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NETWORK THERMODYNAMICS, HEAT AND MASS TRANSFER IN BIOTECHNOLOGY

edited by
K. R. DILLER

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NETWORK THERMODYNAMICS, HEAT AND MASS TRANSFER IN BIOTECHNOLOGY

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FOREWORD

Heat and mass transport processes play a cricial role in the normal function of living systems. Many aspects of these phenomena have been investigated by researchers representing a broad spectrum of academic disciplines. Mechanical engineers have played a key role in the identification, development, and exploitation of this area of research, with many contributions presented in conjunction with the activities of the K–17 Joint Committee on Heat and Mass Transfer in Biotechnology of the ASME Bioengineering and Heat Transfer Divisions.

In recent years a series of symposia and thematic sessions have been sponsored by the K–17 committee at the Winter Annual Meeting, and the present volume is a further contribution in kind. The present symposium on the topic *Network Thermodynamics, Heat and Mass Transfer in Biotechnology* was organized to bring together engineers and life scientists having a mutual interest in problems in this area to present and discuss current research activities. This group of sixteen papers represents a varied array of perspectives on the analysis of numerous problems drawn from this common arena of technology. However, the papers fall naturally into three topical subsets. The first eight papers deal with the new and emerging field of network thermodynamics as applied to problems of biological interest. The next four papers address problems associated with freezing processes in living tissue, and the last four are concerned with heat transfer in physiological function.

The section on network thermodynamics (NT) is particularly novel in that it includes a number of the pioneering workers who have contributed most heavily to development of the field since it was first introduced early in the 1970's by Katchalsky and coworkers [1], based on the bond graph method devised previously by Paynter [2], and by Peusner [3]. Since then, a number of workers have exploited the enhanced ability of NT to offer a graphical,but fully rigorous, display of complex processses that specifies both the energy flows and topology of the system. The governing equations for a given system may be formulated and solved automatically from a specified graph of the system, and the graph provides a readily interpretable, functional representation of the system. Although the utility of NT has been demonstrated in a few specific application areas, much work remains to be performed before its potential will be realized.

The analyses of low temperature processes in cells and tissues and of heat transfer in physiological function are more mature research areas, but are still experiencing significant new developments. Recent years in particular have witnessed an accelerated pace on new contributions to these disciplines from engineers in conjunction with the exploding availability of new tools and techniques for quantitative analysis of systems. Consequently, engineers have been playing an ever increasing integral and important role in extending the state of the art.

The papers in this symposium volume describe a significant step forward for the contributions of engineers in biotechnology. Although they are clearly not comprehensive

in scope, they do illustrate the effectiveness of the collaboration of the engineering and life sciences in solving major problems of heat and mass transfer in biotechnology.

Kenneth R. Diller
Symposium Organizer

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NETWORK THERMODYNAMICS AND COMPLEX SYSTEMS THEORY: AN APPROACH TO UNDERSTANDING THE RELATIONSHIP BETWEEN STRUCTURE AND FUNCTION IN BIOLOGICAL SYSTEMS

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ABSTRACT

Organization and complexity are two themes which are constantly encountered in theoretical biology. The dominant philosophical and methodological approach in modern biology is reductionist in nature, focusing on the molecular level of the hierarchy (Molecular Biology). This approach has had an impressive amount of success. One historical reason for this focus is that theory from physics and chemistry, as well as applied mathematics, was designed for, or at least most appropriate for, the understanding of simple systems. There has been no systematic development of a complex systems theory in these disciplines. On the other hand, because of practical pressures, engineering disciplines have consistently been in the forefront of the development of complex systems theory. It is for this reason that network thermodynamics has become one of the most powerful approaches to the theoretical and practical aspects of the complexity of living systems. In this presentation the utility of network theory will be examined as a "common language" for extending thermodynamic and mechanistic reasoning into the domain of complex hierarchical systems. Examples will be given of simple networks which illustrate how quickly the whole becomes more than the mere sum of its parts in biological systems. The network thermodynamic approach will be shown to be capable of utilizing information obtained from reductionist methodology to synthesize models of complex systems which illustrate the relations between the whole and its parts. Much in the same manner, holistic reasoning has also had its impact on the discipline of thermodynamics itself and some of these parallels will be pointed out. Examples to be discussed will include models of epithelial transport through epithelial membranes and the combination of transport and metabolism in cellular systems.

INTRODUCTION.

The Historical Relationship Of Biology To Other Disciplines. Where Has It Led?

Biology is a discipline which tends to attract researchers of a particular kind. Often, one of the common characteristics of workers in this discipline has been a tendency to avoid mathematics and to utilize more descriptive approaches, even when the problem could better handled by the use of mathematics (1,2). Throughout the history of the discipline there have been noteworthy exceptions to this pattern either at the individual level (3-6, for example) or at the level of subdisciplines such as Mathematical Biology, Biophysics, Biostatistics, and Biomedical Engineering. These subdisciplines are often seen as the entirety of the theoretical component of biology. It can be argued that in each of these cases, the thinking and tools were, in a sense, somewhat foreign to those within the more traditional subdisciplines of biology, and therefore, never really totally integrated into the discipline. It can also be argued (1,2) that theoretical biology exists outside the discipline of biology for all practical purposes. Why bother to try to understand this history? The reason rests in the basis for any serious attempt to predict where theoretical biology is going at this point in time. In particular, it is a good way to anticipate a role for engineering theory, through bioengineering, in theoretical biology. This role will be to provide a basis for the analysis of complex systems. Since this work is focusing on the applications of network thermodynamics to living systems it might be of value to reassess the impact of over ten years of such applications on the discipline (7).

A reassessment can be very revealing and suggests we are in an exciting time. A number of researchers are simultaneously coming to the same conclusion, possibly best stated by Rosen (5,6). It is a conclusion which can be paraphrased as follows: "The relation of biology to our present physics is not that of the particular to the general." In other words, the traditional reductionist hierarchical picture of biology "above" chemistry which in turn is "above" physics may not be the one which is really operative. The alternative is to envision a hole and/or gap in the development of physics and chemistry which resulted from their natural tendency to focus on simple systems and mechanisms. This tendency is one of the best ways of understanding the widespread

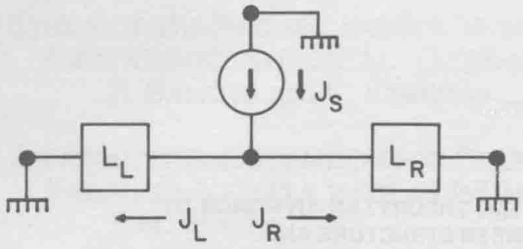

Fig. 1 The simple 1-port current divider network.

reliance of biologists on empiricism over theory and the use of methodological reductionism (8). This methodological reductionism seems rooted in the absence of any viable approach to complex systems coming from Physics and Chemistry and the need to break the complex system down to the level of simple mechanisms in order to use what theory physics and chemistry did have to offer (1,2,5-12).

It is at this point where engineering began to "take over" in a sense. (13). Rather than waiting for physics and chemistry to provide an approach to the complex systems they wished to study, they made one or more of their own. I will not digress into the kinds of snobbery and "more basic than thou" attitudes that grew from this, although it is very tempting to do so. (12) Suffice it to say, in many of the engineering subdisciplines, very complicated systems were being dealt with very successfully. It is my thesis that these approaches are often a better route to whatever a theory of complex systems will become even though they often dealt with very mechanistic concepts and objects which have all the semantic connotations in the word "mechanism". The subdiscipline of bioengineering is a fertile area for this marriage of an approach to complexity with the most complex systems we know about, the living systems. Furthermore, the area of network thermodynamics has made some significant progress in this direction (for a review, see 1). What seems to be needed now, is a conscious effort to overcome some of the barriers to liberating the discipline from the confines of very local, mechanistic thinking toward a more global approach which acknowledges how much more the whole is than the mere sum of its parts.

After recognizing these obstacles, as we have, it then becomes important to develop an application of engineering principles in a systematic and stepwise fashion. The obvious and interesting question, which arises out of Rosen's thesis that mechanisms are at best a local approximation to the complex systems that organisms are, is: "At what point do the mechanisms fail us and how do we make this transition from mechanisms to complex systems with a sound theory?" The following ideas are offered as an approach to the answer to that question.

The particular methodology to be employed comes from network thermodynamics and only some small part of what has been learned in that area will be dealt with here. For more information, the reader is referred elsewhere (1,14).

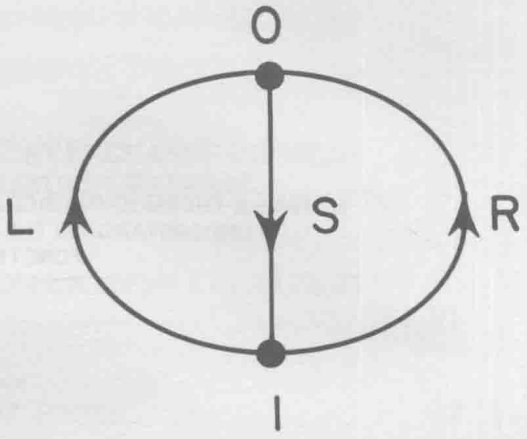

Fig. 2 The linear digraph of the current divider network in Fig. 1

THE CURRENT DIVIDER PRINCIPLE: ONE EXAMPLE OF A COMMON ORGANIZATIONAL THEME FROM NETWORK THERMODYNAMICS:

There begins to emerge a "circuit theory" for biological networks. The idea is somewhat foreign to most researchers in the life sciences, as well as to those in physical network applications. It is an idea that has the potential for providing a set of organizing principles which will have great value (as we try to understand more complex living systems). The elementary building blocks of this theory are the most rudimentary ideas from electronic network theory projected toward more complex physical networks and then, finally, living systems. One very dominant characteristic of biological networks is that they almost invariably involve multiport elements (14-20).

The Current Divider Principle As An Example: 1-port Networks

The simplest manifestations of a general network configuration are the current divider and voltage divider principles. They are found more often than might be expected and for that reason only the current divider will be examined in detail here. For instances of voltage divider application see ref. 21, for example. The current divider involves a flow source feeding into a pair of branching paths consisting of resistive barriers. Its topology is shown in figures 1 and 2. The solution to this simple 1-port network is obtained by standard network methods in the manner described below. The analysis of such networks can be carried out by a variety of standard network and/or graph theoretical techniques all of which combine the use of incidence matrices and Kirchhoff's laws with some set of constitutive relations describing the network elements in the branches (14,19,22-31).

Solution To The Steady State Current Divider Network

For stationary-state networks (networks containing only sources and dissipative elements such as resistors) the node-branch incidence matrix is a convenient way of encoding the topology of the network's linear digraph. In time dependent cases, other, related methods, such as cut set analysis can be reality substituted (27). For the current divider in Figure 2, this is a trivial relation involving one node and three branches. In the general case the incidence matrix is n x m where n is the number of columns and corresponds to the branches in the graph while m is the number of nodes. The "matrix" is now a vector with three components:

$$\underline{A} = (1,-1,1). \tag{1}$$

The flow vector is the vector of the flows in the branches in the order: L=left, S-source, R=right.

$$\bar{J} = (J_L, J_S, J_R), \tag{2}$$

Kirchhoff's Current Law (KCL) is the statement that the product of the incidence matrix, eqn (1), with the flow vector, eqn (2), is the null vector:

$$\underline{A}\bar{J} = 0, \tag{3}$$

or $$J_L - J_S + J_R = 0.$$

Kirchhoffs Voltage Law (KVL) is the statement that the branch forces and node potential are related by the equation

$$\underline{A}^T\bar{c}=\bar{X}, \tag{4}$$

where $\underline{A}^T$ is the transpose of $\underline{A}$. $\bar{c}$ is the vector of node potentials and $\bar{X}$ is the vector of potential drops across the branches, ordered as the flows were in the flow vector above,

$$\bar{X} = (X_L, X_S, X_R). \tag{5}$$

The only other information to be incorporated into the solution are the constitutive relations for the resistors, R_L and R_R. This can be accomplished by defining the conductance matrix $\underline{L}$.

$$\underline{L} = \begin{bmatrix} L_L & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & L_R \end{bmatrix} \tag{6}$$

Using this matrix, the constitutive relations (Ohm's law) can be written in vector-matrix form as:

$$\bar{J} = \underline{L}\,\bar{X} + \bar{J}_s, \tag{7}$$

where $\bar{J}_s$ is the flow source vector which, in this case, looks like:

$$\bar{J}_s = (0, J_s, 0). \tag{8}$$

Using Kirchhoffs laws, eqns. [3]&[4], and the constitutive eqn. [7], and the constitutive relations, the solution of the network can always be expressed by a function relating the node potentials to the source strengths as follows:

First the constitutive relations eqns. [7] are combined with KCL, eqn. [3]:

$$\underline{A}\,\bar{J} = \underline{A}\,\underline{L}\,\bar{X} + \underline{A}\,\bar{J}_s = 0. \tag{9}$$

Then KVL, eqn. [4], is substituted:

$$\underline{A}\,\underline{L}\,\underline{A}^T\,\bar{c} = -\,\underline{A}\,\bar{J}_S \tag{10}$$

or $$\bar{c} = -(\underline{A}\,\underline{L}\,\underline{A}^T)^{-1}\,\underline{A}\,\bar{J}_S \tag{11}$$

From this solution, which has been done in an all too general way to illustrate the way far more complex networks can be solved, it is easy to work out the current divider relations:

$$J_L = L_L\,(L_L + L_R)^{-1}\;J_S, \tag{12}$$

and $$J_R = L_R\,(L_L + L_R)^{-1}\;J_S. \tag{13}$$

For didactic reasons, the solution to linear steady state systems has been presented in detail. Time dependent linear systems are solved through a similar procedure resulting in the state vector equations (27). Nonlinear systems can be dealt with in a similar manner with a few additional considerations. The constitutive relations become nonlinear, multiple argument functions divided into those with flows as inputs (arguments) and those with forces and/or node potentials as arguments. The rest of the formalism has basically the same form so that Kirchhoff's laws still have a linear algebraic format. The solution is found by solving the set of nonlinear equations which results, but, in general, simulation is the method of choice (1,13,32-35).

Extension to n-ports.

In the linear case, it is a simple matter to now extend this result to the class of n-port resistors (dissipators) introduced by nonequilibrium thermodynamics (36-42). A few examples of the multiports used in modeling living systems appear in Table 1.

Table one: Some Multiports Useful in Describing Living Networks.

Common Name	Energy conversion process
Active Transport	Chemical to Osmotic
Bioelectricity (Exitability)	Osmotic to Electrical
Muscle Contraction (Motility)	Chemical to Mechanical
Photosynthesis	Chemical to Chemical
Respiration	Chemical to Chemical

The two-port example is shown in Figure 3.
The equations shown above are still applicable if we recognize the following extensions of the definitions of the symbols:

$$L_i = \begin{bmatrix} L^i_{11} & L^i_{11} \\ L^i_{21} & L^i_{22} \end{bmatrix}$$

and $\bar{J}_i = (J_1^i, \quad J_2^i)$

$\bar{J}_S = (J_S, 0)$

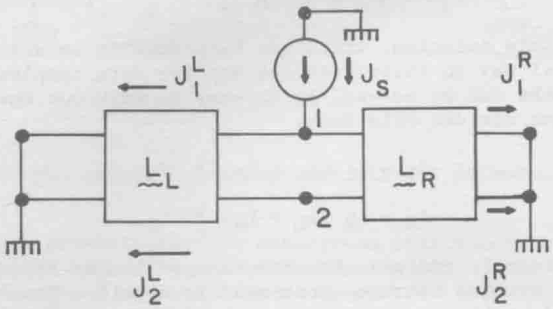

Fig. 3. The 2-port current divider with a 1-port flow source.

Where i = L or R. The expression $(L_L + L_R)^{-1}$ in the current divider equations now represents the inverse of a sum of 2x2 matrices. Thus such structures have a straightforward and general analysis using network theory which easily extends to more complex systems. Also, by using simulation, nonlinear problems involving multiports are easily handled (32-35). The next point is, from the perspective of the application to living systems, the key point-namely, that the actual behavior of n-port current dividers is not a simple intuitive extension of the 1-port case, even though the mathematical analysis is! This is because of a very simple idea which can be seen by inspecting Figure 3. The source feeds into node 1 and therefore KCL allows for the source flow to divide as in the 1-port case. In the case of the other flow, which only has two branches adjacent to node 2, KCL requires that what flows in must flow out, or, in other words,

$$J_2^L = -J_2^R = J_2,$$

a continuous flow across the entire system. Thus, the two port current division has the capacity for the flow coupled to the flow entering the current divider to be driven through the system while the other divides.

Some examples of the application of the current divider principle in stereotypes of biological membrane systems.

The following examples are all simple networks which can be solved completely by the methods illustrated in this paper as long as they are restricted to some steady state around which the flows and forces can be assumed to be linearly related. In the time dependent case or for a wider range of flow-force variation, simulation can be used to replace the analysis as was discussed earlier. The focus for this presentation is a more qualitative set of observations based on a recognition of the topology of the system, namely that of a current divider. Once this recognition is made, the behavior of the systems is readily predicted.

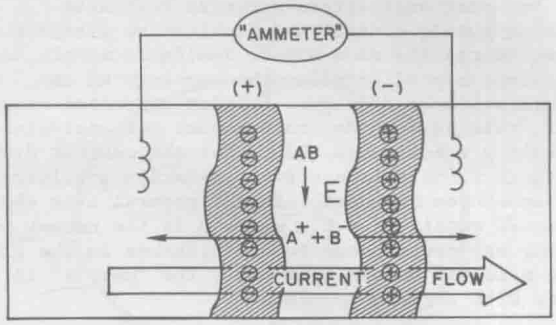

Fig. 4. A 3-port current divider system consisting of a set of two permselective membranes, one for cations (+) and one for anions (-), and an enzyme which catalyzes the breakdown of a neutral substrate AB into charged products A^+ and B^-.

The system shown in Figure 4 can readily be identified with a 3-port version of the current divider having essentially zero conductance for ions of one sign on one side and for the ions of the other sign on the other. The net result of the enzymatic reaction which now acts as a two port source is a flow of current (the third port flow) across the system. The fact that each of the membranes is selective for ions of one sign and that the system is asymmetric (Curies' principle) leads directly to its capacity to convert chemical energy from the chemical reaction to current flow.

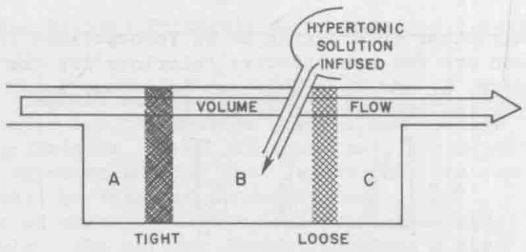

Fig. 5 The Curran - MacIntosh system. The arrangement of tight and loose membranes and the introduction of hypertonic solution into the central compartment, induces volume flow across the system even when solutions A and C have identical composition.

Figure 5 is the system devised by Curran and MacIntosh (37) to explain the phenomenon of isotonic transport across epithelial membranes (32-35,39,42,44-45).

In this case, the two coupled processes are the mechanical flow of volume across the system, driven by a source for solute in the other branch of a 2-port. This case can be matched identically with the 2-port in figure 3. It is easily shown that by recognizing

the current divider principle as a general principle, it can be shown to be simultaneously operative in the space between the cells of the epithelium (the classical explanation for isotonic transport) and across the cell as well (46). The device converts an osmotic flow into the middle compartment (flow of solute) into a volume flow across the system. (Clearly the volume flow has no other alternative since the conservation laws dictate a steady state flow across the system). The direction of the volume flow depends on the direction of the flow source and the asymmetry in the two membranes. Thus, a new, adjacent application of the classical idea appears to be working in holistic harmony with it!

Figure 6 shows an application which illustrates the fact that the macroscopic Curie Principle (47,48) is really an application of the current divider principle. In this case, the asymmetry of the placement of the bound enzyme creates the difference in diffusion resistance between the two stirred baths and the enzyme. The result will be a buildup of gradients of substrate and product in opposite poise across the membrane. Peusner (19,49) showed that this combination of network and symmetry reasoning is an application of group theory to networks.

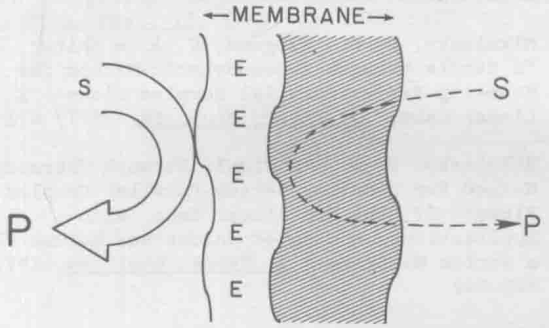

Fig. 6 The Curie Principle as a current divider. This 2-port allows the interconversion of chemical energy to gradients of substrates and products only when an assymmetry exists in the system. "E" represents the enzyme catalyzing the reaction S P and the shaded area is a diffusion barrier between bath R and the enzyme which does not exist between bath L and the membrane due to the assymetric placement of the bound enzyme in the membrane.

SUMMARY

In summary, the multiport current divider principle has been shown to have a number of applications to processes common in living systems. In each case, the result is easily achieved by network analysis, but has meaning which transcends the simple 1-port case. This group of simple examples illustrates the utility of network analysis and its potential for generality in building a complex systems theory for use in biology as well is in chemistry and physics. It also suggests that bioengineering may make an important new contribution to theoretical biology.

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A STUDY OF THE APICAL SODIUM TRANSPORT MECHANISM BY SIMULATIONS OF TRANSEPITHELIAL TRANSPORT USING NETWORK THERMODYNAMICS AND SPICE

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ABSTRACT

Amphibian sodium-transporting epithelia are extensively studied as models of sodium reabsorption in mammalian kidney. In these tissues, sodium is transferred from the external medium (pond water or urine) into the animal. There are two major steps: the sodium ion enters the epithelial cell by crossing its apical (or outer) membrane, it is then transferred from the intracellular medium into the internal medium of the animal across the basolateral (or inner) membrane of the cell. The first step is passive - the electrochemical gradient is favorable for sodium entry into the cell; the second step is active - via the so-called sodium-pump, which requires metabolic energy. The Na-entry step has been characterized as the regulatory step; it is rate limiting and subject to hormonal regulation. The apical sodium flux saturates with increasing sodium concentration in the external medium and is specifically and competitively inhibited by amiloride - a diuretic. Both saturation and inhibition are commonly interpreted as an inhibition of the apical membrane permeability to sodium (P^{ap}_{Na}), but the reasons why P^{ap}_{Na} is inhibited by sodium remain a subject of debate.

This work reports an attempt to clarify this problem. The most commonly used description for the apical sodium flux is an electrodiffusion process, the Goldman-Hodgkin-Katz (GHK) equation which defines an independent P^{ap}_{Na}. As P^{ap}_{Na} is obviously concentration-dependent, *ad hoc* expressions which mimic the observed dependence are often proposed in the literature. On the other hand, the apical sodium flux is thought to be facilitated by an ion-specific channel, which cannot be described by any electrodiffusion process. We have thus replaced the GHK equation by a description of a channel for the sodium entry step in order to test whether such a mechanism could provide an explanation for the experimental observations.

This was done in a model of a typical epithelial cell based on that of Koefoed-Johnsen and Ussing (1) and elaborated by Hviid Larsen (2) and Lew et al. (3). With the model, we simulated experiments such as the effect of the Na-concentration and of the transepithelial potential difference on Na-transport. The model was described as a network, using network thermodynamics, and in the language of the circuit simulation program SPICE. The simulations showed that when the GHK equation is used for the Na-apical flux there is no saturation with increasing Na, unless the concentration-dependence of P^{ap}_{Na} is added by means of *ad hoc* expressions. Thus, the Na-entry step is rate limiting in the model, as thought from experiments. Following this, substitution of a one-site, two-barrier channel for the GHK equation allowed us to simulate the saturation of the Na-flux and its competitive inhibition by amiloride. Moreover, in this channel-containing model, the P^{ap}_{Na} defined by analogy with the GHK equation is indeed inhibited by increasing sodium or amiloride concentrations, and these inhibitions are very simply explained, as P^{ap}_{Na} is tightly related to the fraction of the channels' population having a free site. In other words, a simple channel appears as a possible mechanism for the sodium entry step, giving a simple explanation for the observed saturation and inhibition of the sodium flux. By fitting this model to current-voltage relations from experiments on urinary bladder epithelium, we estimate the Na-site position within the membrane and the Na-site affinity.

INTRODUCTION

Transporting epithelia separate the internal medium of the body from a so-called external medium. Examples are the intestinal wall, the walls of kidney tubules, the gallbladder, and the urinary bladder. These tissues are largely responsible for maintaining the homeostasis of the internal medium by performing selective exchange of water and ions between the internal and external media. As compared to the internal medium, the external medium is generally hypo-osmotic and contains very little sodium. The high internal sodium concentration is maintained by a continuous reabsorption of sodium by the epithelial cells. In mammals, the regulation of the internal hydromineral equilibrium is mainly achieved by the kidney; in amphibians, the kidney is not efficient enough, and the urinary bladder and the skin also participate in this physiological function. Thus, such epithelia as amphibian urinary bladder or skin are extensively studied as models of sodium reabsorption in mammalian kidney.

SODIUM CHLORIDE TRANSPORT ACROSS A TYPICAL EPITHELIAL CELL

An epithelium is made of one or more layers of the so-called epithelial cells which perform sodium transport across the tissue. In multi-layered tissues, each layer corresponds to a different stage of differentiation, the outermost layer being highly specialized in sodium transport. Gap junctions are found

between the different layers and between cells of each inner layer. Thus, the cells of multi-layered tissues are thought to form a syncytium and, as a first approximation, these tissues such as frog skin, are described as functionally mono-layered tissues.

The tissue has two faces, one in contact with the external or mucosal medium, the other with the extracellular space which is identical to the internal or serosal medium. The faces are functionally different. At the outer surface, the cells are connected by tight-junctions which separate the external medium from the extracellular space. In addition to providing mechanical strength, these junctions serve an important functional role. They delimit two regions on the cell membrane: the apical membrane faces the external bath, whereas the remaining membrane, the basolateral membrane, is in contact with the extracellular interstitial space. These two membranes have different functional properties. The basolateral membrane has the usual properties of cytoplasmic membranes: high potassium permeability and the so-called sodium pumps which extrude sodium from the intracellular medium in exchange for potassium. The differentiation of the epithelial cells is manifested in the apical membrane, which has no sodium pumps and is preferentially selective to sodium.

The epithelial cells are thus organized in an asymmetrical structure which offers two parallel pathways to ionic transport: a transcellular pathway which involves two barriers in series, the apical and basolateral membranes, and a paracellular pathway across the tight-junctions. This model, which reduces the tissue to a three-barrier structure, was first proposed by Koefoed-Johnsen and Ussing (1) for the frog skin, and since then has been applied to most of the sodium transporting epithelia. Including the results obtained by intracellular measurements, the description of ionic transport across these tissues can be now summarized as follows.

- The apical membrane is selective to sodium, i.e. it is more permeable to sodium than to any other physiological ion. The sodium ion enters the epithelial cell by crossing the apical membrane. This first step is passive, the electrochemical potentiel gradient being favorable for sodium entry into the cell.
- The intracellular sodium is transferred into the extracellular space by the Na^+,K^+-ATPase (i.e. sodium pump) of the basolateral membrane. This second step is active, the transfer being against the electrochemical potential gradient: it requires metabolic energy supplied by ATP hydrolysis. In exchange for sodium extrusion, the pumps accumulate potassium in the cell, and the intracellular potassium leaks back to the extracellular space across the basolateral membrane. The intracellular concentrations are thus low for sodium ($\simeq$10 mM) and high for potassium ($\simeq$100 mM) as compared to the extracellular concentrations, $Na^+\simeq$100 mM and $K^+\simeq$2.5 mM.
- The transcellular sodium transport is thus said to be active. In the so-called 'tight epithelia' such as amphibian skin and urinary bladder, the total ionic conductance of the tight-junctions is very much lower than that of the two cell membranes. Consequently, the ionic gradients maintained across the two cell membranes by the active transport result in a transepithelial electrical potential difference (PD) of the order of 100 mV, positive in the internal medium. This PD favours chloride diffusion across the tissue. The result is an absorption of sodium chloride from the external medium. In tight epithelia, the apical membrane is almost impermeable to potassium, so that this ion is essentially recycled across the basolateral membrane.
- The active sodium transport across the tissue is the major transport. The three major physiological ions - sodium, potassium and chloride - can diffuse across the tissue, using one or the other pathway, depending on the permeabilities of the different barriers to each of these ions. Numerous studies have shown that the sodium entry step is the regulatory step of sodium reabsorption: it is rate limiting and is under the control of aldosterone and anti-diuretic hormone, the principal hormones regulating the homeostasis of the internal medium. Understanding the apical sodium transport mechanism is thus an important goal.

APICAL SODIUM TRANSPORT. ELECTROPHYSIOLOGY.

The sodium entry step is known to be passive. It is driven by the transapical potential difference and the sodium concentration distribution. The goal is thus to explore the relationships between the apical sodium flux and its driving forces. Since we deal with the whole tissue, a few comments on the experimental methods are necessary.

Steady-state measurements

The measurement of the apical sodium flux is done by short-circuiting, or voltage clamping, the tissue, and using the specific inhibitor of this flux, amiloride. The tissue is placed between two baths replacing the external and internal media. When both baths contain Ringer's solution which mimics the physiological internal medium, there is no transepithelial driving force, apart from the spontaneous PD. By means of a voltage clamp device, the transepithelial PD can be abolished, short-circuiting the tissue. The corresponding short-circuit current which crosses the tissue (I^{sc}) has long been known to be carried by sodium (1). Amiloride decreases I^{sc} to almost zero and the active transepithelial sodium flux is evaluated as the amiloride-sensitive current, i.e. the difference between the current remaining in the presence of amiloride and that measured without the inhibitor. The amiloride-sensitive current, I_{Na}, is thus a measurement of the current carried by sodium through the apical transport mechanism to be characterized.

The electrical driving force for transapical sodium transport, or the transapical potential, V_{mc}, is measured by means of an intracellular microelectrode (4). In order to study the relationships between I_{Na} and its driving forces, the same measurements are made at various sodium concentrations in the external bath. The external sodium concentration (c^m_{Na}) is thus decreased by replacement with an impermeant cation. This leads to a decrease in the current and an increase in the inward-directed electrical driving force for sodium (V_{mc}).

At each c^m_{Na}, typically 45, 15 and 5 mM, the tissue was allowed to reach a new steady-state, which could involve changes of intracellular composition. These short-circuit measurements yield steady-state relationships between I_{Na}, V_{mc} and c^m_{Na}. Typically, as the external sodium concentration increases, the apical sodium flux increases and saturates (5), whereas the electrical driving force decreases (6). The same measurements done with increasing doses of amiloride show that the inhibition results from simple competition between the inhibitor and sodium for the sodium transport mechanism (7).

Instantaneous measurements (*Necturus maculosus* urinary bladder)

The steady-states cited above are defined by the bath composition and the short-circuit. Among the driving forces controlling the apical sodium flux, the external sodium concentration is the only controlled variable. In a given steady-state, it is necessary to evaluate the intracellular sodium concentration (c^c_{Na}). The apical sodium flux being passive, the transapical potential which reverses the flux yields an estimation of c^c_{Na}. In addition, (I_{Na},V_{mc}) curves obtained without inducing changes in the intracellular medium yield information on the apical sodium transport mechanism itself.

Such measurements, which were termed instantaneous, are done by changing the voltage across the tissue for a short period of time and scanning a large range of voltages. Any change in the clamp induces instantaneous changes in V_{mc} and the transepithelial current I_{ms}. The clamp is changed from 0 to 200 mV by 20 mV steps symmetrically applied around the steady-state value 0 mV. Each pulse is applied for 50 ms, a period short enough to avoid changes in the intracellular medium, and yet long enough for capacitive transients within the apical membrane to settle. This yields transepithelial current-voltage (I_{ms},V_{ms}) and transapical-transepithelial voltage (V_{mc},V_{ms}) curves. Such measurements are done at each