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Effects of treatments inhibiting energy transfer and of plasmalemma ATPase inhibitors on the electric potential difference of maize root cells

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Fisiologia. — *Effects of treatments inhibiting energy transfer and of plasmalemma ATPase inhibitors on the electric potential difference of maize root cells* (*). Nota (**) di ANTONIO BALLARIN-DENTI e MAURIZIO COCUCCI, presentata dal Corrisp. E. MARRÈ.

RIASSUNTO. — Si sono studiati gli effetti sul potenziale elettrico transmembrana in cellule corticali di radici di *Zea mays* di vari trattamenti fisici e chimici in presenza o meno di fusicoccina, nota tossina vegetale in grado di produrre iperpolarizzazione del potenziale di membrana. Sono stati utilizzati trattamenti in grado di interferire con il metabolismo energetico cellulare, con le caratteristiche fisiche della membrana o con una ATPasi di membrana plasmatica probabilmente implicata nel trasporto di ioni. I risultati ottenuti hanno confermato che il potenziale è costituito da una componente diffusionale e da una attiva della quale è almeno in parte responsabile un'ATPasi di membrana, Mg^{++}–K^{+} dipendente.

INTRODUCTION

Recent studies have hypothesized that a membrane ATPase constitutes an important factor in directly or indirectly causing the transport of ions and some organic substances across the plasma membrane [9, 12, 16]. This ATPase activity could, by utilizing metabolic energy, extrude protons against an electrochemical gradient, thus determining a proton gradient with electric potential across the membrane. Gradients thus formed could be utilized to make possible many of the observed phenomena of transport. In fact, there have been some indications which suggest that potassium moves along the electric gradient [12, 15], whereas other substances (such as sugars), to be absorbed by the cells, utilize the proton gradient as well as the potential one [6, 12]. Finally, other membrane ATPases could make possible the transport of other substances, such as anions.

The difference in electric potential present on either side of the membrane is the expression of the separation of the charges due to the equilibrium that is established between the active and passive movements of ions and substances across the membrane itself. Moreover, the chemico–physical characteristics of the membrane contribute to the formation and maintenance of the potential.

If the genesis of the potential is at least in part due to the activity of membrane ATPase, the potential should be depressed by substances that

Abbreviations: PD, potential difference; FC, fusicoccin; DNP, dinitrophenol; FCCP, (*p*–trifluormethoxy)-carbonylcyanide-phenylhydrazone; PCMB; *p*-chloromercurybenzoate; PCMBS, *p*-chloromercurybenzosulphanate; DES, diethylstilbestrol.

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interfere with energy metabolism or specifically inhibit the membrane ATP-ases. We have therefore analyzed the action of PD of these two kinds of substances and also studied the effect on the potential induced by FC, a plant toxin which promotes the extrusion of protons, stimulates the uptake of potassium, and increases the transmembrane negative PD, and whose mechanism of action seems to be that of stimulating a membrane-ATPase activity [13].

Material and Methods

Maize seeds (*Zea mays* L. cv. XL 342) were germinated for 3 days on humid filter paper in the dark at 26 °C. The first 10-mm apical sections were removed from the principal roots and incubated for 60 min in agitation in a bath at 26 °C in the control solution. (in mM/liter $CaCl_2$ 0.5, $MgSO_4$ 0.125, K_2HPO_4 0.25 and H_3PO_4 0.192 final pH 6).

Measurements of the potential were carried out by use of the conventional procedure described in previous works [5]. The roots were maintained under flux, and the measurements were taken after at least 30 min to allow

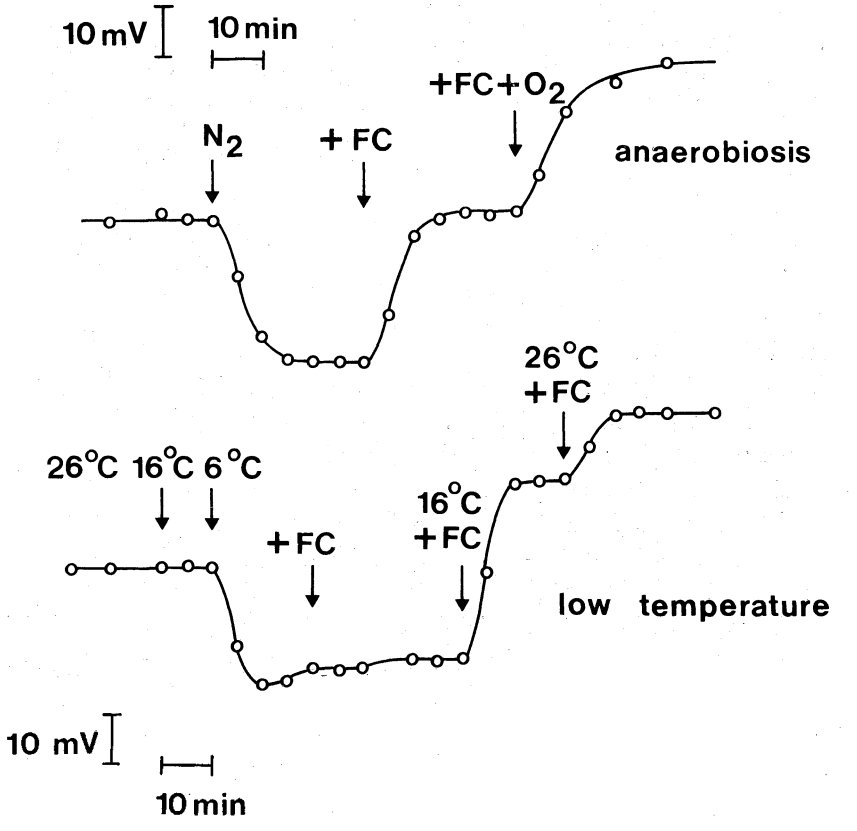

Fig. 1. – Effects of partial anaerobiosis and low temperature on PD in cortical cells of maize roots. PD baseline, 98 mV, S.E. 1.1. Partial anerobiosis was obtained by removing O_2 from the incubation medium and streaming N_2 on the top of the measurement cell. FC, 10^{-5} M.

equilibration of the potassium between the perfusion solution and the free space of the radical cells. The data reported are the average of at least five measurements of PD performed in different cells from the third to seventh layer of the cortical parenchyma of at least four different roots. In these conditions, the measurements were stable and reproducible, and the standard deviations were not more than 3 %.

RESULTS

A) *Physical treatments and uncouplers.*

Fig. 1 shows the effect of a partial anaerobiosis and low temperatures on the PD of maize roots. The data reported indicate that a partial anaerobiosis produces a rapid and marked depolarization; the addition of FC in such conditions produces an increase of about 30 mV in PD, which returns to normal values characteristic of the FC removing the deprivation of oxygen. When the temperature was lowered from the control 26 °C to 16 °C, no variations in PD were observed, whereas at temperatures around 6 °C the fall in PD was about 20 mV, and FC had no effect. Raising the temperature, in the presence of FC, to 16 °C resulted in a considerable increase in PD, which only returned to normal values of the FC when the temperature was raised to 26 °C.

The uncouplers of oxidative phosphorylation, DNP and FCCP, decreased the level of PD as a function of the concentration, which was administered as shown in Fig. 2. It is clearly seen that the FCCP begins to act at a

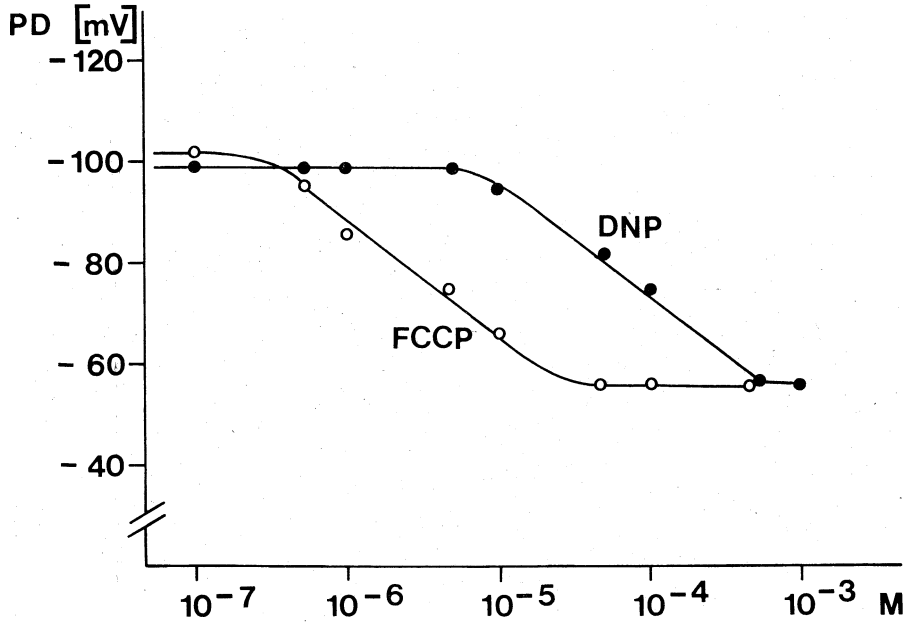

Fig. 2. – Depolarizing effects of the uncouplers FCCP and DNP against their concentration in the incubation medium.

concentration of 3×10^{-7} M until it produces a maximum depolarization (45 mV) at 3×10^{-5} M; the DNP produces a very similar fall in PD but at concentrations about 10 times higher.

The results obtained with anaerobiosis, low temperature, and uncouplers indicate that the PD depends at least in part on the energy metabolism [8, 16]. However, as regards low temperature, we must also keep in mind a probable action of this on the conformation of the membrane, and the uncouplers could act directly on the permeability of the protons of the plasma membrane [10]. The hyperpolarization effect of FC observed in conditions of partial anaerobiosis could be due to the increased capacity of the ATPase induced by FC itself to utilize ATP.

B) *Reagents of the —SH groups.*

PCMB and PCMBS do not have any significant effect on the potential (data not shown), whereas $HgCl_2$ causes a depolarization of about 15 mV, and subsequent administration of FC only partially returns PD values to normal. The lack of an effect of PCMB and PCMBS is probably due to the incapability of these substances to reach the membrane. Another mercurial, mersalyl, which is known to interfere with the transport processes in mitochondria and which has been found to inhibit an anion-sensitive ATPase [9], depolarizes PD by about 15 mV, and when FC is administered PD values return to values almost identical to those of the control (Fig. 3).

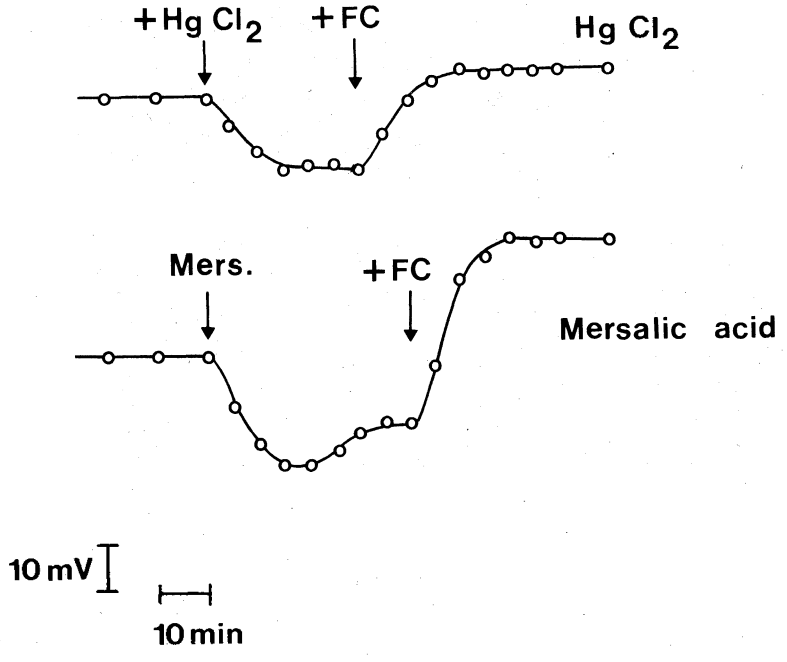

Fig. 3. – Effects of $HgCl_2$ and mersalyl on PD. PD baseline, 98 mV, S.E. 1.2. $HgCl_2$, 10^{-5}M; mersalyl, 10^{-3}M; FC, 10^{-5}M.

The fact that the action on PD of such substances is rapid indicates that they act at the level of the plasma membrane and that the proteins responsible for the genesis of the potential contain —SH groups that are necessary for their function.

C) *Inhibitors of plasma membrane ATPase.*

Fig. 4 shows that DES, a known inhibitor of K^+ transport whose action mechanism probably consists in the inhibition of a Mg^{++}–K^+-dependent plasma membrane ATPase [1], slightly depresses PD (10 mV) at both low and high potassium concentrations: when FC is administered in both cases PD increases to values typical of the control. These data are therefore in accord with the fact that the Mg^{++}–K^+-dependent, DES-inhibited ATPase plays a role in the formation of PD. The hyperpolarization produced by FC is probably due to the fact that the block of ATPase by DES is not total.

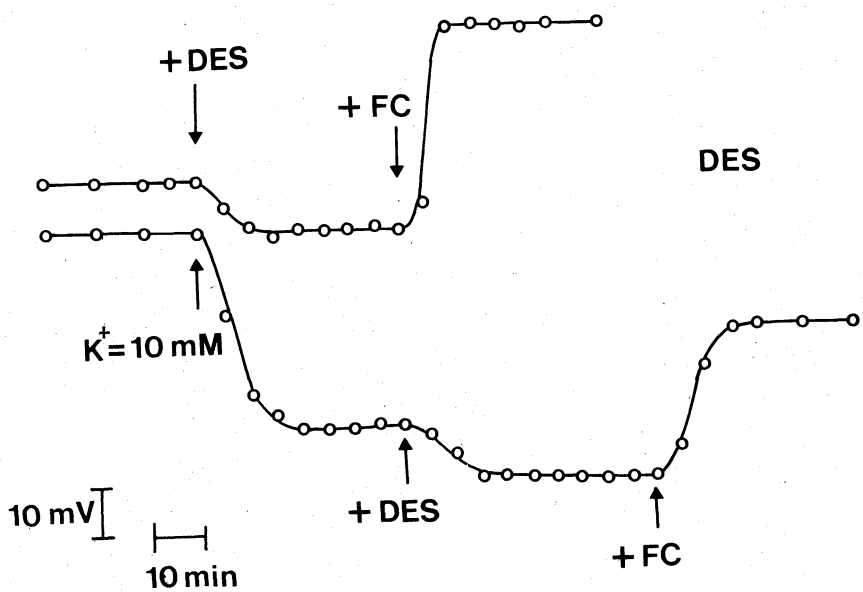

Fig. 4. – Effects of DES on PD. PD baseline, 97 mV, S.E. 1. DES. $3 \cdot 10^{-4}$M; FC, 10^{-5}M.

The effect of octylguanidine, which is known to inhibit the Mg^{++}–K^+-dependent ATPase of the plasma membrane [1], appears to be different; it markedly depresses the basic potential as well as that induced by FC at low concentrations of K^+, whereas it is not efficacious at concentrations around 10 mM (Fig. 5). In this case, the action of octylguanidine could be due, besides the specific effect on membrane ATPase, to the fact that it acts on the electron transport chain of the mitochondria; finally, its behavior as a cation cannot be excluded [2].

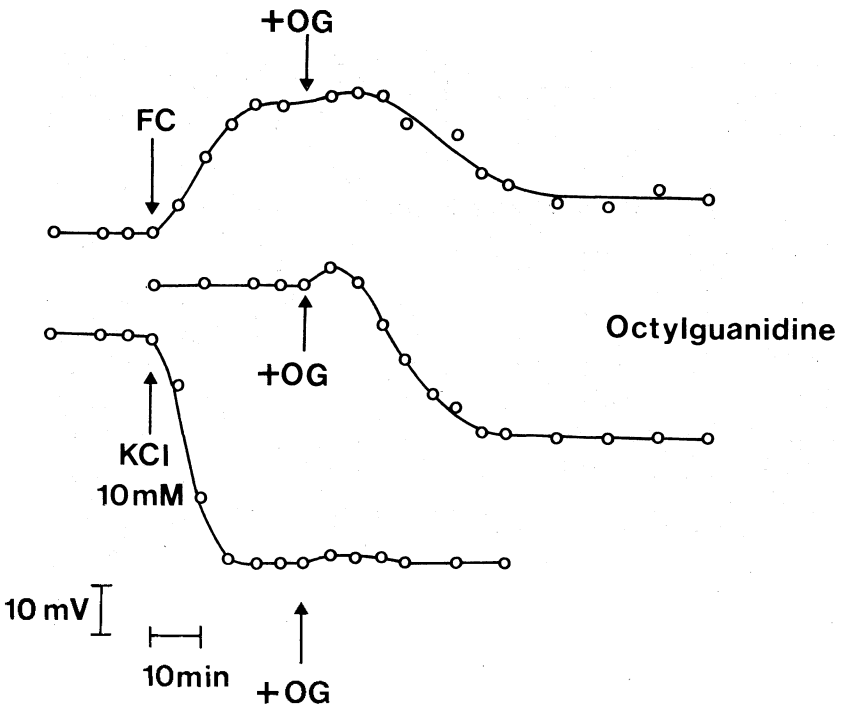

Fig. 5. – Effects of octylguanidine on PD. PD baseline, 98 mV, S.E. 1.2. Octylguanidine, $2 \cdot 10^{-4}$ M; FC, 10^{-5}M.

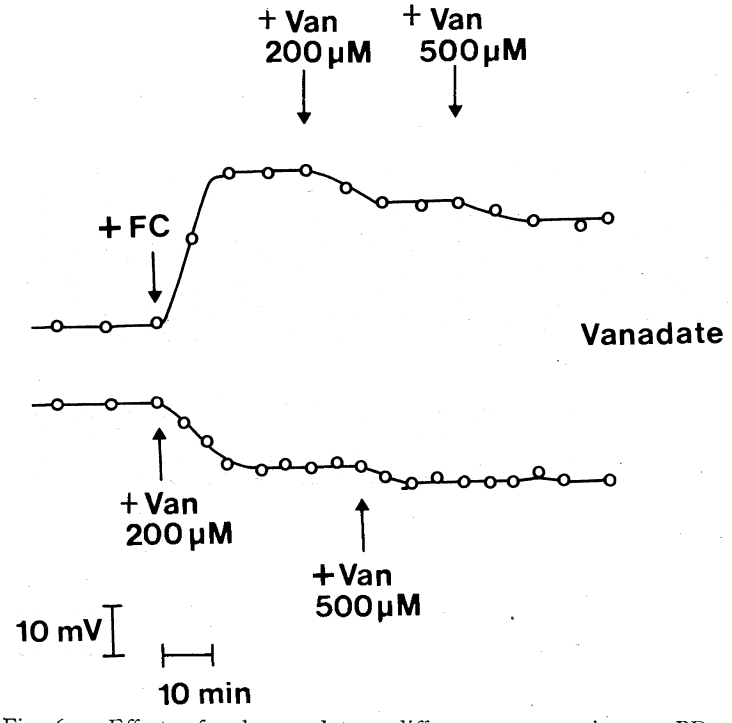

Fig. 6. – Effects of orthovanadate at different concentrations on PD. PD baseline, 98 mV, S.E., 1.3. FC, 10^{-5}M.

Orthovanadate has recently been indicated as a specific inhibitor of plasma membrane ATPase in *Neurospora* [3], and other recent data have also confirmed its action on higher plants [4]. The results in Fig. 6 show that vanadate depresses the basic potential by at least 15 mV at concentrations higher than 200 μM and, although to a slightly lower degree, also the potential induced by FC. Such an effect is therefore in accord with the parallel effect observed on the plasma membrane Mg^{++}–K^{+}-dependent ATPase.

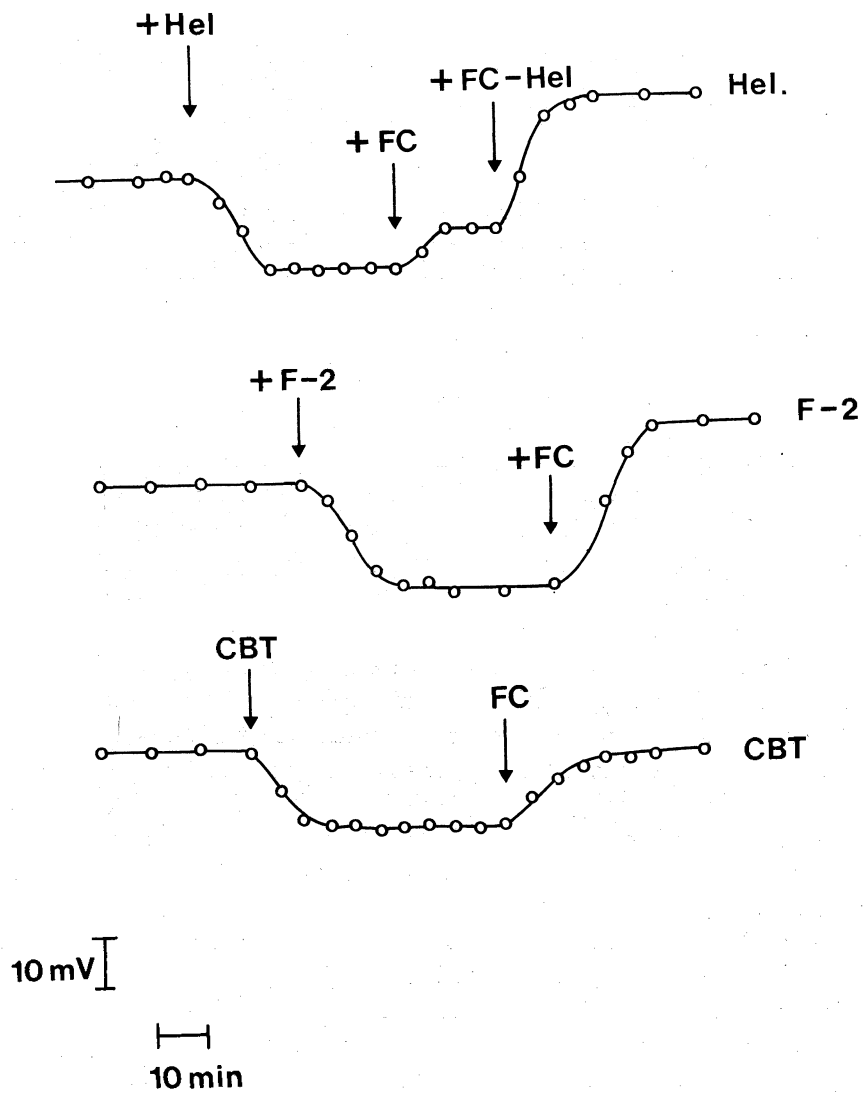

Fig. 7. – Effects of *Helminthosporium maydis* toxin, zearalanone (F–2) and *Cercospora beticola* (CBT) toxin. *Helminthosporium maydis* toxin experiments were carried out with roots obtained from seeds of *Zea mays* cv. W 64A Texas male sterile cytoplasm (PD baseline, 95 mV, S.E. 1.7). For F–2 and CBT experiments, the PD baseline was 98 mN, S.E. 1.1; FC, 10^{-5}M.

D) *Toxins.*

Finally, we have tested the action on PD of some toxins which inhibit ion transport in higher plants [7] and which recent data have indicated to be inhibitors of membrane ATPase (Fig. 7) [17]. The toxin of *Helminthosporium maydis*, in agreement with that reported by Mertz and Arntzen [14], depresses the basic PD. When FC is administered, the potential slightly increases, and removal of the toxin from the media causes the values to return to normal, thus showing a reversible effect of this toxin.

An analogous depression of PD was observed with the other two toxins examined, CBT (produced by *Cercospora beticola*) and zearalanone (F-2) (Fig. 7). These data are in agreement with those reported by other authors [11, 19].

The rapid effect of these substances, together with their effect on plasma membrane ATPase [18], suggests, an action at the plasmalemma level. The increase in potential produced by FC could probably be due to the fact that the inhibition produced by such toxins is only partial.

Conclusions

The data reported seem to confirm the hypothesis already formulated that the transmembrane potential is constituted by a diffusional component which is not influenced by inhibitors and by an active, metabolism-dependent component, which is probably caused by the activity of a membrane ATPase.

None of the treatments carried out resulted in a decrease in PD to below —50 mV, the value which probably constitutes (in the experimental conditions used) the diffusional component of the potential [8]. The physical treatments depressed the active part of the PD as the uncouplers diminished the availability of ATP of the cell. However, the doubt remains that there is a direct action of these treatments on the physical characteristics of the membrane [10]. The depression of PD caused by the sulphydryl reagents, given their rapid action, and the fact that they probably did not penetrate the cells, suggests that membrane proteins with —SH groups necessary for their function contribute to the formation of the potential.

The effect on PD of the inhibitors, which prevalently act on the plasma membrane, Mg^{++}–K^{+}-dependent ATPase, supports the hypothesis that the latter is a determinant factor in electrogenesis [9, 12, 16]. The action on the potential of the substances and the treatments reported in this paper do not allow a single interpretation, since their action is often not sufficiently specific.

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