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TETRAETHYLAMMONIUM ION INHIBITION OF POTASSIUM CONDUCTANCE OF THE NODAL MEMBRANE

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SUMMARY

1. The effect of tetraethylammonium ions at concentrations ranging from 0.1 to 40 mM on the steady-state K^+ conductance of the Ranvier node membrane, which was externally perfused with K^+-rich solutions (50 or 100 mM), has been studied.

2. The dose–response relation is consistent with the supposition that the single conductance channel is blocked by one tetraethylammonium ion and the channel–tetraethylammonium ion complex has a certain dissociation constant (k_{dis}). At a Ca^{2+} concentration of 2 mM and at normal pH, $k_{dis} = 0.52$ mM.

3. Increasing the Ca^{2+} concentration to 20 mM and decreasing the pH to 5.2–5.4 led to an increase of k_{dis} by factors of 1.65 and 1.43, respectively. A 2.5-fold reduction of the ionic strength and a rise of pH to 9.3–9.6 resulted in a decrease of k_{dis} by factors of 1 6 (corrected for tetraethylammonium ion activity) and 1.37, respectively.

4 The changes in k_{dis} are interpreted in terms of changes in the tetraethylammonium ion concentration at the membrane surface as a consequence of variations in the membrane surface potential which in turn undergoes alterations due to changes in Ca^{2+} concentration, ionic strength and pH.

INTRODUCTION

It is assumed by a number of investigators[1–4] that Ca^{2+} is involved in a physicochemical mechanism which may be responsible for changes in membrane permeability. In this context, experiments in which the concentration of external Ca^{2+} and that of selective inhibitors of Na^+ or K^+ conductance are changed simultaneously may by essential If Ca^{2+} interacts directly with the conductance channels competition between Ca^{2+} and the inhibitor molecules may be expected. If the Ca^{2+} effect is caused by alterations in the membrane surface charge, resulting from binding of Ca^{2+} to negatively charged groups in the vicinity of the conductance channels[5–8], competition is unlikely In the latter case, the surface charge may influence the concentration of the inhibitors (if they are ions) at the external membrane surface and the effectiveness of their action.

This paper is concerned with the effect of the tetraethylammonium ion, a selective inhibitor of K^+ conductance[9,10], on the steady-state K^+ conductance of the nodal membrane, externally perfused with K^+ rich solutions at various Ca^{2+} concentrations, pH values and ionic strengths.

METHODS

Single nerve fibres of the frog *Rana ridibunda*, 15-20 μm in diameter, were used for the experiments.

The voltage-clamp technique used was identical with that described in detail previously[11] An operational amplifier for applying feed-back was employed, the voltage gain was 10^4. The membrane currents (I_m) were measured as voltage drops across a constant resistance in series with the membrane resistance. Longitudinal currents along the outer surface of internodes were prevented by a special feed-back system[12]. To obtain a current–voltage relation, a clamping voltage at a speed of 2 mV/s was fed both to the input of the operational amplifier and to the horizontal plates of a cathode-ray oscilloscope to make up a sweep for measured currents. Current–voltage curves so obtained were continuous.

All experiments were carried out at 22 °C.

Two basic solutions had the following ionic compositions. I, 100 mM K^+, 23 mM Na^+, 1 mM $Tris^+$, 2 mM Ca^{2+}, 128 mM Cl^-; II, 50 mM K^+, 76 mM Na^+, 1 mM $Tris^+$, 0.2 mM Ca^{2+}, 127 4 mM Cl^-. All other solutions were prepared by modifying Solutions I and II. The Ca^{2+} or tetraethylammonium concentration was increased at the expense of the appropriate decrease in Na^+ concentration. pH was decreased and increased by substitution of Tris–HCl and, partially, NaCl by biftalic (decrease in pH) or glycine (increase in pH) buffers A solution with 50 mM K^+ was employed when the ionic strength was reduced. The reduction of ionic strength was brought about by substitution of NaCl by sucrose.

The membrane conductance (G_m) was estimated as $I_m/(V_m-V_r)$, where V_m and V_r are membrane potential and resting potential in the given solution, respectively. In all cases the resting potential in solutions without tetraethylammonium was taken as zero for V_m. Negative and positive values of V_m correspond to inward and outward currents, respectively

RESULTS

Fig. 1a shows a set of current–voltage curves from a single node at various concentrations. An increase in tetraethylammonium ion concentration can be seen to reduce I_m within the voltage range from -60 to $+40$ mV. In the absence of tetraethylammonium ion and at low tetraethylammonium ion concentration, the current–voltage curves have a negative resistance component. At a tetraethylammonium ion concentration of 40 mM, the current–voltage relation is almost linear. Increasing the tetraethylammonium ion concentration renders the resting potential more negative, all curves crossing at $V_m = +2$ mV. The data presented here may be explained in terms of Cl^- permeability (P_{Cl}). As the tetraethylammonium ion concentration is increased P_{Cl}/P_K also increases, resulting in a shift in resting potential towards the Cl^- equilibrium potential, and the V_m at which the curves cross should be considered as the K^+ equilibrium potential. The data well fit the Goldman–Hodgkin–Katz constant-field equation[13] for $P_{Cl} = 0.1\ P_K$ and $[Cl^-]_{in} =$ 3–10 mM. The Cl^- current estimated for the above assumption is given in Fig. 1a. Fig. 1b shows a set of conductance–voltage curves at various tetraethylammonium ion concentrations. It is seen that, in agreement with our previous observations[11] and with results of other authors[9,10], tetraethylammonium ion has no effect on leakage conductance (G_{min}) and inhibits the potential-dependent K^+ conductance $\Delta G_m = G_m - G_{min}$.

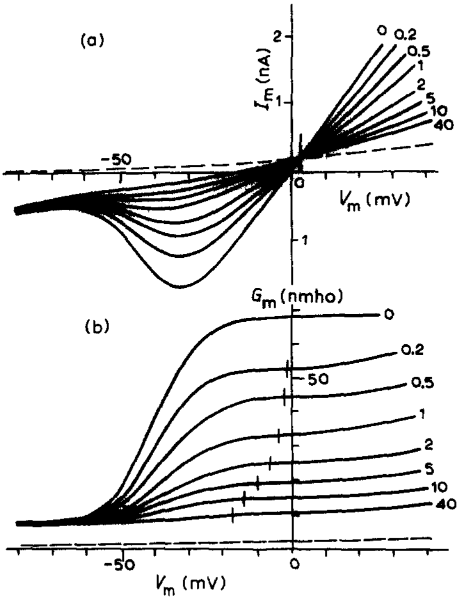
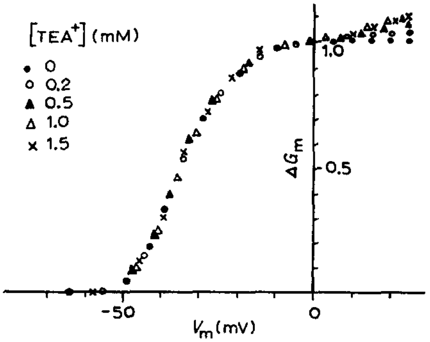


Fig 1. Current–voltage (a) and conductance–voltage (b) relations for the Ranvier node membrane in the presence of tetraethylammonium ion Numbers on curves give the tetraethylammonium ion concentration in mM Dashed lines indicate I_{Cl} (a) and G_{Cl} (b) (see text). Vertical strokes on the G_m–V_m curves indicate resting potential for various tetraethylammonium ion concentrations $[K^+] = 100$ mM, $[Ca^{2+}] = 2$ mM Fibre 43–71

Because the slope of the Cl^- current–voltage curve within the voltage range used is insignificant, Cl^- conductance (G_{Cl}) is small (2–3 % of the maximum values of G_m). It can be assumed that G_m defined under given experimental conditions (at least at low tetraethylammonium ion concentration) is the K^+ conductance.

While in the absence of tetraethylammonium ion, in the region of small negative and positive V_m values, G_m reaches the plateau and obtains its maximum value, in the presence of tetraethylammonium ion in the majority of cases a small second increase in G_m is observed after the plateau. The phenomena observed are hard to explain. To account for the effectiveness of the tetraethylammonium ion, the G_m value of the plateau (near $V_m = 0$) must be taken into consideration.

The G_m–V_m curve analysis (Fig. 2) at various tetraethylammonium ion concentrations indicated that, in spite of the reduction in G_m, the shape of the curves remained unchanged; no curve shift along the voltage axis was observed. $-V_{1/2}$ did not change, where $V_{1/2}$ is the V_m value when G_m is at half-maximum. Tetraethylammonium ion appears to block every conducting channel independently, one channel is likely to be blocked by one tetraethylammonium ion. In such a case the Langmuir formula holds true for the relation between the number of free channels and tetraethylammonium ion concentration. When the conductance of the occupied channels is zero, ΔG_m is proportional to the number of free channels. It is convenient to regard the $\Delta G_0/\Delta G_T$ ratio, where ΔG_0 and ΔG_T are ΔG_m without and with tetraethylammonium ion, respectively, as a degree of tetraethylammonium ion inhibition,



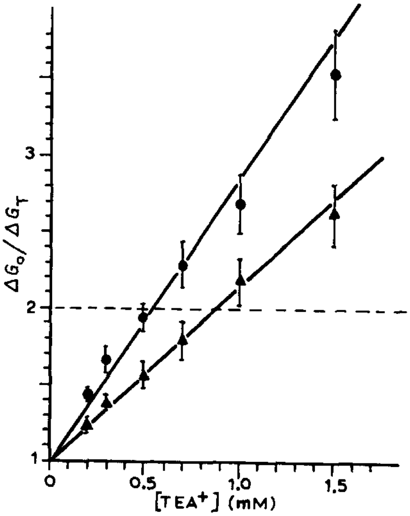


Fig 2 The relation between ΔG_m and V_m with various tetraethylammonium ion concentrations ([TEA+]) ΔG_m is plotted in units of its value on the plateau (see text) $[K^+] = 100$ mM, $[Ca^{2+}] = 2$ mM Fibre 8–71

Fig 3 Relation between $\Delta G_0/\Delta G_m$ and tetraethylammonium ion concentration ([TEA+]) with $[Ca^{2+}] = 2$ mM (●) and $[Ca^{2+}] = 20$ mM (▲) The solid lines were calculated from Eqn 1 with $k_{dis} = 0.52$ mM and $k_{dis} = 0.86$ mM Each value is the mean of 10 experiments ± S E.

since from the Langmuir formula $\Delta G_0/\Delta G_T$ can be expressed as a linear function of tetraethylammonium ion concentration ([TEA+]):

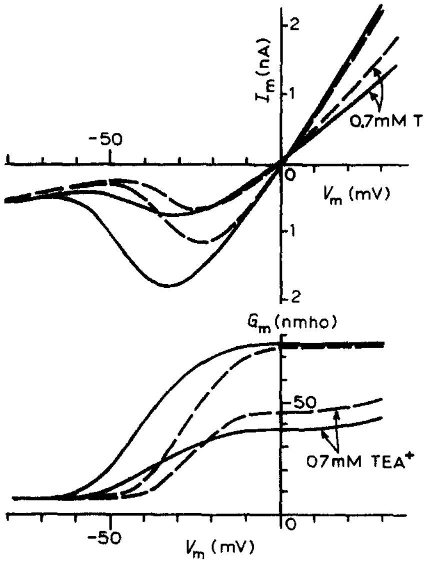
$$\Delta G_0/\Delta G_T = 1 + [\mathrm{TEA}^+]/k_{dis} \qquad (1)$$

where k_{dis} is the effective dissociation constant. As follows from the formula, the slope of the straight line is determined by the value of k_{dis}. When $\Delta G_0/\Delta G_T = 2$, [TEA+] numerically equals k_{dis}.

Fig. 3 illustrates the relation between $\Delta G_0/\Delta G_T$ (mean of 10 experiments) and tetraethylammonium ion concentration at $[Ca^{2+}] = 2$ mM and at $[Ca^{2+}] = 20$ mM. It is seen that within the range of 0 to 1.5 mM tetraethylammonium ion the experimental points approximately follow a straight line. At higher tetraethylammonium ion concentrations ([TEA+]) the slope of the $\Delta G_0/\Delta G_T$–[TEA+] curves decreases. It should also be noted that at high tetraethylammonium ion concentrations the accuracy of $\Delta G_0 \Delta G_T$ calculations falls sharply.

Thus, the Langmuir formula describes the experimental data for moderate tetraethylammonium ion concentrations rather well, and k_{dis} can be used as an adequate criterion of the effectiveness of the inhibitor.

In Fig. 3 the difference in the slopes of the $\Delta G_0/\Delta G_T$–[TEA$^+$] curves (difference in k_{dis}) at different Ca^{2+} concentrations is seen distinctly: an increase in [Ca^{2+}] leads to an increase in k_{dis}. The influence of Ca^{2+} concentration on the tetraethylammonium ion effect is also illustrated in Fig. 4 where the results of one experiment are shown. One can see that increasing the Ca^{2+} concentration leads to an increase in G_m, in the presence of 0.7 mM tetraethylammonium ion, while in the absence of the inhibitor increasing the Ca^{2+} concentration decreases G_m slightly. Thus, increasing the Ca^{2+} concentration decreases the effectiveness of tetraethylammonium ion. Moreover, an increase in [Ca^{2+}] shifts the conductance–voltage curves along the voltage axis in the direction of depolarization ($-V_{1/2}$ decreases). If the $V_{1/2}$ shift ($\Delta V_{1/2}$) is equal to the decrease in negative surface potential (Δ ψ_0)$^{6-8}$ the tetraethylammonium ion concentration at the membrane surface ([TEA$^+$]$_s$) should obviously be reduced by a factor of exp $\Delta V_{1/2}$ $e/\mathbf{k}T$, where e, $\mathbf{k}$, T are the electronic charge, Boltzman constant and absolute temperature, respectively. Eqn 1 includes the tetraethylammonium ion concentration per unit volume of bulk solution. The change in [TEA$^+$]$_s$ which is essential for its action is equivalent to the change in k_{dis}. For example, at $\Delta V_{1/2}$ = 12 mV (as in the experiment shown in Fig. 4) [TEA$^+$]$_s$ should be decreased by a factor of 1.61. In the same experiment for, [Ca^{2+}] = 2 mM, $\Delta G_0/\Delta G_T$ = 2.2, and for [Ca^{2+}] = 20 mM, $\Delta G_0/\Delta G_T$ = 1.76, which corresponds, according to Eqn 1, to the increase in k_{dis} from 0.58 to 0.92 mM. The average (from



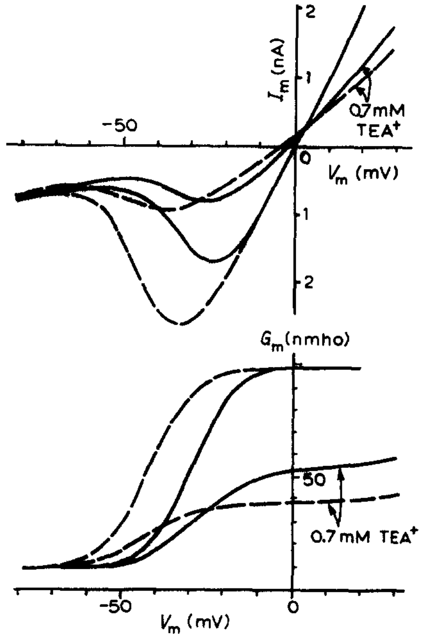


Fig. 4. The inhibition of K$^+$ conductance by tetraethylammonium ions (TEA$^+$) ((0.7 mM) in the presence of [Ca^{2+}] = 2 mM (——) and [Ca^{2+}] = 20 mM (- - -). [K$^+$] = 100 mM. Fibre 44–71.

Fig. 5. The inhibition of K$^+$ conductance by tetraethylammonium ions (TEA$^+$) (0.7 mM) with normal (——) and 2.5-fold reduced ionic strength (- - -). [K$^+$] = 50 mM, [Ca^{2+}] = 0.2 mM. Fibre 17–71.

10 experiments) increase in k_{dis} associated with an increase in [Ca^{2+}] from 2 to 20 mM is 1.65 ± 0.08, and the average value of $\exp V_{1/2}e/\mathbf{k}T$ is 1.79 ± 0.03.

Thus, the quantitative evaluation indicates that the reduction in effectiveness of tetraethylammonium ion when [Ca^{2+}] is increased may be explained on the assumption that the concentration of tetraethylammonium ion at the membrane surface is reduced by a decrease in the negative membrane surface potential.

To verify the above suggestion we compared the tetraethylammonium ion effect at various ionic strengths and pH values of the external solution. These factors are assumed to influence the membrane surface charge[7,8,14,15].

Fig. 5 represents a typical experiment of the tetraethylammonium ion effect (0.7 mM) at normal and at 2.5-fold reduced ionic strength. In this experiment [Ca^{2+}] was reduced to 0.2 mM because at normal and at high [Ca^{2+}] the change in ionic strength has little effect[7,8]. When the ionic strength is decreased the G_m–V_m curves shift towards hyperpolarization ($-V_{1/2}$ increases) and the tetraethylammonium ion effect is more pronounced. For the fibre tested (Fig. 5) k_{dis} decreased by a factor of 2.1 and $[TEA^+]_s$ increased by a factor of 1.68 The respective average values (from 10 experiments) were 1.80 ± 0.07 and 1.56 ± 0.01. The value of 1.8 involves not only the $[TEA^+]_s$ changes resulting from the increase in negative surface potential but also an increase of in tetraethylammonium ion activity due to the reduction of ionic strength. With some corrections 1.6 was obtained instead of 1.8.

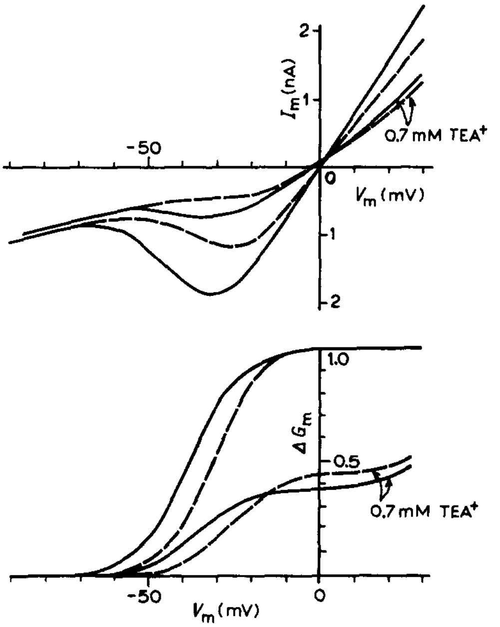


Fig. 6. The inhibition of K^+ conductance by tetraethylammonium ions (TEA^+) (0.7 mM) at pH 7.1 (——) and pH 5.6 (---). ΔG_m is plotted in units of ΔG_{max} in the absence of tetraethylammonium ions $[K^+]$ = 100 mM, $[Ca^{2+}]$ = 2 mM. Fibre 52–71.

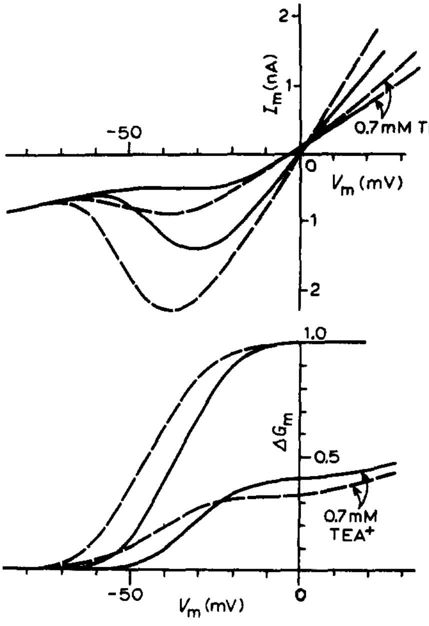


Fig. 7. The inhibition of K^+ conductance by tetraethylammonium ions (TEA^+) (0.7 mM) at pH 7.1 (——) and pH 9.3 (- - -). ΔG_m is plotted in units of ΔG_{max} in the absence of tetraethylammonium ions $[K^+]$ = 100 mM, $[Ca^{2+}]$ = 2 mM Fibre 37–71

Figs 6 and 7 show the effect of pH on the effectiveness of tetraethylammonium ion. The tetraethylammonium ion action (0.7 mM) at normal pH (7.1) was compared with that at lower pH (5 4) and higher pH (9.3) In view of the fact that pH variation may cause marked changes in G_m[7,8,15,16], ΔG_m was plotted in relative units. It is seen that shifts of the G_m–V_m curves towards depolarization when pH decreases are accompanied by a decrease in the tetraethylammonium ion effectiveness, whereas shifts of the G_m–V_m curves towards hyperpolarization when pH increases are accompanied by an increase in the tetraethylammonium ion effectiveness.

The increase in k_{dis} and decrease in $[TEA^+]_s$, when the pH is lowered to 5.2–5.4, are 1.43 ± 0.05 and 1.51 ± 0.06 (means of 19 experiments), respectively.

The decrease in k_{dis} and increase in $[TEA^+]_s$, when the pH is increased to 9.3–9.6, are 1.37 ± 0.05 and 1 31 ± 0.03 (means of 7 experiments), respectively.

It should be noticed that such clear-cut regularity when pH is changed is observed only in cases where the pH deviation from its normal value is not considerable, i e when the changes in G_m are not too great and the plateau of the G_m–V_m curve (see over) is well defined. Besides, when the pH varies greatly from its normal value other, as yet unknown, factors may come into force

DISCUSSION

The data presented here, as well as experiments by Hille[10], are in good agreement with the notion that one tetraethylammonium ion blocks a single channel of K+ conductance without changing the conductance properties of the other K+ channel. From the Langmuir formula used for the dose–response relationship, the following dissociation constants of the K+ channel–tetraethylammonium ion complex were calculated: at 2 mM Ca^{2+} and pH 7.1 $k_{dis} = 0.52$ mM (our data) and 0.4 mM (Hille[10])

In our study, a 10-fold increase of Ca^{2+} concentration enhanced k_{dis} 1.6–1 7 times, whereas Hille did not observe changes in k_{dis} at Ca^{2+} concentrations as high as 22 mM. This discrepancy seems to be presumably due to the high tetraethylammonium ion concentration (6 mM) in Hille's experiments. This concentration is 15 times higher than the k_{dis} obtained by Hille. At such a high tetraethylammonium ion concentration the K+ current is very small and it is difficult to notice its change when the Ca^{2+} concentration is increased. Moreover, Hille observed little or no shift of the K+ conductance relation on elevation of the Ca^{2+} concentration. The cause of such discrepancy is not quite clear, but it should be noted that experiments with K+-rich media are more appropriate for showing these shifts.

The results presented show that the shifts of G_m–V_m curves and the changes in tetraethylammonium ion effectiveness, upon the alteration of Ca^{2+} concentration, pH and ionic strength of solutions, are interrelated. The effectiveness of tetraethylammonium ion decreases when G_m–V_m curves are shifted in the direction of depolarization and increases when the G_m–V_m curves are shifted in the opposite direction.

Previous work[7,8] has shown that shifts in G_m–V_m curves, upon alteration of Ca^{2+} concentration, pH and ionic strength, can be accounted for by changes in the negative surface membrane potential It was supposed that carboxylic groups binding Ca^{2+} and H^+ and amino groups releasing H^+ with increase of pH are not involved in the mechanism of ion transfer control. The results given here support this idea. Factors which decrease negative surface potential (ψ_0), and consequently the concentration at the membrane surface ($[TEA^+]_s$), reduce the effectiveness of the tetraethylammonium ion. Alternatively, factors which increase ψ_0 and $[TEA^+]_s$ increase the effectiveness of the tetraethylammonium ion.

In accordance with the above concept, the energy of the channel–tetraethylammonium ion complex depends on both the chemical properties of tetraethylammonium ion receptor and the surface density of the distribution of charged groups in the vicinity of the channel. If the surface charge density of these groups in the vicinity of each channel is different, different channels may have different binding energy. In such a case, channels with high binding energy will be blocked primarily. This supposition may account for, if only partially the deviation of the relation $\Delta G_0/\Delta G_T$–tetraethylammonium ion concentration from the straight line.

Blaustein and Goldman[17] have found that procaine inhibition of Na^+ conductance decreases with elevation of Ca^{2+} concentration. These data can be explained by a decrease in procaine concentration at the membrane surface due to the decrease in the negative surface potential. Changes of the surface potential can be defined as shifts of the G_{Na}–V_m curves obtained by Blaustein and Goldman[17,18].

Thus, the experimental data of Blaustein and Goldman can be interpreted without using the hypothesis of procaine and Ca^{2+} competition for the "sites" which are involved in the ion transfer control mechanism.

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