2$ gas mixtures. Values recorded in intact turtles are represented by open symbols, those recorded postvagotomy by closed symbols. In c, values recorded while turtles breathed air (0), 5% CO$_2$, (Δ), and 10% CO$_2$ (▽) are shown separately.
simply does not exist.

The plot of $V_T$ against $1/f$ (Fig. 14c) clearly illustrates the marked dependence of increases in $V_E$ on increases in $f$. The negative correlation in this relation is consistent with studies on CO$_2$-ventilation responses in anaesthetized mammals (Euler et al., '70). The use of hypercapnia as a respiratory stimulant does not alter either the $V_E$, $f$ or the $V_T$, $1/f$ relation.

(b) **After Bilateral Vagotomy**

All three relations remain linear following vagotomy (Fig. 14, closed circles). The slope of the $V_E$, $V_T$ relation decreases; that of the $V_E$, $f$ relation increases, and this increased dependency of levels of $V_E$ on changes in $V_T$ is clearly evident from the increased negative slope of the $V_T$, $1/f$ relation. These results indicate that some acceleration of respiratory frequency in response to increased chemical drive remains after the vagi are cut. This is not the case in anaesthetized cats where respiratory frequency remains constant following vagotomy and increases in $V_E$ result solely from increases in $V_T$ (Euler et al., '70).

The slope constant of the $V_E$, $V_T$ relation has the dimension of frequency (Hey et al., '66) and consequently that of the $V_E$, $f$ relation has the dimension of tidal volume. It can be seen from the calculations of Euler et al. ('70) that extrapolation of all these relations, pre and post vagotomy, to zero value will not pass through the origin unless the frequency in the case of the $V_E$, $V_T$ relation or $V_T$ in the case of the $V_E$, $f$ relation remains constant as $V_E$ increases. This is the basis for the $x$ intercept of each of these curves. It should be noted that the respiratory frequency computed during the ventilatory period ($f_{VP}$) remains constant despite CO$_2$-induced ventilation responses (Table 2) and that although vagotomy results in a
decreased $f_{VP}$ this frequency also remains constant as ventilation is increased by hypercapnia.

The Duration of the Breath

(a) **Intact Animals**

During resting, spontaneous breathing, the length of each breath was variable and the lengths of each interval comprising the breath were positively correlated to the total length of the breath (Fig. 15, open circles). The strongest correlation was between $T_{tot}$ and $T_I$ ($r = 0.975$, Fig. 15b) despite weaker correlations between $T_{tot}$ and the component intervals of $T_I$ ($r$ for $T_{tot}$, $T_I' = 0.839$, Fig. 15a, for $T_{tot}$, $T_I - T_I' = 0.655$, Fig. 15d).

The correlation between $T_{tot}$ and $T_E$ was always weaker ($r = 0.844$, Fig. 15c) than between $T_{tot}$ and $T_I$. Stimulation of breathing by $CO_2$ had no effect on these relations (Table 2, Fig. 15).

(b) **After Bilateral Vagotomy**

Following vagotomy $T_{tot}$ was prolonged slightly (Table 2). In mammals there is a lengthening of both $T_I$ and $T_E$ (Clark and Euler, '72), but in the turtles the ranges of $T_I'$ and $T_E$ were reduced and their mean values were relatively unchanged. As a consequence the correlation between $T_{tot}$ and the intervals of active inspiration and expiration were reduced ($r = 0.32$ and $0.30$ respectively) (Fig. 15, closed circles). The breath length was prolonged primarily through the increase in $T_I - T_I'$ which became strongly correlated to $T_{tot}$ ($r = 0.898$) thus the strong linear correlation remained between $T_{tot}$ and $T_I$ ($r = 0.971$). Stimulation of breathing by $CO_2$ still had no effect on these relations (Table 2).
Figure 15. The relationships between $T_{\text{tot}}$ and its component intervals for intact turtles (open circles and solid regression lines) and vagotomized turtles (closed circles and broken regression lines) breathing air and CO$_2$ gas mixtures.
TE, TI and TE, T′I Relationships

(a) Intact Animals

Although TI showed the strongest correlation to Ttot, there was a better correlation between T′I and TE (r = 0.80) than between TI and TE (r = 0.68) (Fig. 16). Further, at higher values of TI, there was an inflexion in this relation so that expiratory duration changed relatively little with further increases in inspiratory duration. These results are similar to results obtained in paralyzed artificially ventilated cats where TI was regulated by electrical stimulation of the severed vagal nerves (Clark and Euler, '72) and provided larger TI values than could be obtained in rebreathing experiments and revealed a similar inflexion. CO2 had no effect on these relations (Table 2).

(b) After Bilateral Vagotomy

Following vagotomy, however, a good correlation remains between TE and T′I (Fig. 16) as in cats (Widdicombe and Winning, '74). This correlation is thus established by some central mechanism and if the expiratory interval is determined by the events terminating the active inspiratory interval in turtles as suggested by some researchers for mammals (Clark and Euler, '72), these events must arise centrally uninfluenced by volume feedback information from the lungs.

Lung volume feedback information is necessary, however, for the positive correlation between TI and TE (Fig. 16). Its influence, therefore, must be on the T′I-I′ interval and must be due to tonic rather than phasic input as changes in volume feedback information resulting from fluctuations in VT induced by hypercapnia in intact animals are without effect on the relation.
Figure 16. The relationship between the expiratory ($T_E$) and inspiratory intervals ($T'_I$ and $T_I$) for intact turtles (open circles and solid regression lines) and vagotomized turtles (closed circles and broken regression lines) breathing air and CO$_2$ gas mixtures.
Neither the $T'_T$, $T_E$ nor $T_I$, $T_E$ relations which do exist after vagotomy are affected by levels of $CO_2$ (Fig. 16).

The Effects of Tidal Volume

(a) Intact Animals

$V_T$ was maintained within narrow limits despite a wide range of spontaneously occurring changes in $T_E$, $T_I$, $T'_I$ and $T_{tot}$ (Fig. 17). Increases in $V_E$ and $V_T$ resulting from $CO_2$ breathing did not alter these relationships (Fig. 17, Table 2). Figure 18 shows a strong linear correlation between the inspiratory interval and the log of the reciprocal of the inspiratory flow rate ($r = 0.845$) as well as between the expiratory interval and the log of the reciprocal of the expiratory flow rate ($r = 0.810$). The linear slopes of these relationships represent constant volume isopleths indicating that $T_I$ and $T_E$ are adjusted to maintain a constant $V_T$ over widely ranging inspiratory and expiratory air flow rates.

This differs markedly from the strong positive correlations shown between $V_T$ and $T_I$ and $V_T$ and $T_E$ in spontaneously breathing steady state conditions in man (Newsom Davis and Stagg, '75), and intact animals (Widdicombe and Winning, '74). $CO_2$ has no effect on these relations in man (Newsom Davis and Stagg, '75) but decreases $T_E$ in intact cats (Widdicombe and Winning, '74).

(b) After Bilateral Vagotomy

When all volume-feedback information was removed, the intervals of active inspiration and expiration were restricted in range and independent of tidal volume (Fig. 17). They no longer strongly correlated with the inspiratory and expiratory flow rates (Fig. 18) and thus tidal volumes increased, presumably in direct proportion to the central respiratory drive.
Figure 17. The relationships between tidal volume and the inspiratory intervals, expiratory interval and total breath length for intact animals (open circles and solid regression line) and vagotomized turtles (closed circles) breathing air and CO$_2$ gas mixtures.
Figure 18. The relationship between the inspiratory interval and inspiratory flow rate (open circles and solid regression lines) and between the expiratory interval and expiratory flow rate (closed circles and broken regression line) in intact (a) and vagotomized (b) turtles breathing air and CO₂ gas mixtures.
Unlike mammals, $T_I'$ and $T_E$ values were not equal to maximum prevagotomy values (Bradley et al., '74a, b). There appears to be some slight lengthening of $T_I'$ and hence $T_{tot}$ at higher values of $V_T$ induced by CO$_2$ stimulation which must have been due to central effects of the CO$_2$. Similar results have been obtained during apneustic breathing in vagotomized animals decerebrate at the level of the pons (Tang, '67; Stella, '38a, b; Lumsden, '22, 23a, b).

The Duration of the Nonventilatory Period

(a) Intact Animals

The $T_{NVP}$ was poorly correlated to $V_T$ ($r = 0.495$), however a general trend for $T_{NVP}$ to decrease and $V_T$ to increase as ventilation was stimulated by CO$_2$ did exist (Fig. 19). $T_{NVP}$ was not correlated to the following or preceding $V_T$. Such a correlation can also not be found in the data of Glass and Johansen ('76) for the snake, although it is argued to be present in the lizard by Jammes and Grimaud ('76).

The $T_{NVP}$ was, however, strongly correlated to $f$ ($\log f = 1.533 - 0.688 \log T_{NVP}; r = 0.899$) and to $\dot{V}_E$ ($\log \dot{V}_E = 2.995 - 0.857 \log T_{NVP}; r = 0.875$) (Fig. 19). The similarity between these two relations depicts the major role of changes in frequency in determining changes in $\dot{V}_E$ in the normal intact animal. Since $T_{tot}$ and hence $f_{VP}$ remained relatively constant as $\dot{V}_E$ increased during CO$_2$ breathing, $T_{NVP}$ was the major determinant of $f$. CO$_2$ did not alter the shape of these relations.

(b) After Bilateral Vagotomy

Following vagotomy the same weak correlation existed between $V_T$ and $T_{NVP}$ ($r = 0.490$), thus the general trend of decreasing $T_{NVP}$ with increasing $V_T$
Figure 19. Relationship between the length of the nonventilatory period and (a) tidal volume, (b) respiratory frequency and (c) minute ventilation in intact (open circles) and vagotomized (closed circles) turtles breathing air and CO₂ gas mixtures.
during CO₂ breathing was independent of volume feedback information and must have resulted from the effects of the CO₂ on peripheral or central chemoreceptors. Vagotomy did not affect the $T_{NVP}$, $f$ relationship but shifted the $T_{NVP}$, $V_E$ curve up and to the right ($\log V_E = 4.549 - 1.194 \log T_{NVP}$; $r = 0.765$). In the absence of lung volume feedback, tidal volume increased dramatically and $V_E$ was met at lower respiratory frequencies. Consequently, mean $T_{NVP}$ rarely fell below a value of 75 seconds.
DISCUSSION

In resting, spontaneously breathing animals, the respiratory pattern was consistent with that recorded by other researchers (see Gans, '78, for review) (Fig. 12). The mean values recorded for \( f \), \( V_T \) and \( V_E \) (Table 2) fall within the lower range of values reported in the literature for these variables in turtles (McCutcheon, '43; Millen et al., '63; Frankel et al., '69; Jackson, '71, '73; Jackson et al., '74). It is more difficult to find reported values for comparison of the other respiratory variables but published values of \( T_{tot} \), \( \text{Breaths VP}^{-1} \), and \( \frac{\text{Breaths VP}}{\text{BP VP + NVP}} \times 100 \) (McCutcheon, '43; Belkin, '68; Frankel et al., '69; Burggren, '72; Lucey and House, '77) encompass those values reported here (Table 2). \( \text{CO}_2 \) has consistently been reported as a respiratory stimulant in turtles leading to increases in both \( f \) and \( V_T \) (Randall et al., '44; Millen et al., '63; Frankel et al., '69; Jackson et al., '74) although there is some discrepancy concerning the sensitivity of turtles to \( \text{CO}_2 \) as a stimulus and the magnitude of the ventilatory response it causes. Millen et al. ('63) report that 6% \( \text{CO}_2 \) introduced into the inspiratory gas mixture of \textit{Pseudemys scripta} (temperature not reported) increased ventilation only slightly (\( V_E \) increased from 31 to 41 ml·min\(^{-1}\), the body weight of the animals is not reported), whereas Jackson et al. ('74), using the same gas mixture and the same animal, report a 10 X increase in ventilation (\( V_E \) increased from 23.8 to 215 ml·min\(^{-1}\)·kg\(^{-1}\)). The 3 X and 7 X increases in pulmonary minute ventilation which we observed when \textit{Chrysemys picta} were exposed to 5 and 10% \( \text{CO}_2 \) respectively in the inspired air (Table 2) support
the contention of Jackson et al. ('74) that CO₂ is a powerful respiratory stimulant. Further, these levels of CO₂ are well within physiological levels considering reported values for Pa_CO₂ of 100 to 130 mm Hg following 2 hr of diving in Pseudemys scripta (Robin et al., '64; Jackson & Silverblat, '74). It should be noted that Pseudemys scripta has now been reclassified as Chrysemys scripta and there appear to be few ecological or physiological differences between this species and the Chrysemys picta used in the present study.

Several studies indicate that O₂ depletion and CO₂ accumulation play a major role in determining the length of the breath hold between ventilatory periods in turtles (Lumsden, '23b, c; Lenfant et al., '70). Although each ventilatory period must then suffice to raise O₂ levels, decrease CO₂ levels and enable breath holding to be resumed, it is difficult to assess how individual breaths are regulated particularly since changes in V̇ appear independent of changes in Ṫ tot, Ṫ i, Ṫ i', or Ṫ e (Fig. 17). In spontaneously breathing, eucapnic animals, tidal volume is held within very narrow limits by adjusting Ṫ e, Ṫ i and Ṫ i (hence Ṫ tot) to the highly variable rates of expiration and inspiration (Fig. 18). Lung volume feedback is necessary to establish the correlations of Ṫ i' and Ṫ e with Ṫ i and Ṫ tot and to maintain the relative constancy of tidal volume (Figs. 15, 16, and 17) as all are eliminated by vagotomy.

Although there is much variability in expiratory and inspiratory flow rates, there is also modulation of the gas flow rates by vagal volume-related information shown by the increase in gas flow rates following vagotomy (Fig. 18). In mammals, after vagotomy the Ṫ i values are prolonged to the maximum
Table 3: Comparison of ventilatory control in mammals and turtles.

<table>
<thead>
<tr>
<th>Mammals</th>
<th>Turtles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>Vagotomized</td>
</tr>
<tr>
<td>---</td>
<td>↑T_I</td>
</tr>
<tr>
<td>---</td>
<td>inspiratory activity unchanged</td>
</tr>
<tr>
<td>---</td>
<td>↑V_T</td>
</tr>
<tr>
<td>V_T, T_I related</td>
<td>unrelated</td>
</tr>
<tr>
<td>V_T, T_E related</td>
<td>unrelated</td>
</tr>
<tr>
<td>T_E, T_I related</td>
<td>related</td>
</tr>
</tbody>
</table>

**CO_2 Effects**

| ↑V_T  | ↑f  | reduced depth exaggerated rate | ↑V_T  | ↑f  | ↑f  | ↑V_T  | ↑f  |
| no effect T_I | some | ↓T_I  | no effect T_I | no effect T_I | no effect T_I | some | ↑T_I  |
| ↓T_E  | some | ↓T_E  | T_E constant | T_E constant | no effect T_E | T_E constant |
| ↑air flow rates | ↑air flow rates | ↑air flow rates | ↑air flow rates | ↑air flow rates | ↑air flow rates | ↑air flow rates |
recorded prior to vagotomy and suggest a maximum interval set by central mechanisms and normally overridden by peripheral inputs. The increase in $V_T$ following vagotomy is due solely to this prolongation of $T_I$, the levels of inspiratory activity remain constant (Euler and Tripenbach, '76a, b) (Table 3). In turtles, however, the values of $T_I'$ and $T_E$ after vagotomy are frequently in the mid-range of those recorded before vagotomy (Fig. 17, Table 3). The large overall increase in $V_T$ and its variability are not due to prolongation of $T_I$ at a constant level of inspiratory activity as in mammals but solely to increased levels of inspiratory activity. The fact that $T_I$ and $T_E$ values are not equivalent to the maximum values measured before vagotomy suggests that volume information prolongs as well as shortens the active inspiratory and expiratory intervals.

When the $F_{ICO_2}$ is increased from 0 to 10%, tidal volume increases roughly 2.5 times yet $T_{tot}$, $T_I$, $T_I'$ and $T_E$ do not change appreciably (Table 2) and the slopes of their relations with $V_T$ remain at zero (Fig. 17). There is an increase in the expiratory and inspiratory flow rates (Fig. 12) and the increase in tidal volume must result from central excitation of the motor output to inspiratory and expiratory muscles. This is linked to both a proportionate rise in the central tidal volume threshold such that an increased tidal volume is ventilated within the same breath length ($T_{tot}$) and to direct inhibition of pulmonary stretch receptor discharge by $CO_2$ (Section I). Such inhibition will lead to increases in tidal volume necessary to restore volume feedback information to previous levels. In mammals $CO_2$ does not change the inspiratory threshold curve and depression of pulmonary stretch receptor discharge is implicated as the sole cause for hypercapnic induced increases in $V_T$ (Bradley et al., '75).
The contribution of the changes in tidal volume to the increase in minute ventilation during hypercapnia, however, is small; the major contribution comes from an increase in respiratory frequency. These predominant changes in respiratory frequency occur despite the constancy of individual breath lengths (Table 2). Thus the frequency of breaths within each ventilatory period \( f_{VP} \) remains constant while the number of breaths per ventilatory period increases 50% and the length of the nonventilatory period decreases; the number of ventilatory periods per minute doubling when \( F_{\text{ICO}_2} \) is increased from 0 to 10% (Table 2). Under conditions of severe hypercapnic stress \( (F_{\text{ICO}_2} \geq 15\%) \) \( f \) approaches \( f_{VP} \) and \( T_{\text{NVP}} \) approaches \( T_I \).

The relative contributions of \( f \) and \( V_T \) to \( V_E \) in turtles are similar to the responses of mammals following brainstem lesions in the reticular formation of the caudal pons and rostral medulla (St. John, '77) which tends to confirm that turtles lack a pneumotaxic centre in the pons (Lumsden, '23c). The Hering-Breuer reflex in mammals normally controls the respiratory rate and, in the absence of the pneumotaxic centre, maintains a relatively constant depth of breathing (Tang, '67). Consequently, in the absence of an integral part of the mammalian respiratory generator, central respiratory integration and the effects of vagal input from pulmonary receptors and of hypercapnia on this integration are bound to be different under steady state conditions in turtles and mammals. The differences, however, are due to more than just the absence of a pneumotaxic centre for although destruction of the pneumotaxic centre in mammals results in larger \( V_T \), \( T_I \) and \( T_E \) values, the \( V_T/T_I \) and \( V_T/(1/f) \) curves are of a shape similar to those found in the intact animal, merely being shifted to the right (Bradley, '77) (Table 3). The
absence of such strong positive correlations in turtles is not due to a lack of central integration of vagal volume related information as shown by the careful regulation of tidal volume and the fact vagotomy drastically changes the correlations which do exist (Figs. 14 and 17). It is also not due to any major differences in the nature of the vagal volume information. The functional characteristics of pulmonary stretch receptors in the turtle differ only quantitatively from those of mammalian pulmonary stretch-receptors (Section I). It seems apparent that central integration of the vagal volume signal must be quite different in these two groups of animals under steady state conditions.

After vagotomy changes in $V_E$ due to hypercapnia are due primarily to changes in $V_T$ (Fig. 14). As in mammals (Widdicombe and Winning, '74; Bradley et al., '74a, b, '75), changes in respiratory rate are greatly reduced (Fig. 14, Table 3). Tidal volume is now primarily determined by the inspiratory and expiratory rates and the length of the active inspiratory and expiratory intervals. The increases in respiratory frequency which occur during hypercapnia are primarily due to decreases in $T_{NVP}$ (Table 2, Fig. 19); the breath hold interval. On vagotomy, this interval in lengthened but since the degree of correlation between $T_{NVP}$ and $V_T$ is unchanged, it must stem either from removal of excitatory tonic vagal influence from the mechanism initiating inspiration following breath holding or a decreased sensitivity of this mechanism to $O_2$ depletion and $CO_2$ accumulation. Hypercapnia still acts to shorten this interval but its effects on respiratory frequency are somewhat offset by the increased breath length and reduced $f_{VP}$ compounded by an increased number of breaths within each ventilatory period (Table 2).
Apneusis in mammals is characterized by a marked prolongation of $T_I$, hence $T_{tot}$, and concomitantly, an elevation of tidal volume and reduction of respiratory frequency (St. John and Wang, '77). During apneusis, inspiratory depth and duration are totally dependent on central effects of $F_{CO_2}$ (Lumsden, '23a,b,c; Stella, '38a,b; Tang, '62). In turtles, following vagotomy, all measured variables behaved in a manner similar to those of mammals during apneusis. In mammals during apneusis, $T_E$ is relatively constant and independent of $T_I$ but somehow related to the duration of the rising phase of brainstem central inspiratory activity rather than the total duration of the inspiratory phase (Euler et al., '76). The expiratory interval in turtles, following vagotomy, also remains relatively constant, is independent of $T_I$ but is correlated to $T_{I'}$, the active inspiratory interval. Thus, in the absence of a pontine pneumotaxic centre (Lumsden, '23a,b,c) simple vagotomy in turtles results in an apneusis comparable to that found in mammals (Table 3).
SECTION III

The Role of Pulmonary Receptor Chemosensitivity in the Ventilatory Response to Inhaled CO₂

INTRODUCTION

In vertebrates, pulmonary receptors with discharge correlated to the rate and degree of lung ventilation are of two major types: intrapulmonary chemoreceptors and intrapulmonary stretch receptors. The intrapulmonary chemoreceptors which have been found in the lungs of birds (Fedde & Peterson, '70; Osborne & Burger, '74) and lizards (Fedde et al., '77) are primarily sensitive to changes in airway CO₂ concentration. Intrapulmonary stretch receptors have been described in the lungs of mammals (Adrian, '33), turtles (Milsom & Jones, '76), lizards (Fedde et al., '77) and frogs (Taglietti & Cassela, '66) and although the adequate stimulus for these receptors is mechanical deformation, they have been shown to possess varying degrees of CO₂ sensitivity (Mustafa & Purves, '72; Milsom & Jones, '76; [Section I]; Fedde et al., '77; Milsom & Jones, '77; [Section IV]). The primary function suggested for these receptors in mammals (Widdicombe, '64) and birds (Osborne & Mitchell, '77) is to reflexly control the tidal volume to respiratory frequency ratio and minimize the work of breathing (Otis et al., '50) or force of contraction of the respiratory muscles (Mead, '60). Recently, however, due to their CO₂ sensitivity, pulmonary receptors have been implicated in the regulation of PaCO₂ during muscular exercise, intravenous CO₂
loading experiments and CO₂ inhalation experiments (Wasserman et al., '67; Wasserman et al., '75; Osborne & Mitchell, '77). To date, this proposed role for intrapulmonary receptors remains unresolved. The following study is a comparison of the ventilatory responses of turtles to changes in the intrapulmonary CO₂ content of vascularly isolated lungs (hence constant \( P_{a\text{CO}_2} \)) with the responses to changes in the intrapulmonary CO₂ content of intact lungs and an assessment of the role of CO₂-related receptor information carried from pulmonary receptors within the vagus nerve in this animal's response to hypercapnia.
METHODS

In one series of acute experiments, turtles (*Chrysemys picta*, 600-1200 g) were single-pithed and tidally ventilated with a constant volume, positive pressure respiration pump. Single and multi-fibre nerve activity in phase with artificial ventilation was recorded in vagal slips using bipolar silver electrodes. Neural activity was amplified (Tektronix FM 122 preamplifier), visually displayed on an oscilloscope and audibly monitored. Receptors were localized by punctate stimulation with a fine probe to major septa within the lungs. The pressure drop across a pneumotachograph during tracheal air flow was recorded with a Hewlett-Packard 268 BC differential pressure transducer and intratracheal pressure generated during ventilation was measured with a Statham P23V pressure transducer. The air flow signal was fed through a Hewlett-Packard 350-3700A integrating preamplifier to give tidal volume and all measurements, pressure, flow, volume and receptor discharge were stored on magnetic tape for later analysis on a Digital PDP Lab 8e mini-computer using conventional software. The ventilating gas mixture was altered from 0 to 10% CO₂ in compressed air using premixed gases and the O₂ and CO₂ composition of the inspired and expired gases was determined either on samples taken through the side arm of the tracheal cannula and measured on a Fisher-Hamilton gas partitioner or by continuous sampling with a Centronic 200 MGA clinical mass spectrometer (sample rate < 10 ml·min⁻¹). For further details, see Section I.

A further series of chronic experiments was performed on unanaesthetized, lightly restrained specimens (500-1500 g) at room temperature (22-23°C).
Surgery was performed under either a combination of cold (1-4 hr at -20°C) and local anaesthesia (2% Lidocaine hydrochloride), or general anaesthesia (1.5-2 ml·100 g⁻¹ 10% MS222 injected I.P.). A window was removed from the plastron above the area of the heart using a necropsy saw allowing both primary bronchi to be cannulated separately. An occlusion catheter was also placed around the left pulmonary artery and the left vagus was exposed and loosely snared for sectioning at a later time. All catheters were led out through the skin at the base of the neck and the window was replaced and sealed with cotton wool and dental acrylic cement. Pneumotachograph cuffs and side arms for tracheal pressure measurement and gas sampling were attached to the bronchial cannulae and the distal ends of these cannulae were attached to T connections. One arm of each T connection was open to atmosphere and the remaining arm was attached to a respiratory gas supply. Using a system of gas flow meters, the composition of the respiratory gas flowing past the end of each bronchial cannula could be independently altered thus separately controlling the composition of the inspired gas going to each lung when the turtle breathed.

Tracheal pressure, air flow and tidal volume were measured as described earlier and continuously recorded on a Sanborn 7700 chart recorder. Gas samples were also measured as has been described for the acute experiments. Animals were allowed to recover fully from anaesthesia (usually 24 hrs) before experimentation began. The turtles were shielded from all activities of the experimenters and while resting quietly, the occlusion cuff was inflated isolating the blood supply of the left lung. The breathing pattern was then allowed to stabilize before presentation of the test gas samples.
Left pulmonary artery occlusion, in itself, did not usually alter the normal breathing pattern. The animals were presented with air to both lungs and 10% $\text{CO}_2$ in air to each lung while the other received room air. Each combination was presented for a one-hour period and the presentation sequence was varied. All variables were recorded continuously during this time but data were selected for analysis only after the responses to each gas mixture had stabilized. Following this series of experiments the left vagus was sectioned under local anaesthesia and on the following day the above protocol was repeated.
RESULTS

Figure 20 shows the discharge profile of a typical receptor in response to lung inflations of varying volumes with 0% and with 10% CO₂ in the ventilation gas mixture. From recordings of 62 fibres in 30 turtles ventilated with room air (Section I) pulmonary stretch receptor discharge rate was best defined by the equation:

\[ \text{Rate} = 3.46 + 11.6 \text{ITP} \]

where ITP is the intratracheal pressure, in kPa, generated during lung inflation. At any level of lung inflation receptor discharge was reduced by approximately 55% when 10% CO₂ was present in the ventilation gas (Fig. 20). Under these circumstances (Section I) pulmonary stretch receptor discharge rate was best defined as:

\[ \text{Rate} = 1.56 + 5.2 \text{ITP}. \]

The relative role of these receptors in the overall response of turtles (n = 12) to CO₂ can be seen from Figure 21. When 10% CO₂ was presented to the vascularly isolated left lung, there was a 47% increase in minute ventilation (\( V_E \)), a 20% increase in tidal volume (\( V_T \)) and a 23% increase in respiratory frequency (f). Following unilateral (left) vagotomy there was an increase in resting minute ventilation, however, there was no longer any respiratory response following introduction of 10% CO₂ to the vascularly, neurally isolated left lung. When the same levels of CO₂ were introduced to the vascularly intact right lung there was a 272% increase in \( V_E \), a 56% increase in \( V_T \) and a 108% increase in f. Although unilateral left vagotomy altered the relative roles of \( V_T \) and f, there was no reduction in the response in minute ventilation to increasing levels of CO₂ in the intact right lung.
Figure 20. Response of a single pulmonary stretch receptor to lung inflation to different volumes with different gas mixtures. The upper trace in each record shows the intratracheal pressure in kPa and the lower trace the receptor discharge for each inflation. The responses at three different inflation volumes (30, 40 and 50 cc) with either 0% CO$_2$ (left hand traces) or 10% CO$_2$ (right hand traces) are shown.
Figure 21. Levels of minute ventilation, tidal volume and respiratory frequency of turtles with blood flow to one lung occluded and both primary bronchi cannulated. Responses are shown of turtles breathing air in both lungs or 10% CO₂ in air in one lung and air in the other, before and after vagotomy. Values are the means ± S.E.
(ml-
\[ \text{min}^{-1} \])

\[ \text{VE} \]

(both lungs)

\[ \text{VT} \]

(both lungs)

\[ \text{f} \]

(both lungs)

\[ \text{CO}_2 \]

(left lung)

\[ \text{CO}_2 \]

(right lung)

\[ \text{unilateral (left) vagotomy} \]

\[ \text{pre} \]

\[ \text{post} \]
DISCUSSION

Our results indicate that turtles increase their minute ventilation slightly in response to increases in the intrapulmonary CO$_2$ concentration of a vascularly isolated lung. The lack of any response to increased levels of CO$_2$ in this lung following vagotomy indicates that pulmonary receptors are the afferent limb of this response.

Intrapulmonary chemoreceptors have not been demonstrated in turtles but the discharge rates of pulmonary stretch receptors are inhibited by increasing levels of intrapulmonary CO$_2$ (Fig. 20; Section I). In spontaneously breathing, intact animals these receptors serve to regulate tidal volume within narrow limits and place the emphasis of respiratory responses to hypercapnia on changes in respiratory frequency. This is clearly illustrated by the major role played by changes in tidal volume in the respiratory response to ventilation of the vascularly intact lung with 10% CO$_2$ following vagotomy. Consequently, the inhibition of the discharge of pulmonary stretch receptors during hypercapnia will necessitate greater tidal volumes to provide the same volume information to the brain and probably accounts for the increase observed in tidal volume when CO$_2$ was inspired by the vascularly isolated lung. The effect of CO$_2$ in increasing tidal volume in this situation will be partially offset by the increased discharge coming from receptors in the intact lung which is being ventilated with air at these increased tidal volumes. However, when the same levels of CO$_2$ were inspired by the intact lung while the vascularly isolated lung inspired air, presumably providing the same total pulmonary receptor input as in the reverse situation, the tidal volume response was more than twice as great. Whether this
increased response is due to central or peripheral effects of the increase in blood $P_{CO_2}$, it indicates that $CO_2$ must also act to functionally raise the central inspiratory volume threshold in turtles, in direct contrast to the situation found in anaesthetized cats (Clark & Euler, '72; Bradley et al., '74).

It has been reported in mammals that pulmonary stretch receptor discharge during expiration prolongs the duration of expiration (Hering & Breuer, 1868; Knox, '73; Misereocchi & Milic-Emili, '75; D'Angelo & Agostoni, '75). It has therefore been suggested that the inhibition of end-expiratory discharge of pulmonary stretch receptors by $CO_2$ is responsible for the increased frequency of breathing obtained when $CO_2$ is inhaled by dogs on cardiopulmonary bypass (Bartoli et al., '74; Bradley et al., '76). This is achieved primarily by shortening the expiratory interval (Bartoli et al., '74). It is possible that the small increase in breathing frequency observed in the turtle when $CO_2$ was inhaled into a vascially isolated lung also resulted from depression of end-expiratory discharge of pulmonary stretch receptors. In the turtle, however, the breathing frequency is increased through a shortening of the periods of intermittent breath holding rather than changes in the rate of active ventilation (Section II), thus if the changes in breathing frequency are the result of decreased pulmonary receptor discharge, the central integration of this information must be slightly different in the turtle and mammal.

The primary ventilatory response of intact turtles to increasing levels of $F_{ICO_2}$ is an increase in respiratory frequency. Since this component of the ventilatory response to $CO_2$ is greatly reduced when $CO_2$ is inspired only by the vascially isolated lung, the total ventilatory response to $CO_2$ under
these conditions is relatively small. Thus it would appear that the CO$_2$
sensitivity of pulmonary receptors does contribute to the increase seen in
tidal volume during hypercapnia but is of little significance to the overall
response of the turtle to increased F$_{ICO_2}$. There is no evidence that turtles
possess intrapulmonary chemoreceptors but if they are present in turtles as
in lizards (Fedde et al., '77) they would appear to have only the same re-
flex functions as intrapulmonary mechanoreceptors.
SECTION IV

Carbon Dioxide Sensitivity of Pulmonary Receptors
in the Frog

INTRODUCTION

The pulmonary receptors of mammals respond primarily to the transpulmonary pressure developed during each breathing cycle but their discharge is partially modified by high levels of alveolar CO₂ (Davis et al., '56; Mustafa & Purves, '72; Bradley et al., '76). Avian pulmonary receptors have little or no mechanosensitivity responding primarily to changes in airway CO₂ concentration throughout the breathing cycle (Fedde et al., '74a,b). Turtle pulmonary receptors are typically mechanosensitive but exhibit a range of variation in their sensitivity to CO₂ which encompasses the different sensitivities to CO₂ found in the avian and mammalian receptor types (Section I). It begins to appear that the divergent receptor types found in birds and mammals may have arisen, phylogenetically, from a common, less specialized receptor type. Amphibia evolved from the evolutionary stem line at an early date, possess structurally simple lungs and represent some of the earliest forms of semi-terrestrial lunged vertebrates. This study was undertaken to determine whether there are receptors present in the lungs of these early forms which are sensitive to CO₂.
METHODS

Frogs (*Rana pipiens*) weighing between 120 and 160 g were used in these experiments. The frogs were double-pithed and unidirectionally ventilated with a continuous gas flow under slight positive pressure, air entering the lung through a tracheal cannula and leaving the lung by a cannula sewn into the caudal tip of the lung. The lung could be inflated during ventilation to any desired volume by altering the resistance of the outflow cannula from the lung. Single and multi-fibre nerve activity were recorded from pulmonary afferent fibres in vagal slips using standard techniques (Section I). The intratracheal pressure was recorded with a Statham P23V pressure transducer and with neural activity were amplified, visually displayed on an oscilloscope and stored on magnetic tape for later analysis on a PDP Lab 8e mini-computer using conventional software.
RESULTS AND DISCUSSION

On the basis of changes in discharge frequency following lung inflation, frog pulmonary receptors have been classified into three groups: rate receptors, proportional receptors and rate plus proportional receptors (McKean, '69). The discharge frequency of rate receptors is modulated solely by the rate of increase in lung volume. Six of 25 fibres recorded from were of this type. Although these fibres were continuously active, their static rate of discharge was unaffected by the volume of the lung; discharge increased only during the period of lung inflation and then returned immediately to the previous level of discharge (Fig. 22b). Addition of 10% CO₂ to the ventilating gas caused a decrease in the static discharge rate of these receptors of 56% (10.6 ± 6.0 Hz falling to 4.7 ± 1.0 Hz) (each value is the mean ± S.E.M.) and the modulation in discharge occurring during lung inflation was reduced by 50% (41.3 ± 7.8 Hz falling to 20.6 ± 2.7 Hz) at an inflation rate of 1.0 ml·s⁻¹ (Table 4). One unit was particularly sensitive to CO₂ showing a 75% reduction in static discharge rate and a 74% reduction in the discharge associated with lung inflation with 3% CO₂ present in the ventilating gas (Fig. 22b) and 67% and 64% reductions in discharge rate during ventilation with 1% CO₂ in air. The discharge rate of proportional receptors increases with increasing lung volume and for any given lung volume discharge is maintained with only slight diminution as long as the lung volume is constant (Fig. 22a). Three of the receptors recorded from fit this category. All units were active during lung deflation when intratracheal pressure approached atmospheric. The addition of 10% CO₂ to
Figure 22.  

a) Proportional receptor response to lung inflation with air (left) and air + 3% CO₂ (right). Upper trace is a time marker, second trace is pulmonary receptor discharge, third trace is analog discharge frequency and lower trace is intratracheal pressure.

b) Rate receptor response to lung inflation with air (left) and air + 3% CO₂ (right). Traces as indicated above.
a.

- **time (s)**
- **vagal discharge**
- **discharge rate (Hz)**
- **intratracheal pressure (kPa)**

b.
the ventilating gas caused a reduction in this discharge rate of 56%.

There was a reduction of 45% in the discharge rate associated with lung inflation to an intratracheal pressure of 0.5 kPa (Table 4). One unit was very sensitive to CO$_2$ and was 75% reduced during deflation and 61% reduced during inflation to 0.5 kPa intratracheal pressure with the addition of 3% CO$_2$ (Fig. 22a) (65% and 43% reduced respectively by 1% CO$_2$). The remaining sixteen fibres recorded were from rate and proportional sensitive receptors which exhibited a peak discharge frequency during inflation as well as an increase in static discharge frequency with increasing lung volume (Fig. 23). Fifteen units were active during lung deflation at an average discharge frequency of 6.9 ± 1.3 Hz which increased to an average discharge frequency of 19.2 ± 3.0 Hz on lung inflation to 0.5 kPa intratracheal pressure. The peak discharge frequency associated with a 1 ml·s$^{-1}$ rate of inflation was 51.6 ± 7.0 Hz. With 10% CO$_2$ present in the ventilating gas these discharge frequencies fell to average values of 5.3 ± 1.0 Hz, 15.4 ± 3.2 Hz and 35.8 ± 5.9 Hz respectively representing 23, 20 and 31% reductions in the discharge frequencies (Table 4). One fibre increased its discharge frequency in the presence of 10% CO$_2$. In all cases the changes in activity began during the first few seconds following a step change in the CO$_2$ content of the ventilating gas. All units were isolated to locations within the lung by punctate stimulation. No fibres could be found which were sensitive to step changes in CO$_2$ content in the ventilating gas but insensitive to mechanical stimuli.

This study has shown that the pulmonary mechanoreceptors in the frog lung are sensitive to CO$_2$ concentrations in the lungs and airways; in some
Figure 23. Rate and proportional receptor response to lung inflation with air (left) and air + 10% CO₂ (right). Upper trace is a time marker, second trace is pulmonary receptor discharge, third trace is analog discharge frequency and lower trace is intratracheal pressure.
Table 4: Effect of rate and degree of lung inflation and of CO₂ on frog pulmonary receptor discharge.

<table>
<thead>
<tr>
<th>Fibre</th>
<th>Receptor Type</th>
<th>Discharge Rate of Receptors (Hz)</th>
<th>Air</th>
<th>Air + 10% CO₂</th>
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<tr>
<td></td>
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<td>Inflation</td>
<td>Deflation to 0.5 kPa at 1 ml.s⁻¹</td>
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<td>6.0</td>
<td>1.0</td>
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<tr>
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<td>4.3</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
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<td>3.8</td>
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<td>X</td>
<td>2.9</td>
<td>5.1</td>
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<td>7.63</td>
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<td>27.5</td>
<td>0.8</td>
</tr>
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<td>20.0</td>
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<td>47.5</td>
<td>10.0</td>
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<td>X</td>
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<td>39.4</td>
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<td>72.5</td>
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<td>5.7</td>
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</table>
instances extremely sensitive to very low levels of CO₂ comparable to the physiological levels recorded in frogs (Emilio, '74). These results are similar to those recorded from the turtle (Section I) and support the suggestion that a pulmonary receptor with distinct mechano- and chemosensitive properties may represent the functional precursor of the variety of more specialized pulmonary receptor types which appear in modern day vertebrates.
Analysis of the arrhythmic breathing pattern in the turtle has shown that the change in minute ventilation during hypercapnia was primarily the result of a change in respiratory frequency. This was not due to a change in the rate of active ventilation but solely to shortening of the nonventilatory period.

At any level of respiratory drive, tidal volume was maintained within narrow limits by adjusting the length of the active inspiratory and expiratory interval to compensate for a large variability in the inspiratory and expiratory gas flow rate. This large variability is probably a consequence of the changes in gravitational and mechanical factors which affect breathing. Turtles lack an independent pulmonary chamber whose volume can be separately varied by muscular activity. Ventilation is thus effected by muscles which alter the volume of the general body cavity and these alterations are in turn transmitted to the lungs resulting in breathing movements. There appear to be four major muscles involved, the serratus major and pectoralis muscles of the pectoral girdle and the transversus abdominus and obliquus abdominus muscles of the posterior flank cavity. With the possible exception of the muscularis transversus abdominus, none of these muscles are purely respiratory in function but subserve other roles, particularly locomotion (Gans & Hughes, '67). Consequently, with each breath, the location of the limbs and limb girdles (Gans & Hughes, '67), the possible depth of the turtle below the water surface from which it is breathing (Gaunt & Gans, '69), and the tonus and position of the viscera

GENERAL DISCUSSION
which hang suspended by connective tissue sheets below the lungs (Gans & Hughes, '67), will all affect the rates of expiration and inspiration for any given level of respiratory motor output.

At any level of respiratory drive, minute ventilation is greater in vagotomized animals although the amount of time spent actively breathing is reduced. Although oxygen consumption was not quantitatively measured and analyzed, it would appear (values in Fig. 12) that $V_{O_2}$ also increases suggesting that control of ventilation primarily through changes in $V_T$ rather than $f$ increases the work of breathing. It is quite feasible that alterations in $T_{NVP}$ are least costly in terms of respiratory work and until breath holding is eliminated changes in $T_{tot}$, $T_I$, and $T_E$, as well as larger changes in $V_T$, will not appear. This argument implies that the change in the force of muscle contraction required to increase respiratory flow rates such that $f$ increases by shortening $T_{tot}$ and $V_T$ increases within this shortened interval, are energetically more costly than taking another breath at the same rate and depth.

It should be noted that under severe hypercapnic stress ($F_{CO_2} > 15\%$), $f$ approaches $f_{VP}$ and breathing becomes virtually eupneic. Although data were not collected and analyzed from animals under such conditions, it is clear that further increases in $V_E$ must either result solely from changes in $V_T$ or $f_{VP}$ must increase. It is possible that once the breathing rhythm becomes continuous, positive correlations of $V_T$ and $f$ with the respiratory intervals may appear as in mammals.

This suggests that the apparent difference in central integration of vagal afferent information between mammals and turtles may stem from the
reduced metabolic demand of turtles. It should be remembered, however, that in turtles, breath holding is a relatively passive phenomenon for at the end of active inspiration the glottis closes and the respiratory muscles relax (McCutcheon, '43; Gans & Hughes, '67). This period is terminated by an active expiration. Models of central integration of respiration in mammals incorporate an inspiratory "off switch" as the major control element determining cessation of active inspiration and the beginning of passive expiration (Bradley et al., '75; Euler et al., '76b). Further, it has been argued that in mammals (Gautier et al., '73) the expiratory interval triggers the next inspiration when lung volume falls to FRC. In turtles there is no correlation between $T_E$ and $V_T$. FRC can be highly variable (Milsom & Johansen, '75), and artificially opening the lungs to atmosphere at the end of each inspiration reduces lung volume to FRC or below during breath holding but does not trigger inspiration. This plus the presence of both an active and a passive inspiratory phase followed by active expiration during steady state spontaneous breathing in turtles indicate that either the inspiratory "off switch" is much more complex in turtles than mammalian models indicate, or that an expiratory "on switch" may exist, or both.

Although the respiratory drive necessary to produce a continuous pattern of breathing in turtles far exceeds physiological levels, answers to these speculative questions may provide insights into the changes which occur in the control of the ventilatory pattern when metabolic demand exceeds the point at which frequent intermittent breath holding is feasible.
SUMMARY

1. CO₂ is shown to be a strong respiratory stimulant in turtles. \( \dot{V}_E \) was increased 3 and 7 times above resting values by the presence of 5 and 10% CO₂ respectively in the inspired gas.

2. Increases in \( \dot{V}_E \) were primarily the result of changes in respiratory frequency. Tidal volume was maintained within narrow limits by adjusting the length of the active inspiratory and expiratory interval to compensate for a large variability in the inspiratory and expiratory gas flow rates. This mechanism was dependent upon lung volume information carried within the vagus nerve.

3. The frequency of breathing within each ventilatory period remained constant during hypercapnia; the increase seen in respiratory frequency was due solely to shortening of the nonventilatory period.

4. Respiratory frequency and tidal volume were controlled separately and independent of the breath length (\( T_{\text{tot}} \)), the inspiratory interval (\( T_I \)), the active inspiratory interval (\( T'_I \)), and the expiratory interval (\( T_E \)).

5. A large increase in \( V_T \) accompanied vagotony and was due to an increase in central respiratory activity. The inspiratory and expiratory intervals were not prolonged.

6. Following vagotony, changes in \( \dot{V}_E \) due to hypercapnia stemmed primarily from changes in \( V_T \) while changes in respiratory frequency were markedly reduced.
7. Single fibre discharge was recorded from slowly adapting pulmonary stretch receptors in single-pithed turtles and the functional characteristics of these receptors were derived from static and dynamic lung inflations. Change in lung volume was the sole stimulus of these receptors; the role and degree of change in transpulmonary pressure were without direct effect on receptor discharge. All other functional characteristics, including sensitivity to CO₂, differed only quantitatively from those recorded in pulmonary stretch receptors of mammals. Most of the quantitative differences may arise from the lower body temperature of the turtle and the location of the receptors in the turtle lung.

8. In several instances both tonic and phasic receptor discharge were totally inhibited throughout the ventilatory cycle by 5 to 10% CO₂ in the inspired gas. The high degree of sensitivity to CO₂ in these few receptors parallels that of the intrapulmonary CO₂ receptors described in birds.

9. Pulmonary mechanoreceptors in the frog lung were also shown to be sensitive to CO₂, in some instances extremely sensitive to very low levels of CO₂. It is suggested that a pulmonary receptor with distinct mechano- and chemosensitive properties may represent the functional precursor of the variety of more specialized pulmonary receptor types which appear in modern day vertebrates.

10. The role of CO₂ sensitivity of pulmonary receptors in the overall response of turtles to inhaled CO₂ was also tested. Isocapnic
hyperpnea associated with inhalation of CO₂ by a vascularly isolated lung was small and abolished by vagotomy suggesting that the ventilatory response of turtles to increasing levels of F_{I CO₂} is primarily dependent on increased arterial P_{CO₂}. It is concluded that both inhibition of pulmonary stretch receptor discharge and a functional increase in central inspiratory volume threshold contribute to tidal volume increases associated with increasing levels of airway CO₂.

11. Careful analysis has been made of the correlations between the breath length, the inspiratory interval, the expiratory interval, the length of breath holding and the tidal volume. Comparison is made of the central integration of the vagal volume signal and the control of the breathing pattern in intact turtles with that of mammals following ablation of the pneumotaxic centre. The effect of vagotomy in producing apneusis is also compared in both these situations.
LITERATURE CITED


PUBLICATIONS:


Jones, D.R., Milsom, W.K. and West, N.H. Reflex role of left ventricular receptors in diving bradycardia in ducks. (Submitted to Am. J. Physiol.).