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LABILITY OF BLOOD VOLUME IN SNAKES AND ITS RELATION TO ACTIVITY AND HYPERTENSION

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SUMMARY

The lability of blood volume and its relationship to locomotor activity was investigated in two species of snakes *Elaphe obsoleta*, Say and *Crotalus viridis*, Rafinesque. Repetitive measurements of blood volume, determined from changes in the specific activity of circulating ^{51}Cr-labelled erythrocytes, indicated that 15 min of locomotor activity reduced blood volume by 21 % due to filtration of plasma from capillaries. This magnitude of plasma translocation exceeds that measured in exercising mammals by factors of 2 to 7, depending on the intensity and duration of muscular activity. Activity produced changes in arterial blood pressure, heart rate and haematocrit that were proportionately similar in both species, increasing approximately 57, 85 and 25 %, respectively, above resting values. Arterial infusion of norepinephrine increased arterial pressure by more than 100 % and reduced blood volumes 8·6 and 17·0 % in *Elaphe* and *Crotalus*, respectively. These data demonstrate that blood volume varies substantially in relation to activity or the hypertensive state of these reptiles. Thus care must be employed in interpreting or comparing reported measurements of blood volume in these and probably other vertebrates.

INTRODUCTION

The importance of blood volume is well established in vertebrate physiology, and a suite of control mechanisms appears to maintain the volume of circulating blood within relatively narrow limits. Blood volumes have been measured in numerous species of reptiles with a variety of techniques, and typically represent 5–8 % of the body mass in restrained or anaesthetized animals (e.g., Bradshaw & Shoemaker, 1967; Thorson, 1968; Lillywhite & Smith, 1981; Pough & Lillywhite, 1984).

We have previously investigated the responses of blood volume in snakes subjected to experimental haemorrhage and found that the ability of snakes to restore blood volume through transcapillary shifts of extracellular fluid apparently exceeds that of other vertebrates studied (Lillywhite & Smith, 1981; Lillywhite & Pough, 1983; Smits & Lillywhite, 1984). These investigations suggest that the capacity for transferring fluid between the vascular and extravascular compartments is relatively great, and that blood volume within an individual snake might be quite labile. We infer that

Key words: Blood volume, hypertension, snakes, exercise, transcapillary fluid shifts, arterial pressure, plasma volume.

if the resistance to fluid movement across capillaries is indeed comparatively small then inducing transient increases in capillary hydrostatic pressure could significantly reduce blood volume by enhancing filtration of plasma. In this paper we report that locomotion and arterial hypertension both induce substantial reductions of circulating blood volume in two species of snakes.

MATERIALS AND METHODS

We measured changes of blood volume in four rat snakes (*Elaphe obsoleta*) and three Pacific rattlesnakes (*Crotalus viridis*) obtained from commercial suppliers. Snakes were prepared for experimentation by cannulating the dorsal aorta just anterior to the vent with heparinized, saline-filled PE-10 or PE-50 occluding catheters. Snakes were anaesthetized by lowering their body temperatures in chipped ice and allowing several inhalations of halothane (Fluothane) vapour. Surgical procedures and exteriorization of the catheter followed those reported by Lillywhite & Seymour (1978). Snakes were allowed 36–48 h for post-operative recovery within transparent acrylic tubes provided with openings for ventilation and exit of the catheter.

Measurements of blood volume were obtained by labelling a snake's own erythrocytes with the isotope ^{51}Cr. Details of the labelling procedure were developed by Smits & Lillywhite (1984). Briefly, approximately 1 ml of a snake's red blood cells were incubated with 10 μCi of isotopic sodium chromate at room temperature for 1 h. The red cells were then repeatedly washed with a plasma-saline solution to eliminate the unbound isotope. Finally, the red blood cells were diluted in saline to a volume originally withdrawn from the snake, and a known volume and specific activity of the labelled blood was infused into the aortic catheter. The labelled cells were allowed to mix with the circulating blood volume overnight.

Because ^{51}Cr binds irreversibly to the haemoglobin within the red blood cells (Jones & Mollison, 1956), and because red cell longevity is apparently long in reptiles (Altland & Brace, 1962; Smeller, Bush & Seal, 1978), the ^{51}Cr marker remained within the vascular space for the duration of the experiments. Therefore, instantaneous blood volumes of snakes were determined at specific times during the experiments by measuring the specific activity of gamma radiation in the blood of the snake. Blood samples (0·3 ml) withdrawn for blood volume determinations were partitioned into microcapillary tubes (0·05 ml) for haematocrit measurement (IEC Microcentrifuge) and into pre-weighed 13 × 100 mm tubes (0·25 ml) for gamma counting. The counting tubes were then reweighed and counted for 5 min each (approx. 1 % sigma) in a Packard Auto-Gamma 800C scintillation counter. Blood volume was obtained by dividing the total activity infused into a snake by the specific activity of a given blood sample, correcting for decay. The specific gravity of whole blood (1·05 g ml^{-1}) was used to convert units of mass to volume.

Changes in blood volume induced by locomotor activity were measured the day following the labelling of the red cells. Aortic pressures were measured by means of a Gould-Statham P23 ID pressure transducer and recorded on chart paper with a Grass Model 7D Polygraph. A blood sample (0·3 ml) was withdrawn for initial measurements of blood volume (BV) and haematocrit (Hct) after a snake exhibited a steady heart rate (HR) and blood pressure (BP) for at least 30 min of inactivi

within the acrylic tube. The blood volume and haemodynamic values measured while the snake was at rest were considered control data, and the cardiovascular responses of each snake to experimental treatments were compared to its own controls.

To induce activity snakes were removed from the acrylic tube and allowed to move across the laboratory floor. Snakes were prodded to move at a steady pace, and both sedentary behaviour and frantic bursts of locomotion were discouraged. Snakes were returned to the acrylic tube following 15 min of activity; measurements of BV, Hct, HR and BP were made immediately and after a 10-min period of recovery. Levels of Hct were remeasured at 5 h, and in one *Elaphe* both Hct and BV were determined at hourly intervals for 7 h.

A second experiment was conducted on each snake to measure responses of blood volume to arterial hypertension. Control values of BV, Hct, HR and BP were determined as before. Blood pressure was then elevated by infusing 100 μg of norepinephrine per kg body mass (Calbiochem DL Arterenol, <50 μl saline carrier) into the dorsal aorta. Heart rate and blood pressure were measured continuously, and blood samples for BV and Hct determinations were taken when BP peaked and at 10 and 20 min thereafter. These measurements were repeated at hourly intervals for 4 h in one of the *Elaphe*.

RESULTS

The resting blood volumes (BV) of the two species of snake measured prior to the experiments were not significantly different ($P > 0{\cdot}2$; *t*-test) and represented $6{\cdot}07 \pm 0{\cdot}40$% and $6{\cdot}41 \pm 0{\cdot}06$% of the body mass (mean ± S.E.) of *Elaphe* and *Crotalus*, respectively (Table 1).

Fig. 1 illustrates the percentage change of blood volume in both species of snake after 15 min of locomotor activity. Both species demonstrated substantial reductions in BV to approximately 21 % below control volumes (mean ± S.E. were $-20{\cdot}8 \pm 2{\cdot}26$ and $-21{\cdot}8 \pm 1{\cdot}86$% for *Elaphe* and *Crotalus*, respectively), indicating that at least one-fifth of the circulating fluid had left the vascular space. BV of both species

Table 1. *Blood parameters in individual, resting snakes before exercise or treatment*

Animal	Mass (g)	Hct (%)	Plasma volume (ml)	BV (ml)	BV (% body mass)
Elaphe obsoleta					
1	1140	16·5	49·7	59·5	5·48
2	331	20·0	15·5	19·4	6·14
3	329	24·0	14·9	19·6	6·26
4	364	20·5	17·6	22·1	6·38
Mean	541	20·3	24·4	30·2	6·07
S.D.	400	3·1	16·9	19·6	0·40
Crotalus viridis					
1	448	19·5	22·1	27·5	6·45
2	325	22·0	15·3	19·6	6·34
3	343	23·0	16·2	21·0	6·43
Mean	372	21·5	17·9	22·7	6·41
S.D.	66	1·8	3·7	4·2	0·06

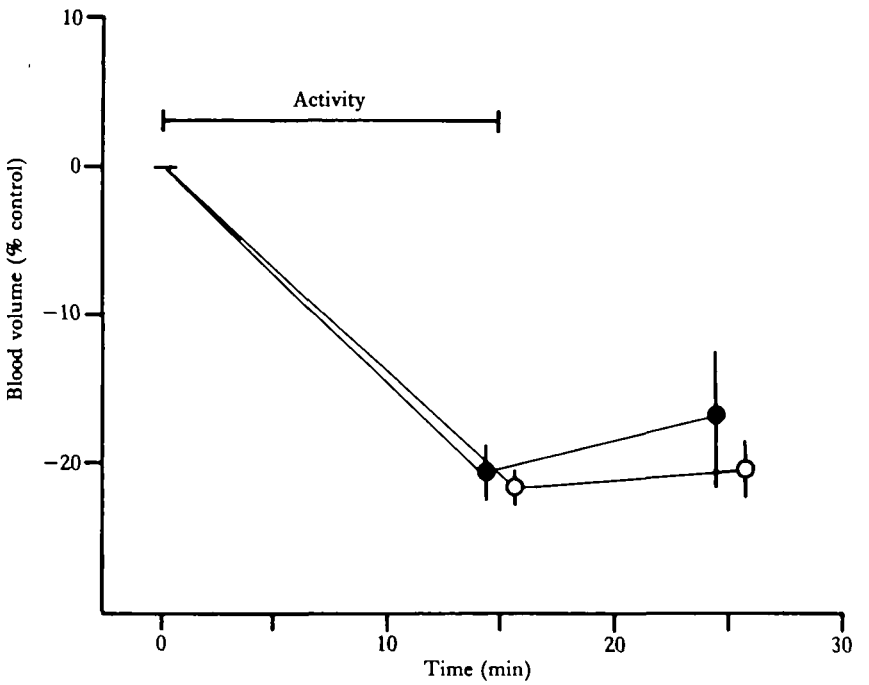

Fig. 1. Percentage change from control (resting) blood volumes in three *Elaphe obsoleta* (closed circles) and three *Crotalus viridis* (open circles) in response to 15 min of locomotor activity, and following a 10 min recovery from activity. Circles depict means; vertical bars represent ±1 S.E. of the mean. The activity period is spanned by the horizontal bar.

remained at these levels during the 10 min of recovery following activity. Measurements of BV made later in recovery indicated that the restoration of control BV required longer than 1 h and varied due to changes in the activity of snakes while they were held inside the acrylic tubes (Fig. 2).

Arterial hypertension caused by the infusion of norepinephrine also reduced BV in both species of snake (Fig. 3). Decreases in BV occurred notably 10–20 min after arterial blood pressure peaked. The maximum BV reductions (at +20 min) in *Elaphe* and *Crotalus* were 8·6 ± 1·9 and 17·0 ± 5·7 %, respectively. The return of BV to control level required 1–3 h but varied according to the activity of snakes, as in the other experiments (Fig. 4).

Haemodynamic responses to activity were proportionately similar in *Elaphe* and *Crotalus* (Fig. 5): HR increased dramatically (83 and 87 %, respectively) while increases in BP were more modest (59 and 55 %, respectively). The significant increases in Hct in *Elaphe* (22 %) and *Crotalus* (29 %) are consistent with the estimates of BV reduction as measured by the ^{51}Cr-labelled erythrocytes (Fig. 1). The infusion of norepinephrine caused proportionately greater hypertension in *Elaphe* than *Crotalus* (154 *vs* 121 % increase in BP). Heart rates remained unchanged, with a slight bradycardia at the peak of arterial pressure in *Elaphe*. Increases in Hct in response to arterial hypertension were smaller than increases due to activity. Maximum increases in Hct above control values in *Elaphe* (7 %) and *Crotalus* (18 %) occurred 10–20 m

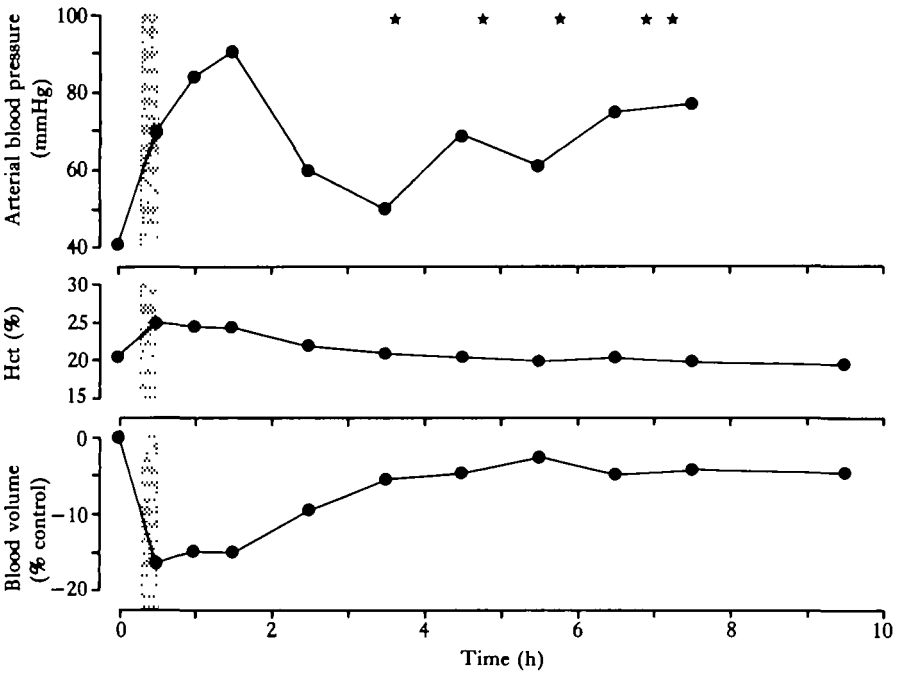

Fig. 2. Patterns of arterial pressure, Hct and BV determined in a single *Elaphe obsoleta* for an extended 'recovery' period following 15 min of locomotion (stippled bar). During recovery the snake was held within an acrylic tube where it rested in an extended position but was free to move forward or backward for a distance of several cm. Note that movements of the snake (indicated by stars in top panel) elevated arterial pressure and prevented BV from returning to the control level measured before exercise.

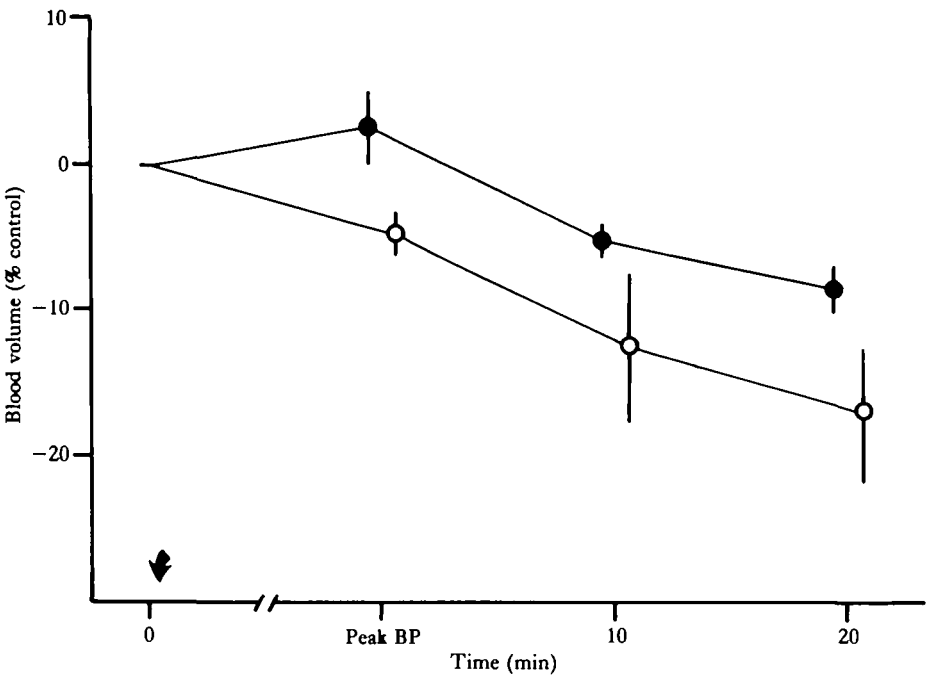

Fig. 3. Percentage change from control (resting) blood volumes in *Elaphe obsoleta* (closed circles) and *Crotalus viridis* (open circles) in response to arterial hypertension induced by norepinephrine (100 $\mu g\,kg^{-1}$, injected at arrow). Blood volume measurements were made after the development of peak arterial blood pressure (Peak BP) and at 10-min intervals thereafter. Symbols as in Fig. 1.

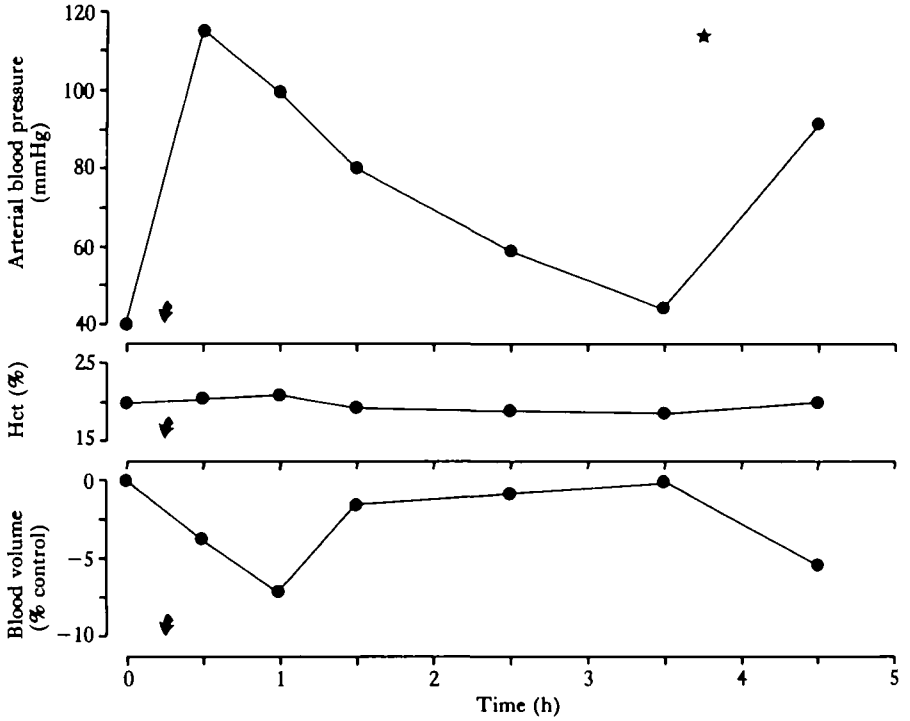

Fig. 4. Patterns of arterial pressure, Hct and BV determined in a single *Elaphe obsoleta* for an extended period following arterial infusion of 100 $\mu g\ kg^{-1}$ norepinephrine (arrows). Note the increase of arterial pressure and decrease of BV that occurred after the snake moved (star in upper panel).

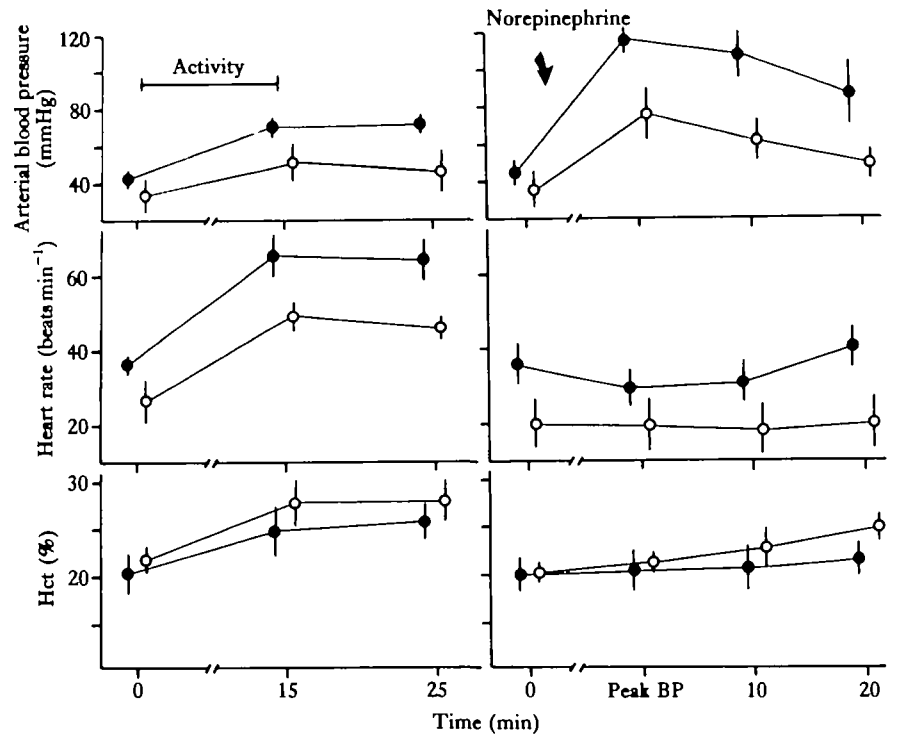

Fig. 5. Haemodynamic responses of *Elaphe obsoleta* (closed circles) and *Crotalus viridis* (open circles) to the two experimental treatments, activity (horizontal bar in left panel) and norepinephrine (arrow in right panel). Sample times and symbols are the same as in Figs 1 and 3.

ter the pressure peak, and corresponded to the degree of BV reduction in each species (Fig. 3).

DISCUSSION

The steady-state distribution of fluid between the vascular and extravascular compartments is determined principally by the hydrostatic and osmotic forces associated with capillaries, and the functional capabilities of lymphatics. Present findings indicate that BV of active or hypertensive terrestrial snakes differs substantially from BV of snakes at rest, apparently due to an increase in the capillary filtration of plasma out of the vascular space. Muscular activity promotes the translocation of plasma more effectively than does hypertension *per se*, for if BP is elevated pharmacologically, the level of arterial pressure is higher but the fluid shift is less than that which is induced by locomotion (Figs 1–5). The state of hypertension promoted by norepinephrine reflects a generalized vasoconstriction in peripheral vasculature, whereas the increase in BP resulting from activity must accompany an increase in blood flow to muscle tissue. It is evident from mammalian studies that elevations of capillary hydrostatic pressure subsequent to a fall in precapillary resistance increase the movement of fluid out of skeletal muscle capillaries during and for varying periods following the activation of muscle in exercise (Haddy, Scott & Grega, 1976). The increased fluid efflux may be augmented by transient increases in tissue osmolality and capillary surface area (Kjellmer, 1964). Thus, skeletal muscle is likely to be a principal site of the transcapillary fluid fluxes observed during locomotion in snakes.

Exercise in mammals reduces plasma volume roughly 5–15 %, depending on the intensity and duration of muscular activity (Ekelund & Holmgren, 1964; Greenleaf *et al.* 1977; Novosadová, 1977; Thomas & Etheridge, 1982). By comparison, the 21 % reduction of BV demonstrated in snakes represents a translocation of plasma which exceeds that of mammals by factors of 2 to 7. This supports our inference that these reptiles possess a comparatively small resistance to fluid transfer between the vascular and extravascular spaces, and may also indicate a limited removal capacity of the lymphatic system.

The potential contribution of the spleen (releasing or sequestering erythrocytes) to our measurements is negligible for several reasons. First, the spleen of the snake is comparatively small (0·086 g wet mass/100 g body mass in *Elaphe obsoleta*, $N = 4$; A. W. Smits, unpublished data) and could not possibly account for the changes in Hct we observed. Secondly, the increases in Hct during activity and hypertension were reversible, and Hct returned to control values in all snakes studied. Finally, we have never previously observed such dramatic increments in Hct in these snakes, even during experiments involving severe haemorrhage when one would expect spleen contraction.

Both species of snake exhibit similar haemodynamics and BV changes with activity, but the responses of each species to arterial hypertension are different. *Elaphe* develops proportionately greater hypertension and reflexogenic response (bradycardia) to norepinephrine and experiences less plasma filtration than does *Crotalus*. These findings are consistent with previous research indicating that rat snakes possess ore potent autonomic adjustments of vascular tone and exhibit less blood pooling

in dependent vasculature than do rattlesnakes when the circulation is disturbed by head-up tilting (H. B. Lillywhite, unpublished data).

Two obvious consequences of the decrease in plasma volume observed during exercise are increases in the oxygen capacity and viscosity of the blood. It is not known whether these changes confer any respiratory (e.g. oxygen delivery) or cardiovascular (e.g. flow changes) advantages or disadvantages to snakes during these dramatic reductions of plasma volume. Our results do indicate a need for caution while interpreting or comparing instantaneous measurements of blood volume in these vertebrates; differences between species may clearly be confounded by variability due to the activity or hypertensive states of animals. This may also apply to other vertebrates as well.

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