Conclusion

Running and jumping are powered by muscles. Athletic performance depends largely on how strong those muscles are and how fast they can contract. So much is obvious. This lecture has stressed the much less obvious point that the elastic properties of tendons and ligaments are also critically important. In running, leg tendons and the ligaments of the foot serve as energy-saving springs, enabling us to bounce along like rubber balls. A sheet of tendon in the backs of quadrupeds saves further energy in galloping, by stretching to store energy as the swing of the legs is halted, and recoiling to swing them the other way. In jumping, tendon recoil makes faster movements possible, and insect jumping depends on built-in catapults. Without springs we and animals would be much less athletic.

Further reading

More information on most of the topics of this lecture can be found in one or other of two books:

Alexander, R.McN. (1992) *Expoloring Biomechanics: Animals in Motion* Scientific American Library, New York.

Alexander, R.McN. (1992) *The Human Machine* Natural History Museum Publications, London and Columbia University Press, New York.

Plenary Lecture 3

Crocodilian Cardiac Dynamics: A Half-Hearted Attempt

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In birds and mammals, the heart is completely divided into paired atria and ventricles and separate circulations serve the lungs and body. The resistances to blood flow, and therefore blood pressures, in the lung and body circuits are very different. High systemic pressures provide a reservoir of potential energy for rapid and effective redistribution of blood flow in response to changing tissue demands and allow a degree of independence from gravitational effects. Gravitational effects are minimized in the pulmonary circulation because the lungs are at the same level as the heart. Furthermore, in the pulmonary circulation, arterial lengths are shorter and the total number of capillaries many less than on the systemic side. Hence, the pulmonary circulation can be driven at low pressure which not only saves cardiac energy but also ensures that even at maximum capillary dilatation Starling forces in the lung capillaries must favour fluid absorption. The major drawback of a completely divided heart is that flow to the lungs and body must be exquisitely matched; even a slight imbalance between the volumes pumped by right and left ventricles cannot be tolerated (Burton, 1972). Hence, in birds and mammals there is independence of pressures in lung and body circuits but not independence of flows.

On the other hand, in the absence of a divided ventricle as occurs in lungfishes, amphibians and many reptiles it is possible to have independence of flows but not pressures in lung and body circuits. Since pressures in the lung and body circulations are derived from the same source (the undivided ventricle) then, at least during systole, they must be similar. Consequently, high systemic pressures mean high pulmonary pressures which in some amphibians and reptiles may be 2 to 5 times greater than pulmonary pressures in mammals. High pulmonary pressures

have severe consequences. In breathing turtles, for instance, the rate of plasma loss from blood to tissues within ventilated lungs is an order of magnitude higher than in mammalian lungs (Burggren, 1982). Hence, pulmonary plasma filtration places a limitation on how high systemic blood pressures can be in lower vertebrates. In contrast to the pressures, flows in lung and body circulations can be vastly different between and during bouts of ventilation. For instance, in *Xenopus laevis* pulmonary flow can fall to less than 20% of systemic flow during breath-holding, rising to exceed systemic flow during breathing (Shelton, 1976). The evolutionary and adaptive significance of such central shunts in independently adjusting blood flow to lungs or body in intermittent breathers is not at all obvious, particularly in view of the spectacular development of intermittent breathing in some diving birds and mammals, in which independent flow adjustments cannot be made.

Independence of pressures in lung and body circulations is seen in two living groups of diapsid reptiles, the varanids and crocodilians. Although, the varanids lack a permanently divided ventricle, an intraventricular partition formed by the atrio-ventricular valves divides the heart in diastole, whereas a muscular ridge within the ventricle divides it at a quite different site during systole (Heisler *et al.*, 1983; Shelton *et al.*, 1995). In the crocodilian heart the ventricle, alone among reptiles and similar to birds and mammals, has a complete interventricular septum. From here on in however, things get complicated. The left ventricle (LV) gives rise to a single aorta which supplies the body (the right aorta (RAo)), whereas the right ventricle (RV), besides supplying the lungs via the pulmonary arteries (PAs), also gives rise to a major vessel supplying the body, the left aorta (LAo) (figure 1). The right and left aortae cross-over one another outside the ventricles and run down the right and left sides of the body, respectively (figure 1). Therefore, this apparent diapsid dyslexia arises from the fact these vessels take their names from their courses and not their origins. The LAo and the RAo join twice; just outside the heart through a hole in their joint wall (the foramen of Panizza) and by a short anastomosis behind the heart (figure 1) which some authors describe as small or minor with respect to its morphology (Webb, 1979; van Mierop & Kutsche, 1985) although this was not the opinion of Shelton and Jones (1991) with respect to its contribution to flow. Therefore, in crocodilians the possibility exists for deoxygenated, right ventricular blood to by-pass the lungs and be recirculated to the body via the LAo. In conventional parlance this is a right to left (R→L) shunt but it also is possible for blood to move between the major arteries (RAo & LAo) through their extra-ventricular connexions, so that a series of central cardiovascular R→L shunts can theoretically occur, although both aortae serve the systemic circulation. Hence, for clarity, it seems best to term the diversion of blood from the pulmonary to the systemic circuit, via the LAo, as a pulmonary to systemic shunt (P→S shunt). When a P→S shunt occurs, not only pressures but also flows in lung and body circuits can be independent, which is a feature unique to crocodilians among extant air-breathing vertebrates.

Given this anatomical arrangement the major questions that arise concerning crocodilian cardiac dynamics are the role played by the extra-ventricular connexions between RAo and LAo, and how and when the P→S shunt occurs. With respect to blood flow through the foramen of Panizza, White (1969) suggested that during systole when the LAo valves were shut, blood flowed into the LAo from the RAo via the foramen. Greenfield and Morrow (1961), in contrast, thought that the foramen was closed during systole by the medial cusp of the right aortic valve, so that blood flow from RAo to LAo occurred during diastole. Grigg and Johansen (1987) suggested that flow through the foramen could occur in both systole and diastole under different circumstances and that the variability of different investigators'

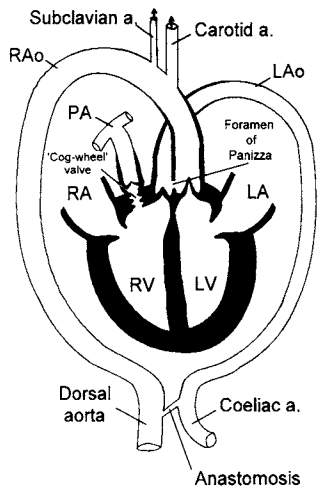


Fig. 1. Diagrammatic representation, in ventral view, of the heart and arterial arches of a crocodilian. The heart, as drawn, is in diastole. PA is the pulmonary artery. LAo and RAo, LA and RA, and LV and RV are the left and right aortae, atria and ventricles, all respectively. The abbreviation "a." represents artery.

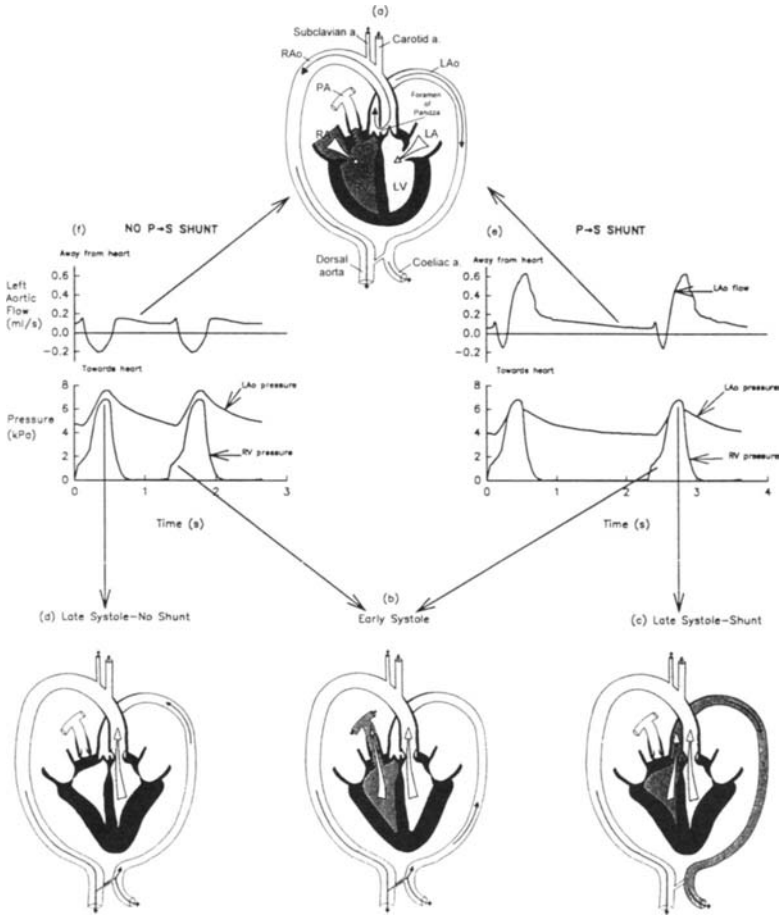
results could be attributed largely to changes in the diameter of the foramen. More recently, direct measurement of flow in the caiman (Axelsson *et al.*, 1989) showed that, in the undisturbed animal, flow rates in the LAo were very low and entirely dependent on movement of blood through the foramen during most of the cardiac cycle. In all of these studies, flow through the anastomosis between the LAo and RAo, behind the heart, and the effect this flow might have on cardiac dynamics has been virtually ignored.

With respect to generation of the P→S shunt, it is obvious that RV pressure must exceed that in the LAo before the shunt can occur. Greenfield and Morrow (1961) proposed that the pulmonary vascular bed became isolated early in systole, by active closure of the pulmonary outflow tract, and that continuing right ventricular contraction caused a further, isovolumic, increase in pressure unless the pressure generated in this second phase exceeded that in the LAo, when blood was ejected into the LAo, establishing the P→S shunt. Such a two-phase increase in RV pressure has been recorded on many occasions since Greenfield and Morrow's (1961) original observation (White, 1969, 1970; Grigg & Johansen, 1987; Axelsson *et al.*, 1989; Shelton & Jones, 1991; Jones & Shelton, 1993) but there has not been universal agreement as to its cause or utility. For instance, White (1969, 1976) envisaged that the resistance of the pulmonary outflow tract from the RV was variable and regulated by a cholinergic mechanism. During forced dives or asphyxia, with accompanying bradycardia, he found that the resistance of the pulmonary outflow tract increased so that RV pressure increased and ultimately blood was ejected into the LAo. White (1969) proposed that the utility of P→S shunting was to redirect cardiac energy expenditure more to systemic than pulmonary perfusion in the submerged animal. Axelsson *et al.* (1989), however, were unable to confirm flow into the LAo from the RV in their diving caimans and found that exogenous injections of acetycholine were necessary to produce a P→S shunt. Acetycholine, injected into the right side of the circulation, caused a marked increase in pulmonary resistance which increased RV pressure and brought about a P→S shunt (White, 1970; Axelsson *et al.*, 1989). The

LAo flow recordings of Axelsson *et al.* (1989) show that during the shunt the LAo flow pulse in systole was monophasic, and away from the heart (anterograde).

Graham Shelton and I recorded pressures in the cardiac chambers as well as pressure and flows in the arterial arches and were able to make a much more complete analysis of the workings of the crocodilian heart (Shelton & Jones, 1991; Jones & Shelton, 1993). We confirmed the two stage pressure rise in the RV with the early stage being associated with pulmonary artery flow. In crocodilians, resistance of the pulmonary circuit is exceptionally low and the second phase of RV pressure increase cannot start until the PAs are closed off from the RV. This occurs when a small subpulmonary chamber situated just behind the pulmonary valves becomes

Fig. 2. Diagrammatic representation, in ventral view, of flow through the heart and arterial arches of a crocodilian during diastole (a) and early (b) and late systole, with (c) and without (d) a P→S shunt. The shunt is the type that develops naturally, in relaxed animals. Schematic representations of RV pressure and LAo pressure and flow traces with (e) or without (f) a P→S shunt, are linked to the corresponding cardiac diagrams. Abbreviations are as described in the legend for figure 1. Light and dark stippling represents oxygenated and de-oxygenated blood, respectively, leaving the ventricles or moving between cardiac chambers.



separated from the rest of the RV. This chamber has a contractile wall with a lining of dense fibrous nodules projecting into and largely occluding the pulmonary outflow tract (Webb, 1979; van Mierop & Kutsche, 1985). These nodules intermesh when the myocardium beneath them contracts, forming a"cog-wheel" type valve (figure 1) which stops flow into the PAs. After cessation of pulmonary outflow, the myocardium of the main chamber of the RV continues to contract and if the pressure generated in the second phase exceeds that in the LAo then a P→S shunt will be established (figure 2c & e). The absolute pressure attained in the second phase will depend on ventricular volume at the start of the second phase, the contractility of the myocardium and the level of activation. An increase in pulmonary resistance, for instance, will reduce the volume flow to the lungs and increase the residual right ventricular volume at the start of the second phase of pressure generation and increase the possibility of producing a P→S shunt. Increasing filling pressure in the pulmonary veins during diastole will also increase ventricular volume and initiate the P→S shunt as Franklin and Axelsson (1994) found in an isolated crocodile heart preparation. To generate relatively high pressures the RV is thick-walled; however, there may be an absolute limit to the pressures it can achieve compared with the LV. Franklin and Axelsson (1994), showed that when the isolated crocodile heart was forced to pump against pressures over 45 mmHg then the RV started to fail. Consequently, a reduction in systemic blood pressure will aid in promoting the P→S shunt and this was the major factor in causing a P→S shunt in awake, chronically instrumented alligators in the absence of any pharmacological intervention (Jones & Shelton, 1993). The lower the systemic pressure then the earlier in the cycle was the second-stage pressure in the right ventricle able to reach levels high enough to open the LAo valves and establish the P→S shunt.

Flow patterns in the RAo and LAo are obviously very different depending on whether there is a P→S shunt in operation or not (figure 2e & f). When blood pressure in the systemic circulation was high and there was no P→S shunt, blood in the LAo moved slowly away from the heart (anterograde flow) during diastole and towards the heart (retrograde flow) during systole (figure 2f)(Jones & Shelton, 1993). The foramen of Panizza is occluded throughout systole by the open RAo valves (figure 2b) and is opened for flow at the start of diastole, when the RAo valves shut. Flow through the foramen as well as elastic recoil of the LAo contribute to forward flow in the LAo in diastole. However, flow through the foramen of Panizza is small being only 2-6% of the output of the left ventricle. In early systole, although the foramen is now occluded, forward flow continues in the LAo due to the elastic recoil of its distended walls. Later, as blood ejected from the LV completes the circuit from RAo to LAo via the anastomosis, flow reverses in the LAo. This flow from the RAo "charges" the elastic reservoir of the LAo (figure 2 b, d, & f); this stored energy is released during diastole. Establishment of the naturally occurring P→S shunt is marked by a change in the LAo flow profile in systole (figure 2e). Flow into the LAo from the RV does not start until later in systole, after active closure of the pulmonary outflow tract. Consequently, in early systole, LAo flow, as before, begins with a period of reversed flow as blood enters the LAo from the RAo via the anastomosis behind the heart (figure 2b,e). This reversed flow is terminated by the ejection of blood through the left aortic valves when RV pressure reaches that in the LAo (figure 2c & e). The naturally occuring P→S shunt could be maintained for substantial periods. However, the shunt is small being, at most, 25% of the output of the RV. Recently, flow has been recorded through the anastomosis and the flow patterns confirm the view presented above and in figure 2 (Karila *et al*, 1995).

Hence, in the naturally occurring shunt the flow pulse is biphasic, qualitatively independent of changing pulmonary resistance and requires low systemic blood

pressures as are seen in relaxed, happy alligators (figure 2e). LAo flow in the naturally occurring shunt is quite different from LAo flow in the P→S shunt envisaged by White (1969, 1976) because establishment of White's P→S shunt depended on a large increase in pulmonary resistance and consequent decline in PA flow, quite unlike the situation in relaxed animals in which the P→S shunt occurs without diversion of blood away from the pulmonary circuit. A large increase in pulmonary resistance can be provoked by injection of acetylcholine into the right side of the circulation, which causes a monophasic, anterograde, LAo flow pulse throughout systole (Axelsson *et al.*, 1989; Jones and Shelton, 1993). This monophasic shunting pattern probably requires all or most of the components of ACh effects (negative chronotropic and inotropic effects, systemic hyptension and pulmonary hypertension, as well as changes in the timing & function of the "cog-wheel" type valve on the pulmonary outflow tract), acting simultaneously, for its full expression. Whether all this can happen in nature is not at all certain.

Hence, crocodilians are unique, not only in their cardiac morphology but also in being the only vertebrates in which there can be independence of both flows and pressures in lung and body circuits. How the ability to shunt blood from a low pressure lung to a high pressure systemic circulation has contributed to the success and survival of crocodilians, however, is a mystery. Because the shunt develops readily without diversion of blood away from the lung, the possible advantages of the shunt may lie in 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 connexions, supplies blood to the stomach and intestines. Flow recordings made by Axelsson *et al.* (1991) from the coeliac artery after pharmacological induction of the monophasic P→S shunt do not contradict Webb's (1979) view. 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 & Hernandez, 1983; Weber & White, 1986) when they are resting in a way that is conducive to the development of P→S shunt. It is certainly possible that the design of the cardiovascular system in crocodilians may be related as much to gastrointestinal as to cardiorespiratory physiology (Jones & Shelton, 1993).

Acknowledgements

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Plenary Lecture 4

Energization of Animal Plasma Membranes by the Proton-Motive Force

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Energization of plasma membranes by ion-motive ATPases

Primary transport processes across biological membranes, those which are linked directly to metabolism, include primary solute translocation by ion-motive ATPases, group translocation and electron translocation (see Harvey and Slayman 1994). The plasma membranes of eukaryotic organisms are usually "energized" by ion-motive ATPases which are molecular pumps that use the energy of scalar ATP hydrolysis to move ions vectorially across the membrane. Osmotic and electrical work, performed by such primary transport, leads to an electrochemical gradient across the membrane which may be used to power secondary transport (Fig. 1; Harold 1986). It is convenient to express the difference in the electrochemical potential ($\Delta\mu k$) for an ion (k) across a membrane in units of voltage. The electrochemical driving force or