

## ACCESSORY LYMPH SACS AND BODY FLUID PARTITIONING IN THE LIZARD, *SAUROMALUS HISPIDUS*

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### SUMMARY

Chuckwalla lizards (genus *Sauromalus*) may accumulate substantial quantities of body fluid in extracoelomic, lateral abdominal spaces called accessory lymph sacs. The lymph sac fluid (LSF) of *S. hispidus* is similar to that of serum in  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations, but the total protein content ( $3.58 \pm 0.20 \text{ g dl}^{-1}$ ) is only half that measured in serum ( $7.05 \pm 0.26 \text{ g dl}^{-1}$ ). These analyses confirm that LSF is an extravascular form of extracellular fluid, similar in composition to true lymph. Measurements of body fluid partitioning by dilution analysis indicate that *Sauromalus hispidus* Stejneger possesses a comparatively large (38.9% body mass) and labile extracellular fluid volume (ECFV), and that the volume of LSF is dependent on the ECFV. Expansion of the ECFV (and subsequent accumulation of LSF) is observed following large, intercompartmental fluid shifts from intracellular to extracellular locations when lizards (1) are kept inactive in simulated hibernation, (2) are injected with KCl in amounts similar to those found in their field diet, and (3) are hydrated with NaCl that is isotonic to their body fluids. These data collectively suggest that the lymph sacs of chuckwallas facilitate expansion of the ECFV, and may be adaptive not only as a means to store body water, but to accommodate transient shifts in body fluid from intracellular to extracellular locations.

### INTRODUCTION

The capacity for animals to store excess body water might represent an important adaptation in arid environments where the availability of water is seasonally limited and highly unpredictable from year to year. Fluid storage has been particularly implicated in several species of plant-eating reptiles that may accumulate significant amounts of fluid within the gut cavity (Louw & Holm, 1972; Grenot, 1976; Lemire, Grenot & Vernet, 1982), urinary bladder (Minnich, 1976, 1982) and in the extracellular fluid compartment (Norris & Dawson, 1964).

Apparently only the chuckwalla lizards (genus *Sauromalus*) accumulate fluid in accessory lymph sacs, extracoelomic membranous sacs that extend along the lateral abdominal folds between the integument and the body wall. The fluid in these sacs

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(often loosely referred to as water) has been assumed to be lymph, based largely on measurements of its chloride concentration ( $122\text{--}136\text{ mequiv l}^{-1}$ ) and the absence of erythrocytes (Norris & Dawson, 1964). Storage of fluid in the lymph sacs by expansion of the extracellular fluid volume was first proposed by Norris & Dawson (1964) and recently has been assumed to occur (Minnich, 1982), despite the lack of any substantive field or laboratory data. Reduction in lymph sac fluid volumes in free-living chuckwallas (*S. obesus* and *S. hispidus*) occurs with dehydration (Nagy, 1972; Smits, 1985), but the conditions under which chuckwallas accumulate sac fluid have not been described.

*Sauromalus hispidus* possesses the largest lymph sacs in proportion to body mass of any species of *Sauromalus* (Norris & Dawson, 1964). Thus, this lizard is the most likely species in which the functional significance of the lymph sacs might be demonstrated.

To clarify the significance of the accessory lymph sacs in fluid storage in chuckwallas, I investigated (1) the composition of the sac fluid, (2) the relationship between the lizard's sac fluid volume and the extracellular fluid volume, and (3) the physiological conditions that result in fluid accumulation in the lymph sacs of *S. hispidus*. The influence of diet and lizard dormancy on body fluid partitioning and storage were also studied using ecologically-relevant information from field studies (Smits, 1984, 1985) of water balance in *S. hispidus*.

#### MATERIALS AND METHODS

##### *Composition of lymph sac fluid*

Blood serum and lymph sac fluid were collected from field-captured *Sauromalus hispidus* on Isla La Ventana, Gulf of California, Mexico. Sac fluid and blood were obtained by syringe aspiration through the lateral sac wall and cardiac puncture, respectively. Blood was centrifuged after clotting, and serum and sac fluid were stored in wax-sealed, plastic vials at  $-20^{\circ}\text{C}$ . Samples were thawed and well mixed prior to analyses and kept frozen at  $-20^{\circ}\text{C}$  at all other times. Total osmolality of body fluid was measured by vapour pressure osmometry (Wescor Instruments, Model 5100C). Concentrations of  $\text{K}^{+}$  and  $\text{Na}^{+}$  were quantified by flame photometry (Instrumentation Laboratory, Model 151). Chloride ion concentrations were determined by microtitration with an Oxford titrator (Sigma Chemical Diagnostic Kit No. 830-T). Total protein was measured using a modification of the Lowry method (Schacterle & Pollack, 1973). Samples were assayed in duplicate and compared to bovine serum albumin standards ( $1\text{--}10\text{ g dl}^{-1}$ ). Absorbance was read at 650 nm after colour development (10 min). Albumin and globulin concentrations were measured in duplicate using Sigma Chemical reagents and procedures no. 560 and 630 and compared to human serum albumin ( $5\text{ g dl}^{-1}$ ) and globulin ( $3\text{ g dl}^{-1}$ ).

##### *Responses to fluid loading*

Total body water (TBW), extracellular fluid volume (ECFV), lymph sac fluid volume (LSFV) and body mass were measured in lizards before and after fluid

loading in the laboratory. Eight adult lizards were given daily intraperitoneal injections of either distilled water (hypotonic group, four lizards) or a solution containing (in  $\text{mmol l}^{-1}$ ) NaCl, 150; KCl, 6 (300  $\text{mosmol kg}^{-1}$ ; isotonic group, four lizards) for 10 consecutive days. The injected volume ( $25 \text{ ml kg}^{-1} \text{ day}^{-1}$ ) exceeded the maximum rates of water loss measured in field-active lizards (approximately  $14 \text{ ml kg}^{-1} \text{ day}^{-1}$ ; Smits, 1985), and was given between 10.00 and 12.00 h CST.

All lizards were in apparent good health. They were kept separate in stainless steel cages and allowed to regulate their body temperatures by proximity to heat lamps that were on for 9 h daily. Lizards were fasted for 3 days prior to and for the duration of the experiment. Measurements were made on the day prior to the start of fluid injections and again on the second day after injections ceased. TBW and ECFV were measured by dilution analysis after injecting known amounts of deuterium oxide ( $2.5 \text{ ml kg}^{-1}$  body mass) and sodium thiocyanate ( $100 \text{ mg NaSCN kg}^{-1}$  body mass), respectively. Analysis of  $\text{D}_2\text{O}$  (infrared spectrometry, Zweens, Frankena, Reicher & Zijlstra, 1980) and thiocyanate ion (Bradshaw & Shoemaker, 1967) in fluid collected at timed intervals from the contralateral lymph sac indicated when each tracer had equilibrated with its respective fluid space, and TBW and ECFV were calculated from the dilution factors. Remeasurement of TBW and ECFV following fluid loading were corrected for residual  $\text{D}_2\text{O}$  and NaSCN that remained in the lizard's body fluids from measurement of TBW and ECFV before fluid loading.

The LSFV was determined by temporarily removing the fluid by aspiration into a graduated cylinder. Lizards were tranquilized by intramuscular injections of ketamine hydrochloride (Ketaset, Bristol Veterinary Products,  $50 \text{ mg kg}^{-1}$  body mass) and allowed to rest for 3 h to ensure the equilibration of fluid between the body and lymph sacs. Each lizard was then held in a vertical, head-up position (causing the fluid to collect in the posterior region of the sac) and the fluid aspirated through a cannula (PE-90) inserted through a 3-mm incision in the anterior sac region. Fluid ( $0.10 \text{ ml}$ ) from either sac was analysed for total osmolality and electrolyte concentration. Aspirated fluid from each sac (measured to the nearest  $0.5 \text{ ml}$ ) was mixed with  $0.10 \text{ ml}$  of sodium heparin ( $250 \text{ units ml}^{-1}$ ) and infused back into the sacs. The incision was closed using cyanoacrylate cement.

### *Responses to dehydration*

Five adult lizards that were in apparent good health and normal state of hydration were held individually in screen-covered plastic boxes ( $0.4 \times 0.5 \text{ m}$ ) for 45 days. The relative humidity was held constant at 10%; the lighting and temperature were cycled to simulate 'Day' (06.00–18.00 h; lizard body temperature at  $36^\circ\text{C}$ ) and 'Night' (18.00–06.00 h; lizard body temperature at  $30^\circ\text{C}$ ). Lizards were fasted and had no access to preformed water for the duration of the experiment.

The distribution of body fluids (TBW, ECFV, LSFV) was measured the day before and after dehydration using methods described above. Deuterium was replaced in this experiment by tritium (THO;  $10 \mu\text{Ci kg}^{-1}$  body mass) as the isotope of water, and the THO activity (c.p.m.  $\text{ml}^{-1}$ ) in the body fluids was used both for the

measurement of TBW and to estimate rates of body water influx and efflux (Nagy, 1975; equations 5 and 6). Tritium activity was determined by liquid scintillation counting of five replicates of 8  $\mu\text{l}$  of LSF, sampled before and after dehydration. The average of the five replicates (minus background activity) was used in the calculations of TBW and water flux. Equations used in water flux calculations (Nagy, 1975) were adjusted to correct for non-aqueous portions of the LSF, determined gravimetrically after drying samples of LSF to constant mass.

Body mass and LSFV were remeasured at approximately weekly intervals throughout dehydration, and LSF (0.10 ml) was obtained each time for later analysis of total osmolality and electrolyte concentrations.

#### *Responses to KCl loading*

The ECFV of five adult lizards was measured before and 24 h after an intra-peritoneal injection of KCl solution (8.5 mequiv  $\text{K}^+$  in 7 ml water  $\text{kg}^{-1}$  body mass) equivalent in volume and  $\text{K}^+$  concentration to that assimilated by lizards eating a single fruit of *Ferocactus peninsulæ*. This  $\text{K}^+$  load was determined by subtracting the weight-specific concentration of  $\text{K}^+$  in the faecal material from the weight-specific concentration of  $\text{K}^+$  in fruits of *Ferocactus peninsulæ*. Both faeces and fruits analysed for  $\text{K}^+$  were collected on Isla La Ventana in June, 1981 when lizards were feeding almost exclusively on *Ferocactus peninsulæ*.

The ECFV of lizards prior to the KCl injections was measured by dilution of NaSCN, as previously described. The ECFV 24 h after the KCl load was calculated by measuring the amount of NaSCN dilution in the extracellular fluid resulting from the treatment (KCl injection, five lizards) or control (0.9% saline injection, two lizards). The amount of NaSCN dilution was determined by comparing the concentration of NaSCN in LSF 24 h after the fluid injection to the predicted NaSCN concentration of the sac fluid (corrected for the slow elimination of NaSCN out of the ECF; Fig. 1). Thus, the difference in ECFV before *versus* after the treatment (or control) injection represented the fluid volume that shifted between the intracellular and extracellular compartments. Electrolyte concentrations of the ECF were measured in LSF at intervals throughout the experiment.

#### *Dormant lizards*

Volume changes in the lymph sacs in response to low-temperature dormancy were measured after placing four adult *S. hispidus* in simulated hibernation, using microclimate conditions measured in hibernacula on Isla La Ventana. Lizards were captured during winter (November, 1982) and transported to a chamber at a constant ambient temperature (18°C) and relative humidity (35%). Animals were kept in separate, screen-covered plastic boxes (0.4  $\times$  0.5 m) with no access to food or preformed water. Lizards were totally inactive throughout the period of dormancy (December 1, 1982 to March 26, 1983). Body mass and LSFV were measured at approximately monthly intervals, and LSF (0.25 ml) was obtained for determination of total osmolality and electrolyte concentrations.

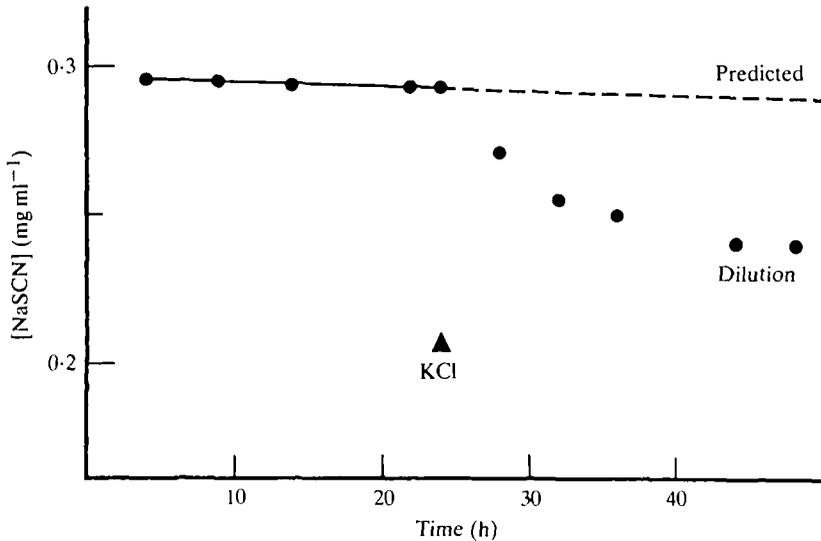


Fig. 1. Concentrations of sodium thiocyanate (NaSCN) measured in extracellular fluid of an adult *Sauromalus hispidus* for 24 h before and following an intraperitoneal injection of KCl. The dilution of the thiocyanate space (ECFV) due to the KCl injection was determined by comparing the measured concentration of NaSCN (closed circles) with the predicted concentration of NaSCN at the same time (dashed line).

## RESULTS

### *Composition of body fluids*

Paired comparisons of the LSF and serum of field-captured lizards indicate that the LSF is slightly hypo-osmotic to the serum (mean  $\pm$  s.e.,  $320.9 \pm 1.36$  mosmol kg<sup>-1</sup> and  $325.2 \pm 1.67$  mosmol kg<sup>-1</sup>, respectively;  $t = 2.48$ ,  $P < 0.02$ ,  $N = 50$  lizards). The concentrations of K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> in LSF ( $6.18 \pm 0.11$ ,  $152 \pm 2.0$  and  $144 \pm 1.6$  mequiv l<sup>-1</sup>, respectively) were similar to electrolyte concentrations measured in the serum ( $6.09 \pm 0.26$ ,  $161 \pm 2.2$  and  $146 \pm 1.6$  mequiv l<sup>-1</sup>, respectively). Pairwise comparisons of ion concentrations in LSF and serum indicated that only Na<sup>+</sup> concentration was significantly different ( $P < 0.01$ ) between these body fluids.

Total protein in LSF ( $3.58 \pm 0.20$  g dl<sup>-1</sup>) was essentially half that of the serum ( $7.05 \pm 0.26$  g dl<sup>-1</sup>). The albumin to globulin ratio (A:G) was higher in the LSF (1.65) than in serum (1.18). Regressions of albumin and globulin concentrations on total protein content (Fig. 2) illustrate that changes in total protein in LSF and serum are primarily due to changes in the albumin concentration.

### *Fluid loading*

Lizards in the hypotonic group lost significant amounts of body mass, due in part to a reduction in TBW (Table 1). Despite this decrease in TBW, lizards partitioned

more fluid to the ECFV, which increased from  $39.4 \pm 0.8\%$  to  $43.8 \pm 0.9\%$  of the body mass. Fluid volume in the lymph sacs after hypotonic fluid loading averaged less than half the LSFV measured before fluid loading.

Lizards in the isotonic group significantly gained body mass (average, +57 g) apparently through fluid retention (Table 1). The expansion of the ECFV represented a 30% increase in absolute volume and an 8% increase with respect to body mass ( $38.3 \pm 1.5\%$  to  $46.4 \pm 1.6\%$ ). The lymph sacs, having accumulated nearly four times the fluid of their original, pre-treatment volume, were grossly distended to an extent that was rarely observed in field-captured lizards.

Total osmolality of LSF and serum of the hypotonic group ( $333.8 \pm 3.06$  mosmol  $\text{kg}^{-1}$  and  $336.8 \pm 2.33$  mosmol  $\text{kg}^{-1}$ , respectively) were not significantly changed by the fluid loading ( $325.3 \pm 2.66$  mosmol  $\text{kg}^{-1}$  and  $331.5 \pm 3.97$  mosmol  $\text{kg}^{-1}$ , respectively). Lizards in the isotonic group increased the osmolality of their LSF and serum significantly (pairwise comparisons,  $P < 0.02$ ) from respective values of  $340.3 \pm 2.84$  mosmol  $\text{kg}^{-1}$  and  $337.8 \pm 4.01$  mosmol  $\text{kg}^{-1}$  to  $356.3 \pm 4.01$  mosmol  $\text{kg}^{-1}$  and  $356.0 \pm 4.29$  mosmol  $\text{kg}^{-1}$ . Cation concentrations measured in LSF before and after fluid loading in both groups indicated that only the  $\text{Na}^+$  concentration in the isotonic group changed significantly ( $155.3 \pm 2.66$  mequiv  $\text{l}^{-1}$  to  $169.5 \pm 3.57$  mequiv  $\text{l}^{-1}$ ).

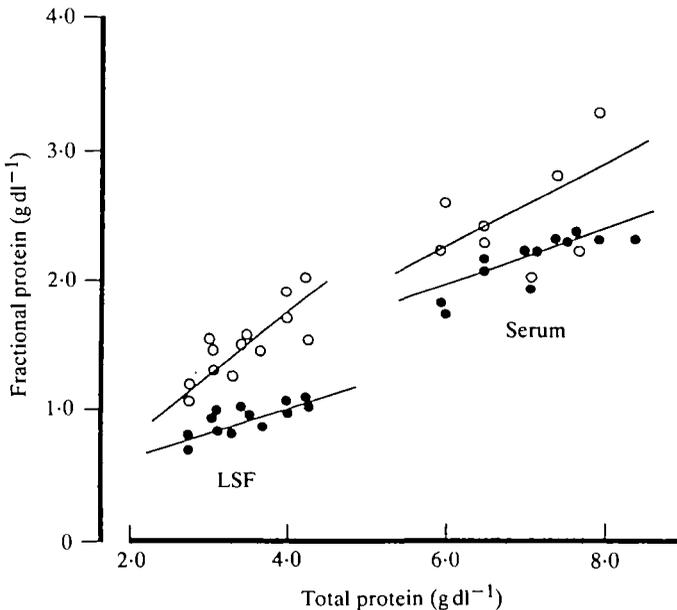


Fig. 2. Total protein content and corresponding fractions of albumin (○) and globulin (●) measured in lymph sac fluid (left) and serum (right) of field-captured *Sauromalus hispidus*. In lymph sac fluid, the regression equation for albumin-total protein is  $y = -0.023 + 0.437x$ ; for globulin-total protein it is  $y = 0.375 + 0.158x$ . In serum, the regression equation for albumin-total protein is  $y = 1.46 + 0.467x$ ; for globulin-total protein it is  $y = 0.555 + 0.226x$ .

Table 1. Changes in body mass, total body water (TBW), extracellular fluid volume (ECFV) and total lymph sac fluid volume (LSFV) of adult *Sauromalus hispidus* that received daily intraperitoneal injections of distilled water (hypotonic group) or a 300 mosmol kg<sup>-1</sup> salt solution (isotonic group) for 10 days

	Before: Mean value (±S.E.)	After: Mean value (±S.E.)
	Hypotonic group	
Body mass (g)	700 ± 33.6	672 ± 26.3*
TBW (ml)	521 ± 29.3	509 ± 15.3
ECFV (ml)	276 ± 10.9	294 ± 16.6
LSFV (ml)	3.8 ± 0.43	1.5 ± 0.50
	Isotonic group	
Body mass (g)	831 ± 42.6	888 ± 52.1*
TBW (ml)	622 ± 51.2	672 ± 30.4
ECFV (ml)	318 ± 21.4	412 ± 24.5***
LSFV (ml)	8.1 ± 0.72	30.5 ± 4.6 **

*N* = 4 lizards per group.  
 \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001.

### Dehydration

Dehydrating lizards lost body water at rates averaging 4.4 times greater than metabolic water production (Table 2). Thus, these lizards lost body mass (approximately -0.4% per day) and averaged a total reduction of 15.7 ± 2.17% of their initial body mass over the 45-day period (Fig. 3). Rates of body mass reduction were greatest during the first 2 weeks of dehydration in four of the five lizards studied.

Significant reductions in LSFV also occurred (pairwise comparisons, *P* < 0.001) in which the fluid volume in the sacs decreased to an average of 17.5% of the pre-dehydration volume (Fig. 3). Lizards lost essentially 75% of their initial sac fluid within the first 2 weeks of dehydration. Lizards that lost body water at the fastest rates (nos 7, 9, 10; Table 2) also demonstrated the greatest reductions in body mass and LSFV (Fig. 3). One lizard (no. 12) possessed comparatively low rates of water flux and mass change and retained significantly more fluid in the lymph sacs than

Table 2. Individual and average rates of body water gain (influx), loss (efflux), and body mass change in five adult *Sauromalus hispidus* during 45 days of dehydration

Lizard no.	Influx (ml kg <sup>-1</sup> day <sup>-1</sup> )	Efflux (ml kg <sup>-1</sup> day <sup>-1</sup> )	% Mass change per day
7	0.71	4.14	-0.47
9	0.51	3.81	-0.38
10	0.89	3.42	-0.49
12	0.10	1.36	-0.18
13	1.27	2.82	-0.39
Mean	0.70	3.11	-0.38
S.E.	0.19	0.49	0.05

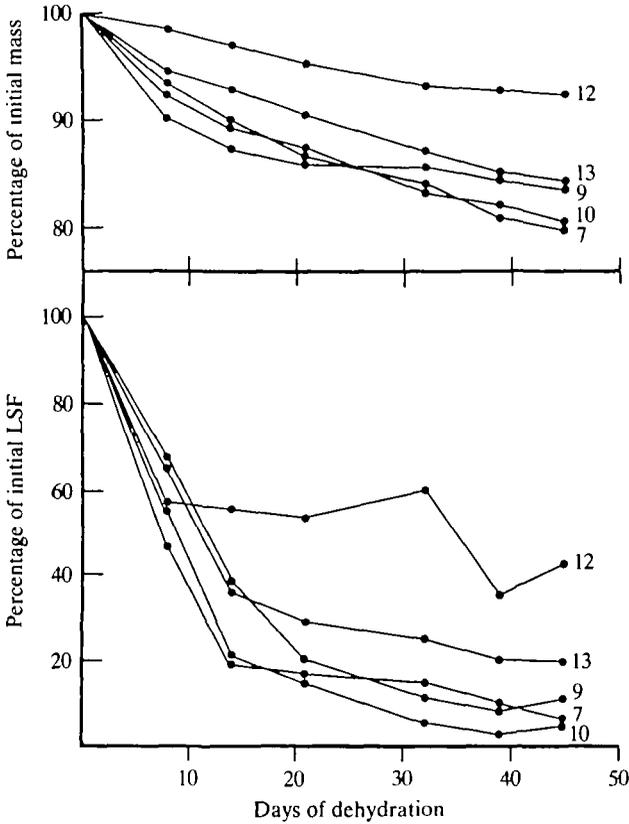


Fig. 3. Changes in body mass and lymph sac fluid volume (LSFV; sum of left and right sacs) in five *Sauromalus hispidus* measured at approximately weekly intervals during a 45-day dehydration. Lizard identification numbers are included for references made in the text and in Table 2.

other lizards studied. This particular lizard was less active and more docile compared to the other lizards in the study.

Although substantial losses in absolute volume occurred from the TBW and ECFV during dehydration, the mass-specific changes in TBW ( $-0.44\%$ ) and ECFV ( $-2.3\%$ ) measured over the duration of dehydration were not significant, thus there appeared to be no preferential reduction of fluid from either intracellular or extracellular fluid compartments. The mass reduction due to fluid loss from the ECFV ( $-2.3\%$  body mass) was similar to the mass change due to loss of LSF ( $-1.5\%$  body mass).

Total osmolality of LSF (Fig. 4) was unchanged from day 8 ( $326.2 \pm 4.05$  mosmol  $\text{kg}^{-1}$ ) to day 39 ( $327.2 \pm 4.31$  mosmol  $\text{kg}^{-1}$ ). Slight but significant elevations in LSF osmolality occurred by day 45 in all lizards studied (average,  $336.6 \pm 4.65$  mosmol  $\text{kg}^{-1}$ ; pairwise comparisons,  $P < 0.05$ ). Increases in total osmolality were presumably due to concomitant changes in both  $\text{K}^+$  and  $\text{Na}^+$  concentrations that increased from  $3.19 \pm 0.11$  and  $174 \pm 3.8$  mequiv  $\text{l}^{-1}$ , respectively on day 39 to  $4.48 \pm 0.51$  and  $220 \pm 3.8$  mequiv  $\text{l}^{-1}$ , respectively on day 45.

*KCl loading*

Lizards significantly expanded the ECFV 24 h following KCl injections by an average of  $27.8 \pm 4.2$  ml, or  $7.6 \pm 1.3$  % of the original ECFV (pairwise comparisons,  $P < 0.01$ ). The major shift in body fluid from intracellular to extracellular locations occurred during the initial 8 h following the KCl load (see Fig. 1). The KCl injections resulted in a three-fold increase in  $K^+$  concentration in LSF (Fig. 5); no significant changes in  $Na^+$  concentration were observed. Extracellular  $K^+$  decreased to approximately 140 % and 120 % of pre-injection levels during the first 24 and 48 h, respectively.

*Dormancy*

Adult *S. hispidus* kept for 75 days in simulated dormancy were totally inactive and lost body mass at constant rates averaging  $0.038 \pm 0.002$  % body mass  $day^{-1}$ . This rate of mass change was exactly an order of magnitude less than the rate of weight loss of lizards in the dehydration experiment (Table 2). However, rather than losing LSF in response to slow dehydration, fluid volumes in the lymph sacs increased an average of 6 ml per lizard over the duration of dormancy. A slight but significant increase in LSF osmolality occurred during dormancy ( $304 \pm 3.6$  mosmol  $kg^{-1}$  to  $314 \pm 4.5$  mosmol  $kg^{-1}$ ;  $X \pm S.D.$ ) that was apparently independent of concomitant increases in the major cations, since concentrations of  $Na^+$  and  $K^+$  were unchanged during dormancy.

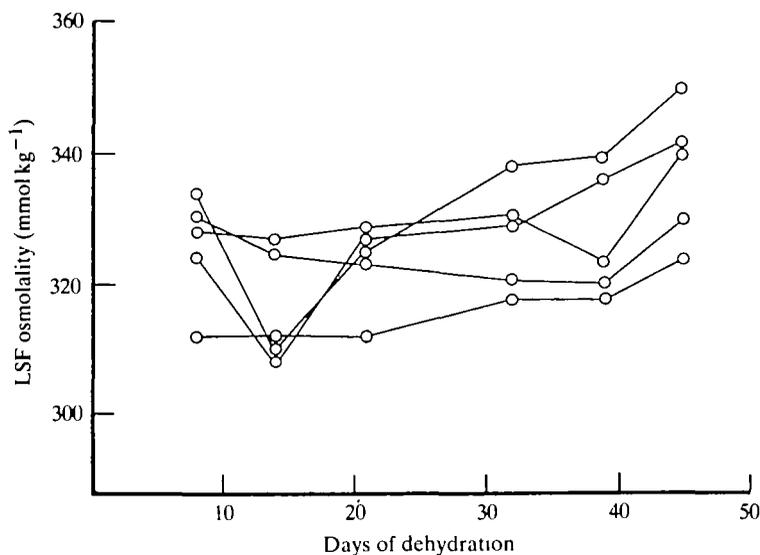


Fig. 4. Total osmolalities of the lymph sac fluid (LSF) sampled from five *Sauromalus hispidus* during 45 days of dehydration. Osmolalities of lizard LSF on day 45 were significantly different from osmolalities measured at all other times ( $P < 0.05$ ).

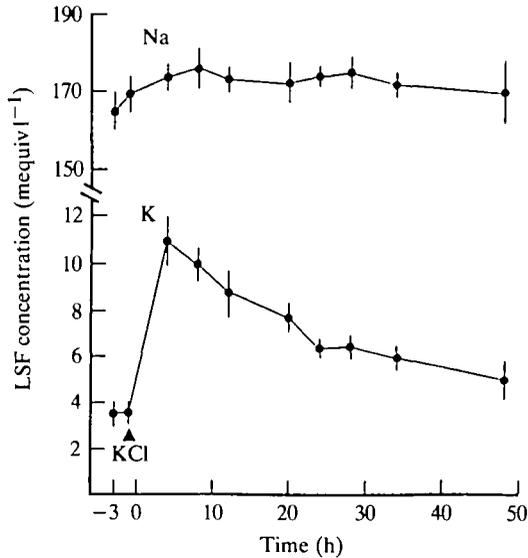


Fig. 5. Average concentrations of  $\text{Na}^+$  and  $\text{K}^+$  measured in lymph sac fluid (LSF) of five *Sauromalus hispidus* before and for 48 h following an intraperitoneal injection of  $8.5 \text{ mequiv K}^+ \text{ kg}^{-1}$  body mass. Vertical lines represent  $\pm 2 \text{ S.E.}$

#### DISCUSSION

The close similarities between blood serum and LSF in *S. hispidus* with respect to total osmolality and the concentrations of major electrolytes confirms that LSF is an extravascular form of extracellular fluid (i.e. lymph or interstitial fluid). The slight hypotonicity of LSF compared to serum and the cation gradient ( $\text{Na}^+$ , serum  $>$  LSF) are consistent with and relate to the Gibbs-Donnan equilibrium established in response to the distribution of protein (serum  $>$  LSF) in these body fluids (Guyton, 1981; Webster, 1982). Lymph sac fluid resists clotting indefinitely if withdrawn slowly and stored without shaking, thus LSF may bear further similarities to true lymph by possessing relatively less fibrinogen and prothrombin than plasma (Mayerson, 1963). Ratios of protein concentration in lymph *versus* plasma (L/P) measured in mammals (review by Renkin, 1979) are very similar to LSF/serum ratios measured in *Sauromalus*. Further, the ratio of protein content measured in interstitial fluid and plasma of the lizard *Varanus niloticus* (approximately 0.5; Hargens, Millard & Johansen, 1974) is identical to the LSF/serum ratio in *Sauromalus*. These data collectively indicate that the LSF is true lymph, and to my knowledge, represent the first description of lymph composition in reptiles.

The ECFV (thiocyanate space) of *S. hispidus* (average, 38.9% body mass) is significantly greater than the thiocyanate space of *Amphibolurus* lizards (33.0%; Bradshaw & Shoemaker, 1967) and *Sauromalus obesus* (35.1%; Nagy, 1972), but is less than thiocyanate fluid spaces measured in other reptiles (*Elaphe obsoleta*, 42.2%; *Crotalus viridis*, 41.9%; *Acrochordus granulatus*, 48.8%; *Chrysemys scripta*, 40.2%) (Smits & Lillywhite, 1985; H. B. Lillywhite, A. W. Smits & M. E. Feder, unpublished; Smits & Kozubowski, 1985). The ECFV of *S. hispidus*

reported here is for lizards with an average LSFV of 6 ml (range 3–9 ml). Because the LSFV is part of the ECFV, lizards with LSFVs different than those reported above may also possess significantly different volumes of ECF. The proportion of body mass representing TBW (74.6 %) is similar to that of other reptiles (Minnich, 1982).

Fluid loading studies indicate that (1) the composition of fluid intake significantly affects the capacity of lizards to accumulate body fluid, (2) accumulated body fluid can be stored in the ECF compartment, and (3) an appreciable portion of the stored ECF is partitioned into the lymph sacs. Lizards receiving water injections regulate their fluid compartments at pre-treatment levels (both in absolute and mass-specific terms), whereas lizards in the isotonic group accumulate body fluid and partition it preferentially into the ECF spaces (Table 1). Because the increase in ECFV averaged twice the stored TBW, half the increase in ECFV was due to a fluid shift from the intracellular spaces, apparently mediated by a retention of  $\text{Na}^+$ .

One might predict that animals with capacious and labile ECFVs might preferentially lose more extracellular than intracellular water when enduring dehydration. However, *S. hispidus* appears to lose water in similar amounts from both the ECFV and ICFV. The volume of ECF in the lymph sacs decreases dramatically and at a rate consistent with the degree of total fluid loss from these lizards (Fig. 3). In fact, average losses of fluid from the lymph sacs (10.2 ml) and non-sac locations (11.6 ml) of the ECFV during the initial 14 days of dehydration were essentially equal. Considering that lymph sacs typically contain less than 5 % of the ECF, a preferential loss of extracellular fluid from the lymph sacs in response to dehydration is indicated.

Changes in body fluid partitioning in response to KCl injections and dormancy strongly suggest that significant shifts in body water may occur when lizards experience osmotic changes of their ECF similar to those realized in field situations. The expansion of the ECFV in response to the KCl load is nearly twice the amount predicted (assuming an instantaneous load to the ECF; Guyton, 1981), and indicates that the degree of fluid shift is facilitated by a mechanism in addition to the increases in extracellular  $\text{K}^+$  and  $\text{Cl}^-$ . Although not yet confirmed in reptiles, a likely explanation is the secretion of adrenalcorticoids (aldosterone) in response to the extracellular hyperkalaemia, that results in a retention of extracellular  $\text{Na}^+$  that osmotically draws water from intracellular to extracellular locations. Expansion of the ECFV and interstitial oedema are well-known effects of aldosterone hypersecretion in response to hyperkalaemia in humans (Guyton, 1981). The fact that reptiles possess similar concentrations of plasma aldosterone to those measured in mammals (Bradshaw & Grenot, 1976) and may elevate plasma aldosterone in response to KCl loading (Bradshaw, Lemire, Vernet & Grenot, 1984) lends support to this hypothesis.

Although lizards in simulated hibernation lost body mass and were in negative water balance, all animals deposited fluid into their lymph sacs. These data appear to be in conflict with results obtained from lizard dehydration where losses in LSFV were prevalent. However, hypothermia may alter ion transport and glomerular filtration in reptiles (Gilles-Baillien, 1974); increases in extracellular organic and inorganic constituents during hibernation have been observed in several species of

reptiles (Minnich, 1982). Dormant *Sauromalus* lost but a small fraction of their body water and extracellular  $K^+$  and  $Na^+$  concentrations were unchanged. This suggests that a metabolite accumulation in the ECF (rather than dehydration) might explain the increased osmolality and subsequent increase in ECFV.

Endotherms possess relatively high sensitivities to both volume and osmotic changes ( $\pm 1\%$ ) within the interstitial spaces (Robertson, Athar & Shelton, 1977; Simon-Oppermann & Simon, 1982) and typically maintain a constant ECFV (Aukland & Nicolaysen, 1981). Bradshaw (1978) proposed that reptiles lack interstitial 'volume receptors' because fluid loading in lizards causes an antidiuresis and subsequent expansion of the ECFV. The lability in ECFV demonstrated by *S. hispidus* in the present study supports this proposal. However, because chuck-wallas may partition a significant portion of the ECF into non-interstitial spaces (lymph sacs), the capacity for ECFV may be enhanced and the formation of interstitial oedema minimized.

In the case of *S. hispidus* where storage of excess body fluid and accommodation of intra-compartmental shifts in body fluid are advantageous, precise regulation of the ECFV as described in endotherms would be non-adaptive, and would result in the loss of precious amounts of body water. Collectively the present results indicate that the accessory lymph sacs are compliant depots for the transient storage of excess extracellular fluid that results during fluid storage or shifts in body water from intracellular to extracellular locations.

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