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CENTRAL BLOOD PRESSURE AND HEART OUTPUT IN SURFACED AND SUBMERGED FROGS

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INTRODUCTION

Those animals which have an incompletely divided double circulation have, for obvious reasons, occupied a dominant role in discussions of the phylogeny of vertebrate circulatory systems. The position of the present-day amphibians, either as representative of an early stage in the progressive separation of two sides of the heart or as a specialized offshoot from ancestors with more effectively divided systems, is in some doubt (Foxon, 1955). Details of the movement of blood through the amphibian heart and arterial arches must be central to the discussion as the only direct evidence to be adduced on the status of the undivided ventricle.

A great deal has been written on this subject and all workers in recent years are agreed that the classical theory of the separation of blood streams (Sabatier, 1873) is totally unsupported by experimental evidence. In spite of this unanimity it is still not clear whether there is indiscriminate mixing of blood from the right and left auricles (Foxon, 1951) or some degree of separation of blood from the body and lung circuits as it passes through the heart. De Graaf (1957), working on *Xenopus*, found a rather odd division with the lung circuit carrying most of the ventricular output. At each ventricular systole the pulmocutaneous arch received all of the blood coming to the heart from the body together with much of that coming from the lungs. Simons (1959) also claimed that in anurans some separation of blood from the two sides of the heart could be seen in the arterial arches, whereas in urodeles complete mixing occurred in the sinus. Recently, Johansen (1963), on the basis of X-ray photography and determinations of oxygen concentration in the arterial arches, decided that in *Amphiuma* separation of the visceral and pulmonary blood can occur under certain circumstances, but that it is not a permanent feature of the circulation.

Since many Amphibia can live for long periods under water as well as in air, they must obviously be able to undertake gross changes in the balance of their respiratory mechanisms. It is unsatisfactory to regard the circulatory system as being stable during these changes, indeed a pronounced diving bradycardia has already been described (Jones & Shelton, 1964). It seems certain that the solution to the problem of function in the amphibian heart must be sought in both the submerged and surfaced animal. In the work which has been done so far on blood distribution the possibility of alterations adaptive to different environmental conditions has not been examined.

Although the effect of submersion on amphibian circulation is largely unknown,

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this is not the case for the less well adapted, diving vertebrates. Diving is known to cause bradycardia in birds and mammals without, however, giving rise to much change in the arterial pressures measured centrally (Irving, Scholander & Grinnell, 1942; Johansen & Aakhus, 1963). This is interpreted as being due to vasoconstriction in certain parts of the periphery such as the skin, viscera and muscles, thus making more blood and oxygen available to other restricted areas, notably the brain and heart. In the alligator a similar peripheral vasoconstriction is thought to occur and accounts for the increase in blood pressure during the early stages of the dive (Andersen, 1961). Later the pressure falls substantially suggesting that blood flow is reduced in all regions. Variations clearly exist in the circulatory responses shown to diving by different animals. The need to maintain adequate skin circulation for gas exchange at the body surface, and the fact that the lung circuit need not carry the same flow of blood as the body (as it must in completely divided systems) lead one to expect that the Amphibia will also differ in some respects from those cases already examined.

METHODS

The majority of the experiments were done on *Rana pipiens*, the American grass frog, but because of supply difficulties *R. temporaria* was also used. The only experiments in which one species (*R. temporaria*) was used exclusively were those of 'simultaneous' pressure measurement. In all other cases both species were used and no qualitative difference could be seen between them. There are some slight quantitative differences in the behaviour of the heart when animals of the two species are submerged (Jones, in preparation), but these are of no significance to the results described in this paper. The text refers to the work done on *R. pipiens* except where it is otherwise stated. The frogs were stored, and the experiments were carried out, at room temperature (20° C.) in the winter and early spring of one year. No obvious differences in activity of the animals could be seen over this period.

Before an experiment the frog was deeply anaesthetized in Sandoz MS 222 solution (300 mg./l.) until the breathing movements had just ceased. The clavicle and coracoid were cut through near their ventral ends and a small part of the sternum was removed. This allowed the conus and the roots of the arterial arches to be exposed. The animal with its ventral side uppermost was then clamped by the four limbs to a wax block and allowed to recover to a lightly anaesthetized state in which the breathing was normal. The frog was kept in this stage of anaesthesia by periodically moistening the skin with MS 222 solution throughout the experiment. The wax block was located in a Perspex tank into which water could be run from a reservoir. To submerge the animal water was run in to the level of the buccal cavity floor, taking care that it did not flow over into the body cavity.

The blood pressures were measured by capacitance manometers of the type designed by Hansen (1949) and manufactured by Ole Dich (Type ATH, new model). They were connected to the artery by means of a short piece of copper tube, and in the majority of cases, a no. 20 hypodermic needle. In some cases no. 15 needles were used, but the experiments were all repeated later using the finer needles. No difference was seen in the results. The manometers were held in manipulators and the needles were inserted into the pulmocutaneous and systemic arches about 1 or 2 mm. beyond

the point of their origin, the openings in the needles pointing downstream. To prevent clots forming in the needles they were coated with silicone, the animal was injected with heparin (2·5 i.u. heparin per 10 g. weight), and the manometers were filled with 5 % sodium citrate. In some later experiments the animals were not injected and the manometers were filled with saline containing 10 i.u. heparin per ml. On the whole this latter method was more effective but, after the initial experiments, very little trouble was experienced with either method even over extensive recording periods. The two manometers were tested by applying a sudden pressure change to the end of the needle. With a no. 20 needle on the slower of the two manometers, the whole equipment, i.e. manometer, capacitance detection unit, amplifier and pen recorder, followed a sudden release of pressure with a time constant of 3·5 msec. The most rapid pressure change recorded (the beginning of the pulmocutaneous pulse) was some five times slower than the instrument's response to a square wave pressure change of similar dimensions.

The electrocardiogram (e.c.g.) was detected by a wire located just under the skin close to the region of the ventricle. The signal was amplified in conventional amplifiers and displayed on the pen recorder. Heart output was measured by means of a cardiometer. A Perspex cup was made to accommodate the ventricle and the open top of the cup was sealed by a thin rubber membrane with a hole through which the ventricle was pushed. The size of the hole was very important and a fresh membrane was prepared for each experiment. The cup was connected directly to the tube of a 1 ml. tuberculin syringe arranged horizontally. Volume change in the saline-filled system was recorded by means of a small metal piston in the syringe tube connected to a mechano-electric transducer. The piston was polished to an easy fit in the tube. The whole moving assembly weighed 1·1 g. and moved through a maximum of 8 mm. In addition the heart had to move about 1 ml. of saline which filled the apparatus. The inertial and frictional losses in the cup and tube were thus quite small, though inevitably in this type of system an extra load was placed on the heart.

In the account which follows 'left' and 'right' always refer respectively to the left and right sides of the animal.

RESULTS

(*a*) *Blood pressures in the systemic arch*

Typical values for pressures measured in the systemic arch when the frog was in air are given in Table 1*a*, and recordings of the pressure pulse can be seen in Figs. 1*b* and 2*b*. The pressure levels were higher than those recorded by Simons (1957) in *R. temporaria*, and slightly higher than those determined by de Graaf (1957) in *Xenopus*. The pulse curve consisted typically of two parts separated by an inflexion in the falling phase. This has been called the incisura by Simons (1957) who thought it was similar to that of the mammalian pulse. In the mammal the incisura is produced by closure of the aortic valves and pressure run-off after this event is due to elastic recoil by the walls of the arterial arch. Standing waves caused by pressure recoil from the periphery also produce secondary pulses after the incisura. In *Xenopus* de Graaf (1957) claimed that the pressure component after the inflexion was due to contraction of the conus arteriosus (bulbus cordis) and our results on *Rana* have confirmed this, though without eliminating the possibility of a small contribution due to standing

waves. The size of the second component in the pressure trace was variable but it became more obvious in animals where, for some reason, there had been appreciable blood loss. Such animals were not used for further experiment but it was noticed that in some cases the second wave could become larger than that due to ventricular contraction, the latter being quite small because of the blood loss. Clearly this type of pulse could not be produced by elastic recoil; active contraction of some part of the

Table 1. *Blood pressures (mm. Hg) recorded from the arterial arches*

	Systolic	Diastolic	Pulse
Systemic pressures			
(*a*) At the surface (16 determinations from 7 animals)			
Average	32·0	20·9	11·1
Range	38·0–25·1	25·1–16·7	16·0– 7·6
(*b*) After 20 min. submersion (10 determinations from 7 animals)			
Average	23·2	15·9	7·3
Range	29·7–16·0	19·4–11·4	12·2– 3·8
Pulmocutaneous pressures			
(*c*) At the surface (13 determinations from 6 animals)			
Average	31·2	16·4	14·8
Range	38·0–26·6	22·8–12·2	19·8–10·6
(*d*) After 20 min. submersion (8 determinations from 5 animals)			
Average	25·8	14·8	11·0
Range	30·4–21·3	19·0– 9·9	15·2– 7·2

system must occur. In the animals in which the inflexion was pronounced and the second pressure component very clear it was possible to relate this component to conus contraction. In normal animals the delay between the conus deflexion of the e.c.g. and the beginning of the second pressure wave was greater than that between the QRS (ventricular) deflexions and the main pulse. There must therefore be a prolonged period of isometric contraction in the conus and it is possible that the active muscle is extended in the early stages by the flow of blood from the ventricle. These observations are in accord with previously expressed views on the function of the conus as a pressure chamber tending to reduce pulse size and maintain diastolic pressure (Johansen, 1963).

Breathing movements did not have much effect on the pulse or diastolic pressures when the breathing was regular. If the breathing was spasmodic then each burst of movement was followed by an increase in pulse pressure. The diastolic pressure was variable under these conditions but did not consistently rise or fall.

When the frogs were submerged the effect on overall systemic blood pressures was surprisingly small (Table 1*b*), even though there was considerable slowing of the heart. In some cases the diastolic pressure hardly changed (Fig. 1), although the systolic pressure invariably fell gradually during the period of submersion. In many frogs, however, the effect was more pronounced and both systolic and diastolic pressures fell (Fig. 2) but only when the heart became arrhythmic after fairly prolonged submersion was the diastolic pressure reduced appreciably. When the heart continued to beat rhythmically it was unusual for changes to be greater than those illustrated in Fig. 2.

At the beginning of the submersion period the systemic pressures increased but the rise was never large or prolonged (Fig. 2) and occasionally was not seen. The heart rate frequently went up during this initial period but the increased pressures were maintained after this when the heart rate had begun to go down.

Since the systolic pressure fell more than the diastolic during the period under water, there was always a change in the pulse pressure (Figs. 1 *a* and 2 *a*). This change together with the altered shape of the pulse curve, was the most obvious effect of

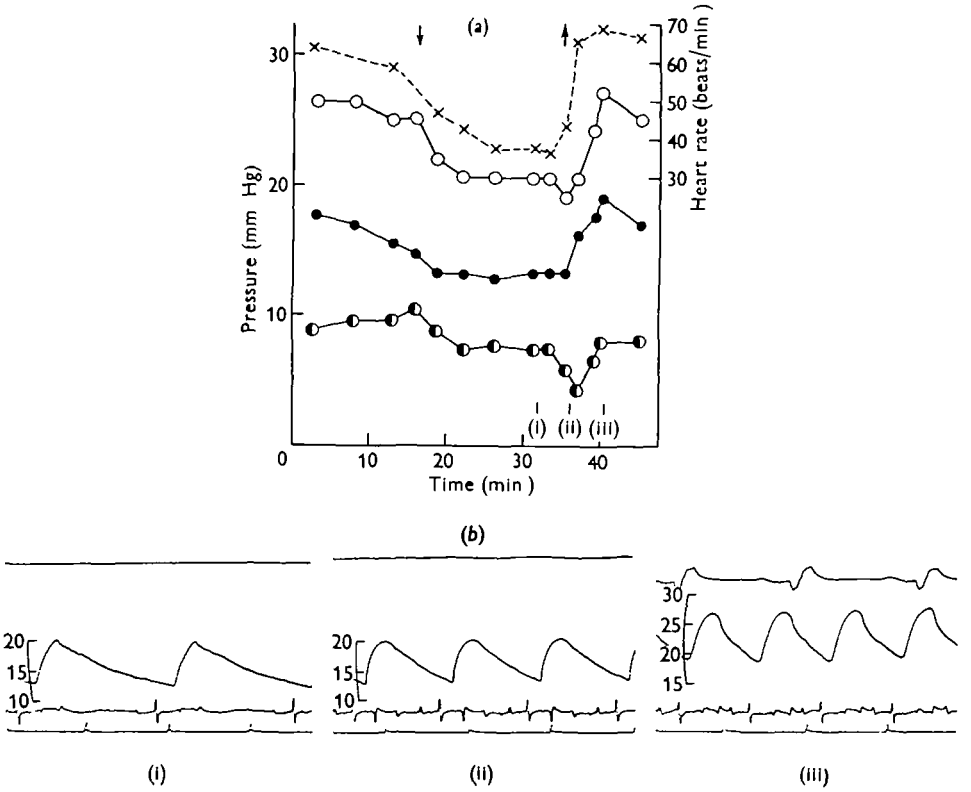

Fig. 1. (*a*) Systemic blood pressures and heart rate in *R. pipiens* (29 g.). Downward and upward pointing arrows indicate submersion and emersion respectively. ×, Heart rate; O, systolic pressure; ●, diastolic pressure; ◐, pulse pressure. (*b*) Pressure pulses recorded at times indicated on graph. Trace 1, breathing movements from buccal cavity; trace 2, systemic pressure (mm. Hg); trace, 3, electrocardiogram; trace 4, time (sec).

submersion. The shape of the pulse can be seen in the superimposed records of Fig. 3 where, in order to compare both the rising and falling phases, the same pulses have been superimposed using (*a*) the beginning, and (*b*) the end of the wave as the point of coincidence. The records have been chosen from experiments in which the diastolic pressures remained approximately constant so that the decline in peak systolic and pulse pressures followed the same course. During submersion the rate of pressure change went down progressively but the time taken for the peak pressure to be developed stayed roughly the same since the level was falling. It seems probable that the period of isometric contraction before the pressure pulse appeared in the aortic arches was also prolonged. Confirmation was obtained by measuring the time

interval between the R deflexion of the e.c.g. and the beginning of the pulse wave. This gave a measure of the change in duration of the isometric contraction, assuming that the time delay between e.c.g. and activation of the muscle and the period for the pulse to reach the manometer remained constant. The results are given in Table 2 and show that submersion did extend the isometric contraction time almost twofold, beyond what might be expected on the basis of an equal expansion of all events of the cardiac cycle as the heart rate decreased. In fact, as Table 2 shows, the timing of the

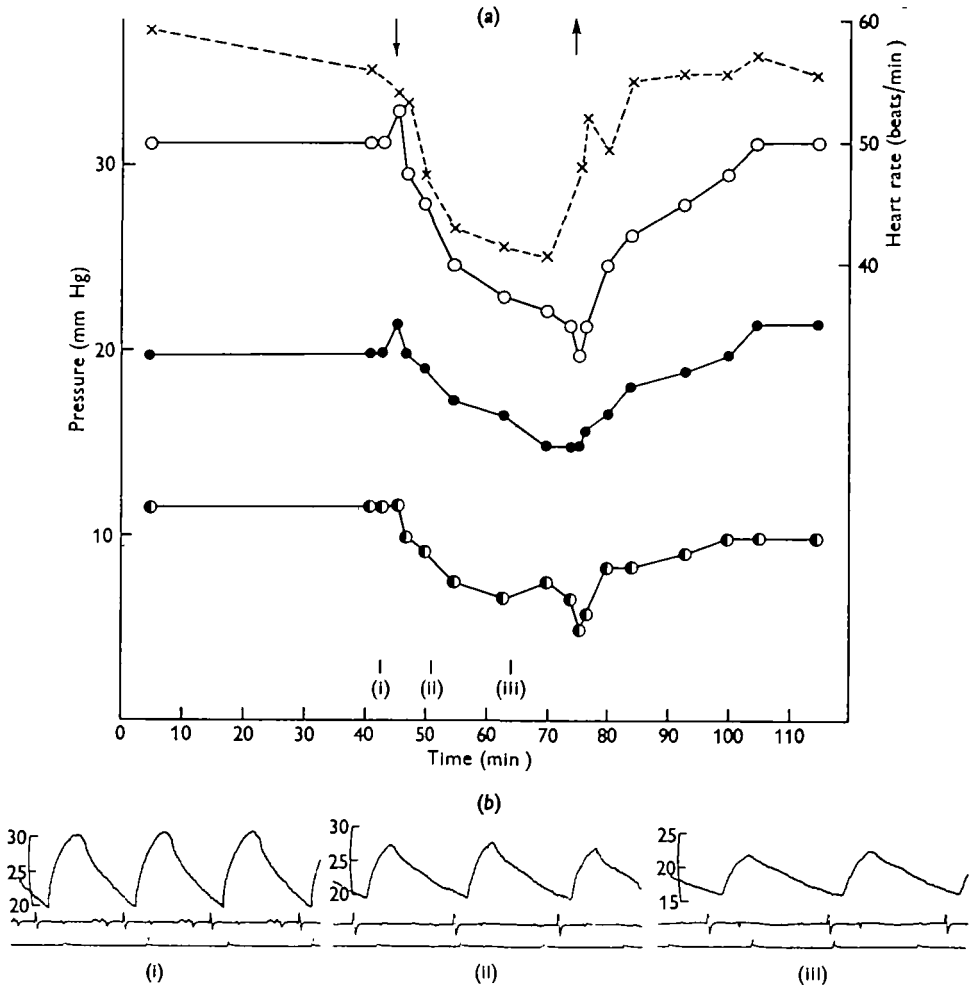

Fig. 2. (*a*) Systemic blood pressures and heart rate in *R. pipiens* (24 g.). Downward and upward pointing arrows indicate submersion and emersion respectively. ×, Heart rate; ○, systolic pressure; ●, diastolic pressure; ◐, pulse pressure. (*b*) Pressure pulses recorded at times indicated on graph. Trace 1, systemic pressure (mm. Hg); trace 2, electrocardiogram; trace 3, time (sec.).

main electrical events of a single activity sequence changed very little, the PR interval increasing and the RT decreasing slightly. In the mammal first-stage anoxia of the heart muscle causes similar changes in the e.c.g. relationships and a displacement of the ST segment which was also seen in many of the recordings from the frog. As the

Table 2. *Time intervals (seconds) between electrocardiogram components and pressure pulse*

(Average values calculated from data obtained from three frogs)

	Cardiac cycle length	R wave to pulse pressure	P to R wave	R to T wave	T to P wave
Animals on surface (30 determinations)	1·02	0·08	0·25	0·63	0·14
Animals submerged for at least 7 min. (24 determinations)	1·36	0·14	0·28	0·58	0·50

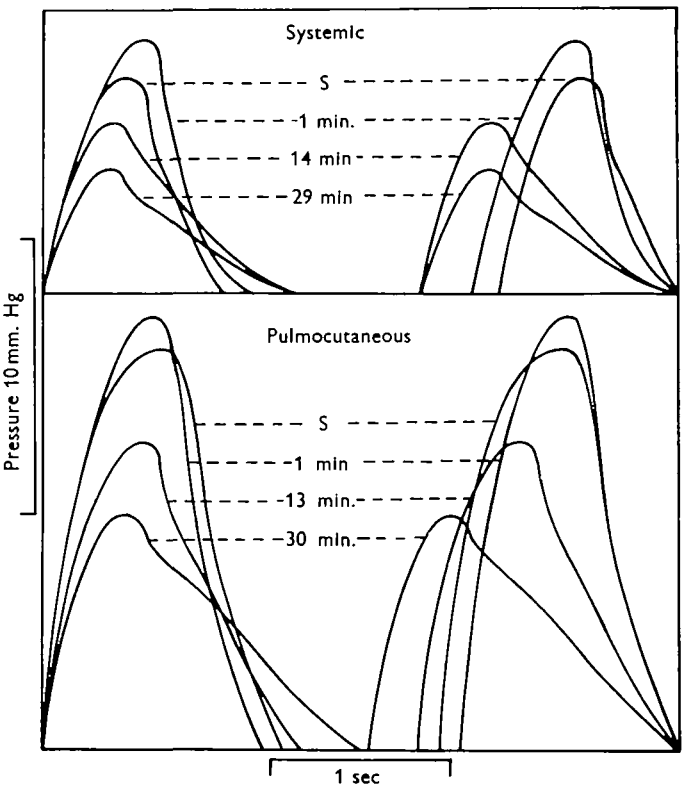

Fig. 3. Pulses recorded from systemic and pulmocutaneous arches in *R. pipiens* at the surface and after the periods of submersion indicated. The same pulses are superimposed using the beginning and end of the pulse wave as the point of coincidence.

heart rate went down the TP interval (i.e. the time between repolarization of the muscle of the ventricle and the beginning of the next auricular contraction) was the factor principally affected.

In the falling phase of the pressure pulse several important changes were seen. The ventricular wave became much smaller in relation to that of the conus and the pressure reversal from rising to falling phase more pointed. The inflexion beginning the conus component moved much nearer to the peak of the diminishing ventricular pulse. Finally there was a very marked decrease in the slope of the whole falling phase

of the pulse as Fig. 3 shows. In general all the changes described above became more marked the longer the frog was kept under water, for periods up to 30 min.

When the animal was brought to the surface these changes were reversed, the diving bradycardia disappearing almost immediately and the blood pressures recovering over a variable period of time. In those animals which began regular breathing soon

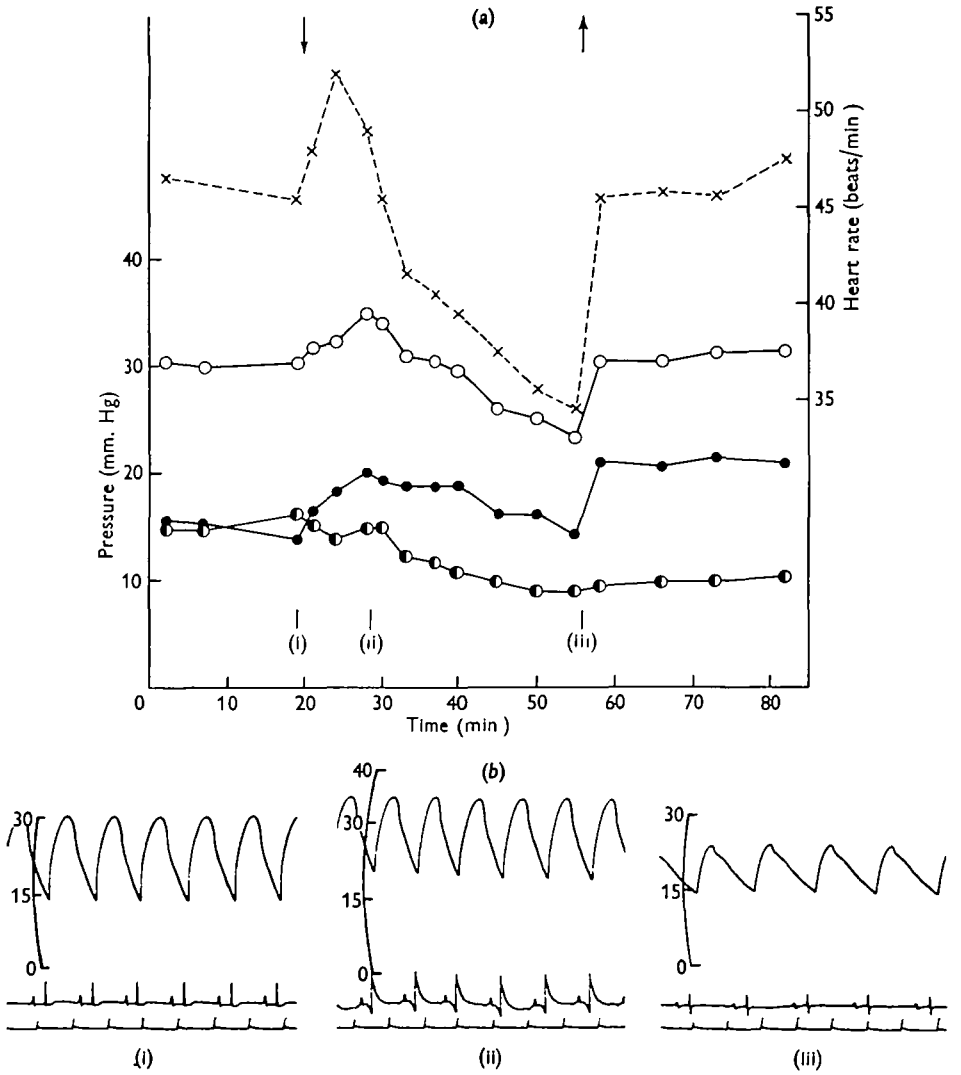

Fig. 4. (*a*) Pulmocutaneous blood pressures and heart rate in *R. pipiens* (23 g.). Downward and upward pointing arrows indicate submersion and emersion respectively. ×, Heart rate; ○, systolic pressure; ●, diastolic pressure; ◐, pulse pressure. (*b*) Pressure pulses recorded at times indicated on graph. Trace 1, pulmocutaneous pressure (mm. Hg); trace 2, electrocardiogram; trace 3, time (sec.).

after the head was clear of water the recovery was rapid and complete in 2 or 3 min. (Fig. 1). If the breathing was intermittent however, the recovery to the original pressure levels took place gradually even though the heart rate was high right from the end of the dive (Fig. 2).

(*b*) *Blood pressures in the pulmocutaneous arch*

The pressures measured in the pulmocutaneous arch when the frogs were in air showed little difference from the systemic pressures in systolic levels but the pulse pressures differed in magnitude (Table 1 *c*) and to some extent in form. The pulse was invariably larger than that from the systemic arches by virtue of the lower diastolic pressures recorded, a difference similar to that described by de Graaf (1957) in *Xenopus*. The rate of change of pressure, both in the rising and falling phases, was greater in the pulmocutaneous arch. In the steeply falling phase of the pulse an

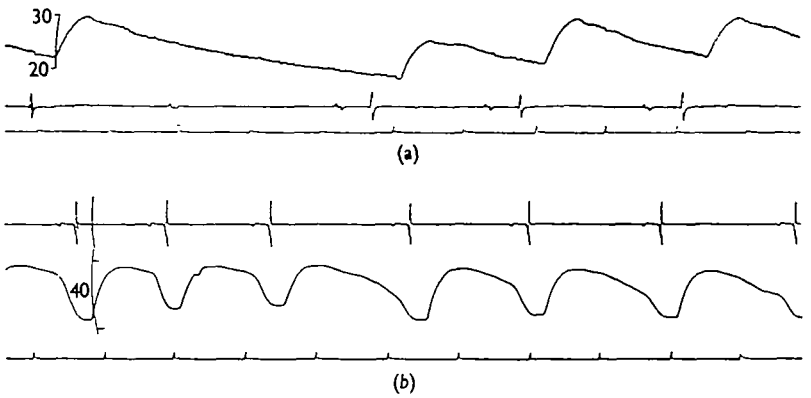

Fig. 5. The effect of arrhythmic heart beat. (*a*) Pulmocutaneous pressure. *R. pipiens* (23 g.). Trace 1, pressure (mm. Hg); trace 2, electrocardiogram; trace 3, time (sec.). (*b*) Stroke volume. *R. pipiens* (21 g.). Trace 1, electrocardiogram; trace 2, volume (μl., up on trace = decrease in volume); trace 3,time (sec.).

inflexion associated with the beginning of the conus pressure wave could not be seen clearly (Fig. 4). There was a slight change of slope in the falling pressure curve but the run-off remained much more rapid than that found in the systemic arch.

When the animal was submerged the pressures changed in the same way as those measured in the systemic arch. After an initial rise the systolic pressure decreased more than the diastolic so that there was again a marked fall in pulse (Fig. 4). It is difficult to make valid comparison between small differences using pressure records from different animals, but it seems likely that the fall, in diastolic pressures particularly, was less marked in this arch than it was in the systemic (Table 1). In the majority of experiments the diastolic pressures varied very little.

In Fig. 3 pulses obtained from the pulmocutaneous arch are superimposed in the same way as for the systemic pulse. Again they were chosen from a series in which the diastolic pressure remained fairly constant and the systolic pressures were at the same level as those of the systemic pulses given in this figure. It can be seen that, as the time under water increased, the pulse changed in shape as before with the rates of change of pressure decreasing, particularly during the falling phase. A clear inflexion frequently appeared, separating the ventricular and conus pressure components, and the peak of the ventricular wave became more pointed. The run-off after the conus pressure became slower. It was not usually as slow as that seen in the systemic arch

even though the diastolic pressure level was on the whole much lower. However, when arrhythmia developed and the heart beat interval was quite long, the slope of the run-off decreased a great deal. The diastolic pressure never declined to zero (Fig. 5*a*).

(*c*) '*Simultaneous*' *measurement of blood pressures*

Because of the range in blood pressures which exists between different individuals some of the differences suggested by the experiments described in the foregoing sections can only be confirmed by determination of pressures simultaneously in

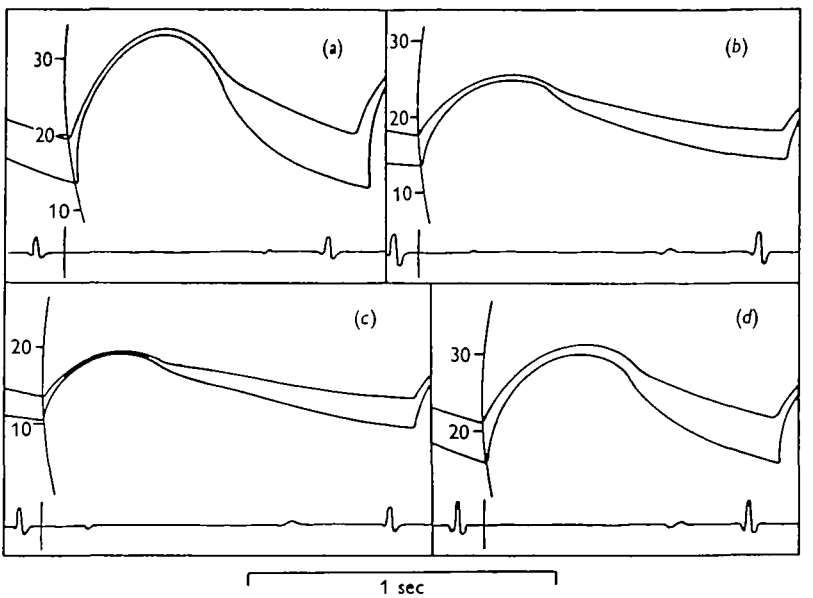

Fig. 6. Pressures (mm. Hg) recorded from systemic and pulmocutaneous arches in *R. temporaria* (32 g.) and superimposed as explained in text. The systemic pressure is the top trace in all cases. The calibration curve and the corresponding curve on the e.c.g. trace show the writing radius of the pens and represent the same point in time: (*a*) at surface; (*b*) submerged 11 min.; (*c*) submerged 22 min.; (*d*) surfaced 10 min.

systemic and pulmocutaneous circuits. Two manometers were used together in three frogs (*R. temporaria*) connected to the pulmocutaneous and either the systemic or carotid vessels, the pressures in these latter being identical. Only one capacity detection unit was available and this had to be switched between the two manometers. The records obtained were therefore not simultaneous but, because the e.c.g. was recorded throughout, the successive traces could be superimposed easily and accurately. Changing from one manometer to the other was done with a switch so that there was rarely any change in pressure characteristics and the timing of events during the short recording periods necessary.

The difference between the systemic and pulmocutaneous pulse pressures was confirmed and further points of difference were found (Fig. 6). Although the diastolic pressures in the two arches were very different, the pulse appeared simultaneously in both. During the rising phase the rate of pressure change in the pulmocutaneous

arch was much greater than in the systemic so that the two pressures approached but never quite reached the same level. The maximum pressure occurred at the same time in both arches but the systemic pressure was the higher by an average 1·6 mm. Hg (Fig. 6*a*, *d*). The two pressures began to diverge during the falling phase and the divergence became most marked after the inflexion in the systemic pulse. These results were confirmed with the manometers reversed to eliminate any difference produced by instrument characteristics.

During submersion of the frogs the same general relationships were maintained. In spite of an increase in the length of time taken for the cardiac cycle, the two pulse waves again appeared simultaneously and the pressure maxima, though lower, were reached together. The systemic pressure also remained the higher one, though by a smaller margin during the later stages of the dive (Fig. 6*b*, *c*). The differences in the two diastolic pressures became less marked.

(*d*) *Heart output*

Since the effect of submergence on aortic pressures, particularly during diastole, was not usually of the same magnitude as the change in heart rate, it must be supposed that some other factors were involved in the relationship between total output and pressure. One of these factors must be the stroke output of the heart; an increase in stroke could obviously offset a decrease in heart rate. Though there has been speculation about this parameter in other diving vertebrates (Eliassen, 1960; Johansen & Aakhus, 1963) direct measurements have not been made. The other factor of major importance which can influence the relationship between overall heart output and aortic pressures is the peripheral resistance.

Observation of the exposed heart suggested that the stroke volume was affected by submersion of the frog though not in the sense suggested above. When the animal was in air auricles and ventricle emptied fairly completely at each systole. The left auricle was more obvious and appeared to be larger than the right as far as could be seen from the rather restricted ventral view. After a period under water the chambers of the heart did not empty to the same extent as on the surface, the stroke volume went down, and the heart became much more distended with blood.

Experiments in which the ventricle was enclosed in the cardiometer have confirmed and extended these observations on ventricular volume. The cycle of activity can be seen in the records of Figs. 7 and 10. The ventricular contraction began after the QRS complex of the e.c.g., there being a short period of excitation and isometric activity before the volume slowly decreased. At this moment the pulse appeared in the systemic arch and, rising steeply, the pressure had increased over at least half its range before the ventricle began to decrease in volume at all rapidly. By the time the pulse reached its maximum value the ventricle had pumped out more than half the total stroke volume. The end of the period of contraction coincided roughly with the T wave of the e.c.g. and the beginning of the conus component in the pressure record. From this point the behaviour of the system was somewhat variable. Usually there was a delay during which the auricles filled and then, following shortly after the P wave, came the period of ventricular filling. In *R. pipiens*, breathing in air, we have no evidence that the ventricle was filled other than by auricular contraction (Fig. 7*a*). In *R. temporaria*, on the other hand, several records suggest that the ventricle was

filling slowly in the period before auricular contraction (Fig. 7*b* ii) and in some cases up to half the diastolic volume entered the ventricle during this period (Fig. 7*b* i). These two records were taken at an interval of 90 min. and though we cannot account fully for the differences it is possible that during the first record the animal had not recovered completely from the operation to expose the heart and arterial arches. In

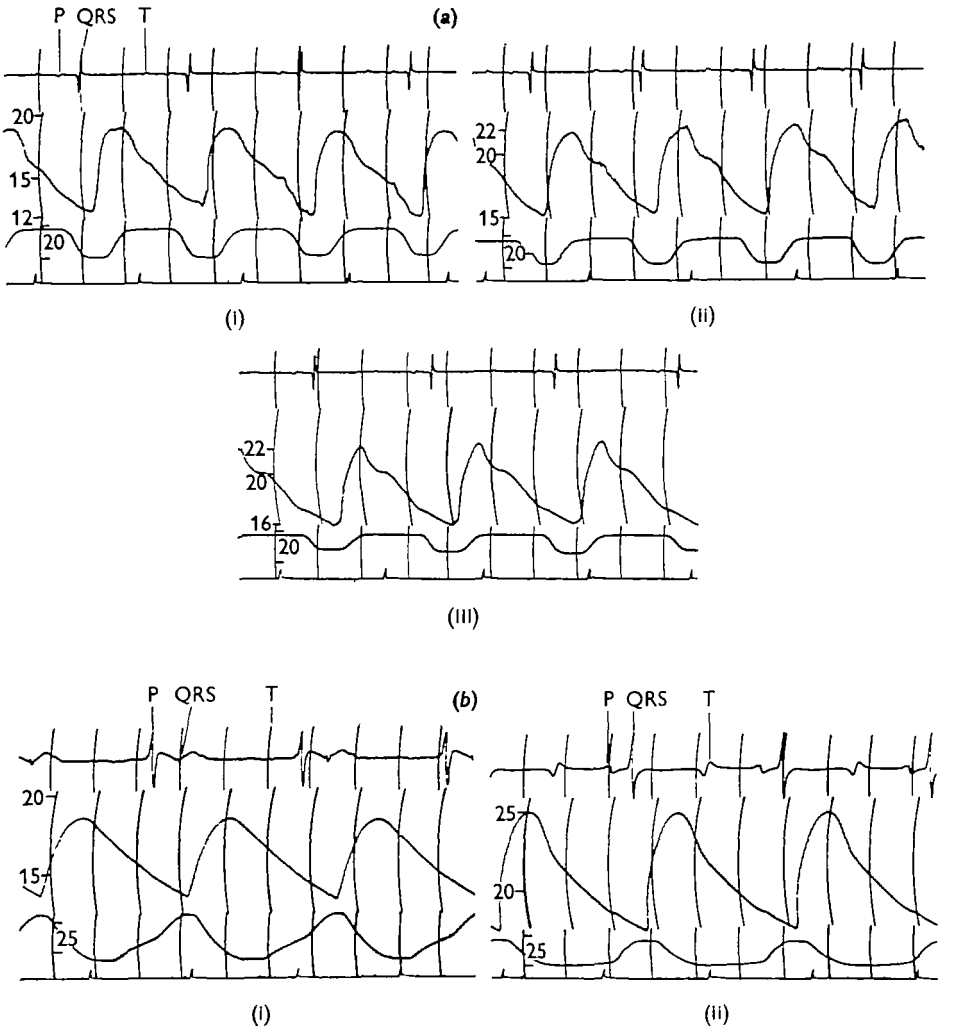

Fig. 7. Ventricular volume and systemic blood pressure in: (*a*) *R. pipiens* (21 g.)—(i) at surface, (ii) submerged 6 min., (iii) submerged 11 min.; (*b*) *R. temporaria* (31 g.)—(i) and (ii) at surface, 90 min. apart. Trace 1, electrocardiogram; trace, 2 systemic blood pressure (mm. Hg); trace 3, ventricular volume ((*μ*l.), in (*a*) up on trace = decrease in volume, in (*b*) down on trace = decrease in volume); trace 4, time (sec.). Curved lines show writing radius of pens and coincident points in time.

R. pipiens ventricular filling before contraction of the auricles was common during bradycardia produced by submersion, and was more likely the lower the heart rate became. It was invariably seen when the heart beat became arrhythmic (Fig. 5*b*).

When the frogs were submerged the stroke volume decreased, usually to a greater extent than the reduction in the heart rate. The combination of these two factors meant that the volume of blood pumped per minute by the heart fell to levels between

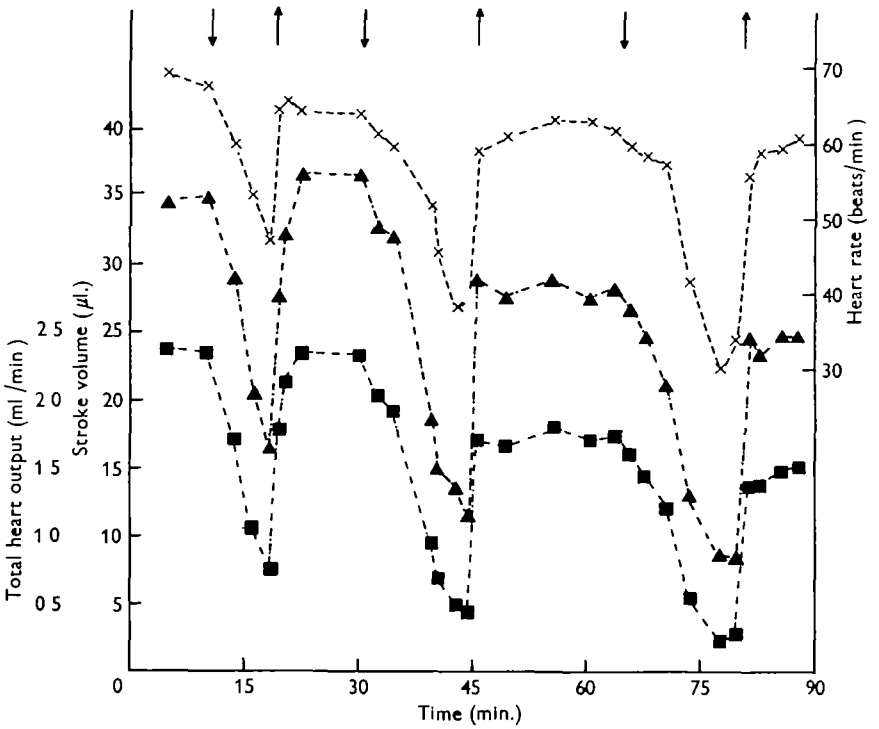

Fig. 8. Stroke volume, minute volume, and heart rate in *R. pipiens* (21 g.). Downward and upward pointing arrows indicate submersion and emersion respectively. ×, Heart rate; ▲, stroke volume; ■, minute volume.

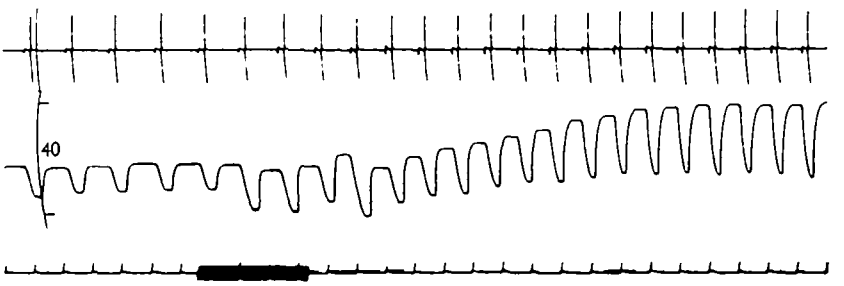

Fig. 9. The effect of surfacing on the total and stroke volume of the ventricle of *R. pipiens* (21 g.). Trace 1, electrocardiogram; trace 2, stroke volume ((μl.), up on trace = decrease in volume); trace 3, time (sec.), mark indicates emersion.

one-half and one-fifth of those found on the surface (Fig. 8). The cardiometer readings showed that as the stroke volume went down the ventricular volume increased not only after systole but also after diastole. This filling took place over a long time course and the DC level of the cardiometer had to be readjusted to make recording possible. This together with long-term fluctuations in level in the equipment made measurement

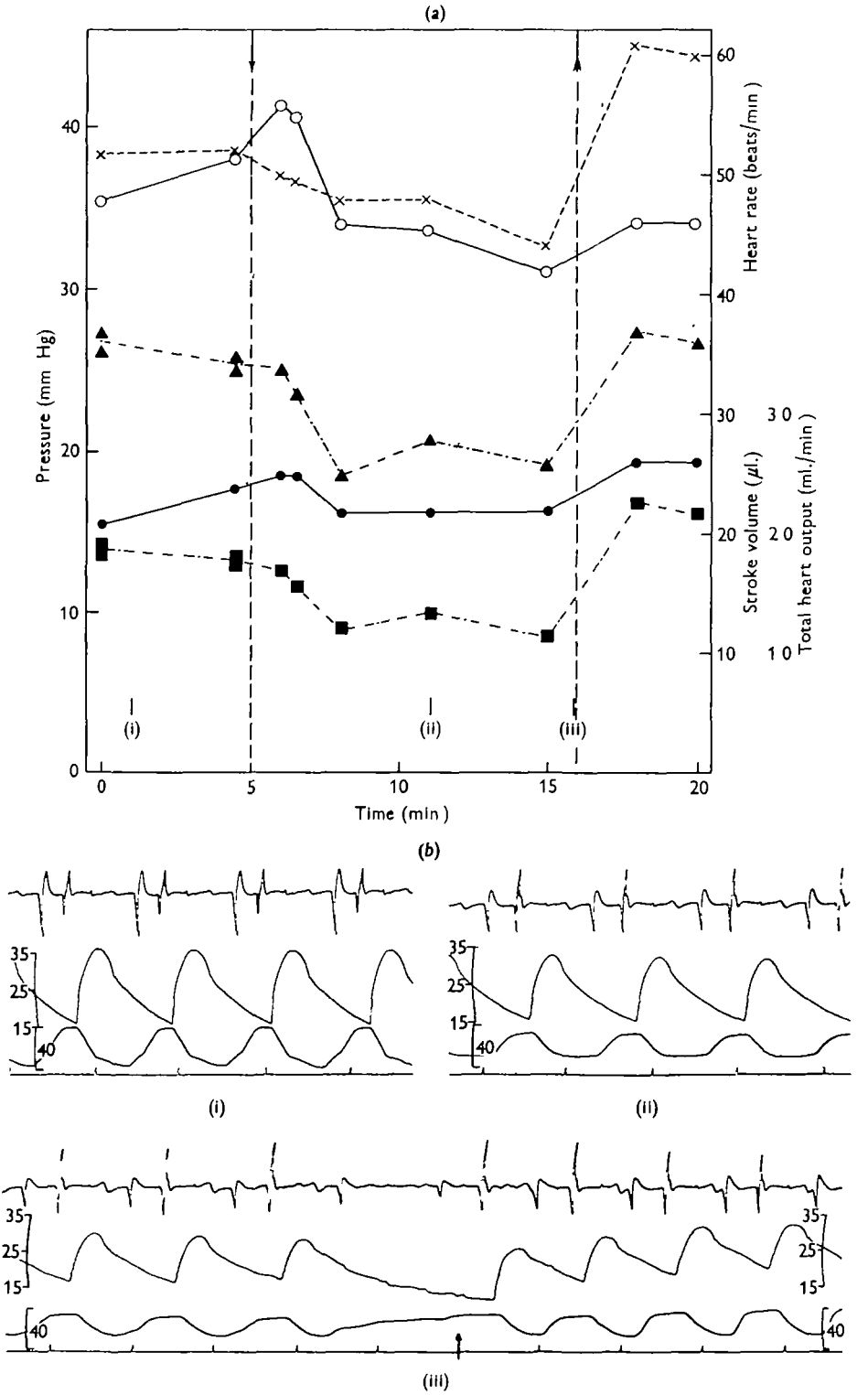

Fig. 10. (*a*) Heart output, carotid blood pressure and heart rate in *R. temporaria* (35 g.). Downward and upward pointing arrows indicate submersion and emersion respectively. ×, Heart rate, O, systolic pressure; ●, diastolic pressure; ▲, stroke volume; ■, minute volume. (*b*) Pressure pulses (mm. Hg) and stroke volumes ((μl.), down on trace = decrease in volume) recorded at times indicated on graph.

of the total volume change impossible. When the animal was surfaced recovery from the bradycardia and low stroke volume was rapid as was the decrease in operating volume of the ventricle (Fig. 9). The first few beats of the recovery usually occurred at a slightly greater ventricular volume. It was estimated that during recovery the total decrease in end diastolic volume was 10–15 μl. in the animal from which Figs. 8 and 9 were obtained.

Finally, to confirm that the large fall in stroke and minute volume was not accompanied by an equally large fall in blood pressure, simultaneous measurements were made of ventricular output and systemic pressures. In both *R. pipiens* and *R. temporaria* these experiments demonstrated that considerable change in minute volume occurred with little or no change in blood pressure. In the experiment from which the records of Fig. 7*a* were taken the diastolic pressure in the systemic arch was no lower than the level recorded at the surface though the minute volume had fallen to one-fifth after 17 min. under water. A smaller fall in output to one-half the pre-submergence level can be seen in Fig. 10, but again there was very little change in the diastolic pressure. The first few minutes of this experiment were noteworthy, in that heart rate and stroke volume were both falling, but systolic and diastolic pressures were rising. This suggests that peripheral vasoconstriction may be a more rapid reaction to submergence than the response of the heart itself.

DISCUSSION

Features of the pulse and ventricular output curves which have some bearing on the movement of blood through the frog heart are:

(1) The simultaneous appearance of the pulse wave in both systemic and pulmocutaneous arches even though the absolute pressure level is different.

(2) The slight difference in pressure which is maintained between the two arches right through systole, the systemic pressures being greater. De Graaf (1957) found a similar difference in *Xenopus*, but Simons (1957) working on frogs and toads claimed that there was no difference between the two pressures. Johansen (1963), recording the pressures somewhat more peripherally in *Amphiuma*, found that the systolic values were similar on both sides of the circulation and that no consistent difference was measurable.

(3) When the ventricular contraction is complete, pressures in the two arches begin to diverge with the pulmocutaneous pressure reaching the lower diastolic level. This pressure pattern was also found by de Graaf (1957) and sometimes by Johansen (1963). Simons (1957) thought that the diastolic pressures were not significantly different.

(4) As most of the recent workers have found, the peak systolic pressure is reached simultaneously in the two arches.

(5) In the air-breathing animal a marked second pulse, attributable to conus contraction, appears in the systemic pressure record. This is absent from the pulmocutaneous pressure wave though it appears here after the animal has been submerged for some time.

It is convenient to think of the cycle of activity as consisting of two distinct periods, the first when the pulmocutaneous and systemic circuits are in continuity with the

ventricle, and the second when the valves at the base of the conus are closed. The first period begins as the pressure rises simultaneously in both arches and continues throughout ventricular contraction. As the output curves show, contraction occurs during the rising and initial falling phases of the pulse curve and stops at the time of the inflexion in the systemic curve. Since the two arches open from the single pressure source the slight difference in recorded pressure during this period will depend on the product of flow rate and the resistance offered by the length of vessel situated between the ventricle and recording needle. The value of this product must be greater for the pulmocutaneous arch than for the systemic, though with the available data it is impossible to say whether flow rate, resistance, or both are the significant factors. In dissection that side of the spiral valve which leads to the pulmocutaneous arch looks small and the opening at the base of the conus is partially occluded by the spiral valve itself. Opening the conus obviously has a profound effect on the spatial relationships of these structures. The simplest explanation of the simultaneous pressure rise at the beginning of the first period is that the ventricle is capable of producing a step-like pressure change at least until the time that blood begins to leave the chamber. There would therefore be no detectable time interval between reaching the diastolic levels in the two arches. It is likely that the pressure rise in the ventricle is not a step function and that it becomes even less so after a period of submergence. The pulses still appear together and this lends support to the alternative suggestion that a valve, perhaps the spiral valve itself, shuts off the opening to the pulmocutaneous circuit until the time that blood begins to flow into the systemic chamber.

The second period begins when the ventricular contraction is completed. During this period the progressive divergence of pressures indicates that the two arches are anatomically separate not only in the truncus and more peripheral vessels but also in the conus since blood is known still to be leaving this part of the system. The only structure which can divide the conus is the spiral valve and the implication is that, although it is attached along one of its sides only, it is nevertheless capable of sustaining some difference in pressure in the two chambers leading to systemic and pulmocutaneous vessels.

In the second period the pressure falls more slowly in the systemic than in the pulmocutaneous arch. De Graaf (1957) suggested that in *Xenopus* this was due to a smaller peripheral resistance in the lung circuit but this is not necessarily the case. Since the propulsive force from the ventricle plays no part at this time, the pressures are maintained above zero level by the muscular contraction of the conus and elastic recoil of extended artery walls. Both of these can be regarded as making the system compliant, though the effect of active conus contraction is to introduce a discontinuity into what would otherwise be an exponential fall in pressure due to elastic recoil. The slope of the falling pressure curves will depend on the combination of the compliance of the system and the resistance of the periphery to flow. Clearly the peripheral resistance of the lung circuit need not be excessively low if the compliance of the conus and arterial reservoir is small on that side, as may be the case. It is the product of these two factors which is sufficiently small to make the run-off rapid and conus pressure pulse negligible in the pulmocutaneous arch. Conversely, the compliance of the post-ventricular reservoir or the resistance of the periphery or both must be greater on the systemic side of the circulation.

Because evidence as to actual flow rates is lacking it is impossible to complete the analysis of this situation in a satisfactory manner. As the left auricle appears to be carrying more blood than the right we would suggest tentatively that the resistance offered by the lungs is lower than that of the body circuit. This agrees with de Graaf's (1957) conclusions. Even if the resistances of the arterial bases are not appreciably different, the flow rates will result in the slightly higher systolic pressures recorded in the systemic arch. Finally, observation of the four major arteries on the body side of the circulation suggests a greater compliance than in the two vessels to the lungs and this, together with the greater peripheral resistance, will produce the relatively slow run-off. These conclusions are summarized diagrammatically in Fig. 11.

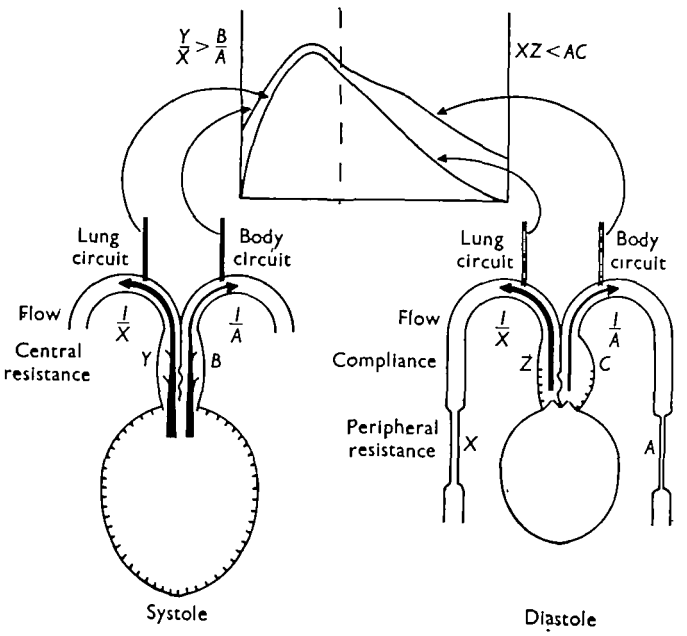

Fig. 11. Diagram to show relationships between resistance, compliance and flow in the body and lung circuits which will result in the characteristic pulse curves. For further explanation see text.

The adjustments which are made when the animal is submerged do not change the overall relationship of the two pulse curves to one another. Ventricular changes, though profound, appear to affect both systems equally. Decreased output in face of increasing diastolic volume argues in favour of a considerable fall in contractility of the cardiac muscle since normally the usual length-tension relationship exists. There is an accompanying increase in the duration of isometric contraction and a gentler slope to the rising pulse wave. We have carried out no experiments to show how these changes are produced though the direct effect of oxygen shortage on the heart muscle may be an important factor as we have suggested previously (Jones & Shelton, 1964). As the diastolic pressure varies surprisingly little during submersion it follows that there must be an increase in peripheral resistance of both lung and body circuits of approximately the same magnitude as the fall in heart output. The purely elastic

compliance in the system will not change during submersion. It is reasonable to expect that the contractile component of the conus may be affected though the evidence suggests that it is still very important. The compliance therefore makes a relatively larger contribution in the submerged animal where blood output is low and peripheral resistance high. Under these circumstances the pulse is small, the run-off prolonged, and a conus pulse appears in the pulmocutaneous arch. In all the cases we have examined these changes have been sufficient to overcome the direct effect of bradycardia, which alone would lead to a larger pulse.

The increased resistance must be produced by peripheral vasoconstriction and this has in fact been seen in the limb muscles during submersion. Other regions have not been examined and the work on peripheral blood pressures is still going on. The pattern looks very similar to that described in other vertebrates and it may be expected that the vasoconstriction is not uniform in all parts of the body. In the pulmocutaneous circuit vasoconstriction and almost complete stoppage of blood flow has been seen in deflated lungs and it is known that the lungs do deflate to some extent when the animal is submerged. From the functional point of view the reduced blood flow should be conducted largely to the cutaneous artery but this has not been confirmed.

Though the present experiments cannot show directly what is the fate of blood entering right and left auricles they do demonstrate that the conus can be regarded as a divided vessel during ventricular diastole and probably during systole. The spiral valve turns clockwise through something more than 180° towards the distal end of the conus and previous work (de Graaf, 1957; Simons, 1959) suggests that it is responsible for conveying blood from the left side of the ventricle to the ventral, carotico-systemic vessel and that from the right side to the dorsal, pulmocutaneous one. During submersion there appears to be no radical reorganization of this pattern of blood flow through the heart and arterial arches. The changes which do occur are due to selective peripheral vasoconstriction and a gradual decrease in heart output. The functional implications of these changes and the mechanisms of their control hold exceptional interest but as yet are largely unexplored.

SUMMARY

1. The systemic blood pressure of *Rana pipiens* and *R. temporaria* is slightly higher than the pulmocutaneous pressure at systole and much higher at diastole. The pulses differ in shape and a conus component can be seen in the systemic wave.

2. Submersion of the animal causes a fall in systolic pressure in both arches, the diastolic pressure remaining relatively constant. The shape of the pulse wave changes, the conus component being accentuated and visible in recordings from both arches.

3. Heart rate and stroke volume fall during submersion so that after 30 min. under water the minute volume may be 20–50% of the value at the surface. The heart becomes increasingly full of blood.

4. The differences in systemic and pulmocutaneous pressures are explained in terms of resistance, compliance and flow in lung and body circuits. The same general relationships persist during submersion but selective increases in peripheral resistance must occur to maintain the central blood pressure in face of falling heart output.

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