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Dynamic Interfacial Electrochemical Phenomena at Living Cell Membranes: Application to the Toad Urinary Bladder Membrane System

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ABSTRACT

The concept of electrochemical information transfer *in vivo* has been studied using the isolated toad urinary bladder membrane system to evaluate a recently developed approach to membrane impedance which emphasized the coupling of interfacial processes to standard transport phenomena. Laplace plane analysis was utilized to examine frequency response from 1 Hz to 1 MHz. The results indicate that, under the linear conditions of this experiment, Na^+ ion is the predominant current carrier and that the rate-determining step is its penetration into the membrane phase from the aqueous phase. Thus, membrane transport appears to be a very rapid step, whereas the interfacial step of phase transfer regulates transport in this system. The implication of this result in terms of potential dependent interfacial steps in the control of cellular function is discussed.

It is becoming increasingly evident that many cellular functions involve a control or regulatory step occurring at the cell's cytoplasmic membrane (1-7). Often this involves the activation of certain membrane-bound enzymes by the specific interaction of charged species such as ions (8-10) and hormones (11, 12). Because of the type of over-all molecular structure of the protein-containing lipid membrane (13-18), it has been possible to postulate that these regulating interactions occur at interfaces or surfaces and that they are dynamic and potential dependent (19-23). This then constitutes the concept of electrochemical information transfer *in vivo*, in which it is supposed that a highly specific dynamic charge injection can cause a selective modification of, *e.g.*, enzyme and/or transport activity, which in turn may modify cellular and tissue function in a beneficial manner. This approach has guided recent studies in which inductively coupled pulsating currents have caused a selective modification of bone (osteocyte) and cartilage (chondrocyte) activity (24, 25). Thus it appears that either Ca^{2+} activity and/or protein synthesis may be selectively elicited by the proper choice of independently different pulse parameters. These parameters were predicted (22, 25, 26) on the basis of an analysis of the different time constants anticipated in either ion or hormone binding at the cell membrane and the subsequent steps to this trigger. It thus appears possible, for bone and cartilage at least, to selectively modify in a beneficial manner two different cellular functions. This approach has already been extended to the clinic wherein it has been possible to heal otherwise incurable bone fractures with approximately 85% success (25).

In order to establish a working hypothesis for the application of electrochemical information transfer *in vivo*, a detailed analysis of the transient electrical behavior of living cell membranes has recently been carried out (22, 23, 26). This approach focuses on the analogy between the electrode/solution interface and those occurring at the living cell's membranes and the intra- or extra-cellular fluid. Of particular importance

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is the addition of potential dependent specific adsorption (binding) and slow phase transfer (partitioning) as coupled steps in the response of the membrane to dynamic changes in interfacial potential. By allowing these to interact with membrane and aqueous transport along with dielectric and surface charge behavior, it has been possible to present a unified approach to membrane impedance (22, 26, 28). The significance of this approach lies in its capability to treat the highly heterogeneous and dynamic behavior of living membranes in a relatively rigorous manner so that theoretical models may be established and experimental tests may be carried out.

The present study involves such a test on the isolated toad urinary bladder membrane system. This system is uniquely suited to a living cell membrane impedance study since, as a membrane system, it consists of a supported single layer of epithelial (skin) cells in tight junction contact (29-31). These mucosal cells (so called because they border the inside surface of the bladder) are approximately 10μ thick and exhibit asymmetrical membranes with respect to both structure and function. Under physiological conditions these cells transport urea, water, and Na^+ (both actively and passively). Tight junctions between the mucosal cells severely limit under normal conditions, transepithelial transport and allow the membrane system to exhibit selective transport properties. The mucosal cells are supported on a loose sheet (electrically resistive only) of connective tissue (collagen) which is widely interspersed with blood vessels and smooth muscle fibers. There is also a discontinuous layer of cells bounding the serosal or external surface of the bladder. These would also not be expected to contribute to the over-all membrane response of this system. It was, therefore, expected that, under the transient electrical conditions of the present study, the bladder system would exhibit a relatively simple response related to passive Na^+ transport. This study will present the application of Laplace plane modeling and analysis to this living membrane system.

Theoretical

In order to construct an impedance model for the toad urinary bladder membrane system, it is necessary to briefly review the application of Laplace plane analysis to a generalized membrane. At the onset it must be pointed out that Laplace plane analysis, in the manner applied here, assumes linear conditions, *i.e.*, the impedance of the system may be defined. Linearity conditions can be established for this complex system, since the modeling may be performed using a piecewise linear approximation, *i.e.*, for any given steady-state behavior the superimposed time varying perturbation may be low enough so that changes from rest or steady state are linear (32). In addition, the very nature of Laplace plane analysis allows the transient impedance of the system to be evaluated (33). In this manner impedance can be obtained using a minimal number of perturbations (usually less than five) with presently available instrumentation, thereby "freezing" the behavior of the system in time.

To establish the impedance of a membrane it is considered that, in addition to the well-known dielectric properties of the lipid-protein system (34), the passage of transient current will satisfy specific adsorption and phase transfer properties which may be coupled to aqueous and membrane transport. This is illustrated in Fig. 1 where it can be seen that in addition to both intra- and extra-cellular diffusion (assumed planar) surface double-layer charging, specific adsorption, and phase transfer (partitioning) can take place. All of these processes can be coupled and are assumed in the first instance to take place at every interface (inner and outer) of each membrane.

Laplace plane analysis is performed in the complex frequency domain defined by the Laplace variable, s, a complex number with the dimensions of frequency and having a real, σ, and imaginary, $j\omega$, part such that $s = \sigma + j\omega$. This variable is utilized in the Laplace transformation wherein the transformed function $F(s)$ is obtained by operating on a time domain function $f(t)$ according to

$$F(s) = \int_0^\infty f(t)\exp(-st)\,dt \qquad [1]$$

All functions in this study will be described in the s plane. The basics of obtaining impedance models in this fashion has been extensively described elsewhere (33, 35).

When a transient current passes across a membrane, a portion of it will satisfy all of the interfacial charging (both specific and nonspecific) and dielectric requirements. This is termed the charging current, $i_c(s)$. Another portion of the total current, $i(s)$, will satisfy all phase transfer phenomena, *i.e.*, that portion of the current carried across the membrane. This is termed the phase transfer current $i_p(s)$. The total current is, therefore, given by

$$i(s) = i_c(s) + i_p(s) \qquad [2]$$

To describe $i_c(s)$ it is convenient to express it in terms of the change in charge, $q(s)$, which may occur as a result of a change in interfacial potential $\eta(s)$ as

$$i_c(s) = sq(s) = q_E s\,\eta(s) + q^e s c^e \Delta c^e(o,s) \qquad [3]$$

where $q_E = (\partial q/\partial E)_{c^e}$ and $q^e = (\partial q/\partial c^e)_E$ representing the variation of q with potential, E and with the concentration of a species c^e which specifically adsorbs at interface e (membrane/extracellular fluid). Note that more than one species may adsorb at this interface, and that specific adsorption may also occur at interface i (membrane/intracellular fluid). However, for

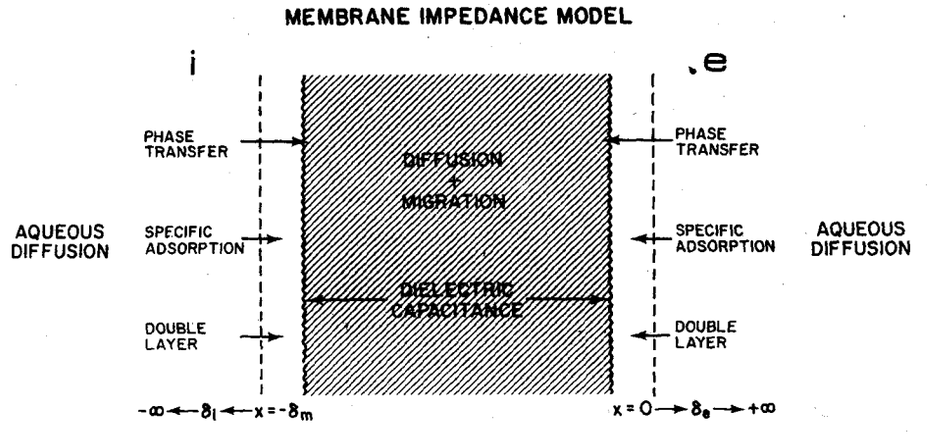

Fig. 1. Schematic diagram depicting the essential elements of the interfacial membrane impedance model described in this study. In addition to aqueous and membrane mass transport finite kinetics are allowed for specific adsorption (binding) and phase transfer (partitioning). All processes are coupled and may involve both the extracellular, *e*, and intracellular *i*, interfaces.

the sake of clarity only a single species at e will be considered. Extension to more complex systems has been described elsewhere (26, 28). Both q_E and q^e are constant coefficients representing the linear conditions of the model; they may change for different resting or steady-state conditions. In Eq. [3] $\Delta c^e(o,s)$ represents the change in concentration with time (frequency) of species c^e at the interfacial region ($x = 0$) as a result of specific adsorption. In other words this quantity represents the change in concentration c^e in the plane $x = 0$ while in the preadsorbed state (*i.e.*, before specific binding has taken place). $\Delta c^e(o,s)$ is defined by $\Delta c^e(o,s) = (c^e(o,s) - c^e)/c^e$, which makes it a relative change (to avoid carrying initial conditions throughout the derivation of the model), and is the reason why c^e appears in the second term on the right-hand side of Eq. [3].

Since specific adsorption has been invoked, it is necessary to establish a relation between the concentration of the specifically adsorbed species Γ_e and $\Delta c^e(o,s)$. This is readily done by assuming first order kinetics (35, 36) which gives

$$\Delta\Gamma_e(s) = v_e/\Gamma_e s[\Delta c^e(o,s) - \Delta\Gamma_e(s) + a\eta(s)] \quad [4]$$

where $\Delta\Gamma_e(s)$ is given by $\Delta\Gamma_e(s) = (\Gamma_e(s) - \Gamma_e)/\Gamma_e$, representing a change with respect to the initial concentration of adsorbed species, Γ_e; v_e is an exchange adsorption rate constant and, a, represents the potential dependence of adsorption (a constant coefficient) and that portion of the total potential change, $\eta(s)$, which acts at interface e. There is a similar relationship for each adsorbing species at each interface. Note that Eq. [4] allows adsorption kinetics to be finite thereby making the model somewhat more general than has previously been the case (37).

It is now necessary to obtain an expression for the phase transfer current, $i_p(s)$. Since $i_p(s)$ represents all of the total current which actually traverses the membrane, in addition to the expected concentration dependence, finite kinetics are also allowed for partitioning (*i.e.*, penetration of the transporting species from the aqueous phase into the membrane phase). Since this is expected to be potential dependent $i_p(s)$ can be written as

$$i_p(s) = I_E\eta(s) + I^{ec^e}\Delta c^e(o,s) + I^{mc^m}\Delta c^m(o,s) \quad [5]$$

in which $I_E = (\partial i_p/\partial E)_{c^e,c^m}$; $I^e = (\partial i_p/\partial c^e)_{E,c^m}$, and $I^m = (\partial i_p/\partial c^m)_{E,c^e}$ representing the variation of i_p with potential and with the aqueous and membrane concentrations of the transporting species, respectively. Again in this linear model these are constant coefficients. The relative concentration change $\Delta c^e(o,s)$ is as for Eq. [4], while $\Delta c^m(o,s)$ represents the relative concentration change of the same species in the membrane phase at the plane $x = 0$ (*i.e.*, after partitioning just inside the membrane). The first term on the right-hand side of [5] represents the finite phase transfer kinetics allowed in this model. The physical significance of this term may be related to the influence of surface charge upon transport (38-41) particularly in the context of specifically adsorbed charge.

Equations [3] and [5] may be employed to provide an expression for the total impedance of the model proposed. For this it is necessary to obtain explicit expressions for $\Delta c^e(o,s)$ and $\Delta c^m(o,s)$ taking into account finite specific adsorption kinetics as given by Eq. [4]. Mass transport in the aqueous phase can be represented by Fick's second law, since the supporting electrolyte in physiological solution is approximately 0.2M. The important quantity is the flux at the plane $x = 0$ which is given by, for finite planar diffusion

$$\left(\frac{d\Delta c^e}{dx}\right)_{x=0} = -\left[\left(\frac{s}{D_e}\right)^{1/2} \coth\left(\frac{s}{D_e}\right)^{1/2}\delta_e\right]\Delta c^e(o,s) \quad [6]$$

Where D_e is the aqueous diffusion coefficient and δ_e is the thickness of the diffusion layer in the extracellular fluid. This quantity reduces to the familiar square root of frequency (Warburg) behavior under semi-infinite conditions.

Membrane mass transport occurs under both concentration and electric field gradients thereby necessitating use of the Nernst-Planck equation which is written here for planar conditions as

$$\Delta c^m(x,s) = \frac{D_m}{s}\left[\frac{d^2\Delta c^m}{dx^2} + \frac{F}{RT}\frac{d}{dx}\left(\Delta c^m\frac{dV}{dx}\right)\right] \quad [7]$$

where D_m is the diffusion coefficient in the membrane phase (not necessarily equal to D_e), dV/dx is the voltage field across the membrane and the other quantities have their usual significance. For most membranes the concentration of the transporting species within the membrane is relatively low (37). In addition, the linear conditions of this model (which are experimentally obtainable) suppose that the change in voltage field is constant through the membrane. For these two reasons, it appears possible to assume constant field conditions, so that $dV/dx = \text{const} = \bar{V}$. Under these conditions Eq. [7] can be solved analytically. Once again the flux at $x = 0$ is the desired quantity. This is given by, for a membrane of biological thickness

$$\left(\frac{d\Delta c^m}{dx} + \frac{F\bar{V}}{RT}\Delta c^m\right)_{x=0} = \left[\frac{F\bar{V}}{2RT} + \frac{1}{\delta_m} + \frac{1}{3}\left(\frac{F^2\bar{V}^2}{4R^2T^2} + \frac{s}{D_m}\right)\delta_m\right]\Delta c^m(o,s) \quad [8]$$

where δ_m is the membrane thickness (usually 70-100Å), and the other terms have been defined previously.

The remaining requirement to solve for $\Delta c^e(o,s)$ and $\Delta c^m(o,s)$ in Eq. [3] and [5] is to provide a mass balance condition which will account for both aqueous and membrane mass transport in the presence of specific adsorption. This can be written as

$$D_e c^e\left(\frac{d\Delta c^e}{dx}\right)_{x=0} - \Gamma_e s\Delta\Gamma_e(s) = D_m c^m\left(\frac{d\Delta c^m}{dx} + \frac{F\bar{V}}{RT}\Delta c^m\right)_{x=0} \quad [9]$$

which states that the actual flux through the membrane (right-hand side, Eq. [9]) is equal to the flux of the same species in the aqueous phase (first term, left-hand side, Eq. [9]) minus the amount specifically adsorbed. Physically this can be pictured by saying that only that quantity of transporting species actually gets through the membrane which does not accumulate at the plane $x = 0$ (the interface) via the process of specific adsorption.

All of the above relationships are sufficient to generate an expression for the impedance of a single membrane where partitioning and specific adsorption occur at one interface. The result is for the charging impedance $Z_c(s)$

$$Z_c(s) = 1/q_E s \quad [10]$$

when $\partial\Gamma_e/\partial E$ is negligible, a condition often applicable when the species undergoing phase transfer is itself specifically adsorbed. The phase transfer impedance $Z_p(s)$ is given by

$$Z_p(s) = \frac{1}{I_E}\left\{1 + \frac{I^e}{nFc^e(D_e s)^{1/2} + s\Gamma_e/(1 + s\Gamma/v_e)} + \frac{I^m}{nFc^m D_m\left[\frac{F\bar{V}}{2RT} + \frac{1}{\delta_m} + \frac{1}{3}\left(\frac{F^2\bar{V}^2}{4R^2T^2} + \frac{s}{D_m}\right)\delta_m\right]}\right\} \quad [11]$$

Both $Z_c(s)$ and $Z_p(s)$ are in parallel as is evident from Eq. [2]. As shown elsewhere (26, 28) $Z_c(s)$ would contain additional terms related to the specific adsorption process, particularly when several species contribute to the current response. For the purposes of this application $Z_c(s)$ is represented by a pure capacitor which is primarily due to the dielectric and double layer response of the membranes in the toad bladder system. On the other hand $Z_p(s)$ contains a phase transfer impedance ($1/I_E$) coupled with semi-infinite aqueous mass transport and specific adsorption (second term in brackets on right-hand side of Eq. [11]) in series with membrane transport which has a more physically meaningful form than reported elsewhere (37).

Inspection of Eq. [10] and [11] shows that it is possible to represent the impedance for this particular model by the aperiodic equivalent electric circuit shown in Fig. 2. The placement of elements in this circuit is directly related to the actual physical pathway (mechanism) which is proposed. No assumption other than the basic model is employed to establish this topology and the frequency behavior described by Eq. [10] and [11] and Fig. 2 can be used to diagnose mechanism and kinetics. Note that this modeling procedure would result in several variants of Fig. 2 if both sides of the membrane and more than one species is involved (22, 26, 28), thereby permitting extensive diagnosis to distinguish among several different proposed pathways.

It is useful to attach physical significance to each of the elements shown in Fig. 2 for the eventual evaluation of kinetic parameters. Referring to Eq. [10] it can be seen that

$$C_d = \partial q/\partial E = q_E \qquad [12]$$

as expected for the charging current pathway. Note that C_d contains (in a series arrangement) contributions from the electrical double layers present at the inner and outer membrane interfaces, as well as that due to the dielectric property of the membrane structure. Although it is in principle possible to isolate the dielectric from double-layer contributions using very high frequency measurements coupled with ion dependences (28, 42), it was not within the scope of the present study to pursue this. Examination of Eq. [11] allows the phase transfer impedance R_p to be defined as

$$R_p = 1/(\partial i_p/\partial E) = 1/I_E \qquad [13]$$

showing that phase transfer kinetics would be expected to exhibit purely resistive behavior corresponding remarkably to the form of the activation process involved in electron transfer at the electrode/solution interface (35). It might, therefore, be expected that ion penetration (partitioning) would involve simple rearrangement of the entry site by, *e.g.*, lipid polar head deformation or displacement (43). The specific

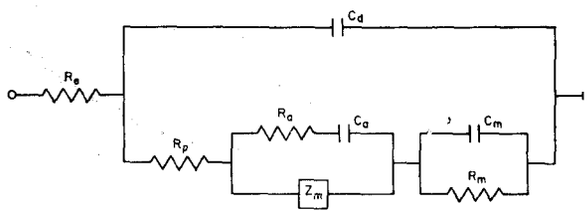

Fig. 2. Aperiodic equivalent electric circuit presented as a shorthand (but exact) notation of the Laplace plane behavior of a membrane (artificial or biological) at which a single species is involved in phase transfer. Note the topology of this circuit corresponds to the physical processes considered in the model and can be examined using the wide frequency range achievable via real axis Laplace transformation. The physical significance of each element is described in the text.

adsorption parameters R_a and C_a are given by

$$R_a = \frac{(\partial i_p/\partial c^e)}{(\partial i_p/\partial E)}\frac{1}{v_e} \qquad [14]$$

and

$$C_a = \frac{(\partial i_p/\partial E)}{(\partial i_p/\partial c^e)}\Gamma_e \qquad [15]$$

where it can be seen that R_a is inversely proportional to the exchange adsorption rate constant v_e and C_a is proportional to the initial concentration of adsorbed species Γ_e as expected. The aqueous transport impedance Z_D is in parallel with the adsorption pathway as required by the mass balance condition given in Eq. [9]. In this application to the isolated toad urinary bladder semi-infinite planar diffusion conditions can be assumed as indicated in Eq. [11]. In this case the movement of a concentration wave to or from the interface is exactly analogous to the propagation of a voltage signal along a semi-infinite R_DC_D transmission line (32). Z_D is therefore given by

$$Z_D(s) = \frac{(\partial i_p/\partial c^e)}{(\partial i_p/\partial E)}\frac{1}{nFc^e(D_e s)^{1/2}} \qquad [16]$$

for which the R_DC_D array has the following physical significance

$$R_D = \frac{(\partial i_p/\partial c^e)}{(\partial i_p/\partial E)}\frac{1}{nFc^eD_e} \qquad [17]$$

and

$$C_D = \frac{(\partial i_p/\partial E)}{(\partial i_p/\partial c^e)}nFc^e \qquad [18]$$

in which R_D is inversely proportional to the diffusion coefficient, D^e, and C_D is directly proportional to the bulk concentration, c^e, of the diffusing species. Membrane mass transport is represented by the parallel combination of R_m and C_m in Fig. 2. Referring again to Eq. [11], these are given by

$$C_m = \frac{(\partial i_p/\partial c^m)}{(\partial i_p/\partial E)}\frac{nFc^m\delta_m}{3} \qquad [19]$$

and

$$\frac{1}{R_m} = \frac{(\partial i_p/\partial c^m)}{(\partial i_p/\partial E)}\frac{n^2F^2D_mc^e\delta_m}{6RT}\left[3\bar{V} + \frac{6RT}{nF\delta_m} + \frac{nF\bar{V}^2}{2RT}\right] \qquad [20]$$

where it can be seen that C_m is proportional to the resting membrane concentration, and R_m is inversely proportional to the diffusion coefficient, D_m, and the voltage field to which the transporting species is subjected. Note that if $\bar{V} = 0$, Eq. [20] reduces to that which would be obtained in the absence of a significant electric field (32).

The above procedure illustrates the manner by which the potential dependent interfacial processes of specific adsorption and phase transfer can be coupled with both aqueous and membrane mass transport in a relatively quantitative manner. For the purposes of illustration a single interface has been modeled. However, it is to be realized that all of the interfaces and membranes in a living system may be treated in an analogous manner, generating equivalent electric circuits similar to that shown in Fig. 2 and added to it, in most cases in series.

In addition, for the interface treated only a single species has been considered. It is straightforward to consider the case of two or more species which may adsorb and/or transport across the same interface even if they act in a coupled manner. It is thus possible in principle to generate models using the above approach which can be employed to distinguish among the physi-

ologically meaningful pathways which can be proposed for a given system.

As mentioned above, the mucosal surface of the isolated toad urinary bladder is known physiologically to selectively transport Na^+ in a passive manner (44-46). In addition, steady-state measurements using microelectrodes have established that the mucosal (inside) membrane exhibits significantly higher resistance to ion transport than the serosal (outside) membrane (47). There is also some indication that the tight junction (cell to cell) contact exhibits a much higher resistance than the same serosal membrane (47). It can, therefore, be expected that current flow through this membrane system will essentially be straight through the mucosal cell (and not be deviated significantly in a parallel pathway across the cell layer). The isolated toad bladder can thus be expected to correspond relatively closely to those portions of the single interface model required for potential dependent Na^+ ion transport.

Experimental

In this study the urinary bladder of the toad (*Bufo marinus*) is subjected to low level current pulses and the voltage response is observed. To perform this type of measurement the bladder must first be isolated from the toad. This is performed using a rapid surgical procedure on a pithed (brain-dead) toad to minimize cellular damage (usually from drying, trauma, and autolysis). First the toad's abdominal cavity is opened in the usual manner exposing the viscera. At this point the bladder is exposed and when full (mostly water since the toad uses this organ for water balance) is seen to be a bilobed structure occupying almost the whole abdominal cavity. At this point the bladder can commence drying if precaution is not taken to maintain the exposed areas moist with amphibian Ringer's solution (an isotonic salt solution described below). The bladder is excised by first severing the dorsal facial layers of each lobe. These are then clamped close to the urinary sphincter region from which the bladder is severed and withdrawn usually while full. If the bladder is completely full, it is in a highly stretched condition and is transparent except in the area of an occasional blood vessel. It is in this stretched condition that the impedance measurements are made. To maintain this situation, the excised full bladder is placed in a petri dish containing aerated Ringer's solution and a cork template. The latter is utilized to hold the bladder in a stretched condition for mounting in a conductivity type chamber. This is achieved by pinning the bladder along one edge of the cork and opening a lobe along the center while pinning the remaining portions of bladder along the other three edges of the cork template. This is the most sensitive part of the isolation procedure since the mucosal cells are now directly exposed. These are fragile and care must be taken not to touch, in any way, that portion of the mucosal surface which will serve as the membrane system when mounted in the measurement chamber. Mounting on the cork template is carried out so as to provide a uniform stretch (monitored by observing for uniform transparency). The bladder is now relatively free of folds and will usually present a single supported mucosal cell layer for study. Great care is exercised during bladder mounting since nonuniform stretch and/or folded cell layers will affect transport properties (48, 49).

Once mounted, the wet bladder is rapidly transferred to the measurement vessel which is essentially a conductivity cell having two identical chambers separated by the bladder. In position between the two chambers, the bladder is then held in place by the pressure applied when the chambers are forced together. The bladder occupies a circular area of approximately 1 cm^2 bounded by O-rings on the face of either chamber. The pressure exerted by the O-rings prevents lateral leakage and minimizes edge damage (50, 51) particularly with the relatively large surface area of exposed bladder. The chambers are then filled simultaneously with Ringer's solution to avoid uneven hydrostatic pressure particularly from the serosal side which can cause morphologic changes such as intercellular space variation thereby modifying transport properties (31, 51).

The conductivity cell (see Fig. 3) consists of two identical chambers each provided with solution and gas inlets and outlets. In addition provision is made for current input and voltage measurement electrodes. These are sintered tantalum slugs providing high surface area for minimal faradaic effects under the experimental conditions employed. An important monitor of membrane integrity is the existence of a transepithelial resting potential due primarily to the active transport systems functioning to maintain intracellular ionic concentration levels. Generally this potential difference is between 10 and 100 mV, mucosal side negative. Provision is, therefore, made in each chamber to introduce a SCE reference electrode (Tacussel Type C-10) to assess membrane integrity, viability, and monitor possible base line changes during the experiment.

The physiological solution utilized to bathe the mucosal and serosal sides of the bladder in the measurement cell is isotonic for the amphibian. It is composed of (in mM/liter): NaCl 109; $CaCl_2$, 0.9; $NaHCO_3$, 2.4; KCl, 2.5; and $C_6H_{12}O_6$, 5.5. The bladder is allowed to stabilize in this solution (while aerated) until the resting potential is invariant (usually after 0.5-1 hr). Current pulses are then applied so that the transepithelial potential change (not including IR_e drop) is restricted to 1 mV or less. In this manner linear conditions are achieved and the active transport systems do not deviate significantly from base line since the net change in intracellular ionic concentration is negligible (<10 $\mu C/cm^2$ of total charge injection is utilized).

The circuit employed for these measurements is shown in block diagram form in Fig. 3. A current pulse (usually <1 $\mu A/cm^2$) is applied across the membrane system by driving the noninverting (+) input of a tunable fast potentiostat, Al, (Tacussel PIT-20-2X) with a pulse generator, SG, (Tektronix PG 405) equipped with an offset voltage source for base line current variations. Resistor, R_C (usually 100-300 kΩ) is

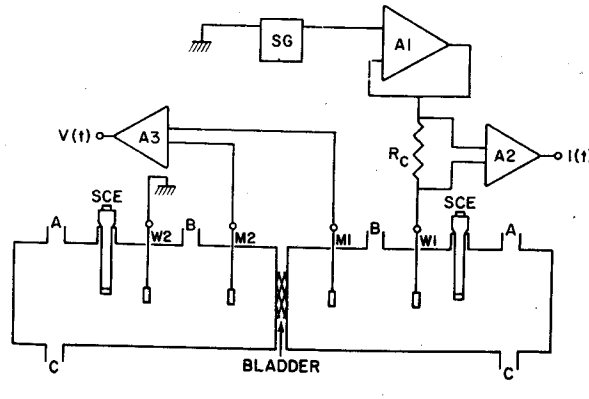

Fig. 3. Block diagram of the circuitry and measurement chamber employed for the transient impedance determination of the isolated toad urinary bladder membrane. The conductivity type chamber is shown in some detail and depicts perturbing (W1 and W2) and measurement (M1 and M2) electrodes along with the saturated calomel electrodes (SCE) employed for resting potential measurements. A, B, and C are solution/gas inlet, gas outlet, and solution outlet, respectively. Al is the tunable amplifier utilized for current injection via signal generator, SG. R_C current limits and allows input current measurement via amplifier A2. Voltage response is obtained via amplifier A3.

current limiting and allows the input current pulse, $I(t)$, to be measured via amplifier, A2. The voltage response, $V(t)$, is measured using electrodes M1 and M2 into amplifier, A3. Both A2 and A3 are the vertical amplifiers (Tektronix 5A22N) of a dual beam oscilloscope (Tektronix 5103N) which is used to display $V(t)$ and $I(t)$, simultaneously. Records of these time domain signals are obtained photographically so that data points required for analysis via digital Laplace transformation (33, 52) are available. Presently the system is being equipped with a transient recorder to increase accuracy and manipulation speed (necessary for living system studies). The total relaxation time of the toad bladder membrane system has a duration of approximately 10-50 msec (with an apparent area of 1 cm^2). In order to obtain data points with sufficient time resolution (from <100 μsec to 50 msec) a series of four to five records in overlapping time ranges are usually obtained.

The experiments reported here have been carried out in isotonic amphibian Ringer's solution. However, it is to be noted that modifications in ionic concentration in either or both mucosal and serosal compartments which will not destroy the viability of the membrane system are in progress. These studies are designed to evaluate the effect of (particularly) Na^+ on the impedance parameters related to transport. In addition it is well known physiologically that antidiuretic hormones such as arginine-vasopressin or oxytocin have a profound influence upon Na^+ (53-55) (as well as urea and water) transport via membrane surface interactions (49, 56, 57). Experiments using the above conditions will be reported elsewhere.

Results and Discussion

In order to obtain the toad bladder membrane system impedance from the experimentally obtained time domain input and response curves the data was first transformed into the Laplace frequency domain. As in previous applications of this technique (58) real axis ($s = \sigma$) transformation was employed. The basics of this approach have been extensively described elsewhere (33, 52). Essentially, digital Laplace real axis transformation is carried out using an algorithm which assumes exponential behavior between successive data points in time. The highest obtainable frequency is limited only by the time at which experimental data is first available. The requirement therefore, is not that the input pulse, here $I(t)$, reach its constant value before data are useful, but that the detection instrumentation be capable of recording the initial rise from zero amplitude. The current pulse risetime in these experiments was approximately 1 μsec allowing the first data point at 100 nsec to be obtained relatively easily. Using the above approach the real axis impedance, $Z(\sigma)$, was evaluated over the frequency range 1 Hz to 1 MHz.

Once $Z(\sigma)$, which is a real function, was available, the next step was to apply diagnostic criteria to ascertain the applicability of the model proposed earlier. This was performed by first examining high and low limiting frequency regions. Referring to Eq. [10] and [11] and Fig. 2 it can be seen that, at high frequencies, the following functional relationship would be expected to hold

$$\underset{\sigma \to \infty}{Z(\sigma)} = R_e + \frac{1}{C_d \sigma} \qquad [21]$$

where R_e is now the electrolyte resistance between the measuring electrodes (M1 and M2, Fig. 3) and the bladder membrane, and C_d is defined in Eq. [12]. Inspection of Eq. [21] shows that at the highest accessible frequencies, a plot of $Z(\sigma)$ *vs.* $1/\sigma$ should be a straight line from which R_e and the dielectric (and double layer) membrane capacitance may be obtained. A typical plot of this type is shown in Fig. 4. It can be seen that indeed the membrane system responds as expected in this frequency range. C_d obtained in this manner is approximately 1.7 $\pm$ 0.5 $\mu F/cm^2$. Note that the dielectric capacitance of the serosal (inner) membrane is in series with that of the mucosal membrane. An attempt to separate its contribution to the observed C_d was not the object of this preliminary study.

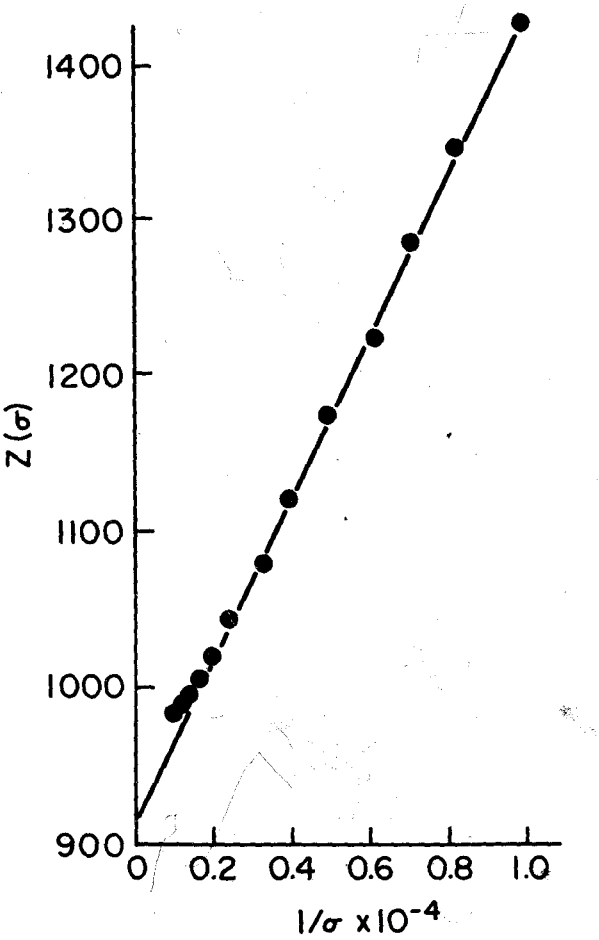

Fig. 4. High frequency diagnostic plot of the real axis impedance $Z(\sigma)$ *vs.* $1/\sigma$ allowing the determination of R_e and dielectric (and double layer) toad urinary bladder membrane capacitance, C_d. See text for details.

Referring again to Eq. [10] and [11] and Fig. 2, it can be seen that in the low frequency limit (prior to steady state conditions) $Z(\sigma)$ would be expected to exhibit the following behavior

$$Z(\sigma) = R_e + R_p + \left(\frac{R_D}{C_D \sigma}\right)^{1/2} \qquad [22]$$

where R_p is defined in Eq. [13], and R_D and C_D in Eq. [17] and [18], respectively. This relationship shows that in the lowest frequency ranges, compatible with that at which steady-state behavior occurs, a plot of $Z(\sigma)$ *vs.* $\sqrt{1/\sigma}$ should be a straight line. Plots of this type over the expected frequency range failed to reveal the anticipated functional behavior. This led to the preliminary conclusion that, under the linear conditions of this experiment, the response could indeed involve the Na^+ ion since its extracellular concentration ($\simeq$0.15M) is too high for significant aqueous diffusion polarization to exist.

Because of the above results and the fact that the bladder membrane exhibits steady-state current behavior it now had to be established whether the transporting species (Na^+) exhibits phase transfer polarization (slow partitioning kinetics) and whether it or another (high aqueous concentration) species exhibits

specific adsorption. Knowledge concerning these two possibilities would reveal whether (Na^+) partitioning depends upon its (or other species) specific interaction with the membrane penetration site, or whether this site undergoes structural (conformational ?) changes or rearrangements as a function of potential to allow (Na^+) entry and/or exit. It has, for example, been proposed (59) that Ca^{2+} adsorption (or binding) at the extracellular entry to the Na^+ pore regulates Na^+ permeability in epithelial cell systems. However, if this were the case, then the response of this membrane system to a linear perturbation should contain a potential dependent adsorption or binding contribution involving Ca^{2+}. This does not appear to be the case for the present study since Ca^{2+} adsorption kinetics would also lead to a limiting low frequency linear function of $Z(\sigma)$ *vs.* $1/\sqrt{\sigma}$ because of its low ($\simeq$ 1.5 mM) aqueous concentration.

It is now necessary to consider whether or not specific adsorption not coupled to aqueous diffusion is one of the kinetic steps in the transient response of the bladder membrane. Examination of the mass balance condition given in Eq. [9] shows that, if specific adsorption of the transporting species (Na^+) is present, then relaxation to steady state would necessitate a new equilibrium surface concentration, Γ'_e. At this point no further steady-state current could pass because this would involve the injection of much more charge than could be compensated by the adjustment in interfacial concentration of the transporting species. Since this is obviously not the case experimentally, it is necessary to invoke the specific adsorption of a species other than that which undergoes phase transfer. This is done by considering that specific adsorption, if present, in this case, contributes to the charging current, $i_c(s)$, only. Thus $i_c(s)$ is now given by

$$i_c(s) = sq(s) = q_E s\eta(s) + q'_e s c'_e \Delta c'_e(o,s) \quad [23]$$

where c'_e is the bulk concentration of the adsorbing species. The relative change in aqueous concentration of the adsorbing species at the plane $x = 0$, $\Delta c'_e(o,s)$, is related to its adsorbed surface concentration change by an equation similar to Eq. [4]. In addition mass balance is given by

$$\Gamma'_e s \Delta\Gamma'_e(s) = D'_e c'_e \left(\frac{d\Delta c'_e}{dx}\right)_{x=0} \quad [24]$$

which states that the flux in aqueous phase (right-hand side Eq. [24]) of the adsorbed species is equal to its change in surface concentration. Using all of the above, the charging admittance $Y_c(s) = 1/Z_c(s)$ is now written as

$$Y_c(s) = q_E s + \frac{q'_e s\Gamma'_e}{1 + (s\Gamma'/v'_e)} \quad [25]$$

where the second term on the right-hand side represents the contribution of finite adsorption (binding) kinetics to the over-all charge requirements of the interface.

In contrast to the charging impedance which has increased in complexity (compare with Eq. [10] because of a possible specific adsorption process, the phase transfer impedance $Z_p(s)$ becomes less complex because Na^+ does not appear to be involved in this process. Thus in the absence of specific adsorption and aqueous diffusion the phase transfer impedance becomes

$$Z_p(s) = \frac{1}{I_E}\left[1 + \frac{I^m}{nFc^m D_m\left[\frac{FV}{2RT} + \frac{1}{\delta_m} + \frac{1}{3}\left(\frac{F^2\bar{V}}{4R^2T^2} + \frac{s}{D_m}\right)\delta_m\right]}\right] \quad [26]$$

in which all of the terms have been previously defined.

Inspection of Eq. [25] and [26] shows that, if the toad bladder membrane system responds in the manner just proposed to linear perturbation, then its aperiodic

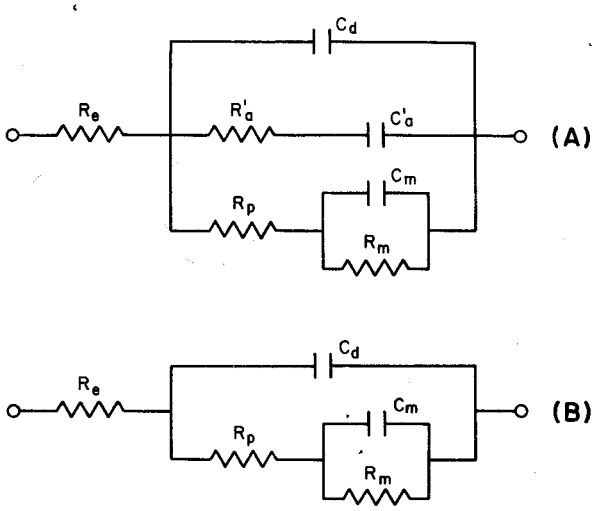

Fig. 5. Aperiodic equivalent electric circuits employed to analyze for the specific phase transfer behavior of the toad urinary bladder membrane system. The top circuit (A) shows a specific adsorption pathway coupled with phase transfer while the bottom circuit (B) shows only the phase transfer process. Finite adsorption was undetected in this study, and the frequency spectrum of $Z(\sigma)$ corresponds therefore, to (B) wherein R_p (partitioning) appears to be the rate-determining step.

equivalent electric circuit would be that given in Fig. 5a. Here all of the parameters have physical significance as defined previously. This model states that a specific adsorption (binding) process not involving phase transfer can occur (R'_a, C'_a) and that a certain quantity of charge can flow to readjust the surface concentration of the adsorbing species to its new resting value. This process does not involve the species undergoing phase transfer, but may, of course have an effect on its kinetics. The species contributing ultimately to the steady-state current through the membrane now follows the R_p, C_m, R_m (Fig. 5a) pathway in its relaxation to steady state. If specific adsorption is absent from the transient response of the bladder membrane to linear perturbation, it can readily be seen that its behavior would be that represented in Fig. 5b. Here the charging impedance is given by Eq. [10] and the phase transfer impedance by Eq. [26].

Analysis for either of the above types of behavior is carried out in a straightforward manner using real axis Laplace plane methods. First, advantage is taken of the fact that both R_e and C_d have been isolated in the high frequency range (Fig. 4). Next, if the adsorption time constant ($R'_aC'_a$) is sufficiently different from that of phase transfer (R_e, R_m, C_m) then R'_a and C'_a, can be isolated from the following

$$\frac{1}{1/(Z(\sigma) - R_e) - C_d\sigma} = R'_a + \frac{1}{C'_a\sigma} \quad [27]$$

which states that a plot of the experimentally known quantity on the left-hand side *vs.* $1/\sigma$ should be a straight line in the intermediate frequency range. This would then allow the evaluation of R'_a and C'_a. These parameters are then employed to determine the remaining quantities in the phase transfer pathway over a lower frequency region via

$$\frac{1}{1/(Z(\sigma) - R_e) - C_d\sigma - (1 + R'_aC'_a\sigma)/C'_a\sigma} = R_p + \frac{1}{C_m\sigma} \quad [28]$$

from which a plot of the left-hand side should be linear in $1/\sigma$. Finally the membrane transport parameter R_m is obtained from knowledge of the steady-state voltage response. If finite specific adsorption kinetics are not present Eq. [28] would no longer be valid. However, the behavior described in Eq. [27] would still obtain, R'_a and C'_a being substituted by R_p and C_m, respectively.

The above diagnostic procedures were employed in the lower and intermediate frequency ranges. As can be seen in Fig. 6 the functional behavior described by Eq. [27] exists over the intermediate frequency range. It was however, not possible to detect another frequency range over which Eq. [28] was obeyed. This appeared to indicate that the over-all transient impedance of the bladder membrane system would be given by Eq. [10] and [26] as depicted in Fig. 5b. In fact R_p and C_m were evaluated from plots such as Fig. 6 and R_m obtained from a knowledge of the steady-state voltage response. These values, along with those determined for R_e and C_d over the high frequency range (Fig. 4) could then be employed to reconstruct the entire $Z(\sigma)$ spectrum obtained experimentally. This was convincing evidence that the impedance of the toad urinary bladder membrane system could be represented by the aperiodic equivalent circuit shown in Fig. 5b. The values obtained in these preliminary experiments for the phase transfer parameters are $R_p = 400 \pm 125\ \Omega cm^2$, $C_m = 2.7 \pm 0.9 \times 10^{-3}\ F/cm^2$ and $R_m = 30 \pm 10\ \Omega cm^2$. Note that, while finite specific adsorption kinetics were not observed (*i.e.*, Fig. 5a), this did not preclude the possibility of very rapid adsorption kinetics. Thus, referring to Fig. 5a, it can be seen that if $R_a \rightarrow 0$ (infinitely rapid kinetics) then the observed value of C_d would in fact be a sum $(C_d + C_a)$ representing electrostatic and adsorption effects. This possibility is presently under study by examination of the effect of various ions on "C_d."

As is common for biological systems the scatter in the data (from toad to toad) is relatively high. This does not preclude, however, analysis of the mechanistic pathway. Thus, for every bladder membrane examined, the behavior could be described by Eq. [10] and [26] as represented in Fig. 5b. This behavior persisted even when the transepithelial resting potential

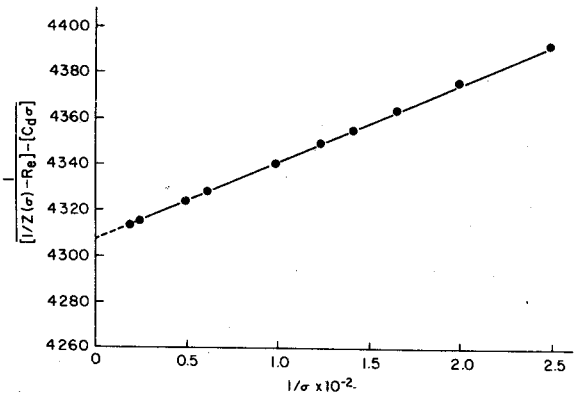

Fig. 6. Intermediate frequency plot of the real axis Laplace plane impedance data using the functional relationship described in Eq. [27] establishing, along with Fig. 3, that the toad urinary bladder membrane exhibited dielectric (and possible infinitely fast specific adsorption) behavior, and that the pathway for Na^+ transport involves slow phase transfer and fast membrane transport.

was lower (<20 mV) than would normally be accepted in biological studies of this membrane system. This is interpreted to mean that the species responsible (Na^+) for the phase transfer portion of the total response did not change its intracellular concentration enough to necessitate compensating action by an active transporting system. This was expected because of the low quantity of charge injected with each perturbation.

Examination of the data obtained reveals that the interfacial (potential dependent) phenomenon of phase transfer (partitioning) of Na^+ is by far the rate-limiting step for its memorane transport. The value of the phase transfer impedance, R_p, is significantly larger than its kinetically relevant counterpart for membrane transport, R_m. This result shows that the interfacial structure (in this case the mucosal interface) determines to a large extent, the passive transport properties of this cell system. Previous analyses using single (38) and bilayer artificial (39-41, 60) membrane systems have dealt with the effect of the diffuse double layer structure upon transport. Remarkably the simple Gouy-Chapman theory quantitatively predicts the shift in current-voltage relationships at steady state in many cases. However, this agreement may be fortuitous even in artificial systems since it is apparent that some divalent cations (*e.g.*, Ca^{2+}) have more specific structural effects than others (61). It is also not clear that the compact double layer, which must exist at interfaces of this type, has been adequately taken into account. Certainly steady-state measurements will not be revealing in this respect. Recent studies involving attempts to isolate the dielectric from double layer capacitance at membranes have been described (42) and may represent the most significant approach thus far in the elucidation of the structure of these interfaces.

The apparent rapidity of membrane transport for this system would appear to favor a pore rather than a carrier interpretation, in agreement with a recent study of membrane noise in a similar epithelial system (62). The determining factors of pore penetration are of course not discernible at this stage. It is not possible, for example, to preclude the involvement of a specific adsorption process which could cause a "gating" phenomenon for Na^+ transport. A suspicion that this may be taking place can be invoked to account for the somewhat high value of C_d observed. In the present study, the adsorption process (if present) appears infinitely fast, but could be separable from the dielectric response by analysis of the system while varying ionic concentration, or by careful high frequency measurements which are presently being carried out.

It is believed that this is the first study of this type on the toad bladder membrane system. Placed in the context of classical studies it is hoped that these results will shed further light on the highly complex transport function of this type of living cell. It is interesting to note that, while short-circuit steady-state studies reveal the existence of a significant intercellular (leak) pathway for current flow (46, 63), this was not detected in the present work. Most probably this is due to the fact that short-circuit conditions are highly nonlinear and usually result in a significant modification in the structure of the intercellular spaces (29). The present study may thus provide an experimental condition in which the bladder is as physiologically undisturbed (electrically) as possible.

Conclusion

This study has invoked the existence of dynamic interfacial electrochemical phenomena, which constitutes the basis of the electrochemical information transfer *in vivo* concept, to provide a model for the elucidation of living cell function. The use of finite kinetics for phase transfer and specific adsorption has allowed a unified approach to membrane impedance to

be applied to the toad urinary bladder membrane system. The modeling approach, which is carried out in the Laplace plane, allows the heterogeneous nature of biological membranes to be treated in a straightforward manner. In this application it has proved successful in a preliminary isolation of the mechanistic pathway for passive Na^+ transport by a careful functional analysis over the wide frequency range available through the use of real axis Laplace transformation. The data obtained reveals that phase transfer and not membrane transport is the rate-limiting step for Na^+ transport. The determining factor for Na^+ entry is not yet clear, but it is intriguing to consider the approach (43, 73) in which the polar groups blocking entry are deformed by the high electric fields present at the interfaces when the penetrating ions interact with, *e.g.*, the lipid structures. The transient injection of charge, as carried out in this study, would then act to provide a sufficient change in interfacial field to allow those Na^+ ions with sufficient energy to penetrate the membrane phase and be literally propelled through to the other side. The deformation of an entry site could of course be caused by a specific adsorption process and would simply constitute a logical extension of the basic premise.

This study has revealed that it is possible to employ modern bioelectrochemical approaches to the study of living membranes. It was begun with the conviction that dynamic interfacial electrochemical processes constitute basic biological steps in cell function. Here a small charge injection has resulted in the modification of a transport function by causing a potential dependent change in interfacial membrane structure. Other biochemical functions appear also to be dependent on similar effects. For example, specific adsorption can influence the activity of membrane bound enzymes (8, 9, 64-66). Transmembrane potential changes as a result of protein synthesis (67) suggest at least a modification in transport properties. The surface charge of differentiated *vs.* undifferentiated (68) and normal *vs.* malignant (69) cells is significantly different, allowing at least the possibility of different interfacial structures which could cause cellular function change. Cell-cell and cell-tissue interaction appears to be greatly dependent upon surface structure and charge (70-72, 74-76). It is hoped therefore, that studies such as this will aid in the elucidation of cellular mechanisms so that even more significant clinical applications will be forthcoming. Certainly, the current success in the healing of otherwise incurable bone fractures by very specifically encoded charge injection (25), the parameters for which were chosen by analyses similar to that given here, warrants significant efforts in this area of bioelectrochemistry.

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Thermodynamic Potential for the Anodic Dissolution of n-Type Semiconductors

A Crucial Factor Controlling Durability and Efficiency in Photoelectrochemical Cells and an Important Criterion in the Selection of New Electrode/Electrolyte Systems

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ABSTRACT

We show that the standard potential for anodic dissolution, $E_D°$, of n-type semiconductors (*e.g.*, $E_D°$ for $CdS \leftrightarrows Cd^{2+}(aq) + S + 2e^-$ is +.0.08V *vs* SCE) plays a key role in ultimate efficiency of thermodynamically stable n-type semiconductor-based photoelectrochemical cells. The selection of redox active substances that can be used for competitive capture of photogenerated holes is limited to those systems where the product does not have the potential to oxidize the semiconductor. Where E_{VB} and E_{CB} represent the position of the valence and conduction band positions at the interface, respectively, we must conclude that $E_D°$ is at a more negative potential (*vs.* a reference) than E_{VB}, if the semiconductor undergoes photoanodic dissolution. Quenching of the photoanodic dissolution by competitive hole capture by some electrolyte component, say A, is possible if $E_{redox}(A^-/A)$ lies at a potential more negative than E_{VB}. But additionally, if the A^-, the oxidation product, is to be incapable of oxidizing the semiconductor, $E_{redox}(A^-/A)$ must be more negative than $E_D°$. Consequently, for the semiconductor to be thermodynamically stable in the A/A^+ electrolyte the maximum photovoltage output, $(E_{CB}-E_{redox})$, can be no greater than $(E_{CB}-E_D°)$ for E_{CB} more negative than E_D.° Naturally, kinetic inertness of the semiconductor to an oxidant with E_{redox} more positive than $E_D°$ may allow its presence and/or use in a more efficient cell. N-type CdS and TiO_2 semiconductors in aqueous electrolytes are treated in detail. Some preliminary comments are made concerning p-type materials.

Much attention has been focused recently on the use of certain redox active electrolytes to "quench" the photoanodic dissolution of small bandgap, nonoxide, n-type semiconductors (1-6). Broadly interpreted, the success realized can be attributed to the ability of the redox active species to capture photogenerated holes before the holes participate in oxidation reactions of the semiconductor. At present there is no detailed understanding of the factors controlling the competi-

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