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Short Communication

Increase in Electrogenic Membrane Potential with Washing of Corn Root Tissue[1]

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WILLY LIN AND J. B. HANSON
Department of Botany, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Washing of corn root tissue increases the electrical potential difference, negative inside, across the membranes of epidermal cells. There is no lag period in the development of the extra potential, and the entire increase is electrogenic as evidenced by collapse with the uncoupler, (*p*-trifluoromethoxy)-carbonyl cyanide-phenylhydrazone.

Proton extrusion by the tissue declines with washing but can be reinstituted by the addition of mersalyl, an inhibitor of the Pi-OH^- antiporter of mitochondrial membranes.

It appears that washing may enhance or augment the activity of an electrogenic ion pump, possibly a proton or cation efflux pump. In addition, there may be augmentation of an anion-OH^- antiporter driven by the proton motive force created.

Macklon and Higinbotham (8) and Pitman *et al.* (10) have reported that aging, or washing, of pea epicotyl and barley root segments is accompanied by an increase in cell potential. In other work, Higinbotham and associates have shown that a portion of the measured potential difference across the cytoplasmic layer of higher plant cells is electrogenic (4). Inhibitors or uncouplers of respiration collapse a significant portion of the cell potential, and this is attributed to failure of the energy supply for a metabolically driven ion or proton pump. There is now considerable evidence for the existence of electrogenic pumps, possibly as proton efflux pumps (3).

As part of an inquiry into the mechanisms underlying the increased rates of solute absorption accompanying the washing of corn root tissue (5, 6), we have now determined the cell potential of epidermal cells of corn root tissue during washing. The entire increase in PD[2] proves to be due to an electrogenic mechanism, the latter being operationally defined as that portion of the PD collapsed by the uncoupler FCCP.

METHODS AND MATERIALS

Two-centimeter segments of primary corn seedling roots [*Zea mays* L., WF9(Tms) × M14] were taken 0.5 to 2.5 cm. from the tip and washed at 30 C in 0.2 mM $CaCl_2$ plus 0.2 mM KH_2PO_4, adjusted to pH 6.0 with KOH, as described previously (5). Identical solutions were used for making cell potential measurements and for measuring pH changes in the medium.

Measurements of PD were made with microcapillary electrodes inserted by micromanipulator essentially by the methods of Etherton and Higinbotham (2) and Pitman *et al.* (10), using a thermostated tissue holder fixed to a microscope stage (S. M. Mertz and C. J. Arntzen, manuscript in preparation). Tissue was washed for varying periods at 30 C and segments were fixed in the holder through which solution at 30 C circulated. Epidermal cells were pierced with microcapillaries containing 3 M KCl, and the potential between the cell contents and the external medium was recorded continuously. When the potential reading stabilized, FCCP was introduced into the circulating medium to give 5 μM final concentration and the PD recording followed (see Fig. 1). For the data of Figure 2, PD determinations were made on a minimum of five cells in each of five root segments before and after adding FCCP.

Determinations of pH were made with a Beckman combination electrode, a Beckman Century SS pH meter and a Heath EU-20B recorder. About 1.5 g of tissue from the washing solution was placed in 200 ml of aerated medium held at 30 C, and changes in pH over 10-min periods recorded. The acid titer of the medium was determined with standard 0.1 N HCl and KOH.

RESULTS

Figure 1 shows PD traces for epidermal cells of fresh and 4-hr washed corn root tissue. Addition of 5 μM FCCP to the irrigation medium (determined to be the minimum concentration for producing maximum collapse) causes a steady decline of PD to a base level which is the same for fresh and washed tissue. The collapse is always more rapid in washed tissue, but the significance of this is obscure. As illustrated for washed tissue, the PD recovers as the uncoupler is washed out. We will refer to that portion of the PD which is reversibly collapsed by uncoupling as the electrogenic PD; the base potential is the diffusion potential (3, 4).

Figure 2 shows a slight, nonsignificant increase in diffusion potential during the course of washing. It appears unlikely that washing has caused marked changes in passive permeability coefficients or salt status. Although not shown here, 0.2 mM KCN is about as effective as FCCP, confirming Higinbotham *et al.* (4).

Figure 2 includes data on rates of phosphate absorption. It can be seen that the increase in electrogenic PD does not exhibit the lag phase characteristic of phosphate absorption

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[2] Abbreviations: PD: potential difference between vacuole and external solution; FCCP, (*p*-trifluoromethoxy)-carbonyl cyanide-phenylhydrazone.

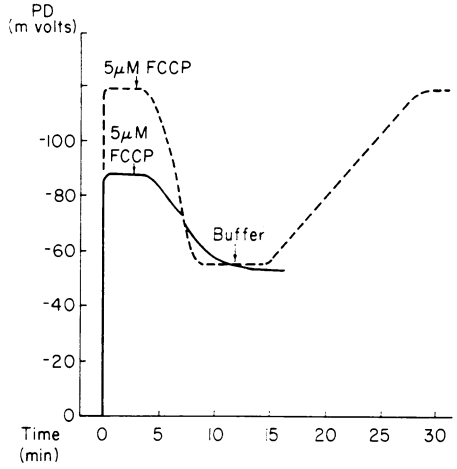

FIG. 1. Partial collapse of corn root cell potential with the uncoupler FCCP. Representative traces from single cells. Fresh tissue (——); 4-hr washed tissue (– – – –). Washing and assay solutions of 0.2 mM $CaCl_2$ plus 0.2 mM KH_2PO_4, pH 6.0. At the point marked "Buffer" fresh solution was started through the tissue holder to wash out the FCCP.

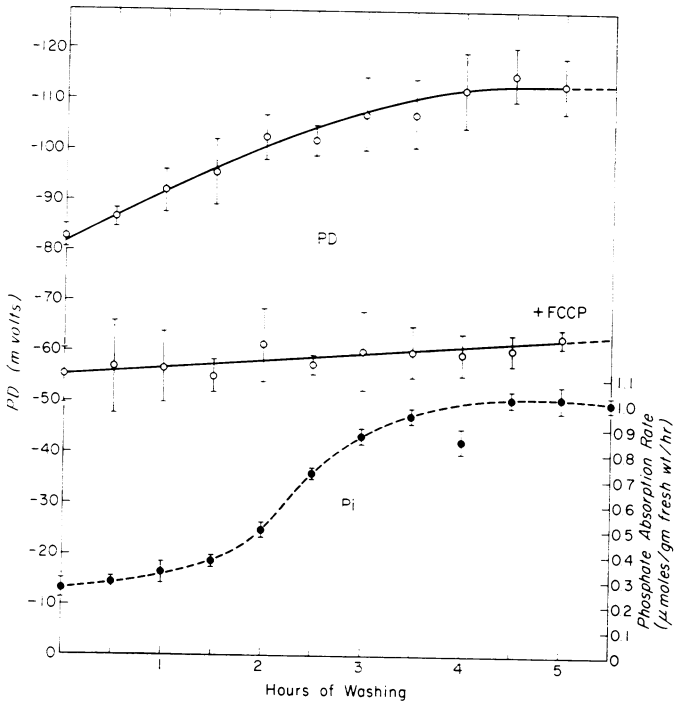

FIG. 2. Time course of increase in cell potential during tissue washing, and the collapse of the electrogenic potential with FCCP. Root segments were removed from the washing medium and determinations made as in Fig. 1. Phosphate absorption rates are from another study (7).

rates. Similarly, there is no lag in the increased PD of pea epicotyl or barley roots (8, 10).

Mitchell's (9) interpretation of uncoupling is that proton conduction by the uncoupler can collapse an electrogenic potential arising from a metabolically driven proton pump (respiratory loops or ATPase). There is a growing belief that proton pumps are the source of the potential for active transport (3, 12). We measured steady state rates of pH change in the medium over 10-min periods during washing. Contrary to what was expected, the net efflux of protons was high with fresh tissue, and fell to a net influx after 4 hr of washing (Fig. 3). The influx of H^+ (or efflux of OH^-) was sensitive to mersalyl (Fig. 4), a mercurial which blocks Pi transport via the Pi^--OH^- antiporter in mitochondria (13) and which will partially block Pi uptake in corn root tissue without lowering ATP levels (7). Mersalyl inhibition is reversible with sulf-

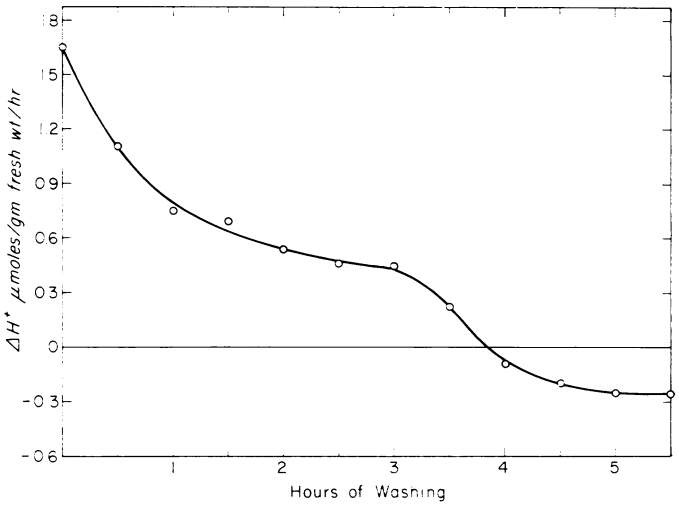

FIG. 3. Decline in rate of net proton efflux from root tissue as a result of tissue washing. See Fig. 4 for examples of ΔH^+ measurement.

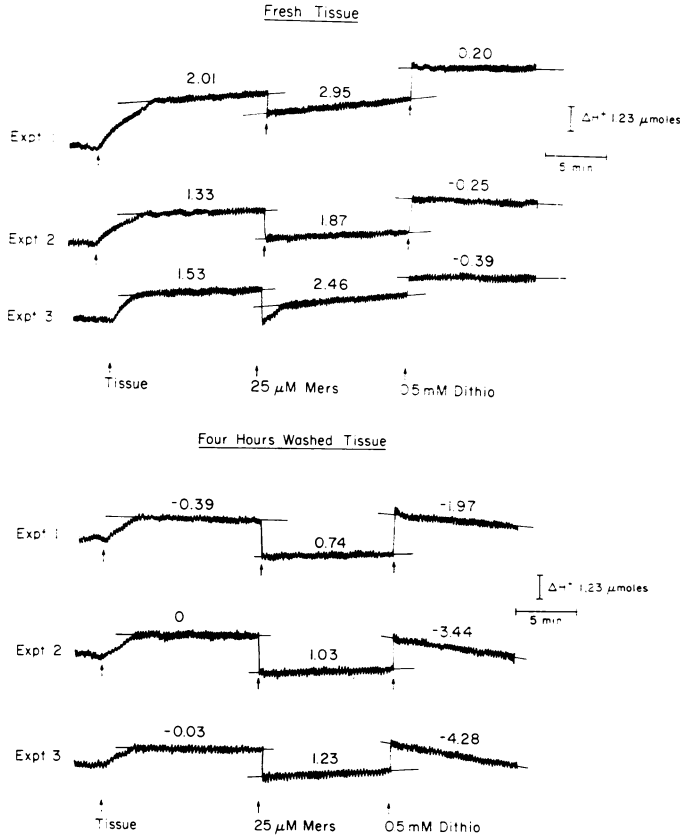

FIG. 4. Increased rates of net proton efflux from root tissue on addition of mersalyl. Mers: mersalyl; Dithio: dithioerythritol. Traces record pH. Upward deflection indicates net proton extrusion. Figures are μmoles H^+/g fr wt·hr.

hydryl protecting reagents (Fig. 4); however, dithioerythritol also induces rapid proton influx in a manner not understood.

We have again determined respiration rates of tissue during washing (5). A slight (10–15%) decline can be detected usually after 4 hr, but it is not statistically significant. The increased electrogenic PD and phosphate absorption rates do not arise from enhanced respiration.

DISCUSSION

The increase in PD with washing (or aging, there is no satisfactory term) is less dramatic in corn than in barley roots (10), but in both the transformation is complete in 4 to 6 hr. We find the entire increase lies in the electrogenic PD, which levels out at the same time as the increase in phosphate absorption. It should be emphasized that the energy-linked absorption rates of a number of solutes are increased by washing, including that of the potassium and chloride ions (5). Hence, the development of an electrogenic PD could be linked to transport processes in general.

But what is the linkage, if any? Because the development of electrogenic PD does not show the pronounced lag typical of ion absorption rates (Fig. 2), it appears that any linkage must be indirect.

An attractive model which accounts for indirect linkage is that of Smith (11), who outlines the history of its development. A metabolically driven proton extrusion pump creates a proton gradient across the plasmalemma, increasing the PD. Anions enter in exchange for OH^- via an antiporter (or exchange carrier) collapsing the proton gradient. Entry of cations, possibly as a counter-current to proton extrusion, partially collapses the electrogenic PD. This model is essentially identical with that for salt transport in the mitochondria (1), and owes much to Mitchell (9) in that two separate systems are involved, a proton pump and an anion-OH^- exchange system.

With this rationale, it might be argued that the primary effect of washing lies with the enhanced activity of a proton pump. Energy for the pump might arise from an ATPase, provided that the membrane-bound system has certain properties (7) and indeed, there is an increase with washing in ATPase of the microsome fraction (6). The problem here is that the increase in ATPase parallels that of phosphate absorption, not that of the electrogenic PD (6; *cf.* Fig. 2). Pitman *et al.* (10) suggested that PD might rise with the removal of a source of hormone, sealing of plasmodesmata, or an increase in the salt concentration in the cytoplasm. We have no data for evaluating these possibilities.

Progressive enhancement of a proton pump during washing should be recognizable in greater rates of acidification of the medium, but the opposite result is found (Fig. 3). However, independent development during washing of increased Pi^--OH^- (or Cl^--OH^-) antiporter activity could accelerate consumption of the proton gradient, giving the result obtained. Assuming that the Pi^--OH^- antiporter of root cell membranes is like that of inner mitochondria membranes (13), the increased net proton efflux on the addition of mersalyl tends to support this concept (Fig. 4). However, the operation of an antiporter depends not only on the proton gradient but also on PD (*i.e.* the proton motive force, or electrochemical gradient of protons, sets the potential with which OH^- exits in exchange for Pi^-). Hence, it is also possible that the mechanisms that develop with washing might increase PD without an increase in the proton gradient; *e.g.*, after washing in a medium which increases K^+ content, the pump might extrude K^+ as well as H^+.

The question of stoichiometry of phosphate uptake as a function of the electrogenic potential is too complex for solution with our limited knowledge of how coupled transport works. For the present, we can only surmise that the mechanisms for creating electrogenic PD are developed independently of those for phosphate transport. If the linkage between the two follows the chemiosmotic model, active transport *per se* lies only in proton-cation efflux pumping; transport of other solutes is coupled transport.

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