was measured as before. In this way, an accurate measure of the difference in MGC as the same composition of the mixture could be obtained. Although this procedure involved gelling the sample twice, no hysteresis was detected; data obtained on once-gelled samples (denoted by open symbols in Fig. 1E) or twice-gelled samples (closed symbols in Fig. 1E) are in good agreement.

13. This result as first sight conflicts with the conclusions of Bertles *et al.* (*6*), who found that deoxygenation of HbS-HbF mixtures apparently led to the complete exclusion of HbF from the solid phase. However, it is likely that under their conditions gelation occurred before complete deoxygenation, and that the HbS was preferentially deoxygenated. Patients homozygous for HbS and with hereditary persistence of HbF have a hemoglobin composition of 70 percent HbS and 30 percent HbF; those who are heterozygous for HbS and with β-thalassemia have a composition of 70 percent HbS, 30 percent HbA. The latter exhibit the symptoms of sickle cell anemia, but the former do not. This result is now explicable: deoxy-HbA tetramers may be incorporated into the gel, but deoxy-HbF tetramers may not.
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Extracellular Acidosis Protects Ehrlich Ascites Tumor Cells and Rat Renal Cortex against Anoxic Injury

Abstract. *The present study indicates that extracellular acidosis protects Ehrlich ascites tumor cells and rat kidney cortex cells against injury from anoxia. Parameters measured included cell potassium, adenosine 5′-triphosphate, and uptake of vital dyes. Cells survived longer at a* p*H of 5.6 to 6.5 than at a* p*H of 7.4;* p*H 7.9 was most detrimental. These findings indicate that production of protons by anoxic cells may be a protective feedback mechanism.*

The tolerance of cells to injury from anoxia varies greatly in different cell types. Morphological and biochemical effects of anoxia have been widely studied both in vivo and in vitro, but methods to protect the function or prevent injury or death of the cells due to anoxia or to other lethal agents are still quite limited and little studied. It is generally accepted that extracellular acidosis is detrimental to the function of many organs. However, little attention has been paid to the effect of acidosis on the viability or death of cells in an anoxic or an aerobic milieu. Recently it has been found that the recovery of heart muscle function following hypoxia was protected by acidosis (*1*). The present experiments were designed to determine the effects of anoxia and acidosis on the viability of Ehrlich ascites tumor cells (EATC) and rat kidney cortical cells in vitro. Vital dye uptake, intracellular potassium, and adenosine 5′-triphosphate (ATP), known to be good indicators of cell injury or cell death caused by several mechanisms (*2*), were used as parameters for EATC, and potassium was used for kidney cells. Ehrlich ascites tumor cells were cultivated in the peritoneal cavity of the mouse by weekly transplantation and handled as reported earlier (*3*). The cells were collected 7 or 8 days after inoculation, washed two times in a modified Krebs-Ringer phosphate (KRP) buffer (*4*), centrifuged at 71*g* for 5 minutes, and resuspended in KRP so that the final concentration was 1.0×10^7 to 1.6×10^7 cells per milliliter. Cells preincubated aerobically for 10 minutes at 37°C were used as preanoxia controls. Renal cortical slices were prepared with a Stadie-Riggs microtome. Slices were cut within 1 minute after removing the kidney and immediately placed into the incubation chamber in KRP or were used as preanoxia controls. Slices were about 0.2 mm thick, as calculated by determining their areas and weights and assuming that the tissue density was 1 (*5*). An airtight chamber, maintained at 37°C, containing 15-ml plastic vessels filled with appropriate incubation media was used for anoxia experiments; a water bath with 45 oscillations per minute was also used. Relative humidity inside the chamber was maintained at 100 percent. The chamber and the incubation media were gassed before being used, and the chamber was gassed during experiments with high-purity grade dry nitrogen in order to totally remove oxygen. Oxygen content was measured before and during experiments with an oxygen monitor (model 53, Yellow Springs Instrument). The trace amount of residual oxygen was consumed during the first 2 minutes of incubation when EATC, preincubated under aerobic conditions and suspended in the buffer vehicle, were injected into an anoxic milieu. Portions were taken from the samples at intervals up to 12 hours. The EATC viability was determined in a hemacytometer chamber with nigrosin as the vital dye stain (*6*); ATP was analyzed in perchloric acid–

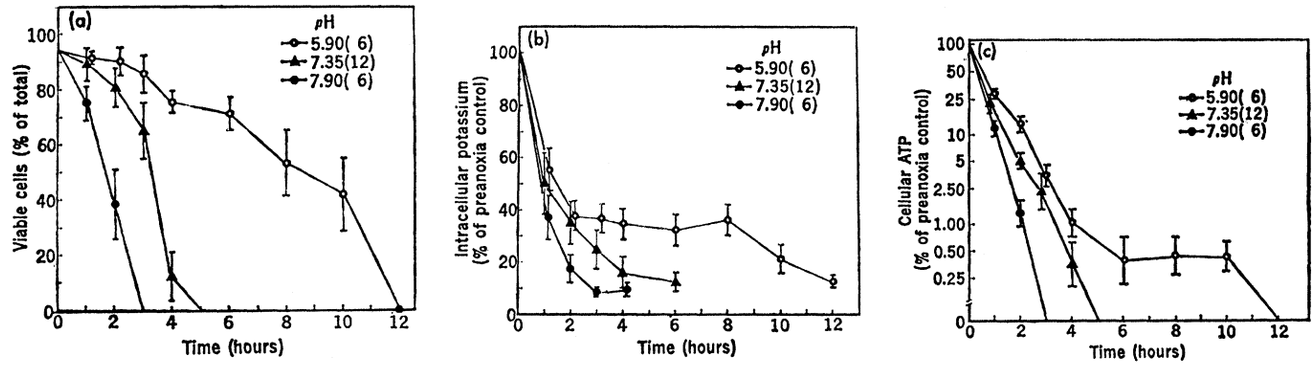

Fig. 1. (a) Viability of Ehrlich ascites tumor cells (EATC) during 12 hours of anoxia at varying *p*H values. The mean preanoxia count of nigrosin-stained cells was 6.6 ± 2.7 percent of the population. The numbers in parentheses following the *p*H values indicate the number of the experiments in each group. Brackets indicate 1 standard deviation. (b) Intracellular potassium of EATC during 12 hours of anoxia. The samples were taken from the same incubations as in (a). The mean preanoxia content of intracellular potassium was 1.84 ± 0.38 μmole per 10^7 cells (100 percent). (c) Intracellular adenosine 5′-triphosphate (ATP) of EATC during 12 hours of anoxia. The mean preanoxia content of cellular ATP was 5.39 ± 8.2 μmole per 10^7 cells.

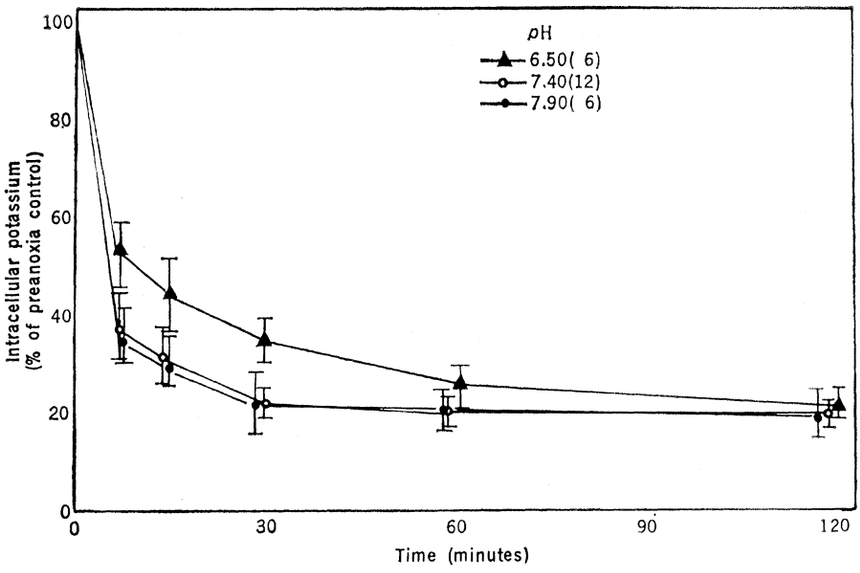

Fig. 2. Intracellular potassium of rat kidney cortical cells during 2 hours of anoxia. The mean preanoxia content of potassium was 315.8 ± 26.6 meq per kilogram of dry tissue. Note the much more rapid decrease in potassium as compared with EATC; this is presumably the result of higher anaerobic glycolysis in the latter cells.

treated samples (*7*). The cells were centrifuged at 1000*g* for 1 minute, and the potassium content of the water-lysed pellets was determined by flame photometry. The amount of potassium trapped by buffer in the pellet was estimated by using [^{14}C]dextran in the determination of the extracellular space. Potassium from kidney slices was determined by acetic acid extraction (*8*); lithium chloride was used as an internal standard in these determinations.

The effect of media at varying *p*H's on the viability of EATC during anoxia was striking (Fig. 1a). About 50 percent of the cells were dead as determined by the ability to stain with a vital dye at about 2 or 3 hours and at 9 hours of anoxia at *p*H 7.9, 7.35, and 5.9, respectively. The respective 100 percent figures were registered at 3, 5, and 12 hours. Cells lost potassium during early anoxic treatment, with the greatest loss occurring at higher *p*H's. Intracellular potassium values were about 22 percent of preanoxia control at *p*H 7.35 and 7.9 and about 30 percent at *p*H 5.9, when 50 percent of the cells were dead (Fig. 1b). Cellular ATP decreased or disappeared more rapidly at higher *p*H's (Fig. 1c).

Similar protective effects were found when EATC were incubated at *p*H 5.6 or 6.5. Analogous results were also found when 25 m*M* HEPES buffer (final *p*H 6.15, 310 milliosmols) or 25 m*M* tris (*p*H 6.30, 310 milliosmols) instead of 25 m*M* phosphate buffer was used in modified Krebs-Ringer solution. Extracellular acidosis also had a marked protective effect on the loss of potassium from kidney cortex cells during anoxia (Fig. 2).

Bing *et al.* (*1*) used functional parameters as indicators of myocardial viability in the reoxygenation phase following hypoxia and showed improved recovery when the cells had been hypoxic at an acid *p*H. The present observations on EATC and kidney cortex cells indicated that they can be protected significantly against deterioration due to anoxia by an acid milieu when cellular potassium, known to be one of the best indicators for the viability of cells in vitro (*2*), was used as a parameter. Also the significant protective effect of extracellular acidosis was demonstrated on EATC by use of vital dye staining commonly applied for differentiation of living and dead cells (*2*, *6*). The identical results obtained here with two quite different cell types suggest that extracellular acidosis may be of paramount importance for elongation of cell survival during anoxia. Unlike the kidney cortex cells, EATC show a high rate of anaerobic glycolysis, which is probably also the principal source of energy during the acid protection phase in an anoxic environment. The rapid loss of potassium and ATP during the early phase of anoxic treatment indicates a rapid adaptation of EATC in an anoxic environment at varying *p*H values, and the minimal amount of cellular ATP in an acid *p*H is one indicator of the minimal rate of cellular metabolism that is capable of keeping the EATC viable for long periods. Observations in some earlier studies support the possibility that the protective mechanism is metabolic. The intracellular *p*H of the EATC have been shown to be essentially dependent on the extracellular *p*H (*9*). Cellular acid-base changes are known to affect enzymes that exert a rate-limiting control upon metabolic reactions. In hypercapnic acidosis, the rate-limiting enzyme of the brain tissue is phosphofructokinase which is inhibited by hydrogen ions (*10*). In EATC at *p*H 6.4 and 7.4 the steady state of aerobic glycolysis is also controlled by phosphofructokinase (*11*). However, the mechanism may be more complicated because phosphofructokinase is activated by potassium (*12*), but the cellular potassium was reduced significantly during the early phase of anoxia. Alternatively, an altered *p*H may exert a direct effect on physical properties of the cell membrane, for example, on membrane permeability through interactions with membrane proteins or lipids, or both. Because two of the most important changes in the cell in connection with injury are alterations of the plasma membrane and interference with bioenergetic systems (*2*), reciprocally dependent on each other, it is possible that the protection mechanism may involve both functions.

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