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STRUCTURE AND FUNCTION IN EPITHELIA

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STRUCTURE AND FUNCTION IN EPITHELIA

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MEMBRANE BIOPHYSICS
STRUCTURE AND FUNCTION IN EPITHELIA

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Introduction

For almost half a century, in methodology, objectivity, and theoretical construct, the study of the movement of ions and water across biological systems has advanced in many directions. Recent achievements in technology have served to motivate and accelerate a stronger quantitative as well as qualitative approach to the problem of ion transport: research which is designed to represent correlations between structural and functional characteristics of biophysical systems evolves more realistic models and mechanisms for the processes involved.

This book represents the proceedings of a conference held at Virginia Polytechnic Institute and State University in Blacksburg, Virginia, May 21–22, 1981. As originally conceived this conference was designed to bring together the ideas and work of investigators from different disciplines whose major thrust is to effect the correlation between structure and function in epithelial physiology, both at the macroscopic and microscopic level. To this end papers presented include studies of the molecular characteristics of specific transport inhibitors, the biochemical substrate and reactions which support ion fluxes, and the relationship between electrophysiological membrane parameters. In a broader sense the conferees were tasked on their ability to assess some of the current epithelial models, including theoretical paradigms and biophysical constructs, particular experimental tissue from different organs, and transport models which reflect discrete anatomical loci in the tissue and/or heterogenous cell populations.

In this volume all of these subjects are addressed with respect to the current body of information on and understanding of epithelial tissues as well as the theoretical principles of ion transport. The topics were selected for the purpose of addressing various aspects of the most commonly studied epithelial membranes; however, the most exciting aspect of these contributions can be attributed to the fact that they achieve a synthesis between classical and recent knowledge on the subject accomplished through a collaborative effort of scientists from various disciplines. Thus it is the editors' hope that this book will be the first volume of a series devoted to up-to-date articles and reviews of membrane biophysics.

The editors wish to acknowledge the Office of Naval Research for its support as evidenced throughout this volume in the contributions of many of its contractors. We would also like to thank each of the participants for the part they played in making the conference a success, and a special word of appreciation is due to Dr. Marie Cassidy, Dr. Steve Hersey, and Dr. William McD. Armstrong for their invaluable advice, especially in the selection of topics. We express our gratitude to Ms. Beth Dillinger for her excellent assistance with the preparations for the conference and these manuscripts. To Paulette Cohen, Vice President/Editor, and Heather Prior, production editor at Alan R. Liss, Inc., special thanks for their expert help.

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INTRACELLULAR SODIUM ACTIVITY AND TRANSCELLULAR SODIUM TRANSPORT IN GALLBLADDER.

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INTRODUCTION

The use, in physiological investigations, of ion-selective microelectrodes (Thomas, 1978) that can measure accurately the intracellular activities of individual ions under various conditions provides a new and powerful tool for investigating the mechanisms of ionic transfer across cell membranes. When combined with measurements of the membrane potential, a knowledge of the outside/inside activity ratio for a given ion yields an estimate of the magnitude and direction of the transmembrane driving force for the transport of that ion (i.e. its transmembrane electrochemical potential difference) and of the way in which this parameter responds to various experimental manipulations. In previous studies with the isolated gallbladder of Necturus (Garcia-Diaz and Armstrong, 1980) we utilized the measurement of intracellular Na^+ and Cl^- activities (a^i_{Na}, a^i_{Cl}) and the apical membrane potential (E_m) under a variety of conditions to explore the energetic relationships involved in the coupled transapical entry of these ions into the epithelial cells of this tissue. In this report we describe some experiments, with the same tissue, that show how the measurement of steady-state a^i_{Na} values can, by appropriate analysis, be used to derive information about the kinetics of transcellular Na^+ transport.

EXPERIMENTAL METHODS

The electrical circuitry and detailed experimental procedures used in the present experiments were similar to those previously described (Garcia-Diaz and Armstrong, 1980). Following excision, gallbladders from Necturus maculosus were mounted as flat sheets in a modified version of the divided chamber used for electrophysiological measurements in isolated frog skin (Nagel, 1976). Both sides of the tissue were superfused at 23 $\pm$ 1°C with identical oxygenated Ringer solutions. Perfusion rates were about 5 ml/min. The control Ringer solution used in these experiments was the phosphate buffered HCO_3^- - free medium previously described (Garcia-Diaz and Armstrong, 1980). It contained 100 mEq of Na^+ and Cl^- and 5.4 mEq K^+ per liter. The pH was 7.2. Alterations of the Na^+ content in the external medium were accomplished by replacing NaCl with Tris (Tris- hydroxymethyl-amino methane) titrated to pH 7.2 with HCl. The Cl^- content of the medium was altered by substituting Na^+ gluconate for NaCl. All changes in the external medium were made bilaterally at the same time.

Transepithelial P.D. (E_{Tr}), Em, a^i_K and a^i_{Na} were measured as previously reported (Garcia-Diaz and Armstrong, 1980). As observed in that study, the average E_{Tr} in the present experiments did not differ significantly from zero. Thus, the tissue was considered to be in an essentially short circuited condition. E_m was measured with conventional microelectrodes filled with 1.0 M KCl and having tip resistances in the range 10-20 megohms. Criteria for the acceptability of impalements with these microelectrodes were as described elsewhere (Armstrong and Garcia-Diaz, 1981). a^i_{Na} was measured with liquid membrane microelectrodes of the type previously described (O'Doherty, Garcia-Diaz and Armstrong, 1979). These contained the neutral Na^+-selective ligand introduced by Simon and his associates (Guggi, Oehme, Pretsch and Simon, 1976) for the measurement of Na^+ activities in aqueous solutions. In common with other types of liquid membrane microelectrodes (Armstrong and Garcia-Diaz, 1980) these Na^+-selective microelectrodes display a wide range of selectivities that depends on minor differences in the method and conditions of their preparation. Thus it is frequently necessary to correct for K^+ interference in the intracellular potentials they register. This is most conveniently done by calibrating the microelectrodes in

solutions containing different amounts of Na^+ together with an amount of K^+ corresponding to the mean a^i_K value measured in the cells under investigation. In an earlier study (Garcia-Diaz and Armstrong 1980) we calibrated these microelectrodes in solutions containing 127mM KCl. This corresponded to the mean a^i_{Ka} value (98 mM) found in that study. Furthermore, in that study and in the present investigation, a^i_K was measured under a variety of conditions with K^+-selective microelectrodes containing Corning 477317 liquid ion-exchanger and was found to remain constant over a wide range of external Na^+ and Cl^- concentrations (Table 1). Therefore, in the present study, 127 mM KCl was incorporated in all solutions used to calibrate Na^+-selective microelectrodes. From the results obtained, a^i_{Na} was calculated as previously described (Garcia-Diaz and Armstrong, 1980).

Table 1

Apical Membrane Potentials and Intracellular K^+ Activities in Necturus gallbladder at various external Na^+ and Cl^- concentration.

$[Na]_o$ (mM)	$[Cl]_o$ (mM)	E_m (mV)	a^i_K (mM)	n
100	100	-51 ± 2	99 ± 3	3
10	100	-51 ± 3	99 ± 2	3
100	0	-49 ± 2	98 ± 5	5

n = Number of gallbladders. Other symbols defined in text. E_m and a^i_K values are means ± SEM.

RELATIONSHIP BETWEEN EXTRACELLULAR AND INTRACELLULAR Na^+ ACTIVITIES.

Table 2 summarizes the relationship between E_m, a^i_{Na} and extracellular Na^+ concentration, $[Na]_o$, for gallbladders mounted in media that contained variable amounts of Na^+ and a constant (100mM) Cl^- concentration (Garcia-Diaz and Armstrong, 1980). Table 3 shows similar results obtained in the present study with Cl^- free media.