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ESTIMATION OF SHUNTING, SYSTEMIC AND PULMONARY OUTPUT OF THE HEART, AND REGIONAL BLOOD FLOW DISTRIBUTION IN UNANAESTHETIZED LIZARDS (*VARANUS EXANTHEMATICUS*) BY INJECTION OF RADIOACTIVELY LABELLED MICROSPHERES

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SUMMARY

Circulatory parameters in a lizard (*Varanus exanthematicus*) were determined using the microsphere method. Microspheres (MS) (slightly larger than the erythrocytes and labelled with different γ-emitting isotopes) were injected into a pulmonary vein or the left atrium for determination of the left-to-right (L–R) shunt and the regional distribution of the ventricular systemic output. Injections were also made into the sinus venosus for determination of the right-to-left (R–L) shunt. The relative blood flow was obtained as the ratio of the MS activity found in the various tissues over the total activity injected. Absolute calibration of the method was performed by introduction of an 'artificial organ' into the circulatory system (Hales, 1973).

Ventricular systemic output (VSO), in five animals, averaged 121 ml/(min.kg) and ventricular pulmonary output 119 ml/(min.kg). The value of VSO was significantly higher than those observed in other lizard species. In all experimental animals both R–L as well as L–R shunting of various extent occurred. The reliability of the microsphere method as applied in lizards is discussed and is considered to be relatively accurate even under conditions of incomplete mixing of shunted and unshunted blood in systemic heart output.

INTRODUCTION

The effectiveness with which the lacertilian ventricle maintains separation of oxygenated and deoxygenated blood is a subject that has been repeatedly studied using various physiological techniques. However, the extent of mixing of the two blood streams remains unclear. By analysing the O_2 content of systemic arterial blood Tucker (1966) found that at a constant body temperature some systemic venous blood was returned to the body (R–L shunt) in half the specimens of *Iguana iguana* studied. Using the same species and technique, Baker & White (1970) failed to observe R–L shunting at a constant body temperature although shunting did occur during rapid

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heating of the animal. However, analysis of the gas content of systemic arterial blood cannot indicate whether there is some pulmonary venous return to the lungs (L–R shunt). Assessment of this possibility requires a comparison of the O_2 content of pulmonary arterial with mixed systemic venous blood. In two studies in which this has been done, the pulmonary artery was often seen to carry more O_2 than mixed systemic venous blood (White, 1959; Khalil & Zaki, 1964). These findings appear to establish that L–R shunting occurs in lizards, but such a conclusion has not been stated in two recent reviews which discuss cardiovascular function in lizards (White, 1968, 1970). The degree of mixing of blood in the heart has also been studied by observing the distribution of radio-opaque substances injected into the circulation. Such work has led to the conflicting conclusions that little mixing of oxygenated and deoxygenated blood occurs in the lizard heart (Foxon, Griffith & Price, 1956) or that a great deal of mixing occurs (Prakash, 1952). The only other evidence bearing upon the existence of shunting in lizards comes from the measurement of systemic and pulmonary blood flows in unanaesthetized *Varanus niloticus* (Millard & Johansen, 1974). The recorded systemic output of the heart was found to equal pulmonary output, if it was assumed that the flow rates in left and right pulmonary arteries were equal.

The present study describes the application of the microsphere technique for estimating shunting and other cardiovascular parameters using radioactively labelled microspheres (MS). Since the technique is, in theory, very accurate, this work was undertaken to elucidate the mechanism of shunting and, in addition, to determine the rates of blood flow through the tissues, including the lungs.

METHODS

Experimental procedure

Specimens of *Varanus exanthematicus* (weight 407–725 g, mean 540 g) were kept in large thermostated boxes for at least 2 days prior to the experiments to acclimate them to temperatures of either 25 or 35 °C. Polyethylene catheters (PE 50) were introduced into the sinus venosus through the caval vein (site 2) and into either the left pulmonary vein or the left atrium (site 1) under general halothane anaesthesia. When a catheter was introduced into the pulmonary vein it was not tied in place to avoid blocking the vessel. The right aortic arch was cut between two intercostal arteries, near the confluence with the left aortic arch, and a stainless steel double Y-piece (inner diameter 2·5 or 3 mm) was tied into upper and lower part of the vessel (Fig. 1). After recovery from anaesthesia the animals, which were loosely fastened to a board, were returned to the thermostatically controlled box and allowed to recover for at least 20 h.

After recovery from surgery microspheres with two different labels were injected into sinus venosus and pulmonary vein or left atrium. Aortic pressure was recorded by means of a pressure transducer which was connected to one of the outlets of the Y-piece. Immediately before injection blood was withdrawn from the right aortic arch, through the second outlet of the metal Y-piece, to determine ventricular systemic output of the heart using the technique of an 'artificial organ' (Hales, 1973). The infusion pump was stopped again 2 min after the injection. This procedure was repeated after 15–30 min. In animals subjected to both temperatures of 25 and 35 °C

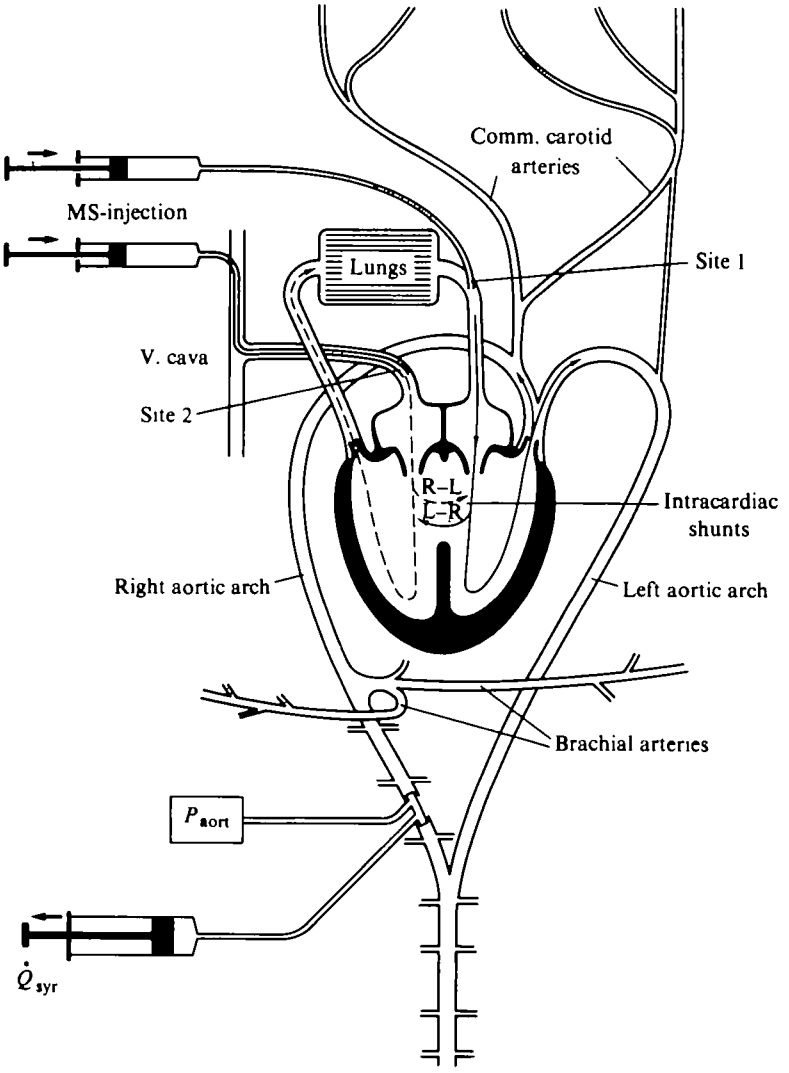

Fig. 1. Schematic representation of the anatomy of the central vascular systems in lizards. Microspheres were injected into a pulmonary vein (site 1) or left atrium for determination of ventricular systemic output (VSO), L–R shunt and regional distribution of VSO, and into the sinus venosus (site 2) for determination of the R–L shunt. Aortal blood pressure was recorded from one outlet of a double Y-piece tied into the right systemic arch, the second outlet was used for withdrawal of blood during the injection procedure for determination of VSO by use of the method described by Hales (1973).

the temperature was changed and the procedure repeated 24 h after the temperature change was made.

Microsphere injection technique

Microspheres (MS) with a diameter of 25 $\mu \pm 2{\cdot}5$ μ (mean $\pm$ S.D.) radioactively labelled with a γ-emitting isotope (either ^{141}Cerium or ^{51}Chrome or ^{85}Strontium or ^{46}Scandium) were properly suspended in 10% dextran solution by means of an ultrasonic homogenizer. Absence of microsphere aggregates and of broken

microspheres was checked with a light microscope. Injection of two differently labelled MS suspensions (each 0·5 ml, containing $3–5 \cdot 10^5$ MS) was started simultaneously at site 1 (left pulmonary vein or left atrium) and site 2 (sinus venosus) and completed within about 10–15 s. The catheters were flushed with 2 ml of dextran solution within about 40–50 s after start of injection. Some minutes after the last of two MS injection procedures the animal was killed by injection of pentobarbital, dissected into small samples, and the activity of the four injected isotopes was determined by use of the gamma scintillation technique. The activity of the isotopes at a given time was calculated from the time that had elapsed before counting of a sample, and from the half lives of the isotopes. The sum of the activity of each label in all tissue samples of the animal was taken as total activity administered.

Microspheres of 25 μ diameter were chosen because the large and small red blood cell diameters were found to be about $19{\cdot}8 \pm 1{\cdot}4\,\mu$ or $10{\cdot}9 \pm 1{\cdot}0\,\mu$ (mean ± S.D.) respectively. It was considered unlikely that capillary diameter would exceed the small red blood cell diameter by as much as 100%, therefore we assume that the MS lodge in the tissues and are not recirculated.

To test the reproducibility of MS distribution two pairs of differently labelled MS were sequentially injected at both injection sites. This was performed in two animals by injection from one syringe in which the two labelled MS suspensions (each 0·3 ml) were separated by 0·3 ml of blank dextran solution. Shunts and regional distribution of MS were determined following the same protocol.

Calculations

Since lizards have an undivided ventricle there is a possibility that some blood returning from the lungs could be returned (L–R shunt). It is also possible that a portion of systemic venous return could bypass the lungs and be returned to the body (R–L shunt). These possibilities can be tested by simultaneous injection of one MS label into the left atrium, and another to the sinus venosus. The size of each shunt can be calculated as following:

$$\text{L–R shunt} = \frac{A_{1L}}{A_{1L}+A_{1S}} \times 100\ (\%) \qquad (1)$$

A_{1L} represents the total activity of MS injected at site 1 (pulmonary vein or left atrium) measured in the lungs; A_{1S} represents the total activity of MS injected at site 1 measured in systemic body tissues.

$$\text{R–L shunt} = \frac{A_{2S}}{A_{2S}+A_{2L}} \times 100\ (\%) \qquad (2)$$

A_{2L} represents the total activity of MS injected at site 2 (sinus venosus) measured in the lungs; A_{2S} represents the total activity of MS injected at site 2 measured in the systemic body tissues.

As emphasized by Hales (1973) an estimate of the systemic output of the mammalian heart can be obtained by introducing an 'artificial organ' into the circulation. If blood is withdrawn, at the rate $\dot{Q}_{syr}$ (with an infusion syringe run in reverse during injection

and until all microspheres have entered the circulation) then the following relation holds:

$$\text{Cardiac output} = \dot{Q}_{\text{syr}} \times \frac{\text{total MS activity in systemic tissues}}{\text{MS activity in withdrawn blood}},$$

where MS activity in systemic tissue includes activity withdrawn in to the syringe as well. When the relationship is applied to lizards and the term ventricular systemic output (VSO) is substituted for cardiac output, then

$$VSO = \dot{Q}_{\text{syr}} \times \frac{A_{1\text{S}}}{A_{1\text{syr}}} (\text{ml/min}). \tag{3}$$

Shunting introduces a complication to the application of this technique in lizards. Only if R–L shunted blood mixes completely with the pulmonary venous return is VSO calculated from formula (3) accurate. If mixing is incomplete and most shunted blood is directed into the catheterized vessel then $A_{1\text{syr}}$ would be artificially low and the calculated VSO consequently greater than the actual VSO. The converse applies if most shunted flow is directed away from the catheterized vessel.

If the shunted and unshunted blood mix completely, then,

$$\text{VSO} = \dot{Q}_{\text{syr}} \times \frac{A_{2\text{S}}}{A_{2\text{syr}}} (\text{ml/min}). \tag{4}$$

If equations (3) and (4) give similar estimates of VSO it is likely that shunted and unshunted blood are well mixed.

The volume of shunted blood in VSO is calculated from (2) and (3):

$$\text{VSO}_{\text{s}} = \text{VSO} \times \frac{\text{R–L shunt}}{100} (\text{ml/min}). \tag{5}$$

$$\text{VSO}_{\text{us}} = \text{VSO} - \text{VSO}_{\text{s}} (\text{ml/min}), \tag{6}$$

where the subscript 's' signifies the shunted and 'us' the unshunted blood component of VSO.

Having calculated the contributions of shunted and unshunted blood to VSO the volume of shunted and unshunted blood received by any tissue (T) can be calculated.

$$\dot{Q}\text{s}_{\text{T}} = \text{VSO}_{\text{s}} \times \frac{A_{2\text{T}}}{A_{2\text{S}}} \times \frac{100}{\text{tissue weight}} (\text{ml/(min} \times 100\text{ g)}). \tag{7}$$

$$\dot{Q}\text{us}_{\text{T}} = \text{VSO}_{\text{us}} \times \frac{A_{1\text{T}}}{A_{1\text{S}}} \times \frac{100}{\text{tissue weight}} (\text{ml/(min} \times 100\text{ g)}). \tag{8}$$

The total blood flow to any tissue is obtained by adding equations (7) and (8),

$$\dot{Q}_{\text{T}} = \dot{Q}\text{s}_{\text{T}} + \dot{Q}\text{us}_{\text{T}} (\text{ml/(min} \times 100\text{ g)}) \tag{9}$$

or can be calculated directly from VSO:

$$\dot{Q}_{\text{T}} = \text{VSO} \times \frac{A_{1\text{T}}}{A_{1\text{s}}} = \dot{Q}_{\text{syr}} \times \frac{A_{1\text{T}}}{A_{1\text{syr}}}.$$

Pulmonary venous return has two destinations; the systemic circuit (VSO_{us}) and

the lungs (L–R shunt). Thus VSO_{us} represents [100-(L–R shunt)] % of pulmonary venous return, and therefore ventricular pulmonary output (VPO):

$$VPO = VSO_{us} \times \frac{100}{100-(\text{L–R shunt})} (\text{ml/min}). \quad (10)$$

The total output of the heart:

$$THO = VSO + VPO. \quad (11)$$

RESULTS

(1) *Blood pressure and effects of injection and withdrawal*

The day after the operation systolic pressure (measured in the descending right aortic arch) ranged from 45–60 mmHg (1 mmHg = 133·3 Pa), diastolic pressure from 32–44 mmHg. Temperature did not influence blood pressure. Differences appear to exist between the cardiovascular physiology of *V. exanthematicus* and *V. niloticus* as reported by Millard & Johansen (1974) which possibly can be attributed to different sizes of the animals used. The mean aortic pressure in *V. exanthematicus* was found to be about 45 mmHg whereas it is about 90 mmHg in *V. niloticus*. Pressures of that magnitude were observed in *V. exanthematicus* only after injection of a pressor substance or as a result of longer periods of handling after the animal had recovered from anaesthesia. After both types of stimulation, blood pressure gradually returned to normal, but remained elevated for longer than 2 h after the stimulus.

Withdrawal of blood from the right aortic arch at rates of 0·6 or 1·6 ml/min in several blank experiments never resulted in changes of blood pressure or irregular beating of the heart. But during the injection procedure in 4 of the 11 experimental animals increases in systolic blood pressure as well as in pulse pressure were observed, and extra beats of the heart occurred.

Such effects are possibly part of a broad cardiovascular reflex response to the experimental procedure and thus the normal distribution of VSO_{us} and VSO_s between the various systemic vessels could be markedly altered and lead to a misestimate of VSO. Thus results from animals showing irregular heartbeats or changes in pulse pressure were discarded.

(2) *Reproducibility of the technique*

Injection of two pairs of MS labels immediately after each other for test purposes resulted in shunt estimates differing only very slightly from each other (Table 1). The R–L shunts were determined to be 7·2 and 7·6% in one, 20·2 and 21·9% in the other animal, while the estimates of L–R shunts came out to be 13·1 and 11·8 in one, 8·7 and 8·2% in the other test animal.

The reproducibility of the regional distribution of MS was dependent upon the fraction of injected MS activity received by a tissue sample. The ratios of the regional blood flow values calculated from the distribution of the two MS labels injected at site 1, $\dot{Q}_{Ta}/\dot{Q}_{Tb}$ (index *a* and *b* for the two MS labels), were in the range of 0·87–1·15 (1·00 ± 0·04, mean ± S.D.) in all tissue samples that received a fraction larger than 0·0009 of the injected MS. The local systemic distribution of the two MS labels injected at site 2 (sinus venosus) and shunted into the systemic circuit showed a greater disparity due to the about ten times smaller number of MS distributed in the systemic

Table 1. *Blood recirculating to the lungs (L–R shunt) and to the systemic tissues (R–L shunt) at constant body temperature (percentage of VPO and VSO respectively)*

Animal	R–L	L–R
1	3	28
2	19	13
	16**	13**
3	3	10
	10**	25**
4*	7·2	13·1
	7·6	11·8
6*	21·9	8·7
	20·2	8·2
8	15	10
9	51**	4**

Note that animals marked with one asterisk were used in testing the technique. Values marked with two asterisks were obtained at 25 °C.

tissues. In tissues receiving a fraction of more than 0·01 of the shunted MS the ratios of the blood flows determined from the distribution of the two shunted MS labels, $\dot{Q}_{Tc}/\dot{Q}_{Td}$ (*c* and *d* for MS labels injected at site 2, were always in the range of 0·80–1·25 (1·00 ± 0·06, mean ± S.D.).

(3) *Shunting*

According to the method of estimation the size of the shunt refers to the percentage of pulmonary or systemic venous return which is sent back to the tissue from which it came. Thus, for example, a R–L shunt of 20% means that 20% of systemic venous return has returned to the systemic tissues or that 20% of VSO is shunted blood. In eleven measurements, the R–L shunt magnitude ranged from 51 to 3% with a mean of 16%. The L–R shunt magnitude ranged from 28 to 4% with a mean of 13% (see Table 1).

(4) *Ventricular systemic output and ventricular pulmonary output*

The estimates of VSO and VPO are presented in Table 2. The sum of VSO and VPO is the total heart output (THO). Part of the relatively large scatter of VSO, VPO and THO is due to different sizes of the experimental animals.

Values of VSO determined in this study for *V. exanthematicus* (mean 121 ml/(min.kg)) were significantly higher than those given by Millard & Johansen (1974) for *V. niloticus* (about 55 ml/(min.kg)), determined with an ultrasonic flowmeter and those reported for *Iguana iguana* by Tucker (1966) with 62 ml/(min.kg) and by Baker & White (1970) with about 43 ml/(min.kg) under control conditions and 55–94 ml/(min.kg), during heating determined by the Fick method. These relatively large differences may be due to the fact, that the animals used in this study were several times smaller than the animals used by the above mentioned authors.

(5) *Regional distribution of blood flow*

The regional distribution of VSO is shown in Table 3 as the mean of estimates in seven animals and expressed as ml/(min × 100 g tissue) and as percentage of ventricular systemic output received by each tissue.

Table 2. *Estimates of ventricular systemic output (VSO), ventricular pulmonary output (VPO), total heart output (THO) and total stroke volume at constant body temperature*

Animal	VSO (ml/min)	VPO (ml/min)	THO (ml/min)	THO/body weight (ml/(min kg))	Total stroke volume (ml)
1	80	107	187	305	2·3
2	77	71	148	197	—
	73*	76*	149*	205*	—
3	66	71	137	281	2·0
	37*	45*	82*	168*	2·0*
8	45	43	88	216	1·1
9	80*	40*	120*	262*	4·0*

Note that estimates of the parameters shown in this table were not made on animals 4 and 6. Note also that results from animals 5, 7, 10 and 11 were discarded because of cardiovascular irregularities during microsphere injection, and that a clot in the systemic catheter allowed only mean pressure to be recorded in animal 2; thus no estimate of total stroke volume was possible. Values marked with * were obtained at 25 °C.

Table 3. *Blood flow and percentage of ventricular systemic output received by various tissues*

Tissue	$\dot{Q}_T$ ml/(min. 100 g)	VSO (%)
Brain	102	1·3
Spinal cord	6·5	0·14
Thyroid	41	0·03
Oesophagus	13	0·4
Stomach	12	2·2
Small intestine	41	5·9
Large intestine	80	6·6
Kidneys	48	4·1
Spleen	194	0·4
Pancreas	70	1·4
Liver	30	8·5
Heart	145	5·1
Abdominal fat bodies	19	5·7
White muscle	5·8	—
Red or mixed muscle	9·3	—
Bone	7·9	—
Skin	7·0	—
Tongue	21	0·3

Each figure is the mean of determinations in seven animals except for white muscle which represents the mean of six estimates. Note that percentages of VSO are not calculated for four tissues because only samples of these tissues were taken.

While most flow rates reported here for certain tissue of *Varanus* fall into the range covered by mammalian species (see Hales, 1973; Altman & Dittmer, 1974; Ruch & Patton, 1974; Wade & Bishop, 1962), kidney blood flow was found to be at least 5 times smaller than in any observed mammalian species. Studies on regional distribution of VSO in reptiles for comparison with our data are not available.

DISCUSSION

Critique of technique

(*a*) *Shunting*

The very good agreement between the two estimates of L–R and R–L shunt in the two test animals suggests that the microsphere technique measures shunt with a high accuracy. It is possible, however, that the technique creates a shunt rather than measuring an existing one. Considering that the mean values of all VPO and VSO measurements are about 65 ml/min, the injected volume represents about 5% of the flow rate past the catheter. An increased ventricular pressure could result from increased filling so that the normal separation of blood in the partially divided ventricle was disrupted during microsphere injection and some of the MS suspension could be shunted because of abnormal ventricular filling. But as the increase in flow rate at the injection sites is small, and the venous pressure low and capacitance high, we believe that injections into the pulmonary vein and sinus venosus do not increase venous pressure significantly.

(*b*) *Ventricular systemic output and ventricular pulmonary output*

In the two test animals the systemic distribution of two MS injected into the pulmonary venous return was almost identical for tissues with flow rates greater than 0·1% VSO_{us}. In all measurements of VSO the syringe, acting as an artificial tissue, withdrew blood at a rate > 1% VSO_{us}. Thus each measurement of VSO contains negligible experimental error so long as the microspheres are uniformly mixed within the whole VSO. But if shunted blood is distributed disproportionally to right and left systemic arches, then the site where blood is sampled must affect the estimate of VSO; the accuracy of the estimate is then likely to be good as long as the R–L shunt size is not large. However, the VSO estimates from shunted and from unshunted MS differ only slightly and even in animal 9 (where the estimates are likely to be relatively inaccurate because of the large R–L shunt (51%)) the VSO calculated from the distribution of shunted and unshunted MS differ by only 4%. Thus obviously shunted and unshunted blood had mixed very well in the heart. VPO is calculated from VSO and L–R shunt. As the determination of the shunt size is considered to be very accurate (see section: Reproducibility of the technique) the VPO estimates can be considered to have almost the same accuracy as estimations of VSO.

(*c*) *Significance of experimental observations*

The present results are the first to show that L–R shunting occurs in unanaesthetized lizards, previous reports having shown that L–R shunting occurs in anaesthetized (White, 1959) or decerebrate animals (Khalil & Zaki, 1964). In all the animals studied in this work R–L shunting was also observed. Previous workers have reported that R–L shunting occurs in about half the animals studied at a constant body temperature (Tucker, 1966) or that it never occurs at a constant body temperature but always does during heating (Baker & White, 1970). It is difficult to reconcile such conflicting observations, but slight differences in experimental conditions might account for the conflict, additionally it may well be that the very sensitive microsphere technique can detect small shunts more reliably than the indirect Fick method.

The anatomy of the varanid ventricle (Webb, Heatwole & DeBavay, 1971) led Millard & Johansen (1974) to conclude that no shunting occurs during systole and that only minor shunting is possible during diastole in varanids. Under resting conditions this conclusion appears to be verified in *V. niloticus* since pulmonary and systemic pressure contours do not overlap at any time during ventricular systole. In contrast, substantial two-way shunting occurs under resting conditions in *V. exanthematicus* where, for methodological reasons, it was impossible to distinguish between systolic and diastolic shunts. Millard & Johansen (1974) also claim that major shunting does not occur in *V. niloticus* during hypoxia, hypercapnia or diving. Thus, even though pulmonary arterial pressure rises during these three states, at no stage of systole pulmonary arterial pressure was found to exceed systemic arterial pressure. But it should be noted that pulmonary arterial pressure was recorded from a small lobar artery. As it has been shown in the lizard *Tiligua rugosa* cholinergic vagal fibres have an extremely powerful constrictor action on the large extrinsic pulmonary arteries and a feeble constrictor action on intrinsic vessels (Berger, 1972). Thus, if the increase in pulmonary resistance observed in *V. niloticus* during hypoxia, hypercapnia and diving was brought about by constriction of large arteries, pressure recording of a small intrinsic artery could not rule out shunting during systole.

The existence of a one-way shunt can be interpreted as serving some useful role (e.g. during heating). In *Iguana iguana* a R–L shunt has been interpreted as a means of increasing the rapidity of heating (Baker & White, 1970). Simultaneous shunting in two directions, however, must involve a waste of cardiac and ventilatory energy. Thus the design which allows the animal to shunt in one direction appears to carry the penalty that constant shunting in both directions is unavoidable. While circulatory inefficiency appears to be demonstrated by the present results another interpretation should be considered. The microspheres were injected into the sinus venosus over a period sufficient for several breathing cycles. It is known that a R–L shunt develops during apnea, and a L–R shunt during breathing in the turtle *Pseudemys scripta* (White & Ross, 1965). If blood were similarly apportioned during the breathing cycle in *V. exanthematicus* the distribution of microspheres injected over several breathing cycles would suggest simultaneous R–L and L–R shunting, whereas, in fact, each shunt might be occurring separately at different phases of the ventilatory cycle.

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