J. Membrane Biol. 38, 347–357 (1978)

Oscillation of Membrane Potential in L Cells: III. K^+ Current-Voltage Curves

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Received 17 May 1977; revised 22 August 1977

Summary. Cultured L cells were found previously to have an oscillating membrane potential. Current-voltage ($I-V$) curves were measured during these oscillations. Two $I-V$ curves were recorded, one at the maximum and one at the minimum of oscillations. Each curve is nonlinear, and when they are subtracted from one another, the result gives the $I-V$ curve for the K^+ current producing oscillations. This I_K-V curve is zero for -85 to -90 mV and saturates for positive and high negative membrane potentials. When the external K^+ is increased the $I-V$ curve is shifted and its zero current potential is reduced. The K^+ zero current potential follows a Nernst relation when plotted against the external K^+ concentration. The I_K-V curves all have a similar shape at different K^+ concentrations, showing a saturation on each side of the zero current potential. The results can be explained satisfactorily in term of a carrier model for K^+ ions.

The membrane potential of large L cells in culture was found by Okada, Doida, Roy, Tsuchiya, Inouye and Inouye (1977*a*) to oscillate between -15 mV and -40 mV. Such large L cells (40–60 μm) are found among small L cells of usual size (15 μm) or can be produced in large number by X radiation (1000 rads). Such oscillations are also observed in small L cells, but only rarely. The membrane resistance was measured during these oscillations: it was found that the resistance changed from 37 to 23 MΩ when the potential changed from -15 to -40 mV. The frequency of these oscillations was 3–4 cpm. Low temperature (3 °C) and metabolic inhibitors abolished oscillations. A constant current was applied to hyperpolarize or depolarize the membrane during oscillations; the amplitude of the oscillations was changed but the membrane resistance was not affected by such currents. Additional experiments by Okada, Roy, Tsuchiya, Doida and Inouye (1977*b*) were performed to

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0022-2631/78/0038-0347 $2.20

determine more clearly the origin of these oscillations. Increasing external K^+ decreased the oscillation amplitude. By applying a constant current, the reversal potential for the oscillations was measured and it was found to be very close to the K^+ equilibrium potential. When external K^+ was changed, the reversal potential also changed; it followed the Nernst equation for the K^+ concentration gradient. Changing external Na^+ or Cl^- had no effect on oscillations. It was concluded that these oscillations were caused by a periodic variation of a K^+ conductance whose frequency and amplitude were independent of membrane potential. The purpose of the following experiments was to determine the characteristic of the K^+ currents producing oscillations. The K^+ current-voltage relation and its dependence on K^+ concentration was measured in order to obtain a more precise understanding of the membrane diffusion mechanism producing this selective K^+ current.

Materials and Methods

Cell cultures, microelectrode preparation and electronic instruments have already been described in Okada et al. (1977*a*). Two different methods were used to measure the current-voltage curves. The first one was the use of a single electrode. Before penetration, the electrode resistance was measured and compensated. The recording amplifier (W-P, M 701) provides a constant current source and electrode resistance compensation. The voltage V_e between electrode and ground is measured and the electrode resistance R_e is easily obtained. Using the compensation adjustment, the voltage output V_e is reduced to zero. This compensation was checked for many amplitudes of currents (positive and negative) in order to verify the linearity of electrodes in the range of currents used for experiments. Usually currents between -10 to $+10$ nA were necessary to provide sufficient membrane potential changes (-150 mV to $+150$ mV). Microelectrodes showing important ($>10\%$) nonlinearities in this range of currents were rejected. Resistance compensation was checked before and after every penetration. If compensation had changed after a penetration, the measurement was rejected. The most important handicap with this single electrode method is the difficulty of obtaining microelectrodes with a sufficiently fine tip and a not too large resistance. The larger the resistance, the more the electrode is nonlinear. Also it often happens that after penetration the compensation has changed. This change of resistance can be caused by some cellular material sticking to the electrode tip. The most suitable microelectrodes were obtained immediately (2–10 hr) after preparation by the glass fiber method (Okada & Inouye, 1976), having 10 to 20 MΩ of electrical resistance and 0 to -5 mV of tip potentials. Storage of microelectrodes for a long time (more than 20 hr), even in the refrigerator, increased those nonlinearities.

Another method, avoiding this problem, is the use of two microelectrodes, one for injecting the current and one for measuring the voltage. This method is more reliable but causes more damage to cell membranes. Also such measurements are more difficult to perform, requiring two penetrations in the same cell. In this case, the W-P was used only as a current source and another independent high impedance amplifier was used to measure voltage.

The current-voltage curves could be measured by applying different constant current pulses and measuring the output voltage or more directly by applying a triangular waveform (negative and positive current ramps) which would provide a continuous linear change of current. Voltage and current probes could be connected to an $X-Y$ recorder, and the current-voltage ($I-V$) curves could be traced directly. These two methods were used to measure $I-V$ curves on oscillating cells. Pulse or ramp signals were applied when oscillations reach a maximum or a minimum as shown in Fig. 1. In order to avoid hysteresis on $I-V$ curves traced directly, it was necessary to use a low frequency triangular wave. But the frequency had to be sufficiently high, so that the oscillating membrane voltage did not have time to change: a value of 1–2 cps was found appropriate. The pulse method was also used. A different pulse amplitude was sent at each peak of oscillation. Both methods gave the same results, but the triangular wave method is better because it gives the $I-V$ curve directly and completely at each peak of oscillation. It avoids changes in membrane and cell interior properties that can occur during long electrode penetrations. Although all the above recording methods were tried and compared, most of the results were obtained with a single compensated electrode using a current ramp signal.

External bathing solutions were phosphate buffer saline (PBS) or Tris buffer saline (TBS). During experiments on the effects of varying K^+, Na^+ was replaced by K^+. When effects of varying Na^+ were studied, a normal constant $[K^+]$ was maintained and Na^+ was replaced by $Tris^+$.

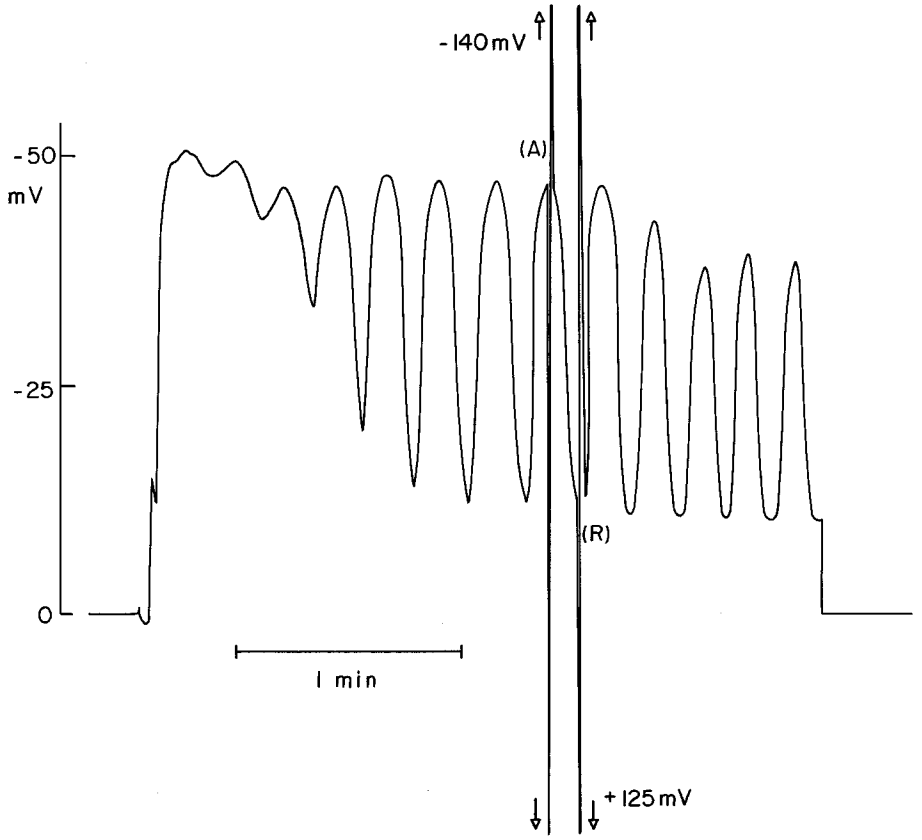

Fig. 1. Typical oscillations of membrane potential showing the measurements of $I-V$ curves

Results

Typical current-voltage ($I-V$) curves are shown in Fig. 2. The first curve (R) represents the total membrane ionic current in the resting state. It is almost linear for negative membrane potentials between 0 and -90 mV, and also linear for positive potentials, but with a reduced slope. The second curve (A) represents the activated state of the membrane and is measured on the high negative potential state (Fig. 1). This current shows a more important nonlinearity for positive membrane potentials,

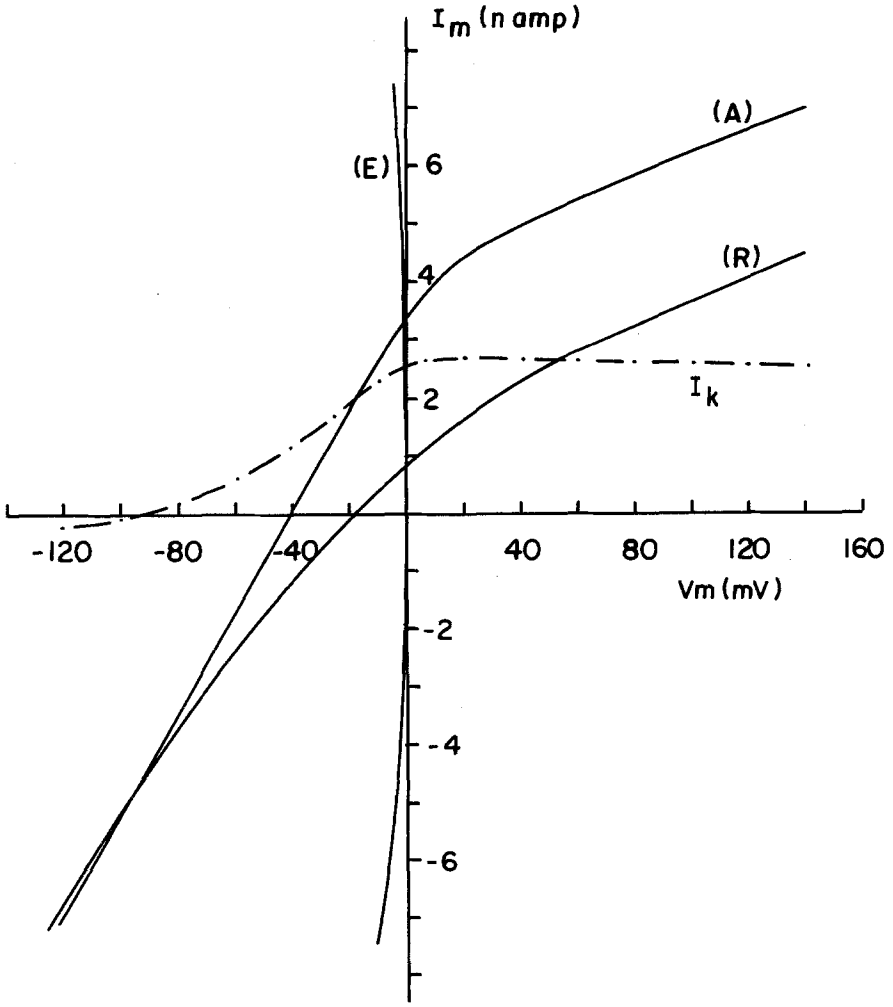

Fig. 2. $I-V$ curves measured at the minimum value of the oscillating potential (R) and at its maximum value (A), as shown on Fig. 1. Curve (E) is the compensated electrode ($I-V$) curve. Curve I_k is the net K^+ current obtained by substrating (A) from (R)

but it appears also linear for negative potentials between 0 and -90 mV. The most interesting aspect of these two curves is their intersection at a high negative potential, around -85 to -90 mV. This means that the difference between these two currents is zero at this potential. From a previous analysis of the origin of oscillations by Okada et al. (1977*a*, *b*), it was found that the change in the membrane potential producing oscillations was caused only by a change in the K^+ conductance. This is confirmed by the above observation showing that the difference between curve *R* and *A* is zero around the equilibrium potential for K ions. Subtracting curve *R* from curve *A* therefore gives the net K^+ current-voltage curve, ($I_K - V$ curve). This is a very important result, because it gives the possibility of characterizing the K^+ current which produces oscillations. An important aspect of all recorded $I-V$ curves *A* and *R* was their parallelism for positive potentials, as shown in Fig. 2. In many cases, it was also possible to observe this parallelism for high negative potential beyond the crossing point. When curves *A* and *R* are subtracted, their parallel section gives a horizontal characteristic to the $I_K - V$ curves, thereby demonstrating that the net K^+ current saturates for positive and high negative potentials.

In order to determine the effect of changing external K^+ concentrations on the characteristic of the net K^+ current, $I-V$ curves were measured for three other concentrations of external K^+. Many $I-V$ curves could be obtained for 10 and 22 mM K^+, but for higher concentrations, it was very rare that oscillations occured. In three cases, small reversed oscillations were seen for $[K^+]=147$ mM, and $I-V$ curves could be measured. Such reversed oscillations had been previously observed (Okada et al., 1977*b*). The crossing potentials for different external K^+ were plotted on semi-log paper as a function of external K^+. The results, shown in Fig. 3, give a straight line with a slope of 60 mV/decade, demonstrating the high selectivity of this current.

The net K^+ current was obtained for each pair of $I-V$ curves, and the average net current was calculated. Results are shown in Fig. 4 for four external K^+ concentrations. It is important to observe that each net K^+ current saturates for positive potentials. The saturation level was found to be the same for $[K^+]=4.2$ mM and 10 mM, but was a little lower for 22 mM and a little higher for 147 mM. Since no attempt was made to determine the current per unit of surface, because of the complexity of surface membrane structure (Lamb & MacKinnon, 1971*a*) it is probably the reason for these differences. Consequently it was decided to scale all the currents to the amplitude of the current obtained with normal K^+.

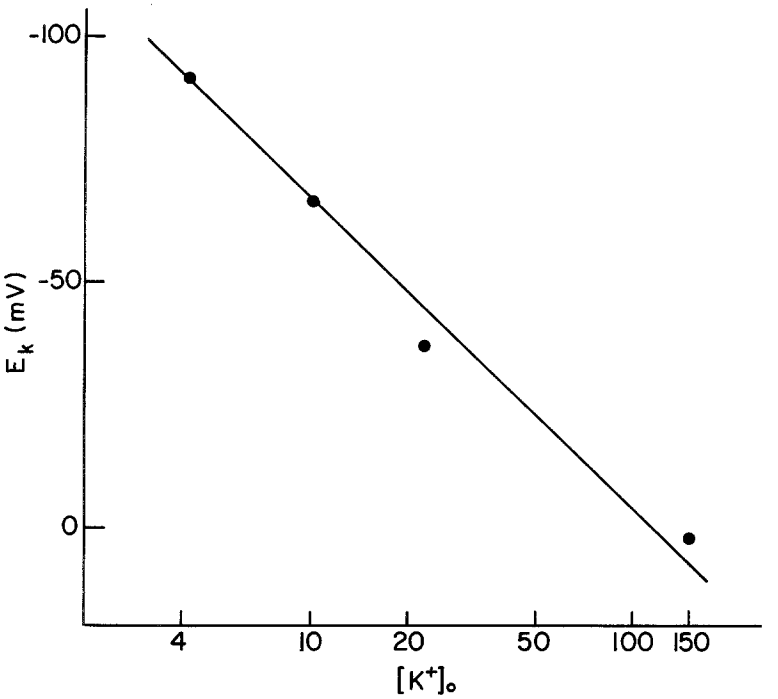

Fig. 3. Relation between crossing potential of curves (*A*) and (*R*), and external K^+ concentration. The straight line has a 60 mV/decade slope

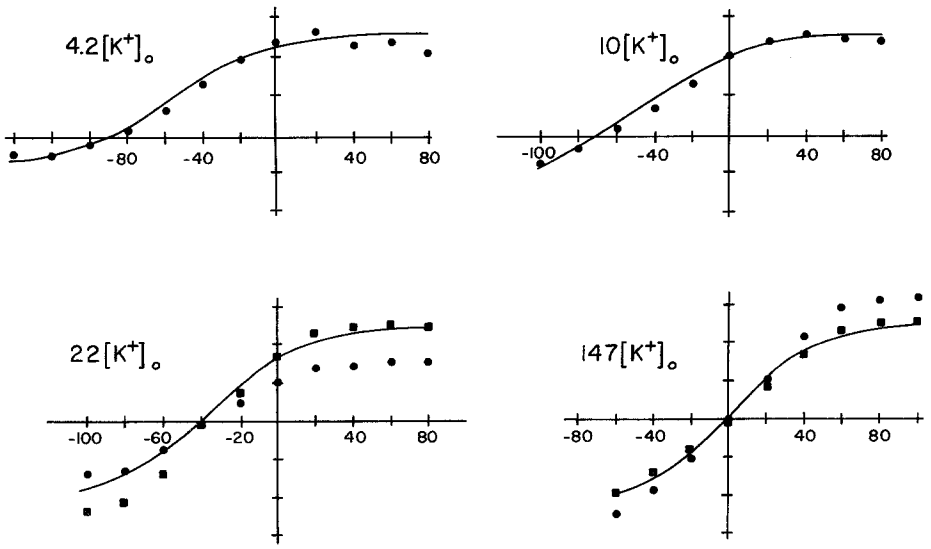

Fig. 4. Average net K^+ current ($I_K - V$) curves. Scales are the same as on Fig. 2. ■ represents corrected data points and ● measured data points. The continuous lines are calculated with Eq. (2)

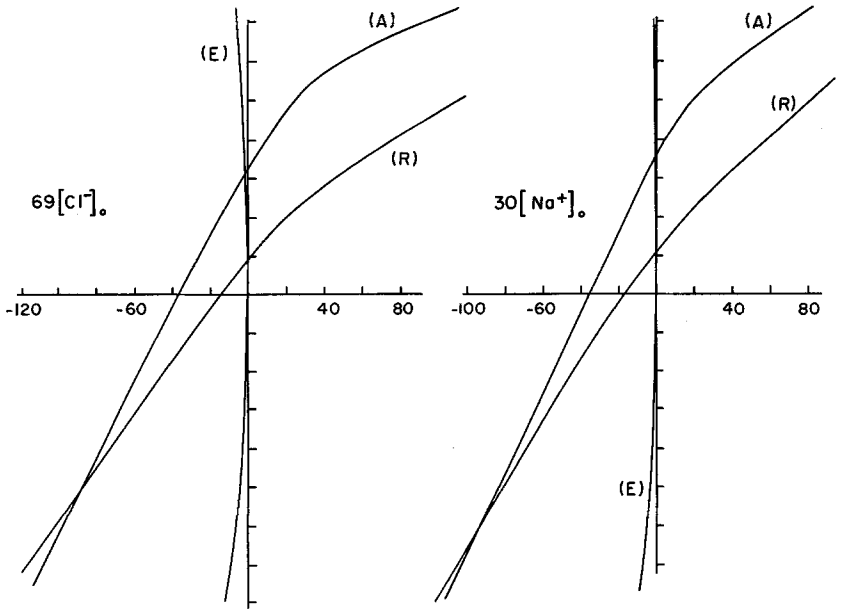

Fig. 5. Two examples of $I-V$ curves obtained with reduced $[Cl^-]$ and $[Na^+]$. Both curves were measured more than 10 min after the concentration change. Scales are the same as on Fig. 2

Other $I-V$ curves were measured, varying external Na^+ or Cl^- concentrations, maintaining a constant K^+ concentration. The measurements were made more than 10 min after the replacement of the medium, in order to avoid the transient effects produced by Cl^- changes (Okada et al., 1977*b*). No significant effect was observed on all measured $I-V$ curves as shown in Fig. 5. This confirms that the net K^+ current is very selective. It is rather surprising that Na^+ has no effect, specially on $I-V$ curves R measured at the resting or low potential state (-15 mV). At first it would have seemed natural that the Na^+ gradient between inside and outside the cell contributes with the K^+ gradient to determine the low value of the membrane potential. From our observations, it seems that the Na^+ gradient does not affect the membrane potential or that $Tris^+$ has a membrane permeability about equal to that of Na^+.

Discussion

The low value of the resting potential (-15 mV) and the $I-V$ curve (R) observed at the resting potential are not easily explained, because re-

placement of Na^+ by $Tris^+$ did not have any significant effect (Fig. 5). Lamb and McKinnon (1971) have proposed on the basis of their ion flux measurements that P_{Na} is about equal to P_K in L cells, thereby explaining the low value of the resting potential. Our results show that Na^+ is not involved at all in determining the resting potential or that $Tris^+$ is as permeable as Na^+. The first possibility is difficult to accept because it would contradict Lamb and MacKinnon's (1971) results and because no other ion except Ca^{++} would have an appropriate chemical gradient. But low or high Ca^{++} did not have important effects on the membrane resting potential (Okada & Tsuchiya, *unpublished observations*). The most reasonable explanation would be that there are pores or carriers in the membrane that are not selective regarding different types of cations, even large ones like $Tris^+$. These unselective pores or carriers could serve as channels for polar molecules like amino acids, which are known to diffuse across membranes with Na^+ ions. Consequently there would be a linear "leakage" current $I_\ell = g_\ell E_m$, where g_ℓ is the "leakage" conductance, superposed on the K^+ selective current. The K^+ current oscillates from a low to a high amplitude, thereby changing the membrane potential. At its low level, the K^+ current is much smaller than the leakage current, and the $I-V$ curve (R) is almost linear with an equilibrium potential of only -15 mV. At its high level in large cells, it becomes about equal to the leakage current and it brings the membrane potential to about -40 mV. The Cl^- current also contributes to the total current during oscillations and during $I-V$ curve measurements.

It would be interesting to interpret the $I_K - V$ curves shown on Fig. 4 in terms of a membrane model for ion diffusion. Basic principles regarding ion diffusion across biological membranes have become rather well established recently through the theoretical and experimental studies on artificial bilayers (Ciani, Eisenman, Laprade & Szabo, 1973; Lauger & Neumcke, 1973). These principles and their applications to excitable membranes were recently reviewed by Roy (1975). It is usually assumed that ions can diffuse across biological membranes through polar pores embedded in the membrane or with a carrier molecule having a polar interior and a nonpolar exterior. In order to diffuse, ions must overcome energy barriers between diffusing sites. In the case of membranes, ions must overcome the surface barriers and also other barriers inside the membrane. Many different specific hypotheses can be introduced within this framework so as to give many different current equations. In order to determine what kind of particular diffusion mechanism should be selected, it is necessary to examine the specific aspects of observed current-voltage curves and their ionic concentration dependence.

The most important aspect of our $I_K - V$ curves is that the current becomes independent of the membrane potential when the latter is sufficiently large (positive or negative).

This means that an increase of the electric field across the membrane beyond a certain value cannot increase the flux of ions any more. It would seem that ion penetration into the membrane is limited to a maximum rate. Energy barriers at each membrane surface could be responsible for such an effect. When the membrane potential is large, the rate of transfer of ions across the membrane reaches the maximum rate of transfer across interfaces, and the current reaches its maximum value.

Many current equations having such properties have been developed for either pores or carriers. Before introducing a specific model, another important aspect of our $I_K - V$ curves had to be taken into account: the ratio of the maximum current for positive and negative potentials. This ratio is about 4 when $[K^+]_o = 4.2$ mM, near 1 when $[K^+]_o = 22$ mM and remaining at 1 when $[K^+]_o = 147$ mM. It can be observed that this ratio of maximum currents does not follow the ratio of $[K^+]_i/[K^+]_o$, especially at low $[K^+]_o$. Many models demonstrate a flattening of the current, but only a few more complicated ones could satisfy the above criteria for the maximum current ratio. Because of that fact, some simple models like the one proposed by Adrian (1969, p. 354) had to be rejected. The multi-site pore models developed by Hill and Chen (1971) could probably be appropriate, but were not tested directly.

The carrier models developed by Ciani et al. (1973) and by Adrian (1969) were found to be satisfactory. Since it was not possible to determine a unique model to explain quantitatively our data, it was decided that the most simple current equation having the appropriate form should be selected. The form of this current equation was obtained from the carrier model of Ciani et al. (1973), after having combined many parameters together. This current equation is given by the following:

$$I_K = \frac{zFK(C_{ki}\, e^{x/2} - C_{ko}\, e^{-x/2})}{v_m^{-1} F(x) + v_s^{-1}[e^{x/2}(1 + A\, C_{ki}) + e^{-x/2}(1 + A\, C_{ko})]} \tag{1}$$

where C_{ki} and C_{ko} are the concentrations of K^+ in internal and external media, $x = zFV_m/RT$, V_m is the membrane potential; v_m and v_s represents ionic velocities in the membrane and at the interfaces, respectively. $F(x)$ is a function depending on the profile of barriers to ions inside the membrane. If ions cross the membrane in a single jump, $F(x) = 1$. If they are continuously distributed in a constant electric field, $F(x) = (2/x)\sinh(x/2)$; in that case the well known Goldman constant field current equation is obtained if $v_s^{-1} \ll v_m^{-1}$. In order that the current becomes limited to a

maximum value when V_m becomes large, v_s^{-1} should not be too small compared to v_m^{-1}, meaning that the interface velocity should not be too large compared to the membrane velocity. If $v_m^{-1} \ll v_s^{-1}$, the form of $F(x)$ is not important, because $v_m^{-1} F(x)$ is negligible. In this case the interface velocity is much slower than the membrane velocity and ions in the membrane can be considered at equilibrium.

The parameter K and A do not have a precise meaning. They would be defined precisely if a particular model is chosen. For example, in the carrier model of Ciani et al. (1973), A is related to the rate of diffusion of carrier molecules in the membrane and at interfaces. When comparing Eq. (1) with the $I_K - V$ curves of Fig. 4, it was found first that v_s should be much smaller than v_m, such that $v_m^{-1} F(x)$ should become negligible. In that case, Eq. (1) was transformed as follows:

$$I_K = \frac{B(C_{ki}\, e^{x/2} - C_{ko}\, e^{-x/2})}{(1 + A\, C_{ki})\, e^{x/2} + (1 + A\, C_{ko})\, e^{-x/2}} \qquad (2)$$

where $B = z F K v_s$.

Parameter B becomes only a scale factor. Since all the four measured curves were scaled to the same amplitude, a single value for B was used. The only remaining parameter is A. A value of $A = 0.1$ was found to give the best fit for all four curves of Fig. 4.

Although it is not possible to decide on the basis of our data if K^+ diffuses through pores or with carriers, it would seem more probable that a K^+ carrier is involved in producing oscillations. The reason is the observation by Nelson and Peacock (1973) that a transmitter is produced by large L cells, after a hyperpolarizing response is provoked. It is possible that the K^+ current producing oscillations or hyperpolarizing responses is caused by the K-carrier complex diffusing out of the cells and reaching nearby cells to hyperpolarize them. The continuous oscillations of some cells could serve as a clock or communicating mechanism.

It is not possible at the moment to propose a precise model to explain these oscillations of the K^+ current. According to the results of Gallin, Wiederhold, Lipsky and Rosenthal (1975), an influx of Ca^{++} ions is necessary to produce the oscillations of potential they observe in macrophage. It was also found in large L cells that an influx of Ca^{++} ions was required in order to obtain oscillations (Okada & Tsuchiya, *in preparation*). As mentioned by Gallin et al. (1975) it is possible that a change of internal Ca^{++} stores is related to these oscillations since it is known

that internal Ca^{++} content modifies the K^+ membrane permeability. More experiments are required regarding the effect of specific metabolic inhibitors, and ATP and Ca^{++} content of these cells before a more detailed model can be proposed. It would be interesting if these potential oscillations are reflecting some specific oscillating function of mitochondria.

We are very much indebted and grateful to Prof. A. Inouye of Kyoto University for providing us with all the appropriate equipment and facilities of his laboratory. We also wish to express our gratitude to Prof. Y. Doida and Dr. W. Tsuchiya for their invaluable assistance in cell culturing and radiation. We are thankful to the Memorial Foundation of Kyoto University and to the National Research Council of Canada for financial assistance.

References

Adrian, R.H. 1969. Rectification in muscle membranes. *Prog. Biophys. Mol. Biol.* **19**:341

Ciani, S., Eisenman, G., Laprade, R., Szabo, G. 1973. Theoretical analysis of carrier-mediated electrical properties of Bilayer membranes. *In:* Membranes, A Series of Advances. G. Eisenman, editor. Marcel Dekker, New York

Gallin, E.K., Wiederhold, M.L., Lipsky, P.E., Rosenthal, A.S. 1975. Spontaneous and induced membrane hyperpolarizations in macrophages. *J. Cell. Physiol.* **86**:653

Hill, T.L., Chen, Y. 1971. Cooperative effects in models of steady-state transport across membranes. *Biophys. J.* **11**:685

Lamb, J.F., MacKinnon, M.G.A. 1971. The membrane potential and permeabilities of the L cell membrane to Na^+, K^+ and chloride. *J. Physiol. (London)* **213**:683

Lamb, J.F., MacKinnon, M.G.A. 1971*b*. Effect of ouabain and metabolic inhibitors on the Na^+ and K^+ movements and nucleotide contents of L cells. *J. Physiol. (London)* **213**:665

Lauger, P., Neumcke, B. 1973. Theoretical analysis of ion conductance in lipid bilayer membranes. *In:* Membranes, A Series of Advances. G. Eisenman, editor. Marcel Dekker, New York

Nelson, P.G., Peacock, J.H. 1973. Transmission of an active electrical response between fibroblasts in cell culture. *J. Gen. Physiol.* **62**:25

Okada, Y., Inouye, A. 1976. Studies on the origin of the tip potential of glass microelectrodes. *Biophys. Struct. Mechanism* **2**:31

Okada, Y., Doida, Y., Roy, G., Tsuchiya, W., Inouye, K., Inouye, A. 1977*a*. Oscillations of membrane potential in L cells: I. Basic characteristics. *J. Membrane Biol.* **35**:319

Okada, Y., Roy, G., Tsuchiya, W., Doida, Y., Inouye, A. 1977*b*. Oscillations of membrane potential in L cells: II. Effect of monovalent ion concentrations and conductance changes associated with oscillations. *J. Membrane Biol.* **35**:337

Roy, G. 1975. Models of ionic currents for excitable membranes. *Prog. Biophys. Mol. Biol.* **29**:57