

THE MECHANICS OF BREATHING
IN THE TURTLE, PSEUDEMYS SCRIPTA

by

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Abstract

Measurements of pulmonary mechanics on anaesthetized specimens of the aquatic turtle Pseudemys scripta, indicate that the static pulmonary mechanics are determined primarily by the mechanics of the body wall of the animal rather than those of the lungs. The mechanics of the body wall are also predominant under the dynamic conditions of pump ventilation, but only at low frequencies. As pump frequency increases, the multicameral lungs of the turtle begin to contribute an ever increasing portion to the total mechanical work required to produce each breath as measured from pressure volume loops. The rise in the work performed on the lungs results from an increase in the nonelastic, flow resistive forces which must be overcome during ventilation. The respiratory system is the major site of nonelastic work. The primary bronchus to each lung branches to seven intrapulmonary chambers, and is the most likely site of flow-resistance. There is also a small elastic component of the work required to ventilate the lung arising from forces in the intrapulmonary septa and the striated muscle surrounding the lungs. The contribution of the body to the total mechanical work required to generate each breath remains relatively unchanged with increasing ventilation frequency indicating that the majority of the forces to be overcome in the body wall are elastic in nature. Since the shell of the animal is rigid these elastic forces must reside in the flanks and pectoral regions. For a constant alveolar ventilation rate (\dot{V}_A) as frequency increases, the elastic work done per minute decreases while the nonelastic work done per minute begins to rise. This results in a 'U'-shaped curve for the total mechanical work done per minute in ventilating the lungs at a

constant \dot{V}_A as breathing frequency increases. There is then, for any given level of ventilation, a combination of tidal volume and frequency at which the rate of mechanical work is minimized. This occurs at a frequency of 35 breaths per minute.

The normal breathing pattern of P. scripta consists of bursts of continuous breathing separated by variable periods of breath holding. Increases in pulmonary ventilation, upon stimulation by hypercapnia (3% CO₂ in air) or hypoxia (4% O₂ in N₂), are caused by increases in the number of breaths per minute due to the shortening of the breath hold period. Tidal volume and breath duration remain unchanged. The instantaneous breathing frequency ($f' = 60/T_{\text{tot}}$) corresponds to the pump frequency which minimizes the rate of mechanical work as measured in anaesthetized turtles. This indicates that turtles breathe at a combination of tidal volume and f' which minimizes the rate of mechanical work required to ventilate the lungs. To increase ventilation, the breath hold is shortened and more breaths are taken at this optimal combination. Bilateral vagotomy drastically alters the breathing pattern producing an elevation in tidal volume, a slowing of breathing frequency and a prolongation of the breath duration, resulting in an increase in the mechanical cost and thus in the oxidative cost of breathing even though total minute ventilation (\dot{V}_E) changes very little. These data suggest that periodic breathing in this species may represent an adaptive strategy which is under vagal afferent control and which serves to minimize the cost of breathing.

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CHAPTER 1

GENERAL INTRODUCTION

The transition from life in water to life on land required the development of two important structures, limbs and lungs (Romer, 1967). Romer (1972) contends that lungs developed in fish, most probably, as a response to shallow, fresh water environments characterized by hypoxia and periodic drought. The appearance of such lungs gave rise to the further possibility of life on land.

Both aquatic air breathing organisms and terrestrial organisms can exploit the use of air as a respiratory medium. Air has the advantage of providing an abundant and stable source of oxygen, but the disadvantage of desiccation posing a constant and serious problem. To combat the problem of desiccation, the integument of the terrestrial tetrapods became increasingly keratinized resulting in a progressive loss of cutaneous gas exchange and an increasing dependence on pulmonary gas exchange (Tenney and Tenney, 1970). Coupled with this increased dependence on pulmonary gas exchange was an increase in the complexity and surface area of the lungs. Several trends also developed in the mechanisms employed for ventilating these lungs.

The first land dwellers were the amphibians. Modern amphibians such as the grass frog, Rana pipiens, rely on cutaneous gas exchange for 34% of their O₂ requirements and 83% of their CO₂ loss (25°C) (Hutchinson et al., 1968). It is unclear, however, whether cutaneous gas exchange in amphibians is a primitive characteristic or has evolved later as a secondary specialization to aid in CO₂ elimination (Romer, 1972). The lungs of modern amphibians are simple sacs with little infolding to increase surface area (Hughes, 1963). The amphibians ventilate their lungs using positive

pressure generated by their buccal cavity (West and Jones, 1975), a process which is dynamically analogous to gill ventilation (Tenney and Tenney, 1970). "This primitive breathing mechanism has a limited ventilatory capacity and never frees the animal from some dependence on extra-pulmonary gas exchange" (Tenney and Tenney, 1970). Ventilatory capacity is limited, not by respiratory frequency but by tidal volume (V_t), which is limited by the size of the buccal cavity. With some modification, this mechanism probably represents a retention of that used by lunged fish where gill and branchial musculature are used for both gill and lung ventilation. By retaining this mechanism a shift only in control mechanisms regulating the timing of various portions of the pumping sequence were required (Shelton et al., 1984).

In reptiles the skin has been almost completely eliminated as a gas exchange organ. The lungs are now the predominant site of gas exchange though aquatic turtles can eliminate considerable amounts of CO_2 through their skin (Jackson et al., 1974). In general, the lungs of reptiles have an increased surface area compared to amphibians but this is not always the case. For instance, some of the less active reptiles such as Sphenodon have only simple bag-shaped lungs (Hughes, 1963). The lungs in all reptiles communicate with the pharyngeal cavity by a cartilage-ringed tracheal tube. In most reptilian species this tracheal tube divides into primary bronchi which connect the several intrapulmonary chambers of each lung (see chapter 2). The positive pressure buccal pump is replaced by aspiration breathing which is characterized by the generation of negative pressure within the lung. Air is now drawn into the lung rather than forced in by positive pressure built up in the anterior buccal cavity. The complex co-ordination

of nasal and glottal opening and closing is no longer essential but both sets of openings still play major roles. During aspiration breathing both sets of openings are open simultaneously allowing for continuous tidal ventilation of the pulmonary gas exchange surface. Once breathing ceases the glottis closes at end-inspiration.

The three classes of modern reptiles all possess different mechanisms of ventilation which, on a morphological basis, are not homologous to one other (Gans, 1976). The variety of ventilatory mechanisms seen in the reptiles suggests that aspiration breathing has evolved many times using different muscles, different mechanics, and different mechanisms to suit the individual problems of each group. Lizards and snakes have long flexible bodies with ribs for support and costal breathing was an easy adaptation. Crocodiles are much too heavy to lift themselves off the ground to breath using rib movement and have developed a liver-piston pump. Turtles live in a box formed by their shell and use their flank cavities and also, partially, their fore-limbs to ventilate their lungs.

Ventilation in lizards (Hughes, 1963) and snakes (Rosenberg, 1973) is powered by a costal pump. Inspiration is an active process brought about by a suction pump powered by the muscles of the ribs. The intercostal muscles contract, moving the ribs and expanding the rib cage and the thorax. This produces a negative pressure in both the thorax and the lungs. Expiration is passive but may be assisted by the contraction of transverse abdominal muscles (Hughes, 1963). The respiratory movements are triphasic while air flow is diphasic. Flow appears to only be triphasic following mistiming of glottis closure in disturbed animals, or due to the effects of anesthesia (Cragg, 1978; Gans and Clark, 1978). A ventilation cycle begins

with expiration. During the period of breath holding which follows inspiration, pulmonary pressure is above atmospheric. Inward movements of the thoracic cage preceding expiration causes the pressure within the lung to rise further until the glottis opens and air flows out of the lungs. The inspiratory phase follows immediately whereupon a subatmospheric pressure is produced in the lungs due to the outward movement of the thoracic cage. At end-inspiration the glottis closes and the inspiratory muscles relax causing a slight compression of the lungs which results in intrapulmonary pressure being above atmospheric pressure during breath holding.

The crocodile, Caiman crocodilus, ventilates its lungs by a hepatic piston pump (Gans and Clark, 1976). The ribs are held in position by the intercostal muscles while the transverse abdominal muscles shift the liver forward thereby reducing the volume of the pulmonary chamber and causing exhalation. If the muscles contract more strongly, or if contraction precedes the opening of the glottis, the lungs may be pressurized leading to a more rapid exhalation. Inspiration occurs after expiration and is brought about by the contraction of the diaphragmatic muscle. This diaphragmatic muscle is not homologous to the mammalian diaphragm but inserts on to the liver from its origin on the ilia and epipubic elements of the pelvic girdle. Contraction of this muscle shifts the liver posteriorly thus increasing the volume of the thoracic cavity and hence the lungs. The negative pressure generated by the increase in lung volume causes air to flow in through the open glottis. The ventral, deep intercostals also contract to maintain the patency of the pulmonary cavity. The pattern of pressure changes generated in the lung during breathing are similar to those seen in lizards and snakes.

The chelonians have a unique mechanism of aspiration breathing which results from the presence of a rigid shell in these animals. The ribs of the chelonians are fused to the dome of the carapace and therefore there are no intercostal muscles available to power or assist in ventilation. A detailed investigation of the mechanism of ventilation has been carried out on three species of chelonians Malaclemmys centata (McCutcheon, 1943), Testudo graeca (Gans and Hughes, 1967), and Chelydra serpentina (Gaunt and Gans, 1969).

Gans and Hughes (1967) and Gaunt and Gans (1969) investigated the breathing mechanism of turtles by correlating electromyographical data from respiratory muscles with pulmonary pressure recordings in the terrestrial tortoise, Testudo graeca, and the semi-aquatic snapping turtle, Chelydra serpentina. Both species show triphasic respiratory movements and diphasic air flows with pauses at end-inspiration, between ventilation cycles. There are differences, however, in the rhythm of ventilation and the muscles powering ventilation. The aquatic turtle ventilates its lungs intermittently with a series of continuous breaths during each breathing cycle. Breathing cycles are separated by long variable periods of breath holding. The terrestrial tortoise, on the other hand, exhibits single ventilation cycles separated by a pause at end-inspiration. The differences are thought by Gaunt and Gans (1969) to be due to the aquatic habitat and predatory life style of Chelydra. A great deal of activity is carried out while the animal is submerged and when it breathes, air has to be recycled very rapidly in preparation for the next dive and presumably this requires multiple breaths.

Ventilation in the tortoise is powered by the muscles of the

pectoral girdle and by the abdominal muscles. The muscles of the pectoral girdle involved in ventilation are the Muscularis (M) pectoralis which induces inward rotation of the pectoral girdle and the M. serratus magnus (= M. testocoracoideus) which produces outward rotation of the girdle. The position of the pectoral girdle determines the anterior limit of the visceral cavity.

The pelvic girdle is immobile and located centrally within the posterior aperture of the shell. The flanks lateral to the hind limbs are formed by skin, connective tissue, and a thin sheet of muscle. The flanks determine the posterior limits of the visceral cavity. Here lie the M. obliquus abdominis and the M. transversus abdominus, referred to by Gans and Hughes (1967) as the respiratory muscles. The connective tissue and muscles of the posterior flanks form an anteriorly concave cup that encloses the visera. Contraction of the M. transversus abdominus flattens the curvature thus exerting pressure on the viscera while contraction of the M. obliquus abdominis increases the curvature and decreases the visceral pressure.

A respiratory cycle begins with a rapid increase in pulmonary pressure which is associated with a high level of electrical activity in the M. transversus abdominus, and a low level of activity in the M. pectoralis, a slight inward movement of the limbs, and electrical activity in the dilator muscle of the glottis. The second phase begins with a reversal of the pulmonary pressure, a sharp increase in the EMG of the M. pectoralis and an acceleration of the inward movement of the forelimbs. The pulmonary pressure drops to atmospheric, the M. transversus abdominus and M. pectoralis cease firing and expiration is complete. The M. obliquus abdominis and M. serratus magnus begin firing at or near the time when

pulmonary pressure approaches atmospheric pressure. The forelimbs begin a phase of outward movement causing the volume of the body cavity to increase which results in a subatmospheric pressure being generated in the lungs. Pressure remains subatmospheric for a variable period and the glottis opener muscle, the M. obliquus abdominus, and the M. serratus magnus cease firing as the pressure equilibrates with atmospheric pressure upon the inflow of air. Inspiration is complete when the pressure reaches atmospheric and the glottis closes. Pulmonary pressure then rises above atmospheric pressure due to a passive compression of the system. Both inspiration and expiration appear to be active processes. The M. transverse abdominus is the most important muscle of respiration with the M. pectoralis playing an accessory role. If ventilation is stimulated by CO₂ inhalation or fright, the pectoral girdle takes on a more active role in ventilation.

The aquatic turtle, Chelydra serpentina shows the same pattern of triphasic pulmonary pressure changes and diphasic air flow as T. graeca. The differences in the mechanism of ventilation seen in the aquatic turtle involve the muscles powering exhalation and inhalation. The muscles that power exhalation are the M. transversus abdominus which internally forms the membrane of the exposed flank, and the M. diaphragmaticus, a muscle not seen in T. graeca. The M. diaphragmaticus originates inside the carapace from the margin of the second costal plate and extends to a position anterior to the lung along the first fused rib. Contraction of these two muscles compresses the visceral mass, increasing the pressure in the lung. The opening of the glottis permits air flow out of the animal. The pectoral girdle does not play an active role in exhalation.

The muscles powering inhalation include the M. testocoracoideus

(M. serratus magnus in T. graeca) and the M. obliquus abdominus. These muscles begin firing as the intrapulmonary pressure falls to atmospheric pressure following expiration. Contraction of these muscles serves to increase the volume of the visceral mass by causing an outward rotation of the pectoral girdle (M. testocoracoideus) and flattening of the concavity of the flanks (M. obliquus abdominis). This causes the pulmonary pressure to drop below atmospheric pressure causing an inward flow of air. Upon closure of the glottis the pulmonary pressure rises above atmospheric pressure due to passive recoil of the system. There is also some low level activity in the M. diaphragmaticus and M. obliquus abdominis.

The actions of the respiratory muscles cause some shell deformation during ventilation. The M. testoscapularis shows low level activity at end-expiration and throughout inhalation which helps restore the curvature of the carapace. The M. testoscapularis originates on the carapace near the lateral tip of the first fused rib and inserts dorsally on the scapula. Contraction of the M. testoscapularis, during the exhalation period when pulmonary pressure drops and the shell's transverse curvature falls, restores the curvature of the carapace.

Chelydra appears to minimize the muscular effort of exhalation by utilizing hydrostatic pressure when submerged. In this situation when the animal is in water expiration tends to be passive. More effort is, however, required of the muscles generating inspiration to overcome the hydrostatic pressure head which tends to keep the pressure in the system above atmospheric. Chelydra also spends time on land and at such times, due to the reduced plastron in this species, the viscera tend to sag resulting in subatmospheric pressures in the lung during the breath-hold period.

Expiration now takes more effort but inhalation tends to be passive.

The presence of the M. diaphragmaticus as a respiratory muscle in Chelydra has freed the pectoral girdle from its active role in expiration which Gaunt and Gans (1969) believe may provide several advantages for this aquatic turtle. First, the limbs are now free for swimming while the animal is ventilating at the surface. While the animal is afloat and stationary, extended limbs would stabilize the animal as it bobs due to buoyancy changes associated with ventilation. Secondly, the freeing of the limbs from powering ventilation also reduces large amplitude movements of the limbs which might cause prey to react suddenly. This is of great importance to a predatory species. The significance of these advantages is questionable, however, as even Testudo has no trouble breathing with its front limbs retracted and most predatory species feed submerged rather than while breathing at the surface.

All these mechanisms of breathing involve muscular work. One can measure the total energy required by the respiratory muscles through oxygen consumption measurements, or the mechanical work done by the respiratory muscles which can be estimated through measurements of pressure changes and volume changes within the respiratory system. The mechanical work divided by the total energy requirements of the respiratory system gives an indication of the efficiency of the mechanism of ventilation. The measurement of pulmonary mechanics allows one to analyze in detail the various forces which contribute to the total mechanical work of breathing. Measurement of the oxidative cost of breathing allows one to examine how much of the energy budget of the animal is required for breathing.

In general, mechanical work is performed when a force moves

through a distance, or in the case of a fluid system such as the respiratory system, when a change in pressure causes a change in volume. During breathing, inspiration requires the respiratory muscles to contract thus increasing the volume of the cavity containing the lungs. To increase the volume of the cavity the inspiratory muscles have to overcome the elastic forces in the tissues of the lungs and body cavity, and the nonelastic forces which include flow resistive forces in the airways, viscous forces due to the movement of tissues and the inertia of the respiratory system. The total mechanical work required to produce a breath consists of the sum of all the elastic and nonelastic forces during inspiration and expiration. Estimates of the total work of breathing and its components can be derived from pressure-volume relationships which are described in chapters 2 and 3.

The work of breathing has been extensively studied in humans (see Otis, 1954; Otis, 1964 for reviews) and other mammalian species (Crosfill and Widdicombe, 1961). Otis et al. (1950) developed a model to estimate the mechanical work of breathing in humans. The model predicts that for a constant level of alveolar ventilation there is a combination of tidal volume and breathing frequency which requires minimum work and thus minimizes the oxidative cost of breathing, since the work per unit ventilation and oxidative cost are proportional (McKerrow and Otis, 1956; Milic-Emili and Petit, 1960). It was found by Otis et al. (1950) that the spontaneous breathing pattern of humans corresponded very closely with the predicted pattern. Crosfill and Widdicombe (1961) showed for several species of mammal that the combination of tidal volume and frequency normally employed at various levels of ventilation also coincided with the model predictions. These correlations gave rise to the suggestion that

regulatory mechanisms must exist to regulate breathing frequency so as to minimize the mechanical work (Otis, 1954). Mead (1960) proposed that regulation occurs at the level of the respiratory muscles to keep the length-tension relationship of the muscles optimal in order to maximize power output rather than to minimize mechanical work. His argument was that force sensed by "appropriately located stretch receptors, represents a more simple case than work rate, which would require sensing, and appropriate manipulation of, three variables; force, distance, and time." (Mead, 1960) Mead (1960) concedes, however, that upon imposed mechanical loads the principal receptors for a reflex controlling breathing frequency cannot lie in the respiratory muscles, but must lie in the lung and this role is probably performed by the slowly adapting vagal stretch receptors. It seems unlikely, however, that the system would regulate breathing frequency by one method at rest (i.e. receptors in the respiratory muscles), and by vagally innervated stretch receptors upon changes in mechanical load. Rabbits subjected to elastic loading have been shown to increase breathing frequency (McClelland et al., 1972) as is predicted. If the animals are vagotomized there is no significant change in breathing frequency upon addition of an elastic load. This seems to indicate that the Breuer-Hering reflex might in some way be responsible for regulating and stabilizing ventilation in order to minimize the work of breathing.

Thus mechanical factors appear to play a major role in determining the nature of the breathing pattern employed by mammals. Given the diversity seen in the mechanisms powering ventilation in the three reptilian groups, a similar diversity might be expected in the mechanics of the various systems described above. This possible diversity in pulmonary

mechanics may play a role in establishing the diversity in breathing patterns seen in reptiles which range from single breaths separated by periods of breath holding, as seen for instance in resting lizards or tortoises, to bursts of continuous breathing separated by periods of breath holding as seen in semi-aquatic turtles and crocodiles, through to continuous or near continuous breathing patterns seen in predatory species of lizards and almost all reptiles when excited.

Little work, however, has been done on the pulmonary mechanics of reptilian systems. Perry and Dunker (1978, 1980) suggested that resting breathing patterns may be related to static mechanics though their analysis was incomplete. Milsom and Vitalis (1984) have investigated the static and dynamic mechanics of the lizard Gekko gekko which has very simple sac-like lungs and breathes with single breaths. It was shown, in this species, that there is an optimal combination of frequency and tidal volume corresponding to minimum mechanical work. In a subsequent study Milsom (1984) showed that the components of time and tidal volume of a single breath from a spontaneously breathing Gekko corresponded to the predicted optimal combination giving minimum work per breath. Upon vagotomy it was shown that the spontaneous pattern deviated from the optimum pattern.

Pseudemys scripta is a semi-aquatic turtle which uses a different mechanism of ventilation from that of G. gekko, due to the presence of a rigid shell enclosing the body cavity as described earlier. This species of turtle also possesses a more complicated multichambered lung compared to the simple sac-like lung of G. gekko. One aim of this present study, therefore, was to investigate how these differences in lung structure and body wall architecture in P. scripta and G. gekko influence the mechanics of the

respiratory system. Another goal was to determine if differences in pulmonary mechanics could account for the differences seen between the single breath pattern of G. gecko and the pattern of P. scripta which consists of bursts of continuous breaths separated by breath-holds of variable duration.

CHAPTER 2

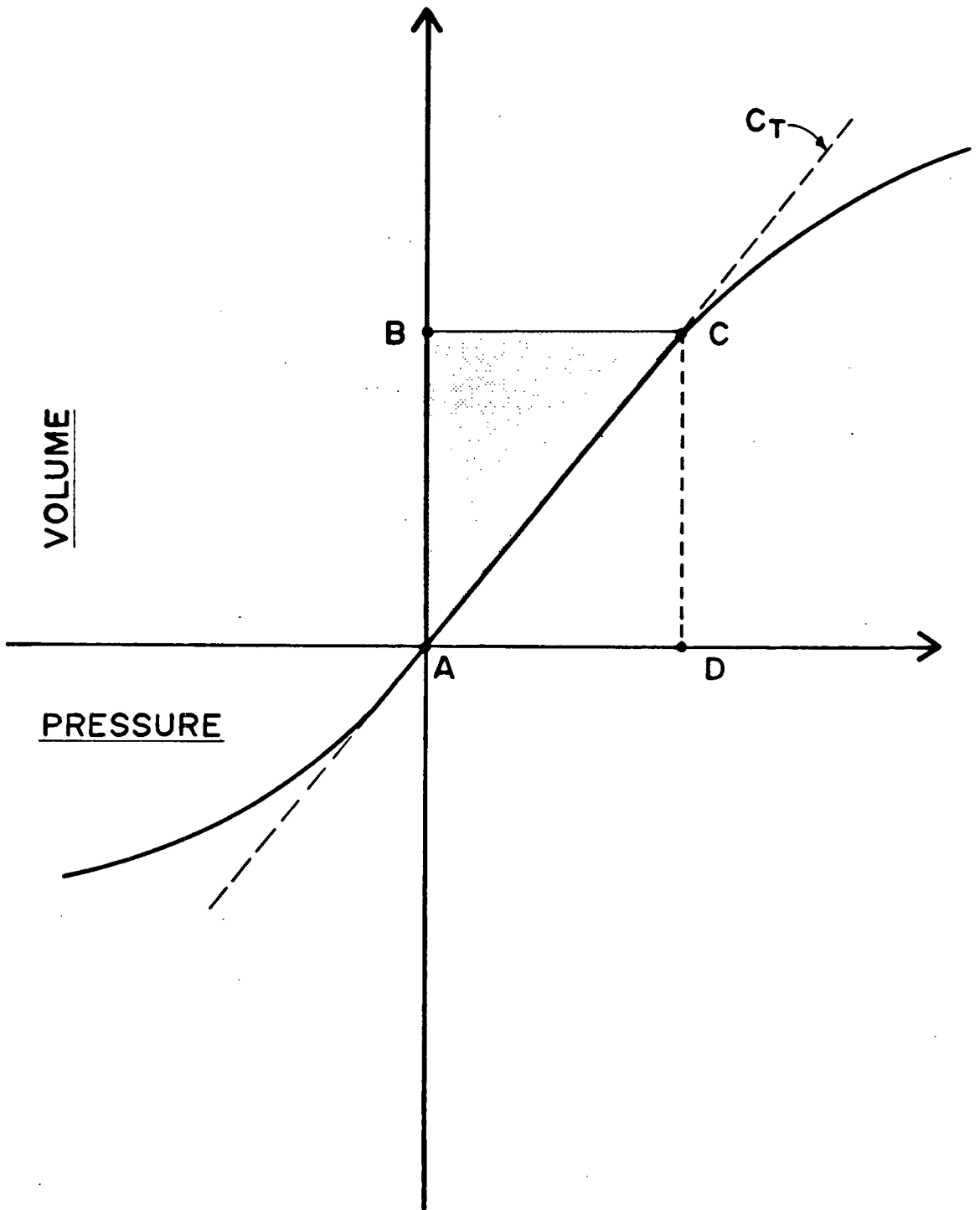
THE STATIC MECHANICS OF THE RESPIRATORY SYSTEM IN PSEUDEMYD SCRIPTA

Introduction

The pressure of a volume of gas contained in the lungs, under static conditions with the respiratory muscles relaxed and the glottis closed, depends upon the surface tension of the lungs and on the elastic and gravitational forces operating on the lung and chest wall (Agostoni, 1970). These are the forces which must be overcome to inflate the respiratory system to this volume. The principal force generating this pressure, which is equal to the difference between intrapulmonary and atmospheric pressure, is that due to elastic recoil. The mechanical work which was required to overcome these elastic forces can be determined from the static pressure-volume relationship of the total respiratory system (the lungs and chest wall).

Figure 2.1 shows a schematic pressure-volume relationship for the total respiratory system of a typical vertebrate (Otis, 1964). Such a curve is generated by measuring the pulmonary pressure following stepwise inflation and deflation of the lungs starting from resting lung volume (V_{LR}). Resting lung volume is the volume of gas in the lung after a normal expiration when intrapulmonary pressure and atmospheric pressure are equal (A in figure 2.1). The mechanical work required to overcome elastic forces resisting an increase in volume from A to B is given by the area ABCA in $\text{ml} \cdot \text{cmH}_2\text{O}$. The slope of the linear portion of the curve (AC which = V/P) represents the static compliance of the total system (C_T). This

Fig. 2.1 Schematic representation of a pressure-volume curve. For a volume change of AB, the pressure change in the system would be AD. The work required to overcome the elastic forces resisting inflation would be represented by area ABCA. The compliance of the system (C_T) is the slope of the pressure-volume curve which is linear over the range shown. The origin of the graph is V_{LR} at atmospheric pressure.



value is a measure of the stiffness of the system. A low compliance indicates a stiff system where the elastic forces are large, and, correspondingly, area ABCA would be large indicating that much mechanical work would be needed to inflate the system. A high compliance on the other hand, indicates a system where the elastic forces are small and thus area ABCA would be small and little mechanical work would be needed to inflate the system.

The mechanics of the total respiratory system can also be analyzed in terms of the mechanics of its component parts, the lung and body wall. Since the forces exerted by the lung and body on the lung contents act in series, the compliance of the total respiratory system is related to the compliance of its component parts by the relationship $1/C_T = 1/C_L + 1/C_B$ where C_L is the compliance of the lung, and C_B is the compliance of the body wall. If the compliance of the lung and body wall are each determined, one can derive the relative contributions of each component to the total elastic forces within the system.

Measurements obtained from such pressure-volume curves also allow comparisons of pulmonary mechanics between animals which exhibit wide variations in lung structure. Reptilian lungs, for instance, have been classified into three major groups according to their gross anatomy (Perry and Dunker, 1978). One group contains the unicameral lungs which each consist of a single chamber lacking any intrapulmonary septation or intrapulmonary bronchi. In these lungs a relatively uniform network of trabeculae arising from the outer lung wall support the faveolar parenchyma. This honey-comb-like parenchyma bears a matrix of capillaries on both surfaces and is the site of gas exchange. Paucicameral lungs are partially

divided into chambers but lack an intrapulmonary bronchus. The faveolar parenchyma now arises from the intrapulmonary septa as well as the lung wall and is deepest immediately caudal to the bronchial entrance. The degree of faveolar partitioning decreases more caudally and disappears in the extreme caudal region which consists of membraneous finger-like projections. Multi-cameral lungs are completely divided into many chambers connected independently by a single cartilage ringed intrapulmonary bronchus. The ventral chambers in the caudal portion of the lung have thin membraneous walls whereas the parenchyma in the dorsal portion and near the intrapulmonary bronchus is densely partitioned. All three of these types of lungs found among the reptiles differ markedly from the alveolar lungs of mammals.

The lungs of mammals are enclosed in a pleural cavity formed by pleural membranes with a muscular diaphragm separating the viscera from the thoracic cavity. This is unlike the reptilian system where the lungs lie in the body cavity with only a delicate mesopneumonium preventing the lung from moving too far cranially or ventrally at low ventilation volumes (Perry and Duncker, 1978). Mammals have a compact lung with a highly branched bronchial tree communicating with a large number of alveoli. Surrounding the alveoli and alveolar ducts are helical networks of collagen and elastic fibers which partially generate the elastic recoil of the lungs (Bouhuys, 1977).

Besides the differences found in lung architecture among various groups of animals there is also tremendous diversity in the structure of the body wall and in the mechanisms employed for ventilation (see chapter 1). One would therefore expect that the static mechanics of the various respiratory systems would also be diverse and reflect these differences. In this

chapter, experiments designed to measure the static pulmonary mechanics of the turtle, Pseudemys scripta are outlined and the values obtained from these experiments are compared to those obtained from other reptiles and mammals.

Materials and Methods

Experiments were performed on eight turtles (726.3 ± 107 grams body weight) which were maintained at room temperature ($20-22^{\circ}\text{C}$) for several days before the experiments. On the day of an experiment an animal was given an overdose of sodium pentobarbital and when all signs of reflex activity were absent, the cloaca was sealed to prevent water loss from the bladder which would have altered body volume, intra-abdominal pressure and thus the mechanics of the body wall. The trachea was cannulated using P.E. 220-240 tubing with a side-arm close to the trachea leading to a Statham P23 Db physiological pressure transducer to measure intrapulmonary pressure. This signal was amplified and recorded on a Gould series 2600 pen recorder.

Compliance of the total respiratory system (C_T)

With the tracheal cannula open to atmosphere the lungs were deflated, then re-inflated to a volume of 11 ml/100 g. body weight with an air filled syringe. This volume corresponded to the resting lung volume (V_{LR}) as determined for this species by Jackson (1971). This volume was used as a common reference point for the total system, body wall, and lungs. The volume in each compartment when the lungs were filled to V_{LR} was referred to as zero volume for each compartment, and the pressure associated with V_{LR} in each compartment was referred to as zero pressure. Starting from V_{LR} , the lungs were then inflated in 5 or 10 ml steps. Following each step inflation the intrapulmonary pressure was allowed to decay to a new steady level. Step inflation continued until the inflation volume approached twice V_{LR} . At this volume the pressure increase for each

further step inflation became very large and the slope of the pressure-volume curve decreased dramatically. The system was then deflated in a similar stepwise fashion until the lungs were completely deflated. The system was then reinflated to V_{LR} .

Compliance of the body wall (C_B)

The preparation of the animals for these measurements required the cannulation of the abdominal cavity in order to measure intra-abdominal pressure rather than intratracheal pressure. The flank of the turtle just anterior to the pelvic girdle was pierced with a 13 gauge hypodermic needle. A saline filled cannula (PE 190) was passed down the barrel of the needle which was then removed and the cannula was secured to the flank with a purse string suture. This cannula was connected to the Statham P23 Db physiological pressure transducer. The trachea was cannulated as in the previous section except there was no side-arm present in the cannula.

The lungs were deflated then inflated to V_{LR} . Stepwise inflation and deflation of the body cavity was achieved by inflation and deflation of the lungs in the same manner as described in the previous section. Pressure inside the body cavity was now monitored instead of intratracheal pressure.

Compliance of the lungs (C_L)

Following the measurements outlined above, the lungs of the turtles were exposed to determine the compliance of the isolated lungs. The plastron of a turtle was removed using a necropsy saw, the pelvic girdle was carefully removed from the shell and all the viscera including the

digestive tract, liver, and heart were carefully dissected away from the lungs. Once the viscera were removed the lungs were inflated to V_{LR} . This volume and its associated pressure are again referred to as zero volume and pressure. Stepwise inflation and deflation of the isolated lungs were then carried out as was done for the total respiratory system.

Results

The pressure-volume curves for inflation and deflation of the total system (total), body cavity (body), and lungs (lung) are shown for a 639 g. turtle in figure 2.2. All curves are generally sigmoid in shape indicating that in all cases increasing or decreasing volume ultimately leads to disproportionate increases in pressure. This signifies that at some volume all air spaces are opened and filled with air and that the elastic components are approaching their limits of extensibility. All three curves showed a pronounced hysteresis upon deflation.

The static compliances derived from the slopes of the straight portions of the various curves originating from V_{LR} and zero pressure are given in Table 2.1 for all animals examined. The mean compliance of the total system (C_T) was $8.4 \text{ ml/cmH}_2\text{O/kg} \pm .65 \text{ S.E.M.}$, that of the lungs alone (C_L) was $35.1 \text{ ml/cmH}_2\text{O/kg} \pm .78 \text{ S.E.M.}$, and that of the body cavity alone (C_B) was $11.6 \text{ ml/cmH}_2\text{O/kg} \pm 1.35 \text{ S.E.M.}$ These values are compared to published values for other reptiles in Table 2.2. The values in Table 2.2 were normalized to body weight and to V_{LR} and grouped according to lung type as classified by Perry and Duncker (1978).

The percentages of the total work required to overcome elastic forces arising from the body and the lungs on inflation of the intact respiratory system are shown in Table 2.3. These values are derived from compliance values given in Table 2.2 in the following fashion. Since the compliance values listed in Table 2.2 are for the linear portion of the pressure-volume curves for each respective animal originating from V_{LR} and zero pressure, Figure 2.1 can be simplified as shown in Figure 2.3. The

Fig. 2.2 A pressure-volume curve for a 639 gram turtle. Curves for the total system (total), the body wall (body) and lungs alone (lung) are shown. Arrows pointing in a positive direction indicate inflation, those pointing in a negative direction indicate deflation. Values for C_T , C_B , and C_L , the compliances of the total system, the body wall, and lungs respectively were measured from the slope of the linear portion of the pressure volume curve upon inflation.

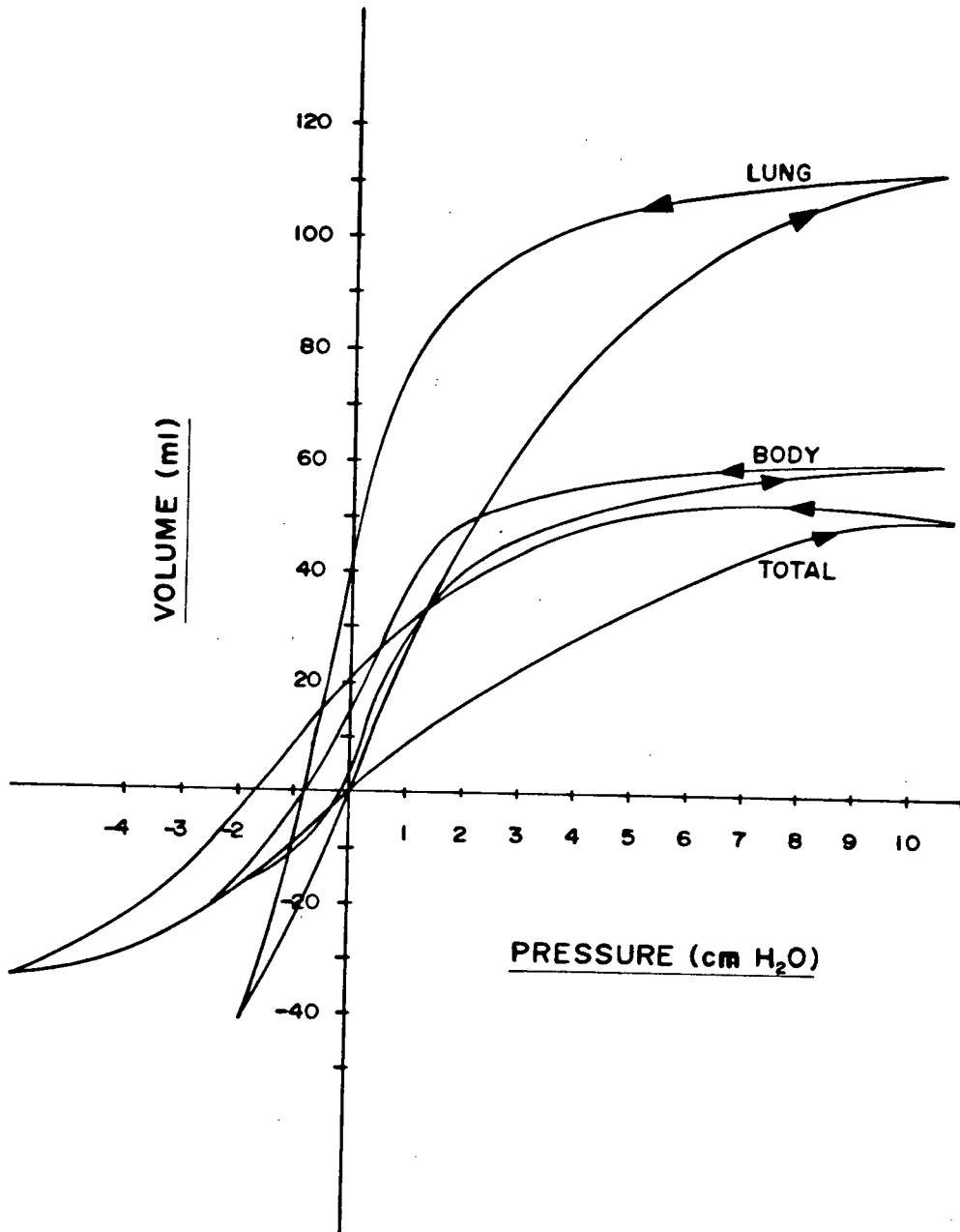


Table 2.1 Compliance of the total system (C_T), body wall (C_B) and lung (C_L) in ml/cmH₂O/kg body weight for 8 animals.

	<u>Weight (kg)</u>	<u>C_T</u>	<u>C_L</u>	<u>C_B</u>
	0.733	9.2	35.8	8.4
	0.437	6.8	37.0	12.1
	0.639	9.1	34.2	12.4
	0.750			9.8
	0.485			17.1
	0.560			17.3
	1.467			10.3
	0.739			5.4
\bar{x}	0.726	8.4	35.1	11.6
<u>+S.E.M.</u>	.107	.65	.78	1.35

Table 2.2 Compliance of the total system (C_T), body wall (C_B), and lung (C_L) for various species. (A) Values standardized to body weight. (B) Values standardized to resting lung volume (V_{LR}).

A.	Animal	Lung Type	ml/cmH ₂ O/kg			Source
			C_T	C_L	C_B	
	Green Lizard (<u>Lacerta viridis</u>)	unicameral	18.20	62.10	26.70*	Perry & Duncker (1978)
	Tokay Gekko (<u>Gekko gekko</u>)	unicameral	47.10	273.40	56.9*	" "
	" "	unicameral	16.0	201.6	14.5	Milsom & Vitalis (1984)
	Chameleon (<u>Chameleo chameleon</u>)	paucicameral	305.70	759.00	511.8*	Perry & Duncker (1978)
	Monitor Lizard (<u>Varanus exanthematicus</u>)	multicameral	66.80	365.50	82.20*	" "
	Red Eared Turtle (<u>Pseudemys scripta</u>)	multicameral	10.00	170.00	10.60*	Jackson (1971)
	" "	multicameral	8.40	35.10	11.60	Present study
	Rat (<u>Ratus sp.</u>)	broncho-alveolar	0.91	1.56	2.20	Crosfill & Widdicombe (1961)
B.	Animal	Lung Type	ml/cmH ₂ O/ml.V _{LR}			Source
			C_T	C_L	C_B	
	Green Lizard (<u>Lacerta viridis</u>)	unicameral	0.53	1.78	0.76*	Perry & Duncker (1978)

Table 2.2 (continued)

Animal	Lung Type	ml/cmH ₂ O/ml.V _{LR}			Source
		C _T	C _L	C _B	
Tokay Gekko (<u>Gekko gekko</u>)	unicameral	0.73	4.07	0.89*	Perry & Duncker (1978)
Tokay Gekko (<u>Gekko gekko</u>)	unicameral	0.25	3.17	0.23	Milsom & Vitalis (1984)
Chameleon (<u>Chameleo chamelean</u>)	paucicameral	2.50	5.73	3.84*	Perry & Duncker (1978)
Monitor Lizard (<u>Varanus exanthematicus</u>)	multicameral	0.66	3.27	0.83*	" "
Red Eared Turtle (<u>Pseudemys scripta</u>)	multicameral	0.10	1.70	0.11*	Jackson (1971)
" " "	multicameral	0.08	0.35	0.12	Present study
Rat (<u>Ratus sp.</u>)	broncho-alveolar	0.09	0.12	0.55	Crosfill & Widdicombe (1961)

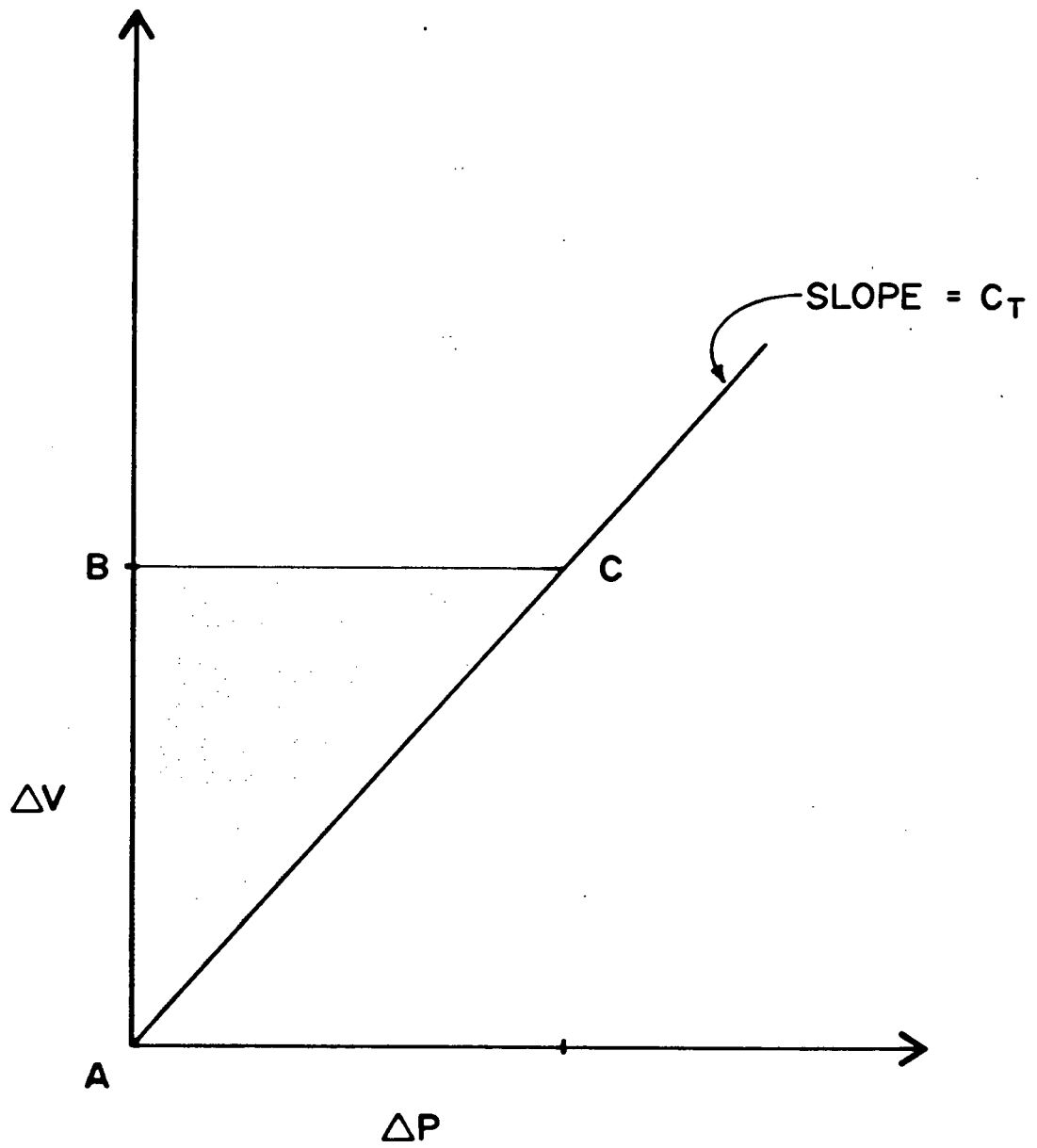
* Values for C_B calculated from the formula $1/C_B = 1/C_T - 1/C_L$.

Other values for C_B were derived experimentally.

Table 2.3 Percentage of the total work required to overcome the elastic forces resisting static lung inflation which arise from the body wall and lung. Calculations were based on compliance values standardized to V_{LR} .

<u>Animal</u>	<u>Lung</u>	<u>Body</u>	<u>Source</u>
Green Lizard	30%	70%	Perry & Duncker (1978)
Tokay Gekko	18%	82%	" "
" "	8%	92%	Milsom & Vitalis (1984)
Chameleon	44%	56%	Perry & Duncker (1978)
Monitor Lizard	20%	80%	" "
Red Eared Turtle	8%	92%	Jackson (1971)
" " "	23%	77%	Present study
Mammal (Rat)	75%	25%	Crosfill & Widdicombe (1961)

Fig. 2.3 Simplification of figure 2.1 to illustrate technique used for calculation of the work required to overcome the elastic forces resisting a 1 ml inflation. For a 1 ml inflation the pressure generated is equal to $1/C_T$ where C_T is the compliance of the system. The work required to overcome elastic forces for a 1 ml inflation is represented by the area ABCA which equals $1/2C_T$.



work required to inflate this system by ΔV is given by the area ABCA as stated earlier. This area is equal to $\Delta V \times \Delta P \times \frac{1}{2}$. Since compliance equals $\Delta V / \Delta P$, ΔP is equal to $\Delta V / \text{compliance}$. As a consequence, for a 1 ml inflation, the area ABCA becomes

$$1 \text{ ml} \times \frac{1 \text{ ml}}{C \frac{\text{ml}}{\text{cmH}_2\text{O}}} \times \frac{1}{2} \text{ or simply } \frac{1}{2C} \text{ ml} \cdot \text{cmH}_2\text{O}.$$

In this way the work required to inflate the lungs alone, the body cavity alone, and the intact respiratory system can be calculated. The values derived this way for the lungs and body cavity are shown in Table 2.3 as percentages of the values derived for the intact respiratory system. Among the reptiles the majority of the elastic recoil resisting inflation arises from the body wall while the highly compliant lungs offer little resistance to inflation. In mammals, on the other hand, the majority of the work performed to overcome elastic forces during inflation is required to expand the relatively stiff lungs. Only 25% of the total elastic work is required to expand the body cavity.

Discussion

The compliance of the total respiratory system of the turtle, Pseudemys scripta, measured in the present study, is in general agreement with results obtained for the same species by Jackson (1971). Jackson (1971) reported values for lung compliance (170 ml/cm H₂O/kg), however, which were much higher than those measured in the present study (35.1 ml/cmH₂O/kg). It is probable that this discrepancy results from differences in methodology. In the study of Jackson (1971), the compliance of the exposed lungs was determined beginning at atmospheric pressure with the lungs completely collapsed. In the present study the static inflation curve was determined beginning with the exposed lungs inflated to V_{LR}. As a consequence C_L in the present study was determined at a greater lung volume which may have corresponded to a stiffer portion of the pressure-volume curve.

Since the lungs and the body are placed in series, the algebraic sum of the pressure exerted by these two component parts must equal the pressure exerted by the total respiratory system (Agostoni, 1970). Since compliance is equal to V/P it follows from this that for any V , the sum of the reciprocals of the lung compliance and body compliance must equal the reciprocal of the compliance of the total system (i.e. $\frac{1}{C_L} + \frac{1}{C_B} = \frac{1}{C_T}$). If the values of C_T and C_B measured in the present study are substituted into this equation a calculated value for C_L of 30.5 ml/cmH₂O/kg is obtained which is close to the experimentally derived value of 35.1 ml/cmH₂O/kg \pm .78 S.E.M. for lung compliance. Thus the compliance of the lung as measured in this study is consistent with the experimentally derived values for body

compliance and total respiratory system compliance.

The results of the present study further indicate that the compliance of the total respiratory system of this species of turtle is determined mainly by the compliance of the body wall. Only 23% of the elastic forces resisting inflation within this system arise from the lungs. Using the values for C_L and C_T reported by Jackson (1971) one obtains an elastic contribution by the lungs of only 8% of the total elastic forces resisting inflation. The percentage of elastic resistance to inflation offered by the lungs, as calculated in this study, lies well within the range of values calculated for other reptiles (8%-44%) while the value calculated from Jackson (1971) lies at the lower extreme.

A comparison of compliance values for the total respiratory system, body wall, and lungs of various species of reptiles indicates that the respiratory system of turtles is relatively stiff (Table 2.2). The lizards for instance, have a highly compliant system two to thirty times more compliant than the chelonian respiratory system. The relatively stiff respiratory system of the turtle can probably be attributed to the animal's shell. The ribs of the turtle are fused to the dome of the carapace and are immobile. The only portions of the animal's body which are capable of distension upon inflation are the flank cavities and the pectoral girdle. The lizards, on the other hand, have articulated ribs capable of moving as the lungs inflate and the volume of the body cavity increases. Table 2.2 also indicates that the lungs of the turtle are somewhat stiffer than those of lizards. This is probably due to the multicameral nature of the lungs of the turtle, which possess intrapulmonary septation. The lungs of most lizards listed in Table 2.2 are unicameral or paucicameral.

Interspecific comparisons of compliance values normalized to body weight show the compliance of the respiratory system of lizards to be much greater than that of chelonians and the chelonians possessing a more compliant system than mammals ($C_{T_{lizards}} \text{ 2 to 30 x } > C_{T_{turtles}} \text{ 9-10 x } > C_{T_{rat}}$). Comparisons made on a volume basis (normalized to resting lung volume) again show lizards having the highest compliance values but now the chelonian system has a similar value to that of the mammalian system ($C_{T_{lizard}} \text{ 5 to 23 x } > C_{T_{turtles}} \approx C_{T_{rat}}$). In mammals, however, C_T is determined primarily by the lungs which contribute 75% of the elastic forces resisting inflation of the respiratory system. Turtles, on the other hand, have relatively compliant lungs compared to mammals and C_T is predominantly determined by the body wall which contributes 77% of the elastic forces within the respiratory system resisting inflation.

Perry and Duncker (1978) claim that high lung compliance is associated with a high degree of development of caudal and ventral dilations of the lung with the development of these membraneous regions being most extensive in the chameleon followed by the tokay gekko and the savanna monitor (Table 2.2). The data of the present study, however, do not support this trend in the relationship between lung volume, general internal architecture of the lung, and lung compliance. Whether the comparisons are made using values standardized to body weight or to resting lung volume the C_T of the turtle is much lower than would be predicted given the lung volume and lung architecture of this species. It may be the case that this trend is only found in the lizards, however, it may also be the case that this trend is

merely a consequence of the small sample size used by Perry and Duncker (1978).

The hysteresis seen in the static inflation-deflation curves has previously been reported for turtles (Jackson, 1971), lizards (Perry and Duncker, 1978, 1980; Milsom and Vitalis, 1984), amphibians (Hughes and Vergara, 1978) and mammals (Butler, 1957; Mead et al., 1957) and has been attributed to viscoelastic effects arising from the lungs and body wall (Agostoni, 1970). In the lungs it has been primarily attributed to surface forces present at the air-alveolar interface. Hughes and Vergara (1978) showed that the hysteresis present during an air inflation-deflation cycle of the frog lung almost completely disappeared when the lungs were filled with saline as was also shown for lizard lungs by Perry and Duncker (1978). This suggests that surface active elements may play a role in reducing the surface tension of the air-alveolar interface, upon inflation of the lungs, in lower vertebrates (Hughes and Vergara, 1978) as in mammals. The hysteresis of the body wall of the turtle is due to extra-pulmonary hysteresis. This extra-pulmonary hysteresis is not due to surface tension forces but to the viscoelastic behavior of the distensible elements of the flanks and body wall of the animal, namely skeletal muscle, connective tissue, and ligaments (Agostoni, 1970).

In conclusion, the compliance of the respiratory system of the turtle is largely determined by a stiff body compartment with little contribution from the lungs. The reverse is true in mammals where the lungs play the predominant role in determining total system compliance. On a weight basis the total system compliance (C_T) of the turtle is ninefold greater than that of the mammalian (rat) system, whereas values standardized

to V_{LR} show the chelonian and mammalian systems to be approximately equal. In all cases, lizards had higher values for C_T than either turtles or mammals due to the presence of a very compliant body wall and lungs. Thus the respiratory system of turtles is stiffer than that of lizards and behaves statically in a similar fashion to the mammalian system. It is of interest then to compare how the turtle system behaves under dynamic conditions with the systems of lizards and mammals and this will be considered in the next chapter.

CHAPTER 3

THE DYNAMIC PULMONARY MECHANICS OF THE TURTHE, CHRYSEMY'S SCRIPTA

Introduction

Alveolar ventilation (\dot{V}_A) is the product of alveolar volume (V_A which equals tidal volume (V_T) minus dead space volume (V_D)) and breathing frequency (f). Any particular level of \dot{V}_A can be produced by a variety of combinations of V_T and f . In mammals, it has been shown that for each level of \dot{V}_A there is a combination of V_T and f which requires a minimum of mechanical work to power breathing (Otis, Fenn, and Rahn, 1950), or, a minimum average force from the respiratory muscles to move the lungs (Mead, 1960). It is more expensive, in terms of mechanical work, to ventilate the lungs at frequencies above or below the values associated with minimum work when V_T is adjusted to maintain a constant \dot{V}_A . This can be understood in view of the fact that at a low f , and therefore high V_T , the work required to overcome the elastic forces within the system is high. At a high f , on the other hand, when V_T is small, the elastic work is smaller but high air flow increases the work required to overcome viscous and turbulent resistance. The result is a U-shaped curve for the work required to overcome the forces within the respiratory system.

The oxidative cost of ventilation is proportional to the mechanical work of breathing (Milic-Emili and Petit, 1960) therefore the combination of V_T and f which requires minimum mechanical work can be considered an optimal pattern in terms of energy expenditure and efficiency.

It has been demonstrated that the spontaneous breathing pattern of many mammals corresponds very closely with their predicted optimal patterns (Christie, 1953; Agostoni et al., 1959; Crosfill and Widdicombe, 1961; Yamashiro et al., 1975). Despite this, it has proven difficult to postulate a control system for the regulation of these optimal patterns in terms of minimal mechanical work. This stems from the fact work is an integral function and therefore would not be sensed by any of the known receptor groups associated with the respiratory system. Pulmonary afferent information does, however, appear to play an important role in pattern regulation since absence of this information results in deviation from the optimal pattern (McIlroy, Marshall and Christie, 1954; McClelland et al., 1972).

In reptiles the normal resting breathing pattern is intermittent unlike the continuous breathing pattern of mammals. The reptilian pattern is characterized by single breaths or bursts of breaths separated by breath holds of varying length (McCutcheon, 1943; Gans and Hughes, 1967; Naifeh et al., 1970; Gans and Clark, 1976; Glass and Johansen, 1976). The primary variable which is regulated in response to respiratory stimulants such as hypoxia, hypercapnia, or increased temperature is the breath hold period (Glass and Johansen, 1976; Milsom and Jones, 1980; Benchetrit and Dejours, 1980) while the response to exercise is characterized by increases in tidal volume (Wood and Lenfant, 1976; Cragg, 1978). Very little is known about the influences of mechanical factors on the breathing patterns or respiratory responses of intermittent breathers. The static compliances of the lung and total respiratory system have been measured in a few species (see chapter 2) and Perry and Duncker (1978, 1980) have attempted to

correlate resting breathing patterns with these static compliances. Such analysis, however, is extremely limited. Since respiration is an active rather than a static process, an understanding of dynamic pulmonary mechanics should provide more useful information for assessing the mechanical factors influencing breathing patterns. To date only one such study has been performed on a non-homeothermic species. Milsom and Vitalis (1984) have shown that for the Tokay lizard Gekko gecko, dynamic pulmonary mechanics were dominated by the mechanics of the body cavity and chest wall. This is unlike the situation in mammals where the pulmonary mechanics are most strongly influenced by the mechanics of the lungs. Despite this there was still a combination of tidal volume and frequency in the gekko which gave minimum values for mechanical work for each level of \dot{V}_A . The aim of experiments outlined in this chapter was to extend such studies on dynamic respiratory mechanics to the turtle, Pseudemys scripta. The results of the previous chapter have shown that although the static mechanics of the total respiratory system of the turtle are very similar to those of mammals, the lungs of turtles are less complex than mammals but more complex than those of lizards. Mechanics of the body wall show that on a volume basis the body wall of the turtle is stiffer than that of either reptiles or mammals due to the presence of the shell. Given this unique combination of features it is of interest to see how the differences in the architecture of the lungs and body wall of turtles is reflected in the dynamic mechanics of the respiratory system.

Materials and Methods

Dynamic Pressure-Volume Relationships

a) Total Respiratory System

Preparation of animals for measurement of the dynamic pulmonary mechanics of the total respiratory system was identical to that used for the determination of the static pressure-volume curve of the total system in chapter 2. These experiments were performed on three animals with a mean weight of 553 g. \pm 81 S.E.M. The tracheal cannula was connected to a Harvard Small Animal Respirator (Model 665 Harvard Inc., Millis, Mass., U.S.A.) with a pneumotachograph (Fleish #00) placed in the airflow line leading from the pump to the tracheal cannula. The pressure drop across the pneumotach screen during tracheal flow was measured with a Validyne differential pressure transducer (DP103-18) and this flow signal was electronically integrated (Gould Integrating Amplifier) to give the stroke volume of the pump. Intratracheal pressure was measured from a side-arm in the tracheal cannula by the Statham P23-Db physiological pressure transducer. Flow, pressure, and volume were recorded on a Gould series 2600 pen recorder. The volume and pressure signal were also recorded simultaneously on an Esterline Angus 575 X-Y plotter. When volume was plotted against pressure on XY coordinates, through a complete pump cycle, a counter clockwise rotating hysteresis loop was produced (Fig. 3.1). The area within the loop was used to calculate the mechanical work required for a pump cycle.

(b) Body Cavity

The dynamic mechanics of the body cavity alone which were associated with lung ventilation were determined for six animals of a mean weight of 553 g. \pm 28 S.E.M. The preparation of animals in this experiment involved the cannulation of the abdominal cavity in order to measure intra-abdominal pressure. The cannulation was identical to the procedure used in determining the static compliance of the body wall (chapter 2). Intra-abdominal pressure was measured by a Statham P23 DB physiological pressure transducer which was connected to the intra-abdominal pressure cannula. The best position for placement of the intra-abdominal pressure cannula was found to be within the urinary bladder of the animal. This large fluid filled sac prevented the viscera from blocking the tip of the cannula yet transmitted changes in intra-abdominal cavity pressure. Placement of the cannula within the urinary bladder always gave consistent pressure recordings.

The trachea was cannulated as described previously. In these experiments, no side-arm was needed for intratracheal pressure recording. The tracheal cannula was connected to the pump and pneumotachograph as described above and tracheal air flow, pump stroke volume, and intra-abdominal pressure were measured and recorded as described above. The intra-abdominal pressure and the stroke volume were also recorded simultaneously on an Esterline Angus 575 X-Y plotter producing hysteresis loops for the changes in intra-abdominal pressure which accompany changes in the volume of the body cavity during inflation and deflation of the lungs.

(c) Protocol

The experimental procedure was identical for measurements carried out on both the total respiratory system and on the body cavity alone. Starting with the lungs open to atmosphere and, therefore, with intra-pulmonary and intra-abdominal pressure equal to atmospheric pressure, the animals were ventilated with volumes ranging from 1 ml to 6 ml at frequencies ranging from 15 to 60 cycles per minute. Pump volume always started at 6 ml and was sequentially reduced to 1 ml in 1 ml steps at each pump frequency. Frequencies were sequentially increased from 15 cycles/min. to 20 cycles/min. and then to 60 cycles/min. in ten cycles/min. steps.

(d) Data Analysis

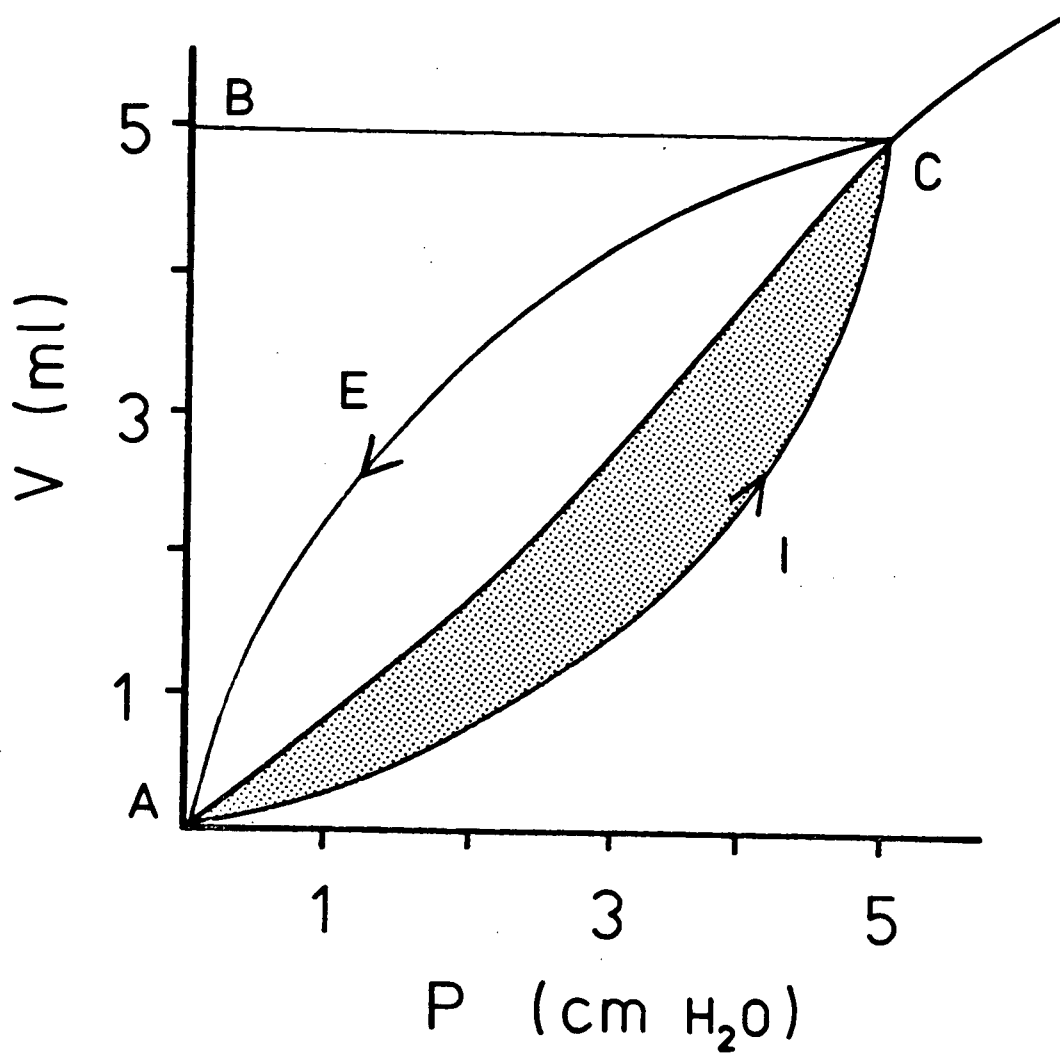
During lung inflation in an intact respiratory system, work is done to overcome flow-resistive, viscous, and elastic forces (Otis, 1964). The magnitude of the nonelastic forces of flow-resistance and viscosity depend on the rates of movement of gas and tissue. Figure 3.1 shows a schematic pressure-volume loop with the arrows indicating the direction of change in pressure and volume for inspiration (I) and expiration (E). The horizontal distance between the line, AC whose slope represents the dynamic compliance, and the curved line AIC, represents the pressure gradient required to overcome the nonelastic forces during inspiration. The area AICA represents the work required to overcome these forces during inspiration. The area bounded by ACEA represents the work required to overcome the nonelastic forces during expiration. The area ABCA represents the work required to overcome the elastic forces for one ventilation cycle. If the area representing the work required to overcome nonelastic forces

during expiration (ACEA) is within the area ABCA then expiration is passive and powered by the stored elastic energy represented by ABCA. The total work for one cycle, the sum of elastic and nonelastic work, is then represented by the area AICBA (Otis, 1964).

For each pressure-volume loop recorded in the present experiments, a perpendicular line was drawn from the volume axis to the point of peak pressure and volume (line BC in Fig. 3.1). The area contained within the pressure-volume loop (AICEA = nonelastic work of inflation and deflation) and the area within AICBA (total work of one ventilation cycle) were measured using an Apple II microcomputer and graphics tablet. Since in these experiments, during pump ventilation of the anaesthetized animals, expiration was always passive, nonelastic work of each cycle was calculated as one half of the area of the pressure-volume loop. The work required to overcome the elastic forces in the total respiratory system were calculated by subtracting the nonelastic work/cycle from the total work/cycle. These calculations of total, elastic, and nonelastic work/cycle were made on loops obtained for pump ventilation of the intact system and ventilation of the body cavity alone. The work required to ventilate the lungs alone was then calculated by subtracting the work/cycle required to ventilate the body cavity from the work/cycle required to ventilate the total respiratory system.

Dynamic compliance, the slope of the line connecting the two points of zero flow on the pressure-volume curve was approximated by calculating the ratio between tidal volume (ΔV) and the corresponding change in pressure (ΔP).

Fig. 3.1 Schematic diagram of the pressure-volume relations of the intact respiratory system during a single ventilation cycle. The loop begins at A and is generated by a point, moving with time, in a counter clockwise direction. Shaded area represents the work required to overcome nonelastic forces during inspiration. See text for details. V = inflation volume, P = intrapulmonary or intra-abdominal pressure.



Effect of changing ventilation frequency at constant minute ventilation
(\dot{V}_E) on blood gas partial pressures

These experiments were performed on six turtles, mean weight 753 ± 81 g., to determine the effect of increasing ventilation frequency, with concomitant decreases in tidal volume to maintain \dot{V}_E constant, on \dot{V}_A and on arterial PO_2 and PCO_2 . The animals were anaesthetized with an injection of sodium pentobarbital (20 mg/kg i.p.) and a midline incision was made along the neck of the animal and the carotid artery exposed. The artery was cannulated with P.E. 50 tubing filled with heparinized saline. The cannula was fed several centimeters down the artery towards the heart to ensure that the blood sampled via this catheter was representative of arterial blood leaving the heart. The P_{aO_2} and P_{aCO_2} of this blood was measured using Radiometer electrodes and a Radiometer PHM 71 blood gas analyzer.

The trachea was cannulated as described previously. The cannula was attached to a Harvard Small Animal Respirator (Model 665) with a pneumotachograph (Fleish #00) in the air flow line to monitor air flow. Air flow and tidal volume were measured and recorded as described earlier. The animal was ventilated at a constant rate of 50 ml/min/kg. Ventilation began at 10 cycles/min. and was increased in steps of 10 cycles/min. to 60 cycles/min. The animal was ventilated with saturated air at room temperature (20-22°C). Small quantities of arterial blood were removed after 15 minutes of ventilation at each frequency and analyzed for PO_2 and PCO_2 . The sample was returned to the animal after each measurement. The pump was returned to 10 cycles/min. after the final measurement and arterial blood was again analyzed to ensure that the blood gases returned to original levels. If this did not occur the data was discarded.

Measurement of dead space volume (V_D)

The anatomical dead space of the respiratory system was determined for six animals with a mean weight of 820 ± 83 g. Anatomical dead space was considered to be the volume of the trachea and primary bronchi from the glottis to the lung hilus and did not include the volume of the intrapulmonary primary bronchi. Two methods were used to determine V_D . One method involved measuring the volume of the trachea by filling it with water after it had been dissected from the animal. The other method involved measuring the length of the trachea and its diameter from which its volume was calculated. These measurements were considered to be minimum values for V_D since they did not include any adjustment for physiological dead space.

All values given in this chapter are mean values \pm S.E.M.

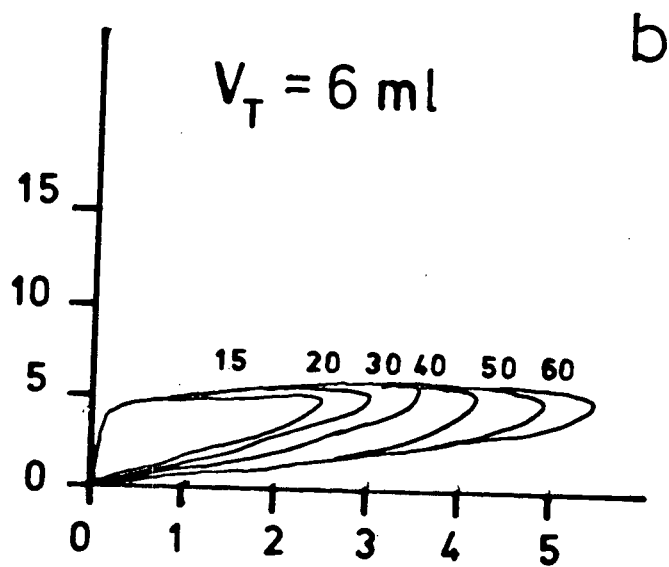
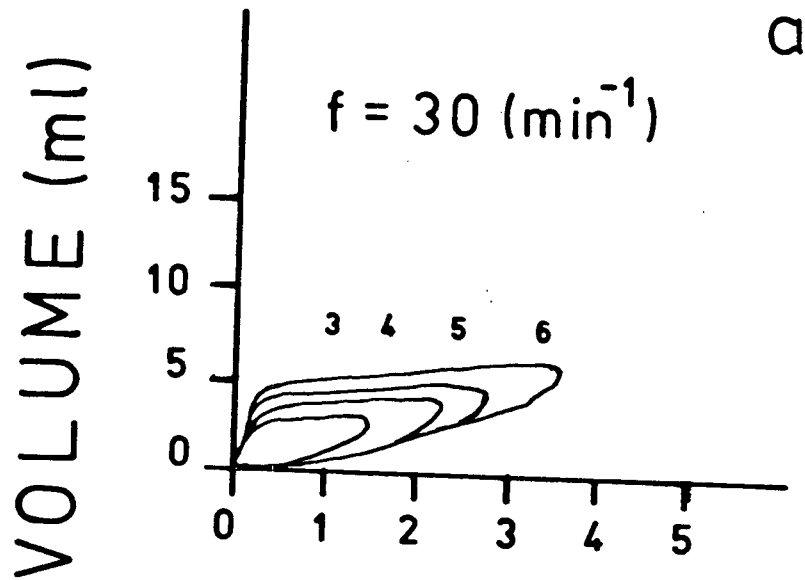
Results

The dynamic pressure-volume relationships for the total respiratory system of a 750 g. animal are shown in Fig. 3.2. These curves are representative of all curves measured. The curves in Fig. 3.2a show that at a frequency of 30 cycles/min., as stroke volume increases, the peak pressure generated within the system rises. The curves in Fig. 3.2b show that at a frequency of 30 cycles/min., as stroke volume increases, the peak pressure generated within the system also rises. Thus the pressure-volume relationships of the total respiratory system are both volume and frequency dependent.

Figure 3.3 shows representative pressure-volume loops obtained for inflation of the body compartment alone in a 468 g. animal. At a constant frequency of 30 cycles/min. there is a strong dependence of the pressure generated on the inflation volume (Fig. 3.3a). For a constant volume of 6 ml, on the other hand, changes vary inconsistently with changes in frequency suggesting that the variation seen is not frequency dependent (Fig. 3.3a). The relationships shown in Figs. 3.2 and 3.3 for the total system and the body compartment alone hold true for all pump frequencies at all inflation volumes.

In Fig. 3.4, the changes in dynamic compliance of the total respiratory system, as a function of pump frequency are shown for volumes of 2, 4, and 6 ml. Each point is the mean value for 3 animals with standard

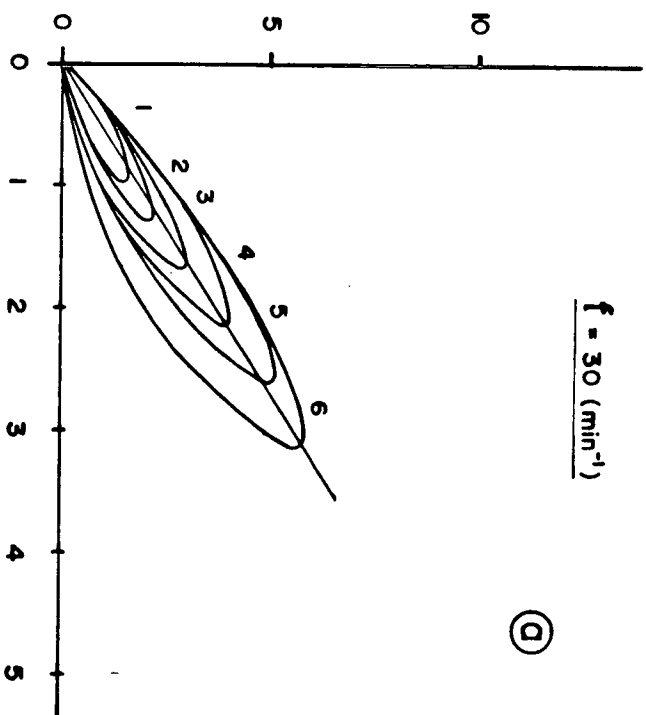
Fig. 3.2 Effects of changes in ventilation frequency (f) and volume (V_T) on the pressure-volume (P-V) relationship associated with a single ventilation cycle of the total respiratory system in a 750g turtle. (a) The effect of increasing tidal volume from 3 to 6 ml on pressure-volume loops at a constant ventilation frequency of 30 cycles/min. (b) The effect of increasing ventilation frequency on pressure-volume loops for a constant tidal volume of 6 ml.



$\Delta P \text{ (cm H}_2\text{O)}$

Fig. 3.3 Effects of changes in ventilation frequency (f) and volume (V_T) on the pressure-volume (P-V) loops associated with a single ventilation cycle of the body compartment alone on a 430 g. animal. (a) The effect of increasing tidal volume from 1 ml to 6 ml on pressure-volume loops for a constant ventilation frequency of 30 cycles/min. (b) The effect of increasing ventilation frequency from 15 cycles/min. to 60 cycles/min. for a constant tidal volume of 6 ml.

$f = 30 \text{ (min}^{-1}\text{)}$



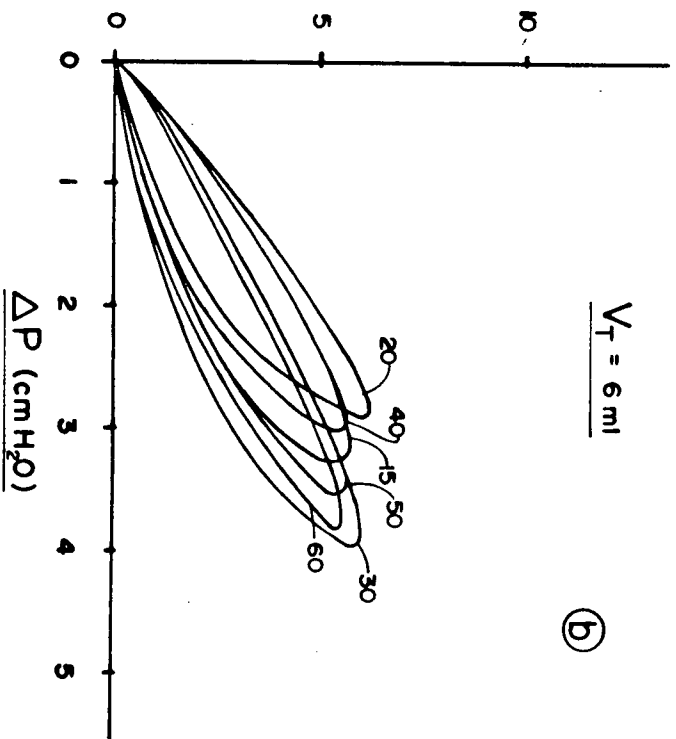
(a)

VOLUME (ml)

$\Delta P \text{ (cmH}_2\text{O)}$

$V_T = 6 \text{ ml}$

(b)



$\Delta P \text{ (cmH}_2\text{O)}$

error bars included. Statistically the dynamic compliance is volume independent over the range of volumes recorded although there is a slight trend for the system to behave in a stiffer fashion at higher inflation volumes. The dynamic compliance is, however, strongly frequency dependent, so that for any given volume, as frequency increases, the dynamic compliance falls. The decrease in dynamic compliance indicates that the system becomes stiffer with increasing frequency thus for any given volume, as frequency increases, the pressure difference generated by inflation also increases.

This apparent stiffening of the respiratory system at increasing ventilation frequency is reflected in the work required to produce each breath. Calculated values of the mechanical work required to produce each breath in the total respiratory system, measured from the pressure-volume loops as described in the methods, are shown in Fig. 3.5. The work/ventilation cycle (W) is plotted against \dot{V}_E in this figure with isopleths illustrating various pump frequencies and pump volumes. Each point is the mean value for three animals standardized for body weight with standard error bars included. For a constant frequency, increases in \dot{V}_E due to increases in V_T result in a linear rise in W on these curves. If \dot{V}_E is increased by holding volume constant and increasing frequency, W also rises but in a nonlinear fashion. Thus the rise in W , with increasing levels of f at constant V_T , is small at $f < 30$ cycles/min. and rises sharply with $f > 30$.

The total work required to produce each breath can be further analyzed in terms of the work required to overcome elastic and nonelastic

Fig. 3.4 The relationship between ventilation frequency (f) and dynamic compliance (C_{dyn}) for constant tidal volumes of 2 (\square), 4 (\bigcirc) and 6 (Δ) ml. Each point represents the mean value for 3 animals. Vertical bars indicate the S.E.M.

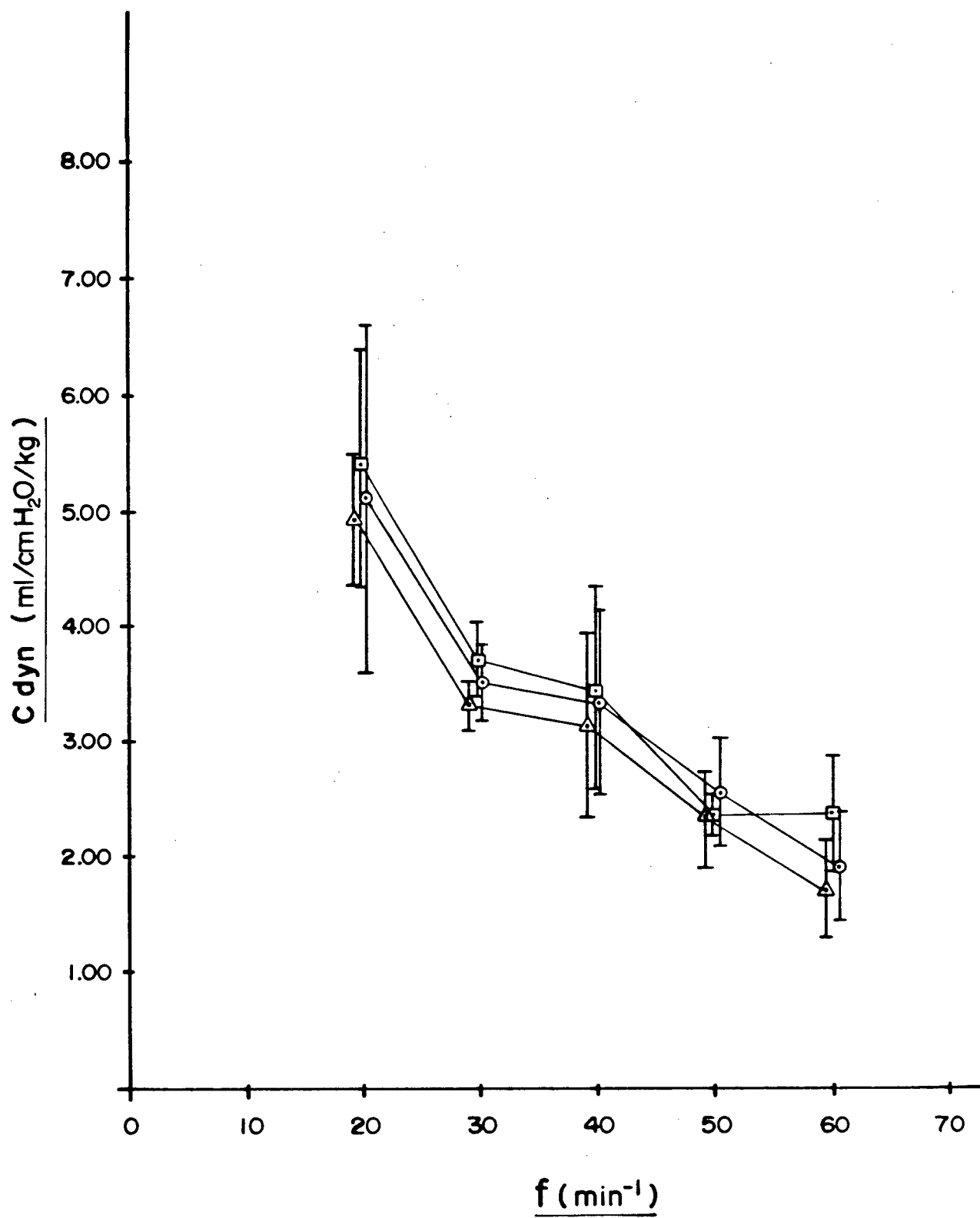
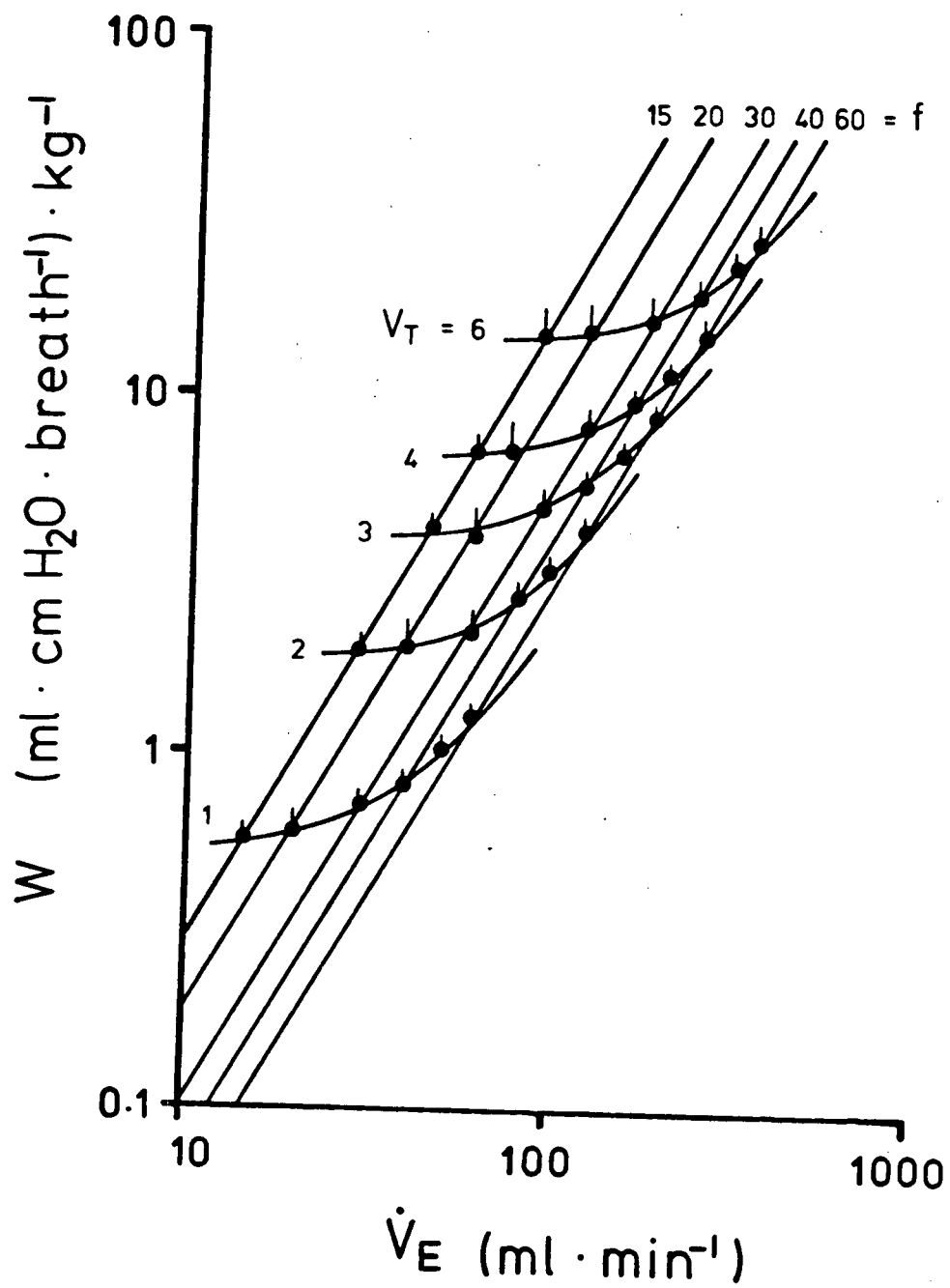


Fig. 3.5 The relationship between the total work/breath (W) and minute ventilation (\dot{V}_E) for different combinations of tidal volume (V_T ml) and respiratory frequency (f in breaths/min.). Data points represent average values from 3 animals with standard error bars.



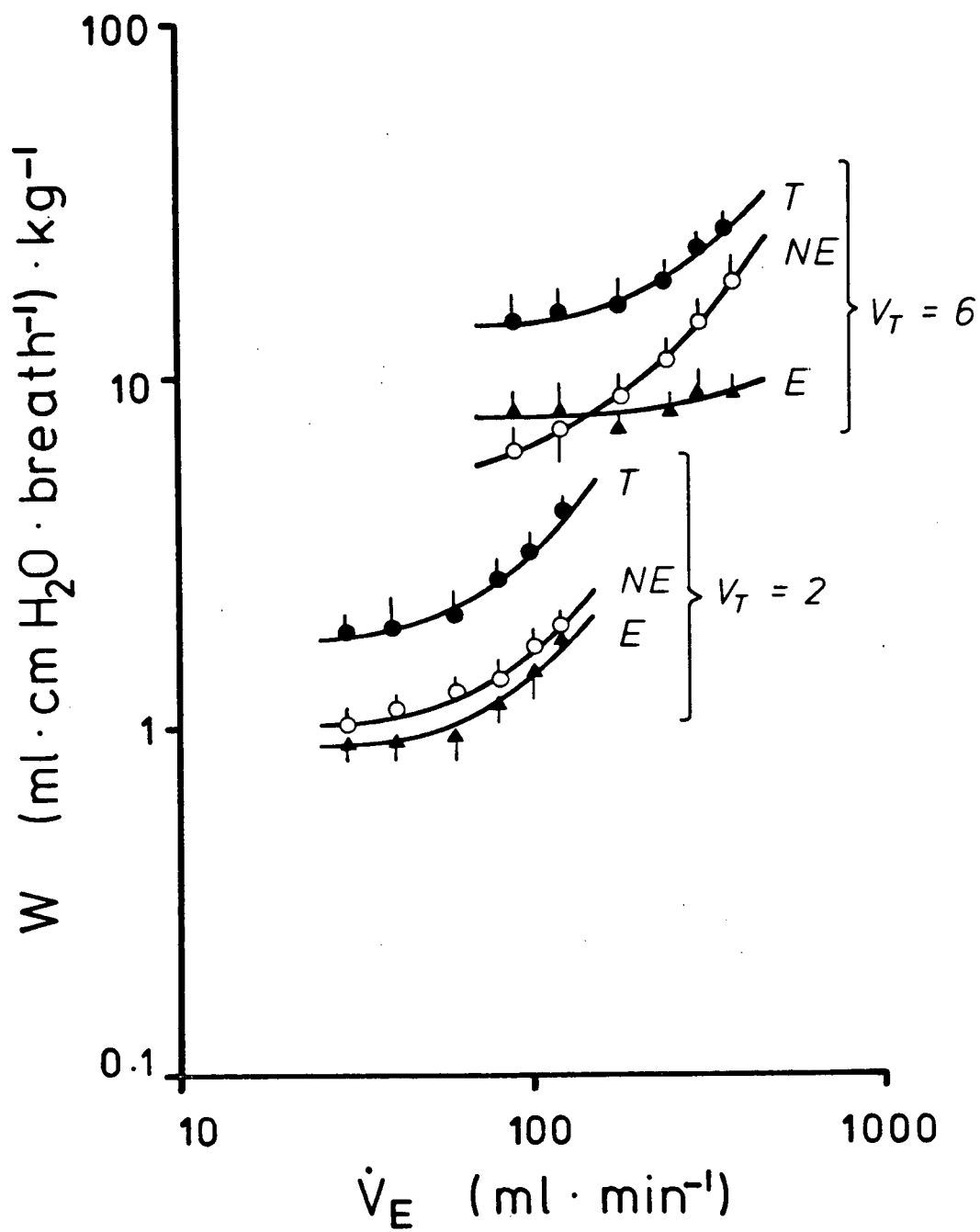
forces. Figure 3.6 shows the elastic and nonelastic components of the total work required to produce a single breath for tidal volumes of 2 and 6 ml at increasing pump frequencies. For a V_T of 2 ml, at low levels of \dot{V}_E , the work required to overcome elastic and nonelastic forces is about equal. At high levels of \dot{V}_E ($f > 30$) with a V_T of 6 ml, the work required to overcome nonelastic forces is the major portion of the total work required to produce each breath.

The work required to produce a single breath can also be analyzed in terms of the work required to inflate the lung and the work required to inflate the body compartment since the pressure within the respiratory system is the sum of the pressure contributed by the lung and the body wall (Agostoni, 1970).

Figure 3.7 shows the contribution of the lung (W_L) and body wall (W_B) to the total work (W_T) required to produce single breaths at tidal volumes of 2 and 6 ml with increasing pump frequencies. The work required to overcome the forces within the body wall are volume dependent. The higher the tidal volume, the larger W_B becomes. W_B is, however, relatively frequency independent. As a consequence, rises in W_T as \dot{V}_E increases are a result of frequency dependent increases in W_L . Thus although W_B is the major component of W_T , for each V_T shown in Fig. 3.7, it is the sharp rise in W_L which produces the rise in W_T during increases in pump frequency.

For purely mechanical considerations the effects of changes in f or V_T on the work of breathing have been referenced in terms of their effect on \dot{V}_E . In terms of gas exchange, however, changes in alveolar

Fig. 3.6 The contribution of elastic (E) and nonelastic (NE) work/breath to the total work/breath (T) for V_T of 2 ml and 6 ml with changing \dot{V}_E . Each point represents the mean value of 3 animals with standard error bars included.



minute ventilation (\dot{V}_A) are of more concern than changes in \dot{V}_E . \dot{V}_A , the product of the alveolar ventilation volume ($V_A = V_T - \text{dead space } (V_D)$) and ventilation frequency (f), decreases as f increases even though \dot{V}_E is held constant by adjustments in V_T . Since the dead space volume of the respiratory system is fixed, anatomically, as V_T decreases, \dot{V}_A becomes disproportionately small and when $V_T = V_D$, \dot{V}_A will equal zero. At this point, although much gas is still being moved in and out of the trachea, there is no gas being turned over in the lungs. As a consequence, there are limits to the extent to which V_T may be reduced before gas exchange is compromised by the fall in \dot{V}_A . This is illustrated in Fig. 3.8 which shows the importance of holding \dot{V}_A constant rather than \dot{V}_E to maintain gas exchange.

For a constant \dot{V}_E of 50 ml/min/kg, \dot{V}_A falls as f increases. \dot{V}_A values were calculated using a V_D of 0.74 ml/kg from Table 3.1. As \dot{V}_A falls with increasing f , arterial PO_2 levels also steadily decrease from 90 mmHg at an f of 30 cycles/min to 30 mmHg at f of 60 cycles/min. Arterial PCO_2 steadily rises from 15 mmHg to 29 mmHg over the range of frequencies used. To maintain blood gas levels \dot{V}_A rather than \dot{V}_E must be kept constant. For \dot{V}_A to be kept constant \dot{V}_E must rise along with increases in ventilation frequency. This has consequences on the minute work of breathing.

The values of work/breath from Fig. 3.5 are replotted in Fig. 3.9 as minute work (\dot{W}), the product of the work required to produce a breath (W_T) and the number of breaths taken each minute (f). For any given level of minute ventilation (\dot{V}_E) \dot{W} initially falls as f increases and V_T decreases until \dot{W} reaches a minimum. Further increases in f and decrease in

Fig. 3.7 The contribution of the lung (L) and body (B) to the total (T) work/breath (W) for tidal volumes (V_T) of 2 ml and 6 ml with changing minute ventilation (V_E). Each point for T and L is the mean for 3 animals \pm S.E.M. Each point for B is the mean of 6 animals \pm S.E.M.

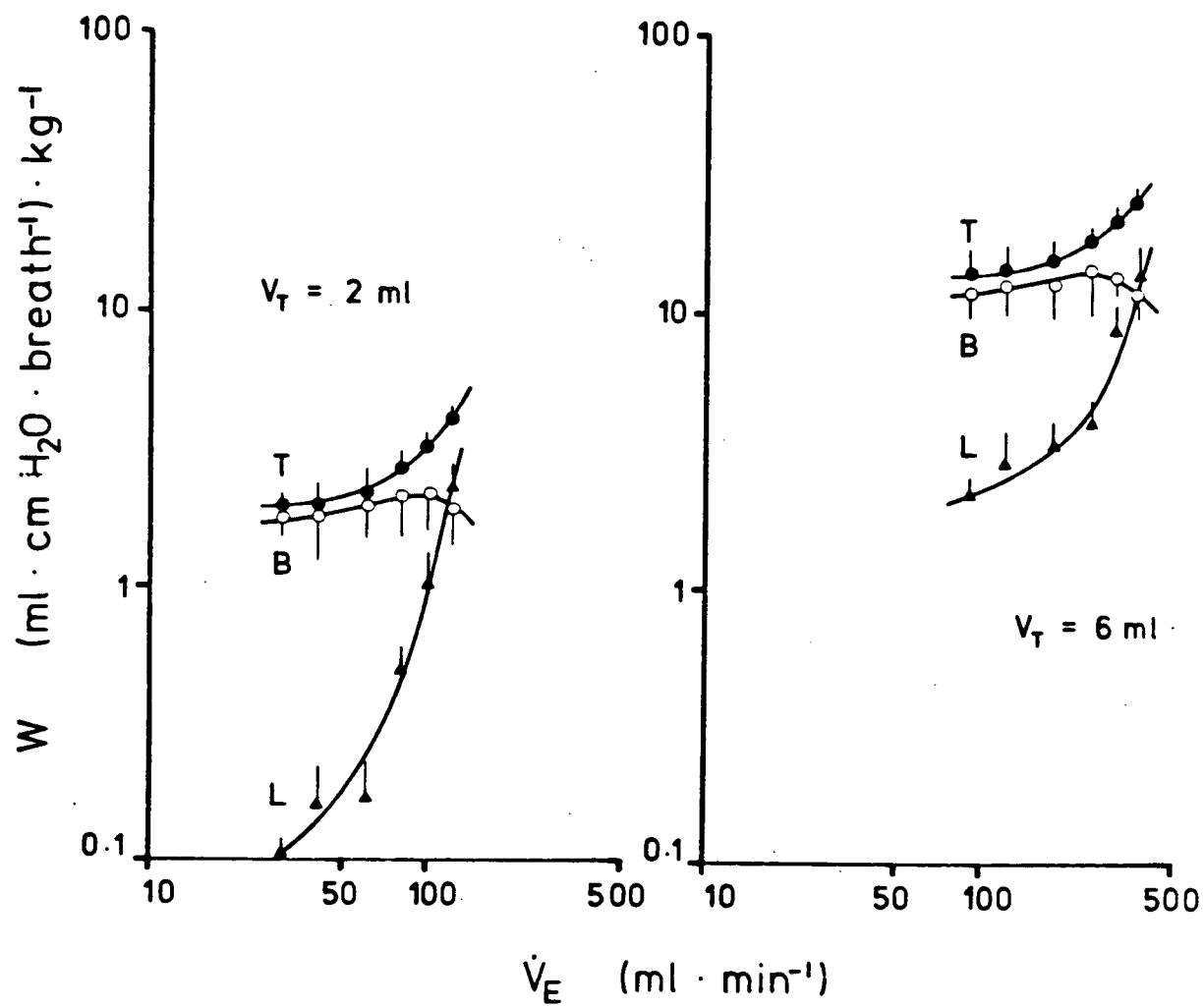


Fig. 3.8 The effect of increasing ventilation frequency (f) at a constant minute ventilation of 50 ml/min/kg. on P_aO_2 (\odot), P_aCO_2 (\triangle) and alveolar minute ventilation \dot{V}_A (\boxtimes). The values for P_aO_2 and P_aCO_2 are the mean for 6 animals \pm S.E.M. \dot{V}_A was calculated using a value of 0.74 ml/kg \pm 0.06 S.E.M. for dead space volume (V_D).

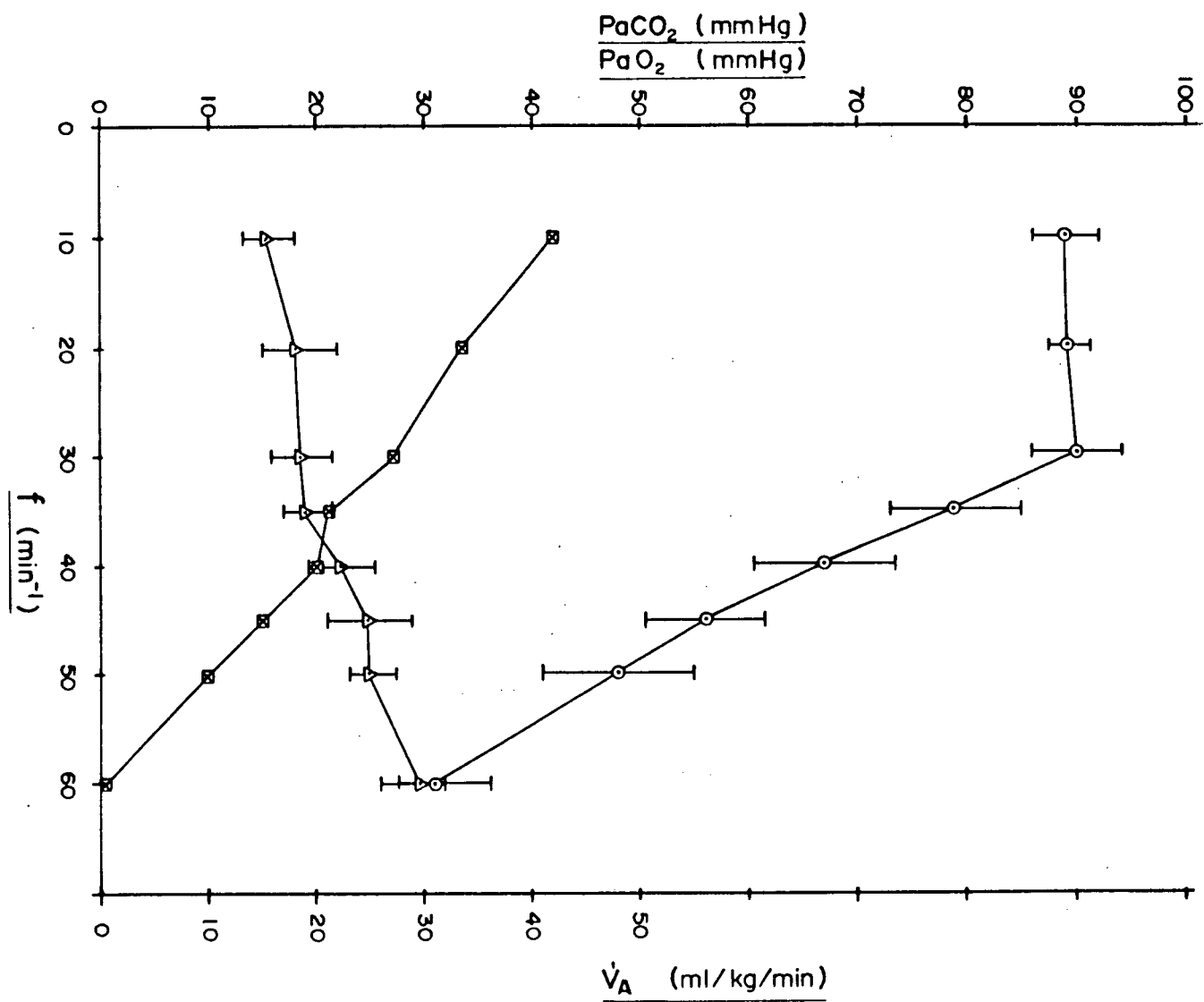
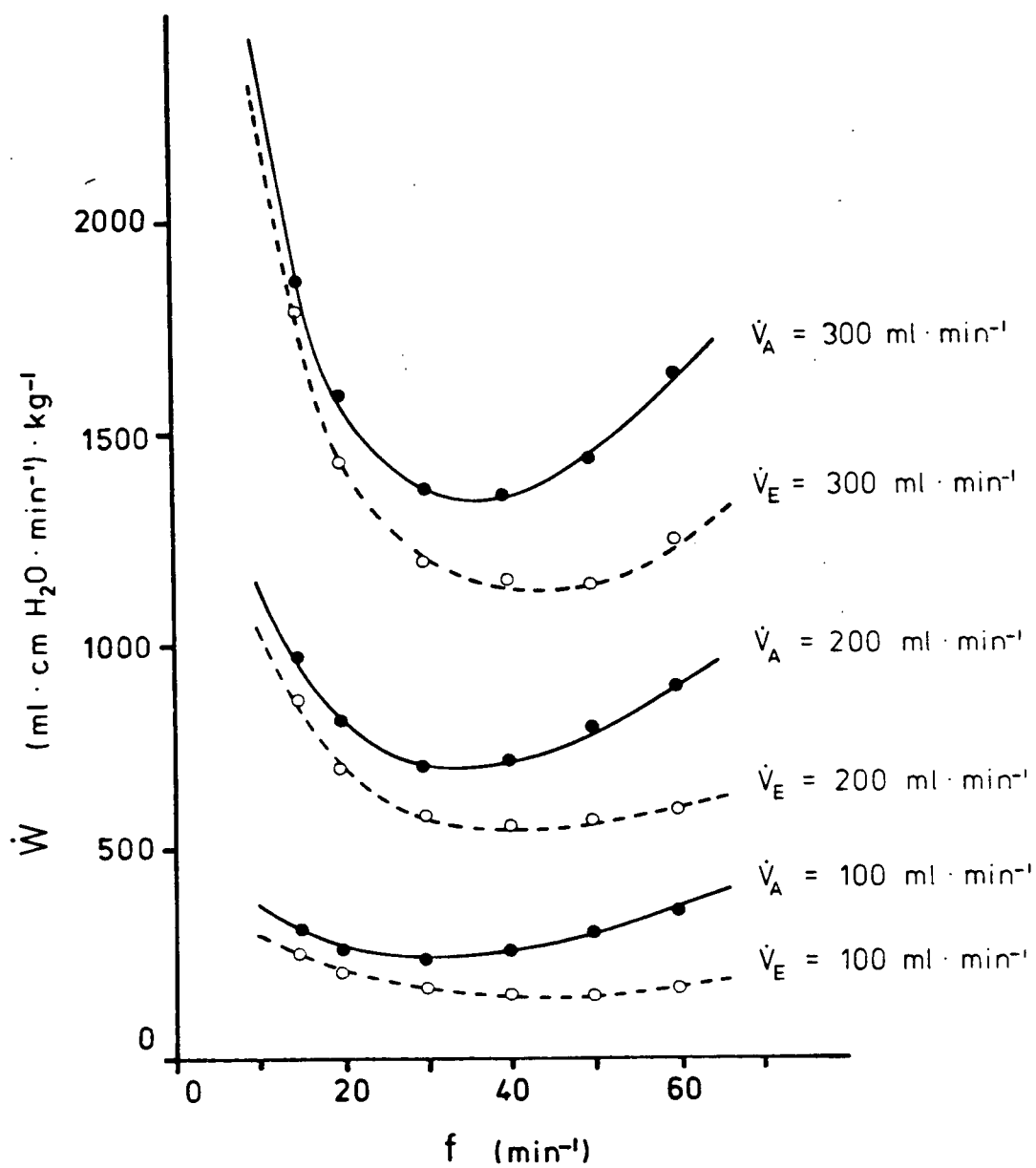


Fig. 3.9 The relationship between the rate of work (\dot{W}) and ventilation frequency (f in breaths/min.) for constant levels of minute ventilation (\dot{V}_E in ml/min.) and alveolar minute ventilation (\dot{V}_A in ml/min.).



V_T produce a rise in \dot{W} . The shape of this curve becomes accentuated and the level of \dot{W} increases as \dot{V}_E rises. For \dot{V}_E ranging from 100 to 300 ml/min., \dot{W} is minimum at frequencies between 35 and 45 breaths/min. Figure 3.9 also shows the effect of changes in f on \dot{W} for various levels of constant \dot{V}_A . Note that \dot{W} is always greater at any given level of \dot{V}_A than it is at a similar level of \dot{V}_E and that this difference increases as f increases. This stems from the fact that at any given level of f , V_T must be greater to produce a level of \dot{V}_A which is similar to any given level of \dot{V}_E since values of \dot{V}_A do not account for dead space ventilation. This also means that as f increases the difference in values of V_T required to produce similar levels of \dot{V}_A and \dot{V}_E also increases. The ventilation frequencies at which \dot{W} is minimum for levels of \dot{V}_A ranging from 100 to 300 ml/min. vary between 30 to 40 breaths/min., slightly lower than the minimum frequencies obtained from the plots of \dot{W} vs. f for constant levels of \dot{V}_E .

Minute work is analyzed in terms of the work required to overcome elastic and nonelastic forces for a \dot{V}_A of 150 ml/min. in Fig. 3.10. As pump frequency increases, the minute work done to overcome elastic forces drops sharply and then rises very slightly as f continues to increase. The minute work required to overcome nonelastic forces steadily rises as f increases. The sum of the work rate required to overcome both elastic and nonelastic forces results in the 'U' shape of the total minute work curve.

In Fig. 3.11 minute work is broken down in terms of the cost of ventilating the lungs (L) and body cavity (B). The minute work required to overcome the forces within the body cavity for a \dot{V}_A of 150 ml/min., steadily declines as f increases. The minute work required to overcome the

Fig. 3.10 The amount of total work (T) required to overcome elastic (E) and nonelastic (NE) forces at a constant alveolar minute ventilation (\dot{V}_A) of 150 ml/min⁻¹ with increasing ventilation frequency (f in breaths/min.).

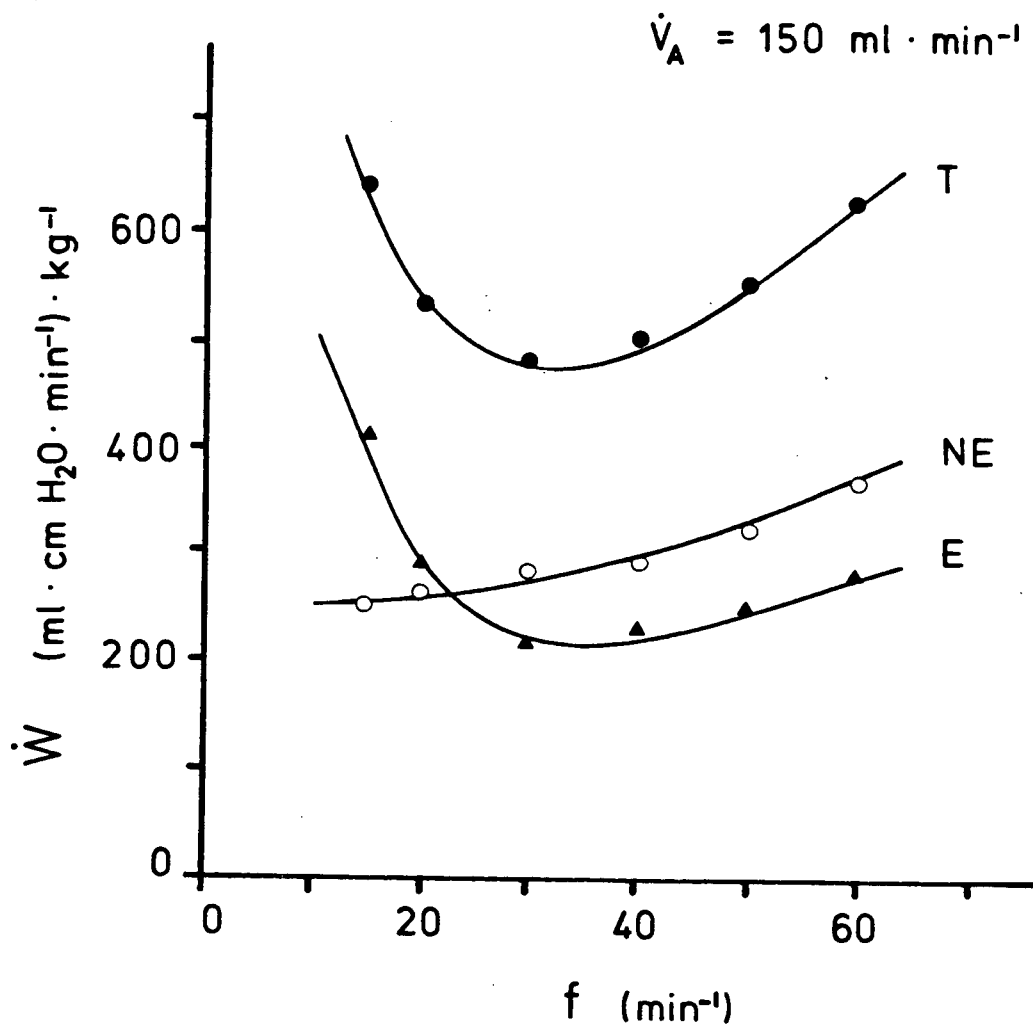
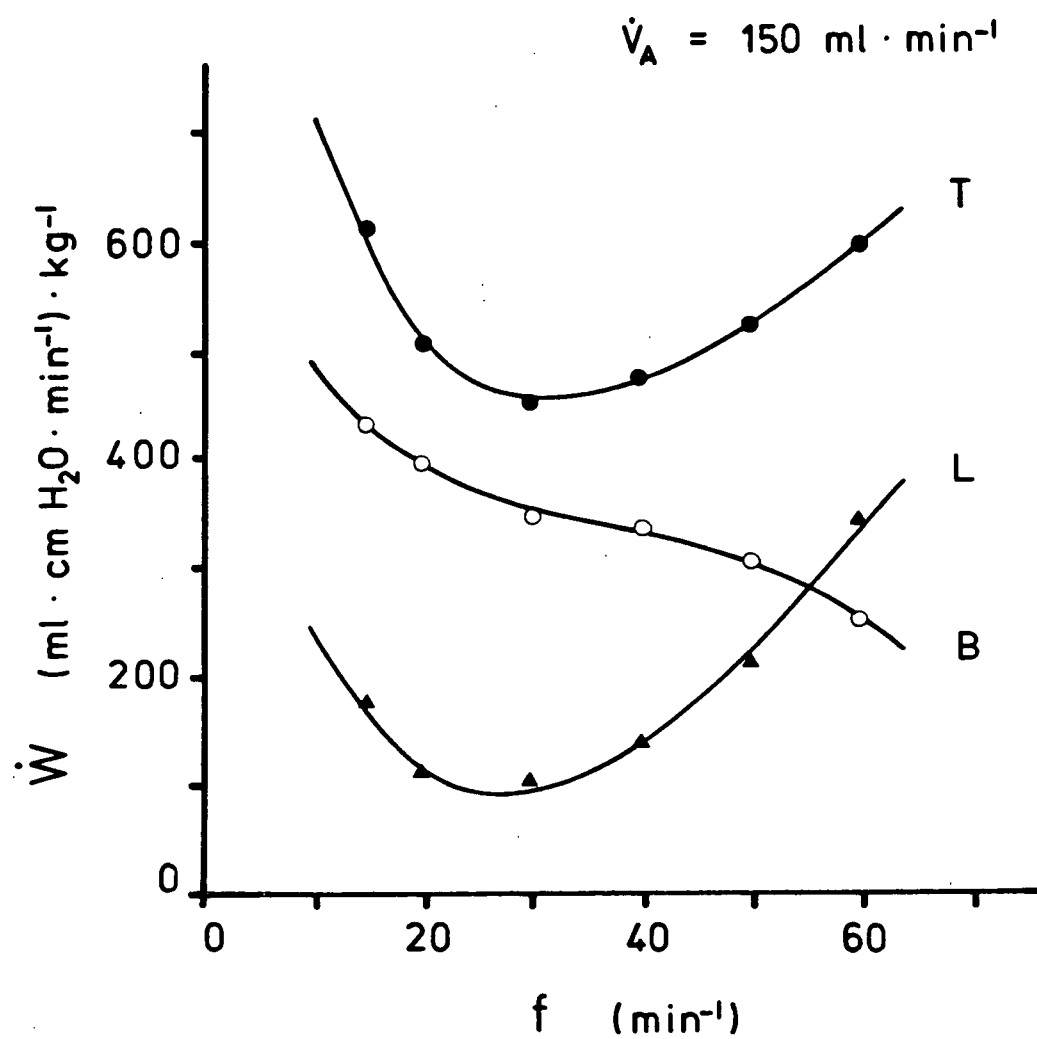


Fig. 3.11 The cost of ventilating the lungs (L) and the body (B) compartment compared to the total (T) rate of work for a constant alveolar minute ventilation (\dot{V}_A) of 150 ml/min. with increasing ventilation frequency (f in breaths/min.).



forces within the lung drops initially but begins to rise sharply after the ventilation frequency increases beyond 27 cycles/min. The steady decline of minute work required to overcome forces within the body cavity and the initial decline in the minute work required to overcome forces within the lung result in the initial decline seen in total minute work. The sharp rise in the minute work required to overcome forces in the lung as ventilation frequency increases, however, causes the total minute work curve to be U-shaped.

The values for the anatomical dead space of the respiratory system (V_D) are shown for all animals in Table 3.1. When V_D was calculated from measurements of tracheal and bronchial length and diameter, a mean value for V_D of 0.65 ± 0.058 ml/kg was obtained. Measurements of V_D based on water displacement yielded a mean value of 0.74 ± 0.063 ml/kg. With 95% confidence, the difference between the two values was found to be insignificant ($\Delta = 0.09 \pm 0.1537$). Since the volume of the trachea is a minimum value for V_D which does not take into account the volume of the intrapulmonary bronchi or physiological dead space it was felt that the larger value of 0.74 ml/kg more closely approximated V_D and was therefore used to calculate \dot{V}_A .

Table 3.1 Values of anatomical dead space determined for specimens of P. scripta by two different methods.

		Dead space	
	<u>Mass (g)</u>	<u>A* (ml/kg)</u>	<u>B+ (ml/kg)</u>
	729	0.74	-
	680	0.66	0.63
	755	0.42	-
	1269	0.63	0.95
	725	0.55	0.65
	759	0.88	0.72
\bar{x}	820	0.65 ml/kg	0.74 ml/kg
S.E.M.	82.7	0.058	0.063

* Calculated from measurements of the length and diameter of the trachea.

+ Determined by filling the trachea with water to determine its volume.

The difference between the two methods of determining the volume of the trachea is insignificant ($\Delta = .09 \pm .1537$) at the 95% confidence limit.

Discussion

Under dynamic conditions the compliance of the respiratory system shows frequency dependence, but little or no dependence on V_T over the range studied. The frequency dependence of the dynamic compliance (C_{dyn}) arises from a change in the stiffness of the lungs as there is no frequency dependence seen in C_{dyn} measured for the body cavity alone (Fig. 3.3). The decrease in C_{dyn} observed with increasing frequency is not affected by changes in ventilation volume over the range of 2 to 6 ml. These results differ from those obtained for the Tokay gekko where the respiratory system showed a frequency dependent decrease in C_{dyn} which was enhanced by increasing ventilation volume at high ventilation frequencies (Milsom and Vitalis, 1984). This dependence of C_{dyn} on tidal volume at high ventilation frequencies in the Tokay gekko was thought to be a result of the visco-elastic properties of the body wall. The absence of such an effect in the present study may be attributable to the presence of the shell in the turtle which does not experience geometric changes with increasing volume and which restricts all visco-elastic properties to the membranes of the flank cavities. The ligaments, cartilaginous elements, and muscle fiber arrangements seen in the articulated ribs of the Tokay are absent in the turtle and this could account for the differences seen in the volume effects on the frequency dependence of C_{dyn} in the two animals. It is also possible that the volume effects on the frequency dependence of C_{dyn} only appear with very high tidal volumes. The tidal volumes in this study were only 2% to 6%

of resting lung volume whereas in the study on the Tokay gekko the tidal volumes used were 10-50% of resting lung volume. The dependence of C_{dyn} on frequency observed in the turtle means that a greater change in pressure will result for any given change in volume as frequency increases. This is reflected in the measurements of the total work/breath obtained for the total respiratory system.

For any given tidal volume the work required to produce a single breath rises as frequency increases, with the slope of the rise in W_T being small at low frequencies but increasing sharply above an f of 30 breaths/min. This is due to an increase in the work required to overcome the forces within the lung which appear to be predominantly nonelastic in nature. Despite this, the work required to overcome the forces within the body wall and body cavity account for the majority of the work required to produce a single breath at all but the highest frequencies studied. The work required to overcome the forces within the body wall and body cavity are relatively independent of frequency indicating that these forces are predominantly elastic in nature.

For any given ventilation frequency, the work required to produce each breath increases as an exponential function as tidal volume increases. This appears to be due to the material properties of the body wall, body cavity, and lungs. Elastic work accounts for approximately 50% of the total work at low tidal volumes, but nonelastic work contributes the major portion of the work at high tidal volumes due to the necessary increase in flow rates and thus flow resistance which are a consequence of the need to move more air in the same time interval.

The results of the work/breath measurements indicate that it

requires less work to increase ventilation by increasing frequency rather than tidal volume at all levels of ventilation studied. This is because the flow resistive forces rise more slowly with increasing frequency over the range studied than the exponential rise which occurs in the work required to overcome elastic forces when tidal volume is increased and frequency held constant. When levels of minute ventilation are high, however, although it still requires less work/breath to increase ventilation by holding f constant, the differences are smaller. This is due to the increase in flow resistive forces associated with high ventilation volumes and frequencies which causes the sharp upturn in the work/breath curves seen in the larger volume isopleths.

The minute work (\dot{W}) required to maintain a constant level of minute ventilation (\dot{V}_E) is a function of the change in the work/breath associated with different combinations of tidal volume and frequency multiplied by the breathing frequency. The slope of the \dot{W} vs. f curve for a constant \dot{V}_E decreases at low frequencies due to an exponential drop in elastic work as V_T falls and f increases. As f continues to rise \dot{W} begins to increase again due to the steady increase in nonelastic work caused by the higher velocity of the air flow. This is also true of the \dot{W} vs. f curve for a constant level of \dot{V}_A but in this curve, \dot{V}_E also has to increase as f increases since the fall in V_T cannot completely offset the increase in f due to the ever increasing ratio of dead space ventilation to total ventilation. This causes a further rise in \dot{W} since \dot{W} is proportional to \dot{V}_E (Milic-Emili and Petit, 1960). Another consequence of having to increase \dot{V}_E when f increases in order to maintain \dot{V}_A constant is a slight left shift in the frequency corresponding to minimum \dot{W} . This shift

occurs as a result of flow resistive forces becoming more important at a lower frequency of ventilation.

This total \dot{W} is the sum of the rate of work required to overcome the forces within the body wall and within the lung. The majority of \dot{W} is required to overcome forces arising from the body wall and cavity at ventilation frequencies below 55 breaths/min. The size of this contribution drops steadily as f increases suggesting that the predominant forces to be overcome within the body wall are elastic in nature. The decline, however, is not exponential as would be the case in a purely elastic system suggesting that there are some viscous forces associated with the movement of tissues within the body cavity also.

Partitioning the amount of the minute work which is required to overcome the various forces within the lung is more complex. The curve describing the relationship between W and f for the lungs alone is also U-shaped with a minimum W occurring at approximately 27 breaths/min. for a \dot{V}_A of 150 ml/min. This curve then rises sharply reflecting the fact that work required to ventilate the lungs becomes the major contributor to the total W required to ventilate the entire respiratory system at frequencies greater than 55 breath/min. The decline seen in minute work with increasing frequency, when frequencies are low, indicates that there are elastic forces which have to be overcome when V_T is high. The sharp rise in W for the lungs above frequencies of 27 breaths/min. is due to nonelastic forces which are flow resistive in nature. The shape of the \dot{W} curve for the lungs in part reflects its anatomy.

The lungs of the turtle are attached to the dome of the carapace dorsally and to the post-pulmonary septum ventrally. The post-pulmonary

septum is in turn attached to the liver and stomach and envelopes the entire lung. The anterior portion of the lung is covered by a sheath of striated muscle, the striatum pulmonale (Perry, 1978). These connective tissues and striated muscle along with the intrapulmonary septa are the probable site of the elastic components of the forces arising from the lungs. The intrapulmonary septa divide the lung into seven chambers which communicate with each other via the intrapulmonary bronchi. These intrapulmonary bronchi along with the extrapulmonary primary bronchi and the trachea are the most probable sites for flow resistance in the lungs of turtles. Even though turtles do not possess a highly branched bronchial tree as in mammals it is not surprising that flow resistance is found in their lungs. It has been shown that due to the large cross sectional area of the lower portion of the bronchial tree in mammals that little flow resistance occurs below the medium sized bronchi with the majority of flow resistance being in the upper airways (Pedley et al., 1970).

In summary, the mechanical work/breath required to inflate the respiratory system in the turtle is divided equally between overcoming elastic and nonelastic forces at low frequencies and tidal volumes with almost all of these forces residing in the body wall and cavity. Work required to overcome nonelastic forces, however, predominates at high frequencies and tidal volumes with the lungs contributing an ever increasing proportion to this work.

This difference in the effect of increasing frequency on the work required to overcome forces arising from the lung and body cavity is responsible for the overall U-shape of the minute work curve which produces an optimum combination of tidal volume and frequency requiring the minimum

work to maintain a constant level of total ventilation (\dot{V}_E or \dot{V}_A).

The nature of the mechanical work of breathing is heavily influenced by the anatomy of the respiratory system. In lizards, turtles and mammals, differences in the architecture of the lungs and body result in differences in pulmonary mechanics which are reflected in the work of breathing. For instance, the lizard, Gekko gekko possesses single chambered lungs which are fused anteriorly, and share a common bronchial opening arising from a wide bore, undivided trachea. They, consequently, lack an intrapulmonary bronchus and internal lung partitioning consists of a series of dorsolateral ridges with a net-like system of tabeculae arising from the lung wall. These lungs are not enclosed in a pleural cavity but lie along the dorsal surface of the pleuroperitoneal cavity, held in place dorsally and ventrally, by mesopneumonia. These connections allow free movement of the lungs and establish resting lung volume when the system is open to atmosphere (Milsom and Vitalis, 1984). The body wall of Gekko gekko also differs from that of the turtle in that it is long, slender and flexible and possesses moveable articulated ribs enclosing the viscera and lungs.

As a result of its anatomy the majority of the work required to produce each breath (70-90%) in Gekko gekko is required to overcome elastic forces at all frequencies and tidal volumes (Milsom and Vitalis, 1984). Furthermore, almost all of these elastic forces arise from the body wall of the animal. The lungs, due to their simple structure, contribute very little to the forces resisting inflation and therefore the total work of breathing in Gekko gekko is almost purely required to overcome elastic forces arising from the body wall. Given this, the minute work required to maintain a constant level of minute ventilation (\dot{V}_E) diminishes

exponentially as a function of increasing breathing frequency. For a constant alveolar ventilation, however, minute work begins to rise after an initial decline due to the increase in \dot{V}_E required to maintain \dot{V}_A constant when ventilation frequency increases. The result is a U-shaped curve for minute work vs. breathing frequency where a specific combination of tidal volume and frequency correspond to the level of minimum work. This 'U'-shaped curve for a constant \dot{V}_A is here due solely to the increase in \dot{V}_E required to account for dead space ventilation when frequency increases and not to rising nonelastic forces as is the case in turtles.

In the case of mammals, the respiratory system differs in two major aspects from the systems found in both the turtle and lizard. These differences also influence the mechanics of the system and are in turn reflected in the work of breathing. The major anatomical difference influencing the mechanics of the respiratory system in mammals is the structure of the lungs. Mammalian lungs are characterized by a highly branched bronchial tree which communicates with approximately 300 million densely packed alveoli which are each approximately .3 mm in diameter (West, 1979). The alveoli and alveolar ducts are surrounded by an arrangement of collagen and elastic fibers in a helical fiber network which provides a large portion of the elastic recoil of the lungs (Bouhuys, 1977). Another important difference is that the lungs are now isolated in a pleural cavity within the thoracic cage which is separated from the viscera by a muscular diaphragm. Within this cavity the lungs are surrounded by pleural membranes and are unattached to the surface of the thoracic cage. Due to the arrangements of the elastic components, the lungs have a tendency to recoil inwards while the chest wall has a tendency to spring outward. These two

forces tend to balance each other under equilibrium conditions and thus the chest is pulled inward and the lungs outward. The result is that during inspiration, because of the natural tendency of the chest wall to spring outwards, work only has to be done to overcome the forces within the lung. Work is stored in the elastic elements of the lung and expiration is passive under resting conditions. In humans 60% to 70% of the work of breathing is required to overcome the elastic forces within the system (Otis et al., 1950) with the majority of these forces residing in the lungs. The lung is also a site of flow-resistance which accounts for approximately 25% of the work of breathing with the remainder of the forces to be overcome ($\approx 5-10\%$) being viscous in nature. Here also, as is the case for lizards and turtles, when W is plotted as a function of f for a constant level of \dot{V}_A , the curve is U-shaped.

In comparing the mechanics of these three respiratory systems it can be seen that anatomical factors play an important role in determining the nature of the work required to inflate the respiratory system. The work required to inflate the lungs as a percentage of the total work of breathing increases in the sequence Gekko < turtle < mammal. This hierarchy also reflects the increasing complexity of the lungs.

It is of interest that an optimal combination of frequency and tidal volume exists in all three groups despite the large diversity seen in the structure and pulmonary mechanics of their respiratory systems. This seems to suggest that optimal combinations exist not only because of anatomical factors but also because of the need to maintain alveolar ventilation (\dot{V}_A) constant rather than total ventilation (\dot{V}_E). It is plausible that anatomical dead space and its magnitude play as important a

role in determining optimal combinations of f and V_T as the material and structural components of the respiratory system.

CHAPTER 4

THE INTERRELATIONSHIP BETWEEN PULMONARY MECHANICS AND THE SPONTANEOUS BREATHING PATTERN IN PSEUDEMYS SCRIPTA

Introduction

In the previous chapter it was shown that a specific combination of tidal volume (V_T) and breathing frequency (f) exists for which a minimum amount of mechanical work per minute (\dot{W}) is required to maintain any given constant level of alveolar ventilation (\dot{V}_A). This holds true for mammals, turtles, and gekkos despite great differences in dynamic pulmonary mechanics.

One assumption inherent in such considerations is that breathing is continuous. Due to their low metabolic rates, however, poikilotherms do not need to breathe continuously but instead exhibit arrhythmic breathing patterns. The arrhythmic patterns of poikilotherms are not all the same but show some variation. These patterns range from single breaths separated by a variable period of breath holding at end-inspiration as seen in the gekko to bursts of continuous breaths separated by variable periods of breath holding as seen in semi-aquatic turtles.

In single breath breathers, it has been suggested that the work required to take each breath is a more meaningful criterium for assessing the mechanical efficiency of breathing rather than minute work as is the case in continuous breathers (Milsom, 1984). Semi-aquatic turtles, being half way between single breath breathers and continuous breathers are

capable of utilizing both patterns. It is of interest, therefore, to analyze the efficiency of the spontaneous breathing patterns seen in the turtle in terms of the mechanics derived in chapters 2 and 3.

Materials and Methods

In total, twelve turtles (Pseudemys scripta) of mean weight 536 ± 40.9 g., were used in this study. The animals were housed in a large circular tank (1.5 m diameter) supplied with flowing tap water (10-15°C) to a depth of 30 cm. Dry areas heated with lamps were provided to allow the animals to bask. Several days before experiments the animals were moved into the laboratory and housed in smaller tanks supplied with water and dry basking areas where they became acclimated to room temperature (20-22°C). All animals were in a post-absorptive state when experiments were conducted.

Measurement of Ventilation

Each turtle was placed in a darkened aquarium with a plexiglass grid covering the surface. A 10 cm. hole was placed in this cover opening into a fitted air filled chamber (150 ml) from which the turtle could breath (Glass et al., 1983). The chamber was flushed with gas at a rate of 500 ml/min. via inflow and outflow ports on the top. The aquarium was filled with water to the level of the chamber opening and the animal was allowed free movement within the aquarium and quickly learned to breath from the chamber. A light was placed near the hole to assist the animal in locating it. The animal was allowed at least 24 hrs. to become accustomed to the experimental set up. The temperature of the aquarium was room temperature (20-22°C).

Inspiratory and expiratory airflows were measured using a Fleish

pneumotachograph (#00) placed over the outflow post in the chamber arrangement. The differential pressure across the pneumotachograph generated by the air flow was measured by a Validyne differential pressure transducer (DP 103-18) and Gould transducer amplifier. The flow of gas flushing the system was offset electronically to read as zero flow. When the turtle breathed in the chamber, inspiratory and expiratory flows were superimposed on this flow of flushing gas and only the inspiratory and expiratory flows were recorded. Inspiratory and expiratory flow were integrated with a Gould integrating amplifier to give tidal volume and recorded on a Tanberg Instrumentation Tape Recorder (Series 115) and a Gould 2600 pen recorder. The system was calibrated by injecting known volumes from a syringe. The rate of injection had no effect on the volume recorded. Ventilation was measured while room air, hypoxic (4% O₂ in N₂), and hypercapnic (3-5% CO₂ in air) gas mixture were used to flush the chamber. These gases were mixed using calibrated flow meters or bottled gases and delivered through the chamber at 500 ml/min. Each animal was allowed to breathe each gas for 1 hour before measurements were taken to ensure that steady state conditions were being monitored (Glass et al., 1983). After this initial one hour exposure to the experimental gas, ventilation was measured for at least 1 hour.

Bilateral Vagotomy

Turtles were mildly anaesthetized with sodium pentobarbital (20 mg/kg i.p.) and restrained in an upright position. A local anesthetic (lidocaine) was also administered to the neck. An incision was made along one side of the neck and the carotid artery was exposed. The vagus nerve was carefully freed from the carotid artery and approximately 5 mm of nerve

was removed. The incision was closed and the procedure was repeated on the other side. The animals were allowed to recover for several days and only those animals that were active, showed good reflexes, and were free of infection were used for experiments. These animals were then subjected to the same protocol described above.

Calculation of Ventilation

All calculations of minute ventilation were based on inspired volume. Breathing frequency (f (breaths/min)) was determined by dividing the total number of breaths by the total length of the recording which was usually one hour or more. The instantaneous breathing frequency (f') was calculated as the total time required to take a single breath (T_{tot} (sec.)) divided into 60 sec. Total ventilation (\dot{V}_E (ml/min)) was calculated as the product of tidal volume (V_T (ml)) and breathing frequency. Average values for V_T and T_{tot} were determined for each animal on each gas by analyzing 15 to 20 randomly chosen breaths and mean values for all the animals involved in the experiments were then determined.

Results

The respiratory pattern of Pseudemys scripta breathing air, under normal resting conditions consists of series of continuous breaths separated by breath holds of variable duration which are usually associated with diving. This is illustrated by Fig. 4.1a which shows a sample trace of respiratory air flow from an intact animal. From such traces the respiratory variables were calculated and are shown in Table 4.1. When respiration was stimulated by hypoxic (4% O₂ in N₂) or hypercapnic (3-5% CO₂ in air) gas mixtures, total ventilation increased by 1.3X and 2.1X respectively. The increase in total ventilation was due solely to an increase in the number of breaths per minute (f) with tidal volume (V_T) and the instantaneous breathing frequency (f') remaining unchanged. To increase ventilation then, the turtle shortened the nonventilatory period (T_{NVP}) and increased f with V_T and T_{tot} and, therefore, f' remaining unchanged. The work/breath (W/b) values shown in this table were taken from Fig. 3.4 in chapter 3 using the spontaneous values for f' and V_T while the work per minute (\dot{W}) is simply the product of W/b and f . Since V_T and f' remained relatively unchanged during respiratory stimulation, W/b also remained unchanged, however, W increased when respiration was stimulated by hypoxia or hypercapnia due to increases in f .

The effects of bilateral vagotomy on respiratory air flow are shown in Fig. 4.1b while the mean values of the respiratory variables measured under these conditions are listed in Table 4.1. During air breathing, vagotomy resulted in large increases in V_T and a reduction in f

Fig. 4.1 Representative flow traces from a turtle spontaneously breathing air; (a) intact animal (b) after bilateral vagotomy.

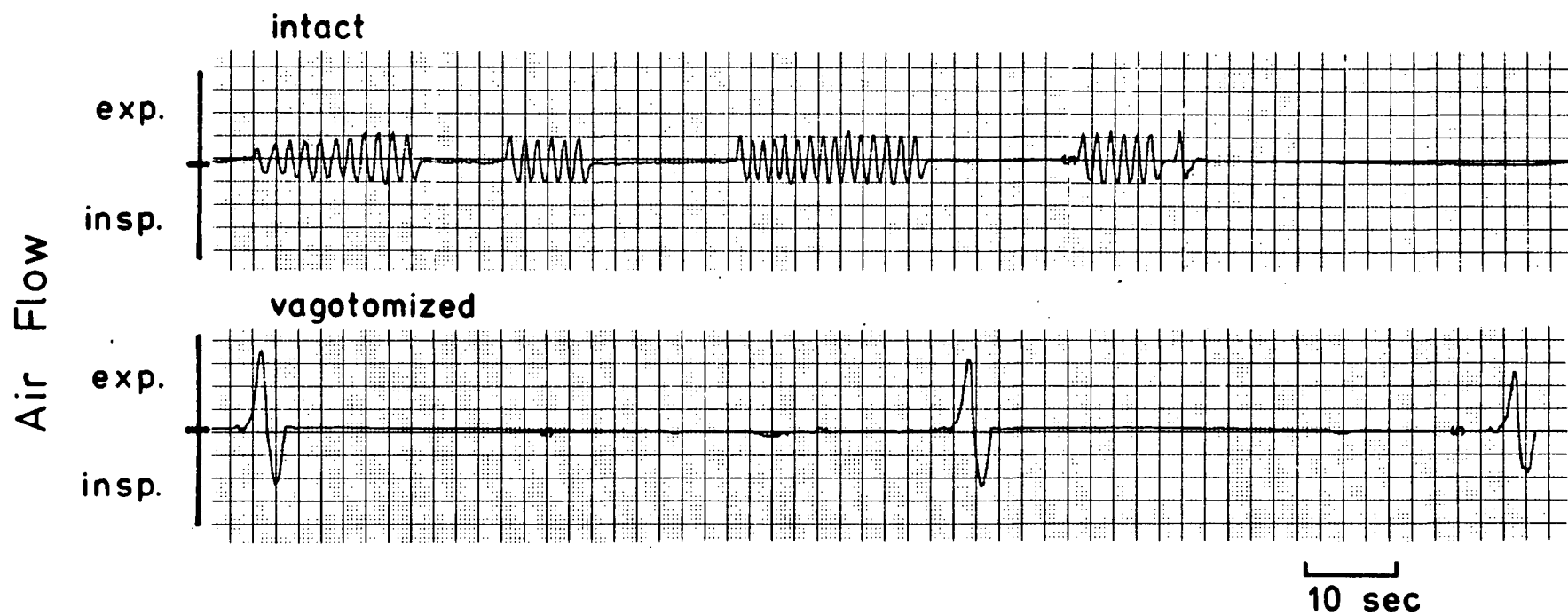


Table 4.1 Respiratory variables for spontaneously ventilating, intact and vagotomized turtles breathing air, 3-5% CO₂ in air, and 4% O₂ in N₂. Tidal volume (V_T), breathing frequency (f) and instantaneous frequency ($f' = 60/T_{tot}$) are expressed as means \pm S.E.M. for 6 animals. Total minute ventilation (\dot{V}_E) is the product of V_T and f. The work/breath (W/b) values are taken from figure 3.5 using spontaneous values for V_T and f' . Minute work (\dot{W}) is the product of W/b and f.

		air	3-5% CO ₂	4% O ₂
<u>Intact</u>				
V_T	ml/kg	6.9 \pm 1.2	6.8 \pm 0.4	6.2 \pm 0.8
f	breaths/min	2.0 \pm 0.7	4.2 \pm 1.3	3.0 \pm 0.4
V_E	ml/kg/min	13.8	28.3	18.6
f'	l/min	35 \pm 2	34 \pm 2	35 \pm 2
W/b	ml.cmH ₂ O/kg	23	22	19
W	ml.cmH ₂ O/min/kg	46	92	57
<u>Vagotomized</u>				
V_T	ml/kg	18.0 \pm 4.0	28.0 \pm 6.0	34.2 \pm 6.0
f	breaths/min	0.6 \pm 0.2	1.6 \pm 0.9	0.8 \pm 0.2
V_E	ml/kg/min	10.8	45.4	27.7
f'	l/min	25 \pm 3	19 \pm 3	21 \pm 2
W/b	ml.cmH ₂ O/kg	110	220	340
W	ml.cmH ₂ O/min/kg	66	352	272

and f' with total ventilation being slightly reduced when compared to values recorded in intact animals. The breathing pattern now generally consisted of single breaths rather than bursts of continuous breathing although this pattern was not seen in all animals.

Under conditions of hypoxia and hypercapnia total ventilation increased 2.6X and 4.2X respectively. The increase in ventilation stimulated by hypoxia was primarily due to a twofold increase in V_T , and a very small increase in f . There was also a lengthening of each breath so that f' was further reduced to 21/min. The increase in \dot{V}_E due to hypercapnia was also due to a large increase in V_T (1.6X) and a 2.7X increase in f . The instantaneous frequency was also reduced to 19/min.

As a consequence of the large increases in V_T and reductions in f' , W/b increased dramatically over the values calculated for intact animals under all conditions. The W/b and \dot{W} values were determined in the same manner as in the intact condition. During air breathing W/b in the vagotomized condition increased 5X compared to values calculated for the intact animals but due to the decrease in f , \dot{W} rose only 1.8X per unit ventilation. Upon exposure to hypoxia and hypercapnia W/b increased 4X and 3X respectively over values obtained for the air breathing condition. Since f also increased upon exposure to hypoxia and hypercapnia in vagotomized animals \dot{W} increased 4X and 5X respectively over the air breathing condition. The increases in \dot{W} under hypoxic and hypercapnic conditions in the vagotomized animals when compared to the intact animals were 3.2X and 2.4X greater per unit ventilation respectively.

Discussion

The breathing pattern recorded in resting, spontaneously breathing Pseudemys scripta in the present study is similar to that recorded by other researchers in semi-aquatic turtles (see Glass and Wood, 1983; Shelton et al., 1984 for reviews). The mean respiratory variables of tidal volume (V_T), breathing frequency (f) and total ventilation (\dot{V}_E) measured in this study are compared to literature values in Table 4.2. The values recorded for f (breaths/min.) fall in the upper range of recorded values for semi-aquatic turtles while values of V_T (ml/kg/min) recorded for P. scripta in this study compare well and are at the low end of previously reported values.

The method of measuring ventilation appears to be an important factor determining the variability of values of V_E found in the literature primarily due to variability in V_T . Almost all previous measurements of ventilation have involved restraining the animal in some fashion, and/or having the animal out of water and, in some cases, catheterized, all of which are unnatural forms of stress. This stress appears to manifest itself in a variable V_T and thus \dot{V}_E . It is felt that the noninvasive method employed in this study provides a close reflection of the true breathing pattern while other studies have tended to overestimate V_T and underestimate f . Glass et al. (1983) using the same method reported identical breathing frequencies, however, V_T was 1.6X larger resulting in \dot{V}_E being 1.5X larger compared to the present study. The differences may have been due to the presence of arterial catheters in the study of Glass et al.

Table 4.2 Comparison of respiratory variables for turtles taken from literature. Standard error shown when reported.

Species	Temp. (°C)	Respiratory gas	V_T (ml/kg)	f (min ⁻¹)	\dot{V}_E (ml/min/kg)	Source
<u>P. floridana</u>	20	Air	12.6	1.4	18.6	Kinney <u>et al.</u> (1977)
<u>P. scripta</u>	20	"	6.9±1.2	2.0±0.7	13.8	Present study
<u>P. scripta</u>	20	"	15.7±2.1	1.6±0.2	23.8±3.4	Jackson <u>et al.</u> (1974)
<u>P. scripta</u>	20	"	8.8*	1.4±0.15	12.3±1.3	Jackson (1973)
<u>C. picta</u>	20	"	10.7±1.2	1.9±0.27	20.3	Glass <u>et al.</u> (1983)
<u>C. picta</u>	22-25	"	13.5±0.9	1.8±0.2	24.8±3.4	Milsom <u>et al.</u> (1980)
<u>T. horsfieldi</u>	23-25	"	8.1	1.4	11.5	Benchetrit <u>et al.</u> (1980)
<u>Hypoxia</u>						
<u>P. scripta</u>	20	3% O ₂	6.8*	2.58±0.25	17.7	Jackson (1973)
<u>P. scripta</u>	20	4% O ₂	6.2±0.8	3.0±0.4	18.6	Present study
<u>C. picta</u>	20	5% O ₂	18.7	2.16	30.0	Glass <u>et al.</u> (1983)

* calculated from reported values of f and V_E .

Table 4.2 (continued)

<u>Species</u>	<u>Temp.</u> <u>(°C)</u>	<u>Respiratory</u> <u>gas</u>	<u>V_T</u> <u>(ml/kg)</u>	<u>f (min⁻¹)</u>	<u>\dot{V}_E</u> <u>(ml/min/kg)</u>	<u>Source</u>
<u>Hypercapnia</u>						
<u>P. scripta</u>	20	4% CO ₂	29.4 \pm 3.6	3.0 \pm 0.4	84.4 \pm 13.3	Jackson <u>et al.</u> (1974)
<u>C. picta</u>	22-25	5% CO ₂	17.5 \pm 2.0	3.9 \pm 0.6	65.5 \pm 11.4	Milsom <u>et al.</u> (1980)
<u>P. scripta</u>	20	3-5% CO ₂	6.8 \pm 0.4	4.2 \pm 1.3	28.3	Present study
<u>T. horsfieldi</u>	23-25	4% CO ₂	15.7	5.3	82.6	Benchetrit <u>et al.</u> (1980)

(1983) or due to species differences.

The ventilatory responses to hypercapnia and hypoxia also differ between various studies. The response to hypercapnia and hypoxia in the present study is a stimulation of ventilation due solely to an increase in f with V_T and breath duration, and therefore f' remaining unchanged. Other studies are in general agreement with hypercapnia (Jackson et al., 1974; Benchetrit and Dejours, 1980; Milsom et al., 1980) and hypoxia (Jackson, 1973; Glass et al., 1983) being ventilatory stimulants and with the former appearing to be the more powerful stimulant of the two. The response to hypercapnia reported in the literature, however, indicates that this response is due to an elevation of V_T as well as an increase in f . A comparison of the results of the present study with those of Jackson et al. (1974), Benchetrit and Dejours (1980), and Milsom and Jones (1980) reveals an absence of the large elevation in V_T in the present study though ventilation appears to increase, more or less, to the same extent. This discrepancy may be due to differences in methodology or it may be due to a lack of equilibrium between blood gases and inspired gas in the present study since blood gas values were not measured to confirm equilibrium had been reached.

The response to hypoxia (3-5% O_2) reported in the literature shows increases in ventilation of 1.5X (Glass et al., 1983) and 1.4X (Jackson, 1973) which are in agreement with the present study (1.3X). The response reported by Glass et al. (1983) was due almost solely to an increase in V_T while Jackson (1973) reported that the increase in V_E upon exposure to hypoxia was due to an increase in f and a drop in V_T . The present study also showed a slight drop in V_T with the response being

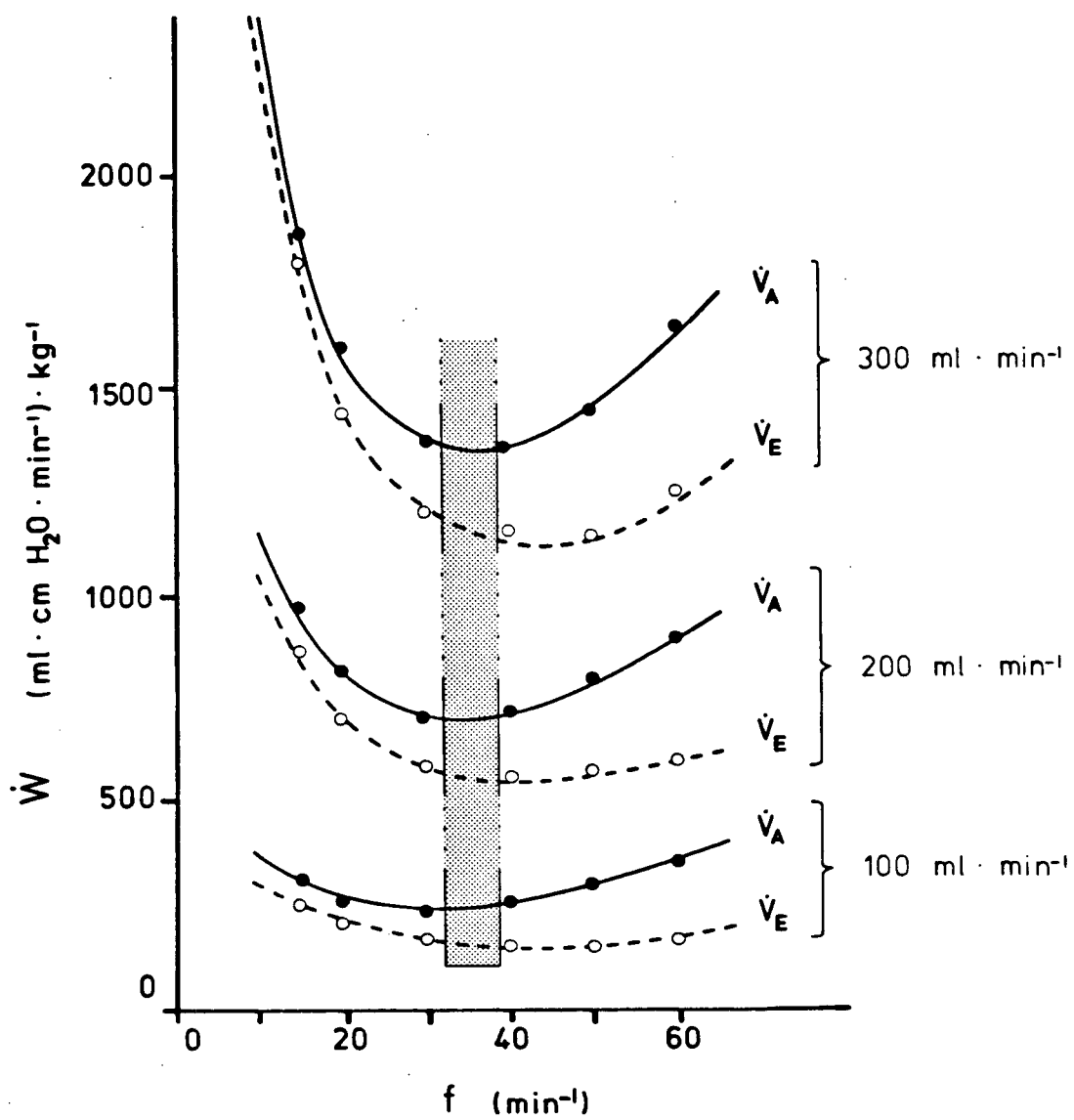
due to an elevation in f as was the case in the study of Jackson (1973).

Thus although there appears to be some variation in the nature of the response to respiratory stimuli reported in the literature it is generally agreed that aquatic turtles increase ventilation by shortening the breath hold period and increasing the number of breaths per minute. This present study confirms this and in addition shows that the characteristics of each breath (i.e. V_T and T_{tot}) remain the same.

Semi-aquatic turtles such as P. scripta spend long periods of time underwater and the nonventilatory period is usually associated with dives. Whatever combinations of respiratory variables initiate and terminate ventilatory periods, the longer a turtle spends underwater, the more breaths it will require to replenish O_2 stores in the lungs and blood and to eliminate CO_2 when the animal surfaces. During such a long bout of continuous breathing, the work of breathing can be analyzed, as in mammals, in terms of W and f , which during the bout of continuous breathing is equal to f' .

Measurements made on continuously pump ventilated animals show that the power required to maintain a constant alveolar ventilation rate (\dot{V}_A) is least at pump frequencies between 30 and 35 cycles/min. (chapter 3). In Fig. 4.2, \dot{W} is plotted against f' for various levels of \dot{V}_E and \dot{V}_A and the shaded area indicates the range of f' calculated for spontaneously breathing animals. Clearly the f' of spontaneously breathing animals corresponds very closely with pump frequencies which generate the minimum rate of work in maintaining a constant level of \dot{V}_A . They correspond less well to those frequencies which correspond to the minimum work required to maintain a constant level of total ventilation (\dot{V}_E). Such considerations

Fig. 4.2 The rate of total work for constant levels of alveolar ventilation (\dot{V}_A in ml/min.) and total ventilation (\dot{V}_E in ml/min). These curves are taken from chapter 3, figure 3.9. The shaded area represents the range of instantaneous breathing frequencies ($f' = 60/T_{\text{tot}}$) measured in spontaneously breathing animals.



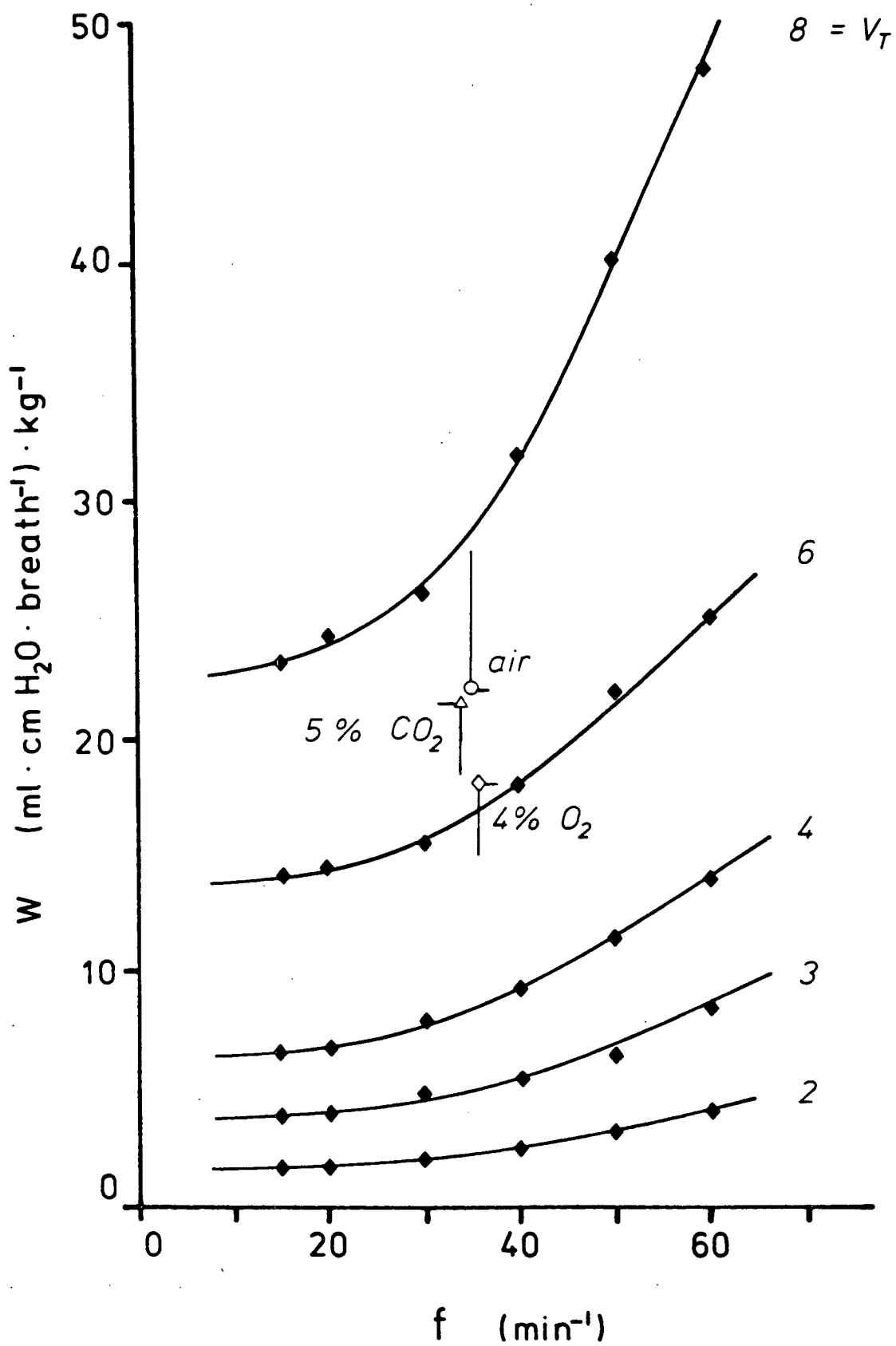
suggest that during the periods of continuous breathing seen in these turtles, as in mammals, spontaneous breathing patterns correspond closely to predicted optimum patterns based on mechanical considerations.

Since marine turtles and tortoises do not breathe in bursts, as seen in P. scripta, but rather take single breaths with periods of breath holding between each breath, it is of interest to analyze the mechanical cost of breathing in terms of the work per breath.

Figure 4.3 shows the mechanical cost required to produce a single breath for various levels of V_T as a function of pump frequency. The values of V_T and f' from intact animals spontaneously breathing various gas mixtures are placed on the graph for comparison. The work/breath curves shown in Fig. 4.3 illustrate that for any given frequency, the smaller the V_T , the lower the mechanical work of each breath. A low V_T , however, compromises alveolar ventilation and gas exchange as was discussed in chapter 3. These two factors, the need to keep V_T sufficiently large in order to maintain alveolar ventilation on the one hand and the increased mechanical work associated with increases in V_T on the other may play important roles in setting V_T .

At any given V_T , the work/breath is then a function of breathing frequency. For a constant V_T the mechanical cost of a breath would be lower the lower the breathing frequency. There are physiological and behavioral factors, however, which would probably restrict breath duration from becoming too long. A very long breath duration in a spontaneously breathing animal would require a slow controlled inspiration while expiration would have to be actively slowed by the braking action of the inspiratory muscles. Since the inspiratory muscles would be working against

Fig. 4.3 The relationship between total work/breath (W) and pump ventilation frequency (f = cycles/min.). Data points for curves represent the mean values for 3 animals taken from figure 3.4, chapter 3. The open symbols represent the mean value \pm S.E.M. of V_T and f' measured from 6 animals spontaneously breathing air (\circ); or 5% CO_2 (Δ) in air; or 4% O_2 (\diamond) in N_2 .



the passive recoil of the system, the stored elastic energy which partially powers both inspiration and expiration would be lost. Although the actual mechanical work of such a spontaneous breath will not be much different from the work recorded when expiration is passive, the oxidative cost would, however, be much greater due to the active muscular work required for the breaking of expiration. This would result in a drop in the efficiency of ventilation. Another important consequence would be the loss of the nonventilatory period. This would result in the semi-aquatic turtle having to shorten its dives and spend more time ventilating at the surface. A further consequence of a long breath duration would be the increased time contribution of the locomotor muscles to their accessory role of ventilation. These factors probably play an important role in restricting the length of breath duration and favouring the retention of a nonventilatory period at the expense of minimizing the work/breath. Inspection of Fig. 4.3 shows that the instantaneous frequency (f') of spontaneous breaths in turtles fall at or near the inflexion point where further increases in pump frequency result in a very sharp rise in the work/breath. The difference in mechanical work between a very low frequency and the f' of a spontaneous breath is however very small compared with the large increase in work/breath which occurs when frequency is elevated above the spontaneous f' . The position of the instantaneous f' on the work/breath curve may represent a compromise which allows for a nonventilatory period and maintains gas exchange and ventilatory efficiency with only a small increase in the work/breath over the breath duration which would minimize the mechanical cost of a single breath.

From the data presented above, it appears that in semi-aquatic

turtles where continuous breathing is not required to meet metabolic demands, but where the oxidative cost of breathing is high (10-30% of total O_2 requirements (Kinney and White, 1977)), the most efficient breathing pattern is an arrhythmic breathing pattern. If oxygen requirements rise or ventilation is stimulated by CO_2 it is better to restrict changes in V_T and f' and take more breaths/burst or shorten the T_{NVP} and take more bursts per unit time. By shortening the breath hold period, or increasing the number of breaths/burst rather than altering V_T or f' , the increase in the rate of mechanical work is minimized.

Pulmonary vagal afferent information appears to play a role in regulating the V_T and f' of each breath (Milsom and Jones, 1980). It could be said then that vagal information plays some role in determining the mechanical work of breathing even though it might be an indirect one. In spontaneously breathing turtles, if vagal information is removed, the work/breath per unit ventilation ($W/b/\dot{V}_E$) increases sixfold due to an increase in V_T and a drop in f' . This must result in an increased oxidative cost for ventilating the lungs. If ventilation is stimulated in a vagotomized animal through hypoxia or hypercapnia, the work/breath increases 2 and 3 fold respectively due to further increases in V_T and decreases in f' . This is unlike the situation in intact animals where there are no appreciable changes in V_T and f' upon ventilatory stimulation.

Although it is not totally clear what combination of sensory inputs initiate and terminate breathing (Lenfant and Johansen, 1968; Lenfant et al., 1970; Toews et al., 1971; Burggren and Shelton, 1979; Ackerman and White, 1979; Boutilier, 1981) once breathing is initiated, the breath length and tidal volume appear to be regulated at levels which minimize the work of

breathing. Whether pulmonary afferent information is directly responsible for the sensing of mechanical work or if minimal mechanical work is simply a fortuitous consequence of tidal volume regulation remains unclear.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Analyses of pulmonary mechanics in anaesthetized turtles have shown that the architecture of the body wall and lung determine the overall mechanics of the respiratory system which in turn appears to influence the spontaneous breathing pattern in the animal. The lungs of the turtle are highly compliant as a result of the presence of large membraneous caudal regions (Perry and Duncker, 1978). The majority of elastic forces which must be overcome in the system during ventilation are located within the body wall of the animal. As a consequence the compliance of the body wall is relatively low and is the key factor determining the compliance of the whole system.

Under dynamic conditions of continuous pump ventilation the dynamic compliance of the system is independent of stroke volume over a range of volumes around the normal V_T of these animals but is dependent on ventilation frequency. For any given volume, as frequency increases C_{dyn} decreases which is reflected in a rise in the work/breath with increasing frequency. Analysis of the components which contribute to the total work of each breath shows that the majority of the work required to produce each breath at low ventilation frequencies is required to overcome forces within the body wall. If tidal volume is held constant and ventilation frequency increased, the proportion of the total work required to overcome forces arising from the lungs steadily rises with these forces being nonelastic, flow-resistive forces. The work required to overcome the

the forces within the body does not change as frequency increases suggesting that these forces are predominantly elastic in nature. If frequency is held constant and volume increased then the work required to overcome the forces resisting lung inflation rises exponentially and is predominantly required to overcome increased elastic forces. These effects of changes in respiratory frequency and tidal volume on the work required to generate each breath influence the relationship between the power required to ventilate the lungs and the breathing frequency for a constant level of alveolar ventilation.

The relationship between the power required to maintain a constant level of alveolar ventilation and breathing frequency takes the form of a U-shaped curve. As frequency increases elastic work decreases due to the necessary drop in V_T required to maintain constant \dot{V}_A . With further increases in frequency, however, minute work begins to rise again due to increasing nonelastic forces and the required increase in total ventilation needed to offset dead space ventilation required to keep \dot{V}_A constant. As a consequence there is a combination of frequency and tidal volume at which the work required to overcome the forces associated with ventilation are at a minimum for each level of \dot{V}_A . The frequency that corresponds to minimum mechanical work is approximately 35 breaths/min.

In spontaneously breathing turtles, the respiratory pattern consists of bursts of continuous breathing separated by a variable breath hold period beginning at end-inspiration. The consequence of this breath hold period is a very low overall breathing frequency of approximately 2 breaths/min. under normoxic resting conditions. The frequency of breathing within the burst, however is 30-35 breaths/min. and is referred to as the

instantaneous breathing frequency (f'). This value corresponds very closely to the ventilation frequency calculated to require the minimum mechanical work to produce similar levels of \dot{V}_A . If respiration is stimulated by hypoxia or hypercapnia V_T and f' change very little and the animal increases ventilation by taking a larger number of breaths at this optimal combination of V_T and f' . If the work of spontaneous breathing is analyzed in terms of a single breath rather than a burst of continuous breaths, f' is still held at a level which tends to minimize the mechanical cost of a single breath.

This mechanical analysis of the respiratory system in the turtle has provided insights which may help explain the adaptive significance of arrhythmic breathing. Since turtles have a very low metabolic rate continuous breathing is unnecessary. Instead these animals appear to have adopted a strategy of periodic breathing which still minimizes the mechanical cost of breathing and thus the oxidative cost of breathing. This is important since the metabolic cost of breathing is already very high (10-30% of resting metabolic rate (Kinney and White, 1977)) and because breath hold periods allow these aquatic animals to spend time underwater and still maintain oxidative metabolism.

This type of arrhythmic breathing which minimizes the mechanical cost of breathing appears to be under neural control. If the animals are bilaterally vagotomized, tidal volume increases, f' decreases along with f so that total ventilation doesn't change appreciably but the mechanical cost greatly increases. While vagal afferent information may not sense mechanical work directly it does regulate V_T and f' in such a way as to minimize the mechanical cost. Under the influence of afferent vagal control

the response to respiratory stimuli is to increase the number of breaths but to maintain V_T and f' . This serves to minimize the mechanical cost and thus the oxidative cost of the increase in ventilation. While the variables which initiate the onset and termination of the ventilatory period in arrhythmically breathing reptiles remain unclear mechanical analysis has provided a framework for better understanding the regulation which does occur within the breathing episode.

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