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THE TEMPERATURE DEPENDENCE OF THE MEMBRANE POTENTIAL AND RESISTANCE IN *NITELLA TRANSLUCENS*

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SUMMARY

1. The aim of the work was to investigate the effect of temperature on the resting membrane potential and resistance of *Nitella translucens* and to determine whether such an effect could be interpreted in terms of changes in the passive ionic permeability of the membrane.

2. The experimental results show (i) a temperature sensitivity of the membrane potential of about 1.6 mV per degree (ii) a four-fold change in the resistance between 0° and 18°.

3. An electrochemical approach to the problem suggests that the changes are due to differential changes in the passive Na^+ and K^+ permeabilities of the plasmalemma. Substantial support for this hypothesis comes from the kinetic theory of rate processes.

4. The kinetic theory enables the free energies of activation for permeation of Na^+ and K^+ through the membrane to be estimated.

INTRODUCTION

The relative permeability of the plasmalemma of the Characeae to Na^+ and K^+ has been the subject of much experimental investigation in recent years. A study of the effect on the membrane potential of changes in the external concentrations of these ions has proved to be a useful approach to this problem. It has been shown that, in calcium-free media, the changes in potential are quite well described by an equation which expresses the functional relationship between potential, ionic concentration, and permeability[1,2]. This equation also contains the temperature of the system in both explicit and implicit terms and it seemed to us to be a worthwhile exercise to investigate the effect of temperature on permeability by studying the temperature sensitivity of both the membrane potential and membrane resistance.

We have used the internodal cells of *Nitella translucens* as our experimental material and the results indicate an almost linear relationship between membrane potential and temperature at temperatures below 18°, but above this temperature the behaviour becomes erratic. The results also show that in the temperature range 0–10° the membrane resistance increases rapidly with decreasing temperature. Using

the equation for the membrane potential, the observed potential changes can be interpreted in terms of changes in the Na^+ and K^+ permeabilities; the resistance changes can be interpreted in a similar way. Substantial support for this hypothesis comes from the kinetic theory of rate processes in which the permeability coefficients are expressed as explicit functions of the temperature.

MATERIALS AND METHODS

Quantities of *N. translucens* were collected from a fresh-water lake and stored in tanks in a cool, sheltered place out-of-doors. During the storage period the cells were bathed in a standard artificial pond water containing 1.0 mM NaCl, 0.1 mM KCl and 0.1 mM $CaCl_2$. The length and diameter of each cell selected for experiment was carefully measured prior to an experiment; the lengths of the cells used were all about 8 cm and the diameters were all close to 1 mm. The cells were pre-soaked in 5 mM NaCl solution at 5° for about 16 h before the commencement of an experiment. The bathing medium used in all the experiments was a calcium-free artificial pond water, *i.e.* its composition was 1.0 mM NaCl and 0.1 mM KCl. The changes in the temperature of the bathing medium were induced quite simply by replacing the existing medium by another of the same composition but at a different temperature. It is, of course, important in this type of experiment to ensure that there is as small a difference as possible between the temperature of the bathing solution and that of the surroundings. For this reason, the experiments were carried out in a cold room, the temperature of which could be controlled to within a degree centigrade.

The electrical measurements made were those of the resting membrane potential and resistance. The method adopted for measuring the membrane resistance has already been described[3]. It is a method which depends on a critical positioning of the current-injecting and voltage-recording electrodes. The positioning is such that the current-injecting electrode is inserted into the cell at its midpoint and the voltage-recording electrode is inserted at a distance equal to 0.42 l from the current electrode, where l is the half-length of the cell. By adopting this procedure the attenuation effects on the membrane current density and potential changes, which are normally observed in the cylindrical cells of the Characeae when the current is injected into the cell at a single point[4,5], are eliminated. The ratio of the change in the resting potential over the applied current then gives the true total resistance, R_t, of the cell. The product of R_t and the surface area of the cell gives the resistance of unit area of the membrane in $\Omega \cdot cm^2$; this quantity is referred to as the membrane resistance R_m. The advantage of using this method of measuring the membrane resistance is in the speed with which the measurement can be made and also in the elimination of a second voltage-recording electrode[3]; we refer to the method as the 'single voltage-probe' method.

The internal current and voltage electrodes were conventional 3 M KCl-filled glass micro-electrodes having resistances of a few $M\Omega$; both electrodes were inserted transversely into the cell and in the relative positions already indicated. The external current electrode was a length of silver wire laid parallel to the length of the cell and close up to it, while the external voltage electrode was a calomel reference electrode. The current was passed into the cell *via* the micro-electrode and a series resistance of 100 $M\Omega$, and the maximum current injected was never more than 0.2 μA;

the actual value of the current was calculated from the measured fall in potential across a series resistance of 10 kΩ. The voltage electrodes were used to measure both the resting membrane potential and the changes in this potential which were induced by the applied current; both quantities were recorded on the calibrated screen of a Tektronix 502 oscilloscope.

It must be emphasised that the tip of the internal voltage-recording micro-electrode was located in the cell vacuole in all the experiments. Thus the resting potential measurements actually determined the potential of the vacuole with respect to the external bathing solution and the measured resistance was the total resistance between the two phases. In other words, the term membrane as used in the present context refers to a composite system made up of the tonoplast, the plasmalemma and the cell wall. It is, of course, possible to determine the separate contribution of the tonoplast to the total potential and resistance by inserting an additional voltage-recording electrode into the cytoplasm[1,6]. However, if a micro-electrode is left in the cytoplasm for longer than about 20 min the tip becomes sealed[7] and it is therefore not possible to obtain continuous recordings of potential and resistance over long periods of time; it is also rather inconvenient to withdraw and re-insert a cytoplasmic electrode at different stages of the experiment. It is for these reasons that we have not attempted to determine the tonoplast contribution to the parameters. In any case, as we discuss later, it is unlikely that large-scale permeability changes take place at the tonoplast.

RESULTS

The initial experiments were concerned with examining the effect of temperature on the resting potential. The time taken for the new steady state to be reached following a change of temperature was of the order of several minutes and the potential was measured after it had attained a steady level. The change in resting

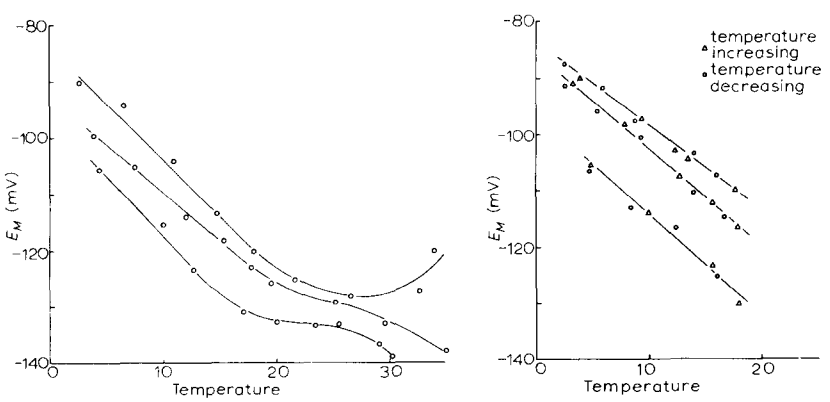


Fig. 1. Typical graphs for three different cells illustrating the linear temperature dependence of the resting membrane potential (E_m) below 18° and the region of erratic behaviour above this temperature. E_m is the potential of the vacuole with respect to the external medium. The composition of the external medium, for all experiments, was 1.0 mM NaCl, 0.1 mM KCl.

Fig. 2. Typical graphs showing the reversibility of the temperature effect on resting potential for temperatures below 18°.

potential in the temperature range from just above 0° to 35° is shown in Fig. 1. The three graphs shown are typical of many others similarly obtained and they illustrate two important features: an almost linear variation of potential below 18° but an unpredictable behaviour above this temperature.

In experiments in which the temperature variations were limited to the range 0–18°, it was observed that the potential changes were reversible; this is illustrated in Fig. 2. If the temperature is raised above 18° and then lowered below 18° the reversibility is lost for several hours.

The mean slope of the potential–temperature graphs for ten cells is about 1.6 mV per degree. The consistency of this slope can be judged from Fig. 3 in which

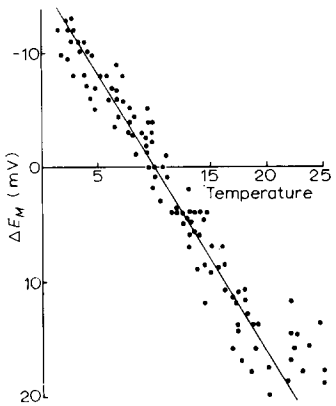


Fig. 3. The plot of ΔE_m against temperature, where ΔE_m is the change in resting potential from a chosen reference level; the chosen level is the resting potential at 10°.

changes in the resting potential have been plotted against temperature. As each cell had a different absolute resting potential, E_m, at a given temperature, comparison of the slopes is made easier by plotting ΔE_m against temperature, where ΔE_m is the change in resting potential from some arbitrary reference level. In Fig. 3 the chosen arbitrary level is the resting potential at 10°.

The practical implications of these results are of considerable importance. Clearly, an unnoticed drift in the ambient room temperature could cause a large error in the measurement of resting potential and if relatively small changes in resting potential were being observed, then the expected changes could easily be masked by temperature fluctuations. It is thus advisable in any series of experiments to maintain a reasonably constant ambient temperature and to ensure that added solutions are at the same temperature as those they are replacing. Again, it would appear that greater experimental consistency can be obtained by working at temperatures below 18° thus avoiding the region of erratic behaviour shown in Fig. 1. The reason for the irregularities above 18° is not known. It may be connected with the adaptation of the cells to the cool water of their native habitat, but this speculation is not entirely convincing as Nitella can be observed growing prolifically near the edges of the lake where the water is shallow and where the summer temperature is certainly greater than 18°.

In another series of experiments with another batch of cells the temperature

variation of both the resting potential and resistance was measured for ten cells in the range 0–10°. Fig. 4 shows the variation of potential with temperature for these ten cells and again the mean slope of the graph is about 1.6 mV per degree. Fig. 5 shows the plot of membrane resistance against temperature. It can be seen that the change in resistance is very marked, there being a two-fold increase in resistance as the temperature falls from 10° to nearly freezing point. The practical implications of these results for resistance measurements are again obvious—a reasonably constant ambient temperature must be maintained.

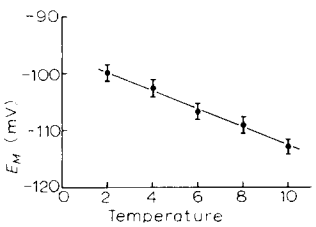


Fig. 4. The relation between resting potential and temperature in the range 0–10° for ten cells. The standard error of the mean potential for the ten cells is shown for each temperature.

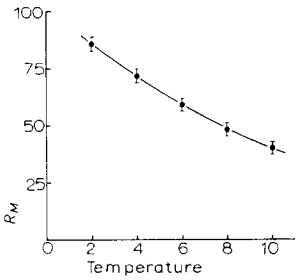


Fig. 5. The relation between membrane resistance and temperature for ten cells. The standard error of the mean resistance is plotted for each temperature. R_M is expressed in $k\Omega \cdot cm^2$.

At this stage it is of some importance to comment upon another important implication of these changes in membrane resistance. It is well known that if an electric current is injected into a Nitella cell at a point along its length, then there will be an attenuation of both the membrane current density and potential change with distance from the point of current injection[3–5]. The so-called 'space constant', λ, is a measure of this attenuation and is given by the expression:

$$\lambda = [R_m/\pi d(r_o + r_i)]^{1/2}$$

where r_o and r_i are, respectively, the resistances per unit length of the external and internal solutions of the cell with respect to the membrane and d is the cell diameter. Clearly the large changes of R_m with temperature mean that there will also be substantial changes in λ under the same conditions and it is therefore necessary to consider the validity of using the single voltage-probe method of measuring the membrane resistance in the present experiments. The mean half-length, l, of the cells used in these experiments was 4 cm and at normal room temperature the value of λ is close to 3 cm (see refs. 4 and 5). Thus the ratio l/λ which we denote by L, has a value of 1.3. It has been shown[3] that with such a value of L there will be no significant error in the value of R_m obtained by this particular method. If the membrane resistance increases four-fold as the temperature decreases from 18° to 0° then, as can be seen from the expression for the space constant, the value of λ will increase by two-fold and the value of L will then be 0.65. Again there will be no significant error arising from the measurement of R_m by the single voltage-probe method (see again ref. 3). It is therefore apparent that we are justified in using this method of measuring the membrane resistance in our experiments. Of course we are assuming that the value of the parameter $(r_o + r_i)$ is constant at all temperatures and this is something that

cannot be tested by the single voltage-probe method. However, in view of the very small magnitude of the measured fluxes of Na^+ and K^+ in *N. translucens*[8], it seems very unlikely that significant changes could take place in the external and internal ionic concentrations of the cell in the time taken to achieve a new steady state after a change in the temperature of the system. In other words, it can be assumed that $(r_o + r_i)$ is reasonably constant over the temperature range of the present experiments.

DISCUSSION

It has been emphasised that the membrane parameters measured in the present experiments are those for a composite system made up of the tonoplast, the plasmalemma and the cell wall, and it is therefore important to try to specify exactly where the permeability changes take place. Measurements of the potential difference across the tonoplast[1] show that it is small compared with the total resting potential of the cell; its resistance is also small in relation to the total resistance[6]. It therefore seems unlikely that the tonoplast is involved to any marked extent in the very large changes which we have observed in the resting potential and resistance, and permeability changes in this membrane are thus probably unimportant. It is also highly unlikely that permeability changes are significant in the cell wall and it is reasonable to suggest that it is at the plasmalemma that the important changes take place.

It has been shown[1], that for cells of *N. translucens* bathed in calcium-free media similar in composition to that used in the present work, the potential of the plasmalemma relative to the bathing medium is quite accurately described by the equation:

$$E_{co} = -\frac{RT}{F} \ln \frac{[K]_o + \alpha[Na]_o}{[K]_c + \alpha[Na]_c} \qquad (1)$$

$[K]_c$, $[Na]_c$, $[K]_o$, $[Na]_o$, are respectively the K^+ and Na^+ concentrations in the cytoplasm and the external medium; α is the permeability ratio P_{Na}/P_K, where P_{Na} and P_K are the permeability coefficients of the membrane to these ions; R, T, and F have their usual significance. E_{co}, though referred to as the plasmalemma potential, is strictly the potential difference between the cytoplasm and the external medium and as such includes contributions from both the plasmalemma and the cell wall. Nevertheless, it was shown[1] that Eqn. 1 describes the response of the plasmalemma to changes in the external K^+ and Na^+ concentrations and that the value of α in the equation is the value which pertains to the plasmalemma. Now before we proceed to apply Eqn. 1 to our results it is relevant to note that both Na^+ and K^+ have been shown to be actively transported at the plasmalemma[1,8] and it is to be expected that the mechanism for this transport is temperature dependent. However, it is assumed in the derivation of Eqn. 1 that the active transport mechanism is electrically neutral and therefore makes no contribution to the net membrane current. This assumption has been amply justified by the measurements of the tracer fluxes of Na^+ and K^+ across the plasmalemma[8] which have provided strong evidence in favour of a one-to-one exchange of Na^+ for K^+. These flux measurements were made at normal room temperature, but since there is no obvious reason to suspect that this very tight coupling would be broken by small temperature changes, then it is probable that the active membrane current is zero over the temperature range of our experiments. In other words, the observed temperature dependence of the membrane

potential is a passive effect. The remainder of the discussion is chiefly concerned with showing that such an explanation is indeed consistent with our experimental results.

In Eqn. 1 the temperature enters explicitly in the RT/F term and implicitly through the temperature-dependent permeability coefficients. A simple calculation shows that the explicit term contributes only 0.5 mV per °K to the change in resting potential. Thus the bulk of the observed change (1.6 mV per °K) must be linked with permeability changes at the plasmalemma. In order to test this hypothesis we have taken values of $[Na]_c = 37$ mM, $[K]_c = 93$ mM (see ref. 1) and a typically observed value for the resting potential, *i.e.* the potential difference between the vacuole and the external medium, of −125 mV at 18°; the value of E_{co} would then be −140 mV since the cytoplasm is some 15 mV more negative than the vacuole, *i.e.* $E_{co} = E_m - 15$ (see ref. 1). If it is assumed that temperature changes do not affect this 15 mV potential difference between the cytoplasm and vacuole to any great extent, and this is not unreasonable, then the E_{co} values can be deduced for different temperatures from the observed values for the resting potential at these same temperatures. Hence the values of α corresponding to these values of E_{co} can be calculated from Eqn. 1. The results of these computations are shown in Table I.

The separate values of the coefficients P_{Na} and P_K at different temperatures can be deduced from an equation which expresses the functional relationship between membrane resistance, ionic concentration, ionic permeability and temperature[2]:

$$R_m = \frac{RT[1/C_o - 1/C_c]}{F^2 \ln (C_c/C_o)} \qquad (2)$$

where $C_o = P_K[K]_o + P_{Na}[Na]_o$ and $C_c = P_K[K]_c + P_{Na}[Na]_c$. In applying this equation we have used the values of α shown in Table I. The values of R_m for temperatures above 10° were obtained by suitable extrapolation of the curve shown in Fig. 5. Strictly these resistance values include contributions from the tonoplast as well as the plasmalemma but for the purpose of these present calculations the tonoplast contribution can be ignored. The calculated values of the permeability coefficients at different temperatures are shown in Table II. It may be noted that the values are appreciably higher than those predicted from tracer flux measurements[8]. This

TABLE I

THE CALCULATED VALUES OF α BASED ON EQN. 1

Temp. (°K):	291	287	283	279	275
E_{co} (mV)	−140	−134	−128	−121	−115
α	0.28	0.37	0.49	0.65	0.88

TABLE II

THE CALCULATED VALUES OF P_{Na} AND P_K BASED ON EQN. 2

Temp. (°K):	291	287	283	279	275
R_m (kΩ·cm^2)	20.0	28.5	40.0	59.0	86.0
P_{Na} (cm·sec^{-1}) × 10^7	17.1	13.0	10.0	7.2	5.3
P_K (cm·sec^{-1}) × 10^7	61.2	35.1	20.4	11.0	6.0

discrepancy between resistance and flux measurements is a familiar one and has been already discussed[4,8].

The results of these computations suggest that the temperature dependence of the membrane potential and resistance can be explained as being due to passive effects arising from differential changes in the Na^+ and K^+ permeabilities of the plasmalemma. But it could also be argued, quite fairly, that such an approach is merely arithmetic juggling and clearly some additional evidence must be produced to substantiate the hypothesis. It seemed to us that supporting evidence might be obtained from the kinetic theory of rate processes in which permeability coefficients are expressed as explicit functions of temperature.

In applying the kinetic theory we have taken the simplest of membrane models in which it is envisaged that the membrane consists of a number of uniform potential barriers under the influence of an electric field[9]. With such a model the permeability coefficient for Na^+ is given by:

$$P_{Na} = \frac{kT}{h} \cdot \frac{\lambda_{Na}^2}{\delta} \exp\left(-\frac{\Delta G^*_{Na}}{RT}\right) \tag{3}$$

where k and h are, respectively, the Boltzman and Planck constants, λ_{Na} is the distance between the potential barriers for Na^+ and δ is the membrane thickness. ΔG^*_{Na} is the free energy change required for Na^+ to surmount a potential barrier, *i.e.* it is the GIBBS free energy of activation for the permeation of Na^+. This quantity can, of course, be expressed in terms of the changes in enthalpy and entropy (ΔH^* and ΔS^*) in the usual way:

$$\Delta G^*_{Na} = \Delta H^*_{Na} - T\Delta S^*_{Na} \tag{4}$$

By substituting for ΔG^*_{Na} in Eqn. 3 and rearranging we get:

$$\ln\left(\frac{h}{kT} \cdot P_{Na}\right) = \left[\ln\frac{\lambda_{Na}^2}{\delta} + \frac{\Delta S^*_{Na}}{R}\right] - \frac{\Delta H^*_{Na}}{RT} \tag{5a}$$

Similarly for K^+ we can write:

$$\ln\left(\frac{h}{kT} \cdot P_K\right) = \left[\ln\frac{\lambda_K^2}{\delta} + \frac{\Delta S^*_K}{R}\right] - \frac{\Delta H^*_K}{RT} \tag{5b}$$

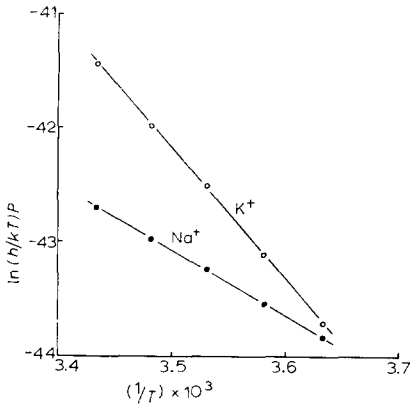
The expression for α is then:

$$\ln\alpha = \ln\left(\frac{\lambda_{Na}}{\lambda_K}\right)^2 + \frac{\Delta S^*_{Na} - \Delta S^*_K}{R} - \frac{(\Delta H^*_{Na} - \Delta H^*_K)}{RT} \tag{6}$$

Eqns. 5a, 5b and 6 imply a linear relationship between $\ln(h/kT)P_{Na}$, $\ln(h/kT)P_K$, $\ln\alpha$, and the reciprocal of the absolute temperature T. In Figs. 6 and 7 we show the plots of these functions for the values of P_{Na}, P_K and α given in Tables I and II. The linearity of these graphs goes a long way to substantiating the suggestion that the main effect of temperature is to induce changes in the passive permeabilities of the plasmalemma to Na^+ and K^+.

The slopes of the graphs in Fig. 6 are proportional to ΔH^*_{Na} and ΔH^*_K and the respective values are: $\Delta H^*_{Na} = 11.2$ kcal·mole^{-1}, $\Delta H^*_K = 22.9$ kcal·mole^{-1}. The terms in brackets in Eqns. 5a and 5b are the respective intercepts on the $\ln(h/kT)P$ axis in Fig. 6 and clearly, from these quantities it is possible to deduce the changes in entropy for both Na^+ and K^+ if δ, λ_{Na}, and λ_K are known. We have assumed

a value for λ_{Na} and λ_K of 3 Å (see ref. 9) and by taking the membrane thickness to be 100 Å then $\Delta S^*_{Na} = -5.0$ cal·degree^{-1}·mole^{-1} and $\Delta S^*_K = 37.5$ cal·degree^{-1}·mole^{-1}. Thus the free energy changes at 18° are: $\Delta G^*_{Na} = 12.7$ kcal·mole^{-1} and $\Delta G^*_K = 12.0$ kcal·mole^{-1}. It is interesting to observe that these values for the free energy changes are comparable with those obtained for the permeation of polyhydroxy alcohols through the membranes of *Chara ceratophylla*[9]. The somewhat surprising result in our present work is the large difference between ΔS^*_{Na} and ΔS^*_K and the fact that these quantities have opposite signs. It should be noted however that the calculated value of ΔS^*_{Na} is dependent upon the values assigned to λ_{Na} and δ. It is possible, for example, to obtain small positive values for ΔS^*_{Na} by taking $\lambda_{Na} \approx 1$ Å and $\delta \approx 200$ Å, but these values are considered to be less realistic than our original choice. Analogous results have been reported for the entropies of activation measured in self-diffusion studies on isomeric pairs of alcohols[10]. The ΔS^* values for *n*-propyl and *n*-butyl alcohols were negative but positive for isopropyl and *tert.*-butyl alcohols.



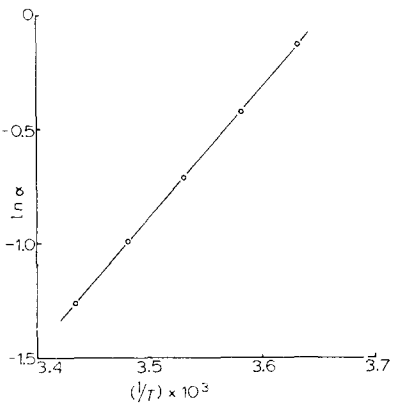


Fig. 6. The plots of $\ln(h/kT)P$ against the reciprocal of T, where P is the permeability coefficient (P_{Na} or P_K).

Fig. 7. The plot of $\ln\alpha$ against the reciprocal of T.

In conclusion it may be said that the observed changes in the resting membrane potential and resistance with temperature in *N. translucens* may reasonably be ascribed to passive effects arising from differential changes in the Na^+ and K^+ permeabilities of the plasmalemma. This hypothesis will be further tested when the necessary tracer flux studies are made at different temperatures.

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