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THE MEMBRANE POTENTIAL AND PERMEABILITIES OF THE L CELL MEMBRANE TO NA, K AND CHLORIDE

BY J. F. LAMB* AND M. G. A. MACKINNON†
From the Institute of Physiology, University of Glasgow, Glasgow, W. 2

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SUMMARY

1. The chloride content and fluxes, and the membrane potential of L cells have been measured.

2. L cells contain chloride, 70 m-mole/l. intracellular water and have a flux of 5·5 p-mole/cm^2 sec.

3. The membrane potential is -15 mV. K-free Krebs causes an increase in E_m and replacing chloride with sulphate causes a temporary reduction in E_m.

4. These values for E_m and chloride, and previously obtained values for Na and K fluxes and contents were used to calculate the permeabilities of the various ions using the Goldman constant field theory. This gave permeabilities of 6·3, 4·2 and 51×10^{-9} cm/sec for K, Na and chloride respectively, a ratio of 1:0·67:8·10.

5. It is concluded that these cells have a low membrane potential because the P_K is some 100 times lower than in skeletal muscle, therefore leading to a P_K of the same order as P_{Na}.

INTRODUCTION

Cells which are electrically excitable are characterized by having a high membrane potential at rest (Katz, 1966) whereas many small inexcitable cells have low membrane potentials (Hempling, 1958; Aull, 1967; Borle & Loveday, 1968). In both kinds of cells chloride is commonly distributed passively across the membrane (but see Keynes, 1963), so that in cells with high membrane potentials the intracellular concentration is low (Boyle & Conway, 1941; Adrian, 1961) and in cells with low membrane potentials

* Present address and address for reprints: Department of Physiology, Bute Medical Buildings, St Andrews, Fife, Scotland.
† Present address: Bio-cult Laboratories Ltd. Glasgow, W. 2.

the intracellular concentration is high (Hempling, 1958; Wickson-Ginzburg & Solomon, 1963; Whittam, 1964).

In excitable cells the high membrane potential arises because of a high K permeability relative to the Na permeability, and therefore the membrane potential is set at a value close to the potassium equilibrium potential. In non-excitable cells the low membrane potential could arise due to the K and Na permeabilities being approximately equal, so that the membrane potential takes up a position roughly midway between the K and Na equilibrium potentials. If this is so this could arise either as a consequence of a low potassium permeability or a high Na permeability compared to excitable cells, or a combination of these factors. It has been argued that these inexcitable cells have a high absolute Na permeability (Schanne & Coraboeuf, 1966; Aull, 1967; Borle & Loveday, 1968). The purpose of the present paper is to show that the Na permeability in L cells is probably very similar to that in excitable cells whereas the K permeability is two orders of magnitude less, so that their observed low membrane potential arises as a consequence of a low K permeability.

METHODS

Two kinds of experiment were done. (1) The chloride contents and fluxes were measured, using ^{36}Cl in a technique identical to that described for ^{24}Na and ^{42}K in the last paper (Lamb & MacKinnon, 1971) and (2) the membrane potentials of the cells were measured, using conventional micro-electrodes. Cells were cultured on plastic Petri dishes, using methods similar to those described in the last paper. Monolayers for both types of experiment were used, those for micro-electrode use were cultured until a complete monolayer was obtained (to increase the chances of the micro-electrode hitting a cell), those for isotopic work were usually rather thinner. We had no evidence that this affected the results. Both types of experiment were done at 20° C.

Membrane potentials. Conventional micro-electrodes, pulled from Pyrex glass and filled with 3 M-KCl, were prepared. These had a resistance in the range of 10–30 MΩ, and small tip potentials. The micro-electrodes were held by a micromanipulator mounted on a rigid steel plate. The output of the electrodes was led to a cathode follower with a grid current of $< 8 \times 10^{-13}$ A and then a Tektronix storage oscilloscope; satisfactory traces were then photographed. Records were calibrated by means of a voltage calibrator between the Petri dish and earth.

To obtain penetration of a cell the tip of the electrode was lowered to the cell surface and then entry obtained by gently tapping on the bench.

RESULTS

In order to calculate the permeabilities of L cells to Na, K and chloride, it is necessary to know the concentration gradients and passive fluxes for each ion and the membrane potential of the cell. The previous paper

gives the necessary data for the Na and K ions, the present paper presents the results for chloride ions and for membrane potentials.

Chloride fluxes and contents

Fig. 1 represents the results of an experiment in which [^{36}Cl]Krebs was applied to plates of cells for varying times, the plates washed in cold inactive Krebs and the ^{36}Cl content measured. The single exponential

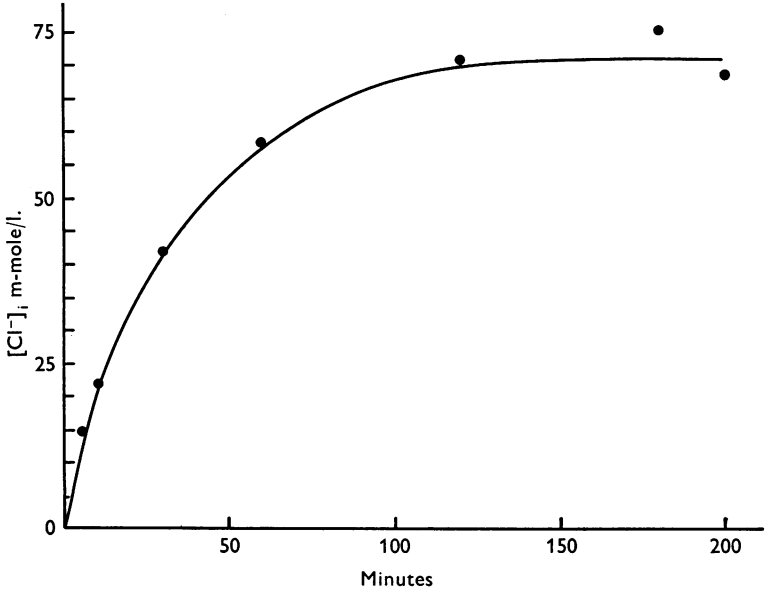

Fig. 1. The influx of ^{36}Cl in L cells. At zero time ^{36}Cl Krebs was added to nine plates of cells. At various times thereafter a plate of cells was washed $\times 6$ with ice-cold inactive Krebs and analysed. Each point represents one such plate. The line was fitted by the method of least squares (after logarithmic transformation; $r = 0{\cdot}999$, $P < 0{\cdot}001$) and represents a single exponential with rate constant of 0·029 min^{-1}. The calculated influx in this experiment was 4·3 p-mole/cm^2 sec.

fitted to these points is shown. The mean results for all experiments were: an intracellular chloride concentration of $69{\cdot}8 \pm 1{\cdot}2$ (S.E., $n = 7$) m-mole/l., an influx of $5{\cdot}99 \pm 1{\cdot}62$ ($n = 3$) p-mole/cm^2 sec, and an efflux of $5{\cdot}02 \pm 0{\cdot}50$ ($n = 3$) p-mole/cm^2 sec. As the influx and efflux were not significantly different an average value of 5·56 p-mole/cm^2 sec was used in subsequent calculations. In one further experiment, ouabain 10^{-3} M, as expected, had no effect on the fluxes or content of chloride. These results therefore show (*a*) that *K* cells have a high intracellular concentration of chloride, in agreement with previous findings on similar cells, and (*b*) that the chloride flux in these cells is several times larger than the Na and K fluxes (Lamb & MacKinnon, 1971).

Membrane potential

Considerable difficulty was experienced in measuring the membrane potential of these cells, presumably because of the small size of the cells and because the cells were in a monolayer on a hard surface. Numerous artifacts were encountered, and only two kinds of record were accepted as giving a true indication of the potential. These (shown in Fig. 2) were when there was an abrupt change of potential on entering the cell and an

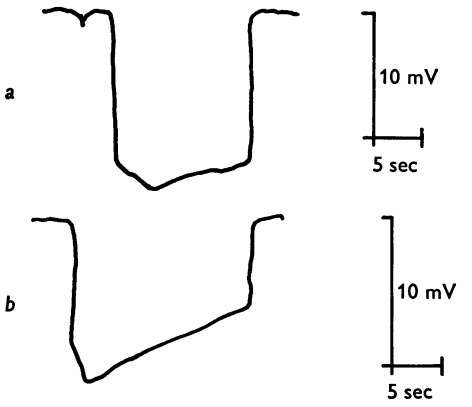

Fig. 2. Examples of acceptable membrane potential measurements. In *a* the potential remains relatively stable; in *b* it decays slowly after penetration, this was the most common pattern (tracings from photographs).

TABLE 1. Membrane potentials in different conditions. The Krebs value is the over-all average; each of the other conditions was tested against appropriate Krebs controls and the significance values apply to this. On removing chloride the cells were initially depolarized but later returned to normal. K-free Krebs caused a hyperpolarization

Conditions	*n*	Mean (mV)	S.E. (mV)	Significant from Krebs
Krebs	78	−15·4	0·5	—
K-free Krebs < 30 min	18	22·8	1·3	$P < 0{\cdot}01$
$Cl \rightarrow SO_4$ Krebs				
< 10 min	10	−3·8	0·7	$P < 0{\cdot}001$
> 20 min	16	−15·9	±0·9	n.s.

abrupt reversal on removal with (*a*) a stable potential once inside (15 sec) or (*b*) a slowly declining potential once inside the cell. This second kind was the more common; in this case the initial deflexion was taken as the true potential, the decline being considered due to injury.

Table 1 shows that an over-all mean value of about −15 mV was obtained for cells in normal Krebs. If this is the actual potential, and not an artifact due to poor technique, it ought to be altered in a predictable

way by changes in the bathing solution. Table 1 shows also that (*a*) chloride removal causes depolarization of the cells followed by recovery to about normal and (*b*) K removal hyperpolarizes the cells. As both of these results are expected, at least qualitatively, on the current views of the membrane potential (e.g. see Hodgkin & Horowicz, 1959) we regard this as good evidence for the validity of our measurements.

DISCUSSION

The present experiments give two pieces of evidence that chloride is passively distributed across the L cell membrane. (1) The observed value of $-15{\cdot}4$ mV, although smaller than the equivalent potential calculated

TABLE 2. The concentrations, fluxes and calculated permeabilities to Na, K and chloride in L cells. Data from this and the previous paper. The permeability is calculated from the constant field equation using an E_m of -15 mv. The ratio of $P_K : P_{Na} : P_{Cl}$ is 1 : 0·67 : 8·10

	Intracellular (m-mole/l. water)	Extracellular (m-mole/l. water)	Passive flux (p-mole/cm² sec)	Permeability of L cells ($\times 10^{-9}$ cm sec)	Permeability of frog muscle
Sodium	8·6	136·58	0·76	4·2	7·9*
Potassium	171	5·65	0·79	6·3	600*
Chloride	70	146·96	5·50	51·0	4000†

* Hodgkin & Horowicz, 1959. † Katz, 1966.

from the Nernst equation of -18 mV, is not significantly different from it ($P > 0{\cdot}05$) and (2) ouabain has no effect on the intracellular concentration or fluxes of chloride, as expected from findings in many other cells.

Table 2 shows the data for all these ions from the present and preceding paper, and derived values for passive flux and permeability. The passive chloride flux has been taken as the average of influx and efflux, the passive K flux as the efflux and the passive Na flux as that equal to the active component of the efflux. The justification for this latter procedure is that part of the Na influx in other cells is commonly an exhange of some kind with intracellular Na, and so carries no net current; the evidence that this also applies to L cells is that the Na influx is markedly increased by a rise in intracellular Na, a result difficult to explain on other mechanisms. In the absence of any more direct measure of the exchange diffusion component, the Na flux chosen to represent the passive leak is that required to balance it actively. If this procedure is not justified then the maximum error introduced is a twofold one.

These values were used to calculate, by the Constant Field Theory

(Goldman, as given by Hodgkin & Katz, 1949), the permeabilities shown in the fourth column of Table 2 (expressed in units of 10^{-9} cm/sec). Before discussing them, three sources of error need to be considered. (1) If all the Na influx represented passive Na movement, then the Na permeability could be twice the value shown (v.s.). This, while not changing the general conclusions, would make the ratio of Na/K permeability different. (2) These results are calculated on the basis that the filopodia take part in the ion exchanges, so if the filopodia do not take part in membrane exchanges then all the values will be at least twice as large per unit cell membrane. This also will not affect the general conclusions, nor will it affect the ratio of Na/K permeabilities, but it would at least double the absolute permeabilities. (3) Observational errors. The real membrane potential might be higher than observed due to faulty technique. Calculations showed that changes over the range of $\pm$ 15 mV would not affect the general conclusion although altering the actual permeability values. Such an error would then require an explanation for the chloride distribution observed. Calculations also showed that errors in observed fluxes would need to be very large ($\times$ 100) to account for the observed P_K being so low compared to skeletal muscle. Therefore it seems unlikely that these results can be attributed to experimental errors.

With these observations in mind it is, nevertheless, clear that the absolute permeabilities of the membrane to Na and K are of a similar order of magnitude and both are low, whereas the chloride permeability is an order of magnitude higher. The corresponding permeabilities of frog skeletal muscle are K, 6×10^{-7}, Na $7{\cdot}9 \times 10^{-9}$ and chloride 4×10^{-6} cm/sec. Therefore the P_{Na} of L cells and frog muscle are similar whereas the P_K of frog muscle is $\times$ 100 greater. This gives a P_{Na}/P_K ratio of 0·67 and this is similar to that calculated by using the abbreviated form of the Goldman equation using only the E_m and the Na and K concentration gradients (0·67, using our results; 0·57, Borle & Loveday, 1968). As pointed out by Hodgkin (1958) the ratio of the Na/K mobilities in aqueous solution is also 0·67. The agreement in these experiments could be fortuitous or it could mean that the membrane of these cells does not have any specially selective mechanism for K but simply has a cation permeability in proportion to the aqueous mobility of each ion.

These experiments show that L cells have a membrane potential of -15 mV with chloride distributed passively across the membrane. The calculated permeabilities show that the ratio of P_{Na}/P_K is 0·67. It is concluded therefore that the low membrane potential of these cells arises because of a low absolute P_K rather than a high P_{Na}.

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