Intracellular Edema and Dehydration: Effects on Energy Metabolism in Alveolar Macrophages

Abstract. *The effects of intracellular edema and dehydration on energy metabolism in alveolar macrophages were studied. Intracellular edema increased lactate production and reversibly decreased oxygen consumption. Dehydration caused no significant change in lactate production but irreversibly decreased oxygen consumption. These phenomena may be applicable to a wide variety of clinical problems including lung and brain edema.*

Although alterations of cellular water content are caused by a variety of disease mechanisms, little is known about the metabolic effects of such phenomena (*1, 2*). Cells such as the pulmonary alveolar macrophage are quite permeable to sodium ions, and the active extrusion of these is important in maintaining intracellular water homeostasis (*3*). A lack of energy for this active transport due to disturbances of oxidative phosphorylation associated with adenosine triphosphate (ATP) depletion results in the intracellular accumulation of sodium and water (*3, 4*). Correction of these disturbances can reverse this edema in certain tissue systems (*5*). The reverse problem, changes in energy metabolism related to changes in cell water, has not been studied extensively. Hypertonic extracellular perfusate prevents the accumulation of intracellular water in hypoxic tissue and thus prevents physiologic changes such as the "no reflow phenomenon" (obstruction of the small vessels of organs subjected to hypoxemia, presumably because of edema of the capillary endothelial cells) (*6*). This suggests that alterations of intracellular water activity per se may cause adverse metabolic effects. We produced alterations of intracellular water content by altering extracellular fluid osmolality in an isolated cell system, the pulmonary alveolar macrophage, to investigate the effects of intracellular edema and dehydration on cell energy metabolism.

Alveolar macrophages were harvested from the lungs of healthy 3- to 5-kg New Zealand white rabbits by methods previously described (*7*). Solutions for osmotic incubation were prepared from a 100 milliosmolar (mOsm) standard solution containing 10 m*M* glucose, 1.0 m*M* $CaCl_2$, 50 m*M* tris(hydroxymethyl)aminomethane, and enough 1*M* HCl to adjust the *p*H to 7.4 at 37°C. More hypotonic solutions were prepared by adding distilled water, more hypertonic solutions by adding 25 percent mannitol. The final osmolality was measured by freezing point depression before use (*8*).

Isotonic controls (300 mOsm) were prepared by adding 200 m*M* mannitol to the standard solution. Sodium and potassium were not included in the medium to obviate changes in permeable cation gradients. Although the incubation medium therefore appears unphysiologic, oxygen consumption ($\dot{Q}O_2$) and lactate production ($\dot{Q}$lact) in this isotonic medium were identical to those measured in a standard rabbit Ringer solution. Macrophages were incubated at 37°C in well-oxygenated solutions of the desired osmolality for 5 minutes. Oxygen consumption was measured for 5 minutes with a polarographic oxygen electrode system (*9*). Isosmotic reconstitution of normal cell water content was then accomplished as follows. For hypotonic solutions, enough 25 percent mannitol was added to return final osmolality to 300 mOsm. Cells in hypertonic media were centrifuged, washed, and resuspended in solutions with a final osmolality of 300 mOsm. After 5 minutes of isosmotic reconstitution (exposure of the cells previously incubated in hypo- and hypertonic media to isotonic media) the $\dot{Q}O_2$ was again measured for 5 minutes. Protein was measured by the method of Lowry *et al.* (*10*). Oxygen consumption was expressed as microliters of oxygen per hour per milligram of cell protein (see Fig. 1). Lactate production was measured in parallel experiments. Lactate in the suspending media was determined by the method of Scholtz *et al.* (*11*) at 0 and 20 minutes. Lactate production was expressed as micrograms of lactic acid per hour per milligram of cell protein. The results are summarized in Fig. 1.

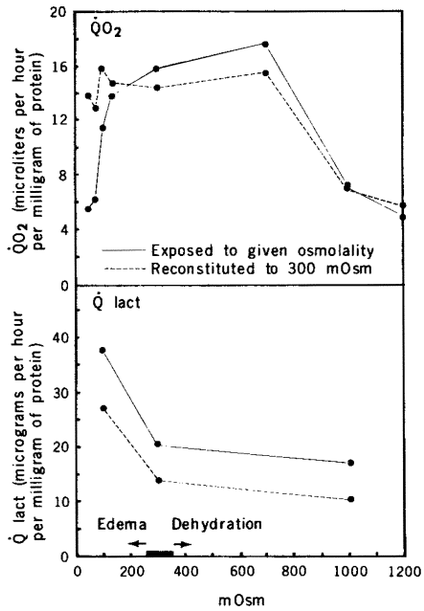

Fig. 1. Oxygen consumption and lactate production at the various osmolalities. The solid lines represent values for the initial incubation, the broken lines values for the same cells after isosmotic reconstitution. For each point $N = 7$ or 8. At 50, 75, and 100 mOsm $\dot{Q}O_2$ was significantly lower ($P < .005$) than at 300 mOsm for the initial incubation. After isosmotic reconstitution, $\dot{Q}O_2$ at these dilutions is not significantly different from that at 300 mOsm. At 1000 and 1300 mOsm $\dot{Q}O_2$ was significantly lower ($P < .001$) than at 300 mOsm and there was no significant change after reconstitution. Lactate production was significantly lower ($P < .05$) after reconstitution at the three osmolalities considered (100, 300, and 1000 mOsm) but $\dot{Q}$ lact at 100 mOsm was significantly higher ($P < .001$) than at 300 mOsm both before and after reconstitution. Lactate production at 1000 mOsm was not significantly different from that at 300 mOsm.

Intracellular edema (incubation media with < 300 mOsm) produced significant decreases in $\dot{Q}O_2$. Isosmotic reconstitution of these cells produced a return of $\dot{Q}O_2$ to control values. Lactate production in these cells was markedly increased compared to the isotonic controls both before and after reconstitution. Intracellular dehydration (incubation media with > 700 mOsm) produced no significant change in lactate production. Oxygen consumption was significantly decreased initially and did not increase after reconstitution.

The reversible reduction in $\dot{Q}O_2$ caused by intracellular edema could not have been due to changes in cation gradients since sodium and potassium were not added to the extracellular media, calcium concentrations were held constant, and the solute used for the reconstitution, mannitol, is nonionic. Pilot studies using variation of the sodium content of the extracellular fluid to change the osmolality produced virtually the same results. The reduced $\dot{Q}O_2$ in both hypotonic and hypertonic media reflects reduced mitochondrial oxygen utilization since approximately 85 percent of total cellular oxygen utilization in the alveolar macrophage represents mitochondrial $\dot{Q}O_2$ (*3*). Electron microscopy has confirmed that osmotically induced intracellular edema is accompanied by mitochondrial edema and that cellular osmotic reconstitution reverses the organelle edema as well (*12*). Edema of isolated liver mitochondria caused by alterations of cation permeability also has been shown to decrease oxygen consumption (*13*). Since cytochrome oxidase is tightly bound to mitochondrial cristae, edema of the organelle could decrease oxygen utilization by distorting the surface geometry of the cristal structure. Restoration of normal mitochondrial architecture would reverse this abnormality. Alternatively, the changes in mitochondrial oxygen consumption could result from an increase in the diffusion pathway of molecular oxygen.

The increase in lactate production presumably represents increased ATP production by the glycolytic pathway in the absence of optimal oxidative phosphorylation (Pasteur effect). Failure of lactate production to return to control values has not been explained in this system. There is evidence suggesting uncoupling of oxidative phosphorylation as a result of brain edema (*14*). This implies oxygen consumption without ATP production and thus an ongoing requirement for glycolytic ATP. Increased lactate production due to intracellular edema has been reported in an intact organ system. Young (*15*) produced edema in the isolated perfused rat lung by increasing pulmonary capillary pressure and by altering alveolar epithelial permeability. Intra-alveolar epithelial edema was present with both pathogenetic mechanisms. Pulmonary edema in this system was associated with increased total lung lactate production. When the lungs were filled with isosmotic NaCl (so that no intracellular edema was present), no increase in lactate production occurred (*15*).

Cellular dehydration appears to produce marked alteration of oxygen utilization. The Pasteur effect was absent, reflecting another aspect of altered metabolic control associated with cellular injury. The reduced $\dot{Q}O_2$ has been reported in isolated mitochondria. Increasing the concentration of mannitol and sucrose in the incubating medium reduces $\dot{Q}O_2$, although this can be reversed in this preparation by adding permeant ions to restore the normal water content of the matrix (*13*).

These studies suggest that regulation of intracellular and subcellular water activity is required for normal cellular energy metabolism. The reversible nature of the changes produced by intracellular edema helps to explain the beneficial effects of administering a nonionic osmotic agent such as mannitol in the treatment of certain disorders associated with cellular swelling. Changes associated with cellular dehydration are profound and in this cell system cannot be reversed.

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Niche Shifts in Sunfishes: Experimental Evidence and Significance

Abstract. *Three species of sunfishes segregate ecologically when stocked together in small ponds. When each species is stocked separately in replicate ponds, it exhibits competitive release through increases in growth rate and average food size. Niche shifts are indicated by convergence of these species to the same food habits in the absence of competitors. These shifts are due to phenotypic (behavioral) plasticity. The significance of niche flexibility is related to seasonal patterns in resource availability.*

The premise that animal communities are shaped primarily by competition serves as a cornerstone of much recent ecological theory (*1*). Morphological changes (character displacement) and ecological changes (niche shifts) occurring in species when the complement of closely related species differs are generally considered the strongest evidence supporting this premise (*2*). The forms and magnitudes of these changes also indicate critical niche dimensions and mechanisms of ecological segregation.

Niche shifts can arise from both genetic changes and phenotypic (behavioral) plasticity (*3*). The latter potentially enables a species to alter its ecology immediately upon removal of a competitor (or competitors). Although data of this sort bear on the development of a theory of community structure (*2, 3*), very little is known about how species differ in these two components of niche flexibility.

Niche shifts are most often revealed when populations of a species inhabiting presumably saturated mainland and depauperate island communities are compared. In the absence of competitors species often increase in abundance or expand the range of habitats, foraging heights, altitudes, or food utilized (*1, 4*). In such comparative studies, however, separating the effects of competition from the effects of relative availabilities of habitats and foods is a major operational problem (*2*). In addition, it is difficult to separate the genetic and phenotypic components of the observed niche shift. Both of these problems can be minimized by removing or adding species to communities in replicate environments and comparing resource utilization. The availability of food and habitat types will be similar and therefore less likely to confound the results. If the experiment is performed over a short time period and reproduction is precluded, any response by a species can be attributed to phenotypic plasticity.

We report a set of experiments designed to examine niche shifts in three species of freshwater sunfishes (Centrarchidae): the bluegill (*Lepomis macrochirus*), pumpkinseed (*L. gibbosus*), and green sunfish (*L. cyanellus*). These species coexist in the littoral zone of small lakes over a broad region of central North America and attain about the same adult body size (150 to 300 g). Data from natural lakes indicate that the green sunfish inhabits regions closer to the shore and in shallower water than the other two species. The bluegill and pumpkinseed are more widely distributed in the littoral; the former predominantly inhabits the water column while the latter remains near the bottom (*5*).

The experimental facility consists of 18 identical circular ponds, 29 m in diameter and 1.8 m deep, located at the Kellogg Biological Station of Michigan State University. Each pond contained a stand of cattails (*Typha* spp.) extending 2 m from shore. Apart from this area, there were patches of submergent vegetation (*Chara* and *Potomogeton* spp.), growing to within 0.5 m of the surface; between these patches were areas of exposed sediments.

Four ponds were selected for uniformity of these characteristics. In one pond, 900 individuals of each species were stocked together (2700 total); in the remaining three, 900 individuals of each species were stocked alone (*6*). Mean size of the individuals of each species ranged from 26 to 28 mm in length ($N > 100$ for each species). These fish were spawned the previous year in local brood ponds. The experiments were initiated in June 1973 and terminated by draining the ponds in October 1973.

At intervals from 3 days to 2 weeks dur-