WATER & ELECTROLYTE TRANSPORT ACROSS CELL MEMBRANES

Principal Investigator: S. Tripathi, Reader.

(No scientific staff, students or visiting fellows in my laboratory)

Laboratory space 600 sq.ft

Background:

The importance of water in a biological system cannot be overstated. A cell needs to regulate its own volume despite osmotic perturbations imposed on it naturally and the composition, osmolality and volume of fluid compartments in a plant or animal are finely regulated. Much of this regulation is accomplished by controls that couple water transport across cell membranes to the transport of a solute in a meaningful way. As a general physiologist I have been interested in this solute-solvent interaction in those cells 'best designed' for this purpose - epithelial cells. While these functions may appear straightforward, many experimental observations are paradoxical and difficult to explain *quantitatively*. Nevetheless, recent technical advances (quantitative ultrastructural stereology of cells; increasingly higher resolution of water fluxes across membranes; spatial and temporal resolution of solute activities in the cytosol; high-resolution current recordings through single cell membranes and single membrane channels) have generated renewed interest in understanding water transport across cell membranes at a molecular level. In addition to water transport I am interested in electrolyte transport and have a general interest in transport phenomena and cellular and molecular physiology.

Progress in the 7th plan period:

The methodology of membrane electrophysiology at a single-membrane and single-channel level was not availbable in India when I joined the Institute in 1986. Thus much time has been spent setting up a laboratory where one could study the physiology of ion and water transport through single membranes and single channels by the patch-clamp and related microelectrode techniques. Preliminary data are just coming in with a variety of cells in primary culture, cell lines and *in vitro* experiments. The experimental facilities that have already been set up successfully are:

1. Cell membrane parameter measurements with intracellular recordings
2. Ion-selective microelectrode techniques for cytosol ionic activity measurements.
3. In vitro microperfusion of small structures
4. Single-channel recording and whole cell recording of ionic currents with real-time computerized data acquisition, digital data storage and analysis.
5. Preliminary work using fluorophores as concentration (and therefore volume) markers has been started.

6. With the assistance of Dr. Padhy I have been growing epithelial cell lines (HeLa and Hep2) derived from the National Facility for Animal Tissue Culture in Pune. I have also learnt to make and culture plant cell protoplasts (*N. tobaccum*) from Dr. A. Mukhopadhya and Dr. M. Padidam at the Tata Energy Research Institute, New Delhi.

OUTLINE OF EXPERIMENTS PROPOSED IN THE 8TH PLAN PERIOD

I have recently reviewed in detail the experiments that need to be done at a single membrane and single channel level to elucidate the transmembrane mechanisms of water transport (Tripathi & Boulpaep, 1989 - ref. 9). In brief, some questions that I will attempt to answer are:

I. Is ion-water interaction in Antibiotic channels revealed by tracer and selectivity studies similar? Why have there been such large disparities in the binding constants of monovalent cations in the Gramicidin channel depending on the methodology used. I will be measuring the binding constants of several monovalent cations in Gramicidins incorporated into planar bilayers and bilayers at microelectrode tips to analyze further the nature of ion-water coupling in gramicidin (Fettiplace & Haydon, 1980; Levitt et al. 1978; see Finkelstein, 1987)

2. I wish to measure the water permeability of several cell types with ion-selective fluorescence techniques and by using impermeant ions as cell volume markers in living cells to test if there is evidence for hydrated channels in these plasma membranes. The regulation of the permeability of these ion channels by sub-cellular mechanisms will also be studied by the same in vitro fluorescence techniques.

3. I will attempt to develop faster techniques for application of osmotic gradients and detect transient early and stedy state volume fluxes to further minimize boundary-layer artifacts (see Barry & Diamond, 1984).

4. I will now have the opportunity to study ion channels in neuronal, epithelial and plant cells that are of mutual interest to colleagues within the Molecular Biology Group.

References:

BARRY, P.H. & DIAMOND, J.M. (1984). Effects of unstirred layers on membrane phenomena. *Physiological Reviews* 64, 763-872.

FETTIPLACE, R., & HAYDON, D.A. (1980). Water permeability of lipid membranes. *Physiological Reviews* . 60, 510-550.

FINKELSTEIN, A. (1987). Water movement through lipid bilayers, pores and plasma membranes: theory and reality. New York: John Wiley & Sons.

LEVITT, D.G., ELIAS, S.R. & HAUTMAN, J.M. (1978). Number of water molecules coupled to the transport of sodium, potassium and hydrogen ions via gramicidin, nonactin or valinomycin. *Biochimica et Biophysica acta* 512, 436-451.

BUDGET:

NO CAPITAL EQUIPMENT IS REQUIRED AT PRESENT

CONSUMMABLE ITEMS REQUIRED DURING 1990-95		Rs.
Glass capillary tubing for microelectrodes		20,000
Flexible precision tubing (polythene tygon polyimide saran etc)		30,000
Valves for rapid fluid exchange		20,000
Liquid Ion exchangers and silanes		30,000
Chemicals		
electrolytes , drugs, antibiotics and blockers	40,000 per yr	200,000
radionuclides for tracer fluxes		50,000
Fluorophores		40,000
Lipids		20,000
Solvents		10,000
Tissue culture plasticware enzymes media tips, pipettes	60,000 per year	300,000
Miscellaneous lab expenditure		50,000
Photography		10,000
Gas mixtures		10,000
gas regulators		10,000
Micromanipulators		150,000
Wires (Ag, Pt-Ir etc.)		10,000
Microelectrode holders		20,000
Syringe pump		30,000
Epi-Fluorescence attachment for a microscope		120,000
Replacement electrodes for pH meter, conductivity meter		17,000
Computer consummables e.g. magnetic media		30,000
Small equipment		33,000
Service contracts for microscope cleaning and computers		20,000
Equipment repair costs		40,000
Electronics component spares		100,000
Analogue to Digital converter and Digital to Analogue converter and data acquisition software		170,000
Publication and reprint costs		40,000
Technical manuals and subscription costs		40,000
Travel International	50,000 and 30,000 domestic	80,000

TOTAL FOR FIVE YEARS: Rs 17 lakhs. 16.00000

The funding requirement is for :

1. a set up for studying single-channel currents in ion-channels incorporated into planar lipid bilayers with simultaneous high-resolution volume flow measurements.

2. a optoelectronic set up for studying cells with quantitative fluorescence microspectrophotometry using ion- sensitive fluorophores.

3.Basic microelectrode and tissue culture consummable requirements.

As in the last plan every effort will be made to improvise equipment whenever possible. I am currently working out the design of the proposed set ups. The technology for data acquisition and analysis and optoelectronic instrumentation is changing very rapidly. I have the committed support of the Instrument Laboratory of the Department of Cellular and Molecular Physiology, Yale University, U.S.A. for data acquisition instrumentation and analysis software. I also have the committed collaboration and assistance of laboratories where these techniques are already established.

1st February, 1990

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Birth:

30 May, 1952 in Berhampur, Orissa, India

Nationality:

Indian citizen

Education:

(i) Medicine (M.B.B.S.) and one year internship at S.C.B. Medical College and Hospital, Utkal University, India, 1976.
(ii) Postgraduate degree in Physiology (M.D.) with Distinction at the V. Patel Chest Institute, Delhi University. India, 1980.

Appointments:

Reader, Molecular Biology Group, Tata Institute of Fundamental Research, Bombay: 1986-
Associate Research Scientist, Department of Physiology, Yale University School of Medicine, U.S.A: 1985-86.
Postdoctoral Fellow and Research Staff Physiologist, Department of Physiology, Yale University School of Medicine, U.S.A: 1982-85.
Research Associate, Department of Physiology, King's College, University of London, England: 1980-82.
Research Fellow, Department of Physiology, V. Patel Chest Institute, Delhi University: 1979-80.

Memberships and honours:

Foreign Member, The Physiological Society of U.K. 1990-
Member, Biophysical Society of U.S.A. 1988-
M. D. in Physiology with Distinction, 1980
R.N. Sen Physiology Prize as medical student, 1970

Fellowships:

Brown-Coxe Fellow, Yale University School of Medicine, 1983-84
Research Associate of the Medical Research Council of U.K., 1980-82
Senior Research Fellow, Indian Council of Medical Research, 1975-79
Wellcome Trust Fellow, Dept of Pharmacology, Cambridge, UK 1990

BIBLIOGRAPHY OF SUBRATA TRIPATHI

PAPERS

(1) S. Tripathi and P. K. Rangachari (1980). In vitro primate gastric mucosa: electrical characteristics. *American Journal of Physiology* **239** (*Gastrointest. Liver Physiol.* **2**): G77-G82.

(2) R. J. Naftalin and S. Tripathi (1985). Passive water flows driven across the isolated rabbit ileum by osmotic, hydrostatic and electrical gradients. *Journal of Physiology (London)* **360**: 27-50.

(3) S. Tripathi, N. Morgunov and E. L. Boulpaep (1985). Submicron tip breakage and silanization control improve ion-selective microelectrodes. *American Journal of Physiology* **249** (*Cell Physiol.* **18**): C514-C521.

(4) R. J. Naftalin and S. Tripathi (1986). The roles of paracellular and transcellular pathways and submucosal space in isotonic water absorption by rabbit ileum. *Journal of Physiology (London)* **370**: 409-432.

(5) S. Tripathi, E. L. Boulpaep and A. B. Maunsbach (1987). Isolated perfused *Ambystoma* proximal tubule: hydrodynamics modulates ultrastructure. *American Journal of Physiology* **252** (*Renal Fluid Electrolyte Physiol.* **21**): F1129-F1147.

(6) A.B. Maunsbach, S. Tripathi and E.L. Boulpaep (1987). Ultrastructural changes in isolated, perfused *Ambystoma* proximal tubule during osmotic water flow. *American Journal of Physiology* **253** (*Renal Fluid Electrolyte Physiol.* **22**): F1091-F1104.

(7) S. Tripathi and E.L. Boulpaep (1988). Cell water permeabilities and streaming currents in *Ambystoma* proximal tubule. *American Journal of Physiology* **255** (*Renal Fluid Electrolyte Physiol.* **24**): F188-F203.

REVIEWS

(1) R. J. Naftalin and S. Tripathi (1983). Routes of water flow across the intestine and their relationship to isotonic transport. In *Intestinal Transport.* eds: M. Gilles-Baillien and R. Gilles. Springer-Verlag, Berlin, Heidelberg. pp 14-25.

(2) S. Tripathi and E. L. Boulpaep (1989). Mechanisms of water transport by epithelial cells. *The Quarterly Journal of Experimental Physiology* **74,** 385-417.

ABSTRACTS

(1) S. Tripathi and P. K. Rangachari (1978). Electrical characteristics of the isolated primate gastric mucosa. Proc. Indian Pharmacol. Soc.

(2) R. J. Naftalin and S. Tripathi (1982). A high-resolution method for continuous measurement of transepithelial water movements across isolated sheets of rabbit ileum. J. Physiol. (Lond.) 326, 3-4P.

(3) R. J. Naftalin and S. Tripathi (1982). Determination of the hydraulic conductivity of the pathways for osmotic and bulk flow across the mucosal and serosal surfaces of isolated rabbit ileum. J. Physiol. (Lond.) 329, 69P.

(4) R. J. Naftalin and S. Tripathi (1982). The effects of changing tonicity of the mucosal solution on fluid transport by isolated rabbit ileum. J. Physiol. (Lond.) 332, 112P.

(5) S. Tripathi and E. L. Boulpaep (1984). Intracellular Na^+ and Na^+ pumping in the isolated perfused *Ambystoma* proximal tubule. Federation Proc. 43, 302.

(6) E. L. Boulpaep and S. Tripathi (1984). Evidence for both paracellular and cellular water flow across the isolated perfused proximal tubule of the salamander *Ambystoma tigrinum* . J. Physiol. (Lond.) 357, 76P.

(7) E. L. Boulpaep and S. Tripathi (1986). Structural models of epithelial solute-solvent coupling. Proc. XXX Congress of Int. Union of Physiol. Sciences. Vancouver, Canada.

(8) S. Tripathi and E. L. Boulpaep (1986). Hydrodynamics of isolated perfused renal tubules. Proc. XXX Congress of Int. Union of Physiol. Sciences, Vancouver, Canada.