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PRESSURE JUMP RELAXATION KINETICS OF FROG SKIN OPEN CIRCUIT VOLTAGE AND SHORT CIRCUIT CURRENT

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Summary

The relaxation kinetics of frog skin open circuit voltage, V_{oc}, and short circuit current, I_{sc}, was studied by analyzing the effects of subjecting the tissue to sudden increments of hydrostatic pressure. Both V_{oc} and I_{sc} are perturbed by the pressure jump. Changes in V_{oc} can be resolved into three components: a rapid decrease (phase I), a second, additional decrease with time constant 2.2 s (phase II), and finally a very slow increase found only in some preparations. The amplitudes of phases I and II are linear in the range of pressures studied (<350 atm) and have respective pressure coefficients of $-1.2 \cdot 10^{-4}$ atm^{-1} and $-3.7 \cdot 10^{-4}$ atm^{-1}.

Under short circuit conditions phases I and II persist. The pressure coefficients of the amplitudes of phases I and II, $-4.3 \cdot 10^{-4}$ atm^{-1} and $-5.0 \cdot 10^{-4}$ atm^{-1}, respectively, are larger than those of V_{oc}, but the time constant of phase II, 2.2 s, is the same. The sum of the amplitudes of phases I and II is directly proportional to I_{sc} when it is inhibited with ouabain. It is argued that in both electrical states pressure perturbs the same transport mechanism giving rise to phases I and II of V_{oc} and of I_{sc}.

The magnitude of the pressure coefficients of these processes implies that they arise from chemical reactions, rather than from simple, physical solution properties. Comparison of the pressure jump kinetics with the previous spectral analysis of the electrical fluctuations of frog skin suggests a common origin for both sets of phenomena.

Introduction

An important insight into the mechanism of active transport would be available if there were detailed knowledge of the kinetics of the underlying phenomena. The kinetic characterization of these processes provides a basis for comparing them to biochemical reactions known to occur within the transporting tissue and for the evaluation of hypothesized mechanisms. Thus far, kinetic

data has been available from three sources: radioactive tracer flux measurement, effect of sudden compositional changes of surrounding media [1,2], and through the analysis of the electrical fluctuations attendant to the transport of charged species [3—5]. The present study was undertaken, first, as a search for macroscopic manifestations of the stochastic events found previously and, second, to find a convenient means for obtaining a kinetic description of active transport outside the scope of existing techniques.

When a system which is in a stationary state is perturbed by a sudden alteration of one of the state variables, it relaxes to, or approaches, a new state with a temporal pattern determined by and revelatory of the kinetic parameters of the underlying processes. I have found that a rapid step of hydrostatic pressure is a conveniently manipulated variable which produces observable kinetic effects on both the open circuit potential across frog skin and its short circuit current.

Brouha et al. [6] and Pequex [7,8] have described and analyzed a steady-state increase of open circuit voltage which occurs during a maintained increment of hydrostatic pressure. Pressurization of their preparation was at a rate at least two orders of magnitude slower than with the present method. It will be seen that the relaxation transients found here would not have been manifest without the rapid compression employed. The focus of these experiments is the rapid phenomena although brief mention is made of confirmatory evidence of the previously reported slow hyperpolarization effect.

Normally metabolizing frog epithelium maintains a potential across itself when placed as a barrier even between solutions of identical composition. This potential and the externally supplied current required to short circuit it to zero are measures of the rate of active sodium transport across the skin. The following direct current equivalent circuit is consistent with both the tracer net flux data [9] and the observed linearity of the current voltage relationship over the range of voltage between open and short circuit conditions [10,11]. If the skin contains a generator of constant sodium current, I^*_{Na}, flowing in the anatomical inward direction in parallel with an electrical resistance, R, then the voltage, V, across the skin is IR, where I is equal to $I_e + I_{sc}$, I_e is the current drawn from or supplied to an external source connected across the skin, and I_{sc} is the passive electrical current flowing through R to compensate for I^*_{Na}. I_{sc} is due primarily to the inward movement of anions, but it can contain a non-metabolically linked sodium component. I_{sc} is the short circuit current of the skin i.e. $-I_e = I_{sc}$ when $V = 0$.

Under open circuit measuring conditions I_e is zero and the voltage, V_{oc}, is equal to $I_{sc}R$. If I_{sc} and R are each functions of hydrostatic pressure,

$$\Delta_p V_{oc} + V_{oc} = (I_{sc} + \Delta_p I_{sc})(R + \Delta_p R)$$

where Δ_p denotes the change in the quantity induced by a pressure increment. For reasonably small Δ_p values, as the present experimental results will be seen to imply, the expression reduces to $\Delta_p V_{oc} = R\Delta_p I_{sc} + I_{sc}\Delta_p R$.

Thus in general, $\Delta_p V_{oc}$ is the consequence of the combined effect of pressure on both R and I_{sc}. Measurements of the effect of pressure on V_{oc} alone are not sufficient to separate the contributions of the two components. However, the component due to R can be eliminated by short circuiting the skin poten-

tial to zero. If the potential is reduced to zero and maintained there by active control, then $IR = 0$ since $I_e = -I_{sc}$. Under these conditions, $\Delta_p I_e = -\Delta_p I_{sc}$ and the pressure dependence of the short circuit current is available directly.

Methods

Samples of abdominal skin of the frog, *Rana pipiens*, were mounted in a miniature version of a Plexiglas holder of conventional design. A circular piece of abdominal skin, 5 cm^2 in area, was tied along its periphery with silk thread to the protruding disk shaped face of one half-chamber in the fashion of a drumtop. The opposing walls of the two half-cells were pierced by coaxial circular holes which delimited the area of skin exposed to measurement to 0.33 cm^2. Sealing of skin to the Plexiglas surfaces was affected with silicone stopcock grease; the two half-cells were lightly clamped together. The volume of solution on either side of the skin was 1 cm^3. Each solution contained a pair of Ag/AgCl electrodes: one for potential measurement and the other for supplying current.

The hydrostatic pressure system consisted of a Parr Instruments (Moline, Ill.) Model 4740 Pressure Bomb modified by the manufacturer to contain four insulated electrical feedthroughs. The bomb was connected to one of the two ports of a high pressure valve (Autoclave Engineers, Erie, Pa.) by means of flexible hydraulic hose. The second port of the valve was connected to an hydraulic hand pump by hydraulic hose. The pressure at the outlet of the pump was monitored with a mechanical gauge (Enerpac Model GP-10). The pump, tubing, and pressure bomb were filled with hydraulic fluid (Enerpac Model HF-102), care being taken to eliminate air bubbles. The skin holder, with sample mounted in place and its two chambers filled with Ringer solution, was submerged directly into the fluid of the bomb. The oil, being less dense than Ringer solution, did not tend to displace it. The bomb, containing the skin holder and its cap sealed in place, was immersed in a large volume of water, the temperature of which was maintained at 20.0°C, except where noted.

The skin sample was subjected to an hydrostatic pressure jump in the following manner. The Autoclave Engineers valve was closed firmly, and the pressure was elevated. The valve was then opened by smartly turning its stem the required half-turn. The respective volumes of the two parts of the system on either side of the valve were such that the pressure within the system equalized to a value of about one-half that of the initial high pressure side of the valve. The time course of the pressure within the bomb was monitored, when desired, by measuring the resistance change of a cracked carbon composition resistor mounted in place of the skin holder. The pressure rose within the bomb along a sigmoidal path, the risetime (time to increase from 10 to 90% of the steady-state level) of which was 40 ms. Provided the valve was opened rapidly, the pressure change was highly reproducible.

The potential across the skin was measured with a direct current coupled differential amplifier (Model 113, Princeton Applied Research Corp., Princeton, N.J.) with input impedance $>10^9$ Ω. The voltage was displayed either by a storage oscilloscope (Telequipment Model DM64, Tektronix, Inc., Beaverton,

Oreg.) or a strip chart recorder (Model 15-6327-57, Gould, Inc., Cleveland, Ohio; Model 127 Sanborn Co., Waltham, Mass.).

Short circuiting of the skin potential was achieved automatically by connecting the amplifier output through two additional stages of amplification (Model P85AU, Philbrick/Nexus Research, Inc., Dedham, Mass.) to a 5 kΩ resistor and thence to one of the current electrodes of the chamber. The sign of the feedback was negative; the total open loop gain of the amplifier chain was 1000—10000. The closed loop frequency response of the system was such that the potential across the skin could be changed in less than 10 ms, i.e. much faster than the rate of increment of hydrostatic pressure. During a pressure jump the deviation from zero of the potential across the skin, as measured at the voltage electrodes, was no more than 0.25 mV. With the skin sample present and the chamber filled with Ringer solution the resistance of the fluid between the voltage probes was 130 Ω, compared to the approx. 4000 Ω of a skin sample. Thus, the short circuiting of the skin potential was about 97% complete. The short circuit current flowing through the skin was measured by the voltage drop it produced across a 1 or 10 kΩ resistor inserted in series with the second, current electrode of the chamber or with an operational amplifier current to voltage converter. The slightly dissimilar junction potentials of the voltage electrodes were compensated for electrically within the feedback loop.

Pressure jumps of the magnitude employed in the study caused slight electrode artifacts (Fig. 1). They were recorded across the voltage probes before and after each experiment by subjecting the empty Ringer-filled chamber to the same series of pressure steps as in the experiment. When the artifacts became excessive the electrodes were replaced by new ones. It seems that the insulating varnish fails after repeated pressure cycling.

The rapid alteration of pressure within the bomb gives rise to temperature changes which must be considered. The kinetics of the change in temperature of the fluid within the skin holder was monitored by observing the alterations in the resistance of the solution between the voltage electrodes when the skin holder contained nothing but 0.1 M KCl. A 1 kHz constant amplitude sinusoidal current was applied through the current electrodes; it was of sufficient amplitude to produce a voltage drop of about 10 mV peak-to-peak. This voltage was sensed by the usual amplifier and the amplitude measured with a phase-sensitive signal detector (Model 822, Keithley Instruments, Inc., Cleveland, Ohio). The signal generator and detector were sufficiently stable so that resistance changes within the chamber of less than 0.1% could be observed easily.

Rapid compression of the contents of the bomb has three descernable effects on electrolyte resistance within the Plexiglas chamber: first, a sharp decrease with a risetime of about 40 ms followed by, second, a smaller, additional decrease with a time constant of about 2 min and, finally, a slow return (5 min time constant) to an asymptotic value between that of the first effect and the resistance at atmospheric pressure.

The initial, rapid resistance decrease is the sum of the steady-state effect of pressure upon resistance and that due to the adiabatic heating of the electrolyte solution consequent to its sudden compression. The second, slower fall in resistance can arise from the compressive heating of the Plexiglas walls of the chamber and/or the hydraulic fluid in which it is immersed. The kinetics of this

effect would be that of the exchange processes between the walls and the fluids which they contact. The resistance of the electrolyte solution attains a final value when all the heat changes have been dissipated, returning the temperature to its initial atmospheric value, that of the water bath in which the bomb is immersed. This equilibration process gives rise to the third phase of resistance change.

Thus, the rate-limiting step in the final attainment of thermal equilibrium following compression should be the conduction of heat from the solution within the chamber, across its walls to the hydraulic fluid, and finally across the walls of the bomb to the isothermal water bath. That this is so is evident from the effect of plunging an electrolyte-filled chamber, initially at room temperature, into a large volume of ice water. The resistance rises along an approximately exponential time course with a time constant of 5 min. The good agreement between this figure and the third time constant of resistance change following the pressure jump implies that the thick steel walls of the pressure bomb do not introduce a significant additional delay in the attainment of thermal equilibrium.

Quantitatively, the initial fractional resistance change is $-1.20 \cdot 10^{-4}$ atm^{-1} and the second is an additional $-0.17 \cdot 10^{-4}$ atm^{-1}. The measured steady-state pressure coefficient of the resistance is $-0.85 \cdot 10^{-4}$ atm^{-1}. These are average values deduced from five determinations following pressure increments between atmospheric and 204 or 314 atm. Symmetric mirror image effects were obtained when the bomb was equilibrated thermally at an elevated pressure and the pressure was suddenly released to atmospheric.

The maximum peak temperature change generated within the electrolyte solution can be calculated by noting that the conductivity of 0.1 M KCl varies by 2.0%/°C and that that portion of the total change in resistance attributable to temperature is $(1.20 + 0.17 - 0.85) \cdot 10^{-4}$ atm^{-1} = $0.52 \cdot 10^{-4}$ atm^{-1}. This is equivalent to $2.6 \cdot 10^{-3}$°C · atm^{-1}. The maximum pressure increment in this study was 374 atm and it would produce, therefore, a temperature rise of 1.0°C within the solution surrounding the skin sample.

The specific conductance of 0.1 M KCl increases fractionally by $0.62 \cdot 10^{-4}$ atm^{-1} [12]; the specific volume of H_2O at 20°C decreases by $0.54 \cdot 10^{-4}$ atm^{-1} [12] within the range of pressure increments employed here. Therefore the total pressure dependence of the resistance of the electrolyte within the chamber should be $-1.16 \cdot 10^{-4}$ atm^{-1}. The figure obtained experimentally, $-0.85 \cdot 10^{-4}$ atm^{-1}, implies that the major portion of the coefficient of resistance change within the chamber is due to the sum of solvent compressibility and the pressure dependence of the specific conductance of KCl. The additional $0.31 \cdot 10^{-4}$ atm^{-1} might arise from slight dimensional changes consequent to compression of the Plexiglas.

Results

Open circuit voltage

The open circuit potential developed by frog epithelium when placed as a septum between volumes of Ringer solution decreases when subjected to a sudden increase in hydrostatic pressure, as illustrated in Fig. 1. Not illustrated

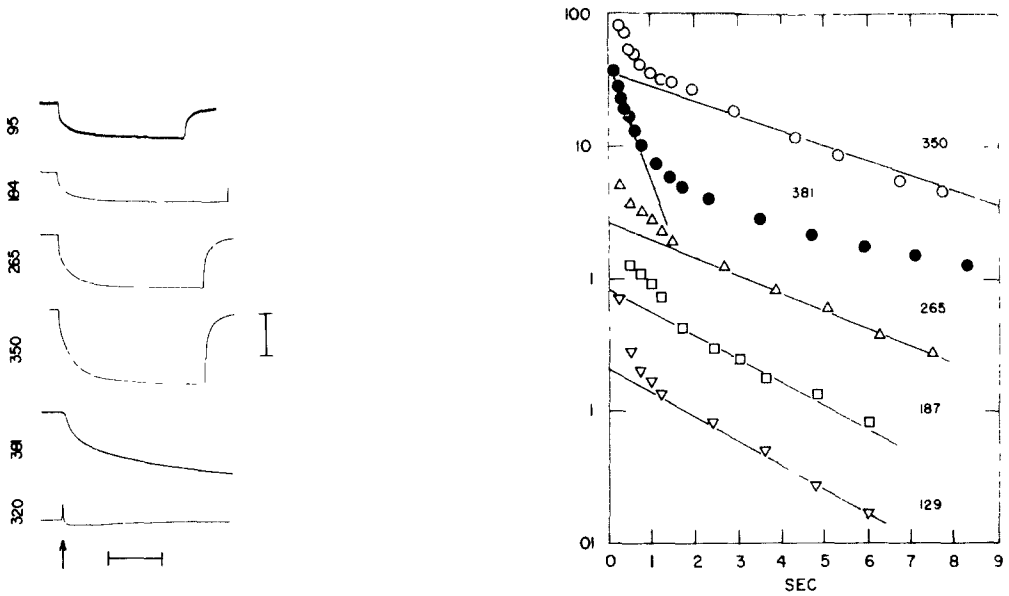

Fig. 1. Effect of a sudden step of hydrostatic pressure on open circuit voltage, V_{oc}, across the frog skin. Arrow denotes moment of application of pressure, number near each record is the increment in pressure in atmospheres above atmospheric. Where included, the rapid upward deflection marks the termination of the pressure step. Downward deflection is a decrease in the magnitude of the voltage. Vertical bar subtends 5 mV for the top record, 12.5 mV for the next four records, and 2.5 mV for the bottom one. Horizontal bar subtends 12 s for the upper four records, 1 s for the fifth and 5s for the bottom one. That record is of the electrode artifact found with no sample present and chamber filled with Ringer solutions, artifact is approximately proportional to pressure. Sample No. 1.

Fig. 2. Logarithm of the absolute value of the time derivative of V_{oc} as a function of time during a pressure step. The step was initiated at zero time and was of the magnitude in atm indicated by the number near each graph. Individual data sets are arbitrarily separated vertically for clarity. The • record (fifth record of Fig. 1) was made at higher speed, for it the abscissa is 3-fold faster ("sec" = 0.33 s). Straight lines were drawn by eye and are those used to extract the kinetic parameters as described in text. Sample No. 1.

is a very much slower sigmoidal voltage increase which follows the initial decrease but is evident in only those preparations of less than average resting potential. This phenomenon is most likely the one whose steady-state features were previously studied [6—8]. An analysis of the transient kinetics of this open circuit voltage increase will be presented elsewhere.

Graphical analysis of the voltage decrease reveals two identifiable components: a rather rapid initial decrease (phase I) and a further, slower decrease (phase II). The graphs of Fig. 2 are plots of the logarithm of the voltage derivatives, obtained manually, as a function of time. Two straight lines can be fitted to the data, as is the case when $\Delta V_{oc} = V_I(1 - e^{-\alpha t}) + V_{II}(1 - e^{-\beta t})$ where t is time, α and β are the rate constants of the respective exponentials, and V_I and V_{II} are constants. Thus, $\Delta \dot{V}_{oc} = \alpha V_I e^{-\alpha t} + \beta V_{II} e^{-\beta t}$ and the graph of log $\Delta \dot{V}_{oc}$ vs. t has limiting slopes of $-\alpha$ and $-\beta$, as t approaches zero and infinity, respectively, if $\alpha t >> \beta t$. The intercept of the second term is βV_{II}. When $\alpha t >> \beta t$, a plot of ΔV_{oc} vs. $\Delta \dot{V}_{oc}$ yields a straight line of intercept $V_I + V_{II}$, which also provides V_I once V_{II} is known from the first graph. This analysis is

rather cumbersome but it is more accurate than others as it does not require the assumption that the asymptote of the $\Delta V_{oc}(t)$ record is equal to $V_I + V_{II}$.

The data of Fig. 2 shows that $1/\alpha$, the time constant of phase I, the fast initial decrease of V_{oc}, is about 150 ms. This is too close to the risetime of the pressure step, 40 ms, for α to be measured at all accurately by the present technique. More complex methods do exist for the production of rapid enough pressurization of the contents of the bomb so that α can be determined [13]. However, the present method does adequately measure the steady-state magnitude of phase I, V_I, as the graphical methods employed do not require knowledge of the value of α, other than knowing that $\alpha >> \beta$. Fig. 3 illustrates the pressure dependence of V_I expressed as the percent change of the voltage just before the pressure step. Data, so normalized, was obtained from several different skin samples, pooled and a least squares straight line fitted to it. The slope of this line is the pressure coefficient of V_I: fractionally, it is $-1.18 \cdot 10^{-4}$ atm^{-1} (Table I). Analysis of the same set of data also yields V_{II}, the amplitude of phase II. Its pressure dependence is illustrated in Fig. 3. The pressure coefficient for the pooled data is $-3.70 \cdot 10^{-4}$ atm^{-1} (Table I). Table I also contains the measured pressure coefficient of $V_I + V_{II}$.

In two of the samples studied the time constant of phase II increased significantly with pressure ($P < 0.005$, $P < 0.001$). The second of these is illustrated in Fig. 3. The third sample had a time constant which was significantly independent of pressure ($r = 0.007$, $P < 0.001$). No inference could be drawn from the fourth sample. Thus, the question of the pressure dependence of $1/\beta$ is unsettled. In addition, samples of low resting potential (<40 mV), not included in Table I, show statistically significant decreases of $1/\beta$. Time constants of all the samples of Table I were pooled and fitted with a regression line, the intercept of which is 2.2 s (Table II); its apparent pressure coefficient is also shown.

Short circuit current

The effect of pressure on the open circuit potential across the skin can arise

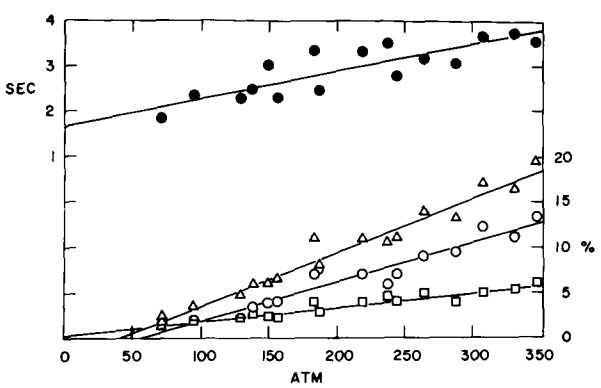

Fig. 3. Pressure dependence of kinetic parameters of $\Delta_p V_{oc}$. Significance of symbols: •, phase II time constant (left ordinate); □, amplitude of phase I (V_I); ○, amplitude of phase II (V_{II}); △, $V_I + V_{II}$. Right ordinate applies to △, ○, and □; it is the percent decrease in V_{oc} measured with respect to the value of V_{oc} just before application of the pressure step. Average resting potential: 106 ± 0.5 mV (± S.E.). Straight lines are least squares regression lines. Sample No. 1.

TABLE I

	Pressure coefficients (10^{-4} atm^{-1})	Samples	Measurements
Open circuit voltage **			
Phase I, V_I	−1.18 ± 0.37 *	4	35
Phase II, V_{II}	−3.70 ± 0.47		
Phase I + phase II, $V_I + V_{II}$	−4.89 ± 0.70		
Short circuit current ***			
Phase I, I_I	−4.28 ± 0.84	4	28
Phase II, I_{II}	−5.01 ± 0.93		
Phase I + phase II, $I_I + I_{II}$	−9.30 ± 0.69		

* Mean ± S.E.
** 99.7 ± 2.5 mV, $n = 35$.
*** (66.9 ± 3.1) × 10^{-6} A/cm^2, $n = 28$.

as the consequence of alterations in I_{sc} and/or R, as described above. When the potential is short circuited and automatically maintained at zero, the electrical manifestation of R is eliminated, but any effect of pressure on I_{sc} remains.

Figs. 4 and 5, illustrating the pressure jump relaxation kinetics of I_{sc}, demonstrate the persistance of phases I and II. Again, the time constant of phase I is about 0.2 s. The short circuit time constant of phase II is comparable to that obtained under open circuit conditions (Table I). Both least squares linear regressions of $1/\beta$ extrapolate to a value of 2.2 s at atmospheric pressure. The pressure coefficient of the short circuit time constant, obtained from the regression line, is not significantly different from zero ($P < 0.25$) (Fig. 6).

There are significant differences between two of the amplitude pressure coefficients, listed in Table I. Both I_I and $(I_I + I_{II})$ (Fig. 6) are larger than the corresponding parameters obtained under open circuit conditions. These differences are significant at the level of $P = 0.01$; the tabulated difference between I_{II} and V_{II} is not significant ($P = 0.4$). As the equivalent circuit analysis shows, such differences between open and closed circuit parameters occur when, first, $\Delta_p R$ is negative, second, $\Delta_p R = 0$, and I_{sc} is not constant but is a function of V or, third, the combination of both $I_{sc}(V) \neq 0$ and $\Delta_p R \neq 0$. Which of the three cases is applicable cannot be decided now, but the matter can be settled by measuring simultaneously $\Delta_p V_{oc}$ and $\Delta_p R$ by a small signal alternating current impedance technique. In any case it is obvious from

TABLE II

	Extrapolation (1 atm) (s)	Pressure coefficients (10^{-4} atm^{-1})	Samples	Measurements
Open circuit voltage				
Phase II time constant	2.16 ± 0.09	15.1 ± 3.1	4	35
Short circuit current				
Phase II time constant	2.18 ± 0.21	−8.58 ± 13.5 *	4	28

* Not significantly different from zero ($P < 0.25$)

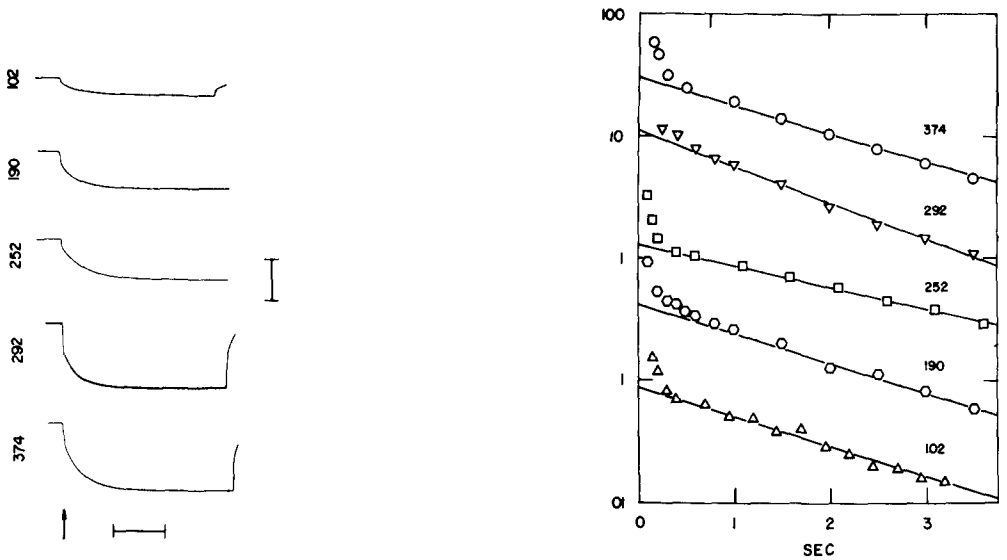

Fig. 4. Relaxation of short circuit current, I_{sc}, during a step of hydrostatic pressure. Arrow denotes moment of initiation of pressure step, rapid upward deflection occurs at end of step. Number near each record is magnitude of the pressure increment above atmospheric in atm. Downward deflection represents a decrease in the absolute value of I_{sc}. Vertical bar subtends $15 \cdot 10^{-6}$ A/cm^2, horizontal bar 5 s. Sample No. 2.

Fig. 5. Logarithm of the magnitude of the time derivative of I_{sc} as a function of time during a pressure step. Numbers near the graphs are the pressure increments above atmospheric in atm, pressure step was initiated at zero time. Data sets are arbitrarily displaced along the vertical axis to improve clarity. Sample No. 2.

the short circuit measurements that $\Delta_p I_{sc} \neq 0$, independent of whether or not $\Delta_p R \neq 0$. Furthermore, since the existing evidence is that I_{sc} does not vary significantly between open and short circuit conditions it follows that a size-

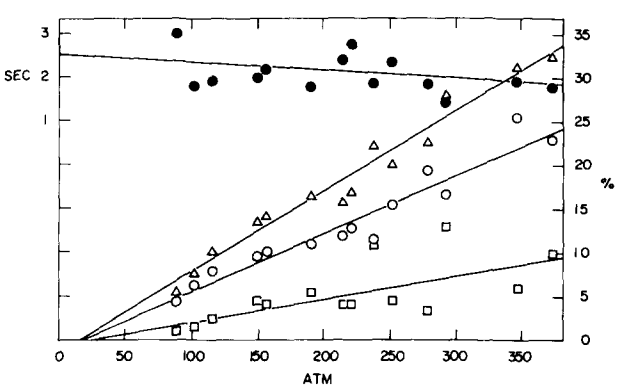

Fig. 6. Effect of pressure on relaxation parameters of I_{sc}. Significance of symbols: ●, phase II time constant (left ordinate); □, amplitude of phase I (I_I); ○, amplitude of phase II (I_{II}); △, $I_I + I_{II}$. Right ordinate applies to ○, △, □; it is the percent decrease in magnitude of I_{sc} measured with respect to I_{sc} just before initiation of the pressure step. Straight lines are least square regression lines. The line for ● is not significantly different from zero ($P < 0.25$). Average value of steady I_{sc}, $(77.1 \pm 1.0) \times 10^{-6}$ A/cm^2. Sample No. 2.

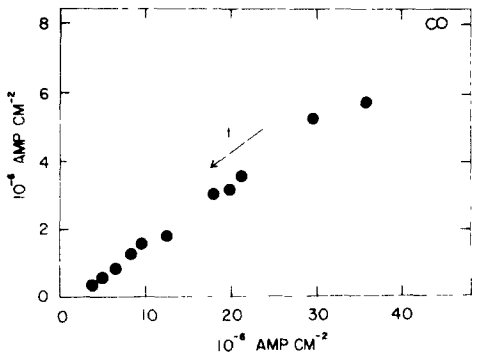

Fig. 7. Effect of ouabain $I_I + I_{II}$ (ordinate) as a function of I_{sc} (abscissa) ○, Ringer solution control just before adding ouabain, ●, after exposing both sides of the skin to 10^{-4} M ouabain in Ringer. I_{sc} decreases with time, direction indicated by arrow, and closely with it, $I_I + I_{II}$. The same pressure step, (244 ± 1.8) atm, was employed throughout the series of measurements. Sample No. 3.

able fraction, at least, of phases I and II of V_{oc} also must be due to an alteration of I_{sc}.

Further evidence that phases I and II arise from a decrease in I_{sc} is the effect of ouabain. As Fig. 7 illustrates, ouabain (0.1 mM) diminishes the short circuit current and, concomitantly with it, phases I and II. The close correlation between $I_I + I_{II}$ and the size of the short circuit current is strong evidence that hydrostatic pressure perturbs I_{sc} directly and is manifest as phases I and II.

Only a slight sign of a phenomenon corresponding to the slow sigmoidal voltage increase could be found under short circuit conditions. Thus, the voltage increase is due most likely to an increase in epithelial resistance.

Discussion

A sudden increase in hydrostatic pressure ought to have a constellation of effects on a system as complex as that of a living, transporting tissue immersed in electrolyte solution. The magnitude and form of these physical and chemical effects can be identified and plausible origins of the relaxation spectrum delimited. It is particularly important to try to extract those events which are manifestations of perturbations of physiological processes normally operative within the skin, and which might be evoked, therefore, by other experimental probes.

The present kinetic data would be of little interest if the relaxation spectrum is no more than an expression of the kinetics of the compression of the tissue. This can be ignored as an explanation of phase II, but not necessarily phase I, as the following calculation demonstrates. The rate at which the tissue sample responds mechanically, by compressing, to the pressure stimulus is determined by the volocity with which the pressure front advances through it [14]. This velocity is approximately K/ρ where K is the coefficient of compressibility and ρ the density of the tissue and is equal to the velocity of sound in the material. If a velocity of 1500 m/s (that through H_2O or castor oil) and a thickness of 0.2 mm is assumed, it follows that the skin will be compacted in about 10^{-7} s when impinged upon by a step increase of pressure.

Phase II is many orders of magnitude too slow to be attributed to dispersion of the pressure stimulus by the tissue. Similarly, the apparent time constant of phase I of 150 ms is not brief enough to be due directly to tissue compression. It is possible that phase I does contain much faster kinetic components which have not been revealed because of risetime limitations of the pressure step. The present experimental results do not permit this possibility to be excluded. If there is a direct, immediate effect of pressure on epithelial transport properties it is concealed within phase I.

A sudden increase in pressure is expected to increase the temperature of the skin, as it does to the solution surrounding it. The possibility that compressive heating of the solutions and/or skin sample attendant to the sudden increase in pressure is the cause of phases I and II is undermined by noting that the temperature coefficient of open circuit voltage is positive [3]. Since phases I and II are both negative changes from the resting level they cannot be due to a temperature rise. Note also that the magnitudes of these effects of pressure are too large for them to originate simply from temperature changes. As noted above (Methods), for example, the maximum temperature change in the surrounding Ringer solution, a representative substance, is only 1°C.

The persistance of the phase I and II pressure jump effects under short circuit conditions and their elimination with ouabain imply that they are manifestations of rate-limiting steps in the mechanism of the generation of I_{sc}. In view of the close link between I_{sc} and active sodium transport and the latter's evident dependence on (bio)chemical reactions it is natural to ask if the relaxation spectrum is a consequence of their perturbation. Consider, as a simple illustrative example of what might be a rate-limiting step, the second-order reaction

$$A + B \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C,$$

where C is a substance the concentration of which is rate limiting for I_{sc}. In general, pressure will affect the reaction in two ways: the concentrations of the reactants and product will rise as a consequence of the compression of the medium in which the reaction is occurring, and the two rate constants can alter. If a_0, b_0, and c_0 are the respective concentrations of A, B and C immediately after pressurization, then c the concentration increment of C during the pressure step satisfies

$$dc/dt = (a_0 - c)(b_0 - c)k_1' - (c + c_0)k_2',$$

where k_1' and k_2' are the rate constants at the elevated pressure. Terms involving c^2 can be neglected for small pressures and the linearized differential equation is solved to yield

$$c = c_\infty(1 - e^{-\delta t})$$

where

$$c = (a_0 b_0 k_1' - c_0 k_2')/[(a_0 + b_0)k_1' + k_2']$$

$$\delta = (a_0 + b_0)k_1' + k_2'$$

and c is seen to vary along a simple exponential time course. Thus, phase II is well explained as a manifestation of a second-order reaction, although it must be noted that a simpler first-order reaction also relaxes exponentially during a pressure step. However, there is an experimentally important distinction between the two reactions. the time constant of the second-order scheme alone is dependent on the concentrations of the reactants and product. The rate of active transport of sodium varies with its concentration, particularly so at low levels [15]. It would be of great interest to see if the kinetics of phase II of I_{sc} is affected by sodium and if such changes can be correlated with alterations in the overall transport rate.

Experiments such as this and others are required for the further dissolution of $\Delta_p I_{sc}$. While it has been firmly established that in the steady state at atmospheric pressure I_{sc} is exactly equal to the net (active) transport of sodium across the tissue, it does not necessarily follow that this identity is valid when the skin has been perturbed by hydrostatic pressure. The identity need not hold, a priori, either during the pressure jump induced relaxation and/or its new steady state. The overall net transport rate is a complex function of the interaction between passive electrochemical processes and metabolically driven ones (e.g. ref. 16). Measurements of the modification of the relaxation kinetic parameters by pharmacological agents (amiloride, ouabain, hormones, etc.) believed to selectively alter these processes should help to identify in greater detail the ion transport mechanisms made visible by pressure jumps.

An important clue to the kinds of phenomena responsible for the pressure jump kinetics comes from a consideration of the sign and magnitude of the pressure coefficients; phases I and II are both negative equilibrium effects of pressure. A variety of biochemical reactions and processes, such as protein denaturation, fall into the category of negative changes of the magnitude of the items in Table I. In general, aqueous reactions in which the products are more ionized than the reactants are favored by increased hydrostatic pressure. Of particular biochemical interest is Penniston's [17] general finding that the activity of multimeric enzymes, including ATPases, are inhibited by pressure. The pressure coefficients for the ATPase inhibition are in the range of $3.7 \cdot 10^{-4}$—$7.1 \cdot 10^{-4}$ atm $^{-1}$ (cf. ref. 8). In view of the well-documented dependence of active transport on ATPase activity, it might be that phase I and/or phase II, in particular, is closely related to inhibition of this enzyme by pressure.

On the other hand the pressure effects seem too great for them to be purely physical in origin. The magnitudes of the pressure coefficients of the solution properties of water, for example, are no more than $6 \cdot 10^{-5}$ atm^{-1} [12]. Included are the following: dielectric constant, specific volume, viscosity, and conductivity and transference numbers of KCl. The pressure coefficients of Tables I and II are seen to be too large to arise from a process dependent solely upon a property of water. In this regard water is an unremarkable substance, the effects of pressure on it being similar to a wide variety of other substances.

It is interesting to consider the extent to which the pressure jump relaxation kinetics is compatible with the electrical fluctuations of the skin. The frequency dependence of the spectral intensity of the open circuit voltage is $1/f^a$, at 20°C, for frequencies below 10 Hz, and where a is 1.7 in *Rana pipiens* [3] and 2.0 in *Rana temporaria* [4]. Upon raising the temperature to 32°C the

spectrum is no longer linear and there emerges evidence of a relaxation process with a time constant of about 0.6 s [3]. I also showed that hyperpolarization of the skin potential with constant external current increases the spectral intensities of the fluctuations. Depolarization diminishes the voltage fluctuation, there being a minimum which persists even as short circuit conditions are closely approached. Thus, it was argued that the open circuit voltage fluctuations are the consequence of concomitant resistance and current fluctuations. The current component disappears when the skin is treated with ouabain.

The two phases discerned in the pressure jump experiments might contribute noise within the frequency range of existing measurements of the voltage fluctuations. Phase II with its atmospheric pressure time constant of 2.2 s would produce a relaxation term with a half-power frequence of 0.07 Hz; assuming the time constant of phase I to be 0.15 s, it would produce a relaxation component of 1.06 Hz half-power frequency. By combining these two spectra it is possible to produce an approximately linear spectrum, as was found within the frequency limits of the measurements, with a slope less than 2.

There is further, preliminary, evidence of the presence of phase II in the noise spectrum. Initial measurements of the temperature coefficient of the time constant of phase II yields a molar Arrhenius activation energy of 16.3 kcal which means that raising the temperature from 20 to 32°C decreases the average atmospheric time constant from 2.2 to 0.7 s. The 32°C noise data evidence a relaxation component with a time constant of about 0.6 s, very close to the phase II time constant of 0.7 s.

Thus there is good reason for thinking that components of the pressure jump relaxation kinetics are reflected in the noise spectrum. More work is required to firmly establish this point, and, in particular, knowledge of the effect of transport affectors on both sets of data would be helpful. Nevertheless, the general consistency between the two types of measurements encourages the important inference that the effects evinced by pressure perturbations are not irrelevent phenomena manufactured by hydrostatic pressure, but, rather, they are manifestations of physiologically significant processes normally operative within the epithelium.

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