$(Na^+ + K^+)$-ATPase Activity in the Liver with Hemorrhagic Shock[1] (36338)

M. A. WURTH, M. M. SAYEED, AND A. E. BAUE
(Introduced by H. T. Blumenthal)
Washington University School of Medicine; and Department of Surgery, Division of Cell Physiology of The Jewish Hospital of St. Louis, St. Louis, Missouri 63110

Injured tissues and tissues subjected to anoxia have been thought to lose potassium and gain sodium (1). Studies performed in man in shock have indicated that plasma sodium levels may be lowered (2). More recently Cunningham *et al.* (3) have reported reduced muscle cell transmembrane potential and elevated interstitial K^+ in shock. In preliminary communications, we have reported a significant increase in $(Na^+ + K^+)$-ATPase activity in the liver of animals with prolonged hemorrhagic shock (4, 5). The electrolyte shifts and membrane potential changes may well be related to changes in $(Na^+ + K^+)$-ATPase activity. The present study was carried out to determine if the alteration in $(Na^+ + K^+)$-ATPase activity could be found in an early stage of shock and if the change can be reversed by restoring blood and fluid volume of the animal in shock.

Methods. Male, albino rats of the Holtzman strain weighing 250–350 g were utilized. All animals were fasted 18 to 20 hr. With ether anesthesia, both femoral arteries were cannulated, one for bleeding into a reservoir and the other for measurement of arterial blood pressure. After the animals had awakened, hemorrhagic shock was produced by bleeding. Details of the procedure have been reported previously (6).

Five categories of animals were studied: (a) Unbled controls: Fifteen animals were anesthetized, cannulated and allowed to awaken, but were not bled. They were killed 1 hr after recovery from the anesthesia. (b) Early shock: Eight animals were bled, maintained at a mean arterial pressure of 35–40 mm Hg and sacrificed when 25% of the shed blood was spontaneoulsy taken up (about 1 hr). (c) Early shock with treatment: Seven animals were bled and maintained at the same pressure as above. When 25% of shed blood was taken back, the remaining blood plus Ringer's lactate solution with 5% dextrose in a volume equal to 50% of the total volume of the shed blood was infused. These animals were sacrificed 1 hr after the infusion. (d) Late shock: Five animals were maintained at 35–40 mm Hg arterial pressure until 50–70% of the shed blood had been returned (about 2 hr) and were sacrificed. (e) Late shock with treatment: Six animals were bled to the late shock stage and then the remaining blood plus Ringer's lactate solution with 5% dextrose in a volume equal to half of total shed blood was infused. Animals were sacrificed 1 hr after the infusion. All animals were sacrificed by decapitation and rapid exsanguination; and the livers were removed immediately.

Approximately 2 g of liver were homogenized in 10 ml of 0.1% deoxycholate in sucrose–Tris–EDTA medium [220 m*M* surose, 10 m*M* tris(hydroxymethyl)aminomethane–HCl, 1 m*M* EDTA] at 4° and pH 7.4. Enzyme containing fractions of liver homogenates were prepared by differential centrifugation. Each sample was successively centrifuged at 8000*g*, 18,000*g*, and 105,000*g*. The first two pellets were discarded; the final pellet was suspended in distilled water by hand-homogenization in a small Teflon–glass homogenizer and used as the enzyme preparation. The protein content of this prepara-

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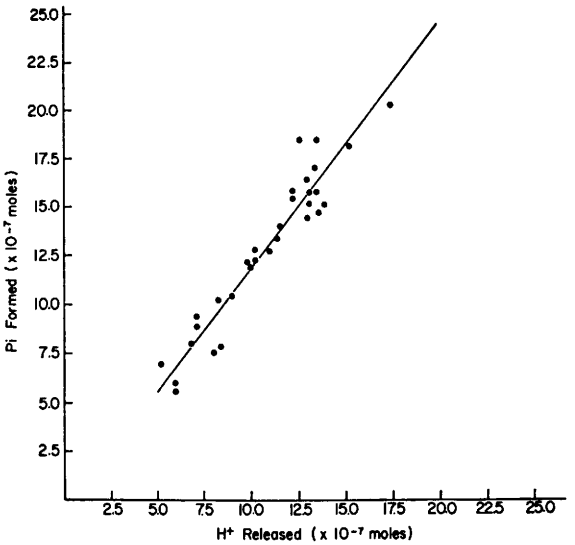

FIG. 1. Proportionality between the amount of P_i formed and the amount of H^+ released during the hydrolysis of ATP: Regression line was calculated by the method of least squares; r (correlation coefficient) = 0.966.

tion was analyzed by the method of Lowry *et al.* (7).

Adenosine triphophatase activity was assayed by quantitating hydrogen ion released from ATP hydrolysis in a Radiometer autotitrator (Radiometer Co., Copenhagen, Denmark) operated in the pH-stat mode. The specific enzyme activity was expressed as the rate of release of hydrogen ion from the hydrolysis of ATP per milligram of protein in the enzyme preparation. At a given pH, the rate of hydrogen ion (H^+) production is a measure of the rate of ATP hydrolysis (8). The pH-stat method for ATPase activities was compared with the more usual assay of release of inorganic phosphate (P_i). At various periods of incubation, aliquots of the titrated reaction media were immediately transferred to cold trichloroacetic acid (final conc, 8%; w/v) and P_i was determined (9). The amount of H^+ titrated was calculated from the pH-stat recorder tracing.

The reaction mixture for the determination of total ATPase activity (Na^+ plus K^+ stimulated Mg^{2+} activated ATPase) consisted of 5 m*M* $MgCl_2$, 20 m*M* KCl, 120 m*M* NaCl, 3 m*M* ATP (disodium salt) and 1.1 to 1.2 ml of enzyme preparation containing approximately 1 mg of protein. The Mg^{2+}-ATPase activity was determined in a reaction mixture from which KCl and NaCl were omitted. Ouabain (0.5 m*M*) was added to the assay medium to test for the sensitivity of the enzyme to this inhibitor. The $(Na^+ + K^+)$-ATPase activity was estimated by subtracting the Mg^{2+} ATPase activity from the total ATPase activity. All reactions were carried out at 30° and pH 7.45.

Results. The comparison of H^+ released to P_i formed demonstrated a high degree of correlation (Fig. 1). The rate of H^+ release during ATP hydrolysis was also examined over a wide range of protein concentration in the enzyme preparation and was found to be linear (Fig. 2). The Mg^{2+}-ATPase activity remained unchanged in the presence of ouabain. However, $(Na^+ + K^+)$-ATPase activity was inhibited by 36% (±17.9 SD; N = 6) in the presence of 0.5 m*M* ouabain.

Figure 3 summarizes the results of the present experiments. The mean $(NA^+ + K^+)$-ATPase activity in control unbled animals was 10.6 ± 1.04 (SEM) nmoles/(mg protein × min), and was increased to 19.4 ± 2.27 in early shock. A further increase in activity to a value of 33.7 ± 2.41 was found in late shock. However, with reinfusion of Ringer's lactate the enzyme activity de-

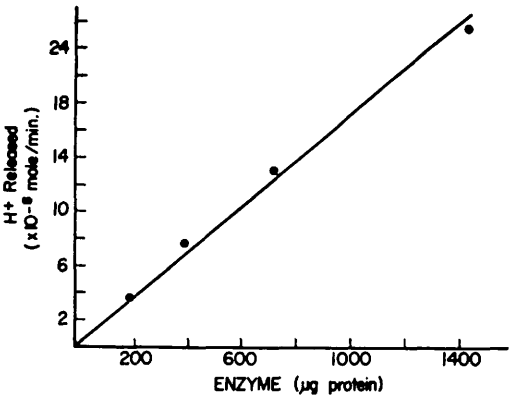

FIG. 2. Rate of H^+ release during ATP hydrolysis as a function of enzyme concentration: The reaction mixture contained Mg^{++}, Na^+, and K^+ in the concentration described in the text. The enzyme concentration, a constant function of the total protein in the sample, was carried as shown.

creased significantly ($p < .01$) to 14.2 ± 1.93 in early shock and to 17.8 ± 2.23 in late shock. The animals' blood pressure returned to the prehemorrhage level during the hour after reinfusion in both groups of animals.

Discussion. It has been postulated that sodium-plus-potassium stimulated and ouabain inhibited adenosine triphophatase of cell membranes is involved in the transport of Na^+ out of cells and K^+ into cells (10). Hence, alterations in $(Na^+ + K^+)$-ATPase activity could represent an important change in cell function in shock. In our experiment, the rat liver $(Na^+ + K^+)$-ATPase activity was only partially inhibited by ouabain. This is in agreement with an earlier report by Bakkeren and Bonting (11) that the $(Na^+ + K^+)$-ATPase from rat liver was relatively insensitive to the cardiac glycoside. These authors have shown an approximately 60% decrease in the Na–K transport enzyme activity in the presence of 0.5 m*M* ouabain. In the present study, we found a 36% decrease at this concentration of ouabain.

Histochemical evidence of increased hepatic ATPase in hemorrhagic shock has been presented by DePalma *et al.* (12). A major finding in our study is the biochemical demonstration of increased $(Na^+ + K^+)$-ATPase activity with shock. Not only is the activity progressively increased as the length and therefore the severity of shock increased, but also the increased activity returned toward normal following treatment of both early and late hemorrhagic shock.

The increase in $(Na^+ + K^+)$-ATPase activity with shock suggests an *in vivo* activation of this transport enzyme to above normal levels. Although the mechanism whereby this change is effected has not been examined, the following factors could be considered. The activation may result from an increased intracellular availability of one or both of the reactant substances, ATP and NA^+. Increased ATP availability seems improbable with the hypoxic conditions which prevail during shock. An increase in intracellular Na^+ consequent to an initially depressed activity of the sodium pump mechanism or to an increased passive entry of sodium into cells, appears to be a more plausible explanation of the increased $(Na^+ + K^+)$-ATPase activity. Increased intracellular Na^+ has been shown to stimulate $(Na^+ + K^+)$-ATPase in erythrocyte ghosts (13, 14). Cunningham *et al.* (3) have suggested similar alternatives as a mechanism responsible for functional alteration in cell membrane parameters with shock. Whether the change in ATPase activity is a primary or a secondary response with shock cannot be ascertained at this time. It is of significance, however, that the change is reversible. No attempt was made to evaluate therapeutic regimens. Blood and Ringer's lactate were given

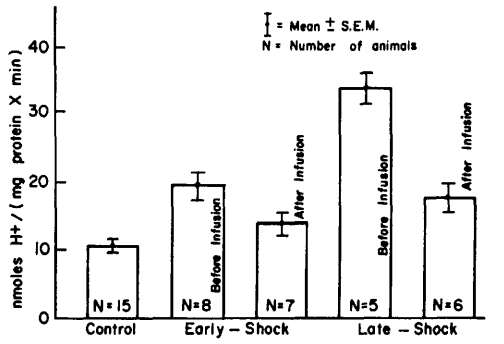

FIG. 3. Liver $(Na^+ + K^+)$-ATPase activities in unbled control, early shock, and late shock. The enzyme activity was measured before and after infusion of the shed blood plus Ringer's lactate in shocked animals.

simply to provide an adequate circulating blood volume.

Summary. $(Na^+ + K^+)$-ATPase activity was measured in the liver of rats subjected to an early stage of hemorrhagic shock (25% of shed blood taken back spontaneously) and a late stage (50–70% of shed blood taken back). In unbled control animals the activity was 10.6 ± 1.04 (mean ± SEM) nmoles H^+/(mg protein × min). An approximately twofold increase in activity in early shock and a threefold increase in late shock was found. When animals were treated by return of the shed blood plus 5–6 ml of Ringer's lactate solution in the early and late stages of shock and allowed 1 hr for recovery, the $(Na^+ + K^+)$-ATPase activity decreased to near control levels. These results also indicate that the increased liver $(Na^+ + K^+)$-ATPase activity in shock is reversible with treatment.

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