

## DYNAMIC ANATOMICAL STUDY OF CARDIAC SHUNTING IN CROCODILES USING HIGH-RESOLUTION ANGIOSCOPY

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### Summary

Prolonged submergence imposes special demands on the cardiovascular system. Unlike the situation in diving birds and mammals, crocodilians have the ability to shunt blood away from the lungs, despite having an anatomically divided ventricle. This remarkable cardiovascular flexibility is due in part to three anatomical peculiarities: (1) an 'extra' aorta (the left aorta) that leaves the right ventricle and allows the blood from the right ventricle to take an alternative route into the systemic circulation instead of going to the lungs; (2) the foramen of Panizza, an aperture that connects the right and left aortas at their base immediately outside the ventricle; and (3) a set of connective tissue outpushings in the pulmonary outflow tract in the right ventricle. Using high-resolution angiography, we have studied these structures in the beating crocodile heart and correlated their movements with *in vivo* pressure and flow recordings. The connective tissue outpushings in the pulmonary outflow tract represent an active mechanism used to restrict blood flow into the lungs,

thus creating one of the conditions required for a right-to-left shunt. We observed that the foramen of Panizza was obstructed by the medial cusp of the right aortic valve during most of systole, effectively differentiating the left and right aortic blood pressure. During diastole, however, the foramen remained open, allowing pressure equilibration between the two aortas. Contrary to current theories, we found that the left aortic valves were unable to cover the foramen of Panizza during any part of the cardiac cycle, supporting the reversed foramen flow hypothesis. This would ensure a supply of blood to the coronary and cephalic circulation during a complete shut-down of the left side of the heart, such as might occur during prolonged submergence.

Key words: *Crocodylus rhombifer*, *Crocodylus porosus*, foramen of Panizza, heart, blood flow, cog-teeth-like valves, diving, right-to-left shunt.

### Introduction

In birds and mammals, there exists an obligatory match between the systemic and pulmonary blood flows. In those birds and mammals that dive, a substantial physiological shunt may develop as the diving depth/time increases. This physiological shunt is due to an inequality in lung perfusion relative to the distribution of alveolar gas, because of lung compression, and is not due to a shunt of blood away from the lungs (Johansen, 1985). Crocodilians, in contrast to other reptiles but like birds and mammals, have a fully divided ventricle separating the systemic and pulmonary vascular beds but retain the possibility of shunting blood between the pulmonary and systemic circuits (right-to-left shunt) *via* a left aorta that opens from the right ventricle (Fig. 1). This unique system give the crocodilians the possibility to match lung perfusion closely with alveolar gas flow without affecting systemic blood flow.

The left aorta communicates with the right aorta through the foramen of Panizza, an opening located in the inter-aortic septum at the base of the outflow tract (Fig. 1). This aperture was described for the first time by the Italian scientist Bartolomeo Panizza in 1833 and it increases the complexity/flexibility of the crocodilian cardiovascular system by permitting a flow between the two aortic arches. The crocodile heart also has two sets of pulmonary valves: (1) leaf-type aortic valves typical of all vertebrate hearts and (2) connective tissue outpushings (Webb, 1979; van Mierop and Kutsche, 1985), termed cog-teeth-like valves, which project from the right ventricular wall into the pulmonary outflow tract just proximal to the leaf-type pulmonary valves (Fig. 1). Collectively, the crocodile circulatory system is probably the most functionally (haemodynamically) sophisticated found among the vertebrates, combining the ability found in

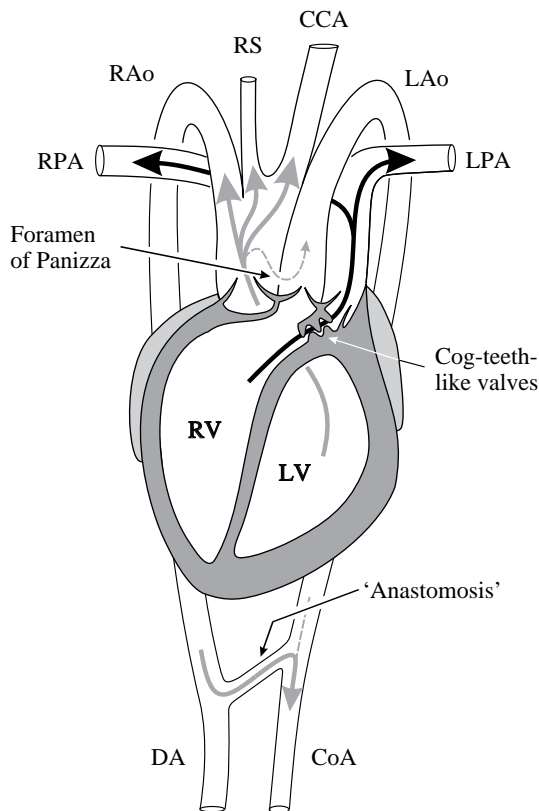


Fig. 1. Schematic representation of the crocodilian heart, outflow tract and major arteries; arrows indicate blood flow pattern during non-shunting conditions, broken arrow indicates blood flow during diastole (foramen and left aorta). Blood is ejected from the left ventricle (LV) into the right aorta/dorsal aorta (RAo/DA), the right subclavian artery (RS) and the common carotid artery (CCA). The right ventricle (RV) ejects blood into the common pulmonary trunk, which divides into the left and right pulmonary arteries (LPA and RPA). During right-to-left shunting, blood is also ejected from the right ventricle into the left aorta (LAo), which continues to the gut as the coeliac artery (CoA). The right and left aortas communicate through the foramen of Panizza located at their base just outside the bicuspid semilunar valves. A second point of communication between the two aortic arches exists in the abdomen *via* the anastomosis. Note the unique set of 'cog-teeth-like valves', found in the pulmonary outflow funnel within the right ventricle just proximal to the bicuspid semilunar valves between the ventricle and the common pulmonary trunk.

mammalian and avian four-chambered hearts to separate oxygenated and deoxygenated blood completely with the shunt capability of the amphibian/non-crocodile reptilians. Ever since it was first described, the crocodilian cardiovascular system has intrigued scientists and, with the application of new techniques, we have gradually investigated the unique anatomy; a series of hypotheses about the functional significance based on these data have been put forward (White, 1969; Grigg and Johansen, 1987; Axelsson *et al.* 1989; Shelton and Jones, 1991; Jones and Shelton, 1993).

The aim of the present study was to investigate the crocodile heart using a combination of high-resolution angioscopy with

blood pressure and flow measurements to obtain information about the functional significance of its unique anatomical structures.

### Materials and methods

Two separate experimental approaches were used to obtain the data presented in this paper. (1) A perfused heart set-up essentially as described by Franklin and Axelsson (1994) was used for the angioscopy. (2) *In vivo* measurements of various haemodynamic data, as described by Axelsson *et al.* (1989), were used for obtaining phasic blood flow and pressure recordings.

#### Perfused heart

Two Cuban crocodiles (*Crocodylus rhombifer* Cuvier), 55 and 78 kg respectively, were purchased from Kolmården Zoological garden and transported to Göteborg, where they were kept at 25–27 °C for 2 days prior to the experiments. The animals were anaesthetised for transportation using a combination of Medetomidin and Telazol (3 and 250 mg, respectively) injected intramuscularly just behind the left hindleg using a blowgun. When full anaesthesia was achieved, a skin incision was made in the left hindleg and a venous catheter was introduced into the femoral vein *via* a smaller branch. This catheter was used to administer intravenous glucose Ringer's solution at a rate of 1 ml min<sup>-1</sup>. The initial anaesthetic injection kept the animals sedated until experimentation (24–48 h). The surgical preparations for the perfused heart set-up have been described in detail by Franklin and Axelsson (1994). Briefly, the animals were killed instantaneously using a bovine captive bolt. They were intubated using a 10 mm silastic tube and immediately ventilated with medical-grade oxygen. Stainless-steel cannulae (internal diameter 6–9 mm depending on the vessel/animal size) were used to cannulate the left and right pulmonary veins and right hepatic vein. The outflow cannulae used for the right and left aortas and the pulmonary artery had an internal diameter of 8–10 mm. The pulmonary and right hepatic vein pressures were adjusted (range 0.15–0.3 kPa) to achieve a flow of approximately 20 ml min<sup>-1</sup> kg<sup>-1</sup> body mass ventricle<sup>-1</sup> (i.e. total cardiac output of around 40 ml min<sup>-1</sup> kg<sup>-1</sup> body mass) against a mean output pressure of 3.5 kPa for the right and left aortas and 2 kPa for the pulmonary artery (see Franklin and Axelsson, 1994). The Ringer's solution used contained 5 nmol l<sup>-1</sup> adrenaline to maintain regular contractility of the heart (Perry and Farrell, 1989). The perfusion experiments were carried out at room temperature (25–27 °C).

#### High-resolution angioscopy

A high-resolution angioscope was used to study the right and left aortic and pulmonary valves and the foramen of Panizza in the beating heart. The lenses used were from Karl Storz, Tutting, Germany, and had an outer diameter of 4 mm and a viewing angle of 0 or 30°. The lenses were used in combination with a Sony 3 CCD camera, model DXC 75 OP, and video

sequences were recorded on a Sony Beta SP video recorder, model BVW 75. For the right aortic sequences, the angioscope was inserted occlusively through the common carotid artery and slowly advanced until the right aortic valves and the foramen of Panizza could be seen. In order to view the left aortic valves and the foramen of Panizza, the angioscope (30° viewing angle) was non-occlusively inserted through the left aortic wall. For the pulmonary valves, the angioscope was inserted into the left pulmonary artery.

The video sequences were fed into a computer (Macintosh, Quadra 800) via a Falcon video capture card (Graphics Unlimited) and stored in Photoshop (version 2.5) format. For the subsequent image analysis and enhancement, Photoshop was used, and the final versions of the images were printed using a Fuji Pictograph Photoprinter.

#### *In vivo measurements*

These experiments were carried out on 10 estuarine crocodiles (*Crocodylus porosus* Schneider), purchased from Edward River Crocodile Farm, Queensland, Australia. The animals were flown to Brisbane and kept at 28–30 °C in outdoor tanks containing shallow water, and they had access to a basking platform. The animals were of either sex with a body mass of 3.4–6.6 kg.

#### *Surgical procedure*

In order to anaesthetise the crocodiles, the animals were restrained, and a piece of plastic tubing was inserted into and secured in the mouth. Lignocaine (20 mg ml<sup>-1</sup>) was applied to a rubber endotracheal tube and sprayed directly onto the glottis for local anaesthesia. The tracheal tube was inserted and connected to a Ohmeda Fluotec 3 halothane dispenser; a concentration of 4–5 % halothane in oxygen was used to induce anaesthesia, which was maintained using 1–2 % halothane during the surgery. The animals were placed ventral side up on a padded surgical table and the thorax was opened using a Surgistat (Valleylab) cautery knife. The outflow tract and the major vessels were freed from surrounding tissue for cannulation and flow probe placement (see below). After surgery, the animals were left to recover for at least 48 h before any recordings were made. The temperature in the experimental holding tanks was 25–28 °C.

#### *Blood flow recordings*

A pulsed Doppler system (Iowa University) was used to record phasic blood velocity profiles. Custom-built flow probes (Perspex) with an internal diameter of 2.5–3.5 mm were placed around the right and left aortas and the left pulmonary artery immediately outside the outflow tract. The leads from the flow probes were exteriorised and secured using skin sutures onto the back of the crocodiles.

#### *Blood pressure recordings*

Polyurethane cannulae (PU 90) filled with heparinized saline (100 i.u. ml<sup>-1</sup> in 0.9 % NaCl) were implanted non-occlusively into the right and left aortic and pulmonary parts of the outflow

tract and secured using purse-string sutures to the wall of the outflow tract. In three animals, right intraventricular pressure was recorded using a cannula (PU 90) inserted through the ventricular wall. The cannula had sideholes close to the tip to ensure that it remained patent throughout the cardiac cycle. All the cannulae were exteriorised and connected to titanium injection ports secured onto the back of the animal. During the experiments, pressures were recorded using needle-tipped polyethylene cannulae (PE 90), filled with heparinized saline and connected to the titanium ports. The polyethylene cannulae were in turn attached to Statham pressure transducers which had been simultaneously calibrated using a static column of water.

#### *Data acquisition and presentation*

The pressure and flow signals were amplified and recorded using a Grass recorder model 7. Signals were also fed into a computer (Toshiba 5200) running Labtech Notebook software version 4.0. The sampling frequency used for the graphs presented below was 250 Hz. AD-Data software (Dr P. Thoren, Department of Physiology, Göteborg) was used for post-sampling treatment of the data. No calibrations of the individual Doppler flow probes were performed, and the blood flow data are therefore presented as relative changes in flow velocity. Video clips of the original video sequences used in the paper can be found on the web-server <http://vivaldi.2001.gu.se/crocodyl.htm>. They will also appear on the Company of Biologists server (<http://www.citscape.co.uk/users/ag64>).

### **Results and discussion**

This is the first high-resolution angioscopy study of the anatomical arrangement of the right and left aortic valves, the foramen of Panizza and the cog-teeth-like valves in the right ventricle in a beating crocodile heart. Complementary phasic blood flow and pressure recordings enabled us to clarify further the functional significance of these structures.

It has been argued that the diameter of the foramen of Panizza is small and that this communication between the right and left aortas may not be physiologically important. In this study, we used a perfused beating heart working at physiological pressures/flows and found that the foramen of Panizza has a diameter of approximately 35–40 % that of the right aorta, indicating that the original arguments for its possible negligible physiological role may be wrong. Moreover, in a recent study of the innervation of the crocodilian heart, it was shown that the area around the foramen of Panizza is heavily innervated and that there might be a sphincter-like muscle arrangement around the foramen, suggesting the possibility of a variation in its diameter (Karila *et al.* 1995). Previous anatomical descriptions of the foramen of Panizza indicated that both the right and left aortic valves could interfere with flow through the foramen (Webb, 1979; Grigg, 1989). Moreover, it was assumed from *in vivo* phasic blood flow and pressure measurements that the right aortic

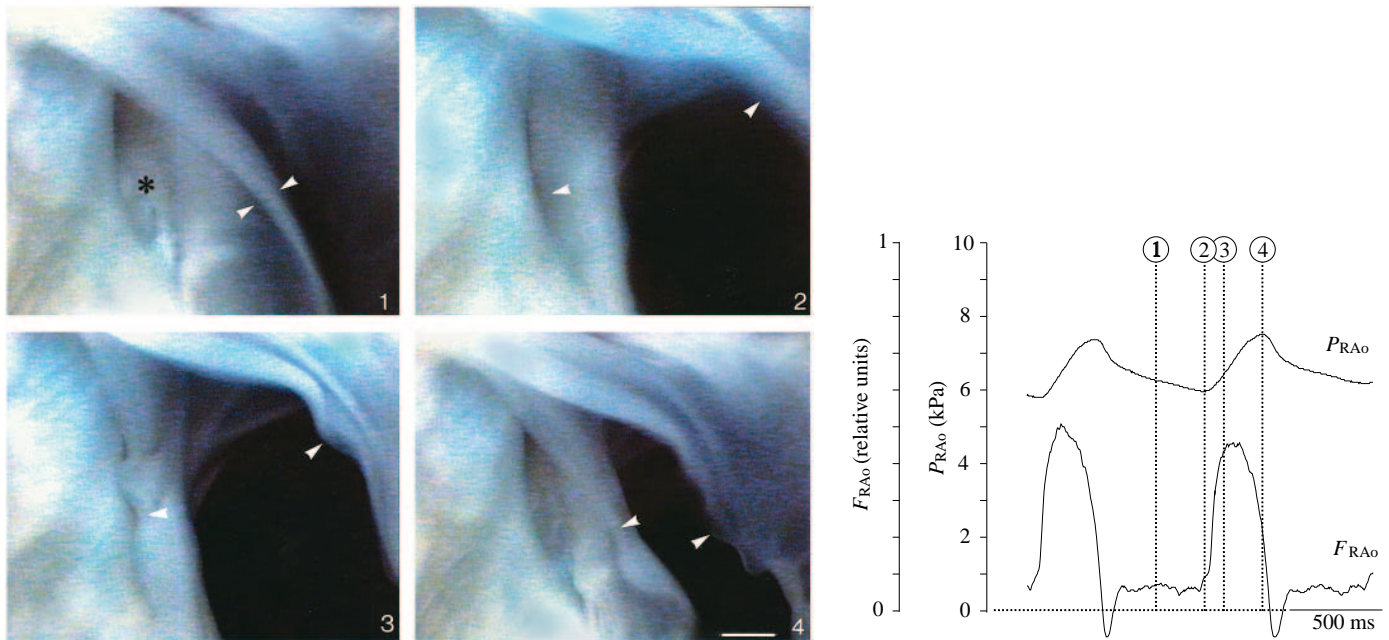


Fig. 2. The photographs show angioscopic images of the right aortic valves and the foramen of Panizza taken at four different stages during the cardiac cycle, starting during diastole with the bicuspid valves closed (frame 1). The graph shows phasic blood pressure recordings from the right aorta ( $P_{RAo}$ ) together with phasic blood flow in the right aorta ( $F_{RAo}$ ). Numbers in circles indicate corresponding times between the angioscopic images and the phasic pressure/flow recordings during the cardiac cycle obtained from different animals. The angioscope was inserted through the common carotid artery and advanced until the foramen of Panizza (marked with an asterisk in frame 1) and the bicuspid valves of the right aorta (marked with white arrowheads) could be seen. Note that the medial cusp of the right aortic valve completely covers the foramen of Panizza during systole (frame 3). Scale bar, 1 mm.

valve covered the foramen during systole and that only during diastole, when the aortic valves were closed, was there free communication between the two aortas (Axelsson *et al.* 1989). High-resolution angioscopy in the present study confirmed that the medial cusp of the right aortic valve does cover the foramen during peak systole (Fig. 2, frame 3, although during early systole, before the right aortic valves are fully open, there is a small flow from the right to the left aorta: this would explain the initial positive flow seen in the left aortic arch (Fig. 3, frame 2 and number 2). The non-shunting flow pattern, which occurs for the majority of time in resting crocodylians (Axelsson *et al.*, 1989; Shelton and Jones, 1991; Jones and Shelton, 1993; Grigg and Johansen, 1987), is shown in Fig. 1. Under these conditions, the heart is functionally similar to the avian or mammalian heart, with blood being ejected from the left ventricle into the systemic circulation (*via* the right aorta in crocodylians) and returning to the right atrium. At the same time, the blood in the right ventricle is pumped into the pulmonary arteries and, after passing the lung vasculature, is returned to the left atrium. Under these conditions, no blood is ejected into the left aorta from the right ventricle and thus the small systolic (early) and diastolic flows through the foramen of Panizza (Fig. 3, frames 2 and 4 and numbers 2 and 4) would be required to prevent clotting of the blood in the left aorta (Shelton and Jones, 1991). The left aorta continues as the coeliac artery and supplies blood to the gut (see Fig. 1). The small blood flow through the foramen of Panizza would be

inadequate to support the function of the gut, and it has been shown that most of the gastrointestinal blood flow is derived from the right aorta *via* the anastomosis (Axelsson *et al.* 1991).

In most diving vertebrates, adjustments to the cardiovascular system during submergence favour blood flow to the heart and the brain (Butler, 1988), thus enabling the animals to stay submerged for longer periods. There is evidence that this is also the case for the diving crocodylian, in which it has been shown that the relative gastrointestinal blood flow, at least, decreases more than the carotid artery blood flow, indicating a selective perfusion of the head region (Axelsson *et al.* 1991). Shunting of blood away from the lungs (the right-to-left shunt) is possible in crocodylians, despite the complete separation of the ventricles. This is accomplished by ejecting blood *from the right ventricle* into the left aorta instead of into the pulmonary arteries. This shunt may be initiated in a number of ways, probably by a combination of the following: (1) increasing the filling pressure of the right ventricle (Starling effect) (Franklin and Axelsson, 1994), (2) increasing the pulmonary resistance, thus increasing right intraventricular pressure (Axelsson *et al.* 1989); and (3) decreasing the systemic vascular resistance (Axelsson and Nilsson, 1989; Jones and Shelton, 1993). A right-to-left shunt has been observed in resting air-breathing crocodylians (White, 1969; Grigg and Johansen, 1987; Axelsson *et al.* 1989; Jones and Shelton, 1993), and this shunt may be even more pronounced during long periods of submergence. In the present study, we used an increased right

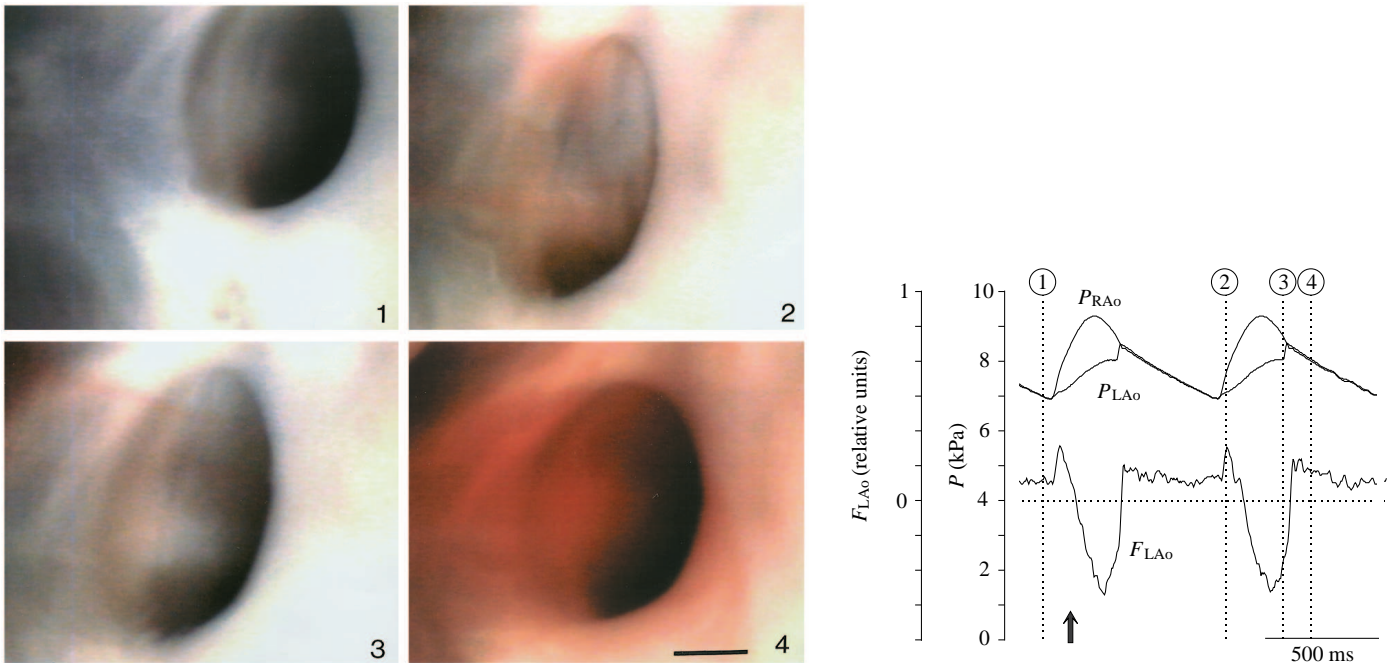


Fig. 3. The photographs show angioscopic images of the foramen of Panizza taken from the left aorta with the angioscope inserted non-occlusively through the left aortic wall at four different stages during the cardiac cycle, starting during diastole (frame 1). The graph shows phasic blood pressure recordings from the right and left aortas ( $P_{RAo}$  and  $P_{LAo}$ ) together with phasic blood flow in the left aorta ( $F_{LAo}$ ). Numbers in circles indicate corresponding times between the angioscopic images and the phasic pressure/flow recordings during the cardiac cycle obtained from different animals. The arrow in the graph indicates the time at which a bolus of haemoglobin solution was injected into the left ventricle (via the left lung vein). The colour coming through the foramen in frame 2 supports the hypothesis that the positive blood flow seen in the left aortic flow profile during early systole (number 2) is due not to a transmural effect but to a small right-to-left flow. During peak systole, no flow was observed; the medial cusp of the right aortic valve completely covered the foramen. At the start of diastole (number 3 and frame 3), the foramen is uncovered (by closing of the right aortic valves) and there is a rapid pressure equilibration between the two aortas (number 3); at the same time, the flow from the right aorta into the left aorta begins (colour appearing through foramen of Panizza in frame 3). Number 4 and frame 4 show the positive flow over the foramen during diastole when the right aortic valves are closed. Scale bar, 1 mm.

hepatic vein pressure, or total occlusion of the pulmonary outflow, to induce a right-to-left shunt in the perfused heart preparation used for angioscopy. We found that even during a complete shunt (pulmonary outflow occlusion) the medial cusps of the left aortic valve did not reach the foramen of Panizza, and flow from the left to the right aorta was

continuous throughout the cardiac cycle (reversed foramen flow, Fig. 4). Functionally, this is a significant finding: during shunting, there is less blood returning from the lungs to the left atrium and, if a complete shunt were to occur, no blood would be returned and the left ventricular output would approach zero. This has been shown experimentally by Petterson *et al.* (1992) in anaesthetised animals and *in vivo* in the present study. The coronary circulation, common carotid artery (including the left subclavian artery), right subclavian artery and right aorta are normally supplied with blood from the left ventricle, but during a large (or total) right-to-left shunt, when the left ventricular output is lowered, the blood to these circuits

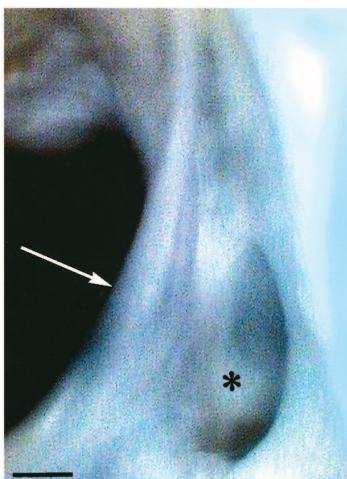


Fig. 4. Angioscopic image of the left aortic medial bicuspid semilunar valve (white arrow) and foramen of Panizza (asterisk) during a right-to-left shunt (systole), resulting from a mechanical occlusion of the lung arteries. The arrow indicates the greatest extent to which the medial cusp of the left aorta can open. Note that, in contrast to the situation in the right aorta, the medial cusp of the left aortic semilunar valve does not reach the foramen of Panizza: thus, there is the possibility of continuous left-to-right aortic blood flow, depending only on the pressure differences between the two aortas. Scale bar, 1 mm.

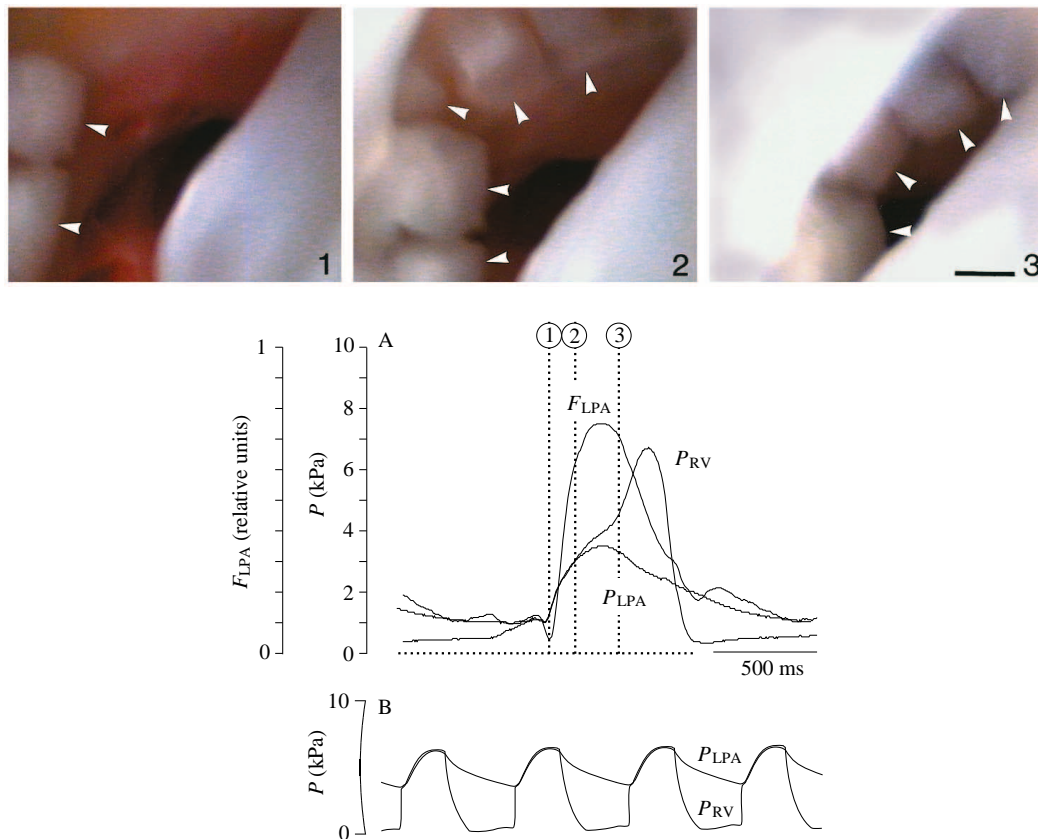


Fig. 5. The photographs show angioscopic images of the unique cog-teeth-like valves in the right ventricle of the crocodile heart at three different times during the cardiac cycle, starting in early systole (frame 1). Arrowheads indicate the individual lateral 'cogs' of the valve. The graphs show (A) phasic blood pressure recordings from the right ventricle ( $P_{RV}$ ) and left pulmonary artery ( $P_{LPA}$ ) with phasic blood flow in the left pulmonary artery ( $F_{LPA}$ ), and (B) the more avian/mammalian-like situation observed in some preparations where right intraventricular pressure lacks the 'extra' (second) peak. Numbers in A indicate corresponding times between the angioscopic images and the phasic pressure/flow recordings during the cardiac cycle obtained from different animals. Owing to the anatomical arrangement of the pulmonary outflow tract, the angioscopic images only show the lateral parts of the cog-teeth-like valve structure. During the early part of systole (frame 1 and number 1), just after the opening of the semilunar bicuspid lung valves, the cog-teeth-like valves are fully open. As systole progresses, the cog-teeth-like valves converge, thereby decreasing the cross-sectional area of the pulmonary outflow tract. This is reflected in the separation of the pulmonary and right intraventricular pressures (number 3). Scale bar, 1 mm.

could be supplied from the right ventricle/left aorta *via* the foramen of Panizza (reversed foramen flow, Grigg, 1989). Since there is evidence for a regional redistribution of blood during diving (Axelsson *et al.* 1991), the functional significance of the foramen of Panizza may be to supply blood to the coronary system and the brain during prolonged submergence.

A question closely linked to that of the role of the foramen of Panizza is the induction of the right-to-left shunt. White (1969) found that in *Alligator mississippiensis* there was a biphasic pressure development in the right ventricle, a finding confirmed in this study (Fig. 5, numbers 2 and 3 and frames 2 and 3). The same type of pressure pattern is seen in the left ventricle and aorta of humans diagnosed with left ventricular hypertrophic cardiomyopathy (Murgo *et al.* 1980) and in the right ventricle and pulmonary artery in patients with pulmonary stenosis (van Mierop and Kutsche, 1985). The most accepted explanation for the intraventricular/aortic pressure

gradients in these patients is a mechanical obstruction to ventricular outflow (Murgo *et al.* 1980). In humans, this is clearly a pathological situation, but the biphasic pressure development in the right ventricle in crocodilians is more likely to be highly adaptive and an effect of the cog-teeth-like valves located in the pulmonary outflow tract of the right ventricle. It represents a unique possibility to control actively the resistance of the pulmonary outflow tract, thus creating the intraventricular pressure required to initiate a right-to-left shunt. High-resolution angioscopy clearly showed individual 'cogs' fitting together during systole, effectively reducing the diameter of the pulmonary outflow tract (Fig. 5, frames 1–3) and resulting in the separation of the two pressure traces (compare numbers 2 and 3 in Fig. 5). *In vivo*, the right intraventricular and pulmonary pressures profiles were found to change spontaneously from that shown in Fig. 5A to more a more avian/mammalian-like profile (Fig. 5B) where the intraventricular/pulmonary pressures follow the same pattern

during the systolic phase of the cardiac cycle, indicating that a control mechanism is present.

In conclusion, the dynamic action of the cog-teeth-like valves together with resistance changes in the pulmonary and/or systemic circuits are responsible for the initiation of right-to-left shunting in crocodilians. The major functional significance of the foramen of Panizza seems to be in supplying blood to the coronary system and brain during periods of right-to-left shunting when the flow from the left ventricle is markedly reduced.

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