

INTRACARDIAC SHUNTING REVEALED BY ANGIOCARDIOGRAPHY IN THE LIZARD *TUPINAMBIS* *TEGUIXIN*

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SUMMARY

1. The central circulation in the lizard *Tupinambis teguixin* (Linné, 1758) was studied using angiocardio-graphic techniques. Contrast medium was selectively injected into the vena cava superior, the sinus venosus, the right atrium, and the ventricular subcompartments [the cavum pulmonale (CP) and cavum arteriosum (CA)], following catheterization of the heart from the right jugular vein.

2. Contrast medium injection in the vena cava, sinus venosus, right atrium or CP showed that there was an exclusive and selective passage to the pulmonary circulation when injections were made during spontaneous or artificial ventilation. Contrast injection during apnoea showed various degrees of right–left shunting to the left aorta but typically not to the right aorta. There was no observable admixture from the CP to the CA.

3. When contrast medium was injected directly into the cavum arteriosum, there was clear selective filling of the right aorta and the cephalad circulation, as well as a lesser but distinct filling of the left aorta. During systole, there was no admixture from the CA to the CP, but a very slight left–right admixture was discernible during ventricular diastole.

4. The selective passage of contrast medium through the heart of *Tupinambis* showed a relationship to breathing in the intermittent ventilation pattern of *Tupinambis*. During apnoea, pulmonary flow appears to be impeded: this may reflect right–left shunting to the left aorta. This vessel becomes important in the alternation between a balance of pulmonary and systemic flow during breathing and a preference for systemic flow during apnoea.

* I am sorry to inform the readers of this journal and the whole scientific world that the famous Professor Kjell Johansen suddenly passed away during a sojourn in France for purposes of study. We will all miss a dear friend and colleague.

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INTRODUCTION

There has been a long-standing and continuing discussion about intracardiac and central vascular shunting in reptiles (Sabatier, 1873; Goodrich, 1919; White, 1970; Johansen, 1985; Burggren, 1985). It appears beyond doubt that bidirectional shunts are physiologically controlled and occur in most reptiles except crocodylians, which probably have unidirectional, right-left shunting only (Grigg & Johansen, 1987). The shunts are of importance to respiratory gas exchange, blood gas transport, the acid-base status and possibly thermoregulation of the organism. The shunts also play an important role in blood pressure regulation in both the pulmonary and systemic circulations (Millard & Johansen, 1973) and thus affect the fluid balance, particularly in the pulmonary vascular bed (Burggren, 1982). The pattern of shunting is considered by most workers to be related to the pattern of breathing, which in most reptiles is intermittent with breathing separated by apnoeic periods of variable lengths.

The present study employs angiocardiographic techniques on the lizard *Tupinambis teguixin* in analysing the intracardiac shunt patterns in a more extensive way than hitherto attempted in ectotherms. The contrast medium was selectively injected into systemic veins, the right atrium and the ventricular cava, from where blood is pumped to the systemic and the pulmonary circulations.

MATERIALS AND METHODS

Six tegu lizards, *Tupinambis teguixin*, were collected in the state of São Paulo, Brazil, and air-freighted to Aarhus, Denmark. The tegu is one of the largest neotropical lizards, it is very active and has a high preferred body temperature. The lizards used in this study weighed between 1.5 and 2.0 kg. They were fed on a diet of mice, eggs and bananas twice a week. The animals were fasted for 5 days prior to an experiment.

The animals were kept at room temperature (25°C) and exposed to infrared lamps allowing basking for 8 h every day.

In preparation for the angiocardiographic examination the tegus were anaesthetized using 5% halothane in air administered by positive pressure ventilation after tracheal intubation. The right jugular vein was exposed through a lateral incision 2–3 cm posterior to the occipital region. A radio-opaque catheter or a PE 60 polyethylene catheter was positioned in the jugular vein without obstructing flow in the vessel. The catheter was tied loosely to the vessel wall and the incision closed without restricting movement of the catheter. The catheterized animals were normally allowed to recover overnight prior to the angiocardiographic examination. Some animals were studied while anaesthetized. These animals were examined when the lungs were not ventilated and studies were also made during positive pressure artificial ventilation and during spontaneous breathing when the lizards had recovered from anaesthesia.

The tegu, like most ectotherm air-breathing vertebrates, practises intermittent ventilation, during which apnoea of variable duration alternates with single breaths

or a series of breaths. Animals allowed to recover prior to examination were studied both during apnoea and during active ventilation of the lungs. The body temperatures of the experimental animals were between 23 and 25°C during the X-ray examinations.

The angiocardiography was performed using the contrast medium Hexabrix (320 mg iodine ml⁻¹). The contrast medium was injected from a syringe by hand, in most cases using 2–3 ml. Aided by fluoroscopy, the catheter was positioned in the superior vena cava close to the sinus venosus (SV) or in the right atrium (RA). It also proved possible to advance the catheter into the ventricle where it could be positioned both in the cavum pulmonale (CP) and the cavum arteriosum (CA). The cavum pulmonale is the ventricular compartment that directly perfuses the pulmonary arterial circulation. This cavum is, however, anatomically incompletely separated from two other ventricular cava: the cavum arteriosum and the much smaller cavum venosum. The cavum arteriosum is predominantly filled from the left atrium and is the main filling pump for the systemic arterial circulation, while the cavum pulmonale is filled mainly from the right atrium.

Since the contrast medium injections were done manually, the exit pressure at the catheter tip was difficult to control. In some cases this, as well as the catheter passage between the valve leaflets, resulted in temporary valve impatency causing reflux from, for example, the right atrium to the sinus venosus, from the ventricular cavum pulmonale to the right atrium, and from the cavum arteriosum to the left atrium. No indication of turbulence or abnormal distribution of contrast medium was apparent during the injections and after contrast injection all the relevant valves were patent: this indicates that the physiological interpretations are valid.

The rate of exposure was usually 4 frames s⁻¹ for 3 s followed by 2 frames s⁻¹ for another 6 s. The exposures were recorded by a Mingograf (Siemens Elema, Sweden). A 24 cm × 30 cm film changer (Siemens Elema Schönander, Sweden) and a Sony U-matic Vo 5630 video cassette recorder were used for all angiocardiograms. The film changer was equipped with a linear grid (FF 100 cm, ratio 1–8) and the screen/film combination was Kodak Lanex screens with Kodak Auto E-films. The Roentgen generator was a triplex automatic CR with a Siemens Bi 150/30/101 R X-ray tube, focus 0.6/1.3 mm; focus-to-film distance was always 100 cm. Exposure voltage was kept between 60 and 70 kV and the exposure time was usually 0.080 s. The examinations were made in biplane exposure. Contrast medium injections were made with the lizards placed in a Plexiglas chamber designed to limit movement of the animal. Each experiment consisted of 2–3 serial injections of contrast medium. The contrast medium was cleared from the heart and central vasculature between injections.

RESULTS

Fig. 1 shows three consecutive frames following injection of contrast medium in the sinus venosus while the animal was ventilated artificially. The exposures were made in the dorsoventral position. In frame A, and again in C, the sinus venosus is

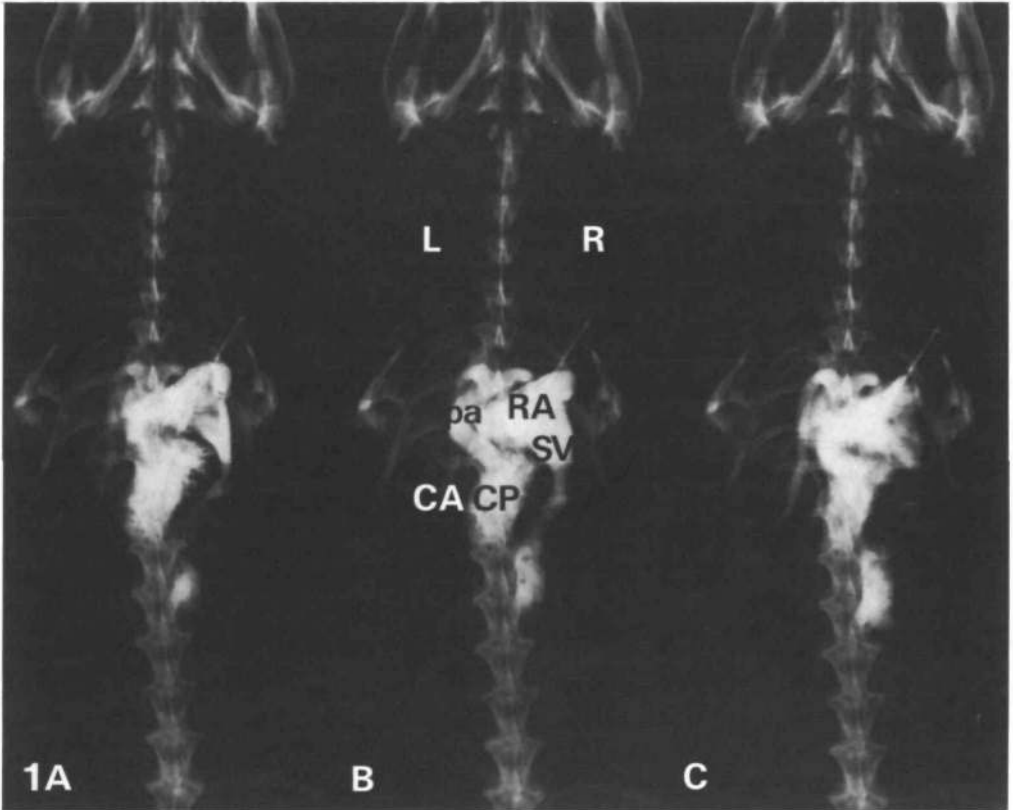


Fig. 1. Angiocardigraphy showing three consecutive frames following injection of contrast medium in the sinus venosus (SV) of *Tupinambis teguixin*. The animal (no. 2) was lightly anaesthetized and artificially ventilated. All experiments were performed with the animals in a dorsoventral position. CP, cavum pulmonale; CA, cavum arteriosum; RA, right atrium; pa, pulmonary arterial trunk; L, left; R, right. Magnification $\times 1.1$.

partially contracted, while in frame B it is distended. Contrast medium has traversed the right atrium and filled the right portion of the ventricle, the cavum pulmonale (CP). An earlier ventricular contraction has ejected contrast medium into the main pulmonary arterial trunk, showing its bifurcation into the right and left pulmonary arteries (frames A–C). Along its right contour, the ventricle (CP) shows a filling pattern of finger-like projections reflecting the spongy trabeculation of the ventricular myocardium. Along its left contour, the filled CP shows a sharp, well-defined demarcation from the ventricular cavum arteriosum (CA). Notably, contrast medium is not observed in the CA. The right and left pulmonary arterial arches and their descending limbs are clearly defined. Frame A also suggests that atrial contraction is in progress since the sinu-atrial connection appears closed and ventricular (CP) filling is in progress. Importantly, there is no contrast visible in the left or in the right aorta or the carotid arteries (see Fig. 5 for filling of these vessels). In frame B ventricular connection is interrupted and the atrioventricular valves are closed. Thus inflow from the right atrium (RA) has ceased and the sinus venosus

(SV) and right atrium are distended. Frame B also clearly defines the ostium between the CP and the pulmonary arterial trunk (pa). In frame C, ventricular diastole must have just started since the atrioventricular connection, superimposed on the pulmonary outflow tract, is re-established and the RA has started to shorten and narrow. The limited filling of the cavum pulmonale at this time and additional evidence from other sequences suggest that the residual volume of the CP following contraction is small, a fact of importance for the extent of intraventricular mixing during diastole.

Following onset of spontaneous breathing, when the contrast medium was injected into the right atrium, the cavum pulmonale (CP) was also the only ventricular subcompartment showing contrast and only the pulmonary arteries were filled with contrast medium following ventricular contraction.

Fig. 2 shows two frames from different animals. Contrast medium was injected in the vena cava superior of the left-hand lizard (Fig. 2A) towards the end of a long apnoeic period (>3 min). Contrast medium is apparent in all the cardiac outflow vessels. While the right aorta and the carotid arteries derived from it show rather faint contrast, the left aorta and the pulmonary arterial outflow trunk show higher contrast density. The right-hand frame shows a situation when contrast medium was injected into the right atrium. This animal (Fig. 2B) was ventilating spontaneously. The frame was exposed near a ventricular systolic termination, showing the ejected contrast medium only in the pulmonary trunk and arches.

Fig. 3 shows another sequence following injection of contrast medium into the sinus venosus. This time the animal was awake and had recovered from anaesthesia, but the exposures were made when the animal was in an apnoeic phase. Under these conditions, there is no ventricular filling from the right atrium that becomes apparent in the cavum arteriosum. Again, the pulmonary arteries (pa) show the highest contrast density, while the left aorta is also filled but to a much lesser extent, making a loop to the left before following its descending course. No contrast medium is apparent in the right aorta or in the carotid or subclavian arteries derived from it.

Fig. 4 shows consecutive frames from another specimen following injection of contrast medium, after the catheter had been advanced through the right atrium and into the cavum pulmonale compartment of the ventricle. Frames A and B indicate that ventricular contraction causes no admixture to the cavum arteriosum nor does contrast appear in the right aorta or the carotid arteries. The pulmonary arteries (pa) are preferentially filled, and later in the sequence the left aorta becomes discernible. The cavum pulmonale is nearly, if not completely, emptied of contrast medium following ventricular contraction (frame D).

Fig. 5 is based on contrast injection directly into the cavum arteriosum after advancing the catheter through the cavum venosum from the cavum pulmonale (CP) into the cavum arteriosum (CA). This sequence clearly shows the cavum arteriosum positioned to the left of the vertebral column whereas the CP overlies the column (Figs 1, 3, 4). Frame A of Fig. 5 was exposed during ventricular systole. No shunting of contrast medium into the CP is apparent during ventricular systole,

while during diastole and expansion of the lumen of the ventricular subcompartments (frames B, C), a small amount of contrast is apparent in the cavum pulmonale. There is a nonequivocal preference for filling of the right aorta, giving rise to circulation in the cephalad direction *via* the carotid arteries. Comparing frames A and D one can clearly see that contrast medium in the carotid arteries has moved into the brain.

During ventricular diastole, the left aorta (la) also becomes discernible (frame C), while the pulmonary arterial arches are only faintly visible.

DISCUSSION

Angiocardiography has played an important role in the detection and evaluation of central cardiovascular shunts in vertebrates. The technique has been used on

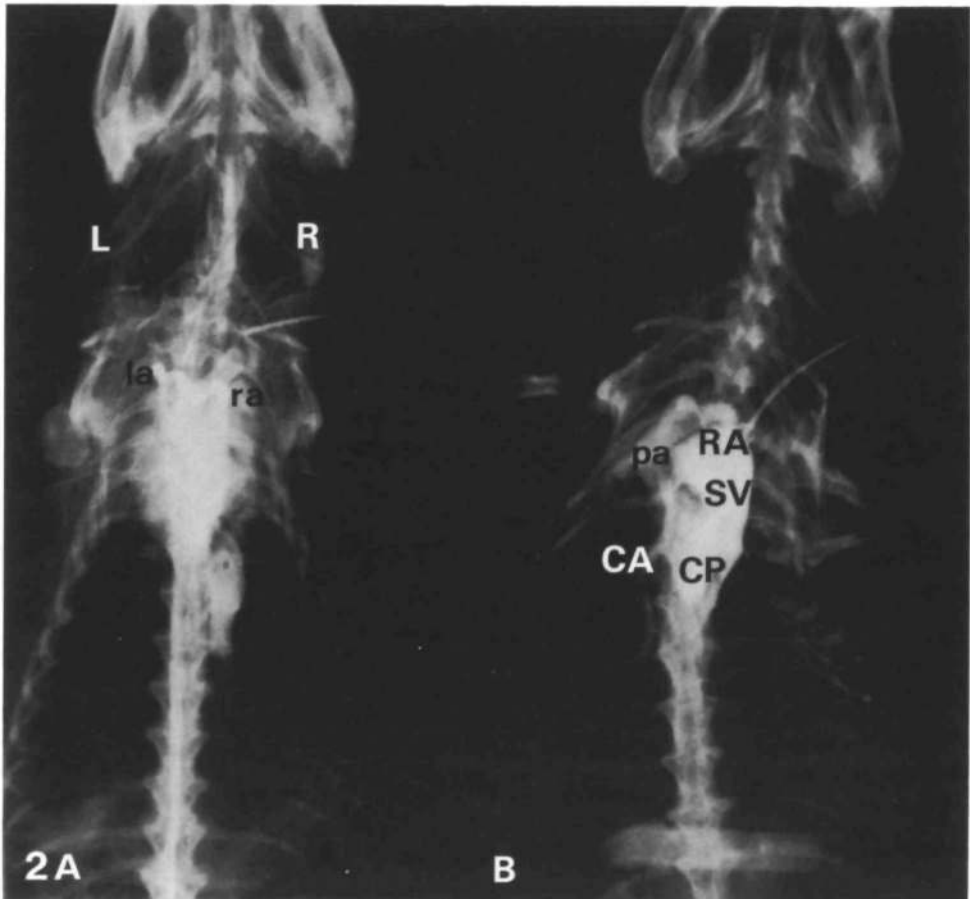


Fig. 2. Two angiographic frames from *Tupinambis* no. 1 (A) and no. 2 (B). (A) After contrast injection into the vena cava superior during a prolonged apnoeic period. (B) Contrast was injected in the sinus venosus (SV) during a period of active lung ventilation in an awake animal. ra, right aorta; la, left aorta; other abbreviations as in Fig. 1. Magnification $\times 1.1$.

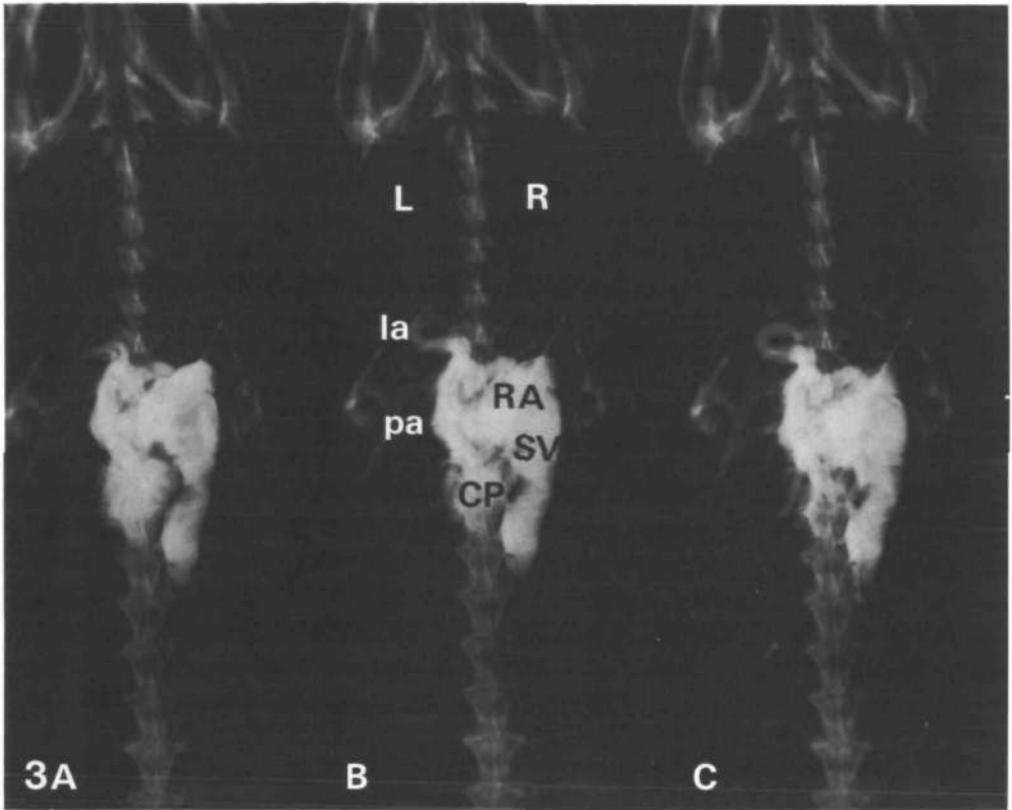


Fig. 3. Injection of contrast medium in the sinus venosus during apnoea in an awake, spontaneously breathing *Tupimambis* (no. 4). Abbreviations as in Figs 1 and 2. Magnification $\times 1.1$.

representatives of all tetrapods as well as in the lungfish *Protopterus* (Johansen & Hol, 1968). The advantage of this method for shunt detection and evaluation is that it allows visualization of the central circulation pattern through several heart beats in animals minimally disturbed by the experimental procedure. For quantitative assessments of shunts, however, analysis of blood gas content in the cardiac compartments and central vessels involved, or indicator dilution techniques, such as dye dilutions or microsphere injections, are useful (Heisler & Glass, 1985).

Due to technical problems related to catheterization without surgical exposure of the heart, particularly in small-sized ectotherm vertebrates, injections of contrast medium for shunt evaluation have only been made in systemic veins, thus marking deoxygenated blood and its possible admixture into oxygenated blood returning from the lungs to the left atrial space (right-left shunting). We know of no angiocardiographic study in which contrast medium has been injected to mark the most oxygenated blood in a pulmonary vein, the left atrium or the cavum arteriosum ventricular subcompartment of a reptile. The latter has been an important objective in the experiments now reported.

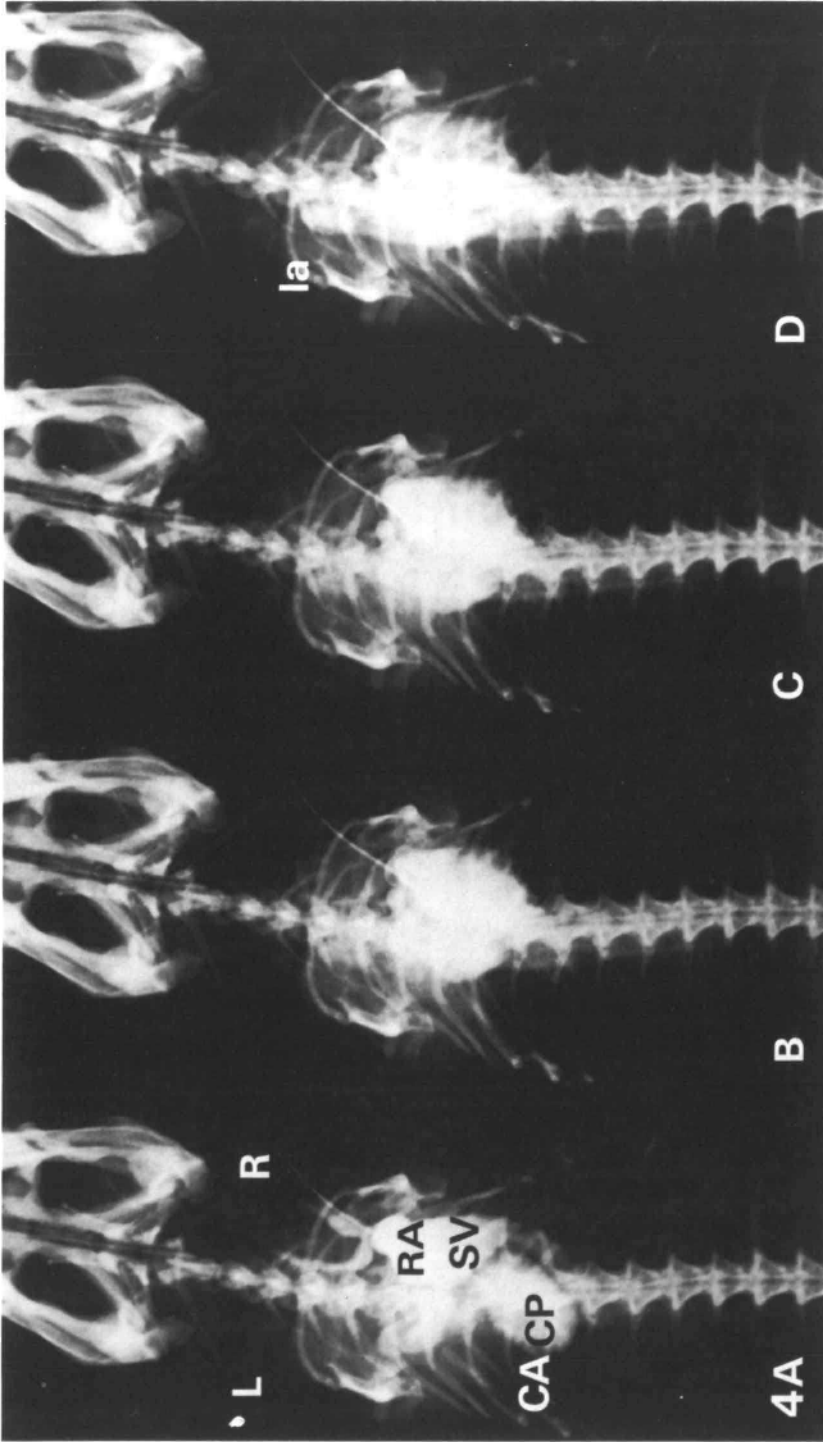


Fig. 4. Consecutive angiographic frames following injection of contrast medium directly into the cavum pulmonale of the ventricle in a non-anesthetized *Tupinambis* (no. 3). Abbreviations as in Figs 1 and 2. Magnification $\times 1.1$.

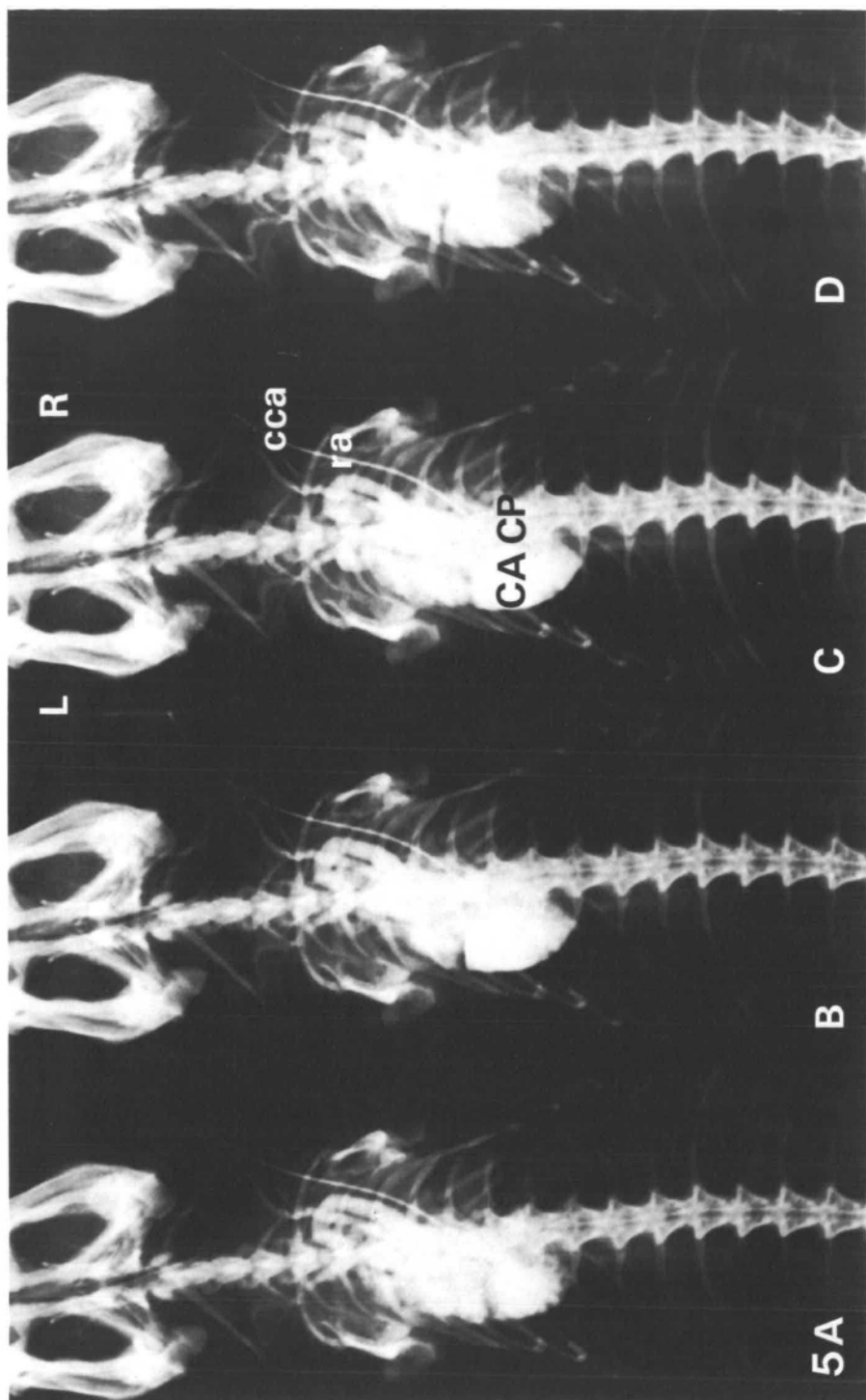


Fig. 5. Four angiocardiographic frames showing passage of contrast medium injected into the cavum arteriosum following catheterization of the cardiac ventricle in a non-anaesthetized *Tupiaimbis* (no. 3). cca, common carotid artery; other abbreviations as in Figs 1 and 2. Magnification $\times 1.1$.

The ventricle of squamate reptiles is a very complex structure. The most important point to be emphasized in the present context is that the inflow from the left atrium to the ventricular cavum arteriosum is likely to be discrete and separated from the right atrial inflow aided by the very large atrioventricular valves. This pattern prevents a large interface between the two qualities of atrial blood entering the ventricular space. For right atrial blood to enter the cavum pulmonale, which during systole will be the exit compartment to the main pulmonary arterial trunk, it has to traverse the cavum venosum and pass across the free edge of the muscular ridge. The relative size of the cavum venosum, which during systole will be the exit compartment for the systemic arterial outflow (right and left aorta), will be important in deciding the extent to which right atrial deoxygenated blood may potentially exit to the systemic arteries. The angiocardigraphic data suggest that this fraction is very small or non-existent, especially when the animal is ventilated either artificially (Fig. 1) or spontaneously. The cavum venosum in *Tupinambis*, like that of the varanids, proved on dissection to be a rather small ventricular compartment compared to the ventricles of chelonians and other squamates (Webb, Heatwole & DeBavay, 1971). If a right-left shunt occurs, the contrast medium typically becomes apparent in the left but not in the right aorta, a pattern mostly seen during periods of apnoea (Fig. 3). The left aorta in squamate reptiles joining the right aorta in its descending course serves mainly the caudal arterial systemic distribution. Depending on the breathing phase, and the end-systolic residual volumes of the ventricular subcompartments, the left aorta may carry blood of mixed gas composition. Earlier studies based on blood gas analysis have arrived at the same conclusion (White, 1976). During very long breath-holdings in *Tupinambis*, injection of contrast medium into a systemic vein did on one occasion show medium in the right as well as the left aorta, although distinctly less was seen in the right aorta and the carotid arteries than in the left aorta, which in turn contained less than the pulmonary arteries (Fig. 2A). In cases when a right-left shunt was apparent, there was no indication of constriction or discrete sphincteric narrowing of the pulmonary arterial trunk, which has been alleged to be the main mechanism behind the increased pulmonary vascular resistance. The control of pulmonary vascular resistance could, however, reside in the more peripheral pulmonary vasculature. Right-left shunting in chelonians and squamates, in which the ventricle generates a uniform pressure in all subcompartments during systole, is likely to depend on an altered impedance in the pulmonary and/or systemic outflow arteries (Shelton & Burggren, 1976; Burggren, 1985). No intraventricular pressure measurements have, however, been performed on *Tupinambis*.

Most importantly this study shows that, when contrast medium was injected directly into the cavum pulmonale or the cavum arteriosum, no – or very slight – admixture between them became apparent (Figs 4, 5). Notably, this clear demarcation between contrast medium in CP (Fig. 4) and CA (Fig. 5) occurred during ventricular systole (Fig. 4), as well as when the CA was maximally filled in diastole (Fig. 5B). Extensive contrast in the right aorta, parent vessel to the cephalad circulation, only occurred when medium was ejected from the cavum arteriosum.

Ejection of medium from the cavum pulmonale predominantly filled the pulmonary arterial trunk but contrast could also appear in the left aorta.

This study has documented a tendency for a right–left shunt to occur from the CP to the left aorta, while a left–right shunt from the CA to the pulmonary arteries is nonexistent or negligible. This indicates that the combined systemic outflow from the heart will at times exceed the pulmonary outflow; a tendency amplified during breath-holding periods in the normal breathing pattern. Since this situation precludes a balanced systemic and pulmonary flow, the left aorta assumes an important role as a flow-adjusting circuit. However, at times when outflow from the CP only reaches the pulmonary outflow vessels (e.g. Fig. 1), a situation associated with the ventilatory periods in the normal intermittent breathing pattern – a double circulation with a balanced systemic and pulmonary flow – must prevail, in spite of the anatomical continuity of the ventricular subcompartments.

The high degree of selective circulation through the heart of *Tupinambis* demonstrated by angiocardiography following catheterization of the cardiac subcompartments must depend on morphological features, such as a well-developed vertical septum and a small cavum venosum as has been described for the varanid lizards (Webb *et al.* 1971). Physiologically, the very small residual volumes documented in both cavum arteriosum and cavum venosum following ventricular ejection will minimize intraventricular admixture during diastole. There was no admixture from CA to CP during systole. A slight tendency for contrast medium to admix from the CA to the CP in diastole (Fig. 5B,C), whilst no admixture could be documented in the reverse direction, suggests that there may occur a pressure separation resulting in higher systolic pressure in the CA compared to the CP during systole. Such pressure separation has been demonstrated for varanid lizards but not for other squamates (Burggren & Johansen, 1982).

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