

CALCULATIONS OF BIOELECTRIC POTENTIALS

IV. SOME EFFECTS OF CALCIUM ON POTENTIALS IN NITELLA

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(Accepted for publication, April 10, 1938)

The action of calcium on the p.d. of *Nitella* is of especial interest in certain cases where it modifies the effect of potassium.

In some cells¹ the negative change produced by substituting 0.01 M KCl for 0.01 M NaCl is not altered by adding to the KCl solution enough CaCl₂ to make its concentration 0.005 M. But in other cases calcium has more effect.

In a previous paper² the values of u_K and u_{Na} , the apparent mobilities of K⁺ and Na⁺ in X, the outer non-aqueous protoplasmic surface layer, were calculated as $u_K = 11.9$ and $u_{Na} = 7.93$. Using these values we may evaluate the effect of calcium by employing Henderson's equation.

This may be written³ (for 20°C.)

$$\text{p.d.} = 58 \frac{(U_I - V_I) - (U_{II} - V_{II})}{(U'_I + V'_I) - (U'_{II} + V'_{II})} \log_{10} \frac{U'_I + V'_I}{U'_{II} + V'_{II}} \quad (1)$$

in which $U_I = u_1c_1 + u_2c_2$

$$V_I = v_1\bar{c}_1 + v_2\bar{c}_2$$

$$U'_I = u_1w_1c_1 + u_2w_2c_2$$

$$V'_I = v_1\bar{w}_1\bar{c}_1 + v_2\bar{w}_2\bar{c}_2$$

where c = concentration of cation and \bar{c} of anion

u = mobility of cation and v of anion

w = valence of cation and \bar{w} of anion

¹ This is more apt to be true of freshly collected cells than of those which have been freed from neighboring cells and kept for some weeks in the laboratory in Solution A at $15 \pm 1^\circ\text{C}$. Regarding Solution A see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87.

² Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312.

³ Cf. Michaelis, L., Hydrogen ion concentration, Baltimore, The Williams and Wilkins Co., 1926, 179. This formula is incorrectly given in some text-books.

The subscripts I and II refer to the two solutions, the subscripts 1 and 2 to the different ions. All values relate to X , the outer non-aqueous protoplasmic surface layer.

In the case of 0.005 M vs. 0.0005 M CaCl_2 it has been pointed out by Longsworth⁴ that when we put $C_{\text{Cl}} = 2 C_{\text{Ca}}$ this equation reduces to

$$\text{P.D.} = 58 \frac{u_{\text{Ca}} - 2v_{\text{Cl}}}{2u_{\text{Ca}} + 2v_{\text{Cl}}} \log_{10} \frac{c'_{\text{Ca}}}{c''_{\text{Ca}}} \quad (2)$$

where c' relates to Solution I (0.005 M CaCl_2) and c'' to Solution II (0.0005 M CaCl_2). The sign of the calculated P.D. is that of Solution II in the external circuit.

It is also pointed out by Longsworth that equation (2) can be derived by starting with the general equation⁵

$$E_L = \frac{RT}{F} \int \sum \frac{t_i}{z_i} d \ln_e a_i \quad (3)$$

in which t is the transference number and z is the valence of the i th ion, account being taken of its sign. In the case of CaCl_2 , assuming a constant transference number and activities equal to concentrations the equation becomes

$$E_L = \frac{RT}{F} \left(\frac{t_{\text{Ca}}}{2} \right) \ln_e \frac{c'_{\text{Ca}}}{c''_{\text{Ca}}} - \frac{RT}{F} (t_{\text{Cl}}) \ln_e \frac{c'_{\text{Cl}}}{c''_{\text{Cl}}} \quad (4)$$

But $t_{\text{Ca}} + t_{\text{Cl}} = 1$ and although $c_{\text{Cl}} > c_{\text{Ca}}$ we have

$$\frac{c'_{\text{Ca}}}{c''_{\text{Ca}}} = \frac{c'_{\text{Cl}}}{c''_{\text{Cl}}} = \frac{c'}{c''} \quad (5)$$

$$E_L = \frac{RT}{F} \left(\frac{t_{\text{Ca}}}{2} \right) \ln_e \frac{c'}{c''} - \frac{RT}{F} (1 - t_{\text{Ca}}) \ln_e \frac{c'}{c''} \quad (6)$$

$$= \frac{RT}{F} \left(\frac{3t_{\text{Ca}}}{2} - 1 \right) \ln_e \frac{c'}{c''} \quad (7)$$

$$= \frac{RT}{F} \left(\frac{u_{\text{Ca}} - 2v_{\text{Cl}}}{2u_{\text{Ca}} + 2v_{\text{Cl}}} \right) \ln_e \frac{c'}{c''} \quad (8)$$

⁴ Longsworth, L. G., personal communication.

⁵ Cf. MacInnes, D. A., and Longsworth, L. G., The potentials of galvanic cells with liquid junctions, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1936. **4**, 18.

At 20°C. this may be written

$$\text{P.D.} = 58 \left(\frac{u_{\text{Ca}} - 2v_{\text{Cl}}}{2u_{\text{Ca}} + 2v_{\text{Cl}}} \right) \log_{10} \frac{c'}{c''} \quad (9)$$

$$= 58 \frac{\frac{u_{\text{Ca}}}{z^+} - \frac{v_{\text{Cl}}}{z^-}}{u_{\text{Ca}} + v_{\text{Cl}}} \log_{10} \frac{c'}{c''} \quad (10)$$

where z^+ and z^- are the valencies of the cation and anion respectively (it should be pointed out that in all the equations u_{Ca} relates to $\frac{\text{Ca}^{++}}{2}$ and not to Ca^{++} ; *i.e.*, it is the mobility per equivalent of calcium ion and not per mole.

Similarly, for Na_2SO_4 we have

$$\text{P.D.} = 58 \frac{2u_{\text{Na}} - v_{\text{SO}_4}}{2u_{\text{Na}} + 2v_{\text{SO}_4}} \log_{10} \frac{c'}{c''} \quad (11)$$

Equations (9) and (10) are identical with equation (2).

No matter how high we put u_{Ca} we find that with $c'_{\text{Ca}} + c''_{\text{Ca}} = 10$ the calculated value of the P.D. does not exceed 29 mv. if the partition coefficient remains constant. But if the partition coefficient S_{CaCl_2} (= concentration in X + concentration in the external solution) increases with concentration (as happens with certain salts in models⁶) so that when $c'_{\text{Ca}} + c''_{\text{Ca}} = 10$ in the external solution we have in X $c'_{\text{Ca}} + c''_{\text{Ca}} = 19.4$ the calculation gives $u_{\text{Ca}} = 20$ for a P.D. of 32 mv. (as reported⁷ in Table I).

As the value of u_{Ca} depends on that assigned to S_{CaCl_2} , its significance is doubtful but the following calculations may be of interest. It has been shown previously⁸ that from the data in Table I we get $u_{\text{K}} = 11.9$ and $u_{\text{Na}} = 7.93$: if the value of $S_{\text{KCl}} + S_{\text{NaCl}}$ is taken as 60 we get the observed value of 95 mv. for 0.01 M KCl *vs.* 0.01 M NaCl. Taking u_{Ca} as 20 we must put $S_{\text{KCl}} + S_{\text{CaCl}_2}$ as 27.5 to get the observed value of 62 mv. given in Table I for 0.01 M KCl *vs.* 0.005 M CaCl_2 .

The value of the partition coefficient probably increases with concentration as already assumed in the case of CaCl_2 . If this increase is more rapid for KCl than for NaCl it would explain why the potassium effect falls off with dilution below 0.01 M.

Since $S_{\text{KCl}} + S_{\text{CaCl}_2}$ is taken as 27.5 and $S_{\text{KCl}} + S_{\text{NaCl}}$ as 60 the value of $S_{\text{NaCl}} + S_{\text{CaCl}_2}$ may be taken as $27.5 + 60 = 0.46$. Calculation on this basis gives for the

⁶ Osterhout, W. J. V., Kamerling, S. E., and Stanley, W. M., *J. Gen. Physiol.*, 1933-34, **17**, 469.

⁷ The experiments were performed on *Nitella flexilis*, Ag., using the technique described in a former paper (*cf.* Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541). Temperature $20 \pm 3^\circ\text{C}$. All measurements of P.D. were made from photographic records.

P.D. of 0.01 M NaCl vs. 0.005 M CaCl₂⁸ 14 mv. instead of the observed 22 mv. (if we put $S_{\text{NaCl}} + S_{\text{CaCl}_2} = 0.25$ we get 22 mv.). This may indicate that CaCl₂ changes the non-aqueous surface layer as it diffuses through it and comes in contact with the NaCl already present.

TABLE I

The sign * of the P.D. is positive unless otherwise stated and is that of the first mentioned solution as observed in the external circuit. (In all cases the change was made in both directions, e.g. from dilute to concentrated and *vice versa* or from NaCl to KCl and *vice versa*.)

Solution	P.D.	No. of observations	No. of cells	Calculated values
Concentration potentials (homoionic junctions)				
0.001 M KCl vs. 0.01 M KCl	49 ± 0.49	95	41	—
0.001 M NaCl vs. 0.01 M NaCl	45 ± 0.69	75	37	—
0.0005 M CaCl ₂ vs. 0.005 M CaCl ₂	32 ± 0.65	60	31	—
0.001 M KCl + 0.0005 M CaCl ₂ vs. 0.01 M KCl + 0.005 M CaCl ₂	45 ± 1.12	75	41	43
0.001 M NaCl + 0.0005 M CaCl ₂ vs. 0.01 M NaCl + 0.005 M CaCl ₂	33 ± 0.73	70	37	28
Chemical potentials (heteroionic junctions)				
0.01 M KCl vs. 0.01 M NaCl	-95 ± 2.0	120	44	—
† 0.01 M KCl vs. 0.01 M KCl + 0.005 M CaCl ₂	-30 ± 2.2	100	44	—
0.01 M KCl vs. 0.005 M CaCl ₂	-62 ± 2.6	38	28	—
0.01 M KCl + 0.005 M CaCl ₂ vs. 0.01 M NaCl + 0.005 M CaCl ₂	-42 ± 2.3	75	40	-28
0.01 M NaCl vs. 0.005 M CaCl ₂	22 ± 1.2	40	31	14

* The P.D. is called positive when positive current tends to flow outward from the sap across the protoplasm to the external solution.

† Very small values have not been considered (in many cases the value was zero).

According to Henderson's equation the addition of solid CaCl₂, without change of mobilities or of partition coefficients, to a solution of KCl should make it more negative but in reality it becomes more positive. The calculation shows that if the ratio of partition co-

⁸ Here CaCl₂ is negative to NaCl.

efficients is $S_{\text{KCl}} \div S_{\text{CaCl}_2} = 27.5$ then 0.01 M KCl should be positive to 0.01 M KCl + 0.005 M CaCl₂ by 0.6 mv. But it is really negative by 30 mv., as shown in Table I. This would result if u_{K} or S_{KCl} were lowered considerably by the action of CaCl₂.

If u_{K} were lowered considerably the concentration effect would fall off greatly but this is not the case. The concentration effect of KCl for a 10-fold dilution is 49 mv. and for KCl + CaCl₂ is 45 mv. (Table I) which would indicate that u_{K} is about the same in both cases. Probably the simplest assumption is that in the presence of calcium the value of S_{KCl} falls off. If in the mixture of KCl + CaCl₂ the ratio $S_{\text{KCl}} \div S_{\text{CaCl}_2}$ fell to 5.4 the calculation would give⁹ for 0.01 M KCl vs. 0.01 M KCl + 0.005 M CaCl₂ a P.D. of 30 mv. with the mixture¹⁰ positive to pure KCl. This agrees with observation (Table I).

It might be suggested that calcium in this case merely has its ordinary antagonistic effect. But it takes very little calcium to form an ordinary balanced solution (*e.g.* 0.01 M KCl + 0.0005 M CaCl₂) which preserves the life of the cell indefinitely. To affect the P.D. in the manner described in this paper much more calcium is necessary (usually about 10 times as much). It is a striking fact that when we employ 0.1 M instead of 0.01 M KCl^{11,12} the addition of 0.05 M CaCl₂ usually has little or no effect on the P.D.

Moreover with certain cells the addition of as much as 0.005 M calcium to 0.01 M KCl has little or no effect on the P.D. although the normal antagonistic action of calcium is seen when its concentration is only 0.0005 M.

Another possibility is that calcium acts by precipitating an organic substance (or group of substances), called for convenience R_p , which sensitizes the cell to the action of potassium. If, as suggested in a

⁹ Presumably this value varies with conditions like that of other partition coefficient ratios.

¹⁰ For purposes of calculation we assume that in the non-aqueous protoplasmic surface we have in arbitrary units for pure KCl $c_{\text{KCl}} = 55$ and for the mixture $c_{\text{KCl}} = 10.8$ and $c_{\text{CaCl}_2} = 1$.

¹¹ In short experiments the effects of 0.1 M KCl are fully reversible in the absence of calcium.

¹² To alter the effect of lower concentrations of KCl (0.01 M to 0.05 M) we need CaCl₂ of the same normality as KCl: less than this usually has little or no effect.

former paper,¹³ the solubility of this substance is decreased by calcium we may expect that as the concentration of calcium increases beyond a certain point the surface will become less sensitive to the action of potassium (unless the latter has too high a concentration¹⁴ as appears to be the case with 0.1 M KCl).

Höber¹⁵ has suggested that in muscle calcium influences the colloidal structure in such fashion as to diminish the effect of potassium on the P.D.

As the result of the foregoing we arrive at the following values: $u_K = 11.9$, $u_{Na} = 7.93$, $u_{Ca} = 20$. The ratios of partition coefficients are, $S_{KCl} \div S_{NaCl} = 60$, $S_{KCl} \div S_{CaCl_2} = 27.5$, $S_{NaCl} \div S_{CaCl_2} = 0.46$: in a mixture of KCl + CaCl₂ we have $S_{KCl} \div S_{CaCl_2} = 5.4$ and in a mixture of NaCl + CaCl₂ we have $S_{NaCl} \div S_{CaCl_2} = 0.46$.

Using these values we obtain the calculated values shown in Table I. For the concentration effect the agreement between calculated and observed values is very satisfactory: for the chemical effect it is less so but here we must allow for complicating factors.

Let us now consider another effect of calcium. Fig. 1 shows that when the concentration of a mixture of KCl + CaCl₂ increases beyond a certain point an action current is encountered after which the level of the curve is higher by a definite amount. This is also found in pure KCl. The latter has been described in a previous paper¹⁶ and as the behavior of the mixture is closely similar it need not be discussed here in detail. The effect of calcium is to raise the concentration at which the action current occurs. This is evident on comparing Fig. 1 with the corresponding figure in the previous paper.¹⁶

Still another effect of calcium is to inhibit the production of rapid action currents when cells are exposed to 0.01 M NaCl, as described elsewhere.¹⁷

¹³ Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 105.

¹⁴ A high concentration of potassium would tend to increase the concentration of R_p if, as suggested in a former paper (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 107), the latter is a compound of potassium.

¹⁵ Höber, R., *Arch. ges. Physiol.*, 1904-05, **106**, 599; 1916-17, **166**, 582; 1929, **222**, 82.

¹⁶ Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541.

¹⁷ Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 91.

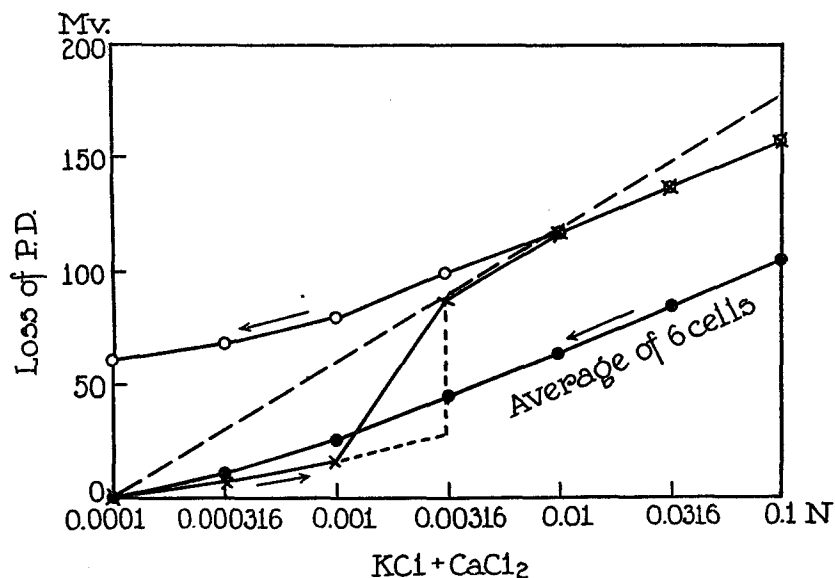


FIG. 1. Changes of P.D. produced by a mixture of equal parts of a solution of KCl and a solution of CaCl₂ of the same normality. The concentrations give the normality of each salt in the mixture: thus 0.1 N signifies a solution containing 0.1 N KCl + 0.1 N CaCl₂.

The broken straight line approximates the theoretical slope of the curve showing change of diffusion potential when the concentration of KCl in contact with *Nitella* increases and the mobility of K⁺ greatly exceeds that of Cl⁻, partition coefficients being constant. The scale of abscissae is logarithmic: each step is made by multiplying by 3.16 (= 10^{0.5}).

The curve with crosses shows measurements on a single cell as the concentration increases (arrows pointing upward). The curve with open circles shows measurements on the same cell as the concentration decreases (arrows pointing downward). The lowest curve shows the average of 6 cells as the concentration of KCl decreases.

The slopes of the curves do not exceed the theoretical except in the curve with crosses where 0.001 is replaced by 0.00316 N. At this point the change in P.D. occurs in two steps as indicated by the dotted line. The first step does not exceed the theoretical; the second is larger and is due to an action current which permanently raises the level of the curve.

In conclusion we may say that in these cells the mobilities of K⁺ and Na⁺ were much closer together than in certain cells studied earlier¹⁸

¹⁸ Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

(the values are 11.9 and 7.93 respectively instead of 85.45 and 2.18). It is possible that this is due in part to the treatment in the laboratory.

We know that mobilities can be influenced in various ways. For example guaiacol greatly lessens the mobility of K^+ in *Valonia*¹⁹ and increases that of Na^+ in both *Valonia* and *Halicystis*.²⁰ Treatment with distilled water greatly lowers the mobility of K^+ in *Nitella*.²¹

We also see that in these *Nitella* cells the partition coefficients differ from those found in earlier studies. For example $S_{KCl} \div S_{NaCl}$ is 60 instead of unity. We find that calcium appears to reduce S_{KCl} from 27.5 to 5.4. This recalls experiments which show that distilled water may lessen the partition coefficient of KCl.²²

We may conclude that mobilities and partition coefficients are variable and can be brought, to some extent at least, under experimental control.

SUMMARY

In *Nitella* the substitution of KCl for NaCl changes the p.d. in a negative direction. In some cases this change is lessened by adding solid $CaCl_2$ to the solution of KCl. This may be due to lessening the partition coefficient of KCl or to decreasing the solubility of an organic substance which sensitizes the cell to the action of KCl.

Little or no correlation exists between this effect of calcium and its ordinary antagonistic action in producing a balanced solution which preserves the life of the cell indefinitely.

$CaCl_2$ is negative to NaCl but positive to KCl.

The effects of mixtures of KCl, NaCl, and $CaCl_2$ are discussed. The concentration effect of a mixture of KCl + $CaCl_2$ shows certain peculiarities due to action currents: these resemble those found with pure KCl.

These studies and others on *Nitella*, *Valonia*, and *Halicystis* indicate that mobilities and partition coefficients are variable and can be brought under experimental control.

¹⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13.

²⁰ Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 707.

²¹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 987.

²² Osterhout, W. J. V., and Hill, S. E., *Proc. Nat. Acad. Sc.*, 1938, **24**, 427.