

FUNCTION OF THE ANURAN CONUS ARTERIOSUS

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(Received 26 February 1974)

SUMMARY

1. The anuran conus arteriosus was studied by direct observation of its structure and function and by observing the effects of artificially induced contraction on the pressure pulse in the arterial arches.

2. The beat of the anuran conus was found to close the outlet to the pulmocutaneous arches. The timing of the beat of the conus in the ventricular cycle is variable; early in the cycle the conus admits less blood to the pulmocutaneous circuit, more blood being admitted when it beats late.

3. The timing of the beat of the conus appears to be adaptively related to beat frequency and to pH of the system. Elementary calculations suggest that these characteristics constitute an important measure of survival value of the anuran conus.

4. The findings of this work are compatible with the theory of selective passage of two streams through the heart, supplementing such passage with additional control of blood volumes entering the pulmocutaneous circuit.

INTRODUCTION

In studies of the function of the amphibian heart there seems to have been a pre-occupation with the selective routing of the two streams of venous return through the atria to the arterial system. The classical theory of this selective distribution is ascribed to Brücke and Sabatier (see deGraaf, 1957).

Several investigators have demonstrated selective distribution of blood in the central circulation of amphibia. Johansen (1963) demonstrated that the oxygen content of blood entering the pulmocutaneous arches in *Amphiuma tridactylum* was slightly greater than that of blood in the sinus venosus. Hence the blood in the pulmocutaneous arches must have been, to some degree, a mixture of that from the two atria, despite a well-developed conus arteriosus and spiral valve.

An imaginative variety of foreign materials has been injected into the blood stream to study flow through the heart, despite the possibility that natural cardiac function and flow patterns may be disturbed (see Johansen & Hanson, 1968). Johansen (1963) demonstrated that selective distribution was 'labile'; 'selective passage could easily be disturbed or abolished'.

Noble (1925) discussed numerous aspects of amphibian evolution and called attention to the fact that the best evidence indicates that cutaneous surfaces for respiratory

exchange, with the necessary circulatory modifications, are of more recent origin than the lungs. The cutaneous drainage enters the right atrium by way of the sinus venosus in all amphibians (Foxon, 1964*a*). During such activities or periods in which the cutaneous system might provide an important source of oxygen, it would seem to be of dubious selective advantage for the oxygen-rich cutaneous return to be sent back into the pulmocutaneous circuit on the succeeding cardiac cycle.

Simons & Michaelis (1953) discussed the possibility that selective advantage might be provided by the amphibian heart not because of the degree to which it could simulate function of that of a mammal, but rather because it offered a unique potentiality for sending more or less blood to respiratory surfaces, as the situation or system might demand. Foxon (1964*a*) credits Jordan (1929) with having initiated this line of thinking, laying great stress on the probable importance of cutaneous respiration to the success of modern Amphibia.

Diastolic pressure in the pulmocutaneous arch is considerably lower than that in the systemic or carotid (deGraaf, 1957). This finding seems to have been in dispute for some years (Simons, 1957; Haberich, 1965) but it was abundantly confirmed by Shelton & Jones (1965*a*, 1968). In a large number of measurements, Shelton & Jones (1965*a*) found that appearance of the pulse was simultaneous in the two arches. DeGraaf observed that the pressure pulse of the systemic arch includes a discontinuity resulting from the conus beat and he thought that a minor discontinuity observed in the pulmocutaneous pulse was also due to conus contraction. However, the work of Shelton and Jones (1965*a*) showed that the discontinuity in the pulmocutaneous is due to arterial recoil.

Shelton (1970) and Jones & Shelton (1972), using techniques directly measuring flow simultaneously in both arches, demonstrated beyond question that anuran circulation is adapted to function in the manner postulated by Jordan (1929).

Although the work of Jones & Shelton (1972) clearly shows that the relative volume of blood entering the pulmocutaneous is directly related to the difference in diastolic pressure between the systemic and pulmocutaneous arches (the pulmocutaneous having the lower), variance of their data makes it clear that at least one other factor must be involved. Those authors consider this factor to be resistance. Unlike deGraaf (1957), who concluded that the low diastolic pressure in the pulmocutaneous reflects a lower peripheral resistance, Jones & Shelton (1972) considered it likely that the conus affects the system, causing the diastolic pressure difference between the two circuits.

By bilaterally occluding either the systemic or the pulmocutaneous arches, generating very high pressure in the system, Jones & Shelton (1972) proved that the conus increment in the pulse wave of the systemic can be taken as an indicator that the systemic arches are isolated from the pulmocutaneous arches early in diastole. In going to the cavum pulmocutaneum from the ventricle, blood must first pass through the cavum aorticum. Hence, if the conus isolates the pulmocutaneous arches from the systemics, it must also isolate the pulmocutaneous from the ventricle.

The present work was undertaken to investigate the possibility that the conus might serve as a variable shunt.

METHODS

Two anurans, the grass frog, *Rana pipiens*, and the cane toad, *Bufo marinus*, were studied. The frogs, numbering about 120, ranged in weight from 43 to 158 g. About 40 toads were used and each weighed about 300 g. All specimens were obtained from commercial dealers. The toads were used in other research which involved decapitation and removal of their urinary bladders; although unsuitable for much of the work, they proved admirable for obtaining isolated heart preparations.

The frogs were deeply anaesthetized by immersion in a 0.1 % solution of MS 222 (Sandoz). Upon removal of the frog from the anaesthetic, the skin, body wall and sternal elements were opened along the midline, from the episternal cartilage to the posterior margin of the xiphoid. The frog was placed on its back with its head and anterior trunk resting on a metal tray which spanned an enamelware pan. The two cut edges of the pectoral girdle were retracted with small hooks and wires. The frog was held loosely in position and water was poured in the pan up to the level of the tray (about 1½ in.).

In the studies of blood pressure the pulse was picked up with a no. 25 hypodermic needle which had been cut from its base and sealed with wax (Vinyl-base red utility wax; ADAK Electronics, 988 Baxter Street, Eugene, Oregon) in the end of a 25 cm length of no. 50 polyethylene catheter tubing. The needles were set manually with forceps in the arterial arches. The tubing led to the reservoir of a pressure transducer (P23AC; Statham Laboratories, Hato Rey, Puerto Rico). The needle, line and reservoir were filled with Ringer to which Heparin sodium had been added, 1 mg/ml. Two of these systems were used and their signals were amplified and recorded with a two channel pen-recorder (Grass Instruments, Quincy, Massachusetts).

It was determined that each of the two complete systems could follow a piston-driven, sine-wave pressure-pulse with the amplitude being down by 2.8 % at 3 Hz and 8.3 % at 6 Hz. After each experiment the system was statically calibrated against a water column with the particular configuration of instrument settings used.

Data have been rounded to the nearest 0.5 torr, except for the statistically treated data in Table 1.

In e.c.g. studies, signals were picked up with pairs of electrodes made of 40 mm lengths of varnished platinum wire (diameter, 0.006 in.). The electrodes were placed either alongside the ventricle or directly on the conus. The signal was filtered and amplified and studied on a storage oscilloscope. In some cases the signals were fed into a channel of the Grass recorder through an a.c. pre-amplifier.

Changes in beat frequency were induced by changes in temperature. The frog was initially bathed with water at 10 °C, which was then warmed over a period of 10–20 min to 25 °C. The original heart rate was restored by substituting crushed ice for water to bring the temperature back to 10 °C. Following such a protocol the effects of temperature upon the rate could not be confounded with levels of anaesthesia or oxygen need.

On occasions a stimulator (Model S 9; Grass Instruments) was used to induce early contractions of the conus. In these studies single-shock, threshold-strength (about 5 V) stimuli of 50 msec duration were delivered to the central wall of the conus through flexible platinum electrodes which rode with the movement of the conus.

The event marker indicated while the key was actuated, so in the figures the left hand margin of the event mark indicates the time at which a stimulus began.

In a study of the effects of pH on the pressure relationships and e.c.g., 12 frogs, 68–109 g, were used. Three ml of Ringer were injected into the dorsal lymph sac of each and the frog was left undisturbed in an empty jar at room temperature for 20 min and then anaesthetized. Five frogs received Ringer which had been buffered to pH 6.5 and seven received Ringer with a pH of 7.5.

Function of excised toad hearts was studied under a binocular microscope by transmitted light with the heart bathed in cold Ringer.

Toad hearts were excised, dissected as necessary, and pinned in a wax-bottom pan. Using appropriately placed steel hooks leading by threads to strain-gauges (Model FTO₃C; Grass Instruments), records were made of the pull of ventricular muscles, and longitudinal and circular conus muscles. The signals from the strain-gauges were amplified and recorded with instruments mentioned earlier. The beat frequency was controlled by the temperature of the Ringer bathing the preparation.

Histological material was fixed in Bouin and, following procedures which in essentials are those of Pantin (1946), stained either with Mallory's triple stain or with Heidenhain's iron haematoxylin.

Composition of the amphibian Ringer was taken from Hoar & Hickman (1967). Shortly before use the solution was buffered as necessary with dibasic or monobasic sodium phosphate.

These studies were carried out during the Summer and Autumn of 1973.

RESULTS

Anatomy

Anatomical terminology used in this work is largely from deGraaf (1957), Sharma (1957) and Shelton & Jones (1965*a*). The literature is rich in synonymy and the reader is referred to the schematic illustration in Fig. 1 to make present usages clear.

Although the anatomy of anuran hearts had been exhaustively studied in histological detail, a search was made for tracts of smooth muscle in the conus that might have been overlooked by earlier investigators. The spiral valve is considered to have evolved from the right member of the four embryonic endocardial ridges (Goodrich, 1930). The arterial trunks are heavily banded with smooth muscle right to their junctions with the conus and it was considered possible that smooth muscle might have attended the evolution of the spiral valve. No smooth muscle was found in the conus arteriosus.

The inner, circular, layer of cardiac muscle is continuous, from the ventricular connexion with the conus, anteriorly to the bases of the arterial trunks. However, the outer, longitudinal layer, is continuous around the conus only near its junction with the ventricle, and as one progresses anteriorly it is confined for the most part to the wall of the cavum aorticum (see Figs. 1, 2(*a*) and (*b*)).

The large, gelatinous, pad-like valve discovered by Sharma (1957) bears across the posterior reaches of the free margin of the spiral valve and with only mild contraction of the circular muscle occludes what would otherwise be a broad passage between the cavum aorticum and cavum pulmocutaneum (Fig. 1).

On the anatomical evidence it is clear that the cavum pulmocutaneum could be iso-

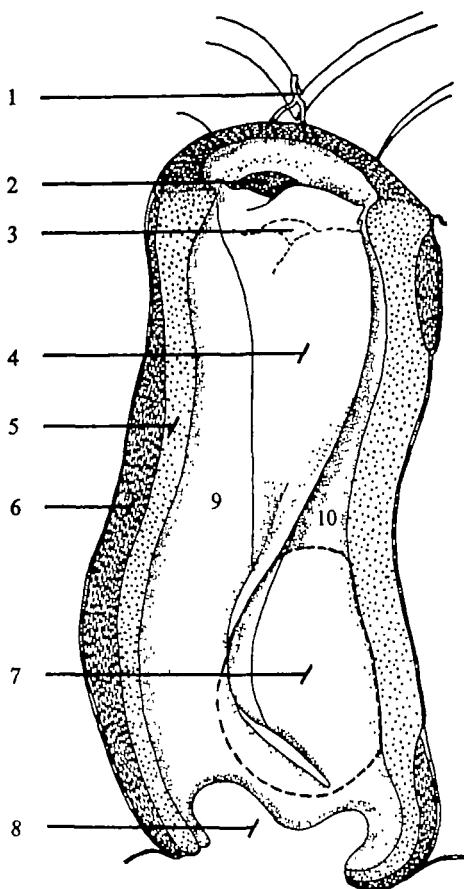


Fig. 1. Semi-diagrammatic ventral view of the anuran conus in frontal section with anterior musculature removed. 1, Coronary artery; 2, outlet to the systemic-carotid circuits; 3, position of the outlet of the pulmocutaneous circuit; 4, spiral valve (fixed margin top and reader's left, free margin at reader's right); 5, circular muscle; 6, longitudinal muscle; 7, position of pad-like valve (in heavy, interrupted outline) on ventrolateral wall, removed; 8, ventral pylangial valve; 9, cavum aorticum; 10, cavum pulmocutaneum.

lated from the rest of the system by contraction of the circular muscles. By shortening the conus, effecting a greater curvature in the spiral valve, it is apparent that the longitudinal muscle could at least narrow the cavum pulmocutaneum.

Since no smooth muscle was found, there appeared to be no mechanism for modulating the general position of the spiral valve. The nature of the cardiac muscles and their patterns of distribution indicated that if the conus were to serve in modulating proportional distribution of blood between the two tracts, it would have to be on the basis of either changes in general tonus or timing of its beat. It was considered quite possible that both of these parameters might be related to beat frequency. A change in beat frequency would be an automatic concomitant of a change in oxygen demand of the system, at least under certain conditions, e.g. with a change in temperature. Therefore it was decided to seek evidence for modulation of distribution that might be related to beat frequency as it is induced by heat.

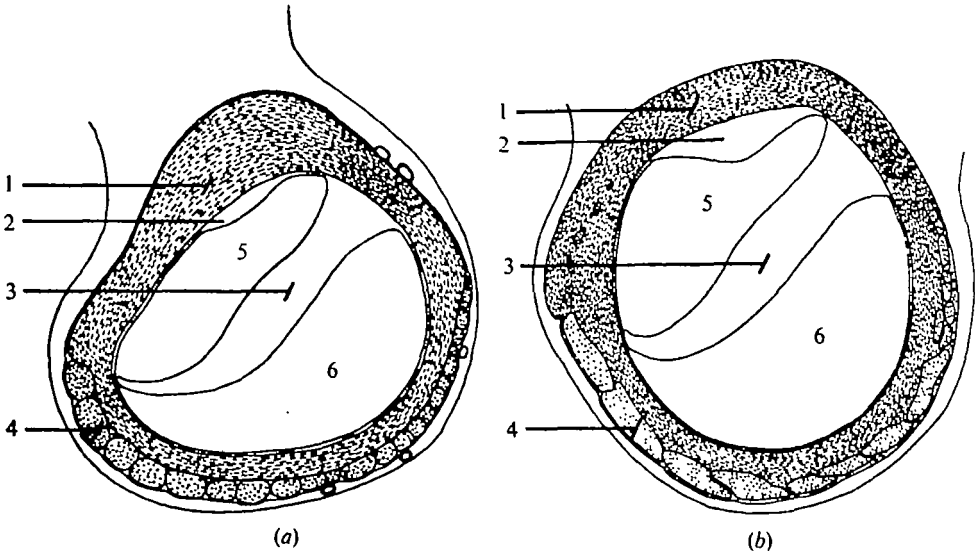


Fig. 2. Cross-section of the middle region of the conus arteriosus. (a) *Bufo marinus*: 1, circular muscle; 2, base of lateral synangial valve; 3, spiral valve; 4, longitudinal muscle; 5, cavum pulmocutaneum; 6, cavum aorticum. (b) *Rana pipiens*: labels as in (a).

Table 1. Data from deeply anaesthetized frogs (pressures in torr)

| | Systemic | Pulmocutaneous | P of difference |
|------------------------------|-----------|----------------|-----------------|
| N . . . | 11 | 12 | — |
| Range of rates (beats/min) | 39.8–60.0 | 31.2–63.0 | — |
| Mean rate | 47.2 | 46.5 | — |
| S.E. of mean rate | 1.78 | 2.73 | — |
| Range of systolic pressures | 21.2–42.4 | 21.4–38.8 | — |
| Range of diastolic pressures | 14.3–32.8 | 8.7–21.4 | — |
| Range of pulse pressures | 3.5–13.1 | 7.9–15.4 | — |
| Mean systolic pressure | 29.03 | 27.83 | > 0.50 |
| S.E. of mean systole | 1.99 | 1.40 | — |
| Mean diastolic pressure | 21.48 | 16.89 | < 0.05 |
| S.E. of mean diastole | 1.50 | 1.30 | — |
| Mean pulse pressure | 7.55 | 10.93 | < 0.02 |
| S.E. of mean pulse pressure | 0.76 | 0.92 | — |

Blood pressures in deeply anaesthetized animals

In testing adequacy of the experimental protocol and studying contours of the pressure pulses, 23 frogs were used. These animals were deeply anaesthetized, one measuring needle was inserted into either the left systemic or the left pulmocutaneous arch and the second measuring needle monitored pressure in the ventricular cavity. Some data from these animals are shown in Table 1. These data are taken from the strip charts during the first 3 or 4 min after setting the needles. The temperature of these preparations was about 20 °C.

Each of the two sets of data was subjected to regression analyses, individually treating systolic, diastolic, and pulse pressure as a function of beat frequency. All of the coefficients were small and none was statistically significant. The beat frequencies were uncontrolled and with only a few exceptions they covered a narrow range (note S.E.).

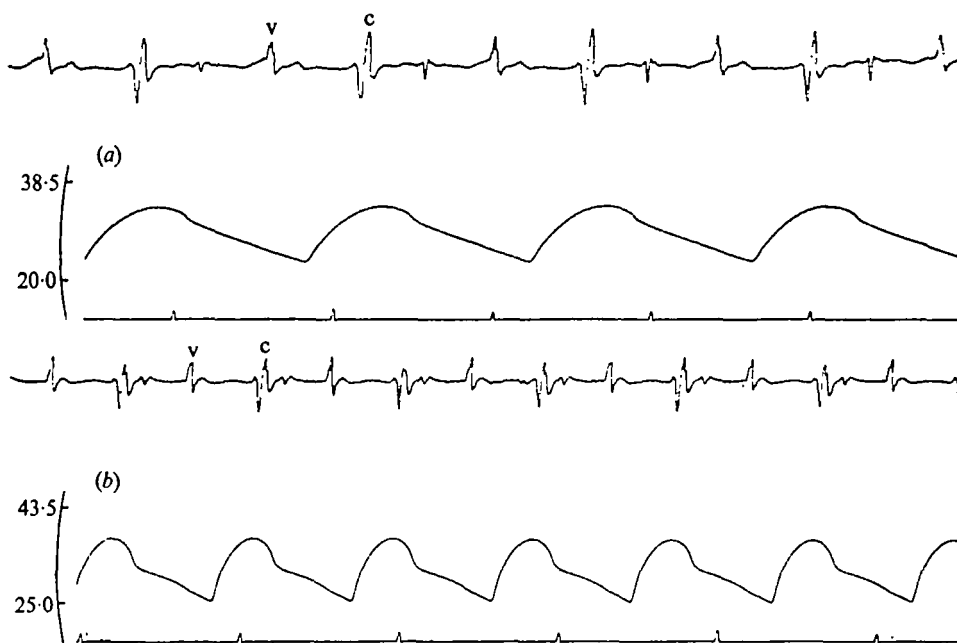


Fig. 3. Electrical and pulse pressure records, the latter from the left systemic arch. Frog treated with Ringer, pH 6.5. Electrical record, top trace of (a) and (b); v = ventricular depolarization, c = conus depolarization. Pulse pressure record, middle trace of (a) and (b); ordinates in torr. Time record, bottom trace in (a) and (b), in seconds. (a) Beat frequency = 43/min; systolic pressure = 34.0 torr; pulse pressure = 10.5 torr. (b) Beat frequency = 68/min; systolic pressure = 37.5 torr; pulse pressure = 12.0 torr.

Studies of the e.c.g. and pressure pulse

Brady (1964) states that 'large delays in conduction occur between the beat of the ...ventricle and the bulbus cordis'. Johansen (1963) ascribed a general depulsing effect of the conus in the amphibian heart. However, this is not the case in some species (Foxon, 1964*b*). Whether or not the beat of the conus results in depulsation would seem to depend upon variation in volume and compliance of the conus, its contractility, or in the duration of the conductance interval between ventricle and conus.

The e.c.g. was studied in several frogs over a range of beat frequencies from about 20/min to 70/min. As would be expected, duration of the period between discharge of the conus and discharge of the atrium of the next beat varied inversely with frequency, reflecting the duration of diastasis. The proportionality between conductance intervals, between ventricle and conus (v-c) and between discharge of the atrium and of the conus (a-c), was studied in several frogs. In a given specimen the proportionality between these two periods did not vary greatly regardless of beat frequency. However, at comparable beat frequencies the proportions were different in different frogs. Among ten such measurements, the ratio v-c/a-c ranged from 0.52 to 0.64 with a mean of 0.57. In a few specimens, a study of electrical cardiac events was made simultaneously with the pressure record in the ventricle or systemic arch. Upon inducing beat frequency changes with heat it was seen that, with an increase in rate, ventricular contraction velocity is affected relatively more than is ventricle-conus conduction

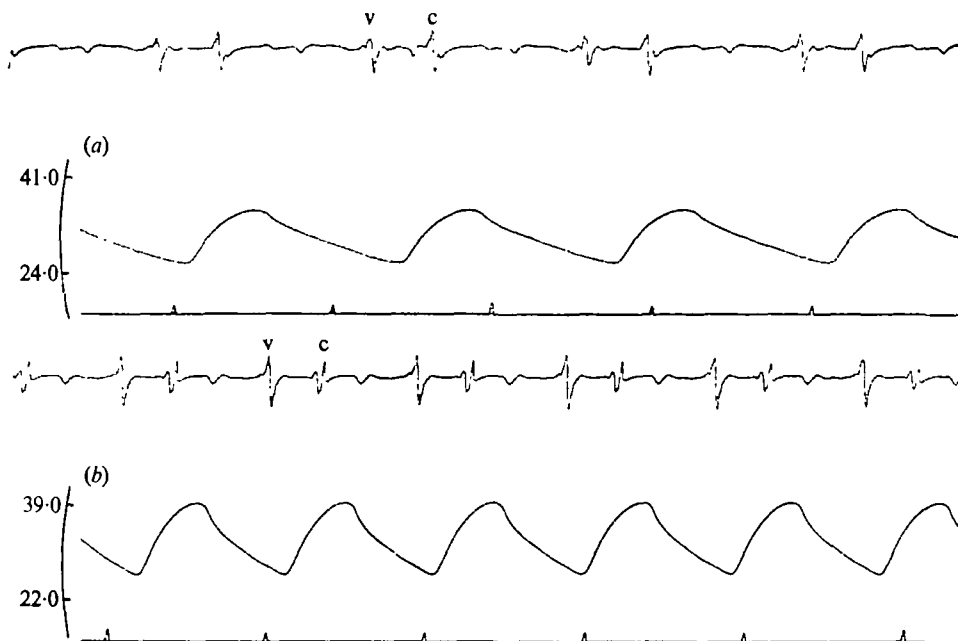


Fig. 4. Electrical and pulse pressure records, latter from the left systemic arch. Frog treated with Ringer, pH 7.5. Display and ordinates as in Fig. 3. (a) Beat frequency = 44/min; systolic pressure = 35.5 torr; pulse pressure = 9.0 torr. (b) Beat frequency = 64/min; systolic pressure = 39.5 torr; pulse pressure = 12.5 torr.

velocity. Hence, with the peak pressure as a relative reference, depolarization of the conus falls later in the cycle as rate increases.

Since the experimental protocol was fixed, the cause of differences in timing of events in the e.c.g. among the frogs was not clear. While the frogs were in the anaesthetic solution (about 30 min) some of them splashed around a lot whereas others were quite composed. These behavioural differences could lead to differences in pH of the system. For this reason, the effects of injecting either acidic or alkaline Ringer were studied.

In all five frogs with acidified systems, depolarization of the conus occurred after the peak of pressure, by the time the beat frequency had reached 60/min. Fig. 3 shows conus depolarization and pressure peak relationships at two rates in an acidified system. Very similar results were seen in all five frogs.

In frogs that had received the alkaline Ringer, increasing rate was accompanied by a less pronounced shift in relative position of conus depolarization, and in none of them did the conus discharge later than the pressure peak, even when the rate was driven well beyond 65/min (Figs. 4, 5).

In comparing Fig. 3 with Fig. 4 we see that with increased temperature and beat frequency there is a substantial increase in systolic pressure in both acidic and alkaline systems. It would appear that a major reason for this increase is the increase in ventricular contraction velocity which expresses itself as a reduction in the pressure rise-time. This factor results in a temporal advance in the pressure peak with respect to the occurrence of the conus discharge and it manifests itself more strongly in the

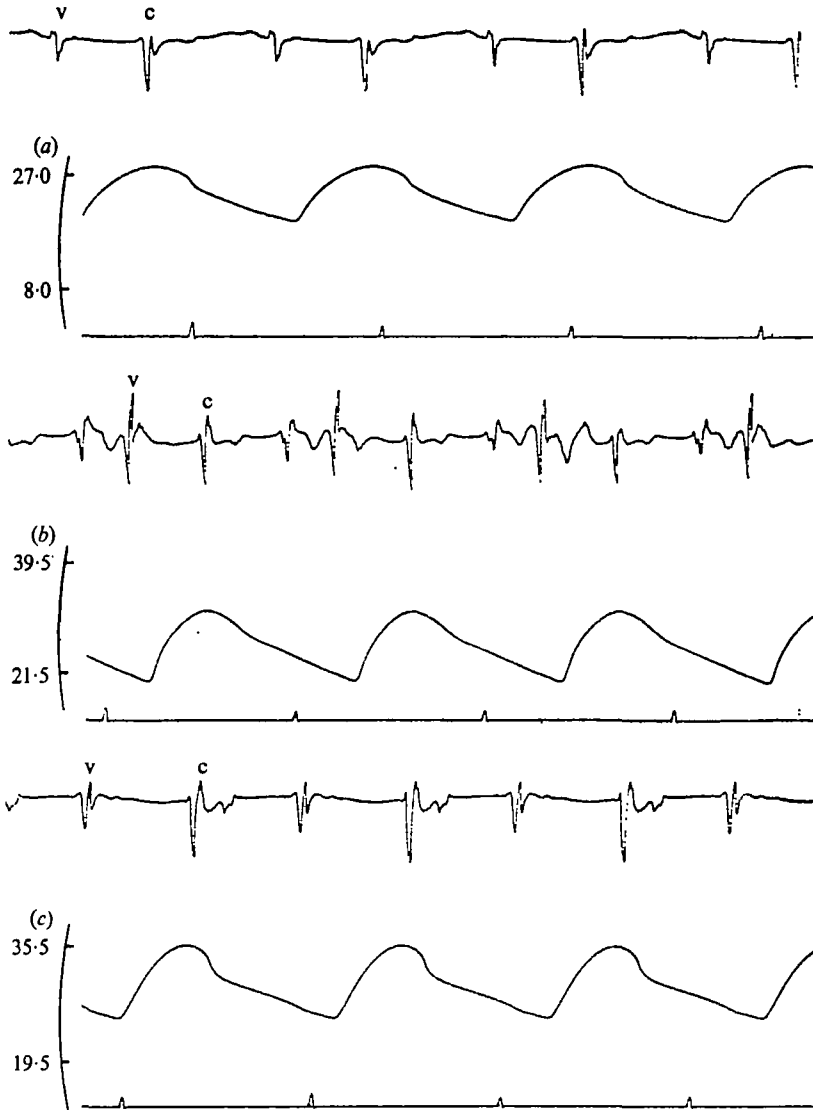


Fig. 5. Records of electrical events and systemic pulse pressures from three different frogs. Display and ordinates as in Fig. 3. (a) Frog treated with Ringer, pH 7.5; systole = 28.5 torr; pulse pressure = 9.0 torr; beat frequency = 53/min. (b) Frog treated with Ringer, pH 7.5; systole = 32.0 torr; pulse pressure = 11.5 torr; beat frequency = 56/min. (c) Frog treated with Ringer, pH 6.5; systole = 35.5 torr; pulse pressure = 10.0 torr; beat frequency = 53/min.

acidic system than in the alkaline (note Figs. 3(b) and 4(b)). If we compare Figs. 5(a) and (c), representing alkaline and acidic systems respectively, and which have cardiac cycles of almost precisely the same duration (1150 msec), we see that the interval between discharge of the ventricle and conus is conspicuously greater in the acidic system.

Responses observed in the alkaline system could be simulated by bubbling the anaesthetic solution vigorously with oxygen during the period the animal was in it.

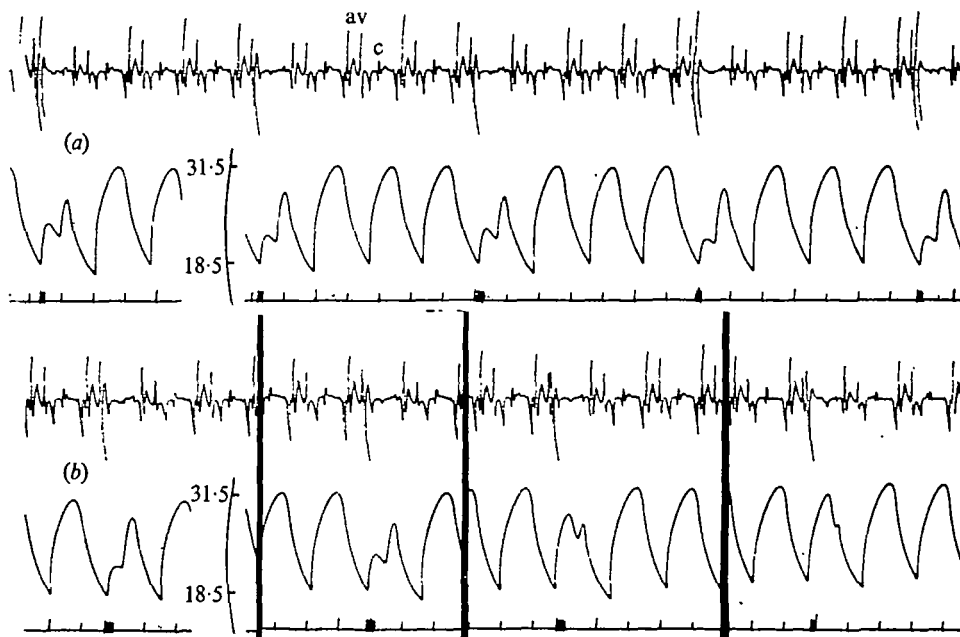


Fig. 6. Pulmocutaneous pressure records and e.c.g. to show effect of induced contraction of the conus. In both (a) and (b), top trace, e.c.g., a = atrial depolarization; v = ventricular depolarization; c = conus depolarization. Time in seconds and stimulation marks, bottom trace of both records; left margin of stimulation mark indicates time at which stimulus was given. Pressure ordinates in torr. Systole = 31.5, pulse pressure = 13.0, torr. (a) Conus stimulated at approximately the same time as spontaneous ventricular depolarization. (b) Conus stimulated at different times in the cardiac cycle. These four traces are from the same specimen represented in (a).

However, administering carbon dioxide during anaesthesia, forcing activity prior to and during anaesthesia, or injecting Ringer containing different concentrations of lactic acid, failed to simulate the effect of acidified Ringer.

Effect of conus contraction on the pressure pulse

In studies of the e.c.g. it was learned that the conus repolarized shortly after depolarization of the atrium. Hence, it appeared possible that one could artificially trigger the conus to beat earlier than it would spontaneously do so. If the conus were to modulate distribution of flow, its premature beat should cause an easily interpreted distortion of the pulse. Fig. 6(a) shows the effect of conus contraction on the pulmocutaneous pulse when stimulated at or shortly after depolarization of the ventricle. Four examples of contraction of the conus, induced at different times up to about that at which it would have contracted spontaneously, appear in Fig. 6(b).

The beat of the conus serves to shut off pulmocutaneous circulation, holding the outlet shut for approximately 20–25 % of the cardiac cycle, even against rising pressure of ventricular systole. Of particular note is the greater diastolic run-out following early contraction, reflecting the smaller volume admitted to the arch during that cycle. The stimulus artifact appears on the e.c.g. and gives a clearer record of the time of stimulation than does the signal marker.

In a similar experiment done on the systemic arch (Fig. 7a) the second stimulus

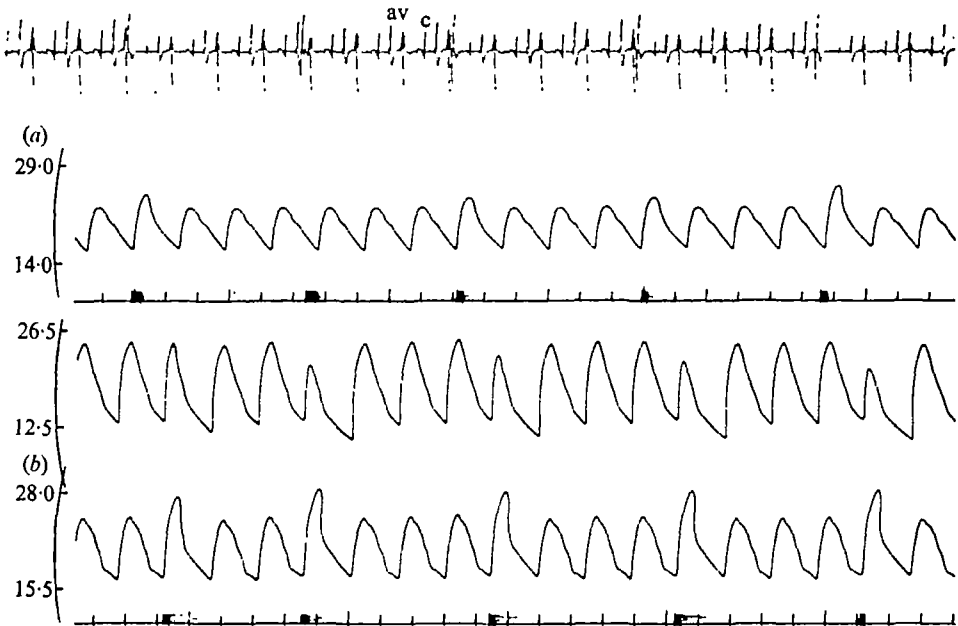


Fig. 7. (a) Pulse pressure record from the left systemic arch showing effect of induced contraction of the conus. Pressure ordinate, e.c.g., time line, and signal marks as in Fig. 6. Systole = 22.5 torr, pulse pressure = 6.0 torr. (b) Simultaneous pulse pressure records from pulmocutaneous arch, top trace (pulse pressure = 11.5 torr); systemic arch, bottom trace (pulse pressure = 8.0 torr). Systolic pressure = 24.5 torr. Time line, signal marks, and pressure ordinates as in (a).

was timed before the conus had repolarized. The four effective stimuli diminished the amplitude of the natural conus discharge but they did not obliterate it. The amplitude of the remaining natural discharge is inversely related to the amplitude of the systolic pressure in that cycle, indicating that the artificial stimulus was not sufficient to trigger all of the musculature. The several bundles of longitudinal muscle are separated from one another and from the circular muscle by connective tissue (Fig. 2), and it appears that such a threshold stimulus as was artificially used did not always spread to all of the bundles. (Increasing the stimulus voltage increases the danger of spread of the stimulus to the atria which billow up on each side of the conus.) Upon early contraction, the thrust of the conus is superimposed on ventricular pressure giving a greater systolic pressure. Knowing what must have happened on the pulmocutaneous side we can deduce that this increased pressure pulse implies a greater volume entering the systemic arch. In both circuits, immediate recovery of the normal pressure pulse on the next succeeding cycle indicates that in spite of the fact that contraction of the conus may have narrowed the cavum aorticum when it was triggered early, the ventricle emptied normally. Unlike the condition in the pulmocutaneous, the systemic pulse shows no great distortion of diastolic level accompanying early contraction of the conus.

Simultaneous measurements of systemic and pulmocutaneous pressures are shown in Fig. 7(b). As a result of early conus contraction there was a dramatic drop (as much as 2.75 torr) in diastolic pressure in the pulmocutaneous arch. Even in the next cycle,

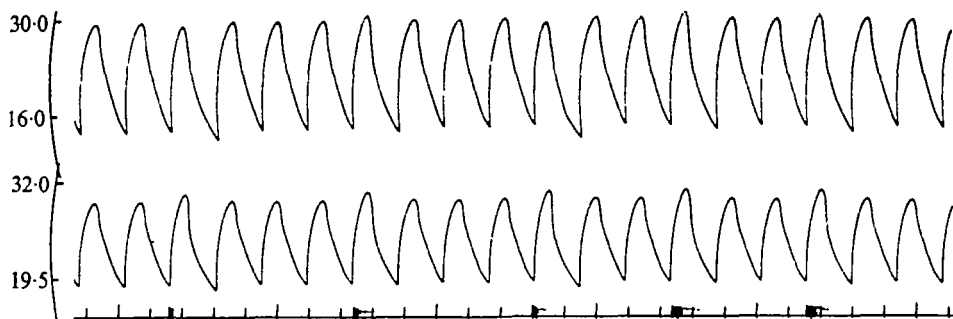


Fig. 8. Pulse pressure records in the pulmocutaneous arch, top trace, and the systemic arch, bottom trace, after fatigue of longitudinal conus muscle. Time line, signal marks, and pressure ordinates as in Fig. 6.

normal pressure relations have not quite been re-established. In the systemic there is no reciprocal upset of diastolic pressure relations during or following the cycle in which the stimulus was given. Without an e.c.g. we cannot determine precisely where in the electrical cycle the conus was being fired, but directly above the event mark it is seen that diastole was not quite complete when the stimuli were usually given, and that diastolic pressure did not reach a normal level as a result. This raises the possibility that the stimulus could have reached and fired the ventricle before it was entirely filled. However, if the trace did represent premature ventricular contraction, then the next natural pulse would necessarily be enlarged, but we see that this is not the case. The appearance of the trace is due to the conus being stimulated to contract just slightly in advance of the ventricle.

In the work concerning induction of early conus contraction, several experiments were terminated when it was seen that the conus was to some degree fatigued as a result of prolonged artificial stimulation. Fig. 8 shows a chart from the same experiment as in Fig. 7(b), but after about 30 min further stimulation (10–15/min). Due to fatigue the beat of the conus was now too weak to close the pulmocutaneous. Since the artificial stimulus brought the weakened beat of the conus more closely into phase with the ventricular beat, there was occasionally a slight elevation in the pulmocutaneous peak resulting from the weak conus contraction.

If the exhausted conus was not closing when artificially stimulated, it seems reasonable to assume that it was not closing in the course of a natural, unstimulated cycle. In the unstimulated cycles as they appear in Fig. 7(b) there are three distinct discontinuities in systemic diastole and one, only vaguely defined, in the pulmocutaneous. The discontinuity during pulmocutaneous diastole apparently results from arterial recoil. In the order in which they appear during systemic diastole, the discontinuities appear to result from: longitudinal conus contraction, arterial recoil, and circular conus contraction. Circular conus contraction does not always manifest itself as clearly as it does in this figure. After the conus is fatigued (Fig. 8) we see an almost complete absence of discontinuities during diastole in natural, unstimulated pulses of both arches. However, onset of systole is still simultaneous in the two circuits, so at least some part of the conus musculature is still able to contract and close the cava late in diastole.

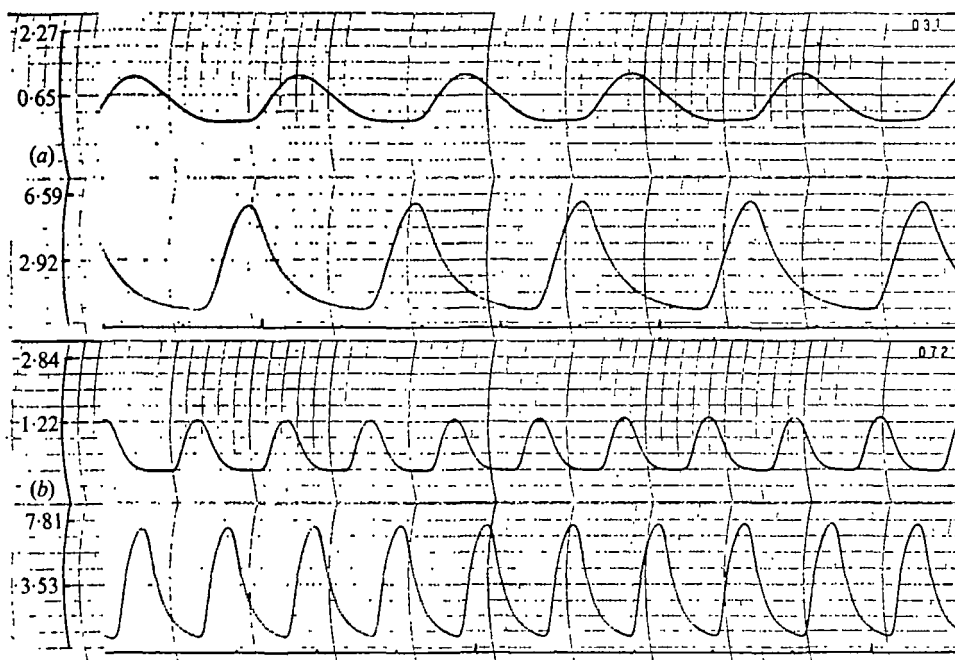


Fig. 9. Strain-gauge records of contractions of conus and ventricular muscles. Preparation was bathed in Ringer, pH 6.5. Time line in seconds, bottom trace in both (a) and (b). Ordinates in g, weight, zeroed at diastolic level. (a) Beat frequency = 28/min. Top trace, longitudinal contraction of the conus; second trace, contraction of the ventricle. (b) Beat frequency = 55/min. Top trace and second trace, same as in (a).

Isolated heart preparations

Studies of the e.c.g. suggested that with a change in beat frequency there was probably a change in temporal relationship between the contraction of the ventricle and that of the conus. Occurring as it does near the peak of ventricular systole, a slight shift in time of the beat of the conus would serve to shunt more, or less, blood into the pulmocutaneous arches. Hence, a lag in the time of discharge of the conus would permit more blood to enter the pulmocutaneous than would otherwise be the case. If, however, such a lag were accompanied by an increase in contraction velocity, then the apparent advantage of the change in timing of discharge might be negated.

In studying function of the heart simply by observing blood pressure relations it was impossible to make a clear distinction between ventricular and conus contributions since the two overlapped so extensively. Using the isolated toad heart preparations it was not only possible to discriminate between ventricular and conus contractions but also between contractions of the longitudinal and circular muscles as well.

Some results from one toad heart experiment appear in Fig. 9. Here we see with a beat frequency of 28/min, and the cardiac cycle spanning more than 2000 msec, that longitudinal contraction of the conus starts about 100 msec after the peak of ventricular contraction. Fig. 9(b), a record taken when the beat frequency had been increased by heat to 56/min and with an entire cardiac cycle spanning only approximately 370 msec, shows initiation of contraction of the conus to be lagging behind the

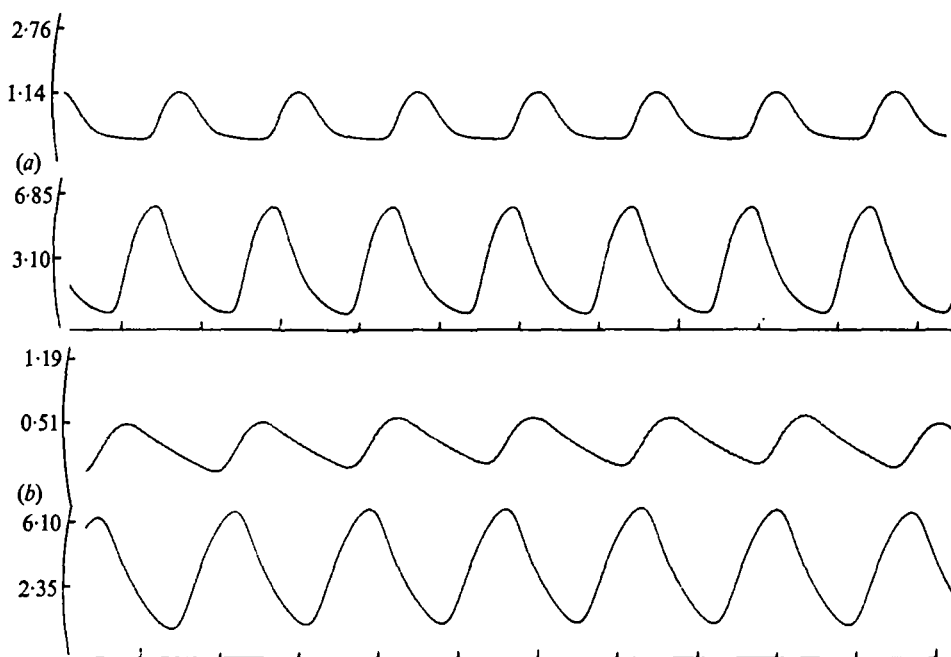


Fig. 10. Strain-gauge records of isolated conus muscles. Preparation was bathed in Ringer, pH 7.5. Time line in seconds, bottom trace in both (a) and (b). Ordinates in g, weight, zeroed at diastolic level. (a) Top trace, record of contraction of longitudinal conus muscle; second trace, record of ventricular contractions. (b) Top trace, record of contraction of circular muscle; second trace, record of ventricular contractions.

peak of ventricular contraction by more than 300 msec. Three of these preparations in alkaline Ringer showed little change in timing of contraction of the longitudinal conus muscle as a function of beat frequency. Five preparations in acidic Ringer showed that contraction of the longitudinal conus muscle was delayed by an average of 159 msec (range: 125–200 msec) when beat frequency was increased from about 30/min to 60/min. This would appear to be conclusive evidence.

Isolation of measurement of contraction of circular and longitudinal conus muscles from each other shows that contraction of the longitudinal muscle is sharp and hammer-like with early relaxation (Fig. 10a), quite like that seen in Fig. 9. The shape of its contraction curve closely matches the shape of the distortion on the pulse pressure curve of the pulmocutaneous when the conus was stimulated to contract early (e.g. Fig. 6a). Contraction of the circular muscle (Fig. 10b) is not as fast and relaxation is even slower. Indeed, the circular muscle is obviously still tonically contracted after the beginning of ventricular contraction of the next cycle.

Observing the conus of an isolated heart in strong transmitted light when it is beating slowly makes it apparent that the longitudinal muscle effects closure of the pulmocutaneous trunk by shortening the conus on the side of the cavum aorticum, bringing the anterior end of the spiral valve to bear against the outlet of the cavum pulmocutaneum. The longitudinal contraction induces an increase in curvature of the spiral valve, which travels in a wave-like motion in an anterior to posterior direction. Hence, closure of the pulmocutaneous outlet does not result in an inflexion in the

Pressure pulse in that arch. The wave-like motion appears to purge the cavum pulmocutaneum posteriorly, its contents going into the cavum aorticum. Further contraction of the longitudinal muscle, and later, of the circular muscle, is expressed entirely in the pulse of the systemic-carotid circuit. The manner in which the circular muscle holds the cavum pulmocutaneum closed is self-evident but the site of subsequent closure of the cavum aorticum cannot be seen.

DISCUSSION

Although the present work deals with a study of only two species, *Rana pipiens* and *Bufo marinus*, gross dissections of two others, *Rana catesbiana* and *Xenopus laevis*, together with the anatomical studies of the hearts of *Rana temporaria* and *Rana pipiens* (Sharma, 1957, 1961) and physiological studies of *R. temporaria*, *R. pipiens*, *X. laevis* and *Bufo bufo* (Shelton & Jones, 1968), strongly indicate that we are dealing with a mechanism common among anurans.

Distribution of blood in the vertebrate system is generally controlled by smooth muscle. Such muscles determine resistance of the arteries, capacity of the veins, and drive the sphincters which regulate flow into the capillary beds. It appears that in anurans we have a most unusual mechanism for regulating distribution; in effect, a switching device driven by cardiac muscle. The classical theory of selective distribution depends on movement of the spiral valve during systole (Foxon, 1955), but the nature of this movement appears to have been thought to be passive.

The data in Table 1, derived from 23 independent measurements, showed that, under the conditions of these experiments, there is a highly significant difference between pulmocutaneous and systemic pulse pressures, and a difference between their diastolic pressures at the 0.05 level of confidence. In other parameters, the data are also in accord with the results of the numerous studies culminating in the work of Jones & Shelton (1972), particularly Shelton & Jones (1965*a, b*, 1968).

Heat-induced beat frequency, as it might affect mode, seems to have been given little attention in most of the more important investigations of anuran haemodynamics. There is little in the literature to indicate that experiments have been conducted on anurans to comparatively assess distribution of blood flow as a function of some parameter which would reflect demands of the system. The presence of oxygen at the pulmonary surfaces is an environmental parameter.

On the basis of the research leading up to that of Jones & Shelton (1972), particularly the work of Shelton (1970) and Shelton & Jones (1965*a*), it seems safe to conclude that a large differential between diastolic pressure in the systemic-carotid circuit on the one hand and the pulmocutaneous on the other is a correlate of an availability of oxygen at the pulmonary surfaces. And the first two mentioned works demonstrated by direct means that this condition leads to increased flow to the pulmocutaneous circuit. There are no indications that the diastolic pressure differential (which is manifest with resumption of air breathing upon emerging from a dive) reflects oxygen need by the system. Jones (1967) showed that submergence for extended periods (well over an hour) resulted in accumulation of no oxygen debt in *Rana pipiens*. It would appear from all of these studies that increased flow to the lungs as it is reflected by relatively low pulmocutaneous diastole is an opportunistic aspect of a reflex

response which enables the animal to charge the blood with oxygen when that commodity is readily available. DeGraaf (1957) considered this diastolic differential to represent a lower peripheral resistance in the pulmocutaneous circuit. It is apparent that he took peripheral resistance to imply some reciprocal of vessel diameters in the circuits, particularly in the terminal arteries, arterioles and capillaries. Shelton (1970), expressing it as an impedance modulus, calculated the value as the quotient of mean arterial pressure, divided by blood flow. The present work indicates that this is not adequate without some expression of the length of time the pulmocutaneous circuit remains open during the cycle and that deGraaf (1957) probably deduced relative peripheral resistances correctly.

If lower diastolic pressure in the pulmocutaneous circuit does imply a lower resistance in that circuit, then simultaneous appearance of the pulse in both circuits would represent a problem. However, Shelton & Jones (1965*a*) pointed out that appearance of the conus component in the systemic pulse but not in the pulmocutaneous demonstrated that the two cava of the conus are isolated from each other during diastole. With the circular muscle of the conus holding both cava closed, not only through late diastole but through early systole as well the ventricular pulse will break the tonic 'hold' and burst simultaneously into both circuits. There is great adaptive significance in this because of the fact that it permits the system to be 'finely tuned' simply by adjustment of the one variable, i.e. the time at which the pulmocutaneous circuit is closed, later in the cycle.

It seems reasonable to assume that in any set of circumstances the animal's source of oxygen, no matter to which atrium the blood is returned, will be blood which went out in the pulmocutaneous circuit. Even the best evidence for selective routing of returns of the two atria indicates that this selection is a labile phenomenon. During periods when ventricular mixing might be complete, elementary calculations demonstrate that when, for example, the body circuit is receiving 70 % of the stroke volume, denying that circuit an additional 10 % of the total and allowing that to join the flow to the low-pressure pulmocutaneous circuit, there would be an actual increase of oxygen sent to the body circuit – an increase of more than 14 % per cycle. In view of the probable importance of cutaneous circulation to anuran respiration (Foxon, 1964*a*) and of the locus of its return, it would seem certain that a peculiarity imparting survival value to the anuran conus would be its ability to proportionally modulate output between the pulmocutaneous and systemic-carotid circuits. Such a mechanism permits immediate and automatic response to demands of the system.

In those quiet times in the frog's life when it is respiring across the lungs and when the heart beats at a modest rate, streams of passage of blood through the ventricle can be separated in time. If one examines the remarkable system of trabeculae in the ventricle, concept of such separation requires little imagination. Although the mechanics in the present work are not in agreement with those proposed by Hazelhoff (1952), the functional results that can be visualized agree very well with those proposed by him. It is easily demonstrated that during ventricular systole, blood bursts through the conus and into the body and pulmocutaneous circuits simultaneously. However, once the cava are opened, the relative volumes of flow must certainly be proportional to resistances and pressure gradients involved. All of the direct evidence indicates that in air-breathing frogs the pulmocutaneous has the lower diastolic

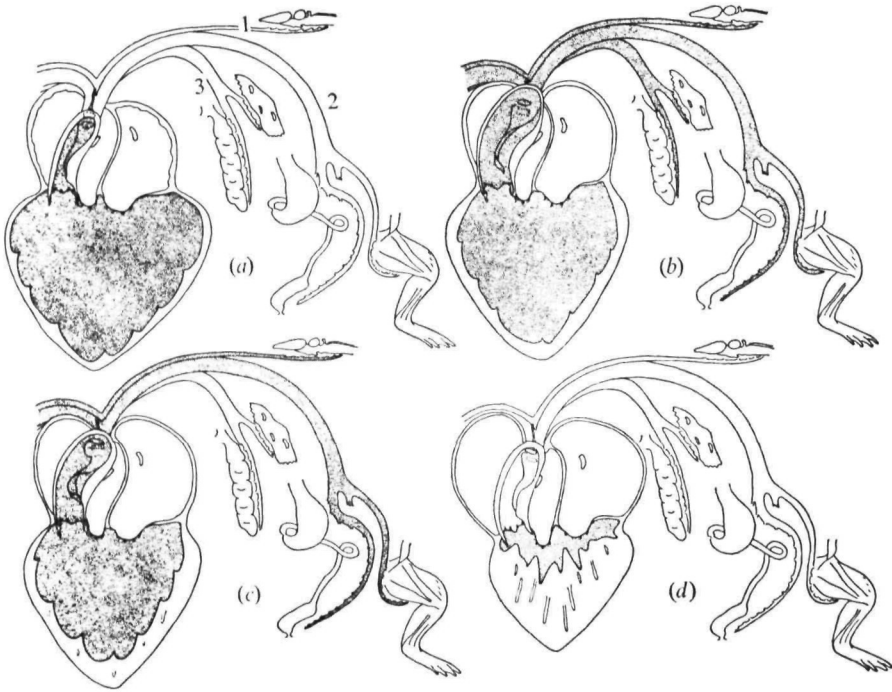


Fig. 11. Diagram showing four stages of conus function. Ventricular contents and flow resulting from ventricular contraction are stippled. 1 = carotid circulation; 2 = systemic circulation; 3 = pulmocutaneous circulation. (a) Early ventricular systole after onset of pressure pulse in the conus but before pressure has overcome the tonic 'hold' of the circular muscle in the synangial region. (b) Later ventricular systole after flow has entered all of the arches and with the conus complying with the ventricular output. (c) Contraction of the longitudinal muscle of the conus closes the pulmocutaneous trunk. (d) Circular muscle of the conus has closed the cavum pulmocutaneum and pressure in the cavum aorticum has closed the pylangial valves, isolating the conus from the ventricle.

pressure and probably the lower resistance. Hence, flow from the initial burst will prefer this channel and this is the blood that will have been positioned in the right of the ventricle because of its arrival from the right atrium. Later, the pulmonary return from the left will join the flow, and it will be shunted into the systemic-carotid circuit when the conus snaps the pulmocutaneous shut. Essentials of such a sequence are summarized in the diagram (Fig. 11).

I am pleased to acknowledge advice given by Mr Marus W. Mumbach and Mr Lee H. Vernon. The toads were provided by Dr Allen R. Thompson from grant funds (AMO3536, NIH) of Dr B. T. Scheer. I am grateful to Dr Sanford S. Tepfer, Head of the Department of Biology, for other materials used in this study. I appreciate the constructive criticism of the manuscript by Dr Graham Hoyle. I am singularly grateful to Dr J. H. Day, Professor of Zoology, University of Cape Town. Ideas leading to this research were conceived during a most rewarding sabbatical year in Professor Day's department.

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