J. exp. Biol. (1981), 94, 275–283
With 4 *figures*
Printed in Great Britain

HAEMODYNAMIC RESPONSES TO HAEMORRHAGE IN THE SNAKE, *ELAPHE OBSOLETA OBSOLETA*

BY HARVEY B. LILLYWHITE AND LAURENS H. SMITH
Department of Physiology and Cell Biology, The University of Kansas, Lawrence, Kansas 66045

(*Received* 30 *January* 1981)

SUMMARY

Haemodynamic responses to graded haemorrhage were studied in unanaesthetized black ratsnakes (*Elaphe o. obsoleta*) wherein fixed percentages of the blood volume were removed at regular intervals over periods which varied from one to several hours. Initially measured whole blood volumes averaged about 6% of the body mass and were determined from plasma dilutions of Evans blue dye or the bleeding and washing out of red cells. In response to haemorrhage, heart rate increased, haematocrit decreased, plasma protein concentration decreased, and arterial pressure remained relatively constant until a critical blood loss at which pressure dropped precipitously. Critical volume deficits occurred at the withdrawal of 63–122% of the initially measured blood volume. It appears that cardiovascular reflexes assist the regulation of arterial pressure during haemorrhage; additionally, there occurs a progressive and significant restitution of plasma volume owing to the absorption of extravascular fluid. Fluid volumes absorbed by the circulation during haemorrhage replaced 20–71% of the haemorrhaged blood loss and were correlated with the magnitude of the critical volume deficit. Body movements increased arterial pressure by improving venous cardiac return.

INTRODUCTION

The cardiovascular adaptations of snakes invite attention because of the long body shape and ecological specializations of these reptiles. Recent studies of terrestrial and arboreal species demonstrated that blood pressure is restored towards normal following disturbance by tilting (Seymour & Lillywhite, 1976; Lillywhite & Seymour, 1978; Lillywhite, 1980*a*). However, relatively little is known regarding cardiovascular regulatory mechanisms in these or other reptiles.

In this paper we report the cardiovascular responses of a terrestrial and partly arboreal snake to losses of blood volume. The observed compensatory responses are notable for their efficacy and invite comparison of the regulatory mechanisms with those of higher vertebrates.

MATERIALS AND METHODS

Sixteen adult black ratsnakes, *Elaphe obsoleta obsoleta* (Say), were captured near Lawrence, Kansas, and maintained in the laboratory at 25–28 °C. Surgical procedures generally followed the methods developed by Seymour & Webster (1975) and

Lillywhite & Seymour (1978). Snakes were allowed at least 36 h to recover from surgery and were held within acrylic tubes having narrow longitudinal openings to permit free exit and movement of catheters. Only healthy snakes in positive water balance were used in these investigations.

Arterial pressures were measured by means of an occluding catheter (PE-50 polyethylene tubing) filled with heparinized saline and tied into the dorsal aorta at a level near the vent. Central venous pressures were measured from a non-occluding, bevel-tipped catheter (usually PE-10) inserted through a small needle puncture made in the inferior vena cava 10–20 cm posterior to the heart. The catheter tip was advanced to within a few cm of the heart and secured by external suture. Blood pressures were recorded from pressure transducers (P–1000 Linear Core) coupled to separate pen-channels of a desk model DMP–4A physiograph (Narco–Biosystems, Inc., Houston, Texas).

(A) *Measurement of blood volumes*

Blood volumes were determined in eleven snakes by either of two methods, hereafter referred to as 'Evans blue' or 'washout' respectively. In the first, blood volumes were estimated from dilutions of Evans blue dye (T-1824) in plasma, using conventional procedures. Either 0·15, 0·25 or 0·5 ml of 1 % Evans blue dye solution was injected into the venous catheter which was then flushed with blood previously withdrawn from the snake. At least four blood samples (0·5–0·9 ml) were subsequently withdrawn from the aortic catheter (excluding dead space) at 10–15 min intervals and centrifuged for 5 min in an International microcentrifuge. Haematocrits determined in micro-capillary tubes were not changed by longer centrifugation. The absorbance of plasma samples, each diluted in water 1:25, was read at 610 μm in a Bausch and Lomb Spectronic 600 spectrophotometer. Readings for the several samples were extrapolated to zero time on a semi-logarithmic time-absorbance plot, and the resulting plasma volume (PV) was read from a standard volume dilution plot determined previously. In extrapolating time-absorbance plots to zero time, it was necessary to assume that mixing in the circulation was completed when the initial period of rapid disappearance was terminated (5–15 min) (See Lawson, 1962). Blood volume (BV) was calculated from the equation

$$\mathrm{BV} = \frac{\mathrm{PV}}{1-(\mathrm{HCT}\times 0{\cdot}843)},$$

where HCT is the haematocrit and 0·843 the 'plasma trapping factor' (see below).

The percentage of plasma trapped in the packed red cell column was determined in four separate tests (different snakes) where the red cell volume of a centrifuged blood sample was resuspended in 4 ml of 0·9 % saline and centrifuged a second time. The absorbance of a diluted sample of the supernatant was read from the spectrophotometer and compared with a standard volume dilution plot determined from dilutions of small volumes of plasma taken from the initially centrifuged blood sample. Subsequently, haematocrits were multiplied by 0·843 in calculations of blood volume using Evans blue dye, the percentage of trapped plasma taken as 15·7 % (four determinations = 13, 16·5, 16·7, 16·7 %).

In the washout method, blood volumes were determined by bleeding and washing out of blood with 0·9 % saline. Blood was withdrawn a few ml at a time, and

haematocrit of the first collected volume was determined. After removal of 10–20 ml of blood, 5 ml volumes of oxygenated saline were periodically infused into the snake via the venous catheter or the arterial catheter. The alternating procedures of infusion and withdrawal were continued until the blood withdrawn showed a haematocrit < 0·05 %. The snake was killed by an overdose of lidocaine and subsequently perfused with saline from arterial to venous catheter. The snake was finally dissected and examined for remaining trapped blood. Small volumes of blood were sometimes evident in the pulmonary vasculature and were collected by a separate washout procedure. The total blood volume was then calculated as

$$BV = \frac{RCV + (FV \times HCT_f)}{HCT_i},$$

where RCV = volume of red blood cells withdrawn, FV = volume of fluid estimated to remain in the circulation, HCT_i = starting haematocrit, HCT_f = ending haematocrit.

In separate tests, samples of blood were centrifuged, and the red cell volume was subsequently resuspended in 0·9 % saline. Haematocrits determined from the resulting suspension differed by no more than 0·1 % from those of the original blood. Similarly, the percent volume of packed red cells in microcapillary tubes was similar to that in test tubes which were used to estimate red cell volumes removed from the snakes.

(B) *Graded haemorrhage*

Snakes were bled individually while lying at rest, unanaesthetized, in horizontal tubes. Fixed percentages (2–8 %) of the initially measured blood volume (IBV) were withdrawn at regular intervals into a clean syringe from the arterial catheter. Blood pressures and heart rate were recorded for 5 min between successive blood withdrawals. In this manner, graded haemorrhage was executed at roughly 6–8 min intervals (withdrawal times varied) until blood could no longer by obtained with reasonable ease. In some experiments the blood was stored at room temperature in heparinized containers and returned to the snake at the conclusion of haemorrhage. These snakes survived.

Experiments were conducted at temperatures of 25–28 °C. Arterial and venous pressures were recorded from nine snakes; only arterial pressure was measured in the remaining snakes. Heart rate was determined from the pulsatile arterial pressure trace or from ECG recordings signalled from two silver electrodes sutured to the skin near the heart. At regular intervals blood samples were used for determinations of haematocrit and plasma protein concentration. The latter was determined from a micro modification of the method of Bradford (1976) using Coomassie Blue G–250.

RESULTS

(A) *Physiological parameters before haemorrhage*

The IBV varied directly with body mass according to the regression equation

$$IBV = 0{\cdot}07^{0{\cdot}98},$$

where r^2 = 0·96. The specific gravity of whole blood (1·04–1·06) was used to convert

Table 1. *Whole blood volume and haemorrhage parameters in* Elaphe obsoleta

(Question marks denote critical volume deficits which may be underestimates owing to the necessity of terminating the experiment.)

	IBV, ml		Cum. absorbed fluid, % IBV*				
Snakes (sex, mass g)	Evans blue	Washout	Hct	Evans blue	Washout	Critical vol. deficit (% IBV)	'Net' critical vol. deficit (% IBV)†
G (♀ 244)	12·73	—	16·84	—	—	89·1 (?)	72
I (♂ 448)	32·85	27·40	—	—	—	—	—
K (♂ 415)	—	25·29	11·90	—	14·39	63·3	54·9
L (♂ 850)	—	45·60	73·60	73·2	67·1	121·5 (?)	47·8
M (♂ 395)	25·46	20·14	46·4	—	40·4	69·8 (?)	22·3
N (♂ 545)	31·24	31·6	60·9	37·0	51·6	88·6	31·6
O (♀ 364)	—	24·28	14·4	14·0	12·3	49·4 (?)	32·5
P (♂ 710)	40·14	32·3	53·2	52·9	48·1	81·3	39·8
Q (♂ 410)	—	20·34	—	—	—	—	—
R (♂ 990)	69·4	61·45	—	—	—	—	—
T (♀ 161)	11·01	9·27	—	—	—	—	—

* Data were computed based on serial decrements of the haematocrit or from volume measurements using Evans blue or washout as described in the text.

† Computed by subtracting the volume of extravascular fluid absorbed by the circulation during the period preceding the critical deficit from the critical volume deficit (whole blood withdrawn) and expressing the remainder (actual volume of fluid in the circulation) as a percentage of the IBV. The absorbed fluid volumes were calculated from the serial decrements of the haematocrit.

volume measurements to percentages of body mass. The mean of these percentages was $6{\cdot}64 \pm 0{\cdot}28$ S.E. ($N = 7$) and $5{\cdot}89 \pm 0{\cdot}22$ ($N = 10$) for measurements determined by Evans blue and washout, respectively. In cases where both methods were employed in the same snake, the mean of the two measurements was used in further data analyses where IBV was a parameter. A summary of these volume measurements and related variables is given in Table 1.

Initially measured haematocrits averaged 27·9% (S.E. = 1·9, $N = 16$). The mean concentration of plasma protein was 75·26 mg/ml (S.E. = 2·70, $N = 9$). Arterial pressure varied from 38 to 75 mmHg in different snakes and was significantly higher in males (mean = $54{\cdot}45 \pm 3{\cdot}65$ S.E., $N = 11$) than in females (mean = $41{\cdot}75 \pm 1{\cdot}50$ S.E. $N = 4$) (Mann Whitney U test, $P < 0{\cdot}05$).

(B) *Responses to haemorrhage*

Graded haemorrhage elicited cardiovascular responses which acted to compensate for the reduction of blood volume. Arterial pressure was generally maintained until a 'critical volume deficit' was reached; following this, the pressure dropped precipitously in response to further blood loss (Figs. 1, 2). Critical volume deficits ranged from 63·3 to at least 121·5% IBV (mean = $85{\cdot}6 \pm 8{\cdot}3$ S.E., $N = 6$) and could not be determined in certain snakes whose behaviour (e.g. curling of the tail) prevented efficient withdrawal of blood even though arterial pressure remained high (e.g. Fig. 1).

Heart rate increased during haemorrhage and typically plateaued for variable periods before the critical volume was removed (Fig. 3). The mean increase in heart rate during haemorrhage was 119% (S.E. = 13·9, $N = 12$). Pulse pressures initially ranged from 2 to 13 mmHg (mean = 6·3, S.E. = 1·1, N = 12), but in all cases diminished by the conclusion of haemorrhage. Central venous pressures were consisten

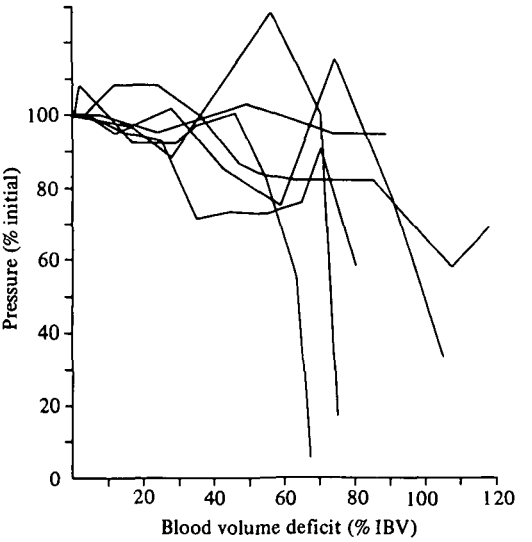

Fig. 1. Relative changes of arterial pressure plotted as a function of increasing blood volume deficit in six snakes. Each curve represents a single animal and a single experiment. In two cases the experiment was terminated before a precipitous pressure drop occurred. The ability of snakes to increase arterial pressure following periods of diminishment was usually attributed to body movements.

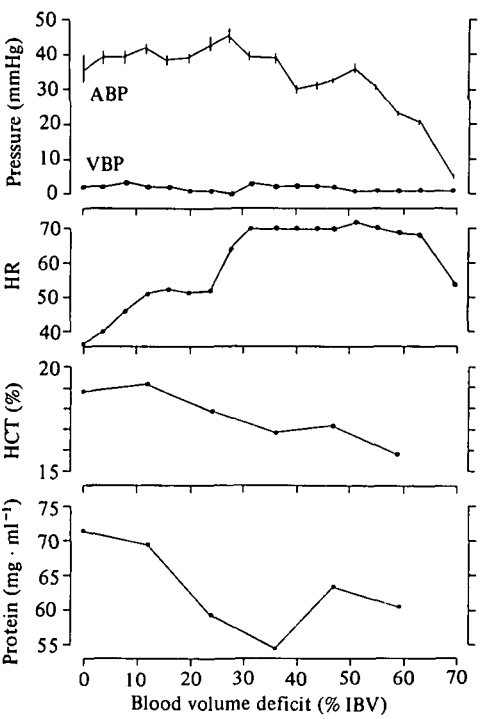

Fig. 2. Relationships of cardiovascular parameters to blood volume deficit during graded haemorrhage of a 415 g male snake. The parameters are mean arterial pressure (ABP), pulse pressure (vertical lines), central venous pressure (VBP), heart rate, haematocrit and plasma protein concentration.

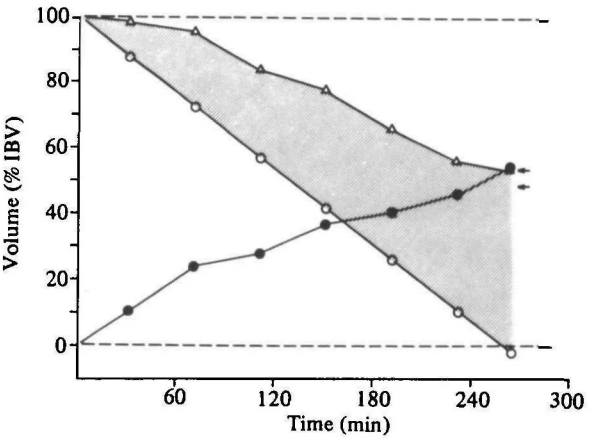

Fig. 3. Time course of haemorrhage and blood dilution in a 710 g male snake from which roughly 100 % of the IBV was removed. Open circles represent cumulative volume deficits in terms of the actual blood volumes removed during haemorrhage. Filled circles represent cumulative volumes of extravascular fluid absorbed by the circulation and determined from the serial reductions of haematocrit. Triangles represent the net volume deficit, determined by adding the absorbed fluid volume (filled circles) to the withdrawn deficit (open circles). The hatched area therefore depicts the extent of haemodilution. The small horizontal arrows denote cumulative dilution volumes determined at the end of the experiment by means of Evans blue measurement (upper arrow) and washout (lower arrow) as described in the text.

between 1 and 4 mmHg in quiescent animals and showed little net change during haemorrhage (Fig. 2).

Haematocrits progressively diminished during haemorrhage (mean total decrement = −30·5 % initial, S.E. = 4·1, N = 12). Plasma protein concentration generally declined, but transient increases sometimes attended movements of snakes and increases of arterial pressure (Fig. 2). Maximum changes in protein concentration varied from roughly 0 to −25·6 % (mean = −10·1, N = 10).

The patterns of haematocrit and plasma protein imply a dilution of the circulating blood volume by extravascular fluids. The magnitude of haemodilution was quantified in six snakes by measuring the circulating blood volume at the conclusion of haemorrhage. This residual volume, when added to the known volume of blood removed from a snake during haemorrhage, should exceed the IBV by an amount equal to the volume of extravascular fluid entering the circulation during the haemorrhage period. Such measurements, using both Evans blue and washout methods, are given in Table 1 along with haemodilution volumes estimated from progressive changes of haematocrit in relation to known haemorrhage volumes and the IBV. Measurements derived from either procedure generally agreed.

A graphic illustration of the haemodilution in a snake which lost 100 % of its IBV is shown in Fig. 3. Regression coefficients of similar time-dilution plots determined from haematocrit changes indicated that rates of haemodilution varied from 5·75 to 18·3 % IBV·h^{-1} (mean = 11·4, S.E. = 1·64, N = 7). The percentage of blood loss that was replaced by haemodilution ranged from 20 to 70·8 % (mean = 49·9, S.E. = 8·4, N = 7). There was a significant correlation between the cumulative (total) haemodilution volume and the critical volume deficit (r = 0·78, P < 0·01). Howe

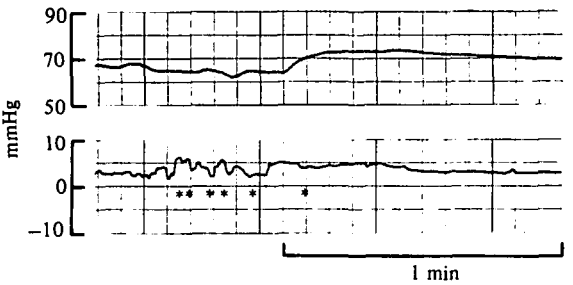

Fig. 4. Changes of arterial pressure (upper panel) and central venous pressure (lower panel) in response to stereotyped 'wiggling' movements described in the text. The movements are denoted by asterisks. Data are from a 591 g male snake responding to the cumulative loss of 14 ml whole blood.

neither variable bore a significant relationship to either the rate of blood withdrawal or duration of experiment (both $P > 0{\cdot}05$).

(C) *Behaviour*

Snakes typically were motionless during most of the haemorrhage period but became active at volume deficits ranging from 28 to 77% IBV (mean = 54). Activity consisted of a snake slowly lifting its head and neck or moving the body more vigorously while crawling forward and backward in the tube. During periods of low arterial pressure snakes engaged in stereotyped 'wiggling' where lateral undulations were advanced sinusoidally along the body. These movements were nearly always propagated headward, usually from the rear of the body to the head but sometimes limited to the region near the heart or neck. Occasionally, the movements were vigorous, causing the body to 'flap' against the tube. The movements produced transient increments of venous and arterial pressures which persisted considerably longer than the behavioural acts themselves (Fig. 4).

DISCUSSION

The abilities of snakes to resist the disturbing effects of blood loss are probably the most potent reported in any vertebrate. Snakes maintained arterial pressure at IBV losses averaging 86%, and one individual maintained pressure during deficits exceeding 100%. Studies of other snakes corroborate these data and demonstrate capabilities to recover from such treatment provided that blood is returned to the circulation or the animal is allowed to drink water (Lillywhite, unpublished data). The only comparable data for other reptiles are those of Hohnke (1975) who reported critical volume deficits ranging from 3·8 to 71% (mean = 39·8) in *Iguana iguana*. It is generally accepted that among mammals blood losses in excess of 35 to roughly 50% result in irreversible shock (Kovach, Szasz & Pilmayer, 1969; Guyton, 1980). Only among avian species do reported haemorrhage tolerances approach that of snakes (Kovach & Szasz, 1968).

The stability of arterial pressure and graded tachycardia observed during haemor-

rhage (Fig. 2) suggest that baroreflexes (Hohnke, 1975; Seymour & Lillywhite, 1976; Lillywhite & Seymour, 1978; Berger, Evans & Smith, 1980; Millard & Moalli, 1980) are activated by the condition of blood loss. Adrenergic mechanisms probably play a dominant role in modulating cardiac performance (Lillywhite & Seymour, 1978; Berger *et al.* 1980) and may also be expected to enhance vascular tone (Lillywhite & Seymour, 1978). Possible regulation of venomotor tone can be inferred from the stability of central venous pressure (Fig. 2) which suggests that venous capacity is reduced in response to blood loss.

Movements of snakes clearly are important in elevating venous and arterial pressures, no doubt by improving the venous cardiac return. It appears also that snakes have evolved specific behaviours which function to control arterial pressure (Fig. 4) (Lillywhite, 1980*b*; unpublished).

Behavioural and reflexogenic mechanisms confer rapidity of response but are not suitable for long-term compensation of large blood losses. Hence, the restoration of blood volume is an important feature of homeostatic adjustment in snakes. Estimates of haemodilution based on haematocrit changes or derived more directly from measurements of intravascular volumes (Table 1) indicate that relatively large percentages of blood loss (20–71 %) were replaced by the absorption of fluids from extravascular compartments. As expected, the degree of haemodilution was correlated with the severity of haemorrhage that was tolerated by a snake. We did not calculate haemodilution volumes based on observed changes in plasma protein because the latter were less consistent than were changes in red cell concentration. Transient increases in plasma protein concentration observed when snakes moved or increased arterial pressure suggest that filtration of plasma was temporarily enhanced in response to the improved haemodynamic state. However, we cannot exclude the possibility that protein entered the circulation from an extravascular source.

If the volume deficits tolerated by snakes are expressed in terms of the volume of blood withdrawn *minus* the volume of extravascular fluid added to the circulation during the same time period, the resulting net losses of vascular fluid are reduced in magnitude (Table 1) and appear more comparable to the critical deficits observed in mammals. The superior tolerance of snakes to blood loss is thus related, in part, to a high fluid transfer capacity as was shown for birds (Djojosugito, Folkow & Kovach, 1968). In birds, the efficient volume compensation was attributed to strong reflex vasoconstriction in skeletal muscle, resulting in increased pre- to postcapillary resistance ratio and attendant fall in capillary pressure. Similar responses seem likely to occur in snakes, since increased peripheral resistance is a dominant reflex in pressure adjustments to tilting (Lillywhite & Seymour, 1978). There appear to be no data indicating differences in the amount of fluid stores that is available for compensating blood loss in various vertebrates. Possibly snakes are capable of mobilizing part of the intracellular fluid which comprises a relatively larger space than in birds or mammals (Thorson, 1968).

The IBV of *Elaphe obsoleta* scales in direct proportion to the body mass, as was shown in a variety of mammals (Sjostrand, 1962). Since we used arterial haematocrits in calculations of IBV, a rather consistent discrepancy between Evans blue and washout estimates of the IBV (Table 1) is expected. Assuming that the ratio of whole body haematocrit to central haematocrit is somewhat less than unity (Gregerson & Raws

959; Lawson, 1962), correction of the present measurements for the distribution of red cells would confer closer agreement since Evans blue calculations would be lowered somewhat and washout calculations similarly elevated. It suffices to conclude that the whole IBV of *E. obsoleta* is roughly 6% of the body mass which is in agreement with data for other terrestrial snakes (Thorson, 1968; Smeller, Bush & Seal, 1978). Aquatic species may have larger blood volumes (Dunson, 1975; Feder, 1980).

This work was supported by NIH Grant HL 24640, Biomedical Sciences Support Grant RR07037, and University of Kansas general research award 3494-0038.

REFERENCES

BERGER, P. J., EVANS, B. K. & SMITH, D. G. (1980). Localization of baroreceptors and gain of the baroreceptor-heart rate reflex in the lizard *Trachydosaurus rugosus*. *J. exp. Biol.* **86**, 197–209.

BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* **72**, 248–254.

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

DUNSON, W. A. (1975). Salt and water balance in sea snakes. In *The Biology of Sea Snakes* (ed. W. A. Dunson), pp. 329–353. Baltimore: University Park Press.

FEDER, M. E. (1980). Blood oxygen stores in the file snake, *Acrochordus granulatus*, and in other marine snakes. *Physiol. Zoöl.* **53**, 394–401.

GREGERSEN, M. I. & RAWSON, R. A. (1959). Blood volume. *Physiol. Rev.* **39**, 307–342.

GUYTON, A. C. (1980). *Textbook of Medical Physiology*. Philadelphia: W. B. Saunders.

HOHNKE, L. (1975). Regulation of arterial blood pressure in the common green iguana. *Am. J. Physiol.* **228**, 386–391.

KOVACH, A. G. B., SZASZ, E. & PILMAYER, N. (1969). Mortality of various avian and mammalian species following blood loss. *Acta physiol. hung.* **35**, 109–116.

KOVACH, A. G. B. & SZASZ, E. (1968). Survival of pigeon after graded hemorrhage. *Acta physiol. hung.* **34**, 301–309.

LAWSON, H. C. (1962). The volume of blood – a critical examination of methods for its measurement. In *Handbook of Physiology*, Sect. 2: *Circulation*, vol. 1 (ed. W. F. Hamilton & P. Dow), pp. 23–49. Washington, D.C.: Americal Physiological Society.

LILLYWHITE, H. B. (1980*a*). Blood volume and arterial pressure regulation in the black ratsnake. *Fed. Proc.* **39**, 1060.

LILLYWHITE, H. B. (1980*b*). Behavioral control of arterial pressure in snakes. *Am. Zool.* **20**, 768.

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

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.

SEYMOUR, R. & LILLYWHITE, H. (1976). Blood pressure in snakes from different habitats. *Nature, Lond.* **264**, 664–666.

SEYMOUR, R. S. & WEBSTER, M. E. D. (1975). Gas transport and blood acid-base balance in diving sea snakes. *J. exp. Zool.* **191**, 169–181.

SJOSTRAND, T. (1962). Blood volume. In *Handbook of Physiology*, Sect. 2: *Circulation*, vol. 1 (ed W. F. Hamilton & P. Dow), pp. 51–62. Washington, D.C.: Americal Physiological Society.

SMELLER, J. M., BUSH, M. & SEAL, U. S. (1978). Blood volume measurements in gopher snakes, using autologous ^{51}Cr-labeled red blood cells. *Am. J. vet. Res.* **39**, 355–356.

THORSON, T. B. (1968). Body fluid partitioning in reptilia. *Copeia*, pp. 592–601.