J. Exp. Biol. (1972), 57, 789–803
With 7 text-figures
Printed in Great Britain

FACTORS AFFECTING DIASTOLIC BLOOD PRESSURES IN THE SYSTEMIC AND PULMOCUTANEOUS ARCHES OF ANURAN AMPHIBIA

BY DAVID R. JONES

Department of Zoology, University of British Columbia, Vancouver 8, B.C.

AND GRAHAM SHELTON

School of Biological Sciences, University of East Anglia, Norwich, NOR 88C, U.K.

(*Received* 10 *April* 1972)

INTRODUCTION

Recent work has made it clear that there are marked differences in physiology between the partially divided circulatory system of the amphibian and the completely double circulation as seen for example in mammals. In the latter, rates of blood flow in the pulmonary and systemic circuits must, in the long term, be equal. Blood pressures in pulmonary and systemic arteries on the other hand are very different because right and left ventricles are, in this respect, completely independent. In amphibia, however, there is only a single pressure source and several workers have shown that blood pressures are the same in both systemic and pulmocutaneous arteries during ventricular contraction (Simons, 1957; de Graaf, 1957; Johansen, 1963; Shelton & Jones, 1965*a*, 1968; Shelton, 1970). Even during diastole Simons (1957) found pressures identical in *Rana temporaria* and *Bufo bufo* but de Graaf (1957) and Shelton & Jones (1965*a*, 1968) described major differences in diastolic pressures between the systemic and pulmocutaneous arches, with pulmocutaneous pressures being lower in *Xenopus laevis*, *R. pipiens*, *R. temporaria* and *B. bufo*. This difference has also been reported for the urodele *Amphiuma* (Johansen, 1963; Toews, 1971).

The solution to the problem of maintenance of a higher diastolic pressure in the systemic than pulmocutaneous arches, since systolic pressures are the same, would appear to be essentially peripheral. In a purely elastic system rate of fall of pressure is proportional to the product of the amount of potential energy stored in the elastic system during systole and the resistance into which the elastic system is feeding. However, these will effect, or be affected by, changes in blood flow, and the shunt between lung and body circuits, offered by a single ventricle, gives an almost infinite capacity for variation in flow to these regions. The problem is further complicated in Amphibia by the presence of the contractile conus arteriosus, although the propulsive energy imparted to the blood by its contraction is small compared with that imparted by the ventricle (Shelton & Jones, 1965*b*). Nevertheless the question arises as to whether this energy contribution is significant as compared with the energy release of elastic recoil. The present study was undertaken to try to determine the precise role of central and peripheral factors in governing the generation of systolic and diastolic pressures in the systemic and pulmocutaneous arches of anuran Amphibia.

METHODS

The experiments were carried out on 70 *Rana pipiens* and 20 *Rana temporaria*. The animals weighed between 25 and 35 g, except those used for blood-flow studies, which ranged in weight from 40 to 125 g. All operations to expose and cannulate parts of the respiratory and circulatory systems were carried out when the animals were anaesthetized by injection of paraldehyde (0·02 ml/10 g) or immersion in Sandoz MS 222 solution (300 mg/l). Measurements were made when frogs had recovered to a lightly anaesthetized state in which breathing was normal, though in some experiments in which the lungs were ventilated mechanically anaesthesia was maintained at a level sufficient to prevent normal breathing. In these experiments the lung was ventilated through a cannula (1–2 mm diam. polyethylene tube) introduced into the tip of the lung through a small slit in the body wall. Single cycles of inflation and deflation were made by hand using a hypodermic syringe. A variable-displacement respiratory pump was used to give rhythmic ventilation. The pump forced fresh air into the lung but on its recovery stroke the lungs were allowed to deflate directly to air by elastic recoil. Movements of the pump were recorded by connecting its crank to a variable resistance which could then be used to provide a signal of appropriate voltage for the recording device. The natural ventilation volume was determined by measuring the volume of air which could be removed from the lungs of frogs after they had filled them by normal breathing movements. The artificial ventilation volume was then adjusted so that the lungs were not over-inflated. The glottis was closed off by sutures to prevent undue air loss. Both lungs were inflated through the single cannula.

Blood pressures were measured by means of Statham, Sanborn or Hansen manometers, connected to blood vessels via copper tubes fitted with 26 G hypodermic needles or flexible polyethylene catheters. The heart and arterial arches were exposed from the ventral surface by cutting through the clavicles and coracoids and removing part of the sternum. All experiments were carried out on exposed heart preparations, though the pericardium was left intact in most experiments. Blood loss was negligible. The blood-pressure techniques have already been described in detail (Shelton & Jones, 1968; Jones, 1970).

Blood flow in the systemic and pulmocutaneous arches was recorded by means of a Biotronix BL 610 electromagnetic blood flowmeter with appropriate flow transducers. The transducers were held in clamps and were selected for size so that vessel constriction during diastole was minimal. The flowmeter and transducers were calibrated *in vitro* using freshly excised blood vessels with both a flow-through and reciprocating pump system (Jones, 1970). The amplitude response of the whole system (transducer–flowmeter–recorder) was within 1% of the calculated maximum amplitude up to 10 cyc/sec and the phase shift was 4·7° at 1 cyc sec^{-1}. Zero flow was established by occlusion of the blood vessel with either fine clamps or cotton snares. 'Pounding' during occlusion zero was not marked. Total peripheral resistance was calculated from the formula

$$\frac{\text{mean arterial pressure (mmHg)}}{\text{blood flow (ml/min)}}.$$

In 22 experiments attempts were made to remove the spiral valve from the conus arteriosus. For this purpose deeply anaesthetized animals were placed in a deep-

freeze (−10 °C) and cooled until the heart rate was less than 1 beat/min. Venous return was then stopped by clamps on the venae cavae and pulmonary vein and a transverse slit was made in the wall of the conus so that the spiral valve could be pulled through and cut free. A polyethylene tube of appropriate diameter and length was then inserted into the conus through the slit and tied in position by ligatures at the base and apex. In this operation the valves at the base or apex of the conus were damaged to some extent and in no case were both sets of valves functional. Pressure measurements were made on the experimental animals after they had been allowed to warm to room temperature and their heart rates had returned to normal.

Recordings were made on a Sanborn 966 six-channel pen recorder, writing on rectangular co-ordinates or on a Beckman RS 4-Channel Dynograph. The electrocardiogram (E.C.G.) was detected by a thin wire near the heart and amplified in the usual way. The experiments were carried out at room temperature (17–20 °C) and the animals were held under the same conditions. Results were statistically analysed by Fisher's *t* test and 5% was considered the fiducial limit of significance. All results in text and figures are given ± S.E.M. (standard error of the mean).

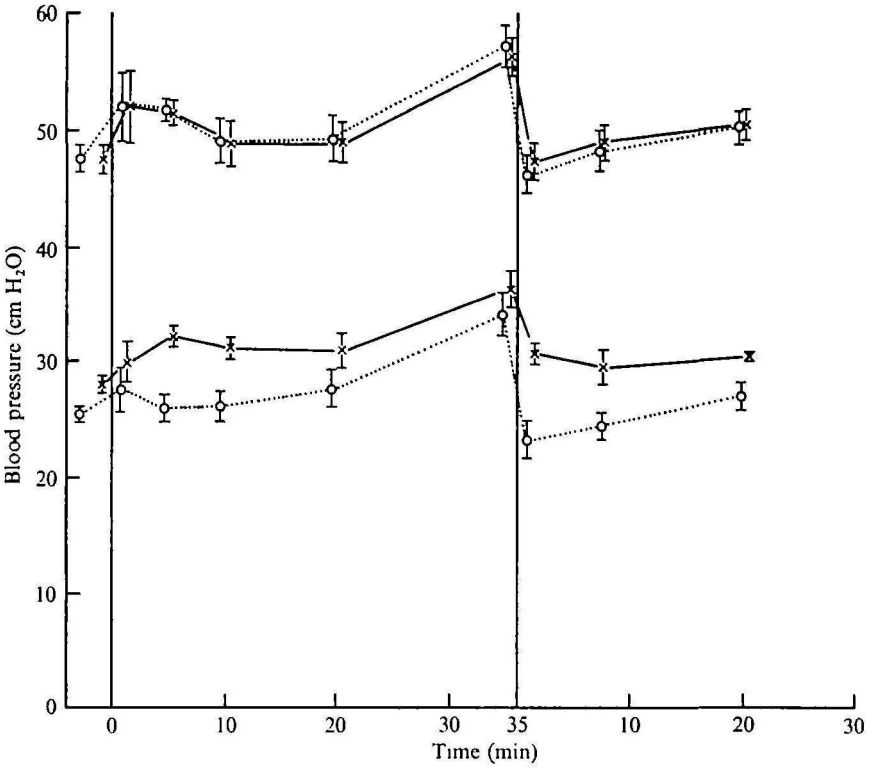
RESULTS

1. *Factors affecting pulse pressures in the systemic and pulmocutaneous arches*

(*a*) *Artificial ventilation of the lung*

Following a period of apnoea, rhythmic ventilation of the lungs with fresh air from the pump almost always resulted in changes of pulse size in either the systemic or pulmocutaneous arch and sometimes in both arches. A period of apnoea following rhythmic ventilation produced the opposite change. The changes were not absolutely consistent from animal to animal; the average responses from the 28 animals examined in these experiments are shown in Fig. 1(*a*). During ventilation, diastolic pressure in the pulmocutaneous arch was, on average, 3–4 cm H_2O below that in the systemic, and the pulse pressure in the former was therefore greater by that amount. In the early stages of a period of apnoea the diastolic pressure differences became greater, though as the apnoea became more prolonged (20–35 min) the differences gradually disappeared and the pulse pressures usually became identical. In the data presented in Fig. 1(*a*) there is no significant difference between the systemic and pulmocutaneous diastolic pressures after 20 min of apnoea. There was a tendency for both the systolic and diastolic pressures to go up during apnoea and to fall rapidly when ventilation was restarted. The fall in diastolic pressure was much greater in the pulmocutaneous arch than in the systemic, thus re-establishing the difference in pulse pressures which was characteristic of the ventilated preparation. The difference was large at first and gradually fell to a steady value. The majority of animals behaved as the average results suggest and in many cases the pulse difference during ventilation was very marked (Fig. 1*b*). In a few animals no changes were seen between the ventilated and non-ventilated state and in one experiment the blood-pressure changes were precisely the reverse of those described above. No differences were seen in the results if the period of apnoea was begun with the lungs inflated or deflated.

Pressure changes during each cycle of lung inflation were slight. In both systemic and pulmocutaneous arches systolic and diastolic pressures rose during the early



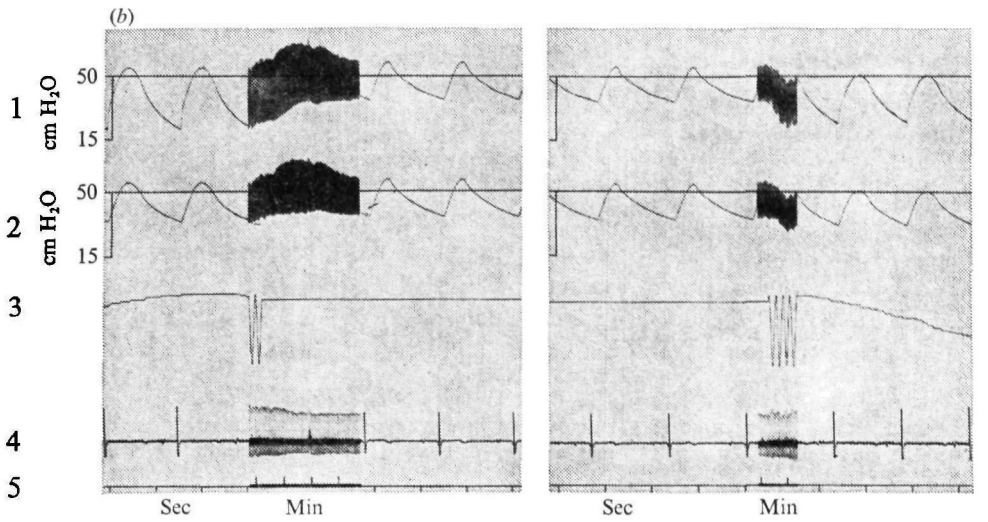


Fig. 1 (*a*). The effect of apnoea on systolic and diastolic pressures in the systemic and pulmocutaneous arches. Average values from 28 frogs, anaesthetized at a level sufficient to prevent normal breathing movements. Artificial ventilation was stopped at time 0 and restarted after 35 min. ×——×, Systemic systolic and diastolic pressures; ○·····○, pulmocutaneous systolic and diastolic pressures.

(*b*) Records from one frog illustrating the changes in pulse pressures in the systemic and pulmocutaneous arches at the onset (left) and termination (right) of 35 min apnoea. Traces (from top to bottom): 1, pulmocutaneous pressure; 2, systemic pressure; 3, artificial ventilation (down on trace = inflation of lungs; 4, E.C.G.; 5, time marks.

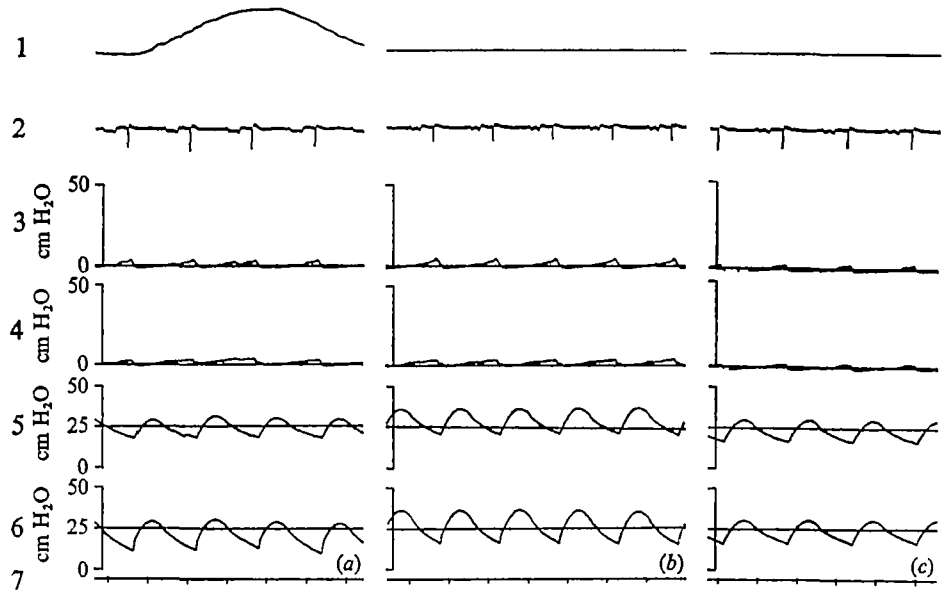


Fig. 2. Blood pressure in the left auricle and posterior vena cava during prolonged apnoea. Records from one animal. (*a*) During artificial ventilation; (*b*) after 10 min apnoea; (*c*) after 30 min apnoea. Traces (from top to bottom): 1, artificial ventilation (up on trace = lung inflation); 2, E.C.G.; 3, pressure in the left auricle; 4, pressure in the posterior vena cava; 5, systemic arch pressure; 6, pulmocutaneous arch pressure; 7, time (sec).

period of inflation but by peak inflation systolic pressures had decreased to a level below that recorded during deflation. More rapid inflation of the lungs (0·5 sec) from a syringe accentuated these pressures changes; increases of the order of 3 cm H_2O were not uncommon, and in these cases the fall in systolic pressure was not apparent until two to three heart beats after peak inflation. Obviously these pressure changes were reversed by deflation of the lungs.

Some of the circulatory changes seen during apnoea caused by submersion in water (Shelton & Jones, 1965*a*) led us to consider the possibility that changes in venous pressures could occur during the change from artificial ventilation to apnoea or vice versa. For example, the left ventricle was frequently much fuller during a period of apnoea caused by submersion, suggesting an increased venous pressure which might affect the arterial blood pressure, particularly diastolic levels. In several of the experiments involving lung ventilation, pressures were therefore measured in the left auricle and posterior vena cava as well as in the arterial arches. The pulmonary vein proved to be inaccessible but its connexion to the left auricle is large and unprotected by valves (Holmes, 1927), so there is probably little pressure difference between the two, at least until auricular contraction when the opening of the pulmonary vein is compressed. As Fig. 2 shows, the venous pressure in both the lung and body circuits was low, increasing slightly as the auricles filled. Pressure rose more sharply in the left auricle after its activation, and peak venous pressures in both circuits occurred soon after the Q.R.S. complex of the E.C.G. (Fig. 2*a*). Both pressures then fell rapidly and minimum venous pressures in both circuits coincided with the appearance of the pressure pulse in the systemic and pulmocutaneous arches.

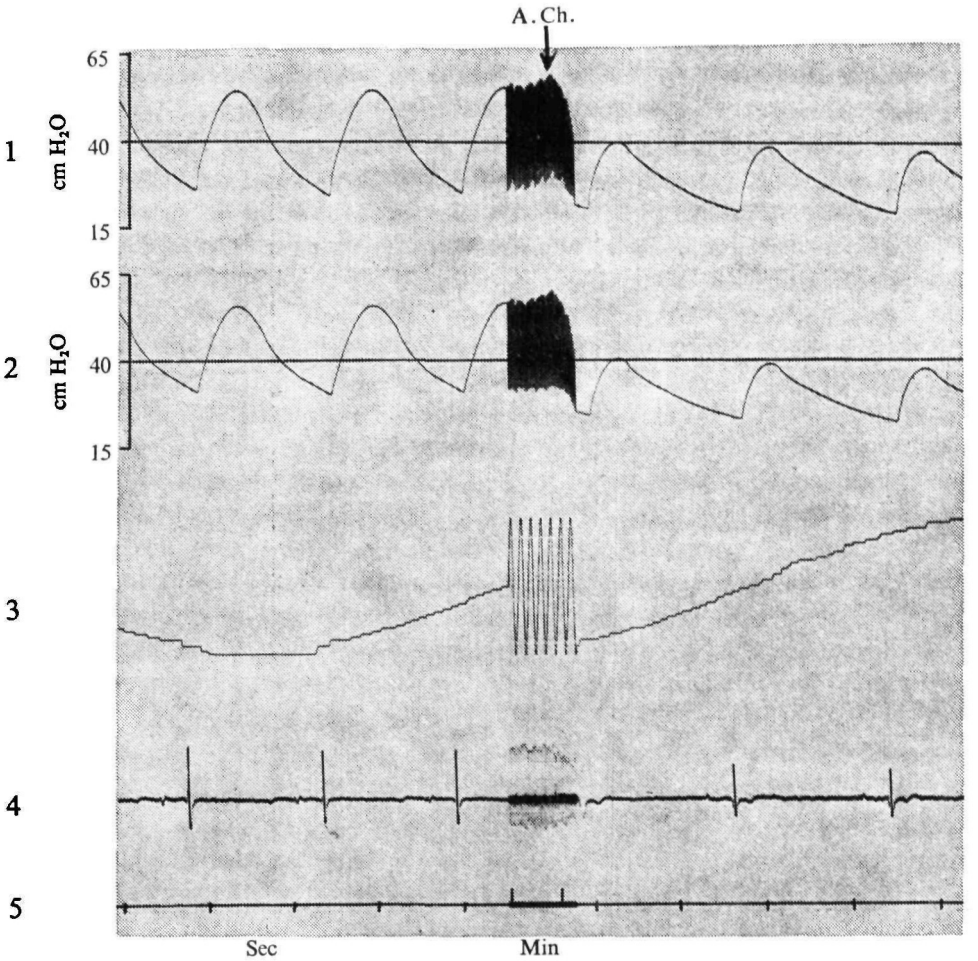


Fig. 3. The effect of application of acetylcholine to the exposed heart (at arrow) on pressures in the pulmocutaneous and systemic arches. Traces (from top to bottom): 1, pulmocutaneous blood pressure; 2, systemic blood pressure; 3, artificial ventilation (down on trace = lung inflation); 4, E.C.G.; 5, time marks.

Venous pressure in both the lung and body circuits was little affected by periods of apnoea up to 45 min; on average, peak pressures were slightly reduced. Nevertheless changes in diastolic pressures in both systemic and particularly pulmocutaneous arches occurred. As Fig. 2 shows, a difference in diastolic pressures of 7 cm H_2O (Fig. 2*a*) during ventilation was reduced to 1 cm H_2O after 30 min apnoea (Fig. 2*c*), the change being accompanied by a reduction in venous pressure in both circuits.

(*b*) *Drugs*

During apnoea systolic blood pressure rose as the pulses became similar and fell rapidly when ventilation was restarted (Fig. 1*a*). This suggested that the overall level of blood pressure might be important in determining differences in diastolic pressure in the pulmocutaneous and systemic arches.

A decrease in systolic pressure was produced by application of acetylcholine to the exposed ventricle or by injection of paraldehyde into the dorsal lymph sac. Although

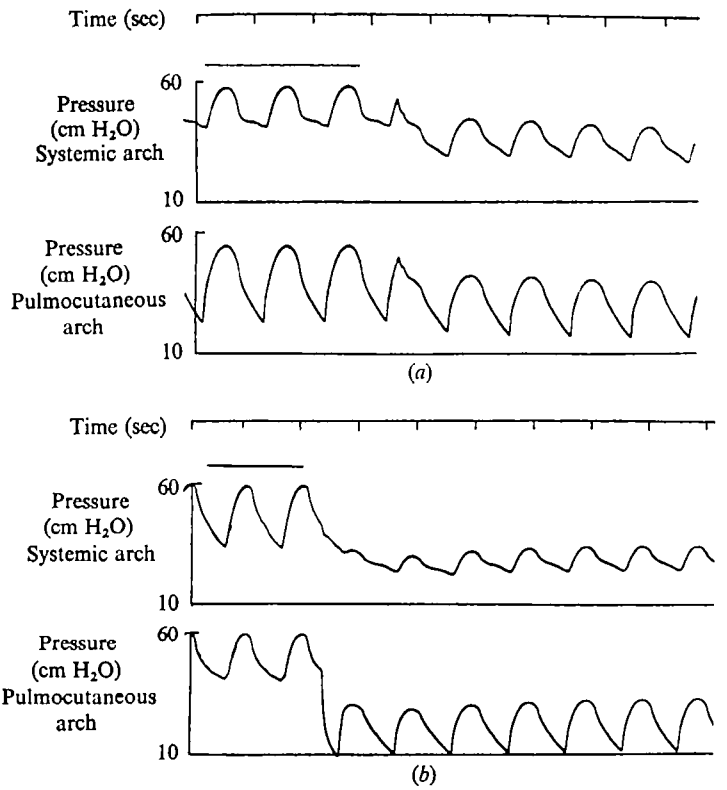


Fig. 4. Records illustrating the effect of bilateral occlusion (*a*) of the systemic arches and (*b*) of the pulmocutaneous arches, on pulse pressures in the two arches. The clips occluding the blood vessels were removed at the end of the period marked by the horizontal marker line.

the former was much more rapid in its effect the net results were the same and may be treated as one. As Fig. 3 shows, a decrease in systolic pressure of 15 cm H_2O caused by application of acetylcholine to the exposed ventricle failed to eliminate the difference in diastolic pressure existing in the pulmocutaneous and systemic arches before treatment with the drug. On average, the difference between diastolic pressures in the systemic and pulmocutaneous arches was 8 cm H_2O, and after treatment with acetylcholine or paraldehyde the difference was halved but maintained. Left auricular pressure changed little after drug application but venous pressure on the body side often increased substantially, on one occasion reaching 15 cm H_2O.

Increases in systolic pressure in both arches were produced by application of adrenaline to the exposed ventricle. Increases of the order of 25 cm H_2O were produced on some occasions but differences in diastolic pressure in the systemic and pulmocutaneous arches existing before treatment with the drug were maintained.

2. *The role of the conus arteriosus and spiral valve*

The pressure difference between the pulmocutaneous and systemic arches is only seen in animals with a well-developed spiral valve and is established early in the cardiac cycle, while the conus is actively contracting (Shelton & Jones, 1965). A second pressure wave during diastole coinciding with conus contraction is usually evident in the

systemic pulse but is seldom seen in the pulse recorded from the pulmocutaneous arch. De Graaf (1957) suggested that the absence of a conus component in the pulmocutaneous pulse was due to the pulmonary circuit offering little resistance to blood flow. In this event the spiral valve, although free along one edge, must be capable of maintaining a pressure difference of 5–10 cm H_2O; furthermore, if pulmocutaneous resistance increases then a conus component in the pulse should become evident.

Occlusion of both pulmocutaneous arches by clamping the walls in fine forceps produced a marked elevation in pulmocutaneous diastolic pressure (Fig. 4*b*) and the diastolic pressure difference between systemic and pulmocutaneous arches existing before occlusion was reversed. Although peripheral resistance was now infinite no conus component appeared in the pulmocutaneous pulse (Fig. 4*b*). Occlusion of both systemic arches enhanced the diastolic pressure difference existing before occlusion but the conus component still appeared in the systemic pulse (Fig. 4*a*).

These results suggested that the energy contribution of the conus might in some way be confined to the systemic (and carotid) arches and that this represented, in part, a reason for the higher diastolic pressures recorded from the systemic arch. Accordingly, we decided to remove the spiral valve from the conus arteriosus and to insert a polyethylene tube, thereby eliminating the conus as an active contractile element.

These experiments were not entirely successful. For instance, it might be predicted that the systolic pressure rise would be more rapid after removal of the conus since central elasticity had been reduced, but this was seldom the case. On the majority of occasions the systolic pressure rise was slower, which suggests, among other reasons, that the resistance of the vessels between the ventricle and pressure-recording sites had been increased by this procedure.

After removal of the spiral valve and conus arteriosus, peak systolic pressures in the systemic and pulmocutaneous arches were 42 ± 3 cm H_2O. This value was not significantly different from peak systolic pressures recorded in animals described in section 1 (average $47 \pm 1{\cdot}5$ cm H_2O) or for *R. pipiens* obtained previously (average $40 \pm 1{\cdot}4$ cm H_2O; Shelton & Jones, 1968). The diastolic pressure in the systemic arch was $21{\cdot}7 \pm 2{\cdot}5$ cm H_2O and was significantly different from diastolic pressure recorded in the frogs described in section 1 (average 28 ± 1 cm H_2O); but was not significantly different from pulmocutaneous diastolic pressure recorded in these animals (average 25 ± 1 cm H_2O).

After removal of the spiral valve, valves at either the apex or base of the conus were intact and functional. Whichever set of valves remained functional systemic and pulmocutaneous pressure pulses were usually identical (Fig. 5*a*). However, when valves at the apex of the conus were functional, differences in the diastolic pressure fall between the two arches could be induced by periods of artificial ventilation interspersed with long periods of apnoea. This was not the case when only the valves at the base of the conus were functional.

In both cases valve closure occurred early in the cardiac cycle, soon after generation of the peak systolic pressure. Valve closure was often marked by a slight inflexion on the pressure pulse and it was confirmed visually that this truly represented valve closure in a number of animals. Only after closure of the valves at the apex of the conus was any divergence between the pressures in the systemic and pulmocutaneous arches observed. This divergence could take the form of a different rate of fall in pressure in

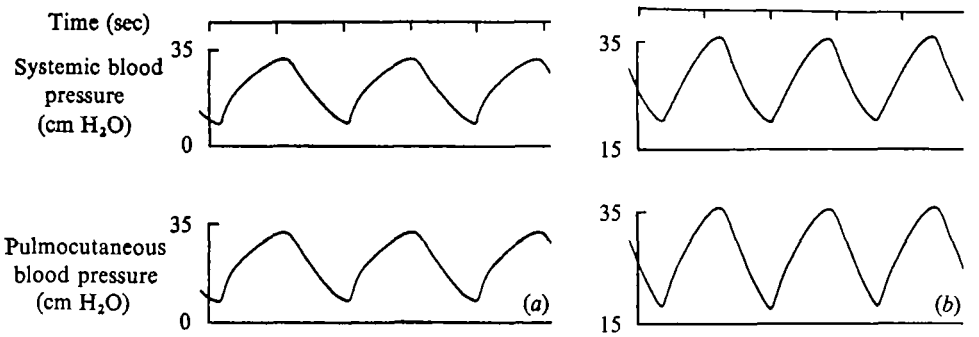


Fig. 5. Pressure pulses in the systemic and pulmocutaneous arches after elimination of the conus as an active contractile element. (*a*) Valves at the apex of the conus destroyed. (*b*) Valves at the apex of the conus functional.

the two arches although ultimately the same minimum diastolic pressure level was obtained, but more often the fall in pressure in the pulmocutaneous arch was faster and minimum diastolic pressure was below that of the systemic arch (Fig. 5*b*). At the next systole the pressure wave first appeared in the pulmocutaneous arch and only after pulmocutaneous pressure had risen to the diastolic level in the systemic arch did the pressure wave appear in the systemic (Fig. 5*b*).

3. *Resistance and compliance of the pulmocutaneous and systemic vascular beds*

The results of the previous section, although suggesting the importance of central cardiovascular structures in maintenance of diastolic pressure (at least in respect of the systemic arch), also indicate that a complete analysis cannot be made in the absence of some measure of the compliance and resistance of the systemic and pulmocutaneous vascular beds.

(*a*) *Indirect measurement of compliance and flow in the carotid, systemic and pulmocutaneous vascular beds*

The occlusion experiments which have already been described suggested to us a means of indirectly determining the product of flow and compliance for the carotid, systemic and pulmocutaneous vascular beds. For instance, if one pair of the arterial arches is occluded then the increase in pressure in the other vascular beds will reflect the ability of these vascular beds to cope with the redirected blood. If either the redirected flow is high or the compliance of the receiving bed is low then the pressure rise will be greater than in the reverse situation.

Occlusion of one pulmocutaneous, systemic or carotid arch caused only a slight increase in systolic blood pressure in the systemic or pulmocutaneous arches even when recording from the occluded vessel. Increase in pressure following occlusion of one carotid arch was only 0·5 cm H_2O and less than that caused by occlusion of one systemic or pulmocutaneous arch (Fig. 6*a*). Occlusion of both carotids caused systolic pressure in the systemic and pulmocutaneous arches to rise by 1 cm H_2O whereas bilateral systemic occlusion caused an increase of 14·5 cm H_2O in both arches and this was about twice as large as the change following bilateral pulmocutaneous occlusion (Fig. 6*a*). Fig. 6(*a*) depicts the average of maximum systolic pressures attained during

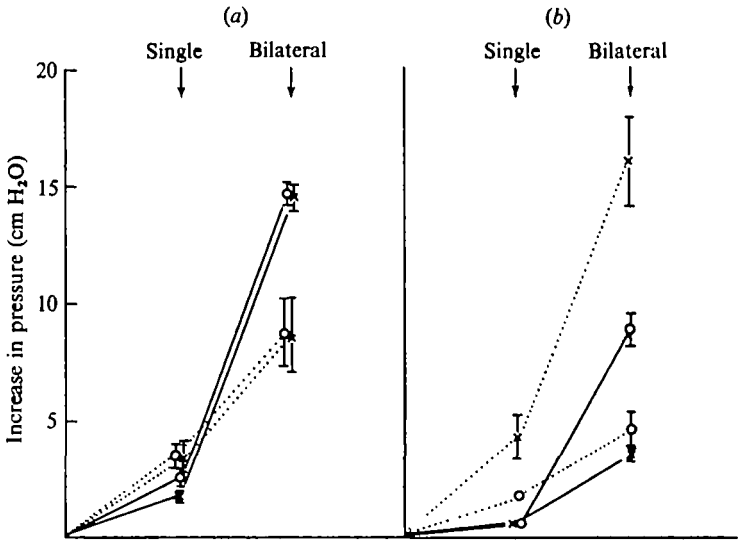


Fig. 6. Change in (*a*) peak systolic pressure, and (*b*) minimum diastolic pressure caused by occlusion of one (1st arrow) and both (2nd arrow) systemic or pulmocutaneous arches. ×, pulmocutaneous pressure; O, systemic pressure; ·····, pulmocutaneous occluded; ——, systemic occluded.

135 occlusions when recording from either the left systemic or left pulmocutaneous arches. The increase in systolic pressure caused by bilateral systemic occlusion was reasonably consistent both from one test to another and from one frog to another; a range of $\pm 2{\cdot}5$ cm H_2O on the mean value shown in Fig. 6(*a*) included 85% of the responses to bilateral systemic occlusion. However, following bilateral pulmocutaneous occlusion increases in systolic pressure were more variable; a range of ± 5 cm H_2O on the mean value shown in Fig. 6(*a*) included only 60% of the total range of values. Furthermore there was a close relationship between the control diastolic pressure in the pulmocutaneous arch and the elevation in peak systolic pressure following bilateral pulmocutaneous occlusion: the lower the control diastolic pressure during any experiment the greater was the increase in systolic pressure on bilateral occlusion.

The responses to bilateral occlusion of the systemic and of the pulmocutaneous arches also differed somewhat in form. Occlusion of both pulmocutaneous arches caused a sudden elevation in systolic pressure followed by a gradual readjustment. Following occlusion of both systemic arches pressure rose more gradually, reaching a maximum after 5–10 heart beats. The peak systolic pressure attained in each case was used in the assessment shown in Fig. 6(*a*).

Bilateral systemic or pulmocutaneous occlusion caused a marked increase in diastolic pressure only when occlusion and recording concerned the same arch. The elevation in diastolic pressure was approximately twice as great in the case of the pulmocutaneous as the systemic arch (Fig. 6*b*). This was not surprising since the systemic arch remains confluent with the carotid arch for most of the cardiac cycle and the latter could act as a drain for blood contained in the blocked systemic arches at the end of systole. The highest diastolic pressure recorded during the period of occlusion was used in the analysis shown in Fig. 6(*b*).

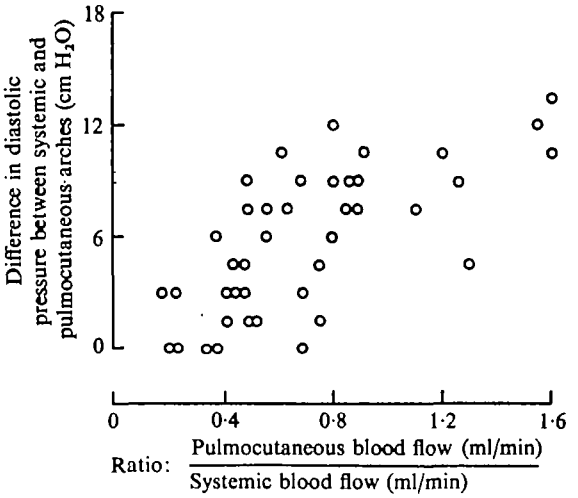


Fig. 7. The relationship between the difference in minimum diastolic pressures in the systemic and pulmocutaneous arches and the ratio of blood flows in these arches.

Only one experiment was successfully completed in which pressures in the systemic and pulmocutaneous arches were measured simultaneously. In this experiment it was noticed that the increase in systolic pressure following bilateral pulmocutaneous occlusion was greater when there was a substantial difference between minimum diastolic pressures in the systemic and pulmocutaneous arches during the control period.

(b) Direct measurement of blood flow in the systemic and pulmocutaneous arches

Simultaneous recordings of blood flow and blood pressure in the pulmocutaneous and systemic arches have only been made from large animals (> 90 g). From frogs smaller than this it has only proved possible to record pressure and flow in either the pulmocutaneous or systemic arches alone. The flow patterns from each arch, however, were of the same form in both large and small animals. The blood-flow pulses in the systemic and pulmocutaneous arches were similar in shape to those reported by Shelton (1970) for *Xenopus laevis*. A more complete description of pressure-flow relationships in *R. pipiens* will be given elsewhere (Jones, in preparation).

Stroke flow to the body side of the circulation was more or less constant during periods of lung ventilation or apnoea, the latter being produced by submersion following light anaesthesia (80 mg/l MS 222;). Peripheral resistance of the systemic circuit varied from 9·16 P.R.U. during submergence to 6·61 P.R.U. during lung ventilation (average of values from all animals). However, stroke flow in the pulmocutaneous arch changed markedly during these procedures. Variations in stroke flow of the order of 400% were not uncommon. Stroke flow in the pulmocutaneous arch was largest following lung ventilation whether effected artificially or by the animal's own efforts. Minimum flows were usually recorded during periods of apnoea between lung ventilation or during submergence. Total peripheral resistance of the pulmocutaneous circuit varied from 20·58 P.R.U. during apnoea to 6·18 P.R.U. just after a period of lung ventilation (average of values from all animals).

Changes in stroke flow in the pulmocutaneous arch were related to changes in diastolic pressure in that arch (Fig. 7). When stroke flow increased, diastolic pressure in the pulmocutaneous arch fell thereby accentuating any difference in diastolic pressure levels between the pulmocutaneous and systemic arches since the latter was largely unaffected by changes in the pulmocutaneous arch. Stroke flows have occasionally been measured in animals in which diastolic pressures in both the pulmocutaneous and systemic arches were identical and, as Fig. 7 shows, stroke flows were always very different when the diastolic pressures in the pulmocutaneous and systemic arches were the same.

DISCUSSION

It is apparent from the present study that stroke flow and diastolic pressure in the pulmocutaneous arch are the major variables in amphibian central cardiovascular dynamics. What remains a matter of speculation is the precise role of the conus arteriosus and spiral valve in contributing to the generation of diastolic pressure differences between the systemic and pulmocutaneous arches. When this pressure difference is large there appears to be no detectable time lag between the appearance of the next systolic pressure wave in both the systemic and pulmocutaneous arches, whereas after removal of the spiral valve and conus arteriosus the systolic pressure wave always appears first in the arch with the lowest diastolic pressure, a similar situation to that seen in *Amphiuma* (Toews, 1971). The spiral valve is not as well developed in urodeles as in anurans but Toews (1971) suggests that it plays a role in occluding the systemic arch until pressures in both the systemic and pulmonary arches are the same. However, the valves at the apex of the conus should be sufficient to achieve this end, as has been confirmed in the present experiments.

For anurans the most reasonable explanation of the simultaneous pressure rise in both systemic and pulmocutaneous arches is that which has already been proposed (Shelton & Jones, 1965*a*): the spiral valve occludes the orifice of the pulmocutaneous arch until pressure in the conus arteriosus has risen to the level of the systemic. The occlusion must be achieved early in diastole since by bilateral occlusion of both systemic arches diastolic pressure differences of the order of 20 cm H_2O have been produced between the systemic (higher) and pulmocutaneous arches. It is difficult to imagine the spiral valve, being free along one edge, maintaining this kind of pressure differential. The occlusion is probably effected by movement of the spiral valve, and active conus contraction must be responsible for occlusion rather than passive movement of the spiral valve in response to a pressure differential, since when pulmocutaneous diastolic pressure is maintained by bilateral pulmocutaneous occlusion a conus component still fails to appear in the pulmocutaneous pulse. However, Shelton & Jones (1965*a*) report the appearance of a conus wave in the pulmocutaneous pulse during prolonged submersion in frogs. The conus component only appears when the systemic and pulmocutaneous pressure pulses are very similar. It may be that the mode of conus contraction is adversely affected by prolonged hypoxaemia, in such a way that movement of the spiral valve is now a passive rather than active function. In the absence of any pressure differential between either side of the conus the arches will not be isolated until late in the cardiac cycle. This supposition would also explain why, apart from the artificial situation of bilateral pulmocutaneous occlusion, the diastolic

level in the pulmocutaneous arch is never higher than that in the systemic, for factors promoting an elevation in the pulmocutaneous diastolic pressure are undoubtedly linked in the normal animal to hypoxaemia.

Simons (1957) considered that the valves at the apex of the conus closed after only 40% of the cardiac cycle. This approximately agrees with the time that closure of the pulmocutaneous arch must occur but not closure of the systemic–carotid arches. The flow pulses recorded from the systemic and pulmocutaneous arches support this interpretation. The systemic flow pulse shows an accentuated second peak associated with conus contraction in the breathing animal whereas diastolic flow in the pulmocutaneous arch is uninterrupted by such a discontinuity (Shelton, 1970; Jones, in preparation). Consequently in the normal animal a portion of the energy of conus contraction, probably the majority, is confined to the systemic and carotid arches. The total energy released by conus contraction is small – at most only 1/20th of that imparted to the blood by ventricular contraction – but after conus removal diastolic pressures in the systemic arch are significantly lower than those in animals with an intact conus.

Obviously, no differences in pulse size can exist between the systemic and pulmocutaneous arch when both the spiral valve and valves at the apex of the conus are removed, since the arches are in contact, by means of low-resistance connexions, throughout the cardiac cycle. When valves at the apex of the conus are functional, differences in minimum diastolic level occur. These differences are never so marked as are recorded in normal animals. The valves at the apex close soon after peak systole allowing the majority of the cardiac cycle for the development of pressure differences. Strictly speaking, early isolation of the arches and not possession of a spiral valve is necessary for this development. In the salamander pressure differences between systemic and pulmocutaneous diastolic pressures never occur even when the ductus Botalli is occluded. The spiral valve is reduced but the arches are not isolated until late in the cardiac cycle (Shelton & Jones, 1968), unlike the situation in *Amphiuma* (Toews, 1971) and in anurans where the pulmocutaneous arch must be occluded soon after the the generation of the peak systolic pressure.

If the energy contribution of the conus to the systemic–carotid vessels is ignored – and this seems justifiable in a simple analysis since differences in diastolic pressure between the systemic and pulmocutaneous arches continue after it is removed – then the amphibian arterial system may be treated as a simple *windkessel* (Frank, 1899). The pressure fall during diastole may then be described by the time constant of the system. The time constant is given by the product of arterial compliance and peripheral resistance. Consequently, to obtain the observed run-off when the diastolic pressures are the same,

$$C_{PC} \times R_{PC} = C_S \times R_S,$$

where C = arterial compliance, R = peripheral resistance, and PC and S refer to the pulmocutaneous and systemic arches respectively.

Both the occlusion experiments and the flow experiments showed that flow in the pulmocutaneous arch is variable and that the difference between stroke flows in the pulmocutaneous and systemic arches was greatest when the pressure pulses were the same. Flow is inversely related to peripheral resistance, so during a period when

diastolic pressures are the same then resistance of the pulmocutaneous circuit must b much higher than that of the body circuit since flow is much lower in the former. So if

$$R_{PC} > R_S \quad \text{then} \quad C_{PC} < C_S.$$

Therefore the elastic reservoir on the pulmocutaneous side of the circuit must be much smaller than on the body side. This seems reasonable since C_{PC} and C_S should be related to the length of elastic vessel involved and this is much greater on the body side of the circulation.

The changes in peripheral resistance of the pulmocutaneous circuit are related to the pattern of lung ventilation. Periods of low resistance are usually associated with lung ventilation or the start of a period of apnoea. During prolonged apnoea the pressure pulses in the systemic and pulmocutaneous arches tend to become identical and peripheral resistance of the lung circuit increases. Shelton (1970) has already discussed the functional significance of these changes to the whole animal. The changes in resistance of the pulmocutaneous circuit are independent of changes in systolic pressure and are not affected by an elevation in venous pressure. Some of the factors influencing pulmonary resistance have been examined (Emilio & Shelton, 1972). A study of the regulation of pulmonary vasomotor changes in relation to the normal diving behaviour of an amphibian should be of great interest.

SUMMARY

1. During rhythmic lung ventilation systolic blood pressures in the pulmocutaneous and systemic arches were more or less the same although diastolic pressures in the former were some 3–4 cm H_2O lower than in the latter. During prolonged apnoea (20–35 min) the pulse pressures became identical. Venous pressures in both lung and body circuits were little affected by these procedures.

2. Raising or reducing systolic pressure by application of drugs to the exposed ventricle caused no change in the relationships of the pressure pulses in the systemic and pulmocutaneous arches.

3. Removal of the conus arteriosus and spiral valve caused a significant reduction in diastolic pressure in the systemic but not in the pulmocutaneous arch.

4. After conus removal differences in diastolic pressures in the systemic and pulmocutaneous arches were only recorded when valves at the apex of the conus were functional.

5. Bilateral occlusion of both systemic arches produced a greater increase in systolic blood pressure in the systemic and pulmocutaneous arches than bilateral occlusion of the pulmocutaneous arches, although pressure changes following the latter were more variable.

6. Blood flow in the pulmocutaneous arch was extremely variable, lowest stroke flows being recorded when the pressure pulses in the systemic and pulmocutaneous arches were identical.

7. The role of the conus arteriosus, spiral valve, arterial compliance and peripheral resistance in maintenance of diastolic pressures in the systemic and pulmocutaneous arches is discussed.

D. R. Jones wishes to thank the National Research Council of Canada for financial support of part of this work.

REFERENCES

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

FRANK, O. (1899). Grundform des arteriellen Pulses. *Z. Biol.* **37**, 483.

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

HOLMES, S. J. (1927). *The Biology of the Frog*, 4th ed. New York: Macmillan.

JOHANSEN, K. (1963). Cardiovascular dynamics in the amphibian *Amphiuma tridactylum*. *Acta physiol. scand.* **60**, suppl. 217.

JONES, D. R. (1970). Experiments on amphibian respiratory and circulatory systems. *Exp. Physiol. Biochem.* **3**, 233–93.

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

SHELTON, G. & JONES, D. R. (1965*b*). 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 pressures in five amphibians. *J. exp. Biol.* **49**, 631–43.

SIMONS, J. R. (1957). The blood pressure and the pressure pulses in the arterial arches of the frog and the toad. *J. Physiol., Lond.* **137**, 12–21.

TOEWS, D. P. (1971). A mechanism for the selective distribution of blood in the amphibia. *Can. J. Zool.* **49**, 957–959.