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INFLUENCE OF INTERMITTENT BREATHING ON VENTRICULAR DEPOLARIZATION PATTERNS IN CHELONIAN REPTILES

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SUMMARY

1. Alterations between two quite distinct patterns of epicardial depolarization are associated with the normal occurrence of intermittent lung ventilation in both lightly anaesthetized and unrestrained, conscious turtles (*Pseudemys scripta*) and tortoises (*Testudo graeca*).

2. During apnoea depolarization sweeps from the left to the right over the ventricular surface at a conduction velocity of 0·15 m/sec. With the onset of lung ventilation the direction of depolarization propagation over the ventricle is reversed, and conduction velocity in the epicardium falls to 0·09 m/sec.

3. Vagal stimulation and acetylcholine produce a shift from the apnoea to the breathing pattern of depolarization in intact animals, while vagal sectioning and atropine abolish all shifts. Acetylcholine reduces conduction velocity but has no effect on the strength of contraction of isolated cardiac muscle strips from turtle hearts. Changes between the two patterns of ventricle depolarization are likely produced by vagal innervation of the rudimentary conduction system of the chelonian heart.

4. Experimental induction of the depolarization pattern of the ventricle normally evident during lung ventilation produces an improved separation of oxygenated and deoxygenated blood within the anatomically undivided chelonian ventricle. It is suggested that changes in ventricle depolarization patterns during intermittent lung ventilation may be an active component of the cardiovascular responses controlling intracardiac blood shunting in reptiles.

INTRODUCTION

Cardiovascular performance in many reptiles is greatly influenced by their pattern of intermittent breathing. Periods of active lung ventilation are often accompanied by large increases in heart rate (Belkin, 1964; Gaunt & Gans, 1970; Burggren 1975), vasodilatation of the pulmonary vascular bed (White & Ross, 1966; Johansen, Lenfant & Hanson, 1970; Shelton & Burggren, 1976; Burggren, 1977) and increases in cardiac stroke volume (White & Ross, 1966; Shelton & Burggren, 1976). Significant alterations in the magnitudes and directions of arterial-venous blood shunting

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within the incompletely divided ventricle of the non-crocodilian reptiles are also associated with these respiratory events (White & Ross, 1966; Shelton & Burggren, 1976).

The mechanisms by which systemic and pulmonary venous return to the non-crocodilian heart is kept separated during ventricular filling and ejection are not fully understood. Total functional division of the anatomically undivided ventricle of the varanid lizards can develop during systole (Millard & Johansen, 1974; W. W. Burggren & G. Shelton, unpublished). However, most other non-crocodilian hearts must depend in part upon the establishment and maintenance of laminar blood flow through the ventricle if gross blood admixture is to be prevented. Any cardiac mechanism which serves to alter the extent to which muscular ridges on opposing walls of the ventricular chambers are apposed during systole or alters their position or even rate of movement during any part of the cardiac cycle may thus affect admixture by changing the intraventricular patterns of blood flow. So might any event which changes the relative dimensions or rate of filling and emptying of the three subchambers of the undivided ventricle. Unfortunately, such changes are exceedingly difficult to observe directly in reptiles during intermittent ventilation, let alone to quantify.

Patterns of myocardial activation of the reptile heart previously have been related to the mechanical events and pumping function of the ventricle (Gray, 1950; Alexa, 1970) and beat to beat variations in the direction of action potential propagation over the chelonian ventricle have long been documented. For example, Meek & Eyster (1912) presented data on ventricular depolarization in the tortoise *Testudo* which indicated that depolarization swept as a uniform wave over the ventricular surface, with depolarization of the left anterior base of the ventricle usually preceding the right. However, in about one-quarter of their observations the right side of the ventricle was reported to depolarize before the left side. Lewis (1916) and Gray (1950) showed similar occasional changes in the depolarization pattern of the ventricle of *Testudo*, *Graptemys* and *Pseudemys*. These variations in the purportedly normal pattern of depolarization from the left to the right side of the chelonian ventricle were attributed to various experimental artifacts. However, many earlier investigators of reptilian cardiovascular physiology appeared to be largely unaware of the striking changes in cardiovascular performance which are now known to accompany intermittent breathing in chelonians. Hence, the present experiments were designed to investigate (1) whether predictable changes in epicardial activation might be associated with intermittent lung ventilation and (2) whether changes in epicardial activation patterns exerted any influence on intraventricular blood admixture.

METHODS

Experiments were performed on twenty-three *Pseudemys scripta*, twenty-one *Testudo graeca* and fourteen *Testudo hermanni*. Experimental work on intact animals was performed at 18–20 °C. *In vitro* experimentation on excised cardiac muscle was performed at 20·0 °C.

In vivo experiments

Animals were initially anaesthetized with cold torpor by exposing them to 1 °C for 12–15 hr before surgery. Anaesthetized animals were restrained ventral side up, surrounded in crushed ice,

and their heart exposed with a circular saw mounted in a dentist's drill (see Shelton & Burggren, 1976, for surgical techniques). The pericardium was carefully opened and care was taken not to disturb the heart unnecessarily. Anaesthetized animals were then subjected to cardiac electrode implantation. After surgery animals were allowed to warm to room temperature. In acute experiments animals remained restrained ventral side up with the heart exposed and were left lightly anaesthetized with halothane vapour to a level at which voluntary intermittent breathing was just maintained. In chronic preparations animals were unrestrained and unanaesthetized.

Bipolar recording was used in all experiments. Electrode pairs were connected to differential AC amplifiers. An earth wire was placed within the opened body cavity. Electrical potentials from electrode pairs implanted in the epicardium were displayed on either a Tectronix 502A dual beam oscilloscope or a Telequipment DM64 dual beam storage oscilloscope and then photographed.

Two different types of electrodes were utilized. The first electrode system, which was used in acute preparations, consisted of pairs of silver wires (0·4 mm diam.) etched to fine points at the tips. Each pair of wires was held in a micromanipulator. The silver wire tips, which were approximately 2 mm apart in each electrode pair, were inserted 1 mm into the myocardium. Electrodes could be implanted, withdrawn and reimplanted in a different location with no apparent damage to the mycocardium, and in most instances there were no signs of myocardial puncture. Control experiments using cotton wick electrodes established that no movement artifact or gross myocardial damage affected the records obtained from these silver wire electrodes.

In order to map the spread of excitation over the surface of the ventricle, two electrode pairs were initially placed in the epicardium at two arbitrary points. Since electrical activity recorded from the two electrode pairs was displayed simultaneously on the oscilloscope, it could readily be determined which of the two regions underneath the electrode pairs first became depolarized. The electrode pair at the position where depolarization first appeared was left in place, the second electrode pair was moved to a different location, and the temporal relations of the depolarization again compared. In each instance, the electrode pair first recording depolarization was left in position, and the other moved to a new location. In this fashion the specific region of the entire ventricle surface which first become depolarized during the cardiac cycle could be readily located. One pair of electrodes was then left permanently implanted to record depolarization at this site, and the time delay between depolarization there and depolarization at all other regions over the ventricle could be ascertained by repeatedly changing the location of the second electrode pair. In each acute preparation a minimum of fifty different recording sites was used in the compilation of depolarization maps (see Results).

A second electrode system was used for recording in chronic experiments lasting up to 4 days. The bared tips of two pieces of insulated copper wire (0·2 mm diam., 20 cm long) were implanted to the depth of 2 mm into an appropriate region of the ventricle wall with a 22 gauge needle (Basmajian & Stecko, 1962). The pericardium was then tightly drawn up and tied around the copper wire leads. Up to four pairs of copper wire electrodes were implanted in a single ventricle without any apparent disruption of heart function. The electrode leads were then brought out through a small notch cut into the excised piece of plastron, which was fastened back into position with rapidly setting expoxy resin. The turtle or tortoise was allowed to recover and then released into an experimental cage which permitted unrestricted movement. Electrodes usually remained functional for a minimum of 2 days. After the conclusion of each chronic experiment, the animal was killed and the electrode position confirmed by dissection.

Cardiac recording electrodes could also be used to stimulate the ventricle by connecting them to a Grass S3 stimulator set to deliver single shocks (0·5–5·0 V, 5 msec duration). In certain acute experiments the vagus and sympathetic trunks were exposed and sectioned on the left or right side of the neck between the superior and inferior cervical ganglia at the level of the third to fifth vertebra. Small fibres dissected from the peripheral end of the vagus or sympathetic nerve were laid over a pair of silver wire hook electrodes, which was then used to stimulate them (0·5–3·0 V, 5 msec duration, 10 c/s). The nerves on the electrodes were covered with paraffin to prevent their desiccation.

In six chronic experiments the left pulmonary artery, the left aorta and the brachiocephalic artery were non-occlusively cannulated with PE60 polythene tubing to permit sampling of central arterial blood (see Shelton & Burggren, 1976). Arterial blood P_{O_2} was measured with a Radiometer BMS3 blood gas analysis system. Cardiac recording electrodes in these preparations were implanted as described above. An additional copper electrode pair was also implanted in the medial ventral wall of the left auricle. This electrode pair was connected to a Grass S3

simulator, and through this system the animal's heart rate could be artificially driven at slightly higher than normal heart rates to allow a complete control of cardiac frequency (18–25 beats/min in most animals). A pair of electrodes were also implanted over the cavum pulmonale on the right side of the ventral surface of the ventricle (see Results) and connected to a second S3 stimulator so that an artificial spread of activation from the right to the left of the ventricle could be induced. The triggering circuits of the stimulators were interconnected so that 150 msec after the left auricle was depolarized by a single shock, a second depolarizing shock was delivered to the ventricle. During experimental periods auricular systole thus preceded induced ventricular systole by an interval just sufficiently long to allow a near normal period of auricular-assisted filling of the ventricle.

Lung ventilation in acute and chronic experiments was monitored visually by observing the conspicuous limb, neck, head and body wall movements associated with breathing. The effects on cardiac function of acetylcholine chloride, adrenaline bitartrate, atropine sulphate and DL-propranolol were determined in both acute and chronic experiments. In acute experiments the drugs were either injected in a subintestinal vein or applied externally to the heart, while in chronic experiments drugs were injected into an arterial cannula.

In vitro preparations

A 20–30 mm long 4 mm diam. muscle strip was excised from the ventricle, starting from one edge of the ventricle base, passing down to the apex, and up towards the other edge of the base. A length of nylon thread was tied around each end of the muscle strip, which was then transferred to a 50 ml. organ bath containing stirred, air-equilibrated reptile saline (de la Lande, Tyler & Pridmore, 1962). The nylon thread at one end of the muscle strip was tied to a calibrated Grass strain transducer and the other end was fixed securely to a hook in the bottom of the bath. A resting tension of about 0·5 g was set and tension changes in the muscle strip were recorded on a Devices two-channel chart recorder. A pair of electrodes were implanted in the muscle near the fixing hook to stimulate the muscle rhythmically, and a pair of recording electrodes were located at each end of the muscle strip. The distance between the two recording electrode sites was carefully measured during several periods of muscle relaxation, and this mean distance, in conjunction with the time delay between depolarization at the two sites, allowed the calculation of conduction velocity in the muscle strip. Drugs were added directly into the 50 ml. organ bath to produce solutions of known concentrations. Drug doses were increased at 5 min intervals by adding additional amounts of the drug without an intervening flushing of the organ bath.

RESULTS

There are three incompletely separated chambers in the chelonian ventricle; the cavum arteriosum, which occupies a small region of the left base of the ventricle; the cavum venosum, which extends from the right dorsal base of the ventricle down to its apex; and the cavum pulmonale, which overlies the cavum venosum and occupies the right ventral region of the ventricle base (see White, 1968; Shelton & Burggren, 1976). The ventricles of the turtle *Pseudemys* and the tortoise *Testudo* are anatomically and functionally nearly identical (Shelton & Burggren, 1976) and the data collected on ventricular depolarization in the turtle and the tortoise were largely similar.

Patterns of epicardial depolarization

Apnoea. Maps of depolarization propagation over the surface of the ventricle were derived from both acute and chronic experiments in ten *Pseudemys scripta* and fifteen *Testudo graeca*, all of which were voluntarily breathing in their typical intermittent pattern. Isochronal lines of depolarization have been drawn relative to the point of the epicardium which depolarizes first during each cardiac cycle (Figs. 1 and 2). The depolarization pattern measured at any time during a period of apnoea was very consistent from cardiac cycle to cycle. The earliest detectable depolarization

of the ventricular surface of both the turtle and tortoise was found in a discrete area of the epicardium overlying the cavum arteriosum. From this point depolarization waves spread out nearly concentrically over the epicardium at a mean velocity in *Pseudemys* of 0·16 m/sec (S.D. 0·04, twenty-two observations in four animals) and in *Testudo* of 0·13 m/sec (S.D. 0·06, twenty observations in four animals), reaching the cavum pulmonale at the right edge of the ventricle after 100–120 msec (Figs. 1*A* and 2*A*). Propagation occurred at an identical velocity from the left to the right

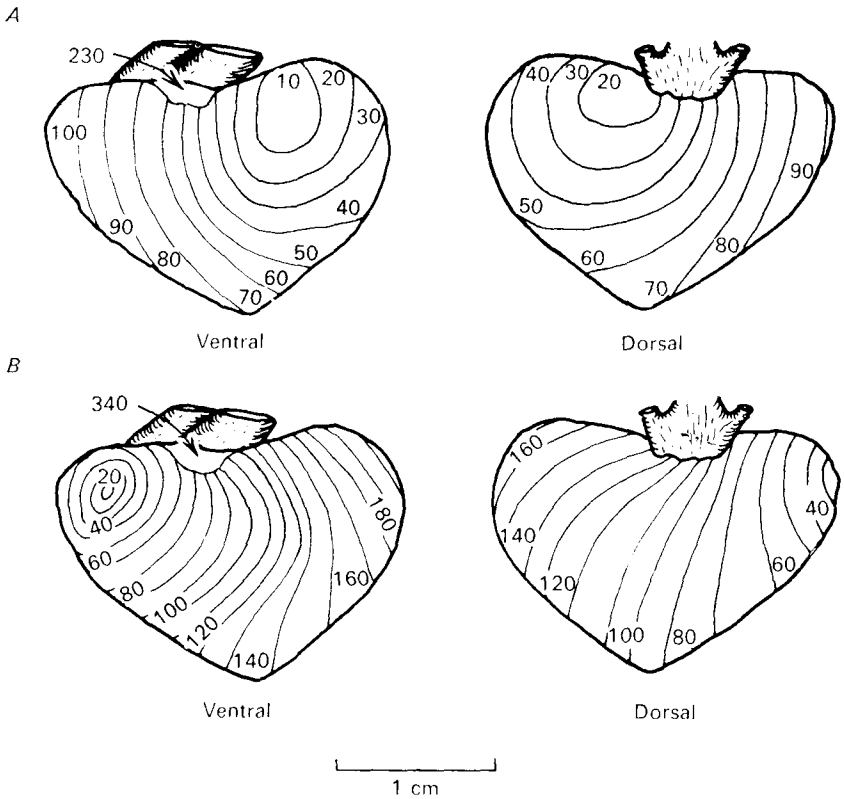

Fig. 1. Patterns of normal *in vivo* depolarization propagation over the ventral and dorsal surfaces of the ventricle of the turtle *Pseudemys scripta* during apnoea (*A*) and during lung ventilation (*B*). The sequence of epicardial depolarization is indicated by 10 msec interval isochronal lines. The numbers indicate msec after the initial appearance of depolarization on the ventral surface of the ventricle.

side over the dorsal surface of the ventricle. The earliest depolarization on the dorsal surface was found immediately beneath the point of earliest depolarization on the ventral surface, although its onset was slightly delayed (10 msec in *Pseudemys*, 40–50 msec in *Testudo*).

During apnoea the bulbus cordis, the small discrete band of cardiac muscle which wraps part way around the base of the common pulmonary artery, depolarized 200–250 msec later than adjacent regions of the ventricle (Figs. 1*A* and 2*A*). Conduction velocity in the narrow transitional zone between the cavum venosum and the bulbus cordis was estimated to be 0·02 m/sec.

Lung ventilation. During periods of lung ventilation in both *Pseudemys* and *Testudo* the point of initial depolarization moved to the right side of the cavum pulmonale (Figs. 1*B* and 2*B*), and depolarization now spread out in concentric waves from the right to the left side of the heart. Conduction velocity over the ventricle during lung ventilation was reduced to only $0{\cdot}09 \pm 0{\cdot}02$ m/sec in *Pseudemys* and $0{\cdot}10 \pm 0{\cdot}04$ m/sec in *Testudo* so that depolarization now took approximately 190–210 msec to reach the left medial edge of the ventricle. A similar shift in depolarization pattern and conduction velocity also developed on the dorsal surface of the

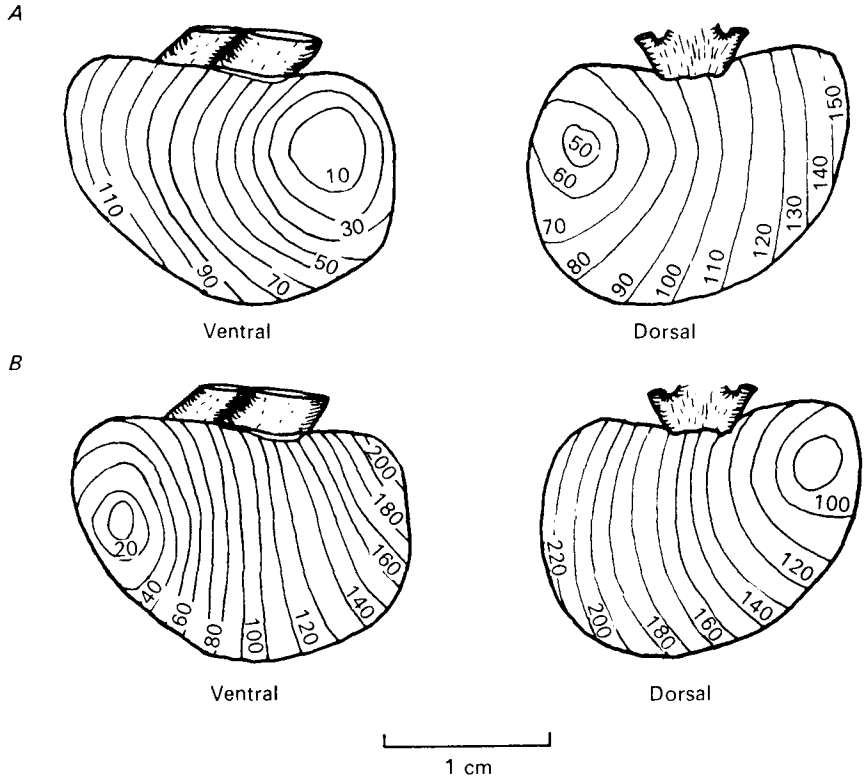

Fig. 2. Patterns of normal *in vivo* depolarization propagation over the ventral and dorsal surface of the ventricle of the tortoise *Testudo graeca* during apnoea (*A*) and during lung ventilation (*B*). Isochronal lines have been drawn as in Fig. 1.

ventricle of *Testudo* and *Pseudemys*. Locomotor activity was often accompanied by similar changes in depolarization direction and conduction velocity even when apnoea continued unabated. Bulbus cordis depolarization during lung ventilation was delayed by an even larger margin of more than 300 msec from depolarization of ventricular epicardium immediately adjacent to it (Fig. 1*B*).

Shifts in the pattern of the spread of activation associated with respiratory movements were instantaneous with no transitional stages occurring. The right-to-left depolarization pattern usually appeared within one cardiac cycle of the first inspiration (Figs. 3 and 4). In *Pseudemys*, in which periods of apnoea are punctuated by a series of closely spaced breaths (Burggren, 1975), the ventilation depolarization

pattern was usually maintained for several successive cardiac cycles (Fig. 4) and if a protracted breathing series occurred sometimes persisted for three or four cardiac cycles after termination of respiratory movements. Occasionally ventricular depolarization patterns towards the end of longer breathing series in *Pseudemys* began to alternate from cardiac cycle to cycle between the two patterns, until at the end of the breathing series the apnoea pattern was once more restored (Fig. 4*A*). In *Testudo*, which characteristically ventilates its lung with only a single breath rather

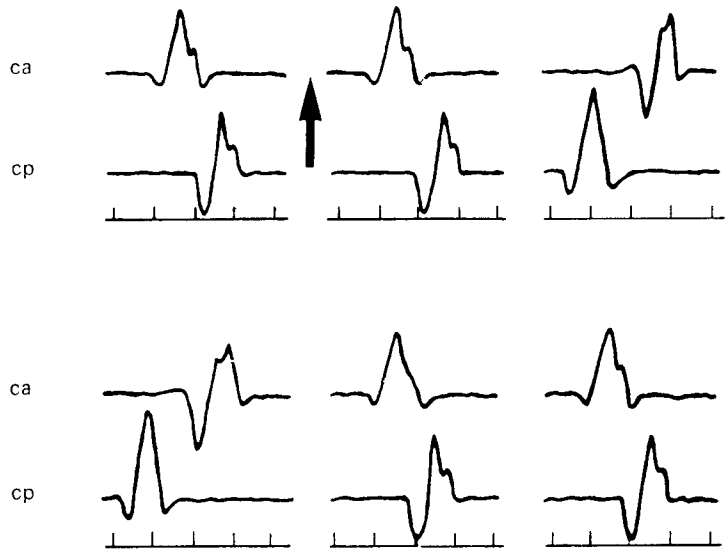

Fig. 3. Simultaneously recorded traces of depolarization of the cavum arteriosum (ca) and the cavum pulmonale (cp) during six consecutive cardiac cycles in unrestrained *Testudo graeca*. The intervals between depolarization have not been included in the records. A single lung ventilation, indicated by the arrow, occurred between the first and second cardiac cycle. The time marker is in 100 msec intervals.

than a series of breaths (Burggren, 1975), the shift from the apnoea to the ventilation depolarization pattern was correspondingly brief (Figs. 3 and 4*B*). In two turtles and one tortoise a left-to-right depolarization propagation was maintained even during breathing, but conduction velocity nonetheless fell during lung ventilation to those levels normally observed during right-to-left propagation.

Experimental factors affecting depolarization patterns

Nerve section and stimulation. In nine out of fifteen animals the changes in direction and velocity of depolarization associated with intermittent breathing were totally abolished by section of the left and right vagus trunks in the neck, and an apnoea pattern was thereafter adopted during both apnoea and lung ventilation. However, if the peripheral ends of the severed vagus nerves of these particular nine animals were then stimulated during apnoea at a frequency of 10 c/s and at a voltage just sufficient to cause an immediate decrease in heart rate (usually 0·75–1·5 V), then the pattern of depolarization abruptly shifted to a right-to-left direction for the first 1–5 heart beats after the onset of bradycardia. Conduction velocity in the

epicardium also fell to values approximately half of those evident before vagal stimulation. All subsequent ventricular depolarizations reverted back to a left-to-right pattern. This phenomenon was reproducible for several consecutive stimul-

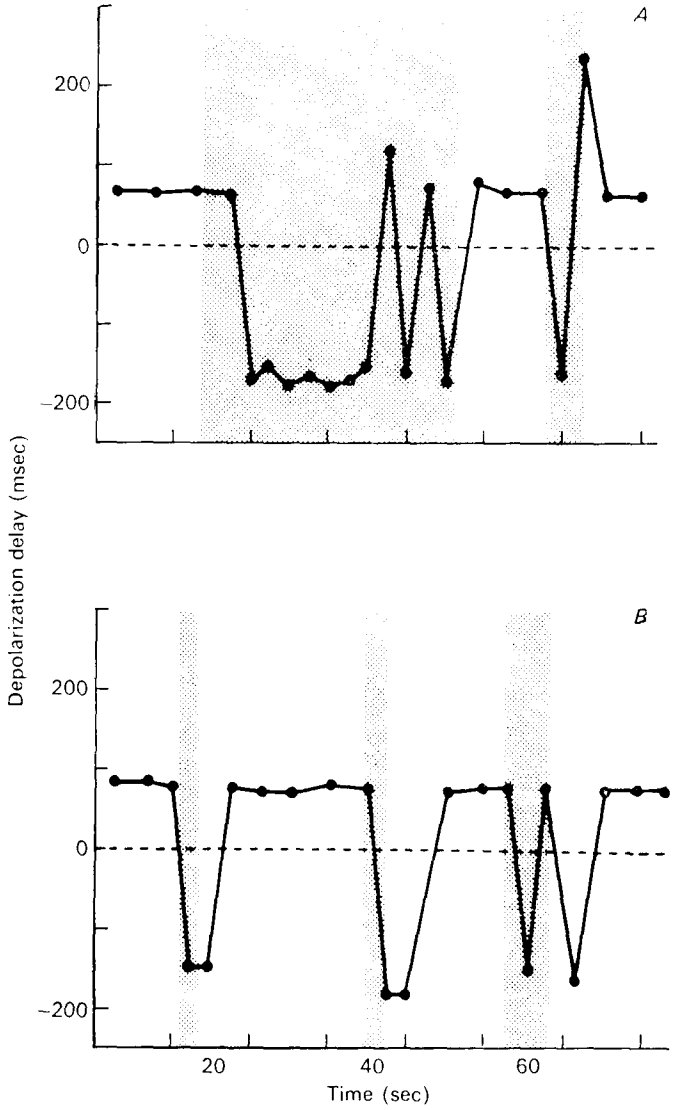

Fig. 4. Changes in patterns of ventricle depolarization during several successive breathing series measured during chronic experiments on unrestrained *Pseudemys scripta* (*A*) and *Testudo graeca* (*B*). The time delay between the occurrence of depolarization at the standard electrode position on the left side of the heart over the cavum arteriosum and on the right side of the heart over the cavum pulmonale is shown for approximately twenty successive cardiac cycles. A positive time delay indicates that the cavum arteriosum depolarized before the cavum pulmonale and reflects a left-to-right depolarization propagation. A negative time delay between depolarization at the cavum arteriosum and the cavum pulmonale indicates that the cavum pulmonale depolarizes first, as during right-to-left depolarization propagation. Lung ventilation occurred continually in the shaded regions.

ations of the vagus. In five of these nine animals, stimulation of the left or right vagus trunk could elicit a pattern shift. In the other four stimulation of only one of the vagi was effective. Both pattern shifts and conduction velocity changes in these animals were completely blocked by atropine (0·5 mg/kg body wt.). Occasionally, peripheral vagal stimulation did not produce a shift in depolarization pattern, but merely resulted in a reduced conduction velocity for several heart beats.

In five of the remaining animals depolarization pattern shifts during intermittent breathing were still seen after complete sectioning of both vagal trunks in the neck. Stimulation of the peripheral ends of the severed vagi, although producing a bradycardia in every animal, never produced any change in the depolarization pattern or conduction velocity. Yet atropine completely abolished all depolarization shifts caused by intermittent breathing in this group of animals as in the other. This suggested the existence in these five animals of an extra-vagal cholinergic innervation of the heart.

One animal showed no changes in depolarization pattern or conduction velocity before or after vagal sectioning or in response to peripheral vagal stimulation.

Stimulation (2·0 V, 5 msec duration, 10 c/s) of sympathetic nerve trunks lying adjacent to the vagus in the neck between vertebrae three and five produced marked inotropic and chronotropic cardiac effects in all fifteen animals, but in each case was completely ineffective in changing the pattern of ventricular depolarization.

Drugs. A distinct shift from the pattern of ventricle depolarization associated with apnoea to that associated with lung ventilation was produced in four out of the six tested animals by the external application to the ventricle of relatively high doses of acetylcholine (10^{-6}–10^{-4} M). A reduction of conduction velocity to those levels evident during both lung ventilation and vagal stimulation also developed. These effects were blocked by atropine (0·5 mg/kg), as were the normally occurring depolarizing changes during intermittent breathing. Neither adrenaline nor propranolol in any dosage produced any change in conduction velocity in these experiments.

Depolarization patterns and arterial blood P_{O_2}

Blood was sampled repeatedly from the left pulmonary artery, the left aorta, and the brachiocephalic artery of six unrestrained *Testudo*. Blood P_{O_2} was determined in both control periods during apnoea when a normal left-to-right depolarization was evident, and during 5 min long experimental periods in which a right-to-left depolarization was artificially generated by stimulation of the epicardium over the cavum pulmonale (see Methods). Changes in depolarization pattern normally generated by the animal itself could not be used in these experiments because they were too brief to allow effective blood sampling. During control periods of normal left-to-right depolarization the P_{O_2} of blood conveyed in the brachiocephalic artery was slightly higher than that in the left aorta, and the P_{O_2} of blood in both of these arteries was considerably greater than that of the blood in the left pulmonary artery (Fig. 5). These P_{O_2} differences between the arterial arches as well as the absolute values of arterial P_{O_2} are typical of *Testudo* (Burggren, 1976). During induced right-to-left depolarization the P_{O_2} of blood conveyed in the brachiocephalic artery was not significantly changed ($P > 0{\cdot}10$) from that during control periods. The P_{O_2} of blood in the left aorta, however, was increased to levels similar to those measured

in the brachiocephalic artery. Also the P_{O_2} of blood in the left pulmonary artery decreased during induced right-to-left propagation. The changes in P_{O_2} in both left aorta and left pulmonary artery blood were both significant at the 0·001 level. These data suggest that there is less intraventricular admixture of pulmonary and systemic venous return during an induced right-to-left spread of activation than during a normal left-to-right progagation.

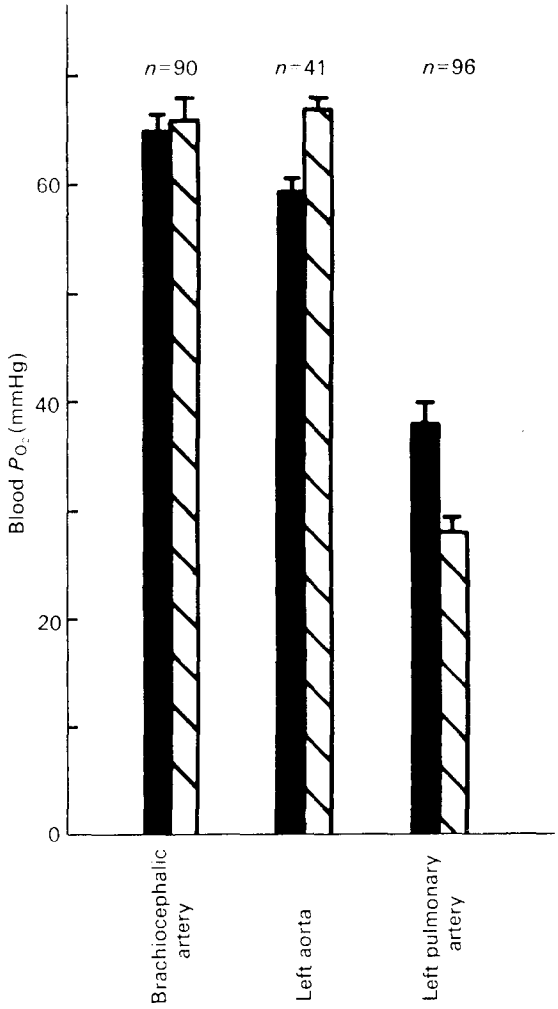

Fig. 5. Arterial blood P_{O_2} measured in the arterial arches of six unrestrained *Testudo graeca*. Mean values ± 1 S.E. are given. Values during normal left-to-right depolarization propagation occurring during apnoea are indicated by the filled bars. Values during experimentally induced right-to-left depolarization propagation over the ventricle are indicated by the diagonally striped bars.

Conduction velocity in isolated mycoardial strips

Because heart rate in the chelonians may double as lung ventilation is initiated (Burggren, 1975), the effects of depolarization frequency on conduction velocity were investigated in 10 isolated cardiac muscle strips. Conduction velocity fell

significantly ($P < 0{\cdot}001$) from approximately 0·10 m/sec at 5 contractions/min to approximately 0·07 m/sec at 40 contractions/min, a decrease of 30%. However, from the lowest frequency (8 beats/min) to the highest frequency (20 beats/min) in the normal heart rate range of *Pseudemys* and *Testudo* during intermittent breathing (Burggren, 1975), conduction velocity only decreased from 0·10 to 0·09 m/sec. Changes in heart rate can account for only a small proportion of the conduction velocity changes evident during intermittent breathing in these chelonians.

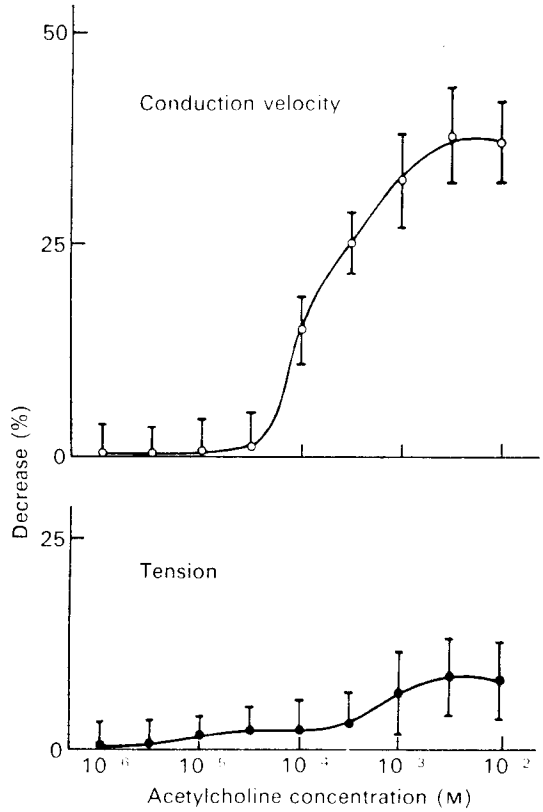

Fig. 6. Effects of acetylcholine on the conduction velocity (*A*) and the tension (*B*) developed during isometric contraction of ventricle muscle strips excised from the hearts of eight *Testudo*. Mean values ± 1 S.D. are given. $n = 8$. The decrease in conduction velocity from 10^{-5} to 10^{-3} M acetylcholine is significant at the 0·005 level. The decreases in tension developed during isometric contraction are not significant ($P > 0{\cdot}1$).

The dose–response curve of conduction velocity in mycoardial strips with increasing concentrations of acetylcholine was sigmoidal in shape (Fig. 6). Conduction velocity during contraction at 12/min in each of the eight preparations examined was unaffected by doses of acetylcholine below 5×10^{-5} M. At higher concentrations, however, conduction velocity fell markedly, until at a dose of 5×10^{-3} M acetylcholine, conduction velocity was reduced by approximately 40% from control levels (Fig. 6). Tension developed during isometric contraction in the same eight muscle strips was virtually unaffected by acetylcholine over a wide range of concentrations.

Tension development in the chelonian ventricle is thus relatively independent of cholinergically induced changes in conduction velocity, at least *in vitro*. (The drug doses required to produce changes in conduction velocity *in vitro* were high. This may reflect the occurrence of slow and passive diffusion of the drug from the organ bath into the relatively thick strip of non-perfused myocardium).

DISCUSSION

Two quite distinct patterns of ventricular depolarization are correlated with the onset and termination of respiratory events in *Pseudemys* and *Testudo*. The fact that (1) depolarization does not arise first in the epicardium immediately adjacent to the auricular-ventricular (A-V) junction and only then spread out to other areas of the ventricle, and that (2) depolarization first appears exclusively in only one of two remotely separated areas of the ventricle during intermittent ventilation (Figs. 1 and 2) provides physiological evidence for specialized cardiac muscle fibres involved with depolarization conduction through the A-V junctional region and into the base of the ventricle. Anatomical evidence of such fibres has been well documented in reptiles (Gaskell, 1883; Keith & Flack, 1907; Laurens, 1913; Robb, 1953). In the general reptilian pattern (including the Chelonia; Laurens, 1913), these specialized fibres take the form of a funnel shaped tube running down from the auricle through the A-V junction. Once into the ventricle the funnel divides into two discrete bands of tissue which run well out into the left and right base of the ventricle to positions which approximately correspond to the two ventricular loci of activation seen in the present study. An abundance of connective tissue intervenes between the funnel and the ventricular myocardium in all but the terminal regions of these two A-V funnel branches, where a rapid assimilation with the fibres of the ventricle finally occurs. The entire A-V funnel is richly innervated by branches of the cardiac vagus and often contains numerous ganglia (Gaskell, 1883; Laurens, 1913; Robb, 1953). The fibres of the A-V funnel are structurally unlike auricular and ventricular cardiac muscle and smooth muscle, while their cellular structure has been likened to the Purkinje fibres of mammalian hearts (Robb, 1953). Incisions in the regions of the A-V funnel have been shown to increase time for A-V conduction or cause A-V block (Lowman & Laurens, 1924; Gilson, 1932).

If it is assumed that the A-V funnel does constitute a rudimentary specialized conduction system in the chelonian heart, how are depolarization pattern shifts mediated? Depolarization arises from only one of two areas of the ventricular surface during any given cardiac cycle (Figs. 1 and 2), suggesting that some influence may be exerted on propagation down a particular funnel branch during apnoea or breathing. The simplest hypothesis is that the spread of activation along the left A-V funnel arm is innately much faster than along the right arm, so that during apnoea depolarization of the left ventricular base will occur relatively rapidly after activation of the A-V funnel. If the difference in conduction velocity in the left and right A-V funnel is great enough, then activation will spread across to the right side of the ventricle before the right funnel branch can initiate an independent activation on this side of the heart. However, if neural activity in the multitude of vagal fibres innervating the left A-V funnel arm increases, then a selective slowing

or blockage of conduction in this arm could be produced. Vagal stimulation and acetylcholine can greatly delay conduction in the A-V region of the heart of turtles (Fischer, 1936) and mammals (see Noble, 1975). Though specific cholinergic effects on the primitive conducting system of the reptile heart are not yet documented, it is conceivable in the turtle heart that the A-V funnel arms, which are simply structural extensions of the A-V node, are similarly affected by vagal activity. During the proposed vagal inhibition of conduction along the left A-V funnel arm, which would be appropriate for the comparatively infrequent periods of active lung ventilation, the spread of activation would be then solely along the right A-V funnel branch to the right base, resulting in the documented right-to-left depolarization pattern. Hence, a rather simple 'on-off' mechanism could operate in which the depolarization pattern produced will depend directly upon whether propagation in the more rapidly conducting left A-V funnel branch is unimpaired or vagally inhibited.

This mechanism could also be activated by non-selective stimulation of the vagus nerves or by the application of acetylcholine to the heart. The experiments on vagal nerve stimulation and section indicate not only that depolarization pattern and conduction velocity shifts can be cholinergically mediated via fibres of vagal origin, but that an extra-vagal cholinergic innervation of the ventricle exists in approximately one third of the animals examined. Mills (1885*a*, 1886) has described 'accessory vagi' in reptiles which diverge from the main vagus trunks in the region of the superior cervical ganglion and follow a separate path to the heart. Great individual variations occur in the pathways of parasympathetic nerves between the chelonian brain and heart (Gaskell, 1883; Mills, 1885*b*; Lee, 1935). It would appear that the two categories of effects of vagal sectioning and stimulation on depolarization patterns in *Pseudemys* and *Testudo* may well be attributable to such vagal nerve pathway variation, in which cholinergic vagal fibres mediating depolarization pattern shifts through their action on the A-V funnel will sometimes be located in the main vagus trunks of the neck, and on other occasions in the much smaller accessory vagi.

The marked change in depolarization conduction velocity during intermittent breathing is apparently an active component of the total cardiovascular response during intermittent breathing, rather than simply a by-product of the depolarization pattern shift. Activation and the start of contraction of myocardial fibres are separated by only a few milliseconds, and contraction of the surface of the chelonian heart generally reflects the spread of epicardial depolarization (Gray, 1950). A marked reduction in conduction velocity could be expected to cause different regions on one side of the heart to begin to contract and change shape and volume before the other side of the heart is similarly activated. During early systolic contraction in the chelonian heart any change in the volume of one ventricular sub-chamber relative to another one containing blood of a different respiratory gas composition may contribute to variations in the admixture of oxygenated and deoxygenated blood during ejection into the arteries. Cholinergic reduction of conduction velocity in cardiac muscle must operate at the level of the individual muscle fibre, and since all regions of the dorsal and ventral surface of the chelonian ventricle exhibit a reduced conduction velocity during lung ventilation, an extensive parasympathetic innervation of the epicardium must therefore exist. It is well documented that neither

vagal stimulation nor acetylcholine directly affects the rhythm, force, and excitability of the chelonian ventricle (Gaskell, 1883; Garrey & Chastain, 1973; Hiatt & Garrey, 1943; Appert & Friedman, 1955) and on this basis vagal innervation of the chelonian ventricle has been repeatedly denied. Yet, morphological evidence of cholinergic vagal nerve fibres within the ventricle myocardium has been well established (Laurens, 1913; Robb, 1953; Okita, 1971; Yamauchi & Chiba, 1973), although until now no function has been attributed to them. It is now clear that this controversy has arisen because acetylcholine and vagal stimulation induce a reduction in conduction velocity of the ventricle via these ventricular nerve fibres which is not accompanied by a substantial reduction in strength of contraction (Fig. 6). This independence of conduction velocity and tension development in the ventricle is crucial during intermittent breathing, for cardiac stroke volume increases enormously during lung ventilation in chelonians (Shelton & Burggren, 1976), a cardiac response which could not be compatible with a marked negative inotropic effect on the myocardium even if end-diastolic volume increased.

Significant changes in arterial blood P_{O_2} occur in the tortoise when the direction of ventricular depolarization is experimentally altered from one pattern to the other. Blood shunts in the incompletely divided chelonian heart cannot be quantified on the basis of arterial blood tensions alone, but if it is assumed that venous oxygen tensions are static under these experimental conditions, the results indicate that during right-to-left depolarization (i.e. during lung ventilation) the functional separation of deoxygenated and oxygenated blood in the ventricle is improved compared to during left-to-right depolarization (i.e. during apnoea). Clearly it would be advantageous to gas transport if improved separation in the heart of oxygenated and deoxygenated blood would occur during breathing when lung P_{O_2} is high and conditions for pulmonary gas exchange are the most favourable. It is not immediately obvious whether the blood P_{O_2} changes are large enough to be of importance. The experimental conditions of heart stimulation were very abnormal (e.g. depolarization conduction velocities were not changing even though direction changes were experimentally induced) and it may be that when this cardiac mechanism functions normally in the intact animals its contribution to blood separation in the heart is enhanced.

The occurrence of an intermittent and predictable alteration in the pattern of ventricular depolarization appears totally without precedent among the vertebrates. Certainly in healthy avian and mammalian hearts the pattern of ventricular depolarization is extremely consistent even during widely varying constraints and demands on cardiac performance. A comparative investigation of cardiac activation in other reptilian orders should prove enlightening.

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REFERENCES

ALEXA, E. (1970). Le méchanique ventriculaire du coeur de la tortue, étudiée par la méthode photomécanographique. (Ventricular mechanics of turtle heart studied by the photomechanographic method.) *Anal. ştiint. Univ. Al. I. Cuza. Iaşai. Sect. II(A) Bio.* **14**, 215–226.

APPERT, H. & FRIEDMAN, M. H. F. (1955). Influence of acetylcholine on turtle auricle and ventricle. *Am. J. Physiol.* **183**, 593–594.

BASMAJIAN, J. V. & STECKO, G. (1962). A new bipolar electrode for electromyography. *J. appl. Physiol.* **17**, 849.

BELKIN, D. A. (1964). Variations in heart rate during voluntary diving in the tutle *Pseudemys concinna*. *Copeia*, 321–330.

BURGGREN, W. W. (1975). A quantitative analysis of ventilation tachycardia and its control in two chelonians *Pseudemys scripta* and *Testudo graeca*. *J. exp. Biol.* **63**, 367–380.

BURGGREN, W. W. (1976). An investigation of gas exchange and cardiovascular dynamics in the chelonian reptiles. Ph.D. Thesis, University of East Anglia.

BURGGREN, W. W. (1977). The pulmonary circulation of the chelonian reptile: morphology, haemodynamics and pharmacology. *J. comp. Physiol.* B **116**, 303–323.

FISCHER, E. (1936). The action of a single vagal volley on the heart of the eel and the turtle. *Am. J. Physiol.* **128**, 596–608.

GARREY, W. E. & CHASTAIN, L. L. (1937). Inotropic and chronotropic effects of acetylcholine upon the chelonian heart. *Am. J. Physiol.* **119**, 315.

GASKELL, W. H. (1883–84). On the augmentor (accelerator) nerves of the heart of cold-blooded animals. *J. Physiol.* **5**, 46–48.

GAUNT, A. S. & GANS, C. (1970). Mechanics of respiration in the snapping turtle *Chelydra serpentina* (Linné). *J. Morph.* **128**, 195–227.

GILSON, A. S. (1932). Vagal depression of the turtle atrium. A study of the integration of effect of nerve impulses. *Am. J. Physiol.* **100**, 459–473.

GRAY, S. (1950). Pattern of contraction and relaxation in tortoise ventricle. *Am. J. Physiol.* **162**, 249–258.

HIATT, E. P. & GARREY, W. E. (1943). Drug actions of the spontaneously beating turtle ventricle indicating lack of innervation. *Am. J. Physiol.* **138**, 758–762.

JOHANSEN, K., LENFANT, C. & HANSON, D. (1970). Phylogenetic development of pulmonary circulation. *Fedn Proc.* **29**, 1135–1140.

KEITH, A. & FLACK, M. (1907). The form and nature of the muscular connections between the primary divisions of the vertebrate heart. *J. Anat.* **41**, 172–189.

LANDE, I. S. DE LA, TYLER, M. J. & PRIDMORE, B. R. (1962). Pharmacology of the heart of *Tiliqua* (*Trachysaurus*) *rugosa*. *Aust. J. exp. Biol.* **40**, 129–138.

LAURENS, H. (1913). The atrio-ventricular connection in the reptiles. *Anat. Rec.* **7**, 273–285.

LEE, H. M. (1935). Distribution of vagus control of the turtle heart. *Am. J. Physiol.* **112**, 207–213.

LEWIS, T. (1916). On the spread of the excitatory process in the vertebrate heart. *Phil. Trans. R. Soc.* B **207**, 221–310.

LOWMAN, E. C. & LAURENS, H. (1924). The physiology of the atrio-ventricular connection. IV. Functional differentiation and innervation of the A-V connection in the alligator heart. *Am. J. Physiol.* **68**, 316–325.

MEEK, W. J. & EYSTER, J. A. E. (1912). The course of the wave of negativity which passes over the tortoise's heart during the normal beat. *Am. J. Physiol.* **31**, 31–46.

MILLARD, R. W. & JOHANSEN, K. (1974). Ventricular outflow dynamics in the lizard, *Varanus niloticus*: responses to hypoxia, hypercarbia, and diving. *J. exp. Biol.* **60**, 871–880.

MILLS, T. W. (1885*a*). On the physiology of the heart of the alligator. *J. Anat. Physiol.* **20**, 549–558.

MILLS, T. W. (1885*b*). The innervation of the heart of the slider terrapin (*Pseudemys rugosa*). *J. Physiol.* **6**, 246–286.

MILLS, T. W. (1886). The rhythm and innervation of the heart of the sea-turtle. *J. Anat.* **21**, 1–20.

NOBLE, D. (1975). *The Initiation of the Heart Beat.* Oxford: Clarendon.

OKITA, S. (1971). The fine structure of the ventricular muscle cells of the soft-shelled turtle heart (*Amyda*), with special reference to the sarco-plasmic reticulum. *J. Electron. Microsc. Chiba Cy* **20**, 107–119.

ROBB, J. S. (1953). Specialized (conducting) tissue in the turtle heart. *Am. J. Physiol.* **172**, 7–13.
SHELTON, G. & BURGGREN, W. (1976). Cardiovascular dynamics of the chelonia during apnoea and lung ventilation. *J. exp. Biol.* **64**, 323–343.
WHITE, F. N. (1968). Functional anatomy of the heart of reptiles. *Am. Zool.* **8**, 211–219.
WHITE, F. N. & ROSS, G. (1966). Circulatory changes during experimental diving in the turtle. *Am. J. Physiol.* **211**, 15–18.
YAMAUCHI, A. & CHIBA, T. (1973). Adrenergic and cholinergic innervation of the turtle heart ventricle. *Z. Zellforsch. mikrosk. Anat.* **143**, 485–493.