Rapid Changes of Potassium Concentration at the Outer Surface of Exposed Single Neurons during Membrane Current Flow

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ABSTRACT K+-sensitive liquid ion-exchanger microelectrodes are shown to be capable of measuring concentration changes which occur on a millisecond time scale. However, some quaternary ammonium ions, such as tetraethylammonium and acetylcholine, are able to block electrode function when present in concentrations as low as $10^{-4}$ to $10^{-3}$ M. Changes in extracellular potassium concentration caused by spike activity or voltage clamp pulses of exposed single neurons of the snail Helix pomatia may be measured by these electrodes. Quantitative analysis shows that the total amount of excess potassium found in the vicinity of the cell a short time after a clamp pulse, is in relatively good agreement with the amount of potassium carried by the membrane current.

Liquid ion-exchanger microelectrodes have been used to determine concentration changes of K+ in a variety of preparations (Walker and Brown, 1970, Khuri et al. 1971; Kunze and Brown, 1971; Russell and Brown, 1972; Hnik et al. 1972; Vyskočil et al., 1972; Prince, Lux, and Neher, 1973; Lux and Neher, 1973). The purpose of this paper is to determine rapid concentration changes that take place in close vicinity of single exposed neurons during spike activity or during depolarizing voltage clamp pulses. It is interesting to compare these values with estimates of [K+] at the outer membrane surface, based on equilibrium potential measurements. Any differences between the two sets of measurements should be due to diffusion barriers in the immediate vicinity of the plasma membrane. (Frankenhaeuser and Hodgkin, 1956; Adelman and Palti, 1969).

The ionic theory predicts that a quantitative amount of K+ flows into the outside medium during outward membrane currents. We were therefore interested in demonstrating whether or not the sensitive microelectrodes, and also the techniques involved in this study, were capable of recovering the predicted amount of K+. This paper shows that with a fast recording tec-
nique a quantitative detection of [K+] increase due to voltage clamp currents is possible.

Parts of the present work have been previously published in abbreviated form (see Lux, Neher, and Prince, 1972; Neher and Lux, 1972 a).

METHODS

Liquid ion-exchanger microelectrodes are glass micropipettes filled with electrolyte; for a short length at the very tip, there is a column of liquid ion-exchanger. Thus, there is a test fluid-ion-exchanger boundary at the tip of the electrode which develops a boundary potential proportional to the logarithm of ion concentration. The electrode measures a potential which is the sum of this boundary potential and the actual electric potential existing at the tip. The electric contribution can be cancelled by selecting an appropriate reference electrode.

Electrode Fabrication

In order to hold the exchanger column in a stable position, the inner surface of the glass wall at the tip must be rendered hydrophobic. This is achieved by siliconization, a procedure which is described in the papers above. It involves treatment with silicon oil and subsequent baking (see Walker and Brown, 1970). After some experimentation, we arrived at an alternative procedure which is less time consuming and has so far shown equivalent results. This process consists of the following steps:

(a) Glass micropipettes are pulled in the normal way using pyrex glass.

(b) The electrode tips are broken under the microscope with the help of a glass rod until tip diameters of 2-6 \( \mu \)m are obtained. The edges of the tip are smoothed in a flame in order to prevent cell damage when the electrodes occasionally touched the cell surface.

(c) The tips of the pipettes are dipped into a 4\% solution of dichlorodimethylsilane in CCl\(_4\) for 1-5 s. Because of capillary action, a column of liquid between 200 and 500 \( \mu \)m is drawn into the tip.

(d) The electrodes are immediately submerged in methanol and are filled with the aid of a vacuum pump. This is a standard method of filling electrodes. The methanol is exchanged with water as soon as possible. The water is allowed to diffuse into the electrode for a few hours to ensure complete removal of the methanol from the tip; it is then exchanged with 0.1 M KCl. At this point the electrodes can be stored for 2-3 days.

Before the experiment, the electrode tips are dipped into the ion-exchanger oil, which in our case is Corning K\( ^+ \)-ion-exchanger no. 477317 (Corning Glass Works, Corning, N. Y.). The tips should fill spontaneously with the oil, but if they do not, suction can be applied by means of a syringe. The electrodes should be stored with the tips immersed in saline.

Amplifier

Connection to the amplifier is made by an Ag-AgCl wire. For preamplification we used an Ancom 15A71a operational amplifier (or Teledyne Philbrick 1029 Teledyne
Philbrick, Dedham, Mass.) which provided a low capacitance input and $10^{10}$ Ω input resistance (MOS-FET input). Negative feedback was applied giving a total amplification factor of 10 at the output. Standard C-neutralization provided a fast response (see Fig. 1 A). In most cases, a remotely located Ag-AgCl wire connected to the bath via an agar bridge was used as a reference electrode. For this reason, a small contribution because of the extracellular clamp current field could be seen in some recordings. This was small compared to the potassium signal. In the case of Fig. 5, a double-barreled electrode, according to Lux and Neher (1973) was used, one barrel being the sensitive electrode and the other the reference electrode. Any contribution of diffusion potentials at the reference electrode can be shown to be small, as long as measurement is restricted to small changes in KCl concentrations in the presence of large NaCl concentrations.

**Preparation**

Exposed ganglion cells from the visceral ganglion of the snail *Helix pomatia* were used for the experiments. The inner connective tissue capsule was opened by microdissection. Cells prepared in this way could be penetrated smoothly for intracellular recordings without major dimpling. Recording electrodes, voltage clamp setup, and details of the preparation have been described in a previous paper (see Neher, 1971). Ringer solution had the following composition (millimoles/liter): NaCl, 80; CaCl₂, 10; KCl, 4; MgCl₂, 5; Tris-Malein buffer, 5; glucose 2 mg/ml.
RESULTS

Electrode Response

Electrodes were calibrated by using a series of Ringer solutions, in which different amounts of NaCl had been replaced by KCl. The calibration curve is given in Fig. 1 B. (for details of electrode calibration see also Lux and Neher, 1972). According to the electrode resistance of around 10 GΩ and an input capacitance of 10–20 pF, a time constant for the input circuit of 0.1 s is expected. With C-neutralization optimally adjusted, this could be reduced to a few milliseconds as illustrated in Fig. 2 (left side). Here rec-

![Figure 2](image-url)

**Figure 2.** Left side, signal measured in response to a triangular voltage applied to the ramp input (2 GΩ, 2 μm electrode connected; three different settings of C-Neutralization). Right side, upper trace shows current, which is applied to a K⁺-iontophoresis electrode. Lower trace shows signal of a K⁺-sensitive electrode, the tip of which is about 5 μm away from the iontophoresis electrode.

tangular current pulses were injected into the input lead of the amplifier by means of a voltage ramp, with the electrode connected. The resultant signal was recorded for three different settings of C-neutralization.

From this experiment, one can see that the electrical response time of the input is definitely well within the millisecond range. There is, however, still the question of whether or not actual changes in concentration are recorded with the same speed, or more specifically, whether the boundary potential across the test fluid-ion exchanger interface equilibrates with comparable speed. To test this, we observed the accumulation and dispersion of K⁺-concentration gradients in the immediate vicinity of a K⁺-iontophoresis electrode. Fig. 2 (right side) illustrates a constant current step injected via a 1 GΩ resistor into a K⁺-iontophoresis electrode (filled with 2 M KCl) with a resistance of 30 MΩ (upper trace). The lower trace shows the signal which was measured with a K⁺-sensitive electrode, the tip of which was separated...
about 5 μm away from the iontophoresis electrode. The lower trace shows a small immediate jump caused by the electrical field of the iontophoresis (the reference electrode was a long distance away in this case), and after this is a sigmoid rise caused by the increase of \([K^+]\). The diffusion equation predicts a time-course for the rise of \([K^+]\) which can be calculated in analogy to Carslaw and Jaeger (1959):

\[
\Delta c(t) = \frac{it}{4\pi FrD} \text{erfc} \left( \frac{r}{2\sqrt{Dt}} \right)
\]

where

- \(i = \text{current}\),
- \(r = \text{tip separation}\),
- \(D = \text{diffusion coefficient}\),
- \(F = \text{Faraday's constant}\),
- \(t = \text{transport number}\).

The half-value for the rise in \([K^+]\) calculated from this formula, approximates very closely the experimental value obtained. Measurements of tip separation could not be made very accurately in the present experimental setup. For this reason, steady-state values for \(\Delta c\), which according to (1) have a linear dependence on \(1/r\), were used as a measure for inter-tip separation. In Fig. 3, half-times \((t_{1/2})\) for the rise of the \(K^+\)-signals are plotted.

![Figure 3](image)

**Figure 3.** Comparison of experimental \(t_{1/2}\) values with those expected from diffusion equations (1) for different inter-tip distances. \(t_{1/2}\) is the time in which the electrode signal (not the concentration) reaches half of its final steady-state value. \(t_{1/2}\) is plotted against the steady-state increment of concentration. Open circles with numbers represent theoretical points. Numbers give the tip-separation in micrometers. The experiment was performed in normal Ringer solution.
against steady state \([K^+]\)-values \((\Delta c_a)\), which were obtained by a series of 150 nA iontophoresis pulses with inter-tip separations varying randomly. Theoretical points corresponding to \(t - \Delta c_a\) pairs obtained from formula (1) with \(t = 0.6\) are also included in Fig. 3 with numbers indicating tip separation in \(\mu\text{m}\). One can see that experimental values parallel the curve for the theoretical values except in those cases where theoretical \(t\) values are below 5 ms. In this range, there is some scatter in the experimental points due to switching artefacts. One can conclude that changes in \(K^+\)-concentration which take place in a time span longer than 10 ms are recorded faithfully by the electrodes.

It should be mentioned, however, that in approximately 1 out of 10 electrodes upon immersion in saline or tissue the ion-exchanger column moved 10–50 \(\mu\text{m}\) upwards into the electrode. This caused electrodes to have very slow response times, as any change in \([K^+]\) must then diffuse to the recessed resin surface. These electrodes can be recognized easily and eliminated during the calibration process, by watching the rate of \([K^+]\)-change on the oscilloscope when changing standard solutions. Fast fluctuations which normally occur are markedly reduced if the resin is not at the tip.

**Interference of Large Cations**

An attempt was made to measure \(K^+\) in the presence of tetraethylammonium chloride (TEA). We found that concentrations of TEA as small as \(10^{-4}-10^{-3}\) M can drastically impair electrode function. When TEA concentration is increased in the presence of a constant 10 mM KCl, a correct \([K^+]\) reading is obtained only for TEA concentrations of less than \(5 \cdot 10^{-5}\) M. With TEA concentrations between \(10^{-4}\) and \(10^{-3}\) M, the electrode signal rises sharply, with changes of 150–200 mV per 10-fold change in \([\text{TEA}]\). At the same time the electrode becomes very sensitive to movements of the test fluid and is also polarizable by currents as small as \(10^{-12}\) A. In this transition region equilibration toward a steady-state value sometimes takes as long as 10–20 min. Above \(10^{-2}\) M, the electrode behaves as a Nernstian electrode for TEA.

Effects similar to those caused by TEA could be observed with other substituted amines. Table I shows the changes in electrode readings when 1 mM of the cation in question was added to a standard solution of 10 mM KCl.

**\(K^+\)-Elevation during Spike Activity**

If \(K^+\)-electrodes are advanced toward the surface of exposed neuronal somata, \(K^+\)-waves corresponding to cell activity can be observed. In Fig. 4 A a depolarizing current pulse was applied in order to elicit a volley of spikes. The intracellularly recorded potential is shown in the uppermost trace. The middle trace illustrates the current density flowing through the membrane
and the lower trace shows the signal picked up by the K+-sensitive electrode. Current density is measured by means of a pipette as described previously by Neher and Lux (1969). The K+-electrode was just touching the cell surface in this case. The tip had a rather large diameter (approximately 4 μm, flame polished) in order to obtain high resolution. In some cases a response generated by single spontaneous spikes could be resolved. (Fig. 4 B)

**K+-Signals during Voltage Clamp**

Large and long lasting depolarizations applied in the voltage clamp experiment are reproducibly coupled with relatively high concentration changes of potassium. This is illustrated in Fig. 5. A plot of the maximum K+-signal

| Substance                        | ΔV° (mV) |
|----------------------------------|----------|
| Tetramethylammonium chloride     | 115      |
| Tetraethylammonium chloride      | 205      |
| Tetrapropylammonium iodide       | 310      |
| Triethanolamine-HCl              | 0        |
| Trimethylamine-HCl               | 54       |
| Dimethylammonium chloride        | 18       |
| Methylammonium chloride          | 5        |
| Acetylcholine                    | 95       |
| Ammonium chloride                | 0        |
| Cetyl trimethylammonium chloride | 300      |

* ΔV : 10 mM KCl → 10 mM KCl + 1 mM TEACl.

Figure 4. Response of K+-electrode to a volley of spikes (A) and to a single spike (B). Middle trace is current density with outward current recorded as a downward deflection. The electrode was in closest vicinity to the cell surface. 2 mV K+-signal is equivalent to a 0.5 mM change in concentration.
Figure 5. K\(^+\)-movements during voltage clamp: (A) shows clamp currents for depolarizing test pulses. Amplitudes of test pulses are indicated in millivolts. Current calibration: 20 nA or 0.4 mA/cm\(^2\), outward currents positive. Inward currents are not visible because of low time resolution. (B), K\(^+\)-traces measured simultaneously with the current traces from (A). Calibration: 4 mV or 1 mM change in concentration. (C), Peak values of clamp current •—• and K\(^+\)-signal ○—○ plotted vs. amplitude of clamp steps. The left ordinate axis is calibrated both in millivolts (reading of K\(^+\)-electrode) and in millimoles/liter total K\(^+\)-concentration. The abscissa gives amplitudes of voltage clamp steps from resting potential (45 mV). (D), Time-course of the changes of the outward current equilibrium potential (measured from resting potential) during and after a 1200 ms voltage clamp pulse of 60 mV amplitude. Parts A, B, C, D taken from the same cell.

versus amplitude of the clamp pulse (Fig. 5 C), reveals a threshold behavior similar to that of the clamp outward currents. The strong K\(^+\)-inactivation, seen in the clamp current recordings, appears in the K\(^+\)-signal as a slow sag toward the end of clamp pulses.

The voltage clamp method allows measurement of the K\(^+\)-equilibrium potential. This has been shown to shift during the course of a clamp pulse because of K\(^+\)-accumulation on the outside of the cell membrane (Alving, 1969). This shift was found to be much larger than the signal picked up by the K\(^+\)-sensitive electrode. The time-course of the K\(^+\)-equilibrium potential is shown in Fig. 5 D. For the ascending part of the curve, values were obtained by measuring the equilibrium potentials after termination of 60 mV clamp pulses of different lengths (see Neher and Lux, 1972 b). For the descending part, values were obtained by giving a 60 mV clamp pulse of 1200
ms duration, and after its termination, measuring the equilibrium potential at different times by means of a second short pulse pair.

K⁺-signals measured with sensitive microelectrodes as described above are reproducible in their time-course, however, they vary in amplitude up to a factor of 5 when using different cells or different locations on one cell. Sometimes the K⁺-signal increased suddenly during an experiment when an electrode had touched the cell for a long time or had been slightly pressed on it. In these cases K⁺-signals no longer showed a threshold behavior, i.e., there were K⁺-elevations for subthreshold clamp pulses without active K⁺-currents flowing. Also, K⁺-values rose to constant levels during a pulse in spite of appreciable K⁺-inactivation. These observations suggest the formation of additional leaks. However, any increase in leakage conductance larger than 10% can be ruled out by the fact that there were no detectable changes in resting potential or resting conductance.

Quantitative Assay of Potassium Loss from a Neuron during a Depolarizing Voltage Clamp Pulse

An experiment was done in order to show if there is quantitative agreement between the K⁺ lost from the cell (measured by clamp outward current) and the K⁺ which could be detected by the K⁺-electrode. For this purpose, 60 mV depolarizing clamp pulses were given repeatedly, and simultaneously the K⁺-electrode tip was advanced towards the cell in 10 μm steps. Special care was taken to record only when actually approaching the cell, i.e., all the original traces except the one for \( d = 0 \) were obtained before the K⁺-electrode touched the cell. In this way damaging effects are avoided.

The resultant curves \( c_i(t) \) are shown in Fig. 6 together with a representative current trace. From these curves another family of curves was obtained (Fig. 7) which represent the spatial distribution of "extra"-[K⁺] at different times (curves \( c_i(x) \)). "Extra"-[K⁺] is the term used for the [K⁺] which appears in addition to normal Ringer potassium. Once this is established it is easy to calculate the total amount of extra-potassium in the vicinity of the cell at a certain time \( t \):

\[
m_t = \int c_i(x) \, dv
\]

The integration process extends over the whole extracellular space. In order to evaluate Eq. (2), radial symmetry was assumed. This is a close approximation, as most of the cells are almost spherical. The cellular surface is in contact with free Ringer solution except for an angular section consisting of about one-third of its total area. The procedure does not account for the existence of extracellular tissue, however, errors were minimized by placing K⁺-electrodes so that they were in the central part of the free cell section. The total amount
of "extra"-K\(^+\) in the extracellular space then becomes:

\[
m_i = \int_{r_o}^{\infty} c_i(r)4\pi r^2 \, dr,
\]

where \(r_o = \) cell radius.

Experimental values from Fig. 7 were multiplied by their corresponding factor \((4\pi r^2)\) and plotted. Finally the area under the resultant curve was determined.

In order to obtain the total amount of K\(^+\) which was lost from the cell, the outward current density close to the location of the K\(^+\)-measurement was determined using the pipette method (80 \(\mu\)m diameter pipette, Neher and Lux, 1969). From this, the current was calculated by multiplying the density by the area of a sphere that had the same radius of curvature as the cell at the point of interest. The current was integrated to obtain the charge lost from the cell, which was then converted into the amount of K\(^+\) involved, by multiplying with the inverse of Faraday's constant. This method was preferred to that of measuring total clamp currents, because many of the errors caused by
inhomogeneity and nonradial symmetry cancel out, with respect to the comparison of both K⁺-values. Contributions of Na⁺-current are negligible in the case of voltage clamp pulses of 500 ms duration or more. Values for total amount of extra-K⁺ at different times during and after a clamp pulse are plotted against time in Fig. 7.

A comparison of the K⁺ lost from the cell (or more precisely from an ideal sphere with homogeneous properties) to that of the amount of K⁺ found in the vicinity of the same ideal sphere, 1–2 s after the pulse, is given in Table II. It is seen that an average of 105% of the potassium which had been released from the cell can be detected by the electrode.
TABLE II
COMPARISON ON K⁺ LOST FROM CELL TO K⁺ FOUND IN VICINITY OF SAME IDEAL SPHERE 1-2 S AFTER PULSE

| Cell number | K⁺ current (mol 10⁻¹²) | K⁺ electr. (mol 10⁻¹²) | Percent |
|-------------|------------------------|------------------------|---------|
| 2           | 0.95                   | 1.65                   | 170     |
| 3           | 0.6                    | 1.02                   | 170     |
| 3           | 1.18                   | 1.17                   | 99      |
| 5           | 1.49                   | 1.57                   | 105     |
| 5           | 0.63                   | 0.65                   | 105     |
| 7           | 5.73                   | 3.91                   | 68      |
| 8           | 1.63                   | 1.41                   | 86      |
| 9           | 2.32                   | 2.04                   | 87      |
| 9           | 1.17                   | 0.78                   | 66      |
| 9           | 3                      | 2.2                    | 73      |
| 10          | 0.83                   | 1.0                    | 121     |
| 11          | 1.25                   | 1.07                   | 85      |
| 5           | 1.65                   | 2.36                   | 143     |
| 5           | 1.65                   | 1.57                   | 95      |
| Mean        |                        | 105                    |         |
| rms         |                        | 35                     |         |

DISCUSSION

Liquid ion-exchanger microelectrodes have been shown to be reliable when measuring K⁺-activities in some biological fluids (Cornwall et al., 1970). We found that some physiologically important substances, e.g. tetraethylammonium chloride and acetylcholine, impair electrode function at very low concentrations. This indicates that one should be very cautious when interpreting results measured with these electrodes, especially in cases when abnormally high values are obtained. In this paper interference of any TEA+-like substances is not likely, as the known relatively low K⁺-concentration in the Ringer fluid was measured correctly in the presence of tissue.

It is noticeable that not only does TEA⁺ impede K⁺-electrode function, but also affects K⁺-outward currents in nerve. However, these TEA⁺-effects differ in that TEA⁺'s action on nerve is highly specific, whereas with K⁺-electrodes it is not specific. Compounds with hydrophobic side-chains in combination with an -N⁺- seem to be the only requirements needed to cause this effect on electrodes.

The limitation of the electrode response time (≈5 ms) was most probably set by the time constant of the input circuit. In order to obtain faster responses, electrodes with wider tips (>3 μm) should be used. However, in this case, diffusional equilibration along the tip diameter takes place on the millisecond time scale. Whether an attempt to decrease electrode response time
further is worthwhile or not, seems doubtful. Any dead space around the electrode tip larger 3-5 μm or any equivalent incorrect placement of the electrode will cause delays in measurement, which are longer than 5-10 ms.

Measurements on snail neurons were made with the cells exposed to saline as much as possible. Electron micrographs of cells prepared in this way showed that there was a thin layer (2-5 μm) of partially torn glia-like lamellae, which irregularly covered the plasma membrane. This layer is most probably the cause of the prominent discrepancies between the \([K^+]\) which was measured by the electrode (Fig. 5 B), and the \([K^+]\) at the outer membrane surface, calculated from measurements of equilibrium potential (Fig. 5 D). The equilibrium potential had a \([K^+]-\)sensitivity of approximately 48 mV/decade in the low \([K^+]\) range (Neher and Lux, 1972 b). \(K^+\)-sensitive electrodes on the other hand, at normal Ringer concentrations had a differential sensitivity of 40 mV/decade. Therefore millivolt readings of similar magnitudes would be expected in Figs. 5 B and 5 D unless there is a barrier to diffusion near the outer membrane surface.

Under the simplest assumption of just one diffusion barrier in a certain distance \(d\) from the membrane and an otherwise free solution, the value of \(d\) can be estimated from the initial slope of the equilibrium potential shift. Between 50 and 100 ms after onset of the clamp pulse, there is a shift in equilibrium potential of 5.5 mV. This gives an initial \(dc/dt\) of 2.6·10^{-8} mol/cm^3 per s. With an average current density during this period of 0.3 mA/cm^2, which corresponds to 0.32·10^{-8} M released per cm^2 and s, a width of \(d = 1.4 \mu m\) is calculated for the space. With different cells, this parameter varies between 1 and 4 μm.

For the above calculation spatial uniformity of concentration in the cleft was assumed. This is justifiable, since diffusional equilibration takes place in about 1 ms. This is quite fast in relation to the time-course of the potential shift.

The existence of a diffusion barrier can also be inferred from the delayed time-course of the \(K^+\)-signal (Fig. 5). Rise in \([K^+]\) does not reflect the relatively fast current peak (Fig. 5 A), but is delayed by about 200 ms. A distance from the cell surface of more than 20 μm would be required if this delay were due to diffusional spread in Ringer solution only. On the other hand the kinetics of the \(K^+\) signal closely resembles the time-course of the \(K^+-\)equilibrium potential.

Frankenhaeuser and Hodgkin (1956) and Adelman and Palti (1969) postulated an extracellular space of 200 Å in squid giant axon. This is about 100 times smaller than the value reported here. Therefore, it can be concluded that the diffusion barrier in our preparation does not consist of a continuous layer of satellite cells as in squid, but is rather more a loose arrangement.
Electron microscopic studies on Aplysia neurons by Coggeshall (1967) and Rosenbluth (1963) lead to similar conclusions. However, it is possible, that the extensive dissection used in our experiments could have loosened the cell layers around the plasma membrane. For instance Baylor and Nicholls (1969) found very different redistribution properties of extracellular potassium after spike activity in leech neurons which depended on whether the tissue surrounding the cells was intact or not.

Alving (1969) reported shifts in equilibrium potential in Aplysia cells between 0 and 25 mV and found noticeable differences between pacemaker and nonpacemaker cells. We found similar differences in shifts of $V_{\text{equil}}$, ranging from 7 to 20 mV. However, no attempt was made to correlate this with pacemaking, as most of the cells were silent after an initial period of firing. All the cells could be made repetitively active by application of a constant depolarizing current.

Differences in the degree of covering, and also in homogeneity of the layer of satellite cells, seems to be the cause of the wide variation (factor of 5) of K+-signals in those cases when the electrode tip touched the cell surface. An alternative explanation could be the possibility of localized areas of increased K+-permeability. At the moment we have no way to elucidate the causes of the discrepancies. In some cases, the K+-signal increased with time, which is to be expected if the electrode had penetrated into the cleft system. However, the possibility exists that K+-carriers diffused from the electrode into the adjacent membranes, and thereby produced K+-specific leaks. This might also explain the side effects which were observed in these cases. However, neither a K+-specific leak which would increase resting potential, nor an injury leak which would depolarize the cell, could be detected.

If $[K^+]$ is measured at a larger distance from the cell, local inhomogeneities should be averaged out. The same should be true for measurements of current density with an 80 $\mu$m pipette. It turned out that the total amount of potassium which is lost from the cell can be detected relatively accurately about 0.5-1 s after termination of a clamp pulse. It is within $\pm 35\%$ (rms) of that amount which is estimated from the charge displacement across the membrane. At the time of this measurement, according to Fig. 5 D most of the potassium in the area of restricted diffusion has disappeared. In Fig. 7 it is seen that at this time the bulk of K$^+$ is found at distances up to 60 $\mu$m away from the cell boundary. This result independently proves that outward currents in nerve are largely carried by K$^+$-ions, although if there were a 10-20% contribution from other ions, it would not have been detected using this method. It also shows that most of the K$^+$ is released into the bath under these conditions (i.e. with most of the connective tissue removed).

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