DYNAMICS OF BLOOD FLOW THROUGH THE HEARTS AND ARTERIAL SYSTEMS OF ANURAN AMPHIBIA

By B. LOWELL LANGILLE* AND DAVID R. JONES†

Department of Zoology, University of British Columbia,
Vancouver, B.C., Canada

SUMMARY

1. Blood pressures in the ventricle, conus arteriosus, systemic arch and pulmocutaneous arch along with blood flows in the systemic and pulmocutaneous arches have been recorded in *Rana catesbeiana*, *R. pipiens*, *R. temporaria* and *Xenopus laevis*. Central pressure and flow waveforms exhibited the same qualitative features in all species.

2. Blood pressure in both the systemic and pulmocutaneous arches passively followed ventricular pressure throughout ventricular ejection. Since diastolic pressure run-off was faster in the pulmocutaneous arch, pressure rose and ejection started in this arch first. The subsequent delay before the onset of ejection and pressure rise in the systemic arch was directly attributable to the time taken for ventricular pressure to rise from pulmocutaneous to systemic levels.

3. Details of arch pressure profiles previously attributed to contraction of the conus, and a consequent occlusion of pulmocutaneous outflow, persisted when conus contraction was eliminated by ligature of the coronary blood supply to the conus for one week. Thus descriptions of a central (cardiac) mechanism for shunting blood to or from the lungs, based on such an active occlusion of pulmocutaneous outflow, are disproven. It is concluded that distribution of blood by the heart to the systemic and pulmocutaneous systems is controlled only by vasomotion in the vascular beds.

4. The cardiac pulse travelled through major arteries with a velocity of 3·1 m/s and thus traversed the complete arterial tree in a very small fraction of a cardiac cycle. Consequently wave transmission phenomena are not important in the arterial systems of Anura. Pressure pulses recorded in peripheral arteries were virtually identical to the central pulse. In addition a simple windkessel model, depicting the arterial tree in terms of a central elastic reservoir supplying peripheral resistance vessels, gave reasonable predictions of arterial pressure-flow relationships.

INTRODUCTION

The description of central haemodynamics of Amphibia has been substantially revised in the past two decades. Early anatomical investigations (Brücke, 1852; Sabatier, 1873) led to the 'classical theory' according to which the ventricle first

---

* Present address: Department of Biophysics, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

† Killam Senior Fellow.

ejected blood into a low pressure pulmocutaneous system then, as ventricular pressure exceeded the higher systemic pressure, the remainder of cardiac ejection was sent into the systemic system. At the onset of systemic ejection it was proposed that pulmocutaneous outflow was occluded by deflection of the spiral valve, a longitudinal internal septum within the conus arteriosus. Consequently no coincident flow in the systemic and pulmocutaneous arches was envisaged. However, it has now been well established that pressures in both the systemic and pulmocutaneous circuits follow ventricular pressure throughout ventricular ejection (de Graaf, 1957; Simons, 1959; Shelton & Jones, 1968) and therefore sequential distribution of blood does not occur.

Although the classical theory is no longer accepted, the view that rapid deflexions of the spiral valve within the conus play an important role in central blood distribution has persisted. Shelton & Jones (1965a) were unable to detect a time difference between the onset of pressure rise in the two arches even though pulmocutaneous arch pressure falls to a significantly lower level than systemic pressure and, hence, should be exceeded earlier by the rising front of the ventricular pressure pulse. They suggested two possible explanations for this observation: (a) ventricular pressure rises so rapidly during early systole that no detectable delay occurs between the time when ventricular pressure exceeds pulmocutaneous and systemic levels (ventricular pressure measurements were not made in this study); (b) contraction of the conus arteriosus actively deflects the spiral valve to occlude pulmocutaneous outflow in late ventricular systole. This occlusion persists until the start of the next cardiac ejection which passively returns the spiral valve to its original position, thus allowing pulmocutaneous outflow. The latter explanation was favoured.

This description of active deflexions of the spiral valve during conus contraction has acquired considerable importance since Morris (1974) has incorporated it into a theory of central shunting of blood, to or from the pulmocutaneous circuit, in response to changes in respiratory activity (e.g. during diving apnoea). He argued that the timing of conus contraction (normally intitiated late in ventricular systole) is related to coronary blood pH and heart rate, both of which are sensitive to respiratory activity. Thus it is proposed that the animal varies the timing of occlusion of pulmocutaneous outflow, and the proportion of blood going to the gas exchangers, in response to oxygen availability. Presumably this mechanism complements the controls afforded by vaso-motion in the peripheral beds (Shelton, 1970; Emilio & Shelton, 1972). That the coronary circulation of the frog heart has persisted only in the conus arteriosus suggests an important functional significance, and since Shelton and Jones (1965a) failed to observe obvious detrimental effects of acute ligation of the coronary supply of frogs under controlled conditions, the suggestion that it plays a role in regulatory adjustments to changing environments merits further investigation. Certainly White's (1969) conclusion that a central shunting mechanism operates in the hearts of crocodilian reptiles during diving lends credibility to Morris' theory. On the other hand, the absence of marked pressure gradients between the ventricle and pulmocutaneous arch during late systole does not support this argument. In addition, a central shunting mechanism is a curious strategy for an amphibian to employ since such a mechanism would reduce blood flow to the skin gas exchanger as well as to the lungs during apnoea.

Recently, Shelton (1976) has reported that, in *Xenopus*, a small but detectable time

lag does exist between the onset of pressure rise in the pulmocutaneous and systemic arches and he questioned earlier conclusions regarding occlusion of the pulmocutaneous outflow by the spiral valve, at least in this species. However, it is possible that this time lag represents the response time of the pulmocutaneous circulation to the sudden removal of such an occlusion. The resolution of this question has awaited accurate, simultaneous, recordings of pressure in the ventricle and the systemic and pulmocutaneous arches to determine if arch pressures simply follow ventricular pressure during early ventricular contraction or if pulmocutaneous pressure is indeed responding to the removal of an occlusion by suddenly jumping to ventricular and systemic levels. Thus, one goal of the current study has been to examine pressures and flows in the central circulation of a number of amphibian species with high-speed recording techniques to establish precisely the sequence of events in the cardiac cycle. Additional experiments are directed specifically at determining the role of conus contraction in influencing these events.

While many studies have examined blood flow through the amphibian heart we are aware of no detailed descriptions of haemodynamics in the major arteries. Presumably it is widely believed that the amphibian arterial tree is dynamically similar to the mammalian system and that the extension of mammalian studies to Amphibia is valid. This assumption requires close inspection. In mammals (McDonald, 1974) and birds (Langille & Jones, 1975) each cardiac ejection sends out a pulse wave which arrives at different arterial sites at significantly different times. This time difference depends on the relative distances of the recording sites from the heart and the speed at which the pulse wave propagates along arteries. Consequently, haemodynamic phenomena are influenced by wave propagation effects (e.g. wave reflexion and attenuation (McDonald, 1974)). In smaller mammals the transit time of the pulse travelling through the arterial tree is predictably shorter and one might expect, at first inspection, that such effects are reduced. However, the heart rates exhibited by these species are high and transit times represent approximately the same fraction of a cardiac cycle as in larger species (Kenner, 1970). Thus wave propagation phenomena are of comparable importance in virtually all birds and mammals studied. In small poikilotherms (including most Amphibia), on the other hand, a short arterial tree coincides with a low resting heart rate and it is possible that the pulse takes a negligible fraction of the cardiac cycle to traverse the arterial tree. If this is true then wave transmission phenomena will not occur and simpler models of the arterial system may apply. The most commonly studied of these, the windkessel model, describes the arterial system in terms of a simple elastic reservoir, the lumped compliance of the major arteries, which feeds terminal resistance vessels, the peripheral vasculature. If such a model applies to Amphibia then these species should not exhibit wave transmission phenomena, such as pressure wave amplification and distortion (Remington, 1960) or the oscillating pattern of arterial impedance at physiological frequencies (O'Rourke, 1967; O'Rourke & Taylor, 1967), which play such a pre-eminent role in the mammalian system. Consequently an additional goal of this study has been to investigate the effects of pulse wave propagation in the amphibian circulation and to examine pressure–flow relations quantitatively in terms of windkessel and wave propagation models.

METHODS

Physiological studies

The majority of the experiments were performed on *Rana catesbeiana* (31 individuals weighing from 175 to 575 g) and *Rana pipiens* (14 individuals weighing from 90 to 195 g). However, pressure and flow measurements were also made in *Xenopus laevis* (5 animals weighing from 50 to 150 g) and *Rana temporaria* (3 animals weighing from 18 to 25 g). These experiments indicated no qualitative differences in the mechanical events of cardiac pumping in the four species. The frogs were anaesthetized by immersion in Sandoz MS 222 anaesthesia (0·3–2·0 g/l) and restrained on their backs. The heart and arterial arches were exposed by a midline incision through the sternum. The lungs were cannulated through an incision in the left abdominal wall which was subsequently sutured around the cannula and the animal was ventilated with a Harvard 670 positive pressure respirator, although some animals breathed spontaneously when the pump was shut off. The lungs of the *R. temporaria* were ventilated with a low-displacement syringe pump. The frogs were allowed to recover to a more lightly anaesthetized stage which was maintained by frequently wetting the skin with the anaesthetic solution.

Pressures were recorded in the ventricle, conus arteriosus, one systemic and one pulmocutaneous arch with Bio-tec BT-70 and Hewlett-Packard 267 BC pressure transducers which were connected to the blood vessels with 6–10 cm lengths of PE 50 tubing. Dynamic calibration of the manometers and recording systems was performed by applying a step change in pressure to the tips of the manometer cannulae and recording the free vibrations of the system. Since the natural frequency of the system always exceeded 50 Hz, which is far in excess of the frequency of the physiological signals recorded, no correction for manometer distortion was required.

Blood flows were recorded in the systemic and pulmocutaneous arches (opposite side to pressure recordings) with Biotronix BL 610 electromagnetic flowmeters utilizing cuff-type flow probes. Flow probes were calibrated by excising a portion of the artery to which they were attached and perfusing this vessel under pressure with physiological saline. The saline was collected in a calibrated cylinder and the time taken for a given volume to pass through the vessel was recorded with a stopwatch. The volume flow rate thus determined was compared with the output voltage of the flowmeter. Flowmeter outputs during isotonic saline perfusion differ from outputs during blood perfusion by at most 2% (Pierce, Morrow & Braunwald, 1964; Greenfield *et al.* 1966). Care was taken to site pressure and flow probes the same distance from the conus in both the systemic and pulmocutaneous arches and in all cases this distance was from 0·5 to 2·5 cm.

Pressures and flows were recorded during lung ventilation and during apnoea to examine respiratory influences on central haemodynamics and the distribution of cardiac output. In a few experiments spontaneously breathing animals were restrained in a tank and apnoea was induced by raising the water level in the tank until it was just above the mouth and nares.

In some experiments a stimulator was connected to the conus by two fine copper wires sewn into the conus wall and triggered by the QRS wave of the ECG so that the ventricle and conus contracted simultaneously. Normally conus contraction is not

initiated until late in ventricular systole (Brady, 1964) and it was hoped that these experiments would provide insight into the importance of this delay.

Data was recorded on a Techni-Rite TR 888 chart recorder writing on rectilinear co-ordinates, or on a Beckman RS 4-channel, Dynograph and in most cases was simultaneously recorded on an Akai 280 D-SS four channel tape recorder. F.M. adaptors (A. R. Vetter and Co., Rebersburg, Pa.) frequency modulated the data for storage on the audio recorder and demodulated the output when the recorder was in the playback mode. Vascular impedance at the input to both the systemic and pulmo-cutaneous circulations was determined by playing tape recorded pressure and flow signals into an A–D converter interfaced with a LAB 8/e computer (Digital Equipment, Maynard, Mass). The computer performed a Fourier analysis of the pressure and flow signals and printed out the ratio of pressure to flow (impedance modulus) and the phase difference between pressure and flow (impedance phase) for each of the first ten harmonics. These experimentally determined impedance curves were compared with curves calculated from a two component windkessel model (see Taylor, 1964) for which impedance modulus and phase are given by

$$\text{impedance modulus} = R/\sqrt{[1 + (2\pi f T)^2]},$$

$$\text{impedance phase} = -\arctan (2\pi f T).$$

Here R is the vascular resistance (mean arterial pressure divided by mean flow), f is frequency and T is the time constant of the windkessel which is determined from the diastolic portion of the pressure profile according to the equation

$$T = t/\ln (P_0/P(t)),$$

where t is the duration of diastole, $P(t)$ is pressure at the end of diastole and P_0 is pressure at the beginning of diastole. In addition, pressure was recorded in a systemic arch and the sciatic artery in two experiments to examine the transit time and distortion of the pressure pulse as it travelled through the arterial system. All pressure cannulae except those in the sciatic artery were non-occlusive.

In eight frogs (seven *R. catesbeiana*, one *R. pipiens*) the sternum was opened with a small incision directly ventral to the atrio-ventricular junction and a ligature was placed around the coronary artery. The wound was then closed and the frog left to recover for one week. The chest was then reopened and arch, ventricular and conus pressures recorded to assess the effects of long term coronary occlusion on conus function. All eight frogs survived the full week.

Anatomical investigations

The anatomy of the conus arteriosus was examined *in situ* by opening the conus with a midline incision. The cut edges were drawn back and trimmed to permit direct observation of the spiral valve, the inflow and outflow regions of the conus and the cavum aorticum and cavum pulmocutaneum. The exposed conus interior was photographed using an Asahi Pentax ES camera mounted on a Leitz dissecting microscope.

In addition serial sections of the conus were prepared. Standard fixing techniques (using Bouin's solution) were unsatisfactory for these preparations as marked tissue shrinkage occurred. Similar shrinkage problems have been cited in previous studies

(de Graaf, 1957) and no doubt contribute to the controversy surrounding the structure of the spiral valve (de Graaf, 1957; Sharma, 1961; Morris, 1974). Consequently in the present study frozen sections were prepared (12 μm sections) which indicated a structure compatible with direct observations made during dissection.

<div align="center">RESULTS</div>

Anatomical investigations

The hearts of anuran amphibians have been described in detail in a number of studies (e.g. Sabatier, 1873; Sharma, 1957, 1961; Morris, 1974) and in most respects our results confirm those of previous investigations. An important exception, however, lies in defining the structure of the spiral valve. Most commonly the spiral valve has been described as a rather narrow septum with a flimsy attachment to the conus wall (i.e. a structure which could easily be deflected (Sabatier, 1873; de Graaf, 1957; Sharma, 1957, 1961; Morris, 1974)). Similar results were obtained in the present investigation when standard histological preparations were employed but it was obvious, when comparing the resulting slides with the spiral valve in fresh preparations, that marked shrinkage had occurred during preparation of the sections. de Graaf (1957) has commented on this problem previously. However, when frozen section techniques were employed results were consistent with direct observations made during dissections. Fig. 1 illustrates selected sections through the conus. Also shown is a ventral view of the conus which shows the positions from which the frozen sections were selected. In the proximal regions of the conus (sections 3–5) the spiral valve is a narrow structure with an even narrower attachment to the conus wall and consequently resembles previous descriptions (de Graaf, 1957; Morris, 1974). However, in the outflow regions of the conus (sections 1 and 2) the spiral valve forms a wide septum with a thick attachment to the conus wall, not at all suggestive of an adaptation to rapid deflections. Thus any displacements of the spiral valve would appear to be limited to a minor bending of the valve rather than a flapping movement from a hinge-like attachment.

Blood pressures from ventricle, conus and arterial arches

The pressure pulses recorded in the central circulation confirmed the general shape of pulses reported for amphibia in other studies (Shelton & Jones, 1968; Emilio & Shelton, 1972). However, by recording arch pressures and ventricular pressure simultaneously at a high recording speed, it was possible to establish, in detail, the relationships between these three pressures throughout the cardiac cycle. During the phase when ventricular pressure was rising diastolic pressure in the pulmocutaneous arches was exceeded before systemic pressure and pulmocutaneous pressure showed an initial sharp rise (Fig. 2). In the bullfrog it took approximately 50 ms for ventricular pressure to go from pulmocutaneous to systemic end-diastolic pressure (the interval between the vertical lines in Fig. 2) when the lungs were being ventilated either artificially or spontaneously. During this interval, pressure in the pulmocutaneous arch closely followed ventricular pressure and did not suddenly jump to ventricular levels when systemic pressure was reached. At the end of this interval the synangial valves between the conus and systemic channels of the truncus opened and
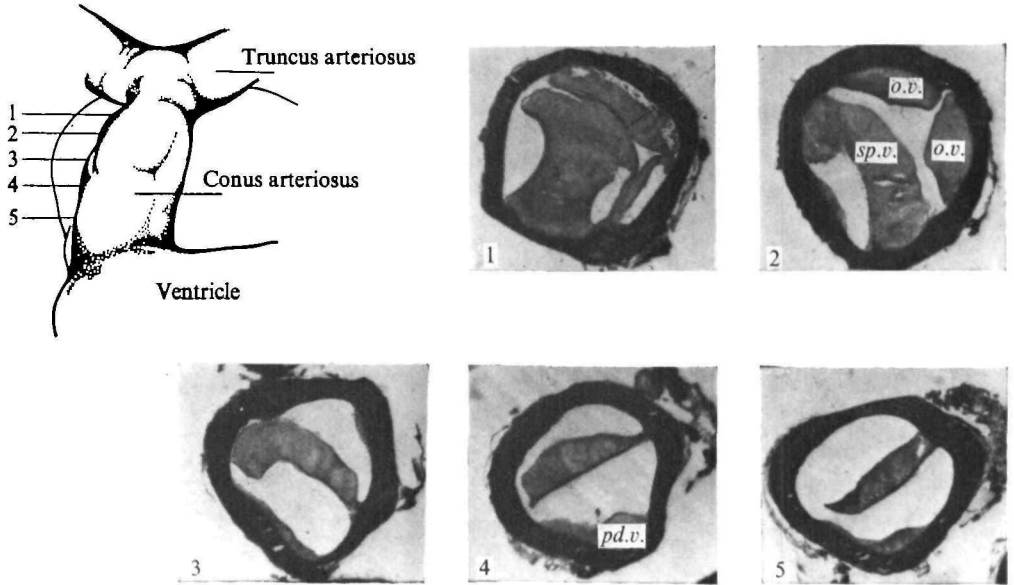
Fig. 1. Transverse cross-sections of the conus arteriosus prepared using frozen section techniques. Slides are chosen from 12 μm serial sections at the sites indicated in the line diagram. Sections display the cross-sections as seen from an upstream viewpoint with ventral side of conus uppermost. *pd.v.*, pad-like valve; *o.v.*, outflow valves; *sp.v.*, spiral valve. The pad-like valve (Sharma, 1957) is a protuberance from the proximal, right dorsal conus wall.
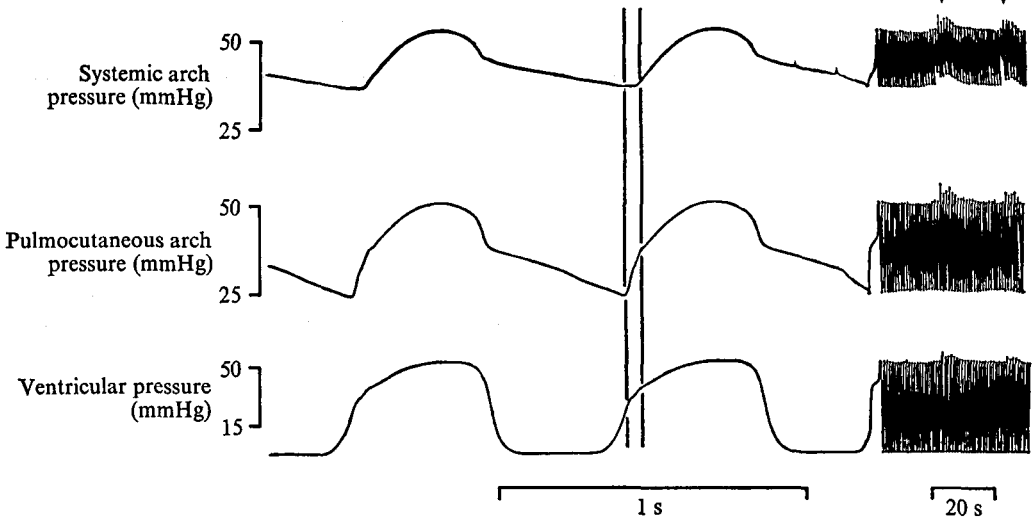
Fig. 2. Blood pressures recorded in the systemic arch, pulmocutaneous arch and ventricle of the bullfrog. Vertical lines link coincident events at the onset of ejection into the pulmocutaneous and systemic circulations. Arrows indicate spontaneous breathing movements and concomitant changes in pressures.

systemic pressure started to rise. This pattern of events is in contrast to earlier reports (Jones & Shelton, 1972) in which the initial pulmocutaneous pressure rise was described as instantaneous and coincident with the pressure rise in the systemic arch. In the present study the same sequence of events as seen in the bullfrog was observed in other species although ventricular pressure rose more quickly in the smaller species. Typically only 20 ms elapsed between the onset of ejection into the pulmocutaneous and systemic arches, a time interval which can only be detected at very high chart speeds. During lung ventilation the inflexion on the rising front of the ventricular pressure pulse most often occurred when pressure reached pulmocutaneous arch levels whereas during apnoea it occurred when ventricular pressure reached systemic levels. On a few occasions a distinct inflexion was seen at both of these times (Fig. 2).

The effect of conus contraction on arch pressures is slight because of the small ejection volume of this chamber (Shelton & Jones, 1965b) and is often evident only as a small notch signalling conus relaxation. In the present study this conus component was observed in both arches although it occasionally did not appear in the systemic arch pressure trace during lung ventilation, a response attributed to marked lung vasodilation which may decrease outflow resistance to the lung-skin circuit to the extent that the conus is no longer capable of reaching systemic pressures. Although there must be a resultant elevation in the volume of conus ejection to the pulmocutaneous system, pressure in the pulmocutaneous arch often showed a reduced conus component and this is attributed to the marked fall in pulmocutaneous vascular impedance during lung ventilation (discussed later) which reduces pressure increments produced by a given inflow. Diastolic decline of pulmocutaneous pressure was more rapid than that of systemic pressure although when both pressures displayed a
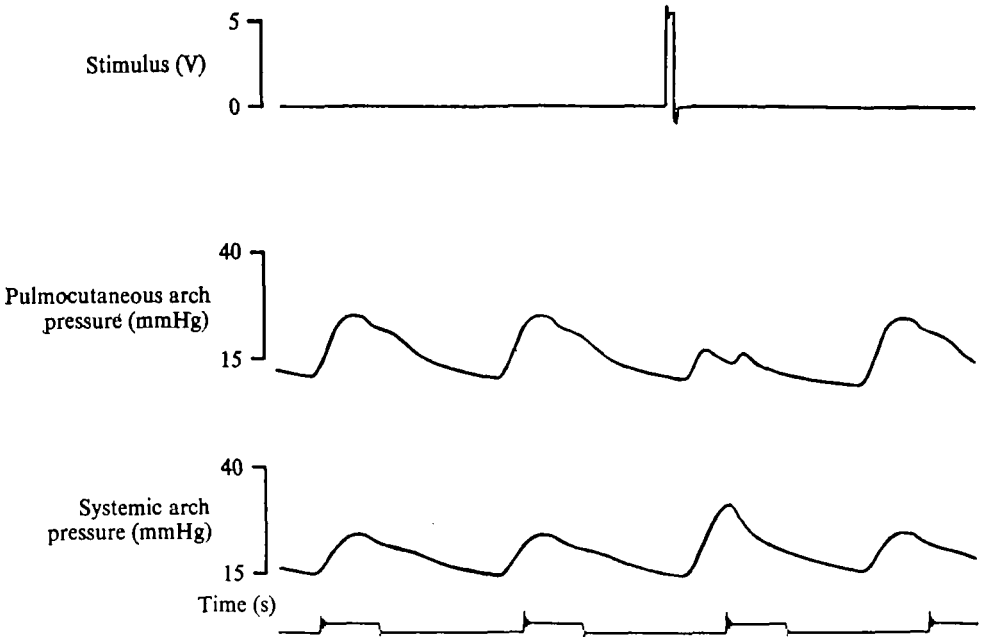
Fig. 3. Result of stimulating conus contraction in time with ventricular contraction (stimulator triggered from QRS wave of the ECG). The decrement in pulmocutaneous arch pressure during systole and coincident rise in systemic pressure indicate at least partial occlusion of the pulmocutaneous outflow.

conus component this more rapid decline was restricted to late diastole. Consequently pulse pressures were always greater in the pulmocutaneous circulation although, as previously reported (Jones & Shelton, 1972), this difference was diminished during apnoea. Fig. 2 also illustrates the immediate effects of lung ventilation on central pressures. Spontaneous breathing movements (at arrows) in frogs not artificially ventilated resulted in elevated ventricular pressures which caused an increase in mean systemic pressure with little change in pulse pressure whereas in the pulmocutaneous arches both mean and pulse pressure increased distinctly. This increase in pulmocutaneous pulse pressure has previously been investigated in *Xenopus laevis* (Shelton, 1970) and was found to result from lung vasodilation which causes a drop in the time constant of the pulmocutaneous circulation (i.e. an increase in the rate of decline of diastolic pressure).

Elimination of the ventricle to conus conduction delay by stimulating the conus in phase with ventricular depolarization had a marked effect on arch pressure profiles (Fig. 3). Pulmocutaneous arch pressure showed an initial small rise indicating early systolic flow; however, pressure suddenly fell and remained low, indicating at least partial occlusion of pulmocutaneous outflow until late in systole when pulmocutaneous pressure rose slightly again. At the same time systemic pressure reached abnormally high levels during systole as a result of the redirection of blood to the systemic circuit. Apparently the normal conduction delay is necessary to allow the ventricle to fully distend the conus before conus contraction and when the delay is eliminated the unfilled conus closes down on the spiral valve and occludes the cavum pulmocutaneum. Our findings confirmed similar experiments by Morris (1974) although he interpreted
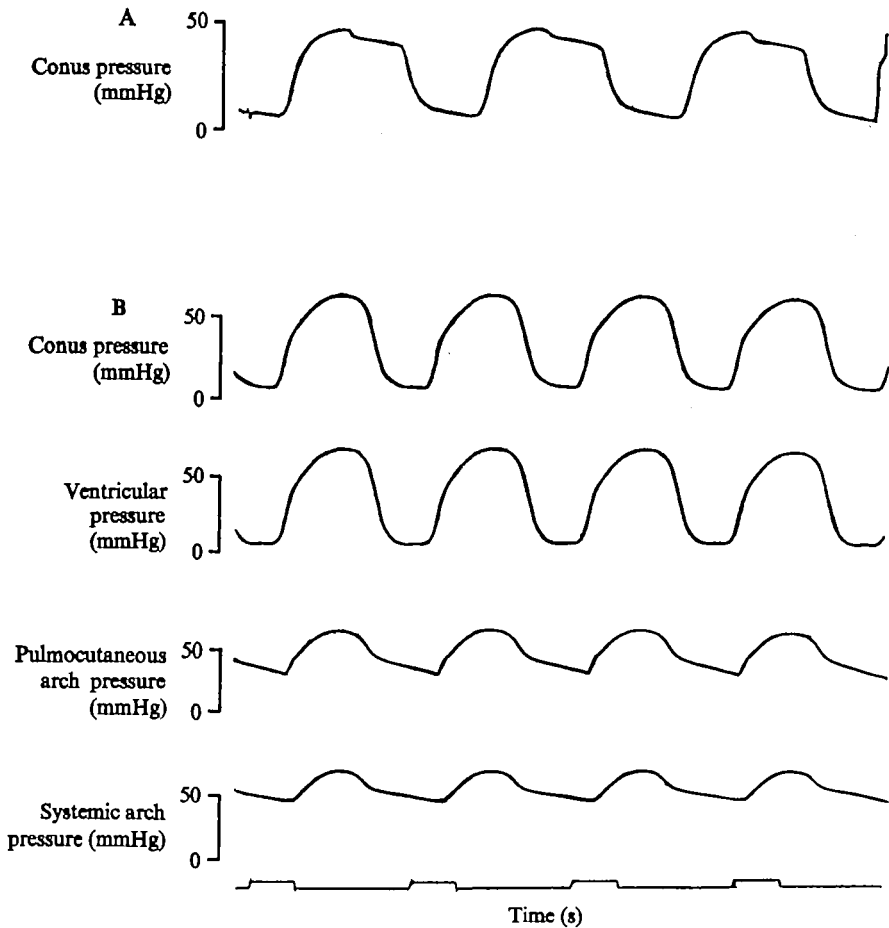
Fig. 4. (A) Pressure recorded in the conus arteriosus of a 'normal' bullfrog. (B) Simultaneous pressures recorded in the conus, ventricle and the two arches of a frog following 1 week of coronary ligation.

the results as evidence that normal conus contraction occludes pulmocutaneous outflow.

Pressure recorded in the conus arteriosus after ligation of the coronary artery for 1 week indicated a complete loss of conus contractility. Conus pressures fell quickly to diastolic levels following ventricular relaxation and, aside from a slightly slower elastic recoil during relaxation, pressure followed that within the ventricle (Fig. 4 B). The normal pattern, in which pressures are maintained at arterial levels until late diastole (Fig. 4 A), was lost completely, indicating that coronary vascularization is required for adequate blood supply even though the conus may survive short-term coronary occlusion (Shelton & Jones, 1965 a). Although there was a complete loss of conus contraction, pulmocutaneous arch pressure still exhibited a sharp initial rise to systemic pressure. Obviously the appearance of this pressure rise in normal animals did not signal the removal of an active conus occlusion of pulmocutaneous outflow.

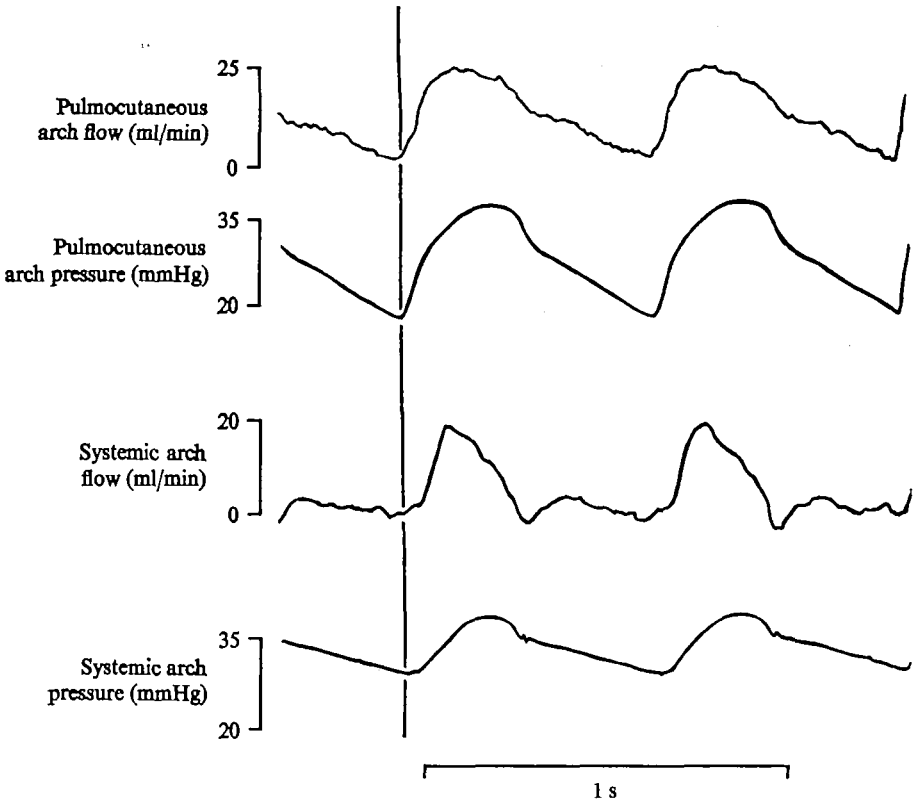One final aspect of conus function has been investigated. In the conus of elasmo-

Fig. 5. Pressures and flows recorded in the systemic and pulmocutaneous arches of the bull-frog. The vertical line indicates onset of pulmocutaneous outflow.

branch fishes there are three tiers of watch-pocket valves (no spiral valve) and con-traction of the conus is required to bring opposing valves in each tier sufficiently close together to ensure competency (Satchell & Jones, 1967), a situation permitting smaller valves which require less backflow to close them. In the present experiments the frog conus was examined for a similar function. Frogs were deeply anaesthetized, the sternum opened as described above, and a stout ligature was tied around the sino-atrial junction which stopped the heart beating. Remaining venous return to the atria, and all arterial arches except one systemic arch, were ligated and two pressure reservoirs were then connected via stopcocks and cannulae to the remaining systemic arch and the ventricle. Ventricular pressure was set at 10 mmHg to fill the cardiac chambers and systemic pressure was raised in steps while recording pressure in the ventricle and conus. Competency of the conus valves consistently broke down, as indicated by a sudden jump in conus and ventricular pressures to reservoir levels, at reservoir pressures of 50–80 mmHg (i.e. at high physiological pressures). Thus it appears that conus valves are competent at normal physiological pressures even when the conus is not contractile (hence the survival of frogs with conus dysfunction induced by coronary ligation). However, during those times when arterial pressures are high it appears that conus contraction is required for proper valve function. In one animal atrial contraction persisted throughout the experiment although the contractions did
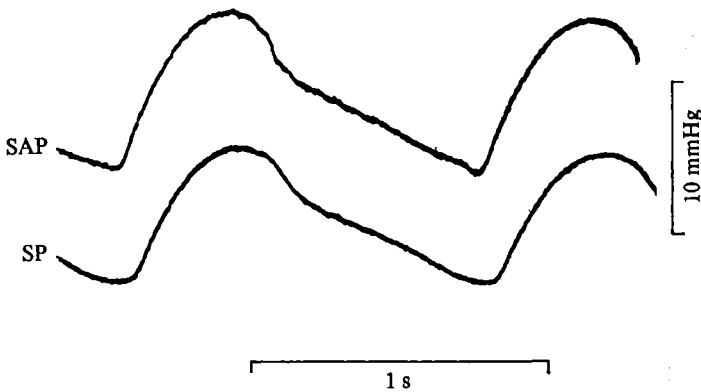
Fig. 6. Near identical pressures recorded in the systemic arch (SAP) and sciatic artery (SP) of the bullfrog. Distance between the recording sites is 14 cm (recorded at the level of the heart and midway along the thigh). Mean pressure at the two sites is the same and profiles have been shifted apart for clarity.

not propagate to the ventricle. Immediately upon breakdown of the conus valves atrial pressure waves were superimposed on conus and ventricular pressures and the atria immediately increased in volume several-fold. This breakdown of the atrio-ventricular valves indicates that contraction of the ventricular myocardium around these valves may also play a role in ensuring their competency.

Arterial flows and pressure–flow relations

The systemic flow pulse in all species was concentrated mainly in ventricular systole (Fig. 5). Ventricular systole was terminated by a reversal of systemic flow which served to close the pylangial valves and possibly caused the synangial valves between the conus and systemic truncus to close briefly. This backflow was generally followed by a period of positive flow due to conus contraction. The onset of ejection into the pulmo-cutaneous arches was synchronous with the initiation of the pressure pulse and consequently preceded ejection into the systemic arches. Marked pulmocutaneous diastolic flows were observed (Fig. 5) which typically contributed 35–40% of stroke flow.

At the end of diastole the steady decline in systemic pressure was interrupted at the point when pulmocutaneous outflow started and pressure levelled off or rose slightly before systemic ejection. At the same time a slight positive flow was recorded in the systemic arch (Fig. 5) although ventricular pressure was still clearly below systemic pressure at this point. This small disturbance in the systemic system is attributed to deflexion of the septum between the systemic and pulmocutaneous channels of the truncus during the initial rise in pulmocutaneous pressure. This rise decreases the pressure gradient between the systemic and pulmocutaneous channels and a resulting displacement of the truncus septum must generate a small positive systemic flow. Although probably of little functional significance this phenomenon again reflects the time difference between ejection into the two circulations.

Apnoea caused a sharp drop in pulmocutaneous flow whereas systemic flow was
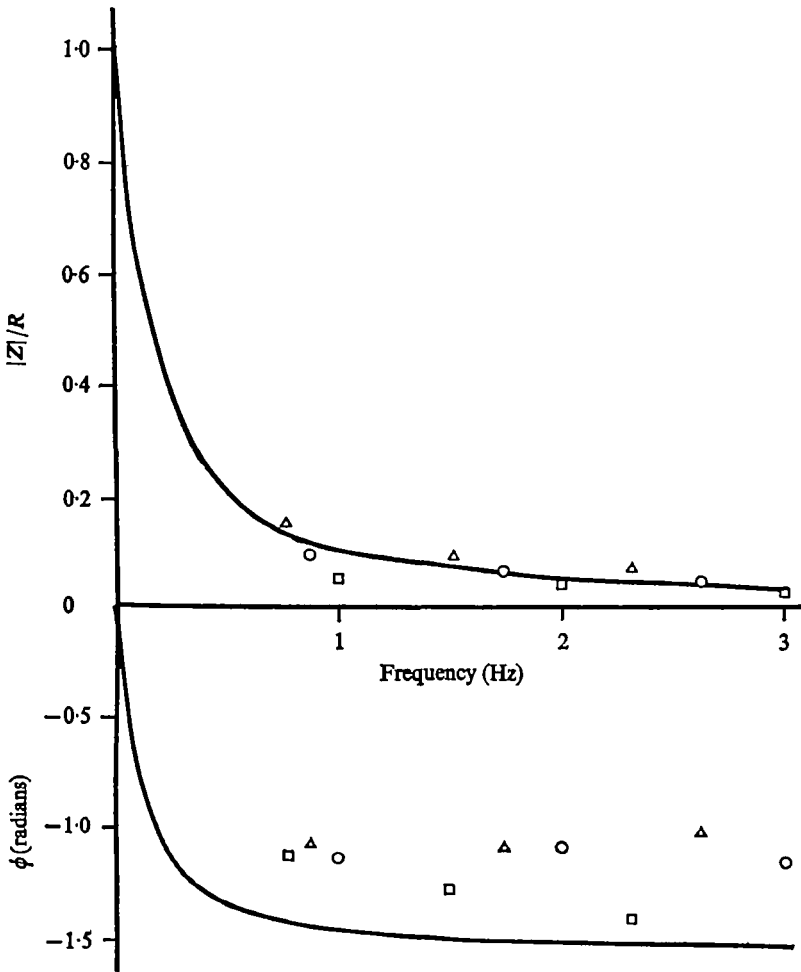
Fig. 7. Input impedance modulus (Z) and phase (φ) of the systemic circulation in three bull-frogs. The solid curves illustrate theoretically predicted (windkessel) impedances for one of the frogs (triangles). Impedance modulus is normalized by dividing by peripheral resistance (R) to allow comparison of different individuals.

relatively unaltered in the short term. In ventilated animals pulmocutaneous flow exceeded systemic flow by up to 50% whereas during apnoea pulmocutaneous flow fell to less than half systemic flow despite a rise in mean pulmocutaneous pressure. Although systemic flows were not immediately influenced by lung ventilation main-tained apnoea caused a marked reduction in systemic flow, with flow declining gradually by some 40% after 10 min of apnoea. During long-term apnoea (more than 30 min) the development of pronounced bradycardia further decreased flow to both arches. These patterns were similar in all species examined and are similar to the findings of Shelton (1970).

Fig. 6 illustrates the effects of transmission through the arterial tree of the bullfrog on the pressure waveform. The pressures shown were recorded in the proximal systemic arch 2 cm from the heart and in the sciatic artery some 14 cm away. The pressure
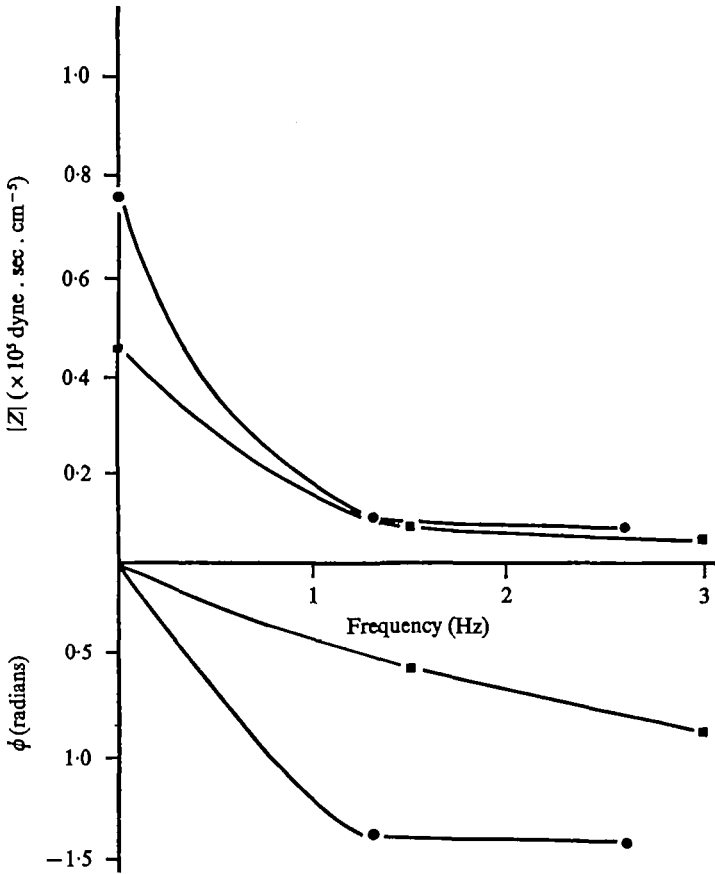
Fig. 8. Input impedance to the pulmocutaneous circulation of the bullfrog during artificial lung ventilation (■) and after 10 min apnoea (●).

pulse was unchanged during propagation between the recording sites, aside from very slight damping, and, in this individual, arrived at the distal site some 45 ms (3·6% of the cardiac cycle) after it passed the proximal transducer, thus travelling with a mean pulse wave velocity of 3·1 m/s. This finding is in marked contrast to pressure wave propagation in mammals (Remington, 1960; McDonald, 1974) and birds (Langille & Jones, 1975) in which a marked amplification and distortion of the pressure wave is observed.

The relationships between arterial pressures and flow waves are most conveniently described by breaking the two waves down into their harmonic (sinusoidal) components and determining vascular impedance *v.* harmonic frequency curves (see McDonald, 1974). Impedance modulus is the amplitude of a pressure harmonic divided by the amplitude of the corresponding flow harmonic and is thus analogous to vascular resistance for steady flow. Impedance phase describes the degree to which the pressure and flow oscillations are out of synchrony. Fig. 7 shows systemic impedances calculated for three bullfrogs. Impedance modulus, normalized by dividing by peripheral resistance (zero frequency impedance) to allow comparision of results from different frogs, displayed a sharp initial fall followed by a more gradual but steady

decline towards zero at higher frequencies. Impedance phase was negative (pressure lagging behind flow oscillations) being between -1 and $-1\cdot57$ radians (57° and 90°) at all pulsatile harmonics examined. Only three harmonics are used to compute these curves because higher harmonics of pressure were consistently below 1 mmHg in amplitude and therefore contributed a negligible component of the total wave. The solid curves in Fig. 7 are theoretical impedance curves for one frog (triangles) calcu-lated from a windkessel model. Fig. 8 illustrates the effects of apnoea on impedance of the pulmocutaneous vascular beds. Apnoea caused an increase in impedance modulus which is significant only at the low frequency limit while impedance phase consistently decreased (became more negative). Similar but less drastic changes in systemic impedance were observed during apnoea.

<center>DISCUSSION</center>

Function of the conus arteriosus

The present study has established that active occlusion of pulmocutaneous outflow by conus contraction, resulting from deflexion of the spiral valve, does not occur in any of the species examined. The strongest evidence for this conclusion is the marked diastolic flow recorded in the pulmocutaneous arches, for which the only potential sources are diastolic recoil of the truncus arteriosus and/or conus ejection, the latter clearly requiring a patent pulmocutaneous outflow. Shelton & Jones (1965b) have measured volume changes in the ventricle, conus arteriosus and truncus arteriosus of *Rana pipiens* throughout the cardiac cycle and found that truncus volume changes are very small. If similar (percentage) changes apply to other amphibians then even liberal estimates of truncus volumes indicate that truncus recoil generates a small fraction of pulmocutaneous diastolic flow (20 % at most, if all truncus run-off were directed down the pulmocutaneous circulation). On the other hand, the volume of the conus is halved during contraction (Shelton & Jones, 1965b) and the resultant outflows are in the range of pulmocutaneous diastolic flow. The appearance of a small conus wave on the pulmocutaneous pressure profile (Fig. 2) supports this interpretation. Previous conclusions to the contrary, i.e. that pulmocutaneous outflow is occluded (Shelton & Jones 1965a, 1972; Morris, 1974) are based on the absence of such a conus wave and also on the lack of a detectable time difference between the systolic rise in pressure in the systemic and pulmocutaneous arches. Certainly the rising portion of the pulmocutaneous arch pressure (Fig. 2), which exhibits a sharp initial rise followed by a slower rise to peak pressure, is suggestive of the sudden removal of an occlusion. However, high-speed recordings of simultaneous pressures in the arches and the ventricle (Fig. 2) clearly demonstrate that pressure in both arches simply follows ventricular pressure throughout systole. In addition, the inflexion on the rising front of the pulmocutaneous pressure wave is also observed on the ventricular pressure profile indicating that this inflexion is simply a characteristic of ventricular con-traction, probably produced by the sudden release of systemic outflow. In this regard it is worth noting that a similar inflexion in ventricular pressure often occurred at the start of pulmocutaneous outflow when the lungs are ventilated, and pulmocutaneous flows are high (Fig. 2). Finally the fact that these pressure wave characteristics were maintained when the conus was rendered non-contractile by coronary occlusion

guarantees that they are not the result of active conus contraction. Since previous descriptions of such active occlusion were based on studies of *Rana pipiens* and *Rana temporaria*, both of which were examined in this report, species differences are not playing a role. We must conclude that present evidence is against any active occlusion of pulmocutaneous outflow in Anura, or any consequent centrally controlled shunting of blood between the systemic and pulmocutaneous circulation. Instead, our findings indicate that distribution of blood by the heart is controlled only by the relative magnitudes of vascular resistances in the systemic and pulmocutaneous beds.

Thus, according to present findings, the conus is pictured as a contractile chamber beating with a time delay with respect to the ventricle, this time delay serving to allow the conus to distend with the initial ventricular ejection so that outflow is not impeded. The pumping action of the conus drives a small fraction of total cardiac stroke volume although during lung ventilation this flow is distributed preferentially to the pulmocutaneous circulation (conus pressure occasionally being insufficient to open systemic outflow valves) and contributes a significant fraction of this flow. Under acute experimental conditions pulmocutaneous diastolic pressures never exceed systemic levels even during prolonged apnoea and hence large conus ejections into the systemic circuits are not favoured. However, when *Xenopus* is allowed to freely surface and submerge diastolic pulmocutaneous pressures are well above systemic levels (Emilio & Shelton, 1972) and under these conditions it seems likely that conus ejection would be preferentially distributed to the lower pressure systemic circulation. Contraction of the conus also draws opposing synangial (outflow) and pylangial (inflow) valves sufficiently close together to ensure competency at high physiological pressures. Since the conus does not fill before the next ventricular ejection, conus pressure exceeding ventricular pressure throughout diastole, the conus presents undistended valves to both the arterial circulation and the filling ventricle, a situation not possible if this chamber were missing. The initial surge of flow to the pulmocutaneous circulation may cause minor deflexions of the spiral valve to widen the cavum pulmocutaneum and bring the free edge into closer proximity with the conus wall (Sharma, 1957, 1961); however, no major deflexions of the spiral valve are envisaged and no valvular function appears to be served by this structure.

It is tempting to ask why the spiral valve does not form a complete septum within the conus since homologous structures in higher vertebrates, e.g. the foetal bulbus cordis of mammals, are totally divided. Certainly communication between the two sides of the conus is necessary if, as suggested above, the major portion of conus blood volume is ejected to the pulmocutaneous circuit during lung ventilation and to the systemic outflow during spontaneous submersion. In addition, it is possible that during the course of normal activity, when blood is shunted from the lungs to the tissues or vice versa, the site of redistribution is within the conus. Simons (1959) observed that dye streams injected into the right atrium would occasionally divide on entering the conus with part of the stream passing into the cavum aorticum. A reverse division could, at times, be seen in dye streams injected into the left atrium (in Simons' study no control over lung ventilation was exercised). If shunting does occur within the conus this would allow a variable distribution of blood to the vascular beds without disrupting flow streams along the ventricular outflow tract where no physical separation exists. During prolonged submergence, when flow to the lungs is

drastically reduced, such a mechanism might be difficult to maintain, and in any event would convey no advantage since systemic and pulmocutaneous arch P_{O_2}'s are the same in this instance (Toews, 1969; Emilio & Shelton, 1974). Flow redistribution within the ventricle might be more likely in this situation. Foxon (1947, 1955, 1964) has suggested that the completely undivided ventricle in the frog is an amphibian specialization associated with the shift from a terrestrial to amphibious environment (and concomitant blood shunting requirements). It is possible that the partial division of the conus is a similar specialization and in this regard amphibians which have returned to a more aquatic environment, the urodeles, commonly exhibit less well developed spiral valves than anurans. In any event these considerations serve to stress the importance of firmly establishing stream patterns through the amphibian heart and their relationship to respiration.

Pressure–flow relationships in arteries

It is apparent from the present study that wave transmission phenomena are of little significance in the arterial system of the frog since pressures measured in distal arteries are virtually identical to those measured near the heart and vascular impedance shows no evidence of the oscillations which wave reflexions produce in mammals. Undoubtedly the chief reason for this is the brief time taken for the pulse to travel from the heart to peripheral arteries, a result of a short arterial tree and a sufficiently high pulse-wave velocity (approximately 3 m/sec). Wave transmission effects, of which reflexion phenomena are most significant, become manifest when pressure (and flow) oscillations are appreciably out of phase at different sites within the arterial tree. In this regard the absence of significant higher harmonics of the pressure wave contributes to the lack of wave transmission effects since a given time lag between the arrival of pressure waves at two different sites represents a greater phase difference for higher frequencies. Other wave transmission phenomena, dispersion effects, for example, are similarly of less significance when transmission times are short. It is believed that wave transmission effects contribute to the mechanical efficiency of the mammalian circulation by reducing the energy expended in driving the pulsatile component of arterial blood flow; (O'Rourke, 1967; McDonald, 1974). Apparently in frogs the costs of higher heart-rates or very low pulse wave velocities outweigh these advantages.

The absence of wave transmission effects in the frog circulation implies that simple 'lumped-parameter' models should apply and the findings of this study indicate that the simplest of these models, the two-parameter (compliance and resistance) windkessel, yields a reasonable estimate of pressure–flow relationships as measured by arterial impedance. Although there is a consistent deviation between predicted and observed values for impedance phase which cannot be explained at present these deviations are far less than those observed when applying a windkessel model to mammals for which measured impedance phases fluctuate widely (O'Rourke & Taylor, 1967). The variations in pulmocutaneous impedance associated with apnoea can be described, according to a windkessel model, in terms of a simple change in time constant of the system associated with peripheral vasoconstriction. Thus an increase in peripheral resistance produces a larger time constant which in turn results in impedance curves of the same shape but with impedance modulus and phase declining

more rapidly with frequency. Consequently the present study has established the viability of a windkessel approach to modelling arterial haemodynamics in Amphibia.

REFERENCES

BRADY, A. J. (1964). Physiology of the amphibian heart. In *Physiology of the Amphibia* (ed. J. A. Moore). New York, London: Academic Press.

BRÜCKE, E. VON (1852). Beiträge zur vergleichenden Anatomie des Gefäss-Systemes. *Denkschr. Akad. Wiss., Wien* 3, 335–67.

EMILIO, M. G. & SHELTON, G. (1972). Factors affecting blood flow to the lungs in the amphibian, *Xenopus laevis*. *J. exp. Biol.* 56, 67–77.

FOXON, G. E. H. (1947). The mode of action of the heart of the frog. *Proc. Zool. Soc. Lond.* 116, 565–74.

FOXON, G. E. H. (1955). Problems of the double circulation in vertebrates. *Biol. Rev.* 30, 196–228.

FOXON, G. E. H. (1964). Blood and respiration. *Physiology of the Amphibia* (ed. J. A. Moore). New York and London: Academic Press.

DE GRAAF, A. R. (1957). Investigations into the distribution of blood in the heart and aortic arches of *Xenopus laevis* (Daud.). *J. exp. Biol.* 34, 143–72.

GREENFIELD, J. C., PATEL, D. J., MALLOS, A. J. & FRY, D. H. (1966). Evaluation of the Kolin type electromagnetic flowmeter and the pressure gradient technique. *J. appl. Physiol.* 17, 372–4.

JONES, D. R. & SHELTON, G. (1972). Factors affecting diastolic blood pressures in the systemic and pulmocutaneous arches of anuran amphibia. *J. exp. Biol.* 57, 780–803.

KENNER, T. (1970). Flow and pressure in arteries. In *Biomechanics* (ed. Y. C. Fung, N. Perrone and M. Anliker). Englewood Cliffs (U.S.A.): Prentice-Hall.

LANGILLE, B. L. & JONES, D. R. (1975). Central cardiovascular dynamics of ducks. *Am. J. Physiol* 228, 1856–61.

MCDONALD, D. A. (1974). *Blood Flow in Arteries*. Baltimore: Williams and Wilkins.

MORRIS, R. W. (1974). Function of the anuran conus arteriosus. *J. exp. Biol.* 61, 503–20.

O'ROURKE, M. F. (1967). Steady and pulsatile energy losses in the systemic circulation under normal conditions and in simulated arterial disease. *Cardiovas. Res.* 1, 313–26.

O'ROURKE, M. F. & TAYLOR, M. G. (1967). Input impedance of the systemic circulation. *Circulation Res.* 20, 365–80.

PIERCE, D. E., MORROW, A. G. & BRAUNWALD, E. (1964). Intra-operative studies of the mechanism of obstruction and its haemodynamic consequences. *Circulation*, 30, Suppl. 4, 152–213.

REMINGTON, J. W. (1960). Contour changes of the aortic pulse during propagation. *Am. J. Physiol.* 199, 331–4.

SABATIER, A. (1873). Etudes sur le cœur et la circulation centrale dans la série des vertébrés. Paris: Montpellier.

SATCHELL, G. H. & JONES, M. P. (1967). The function of the conus arteriosus in the Port Jackson shark, *Heterodontus portusjacksoni*. *J. exp. Biol.* 46, 373–82.

SHARMA, H. L. (1957). The anatomy and mode of action of the heart of the frog, *Rana tigrinia*. *J. Morph.* 100, 303–34.

SHARMA, H. L. (1961). The circulatory mechanism and anatomy of the heart of the frog, *Rana pipiens*. *J. Morph.* 109, 323–49.

SHELTON, G. (1970). The effect of lung ventilation on blood flow to the lungs and body of the amphibian, *Xenopus laevis*. *Resp. Physiol.* 9, 183–96.

SHELTON, G. (1976). Gas exchange, pulmonary blood supply and the partially divided amphibian heart. In *Perspective in Experiment Biology*, vol. 1 (ed. S. Davies). Oxford: Pergamon.

SHELTON, G. & JONES, D. R. (1965a). Central blood pressure and heart output in surfaced and submerged frogs. *J. exp. Biol.* 42, 339–57.

SHELTON, G. & JONES, D. R. (1965b). Pressure and volume relationships in the ventricle, conus, and arterial arches of the frog heart. *J. exp. Biol.* 43, 479–88.

SHELTON, G. & JONES, D. R. (1968). A comparative study of central blood pressure in five amphibians. *J. exp. Biol.* 49, 631–43.

SIMONS, J. R. (1959). The distribution of blood from the heart in some amphibia. *Proc. Zool. Soc. Lond.* 132, 51–64.

TAYLOR, M. G. (1964). Wave travel in arteries and the design of the cardiovascular system. In: *Pulsatile Blood Flow* (ed. E. O. Attinger). New York: McGraw.

TOEWS, D. P. (1969). Respiration and Circulation in *Amphiuma Tridactylum*. Ph.D. thesis, University of British Columbia.

WHITE, F. N. (1969). Redistribution of cardiac output in the diving alligator. *Copeia* 3, 567–70.