

ELECTROKINETIC MEMBRANE PROCESSES IN RELATION TO PROPERTIES OF EXCITABLE TISSUES*

I. EXPERIMENTS ON OSCILLATORY TRANSPORT PHENOMENA IN ARTIFICIAL MEMBRANES

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ABSTRACT

An artificial system is studied consisting of salt solutions of different concentrations separated by a porous, "charged" membrane, through which a constant electric current is passed. Experiments on such systems demonstrate rhythmic variations of the transmembrane potential and the membrane resistance, which are concomitant with an oscillatory streaming of water solution across the membrane. The repetitive oscillations can be of a damped or undamped type dependent on the "stimulating" current density.

A qualitative discussion of the mechanism of the oscillations is given. It centers around the periodic resistance changes in the membrane, which result from a complicated interplay between the driving forces present. The importance of electro-osmotic effects is emphasized.

A few comparisons relating to possible electrophysiological implications are presented. In the metastable state of this *membrane oscillator*, "make" and "break" responses can be triggered by electric as well as by mechanical (pressure) "stimuli."

INTRODUCTION

1. In this and subsequent papers an attempt will be made to analyze the behavior of excitable tissues in terms of electrokinetic properties. Typical excitable tissues are the nerves, the end-plates, and the conductive elements of the heart. In the plant world there exist several excitable algae like *Nitella*. These tissues seem to be in a *metastable* state, because an external disturbance or stimulus can induce a single or repetitive, oscillatory response, the well known "action potentials." Usually electrical currents are employed as external stimuli, but mechanical disturbances (pressure changes) seem to elicit similar effects. External stimuli can also be of chemical nature (acetylcholine,

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etc.) as well as changes in temperature. These latter types will, however, not be considered in this discussion.

The vast amount of experimental facts emerging from electrophysiology has described the properties of excitable tissues mainly in terms of action potentials. Although it has been realized that the potential changes are only one type of the many concomitant processes underlying the excitation phenomenon, comparatively few experiments have been conducted on other equally important quantities, such as the action currents and the electrical impedance changes. Other physical events, such as swelling, have been studied only occasionally and incompletely. This is to be regretted, but it can be explained by the fact that there are considerable technical difficulties in studying several other properties simultaneously with the more easily recorded action potentials.

2. All modern views on the mechanism of nerve action are based on considerations of ionic transport processes in cell or tissue membranes. Most successful of these views is the well known "ionic hypothesis" of Hodgkin-Huxley-Keynes (1952, 1955) and of Cole (1953, 1954) which covers remarkably well a large number of experimental observations. The basic transport equation used in these concepts can be expressed, for a single ion species, as

$$\text{Transport ("flux")} = \text{concentration} \times \text{"permeability"} \times \text{sum of driving forces.}$$

Usually the force sum has been identified with the "electrochemical potential gradient." This force consists of two terms, *i.e.* the force arising from differences in concentration across the excitable membrane (the "chemical" or diffusion term) and the force created by the presence of an electrical potential difference (the "electrophoretic" term). Strict electrochemical theories of "two force" electrolyte diffusion founded by Nernst and Planck, as well as modern extensions (for instance by Meyer and Sievers (1936), Goldman (1943), Schlögl (1954, 1955), Schlögl and Schödel (1955), and Teorell (1935, 1951, 1953)) have failed to prove the existence of rhythmical or oscillatory transport processes. Kirkwood (1954) mentions impedance quantities which might give rise to periodic events (*cf.* also Bloch, 1947). In the applications to the nerve processes one has therefore been forced to invoke a number of extra assumptions. Many of these are difficult to define rigorously, like the terms "permeability changes" and "active transport."

The partial failure of the "two force" theories to account for more than "monotonic" electrochemical responses has stimulated the author to re-investigate the effects of the introduction of a *third* driving force, namely a *hydrostatic pressure difference*. A very essential novelty in this extension of membrane transport kinetics is the special attention paid to fixed ionic groups or "fixed charges" in the membrane. This has led to the consideration of *electro-osmosis* as an integrating part of the transport mechanism. It has been

found that the simultaneous interplay, in a "charged" membrane, of the three forces arising from gradients of concentration, membrane potential, and hydrostatic pressure can result in *oscillatory transport processes* of the ions and of the water, thereby creating a repetitive "firing" of the transmembrane potential (*cf.* Teorell (1958 *b*)).

In this manner it has been possible to reproduce many of the characteristic features of the excitable tissues. This paper will deal with some appropriate model experiments on artificial membranes. Subsequent papers will deal with the theory of the "membrane oscillator" and with applications relating to electrophysiology.

Basic Considerations

It should be emphasized at once that the following working hypothesis and basic considerations certainly involve oversimplifications and serious deficiencies. This hypothesis is meant only as a tentative attempt to find out to what extent the application of straightforward electrokinetic principles could describe some essential features exhibited by excitable tissues. In the present state we deal with a strict model conception.

Electrophysiology places the excitatory ionic and electrical events within a membrane unit. The membrane processes are also dependent on the state and composition of the two compartments which surround the membrane. In what follows the term "membrane" will be equivalent to the cell or tissue membrane separating the cell interior ("the inside") from the extracellular phase ("the outside"). The model membrane unit to be considered has the following characteristics:

1. A different ionic composition inside and outside. In living matter the most conspicuous feature is the fact that the cell interior is rich in potassium, while the extracellular space is rich in sodium. It will be assumed that this difference in composition can induce changes of electrical conductance *within* the membrane, depending on the direction of an applied current flow or a bulk flow. In short, the membrane should exhibit an electrical *rectification*, which somehow is related to the ionics of the surrounding media.

2. The membrane is of a porous nature and the pore walls contain *fixed charges* arising, for instance, from dissociable groups in the matrix of the membrane (in living matter probably consisting of carboxyl groups, or amino groups of the proteins, which generally are assumed to form the skeleton of the cell wall).

The existence of the "charged" wall in the sense advocated by the author, Meyer-Sievers, Sollner (1950, 1955), and others, is essential for the model conceptions to be discussed in this and the following papers. In several earlier papers the author has pointed out a number of consequences of the fixed charges as to the electrical potential, the conductance etc., which can modify markedly

the simple diffusion processes of electrolytes, with the assumption that movement of water was negligible (Teorell, 1935, 1951, and 1953). In this paper, however, the electro-osmotic effects of the fixed charges of the pore walls will be especially studied. This implies the important consequence that the bulk solution, the water, will be subject to transport across the membrane wall, a concept which has generally been neglected hitherto. The movements of the ions in relation to the membrane have usually been thought of as taking place in a more or less immobile water medium.

3. It will be supposed that an *unidirectional electric current* flows across the membrane. For simplicity it will be assumed that the current is of a constant strength (or subject to stepwise changes).

For the sake of the theory, to be presented later, it is immaterial how the membrane current is generated. In actual living cells it is, of course, a crucial problem, how chemical energy coupled with respiration can give rise to the ionic events, which ultimately might create a steadily flowing current leading to the "polarization" and metastability of the membrane conditions.

EXPERIMENTAL

The experimental realization of the oscillating membrane unit (the "membrane oscillator") was achieved as follows:—

1. The required electrical rectification was most easily induced by placing a more concentrated and a less concentrated solution of the same alkali salt on opposite sides of the membrane.

2. The porous, "charged" membrane corresponding to the excitable cell membrane was a porous glass or porcelain diaphragm. At neutral or alkaline pH this is negatively charged owing to the silicic acid ions (SiO_3^-), which may simulate, say, proteinic carboxyl groups of the living membranes.

3. A "polarizing" current, and the stimulating current (a "shock"), were introduced with the aid of an external battery feeding a potentiometer.

The general principle of the experimental arrangement is shown in Fig. 1. Two small and equal chambers (1) and (2), containing a stirred solution of approximately $N/10$ and $N/100$ NaCl respectively, are separated by a porous glass or porcelain membrane. With the aid of two reversible AgCl electrodes a constant current (mA) is applied across the membrane from a D.C. source, with the anode placed in solution (2). A flow of solvent and solute takes place causing (a) A difference in the height of the free levels, because the level of solution (1) rises and the level of (2) diminishes; *i.e.*, a *water pressure difference* (P) is created. (b) A change in the *membrane resistance* (membrane Ω). (c) A change in the *transmembrane potential* (E) (membrane mv.). (d) A *net transport of the solute NaCl* indicated by a change of the conductance in solution (2) (Ω solution (2)).¹

¹ Experiments on ion transport will be treated in another publication.

All the changes (*a* to *d*) were simultaneously recorded with respect to time. Typical results are depicted in Figs. 2 and 3.

Technical Details

Chambers.—The actual chambers employed were made of perspex and are similar to those previously described by the author (Teorell, 1954, 1955), although only a half

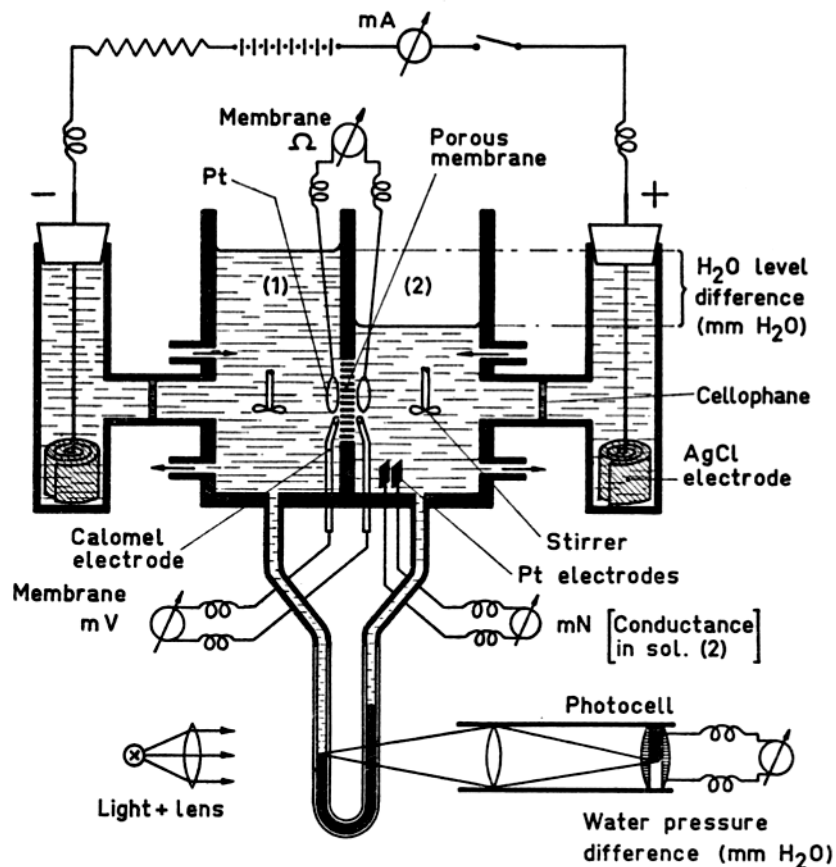


FIG. 1. A scheme of the experimental arrangement (for description, see text).

of the previous device was used here (see Fig. 1). Each half-chamber had a horizontal cross-section of 2.12 cm^2 and an initial free level about 40 mm. from the bottom, corresponding to an initial volume of about 18 ml. in each chamber. Thus the transfer of 1 ml. of solution from chamber (2) \rightarrow chamber (1) raises the pressure difference by approximately 10 mm. H₂O.

Good stirring was important and was produced by glass rods fastened to a magnetic vibrating device. In order to maintain approximate constancy of the concentrations in the chambers, these chambers were connected to large reservoir flasks

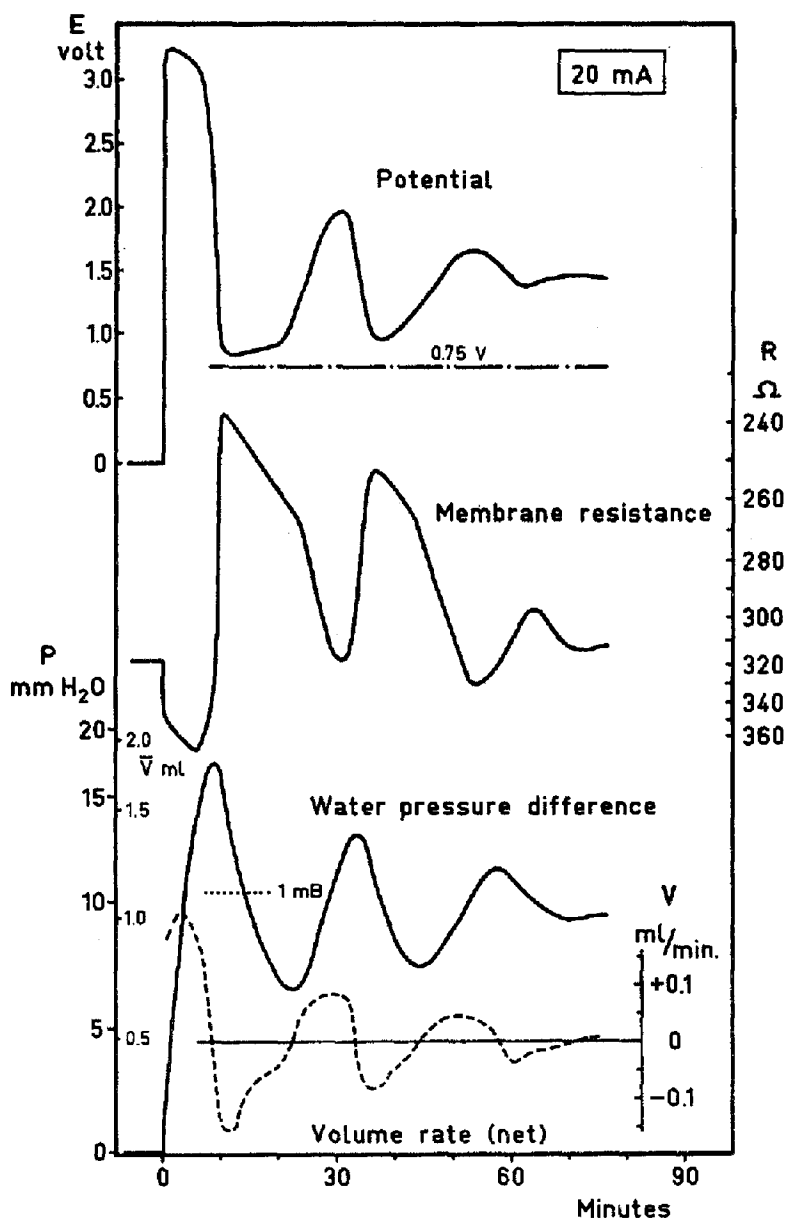


FIG. 2. Damped oscillatory processes in the system 100 mM NaCl/porous porcelain membrane/10 mM NaCl after the application of a constant electric current from time 0. Current strength 20 ma. (Original tracings, with exception of V , hence the slightly non-linear scales of R and P .)

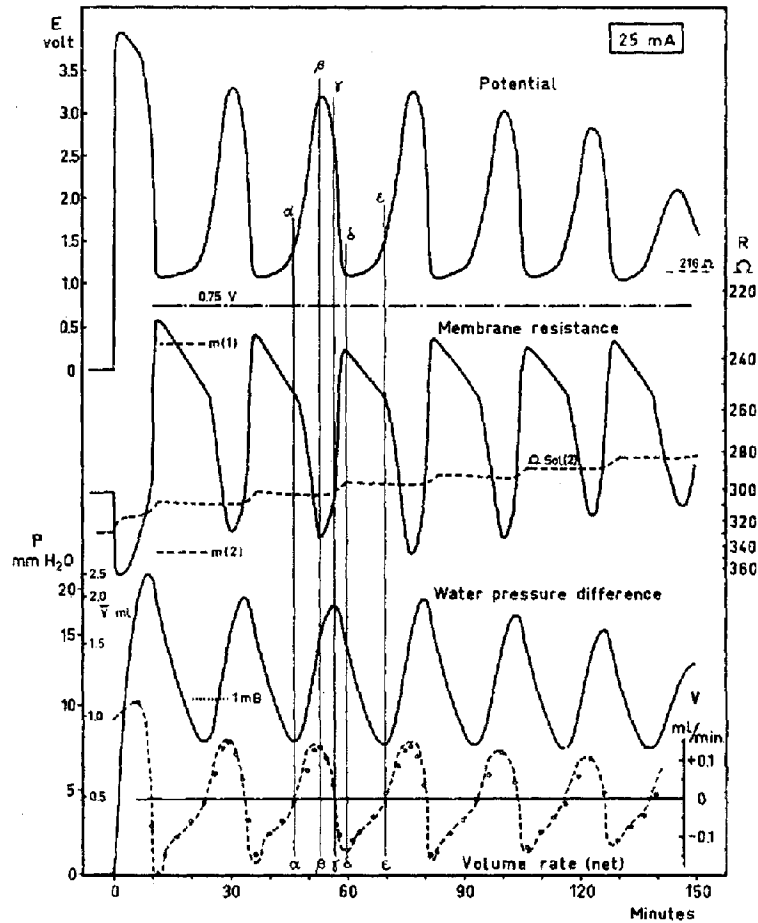


FIG. 3. Undamped oscillatory processes in the system 100 mM NaCl/porous porcelain membrane/10 mM NaCl after the application of a constant electric current from time 0. The current strength 25 ma. is slightly higher than in the otherwise identical experiment of Fig. 2. $m(1)$ and $m(2)$ denote membrane resistance before application of the current but with a free level excess in solutions (1) and (2), of about 20 mm. H₂O. The open circles on the V curve are values calculated from theory. Other symbols are explained in the text.

(3 litres) containing the salt solutions. Rapid circulation between the chambers and reservoirs was maintained with the aid of modified aquarium pumps. The pump-reservoir system was entirely closed and thermostated, so that any change in the free levels of the solutions in the chambers was due only to a volume transfer across the separating membrane.

The $AgCl$ -electrodes were heavily chloridized and employed in two different fashions.

(a) In the experiments of Figs. 2 and 3 the AgCl electrodes were placed in separate vessels attached to the sides of the chambers through holes covered with thick cellophane as shown in Fig. 1. The electrodes were large and were immersed in $N/20$ NaCl (they could therefore support a long period of current flow without disappearance of the AgCl deposit on the cathode). (b) In experiments on ionic flux (to be described in another publication) small, square AgCl electrodes were inserted directly in chambers (1) and (2). The connection to the reservoir vessel (2) was closed thereby confining solution (2) to the small volume of about 18 ml.

The current supply was delivered to the AgCl electrodes from a high tension battery or an electronic d.c. source (150 to 300 volts) through a large resistor. Provided the resistor was sufficiently large, a practically constant current could be drawn for a whole experimental period, only minor adjustments being occasionally required. These adjustments, as well as the choice of the current strength, were made with the aid of a potentiometer in the output of the d.c. supply source. The positive electrode was placed adjacent to the more diluted NaCl solution.

Membranes.—A great number of trials have shown that a proper choice of membrane (material, porosity, thickness, electrical charge, etc.) is of the utmost importance for the production of undamped oscillations. However, most porous membranes give highly damped oscillations with ease. Usually sintered glass filters or porcelain filters have been employed.

In the experiment of Figs. 2 and 3 a porcelain membrane was used with an average pore radius of 0.7 micron according to the manufacturer's specification (Standard microporous filter disc, Type 015, from Selas Corporation, Philadelphia). The dimensions: 13.2×13.2 mm. of exposed surface and the thickness 0.65 mm. The "free" pore volume was 50 to 70 per cent. The "half-time," *i.e.* the time necessary for a given water pressure difference, at zero electrical current, to be reduced to 50 per cent, was of the order of 5 minutes for both the glass and the porcelain membranes and is a measure of the "hydrostatic permeability." Further means of characterizing these membranes are "electroosmotic permeability" and "membrane conductance". These parameters will be dealt with in a forthcoming theoretical paper.

The potential measurements were made *via* calomel electrodes filled with a jelly mass as described in a previous paper (Teorell, 1954). The electrode orifices were, however, extended up to the center of the membrane with the aid of thin polyethylene tubes. The potential was fed to the high resistance input of a d.c. amplifier-recording channel. The accuracy was ± 2 per cent.

The membrane resistance was recorded across platinized platinum rings pressed close to the membrane surfaces. An alternating current of 1000 cycles and about 100 mv. across the electrodes was employed and fed to a Wheatstone bridge-rectifier-recorder channel. The measuring accuracy is ± 5 to 10 ohms. A similar electrode pair was used for the measurement of the conductance of solution (2).

The calibrations of the potential and resistance scales were made by substitution with known values from a millivolt potentiometer and a decade resistor box respectively.

In both the potential and membrane resistance measurements a certain amount of "blank" value is unavoidable owing to the fact that the electrodes can not be placed in ideal juxtaposition to the membrane. A certain amount of voltage and resistance drop of the bulk solutions between the electrodes and the membrane is therefore in-

cluded in the records. The "blanks" or "real baseline" values can, however, be deduced in several ways. In the experiment of Figs. 2 and 3 they are assigned values of 0.75 volt and 216 ohm respectively. The differences as to blanks, etc., of the potential and the resistances are due to the fact that the potential electrodes were located closer to the membrane than the platinum electrodes (*cf.* the figures).

The *water pressure difference* was measured with the aid of a narrow U-tube differential manometer filled with a non-aqueous liquid (a mixture of kerosene and acetylene bromide of specific gravity 1.60 or 2.0, stained with a trace of Sudan III). The excursions of one meniscus were projected on a photocell as indicated in Fig. 1. The light source was fed from a constant voltage device.

The calibration of the photocell-manometer was performed by addition, or withdrawal, of measured volumes into the chambers. Usually 0.5 to 5 ml. was rapidly transferred by means of a syringe from solution (2) to solution (1), starting every time from pressure equilibrium. In this manner it is possible to express the pressure difference recorded in terms of milliliters of liquid transported from (2) to (1) (indicated in Figs. 2 and 3 as \bar{V} ml.). From knowledge of the cross-sectional areas of the chambers it was possible to calculate the manometer excursions in terms of millimeters H_2O pressure difference, or any other measure desired (the pressure difference of 1 millibar is marked on Fig. 2 as "1 mB").

The *electric recording* of the five variables of interest, *i.e.* mA, volt, membrane resistance, solution (2) resistance, and water pressure difference was performed with the aid of five different recording channels of a multipoint ink writer (the principle identical with that described by Teorell, 1954). The same d.c. amplifier (input impedance 1 to 10 megohm) was used for all five channels. These in turn were switched in successively by means of a selective switch mechanism at a speed of about three "point" measurements per minute of each of the variables.

The Compositions of Solutions (1) and (2).—In the experiments of Figs. 2 and 3 the NaCl concentrations of the solutions were approximately $N/10$ and $N/100$ NaCl in chambers (1) and (2) respectively and they were slightly buffered with phosphates. The pH was 6.4.

Various types of *control experiments* have been performed to study possible interfering factors, in particular heating effects within the membrane. Miniature thermistors drilled into the membrane indicated a maximal temperature rise during the current flow of a few degrees C. Liberation of dissolved gases within the membrane was also excluded as a cause of disturbance.

RESULTS

The main observations discussed below refer to the experiments recorded in Figs. 2 and 3. They are, however, corroborated by a large number of other experiments not reported. The results will be condensed here to qualitative statements only, particularly with regard to the time relations between the different phenomena studied.

Before Application of Any Current.—Solutions (1) and (2) were in an approximately steady state of pure diffusion, *i.e.* the transmembrane potential E was low, about -10 mv. (the sign in (2)), the membrane resistance at an intermediate value, and the water pressure difference between (1) and (2)

equal to zero. A slow conductance increase in the dilute solution (2) was observed owing to a slow diffusion of NaCl from (1) to (2).

During a Steady Current Flow.—(a) *Influence of the Current Density.*—After the application of a steady current all the measured variables showed an *oscillatory* variation with time. Two different responses could be observed, *damped* or *undamped* oscillations. These two types of responses can be seen in Figs. 2 and 3 respectively. Experience has shown that there exists a “threshold” value of current density. Below this value one obtains highly damped, or moderately oscillatory responses (Fig. 2). Above the threshold (in this experiment about 20 ma.) the oscillations were transformed into the undamped type (Fig. 3).

In the damped cases the oscillations die away with time and emerge into final steady state levels. There is a pressure excess in (1) corresponding to the equilibrium “electro-osmotic pressure.” In the undamped cases the oscillations can go on for hours and start to die away only when the current supply accidentally becomes too low, the AgCl electrodes become exhausted, or, usually, due to the fact that a critical concentration change has taken place in solution (2). During the whole period there is a pressure excess in (1) oscillating around an average of about 10 mm. H₂O.

(b) *Influence of the Concentration Ratio.*—In this connection it should be emphasized that certain rather narrow concentration ratios or rather concentration “pairs,” are optimal for the obtaining of undamped oscillations. In these experiments optimal values were in the range 100 to 90 mN NaCl in solution (1) and correspondingly 12 to 8 mN NaCl in solution (2). When 12 mN NaCl in (2) was exceeded the oscillations changed over to the damped type.

(c) *The Influence of the Ionic Composition of the Bulk Solutions.*—This has been only preliminarily explored so far. The results of experiments with KCl and LiCl were quite similar to those with NaCl. The hydrogen ion concentration plays, as expected, a decisive role. Alkaline solutions favor electro-osmotic solvent transport; *i.e.*, higher maximal water pressure differences can be obtained with a given current density. Similar effects have been observed with the addition of some other strongly adsorbable ions, or dyes. Apparently there are various means of “conditioning” the membrane properties in any desired direction.

(d) *The “Initial Wave.”*—It should be noted that the first oscillation (the “*Einschwingung*”) of all the variables is usually of a considerably higher amplitude than the succeeding ones.²

(e) *The Oscillation Frequency and Wave Form.*—In the cases of Figs. 2 and 3

² This applies only when the experiment is started at $P = 0$. If one starts, however, with a “preset” value of $P \neq 0$, one can, for instance, obtain initially “growing” oscillations, often with a time delay, provided the “stimulating” current density is appropriate for the elicitation of undamped waves.

the frequency was of the same order, two or three full waves per hour. There are several factors which seem to influence this frequency, such as the thickness of the membrane, the hydrostatic permeability, the ratio of the "effective" membrane area to the horizontal cross-section of the chambers (the "geometry factor"), and other factors. In any one experiment the rhythm is remarkably constant over long periods.

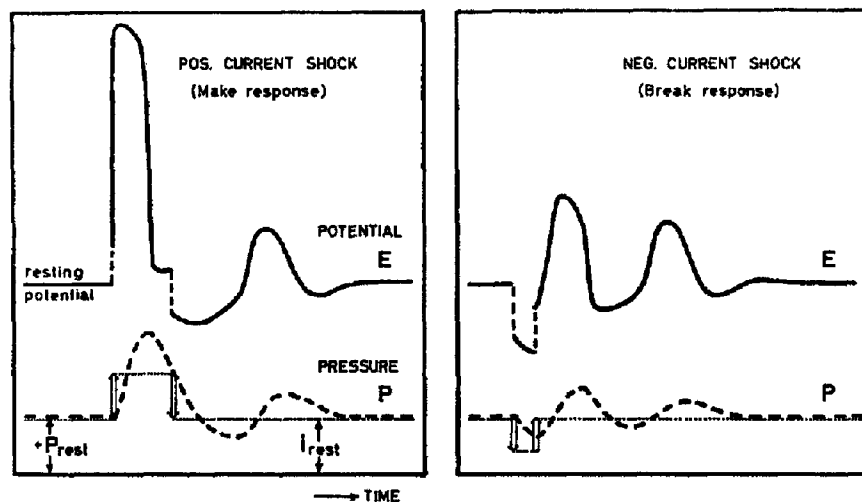


FIG. 4. Effects of electric current shocks on the artificial membrane oscillator. The system was similar to that of Fig. 2 (sintered glass membrane) and was polarized with a weak positive current of 5 ma. for 1 hour before this experiment began. Thus the initial, damped responses had subsided to an equilibrium state with a "resting potential" as well as a "resting pressure difference." This state was, however, *metastable*, because brief *current* shocks (marked by the vertical, open arrows) elicited an oscillatory response. Note the "make" and "break" responses being dependent on the direction of the shock current, which was superposed on the "resting current" (i_{rest}).

The wave form is roughly that of distorted sinusoidal waves or "relaxation oscillations," damped or undamped as the case may be. An interesting feature is shown by the potential waves, which are in general shape similar to the well known "action potential spikes" of nerves (see also Figs. 4 and 5).

(f) *Phase Relations between the Various Waves.*—Conclusions as to the relations of the curves of the transmembrane potential, membrane resistance, water pressure difference, and liquid transfer can be summarized as follows:—

If the *potential* curves, E , are chosen as a frame of reference, a closer study of the oscillatory waves recorded in Figs. 2 and 3 reveals (1) The *membrane resistance* (R) varies closely in parallel with the potential; *i.e.*, R is in phase with E . (2) The *water pressure difference* (P) curve has a marked time lag with respect to the E curve, the "tops" always occurring at the sharp decline

of the E "spikes" and the "trough" always timed with the first rising portion of an E spike (note the vertical reference lines labelled γ and α respectively on Fig. 3). (3) The net rate of liquid transfer (= "volume rate," V ml./minute) is an important variable, which can be deduced by graphical differentiation of the experimental P curves.³ In the experiment on the porcelain membrane reproduced in Figs. 2 and 3, the graphically deduced volume transfer rates, V , are given as a dotted curve in the lower part of the figure (note that the positive sign means flow in the direction (2) to (1) and a minus sign a reversed flow). Apart from the self-evident fact that the zero values of V coincide

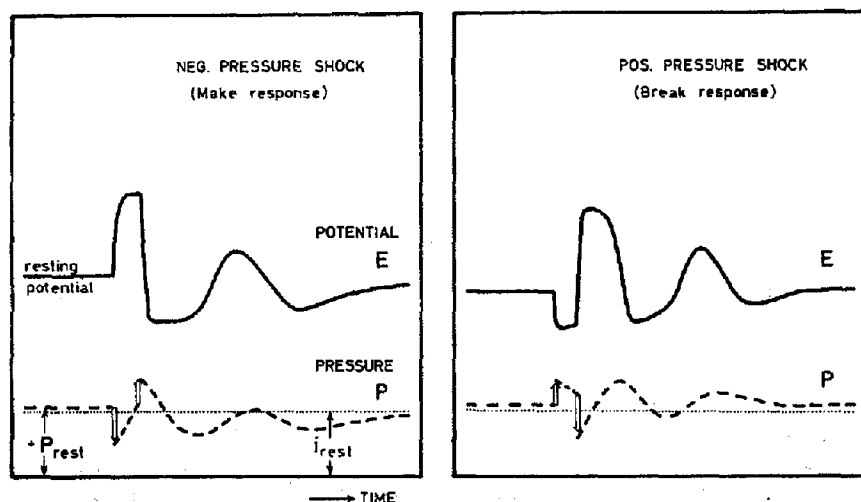


FIG. 5. Effects of pressure shocks on the artificial membrane oscillator. The system was identical with that of Fig. 4, but here the oscillatory responses were triggered with pressure shocks (marked by the vertical, open arrows). Note again the "make" and "break" effects, now dependent on the direction of the external pressure changes.

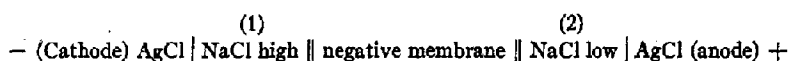
with the maxima and minima of P , one finds that the V curve is only approximately in phase with the potential curve E and the membrane resistance curve, R . Close examination reveals that the maximal V flows in either direction occur somewhat earlier than the corresponding maxima and minima of the E , or R , curves (*cf.* reference lines β and δ).

The observed appreciable time lag of the pressure and the small lead of the volume rate in relation to the potential, or resistance curve, are in complete accord with the theoretical predictions as will be shown in Fig. 3 of Part II of this paper (Teorell, 1959).

³ V is defined as the time derivative of the pressure difference values (P) as $V = q \cdot dP/dt$ and is expressed in terms of the actual volume transfer of liquid from solution (2) to (1). The relation between V and P (millimeters H_2O) has been discussed in connection with the calibration procedure of the P records (*cf.* p. 839).

DISCUSSION

The experiments discussed above demonstrate that the superposition of an electric current on a diffusion system within a porous, negatively charged membrane can induce rhythmical transport processes of the solutes and the solvent, provided certain conditions are fulfilled. Here these processes have been recorded in terms of *transmembrane potential* (E), total *membrane resistance* (R), *hydrostatic pressure difference* (P), and the *net rate of volume transfer* (V) of the bulk liquid across the membrane. The system was built up as a "concentration chain:"



The essential phenomena taking place will be discussed only qualitatively in this paper. The quantitative analysis will be given in the following paper (Teorell, 1959). The main processes coming into operation are the following:—

Owing to a negative charge on the membrane pore walls the application of a steady electric current will cause an *electro-osmotic flow* of the liquid across the membrane towards the cathode (in this case from the dilute NaCl solution (2) to the strong NaCl solution (1)). This flow will soon elevate the free level of (1) and diminish that of solution (2) and thus create a pressure difference, P . This "back pressure" P in turn will tend to send solution (1) in the opposite direction across the membrane towards solution (2) as a *hydrostatic flow*. As a result of these two opposite tendencies of streaming a *net flow of volume* of liquid will take place. Dependent on the direction and rate of this liquid flow, the membrane will be more or less filled with either dilute NaCl solution (2), or concentrated NaCl solution (1). In this way the membrane resistance will vary with time; *i.e.*, exert "time-varying rectification" properties (*cf.* Teorell, 1951, 1953).

Expressed differently, the "concentration profile," *i.e.* the spatial distribution of the salt concentration within the membrane, will be distorted by the *bulk flow* one way or the other from its resting shape, which is a straight line (in the steady state). There will always be present a "restoring process" in the form of simple diffusion, which will strive to readjust the distorted profile (concave or convex) to the straight line. This diffusion process is slow and therefore it lags behind the distorting effects. In this way it may be understood how a "time delay" or "inertia factor" is introduced in the membrane system, operating on its total electrolyte content and thereby affecting the membrane resistance in a variable way. These variations of the resistance in turn cause corresponding variations of the voltage drop (the potential) across the membrane, because of the constant current present. The potential variations finally affect the drive on the electro-osmotic part of the bulk flow, giving it a varying net rate which again distorts the membrane concentration profile. The result will be an oscillating process. The reason is the *coupling between the three driving forces* (the gradients of concentration, membrane potential, and hydrostatic pressure) within

a *charged* membrane and the presence of the time delay of the membrane resistance. Further discussions will be given in the theoretical paper.

The electrophysiological implications of the findings presented will be postponed to later papers, in which the properties of the "membrane oscillator" will be more profitably studied on an electronic analog computer, simulating the physical system examined in this paper. Only a few comparisons with actual excitable tissues should be mentioned here.

1. The general *shape* of the potential spikes is similar to that of the natural action potentials. The model potential spikes, as produced here, are accompanied by a conductance *minimum* (*R* maximum). The biological spikes are, however, usually in parallel with a conductance *maximum*. This divergence will be discussed in a later publication on the impedance properties of the membrane oscillator.

2. The membrane model shows a definite "threshold" with regard to the relation stimulus-response. It has been found that a constant current (the "stimulus") triggers the system to a repetitive response with undamped oscillations only above a certain current density (*cf.* Figs. 2 and 3 where the change from a strength of 20 to 25 ma. transforms a damped into an undamped response).

3. Still smaller current stimuli may produce a single "overshoot" of the potential response, followed by a long lasting train of small and frequent "miniature" waves resembling the "local response" or "*subthreshold activity*" well known in nerve physiology.

4. If one maintains the membrane oscillator at, or slightly below, subthreshold activity by means of a weak current ("resting current"), it is possible to trigger typical spike responses by applying a brief "shock" in the form of an increased or decreased current flow. Typical "*make*" and "*break*" responses will be obtained not only of the potential, but also of the pressure difference (see Fig. 4).

Corresponding brief changes of the *pressure* difference (simply produced by dipping and withdrawing a glass rod in chamber (1) or (2) of the model apparatus) will also elicit oscillatory potential and pressure responses (see Fig. 5). This equivalence of electrical and "mechanical" (pressure) stimuli is interesting in relation to the problem of mechano-electrical transduction in pressure-sensitive organs and smooth muscles. In the model system presented it is a direct consequence of the coupling between the driving forces.

The experiments of Figs. 4 and 5 illustrate clearly the *metastable* state of this membrane system when it is "polarized" (in this case by a steady *positive* current flow⁴). Any other extrinsic (or intrinsic) "disturbance" will actually act as a "stimulus" eliciting oscillations.

⁴ In cases of a steady *negative* current polarization the system will give *non*-periodic responses of a monotonic type, or as a single "overshoot" or "undershoot," regardless of the current density applied.

5. Finally the following facts may be mentioned. If symmetrical square wave currents are applied to the present electrokinetic membrane model, very characteristic potential response patterns at the higher current densities are observed. Closely related patterns have recently been demonstrated on the excitable *Nitella* algal cell, *suggesting that oscillatory electrokinetic processes of the type proposed in this paper can have real biological counterparts* (Teorell, 1958 a). At any rate, it seems true that flowing electric currents exist in some biological tissues of a density quite comparable with those eliciting periodic events in the experiments with artificial membranes as presented in this paper. Here undamped oscillations could be elicited above a threshold of the order 5 to 25 ma./cm². Tasaki and Bak (1958) recently reported that the nerve nodal membrane can take a current density of about 100 ma./cm² without damage (although it seems probable that the actual action current densities may be about one-tenth of this value).

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