THE PURPOSE of this writing is to point out a possible role of cell swelling in ischemic damage to tissues.

Krebs and his associates\(^1\) first showed that the water content of tissues \textit{in vitro} was dependent on tissue metabolism. With inhibition of metabolism tissues swell and with restoration of metabolism, if not too delayed, the water content returns to normal. It has been shown that the changes affect the intracellular fluids rather than the interstitial fluid of the tissue and the swelling and shrinking of the cells is secondary to their changes in content of solutes.\(^2\), \(^3\), \(^4\)

The concentration of water is the same inside and outside of cells.\(^5\), \(^6\), \(^7\) Cell membranes are generally so permeable to water that a uniform chemical potential of water or tonicity exists throughout intra- and extracellular fluids. This means that the volume of cells is determined by the quantity of solutes which they contain. The major osmotically active solute within cells is potassium ions. Sodium which is high in the plasma and extracellular fluids is present at low concentrations within most cells, e.g., at about one-tenth its extracellular fluid concentration within muscle cells. But cell membranes are permeable to sodium, and positive sodium ions, abetted by the electrically negative cell interior, constantly diffuse down their chemical concentration gradient. Under usual circumstances, this inward diffusion of sodium is just balanced by its outward extrusion so that the amount of sodium within the cell is kept low and constant. The outward extrusion of sodium takes place against both a chemical and electrical gradient, and therefore, must be an active process deriving its energy from the metabolism of the cell. The view that the cytoplasm of cells was similar to a cation exchange resin which favored potassium over sodium is incorrect. Sodium and potassium within cells behave as though they are in free solution\(^8\) and the low intracellular concentration of sodium is preserved by active transport processes within cell membranes. This means of regulating cell volume, although at the cost of metabolic energy, allows animal cells to maintain very low membrane tensions, a condition essential for motility.

When the metabolism of a tissue is inhibited by toxins, cold, anoxia, or when the cell membrane is made so permeable to sodium that the extrusion process can no longer keep pace with the rate of entry of sodium into cells, then sodium accumulates within cells. But the accumulation of positive sodium ions within the cells reduces the intracellular electronegativity. This depolarization of cell membrane potential allows negative chloride ions now to enter cells and positive potassium ions to

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leak out. However, the accumulation of sodium and chloride will exceed the loss of potassium so a net gain of intracellular solute occurs which osmotically draws water into the cells. As a result cells swell.

If normal metabolic processes can be restored before the cells are irreversibly damaged, energy for sodium transport will cause extrusion of the accumulated sodium and cell volume will return toward normal. It was first suggested by Ames and his associates that swollen cells may block the blood supply within an organ so as to prevent return of nutrient blood flow to a tissue, brain in this instance, after an initial transient vascular obstruction. This secondary vascular obstruction may prolong the tissue ischemia and increase the ultimate tissue damage.

Two recent studies have given substance to the hypothesis that cell swelling during a transient ischemic episode may obstruct small vessels and prolong the actual ischemia and increase tissue damage. Flores et al. studied the "no-reflow" phenomenon in rat kidneys. They found, as many others had previously, that after a one to two hour period of obstruction to a renal artery, release of the obstruction is not accompanied by a prompt return of blood flow to the kidneys. Arterial injection of silicone rubber revealed a patchy ischemia throughout the kidney but perhaps most pronounced in the subcortical zone. This patchy ischemia was found to be associated with swelling of all cellular elements in the kidneys. A striking feature was the swelling of endothelial cells which appeared to narrow the lumen of small vessels so as to trap red cells with complete obstruction of the lumen. Since the animals were fully heparinized during these studies, thrombosis did not occur but small vessels appeared to be blocked by erythrocytes. Weed et al. have shown that red blood cells lose their remarkable flexibility when their adenosine triphosphate (ATP) levels become depleted and such depletion may be anticipated when metabolic substrates are depleted in the ischemic tissue. Summers and Jamison have demonstrated the role of the erythrocyte in the "no-reflow" phenomenon. Thus, if all blood were eluted from the renal circulation prior to obstruction of the renal artery, subsequent injection of carbon black suspension would fill all the vascular channels of the kidney. Injecting red blood cell ghosts rendered rigid by soaking in 10 mM calcium solution occluded small blood vessels reproducing the typical postischemic patchy obstruction of small vessels. The same findings have been demonstrated using the silicone rubber technique to visualize the patency of the renal vasculature. Apparently the lumen of small vessels, although narrowed by compression from surrounding swollen cells and by its own swollen endothelium, is still sufficient to permit passage of the fine carbon black or silicone rubber particles but insufficient for rigid erythrocytes and the latter apparently contribute to the vascular obstruction.

Intravascular injection of hypertonic mannitol, or other solutes which penetrate cell membranes poorly, was effective in osmotically shrinking the swollen cells, promptly reestablishing a normal vascular pattern (as visualized by the silicone rubber injections), and reducing the ultimate degree of tissue damage and failure of renal function resulting from transient compression of the renal artery. More recently a similar role of cell swelling in the "no-reflow" phenomenon following a reversible episode of hemorrhagic hypotension has been established, although the rat kidney is not selectively vulnerable to this form of ischemia and other factors, humoral and neurogenic, are also etiologically important.

Evidence that cell swelling may contribute to ischemic tissue injury in experimental myocardial infarction has been presented by Willerson, Powell, and their associates. Reversible occlusion of the left anterior descending coronary artery in the dog was associated with elevation of ST-segments in electrocardiograms obtained with electrodes placed directly over the ischemic myocardium. A prompt diminution of contractile force in the ischemic muscle was also demonstrated. Infusion of hypertonic mannitol during or prior to the coronary occlusion, lowered the ST-segment elevation over the ischemic muscle, increased the force of contractility of the muscle, and enhanced the collateral blood flow into the ischemic area, as measured by the rate of wash-out of krypton-85 injected into the coronary artery just distal to its point of obstruction. Recently Powell et al. have produced evidence compatible with the hypothesis that swelling of cells in the area of coronary occlusion contributes to the tissue ischemia and that hypertonic mannitol may shrink such swollen cells, improve blood flow, and protect cells from irreversible damage.

The swelling of cells with resultant vascular obstruction need not be associated with a generalized increase in tissue pressure in the affected organ. Swelling of cells only at critical points where small vessels may become obstructed is all that may be required. Transfer of interstitial fluid to the
intracellular compartment of critically located cells may suffice.

Not all cells swell at an equal rate. Red blood cells have a very low rate of permeability to sodium so that even with depletion of their energy stores swelling might be expected to occur very slowly. In the kidney, obstruction of the renal artery causes a drop of ATP levels in cortical tissue to less than 1/3 in 30 sec. However, compression of a renal artery for only 30 min is associated with very little, if any, "no-reflow," whereas 60 min of arterial obstruction produces this phenomenon with regularity. Not only must time be required for sodium to enter the cells but a sufficient quantity must enter to allow potassium to be discharged and the cell membrane potential to diminish. The cell membrane potential changes only as the logarithm of the intracellular to extracellular concentration of potassium or of the extracellular to intracellular concentration of chloride. Time is required for sufficient changes in the intracellular concentrations of these two ions to occur and the time will vary from tissue to tissue and from one cell type to another.

The current studies to which reference is made are supported by a huge literature in which pathologists and investigators have noted cell swelling in ischemic tissues but have regarded it solely as a consequence of the ischemia not as a link in a self-perpetuating vicious cycle which may lead ultimately to cell and organ death. It is perhaps surprising that the proximate cause of cell death remains unknown. With inhibition of cell metabolism many potentially deleterious effects occur. Under aerobic conditions the redox state of cells becomes reduced, cellular acidification results from lactic acid formation, synthetic processes stop, among other responses. But cell swelling may also be an important factor in cell death. There is an important principle, the Curie Principle, which states that scalar processes occurring in an isotropic medium cannot give rise to directed flows. Chemical reactions are scalar, namely undirected, and yet they give rise in living systems to highly oriented phenomenon such as unidirectional ion transport or complex metabolic sequences. Such oriented phenomena could not result from scalar chemical reactions occurring in isotropic aqueous solution, as in a test tube. But the cell interior is not a bag of fluid but a multicomparted system in which an abundance of membrane structures provide an anisotropic or nonhomogeneous environment for chemical reactions. Enzymes fixed to membrane structures determine the flow of substrates or reactants in an orderly manner consistent with the multistage sequential reactions or oriented transport upon which the life processes depend. When cells swell, the orientation of these fixed reactive sites become distorted and the orderly flow of reactants or transport phenomena is disturbed.

Shrinking cells back to their normal volume before irreversible changes occur within the internal structures of the cell may permit the return of a normal flow of reactants from one fixed site to another and assure the viability of the cell. If cell swelling is too pronounced or structure too disorganized then even shrinkage of the swollen cell may be insufficient to reestablish vital reactions in the cell. Prior to this irreversible stage, however, shrinking swollen cells may realign reactive sites so that normal metabolic processes are reestablished and the cell and tissue survive. It appears that the time available to reestablish normal cell activities following a period of transient ischemia may be considerably longer than recognized before the perpetuating and amplifying effect of cell swelling on tissue ischemia was recognized. This raises hopes for possible therapeutic intervention when cell swelling contributes to tissue ischemia.

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References
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