

# Changes in Red Blood Cell Electrolytes and ATP in Newborn Shock<sup>1</sup>

JURETA W. HORTON AND DALE COLN

*Division of Pediatric Surgery, Department of Surgery, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235*

**ABSTRACT.** Previous studies show an inability of the skeletal muscle cell and red blood cell to maintain sodium, potassium, and calcium homeostasis during hemorrhagic shock in adults. However, there is no information on the cellular effects of shock in the neonate. This study examined the effects of hemorrhagic shock on red cell membrane function in a newborn canine model. Changes in sodium, potassium, calcium, and magnesium concentrations in red blood cells and plasma were correlated with changes in intracellular and plasma ATP levels. Newborn dogs ( $n = 36$ ), 10 to 14 days of age and weighing 501 to 707 g, were studied. After baseline studies (blood pressure, heart rate, temperature), electrolyte and ATP concentrations in red blood cells and plasma were measured. The dogs were then bled 40% of their estimated blood volumes. All parameters were measured after 1 h of shock. This shock model produced hypotension, bradycardia, and acidosis. The red blood cell sodium, calcium, water, and ATP content increased in shock, whereas intracellular magnesium fell. Red blood cell potassium levels, plasma sodium, and calcium concentrations were not significantly altered in shock, although plasma potassium and magnesium levels rose. Our data show that shock in the newborn disrupts cell membrane integrity. Intracellular accumulation of sodium and calcium occurred despite cellular ATP uptake, suggesting that high energy deficits are not the primary mechanism contributing to electrolyte imbalance in newborn shock. (*Pediatr Res* 24: 438-441, 1988)

Previous studies in our laboratory show that hemorrhagic shock in the adult dog alters cell volume regulation, resulting in increased intracellular water and sodium and a loss of cell potassium (1, 2). Shock-induced redistribution of fluid and electrolytes has been attributed to loss of cell membrane integrity and depletion of intracellular ATP (3-9). Altered electrolyte transport in shock is thought to represent generalized cellular dysfunction in a low flow state. Although derangements in skeletal muscle and red blood cell function have been well characterized in adult hemorrhagic shock (7), there are no studies of the cellular effects of hemorrhagic shock in the neonate. The ease of obtaining isolated cells make the red blood cell model attractive for serial assessment of cellular dysfunction in newborn shock.

Dog red blood cells differ from those of many mammalian

species in that they have a high intracellular sodium concentration and lack a classical sodium-potassium pump (10, 11). Numerous studies by Parker showed that the dog red blood cell regulates internal volume by accumulating or extruding sodium. The outward sodium transport occurs against an electrical gradient and requires external calcium. Much evidence supports the presence of a red blood cell ATP-dependent calcium extrusion pump. Despite the differences between canine somatic and red blood cells, both require high energy phosphate stores (ATP) to maintain electrolyte homeostasis and intracellular volume (8-18).

The purpose of the present study was to examine the effects of acute blood loss on red blood cell sodium, potassium, and calcium concentration in a newborn canine hemorrhagic shock model and to correlate electrolyte shifts with the concentration of intracellular ATP. We hypothesized that shock-induced depletion of intracellular ATP would impair the active calcium extrusion pump, resulting in progressive calcium accumulation by the dog red blood cell. In addition, shock was expected to alter volume regulation of the newborn canine erythrocyte.

## MATERIALS AND METHODS

A total of 36 newborn dogs, 10-14 days of age and weighing between 501 and 707 g were anesthetized with pentobarbital (15 mg/kg). Each dog was restrained and a warming blanket was used to maintain a body temperature of 37.5° C. Respiration was spontaneous. Cannulae were placed in the femoral arteries for continuous blood pressure monitoring (Statham, model PE23ID, Statham Gould Medical Products, Oxnard, CA) and collection of blood samples. Arterial blood gases and arterial oxygen content were measured using a Radiometer blood gas analyzer (model ABL3, Radiometer American Inc., Dallas, TX). Each animal received heparin (100 U/kg) to prevent clotting of catheters during the experimental period.

Blood pressure, heart rate, respiratory rate, and temperature were recorded after induction. A baseline blood sample (4 ml) was obtained for determination of sodium, potassium, calcium, magnesium, and ATP concentrations in plasma and red blood cells.

Blood samples were centrifuged at 30,000 rpm (Sorval, model RC2B, Sorvall Instruments, Wilmington, DE). The plasma and buffy coat layers were removed. Approximately 100- to 150-mg aliquots of the red blood cell pellet were transferred to each of three weighed containers and reweighed to five decimals (Cahn, TA450, Cahn Instruments Inc., Cerritos, CA) for determination of cell water content. One aliquot was diluted with 5 ml of 5 mM LiNO<sub>3</sub> for flame photometry. The sodium and potassium concentrations in plasma and red blood cells were measured in duplicate by flame photometry as previously described (5, 11, 14, 19). The remaining aliquot of red blood cells was washed twice with isotonic media (5° C) and recentrifuged as described above. For calcium and magnesium measurements, red blood

Received September 14, 1987; accepted June 1, 1988.

Correspondence and reprint requests to Jureta W. Horton, Ph.D., Division of Pediatric Surgery, U.T.S.M.C.D., 5323 Harry Hines Boulevard, Dallas, TX 75235-9031.

<sup>1</sup>In conducting the research the investigators adhered to the National Institutes of Health and American Physiological Society guidelines for use of experimental animals.

cells were washed with LiCl (120 mM) at a cell to medium volume of 1/20 and centrifuged at 0° C as previously described (16). After the last wash, cells were resuspended in an equivalent volume of LiCl wash. A total of 10  $\mu$ l of the resuspended, washed cells was aspirated by an automatic sampler into a Perkin-Elmer atomic absorption spectrophotometer (model 305B, Perkin Elmer Corp., Norwalk, CT). Calcium and magnesium concentrations were measured. Preliminary studies in our laboratory confirm previous reports that the procedures used in cell washing, incubation, and centrifugation did not alter water or electrolyte content of the red blood cells (16, 17, 19, 20). The electrolyte content in packed red blood cells was expressed as mM/liter cell water.

ATP concentrations in red blood cells were determined using enzymatic techniques. The enzyme phosphoglycerate phosphokinase catalyzes the reaction between ATP and 3-phosphoglycerate. The reaction is coupled with glyceraldehyde 3-phosphate dehydrogenase-catalyzed dephosphorylation and oxidation of NADH. Changes in absorption at 340 NM (Gilford Spectrophotometer, model 240, Gilford Instrument Laboratories, Oberlin, OH) which occur with oxidation of NADH to NAD provide a sensitive and specific measure of ATP. The ATP content in packed red blood cells was calculated as previously described (4, 7).

After completing baseline measurements, hemorrhagic shock was produced by withdrawing 40% of the estimated total blood volume into a heparinized reservoir over a 15-min period. Total blood volume was calculated as 85 ml/kg body weight. The initial volume of blood collected for measurement of pH, blood gases, and electrolyte concentrations was calculated as part of the 40% hemorrhage. Vital signs were recorded every 15 min after the withdrawal of blood and the second blood sample was drawn after 1 h of sustained shock.

All values obtained during shock were compared to values measured in the control period. All values are reported as mean  $\pm$  SEM. Statistical analysis included an analysis of variance and repeated measures procedure (Student Newman-Keuls). Differences were considered significant at  $p < 0.05$ .

## RESULTS

Removal of 40% of the calculated total blood volume and 1 h of shock caused mean arterial blood pressure to fall 33% (from  $80 \pm 3$  to  $50 \pm 3$  mm Hg) whereas heart rate fell 11% (from  $166 \pm 4$  to  $145 \pm 6$  beats/min) (Fig. 1). Hypotension was associated with acidosis (mean pH =  $7.16 \pm 0.04$ ;  $\text{HCO}_3^- = 11.0 \pm 1.4$  mEq/liter). An increased respiratory rate in the newborn dog subjected to hemorrhage (from  $11 \pm 1$  to  $16 \pm 2$  breaths/min) caused arterial  $\text{pCO}_2$  to fall (from  $42.5 \pm 1$  to  $32.0 \pm 2$  mm Hg) whereas  $\text{pO}_2$  increased (from  $78 \pm 4$  to  $94 \pm 4$  mm Hg). These shock-induced changes in acid-base balance and hemodynamics were significant at  $p < 0.05$ . Arterial oxygen content remained stable at 11.5 ml/100 throughout the study period. Hematocrit fell from  $30 \pm 0.5$  to  $25 \pm 0.5$  after hemorrhage.

The sodium and calcium content of red blood cells (mM/liter cell water) increased significantly during shock in the newborn (sodium from  $119 \pm 3$  to  $128 \pm 2$  mM/liter cell water,  $p < 0.05$ , calcium from  $1.55 \pm 0.05$  to  $2.65 \pm 0.04$  mM/liter cell water,  $p < 0.05$ ) whereas intracellular magnesium fell from  $7.62 \pm 0.12$  to  $6.04 \pm 0.10$  mM/liter cell water,  $p < 0.05$  (Fig. 2). Red blood cell potassium content was unchanged during shock. Red blood cell water increased significantly from a baseline value of  $626 \pm 1.2$  g/kg red blood cell to a value of  $636.8 \pm 2.9$  g/kg red blood cell after 1 h shock ( $p < 0.05$ ). Although there was a tendency for the ATP content of red blood cells to increase and plasma ATP concentration to fall, these changes were not statistically significant. In addition, the mean plasma sodium, calcium, and potassium (mM/liter) concentrations measured in shock were not statistically different from control values. However, plasma magnesium concentration in shock ( $1.81 \pm 0.03$ ) was signifi-

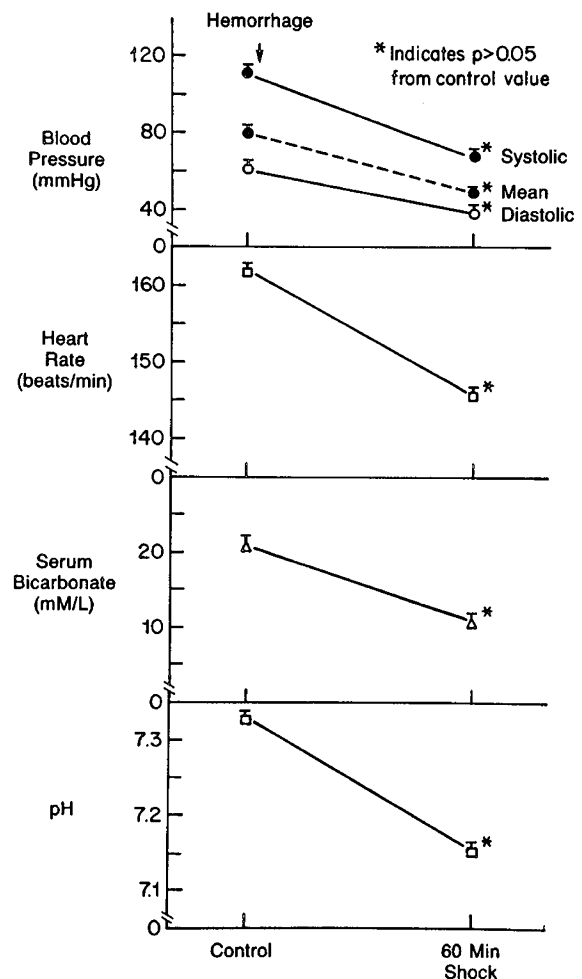


Fig. 1. Changes in blood pressure, heart rate, and acid-base balance in newborn shock. All values are mean  $\pm$  SEM. \* denotes significant change from control values at  $p < 0.05$ .

cantly higher than control values ( $1.26 \pm 0.04$  mM/liter,  $p < 0.05$ ).

## DISCUSSION

Whereas previous studies confirm cellular dysfunction in adult hemorrhagic shock as indicated by intracellular-extracellular redistribution of water and electrolytes in skeletal muscle and myocardium and a fall in skeletal muscle transmembrane potential (1, 2, 8), there are no studies of the cellular effects of hemorrhage in the newborn. The ease of obtaining isolated cells make the red blood cell model attractive for repetitively assessing cellular function in newborn shock. In this study, the dog was chosen as a model for cellular dysfunction in neonatal hemorrhagic shock. Dog red blood cells differ from other mammals in their regulation of cellular electrolytes, suggesting that an alternative animal model would be preferable for assessing cellular dysfunction. However, the hemodynamic responses and fluid derangements in newborn shock have been well characterized by our laboratory using a canine model (21). Furthermore, the ready availability of the dog led us to continue our study of shock-related changes in cellular function in this model.

In our present study, newborn shock altered cell volume as indicated by the rise in red blood cell water content. Cellular edema was accompanied by a significant increase in red blood cell sodium and calcium content. These data are consistent with shock-induced sodium influx in canine somatic cells (1), human (22), rat (5, 23), and baboon red blood cells (7). It was of interest that newborn shock caused a significant fall in bicarbonate, a

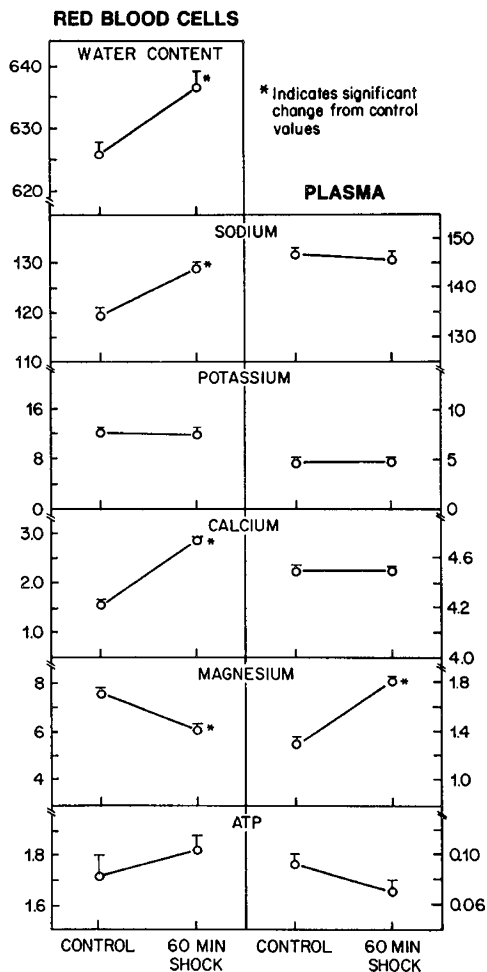


Fig. 2. Changes in electrolyte and ATP concentrations of red blood cell (mM/liter cell water) and plasma (mM/liter) in newborn shock. All values are mean  $\pm$  SEM. \* denotes significant change from control values at  $p < 0.05$ .

factor that should decrease cell membrane permeability. The fall in bicarbonate concomitant with increased cell volume was expected to decrease sodium influx. Therefore, an increase in sodium influx mediated by shock would have to be sufficient in magnitude to overcome these two physiological mechanisms.

The increased sodium content of the red blood cell in newborn shock could result from a loss of cell membrane integrity and a selective increase in membrane permeability to sodium; in this situation, sodium would flow down the concentration gradient into the red blood cell. Alternatively, sodium influx in shock could result from a decrease in external calcium because active sodium extrusion by the canine red blood cell requires external calcium. In our study, plasma calcium and sodium concentrations did not change in shock. In addition, sodium influx was not associated with any change in intracellular potassium content. Our data suggest that the significant rise in red blood cell sodium content was related to a selective increase in sodium permeability induced by the low flow state.

In the newborn red blood cell, calcium influx occurred in shock despite a profound acidosis, a condition that does not favor calcium accumulation by the canine erythrocyte. Although intracellular-extracellular electrolyte gradients are important in red blood cell volume regulation, the mechanism by which calcium alters transmembrane sodium movement and volume in the dog red blood cell remains unclear. In our study, the increased intracellular calcium during shock is consistent with studies by Parker (16) who showed that cellular swelling or a high intracellular sodium favors calcium influx. These data in-

dicate coupled sodium-calcium transport by dog red blood cells or alternatively, competition for a cell surface transport site. It is likely that transmembrane calcium movement in the dog erythrocyte includes passive calcium flux and an ATP energy-dependent pump that actively extrudes calcium, maintaining a low cytoplasmic calcium concentration.

Several studies have described depletion of intracellular ATP stores in hemorrhagic shock (24–26). One might expect that if shock depleted intracellular ATP stores, the calcium extrusion pump would be inhibited, resulting in intracellular calcium accumulation. However, in this study, ATP content of the newborn erythrocyte was unchanged, excluding high energy deficits as the primary factor for cellular dysfunction in shock. It is likely that the tendency for cellular ATP to increase in newborn shock reflects decreased energy use in a low flow state (27). Brown (10) reported that calcium extrusion by the dog red blood cell was dependent on both intracellular ATP and magnesium (10). Therefore, we must consider the possibility that increased calcium content of the erythrocyte in newborn shock was related to the loss of intracellular magnesium. In our study, intracellular magnesium content fell during shock, suggesting that shock-altered calcium transport by the canine erythrocyte was related in part to red blood cell magnesium content.

In this study, the hemodynamic and acid-base response to shock confirm a previous report from our laboratory of bradycardia and acidosis in newborn shock (28). In contrast, hemorrhagic shock in the adult dog impairs hemodynamic function and produces a profound tachycardia (21).

In summary, our data indicate that a pathophysiological situation such as shock alters erythrocyte water and electrolyte content in the newborn dog. This study does not clarify the mechanism by which sodium-calcium exchange regulates red blood cell volume in the dog model. The intracellular accumulation of sodium and calcium occurred despite cell ATP uptake, suggesting that high energy deficits are not the primary mechanism contributing to electrolyte imbalance in shock.

*Acknowledgments.* The authors thank Sheila Taylor for her excellent technical assistance and Janine Franklin for manuscript preparation.

#### REFERENCES

- Horton JW, Tuggle DW, Vaught MC, Taylor SE 1983 Effects of Verapamil on cellular function in hemorrhagic shock. *Circ Shock* 10:239
- Horton J 1987 Hemorrhagic shock impairs myocardial cell volume regulation and membrane integrity in dogs. *Am J Physiol* 252:H1203–1210
- Holliday RL, Illner HP, Shires GT 1981 Liver alterations during hemorrhagic shock in the rat. *J Surg Res* 31:506–515
- Illner HP, Shires GT 1981 The membrane defect and energy status of rabbit skeletal muscle cells in sepsis and septic shock. *Arch Surg* 116:1302–1305
- Kreis DJ Jr, Chaudry IH, Schleck S, Baue AE 1981 Red blood cell sodium, potassium, and ATP levels during hemorrhagic shock. *J Surg Res* 31:225–228
- Sayed MM, Adler RJ, Chaudry IH, Baue AE 1981 Effect of hemorrhagic shock on hepatic transmembrane potentials and intracellular electrolytes, in vivo. *Am J Physiol* 24:R211–R219
- Shires GT III, Peitzman AB, Illner H, Shires GT 1983 Changes in red blood cell transmembrane potential, electrolytes, and energy content in septic shock. *J Trauma* 23:769–774
- Shires GT, Cunningham JN, Baker CRF, Reeder SF, Illner H, Wagner IY, Maher J 1972 Alterations in cellular membrane function during hemorrhagic shock in primates. *Ann Surg* 176:288–295
- Vincenzi FF 1981 Red blood calmodulin and  $Ca^{2+}$  pump ATPase. Preliminary results of a species comparison. *Prog Clin Biol Res* 55:363–383
- Brown AM 1979 Evidence for magnesium and ATP-dependent calcium extrusion pump in dog erythrocytes. *Biochem Biophys Acta* 553:195–203
- Muller-Soyano A, Glader BE 1977 Cation specificity of propranolol-induced changes in RBC membrane permeability: comparative effects of human, dog and cat erythrocytes. *Cell Physiol* 91:317–322
- Parker JC 1983 Hemolytic action of potassium salts on dog red blood cells. *Am J Physiol* 244:C313–C317
- Parker JC 1983 Volume-responsive sodium movements in dog red blood cells. *Am J Physiol* 244:C324–C330
- Parker JC 1981 Heterogeneity among dog red blood cells. *J Gen Physiol* 78:141–150

15. Parker JC, Harper JR 1980 Effects of aminone, a cardiotoxic drug, on calcium movements in dog erythrocytes. *J Clin Invest* 66:254-259
16. Parker JC 1979 Active and passive Ca movements in dog red blood cells and resealed ghosts. *Am J Physiol* 237:C10-C16
17. Parker JC 1978 Sodium and calcium movements in dog red blood cells. *J Gen Physiol* 71:1-17
18. Trunkey DD, Illner H, Wagner IY, Shires GT 1973 The effect of hemorrhagic shock on intracellular muscle action potentials in the primate. *Surgery* 74:241-250
19. Parker JC, Castranova V, Goldinger JM 1977 Dog red blood cells: Na and K diffusion potentials with extracellular ATP. *J Gen Physiol* 69:417-430
20. Parker JC, Gitelman HJ, Glosson PS, Leonard DL 1975 Role of calcium volume regulation by dog red blood cells. *J Gen Physiol* 65:84-95
21. Horton J, Landreneau R, and Tuggle D 1985 Cardiac response to fluid resuscitation from hemorrhagic shock. *Surg Gynecol Obstet* 106:444-452
22. Cunningham JN, Shires GT, Wagner IY 1971 Changes in intracellular sodium and potassium content of red blood cells in trauma and shock. *Am J Surg* 122:650-654
23. Day B, Friedman SM 1980 Red cell sodium and potassium in hemorrhagic shock measured by lithium substitution analysis. *J Trauma* 20:52-54
24. Baue AE, Chaudry IH, Wurth MA, Sayeed MM 1971 Cellular alterations with shock and ischemia. *Angiology* 25:31-42
25. Chaudry IH, Sayeed MM, Baue AE 1974 Depletion and restoration of tissue ATP in hemorrhagic shock. *Arch Surg* 108:208-211
26. Chaudry IH, Sayeed MM, Baue AE 1972 Alterations in adenosine nucleotides in hemorrhagic shock. *Surg Forum* 23:1-3
27. Illner HP, Cunningham JN, Shires GT 1982 Red blood cell sodium content and permeability changes in hemorrhagic shock. *Am J Surg* 143:349-355
28. Horton JW, Coln D 1985 Cardiovascular function and fluid compartments in newborn canine hemorrhagic shock. *Am J Physiol* 248:R724-R731