

THE MECHANISM OF LUNG VENTILATION IN THE TORTOISE *TESTUDO GRAECA* LINNÉ

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(Received 6 February 1967)

INTRODUCTION

Air-breathing vertebrates use at least two fundamentally distinct gas-pumping mechanisms. In the 'mammalian' pattern the animal reduces the lung pressure below atmospheric; inflow thus occurs as a result of suction through a completely open channel. In the 'frog' pattern, however, air does not pass directly into the lungs which are filled by the action of a buccal force pump. The animals relying upon the second pattern presumably lack an arrangement for creating subatmospheric pressures in the lung cavity. They pump air by contracting the muscles of the buccal cavity, alternately opening and closing the nasal and glottal valves (Hughes, 1963).

Recent reptiles are surviving members of a group that was presumably intermediate between the early amphibians and mammals. The nature of their respiratory cycle might then offer some clues regarding the development of air-breathing mechanisms. The situation in chelonians is of further interest since their rigid and box-shaped skeleton imposes exceptional demands upon the filling mechanism; the placement of the lungs within a rigid frame suggests analogies with the avian condition which have been reflected in some of the physiological explanations offered by authors (cf. Wolf, 1933).

The literature contains a variety of 'functional' extrapolations regarding the mechanisms by which tortoise lungs are filled and emptied. The older authors (Malpighi, 1719; Agassiz, 1857; Milne-Edwards, 1857) tended towards unequivocal statements that filling occurred by means of a buccal pump and cited, in support, the easily observed pumping movement of the throat. This view, retaining Morgagni's claim (1719) that turtles breathe like frogs, was still perpetuated in some recent textbooks (Hansen, 1941), even though Townson (1799), Mitchell & Morehouse (1863) and again McCutcheon (1943) provided strong evidence that ruled out the swallowing of air as the major inspiratory mechanism. These authors considered that changes in lung volume were produced by the alternate contraction of the 'respiratory muscles', two sets of antagonistic muscles spanning the flank cavities lateral to the limbs. Tauverý's (1701) claim that turtles breathe only in walking was apparently based upon his observation of the marked pumping movements of the limbs, which led Wolf (1933) to claim that these powered the respiratory cycle. Lüdicke (1936) accepted the function of the respiratory muscles, but stated that *Emys* (and 'freshwater' turtles in general) were able during emergencies to 'swallow' air by convulsive contraction of the air-filled buccal region. McCutcheon (1943) could not confirm this on *Malaclemmys* and some other North American species.

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More recently George & Shah (1954, 1955, 1959*a, b*) and Shah (1962), in a series of studies, have pointed out the marked variation of the respiratory musculature in different groups of turtles. Their investigation was started after the discovery of a striated muscle sheath completely enveloping the lungs of the 'soft-shelled' turtle, *Lissemys*. The investigation demonstrated major differences between, for instance, *Emys* and *Malaclemmys* which might reconcile the conflicting views of some earlier investigators. The authors further suggested that a major physiological change had taken place within the turtles. In advanced forms, including the testudinids, inspiration alone is actively powered, while expiration occurs passively as the respiratory muscles relax.

All previous conclusions regarding muscle action were derived by extrapolation from anatomy, coupled with the observation of very artificial preparations. Hence there was an evident need for studies using more direct methods of the type here described for a species of *Testudo*, the most highly specialized genus in the George & Shah scheme. The aim of the present work was to determine by means of electromyography the pattern and timing of activity of the muscles important in respiration in relation to simultaneous recordings of lung pressures and forelimb movements. The methods used were intended, as far as possible, to yield records from unanaesthetized and unrestrained animals. The ease with which they could be applied suggests the utility of such preparations both for teaching demonstrations and for a variety of physiological studies. A preliminary report of this work has already been given (Hughes & Gans, 1966).

MATERIALS AND METHODS

The observations were made on eighteen individuals (200–900 g.) of both sexes of *Testudo graeca* Linné, obtained from an animal supply house and presumably of different provenance. No differences pertinent to the present studies were observed. All observations were made at temperatures between 20° and 22° C.

The shell of a tortoise is formed of a series of bony elements which are overlain by the external, keratinous shields. The two sets of elements are independent; they differ in number, size and shape so that their sutures do not coincide. Their relations are, however, constant to each other and to the underlying blood vessels and organs. This makes it possible to map on the carapace of a particular species the attachments of a number of limb muscles and respiratory muscles and of the diaphragmatic membrane, as well as of specific regions of viscera and lungs. Holes drilled for the placement of cannulae and electrodes can be positioned with accuracy and experiments can be repeated with relative ease.

Tortoises were anaesthetized in mixtures of CO₂ and Halothane, administered in a desiccator. A 2.5 mm. hole was drilled into the left second costal of most specimens and a cannula was attached to the hole. Various tests suggested that the holes remained open for days when the perforations through the internal periosteum and adherent soft tissues were immediately cauterized. The best method of lung cannulation was by cementing 2 cm. lengths of 4 mm. glass tubing on to the hole in the shell with a thick and wide layer of epoxy resin. The tubes were closed between experiments either by a silicone rubber seal or by clips on short lengths of rubber tubing. Visceral pressures were recorded from a catheter made of a length of polyethylene tubing which was brought out through a sealed opening near the ventro-lateral edge of the carapace.

Pressure recordings were made using two Sanborn 268B pressure transducers. The output of their 350-1100C Carrier preamplifiers was fed into a Tektronix 565 oscilloscope and a Devices six-channel pen-recorder.

Movement records of the forelimb of quietly breathing animals were obtained by means of an RCA 5734 mechano-electric transducer valve. A slender rod attached to the anode of this valve was lightly pressed against the forelimb so that the movements were not hindered. The output of the transducer was amplified and fed to the oscilloscope and pen-recorder.

Electromyographic records were led off with two types of electrode systems. Shielded needle electrodes were found to be most useful for the initial location of muscles and for records taken through the shell. These electrodes contained one or two strands of fine copper wire which had been cemented into a no. 8 hypodermic needle. For longer-term observations and for records of the glottis muscles two strands of fine wire were inserted by means of a no. 22 hypodermic needle that was then withdrawn (Basmajian & Stecko, 1962). The wires from the glottis muscles were run out through the angle of the mouth and fixed externally to the skin by means of rapidly drying cement (Eastman 910). The electrical activity of the muscles was amplified using Tektronix 122 preamplifiers.

Electrocardiograms were most consistently obtained by recording from stainless pin electrodes placed in the right axilla and left groin immediately below the carapace.

Through the courtesy of Dr G. M. Ardran of the Nuffield Institute for Medical Research at Oxford, we were able to make an X-ray film of a rapidly breathing tortoise. The record was made on 35 mm. film at 2 frames/sec. in a dorsoventral direction and showed the girdle movements.

RESULTS

A. Morphological

The respiratory system

During normal respiration the mouth is held closed and air enters through the nostrils which appear to be always open and lack valves (cf. Lüdicke, 1936). The nares open into the dorsal part of the buccal cavity immediately dorsal to the tongue, behind which lies the slit-like glottis. The soft and highly muscular nature of the posterior region of the buccal cavity, together with the mechanics of the branchial elements, permit marked antero-posterior and dorsoventral movement of the glottis itself.

The cricoid and arytenoid cartilages form the skeletal framework of the glottis. The inherent elasticity of the arytenoid and the adhesive nature of its mucous membranes tends to keep the glottal slit closed and closure appears to be reinforced by some circular muscle fibres. Very large opening muscles are attached to the dorsal horns of the arytenoid and run backward along its sides.

The trachea is short and soon divides into paired bronchi which remain separate along the sides of the neck, which probably keeps them out of the way of the medial retractor muscles of the head and neck.

The bronchi extend the length of the lungs, entering at the level of the third chamber. Each bronchus here bifurcates, one branch running posteriorly and the

other anteriorly to end in the most anterior of the lung's main chambers. These branches remain patent and are perforated along their length so that air can enter or leave them anywhere.

The very large lungs occupy the dorsal half of the body cavity with the animal in a resting position. Their volume is reduced to one-fifth when head and limbs are fully retracted (Pl. 1). There is a suggestion that the right lung is somewhat larger than the left. They extend from the pectoral to the pelvic girdles and their anterior aspects reach forward on both sides of the pectoral apparatus. Both anteriorly and posteriorly the pleura fits against the muscles covering the girdle elements; dorsally it attaches intimately to the periosteum. Ventrally the pleura attaches closely to the more or less horizontal non-muscular connective tissue sheet ('diaphragm') which separates the body cavity into pleural and peritoneal spaces. The walls of the lungs appear closely attached to all of these limiting membranes. Consequently in spite of their inherent elasticity the lungs do not collapse when the pleural cavity is opened.

In longitudinal section each lung appears divided into five or six major chambers, separated from one another by vertical membranous plates. The anterior two or three chambers are the smallest, and the posterior one is very much the largest. Within each major chamber partial subdivisions form an irregular meshwork. Connexion between the main chambers occurs only via the bronchi. Wolf (1933) suggests that other connexions 'should' exist and are occasionally found, but we have been unable to confirm their existence.

The remaining viscera lie within the shell, between the girdles and in the space defined by diaphragm and plastron. The heart is situated in a pericardial cavity in the anterior part of this space. A portion of the liver, and of various sections of the alimentary canal, shows a firm connexion to the diaphragmatic sheet. Since these elements are otherwise more loosely placed, without firm connexions either to ventral or lateral aspects of the shell, their weight would tend to keep the diaphragm stretched downwards.

Musculature

All muscles are described on the basis of gross dissections and frozen sections cut from the whole animal. The detailed fibre connexions were not checked histologically.

The anterior aperture of the shell is almost completely taken up by skeleton and muscles which suspend the head and forelimbs.

Each triradiate half of the shoulder girdle is cradled in a complex of red-fibred muscles that can freely rotate it around top and bottom connexion of the angled scapula to shell, moving the forelimbs into and out of the shell (Walker, 1963). The most obvious muscle inducing inward rotation is the fan-shaped *M. pectoralis* which originates over a wide area of the plastron, and inserts via a tendon on the lesser tuberosity of the humerus. Outward rotation is produced by the *M. serratus magnus* (= *M. testocoracoideus*) which originates near the anterior edge of the carapace, dorsal to the limb position, and inserts, on the antero-dorsal aspect of the flattened coracoid. Rotation of the limb girdles shifts the anterior limits of the visceral cavity.

The pelvic girdle and limbs are more centrally located within the posterior aperture of the shell; the girdle itself is immobile. A relatively great area of each flank lateral to the hind limbs is formed only of the skin and a thin sheet of muscle and connective

tissues, the complex posterior limiting membrane of the visceral cavity. Here lie the *M. obliquus abdominis* and the *M. transversus abdominis*, the so-called respiratory muscles.

The short-fibred, brownish *M. transversus* originates from an approximately elliptical area on the inner surface of the carapace corresponding to the postero-ventral portion of the third costal and the dorsal parts of the seventh, eighth and ninth marginals. It inserts on a fairly dense sheet of connective tissue which forms the posterior closure of the visceral cavity. The fibres of the *M. transversus* passing posteroventrally continue as a series of connective tissue fibres which join and attach near the pubic symphysis.

The *M. obliquus abdominis* originates in various slips from the postero-dorsal margin of the flank aperture and crosses this, just deep to the skin, to insert on the posterior limiting membrane immediately postero-ventral to the insertion of the *M. transversus*. This red-fibred muscle can thus be reached inward of the dorsal half of the eighth, ninth and tenth marginals.

The muscle connective-tissue system of the posterior limiting membrane then forms an anteriorly concave cup that encloses the viscera within the concavity. Contraction of the *M. transversus* tends to flatten the curvature and thus exert pressure on the contained viscera; contraction of the *M. obliquus* increases the curvature and decreases the visceral pressure.

Respiratory movements

B. Experimental

A resting tortoise exhibits two kinds of rhythmic movements. The first is an occasional buccal pulsation which can, upon close observation, be seen to result from ventral shifts of the branchial elements posterior to the lower jaws. The second is a slow inward-outward movement of the forelimbs. Only extremely low amplitude movements of the hind limbs are noticeable. The mouth generally remains closed and only rarely are the breathing noises audible.

The buccal movements of *Testudo* seem to be olfactory rather than respiratory (Mitchell & Morehouse, 1863; Lüdicke, 1936; McCutcheon, 1943). The movements do not correspond to intrapulmonary pressure changes, but they are insignificantly correlated with epithelial gas absorption (Vos, 1936) and their frequency and amplitude are affected by the presence of food.

The reciprocating forelimb movements, which will here be shown to be associated with ventilation, have been described by many previous workers, and Wolf (1933), among others, claimed that they (and the associated hind-limb movements) were primarily responsible for ventilation. This is only acceptable if it is assumed that these animals undergo periods of apnoea whenever they wedge themselves into tight corners, precluding any movements of the head and neck. The predilection of many specimens for long periods of rest in tight crevices suggests that the forelimb movements have an accessory, rather than a primary, respiratory function.

An increase in the breathing rate by fright, exercise, or CO_2 induces a much more noticeable ventilation cycle. The amplitude and frequency of limb movements increases. The head and neck become involved, pumping in and out synchronously with the limbs. The mouth starts to open and the opening and closing of the glottis may be observed. A highly disturbed tortoise gasps with extended neck and its breaths are quite audible.

Pressure recordings

Ten tortoises were fitted with lung cannulae and their intrapulmonary pressures were recorded for varying periods. The most characteristic features found in all records were the intermittency of the lung ventilating movements and the flexibility of the 'baseline', i.e. that pressure maintained during periods of apnoea.

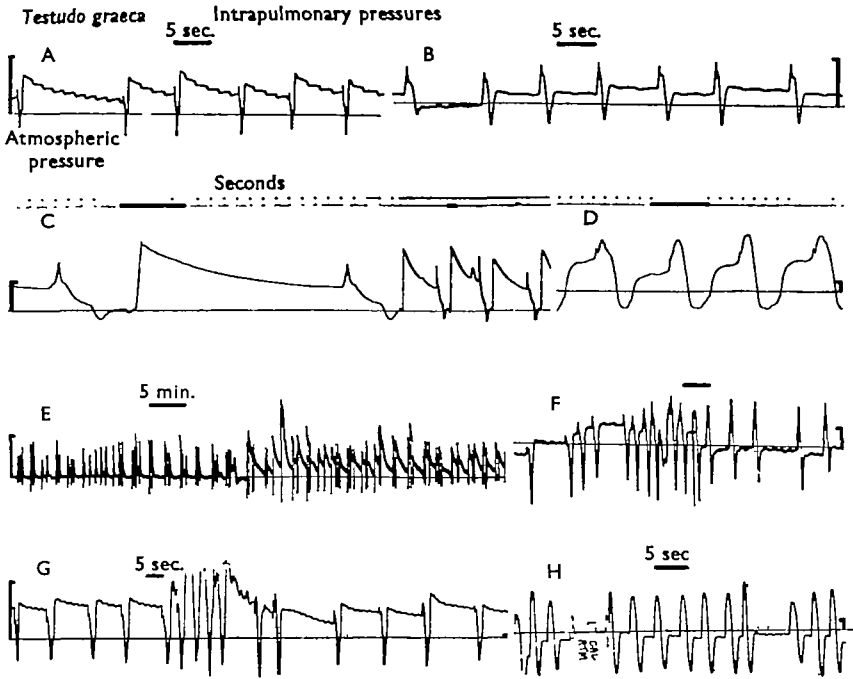
The breathing 'rhythm' of tortoises consists of pauses, interrupted by ventilating movements, here referred to as ventilating cycles. The pauses lasted between 4 sec. and 23 min.; in the first case the tortoise had been severely disturbed, in the latter it may have been disturbance that terminated the pause. The interval between cycles was more or less variable; only during the recovery from CO₂ anaesthesia did the cycles immediately follow one another. We never observed the bursts of some half-dozen cycles followed by a long pause as described by Shaw & Baldwin (1935) and McCutcheon (1943); perhaps they are characteristic of diving turtles as Vos (1936) did not report them in *Testudo*. Shaw & Baldwin (1935) never assigned their observations to any particular species, but their published record suggests that it was taken on a severely disturbed animal.

The intrapulmonary pressure varies both between successive pauses and during any single pause. It is maintained near, normally above, atmospheric pressure, with a range of -0.8 to $+7$ cm. of water though much higher pressures are observed during dyspnoea following the inhalation of carbon dioxide. This is in good agreement with the values of $2.7-5.6$ (momentarily $20-24$ cm.) of water reported as tracheal pressure measurements by McCutcheon (1943). When the intrapulmonary pressure is artificially dropped, by opening the sampling connexions, the pressure remains near atmospheric until the next cycle.

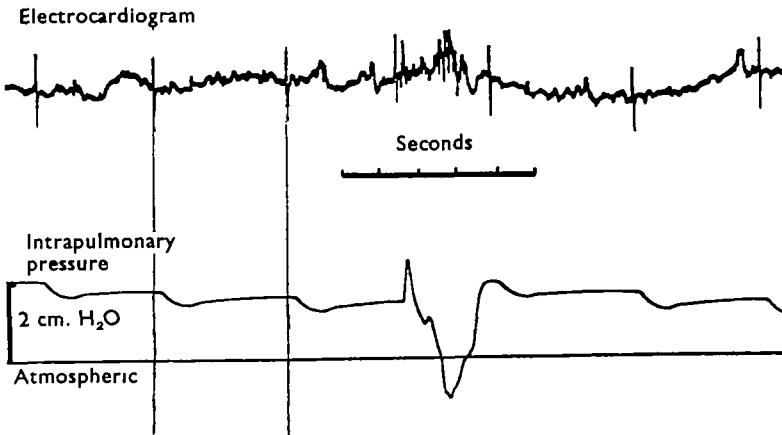
Whenever the intrapulmonary pressure is maintained above atmospheric the pressure will tend to be higher after the completion of each ventilating cycle than at its start (Text-fig. 1 A, C, G). There is a subsequent decrease of pressure before the next cycle. If the positive pressure at the beginning of a pause exceeds $2-4$ cm. H₂O there occurs a characteristically rapid and logarithmic fall in pressure toward the baseline (Text-fig. 1 A, C). Indications of this logarithmic decay are also seen at lower pressure levels and its causes will be discussed below.

The pulmonary pressure record reflects the pulsing heart-beat as a sharp drop of about 0.2 cm. of water in pressure some constant period after the PQRS of the electrocardiogram (ECG) (Text-figs. 1 A, 2). A very slight but equivalently sharp pressure recovery is followed by a gradual increase which ceases just before the next PQRS. The constant interval between electrical and pressure records is about 0.2 sec. with an average interval between heart beats of 5.5 sec. The pressure drop thus correlated with maximum ventricular contraction. Variations in heart rate are accompanied by changes in the recovery rate and not in the time required for the sharp decrease in pressure.

Fluctuations in base-line pressure also reflect other non-respiratory events. Any shift of the head and neck or of the limbs causes major and simultaneous fluctuations (Text-fig. 1 G). Their magnitude often exceeds and may be more than twice the maximum observed during normal ventilating cycles. The position at which the girdles come to rest determines the new base-line, which often differs from the preceding value. (Text-figs. 1 E, F; 3).

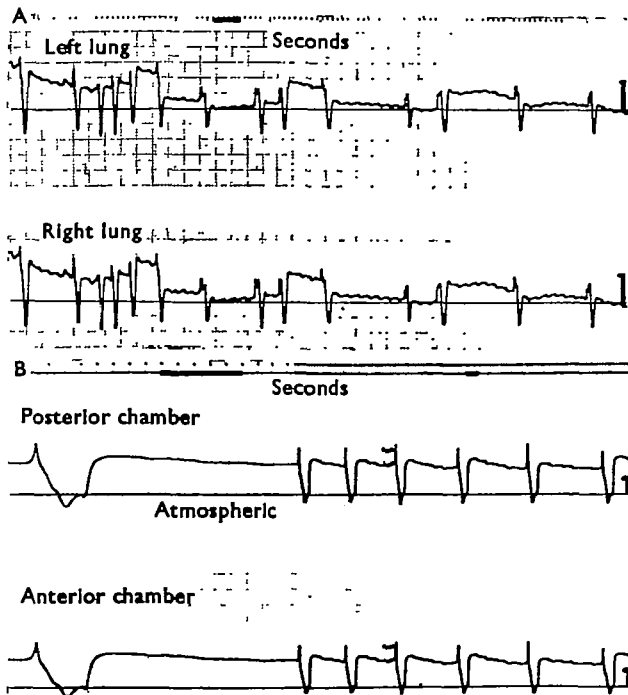


Text-fig. 1. *Testudo graeca*. Records of intrapulmonary pressures during ventilation in a variety of preparations. Atmospheric pressure is shown by a horizontal line in each case. A, Showing decline in overshoot pressure with superimposed changes due to heart contraction. B, Fairly regular pressure changes with little overshoot. C, Very marked overshoot and decline to base-line level, which is above atmospheric. D, Biphasic pressure changes following inhalation of CO_2 . E, Part of prolonged recording showing transition in type of pressure waveform involving a shift in the base-line. F, Similar change as in E but faster time-scale (arrow indicates artifact due to pause in pen recorder). G, Recordings showing larger pressure changes associated with movement of the animal. H, Large pressure changes following CO_2 ; base-line level is here below atmospheric. Block time-scales = 5 sec. Calibration pressure = 5 cm. water.



Text-fig. 2. Simultaneous recording of electrocardiogram and intrapulmonary pressure. Notice large PQRST precedes reduction in pressure record. A single ventilation cycle is shown and is associated with an electromyogram on the ECG record.

The pressure records from the left lung were compared with simultaneous records of the right lung of the same specimen. They appeared identical (Text-fig. 3) and records of the left/right pressure differential show extremely minor and transient pressure differences between the two lungs. The small differences suggest that the pressures in the two chambers are determined by a single external event rather than each being directly and independently induced. Pressure equalization of transient differentials might occur by movement of the medial interpulmonary membrane, or by mass flow through the bronchial bifurcation at the trachea. When the pressure in one lung was dropped to atmospheric experimentally there was a slow drop of the pressure in the other lung.



Text-fig. 3. Simultaneous records from different parts of the pulmonary system during ventilation. A, Left and right lungs show almost identical waveforms. B, Similar recordings from anterior and posterior chambers of the same lung. Calibration pressure = +5 cm. water. Block time-scales = 5 sec.

Recordings of the intrapulmonary pressure and of the pressure in the peritoneal cavity suggested that the latter is at a level less than 70% of the former. Initially records showed parallel fluctuations during a series of ventilating cycles, but the peritoneal recording was much less sensitive to minor changes and showed no trace of the cardiac pulse. The visceral connexions ceased to record any pressure changes after a brief period, presumably because the cannulae were blocked by the intestinal folds. It is probable that the reduced pressure recorded from the peritoneal cavity represents an artifact; otherwise it would be necessary to assume a pressure transmission with local displacement of the diaphragm by viscera acting as linkage elements.

Wolf (1933) has suggested that the chelonian lung incorporates an 'avian' flow

pattern in which the inspired air first enters the posterior chamber and then passes to the more anterior ones where most of the gas exchange takes place. Simultaneous recordings of the pressure in the posterior chamber and of the differential between it and the most anterior chamber on the same side did not provide any evidence for this hypothesis (Text-fig. 3). The transient pressure differentials are equivalent to those between the two lungs, and with amplitudes of about 1 mm. water are probably too small to induce significant intrapulmonary flow. Pressure equalization may occur both by mass flow along the bronchi and by deflection of the septa between the chambers.

The ventilation cycle

The pressure changes during a typical cycle are triphasic and include successively a pressure increase, a decrease to below atmospheric pressure, and a secondary increase (Text-fig. 8). A considerable number of variations occur on this pattern; the most important concern the nature of the initial pressure increase, the period and nature of the pressure change during the time the lung is held below atmospheric pressure, and the height of the 'overshoot' of the terminal increase above the new baseline.

(i) The initial pressure increase is of variable height. Normally it rises to a sharp peak from which it falls equally rapidly. In other cases the peak is truncated or has several subpeaks. Sometimes there is only a very minor peak which is often preceded by a period in which the pressure is slightly and irregularly modified. In animals recovering from anaesthesia the pressure increase seems to be more gradual and the zone of maximum pressure forms a rounded rather than a very sharp peak.

(ii) The second phase consists of a decrease in pressure to below atmospheric. The initial fall-off is generally very sharp and is followed by a shoulder in the curve, usually above or near the pre-cycle base-line and always above atmospheric pressure. Following this the pressure again drops very sharply to near minimum. A small number of recordings show a secondary and more gradual depression to a still lower level at or near which the pressure is maintained for a varying period.

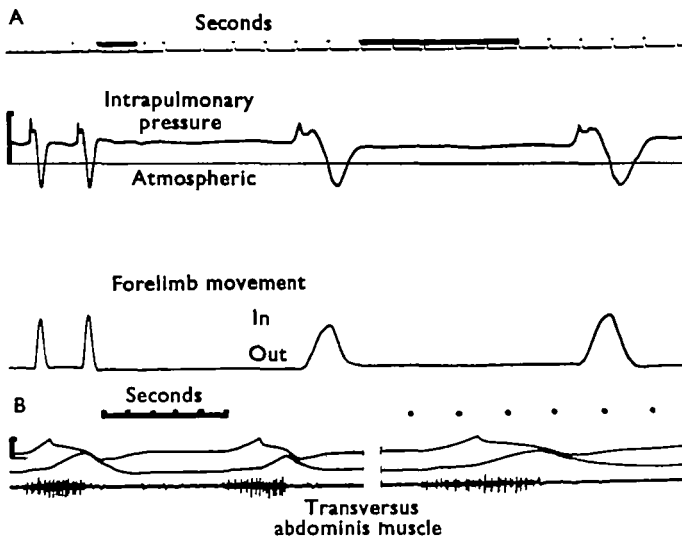
(iii) The third or recompression phase starts with a gradual increase in pressure which becomes very steep. The curve almost always shows a shoulder just below atmospheric pressure. It then rises even more steeply to the level of, or slightly above, the new base-line.

The amplitude of the positive pressure at the end of phase (i) is generally at least twice that of the negative pressure at the end of phase two. The lung pressure appears to remain below atmospheric for more than 60 % of each cycle.

In a limited number of records the recompression markedly exceeds the post-cycle base-line. The pressure first rises to a new peak and then falls to the new baseline. This breathing pattern was observed in some tortoises in which the baseline was temporarily maintained at a very constant level slightly above atmospheric pressure. They breathed with an essentially quadriphasic sequence involving a primary increase and decrease, followed by a secondary sequence (with the glottis closed). This relatively rare behaviour may account for Shaw & Baldwin's (1935) observation that each cycle is terminated by a partial 'exhalation'.

Forelimb movements

The very noticeable movements of the forelimb suggested a role in ventilation and this was confirmed by recording them simultaneously with the pressure changes (Text-figs. 4-6). It was observed that specimens would often rest quietly for hours if they were given the opportunity of wedging the shell against a wall of the cage. The force was exerted by the hind limbs and the movements of the forelimbs were almost completely abolished when the body rested at right angles to the wall. If the tortoises approached the wall at an angle of more than 45° they would still engage in the wedging action but would rest at an angle with one forelimb free. The free limb continued the ventilating movements with full amplitude and could be used to record them.

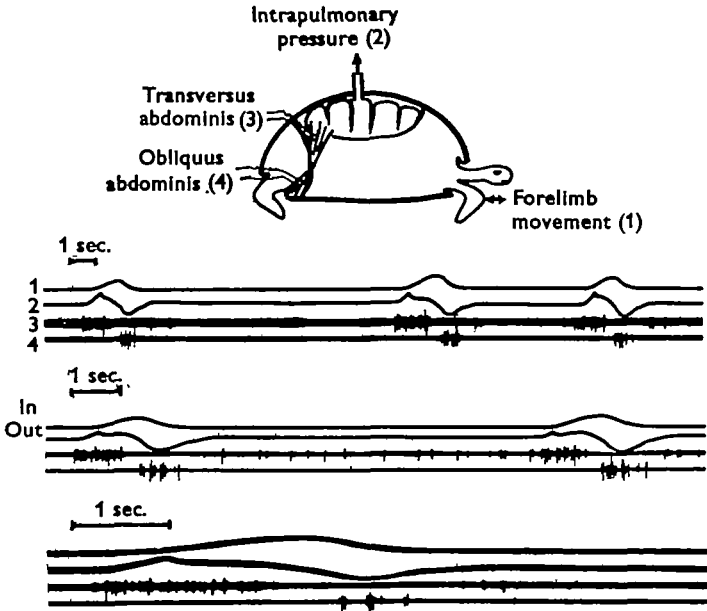


Text-fig. 4. A., Simultaneous pen recording of intrapulmonary pressure and associated forelimb movements. B., Oscilloscope records of pressure and movements together with electromyograms of transversus abdominis. Note movement and pressure changes occur simultaneously at beginning of cycle. Calibration = +5 cm. water. Block time-scales = 5 sec.

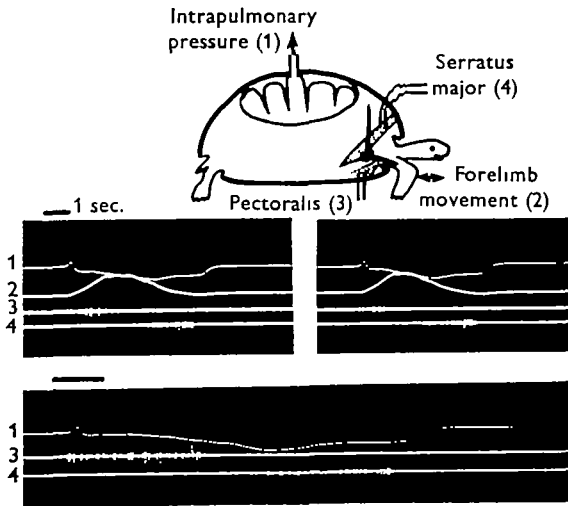
The recordings in Text-figs. 4 and 5 show that a limb movement and a ventilating cycle always accompanied each other. The terminal positions of the limbs did not vary too much; they seemed far more constant than the pressure base-lines. The limb movements consist of an inward (into the shell) and an immediate return outward movement, both of which appeared to start very rapidly and slow down near their ends. Initial analysis of the recordings suggested that the start of the inward movement coincides with the peak of the initial pressure increase, i.e. with the end of the first ventilation phase. Further analysis of oscilloscope records, and with careful placement of the recording lever, showed that the limb movements occurred throughout the whole cycle (Text-fig. 4). Thus the inward movement begins at the start of the first phase when the pressure starts to rise and the maximum pressure peak coincides with a sudden acceleration of the movement.

The reversal in direction of limb movement coincides with the shoulder in the

decreasing pressure curve of the second phase, marking the point at which the pressure starts to fall very sharply. The outward movement is completed shortly before the rising pressure of the third phase reaches the atmospheric level. It may overshoot slightly so that there is often a terminal inward movement and it finally comes to rest after atmospheric pressure is reached.



Text-fig. 5. Simultaneous recordings of (1) forelimb movement, (2) intrapulmonary pressure, electrical activity in (3) transversus abdominis and (4) obliquus abdominis muscles. Recordings at three speeds are shown. In B, note activity in the transversus abdominis between individual ventilation cycles.



Text-fig. 6. Simultaneous recordings of (1) intrapulmonary pressure, (2) forelimb; movement and electrical activity in (3) the pectoralis and (4) serratus major muscles. Inward movement of the forelimb is upward on the tracing.

X-ray ciné films showed that these forelimb movements are due to movements of the girdle rather than to intra-limb movements. The girdles rotate inward, carrying the glenoid cavity with them, so that the limbs are pulled into the shell (Text-fig. 5). As long as the forelimbs are incompletely retracted the movement remains possible; it would be impossible in a frightened or resting tortoise that had achieved maximum inward movement of limbs and girdles.

Respiratory muscle activity

Electromyograms were obtained using fine wire and/or shielded bipolar needle electrodes placed at various sites within the *M. transversus abdominis* and the *M. obliquus abdominis*. Activity recorded simultaneously with lung pressures and forelimb movements (Text-fig. 5) showed that these muscles fire during each ventilating cycle. The two muscles appear to be truly antagonistic in their respiratory function as suggested by the apparent inhibition of the *M. transversus* when the *M. obliquus* fires.

The *M. transversus abdominis* starts to fire when the first phase of the cycle begins (Text-fig. 4) and continues as far as the shoulder in the descending pressure curve, when it cuts off sharply. A secondary burst of signals of somewhat lower amplitude sometimes starts at or very close to the point at which the ascending pressure curve passes atmospheric pressure (Text-fig. 5). Its firing rate falls rapidly when the new baseline pressure is reached and a gradual decay in firing follows until only intermittent discharges are heard or until the discharge ceases entirely.

The magnitude of the initial burst appears to be roughly proportional to that of the pressure build-up during the first phase of ventilation. Several records also show a sudden discharge of this muscle during the pause; the firing rate in each case corresponds to the transient pressure build-up, and the pressure returns to the base-line as the firing ceases. Some parts of the muscle (or perhaps some preparations) show a much greater secondary or recompression discharge than others. The *M. transversus* never fires between the first and secondary discharges. The hypothesis that this is due to an active inhibition is supported by observations on a preparation in which the muscle had been accidentally damaged during placement of electrodes. This showed an almost continuous discharge from the muscle throughout the pause, but total silence while the lung was at subatmospheric pressure.

The response obtained from the *M. obliquus* varied slightly in different sites along its length both in amplitude and time of onset of its discharge. The muscle fired most strongly during the inactive period of the *M. transversus*, starting as the pressure curve crossed the atmospheric level and stopping about $\frac{1}{10}$ sec. before the pressure again rises to this level. High-speed records show that there are two brief periods when neither muscle fires; there is thus no overlap (Text-fig. 5). The discharge of the *M. obliquus* did not appear to be proportional to the negative pressure generated. This would be expected if we were sampling a restricted region of a long muscle in which the motor units are arranged regionally. The *M. obliquus* also fires during some limb movements, though details of correlation are beyond the scope of this paper.

Activity in girdle muscles

Records were taken from a variety of sites within the *M. pectoralis* and from sites near the origin of the *M. serratus magnus*. Both muscles were clearly locomotory, firing regularly during any movement of the animal's forelimbs and their firing rate differed when the animal moved more toward one side than the other. Both muscles also fire when the girdles move in synchrony with the ventilation movements.

The major portion of the *M. pectoralis* starts to fire at the beginning of the inward movement of the limbs and stops sharply as they reach their reversing point. (Text-fig. 6). A response of lower intensity, perhaps 30% of the final level, begins at or very close to the rise of the primary compression phase of the cycle. This activity is only noticeable in certain portions of the muscle, suggesting that only specific motor units are involved. The regions that do not show this response also cease firing for a brief period earlier in the cycle. The firing sequence of this complex muscle presumably meets its primary functions in locomotion. Very few fibres are active if the limb movement curve shows overtravel outward and is returning to base-line.

The *M. serratus magnus* becomes active after the lung pressure has dropped to atmospheric and continues firing until just before the rapid pressure rise (Text-fig. 6). The firing rate increases gradually until just before the end when there is generally a marked high-frequency burst of signals of larger amplitude. This increased firing occurs when the pulmonary pressure has nearly reached its lowest level. In relation to the movements this muscle fires after the outward movement of the forelimb has started, but continues until it is fully extended.

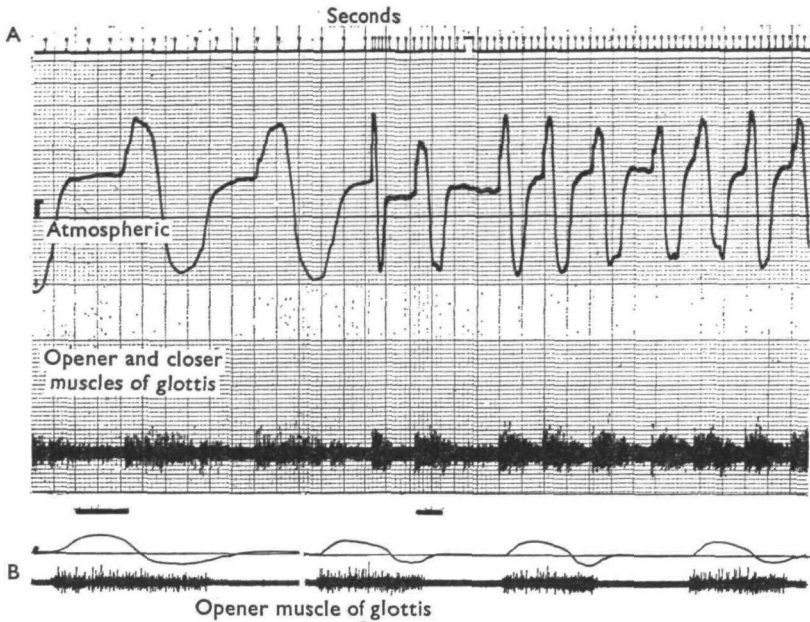
It must be mentioned that the other muscles of the girdle suspension are bound to be involved in these movements. The present experiments were not intended to be exhaustive, but were designed to test whether the movements associated with ventilation are active or passive.

Activity of the glottis muscles

Very fine wire electrodes could be placed into the glottis opener muscles but it proved impossible to place them within the semicircular fibres surrounding the anterior end of the glottis which presumably produce closing. Electrodes in the immediate vicinity gave a mixed record which when compared with that from the opener alone made it possible to suggest the firing times of the semicircular fibres. Direct observation of the glottis in 'gaspings' tortoises confirmed that the firing times corresponded respectively to opening and closing of the glottis.

The opening muscles show a sharp onset of firing just as the lung pressure begins to rise (Text-fig. 7). This continues until the pressure has passed into the tertiary phase, just before atmospheric pressure is reached. They are then completely silent until the following cycle. (The records in Text-fig. 7 are from specimens whose breathing had been stimulated with CO₂ but they are very similar in more slowly breathing tortoises.)

Some recordings showed activity of both muscles. The sharp onset is also clear in these mixed records, but the signal does not die out just before atmospheric level is reached. There is, however, a sharp drop in frequency. This low-level signal continues at a constant rate for some four or five heartbeats beyond the time when the base-line



Text-fig. 7. A, Simultaneous record of intrapulmonary pressure and electrical activity from two electrodes placed close to the glottis. Under these conditions potentials were picked up from both the opener and closer muscles. B, Oscilloscope records of intrapulmonary pressure changes and electrical activity in the opener muscle of glottis. Both A and B were recorded from specimens which were hyperventilating following the administration of carbon dioxide. Calibration pressures = 5 cm. H_2O . Block time-scales = 5 sec.

pressure is attained. These potentials appear to be due to activity of the circular fibres and indicate the time of active glottis closure.

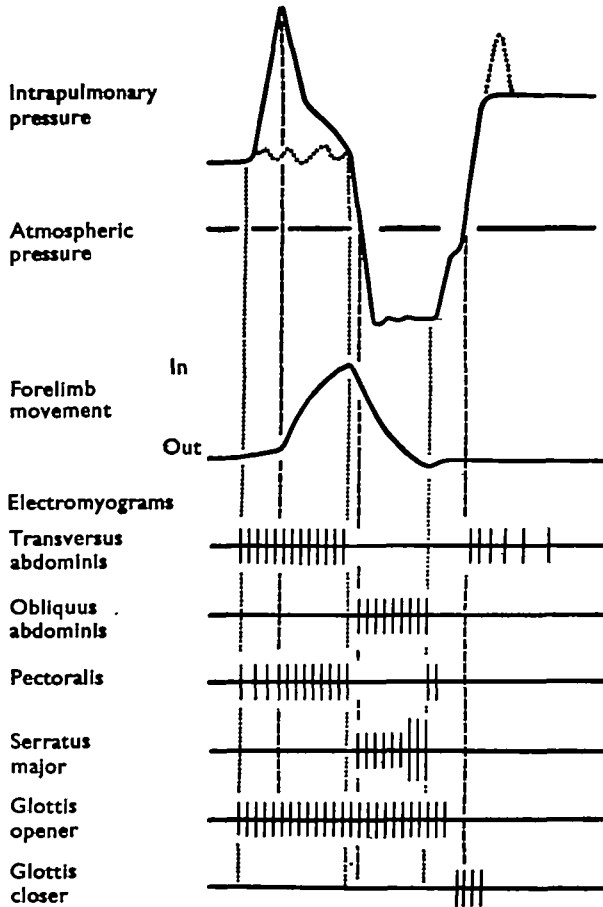
DISCUSSION

As a result of these experiments the various events of a typical ventilation cycle may be summarized as in Text-fig. 8. On this basis it is possible to suggest the causal relationships between some of the observed events.

The start of a cycle is marked by (1) a rapid increase in intrapulmonary pressure, (2) a high level of electrical activity in the *M. transversus*, (3) a low level of electrical activity in the *M. pectoralis*, (4) a very slight inward movement of the forelimbs, and (5) electrical activity in the opener muscles of the glottis (apparently synchronized with glottis opening). It is suggested that the pressure increase of the first phase is mainly due to contraction of the *M. transversus*, coupled with a slight inward movement of the forelimb induced by the *M. pectoralis*. Since the glottis appears to open when the first pressure changes occur these seem to be primarily induced by the relation between the repositioning velocity of the bone/muscle systems and the flow restrictions of the trachea and bronchi.

The second phase begins with (1) a sudden reversal of the pressure curve, (2) a sharp increase in firing rate of *M. pectoralis*, and (3) a marked acceleration of the inward movement of the forelimbs (produced by rotation of the shoulder girdles).

The inward velocity of the forelimbs gradually decreases though the firing rate of the *M. pectoralis* remains apparently constant. The explanation of the velocity change (and of the equivalent change in velocity of the outward moving limb) needs to be sought in terms of (1) the reduction of contractile force as the muscle shortens, (2) the linkage pattern of humerus, girdle, and shell, (3) the relation of unit angular



Text-fig. 8. Diagram summarizing the time relationships between intrapulmonary pressure changes, forelimb movements and electromyograms from the muscles investigated. A common variation on the pressure record is shown by a dotted line.

girdle rotation to unit volumetric change of the intrashell space, (4) the decrease of the internal pressure level opposing the movement, and (5) the activity of other muscles rotating the girdle (cf. Gans, 1966).

Both the *M. transversus* and the *M. pectoralis* cease firing a short time before the lung reaches atmospheric level. The cessation of firing corresponds to the reversal of the limb movement and the shoulder on the descending pressure curve. Following this shoulder the pressure again drops rapidly without a significant discontinuity at the atmospheric level. Both the *M. obliquus* and the *M. serratus* start to fire during this fall in pressure though their time of onset is variable. The *M. obliquus* generally starts as the pressure falls and always fires before the atmospheric level is passed.

The *M. serratus* is apparently stimulated to fire at or after the atmospheric level has been passed.

The lung pressure is maintained at a subatmospheric level for a relatively long period though the course of the pressure curve is quite irregular during the end of the second phase. The firing rate of the *M. obliquus* is relatively constant while that of the *M. serratus* increases markedly during its terminal phase. Both of these muscles, as well as the glottis opener, cease firing just before the pressure starts to rise toward atmospheric at the beginning of the third phase. The cessation of firing of the *M. serratus* corresponds with the end of the slowed down outward movement of the forelimbs. The gradual decrease of their outward velocity at a time when the firing rate of the muscle increases will be discussed in the next section.

The ascending pressure curve also has a shoulder just below atmospheric pressure that is passed during the time that the glottis closing muscles are active. Some of the fibres of the *M. transversus* again fire at a low rate which gradually declines.

The flow of air during ventilation

Consideration of the ventilation cycle in terms of pressure changes provides only an indirect indication of events. Pressure changes were recorded because they involved minimal disturbance of the animals' behaviour, yet it is the flow rate rather than the pressure change that is of importance to the animal.

The volume flow of respiratory gases will be a function of the pressure differential and the resistance of the flow path. Some measurements have been made of the pressure differential, but none of the flow path resistance. The latter will be influenced by autonomic input to the smooth-muscle coat of the trachea and bronchi, the folding or relative patency of the pathway, the degree of glottal dilation, and the flow resistance of the gaping mouth as opposed to the nostrils. All of these factors are neglected here, as the present study only provided an indication of the times in the cycle when the glottis opened and closed and not its degree of opening.

While the course of pressure changes during each cycle is triphasic the direction of flow is diphasic. Throughout the first part of the second phase the lung pressure is above atmospheric and air will flow out of the lungs. During the latter part of the second and the start of the third phase the lung pressure is below atmospheric and air will flow into the lungs. This flow is terminated by the firing of the glottis-closing muscles. The overall pattern is thus one of expiration–inspiration–pause. The recompression of the lungs (by the secondary low-level firing of the *M. transversus*) is not associated with any flow of air. This diphasic pattern is in agreement with Mitchell & Morehouse (1863) and of McCutcheon (1943) and in opposition to the concept of a terminal partial expiration claimed by Shaw & Baldwin (1935). Furthermore there can be no question that both inspiration and expiration are active (= powered) processes in *Testudo graeca*. The concept of passive expiration extrapolated from anatomical findings by George & Shah (1959) is in error, but of course the passive elastic properties of the system must play some part.

The indication that the glottis is open throughout the cycle means that the observed pressure changes, or rather the pressure differentials in an open system, must be maintained by continuous muscular effort. This raises some questions regarding the energetics of ventilation. A pressure change of 7 cm. of water will in a closed system

be achieved by a volume change of less than 1% (this accounts for the low firing rate during recompression). In a system open to the air the differential is only maintained as long as the rate of volume change exceeds the flow rate through the trachea.

An increase in the rate of pressurization implies a logarithmic increase of energy consumption by muscle. Furthermore the flow resistance of the trachea will rise as the second or third power of the velocity. These two factors must be balanced against the energy consumption involved in *maintaining* a particular pressure level for a unit of time. This suggests that there may be optimal inspiration and expiration pressure levels for minimum expenditure of energy during ventilation. It remains to be seen to what extent the suppression of the initial pressure peak by some specimens and the maintenance of an irregular subatmospheric plateau during the latter portion of the second phase reflect these considerations. The sequential firing and discharge patterns of the posterior and anterior muscle systems certainly appear designed to maintain pressure plateaus rather than to achieve pressure peaks of short duration.

The observation that the high recompression levels achieved by certain tortoises decayed fairly rapidly raises further interesting questions. It would be useful to discover what determines the pressure level in the lung, what proprioceptors monitor it, and whether the decay reflects their adaptation to new conditions. Since the changes in volume are relatively small, it is for the moment difficult to eliminate the effects of leakage through the glottis. More interesting is the fundamental question of the signals that trigger the reversals of muscle activity. Equally important is the nature and effect of the inherent pulmonary elasticity and contractility in this form (cf. Carlson & Luckhardt, 1921).

Transmission of pressures

The lungs of *Testudo* are limited by, and attached to, a rigid box on three sides; lung patency is thus assured and only the ventral diaphragmatic membrane is free to move. Pulmonary volume change therefore implies diaphragm deflections.

The remaining viscera lie below and some are attached to the ventral membrane; they are rigidly supported by the plastron, by its bridges with the carapace and by the ventral edge of the carapace. Only the anterior and posterior apertures of the shell provide escapements. Rotation of the pectoral girdles tends to shift the anterior limits of the visceral cavity and to displace the visceral mass. Only the pericardial cavity lying near the mid-line remains unshifted during girdle rotation.

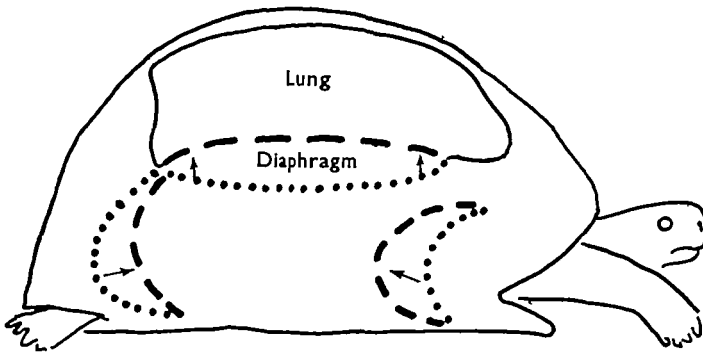
The pelvic girdle shows only very slight motion relative to the shell but expansion is possible lateral to it. The posterior aspect of the visceral space is formed as a muscle connective-tissue cup with changes of its convexity affecting the position of the viscera (Text-fig. 9).

Inward rotation of the girdles (*M. pectoralis*) and flattening of the posterior cup (*M. transversus*) reduce the volume available for the viscera. Outward rotation (*M. serratus*) and deepening of the cup (*M. obliquus*) increase it. The viscera are semisolid, of irregular shape, and surrounded by liquid. A tendency to reduce the volume available for them will therefore build up a hydrostatic pressure. The relatively incompressible mass will bulge upward against the diaphragmatic membrane. The greater compressibility of the air contained in the lungs will emphasize the deflection of the diaphragmatic membrane.

This concept of the suggested mechanism is supported by the minimal differential

pressures recorded between either the front and rear chambers on one side or between the left and right lungs. The observed differentials were less than one-hundredth the absolute pressure changes. This argues for an induction of lung pressures by a single external event or by transmission via a single channel.

The results presented suggest that the concepts of 'respiratory' and 'non-respiratory' muscles are probably misleading. Certainly all of these muscles participate in the respiratory activity. Only the pale-fibred *M. transversus abdominis* did not appear to be associated with locomotor events; yet this negative finding is to be treated cautiously as we made no effort to study locomotion *per se* nor did we curarize the animals.



Text-fig. 9. Diagram of changes in volume of the visceral cavity produced by changes in shape of its anterior and posterior borders during a ventilating cycle. These result in movement of the 'diaphragm' and alterations in lung volume. Dashed lines, expiration; dotted lines, inspiration.

The mechanism described above applies only to *Testudo graeca* and perhaps to some related species. These would seem to be highly specialized animals which have developed a domed shell deprived of hinges and constructed as a rigid box. The marked morphological differences between different chelonians described and implied in the literature suggest the need for checking these conclusions on other species, especially those belonging to other lines of the testudinate radiation. Even in *Testudo* we still lack comparable records for other muscles, as well as information on such questions as possible gas flows within the lung during prolonged pauses, and the effects of recompression on the gas exchange rate.

SUMMARY

1. An account is given of the gross structure of the respiratory system and of the principal muscles involved in ventilation of the tortoise, *Testudo graeca*.
2. The mechanism of ventilation was investigated by electrophysiological methods. Cannulae inserted into the lung through holes drilled in the shell recorded pressure changes during the cycle of ventilation and simultaneous recordings were made of the forelimbs movements. The pressure changes were triphasic in form, consisting of an initial increase in pressure followed by a fall to a level of 7 cm. water below atmospheric, and again returned to the atmospheric level or usually slightly above. During the pause between individual ventilation cycles, any overshoot gradually declined to the base-line.

3. Electromyograms showed that all phases of the respiratory cycle were active. The increase in pressure was accompanied by activity in the transversus abdominis and in the pectoralis muscle which draws the shoulder girdle back into the shell. These activities increase the pressure in the peritoneal cavity, which is transmitted to the lung. During the opposite phase of the cycle, the obliquus abdominis and serratus major muscles are active. This increases the volume of the peritoneal cavity and leads to the reduction in intrapulmonary pressure below atmospheric.

4. Activity recorded from the opener and closer muscles of the glottis showed that the glottis is dilated during the first two parts of the cycle but not during the final recompression phase.

5. The respiratory rhythm does not appear to be composed of brief periods of ventilation activity followed by prolonged pauses, as supposed by many authors. These differences may relate to the species investigated.

6. No evidence was found for significant differences in pressure between the right and left lungs or between the anterior and posterior chambers of the lung on one side.

7. It is concluded that the mechanism producing ventilation in the tortoise takes advantage of the only possible way of changing the volume of the thoracico-peritoneal cavity, namely by altering the position of the limb flanks. Both expiratory and inspiratory movements are active.

Messrs John Kramer and Herbert I. Rosenberg furnished technical assistance. The work of one of us (C.G.) was partially supported by NSF grant GB-2460. Most experiments were performed using equipment provided by a NERC grant to G.M.H.

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EXPLANATION OF PLATE

Cross-section at the level of the anterior third of the shell (top), mediad view of parasagittal section (middle) and lateral view of parasagittal section (bottom) of *Testudo graeca* to show the relative positions of the lung and other organs. The cross-section is of a specimen fixed in maximal retraction of head and limbs so that the lung in section appears compressed into a narrow dorsal crescent.

