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WORKSHOP REPORT

Report of Workshop on Role of Water and Ions in Biological Membrane Function

D. W. Deamer[1]

A joint U.S.–Romanian exchange workshop on the role of water and ions in biological membrane function was held in conjunction with the Biophysical Society meeting in San Antonio, Texas. The meeting was sponsored by the National Science Foundation and by the National Council of Romania. American participants included Drs. Wally Boron, Ivan Cameron, James Clegg, John and Lois Crowe, David Deamer, Laura Eisenstein, Sidney Fleischer, Philip Morse, Richard Nuccitelli, Lester Packer, Elizabeth Simons, and Stephen Scheiner. Romanian participants included Drs. Ion Baciu, Constanta Ganea, Eva Katona, Doru-Georg Margineanu, Ioan Nicolascu, Valeriu Rusu, Mioara Tripsa, Vasilie Vasilescu, and Cornelia Zaciu. This report provides an overview of the research themes and observations reported at the conference.

Membrane Water and Cellular Energy

The first group of papers focused on our growing knowledge of the role of water in cell function. Dr. James Clegg reported recent studies in which water contents of L-cells were manipulated by immersion in buffered salt solutions containing increasing osmotic concentrations of sorbitol. It was found that L-cells can lose a remarkable amount of water without lethal damage. For instance, cells incubated in 0.3 M sorbitol lost 65% of their initial volume, reaching a water content of about 0.7 g water per gram dry weight. Metabolic parameters measured in such cells occurred at rates similar to those of controls. These observations provide additional support for the

[1]Department of Zoology, University of California, Davis, California.

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proposal that most metabolism occurs on relatively structured enzyme matrices in cells, rather than on randomly dissolved "soluble" enzymes.

In past work, John and Lois Crowe have shown that several highly adapted organisms such as nematodes and Artemia are able to survive essentially complete loss of water. Under these conditions, the cells of such organisms accumulate protective agents, particularly glycerol and trehalose, and in their most recent studies the Crowes are attempting to determine the molecular mechanisms of the damage process itself as well as protective agents. In the work reported at the workshop, it was shown that some forms of damage involve phase separation of membrane lipid components which are normally present in bilayer phase. During drying, it was observed that a significant fraction of the lipid underwent a transition to hexagonal phase in a model membrane system such as sarcoplasmic reticulum, and that this had marked deletory effects on the function of the calcium transport ATPase in SR. Addition of trehalose to the membranes before drying both prevented the phase separation and protected enzyme function.

Drs. Ganea, Katona, and Tripsa, working at the Biophysics Department in the Faculty of Medicine, Bucharest, under the direction of Dr. V. Vasilescu, have maintained a long-term research interest in using deuterium oxide to probe the role of water in cell function. At the workshop, Dr. Ganea reported that ATP pools in several excitable tissues (nerve, muscle, retina, heart) were substantially decreased in the presence of deuterium oxide. This suggested that deuterium oxide may interfere with energy production, perhaps through inhibition of coupling steps involving protons. Dr. Katona reported on the distribution and molecular mobility of water in nerve, muscle, and heart tissue. It was found that water does not exchange freely among the cells of such tissues, but instead that there exist water compartments of different accessibilities. Dr. Tripsa has been able to demonstrate that local anesthetics such as procaine, benzocaine, lidocaine, and tetracain are able to modify the properties of the aqueous compartments in frog sciatic nerve.

Dr. Philip Morse has applied electron spin resonance methods to study intracellular viscosity. He uses charged spin labels such as TEMPAMINE which cross the cell membrane. The signal from the extracellular spin label is removed with transition metal line broadening agents such as potassium ferricyanide. Dr. Morse found that TEMPAMINE equilibrates between the intra- and extracellular space in red blood cells in about 10–15 sec. His results indicate that red blood cell ghosts have an internal viscosity about twice that of water while intact red cells have an internal viscosity about five times that of water.

Membrane Structure and Function

In earlier studies, Ion Baciu and co-workers have established that erythropoietin binds to red cell membranes through specific receptor sites which are trypsin sensitive. At the workshop, results from studies of erythropoietin binding to red cells at different stages of maturation were reported. It was found that there were distinct differences in binding among erythrocytes, reticulocytes, and erythroblasts, with erythroblasts binding nearly three times more erythropoietin than erythrocytes after one hour incubation. Reticulocyte binding was intermediate. This result confirms an earlier conjecture that stem cell lines become sensitive to erythropoietin when receptors appear during cell maturation. Finally, results were presented in which alpha and beta receptors were blocked with dibenilin, propranolol, and isoproterenol. The data suggested that the adenylcyclase system is involved in mediating the effect of erythropoietin on hematopoeisis.

Dr. Margineanu reported on his investigation of bifunctional cross-linking agents such as glutaraldehyde on several membrane-mediated functions, including action potential in frog sciatic nerve, ion transport in frog skin, and hemolysis in erythrocytes. In general, inhibitory effects were produced by mild cross-linking, and these were interpreted within the context of membrane fluidity changes and chemical effects on the enzymes involved. Dr. Rusu reported recent results on freeze-fracture electron microscopy of erythrocytes, particularly during erythrocyte aging and in relation to certain disease states such as essential hypertension.

Dr. Sidney Fleischer described recent observations on the orientation of the calcium transport ATPase in sarcoplasmic reticulum. There has been considerable progress in organizing this enzyme into two-dimensional crystals suitable for analysis by electron and optical diffraction methods, and Dr. Fleischer reported on the use of vanadate for this purpose. At a specific pH range, vanadate is present as decavanadate, and this form apparently is the active species in organizing the enzyme. Both freeze-fracture and negative stain specimens show rows of organized particles within the plane of the membrane which represent the enzyme.

Ionic Conditions of the Intracellular Volume

Reports on proton transport and intracellular pH as related to cell function were dominant themes in this session. Dr. Deamer reported on recent measurements of passive proton flux across both model membranes (liposomes) and biological membranes. In general, measurements in both

systems produce permeability values that are orders of magnitude greater than expected from comparison with other monovalent cations. A working hypothesis is that proton equivalents may move along low resistance pathways within membranes which are composed of associated clusters or strands of hydrogen-bonded water molecules. Such pathways are not available to other ions and can at least qualitatively account for the intrinsic high permeability measured for proton flux.

Dr. Scheiner reported on quantum mechanical calculations for such hydrogen-bonded proton conductance pathways, showing that the conductance will be highly dependent on the chemical groups involved and their spacing relative to one another in the plane of the membrane. It was also possible to show that movement of point charges toward a hydrogen-bonded chain markedly affects the ease by which protons can move along the chain. This leads to a useful and interesting mechanism by which a protein could control the rate of proton transfer across a membrane.

Dr. Boron has established techniques by which active proton transport across cell membranes can be monitored. The proton electrochemical gradient in cells favors inward proton flux, so there must be mechanisms available to pump protons outward in order to maintain intracellular pH in the neutral range. Dr. Boron reported results from perfused squid axon and renal tubules. The squid axon actively pumps protons outward after an acid load, and the mechanism was shown to involve uptake of bicarbonate and sodium ions, accompanied by chloride efflux. Results from renal tubules were also presented, and in the salamander proximal tubule it was demonstrated that sodium-proton exchange occurs across the luminal membrane.

Dr. Elizabeth Simons discussed recent advances in her studies of platelet-activating mechanisms. Dr. Simons' group has found that activation of platelets by thrombin causes a rapid sodium influx which is maximal after 30 sec. The influx of sodium ion is accompanied by proton efflux, so that internal pH rises from near 7.0 to 7.3. Both ion fluxes are blocked by amiloride. Significantly, even when blocked, granule release by platelets is unaffected. However, the release can be blocked by conditions which collapse potassium gradients (valinomycin, high external potassium) indicating that the release response is entirely dependent on existing potassium gradients.

Dr. Nuccitelli has measured the intracellular pH shifts involved in activation of amphibian eggs following fertilization. Using both NMR data obtained from the inorganic phosphate in the cell and intracellular pH microelectrodes, he found that intracellular pH increases 0.3 pH units following fertilization, and related this effect to other events of the activation sequence.

Dr. Ivan Cameron uses the electron microprobe X-ray analysis method to monitor the distribution of ions in cell components. In the work reported

at the conference, amphibian oocytes were mounted in such a way that they could be frozen and sectioned, then examined for the distribution of monovalent cations. It was found that the total concentration of monovalent ions (sodium and potassium) in the nucleus is 123 mM. However, from exchange measurement with rubidium, only about 14% of the potassium is free in solution, and together with the free sodium, provides a total free concentration of 26%. The amount of free and bound cations was related to the known requirements for stability of chromatin, and it was concluded that the chemical activities are in appropriate ranges for stabilizing chromatin structure.

In the last paper of this session, Dr. Nicolaescu reported observations of the ionic composition of synovial fluid in arthropathies. It was determined that sodium ion was increased 2- to 3-fold in synovial fluid from two arthropathies, while calcium ion was greatly decreased.

Bioenergetics in Cell and Membrane Function

Dr. Cornelia Zaciu discussed her investigations of threshold functions in excitable membranes. In these studies noise analysis is used to establish theories relating the filter properties of such membranes at their excitation threshold to characteristics of the signals commonly used as physiological stimuli. Dr. Vasilescu reported observations of ATP concentrations in different physiological compartments of human subjects (plasma and cerebrospinal fluid) and attempted to relate these to bioenergetic changes induced by the hypertensive state. There were apparent differences between normal controls and patients with essential hypertension, with significant loss of ATP from erythrocytes to plasma and CSF in the latter.

Drs. Lester Packer and Laura Eisenstein described recent results on bacteriorhodopsin, the proton transport protein of the purple membrane in Halobacteria. There is still considerable uncertainty about the mechanism of proton transport, and Dr. Packer's laboratory has been utilizing chemical modification and pH/pK shifts to determine which groups on the bacteriorhodopsin molecule might be involved in the uptake or release of protons. In particular, the epsilon-amino group of lysine, the phenolic group of tyrosine, the carboxylate groups of aspartate and glutamate, and the guanidinium group of arginine were tested as potential candidates. These results were compared with Dr. Eisenstein's studies in which FTIR methods were used to follow changes occurring in bacteriorhodopsin during proton translocation. The combined data indicate that as many as three tyrosine residues are involved in proton translocation. The carboxylate and guanidinium groups are also essential, but the epsilon-amino group of lysine does not play a role in the transport process.