

ELECTRIC IMPEDANCE OF THE FROG EGG

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INTRODUCTION

The analysis of measurements of the alternating current impedance of tissues, cell suspensions, and single cells has shown that the cell membrane has a capacity of about $1 \mu\text{f}/\text{cm}^2$ and an associated dielectric loss similar to that found in solid dielectrics. Because the capacity is relatively constant for many cells and under various conditions, it and the loss are probably characteristics of the molecular structure in the ion-impermeable part of the membrane. In the case of a few cells it has been possible to measure a leakage resistance of the membrane. This resistance is dependent upon the condition of the cell and may be ascribed to the transfer of ions across the membrane. The ion permeability of the membrane is an important factor in the functioning of the cell but its electrical measurement as a leakage conductance is dependent upon a favorable combination of the size and shape of the cell and the resistance of the medium (Cole, 1937, 1940). The ideal material for physiological work of this nature is a single large cell of regular shape which is normally found in an environment of low salt concentration. The frog egg seemed to meet these requirements and this work was undertaken to determine the plasma membrane resistance of the unfertilized and fertilized egg. In addition it has been possible to determine the membrane capacity and phase angle, and the internal resistance for the unfertilized and fertilized egg in spring water and in diluted amphibian Ringer's. The stability of the impedance was also of interest because of the spontaneous, rhythmic impedance fluctuations observed in the trout egg by Hubbard and Rothschild (1939).

Material and Apparatus

Single eggs of the leopard frog, *Rana pipiens*, were used throughout. The eggs were stripped from the frog and immediately placed in the measuring cell. Eggs which were to be fertilized were stripped from females previously injected with frog pituitaries (Rugh, 1934) so that they were sexually mature in the fall and winter months. These eggs were allowed to fall into spring water or 10 per cent Ringer's solution (1 part amphibian Ringer's to 9 parts of distilled water), artificially inseminated, and placed in the measuring cell. Only those eggs, eight in number, which showed subsequent development to at least the two-cell stage were designated as fertilized eggs. Checking the fertilizability of the unfertilized eggs (two in number) after a run would

not have had any particular value because it is well known that these eggs lose their fertilizability very soon after removal from the body.

In addition to the fertilized and unfertilized group, there was another group of eggs designated "undetermined" (nine eggs). These eggs were treated with sperm solution prior to measurement but whether or not their insemination was successful could not be proved, inasmuch as when it was next possible to examine them some days later, they had undergone decomposition. Thus it was impossible to determine whether they had died because of injury subsequent to fertilization and early development or whether they had been unsuccessfully inseminated and never developed at all.

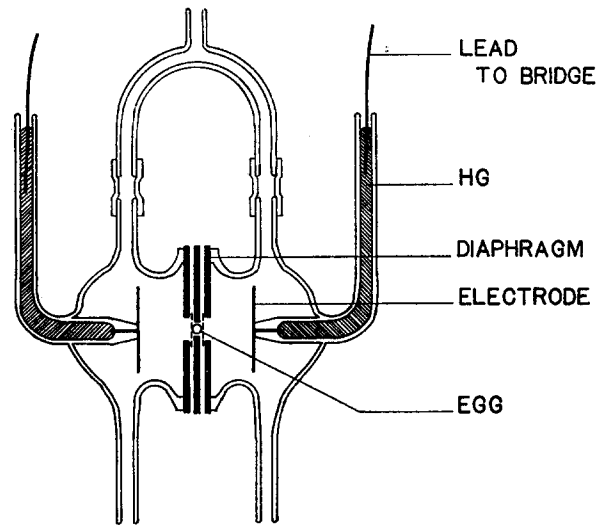


FIG. 1. Chamber for measuring impedance of single frog eggs. When assembled the diaphragms are in contact. (The broken lines on either side of the egg represent pieces of cheesecloth which hold the egg in place.)

A few measurements were made upon eggs which were known to be dead. In these cases the impedance was independent of frequency and close to that obtained for the medium alone. Measurements were also made on the vitelline membrane alone which showed that it had approximately the conductivity of the medium.

After preliminary experiments with other types, a measuring cell of the form used by Frick and Morse (1925) was adopted for these experiments. As is shown in Fig. 1, each platinized platinum electrode of this cell was sealed into a cup with a ground lip. When the two cups were clamped together directly or with flat diaphragms between them a watertight joint was formed. The egg was placed in a circular hole, 0.216 cm. in diameter, drilled in the center of a hard rubber diaphragm, 0.164 cm. thick. The adhering jelly served to center the egg in the hole and a small piece of moistened cheesecloth on each side kept the egg from being forced out of the hole by momentary pressure differences. Each piece of cheesecloth in turn was held in

place by another diaphragm having a center hole, 0.6 cm. in diameter. These three diaphragms were placed between the ground lips of the electrode cups, the whole clamped together and filled with spring water or 10 per cent amphibian Ringer's solution through the tubes at the top and bottom of each cup.

An alternating current Wheatstone bridge similar to that described by Cole and Curtis (1937) was used for the measurements. A set of measurements was made at eight frequencies from 50 cycles to 10 kc. first with and then without an egg in place. At each frequency the parallel resistance, R_p , and capacity, C_p , were measured. The temperature was not controlled but several check measurements were taken, usually at 1 kc., during the course of each run and correction made for the effect of temperature change when necessary.

Corrections and Calculations

In other work it has usually been necessary to apply a correction for the polarization impedance of the electrodes but in this case with electrodes 3.3

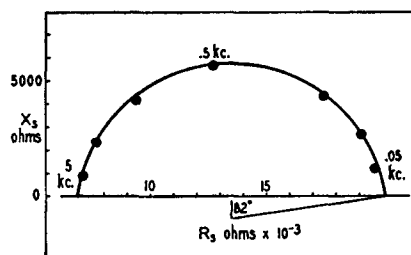


FIG. 2 a

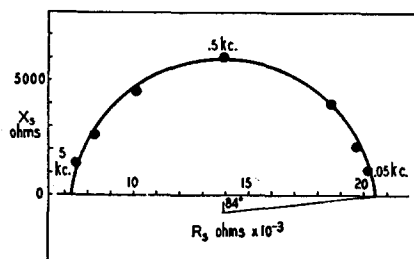


FIG. 2 b

FIG. 2. Frequency impedance locus, *i.e.* series resistance, R_s , vs. series reactance, X_s , of (a) unfertilized and (b) fertilized frog egg. Frequencies indicated are in kilocycles per second.

cm. in diameter this impedance was negligible. The series resistance, R_s , and series reactance, X_s , at each frequency were then computed from the measured resistance and capacity and plotted as abscissae and ordinates, respectively, of the impedance locus (Cole, 1928 a). A typical locus for an unfertilized frog egg is shown in Fig. 2 a and for a fertilized egg in Fig. 2 b.

End Effect

Before these data can be interpreted, it is necessary to consider the current flow between each electrode and the end of the cylindrical hole in the diaphragm facing it. This resistance of this region depends upon the specific resistance of the electrolyte, the separation between the electrode and the diaphragm, and the diameters of the hole, the vessel, and the electrodes. The current from the electrode converges rapidly in the electrolyte outside the diaphragm as it approaches the hole and encounters a resistance which is practically independent of the length and contents of the hole. This resistance could be

measured directly if the central hole were filled with metal or if a very thin diaphragm having a central hole of the same diameter were used. However, these measurements would be complicated by the polarization impedance of the metal faces in the first case and the dielectric capacity of the diaphragm in the second case. A third possibility is to vary the length of the hole by using diaphragms of different thickness and obtain the resistance for zero length by extrapolation. The results of this latter experiment with five diaphragms having holes 0.216 cm. in diameter are shown in Fig. 3. The measured resistance with the diaphragm 0.164 cm. thick used for the eggs is 2,600 ohms and the extrapolated resistance for zero thickness is 1,300 ohms. Consequently the end effect is 50 per cent of the resistance observed for the experimental

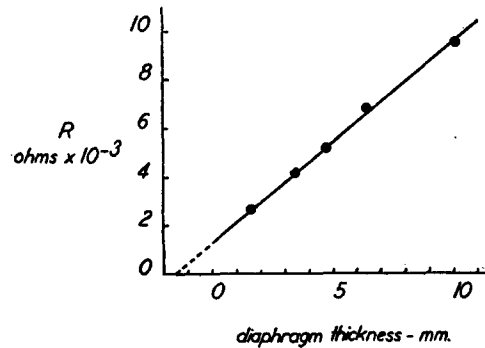


FIG. 3. The overall resistance, R , in thousands of ohms, of measuring chamber and diaphragm plotted against diaphragm thickness in millimeters. Each diaphragm had a central hole 0.216 cm. in diameter. The medium was spring water.

diaphragm without an egg in place. This value was then subtracted from the series resistance obtained at each frequency to correct for the resistance of the electrolyte on each side of the diaphragm.

From the extrapolation to the axis of abscissae, it is found that the end effect is equivalent to an added hole length of 0.16 cm. If the equivalent hole length l' is $l' = l + \gamma a$, where l is the actual length and a the radius of the hole, then $\gamma = 1.48$ experimentally. An approximate calculation for extensive electrolyte and large distant electrodes gives $\gamma = 1.57$ and Rayleigh gives $\gamma = 1.64$ as a better value (Jeans, 1927, page 358). However, the electrodes are only 0.75 cm. from each side of the disc and this reduces the computed value of γ by about 0.10 (Gray, Mathews, and MacRobert, 1922, page 145). The finite diameter of the cups and the electrodes increases γ but only by a negligible amount (Gray, Mathews, and MacRobert, 1922, page 147). The theoretical value is then in the neighborhood of $\gamma = 1.5$ and the agreement with the experimental value of $\gamma = 1.48$ is better than should be expected.

Effective Volume Concentration

The next step in the analysis is to consider the distribution of current flow around and through the egg in the cylindrical hole. The resistance, r , of a cubical cell containing a small sphere is most easily obtained by the assumption that the field strength at the boundaries of the cell is not modified by the sphere. On this basis Fricke (1924) found

$$\frac{1 - r_1/r}{3} = \rho \frac{1 - r_1/r_2}{2 + r_1/r_2}$$

where r_1 and r_2 are the specific resistance of the medium and the sphere respectively, and ρ is the fraction of the volume occupied by the sphere. Obviously this equation will apply for any shape of boundary if it is everywhere sufficiently remote from the sphere. It will also apply for a dilute suspension of spheres in which the field at a single sphere is not affected by the presence of the others. As the fractional volume ρ is increased, the field inside a sphere is altered by the walls or by the other spheres of a suspension. If this internal field remains uniform and is determined by the average field in the system, the next approximation is the Maxwell (1873, section 313) equation for a random suspension

$$\frac{1 - r_1/r}{2 + r_1/r} = \rho \frac{1 - r_1/r_2}{2 + r_1/r_2} \quad (1)$$

This equation also applies to an approximately cubical arrangement of spheres (Rayleigh, 1892) and consequently to a single sphere in a nearly cubical cell. We then expect that the exact shape of the measuring cell is not particularly important and that equation (1) will also apply to a sphere in a short cylindrical hole. This is easily shown to be true if the internal field is uniform and determined by the average field in the system. However, when the effect of the sphere becomes too large, this assumption will not be valid and a more complete theory is necessary to give these limits. Since this theory is not available the validity of equation (1) can only be established experimentally.

In the case of a non-conducting sphere, which is the most critical theoretically and the most easily tested, equation (1) becomes

$$(1 - r_1/r)/(2 + r_1/r) = \rho/2 \quad (2)$$

To test this equation for a non-conducting sphere, one end of a fine glass rod was melted down until it became a sphere of the proper size. This sphere was then held in the center of the hole by the attached rod which in turn was cemented to a glass bridge on one side of the diaphragm. The effect of the rod and bridge was negligible because they were small and in regions of low current density. In Fig. 4, the left hand side of equation (2) is computed from the

measured resistances and plotted against the volume, v , of the sphere. The linear relation for small values of v justifies the use of equation (2).

The linear relation between these two quantities is expected according to equation (2) for small values of v . With the larger values of v , the assumptions of equation (2) are no longer valid, and the measurements do not lie on the straight line. For example, if the diameter of the sphere is equal to that of the cylindrical hole the resistance is infinite and $(1 - r_1/r)/(2 + r_1/r) = 0.5$. But according to equation (2), the resistance should not become infinite until $v = V$, the volume of the cylinder. Then by extrapolation of the straight line obtained for small v we find $V = 6.3 \text{ mm}^3$. Although the actual volume of the cylinder was 6.0 mm^3 , the extrapolated value may be used as an apparent

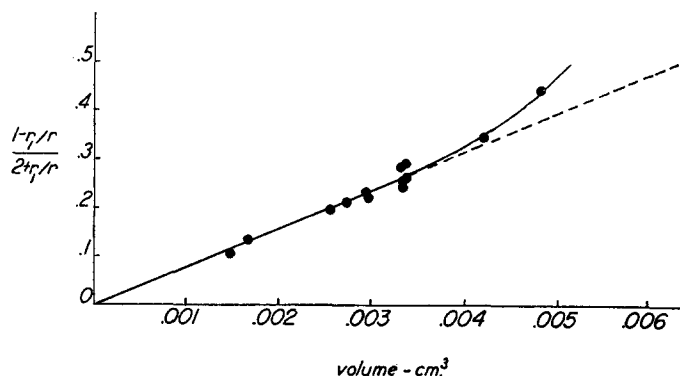


FIG. 4. The volume of the glass sphere in cm^3 plotted against the expression $(1 - r_1/r)/(2 + r_1/r)$, where r_1 is the resistance in ohms of the medium and r , the resistance in ohms with the sphere in place. The experimental points are joined by an unbroken line, while the extrapolation of the straight line is represented by a broken line.

or effective volume. Equation (2) may then be used for values of ρ up to 50 per cent and $\rho = v/V$, where v is the volume of the egg computed from its diameter and V is the effective volume of the cylindrical cell.

Internal Resistance

The impedance loci of Fig. 2 are approximately circular arcs and may be extrapolated to the resistance axis to give r_0 , the zero frequency resistance at one end and r_∞ , the infinite frequency resistance at the other end. If we assume that the membrane impedance is negligible at infinite frequency, the internal resistance r_2 may be computed by equation (1) where $r = r_\infty$

$$\frac{1 - r_1/r_\infty}{2 + r_1/r_\infty} = \rho \frac{1 - r_1/r_2}{2 + r_1/r_2} \quad (3)$$

The values for r_2 so obtained are given in Table I.

Membrane Resistance

In a similar manner the resistance, \bar{r}_2 , of an equivalent uniform sphere at zero frequency may be computed when $r = r_0$,

$$\frac{1 - r_1/r_0}{2 + r_1/r_0} = \rho \frac{1 - r_1/\bar{r}_2}{2 + r_1/\bar{r}_2}. \quad (4)$$

If the egg has an electrically homogenous interior of specific resistance r_2 surrounded by a thin membrane of resistance r_3 per unit area, the equivalent resistance is given by

$$\bar{r}_2 = r_2 + r_3/a \quad (5)$$

where a is the radius of the egg (Cole, 1928 *a*). The membrane resistances may now be obtained from the values of \bar{r}_2 , r_2 , and a previously found. These results are given in Table I.

Membrane Phase Angle and Capacity

The formulae previously used to calculate the membrane capacity cannot be used because they have been based on the assumption that the membrane conductance was negligible. The fact that the zero frequency resistances were usually larger for the medium alone than with an egg in place shows that the equivalent resistance of the egg is less than the resistance of the medium and that the assumption of a negligible conductance is not valid.

On the basis of previous work, summarized by Cole (1940), it will be assumed that the membrane resistance, r_3 , and the dielectric impedance, z_4 , are in parallel. The membrane impedance z_m is then given by $1/z_m = 1/r_3 + 1/z_4$. The equivalent egg resistance, \bar{r}_2 , in equation (1) is now replaced by the equivalent impedance $\bar{z}_2 = r_2 + z_m/a$ and by rearrangement equation (1) becomes

$$z = r_1 \frac{(1 - \rho)r_1 + (2 + \rho)r_2 + [(1 - \rho)r_1 + (2 + \rho)(r_2 + r_3/a)]z_4/r_3}{(1 + 2\rho)r_1 + 2(1 - \rho)r_2 + [(1 + 2\rho)r_1 + 2(1 - \rho)(r_2 + r_3/a)]z_4/r_3} \quad (6)$$

This equation is of the same form as for a negligible membrane conductance. For a constant phase angle dielectric impedance $z_4 = |z|(j\omega)^{-\alpha}$ the impedance locus of z should be a circular arc of central angle, $2\varphi = \alpha\pi$ (Cole, 1928 *a*, 1932). As seen in Fig. 2 this is approximately true. The values of φ found are between 80° and 88.5° and the average value is 86° .

Solving equation (6) for z_4 and introducing the values for r_0 and r_∞ from equations (3) and (4), we obtain

$$z_4 = \frac{[(1 + 2\rho)r_1 + 2(1 - \rho)r_2]r_3}{(1 + 2\rho)r_1 + 2(1 - \rho)(r_2 + r_3/a)} \cdot \frac{z - r_\infty}{r_0 - z} \quad (7)$$

The frequency for which the reactance component of the measured impedance z is a maximum may be called the characteristic frequency, \bar{n} , (Cole and Curtis, 1936). At this frequency, the absolute value

$$\left| \frac{z - r_\infty}{r_0 - z} \right| = 1$$

and if z_4 were that of a pure capacity $z_4 = 1/c_m \omega$ we have

$$c_m = \frac{1}{a\bar{\omega} \left(r_2 + \frac{1 + 2\rho}{2(1 - \rho)} r_1 \right)} + \frac{1}{\bar{\omega} r_3} \quad (8)$$

where $\bar{\omega} = 2\pi\bar{n}$.

This equation is not strictly valid because the phase angle of z_4 is somewhat less than 90° , but it gives the capacity component of the membrane impedance to within 1 per cent in the present experiments. The values of c_m obtained under various conditions are given in Table I.

DISCUSSION OF RESULTS

The results of the study of the electrical parameters of the frog egg are summarized in Table I.

Internal Specific Resistance.—The internal specific resistance of the cell, r_2 , is dependent upon the concentration and mobilities of ions in the cell interior. It was calculated on the assumption that the membrane has a negligible resistance at the infinite frequency extrapolation. The average value obtained for the nineteen eggs studied was 570 ohm cm. This value can be compared with the value of 200 ohm cm. obtained by Holzer (1933) for unfertilized trout eggs, which are also fresh water eggs and with the values obtained by Cole *et al.* for various marine eggs (*Arbacia* (1928 *b*, 1936 *b*, 1938), *Asterias*, (1936 *a*), *Hipponoë* (1935), *Cumingia* and *Chaetopterus* (1938)) which also are approximately 200 ohm cm.

Membrane Resistance.—The membrane resistance is probably a measure of the membrane permeability to ions. It is dependent upon the condition of the cell, being altered during cell activity (Cole and Curtis, *Nitella* (1938), squid axon (1939)) and during narcosis (Guttman, 1939). The average membrane resistance value obtained for the frog egg was 170 ohm cm². Because of technical difficulties it has in the past been impossible to measure the membrane resistance of most cells studied with impedance methods. There are therefore few data with which the frog egg value may be compared. Holzer (1933) obtained a value of 6000 ohm cm². for the membrane resistance of unfertilized trout eggs. The value obtained for the frog egg lies in the general range of resistances found by Blinks for *Valonia* (1930) and *Halicystis* (1938), by Cole and Hodgkin for the squid axon (1939), and by Bozler and Cole (1935)

for frog sartorius muscle. This suggests that the ion permeabilities of the membranes of these cells are comparable.

Membrane Capacity.—Unlike membrane resistance, membrane capacity is a fairly inert characteristic of the cell membrane, changing neither during activity nor narcosis of the cell. It probably is associated with the ion-impermeable aspect of the cell membrane. The average value obtained for the frog egg was $2.0 \mu\text{f}/\text{cm}^2$. Almost all living cells thus far measured have

TABLE I

Calculation of Electrical Parameters of Single Frog Eggs under Various Conditions

r_2 , internal specific resistance in ohm cm.; r_3 , membrane resistance in ohm cm.²; c_m , membrane capacity in $\mu\text{f}/\text{cm}^2$. Numbers in parentheses indicate number of eggs in each case.

r_2	10 per cent Ringer	Spring water	Average
Fertilized	625 (6)	510 (2)	620 (8)
Unfertilized	480 (1)	580 (1)	540 (2)
Undetermined	590 (8)	320 (1)	560 (9)
Average	600 (15)	430 (4)	570 (19)
r_3	10 per cent Ringer	Spring water	Average
Fertilized	140 (6)	390 (2)	200 (8)
Unfertilized	70 (1)	430 (1)	225 (2)
Undetermined	120 (8)	190 (1)	130 (9)
Average	120 (15)	350 (4)	170 (19)
c_m	10 per cent Ringer	Spring water	Average
Fertilized	1.8 (6)	1.4 (2)	1.7 (8)
Unfertilized	2.7 (1)	1.7 (1)	2.2 (2)
Undetermined	2.3 (8)	2.2 (1)	2.3 (9)
Average	2.1 (15)	1.7 (4)	2.0 (19)

surprisingly constant membrane capacities of about 1 or $2 \mu\text{f}/\text{cm}^2$. Into this general scheme the membrane capacity of the frog egg fits rather well.

Effect of Alteration of External Medium.—Since frog eggs develop equally well in spring water and in a 10 per cent amphibian Ringer solution, it was possible to study the effect of the external medium upon the cell parameters by making observations on eggs in each type of medium and then comparing the results.

It is of interest that the membrane capacity, c_m , which is, as we have seen, in general a rather inert characteristic of the cell, is not significantly different when the egg is in 10 per cent Ringer ($2.1 \mu\text{f}/\text{cm}^2$) from the value when the egg is in spring water ($1.7 \mu\text{f}/\text{cm}^2$) (Table I). On the other hand, the membrane resistance, r_3 , is definitely higher in the case of the spring water values (350 ohm cm^2) than in the 10 per cent Ringer values (120 ohm cm^2). That

the membrane resistance should change is not very surprising since this value probably represents an equilibrium with both the external and internal media. The internal specific resistance, r_2 , may also be dependent upon the external medium, being 600 ohm cm. when the eggs were in 10 per cent Ringer and 430 ohm cm. when the eggs were in spring water. If this difference is significant it indicates an accumulation of ions in the cell interior from the more dilute medium.

Effect of Fertilization.—Fertilization of the eggs had little effect upon the cell parameters investigated (Table I). Of the nineteen eggs studied, eight were successfully inseminated just before measurement, as was shown by their subsequent normal development. These are designated "fertilized" in Table I. Two eggs were unfertilized and are so designated. Nine eggs whose fertilization was uncertain are listed as "undetermined" in Table I.

The data obtained for the "undetermined" group of eggs do not fall into two distinct classes. This means that either (1) these eggs were all fertilized eggs or all unfertilized eggs or (2) the group includes both fertilized and unfertilized eggs, and the membrane resistance, membrane capacity, and specific resistance of the egg interior do not change upon fertilization. While it is possible that the nine eggs were either all fertilized or all unfertilized, the second assumption is more probable. In any case, there is no marked difference in membrane resistance, membrane capacity, and specific resistance of the egg interior in those eggs definitely known to be fertilized or unfertilized.

McClendon (1910) and later Gray (1916) demonstrated a decrease in the resistance of centrifuged echinoderm eggs on fertilization. On the other hand no marked change in membrane resistance was found after fertilization in *Arbacia* eggs (Cole and Spencer (1938)), or in *Hipponoë* eggs (Cole, 1935). Both the *Arbacia* and the *Hipponoë* membrane capacities increased on fertilization, unlike the frog egg. No such change in membrane capacity on fertilization occurs in *Cumingia* or in *Chaetopterus* eggs (Curtis and Cole, 1938).

No Spontaneous Rhythmical Impedance Changes in Frog Egg.—Although Hubbard and Rothschild (1939) have reported small spontaneous rhythmical impedance changes in the unfertilized and fertilized trout egg after soaking for 12 hours in running tap water, no similar phenomenon was observed in the frog egg.

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SUMMARY

Electrical impedance measurements were made upon unfertilized and fertilized eggs of the leopard frog, *Rana pipiens*, over a frequency range of

0.05 to 10 kc. Average values of 170 ohm cm.² were obtained for the plasma membrane resistance of the egg, 2.0 μ f/cm.² for the plasma membrane capacity, 86° for the phase angle of the membrane, and 570 ohm cm. for the specific resistance of the interior. These values did not change upon fertilization. No spontaneous rhythmical impedance changes such as have been found by Hubbard and Rothschild in the trout egg were found in frog eggs.

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