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THE MECHANISM OF INTRACARDIAC SHUNTING IN THE LIZARD *VARANUS EXANTHEMATICUS*

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SUMMARY

Intracardiac shunting was studied in unanaesthetized and unrestrained specimens of *Varanus exanthematicus* by simultaneous injection of radioactively labelled microspheres (15 μm) into the right and left atria. Lung ventilation was monitored by intratracheal pneumotachography. It was found that intracardiac shunting was not significantly affected by the spontaneously occurring periods of ventilation and apnoea: the right-to-left shunt averaged 29 and 31 %, respectively, and the left-to-right shunt was 11 % in both conditions. The observed shunting, although rather constant with time and independent of the ventilatory state, varied in different individuals. Anatomical studies and intracardiac pressure measurements revealed that, in spite of crocodilian-like systolic pressure separation between pulmonary and systemic circulation (based on the muscular ridge, 'Muskelleiste', between cavum venosum and cavum pulmonale), the cavum venosum is shared by both the pulmonary and the systemic circulation. Intracardiac shunting appears to be mainly due to wash-out of the cavum venosum: blood remaining in this chamber at the end of systole (oxygenated) or at the end of diastole (deoxygenated) is washed into the respective 'inadequate' vascular bed during the next half-cycle of heart action. Thus the extent of intracardiac shunting is expected to depend primarily on the volume and the changes in volume of the cavum venosum during the cardiac cycle.

INTRODUCTION

Intracardiac shunting and the mechanisms of systemic and lung blood flow separation in the incompletely divided ventricle of non-crocodilian reptiles have been the subjects of various physiological studies (e.g. Prakash, 1952; Foxon, Griffith & Price, 1956; White, 1959; Khalil & Zaki, 1964; Tucker, 1966; Baker & White, 1970; Millard & Johansen, 1974; Berger & Heisler, 1977), leading to rather conflicting results between the two extremes of no intracardiac shunting at all, and shunting to a large extent in both directions (R→L, L→R, or both simultaneously). Attempts have been made to explain these extreme discrepancies by anatomical or other species differences, differences in experimental conditions (constant temperature; heating or

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cooling), or to a modulation of shunting with the aim of conserving energy or serving gas exchange requirements.

In fact anatomical differences are found between different orders of reptiles (Chelonia and Squamata), between suborders (Lacertilia and Ophidia), and even within suborders (Varanidae and non-varanid Lacertilia) (for references see: Webb, Heatwolfe & DeBavay, 1971; Webb, 1979; Mathur, 1944). Also, some physiological measurements have suggested that the intracardiac haemodynamics may differ between species. The turtle *Pseudemys scripta* (Chelonia) (White & Ross, 1966; White, 1968) and the grass-snake *Tripodonotus natrix* (Ophidia) (Johansen, 1959) exhibit almost identical peak blood pressures in aortic arches and pulmonary artery, whereas in *Iguana iguana* (non-varanid Lacertilia) peak pressure in the aortic arches is about 50% higher than in the pulmonary artery (White, 1968). Pressure separation in varanids is even better, resulting in 2·5 to 3·5 times higher aortic than pulmonary peak blood pressures (Harrison, 1965; Millard & Johansen, 1974; Burggren & Johansen, 1982). These factors are about the same as those found in crocodiles (White, 1968) which, with their completely divided ventricle, are considered to be the most advanced reptiles in this respect (Webb, 1979).

Differences in functional anatomy of reptiles, however, cannot explain the extreme variability of shunting, which has been observed in individual species (*Iguana iguana*: Tucker, 1966; Baker & White, 1970; *Varanus exanthematicus*: Berger & Heisler, 1977). In the turtle, *Pseudemys scripta*, the direction of net shunting appears to be correlated to ventilatory (L→R shunt) or apnoeic periods (R→L shunt) (White & Ross, 1965). If shunting in Lacertilia was correlated to the ventilatory period in a similar way, this modulation could at least partially be responsible for the variability of intracardiac shunt measurements in *Iguana* and *Varanus*.

The present study was performed in order to evaluate the influence of the respiratory state, ventilation or apnoeic period, on intracardiac blood flow separation and to shed more light on the mechanisms of intracardiac shunting in reptiles.

MATERIALS AND METHODS

Savannah Monitor lizards (*Varanus exanthematicus* Bosc, weight 1140–1970 g) caught in the wild were imported from Kenya and kept in the laboratory for at least 8 weeks prior to experimentation. As they were massively infested with internal (mainly nematodes) and external parasites (mainly ticks) as well as infected with salmonellae they were repeatedly treated with anti-helminths, insecticides and chloramphenicol. After treatment they fed well on chopped liver and heart as well as on rats and mice. They were acclimated to a temperature of 30 ± 0·5 °C in large terraria equipped with hiding caverns and water pools for at least 4 weeks. The terraria were flushed with thermostatted air (30 °C) at a rate of more than 4 l min^{-1} $animal^{-1}$. Three-to-five days before surgery the respective animals were isolated in separate terraria and remained unfed.

Surgical preparations

At least 2 days before the experimental procedure, general anaesthesia was introduced by evaporation of halothane in a closed terrarium. After loss of reactivity

The animals were intubated intratracheally through the glottis with an appropriate polyethylene tube and artificially ventilated with oxygen-enriched air/halothane gas mixture (30–40 % O_2, 0·5–2 % halothane) throughout surgery. With the animal laid on its back, the thorax was cut open mid-ventrally and the pericardium was opened in the area of the auricles in order to implant indwelling catheters into both atria (Fig. 1). Under microscopic control the atria were lifted with a very small atraumatic surgical clamp, and within 10–15 s a fine hole was cut into the wall of the atrium and a PE 50 catheter mounted with a metal tip (enhancing the mixing of injected microspheres with the blood) was introduced into the atrium and secured with a circular very fine polyamide suture. The metal tip consisted of a 5 mm length of 0·55 mm o.d. stainless steel tube with a drop of silver solder on one end (diameter of 0·95–1 mm) with five holes of 0·4 mm diameter drilled to the lumen of the stainless steel tube (Fig. 1). The cannulation procedure was repeated at the other atrium. The atrial catheters were fed out of the pericardium in large loops in order not to disturb the movements of the heart and the pericardium was closed again with extremely fine atraumatic sutures under microscopic control. Both catheters were led out of the body cavity through an intercostal space at the

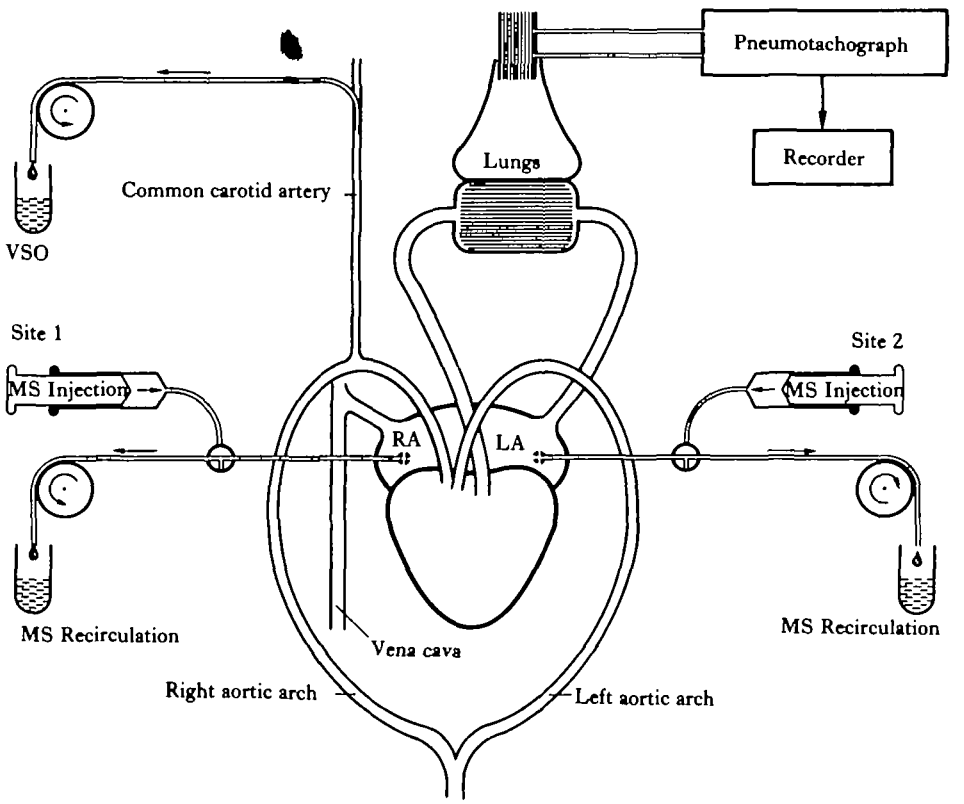

Fig. 1. Schematic representation of the central vascular system of *Varanus* and of the microsphere (MS) injection technique. Indwelling catheters are implanted into right and left atria for injection of microspheres and withdrawal of blood for estimation of microsphere recirculation. Withdrawal of blood from the catheter in the right common carotid artery yields ventricular systemic output (VSO). Ventilation is monitored by a pneumotachograph sensor implanted into the trachea (for details see text). RA = right atrium, LA = left atrium.

side of the animal and under the skin to a skin hole in the midline on the back of the animal. The thorax wall was closed with three layers of atraumatic sutures.

The right common carotid artery was cannulated occlusively *via* a mid-ventral incision at the neck of the animals. Through the same incision, a pneumotachograph sensor, made of a thin-walled stainless steel tube with two connectors for pressure difference measurements, was implanted into the trachea. With a set of different sensors the inner diameter of the trachea could be matched to the nearest 0·5 mm. The three PE 50 catheters (two from the pneumotachograph, one from the carotid artery) were fed under the skin to the same hole as the two atrial catheters and all five were armed with a light stainless steel spring sewn to the back skin of the animal (Fig. 2). The neck incision was closed again with atraumatic sutures. After surgery the animals were artificially ventilated with room air until they had completely recovered and were then transferred into the experimental apparatus.

Experimental apparatus

The experiments were performed in an 80 l terrarium which was thermally insulated and shielded against visual disturbances except for the Plexiglass lid (Fig. 2).

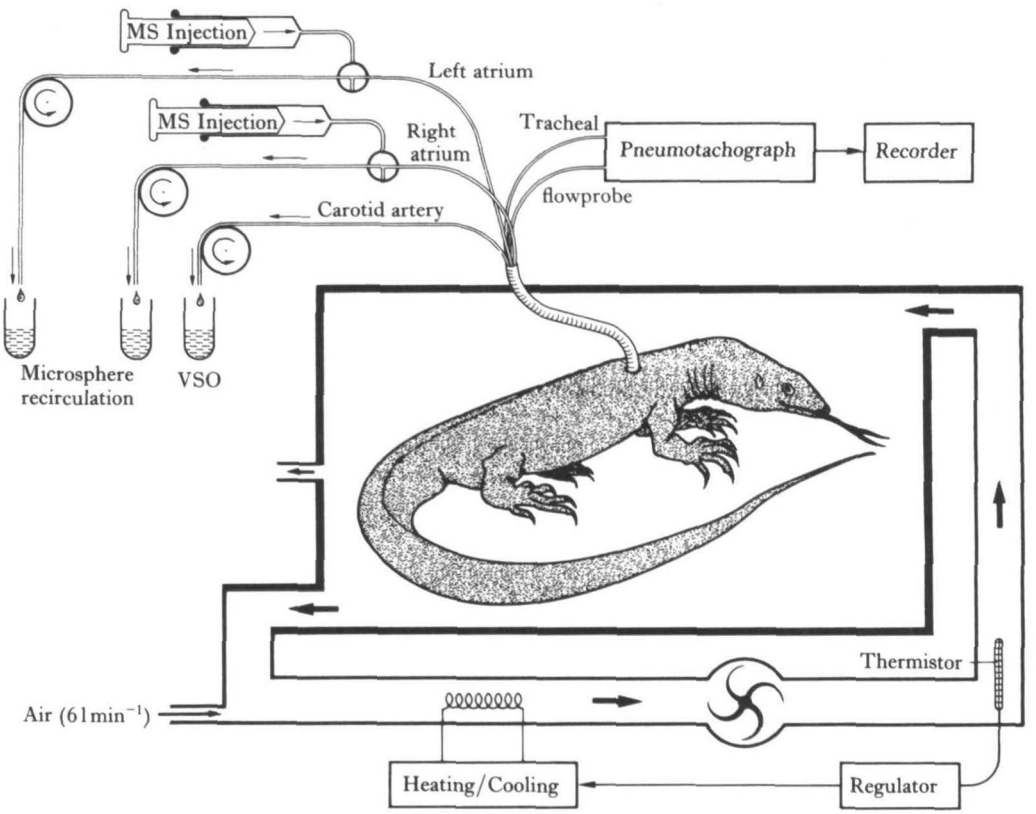

Fig. 2. Experimental set-up. Unanaesthetized and unrestrained specimens were kept in a thermostatted and shielded terrarium. Five indwelling catheters (from the right and left atria, the carotid artery and the tracheal pneumotachograph sensor) are fed under the skin to a skin hole and through a light stainless steel spring out of the terrarium to the injection and withdrawal devices and to the pneumotachograph.

Temperature was kept constant (30 ± 0·2 °C) by an external control circuit consisting of a fan, a heating/cooling unit and a temperature regulator. The terrarium was flushed with fresh air at a rate of 6 l min^{-1}. The stainless steel spring surrounding the five catheters from the animal (Fig. 2) was clamped in the lid in such a manner that the animal could move quite freely and with little disturbance in the terrarium. The two catheters from the tracheal flow probe were connected to a pneumotachograph differential pressure transducer (Godart Statham) and allowed semiquantitative monitoring of lung ventilation (Fig. 2). The atrial catheters were connected *via* three-way valves either to syringes for injection of radioactively labelled microspheres or to constant flow roller pumps for withdrawal of blood for the determination of microsphere recirculation. The arterial catheter was directly connected to a roller pump (Figs 1, 2) for determination of systemic ventricular output.

Procedure

After at least 2 days of recovery from anaesthesia and surgery, two batches of differently-labelled microspheres (2–6 × 10^5 spheres, ^{141}Ce, ^{51}Cr, ^{85}Sr or ^{46}Sc, nominal diameter 15 μm) suspended in 0·5 ml 10 % dextran solution (for details of the procedure see Neumann, Holeton & Heisler, 1983) were injected, by a pneumatic syringe drive, simultaneously into the left and right atria. Thereafter, the catheters were flushed with 0·7–1 ml dextran solution within about 20 s. Immediately after injection, the three-way valves (Figs 1, 2) were actuated and blood was withdrawn from the atria at a rate of either 0·5 or 0·6 ml min^{-1} for 8 min for the correction of shunt values according to microsphere recirculation. Simultaneously with the microsphere injection, blood was withdrawn at the same rate from the carotid artery for determination of ventricular systemic output according to the method of the 'artificial organ' (for details see: Berger & Heisler, 1977).

Microspheres with different label were injected simultaneously into the two atria (sites 1 and 2, Fig. 1), once during a period of intense breathing and once again with a second set of two different labels during apnoea which had lasted for at least 2 min. Only experiments in which apnoea continued for at least another 2 min were used.

About 15 min after the final microsphere injection and the correlated blood sampling procedure the animals were killed by injection of an overdose of pentobarbital. The animals were completely dissected into 1–3 g samples which were analysed together with the blood samples for radioactivity by gamma scintillation counting with a multichannel analyser (Model 5986, Packard Instruments, Inc.). The activity of the four individual labels was recalculated by a computer programmed with an efficiency matrix obtained from standard countings, immediately before counting of the samples (for further details see Berger & Heisler, 1977; Neumann *et al.* 1983).

Calculations of shunts, heart output etc. have been reported before (Berger & Heisler, 1977).

Anatomical preparations

For the precise evaluation of the anatomical arrangement of the chambers, valves and effluent vessels in the heart of *Varanus*, 15 heart/lung preparations were fixed in 16 % formaldehyde which was later washed out with 70 % ethanol. Transverse sections were made through the valve plane and through the chambers at about one

quarter of the distance between valves and the tip of the heart (Fig. 4). In addition, various other sections were performed, the chambers opened, and thin colour-coded wires introduced along the preferential blood streams from the sinus venosus *via* the right atrium into the pulmonary artery and from the pulmonary veins *via* the left atrium into the left and right aortic arches.

Intracardiac pressure measurements

In order to evaluate the degree of pressure separation between the various chambers of the heart, thin stainless steel cannulae (0·55 o.d.) were inserted into the cavum arteriosum, the cavum venosum close to the caudal end of the muscular ridge (see anatomy of the hearts), the cavum pulmonale, the arteria pulmonalis, and the left aorta of eight varanid hearts in open chest preparation. The cannulae were connected to five Statham pressure transducers and the pressures as well as pressure differences between cavum arteriosum and cavum venosum were recorded simultaneously. The position of the cannula tips was verified after the end of the experiment.

RESULTS

Ventilation, ventricular output and intracardiac shunting

Ventilation, measured by pneumotachography in the trachea, was fairly regular with decreasing frequencies from 8–10 min^{-1} to 3–5 min^{-1} during the first 12–18 h after recovery from anaesthesia, with no apnoeic intervals longer than 30 s between breaths. When the animals had become acquainted with the terrarium and the slight resistance of the catheter assembly on their back, they gradually assumed an intermittent type of breathing with periods of large ventilatory movements, periods of only slight ventilation, and periods of total voluntary apnoea of up to 8 min (Fig. 3). Visual or acoustic disturbances resulted in immediate termination of apnoea and continuous ventilation for several hours. The same disturbances during periods of continuous ventilation had little immediate effect on ventilation, but resulted in regular breathing without apnoea for several hours. The pneumotachograph sensors could in principle

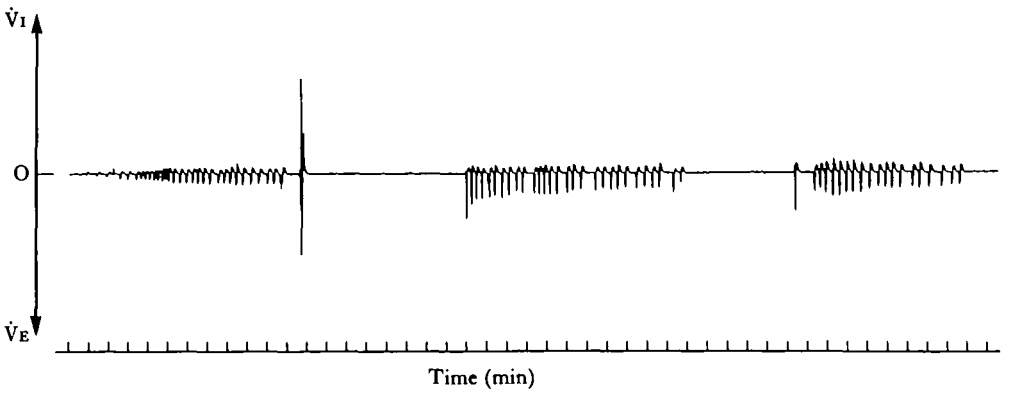

Fig. 3. Typical pneumotachograph recording. Three types of ventilatory periods are observed: deep breathing movements with frequencies of 10–15 min^{-1}, slight ventilation with frequencies of 3–4 min^{-1} and complete apnoea.

be calibrated; mucous covers on the inside of the probe, however, changed the calibration during the experiment in an unpredictable manner. Therefore, no measurements of lung ventilation are reported.

Ventricular systemic output (VSO), ventricular pulmonary output (VPO) and the sum of both, total heart output (THO) were neither significantly different with unpaired or paired *t*-test nor showed any trend between breathing periods and apnoea (Table 1). The mean values (±S.D.) from both periods were 35·3 ± 10·5 ml min^{-1} kg^{-1} for VSO, 26·9 ± 7·2 ml min^{-1} kg^{-1} for VPO, and 62·2 ± 16·0 ml min^{-1} kg^{-1} for THO. The observed difference between VSO and VPO is the result of pronounced shunting of deoxygenated blood past the lung circulation (R→L shunt), averaging about 30% of systemic venous return (Table 1), whereas only about 11% of oxygenated pulmonary venous blood was returned to the lung circulation in the incompletely divided ventricle of *Varanus* (L→R shunt). The shunt pattern was not significantly different between apnoeic and ventilatory periods (Table 1). The magnitude of shunting was extremely variable between individual animals; within individuals, shunting remained rather constant. This is especially evident in animals 3–6 for the R→L shunt and in animals 2–5 and 7–9 for the L→R shunt. The constancy of the shunt pattern in individuals was more closely examined in three additional animals. All the four types of microspheres (labelled with ^{141}Ce, ^{51}Cr, ^{85}Sr and ^{96}Sc) were injected consecutively into the same atrium at 1 h intervals. In two animals, in which the microspheres were injected into the right atrium (site 1, Fig. 1), the R→L shunts were 22·5, 24·6, 23·2, 21·2 and 35·6, 36·8, 39·2, 36·3% for the four labels, respectively. In one animal, in which the four labels were injected into the left atrium (site 2, Fig. 1), L→R shunt values of 8·2, 9·5, 8·7, 7·9% were determined.

The microspheres used for determination of heart outputs and intracardiac shunting were only slightly larger (14·9 ± 1·8 μm for ^{141}Ce, 15·3 ± 1·6 μm for ^{51}Cr, 15·4 ± 1·8 μm for ^{85}Sr and 14·9 ± 1·7 μm for ^{46}Sc, measured microscopically, $\bar{x}$ ± S.D., N = 100 for each label) than the smaller diameter of the *Varanid* erythrocytes (10·9 μm), but smaller than the larger erythrocyte diameter (19·8 μm; Berger & Heisler, 1977).

Table 1. *Central vascular shunting during ventilatory and apnoeic periods in* Varanus exanthemicus

Animal	R→L		L→R	
	Ventilation	Apnoea	Ventilation	Apnoea
1	18·8	9·6	12·7	4·8
2	39·5	27·7	20·1	23·0
3	41·3	40·9	4·7	3·8
4	14·0	16·3	2·5	3·8
5	15·0	13·8	12·3	10·1
6	46·2	42·4	15·3	21·2
7	53·9	63·5	10·4	10·7
8	19·3	25·5	4·7	3·4
9	10·2	37·9	15·5	19·8
$\bar{x}$	28·7	30·9	10·9	11·2
S.D.	± 16·4	± 17·0	± 3·3	± 8·1

R→L shunt: % of VSO; L→R shunt: % of VPO.

Table 2. *Percentage of microspheres (MS) not trapped in tissues during the first passage (recirculation)*

Animal	Ventilation		Apnoea	
	RA → LA	LA → RA	RA → LA	LA → RA
1	0·2	0·3	0·1	0·4
2	0	1·1	0	0·3
3	0	0·1	0·4	3·3
4	0·1	1·3	0·2	1·5
5	0·2	1·1	0·2	0
6	0·1	3·2	0·3	2·5
7	0	1·8	0	1·1
8	0	1·4	0·4	0·9
9	0·4	1·9	0	3·6
$\bar{x}$	0·11	1·36	0·18	1·51
S.D.	± 0·14	± 0·92	± 0·16	± 1·33

RA → LA = MS injected into the right atrium and detected in the left atrium.
LA → RA = MS injected into the left atrium and detected in the right atrium.

Accordingly, it could not be expected that all microspheres would lodge in the tissues during the first passage. Therefore, recirculation of microspheres (Table 2) was measured by withdrawal of a small percentage of the respective venous tissue return (see Methods) and heart outputs and shunting were corrected accordingly.

Anatomical studies

Inspection of the hearts during surgery and of the dissected hearts of freshly killed animals as well as our anatomical studies of fixed hearts revealed that size and internal configuration were much more variable than in any other animal species investigated in our laboratory. The variability was not correlated with weight, sex or any other obvious parameter of the animals. Nor was it related to the health status of the animals, because only apparently healthy and active animals, which were free of any kind of parasitosis, were used in the experiments.

A transverse section through fixed hearts slightly above the valve plane (Fig. 4A, solid line) shows the arrangement of the valves between atria, chambers and effluent vessels (Fig. 4B, solid lines). This arrangement is rather constant, whereas other features of the hearts are variable. The left and right atrio-ventricular valves are situated posteriorly, whereas the right aorta originates at the right lateral side, the left aorta and the pulmonary artery in the anterior part of the heart. A second transverse section at about one-third of the distance between valve plane and tip of the heart (Fig. 4A, B, dashed lines) presents the U-shaped vertical projection of the chamber sequence. Superposition of these two sections (Fig. 4B) indicates the sequence of valves in relation to the general blood stream: left atrio-ventricular valve, right atrio-ventricular valve, aortic valves and finally the valve of the pulmonary artery.

Unfolding of the U-shaped chamber arrangement (Fig. 5A) reduces the complex three-dimensional configuration to a more easily comprehensible two-dimensional picture.

The cavum arteriosum (CA), characterized by the connection to the left atriu

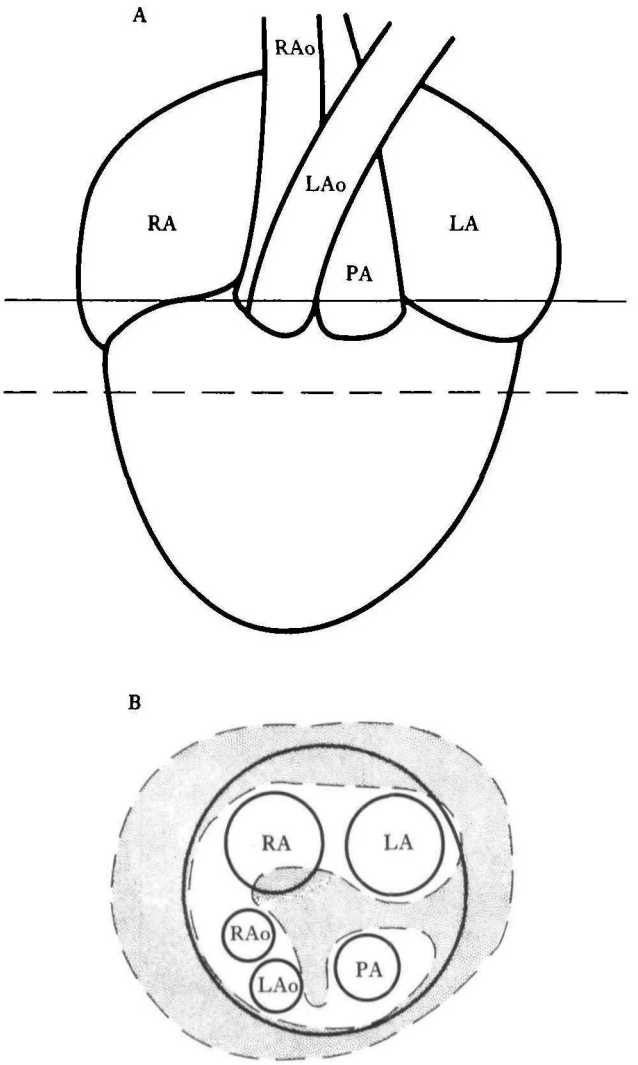

Fig. 4. (A) Ventral aspect of the heart of *Varanus*. Horizontal solid line: transverse section slightly above the valve plane. Horizontal dashed line: transverse section at about one quarter of the distance between valve plane section and apex of the heart. (B) Schematic drawing of the valve plane of the heart with the typical arrangement of valves orifices (solid lines). Projection of the second section through the cava (dashed lines, shaded area = heart wall). RA = right atrium, LA = left atrium, RAo = right aortic arch, LAo = left aortic arch, PA = pulmonary artery.

(LA) *via* the left atrio-ventricular valve is a relatively deep, largely trabecularized chamber (Fig. 5). The medial wall of this chamber is formed by the vertical septum. This wall is usually in line with the atrial septum (Fig. 5), but may also be shifted to the right in the direction of the cavum venosum (in one-third of the cases), being then in line with about the midpoint of the right atrio-ventricular valve. The heart cavern is continued over a flat ridge on the septum (lacking in one-third of the cases) into the cavum venosum (CV), which is much smaller than the CA (by about 60–70% in volume). The first part of the CV below the atrio-ventricular valve is formed as a

Varanus exanthematicus

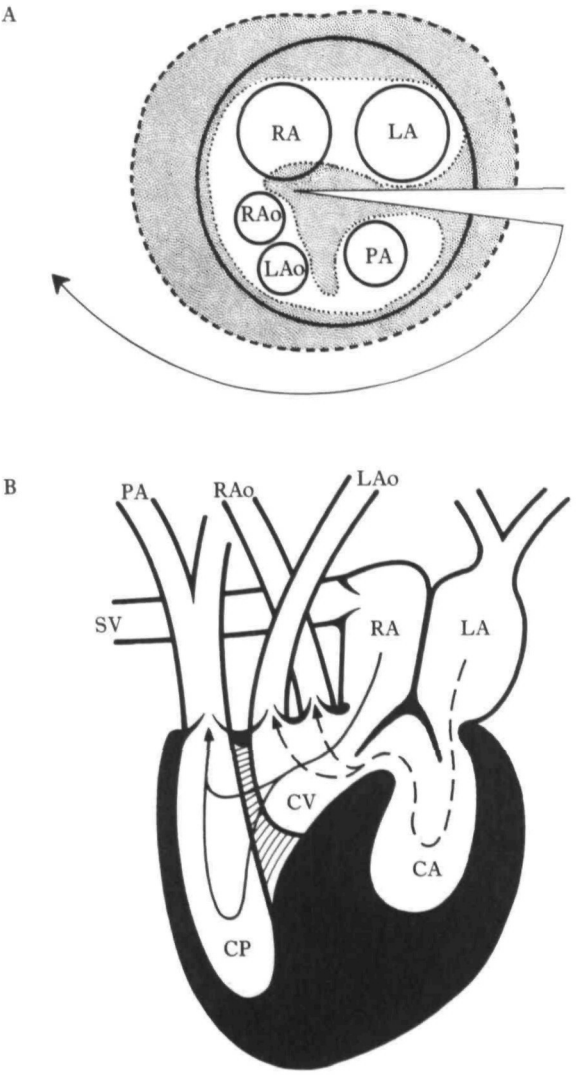

Fig. 5. (A) Unfolding of the three-dimensional chamber arrangement of Fig. 4. (B) Highly schematic two-dimensional presentation of the heart chamber and vessel arrangement after unfolding according to (A). The muscular ridge (striped area) between cavum venosum (CV) and cavum pulmonale (CP) is projected on to the outer heart wall for clarity. CA = cavum arteriosum, SV = sinus venosus, PA = pulmonary artery, RAo = right aortic arch, LAo = left aortic arch, RA = right atrium, LA = left atrium.

narrow interventricular channel, before it widens again near the muscular ridge, which separates CV and cavum pulmonale with the ostia of the aortic arches and of the pulmonary artery (Fig. 5). The lower, widened part of the CV near the muscular ridge is trabecularized, whereas the cavum pulmonale (CP) on the anterior side of the muscular ridge lacks any trabeculae.

The left atrio-ventricular valve is capable of covering the entrance to the inter-ventricular channel in only half the cases, the right atrio-ventricular valve can occlud

Table 3. *Peak blood pressures in the large vessels and the heart of* Varanus exanthematicus *(mmHg)*

Animal	Cavum arteriosum (CA)	Cavum venosum (CV)	Left aorta (LAo)	Cavum pulmonale (CP)	Pulmonary artery (PA)
13	48	48	48	22	22
14	52	50	52	22	20
15	74	76	72	18	14
16	72	72	60	14	14
17	50	48	42	28	28
18	54	64	67	13	12
19	58	66	72	11	16
20	60	64	70	14	13
$\bar{x}$	59	61	60	18	17
S.D.	± 10	± 11	± 12	± 6	± 6

the other end of the interventricular channel in 80 % of the cases. Always one or other atrio-ventricular valve can occlude the interventricular channel during diastole (correlated with the position of the vertical septum).

This type of arrangement of valves and intracardiac chambers results in a cross-over of the preferential blood streams of oxygenated and deoxygenated blood, which became particularly evident when windows were cut into the heart wall of the cavum venosum and of the cavum pulmonale, and colour-coded wires were fed from the atria through the heart chambers to effluent vessels.

Blood pressures

Pressure measurements in the large vessels and the chambers of the heart resulted in identical values for peak pressures in cavum arteriosum, cavum venosum and left aortic arch (Table 3). Except for a very short time during the systole (less than 10 % of the systole), the pressure difference between cavum arteriosum and cavum venosum did not exceed 3 mmHg.

Peak pressures in cavum pulmonale and pulmonary artery were lower than those in cavum arteriosum and cavum venosum by a factor of about 3·5 (Table 3), thus indicating a mammalian-like pressure separation between pulmonary and systemic circulation, which is presumably achieved by pressing the muscular ridge between the cavum venosum and the cavum pulmonale (Figs 5B, 6 Diastole, striated area) against the heart wall.

The average heart frequency was 29·2 ± 8·7 ($\bar{x}$ ± S.D., $N = 8$).

DISCUSSION

Critique of methods

There are two important prerequisites for the application of the microsphere (MS) method for an accurate determination of central vascular shunting. The first – good mixing of the MS with a representative portion of the respective venous return – was provided by jet-injection into five different (by 90°) directions *via* the metal tip of the

atrial catheters (see Methods) and the contractile action of the atria. How well microspheres and blood were mixed is documented by the constancy of the shunting fractions, and of tissue MS distribution. The second – trapping of MS during the first passage of the tissues – could not be completely achieved due to the small size of the MS (15 μm) when compared to the size of the erythrocytes (see Berger & Heisler, 1977) (Table 2). Therefore the extent of recirculation was determined in every single experiment and taken into account for all calculations. The magnitude of recirculation through lung tissue (Table 2) was much smaller than through systemic tissues, which could be due to smaller average capillary diameter in the lungs, or could be the result of larger diameter vascular bypasses of systemic tissues.

Recirculation was still small enough so that neglect of this factor would have had considerable influence on the results only for the L→R shunt in animals 3 and 4 (Tables 1, 2). This conclusion, however, cannot be extended to other species, even if related, as only small increases in erythrocyte diameters (<10 %) may result in 4–16 % MS recirculation with 15 μm MS (M. L. Glass & N. Heisler, unpublished).

When good mixing of the MS with the blood is ensured and correction for MS recirculation is made, the MS technique is the most direct, and probably the most reliable, method for quantitative determination of intracardiac shunting. Shunt estimates based on measurements of blood oxygen content require simultaneous withdrawal of blood samples from at least four selected sites, a procedure which has never been performed in unanaesthetized unrestrained reptiles. Moreover, this method is also rather insensitive for the detection of small shunts. Direct determination of flow rates in the effluent vessels of the heart with electromagnetic flowmeters implies simultaneous flow recording in at least three, or – because of the anatomical arrangement – usually four, vessels, with calibrated flow probes perfectly matched to the vessel diameter.

Functional anatomy of the heart

In spite of the considerable variability in size of the hearts, in the volumes of the heart chambers and in other internal features, the arrangement of the valves in relation to each other and to the cava was constant in all 15 hearts examined. The valve arrangement is similar to that reported for other squamate reptiles (non-varanid Lacertilia and Ophidia: White, 1959; varanids: Webb *et al.* 1971; Webb, 1979). Size and arrangement of the chambers, of the vertical septum and of the muscular ridge 'Muskelleiste', (a term introduced by old German authors and used by Webb), however, are extremely variable between reptilian groups (for references see Webb *et al.* 1971; Mathur, 1944; White, 1959), and, as indicated by the present study, to a certain extent even in a single species. This variability, together with the complex three-dimensional arrangement and terminological problems, are probably responsible for the conflicting results of a number of anatomical studies (e.g. Greil, 1903; Goodrich, 1916, 1919, 1930; O'Donoghue, 1918; Thapar, 1924; Brücke, 1852; Leene & Vorstman, 1930; Vorstman, 1933; Benninghof, 1933; Mertens, 1942; Mahendra, 1942; Mathur, 1944; Webb *et al.* 1971).

We have re-examined the gross anatomy of the varanid heart in order to gain better insight into cardio-vascular haemodynamics in connection with physiological studi

and have put more emphasis on the features apparently important for the function of the heart rather than performing a phylogenetically-orientated study.

The cavum arteriosum (functionally characterized by the connection to the left atrium through the left atrio-ventricular valve) is not directly connected to any of the large effluent vessels of the heart (Fig. 5) and blood has to leave the chamber over the vertical septum through the interventricular channel of the cavum venosum (which is functionally characterized by connection to the right atrium through the right atrio-ventricular valve). This observation is in accordance with the reports by Webb *et al.* (1971) and Webb (1979) on the heart anatomy of nine species of varanids, but in contrast with Burggren & Johansen's (1982) schematic diagram showing the aortic arches arising from the cavum arteriosum, and their statement that 'the cavum arteriosum . . . perfuses the systemic arteries, a function which in other non-crocodilians is achieved by the cavum venosum'.

The interventricular channel part of the cavum venosum below the ostia of the atrio-ventricular valve is probably closed by at least one atrio-ventricular valve during diastole, thus effectively preventing diastolic mixing of oxygenated and deoxygenated blood (Fig. 6). During systolic contraction of the heart, the blood from the cavum arteriosum is ejected through the cavum venosum into the aortic arches past the right atrio-ventricular valve, which is situated in close vicinity to the left atrio-ventricular valve (Fig. 4), an arrangement similar to that reported for other varanids (Webb *et al.* 1971; Webb, 1979), but in contrast to the transposition of right atrio-ventricular valve and aortic arches recently reported for *V. exanthematicus* by Burggren & Johansen (1982), whose model provides well separated bloodstream beds for oxygenated and deoxygenated blood (which may still, as they admit, mix to a certain extent at the incomplete vertical septum during diastole). However, as shown by our studies using colour-coded wires fed through the heart chambers following the preferential blood streams, the stream beds of oxygenated and deoxygenated blood cross in the cavum venosum (Fig. 5). Accordingly the cavum venosum has to be considered as a heart chamber common to both the pulmonary and systemic circulation and as a potential source for intracardiac shunting.

Pressure separation in the heart

Millard & Johansen (1974) were the first to report that pulmonary and systemic pressure contours for a reptile do not overlap at any time during systole, and that systemic blood pressure exceeds pulmonary pressure by a factor of five. Pulmonary arterial pressure, however, was recorded from a small lobal artery and the low pressure recorded could have been the result of an increased proximal pulmonary resistance induced by constrictor action of cholinergic vagal fibres (Berger, 1972). Recently Burggren & Johansen (1982) confirmed the pressure separation in varanid hearts by pressure measurements in the large effluent vessels, and in cavum pulmonale and cavum arteriosum. They found that blood pressure in the systemic circuit and cavum arteriosum exceeded the pressure in pulmonary artery and cavum pulmonale by a factor of about two. Burggren & Johansen (1982) concluded that pressure separation was achieved by pressing the vertical septum against 'the aortico-pulmonary septum bringing the cavum arteriosum in direct contact with the right and left systemic arteries'. This model is not compatible with our pressure measurements in effluent

vessels, cavum arteriosum, cavum pulmonale, and also in the lower extension of the cavum venosum near the muscular ridge (Muskelleiste). Our results did not show any significant pressure separation between cavum arteriosum (CA) and cavum venosum (CV) and left aortic arch but a considerable reduction of pressure from about 60 mmHg to 18 mmHg from cavum venosum to cavum pulmonale. Thus, pressure separation is achieved by pressure-tight contact between the muscular ridge (Muskelleiste) and the external wall of the heart during systole. This mechanism was first suggested about 80 years ago (Greil, 1903) and has been discussed again several times (e.g. Thapar, 1924; White, 1959; Webb *et al.* 1971); physiological evidence, however, for the role of the muscular ridge as the pressure separating structure has not been provided before.

Mechanisms of intracardiac shunting

Based on the anatomical configuration of the varanid heart with its functional crossover of oxygenated and deoxygenated bloodstreams (Fig. 5), the occurrence of intracardiac shunting appears to be unavoidable. It is surprising, however, how constant in time and unaffected by ventilatory periods both R→ L and L→ R shunts are in individual animals, whereas between individuals, shunting may vary enormously in spite of the fact that our animals were unanaesthetized, well recovered, unrestrained, undisturbed and not affected by any apparent disease.

In the past, shunting in reptile hearts has always been considered to be the result of blood movements between the respective bloodstream beds by pressure differences during either systole or diastole. For varanid hearts, however, during diastole very good separation between oxygenated and deoxygenated blood can be expected resulting from the occlusion of the interventricular channel by the atrio-ventricular valves (see Results and White, 1959, 1968; Webb *et al.* 1971). Also, systolic shunting is unlikely, since pressure measurements in the cavum arteriosum and cavum pulmonale show no overlap at all during systole (Burggren & Johansen, 1982). These results provide evidence for another mechanism which more easily explains the highly constant shunt pattern observed in individual animals: the wash-out of blood remaining in heart chambers at the end of systole and diastole.

This model is based on two main features of the functional anatomy of the heart of *Varanus*: the pressure separation during systole between lung and systemic circulation at the muscular ridge (Muskelleiste) as the interface between the cavum pulmonale and the cavum venosum, and the fact that the cavum venosum is part of the stream bed of both the oxygenated (systemic circulation) and deoxygenated blood (pulmonary circulation).

As a result of the pressure separation during systole, the blood from the cavum pulmonale is pumped exclusively into the pulmonary artery, and the blood from the cavum venosum and cavum arteriosum is ejected into the left and right aortic arches. At the end of systole, therefore, the cavum venosum is still filled with oxygenated blood. During diastole, the oxygenated blood remaining in the cavum venosum is then flushed by deoxygenated blood from the right atrium into the cavum pulmonale (Fig. 6), and the cavum venosum then remains filled with deoxygenated blood. At the same time, the cavum arteriosum is filled with oxygenated blood from the left atrium, which, during the next systole, again flushes the cavum venosum and carries the

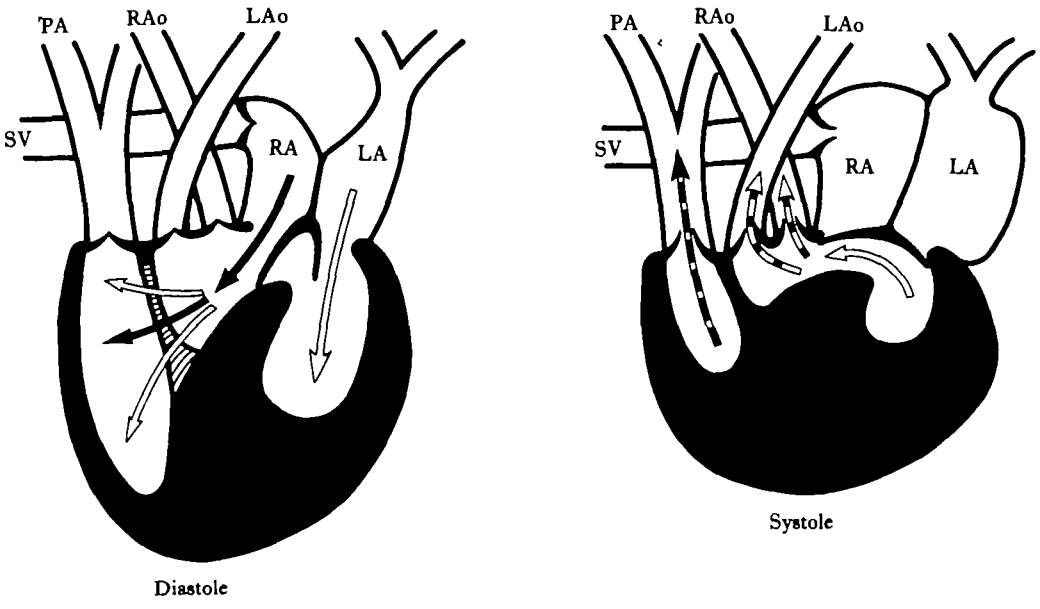

Fig. 6. Schematic representation of intracardiac shunting due to wash-out of cavum venosum. Left: *Diastole*. Oxygenated blood (open arrows) remaining in the cavum venosum from the preceding systole is washed into the cavum pulmonale by deoxygenated blood (black arrows). The cavum arteriosum is filled with oxygenated blood. Separation between the cavum arteriosum and the cavum venosum is provided by at least one atrio-ventricular valve. Striped area, see Fig. 5. Right: *Systole*. Deoxygenated blood remaining in the cavum venosum from the preceding diastole is flushed into the aortic arches by the oxygenated blood from the cavum arteriosum. Deoxygenated blood with admixture of oxygenated blood is expelled from the cavum pulmonale into the pulmonary artery. The muscular ridge is pressed pressure-tight against the outer heart wall during systole. RA = right atrium, LA = left atrium, LAo = left aortic arch, RAo = right aortic arch, PA = pulmonary artery, SV = sinus venosus.

deoxygenated blood remaining in this chamber from the diastole into the systemic circulation (Fig. 6). According to these haemodynamic events, the volumes of blood which are spilled into the 'wrong' circulation are largely dependent on the volume of the cavum venosum at the end of diastole (large) or systole (small). These relative volumes are in accordance with the intracardiac shunts determined in this study; i.e. large R → L shunt (~30 %) and smaller L → R shunt (~11 %). Also, the variability of shunting between individuals can be explained by anatomically predetermined, and in the course of this study actually observed, individually different volumes of the cavum venosum.

Based on this wash-out model, intracardiac shunting can be modulated only by changes in end-diastolic and end-systolic volumes of the cavum venosum. Pressure changes in the pulmonary artery induced by changes in lung inflation or pulmonary vessel vasomotor activity will have only little, if any, effect on intracardiac shunting, because of the complete pressure separation. Only large changes in cardiac output (which in the present study did not occur between ventilatory and apnoeic periods) are usually associated with changes in end-systolic ventricular blood volume and diastolic blood filling. Under conditions of activity with increased cardiac output, a reduction in end-systolic ventricular volume and an increase in end-diastolic volume

can be expected. The implications of these haemodynamic changes (a reduction of the relatively small L → R shunt and a further increase of the R → L shunt), however, can hardly be considered as advantageous for gas exchange and circulatory efficiency. Therefore intracardiac shunting in varanids has to be considered as the inevitable consequence of the functional heart anatomy rather than as a mechanism for conservation of energy during periods of low gas exchange and gas transport requirements.

In spite of the fact that pressure separation between lung and systemic circulation during systole occurs in varanids, as in crocodiles, the general arrangement of atrioventricular valves, heart chambers and effluent vessels in varanids is still similar to that of other squamate reptiles, with the intermediate ventricular chamber and the cavum venosum common to the stream beds of both the oxygenated and the deoxygenated blood. The unavoidable intracardiac shunting is due to the wash-out of blood remaining in the cavum venosum at the end of systole (oxygenated) or diastole (deoxygenated) into the respective 'wrong' vascular bed (lungs or systemic tissues). The extent of shunting is largely determined by the anatomical size of the cavum venosum and, because of the pressure separation by the muscular ridge (Muskelleiste), is not modulated according to the changes in pulmonary arterial blood pressure during ventilation and apnoeic periods.

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