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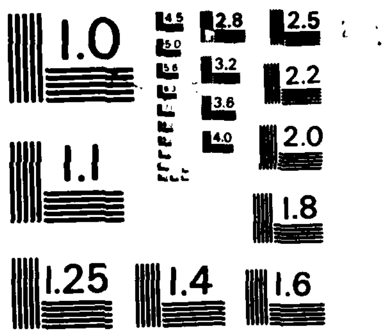

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The scientific sessions began with three lectures which provided a broad overview of our current understanding of cellular structure and function. These lectures set the stage for the remainder of the workshop which involved the presentation of data related to the interrelationships between cellulr function and structure, the latest concepts of cellular transport processes, the physical state of water and ions in cells, and divergent view of cellular function. In addition to these, the last session

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) dealt with the applications of new technologies to the broad range of bio-physical problems which were discussed throughout the workshop.

The role of membranes and the cytoplasm in the control of the cellular environment were of major focus during the Tuesday and Wednesday sessions. The latest information on membrane transport proteins were presented by leading authorities in this field. New information on the role of the cytoplasmic matrix in the control of the cellular environment was presented and extensively discussed. There emerged what appears to be a consenses that a significant amount of ion binding is present in cell membranes as well as the cytoplasmic matrix. In addition, new information was presented on the diffusive motion and the relaxation processes of water molecules in various cellular systems. It was concluded that more studies are needed in this area in order to obtain self consistency of information obtained by different technologies such as nuclear magnetic resonance spectroscopy, dielectric relaxation, and quasielastic neutron scattering. Optimism was expressed that a coherent view on the physical properties of water in biological systems is emerging.

International Workshop on Biophysical Correlates of Cellular Function UNESCO. ONR Contract N00014-83-G0056

The program for the workshop is attached. The list of invited participants for the workshop totaled 30, with 11 from European countries, 2 from third world countries, one from the Peoples Republic of China, (Dr. Zhang Zhen-Lian, Institute of Biophysics of the Academia Sinica of China, Beijing, China), 2 from the Soviet Union, and 15 from the United States. Of all those invited, only the Soviets were unable to attend.

The workshop was opened with the appropriate welcoming addresses which included a message of support from the United States Academy of Science delivered by professor Norman Hackerman (Member of the Academy, and President of Rice University).

The scientific sessions began with three lectures which provided a broad overview of our current understanding of cellular structure and function. These lectures set the stage for the remainder of the workshop which involved the presentation of data related to the interrelationships between cellular function and structure, the latest concepts of cellular transport processes, the physical state of water and ions in cells, and divergent view of cellular function. In addition to these, the last session dealt with the applications of new technologies to the broad range of biophysical problems which were discussed throughout the workshop.

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The Thursday sessions were held at the L.B. Johnson Space Center (National Aeronautics and Space Administration). The latest studies on disuse osteoporosis, image enhancement of cells, and biophysical problems associated with space flight were discussed.

A unanimous opinion was expressesd, on the part of the participants, that the workshop was extremely successful. The participants also expressed their thanks to UNESCO as well as the other supporting institutions for making the workshop possible. The organiztion of future international workshops of this type were enthusiastically encouraged.

Because of the diverse disciplines and points of view represented and because of the controversial nature of many of the topics covered, one participant was designated to provide a summary of the work presented and of the lengthy discussions held at the workshop. Professor Leo Neuringer assumed this most difficult task. His summary and extended abstracts from each of the participants are being bound as a proceeding of the workshop. The proceedings should be completed within the next two months, and, when completed, will be forwarded to complete the final report.

In order to provide the Office of Naval Research with some more details of the workshop, I am enclosing a copy of Professor Neuringer's summary of the workshop as well as my foreword for the workshop. The workshop was exciting, stimulating and most informative and the importance of this general area of research seems evident.

SUMMARY OF WORKSHOP

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Almost all cell types are in continuous contact with an external medium, whose composition may vary over a considerable range. This is especially true for free-living unicellular organisms and for cells of absorptive tissues such as renal tubules or intestinal epithelium. The cell, however, maintains a fairly constant composition of the principal physiological solutes. These are ionic in nature, mainly Na^+, K^+, Ca^{2+}, Mg^{2+}, and non-ionic such as free amino acids and low molecular weight sugars. The maintenance of the intracellular composition and the mechanisms whereby it is achieved were the main topics of this workshop. The papers which were presented fell into two major groups dealing with membrane associated phenomena or with intracellular phenomena.

Contact with external medium is provided by the cell's plasma membrane which permits the passage of solutes. In some cases no energy is required for such passage. However, in a majority of the cases involving transport of physiologically important ions (H^+, Na^+, K^+, Ca^{2+}, phosphate, sulfate) and nonelectrolytes (sugars, nucleic acid bases, vitamins, amino acids) a source of energy is required. In primary transport processes this energy is derived from either a chemical or photochemical reaction such as oxidation of NADH in mitochondria, or hydrolysis of ATP, or relaxation of the photo-excited state of pigments in chloroplasta. In secondary transport, on the other hand, the immediate source of energy is the electrochemical potential gradient either of H^+ or Na^+, which has been established by the activity of one of the primary mechanisms. In this type of transport a role is ascribed to both the electrical potential across the membrane and to the chemical potential difference of the ion on each side of the membrane.

At this meeting the validity of the "macroscopic" values of the components was questioned both with respect to ATP synthesis and with respect to the secondary transport of sugars in some membranes. To an increasing extent it appears that (a) the H^+ concentration within or very near to the membrane is important (Kotyk, Kell), and (b) the potential may have effects on half-saturation constants as well as on rates of transport (Semenza, Maloney, Kotyk).

The validity of Na^+, K^+ transport mechanisms was also examined in an extensive series of experiments on human peripheral blood lymphocytes (Negendank). These were interepreted in terms of a one-dimensional Ising model and the cooperative interaction between sites and their association with ions. Studies of the animal cell ATP-driven calcium pump protein were also presented (Fleischer). These experiments, as well as others, have documented the mechanism whereby the calcium pump operates and its existence is now generally accepted. However, the existence of a membrane pump for the active transport of Na^+ continues to be opposed (Ling). In Ling's presentation, the concepts underlying the association-induction (AI) hypothesis and his generalized theory for the living cell were reviewed. Many specific questions remain to be answered about this particular model which is not universally accepted. The AI hypothesis, however, has served as a goad and stimulus to work on the state of ions and water in cells. For example, results were reported of an experiment designed to test explicitly the AI hypothesis that solutes of cell and media are at equal chemical activity even though concentrations may differ from unity (Horowitz). It would appear that his results raise a serious question about the validity of the AI hypothesis.

A number of papers dealt with another complication of any quantitative assessment of intracellular concentrations, i.e., the state of intracellular water and the state of solutes in cells. Various techniques were employed to show that a good fraction of intracellular water exists in a form different than free, bulk water; i.e. structured water, polarized water and bound water. With the possible exception of dielectric relaxation measurements (Schwan), other studies such as proton NMR relaxation times (Cameron) and chemical shift (Kasturi), quasi-elastic neutron scattering experiments (Rorschach) and the density and diffusion measurements of Hansson-Mild all support the idea that water in cells has difference physical properties than freely mobile water. However, there does not exist a generally accepted model for describing the interaction of water with macromolecules. This is not surprising in view of the different time and distance scales of the various techniques and also to the complexity of the interaction with macromolecules in heterogeneous biological systems. In fact, this complexity was pointed out and invoked to account for glucose metabolism in permeabilized cells (Clegg). It was emphasized that enzymatic action within the cytosol occurs at sites associated with the microtrabecular lattice. Furthermore, the existence of this extensive cytoskeletal structure provides the basis for understanding the observed changes in cell water properties. The potential use of synchrotron radiation to study the chemical state of metal ions in cells was discussed (Huang). Study of K^+ in frog blood cells indicates that its state is substantially different from that in free solution.

There is evidence that in addition to the aforementioned intracellular surfaces, the macromolecules can provide an adsorption surface, especially for cations such as K^+. This property is especially pronounced in protein-loaded cells such as the muscle cell (Edelmann) or in cell nuclei with their high content of proteins and nucleic acids (Kellermayer).

The lively discussions throughout the workshop served to identify gaps in our knowledge and to point the way towards future experiments. In my view, a focused approach should be adopted. Serious attention should be given to obtaining a clear understanding of the electrochemical potential and how to measure accurately and unambiguously each of its constituent terms. Better or more accurate tracking of the energy involved in active transport systems should be carried out to determine quantitatively and conclusively whether cellular processes can in fact maintain ion pumps, in particular the sodium pump. Experiments should be performed to measure the diffusion constants of sodium and potassium in protein solutions in the presence and absence of ATP, or in some other system where "structured" or "bound" water is believed to exist. As a further direct test of the AI hypothesis it is necessary to examine and test by critical experiments the notion of cooperative interactions between ion binding sites and their association with ions. Finally, other types of experiments should be designed specifically to measure the intracellular concentrations of free cations (Na^+, K^+) without electrodes to confirm or refute the results reported by Horowitz. Conceptually it may be difficult to achieve this. However, without such efforts it will be impossible to address membrane and intracellular phenomena in a quantitative well-defined fashion.

In closing it is a pleasure to thank the sponsors and organizers for arranging a stimulating conference on a topic of high scientific interest in such wonderful surroundings.

While many of the discussions were heated at times they nevertheless were always carried out in the spirit of a search for truth. The participants are to be congratulated in this endeavor. We look forward to future, new results with the hope that they will reveal deeper insights into cellular function.

FOREWORD

In June of 1981 Professor Jaz and I met in Houston and initiated the first discussions for the current workshop. In June of 1982, at the 4th General Meeting of UNESCO's Expert Committee on Biophysics held at Johns Hopkins University in Baltimore, Maryland, a general proposal for the workshop was approved by that committee. The organizing committee was formed at that meeting and my attentions then turned to finding funds for the workshop. We were most fortunate in that UNESCO, The Office of Naval Research, The National Aeronautics and Space Administration, The National Science Foundation, I.B.M. and some private concerns provided funds for the workshop.

It is well known that ions and water are necessary for the support of life. Furthermore, all theories developed to describe or explain the living state require assumptions about the physical state of ions and water within living cells. Although these matters are well recognized, it has been difficult to obtain a consensus as to whether the physical properties of cellular ions and water are like those of non-cellular dilute solultions, or significantly different. Part of this difficulty arises from the lack of fundamental knowledge of the microscopic structure of pure water, of simple solutions of water and ions, and of water and ions in biological systems. A second part of this problem arises from the fact that the advantages and disadvantages of the various technologies used to study the macro- and microscopic properties of water have not been fully evaluated to determilne whether the disagreements in the interpretations of the data reported in the literature are more apparent than real. Similar problems hamper our understanding of the physical state(s) of solutes in cells and how the physical properties of various solutes and water are related to cellular structure and function.

During the past ten years, scientists have amassed considerable data by way of diverse technologies, which should be accounted for in our various models of the living cell. These data include some insights as to the microscopic properties of water and ions - more, however, is needed. The technologies include high voltage electronmicroscopy, electron neutron scattering, synchrotron radiation, etc. In addition, through more conventional technologies, new knowledge has emerged that increased our understanding of selective accumulation and exclusion of ions and kinetic aspects of solute movement into and out of cells. Structural changes in cells and changes in the physical properties of cellular ions and water have been correlated with changes in the functional state of cells and organs. New insights into cellular structure and function and their relationships to the physical properties of water and ions are emerging now as a result of advances in technology and in the applications of those technologies. There is a great need to address the overall problem of these interrelationships.

Because of the items mentioned above, the organizing committee and UNESCO's Expert Committee on Biophysics concluded that there is a need to bring together a rather small group of international scientists, of diverse backgrounds, who share a common interest. A common interest is to undserstand the role(s) of the physical properties of ions and water in the interrelationship between cellular structure and function. Progress in this area was evaluated and attempts were made to identify areas of research that will enhance our understanding of cellular structure and function.

The interdisciplinary group that was brought together, came from diverse backgrounds with broad ranging view points concerning this important subject. It was, therefore, decided to designate one participant to provide a summary of the work presented and the lengthy discussions held at the workshop. Professor Leo Neuringer agreed to assume this most difficult task. I, as well as all of the participants, are indebted to him for the outstanding job he did. His ever presence and probing questions stimulated much discussion and clarity of expression on the part of the participants.

In conclusion, I express my thanks to the sponsors, the organizing committee, and to each of the partaicipants for all the ingredients of a most stimulating and informative workshop.

Carlton F. Hazlewood, Ph.D.

Houston, Texas
May 1984

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