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PARTITIONING OF BODY FLUIDS AND CARDIOVASCULAR RESPONSES TO CIRCULATORY HYPOVOLAEMIA IN THE TURTLE, *PSEUDEMYS SCRIPTA ELEGANS*

BY ALLAN W. SMITS* AND MARK M. KOZUBOWSKI

Department of Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045, U.S.A.

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SUMMARY

Investigations were conducted (1) to measure the steady state compartmentation of body fluids and (2) to assess the efficacy of blood volume and pressure maintenance during haemorrhage-induced hypovolaemia in the pond turtle, *Pseudemys scripta elegans*. The pre-haemorrhage blood volume, as determined by tracer dilution of ^{51}Cr-labelled erythrocytes, averaged $6{\cdot}89 \pm 0{\cdot}33\,\%$ of the body mass, and was part of comparatively large extracellular ($40{\cdot}2 \pm 0{\cdot}70\,\%$) and total body fluid volumes ($75{\cdot}25 \pm 1{\cdot}48\,\%$). Turtles exhibited progressive reductions in systemic arterial pressure throughout a cumulative haemorrhage of $-48\,\%$ of their original blood volume, despite dramatic increases in heart rate and comparatively large magnitudes of transcapillary fluid transfer from interstitial to intravascular spaces. Arterial blood pressure returned to pre-haemorrhage values 2 h after experimental haemorrhage ceased, concomitant with the restoration of the original blood volume. Our results support arguments made in previous studies that the resistance to fluid movement between vascular and extravascular locations in reptiles is comparatively low. Furthermore, the haemodynamic responses of turtles to experimental hypovolaemia suggest that barostasis through adjustments in vascular tone is less effective than that observed in other reptiles.

INTRODUCTION

Vertebrate animals typically maintain a relatively constant blood volume and pressure through regulatory mechanisms that are both numerous and inter-related (see review by Little, 1981), resulting in a circulatory homeostasis upon which many bodily functions depend. The efficacy of these regulatory mechanisms has been largely demonstrated in experiments that induce changes in circulation volume (through haemorrhage or transfusion) in mammalian models; comparatively little is known concerning similar regulatory mechanisms operating in the cardiovascular systems of other vertebrate taxa.

* Present address: Department of Zoology, University of Massachusetts, Amherst, MA 01003, U.S.A.
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A unique tolerance to circulatory hypovolaemia has been demonstrated in snakes in which cardiovascular and respiratory homeostasis are maintained despite haemorrhages exceeding 80–100 % of the snake's initial blood volume (Lillywhite & Smith, 1981; Lillywhite, Ackerman & Palacios, 1983). The primary mechanisms that counteract hypovolaemia in these reptiles are a rapid and extensive mobilization of interstitial fluid to the intravascular space (Lillywhite & Smith, 1981; Lillywhite & Pough, 1983; Smits & Lillywhite, 1985) and reflexive adjustments of cardiac performance and vascular tone (Lillywhite & Seymour, 1978; Lillywhite & Smith, 1981). The volume of interstitial fluid is also proportionately greater in snakes than in mammals, and essentially all the fluid absorbed into the vascular space during haemorrhage comes from the interstitium (Smits & Lillywhite, 1985).

Turtles represent an evolutionarily distinct taxon of reptiles and have served as model animals in cardiovascular research for decades. However, comparatively few data exist that describe the compartmentation of body fluids between the vascular and non-vascular locations in these reptiles, or the capacity for fluid movement between these locations to maintain circulatory homeostasis.

In this paper we report how the intracellular and extracellular body fluid is partitioned within the pond turtle, *Pseudemys scripta*. Further, the capacity for blood volume regulation in these reptiles has been tested by employing tracer dilution techniques to measure the extent and source of transcapillary fluid transfer during hypovolaemia induced by haemorrhage.

MATERIALS AND METHODS

Animals and surgical preparations

Pond turtles (*Pseudemys scripta elegans*) were obtained through a commercial supplier (Lemberger Associates, Wisconsin) and were kept in fresh water (24–28 °C) on a diet of vegetables, fish and meat. The 14 adult turtles (body mass, $\bar{X} \pm$ S.D. = 1466 ± 173 g) used in this study were in apparent good health and normal state of hydration prior to experimentation.

Surgical anaesthesia was induced by lowering the turtles' body temperature in an ice bath for 1 h; cold narcosis was maintained during surgery by covering the animals with chipped ice. A hole (4 cm diameter) was drilled through the plastron to allow access to the major blood vessels and the heart. The left subclavian artery was occlusively cannulated (PE-90, i.d. = 0·860 mm, o.d. = 1·270 mm) and the catheter was advanced to the most proximal portion of the artery. The catheter passed through the body cavity *via* a stab wound through the loose skin between the neck and left forelimb. The patency of the catheter was maintained by occasional flushing with small volumes (< 0·2 ml) of heparinized (100 units ml^{-1}) turtle Ringer's solution (Rogers, 1938).

Tracers and labelling procedures

The compartmentation of body fluid in each turtle prior to haemorrhage was assessed by tracer dilution analysis. Deuterium oxide (D_2O), ^{51}Cr-labelled erythrocytes

and thiocyanate ion (SCN^-) were used to measure total body water (TBW), whole blood volume (BV) and extracellular fluid volume (ECFV), respectively.

The D_2O (approx. 2·5 ml kg^{-1} body mass) was injected intraperitoneally at 16.00 h the day prior to the experiment. Turtles were kept overnight in dry but covered boxes to reduce both cutaneous D_2O exchange and evaporative water loss. A blood sample taken immediately preceding experimental haemorrhage was vacuum distilled to remove organic components, and the D_2O concentration of this body fluid was determined by comparison with D_2O standards (1·0–5·0 ml D_2O l^{-1}) using infrared spectrometry (Zweens, Frankena, Reicher & Zijlstra, 1980).

Turtle BV was measured by the dilution of autologous, ^{51}Cr-labelled erythrocytes infused into the arterial catheter approximately 3 h prior to experimental haemorrhage. Details of the labelling procedure are described by Lillywhite & Smits (1985). Briefly, 1 ml of each turtle's erythrocytes was incubated with 10 μCi of isotopic sodium chromate at room temperature for 1 h. Following incubation the erythrocytes were repeatedly washed with a plasma-saline solution to eliminate the unbound isotope. The erythrocytes were then diluted in normal saline to the volume originally withdrawn from the turtle, and a known volume and specific activity of the labelled blood was infused into the arterial catheter. The blood volume was obtained by dividing the total activity infused (counts per minute) by the specific activity of the turtle blood (c.p.m. ml^{-1}) sampled 3 h following tracer infusion. Radioactivity in all blood samples drawn for BV determination was assayed by counting each sample for 5 min (approx. 1 % sigma) on a Packard Auto-Gamma 800C scintillation counter. The specific gravity of whole blood (1·05 g ml^{-1}) was used for conversions of mass to volume (Thorson, 1968).

Dilution of the thiocyanate ion was used to estimate the ECFV of turtles. NaSCN was infused into the arterial catheter (0·025 g NaSCN kg^{-1} body mass) 4 h prior to experimental haemorrhage. Concentrations of the thiocyanate ion in turtle plasma were measured colourimetrically (Bradshaw & Shoemaker, 1967) using a Zeiss PM QII spectrophotometer. The pre-haemorrhage ECFV was calculated from concentrations of the thiocyanate ion in four plasma samples (0·2 ml) collected at 15-min intervals in the hour preceding haemorrhage. The exact quantity of each of the three tracers injected was determined gravimetrically.

Tracer equilibration times were determined on one turtle (1290 g), in which the tracer concentrations were measured in the turtle's blood sampled at intervals up to 3 h (^{51}Cr) and 6 h (NaSCN) following tracer infusion (Fig. 1). These data confirmed that adequate time was given (3 h for ^{51}Cr; 4 h for NaSCN) for these tracers to equilibrate with their respective fluid spaces. Deuterium oxide was found to equilibrate with the TBW of turtles in 5–6 h at 25 °C; thus, adequate equilibration time was given for this tracer by administering it the day previous to the TBW determination.

Haemorrhage and fluid sampling

Immediately following measurements of steady-state fluid volumes, turtles were subjected to a graded haemorrhage to assess the extent of inter-compartment fluid transfer in response to hypovolemia. Turtles were conscious and at room temperature

(22–26°C) during haemorrhage, and rested in a horizontal, ventral up position. Arterial blood pressures and heart rates were measured from the subclavian arterial catheter using a Narco P1000A Linear Core pressure transducer coupled to a DMP-4A physiograph (Narco Biosystems, Inc.). Haemorrhage was begun only after arterial blood pressure and heart rate had appeared to stabilize.

Graded haemorrhage was accomplished by withdrawing 4% of the turtle's estimated BV at 10-min intervals until a total of 48% of the pre-haemorrhage BV had been removed. Initial blood volumes were assumed to equal 7% of the turtle's body mass based on previous BV measurements in the same species (Hutton, 1961; A. W. Smits & M. M. Kozubowski, unpublished observations). Each interval blood sample was withdrawn from the subclavian arterial catheter into a clean syringe and partitioned approximately equally for the separate analyses of whole blood (^{51}Cr) and plasma (NaSCN). Samples of whole blood were delivered directly from the withdrawal syringe into pre-weighed counting tubes. These tubes were reweighed, sealed with Parafilm, and counted the following day for gamma radioactivity. Plasma fractions from each withdrawal were obtained by immediate centrifugation (5 min in IEC Clinical centrifuge), and were pipetted into sealable plastic vials for later analysis of NaSCN concentration. The total osmolality of this plasma was also determined using vapour pressure osmometry (Wescor, Model 5100C). Haematocrits were measured on every blood sample by spinning blood-filled microcapillary tubes for 5 min in an IEC MB microhaematocrit centrifuge (13 000 ***g***). Arterial blood pressures and heart rates were measured continuously between consecutive withdrawals from the subclavian arterial catheter; mean pressures and heart rates reported were those measured during the last minute of each 10-min sampling interval. Analysis of tracer

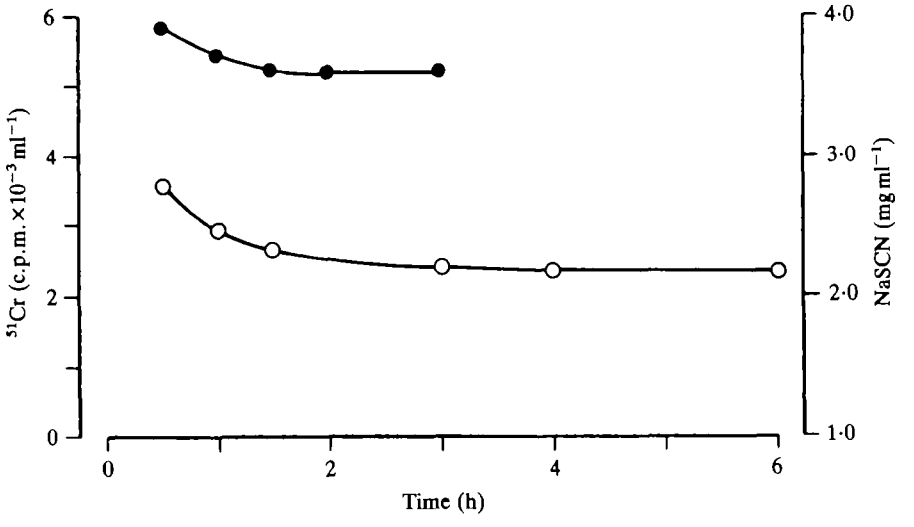

Fig. 1. Time-dependent changes in the activity of ^{51}Cr in whole blood (closed circles) and thiocyanate concentration in plasma (open circles) measured in a 1290 g female *Pseudemys* following intravascular infusion of these tracers at time zero.

concentrations in 1-ml blood samples taken 0·5, 1·0 and 2·0 h after haemorrhage ceased (−48 %) were used to assess body fluid distribution during post-haemorrhage recovery.

The volume and source of fluid mobilized into the vascular space were also determined by tracer dilution analysis, based on the assumption that the dilution of a particular tracer within a fluid space was proportional to the amount of unlabelled fluid entering that space. Decreases in the specific activity (c.p.m. ml^{-1}) of the ^{51}Cr within blood samples withdrawn during haemorrhage were used to calculate the volume of extravascular (unlabelled) fluid transferred into the vascular space. Because the calculation of BV by ^{51}Cr-labelled erythrocytes requires a knowledge of the total radioactivity infused (see above), all calculations of BV subsequent to the initial BV measurement were corrected to account for ^{51}Cr activity withdrawn from the turtles. The concentration of the thiocyanate ion was used to determine the source of fluid entering the vascular space during haemorrhage. Because both the plasma and the interstitial fluid contained the same thiocyanate concentration at equilibrium, the thiocyanate concentration was assumed to remain unchanged if the source of absorbed fluid was solely from the interstitium. Reductions in the plasma thiocyanate concentration during haemorrhage were considered to indicate movement of intracellular (unlabelled) fluid into the extracellular space. The thiocyanate tracer is not a permanent marker within the ECF, but is normally eliminated from the extracellular space of reptiles at a very slow rate (A. W. Smits, unpublished observations). The time constant of NaSCN elimination was sufficiently large (Fig. 1) to support the assumption that natural decay of this tracer during experimental haemorrhage (2 h) was negligible.

In addition to these experiments where a discrete volume of blood (48 % BV) was removed, five turtles were subjected to an extended haemorrhage. Four percent of the pre-haemorrhage BV was removed every 10 min until arterial pressure could no longer be regulated, or until blood could no longer be withdrawn through the arterial catheter. All procedures (surgery, tracers, haemorrhage rate, fluid sampling and analyses) except the extent of haemorrhage were the same as described above. Albumin and globulin fractions of protein within the blood plasma (sampled throughout haemorrhage) were assayed using Sigma Chemical Diagnostic Kits Nos. 630 and 560, respectively. Following the experiment turtles were held overnight in dry, plastic containers after their plastrons had been sealed with a wooden plug and medical grade silicone rubber (Elastomer Silastic, Dow Corning).

RESULTS

Distribution of body fluid

The compartmentation of body fluid measured within turtles by tracer dilution analysis is schematically illustrated in Fig. 2. The whole blood volume (PV+RCV) averaged 6·89 ± 0·33 % of the body mass ($\bar{X}$ ± S.E.), and ranged from 5·74 to 8·15 %.

Physiological responses to haemorrhage

The maximum net decrease in circulating blood volume (BV) demonstrated by turtles experiencing a graded removal of whole blood averaged only 14·4 % of their pre-haemorrhage BV (Fig. 3), despite a cumulative withdrawal of 48 %. The dramatic departure of the lines in Fig. 3 depicting changes in BV (solid circles) and the predicted BV changes in the absence of autotransfusion (dashed line) indicates that turtles mobilized substantial quantities of fluid from extravascular to vascular locations. The rate at which this fluid was transferred into the vascular space accelerated as haemorrhage progressed, until the later stages of the experiment (−40 to −48 % BV deficit) when rates of autotransfusion equalled the rates of experimental haemorrhage (Fig. 3). Restoration of the BV to pre-haemorrhage levels was accomplished within 1–2 h after bleeding was discontinued.

Transcapillary fluid transfer determined by ^{51}Cr dilution (Fig. 3) was consistent with the degree of haemodilution observed in the haematocrit (Fig. 4). Haematocrits decreased significantly (paired comparisons *t*-test; $P < 0{\cdot}001$) from ($\bar{X} \pm$ S.E.) $22{\cdot}1 \pm 1{\cdot}89$ % to $13{\cdot}6 \pm 1{\cdot}41$ % during the course of haemorrhage, representing a mean reduction of 38·5 %. Following 2 h of post-haemorrhage recovery, the haematocrit decreased further to $11{\cdot}8 \pm 1{\cdot}22$ %. Thus, the cumulative decrease in

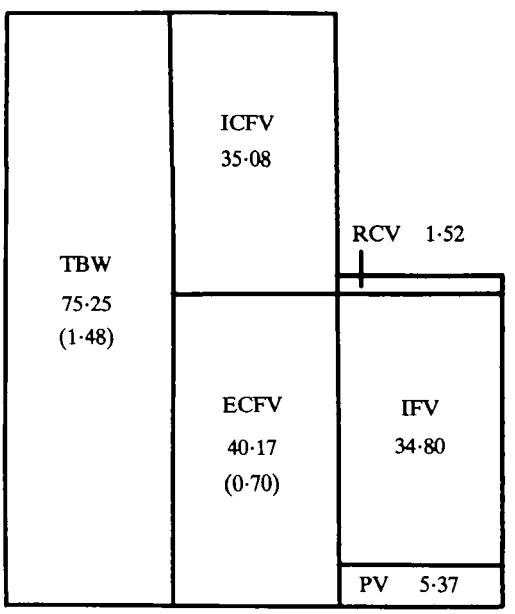

Fig. 2. Compartmentation of body fluids measured in nine adult *Pseudemys scripta elegans* (body mass = 1475 ± 95 g, $\bar{X} \pm$ S.D.), expressed as percentages of the body mass (numbers in parentheses represent the S.E.M.). Red cell volumes (RCV) and plasma volumes (PV) were calculated from the whole blood volume and the haematocrit. Intracellular fluid volumes (ICFV) and interstitial fluid volumes (IFV) were calculated by subtraction of the extracellular fluid volume (ECFV) from the total body water (TBW) and subtraction of the PV from the ECFV, respectively.

haematocrit represented a haemodilution of 46·6 %, a volume essentially identical to the haemorrhage deficit (−48 %).

There were no measurable changes in plasma NaSCN concentration in any of the turtles during the course of haemorrhage, indicating that the source of fluid mobilized into the vascular space was entirely extracellular. The total osmolality of the plasma also appeared unaffected by the experimental haemorrhage, as average osmolalities before (298 ± 16 mosmol kg^{-1}) and after haemorrhage (298 ± 21 mosmol kg^{-1}) were identical.

Arterial blood pressures (Fig. 5) were significantly reduced (33 %) as a result of experimental haemorrhage (paired comparisons t-test, $P < 0{\cdot}01$), from the initial mean (±S.E.) of 39·2 ± 3·27 mmHg (range, 25·0–57·5 mmHg) to 26·1 ± 3·17 mmHg (range, 16·5–45·6 mmHg). Initially-measured pulse pressures (5–10 mmHg) typically decreased to 3–5 mmHg in response to the bleeding. Turtles raised arterial blood pressures during the 2-h recovery period to a level (34·8 ± 3·14) that was not significantly different from that of the pre-haemorrhage value ($P > 0{\cdot}2$). The average

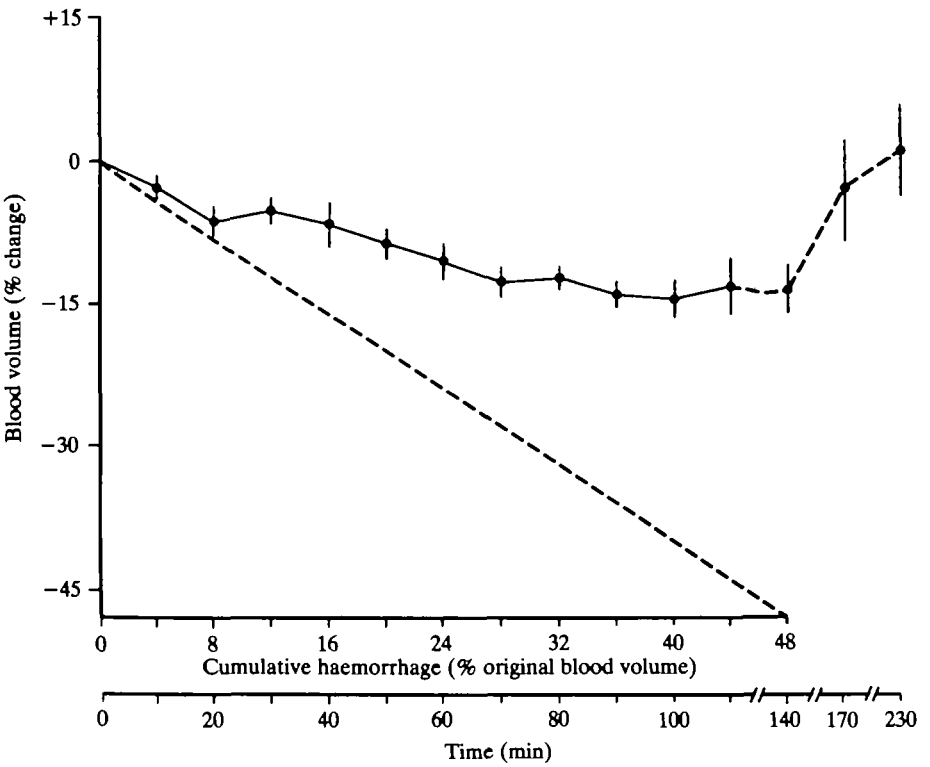

Fig. 3. Mean changes in blood volume (±1 S.E.) as a function of time and the deficit of blood volume incurred by haemorrhage. The ordinate is the actual volume expressed as a percentage of the pre-haemorrhage volume. The abscissa is the deficit of volume removed by cumulative haemorrhage, expressed as a percentage of the original blood volume. The dashed line represents the cumulative blood volume deficit of turtles in the absence of transcapillary fluid transfer into the vascular space. ($N = 9$ turtles).

heart rate of turtles (Fig. 5) at the cessation of haemorrhage ($41{\cdot}9 \pm 2{\cdot}19$ beats min^{-1}) was 120% higher than the initially-measured heart rate ($19{\cdot}1 \pm 3{\cdot}43$ beats min^{-1}). Although heart rate slowed significantly ($P < 0{\cdot}02$) during the recovery period to $36{\cdot}1 \pm 2{\cdot}49$ beats min^{-1}, this rate was still significantly greater (86·5%, $P < 0{\cdot}001$) than that measured just prior to haemorrhage.

Extended haemorrhage

The changes in BV measured during the initial 48% haemorrhage deficit in turtles subjected to an extended haemorrhage (Fig. 6) were similar in pattern and magnitude ($-10{\cdot}6 \pm 3{\cdot}5$%; *t*-test following arcsin transformation, $P > 0{\cdot}35$) to those observed in turtles in the previous experiment (Fig. 2). Despite continued haemorrhage, BV in these turtles increased dramatically during the later stages of the experiment to levels that exceeded pre-haemorrhage values by 30% (Fig. 6). The greatest increments in BV occurred following the removal of 80% of the initial BV and concomitant with an accelerated decline in arterial blood pressure. The maximum heart rate ($42{\cdot}3 \pm 1{\cdot}07$

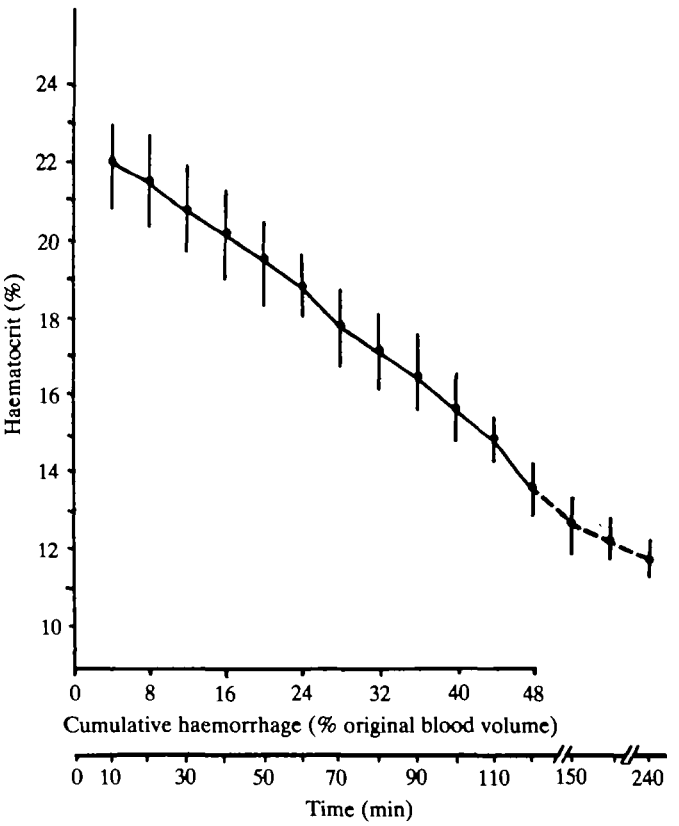

Fig. 4. Mean changes in turtle haematocrits (±1 S.E.) plotted against time and the deficit of blood volume incurred by haemorrhage. The abscissa is the same as described in Fig. 3 ($N = 9$ turtles).

beats min^{-1}) was observed at an 80 % BV deficit and declined from this point until the cessation of haemorrhage.

Concentrations of albumin ($16{\cdot}9 \pm 0{\cdot}51\,g\,dl^{-1}$) and globulin ($28{\cdot}5 \pm 1{\cdot}78\,g\,dl^{-1}$) within the plasma decreased gradually throughout extended haemorrhage to values

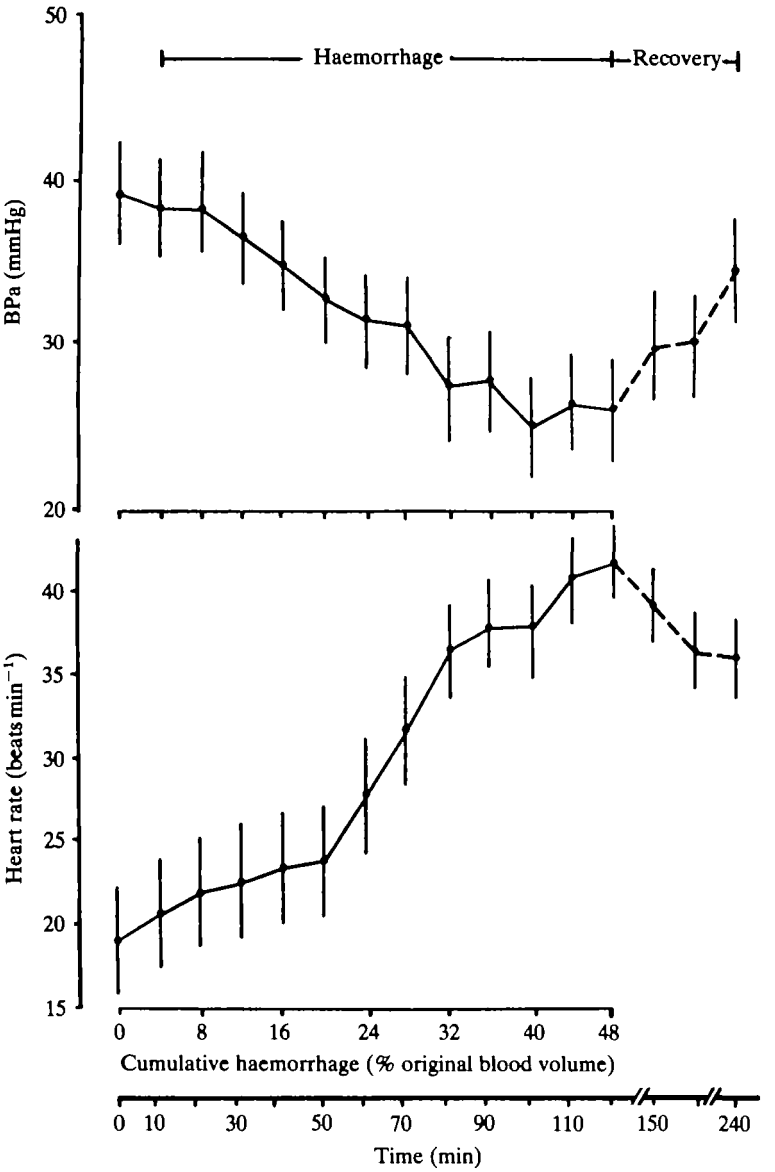

Fig. 5. Average changes in arterial blood pressure (BPa) and heart rate in nine turtles (±1 S.E.) in response to hypovolaemia induced by haemorrhage. The abscissa is the same as described in Fig. 3.

that were 33 % ($11{\cdot}3 \pm 0{\cdot}96\,g\,dl^{-1}$) and 38 % ($17{\cdot}6 \pm 1{\cdot}38\,g\,dl^{-1}$) of the pre-haemorrhage levels, respectively. All turtles survived the extended haemorrhage to the following day without access to water, and several were kept alive for 1–2 months in the laboratory in plastic tanks containing fresh water.

DISCUSSION

Body fluid compartmentation

The mean BV of turtles at steady state (6·89 % body mass) is similar to the BV assumed (7 %) for the subsequent haemorrhage protocol, and is consistent with BV values reported for *Pseudemys scripta* and *Chrysemys picta* (combined; 8·1 %) measured by the dilution of ^{51}Cr-labelled erythrocytes (Wilson, Hansard & Cole,

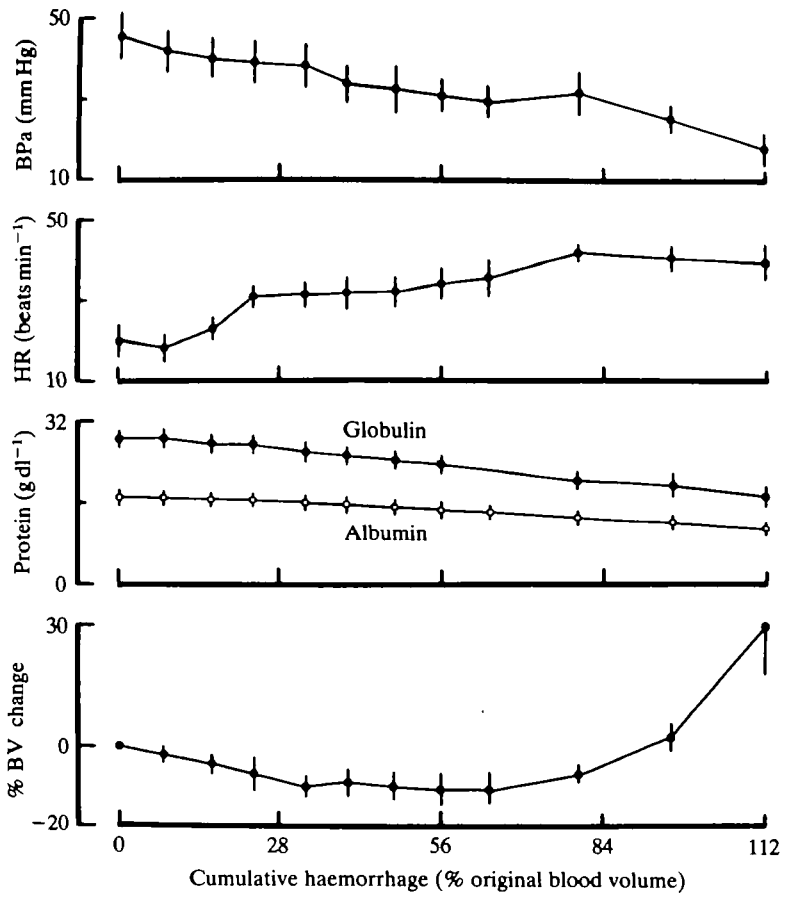

Fig. 6. Changes in arterial blood pressure (BPa), heart rate (HR), fractional protein content, and the percentage change from original blood volume (BV) measured in five turtles ($\bar{X} \pm$ S.E.) in response to an extended haemorrhage where greater than 100 % of the pre-haemorrhage BV was removed.

1960). The small discrepancy in the BV of *Pseudemys scripta* reported here and that measured by Wilson *et al.* (1960) or Hutton (1961; 8·75 % body mass) presumably reflects differences in body sizes of the experimental animals, as plasma volume appears to be inversely related to body mass in *Pseudemys* (Hutton, 1961).

Apparently no marker substance used in the dilution analysis of the ECFV is universally acceptable (Law, 1982), thus, only studies utilizing similar techniques and tracers may be compared. The ECFV (thiocyanate space) of *Pseudemys scripta* (Fig. 2) exceeds the thiocyanate space measured in rats (33 % body mass; Huang & Bondurant, 1956) and is intermediate in volume compared to the thiocyanate spaces of other reptiles (Coulson & Hernandez, 1953; Minnich, 1982; Smits & Lillywhite, 1985).

Total body water in *Pseudemys* (75·3 % body mass; Fig. 2) is greater than that measured in other freshwater or marine turtles (64·0–72·9 %; Thorson, 1968), but is very similar to values of TBW in other species of reptiles (review by Minnich, 1982).

Experimental haemorrhage

Decreases in systemic arterial pressure induced by haemorrhagic hypovolaemia in higher vertebrates initiate a baroreceptor-controlled stimulation of the sympathetic nervous system that primarily increases peripheral resistance and venous return through vasoconstriction, and secondarily improves cardiac output (Guyton, 1981; p. 333). Compensation for hypovolaemia *via* fluid resorption is typically considered a more passive process resulting from a reduction in capillary hydrostatic pressure (Djojosugito, Folkow & Kovach, 1968) and from the osmotic gradients caused by a hyperglycaemic hyperosmolality of the plasma (Ware, Norberg & Nylander, 1980), although activation of β-adrenoreceptors (Hillman, Gustafsson & Lundvall, 1982) appears to contribute significantly to fluid resorption.

The regulation of systemic blood pressure in *Pseudemys* in response to hypovolaemia appears poor despite dramatic increases in heart rate (Figs 5, 6). Systemic pressure seems largely dependent on the BV deficit (Figs 3, 5). The dependence of

Table 1. *Absolute and proportional changes in heart rate, blood pressure and blood volume in three species of reptiles subjected to a graded haemorrhage of 32 % of their initial blood volumes*

	*Elaphe obsoleta**	*Crotalus viridis**	*Pseudemys scripta*
Heart rate (beats min^{-1})	25→38 (+56 %)	19→32 (+67 %)	19→37 (+92 %)
Arterial blood pressure (mmHg)	49→44 (−11 %)	36→35 (−3 %)	39→27 (−30 %)
% Change in blood volume	−13·6 %	−14·4 %	−12·4 %
Source of absorbed fluid	Extracellular	Extracellular	Extracellular

* Data from Smits & Lillywhite (1985).

pressure regulation on BV is perhaps best exemplified by the return of arterial blood pressure to pre-haemorrhage values during the recovery period, concomitant with a complete restoration of pre-haemorrhage BV (Figs 3, 5). This recovery of blood pressure indicates that turtles can withstand a gradual removal of at least 48 % of their resting BV without entering severe and irreversible circulatory shock.

The experimental rate of haemorrhage used to demonstrate the tolerance of terrestrial snakes to hypovolaemia (Lillywhite & Smith, 1981; Smits & Lillywhite, 1985) was duplicated in the present study, thus the haemodynamic responses and changes in BV during hypovolaemic stress in turtles and snakes may be quantitatively compared (Table 1). Turtles and snakes are similar in their capacity to mobilize substantial volumes of extracellular fluid from extravascular to vascular locations during hypovolaemia; this supports the inference that the resistance to fluid movement across the capillary wall in reptiles is comparatively low (Lillywhite & Smits, 1984; Smits & Lillywhite, 1985). Unlike mammals, turtles and snakes do not demonstrate an increase in plasma osmolality during haemorrhage. Thus, the transfer of fluid between extravascular and vascular locations does not appear to be facilitated by a hyperglycaemic hyperosmolality in these reptiles. The observed stability of extracellular osmolality also indicates that turtles do not resorb significant volumes of dilute urine ($< 100\,\text{mmol}\,\text{kg}^{-1}$) during acute hypovolaemia.

Turtles do not maintain systemic arterial pressure as effectively as snakes in response to hypovolaemia, despite a proportionately greater increment in heart rate (Table 1). Baroreceptors located at the base of the pulmonary artery in *Pseudemys* have been characterized by recording afferent vagal nerve activity synchronous with the heart rate (Faraci, Shirer, Orr & Trank, 1983), but the primary effector(s) of these mechanoreceptors remain in question. The haemodynamic responses of *Pseudemys* to hypovolaemia in the present study (Figs 5, 6) and to drug-induced changes in blood pressure (Millard & Moalli, 1980) suggest that the heart, rather than the peripheral vasculature, may be the primary effector of barostasis.

Because measurements of BV in turtles are based on changes in the concentration of ^{51}Cr-labelled erythrocytes, a rapid change in haematocrit influenced by contraction of the spleen during haemorrhage may invalidate BV estimates. However, experimental turtles did not demonstrate transient increases in haematocrit during any stage of the haemorrhage (Fig. 4), thus we feel that the contribution of the spleen to erythrocyte recruitment and changes in BV during the experiment is negligible.

Turtles enduring an extended haemorrhage over-compensate for their initial BV deficits and become increasingly hypervolaemic (Fig. 6). The restoration of BV in these turtles does not augment the regulation of systemic blood pressure or significantly reduce the heart rate, in contrast to turtles bled 48 % of their initial BV (Fig. 5). This dramatic shift of fluid into the vascular space appears more related to changes in the hydrostatic than osmotic pressures of the Starling equilibrium, as the proportional decreases in protein fractions that contribute to plasma oncotic pressure are constant throughout the experiment (Fig. 6). We have also measured dramatic increases in the concentrations of plasma epinephrine and norepinephrine at times concurrent with the accelerated vascular resorption (−84 % to −96 % deficit; A. W.

Smits, M. M. Kozubowski & K. J. Renner, in preparation), and suggest that this dramatic change in circulating BV may be related to the humoral effects of these catecholamines.

In summary, the capacity of aquatic turtles to counteract hypovolaemic stress through fluid recruitment (resorption) into the vasculature is exceptional and equals (if not exceeds) rates of autotransfusion measured in terrestrial snakes (Smits & Lillywhite, 1985). As in snakes, the ECFV is comparatively large and is the primary source of fluid that is apparently mobilized across a low resistance between the interstitial and intravascular spaces. Although turtles tolerate and survive substantial losses of their blood, their haemodynamic responses to experimental hypovolaemia suggest that barostasis through reflexogenic adjustments in vascular tone in these reptiles is comparatively weak.

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REFERENCES

BRADSHAW, S. D. & SHOEMAKER, V. H. (1967). Aspects of the water and electrolyte changes in a field population of *Amphibolurus* lizards. *Comp. Biochem. Physiol.* **20**, 855–865.

COULSON, R. A. & HERNANDEZ, T. (1953). Glucose studies in Crocodilia. *Endocrinology* **53**, 311–320.

DJOJOSUGITO, A. M., FOLKOW, B. & KOVACH, A. G. B. (1968). The mechanism behind the rapid blood volume restoration after hemorrhage in birds. *Acta physiol. scand.* **74**, 114–122.

FARACI, F. M., SHIRER, H. W., ORR, J. A. & TRANK, J. W. (1982). Circulatory mechanoreceptors in the pond turtle, *Pseudemys scripta*. *Am. J. Physiol.* **242**, R216–R219.

GUYTON, A. C. (1981). *Textbook of Medical Physiology*. Philadelphia: W. B. Saunders.

HILLMAN, J., GUSTAFSSON, D. & LUNDVALL, J. (1982). β2-Adrenergic control of the plasma volume in hemorrhage. *Acta physiol. scand.* **116**, 175–180.

HUANG, K.-C. & BONDURANT, J. H. (1956). Simultaneous estimation of plasma volume, red cell volume and thiocyanate space in unanesthetized normal and splenectomized rats. *Am. J. Physiol.* **185**, 441–445.

HUTTON, K. E. (1961). Blood volume, corpuscular constants, and shell weight in turtles. *Am. J. Physiol.* **200**, 1004–1006.

LAW, R. O. (1982). Techniques and application of extracellular space determination in mammalian tissues. *Experientia* **38**, 411–421.

LILLYWHITE, H. B., ACKERMAN, R. A. & PALACIOS, L. (1983). Cardiorespiratory responses of snakes to experimental hemorrhage. *J. comp. Physiol.* **152**, 59–65.

LILLYWHITE, H. B. & POUGH, F. H. (1983). Control of arterial pressure in aquatic sea snakes. *Am. J. Physiol.* **244**, R66–R73.

LILLYWHITE, H. B. & SEYMOUR, R. S. (1978). Regulation of arterial pressure in Australian tiger snakes. *J. exp. Biol.* **75**, 65–79.

LILLYWHITE, H. B. & SMITH, L. H. (1981). Haemodynamic responses to haemorrhage in the snake, *Elaphe obsoleta obsoleta*. *J. exp. Biol.* **94**, 275–283.

LILLYWHITE, H. B. & SMITS, A. W. (1984). Lability of blood volume in snakes and its relation to activity and hypertension. *J. exp. Biol.* **110**, 267–274.

LITTLE, R. C. (1981). *Physiology of the Heart and Circulation*. Second Edition. Chicago: Year Book Medical Publishers, Inc.

MILLARD, R. W. & MOALLI, R. (1980). Baroreflex sensitivity in an amphibian, *Rana catesbeiana*, and a reptilian, *Pseudemys scripta elegans*. *J. exp. Zool.* **213**, 283–288.

MINNICH, J. E. (1982). The use of water. In *Biology of the Reptilia*, (eds C. Gans & F. H. Pough). New York: Academic Press.

ROGERS, C. G. (1938). *Textbook of Comparative Physiology*. New York: McGraw-Hill.

SMITS, A. W. & LILLYWHITE, H. B. (1985). Maintenance of blood volume in snakes: transcapillary shifts of extravascular fluids during acute hemorrhage. *J. comp. Physiol.* **155**, 305–310.

THORSON, T. B. (1968). Body fluid partitioning in Reptilia. *Copeia* **1968**, 592–602.

WARE, J., NORBERG, K.-A. & NYLANDER, G. (1980). Osmolar developments and altered thoracic duct lymph flow in hypovolaemic shock. *Europ. Surg. Res.* **12**, *Suppl.* **1**, 48.

WILSON, B., HANSARD, S. L. & COLE, B. T. (1960). Total blood volume of the turtle and the frog. *Louisiana Acad. Sci.* **23**, 45–52.

ZWEENS, J., FRANKENA, H., REICHER, A. & ZIJLSTRA, W. G. (1980). Infrared-spectrometric determination of D_2O in biological fluids. *Pflügers Arch. ges. Physiol.* **385**, 71–77.