

IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

V. THE ACTION POTENTIAL

By A. B. HOPE*

[Manuscript received February 13, 1961]

Summary

The effect of various ions on the resting and action potential differences in single cells of the alga *Chara australis* was studied. When the concentration of calcium ions in the external medium was changed by replacement with magnesium ions, keeping the total concentration constant, the peak level of the action potential changed reversibly. The change was about +29 mV per tenfold increase in calcium concentration, over a restricted range of the latter.

Changes in concentration of other ions did not change the peak level of action potential except insofar as the concentration of calcium in the immediate neighbourhood of the plasmalemma was modified by ion exchange.

It is suggested that the plasmalemma becomes specifically permeable to calcium during the action potential so that the peak level of potential reached is related to the ion equilibrium potential for the calcium ion. This is determined by the ratio of calcium ion activity in the cell cytoplasm to that external to the plasmalemma. Earlier published results, suggesting the chloride ion as determining the peak of the action potential, are explained in terms of the present scheme involving calcium.

I. INTRODUCTION

In previous papers (Walker 1955, 1960; Hope and Walker 1961) the resting potential difference and resistance, measured between the vacuole and external medium, have been attributed to a thin membrane (the plasmalemma) bounding the outside of the cell cytoplasm. The main factors setting the magnitude and sign of the resting potential were found to be the concentration of potassium ions and, to a lesser extent, sodium ions in the medium and the permeability of the plasmalemma to these ions. The same parameters were found to describe reasonably accurately the resistance of the cell and its change with external concentration and with current density, assuming in the analysis a linear potential gradient across the plasmalemma (Goldman 1943).

The action potential, a reversible depolarization of this membrane, occurs on stimulation by a pulse of current or by abrupt change in temperature, etc. The magnitude and form of the action potential has been the subject of much study (Osterhout 1955; Umrath 1956; Gaffey and Mullins 1958; and Findlay 1959).

The action potential in the Characeae is qualitatively similar to that in the nerve axon, which has been shown to be associated with quick changes in permeability of the membrane first to sodium and then to potassium (Hodgkin and Huxley 1952). It is of great interest to establish whether similar changes occur in cells of the

* Plant Physiology Unit, Division of Food Preservation, C.S.I.R.O., and Botany School, University of Sydney.

Characeae during activity. Gaffey and Mullins (1958) reported that extra fluxes of potassium and chloride accompanied the action potential in *Chara globularis* (a corticated species) and that the potential difference (p.d.) reached at the peak of the action potential was influenced by the concentration of chloride in the external medium. They therefore suggested that the permeability of the cells to chloride increased during the action potential so that the potential then approached the equilibrium potential for the chloride ion.

The present investigation is concerned with the effects of change in concentration of various ions in the medium on the action potential in *C. australis* and an alternative explanation of the results of Gaffey and Mullins is offered.

II. MATERIAL AND METHOD

Single cells of *C. australis* R.Br. var. *nobilis* A.Br. were cut from a growing culture and used either shortly after cutting or within 24 hr. Shorter and more transparent cells were chosen to enable the position of inserted micro-salt-bridges to be seen under the microscope. Cell length was usually 1–3 cm and diameter 1 mm. Internodal or, occasionally, whorl cells were used.

The experimental arrangement was essentially that used by Walker (1957) and Findlay (1959, fig. 1) where two fine glass probes filled with 0.3N KCl were inserted into the vacuole, one to record potential relative to a salt-bridge in the external medium and the other, in this case, to pass a current pulse to depolarize the resting potential until the action potential occurred. The pulse was usually of such a magnitude as to cause about 30–100 mV change in potential and of 0.3 sec duration. The time courses of the resting potential and of the action potential were recorded on a chart recorder with a full-scale response of less than 0.3 sec. Potential difference could be read from the chart to within ± 1 mV. The cells were uniformly illuminated and in a bath of flowing solution at a temperature which was constant to within $\pm 1^\circ\text{C}$ in a given experiment, and was in the range 17–21°C.

In order to test the effect of variations in concentration of a given ion it was necessary to keep the p.d. between the cell wall and medium constant, since it has been found (Hope and Walker 1961) that following an external change of total concentration, the wall potential changes quickly and the protoplast surface comes to equilibrium more slowly (half-time several minutes). The quick changes in wall potential can be eliminated by keeping the total concentration constant and increasing the concentration of one ion while decreasing that of another. The standard artificial pond water (A.P.W.1) mentioned below contained 0.1 mN KCl, 1.0 mN NaCl, and 0.5 mN CaCl_2 .

III. RESULTS

(a) *Effect of Calcium Concentration*

Figure 1 shows the general form of the action potential in A.P.W.1, the form being qualitatively similar to that found by other investigators. E_{oi} is the potential difference between inside and outside, with the sign of i relative to o . The level of the resting potential and the peak of the action potential are steady over a long time

interval, if stimulation is at intervals of not less than 8–10 min. Figure 2 shows the resting potential and peak of the action potential in solutions containing 0.1 mN KCl, 1.0 mN NaCl, and 5 mN ($\text{CaCl}_2 + \text{MgCl}_2$) where the calcium concentration varied from 0.3–5 mN (magnesium from 4.7–0 mN). Changes were made in the direction of increasing $[\text{Ca}^{++}]_o$ followed by a return in steps to the lowest concentration. The figure shows that, as found consistently, the resting potential is not affected by calcium, there being a steady drift from -142 to -148 mV over the duration of the experiment. However, the peak of the action potential was made more positive by increased $[\text{Ca}^{++}]_o$ in a reversible way, the new value being reached within about

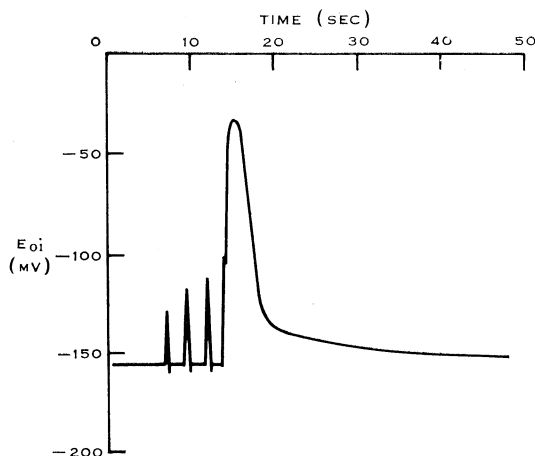


Fig. 1.—Time course of the action potential in a cell of *Chara australis*. The three spikes up to 12 sec on the time scale are the responses in p.d. to increasing pulses of current. The action potential was initiated by a fourth pulse at 14 sec which caused about 55 mV depolarization.

10 min of changing the solution. Since the points follow quite closely a line with a slope of $+29 \text{ mV}/(10 \times [\text{Ca}^{++}]_o)$, it is concluded that the cell acts as a calcium electrode at the peak of the action potential. This behaviour is found when the total (calcium+magnesium) concentration does not exceed about 6 mN and when the calcium/magnesium ratio is not less than about 1/15. Beyond those limits, with low calcium concentrations, the peak of the action potential does not remain constant but tends to become more negative and the cell may become refractory (unable to be stimulated to give an action potential). When concentrations of calcium greater than 6 mN are applied the slope of the curve of action potential *v.* $\log_{10} [\text{Ca}^{++}]_o$ becomes less than 29 mV and may be zero.

(b) Effect of Chloride Concentration

(i) *Choline Chloride*.—Changes in total concentration of choline chloride caused large changes in wall p.d. followed by slow drifts. The action potential was not steady but declined. After sufficient time the cell would become refractory. The first effect

is illustrated in Figure 3 which shows the resting potential and the action potential in 1, 3, and 10 mN choline chloride. The drift with time of the peak potential is not well illustrated here. Figure 4 shows the action potential peak over longer times, and suggests that the rate of drift of the peak of the action potential is increased by increased external concentration.

(ii) *Anion Substitutions.*—To eliminate changes in wall p.d., part of the chloride in an artificial pond water was replaced by other anions such as glucuronate, bromide, and benzenesulphonate. Table 1 records the resting potential and peak value reached during the action potential in solutions of the composition shown, after sufficient

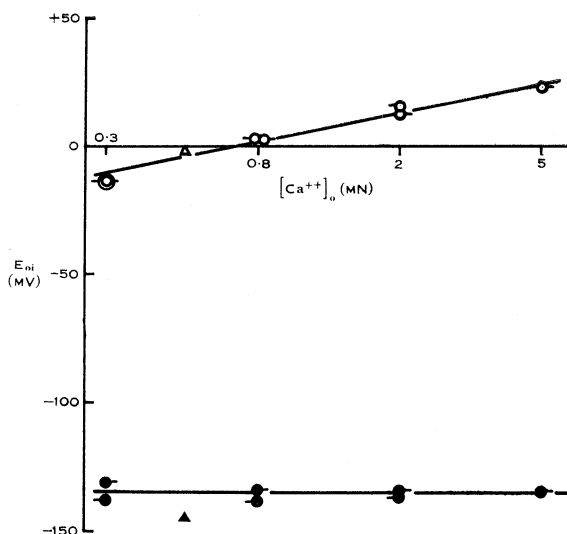


Fig. 2.—Resting potential (●) and peak level of the action potential (○), plotted against calcium concentration on a logarithmic scale. The triangles are corresponding values in A.P.W.1 (see text). Bars on symbols show direction of increasing or decreasing concentration, starting and finishing at 0.3 mN. The full line through points for action potential has a slope of 29 mV per tenfold $[Ca^{++}]_0$ change.

time (20–30 min) to reach a steady value of both, after changing a solution. Other experiments were consistent with this in that while the resting potential could be changed by anion substitutions (see also Hope and Walker 1961) the peak of the action potential was not a function of external chloride concentration.

(iii) *Changes in Chloride Concentration with Calcium Present.*—Choline chloride added to A.P.W.1 caused smaller changes in wall p.d. than those in Figure 3 and the resting potential drifted back to a value close to that in A.P.W.1. After about 20 min both resting potential and action potential kept constant values, in contrast to the results shown in Figure 4 where calcium (and some sodium and potassium chloride) were absent. The peak level of the action potential was again not a function of external chloride concentration. Such an experiment is illustrated in Figure 5.

(c) *Effect of other Cations*

(i) *Bivalent Cations*.—When calcium was completely replaced by magnesium the peak level of the action potential became more negative with time, at a rate

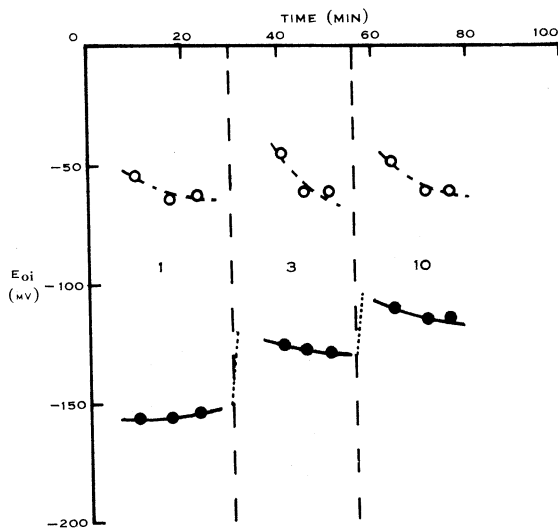


Fig. 3.—Resting (●) and action (○) potentials in solutions of choline chloride of the concentrations (mN) shown, plotted against time. Concentration was changed at times indicated by vertical broken lines.

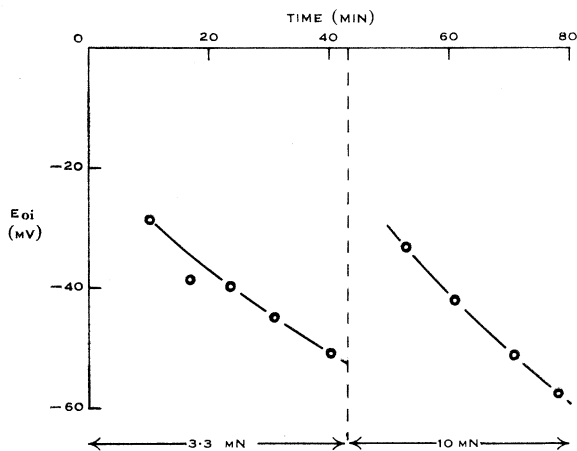


Fig. 4.—Peak value of the action potential of a cell in 3.3 mN and 10 mN choline chloride, plotted against time.

greater than with choline chloride of the same concentration. This is shown in Figure 6. The cell became refractory (*R* in the figure) to depolarizations as great

as 100 mV. The action potential was restored after replacing the calcium, either in the form of A.P.W.1 or CaCl_2 alone.

In *C. australis*, partial replacement of calcium in A.P.W.1 with barium or strontium gave a change in the action potential peak as expected on the basis of calcium electrode behaviour*.

(ii) *Monovalent Cations*.—Neither sodium nor potassium affected the peak level of the action potential in experiments where total concentration was kept constant and the metal cation replaced by choline chloride, in the presence of 0.5 mN CaCl_2 . The resting potential was little affected by sodium concentration in the range 0.3–3.0 mN, the form of the graph of p.d. *v.* $[\text{Na}^+]_o$ resembling Figure 5. Potassium affected the resting potential but the change with concentration in the presence of calcium was less than in its absence.

TABLE 1

EFFECT OF ANION SUBSTITUTIONS ON RESTING AND ACTION POTENTIALS IN CELLS OF CHARA AUSTRALIS

Time sequence from left to right. All solutions contained, in addition, sodium (4 mN), potassium (0.1 mN), and calcium (0.5 mN)

Anion	Cl^-	Cl^- Glucuronate	Cl^-	Cl^- Br^-	Cl^-	Cl^- $\text{C}_6\text{H}_5\text{SO}_3^-$	Cl^-
Concn. (mN)	4.6	0.6 4.0	4.6	0.6 4.0	4.6	0.6 4.0	4.6
Resting potential (mV)	−149	−185	−147	−149	−142	−184	−149
Action potential peak (mV)	+15	+5	+11	+5	+14	+12	+16

IV. DISCUSSION

(a) *Effect of Calcium*

Of all the ions tested only calcium was found to produce consistent changes in the peak level of the action potential in *C. australis*. The changes were of such a sign and magnitude as to suggest that, under the conditions of experiment, the cells became specifically permeable to calcium about 1–2 sec after stimulus, returning to their normal resting permeability during the next 30 sec. At the peak of the action potential the p.d. between inside and out obeyed an equation of the type:

$$E_{oi} = \text{constant} + 29 \log_{10} [\text{Ca}^{++}]_o, \quad (1)$$

which is that for a calcium electrode at 19°C.

* Specific effects of bivalent cations on the shape of the action potential, i.e. duration and rate of change of p.d., have been found here and also in *Nitella* by Findlay (personal communication).

If it is assumed that the measured p.d. contains no additive component due to the cell wall and that the cytoplasmic ion activity remains constant, equation (1) can be rewritten:

$$E_{oi} = 29 \log_{10} \{ [Ca^{++}]_o / [Ca^{++}]_i \}. \quad (2)$$

$[Ca^{++}]_i$ might then be calculated from the results. In the present experiments the peak of the action potential was zero when $[Ca^{++}]_o$ was on the average 1.5 ± 0.3 mN

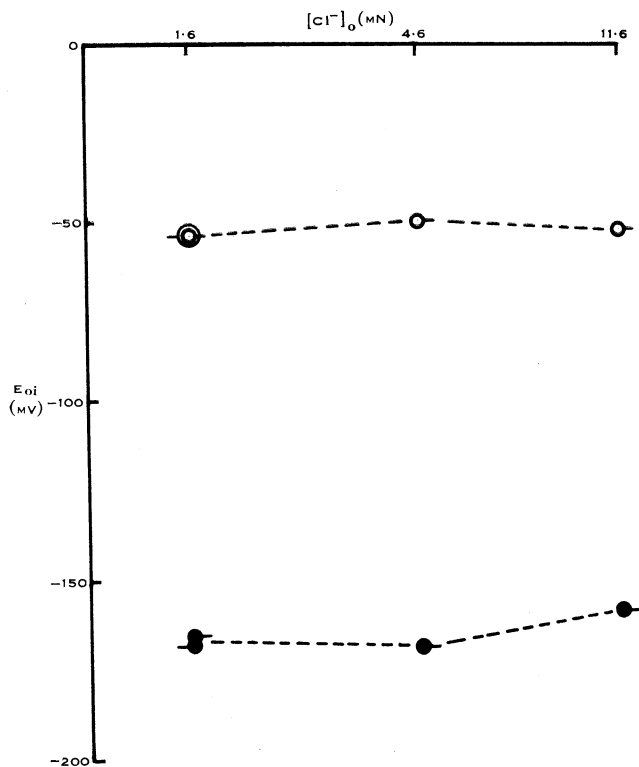


Fig. 5.—Resting potential (●) and peak of the action potential (○) of a cell in a medium in which chloride was varied by adding choline chloride to A.P.W.1 (containing 0.5 mN calcium).

in four experiments similar to that of Figure 2 (omitting one in which $[Ca^{++}]_o$ was greater than 20 mN). According to equation (2) this value can be identified with the internal (presumably cytoplasmic) calcium ion activity.

While it is not strictly permissible to compare this with measured vacuolar concentrations of calcium, it is worth mentioning that the latter was found to be 2.6 mN (average of 10 sap samples pooled) by Hope and Walker (1960).

Since the effect of calcium was obtained in the presence of concomitant changes in magnesium (or barium or strontium) concentration, the supposed increased permeability during the action potential is a very specific one and not merely an effect of bivalent cations. None of these ions had a large effect on the resting potential.

(b) *Effect of Chloride*

Increasing concentrations of choline chloride give swift changes in wall potential and also semipermanent depolarization of the resting potential (the p.d. some time after the wall change has occurred). The action potential peak was not affected by the concentration of chloride present (Figs. 3 and 5; Table 1). This is in complete contrast to the results reported by Gaffey and Mullins (1958, fig. 4) who found that the resting potential was approximately constant as the choline chloride concentration was increased from 1 to 10 mN, while the peak of the action potential became more

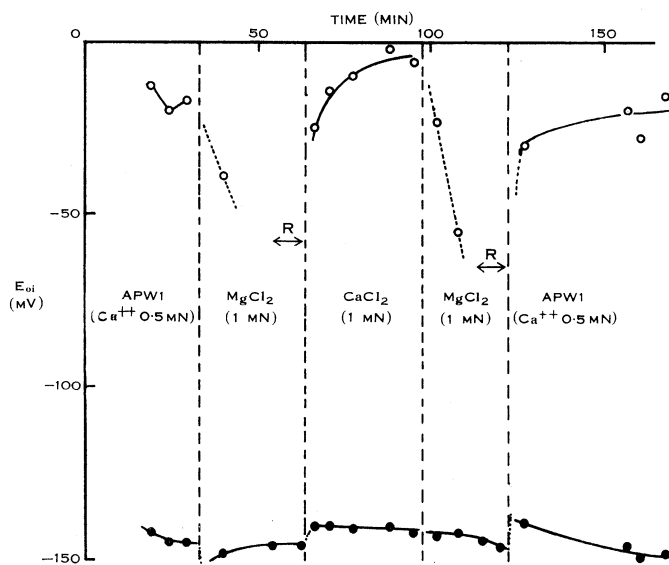


Fig. 6.—Effect on the resting potential (●) and peak of the action potential (○) of substituting magnesium for calcium. At times marked *R*, the cell was refractory to large depolarizations.

negative by about 40 mV. (Its magnitude is shown as declining from 120 to 80 mV.) Such behaviour was stated as indicating that the cell became specifically permeable to the chloride ion, during the action potential.

In the present experiments, a similar decline in the action potential was found in a constant concentration of choline chloride (Fig. 4). The explanation of this, on the basis of the effect of calcium ions, is as follows. The level of the peak of the action potential is governed by the activity of calcium ions just outside the plasma-lemma. On removal of a cell from a culture solution containing calcium ions into one of choline chloride (or indeed any other solution not containing calcium) the calcium ions are removed from near the plasmalemma by exchange via the cell wall Donnan phase. Exchange of bivalent counterions with monovalent ions was shown to be slow, and rates of isotopic exchange were increased by increasing the external concentration in earlier experiments with *Chara* (Dainty and Hope 1959).

The results described here are completely consistent with these considerations. The decline of the action potential reflects the decrease in calcium, not that in the

external medium, but in the immediate neighbourhood of the plasmalemma. In Figures 4 and 6, the peak potential is reduced by an exchange of calcium with choline and magnesium ions respectively. The rate of decrease of this potential is greater in 10 mM choline chloride than in 3.3 mM and, for a given equivalent concentration, is greater for magnesium than for choline. This agrees qualitatively with comparative rates of exchange in isolated wall segments of *Chara* (Dainty and Hope 1959, Table 3).

If the effective concentration of calcium is made low enough by prolonged exchange, say with magnesium (Fig. 6), the peak of the action potential would be expected to approach the level of the resting potential; stimulation while depolarizing the membrane does not initiate an action potential—the cell becomes refractory, as is observed.

When increasing concentrations of choline chloride are added in the presence of a fixed concentration of calcium, the calcium concentration next to the plasmalemma is maintained even though the wall counterions may increase in their ratio of choline to calcium. Thus the action potential peak stays almost constant with increasing choline chloride concentrations (Fig. 5).

It is thought, therefore, that the result of Gaffey and Mullins (1958) can be explained if the time sequence had been in the direction of increasing choline concentration, since the magnitude of the action potential would then appear to decrease with increasing concentration. Since, in the cells these authors used, choline did not affect the resting p.d. greatly, the net effect would be to make the peak of the action potential more negative with increasing $[Cl^-]_o$.

However, the postulated large permeability to chloride during the action potential is also indicated by the extra efflux of labelled chloride found by Gaffey and Mullins (1958) over and above the resting efflux. Extra efflux of potassium was found also. These results need to be confirmed with an uncorticated species and with "physiological" concentrations of chloride. It is entirely possible, on the other hand, that in the absence of calcium other ions may control the action potential in species other than *C. australis*.

The present experiments would predict a large current of calcium ions entering the cytoplasm during the action potential. The current flows during the action potential are being investigated by Findlay (unpublished data) under a voltage clamp, and fluxes of calcium will be measured with the aid of tracers.

(c) Mechanism of the Action Potential

The shape of the action potential trace has been given a detailed explanation (see Osterhout 1955) in terms of movements of potassium ions. This description is entirely theoretical and depends on the assumption that the potential change takes place at an inner "non-aqueous layer" (presumably the tonoplast) and not at the plasmalemma where it in fact occurs. Furthermore, potassium is almost in electrochemical equilibrium between the vacuole and external medium in several genera of the Characeae, which means that a sudden increased permeability to this ion would not be expected to increase the potential, as observed. The concentration of external

potassium does not affect the action potential peak but does affect the resting potential, in agreement with this view.

It is apparent from the above that the action potential can be qualitatively explained by a sudden, reversible change in the permeability of the plasmalemma to calcium. One conjecture (Mullins 1959) is that the membrane contains long narrow pores, the majority of which are filled in the resting state, in muscle or nerve, with potassium ions. The pore size is then said to change during excitation so that the majority are filled with sodium. Thus the membrane acts as a potassium electrode in one state and resembles a sodium electrode in the excited state, as observed. The ions in the pores are supposed to retain only one shell of solvation so that their sizes are related to crystallographic radii. Relative permeability of the membrane is related to relative numbers of pores filled by the various ion species, as well as ionic mobility in the pores.

This idea could be applied to *C. australis*, with calcium ions taking the place of sodium, but it is difficult to see how it could be tested, or by what molecular means the membrane effects the supposed change in pore size. Any proposed mechanism, as well as providing a reasonable explanation for the potential differences, must also account for the large increase in overall conductance (Cole and Curtis 1938) during the action potential. It is as if *more* pores (all calcium-filled) are opened and then closed, rather than as if a change in occupation of existing pores occurred.

Use of equations as simple as (1) or (2) may not be permissible if changes in permeability to other ions besides calcium are involved. Under these conditions the potential reached at the peak of the action potential would not correspond to the equilibrium potential difference for any single ion. For example, if the permeability to potassium increased sufficiently as the potential approached the equilibrium potential for the calcium ion, the observed p.d. would then tend to reach a value somewhere between the ion equilibrium potentials for these two ions, returning finally to the resting potential as the permeability to calcium declined. Experiments to test this possibility are being designed.

V. ACKNOWLEDGMENTS

The author is indebted to Mr. G. P. Findlay, formerly of the Physics Department, University of Tasmania, for helpful discussions.

Thanks are due to Dr. J. R. Vickery, Chief, Division of Food Preservation, C.S.I.R.O., and Professor R. L. Crocker, Botany School, University of Sydney, in whose Laboratories the work was carried out.

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