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AN ABSTRACT OF THE THESIS OF Andrew Christopher Zygmunt for
the Masters of Science in Biology presented August 3, 1984.

Title: Effects of Dehydration on Hemoglobin Oxygen Affinity
and Red Blood Cell Volume in Two Anurans.

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The degree of terrestriality in anurans is correlated
with a differential tolerance to desiccation.
Cardiovascular insufficiency and reduced oxygen delivery to
the tissues may be the mechanism of dehydrational death in
amphibians.

Two aspects of possible adaptation in cardiovascular performance caused by increased plasma electrolytes were examined. Cells in anisotonic plasma may either act as osmometers or volume regulate. Blood flow rate is dependent upon cell viscosity, which in turn is a consequence of cell volume and membrane deformability. Cell volume changes which increase membrane deformability will thus potentially extend the limits of dehydration tolerance. It was found in R. catesbeiana and B. marinus that red blood cells maintain constant volume during dehydration. Cells in vitro initially lose water, but then sodium, potassium and water move into the cell. Cell viscosity within the physiologic range of hematocrits was higher in salt loaded non-regulating cells of B. marinus than in regulating isotonic cells.

A consequence of water loss in non-regulating cells, or uptake of ions in regulating cells, is an increase of intracellular ion concentration. Hyperosmolality influences oxygen loading characteristics of blood. Ionic interactions are known mediators of hemoglobin function. It was found in Rana and Bufo that increasing intracellular ionic concentration failed to influence the oxygen dissociation curve. Adaptation was therefore not made to increase oxygen delivered to the tissues during a time of general circulatory insufficiency.

EFFECTS OF DEHYDRATION ON HEMOGLOBIN
OXYGEN AFFINITY AND BLOOD CELL
VOLUME IN TWO ANURANS

by

ANDREW CHRISTOPHER ZYGMUNT

A thesis submitted in partial fulfillment of the
requirements for the degree of

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In
BIOLOGY

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1984

TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

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INTRODUCTION

Anurans are a diverse group of some 3000 species which occupy habitats ranging from rain forests to deserts. Although the vast majority of species are freshwater, their number includes species adapted for marine environments. Modern anurans are descendants of the first vertebrates to face the desiccating environment of a terrestrial habitat. The examination of the physiological correlates associated with their degree of terrestriality has been a fruitful inquiry.

Water balance in anurans has been described by a number of workers. With a few exceptions, (Phyllomedusa, Chiromantis, and Hyperolius), frogs and toads have cutaneous evaporative water loss rates equal to a free water surface (Bentley 1966a). Thorson (1955) found no correlation between evaporative water loss and degree of terrestriality, but did find a high correlation between increased tolerance to water loss and xeric habitats. Hillman (1980) found the aquatic species Xenopus laevis to tolerate 34% loss of initial body mass, whereas Scaphiopus couchii, an animal found in desert areas with access only to temporary ponds and soil moisture, tolerated a loss of 45% before dehydrational death.

The physiological basis for interspecific dehydration tolerance has recently begun to emerge. A terrestrial vertebrate in negative water balance is necessarily dependent upon stored water. A positive correlation exists between increased capacity of anuran urinary bladders and terrestriality. Bladder capacity ranges between 1% body mass for X. laevis to 44% in B. cognatus (Bentley 1966b). Ruibal (1962), Shoemaker (1964), and McClanahan (1967) have shown that bufonids and pelobatids maintain normal body fluid concentrations until bladder stores are depleted. Since anurans lack salt glands and kidneys stop urine formation during dehydration, the osmotic concentration of body fluids must increase after depletion of bladder water. Shoemaker (1964) has formulated the following equation to describe the increase in plasma electrolytes with dehydration:

$$C_f = C_o * (B_{w0} / (B_{w0} - WD))$$

where C_f = final plasma osmolarity
 C_o = original plasma osmolarity.
 B_{w0} = original body water content
 WD = water deficit

Further bases for graded dehydration tolerance were found by Hillman (1976, 1978). Terrestrial species

B. cognatus and S. couchii have increased oxygen storage capabilities and maximum oxygen consumptions ($\dot{V}O_2$ max). He found positive correlations between ventricle mass and $\dot{V}O_2$ max, a significant decrease in $\dot{V}O_2$ max with dehydration, and an increase in whole animal lactate at critical activity point (loss of righting response). The implication is that terrestriality is associated with selection for increased aerobic capacity and that dehydrational death in the species investigated is brought on by a reduction in circulatory oxygen delivery.

Possible mechanisms for reduction in O_2 delivered to the tissues include the effects of hyperosmolality, hypovolemia, and viscosity. Investigations of the contractile performance of cardiac muscle bathed in hyperosmotic solutions have shown both positive (Koch-Weser 1963; Atkins et al. 1973), and negative inotropic effects, (Wildenthal 1975; Hillman 1984). Hillman (1978, 1980) demonstrated increased tolerance for plasma sodium in terrestrial species and suggested that a necessary adaptation for extending dehydration tolerance is to extend the intrinsic osmotic limit. This effect of electrolytes was found to be independent of hematocrit and thus viscosity effects.

Compensation associated with hypovolemic stress was shown by Hillman and Sommerfeldt (1981) to include a redistribution of blood flow to the head, enhanced return

of lymph to the plasma space, (Hillman and Zygmunt, unpublished), and an increase of resting heart rate (Hillman 1978). In this manner brain function and cardiac output is maintained.

Blood flow rate is inversely proportional to blood viscosity. During dehydration, blood viscosity increases after bladder and lymph fluid volumes have been exhausted. Any compensation in circulatory resistance involving dilation of peripheral vessels is counter-productive since the appropriate response to hypovolemia is constriction of the peripheral vessels.

To summarize, dehydrational death in anurans appears to be the result of curtailment of oxygen delivery to the tissues, resulting from hypovolemia, hyperviscosity, and hyperosmolality. Consequently, adaptations which facilitate oxygen delivery with dehydration should enhance tolerance. The objectives of this study were to investigate aspects of the effects of increased plasma electrolytes during dehydration on RBC function in R. catesbeiana and B. marinus.

Two aspects of red cell function under hyperosmotic stress are germane to the question of oxygen delivery. The first is whether the red blood cells volume regulate or not with hyperosmolality. Cell deformability is a functional consequence of cell volume. Blood viscosity is related to cell deformability, hence cell volume. Mechanisms which

reduce blood viscosity with dehydration will enhance oxygen delivery and be adaptive relative to dehydrational tolerance. The second question is whether the hyperosmolality influences the oxygen loading characteristics of the blood. Ionic interactions are known to mediate hemoglobin oxygen loading. During dehydration intracellular ionic strength increases, therefore the effects of hyperosmolality on oxygen dissociation curves is central to understanding hyperosmotic stress on oxygen delivery.

One aspect of the inability to regulate plasma electrolytes is that cells will be bathed in hyperosmotic fluids. Net movement of water is dependent upon extracellular osmolality. In hypertonic solutions, cell volumes will decrease unless intracellular osmolyte concentrations are increased to match extracellular osmolality. A number of investigators have examined volume regulation of cells in anisotonic media. Shoemaker (1964) found retention of water in skeletal muscle, liver, kidney, lung, and heart in the toad B. marinus after dehydration to 80% of original mass. He associated this volume regulation with an increase in cellular electrolytes, (Na^+ , K^+ , Cl^-). Katz (1978) found differential volume regulation in tissues of B. viridis. A reduction in water content between 15-30% was found for erythrocytes, muscle, and liver. Heart water content changed very little after adaptation to 500 mOsm

NaCl. Oubain (10^{-3} M), an active transport blocker, affected ionic composition, but not cellular water content. Studies of erythrocytes in hypotonic media have shown volume regulation to be the result of either reduction in cell free amino acids (Fugelli 1967; Costa and Pierce 1983), or cell electrolytes (Kregenow 1971a). Duck red cells in hypertonic media increase cellular Na^+ and K^+ content after an initial period of cell shrinking (Kregenow 1971b).

Red cells in anisotonic solutions change surface to volume ratio and packing of cell hemoglobin. These factors potentially affect blood viscosity. Investigators have reported conflicting results. Meiselman et al. (1967) found hypertonic suspensions of red cells to have increased viscosity, whereas cells in hypotonic solution had reduced viscosity relative to controls. Rand and Burton (1964) found red cell membranes to be more easily deformed in hypertonic solutions. Increased deformability should yield a reduced viscosity (Braasch 1971). I have investigated blood viscosity over a range of hematocrits in salt loaded red blood cells. These experiments answer questions regarding possible viscosity advantages in regulating vs nonregulating nucleated red cells. A reduction in viscosity offers an obvious advantage in oxygen delivery.

If erythrocytes maintain volume as a consequence of intracellular uptake of electrolytes, or if they simply

lose water, it would be expected that the oxygen dissociation curve would be shifted to the right, due to an increase in intracellular ion concentration (Rossi-Fanelli et al. 1961; Brunori et al. 1975). A rightward shift of the curve would be a useful adaptation for promoting the oxygen supply to the tissues by decreasing hemoglobin affinity for oxygen (Lenfant et al. 1970; Metcalfe and Dhindsa 1970). Facilitation of O_2 delivery at the tissues would offset general circulatory insufficiency which accompanies dehydration. I have undertaken experiments to determine the half saturation point of hemoglobin in control and dehydrated animals. The half saturation point of hemoglobin, (P_{50}), is a measure of the position of the oxygen dissociation curve.

In summary, the thrust of this study is to elucidate factors for possible compensation of problems involved with O_2 delivery during dehydration in anurans.

These factors arise from reduced cell volume in hypertonic media, or from increased intracellular ionic concentration with dehydration. A comparison between species of differing tolerance to dehydration, (Bufo>Rana), may identify adaptations for terrestrial radiation in anurans.

Materials and Methods

Animals

Bufo marinus (mean mass=253g) and Rana catesbeiana (mean mass=438g) were purchased from commercial suppliers. B. marinus were maintained in the lab between 17-20°C on a sand substrate. Pans of distilled water were included within the enclosure for rehydration. R. catesbeiana were kept in a sheet metal enclosure with available water and were used within two weeks of arrival in the lab.

Mean Cell Volume

Mean cell volume (mcv) determinations were made using a Coulter Counter, (model Zbl), and Channelizer, (model C-1000), interfaced with a Tektronix 4051 computer. The counter produces a pulse which is in principle proportional to the volume of suspension electrolyte displaced by a cell within the counting aperture. The current pulse is theoretically assumed to be caused by an insulating particle moving within a conducting medium, and this predicts that mcv measurements will be affected by cell membrane charge. Adams and Gregg (1972) found that errors introduced by cell charges are insignificant due to the

comparatively large resistive current of the electrolyte. Additional errors due to cell path, tumbling of the cell, and adherent cells passing through the aperture as doublets, were reduced by use of an electronic editor on the model C-1000 Channelizer. Coulter Electronics suggest cell counts be less than 40,000 cells/ml for the 100 micron aperture tube to reduce counting errors, a procedure always followed in these experiments. Further correction for the above anomalies was made by using human red blood cells in isotonic solution to calibrate the counter and channelizer.

Since experiments required cells to be suspended in electrolyte of increasing tonicity, mcv determinations for a 23.2 micron diameter latex bead (Coulter Electronics) were made in salt solutions covering the range experienced by dehydrated individuals. For sodium concentrations between 100-250 mM, mcv as determined on the Coulter counter was within 1% of its calculated volume assuming the latex particle to be a sphere.

Volume regulation experiments fell into two categories. Red blood cell (rbc) volume was followed during dehydration in B. marinus (n=6) and R. catesbeiana (n=6). Animals were weighed after their bladder was drained by cloacal cannulation. After control blood samples were obtained by ventricular puncture, animals were placed in screened plastic cages and subsequently lost 6-9% of initial body mass/day. Daily blood sampling continued

until water losses of 35% initial body mass in Bufo and 24% in Rana were achieved. Control and all subsequent blood sampling was made by ventricle puncture. Blood was collected in heparinized tubes and a quantity of whole blood was centrifuged at 4,000 rpm for 2 min. Hematocrit was recorded and plasma sodium and potassium determined on an IL model 143 flame photometer. Red cells which were to be analysed by the Coulter counter were suspended in Ringer's Isotonic for the nonpermeant sodium ion, and containing 5 mM KHCO_3 , 1 mM CaCl_2 , 5 mM glucose.

Additional experiments were performed to describe the effects of temperature, extracellular potassium, and the function of the Na^+/K^+ pump on cell volume regulation. Blood obtained by heart puncture from hydrated B. marinus and R. catesbeiana was suspended in Ringer's solutions ranging from isotonic to solutions containing 225 mM Na^+ . These suspensions were maintained at either 20°C or 9°C and mcv determinations made every 15 minutes for 2 hours. A comparison of the degree and time course of volume regulation was made between cells maintained at the two temperatures.

In order to investigate possible ionic contributions to volume regulation, red cells from hydrated B. marinus and R. catesbeiana were placed in hypertonic media which were either potassium free or contained ouabain at a concentration of 10^{-3}M . At this concentration, ouabain

blocks the operation of the Na^+/K^+ pump (Kregenow 1971b). Mean cell volume comparisons were made between cells in hypertonic normal, K^+ free, and ouabain media.

Hemoglobin Oxygen Affinity

Hydrated B. marinus (n=12) and R. catesbeiana (n=12) were doubly pithed, after which the ventricle was exposed. A 4 ml sample of blood was collected in a syringe containing heparin. Blood from dehydrated Bufo (35% loss initial body mass, n=9) and Rana (20% loss initial body mass, n=6) was similarly collected.

Measurement of the half saturation point of hemoglobin (P_{50}) was made using the technique of Edwards and Martin, (1966). Four ml of blood was divided between 2 test tubes and each tube sealed by rubber stopper. Two 18 gauge needles on which 3-way stopcocks had been mounted allowed filling of each test tube with gas mixtures under a positive pressure. Within these equilibration tubes, whole blood was fully oxygenated (gas mixture 20% O_2 , 5% CO_2 , balance N_2) or fully deoxygenated (gas mixture 5% CO_2 , balance N_2). After an equilibration of 45 min, 150 ul oxygenated blood was injected in a 300 ul capillary tube, followed by immediate injection of 150 ul deoxygenated blood into the column of saturated blood. This volume of blood was mixed anaerobically and analyzed for Po_2 , pH, and Pco_2 at 19°C using an IL model 313 blood gas analyzer.

Since equilibrium oxyhemoglobin saturation is dependent upon the relative volume contribution of saturated Hb to the total volume of mixed blood, the P_{O_2} of a mixture of equal volumes of oxygenated and deoxygenated blood is the P_{50} . Determinations were made at a temperature of 19°C, pH 7.55, and P_{CO_2} of 40 mmHg.

Intracellular Sodium and Potassium

Approximately 2 ml of blood was collected by heart puncture from fully hydrated B. marinus (n=9) and R. catesbeiana (n=7). Individuals were subsequently dehydrated until they lost 35% of initial body mass in Bufo and 25% in Rana. Animals were doubly pithed and the ventricle exposed for blood collection in heparinized syringes.

Blood was centrifuged at 4,000 rpm for 10 min in 300 ul capillary tubes. Plasma and white cell fraction were removed and the red cells were blown into test tubes weighed to the nearest 0.1 mg, (Sauter model 414 balance). Red cells were dried to constant mass at 40°C. Two ml of 0.8N HNO_3 was used to extract Na^+ and K^+ from the cells. Aliquots of 100 ul, 500 ul, and 1 ml were evaporated to dryness in order to concentrate ions. Lithium diluent was added and determinations made on an IL model 143 flame photometer. After correction for trapped plasma, (10% wet

mass), intracellular concentrations were expressed as meQ/Kg dry mass cells.

Viscosity

Bufo marinus (n=3) were doubly pithed and the ventricle exposed. Blood was collected in test tubes containing ammonium heparin and centrifuged at 4,000 rpm for 5 min. Separated plasma was salt loaded to a concentration of 180 meQ NaCl. Red cells were then suspended in salt loaded plasma in order to manufacture hematocrits between 0-77%. A 200 ul sample of suspended cells was immediately analysed at 20°C with a Wells-Brookfield cone/plate viscometer model LVTDCP. Viscosity reported was for a shear rate of 450/sec. RBC's in hypertonic media initially behave as osmometers before gaining water (Figure 2). Cells placed in salt loaded plasma for immediate viscosity determinations are thus termed nonregulating. Comparison was made with viscosity of red cells in isotonic plasma of hydrated Bufo (Hedrick, unpublished).

RESULTS

Mean Cell Volume

The results of in vivo regulation of red cell volume in R. catesbeiana and B. marinus are shown in Figure 1. Mean cell volume of hydrated R. catesbeiana (n=4) was $770 \pm$

20 μ^3 ($\pm$ se). Loss of 14-20% of initial body mass resulted in a mcv of $771 \pm 18 \mu^3$. Mean cell volume of B. marinus (n=4) red cells was $460 \pm 11 \mu^3$, and $451 \pm 11 \mu^3$ after water losses ranging from 28-36% of initial body mass. These data indicate volume regulation since predicted reduction of initial volumes should equal 15% in R. catesbeiana and 28% in B. marinus if these cells acted as perfect osmometers in hypertonic plasma.

The mechanism of volume regulation is temperature dependent as shown in Figure 2 for both species. Recovery of cell volume after challenge by hypertonic medium, (180 mEq Na^+), occurs within 45 minutes at 20°C, whereas at 9°C, recovery is incomplete after 2.5 hours.

The results of in vitro experiments involving regulation in potassium free solutions and normal Ringer's containing ouabain (10^{-3} M) is shown for R. catesbeiana (Figure 3) and B. marinus (Figure 4). The relationships between percent decrease of initial cell volume and media tonicity were similar in both species. Cells placed in K^+ free media behaved as simple osmometers and did not volume regulate ($p > 0.05$). In a similar fashion, slopes of regression lines for cells in normal and ouabain Ringer's were not different ($p > 0.05$). A significant decrease in slopes was found between predicted osmometer and cells in normal Ringer's ($p < 0.01$), or cells in Ringer's plus ouabain.

Evidently, extracellular potassium is required for the observed volume regulation, but ouabain has no effect.

Hemoglobin Oxygen Affinity

No difference ($p > 0.05$) was found between hydrated and dehydrated hemoglobin oxygen affinities in either species. P_{50} was determined to be 48.8 ± 1.4 torr ($n=12$) and 50.6 ± 1.6 torr ($n=6$), respectively, in hydrated and dehydrated B. catesbeiana. Oxygen affinity was greater in B. marinus; 41.6 ± 1.4 torr ($n=12$) in hydrated animals and 43.0 ± 0.5 torr ($n=9$) in dehydrated individuals.

Intracellular Sodium and Potassium

The relationships between plasma sodium and intracellular ion concentration were similar for both species (Figures 5 and 6). A significant increase of intracellular K^+ and Na^+ concentrations accompanied dehydration in both species ($p < 0.05$). Results are listed in Table 1.

Viscosity

Figure 7 shows the viscosity relationships for B. marinus red cells in isotonic and hypertonic (180 mEq Na^+) solutions. The slope is significantly lower ($p < 0.005$) and the intercept higher ($p < 0.001$) in salt stressed cells. A higher intercept may be the result of salt interactions

with plasma proteins. Control plasma viscosity ($1.8 \pm 0.04\text{cP}$) was lower than viscosity of plasma loaded to 180 mEq Na^+ ($2.6 \pm 0.07\text{cP}$).

TABLE 1
 INTRACELLULAR SODIUM AND POTASSIUM CONCENTRATIONS
 (MEQ/KG DRY MASS) IN THE ERYTHROCYTES OF
B. MARINUS (N=9) AND R. CATESBEIANA (N=9).
 VALUES ARE MEANS $\pm$ STANDARD ERROR ASSUMING
 10% TRAPPED PLASMA.
 ASTERISK INDICATES SIGNIFICANT DIFFERENCE ($P < 0.025$).

SPECIES	CONTROL	DEHYDRATED
<u>B. marinus</u>	Na ⁺ 40.4 $\pm$ 1.7	73.2 $\pm$ 8.7*
	K ⁺ 234.8 $\pm$ 2.3	260.6 $\pm$ 6.0*
<u>R. catesbelana</u>	Na ⁺ 34.1 $\pm$ 2.3	58.3 $\pm$ 3.7*
	K ⁺ 229.0 $\pm$ 3.4	269.0 $\pm$ 8.8*

Figure 1. In vivo volume of erythrocytes of B. marinus (water losses 28-36% initial body mass) and R. catesbeiana (water losses 14-20% initial body mass).

Figure 2. Temperature dependence of RBC volume regulation in *B. marinus* and *R. catesbeiana*. *Rana*, (▲ 9°C isotonic, ● 20°C isotonic, ▲ 9°C hypertonic, ● 20°C hypertonic). *Bufo*, (▼ 9°C isotonic, ● 20°C isotonic, ▼ 9°C hypertonic, ● 20°C hypertonic).

Figure 3. Volume regulation of RBC's in R. catesbeiana after 1.5 hours. Top line indicates behavior as osmometer, $y=71.5 (\pm 0.4)x-0.1 (\pm 0.1)$, $r=1.00$. Bottom line RBC's in normal Ringer's, $y=21.0 (\pm 5.3)x-0.4 (\pm 2.0)$, $r=.78$.
 (▲ , potassium free media; ▼ , ouabain media;
 ■ , normal Ringer's)

Figure 4. Volume regulation of RBC's in *B. marinus* after 1.5 hours. Top line is behavior as osmometer, $y = 66.7 (\pm 0.5)x + 0.1 (\pm 0.1)$, $r = 1.00$. Bottom line RBC's in normal Ringer's, $y = 26.9 (\pm 2.8)x - 0.3 (\pm 1.1)$, $r = .94$. (▲, potassium free media; ▼, ouabain media; ■, normal Ringer's)

Figure 5. The relationship between red cell intracellular ion concentrations and plasma electrolytes in *R. catesbeiana* corrected for 10% trapped plasma.

(● sodium $y=0.5x-21$, $r=.75$; ▲ potassium $y=1.1x+97$, $r=.93$).

Figure 6. The relationship between red cell intracellular ion concentrations and plasma electrolytes in *B. marinus* corrected for 10% trapped plasma.

(● sodium $y=0.5x-14$, $r=.73$; ▲ potassium $y=0.4x+190$, $r=.79$).

Figure 7. The relationship between B. marinus hematocrit and the $\ln$ transformation of red cell viscosity at a shear rate of 450/sec and 20°C. Solid line is salt loaded (180 mEq/L), $\ln y = 0.028 (\pm 0.001)x + 0.90 (\pm 0.04)$, $r = .98$. Dashed line is isotonic plasma. $\ln y = 0.035 (\pm 0.002)x + 0.43 (\pm 0.07)$, $r = .93$.

DISCUSSION

R. catesbeiana and B. marinus regulate red blood cell volume during dehydration, (Figure 1). In vitro experiments in hypertonic media indicate similar responses for red blood cells of both species and a common mechanism for volume regulation. Figure 2 shows the biphasic nature of volume regulation in RBC's. Initially, cells in hypertonic media shrink, followed by volume recovery within 45 min for cells maintained at 20°C. The initial shrinkage is not significantly different from cell behavior as an osmometer. Kregenow (1971b), Cala (1977), and Amende and Pierce (1980), demonstrated a similar biphasic volume regulation for red cells in ducks, flounder, and molluscs. Shown by Figure 2 is the temperature dependence of volume regulation, since cells maintained at 9°C require a longer time to regain volume in hypertonic media than cells at 20°C. Temperature dependence indicates a mechanism involving an active uptake of plasma osmolytes. Additional experiments were designed to test volume regulation in the face of a Na⁺/K⁺ pump blockade by ouabain (10⁻³M), as well as cell osmotic behavior in potassium free Ringer's. R. catesbeiana (Figure 3) and B. marinus (Figure 4) show a regulatory behavior in K⁺ free solution which is not

significantly different from an osmometer ($p > 0.05$). Oubain fails to affect volume regulation since this treatment is not significantly different from cell behavior in normal media. These results are in agreement with data presented for red cells of ducks and polychaete worms by Kregenow (1971b) and Costa and Pierce (1983).

Figure 5 (Rana) and Figure 6 (Bufo) demonstrate sodium and potassium uptake during dehydration, indicating the importance of these cations in maintenance of RBC volume. Schmidt-Nielsen (1975) found sodium permeability increased for flounder red blood cells in hypertonic media. Kregenow (1971b) showed an increase of intracellular potassium in duck red cells exposed to hyperosmotic shock. Volume regulation requires extracellular K^+ availability. Potassium is accumulated against a concentration gradient in both species. Schmidt and McManus (1974) describe a Na^+ and K^+ uptake which is oubain insensitive and facilitates cation movement during volume regulation. Nakao et al. (1963) isolated from human red blood cells a Na^+ , K^+ ATPase which was not inhibited by oubain.

To summarize, volume regulation of RBC's in the two anurans studied is achieved by active uptake of plasma potassium via an oubain insensitive pump and movement of plasma sodium down a concentration gradient. These results are in agreement with previous studies. In addition, this study demonstrates volume regulation and ionic uptake

during dehydration. Previous studies have largely involved mammalian or avian cells which do not normally experience markedly increased plasma electrolytes with dehydration.

Volume regulation achieved by uptake of ions offers an opportunity to offset general circulatory insufficiency accompanying dehydration. The effect of ionic mediators of hemoglobin function is to reduce Hb oxygen affinity. The effect of salts in lowering oxygen affinity is thought to reflect preferential binding of salts by deoxygenated as opposed to the oxygenated form of hemoglobin (Tyuma 1974). A shift of the oxygen dissociation curve to the right has been implicated in the adaptation to anemia and hypoxic hypoxia in sheep (Lenfant et al. 1970). In this manner, increased intracellular electrolytes could substantially increase delivery of oxygen to the tissues and be adaptive in terrestrial species to extend tolerance to dehydration. Control P_{50} determinations for both species are in agreement with published values established under similar conditions (Hall, 1968; Tazawa et al., 1979).

Dehydrated individuals do not have a significantly different oxygen affinity. Increased intracellular concentrations do not prove to be adaptive for delivery of oxygen during dehydration in either species. This seemingly anomalous finding may be explained in terms of increased oxygen affinity caused by other modifiers of Hb

function such as organic phosphates which override the effects of salts.

Blood flow rate is inversely proportional to blood viscosity. Blood viscosity is a combined term which includes the viscosity of the two components of whole blood, blood plasma and red blood cells. The non-Newtonian behavior of whole blood is associated with substantial protein concentrations and suspended red cells.

B. marinus plasma viscosity increased with increased plasma sodium. Ionic interactions with plasma protein were presumably responsible for the significantly higher intercept for salt loaded blood (Figure 7).

Red blood cell viscosity is dependent on shape, volume, membrane rigidity, and mean corpuscular hemoglobin concentration (MCHC). Erslev and Atwater (1963) found nearly a doubling of viscosity as MCHC increased from 24%-38%. In B. marinus, the slope of the line in Figure 7 for salt loaded cells, (non-regulating), is significantly lower than the slope of the line for cells in normal plasma. These slopes represent cell viscosity since the second component of the slope, plasma viscosity, remains constant. I therefore argue that nonregulating red blood cells in hypertonic plasma are more distensible and therefore less viscous than normal cells in isotonic plasma, (dashed line, Figure 7). Rand and Burton (1964) found human red cell membranes in hypertonic solution (1.2%

NaCl), to be more distensible than membranes in isotonic or hypotonic media. Meiselman et al. (1967) found human red cells in hypertonic plasma to be more viscous than cells in hypotonic plasma. Their reported values lack estimates of variability and may merely reflect increased plasma viscosity.

Within a physiologic range of hematocrits, blood viscosity in B. marinus is lower for red blood cells which regulate volume. This may not represent a true viscosity advantage for regulating cells, since if correction is made for plasma viscosity, the previous advantage disappears. Constraints for regulation of cellular volume in red blood cells may therefore include factors other than viscosity. One such selective pressure for maintenance of cell volume might be the proper function of membrane bound enzyme systems.

In summary, R. catesbeiana and B. marinus maintain red blood cell volume during dehydrational stress by uptake of sodium and potassium. Increased intracellular ionic concentrations do not alter oxygen delivery by shifting the oxygen dissociation curve to the right. Hypertonic plasma does not appear to increase red cell viscosity although whole blood viscosity is higher for hematocrits less than 70%. Increased viscosity in salt loaded blood may be largely attributed to increased plasma viscosity.

Volume regulation of red cells occurs but is insufficient to cancel the effect of increasing plasma viscosity.

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