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THE PHYSIOLOGY OF THE ALLIGATOR HEART: LEFT AORTIC FLOW PATTERNS AND RIGHT-TO-LEFT SHUNTS

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Summary

Blood pressures have been recorded in the heart along with pressures and flow in the aortic arches of anaesthetized and awake alligators. Systemic blood pressures were significantly lower [5.22±0.57kPa (*N*=3) *versus* 9.85±0.46kPa (*N*=5)] and cardiac outputs higher [51.6±3.5ml$min^{-1}kg^{-1}$ (*N*=3) *versus* 25.5±8.2ml$min^{-1}kg^{-1}$ (*N*=5)] in awake compared with anaesthetized animals. Using pharmacological interventions, two types of right-to-left shunt could be induced in all alligators. In one, established after acetylcholine (ACh) injection into the right side of the circulation, left aortic flow was an anterograde monophasic pulse which occurred when pulmonary pressure exceeded systemic blood pressure. Hence, this left aortic flow pattern could also be induced by mechanical occlusion of both pulmonary arteries. About one-quarter of cardiac output could bypass the lungs during this shunt. However, this left aortic flow pattern was never seen under any conditions other than pharmacological intervention. In the other type of shunt, induced pharmacologically by ACh injection into the left side of the circulation, left aortic flow was biphasic with a period of backflow, initiated during systole, being progressively shortened by the onset of forward flow from the right ventricle. Establishment of this type of shunt depended on the magnitude of both the systemic pressure and the pressure generated by right ventricular contraction after closure of the pulmonary outflow tract. The amount of blood bypassing the lungs during this shunt was small (13.7±5% of cardiac output) but, at maximum, could be almost 25% of cardiac output. This shunt occurred naturally in resting animals and could be maintained for substantial periods (13.2min). The present observations confirm those made previously on anaesthetized alligators and extend previous work by showing two potential types of shunt. Finally, we suggest that right-to-left blood shunting in crocodilians may be related to the ‘alkaline tide’ that occurs after feeding, so the unique design of the central cardiovascular system in crocodilians could relate to both gastrointestinal and cardiorespiratory physiology.

Key words: crocodilians, alligator, blood shunt, cardiac output, blood pressures, blood flows, acetylcholine, gastrointestinal, cardiorespiratory, *Alligator mississippiensis.*

Introduction

The strange anatomy of the heart and arterial arches of the Crocodilia has interested anatomists and physiologists for a long time (see reviews by Grigg, 1989, 1991). Both right aorta (RAo) and left aorta (LAo) exist as important central blood vessels, joining at the dorsal aorta (DAo) and also connecting centrally through a small aperture, the foramen of Panizza, found just outside the aortic valves. The RAo arises from the left ventricle (LV) in the normal way, but the LAo opens with the pulmonary artery (PA) from the right ventricle (RV). The functional significance of the left aortic connections and of the foramen of Panizza is still far from clear. The important studies of White (1956, 1969) showed that the left aortic valves remained closed during normal cardiac cycles so that there was complete separation of the lung and body circuits. He suggested that blood flowed through the foramen from RAo to LAo aorta during ventricular systole. However, Greenfield and Morrow (1961) claimed that the foramen was closed during systole by the medial cusp of the RAo valve. Grigg and Johansen (1987) proposed that active changes occurred in the diameter of the foramen and that these could cause flow patterns to alter under different conditions.

White (1969) pointed out that, though the LAo valves must be closed when the systemic blood pressures were higher than those in the RV, there were some conditions under which the RV pressure could equal or exceed that in the LAo so that the valves opened. He believed that an increase in pulmonary circuit resistance, due to constriction of the pulmonary outflow tract, was the significant change and that it occurred when the animal was made to dive. In addition, intravenous injections of acetylcholine (ACh) were found to be effective in causing ejection of blood from RV to LAo (White, 1969; Axelsson *et al.* 1989). It was suggested that the increase in resistance in the pulmonary circuit was mediated by cholinergic innervation involved in the diving response.

In a previous paper (Shelton and Jones, 1991) we recorded both pressures and flows in the heart and central arterial system and were able to produce an extensive interpretation of the events of the cardiac cycle. When systemic pressures were high, we showed an alternation of blood flow at very low rates in the LAo, confirming the earlier flow recordings of Axelsson *et al.* (1989). We found that the foramen had little importance in the cardiac cycle, merely serving to prevent blood stagnation in the LAo. We did not use any experimental intervention to cause flow from the RV to the LAo but, under some conditions when systemic pressures were low, such flow was observed without any obvious changes in the pulmonary circuit. The outline of the flow pulse was unlike that described by Axelsson *et al.* (1989) as produced by intravenous ACh, and the LAo flow did not involve the high RV pressures suggested by White (1970).

Our experiments (Shelton and Jones, 1991) were carried out on anaesthetized alligators. The present experiments were done to extend this work by looking at the differences between the patterns of LAo flow induced by drugs and vessel closures and those that we had found occurring spontaneously in anaesthetized animals. We also examined the different types of LAo flow patterns in freely moving, unanaesthetized animals in order to define the circumstances under which they too would show spontaneous or experimentally induced LAo flow. In addition, we were concerned to

establish whether the general cardiovascular relationships described in this and the earlier paper could be seriously affected by the anaesthesia.

Materials and methods

Observations were made on eight alligators (*Alligator mississippiensis* Daudin) obtained from commercial suppliers in the USA and shipped by air freight to the University of East Anglia (U.E.A.) or the University of British Columbia (U.B.C.). They were kept in animal care facilities on a natural photoperiod and at temperatures between 25 and 30°C. The animals were fed on a high-protein fish and meat diet, supplemented by solid dog chow pellets *ad libitum*.

In this paper data are reported from studies on five anaesthetized (U.E.A.) and three unanaesthetized alligators (U.B.C.). Methods used with anaesthetized alligators have been described previously (Shelton and Jones, 1991). In addition, for the experiments reported here, changes in blood vessel resistance were achieved by occluding vessels with clamps or forceps. Acetylcholine (ACh) was administered through cannulae in the inferior vena cava (IVC), RV and RAo in the anaesthetized crocodiles.

At U.B.C. the method used for initial anaesthesia was different from that used at U.E.A. (Shelton and Jones, 1991). An alligator was caught, weighed and injected with $10\,mg\,kg^{-1}$ Ketalean intramuscularly (MTC Pharmaceuticals, Cambridge, Ontario). It was then placed in a dustbin containing iced water and transported from the Animal Care Facility to the Zoology Department, U.B.C. These preliminary treatments produced sedation and made the animal relatively easy to handle. The alligator was then fastened, ventral side uppermost, to an operating table with a pad, between table and animal, through which cooled water was circulated. This arrangement held the body temperature between 15 and 20°C. A close-fitting tracheal catheter was inserted through the glottis, which had previously been sprayed with xylocaine. The catheter was connected to an intermediate animal ventilator (Harvard Apparatus) which was used to ventilate the animal throughout surgery with halothane, at an adjustable level of approximately 1%, in a 1:1 N_2O/O_2 mixture. Ventilation was set at $2.5–4.0\,breaths\,min^{-1}$ and tidal volume at $15–20\,ml\,kg^{-1}$. This treatment induced surgical levels of anaesthesia. After surgery, pump ventilation was continued with air until the alligator produced spontaneous breathing movements.

The heart and arterial arches were exposed as described previously (Shelton and Jones, 1991). Vinyl tubing (V3, 4 or 5: Bolab, Arizona, USA) treated with TDMAC heparin complex (Polysciences Inc., Warrington, PA, USA) as an anticoagulant, was used to connect blood vessels to pressure transducers. The tubing was flared at the blood vessel end, filled with heparinised saline and inserted into the PA, the subclavian artery (SA), the LAo and, in one animal, the RAo. The flared end was introduced into the lumen of the blood vessel through a pin hole made in the vessel wall and a purse-string suture was used to fix the tubing in position. It was retracted until the flared end abutted the vessel wall so that the lumen was not occluded, and side pressures were measured. Blood flows were detected with Biotronix electromagnetic flow probes in the experiments on anaesthetized animals (Shelton and Jones, 1991) and with pulsed-Doppler flow transducers (TMI Iowa

City, IA, USA) on unanaesthetized animals. The Doppler transducers were small and flexible and had very light leads. They were relatively easy to secure in position around the PA, LAo and RAo for chronic implantation. Aquasonic gel (Parker Laboratories Inc., Orange, NJ, USA) was used to achieve a good acoustic contact between the crystal and the blood vessel wall. The transducers gave good and consistent results over many days of experimentation.

The chronic implantation of catheters and flow probes was completed by forming the tubing and transducer leads into a loop in the chest to allow for substantial movement of the underlying viscera during the normal breathing movements (Gans and Clark, 1976). The catheters and leads were then brought out through the abdominal body wall, secured with sutures to abdominal muscles, and taken to the dorsal surface of the animal under the skin, emerging finally through a small slit. They were tied firmly and cemented with tissue cement (Histoacryl, B. Baun, Germany) at their exit from the dorsal surface. The two halves of the sternum were sutured together and the ventral incision in the body wall and skin was also closed with sutures and made watertight with tissue cement. Finally, catheters and leads were wound in a circular loop and loosely tied to the sutures on the dorsal surface.

After recovery from anaesthesia, animals were placed in large plastic tanks (1m long, 0.5m wide, 0.5m deep) containing 5–10cm of water. The tanks were covered with sheets of 2.5mm Perspex. No recordings were made until the animals had had at least 24h to recover from anaesthesia. The catheters and leads were untied from the animal's back and connected to pressures transducers and flowmeter inputs and recordings were made over long periods (5–10h). Frequently, when disturbance was to be kept to a minimum, the animals, in their tanks, were placed behind black polythene screens and observed remotely by video camera. The depth of the water was increased to encourage diving and lowered to make submergence impossible. The lids of the tanks could be sealed with modelling clay to enable the composition of gas in the tank to be changed by connecting holes in the lid to gas cylinder sources of 100% O_2 or 100% N_2. Oxygen levels were monitored using a mass spectrometer (Centronix, MGA200). Forced dives were effected by lowering a mesh screen just below the water surface while the animal was under water and holding it in position for periods up to 10min. Exercise was induced by chasing the animal in its tank. Drugs (ACh 1–20 μg; phenylephrine 1–20 μg) were injected into systemic or pulmonary circulations through catheters in the RAo or PA.

Blood pressures were measured with saline-filled Bio-Tech BT70, Narco or Gould miniature pressure transducers attached to the catheters through three-way stopcocks. Transducers were powered by Gould universal amplifiers (Gould Inc., Cleveland, OH, USA) and output was displayed on a Gould ES1000 electrostatic chart recorder. Calibration and zero pressures were imposed on the transducers by connecting them through the stopcocks to saline columns fixed at appropriate levels. As detailed above, pulsed-Doppler flow transducers were used to measure blood flow. They were connected to an Iowa Biomedicine flowmeter and interfaced to the recorder through Gould universal amplifiers after suitable attenuation. The flowmeters were adjusted for maximum output *in situ* when first applied to the blood vessel in an animal. The setting was measured on a digital voltmeter and thereafter held constant for that animal and calibrated at the end of

the experiments. Calibration was carried out by cannulating the vessels above and below the transducer. Blood was allowed to flow through the system from a reservoir held above the animal at a level equivalent to mean systolic blood pressure. The flow rate was controlled by an adjustable clamp on the outflow vessel and was measured by collecting the outflow over a fixed period. Some recordings of both pressure and flow were made on a Techni-Rite TR222 pen recorder, writing on rectilinear coordinates. This machine was used for long-term recordings of LAo flow patterns when the animal was being observed remotely.

Pressure and flow data were extracted from the chart records using a Jandel digitizing tablet and SIGMA SCAN (Jan Scientific, Corte Madera, CA, USA). Data were analyzed statistically using paired or unpaired *t*-tests, where appropriate. Replicated values from one animal during a given condition (i.e. anaesthetized, alert, resting, time after forced submergence, time after drug injection) were averaged. Grand means for each condition were obtained for five anaesthetized and three unanaesthetized crocodiles. In all statistical tests 5% was taken as the fiducial limit of significance. In the text data are presented as the mean or grand mean ± standard error.

Results

Experimentally induced shunting patterns in anaesthetized alligators

A total right-to-left (R-to-L) shunt was produced in four anaesthetized animals by occluding both PAs. Pressure in PAs central to the occlusion increased substantially and the pressure waveform in LAo changed in outline, acquiring a high peak that fell abruptly to the much lower pressure in the RAo or SA when the LAo valves closed (Fig. 1B,C). The two-phase pressure change, typical of the RV, was lost and a more uniform waveform appeared, similar to that seen in the LV before occlusion (Fig. 1A,B). This single-stage pressure generation caused a monophasic flow pulse in the LAo throughout RV ejection (Fig. 1C). Cardiac output fell substantially during these very brief occlusions because no venous return occurred to the left atrium and the LV was unable to produce pressures high enough to open the RAo valves. Systemic pressure in the LAo and RAo was maintained solely by RV discharge.

Injection of ACh (5–20 μg) into the right heart circuit *via* the IVC or the RV caused an increase in PA pressure and a fall in systemic blood pressure, usually starting before the injection was completed (Fig. 2). The heart rate decreased (Fig. 2) and at higher ACh concentrations the heart stopped. Though the range of ACh doses was restricted to a single order of magnitude, it was clear that the effects of ACh on pressure and rate were dose-dependent (Fig. 3). The time courses of the changes caused by ACh injection were different. The effects on blood pressures lasted for 130–220s. Recovery from the chronotropic changes was more rapid, being complete in 40–65s following ACh injection (Fig. 3).

As the PA pressure began to increase after ACh administration, the small pressure gradient between the RV and the PA during the initial phase of pulmonary ejection decreased and eventually disappeared. The second component in the RV pressure trace, occurring after the inflection caused by closure of the pulmonary outflow tract, was

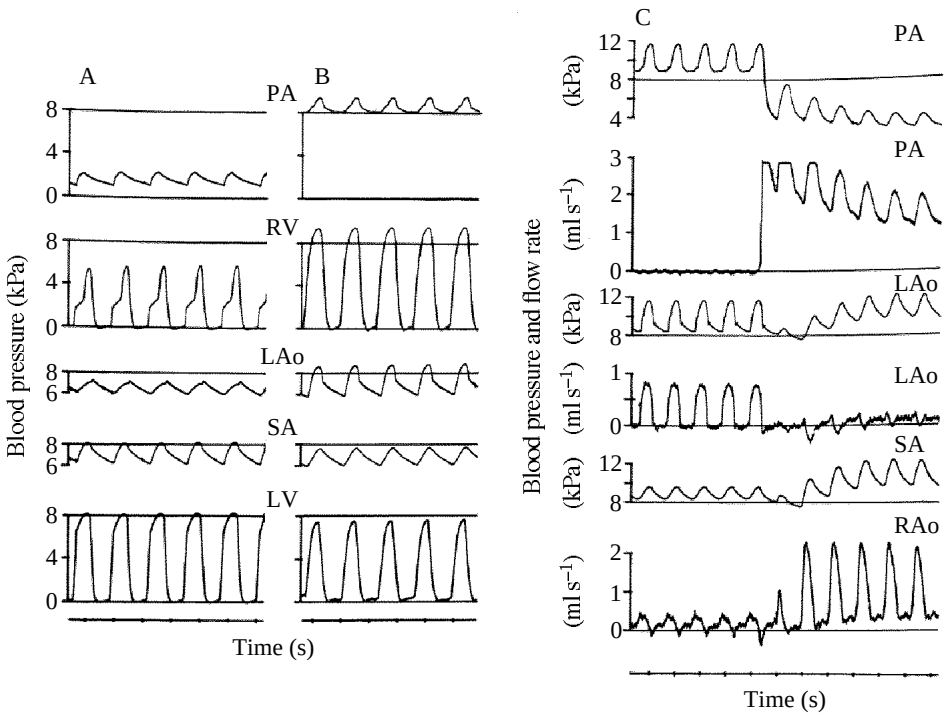

Fig. 1. The effects of pulmonary artery occlusion on blood pressures in the right ventricle (RV), left ventricle (LV), pulmonary artery (PA), left aorta (LAo) and subclavian artery (SA), whose pressures are equivalent to those in the right aorta (RAo). Pressures are shown immediately before (A) and immediately after (B) occlusion and are from a 3.8kg anaesthetized alligator. (C) The effects of a sudden removal of pulmonary artery occlusion (marked by the establishment of pulmonary flow). Pressures and flow rates in the right and left aortae, the subclavian artery and the pulmonary artery are from a 4.6kg anaesthetized alligator.

initially somewhat less affected by ACh injection than was LV pressure so that it soon exceeded the falling pressure in the LAo. Ejection of blood from the RV to the LAo, marked by a change in the rising slope of the LAo pressure trace (Fig. 2), then began. The continuing rise in PA pressure, due to peripheral vasoconstriction of the lung vasculature, together with the falling systemic pressure, caused the outflow tract inflection to disappear in the RV trace and ejection to the LAo to occur earlier in the cycle. Ultimately, as the pressure effects of intravenous ACh became maximal, blood flow occurred simultaneously in the PA, LAo and RAo (Fig. 4). By this stage the chronotropic changes had disappeared. Recovery from the pressure changes began soon after these maximal effects and was complete in 40–80s, with systemic and pulmonary flows rising and the LAo pattern reverting to the alternation of reversed and forward flow (Fig. 4).

The pattern of LAo flow seen in these experiments was unlike that occurring spontaneously in anaesthetized alligators (see Fig. 9, Shelton and Jones, 1991). The spontaneous outflow was always smaller in volume and later in the cycle than that seen

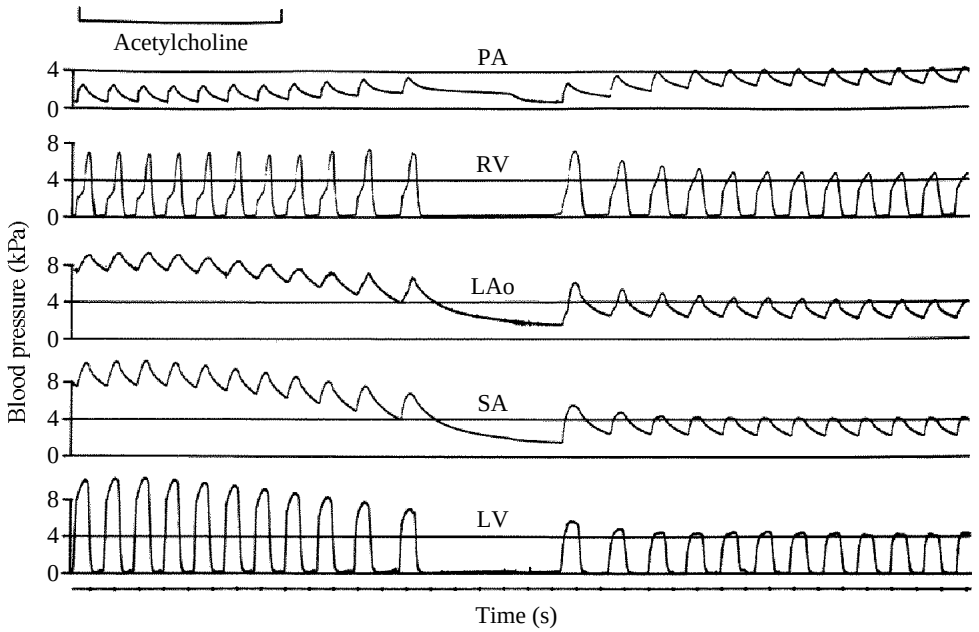

Fig. 2. The effects of injection of 10 μg of acetylcholine into the right ventricle of a 3.8kg anaesthetized alligator, showing changes of blood pressure in the right ventricle (RV), left ventricle (LV), pulmonary artery (PA), left aorta (LAo) and subclavian artery (SA), whose pressures are equivalent to those in the right aorta. The acetylcholine was injected through a second cannula in the right ventricle during the period shown by the marker.

after the full development of ACh-induced flow and was achieved with no changes in pulmonary resistance. The effects of ACh injections into the right heart circuit are complex, changing pulmonary and systemic pressures in different directions as well as reducing heart rate. Experiments in which 20 μg of ACh was injected into the RAo were carried out in one alligator and were found to reduce the systemic blood pressure while leaving the PA pressure and the heart rate largely unchanged. This treatment caused a small ejection of blood from the RV into the LAo but the records are inadequate for clear analysis. The experiments were repeated on unanaesthetized animals and will be dealt with in the following section.

Drug-induced shunting patterns in unanaesthetized alligators

Injection of ACh (1–3 μg) into the right heart circuit of one unanaesthetized animal *via* the PA caused exactly the same changes as those already described in the previous section, as Fig. 5 shows. The dose levels required to cause equivalent effects were about five times lower than those on anaesthetized animals. As we found in the experiments on anaesthetized alligators, pulmonary blood pressure increased from 1.8kPa before injection to 2.75kPa some 30s after injection, while pulmonary flow was halved. Hence, total vascular resistance of the pulmonary circuit was increased threefold by ACh injection. Systemic pressures fell from 6 to 2.3kPa, 30s after ACh injection, which was a

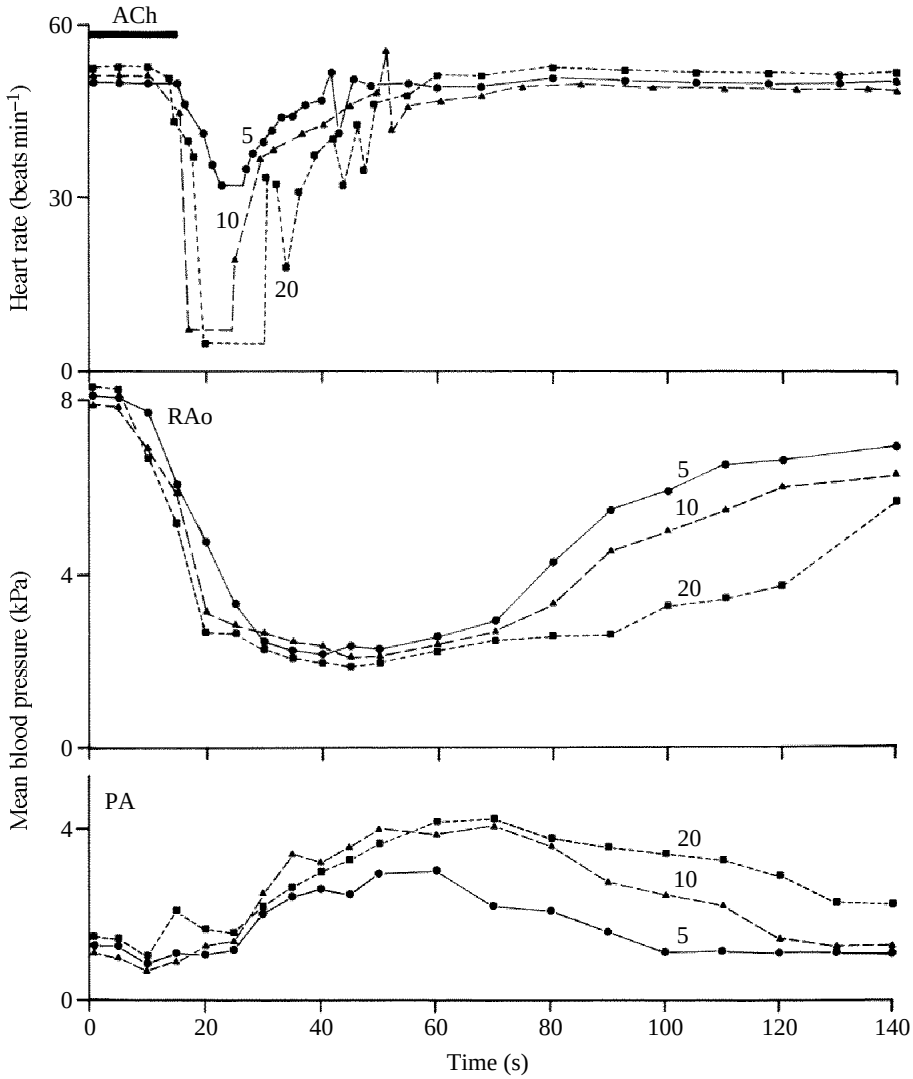

Fig. 3. The effects of intravenous injection of 5, 10 and 20 μg of acetylcholine (ACh) on mean blood pressures in the pulmonary (PA) artery and right aorta (RAo) and on instantaneous heart rate of a 3.6kg anaesthetized alligator. The marker indicates the time of injection of ACh.

proportionately greater decline than the fall in cardiac output ($57 ml kg^{-1} min^{-1}$ to $44 ml kg^{-1} min^{-1}$). Total systemic resistance was halved.

The second component of the RV pressure trace, following active closure of the pulmonary outflow tract, now became high enough to eject blood to the LAo. In the preliminary stages of shunt development, illustrated in the first two cycles of Fig. 5, RV flow to the LAo prematurely ended the small reversed flow in LAo caused by blood moving round the DAo loop from the RAo. The main flow in the LAo then occurred as a forward pulse of blood following the flow recorded in the PA. However, the increasing

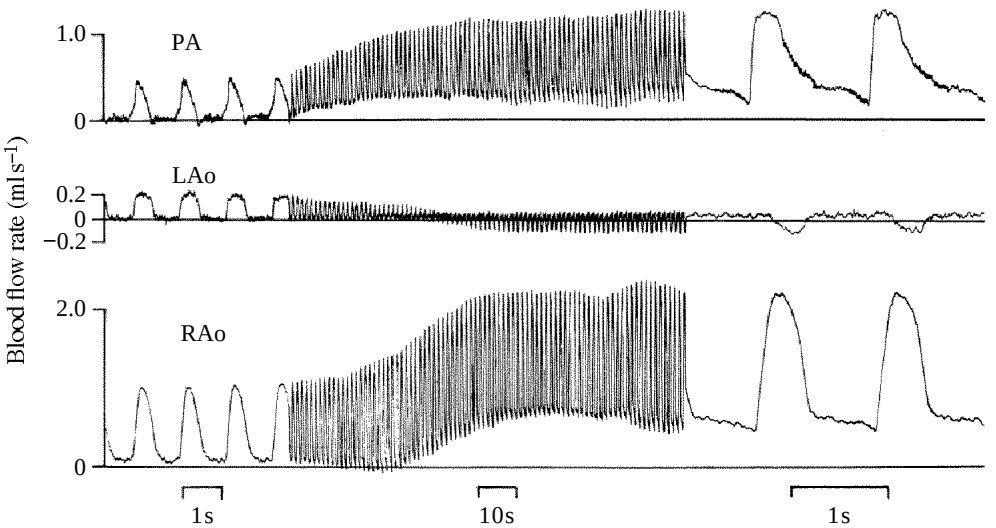

Fig. 4. Blood flows recorded from the right aorta (RAo), left aorta (LAo) and pulmonary artery (PA) of a 3.3kg anaesthetized alligator, showing recovery from the effects of an intravenous injection of acetylcholine given 60s before the start of the record. Recovery from the chronotropic effects of the injection occurred 30s before the start of the record and cardiac output had been falling steadily from the time of the injection. Note the transition in the LAo from forward flow at start of record, due to right ventricular ejection, to alternation of forward and reversed flows at end of record, after the LAo valves have closed.

pulmonary pressures caused LAo flow to start progressively earlier in the cardiac cycle and ultimately to occur throughout the whole period of RV ejection. PA and LAo flow pulses were then concurrent instead of consecutive (Fig. 5). Finally, all evidence of the two-stage pressure waveform in the RV or LAo disappeared.

When the ACh-induced shunt was fully established, there was no reversed flow in the LAo, and cardiac output could be estimated as:

$$\text{Cardiac output} = (2\times\text{PA flow}) + \text{LAo flow (all forward)}, \quad (1)$$

so that:

$$\text{Right-to-left shunt} = \text{LAo flow (all forward)/cardiac output}. \quad (2)$$

In the final cycle shown in Fig. 5, cardiac output was 46.2ml$\text{min}^{-1}\,\text{kg}^{-1}$ and LAo flow was 10.8ml$\text{min}^{-1}\,\text{kg}^{-1}$, equivalent to a 25% R-to-L shunt. In fact, this degree of shunting was quite typical in the period 15–30s after drug injection. In 10 trials on this animal the mean value of the shunt was 22%.

Injection of ACh (1–3μg) into the left heart circuit *via* the RAo also caused changes in the heart rate and systemic blood pressure. Systemic pressures fell from 4.5±0.9kPa before injection to 3.6±0.5kPa (N=3) 10–15s after injection, whereas changes in pulmonary pressures and flows (Fig. 6) were quite different from those occurring after ACh injection on the right side of the heart. Cardiac output 10–15s after injection (43.7±6.3ml$\text{min}^{-1}\,\text{kg}^{-1}$) was not significantly different from output before injection (47.7±6.8ml$\text{min}^{-1}\,\text{kg}^{-1}$) yet pulmonary pressure fell, indicating pulmonary

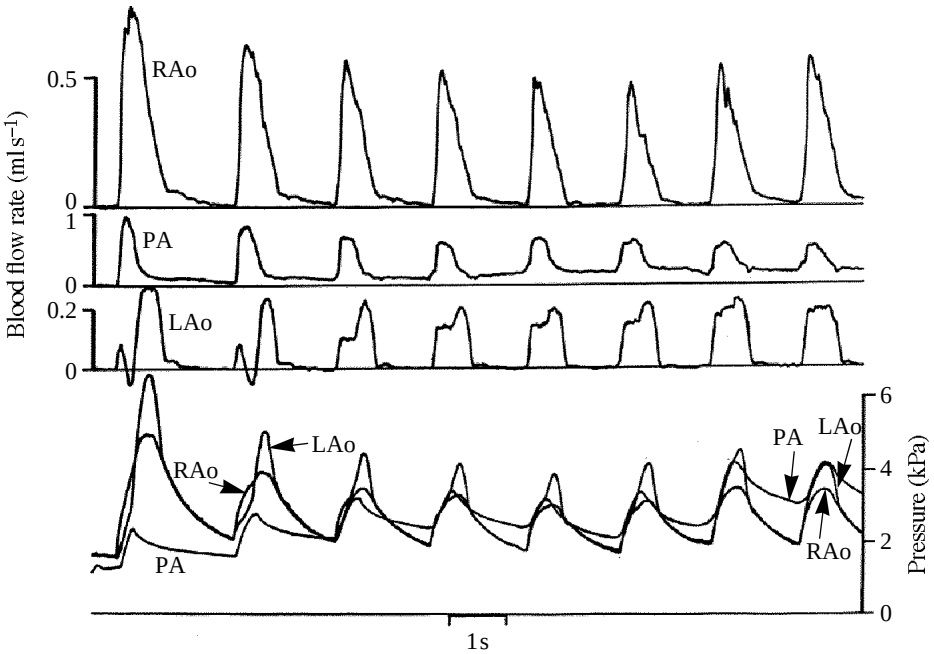

Fig. 5. The effects of injection of 2 μg of acetylcholine into the PA of a 2.8kg unanaesthetized alligator. The record starts 15s after injection and during recovery from the chronotropic effects. The R-to-L shunt in the final cardiac cycle was 25% of RV output (see text for details). Abbreviations as in Fig. 1.

vasodilation. However, the fall in systemic pressure was sufficient to allow the second phase in the RV pressure trace to reach levels that caused LAo ejection. LAo flow changed smoothly from the usual pattern of alternation of diastolic forward flow and systolic reversed flow to one in which the reversed flow was curtailed by RV ejection (Fig. 6). This pattern, with blood flow from the RV going sequentially into the PA and then into the LAo, persisted throughout the response to ACh injection. Flows in the PA and LAo were never concurrent as they were when ACh was injected into the right side of the heart. Reversed flow in LAo averaged 1.4±0.5ml$\text{min}^{-1}\text{kg}^{-1}$ and forward flow was 4.8±1.6ml$\text{min}^{-1}\text{kg}^{-1}$ 10–15s after injection. Reversed flow in the LAo derives from the left side of the heart *via* the dorsal aorta and calculations of cardiac output can take this into account if it is assumed that this blood volume is added to the forward flow ejected from the RV to the LAo without further contribution through the foramen:

$$\text{Cardiac output} = (2\times\text{PA flow}) + \text{LAo flow} - \text{LAo reversed flow}, \quad (3)$$

so that:

$$\text{Right-to-left shunt} = (\text{LAo forward flow} - \text{LAo reversed flow})/\text{cardiac output}. \quad (4)$$

The mean cardiac output shown in Fig. 6 was 47ml$\text{min}^{-1}\text{kg}^{-1}$ and the R-to-L shunt was 13% of the output. The size of this shunt was almost twice the mean value (7.6±1.6%) obtained in three alligators, 10–15s after drug injection.

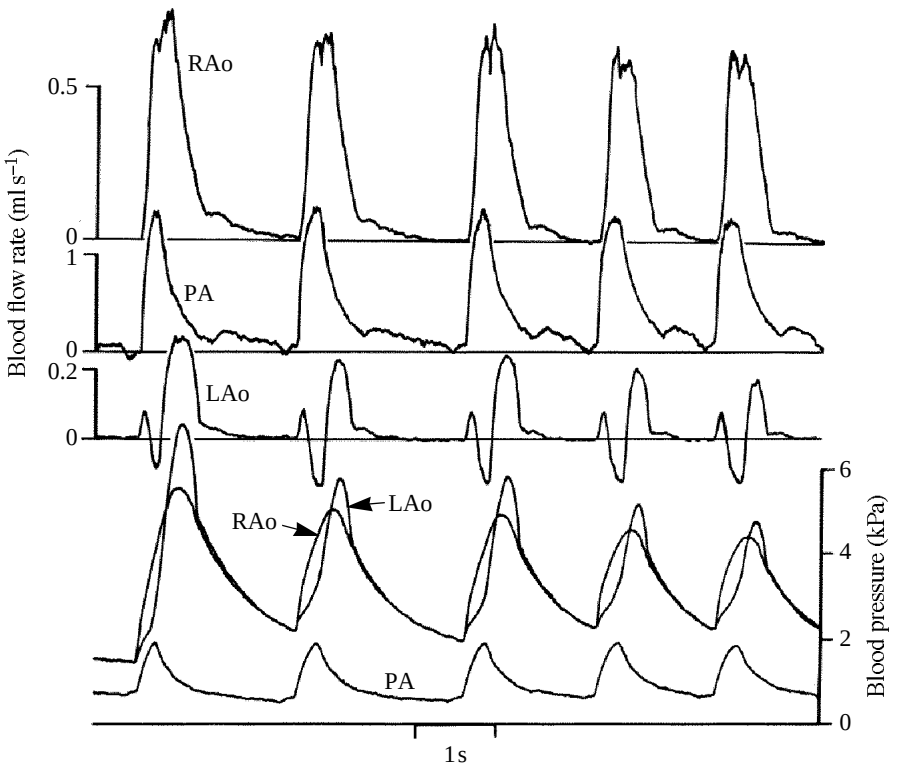

Fig. 6. The effects of injection of 2 μg of acetylcholine into the RAo of a 2.8kg unanaesthetized alligator. The record starts 10s after drug injection and during recovery from the chronotropic effects. Abbreviations as in Fig. 1.

Injection of phenylephrine (1–20 μg) into the RAo caused a substantial increase in systemic pressure (Fig. 7). Flow in the LAo became continuous in the forward direction with small variations during ventricular systole. Such continuous, forward flow could also occur intermittently in unanaesthetized alligators without drug injection and usually when systemic pressure was high (8–10kPa). In addition, it was found in one anaesthetized alligator throughout all the experimental observations (Shelton and Jones, 1991). We attributed it to a failure of the medial cusp in the right aortic valve to close the foramen completely during ventricular systole. This failure would be particularly likely when blood pressures were high and the vessel walls were stretched, but a few individuals may have leaky foramina at normal pressures. We have suggested that the foramen has little importance in the overall cardiac cycle apart from preventing blood stagnation in the LAo and such leakage would have no selective significance (Shelton and Jones, 1991).

Naturally occurring left aortic flow patterns in unanaesthetized alligators

RAo blood pressures were significantly lower in alert, unanaesthetized alligators (5.22±0.57kPa) than in anaesthetized animals (9.85±0.46kPa). PA pressures were similar in the one alert, unanaesthetized animal in which they were measured (2.10kPa)

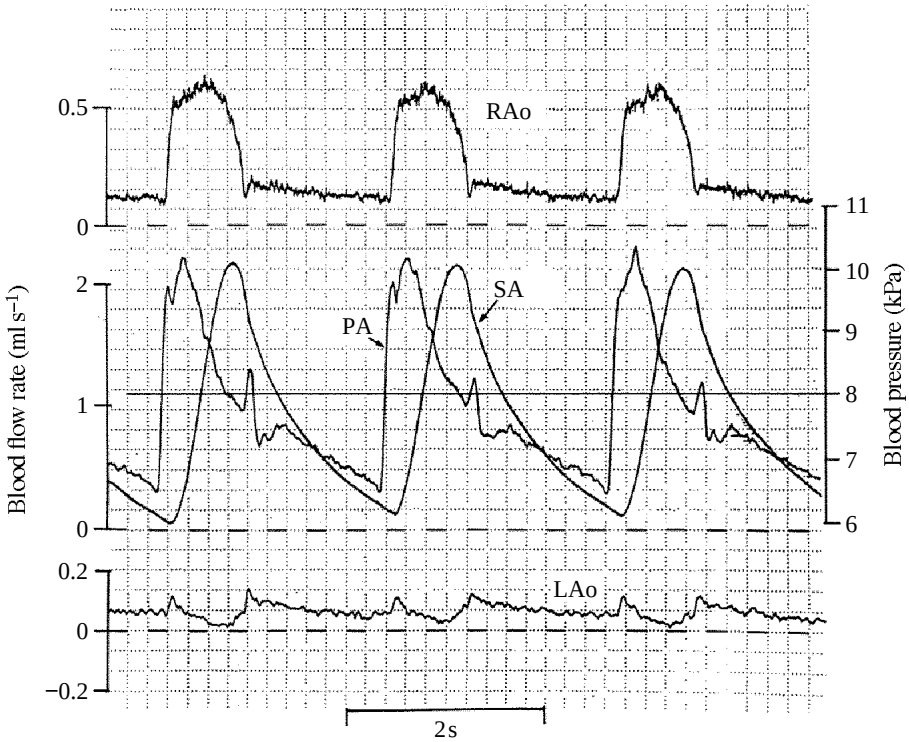

Fig. 7. The effects of injection of 20 μg of phenylephrine into the subclavian artery (SA) of a 2.4kg unanaesthetized alligator. The record starts 3min after drug injection. Abbreviations as in Fig. 1.

and three anaesthetized animals (2.18±0.09kPa). Cardiac outputs were lower in anaesthetized animals (25.5±8.2ml$min^{-1}kg^{-1}$) than in alert, unanaesthetized ones (51.6±3.5ml$min^{-1}kg^{-1}$) although, as a result of considerable variation and low *N* numbers, this difference was not significant.

In animals that were alert and in full view in the laboratory, the events of the cardiac cycle and the patterns of LAo flow were identical to those characteristic of anaesthetized animals (Shelton and Jones, 1991). Forward LAo flow began at the end of systole and declined slowly until the flow reversed at the start of the following LV ejection (Fig. 8A). The slow decline in flow during diastole matched a similar run off in the RAo, though the flow rates were much higher in the latter. The LAo appears to be much less compliant than the RAo and flow through the foramen of Panizza must be low. On average, forward LAo flow (3.43±0.6ml$kg^{-1}min^{-1}$) was not significantly different from backflow (2±0.8ml$kg^{-1}min^{-1}$). However, in one animal, forward flow was markedly greater than reversed flow, requiring some supplementation of the flow from the elastic reservoir with that through the foramen from the RAo.

All the unanaesthetized alligators we examined produced R-to-L shunts quite readily when they were calm, resting and totally undisturbed. These shunts were always of the consecutive type, with LAo flow following PA flow (Fig. 8B). Concurrent LAo and PA

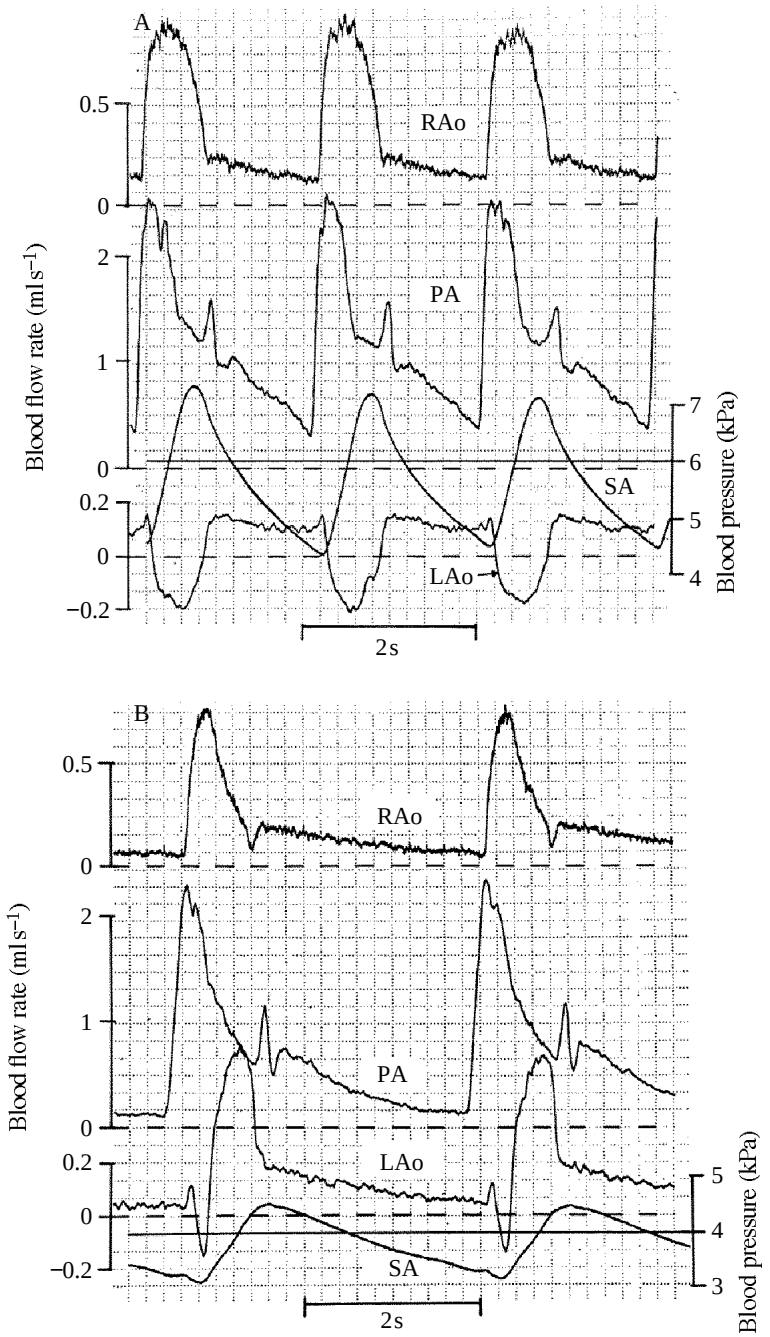

Fig. 8. Systemic blood pressures in the subclavian artery (SA) and blood flows in the pulmonary artery (PA), right aorta (RAo) and left aorta (LAo) to show the differences between (A) an unanaesthetized alert animal (2.4kg), in full view in the laboratory, and (B) the same animal, calm and resting behind a screen. A R-to-L shunt is established in B and is associated with bradycardia and systemic hypotension.

flow, as seen after ACh injection into the right heart circuit, never occurred. The development of the shunting pattern could be recognized as a change in the reversed flow pulse before the end of LV systole. The earlier that this change occurred in systole the greater was the shunt. Fig. 8B shows the early development of a shunt that was sufficiently large to bring reversed flow to an end and quickly establish a large forward flow in the LAo during late systole. LAo flow fell rapidly at the end of RV systole.

Quantification of the R-to-L shunt is somewhat complicated because, during diastole, blood flow down the LAo could come from the RV or from the RAo *via* the foramen of Panizza. Using equations 2 and 3, the mean shunt in three alligators, at rest and showing stable shunts, was 13.7±5%. Cardiac output averaged $38.9\pm7 ml min^{-1} kg^{-1}$ during shunting but this value was not significantly different from that seen in alert animals. Moreover, although systemic pressures during shunting (3.3±0.75kPa) were considerably below those of alert, non-shunting, alligators (5.22±0.57kPa), the difference was not significant. However, there was always significantly more forward ($5.5\pm1.5 ml kg^{-1} min^{-1}$) than reverse flow ($0.5\pm1.6 ml kg^{-1} min^{-1}$) in the LAo when shunts were occurring.

In all animals, it was possible to track the early development of a shunt in the LAo as a small, late systolic, flow pulse that gradually broadened as the shunt developed. The reversed flow found during LV systole was thus ended prematurely. The records in Fig. 9A were taken when the systemic pressures were too high for the LAo shunt and those in Fig. 9B were made when pressures were lower and the incipient shunts caused a variable reduction in the duration of the reversed flow pulse in the LAo. The LAo pressure also showed characteristic changes in profile similar to those seen in anaesthetized animals. When there was no shunt (Fig. 9A), the LAo pressure rise was delayed and slightly damped because it was due to flow around the DAo loop, the foramen being closed. Consequently, LAo pressure never reached RAo levels during systole, though the two were identical during diastole when the foramen was open (Fig. 9A). After the shunt had been established, LAo pressure increased in two stages (Fig. 9B); the first was due to flow around the DAo loop as before and the second was caused by opening of the LAo valves. The blood flow thus established varied enormously and depended on the magnitude of the second-phase pressure in the RV compared to the LV pressure. In the largest shunts, LAo pressure substantially exceeded that in the RAo. Mean pressures and flows in the PA were not greatly affected by the establishment of a R-to-L shunt.

We have already suggested that an important condition for the initiation of spontaneous R-to-L shunts is low systemic blood pressure (Shelton and Jones, 1991). This conclusion was confirmed in the experiments on unanaesthetized animals. Undisturbed animals hidden from view and motionless had low blood pressures and always produced shunts (Fig. 8B). Alert animals in full view, even when not reacting to the presence of people in the laboratory, had higher blood pressures and did not show shunts (Fig. 8A). Attempts to define the boundaries between the two states more precisely show that there are some difficulties. At the borderline between shunt and no shunt there are slight fluctuations in both RV and LV pressures, as Fig. 9 shows. A shunt could be produced as LV pressure fell or as RV pressure increased. Avoiding times of experimental intervention such as

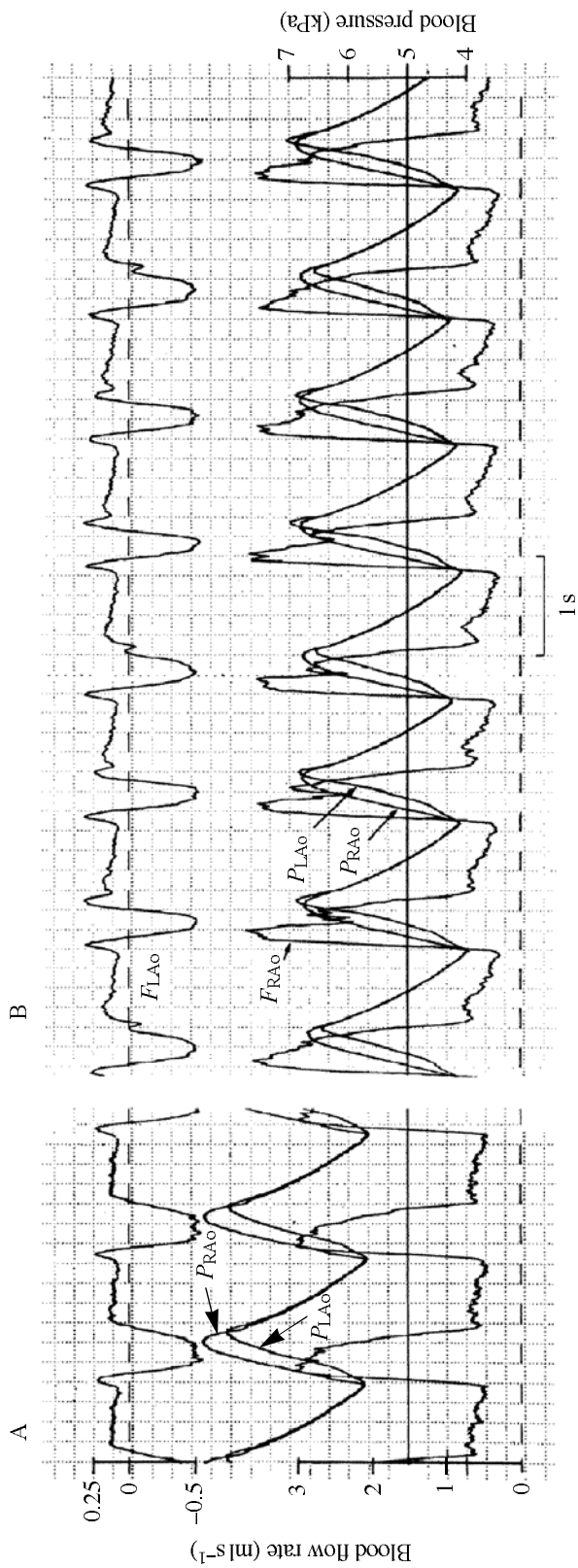

Fig. 9. Blood pressures in the right aorta (P_{RAo}) and left aorta (P_{LAo}) and blood flows in the same vessels (F_{RAo} and F_{LAo}) in an unanaesthetized alligator (2.4 kg) to show relationships (A) before and (B) during a period of low-level R-to-L shunt development. Note that there is systemic hypotension in B compared with A, and that the size of shunt depends on the extent to which P_{LAo} exceeds P_{RAo} at the end of systole.

drug injection, pressure data collected over several days from three alligators showed that R-to-L shunts can appear and disappear over a wide range of systemic pressures (Fig. 10). However, shunts predominate at very low RAo pressures and are never seen when those pressures are at their highest.

Long-term recordings made from animals in tanks concealed behind curtains showed that shunt duration could vary from a few heart beats to approximately 15min. In one alligator, monitored for 125min, there were equal periods of shunting and non-shunting with a maximum shunt duration of 5.2min. Another animal, monitored for 200min, showed R-to-L shunts for only 40% of the time but the longest lasted for 13.2min. The beginning of a shunt was often associated with slight bradycardia. The shunts could occur during voluntary dives but could also be seen at other times in resting animals. Grigg and Johansen (1987) also found that shunts were not invariably correlated with dives. They showed that pressures in the RV, high enough to cause flow in the LAo, could be recorded during some, but by no means all, voluntary aerobic dives. However, White (1969) found that forced submersion of restrained alligators induced bradycardia and ultimately (after about 10min of submersion) led to RV pressures that were sufficient to cause R-to-L shunts.

We carried out forced-dive experiments on two unrestrained alligators by placing a mesh screen just below the water surface for periods up to 10min. This time is well within the diving capability of these animals and corresponds to that used by White (1969). In neither animal did a R-to-L shunt appear. In one animal, the systemic pressures became very high and there was no possibility of RV outflow to the LAo. PA pressure increased by 0.6kPa but it never reached RAo pressure, which at one stage in the forced dive was 13.3kPa. In the second alligator, a substantial bradycardia was seen. Mean RAo pressure

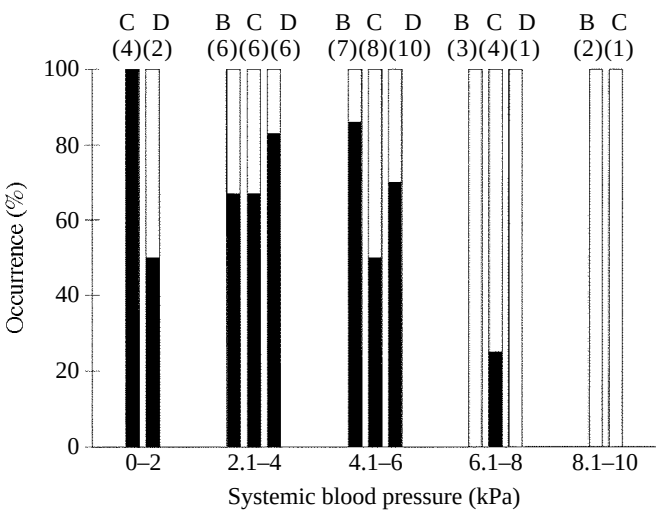

Fig. 10. Percentage occurrence of shunting (filled columns) and non-shunting (open columns) in three alligators (B, C and D), during long-term observations, in relation to the prevailing systemic pressure. The total number of observations, at the indicated pressure ranges, is shown in parentheses over the histogram for each alligator.

increased from 3.9 to 4.8kPa and heart rate fell to one-tenth of the surface value. Stroke volume changed very little so that cardiac output fell in proportion to the change in heart rate. The increase in blood pressure was clearly due to substantial peripheral vasoconstriction. During these changes, which are characteristic of the diving response in many animals, the LAo flow pattern was progressively modified. From the first minute of the mesh being placed in position, reversed flow began to exceed forward flow (Fig. 11). After 4 or 5min no forward flow was detectable during the early stages of diastole following a heart beat. In other respects, the flow pattern was not substantially different from that seen before the forced submersion. Clearly, there was no evidence of a R-to-L shunt.

Breathing 50–60% O_2 made R-to-L shunts more likely to occur in undisturbed alligators, whereas hypoxia between 5 and 10% O_2 caused disturbance and prevented shunts developing. In one alligator, cooling to 16˚C caused a slight reduction in RAo pressure and a R-to-L shunt developed briefly.

Discussion

The present observations on unanaesthetized alligators have confirmed that the functional relationships between heart and major arterial trunks are identical to those worked out in experiments on anaesthetized animals and described in our earlier paper (Shelton and Jones, 1991). The two-phase nature of RV systole was established by Greenfield and Morrow (1961), who suggested that active obstruction of the pulmonary outflow tract in the RV caused the transition from a first phase of blood ejection to the PA to a second phase of pressure increase that, if sufficiently large, led to ejection of blood to the LAo. White (1969) agreed that LAo flow was determined by the nature of the pulmonary outflow tract but thought that it constituted a variable resistance under vagal control and, when activated by ACh, could divert blood from the PA to the LAo. Our evidence confirms the two phases of RV contraction and shows that the outflow tract acts as an on–off valve whose active closure ends the outflow to the PA during the initial phase of RV systole (Fig. 12B,C), confirming the observations of Greenfield and Morrow (1961). The tract offers little resistance to flow before closure; in fact, the resistance decreases as PA pressures increase after ACh injection.

In alert alligators (Fig. 8A), as in anaesthetized animals, RV pressure in the second phase was never high enough to equal or exceed that in the systemic circulation; the LAo valves remained closed, RV contraction was isometric and there was no R-to-L shunt (Fig. 12C). In these conditions, flow in the LAo oscillated at very low levels. During systole, a reversed flow occurred from the DAo into the elastic reservoir of the LAo, the foramen of Panizza being closed by the medial cusp of the RAo valve (Fig. 12B,C). In diastole, forward flow was established by a contribution from the LAo elastic reservoir and sometimes by movement of blood from the RAo through the open foramen (Fig. 12A).

The necessary conditions for RV outflow to occur to the LAo are simple in concept, requiring only that second-phase RV pressures equal or exceed those in the systemic circulation. However, they are more complicated in physiological practice. The simple

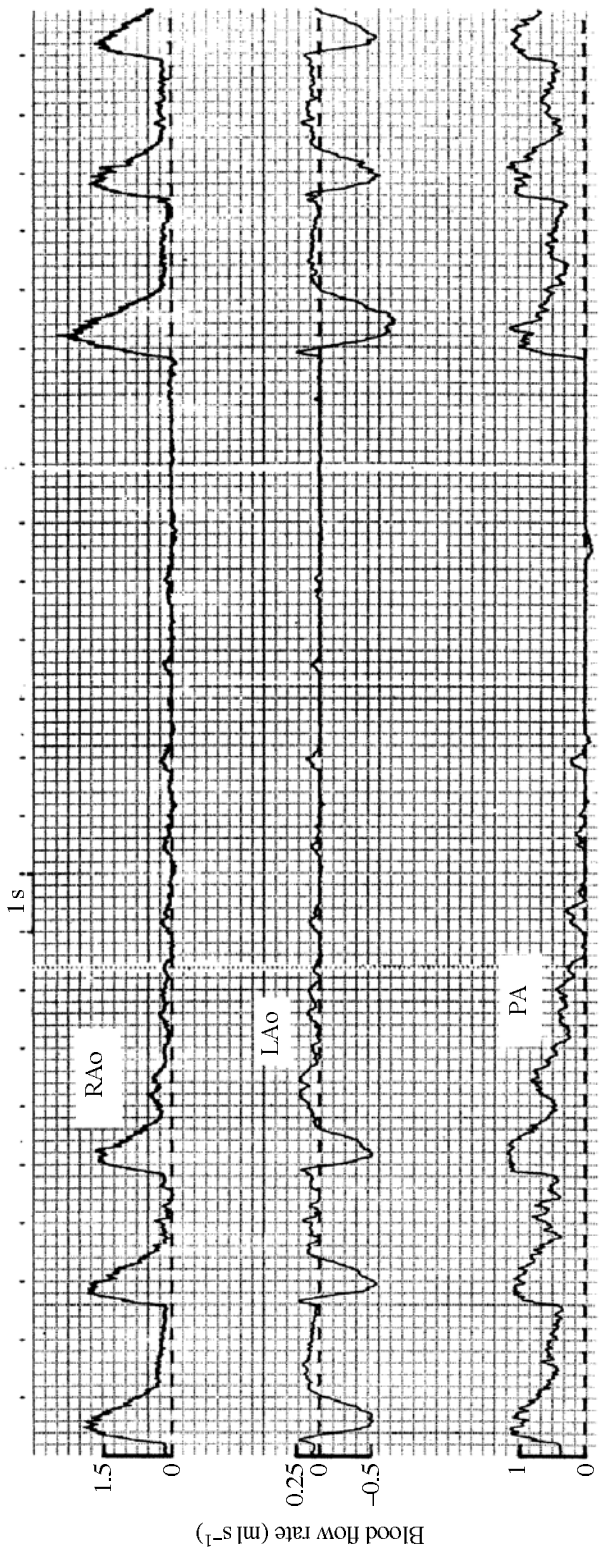

Fig. 11. Blood flow in the right aorta (RAo), left aorta (LAo) and pulmonary artery (PA) of an unanaesthetized alligator (2.7 kg) during an experiment in which access to the surface was prevented for 10 min by a mesh screen. The record was taken after the screen had been in place for 1 min and shows pronounced cardiac arrhythmia.

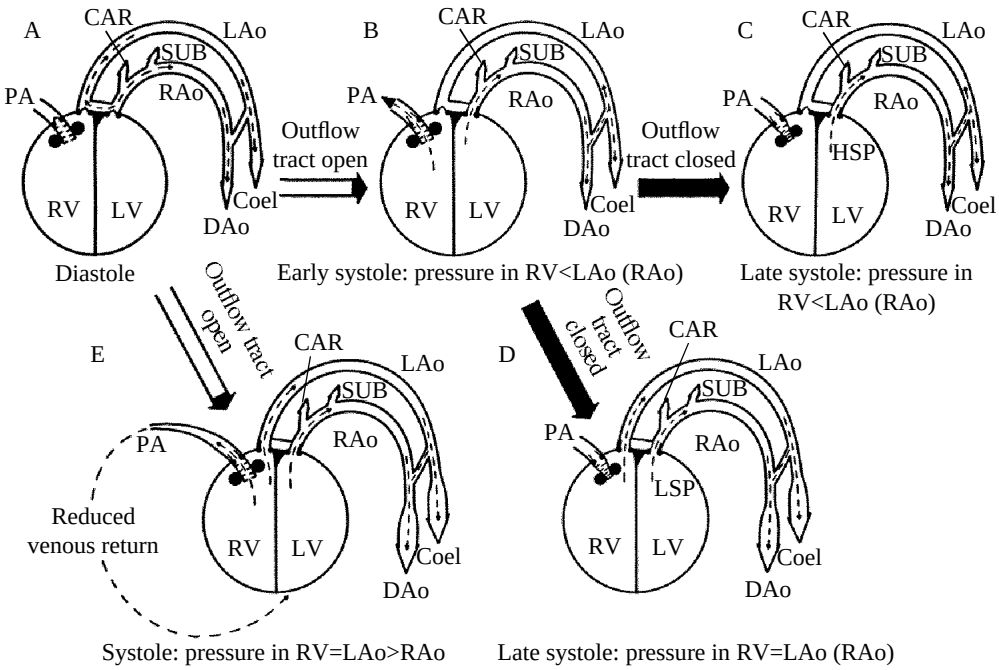

Fig. 12. A diagrammatic representation of events and flow patterns in the heart and central arteries of alligator during (A) diastole with heart filling and arterial flow maintained by energy stored in central elastic reservoirs, foramen of Panizza open; (B) the initial phase of systole with the pulmonary outflow tract open and the foramen closed by the cusp of the right aorta (RAo) valve; (C) the second phase of systole with the outflow tract closed and high systemic pressures as in the alert animal, foramen closed, no right ventricle (RV) ejection to the left aorta (LAo); (D) the second phase of systole with the outflow tract closed and low systemic pressures as in a calm animal, foramen closed, RV ejection to LAo; (E) both phases of systole with lung vasculature constricted and systemic vasculature dilated, as after intravenous injection of acetylcholine, RV and pulmonary artery (PA) pressures as high as systemic, monophasic ejection from RV to LAo occurring though the initial and second phase, foramen closed. CAR, carotid artery; Coel, coeliac artery; DAo, dorsal aorta; LV, left ventricle; SUB, subclavian artery; HSP, high systemic pressure; LSP, low systemic pressure.

PA occlusion experiments show that total prevention of pulmonary flow resulted in the development of very high RV pressure by the thick myocardium during the whole of systole (Fig. 1). There was no differentiation into two phases. This gave rise to levels of flow from the RV to the LAo that were high enough to maintain systemic blood pressures though they were not as high as normal RAo flows. Clearly, the RV is capable of generating high pressures and of causing substantial flows in the LAo.

Intravenous injection of ACh is effective in causing the overlap of RV and LAo pressures necessary for blood outflow to the LAo, as White (1969) showed. He attributed the changes to increased resistance of the outflow tract. Axelsson *et al.* (1989) were able to show that such injections did cause blood flow into the LAo; in fact, these were the only conditions that produced R-to-L shunts in their experiments. Voluntary dives were

not effective and their caimans would not perform 'fright' dives. They suggested that ACh acted by causing constriction of the pulmonary vasculature.

However, the effects of ACh injections into the right side of the circulation, using intravenous, RV or PA catheters, are more complex than this (Fig. 12E). In both anaesthetized (Fig. 2) and unanaesthetized animals (Fig. 5), the initial chronotropic effects were outlasted by rising pulmonary and falling systemic pressures (Fig. 3). The former were clearly due to a peripheral pulmonary vasoconstriction and the latter to some systemic vasodilation as well as to probable inotropic effects on the myocardium. Both were conducive to the development of LAo flow. As the shunting pattern developed, pulmonary vasoconstriction decreased PA flow, so that the RV contained more blood and continued to produce high pressures during the second-phase of systole. Ejection to the LAo began as a second-phase phenomenon therefore, but a continued increase in pulmonary and decrease in systemic pressure caused a rapid transition to forward flow throughout the first and second phases (Figs 5, 12E). For a few beats, contraction of the outflow tract still caused inflections in both pressure and flow traces from the LAo, but PA pressures increased further and LAo flow finally became monophasic and synchronous with RAo and PA flow (Fig. 5). Some similarities could be seen with pressures and flows found after total PA occlusion (Fig. 1). This pattern persisted for as long as the ACh pressor effects were seen. Axelsson *et al.* (1989) show equivalent flow patterns after intravenous ACh. The pressure records of White (1969, 1970) are taken from the period, following intravenous ACh, when the chronotropic effect was maximal and ejection to the LAo still occurred in the second phase of RV systole (Fig. 1), but it seems likely that the pressures were becoming monophasic and that, again, the pattern is as we describe.

We believe that this monophasic shunting pattern (Fig. 12E) is unique and different from naturally occurring shunts. It probably requires all or most of the components of ACh injection (chronotropic, inotropic, systemic pressure and pulmonary pressure changes), acting simultaneously, for its full expression. Whether this can happen in nature is not at all certain. We have not seen the pattern, in the absence of intravenous ACh, in any of our experiments on anaesthetized or unanaesthetized alligators.

Injections of ACh into the left side of the circulation *via* the RAo produced much simpler changes (Fig. 6), minimizing chronotropic and inotropic effects, causing no vasoactive modifications of the peripheral vasculature in the lung, but giving rise to a marked systemic hypotension. In these experiments a R-to-L shunt occurred because the second phase of pressure generation in the RV, after active closure of the pulmonary outflow tract, exceeded the lower pressures in the LAo. The shunt was seen in spite of the fact that PA flow continued during the first phase with little change. This pattern of shunt was characterized by the ejection of blood from the RV to the LAo only during the second phase of systole (Figs 6, 12D). It resembles the spontaneous shunt that we reported in anaesthetized alligators in every respect (Shelton and Jones, 1991) but is one that has not otherwise been reported.

This shunting pattern could be most reliably produced in unanaesthetized animals by leaving them undisturbed in a tank of water in which they could move and surface at will (Fig. 8B). It was a far more frequent and more easily produced phenomenon than in our

experiments on anaesthetized animals, probably because the blood pressures were lower in unanaesthetized alligators. Long-term recordings showed that the shunt could be maintained for substantial periods. LAo flow during shunting averaged $5.5\pm1.5 ml min^{-1} kg^{-1}$ and, even at the extremely low cardiac outputs occasionally found in undisturbed animals, only approached 25% of the cardiac output. We found a mean R-to-L shunt of 13.7±5%, about half that obtained by Powell and Gray (1989) using a multiple inert-gas technique on anaesthetized animals. Powell and Gray (1989) suggested that the shunt they recorded was due to perfusion of unventilated regions of the lung rather than to central cardiovascular effects. However, systemic blood pressures were low in their animals, as was cardiac output, and it seems possible that there was some LAo component in the shunt.

In our experiments, forced diving did not cause a shunt either of the type induced by ACh injection or that seen in the animal at rest. There was little change in PA pressure. Peripheral systemic vasoconstriction associated with bradycardia in the diving response maintained high systemic blood pressures that prevented the development of a shunt. The high levels of excitement during forced dives and during the chasing experiments were also not conducive to shunt development. The pressures recorded by White (1969, 1970) suggest that LAo flow can occur during a forced dive with bradycardia. His animals were restrained on a board with straps but did not become excited, as they did when made to breath nitrogen. It seems conceivable that an element of habituation to the forced dive may have been important in maintaining a tranquillity that was certainly absent in our experiments.

LAo flow patterns were unusual during our forced-dive experiments in that reversed flow began to exceed forward flow and after approximately 5min of submergence there was little or no forward flow (Fig. 11). The excess of reversed flow over forward flow could be due to movement of blood, during diastole, from the LAo to the RAo through the foramen. This would require the output resistance of the RAo to be lower than that of the LAo, which could be achieved by constriction of the gut vasculature.

The present experiments do little to foster our understanding of the importance of the LAo connections to the survival and success of crocodilians; certainly this is more of a problem than we thought at the time of writing our earlier paper (Shelton and Jones, 1991). The mechanisms of the R-to-L shunt and of blood flow in the LAo are now clear. We are convinced that development of a shunt depends on conditions seen in resting, relaxed animals and that it is a different pattern from that seen after injections of ACh into the right heart. It is clearly unrealistic to suppose that the LAo connections are an atavistic relic. The system requires both ventricles to pump blood at high pressure and they both have thick, muscular walls. It also requires an active valve to cut off pulmonary flow and allow high pressures to be generated in the RV. It seems inefficient in that the energy expended in generating these high pressures is often wasted because there is no ejection to the LAo and no mechanical work is done. The benefits that accrue to a resting animal from the ability to shift some of the systemic output of the heart from the LV to the RV and to pump deoxygenated blood to the body have to be weighed against the metabolic and evolutionary costs of the thick RV myocardium and the active valve. The reductions in PA flow are small in the resting animal, but the progressive increase in venous return to

the right atrium allows second-phase ejection from the RV to develop without seriously depleting first-phase outflow. The RV can thus begin to assume a more dominant role than the LV as the shunt develops, while pulmonary flow remains relatively constant. However, some adjustments to lung blood flow may also occur. Further experiments to see whether calm animals can remain apnoeic for periods long enough to cause hypoxia and pulmonary vasoconstriction would be of considerable value. The advantages that accrue from using a complex mechanism to divert blood from a low-pressure lung to a high-pressure body circuit are difficult to visualize and even more difficult to measure.

Since the shunt develops most readily without diversion of blood away from the lung, it seems reasonable to consider the possible advantages of supplying some part of the systemic periphery with hypoxic, hypercapnic and acidic blood. Webb (1979) has suggested that the LAo, because of the anatomy of its connection, supplies much of the blood to the stomach and intestines. This view clearly needs testing with some physiological experiments, but some conditions, such as dilation of the gut vasculature and constriction of the DAo connection, could produce a flow pattern of that type. An ability to supply shunted, acidic blood to the gut may be advantageous in the secretion of HCl into the stomach. A prolonged alkalosis is known to occur in these animals following a meal (Coulson *et al.* 1950; Coulson and Hernandez, 1983; Weber and White, 1986) when they are resting in a way that is conducive to the development of R-to-L shunts. It is certainly possible that the design of the cardiovascular system in crocodilians may be related as much to gastrointestinal as to cardiorespiratory physiology.

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