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VENTRICULAR OUTFLOW DYNAMICS IN THE LIZARD, *VARANUS NILOTICUS*: RESPONSES TO HYPOXIA, HYPERCARBIA AND DIVING

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INTRODUCTION

The typical squamate and chelonian hearts possess a ventricular chamber with three compartments in anatomical continuity (Webb, Heatwole & De Bavay, 1971) but with a high degree of preferential selective distribution of arterial and venous blood (White, 1968, 1971). The hearts of varanid lizards show a number of anatomical features unlike those in other lacertilians, and several of these are suggestive that central cardiovascular dynamics in varanids may differ from the typical squamate pattern. The cavum latero-dorsale (Webb *et al.* 1971), earlier synonymous with cavum dorsale (Mathur, 1944), has two compartments (the cavum arteriosum and the cavum venosum) and is in varanids a distinct chamber surrounded by a cone of dense trabeculae. The cavum arteriosum is functionally separated from the cavum venosum by a partial vertical septum, and during the inflow of blood from the left atrium into the cavum arteriosum the large left septal atrioventricular valve opens against this septum and prevents arterialized blood from filling the cavum venosum. Venous blood from the right atrium is directed through the cavum venosum into the much larger cavum pulmonale (*sic.* cavum ventrale) over a muscular ridge (Muskelleiste) (Webb *et al.* 1971). The outflow from the cavum pulmonale into the pulmonary artery is completely separated from the ostia of the right and left aortic arches emanating from the cavum venosum by the muscular ridge and its anterior continuation into the 'Bulbus lamelle' (Webb *et al.* 1971). The two aortic ostia are in communication with each other, the left being closest to the 'Bulbus lamelle'.

More complete accounts of the course of blood flow inside the ventricle during the filling and ejection phase as deduced from cardiac anatomy, have been offered by Webb *et al.* (1971) for the squamate heart and by White (1959, 1968, 1970) and by White & Ross (1966) for squamate and chelonian hearts, based on physiological data also.

It has been proposed (White & Ross, 1966) that in turtles a large increase in resistance to pulmonary outflow occurring during diving and during breathing hypoxic gas mixtures causes the prevailing left–right shunt during normal breathing to be altered into a marked right–left shunt. No direct measurements of blood flow and blood pressure in the vessels leaving the lacertilian heart have been reported, nor have studies been made of the dynamics of cardiac outflow during hypoxia, hypercarbia and diving. This study was undertaken to supply such information for the large varanid lizard, *Varanus niloticus*.

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METHODS

Five specimens of the lizard, *Varanus niloticus*, weighing from 1·250 to 3·250 kg, were studied. The animals were flown from Kenya, East Africa, and were kept in a terrarium regulated to 25–28 °C. The animals were fed on chicken eggs and laboratory mice.

The animals were cooled in iced water for 20 min prior to surgery. This precooling and local injection of procaine provided the anaesthesia necessary for surgery. Access to the heart and major vessels was gained through a ventral midline incision. Doppler ultrasonic flow transducers of appropriate size were secured on the right and left aortic arches, common carotid and left pulmonary arteries. The right aortic flow transducer was placed distal to the origin of the common carotid artery. Small polyethylene catheters (PE 50) were inserted into the right carotid artery via a lateral cervical incision and in a small lobar branch of the right pulmonary artery. The catheters were filled with heparinized Ringer solution. The thoraco-abdominal incision was closed and wire leads from the flow transducers and the catheters were led out through the lateral chest wall.

All animals recovered from the hypothermia and surgery. Measurements of cardiovascular data were not started until at least 6 hours of rest at room temperature. Recordings of blood flow and blood pressure were obtained during breathing of normal room air, during hypercarbia (breathing 10% CO_2 mixed with 90% air) and during hypoxia. During exposure to hypoxia the lizards were tied to a board in the prone position. Additionally, similar cardiovascular measurements were made during spontaneous diving in water at 22–25 °C. Room temperature during all studies ranged from 20 °C to 23 °C.

Blood flow was measured using an ultrasonic Doppler-shift system as described by Franklin *et al.* (1966). Flow calibrations were made by timed volume collections through excised vessels. Blood-pressure catheters of similar lengths were connected to strain-gauge manometers (Statham, P23Dd, P23BB) previously calibrated with a static column of water. Flow and pressure data were recorded on a direct-writing oscillograph (Brush Mark 260).

RESULTS

Cardiovascular dynamics during breathing of normal air

Heart rate averaged 29+3 beats/min at 20 °C in resting restrained lizards. Mean pulmonary arterial pressure was 19·5 ± 2·0 cmH_2O compared to a right aortic pressure of 118·0+3·0 cmH_2O. The pressure rise on ventricular contraction in the pulmonary artery preceded the right aortic pressure rise by 120 msec at a heart rate of 25 beats/min. While flow acceleration was of equal duration in the two vessels, the outflow through the pulmonary artery decelerated much more slowly, with flow continuing 640 msec after right aortic flow had ceased. The ejection periods therefore lasted 50% of the total length of the cardiac cycle in the pulmonary artery against 25% for the right aortic arch. Whereas flow in the right aortic arch, the common carotid or the left pulmonary artery was readily detectable with peak flow values of 80–90 ml/min in the right aorta and 40–50 ml/min in the left pulmonary artery during breathing of normal air (Fig. 1), the amount of blood passing through the left arch was barely

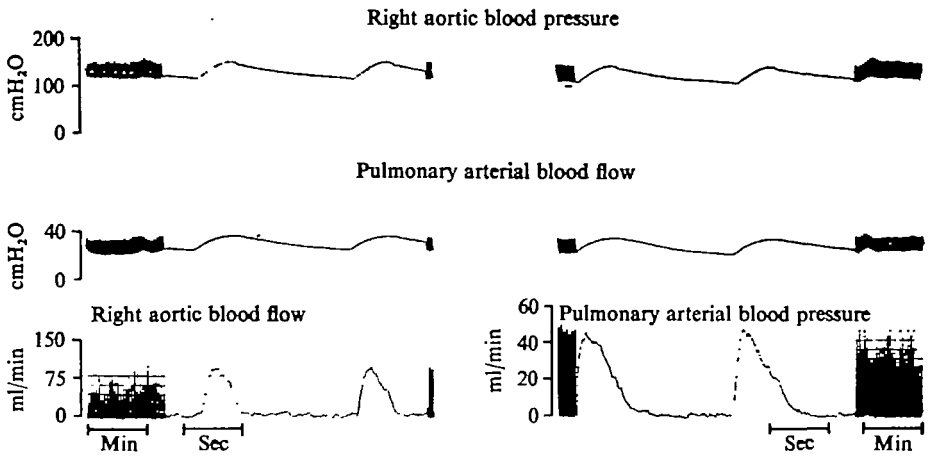

Fig. 1. Simultaneously recorded tracings of blood flow and blood pressure from the right aorta and left pulmonary artery of *Varanus niloticus* during normal undisturbed breathing in air.

detectable during these conditions and quite obviously much smaller than through the right aortic arch. Assuming negligible flow in the left aortic arch, the flow in the right aortic arch and carotid artery combined (120 ml/min) balanced the flow in the two pulmonary arteries, assuming that the right pulmonary artery carried the same flow as was measured in the left pulmonary artery. Using these flow values for an estimation of systemic and pulmonary vascular resistance reveals that the systemic vascular resistance is about 6 times higher than the pulmonary during normal breathing with balanced flow in the two circuits.

Responses to breathing of CO_2-enriched air

Breathing a mixture of 10% CO_2 in air for 10–15 minutes produced marked changes in vascular outflow resistance. The right aortic resistance increased sharply by about 120% of the pre-hypercarbic value while left pulmonary arterial resistance increased moderately by 30%. Meanwhile CO_2 breathing reduced carotid artery vascular resistance to about one third of the pre-hypercarbic value indicating that the tissues perfused by this vessel and its derivatives receive proportionately much more blood during CO_2 breathing. Notably this occurs while the vascular resistance in the parent vessel, i.e. the right aorta, increases markedly.

Heart rate did not change significantly during CO_2 breathing. During prolonged CO_2 breathing the increase in right aortic resistance again decreased to pre-hypercarbic values after 25 min of CO_2 breathing, while the pulmonary resistance also remained moderately increased after 25 min.

Fig. 2 presents the relationships between systolic pressure values in the right aorta and left pulmonary artery during breathing of normal air and during CO_2 breathing. Each point on the graph was obtained from spontaneous pressure changes occurring in both situations and representing the systolic pressures in the same heart beat. The noteworthy feature in the figure is the dramatic shift to the right, i.e. an increase in the pulmonary systolic pressure during CO_2 breathing while the corresponding right aortic pressures remained essentially the same.

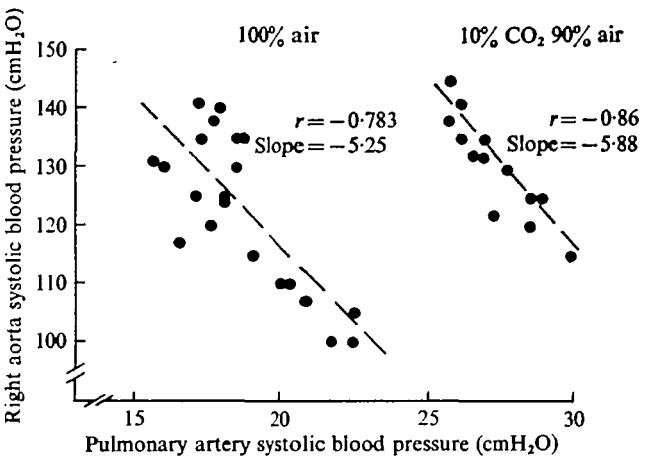

Fig. 2. A comparison of systolic blood pressure values in the right aorta (ordinate) and left pulmonary artery (abscissa) during normal air breathing (to the left) and during breathing 10% CO_2 in air. Each plotted point represents the systolic blood pressure value recorded during the same cardiac cycle.

The ratio of systemic to pulmonary systolic pressures ranged between 8·1 and 4·4 during breathing of normal air and between 5·6 and 3·8 during CO_2 breathing. At no time was there an overlap between the pressures in the right aorta and in the pulmonary artery.

Responses to hypoxia

Marked changes in heart rate, blood pressure and stroke flow in the ventricular outflow vessels took place when the lizards were breathing hypoxic gas mixtures. Apnoea occurred immediately, which gradually yielded, however, to tachypnoea and arrhythmic tachycardia. Fig. 3 illustrates the blood-pressure changes in the pulmonary artery and right aorta during hypoxic breathing. Pulmonary pressure rose throughout the exposure period to a maximum of $36{\cdot}0 \pm 4{\cdot}0$ cmH_2O while right aortic pressure fell to $100{\cdot}0 \pm 10{\cdot}0$ cmH_2O (Fig. 5). Peak systolic flow in the common carotid decreased by 50% during hypoxic breathing and returned to control levels after 10 min recovery in air. Ejection time expressed as fraction of the total cardiac cycle remained unchanged at 45–50% in the right aorta during hypoxic breathing while ejection in the pulmonary artery was extended with flow lasting throughout diastole. Stroke-flow changes in the two vessels during hypoxic breathing are illustrated in Fig. 4. The stroke-flow changes are seen to roughly parallel each other. The relatively greater flow increase in the right aorta as apparent in Fig. 4 does not indicate that total systemic flow is changed relative to total pulmonary flow since the right aortic flow transducer was placed distal to the branching point of the common carotid from the right aorta.

The resultant effect of the changes in pressure and flow on the vascular resistance in the right aorta and left pulmonary artery associated with hypoxic breathing is one of making the pulmonary vascular resistance markedly higher than control values whereas the vascular beds perfused by the right aorta maintain nearly the same resistance. Notably, however, this change did not cause a right to left (venous to arterial) intracardiac shunting from the cavum pumonale to the outflow orifices of the

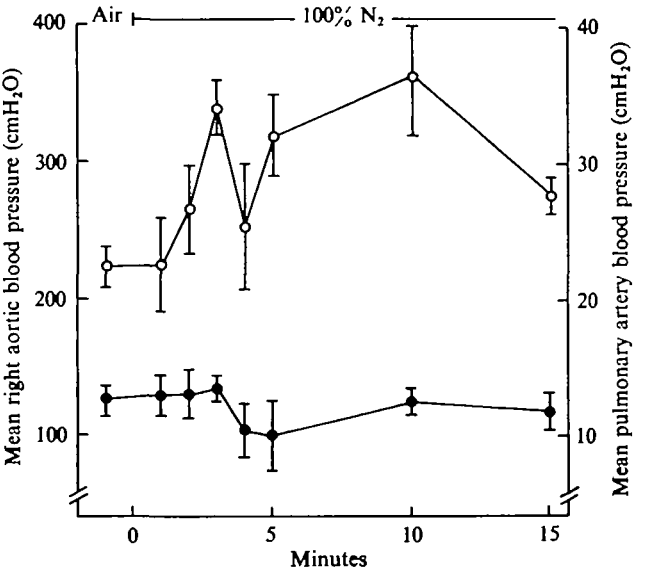

Fig. 3. Mean values of blood pressure (± S.E.) in the right aorta (open circles) and left pulmonary artery (filled circles) during nitrogen breathing. Each value represents a minimum of five experiments.

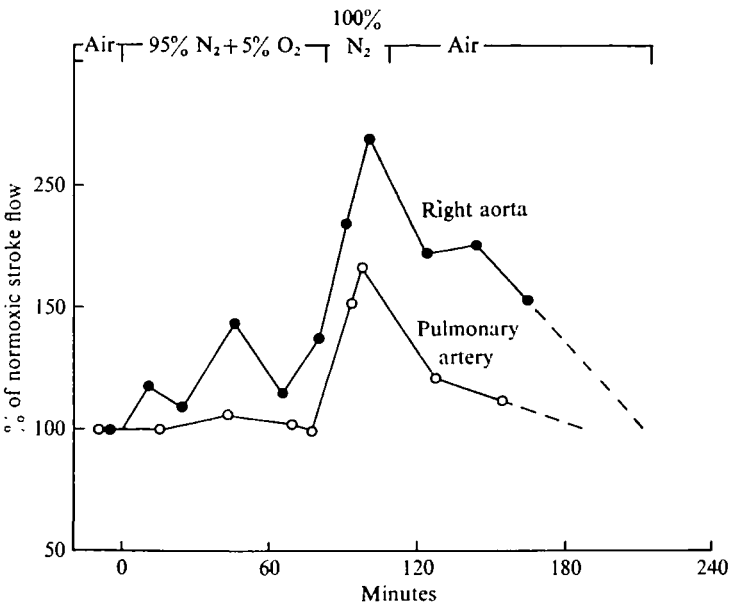

Fig. 4. Changes in stroke flow in the right aorta (filled circles) and left pulmonary artery (open circles) during hypoxic breathing and recovery in air. The values are expressed as percentage change from conditions at normoxic breathing.

aortae in the cavum venosum during systolic contraction, since the pulse pressures in the outflow vessels of the two circuits never overlapped at any time during the cardiac cycle. With an increase in heart rate and stroke flow in both the systemic and pulmonary vascular circuits the cardiac output must have been elevated in response to

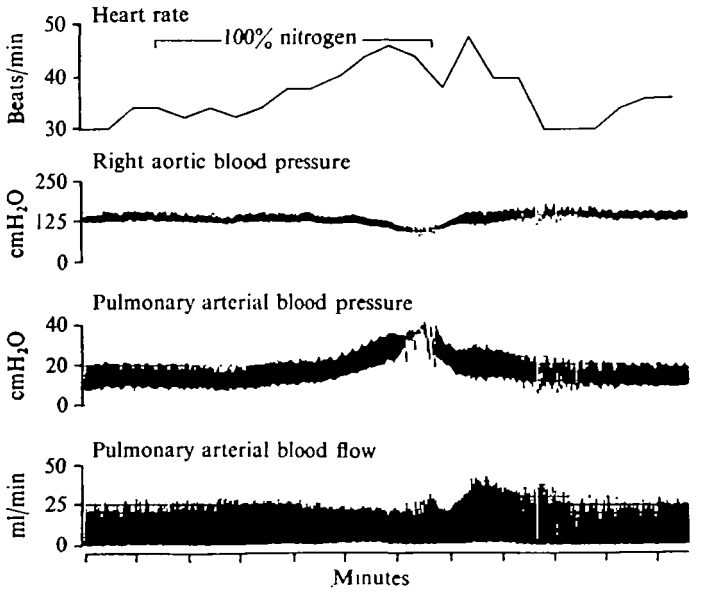

Fig. 5. Heart rate, right aortic blood pressure and left pulmonary blood pressure and flow during nitrogen breathing.

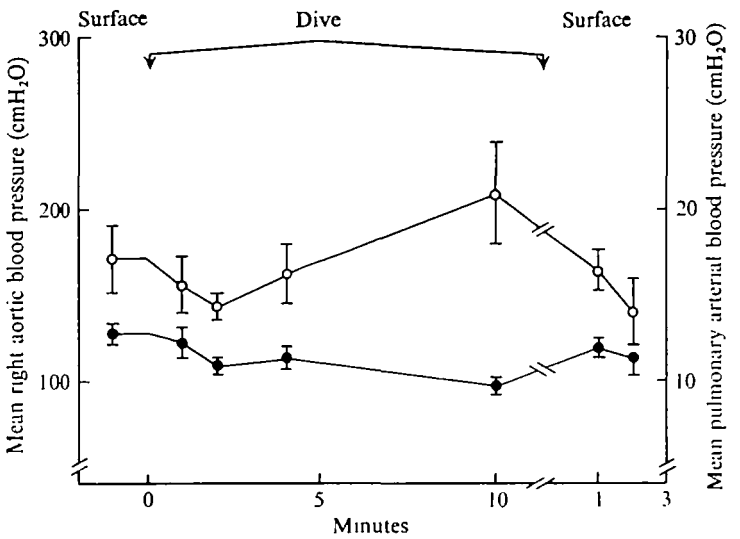

Fig. 6. Mean values of blood pressure (±S.E.) in the right aorta (filled circles) and left pulmonary artery (open circles) during voluntary diving. Each value represents a minimum of five experiments.

hypoxic breathing. During recovery from hypoxic breathing heart rate rose transiently to even higher levels, while right aortic pressure (Fig. 5) returned to about control values. The decline in pulmonary systolic flow during hypoxic breathing as apparent in Fig. 5 does not reflect a decline in overall flow since the diastolic flow was greatly increased resulting in an increase in total flow.

Circulatory changes during voluntary diving

Heart rate during voluntary diving fell in proportion to the duration of the dive. Pulmonary arterial pressures rose throughout the course of a 10 min dive to reach a mean value of 21 cmH_2O, while right aortic mean pressure simultaneously fell markedly to 98 cmH_2O (Fig. 6).

DISCUSSION

The blood pressures recorded in *Varanus niloticus* stand out among earlier findings in other reptiles by the very high pressures in the systemic arteries coupled with low pulmonary pressures. Typically in chelonians and crocodilians the peak systolic arterial pressures range between 30 and 50 mmHg, with pulmonary pressures showing far larger pulse pressures but lower values of peak systolic pressures (Andersen, 1961; Greenfield & Morrow, 1961; White, 1970). Snakes may show high systemic arterial pressures but show also higher and more variable pulmonary arterial pressures (Standaert & Johansen, unpublished). The double aortic arch system in all reptiles shows no differences in the absolute pressure value or in the synchrony of pulses between the right and left aortae during normal breathing. It is, moreover, a common feature that the systolic pressure rise in the pulmonary artery always precedes the systolic pressure rise in the two aortae (Woodbury & Robertson, 1942; Johansen, 1959; White, 1968).

The phylogenetical development of the systemic and pulmonary (gill) vascular circuits, as high-pressure and low-pressure circulations respectively, place amphibians with ratios of systemic to pulmonary vascular resistance around 2·0, and fishes slightly higher between 2·5 and 3·5. Reptiles studied earlier (alligators, snakes, turtles) similarly show resistance ratios between about 2·0 and 3·0 (Johansen, 1972). The presently obtained data on *Varanus niloticus* yield resistance ratios for varanids far higher than for other reptiles studied, with values during normal breathing between 4·0 and 6·0, approaching the typical mammalian values of 7·0–10·0; and this again is exceeded by birds, showing systemic to pulmonary vascular resistance ratios between 10·0 and 12·0. A comparison of mean pressure values in the systemic and pulmonary arteries also places *Varanus niloticus* closer to values in homeotherm vertebrates (Fig. 2) than other reptiles and suggests that the varanid heart is haemodynamically advanced in the direction of the homeotherm condition.

The central and much discussed problem of the outflow distribution of arterial and venous blood from the incompletely divided ventricle of non-crocodilian reptiles also place the varanids, based on the present results, distinct from other squamate and chelonian reptiles. It now seems generally recognized that during normal breathing the two aortae carry blood of similar oxygen content and that the O_2 saturation of left atrial blood is closely reflected by the saturation values in the right as well as the left aorta (White, 1959; Tucker, 1966). A claim from Khalil & Zaki (1964) that admixture of arterial and venous blood occurs in both directions inside the ventricle during cardiac contraction can be criticized because they used decerebrate animals ventilated artificially. Meanwhile there are reports from work on squamate, chelonian and crocodilian reptiles that directional shunting of blood inside the heart may occur in response to an altered resistance of the pulmonary and/or systemic

vasculature. Thus an increase in pulmonary resistance associated with diving in turtles will cause a right-to-left shunt (White & Ross, 1966). A partial by-pass of the lungs and a resultant increase in systemic blood flow has also been suggested as a regulatory measure to increase heat transport (Tucker, 1966; Baker & White, 1970). Such right-to-left shunting usually becomes manifest by the appearance of lowered O_2 saturations in the left aortic blood relative to the right aortic and the left atrial blood. The alteration of the cardiac output distribution in turtles was associated with increased pulmonary arterial pressure to equal the pressures of those in the aortae, both in magnitude and time course, during the period of ejection through the aortic valves (White & Ross, 1966). Such overlap in pressures during cardiac ejection is probably the force behind the directional shunting. Millen *et al.* (1964) working on turtles have similarly demonstrated, using dye dilution technique, that a normally prevailing left-to-right shunt is reversed during diving when pulmonary blood flow is markedly reduced.

The present results on *Varanus niloticus* show that although the pulmonary vascular resistance is increased in response to hypoxia as well as to hypercarbia and to diving the pulmonary and systemic pressures never become super-imposable, indicating that the right aorta and the pulmonary arteries are perfused by functionally different pumps and that no intra-ventricular mixing can occur during systole and cardiac ejection. Most importantly, Harrison (1965) has demonstrated in *Varanus varius* that pressure traces obtained from the cavum venosum have much higher systolic values than those recordable from the cavum pulmonale, a finding implying that the two chambers are functionally distinct.

An increase in the pulmonary vascular resistance during hypoxia or diving is a common attribute of all vertebrates studied for this effect, including the branchial circulation in fishes (Satchell, Hanson & Johansen, 1970). White & Ross (1966) demonstrated that the increased pulmonary resistance in diving turtles persisted if the animal surfaced into CO_2-enriched or hypoxic air but was rapidly reversed when the animal returned to breathing normal air. Similar changes in the pulmonary resistance were demonstrated in the present study in which increased pulmonary resistance persisted during hypercarbia while systemic resistance was only transiently increased.

The data obtained on *Varanus niloticus* exclude the possibility that intracardiac mixing can occur during systolic contraction and thus distinguish between varanids and other squamates and chelonians studied. Intracardiac shunting of arterial and venous blood can hence only occur during cardiac filling or from systolic residual blood. A right-to-left shunt by ejection of venous blood located in the cavum venosum to the aortae after complete filling of the cavum pulmonale could occur, for instance, if this venous blood is not diverted into the cavum pulmonale as a result of the earlier ejection through the pulmonary arteries but is instead ejected through the systemic arteries at the start of systole. Blood entrapped in this way in the cavum venosum during systole, when the muscular ridge has separated the cavum pulmonale from the cavum venosum and made these compartments functionally distinct pumps, would have to mix with arterialized blood early in systole and thus be ejected to the systemic side. A possible left-to-right shunt could result if there was a substantial residual volume in the cavum venosum after completion of systolic ejection. This blood, being arterialized and coming from the left atrium via the cavum arteriosum, could hence

fill part of the cavum pulmonale during the early part of the succeeding diastolic filling. These shunt possibilities, particularly the latter, are likely to play minor roles in the overall distribution of blood from the heart since both the capacity of the cavum venosum and the residual volumes remaining after ejection are very small. A complete assessment of the shunt patterns inside reptilian hearts must, however, await simultaneous measurements of flow and pressure in all the outflow vessels leaving the heart as well as measurements of blood-gas levels in blood returning to and distributed from the heart.

The present results imply, however, that the variable pulmonary by-pass (right-to-left shunting) occurring in other reptiles when alveolar O_2 tension goes down during diving or hypoxic breathing is much reduced and is probably absent in *Varanus niloticus*. The same applies to the partial systemic by-pass (left-to-right shunting) occurring shortly after a breath when alveolar O_2 tension is maximum. These shunt possibilities, assumed to be controllable, constitute an important means by which pulmonary perfusion can be matched to the O_2 availability in the lung. The loss of such shunt possibilities in the birds and mammals, as a consequence of the complete anatomical separation of the systemic and pulmonary circuits, appears also to have been reached or approached in *Varanus*, but most importantly before a complete anatomical separation has become established.

This important distinction between *Varanus* and other reptiles studied is accompanied by a much higher value of the normal ratio between systemic and pulmonary vascular resistance. From this accrues a high-pressure systemic circulation and a low-pressure pulmonary circulation, both of which are essential to efficient operation of the double circulation in the homeotherm vertebrates.

SUMMARY

1. Blood flow and blood pressure have been measured in the right aorta and left pulmonary artery of the semi-aquatic lizard, *Varanus niloticus*, during normal breathing, during hypercarbic and hypoxic breathing and during voluntary diving.

2. Mean pulmonary blood pressure during normal breathing was $19{\cdot}5 \pm 2{\cdot}0$ cmH_2O while right aortic pressure was $118{\cdot}0 \pm 3{\cdot}0$ cmH_2O. The high systemic blood pressure and high ratio of systemic to pulmonary vascular resistance (4·0–6·0) stand out among reptiles and approach values in homeotherm vertebrates.

3. Pulmonary pressure rise preceded right aortic pressure rise by 120 msec at a heart rate of 25/min. Pulmonary ejection lasted 50% of the cardiac cycle compared to 25% for aortic ejection during normal breathing.

4. CO_2 breathing increased right aortic vascular resistance by 120% while pulmonary resistance increased moderately by 30%. Carotid vascular resistance decreased during CO_2 breathing. The pulmonary blood pressure increase was however much higher than the systemic, but at no time did systemic and pulmonary blood pressures overlap.

5. Hypoxic breathing increased pulmonary blood pressure to $36{\cdot}0 \pm 4{\cdot}0$ cmH_2O while right aortic pressure fell to $100{\cdot}0 \pm 10{\cdot}0$ cmH_2O. Ejection time remained unchanged in the right aorta while pulmonary flow became continuous. Overall pulmonary vascular resistance increased markedly while systemic resistance changed little.

6. Voluntary diving increased pulmonary blood pressure while the systemic blood pressure fell markedly.

7. The results obtained are discussed in the light of ventricular outflow distribution in reptiles. Directional shunting of blood inside the heart of *V. niloticus* during cardiac systole must be reduced or absent. Intracardiac shunting during cardiac filling or by systolic residual volumes is small, placing varanid lacertilians haemodynamically closer to homoetherm vertebrates than other reptiles studied.

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