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Cell Potentials, Cell Resistance, and Proton Fluxes in Corn Root Tissue

EFFECTS OF DITHIOERYTHRITOL[1]

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ABSTRACT

Studies were made of the effect of dithioerythritol on net proton flux, potassium influx and efflux, cell potential, and cell resistance in fresh and washed corn (*Zea mays* L. WF9XM14) root tissue. Dithioerythritol induces equal proton influx and potassium efflux rates, decreases membrane resistance, and hyperpolarizes the cell potential. Greater effects on H^+ and K^+ fluxes are secured at pH 7 than at pH 5. Other sulfhydryl-protecting reagents produced the same responses. No evidence could be found that dithioerythritol affected energy metabolism or membrane ATPase, and proton influx was induced in the presence of uncoupling agents.

We deduce that dithioerythritol activates a passive H^+/K^+ antiport, driven in these experiments by the outwardly directed electrochemical gradient of K^+. The net effect on H^+ and K^+ fluxes is believed to reside with the combined activity of a polarized H^+/K^+ exchanging ATPase and the passive H^+/K^+ antiport. A model is presented to show how the combined system might produce stable potential differences and K^+ content.

We recently reported on the increase in potential difference across the cytoplasm of epidermal cells (PD)[3] which accompanies the washing of corn root tissue (19). The increase starts upon cutting and submersion, and levels out at about the same time that ion absorption rates reach a maximum. Mertz and Higinbotham (22) point out that increases in PD of this type are not associated with recovery from injury, and we have verified this for corn root tissue (epidermal cells of intact roots have the same PD as cells of freshly excised root sections [18]). The extra potential produced by washing is collapsed by the uncoupler FCCP, which suggests that an augmented electrogenic proton efflux may be responsible for the increase in PD. Uncouplers also inhibit ion absorption by the tissue (16, 20). In barley roots, the simultaneous inhibition of ion absorption and proton release by uncouplers leads Pitman (26) to believe that both processes are linked to oxidative phosphorylation.

However, there is an observation which is superficially inconsistent with the washing-induced augmentation of an electrogenic proton-pumping mechanism as the source of enhanced PD and ion absorption; net proton efflux rates from corn root tissue decline rapidly during washing (19). Several explanations are conceivable. Potassium phosphate is accumulated during washing, and low rates of proton efflux have been observed in barley roots which have accumulated salt (25). Perhaps efflux pumping of K^+ supplants that of protons in K^+-loaded cells; or possibly the energy-linked proton efflux continues, but is masked by the development of ion exchange mechanisms which consume protons, such as H^+/K^+ or OH^-/anion antiporters (23). Active influx pumping of anions is not eliminated as the source of electrogenic PD (10).

With respect to "masking," we found that the water-soluble sulfhydryl-binding mercurial, mersalyl, which blocks phosphate transport in corn mitochondria (7, 9), and partially so in corn roots (20), did in fact increase net proton efflux rates (19) with 40% greater effect on washed tissue. Reversal of the mersalyl inhibition with DTE, however, produced an unexpected result: proton influx was more than recovered, particularly in washed tissue (19). It appeared that if sulfhydryl-sensitive H^+/cation or OH^-/anion antiporters exist, they are at least partially suppressed endogenously.

In more recent experiments, we have attempted to get data which would clarify the rise in electrogenic PD with washing at the same time that net proton efflux declines. Particular attention has been paid to the action of DTE. The energy-linked mechanism which produces the electrogenic PD does not appear to be much altered by DTE, but there is considerable evidence for the existence of a DTE-sensitive H^+/K^+ exchange mechanism driven by K^+ efflux.

MATERIALS AND METHODS

Plant Material. Corn seeds (*Zea mays* L. WF9XM14) were germinated and 0.5- to 2.5-cm sections of primary roots collected and washed as described by Leonard and Hanson (16). In this paper, the term "washed tissue" always refers to root tissue washed for 4 hr at 30 C in the standard medium of 0.2 mM K phosphate (pH 6) plus 0.2 mM $CaCl_2$.

Solutions. Unless otherwise indicated, the above standard medium was used in all experiments. Where pH was modified, K^+ was varied, keeping phosphate constant at 0.2 mM. For pH 4, 0.2 mM KH_2PO_4 was acidified with HCl. DTE (Sigma) was used throughout at the optimum concentration of 0.5 mM. In one series of experiments (see Fig. 9), Na phosphate and K phosphate were used in varying proportions, keeping phosphate at 0.2 mM, pH 6.

Respiration, ATP, and ATPase. Respiration of the root tissue was determined with the O_2 electrode at 30 C on 10 root sections in 10 ml of air-saturated standard medium ±0.5 mM DTE. Comparison with the standard Warburg technique showed no difference in measured O_2 consumption. DTE was introduced after 10 min of control respiration. ATP determinations were made as previously described (20) on tissue preincubated in

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[3] Abbreviations: DTE: dithioerythritol; DTT: dithiothreitol; FCCP: (*p*-trifluoromethoxy)-carbonyl cyanide phenylhydrazone; PD: Electrical potential difference between vacuole and external solution.

standard medium ±0.5 mM DTE for 30 min. Microsomal ATPase activities were assayed as described by Leonard *et al.* (17) for oat roots using the membrane fraction sedimenting between 13,000*g* and 80,000*g* for 30 min, and assaying in a medium containing 3 mM ATP, 33 mM tris-MES buffer, pH 6, 1.5 mM $MgSO_4$, and 50 mM KCl with and without 0.5 mM DTE.

Cell Potentials and Resistance. PD was measured with the apparatus and techniques described in the earlier paper (19). Resistance measurements were made with the single electrode technique and apparatus of Anderson *et al.* (1), using a Tektronix PG 505 pulse generator (2 msec, pulse-period ratio 0.5, pulse height 4 namp, 10 KH_z, 10^9 ohm resistor). Signals were displayed on a Tektronic model 5103 N/D15 storage oscilloscope with 5A 18N dual-trace amplifier and 5B 10N time base amplifier coupled through a high impedance, capacity compensated amplifier designed and built by Dr. J. A. Conner, Department of Biophysics, University of Illinois. Membrane resistance at any time was taken as the means of values found with positive and negative current pulses. Intercellular electrodes with tip resistances of 3 to 10 megaohms were used.

For calculation of E_K, K^+ was extracted by the boiled tissue method of Higinbotham *et al.* (12). Combined extracts were brought to volume and assayed for K^+ with an Orion potassium electrode 92-19, using KCl standards.

Proton Flux. Eighty root segments (about 1.5 g) were placed in 200 ml of aerated standard medium at 30 C with pH adjusted as indicated, and pH was continuously recorded as previously described (19). The acid titer of the medium in the presence of the tissue was determined with standard HCl, and proton flux calculated as μmol H^+/g fresh wt/hr. After experimental additions, new rates of flux were determined for at least 10 min.

K^+ Efflux and Influx. The technique for measuring efflux was adapted from Macklon and Higinbotham (21). Tissue (about 1.5 g) was washed in 400 ml of standard medium containing 20,000 cpm/ml ^{86}Rb for 4 hr at 30 C, rinsed with distilled H_2O for a few seconds to remove adhering solution, and transferred into flasks containing 200 ml aerated unlabeled standard medium ±0.5 mM DTE at 30 C, the pH of which had been adjusted as indicated with the data. Periodically, 10 ml of solution were removed for determination of radioactivity, and were replaced with 10 ml of standard medium of like pH. At the end of 3 hr of exchange, the root segments were blotted dry, weighed, and the remaining radioactivity measured. An efflux curve was plotted from the calculated radioactivity of the tissue at each time interval (see Fig. 6), and the rate of K^+ (^{86}Rb) efflux from the cytoplasm estimated by assuming that the cytoplasmic compartment had equilibrated with the external solution during the 4-hr uptake period.

For influx measurements, tissue washed in unlabeled medium for 4 hr was placed in identical solutions for the efflux studies for 30 min but labeled with 20,000 cpm/ml ^{86}Rb. After a 10-sec rinse in distilled H_2O, the tissue was placed in ice-cold standard medium (pH 6) for exchange, and radioactivity determined (16). Separate determinations showed ^{86}Rb absorption to be linear for over 1 hr, and the absorption is known to be subject to inhibition by uncouplers and inhibitors of respiration (16).

Kinetic analysis of phosphate influx was obtained by varying the K phosphate content of the standard medium, retaining $CaCl_2$ at 0.2 mM and pH at 6. For the kinetics of K^+ absorption, variable KCl was substituted for K phosphate. Ion absorption was determined for 30 min as above, and Lineweaver-Burk plots made by least squares used to determine K_m and V_{max}.

All experiments were verified by repetition, usually several times.

RESULTS

Energy Metabolism and DTE. No effect of 0.5 mM DTE could be found on tissue respiration rate, on the tissue ATP concentration after preincubation for 30 min, or on the Mg-requiring, K-stimulated ATPase of the microsome fraction (*e.g.* −DTE/+DTE values for washed tissue were 23/23 μmol O_2/hr^{-1}·g fresh wt^{-1}; 73/75 nmol ATP·g fresh wt^{-1}; 20.4/19.6 P_i released hr^{-1}/mg microsomal $protein^{-1}$). Under the same conditions as for measuring respiration, it could be readily verified that DTE causes net proton influx (*e.g.* 0.11/3.23 μmol H^+ influx/hr^{-1}·g fresh wt^{-1} in washed tissue). The DTE-induced proton influx continued unabated up to 1 hr.

Cell PD and H^+ Influx. DTE produced hyperpolarization in both fresh and washed tissue (Fig. 1). However, FCCP did not depolarize these tissues to the same basal PD as is the case with FCCP in the absence of DTE (19), or with DNP or cyanide (Fig. 2). Here, we assume that the basal PD which is resistant to uncouplers or inhibitors represents a diffusion potential. The DTE hyperpolarization (Fig. 1) is thus indicated to be due to an increase in diffusion potential, with greater effect on washed tissue (that is, after DTE + FCCP, the diffusion potential of washed tissue is higher). After washing for 4 hr in 0.2 mM K phosphate, the tissue has come to a steady state K^+ concentration of about 50 μmol/g fresh wt (18), and it is possible that the increase in diffusion potential elicited by DTE is due to an increase in P_K, the permeability coefficient for K^+.

If P_K is increased by DTE, cell resistance should be lower, and this proved to be the case (Fig. 3). Unlike the experiments of Figures 1 and 2, the initial equilibration after impalement is shown. The slow establishment of stable resistance after impalement compared to PD is not clearly understood. Anderson *et al.*

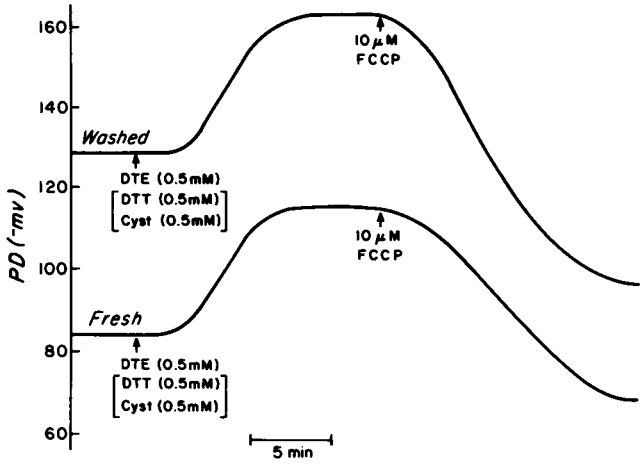

FIG. 1. Hyperpolarization of epidermal cells produced by dithioerythritol. Almost identical hyperpolarization was obtained with dithiothreitol and cysteine. FCCP uncouples the electrogenic potential.

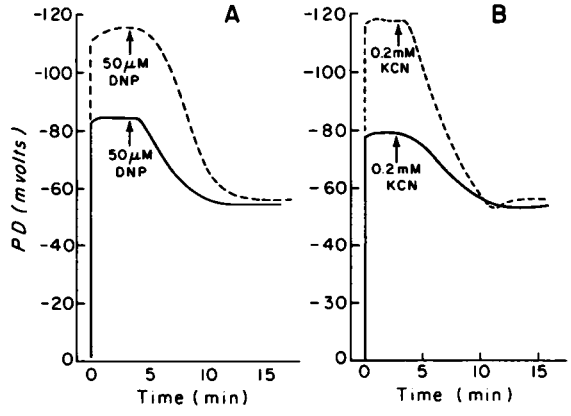

FIG. 2. Collapse of electrogenic cell potential by 2,4-dinitrophenol and potassium cyanide in fresh (——) and washed (- - -) corn root tissue.

(1) suggest that the electrogenic ion current can vary, initially increasing and then falling as the cell membrane heals and resistance rises, giving constant PD. DTE does not appear to affect these processes, but rather to lower resistance independently in association with increased PD. It is of interest that washing does not alter resistance. Hence, increase in electrogenic PD with washing must lie with increased ion current.

The optimum concentration of DTE for causing net proton influx and hyperpolarization is about 0.05 mM (Fig. 4), and this concentration was used throughout these experiments. The close correspondence of the curves in Figure 4 suggests some linkage between proton influx and hyperpolarization due to DTE.

Substitution of dithiothreitol or cysteine for DTE gave essentially the same net proton influx and hyperpolarization (Table I).

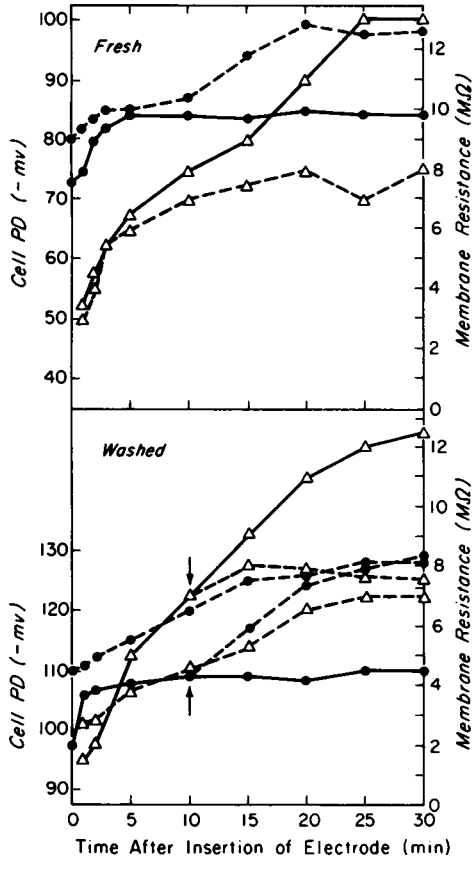

FIG. 3. Cell membrane resistance and PD in epidermal cells of fresh and washed tissue. (△), membrane resistance; (●), membrane potential; (——), control; (- - -), with 0.5 mM DTE; (↑), delayed addition of DTE.

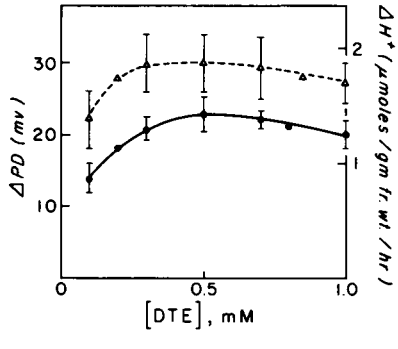

FIG. 4. Effect of DTE concentration on hyperpolarization and proton influx for washed tissue. (——), ΔPD; (- - -), ΔH^+.

Studies were made of pH change on addition of various sugars, including erythritol and glucose, but none were detected, indicating that H^+-cotransport with sugars (30) cannot account for the DTE-induced proton influx (data not shown). The increase in P_K and proton influx with DTE must lie with disulfide reduction or reversal of some endogenous sulfhydryl binding.

pH Effects. In his studies of aged (washed) beet tissue slices, Poole (28) found increasing pH to increase H^+ efflux, K^+ influx, and PD, and it appeared to us that similar experiments would be useful in washing studies on corn root tissue. We found corn root cell PD to be independent of pH from 4 to 7 (Table II). There have been other observations of PD stability with changing pH in higher plant cells (11, 15, 27). The failure of large changes in proton gradient to alter the cell potential is indicative of the conservative and independent controls on PD noted by Jones *et al.* (15). There is evidently some mechanism(s) controlling proton fluxes which stabilizes PD as pH changes. An obvious mechanism would be to exchange protons for equivalent cations (or hydroxyls for anions) equalizing charge transfer. Exchange mechanisms of this type have been proposed for ion absorption by roots (14).

The DTE-activated proton influx was studied between pH 4 and 7 (Table III). Without DTE, the mechanisms involved in H^+

Table I. *Effect of DTE, DTT, and L-Cysteine on Proton Flux and Membrane PD*

Reagents were added at 0.5 mM. Hyperpolarization was determined at steady state (Fig. 1). Net proton fluxes determined from linear rate of pH change (ref. 19 and Fig. 7).

	Control	DTE	DTT	L-cysteine
	μmoles/gm fr wt·hr[1]			
A. Proton Flux				
Fresh tissue	-1.87	-0.45	-0.53	-0.37
Washed tissue	0.05	2.14	2.20	1.98
B. Membrane PD	-mv			
Fresh tissue	85 ± 7	108 ± 5	112 ± 7	105 ± 7
Washed tissue	123 ± 5	150 ± 6	153 ± 6	149 ± 8

[1] negative sign indicates proton efflux.

Table II. *Epidermal Cell PD as a Function of pH*

Standard medium was adjusted to the pH indicated, and after impalement and stabilization at pH 6, medium at other pH was substituted in the irrigation chamber.

pH	4.0	5.0	6.0	7.0
	mV			
Fresh	−75 ± 7	−78 ± 6	−80 ± 4	−77 ± 3
Washed	−117 ± 9	−120 ± 5	−115 ± 7	−121 ± 4

Table III. *Effect of DTE and pH on Net Proton Fluxes in Fresh and Washed Tissue*

Standard medium adjusted to indicated pH was used. Net proton fluxes were determined from linear rates of pH change before and after adding 0.5 mM DTE (see ref. 19 and Fig. 7). Negative sign indicates proton efflux.

pH	4.0[1]	5.0[1]	5.5[2]	6.0[1]	6.5[2]	7.0[1]
	μmoles H+/g fr wt·hr					
Fresh tissue:						
Control	0.96	-1.20	-1.42	-1.98	-3.29	-4.78
DTE	1.01	-0.65	-0.29	-0.27	-1.32	-1.90
ΔH+	0.05	0.55	1.13	1.17	1.97	2.88
Washed Tissue:						
Control	0.61	0.33	0.20	0.09	-2.08	-3.48
DTE	0.63	1.19	1.28	1.51	-0.16	-1.39
ΔH+	0.02	0.86	1.08	1.42	1.92	2.09

[1] mean of 4 experiments
[2] mean of 2 experiments

influx and efflux balance between pH 4 and 5 for fresh tissue (pH changes are difficult to determine reliably below pH 5), and at about pH 6 in washed tissue. Beyond pH 6, there is a dramatic rise in net proton efflux in both fresh and washed tissues. Thus, the maximum effect of washing in depressing net proton efflux is at pH 6.

Proton influx in response to DTE increases with increasing pH (Table III), which is contrary to what one would expect if DTE only opened channels for passive H^+ penetration. There must be a pH-sensitive, sulfhydryl-sensitive transport mechanism for influx of H^+ which is activated by DTE, and which acts in opposition to the normal pH-sensitive H^+ efflux mechanism. As an alternative explanation, DTE could act to inhibit the H^+ efflux mechanism, giving an apparent H^+ influx.

If the DTE-activated H^+ influx mechanism is independent of the H^+ efflux mechanism, the H^+ influx should be charge-compensated; that is, entering H^+ should exchange for a cation (K^+ here) or be accompanied by an anion. On the other hand, if DTE partially blocks an energy-linked H^+/K^+-exchanging enzyme, such as the ATPase visualized for active ion transport in roots (13), there should be lowered K^+ absorption rates corresponding to the inhibition of H^+ efflux.

Table IV shows that the often noted increase in K^+ influx with increasing pH is roughly paralleled by increasing depression of K^+ absorption by DTE. (The 1.5-fold increase in K^+ concentration between pH 4 and 7 will not account for the 70% increase in K^+ absorption rates—see kinetic data below.) However, the inhibition of K^+ absorption by DTE is much smaller than the net proton influx DTE produces (*cf.* washed tissue, Table III). Without DTE, there is little correspondence between net proton efflux rates and K absorption rates except at pH 6.5 and 7. The postulate that DTE inhibits an H^+ efflux system responsible for K^+ uptake is only partially supported at best.

Efflux studies of K^+ (^{86}Rb) accumulated during the washing period prove to be highly complicated due to the changing rate of K^+ loss with time. We used graphic compartment analysis to estimate rates of loss from the cytoplasm through the plasmalemma under the same conditions of pH and DTE as in the experiments of Table III. Since DTE has an immediate effect in causing net proton influx (19), it was believed that if entering H^+ were to exchange, it would be for cytoplasmic K^+. Figure 5 shows an example of the graphic analysis for pH 6, the standard pH for our investigations.

Under control conditions, there is a rapid and steady decline in the K^+ efflux from the cytoplasm as the pH increases (Table IVB). At pH 7, the graphic analysis indicated no K^+ efflux from the cytoplasm (Fig. 6)! We have not yet investigated this phenomenon in detail, but it probably means that efflux through the plasmalemma is rate-limited by a slower component (*e.g.* tonoplast).

Table IV. *Effect of DTE and pH on K^+ Absorption Rates and on K^+ Effluxes in Washed Corn Root Tissue*

Figures 5 and 6 give samples of the graphic analysis for estimating K^+ effluxes from the cytoplasm. Data for K^+ absorption rates are from two experiments, that for K^+ efflux from two (pH 5.5 and 7.0) and four (pH 4.0, 5.0, 6.0, 6.5) experiments. DTE was 0.5 mM. Except for the ^{86}Rb label, conditions are exactly comparable to those for washed tissue, Table III.

	4.0	5.0	5.5	6.0	6.5	7.0
			μmoles $K^+(^{86}Rb)$/g fr wt·hr			
A. K^+ absorption rates						
Control	1.78	2.07	2.23	2.58	2.70	3.02
+DTE	1.75	1.97	1.99	1.97	2.03	2.26
ΔK^+	-0.03	-0.10	-0.24	-0.61	-0.67	-0.76
B. K^+ effluxes			μmoles K^+ (^{86}Rb)/g fr wt·hr			
Control	5.25	4.27	3.58	2.84	1.63	-0.08
+DTE	5.34	5.27	4.68	4.22	3.52	2.05
ΔK^+	0.09	1.00	1.10	1.38	1.89	2.13

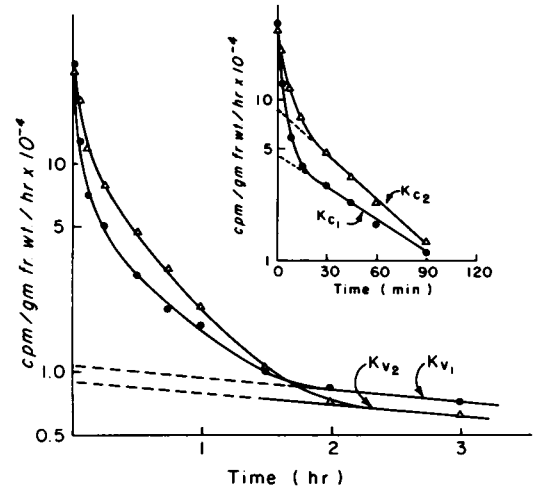

FIG. 5. Example of graphic compartment efflux analysis for washed tissue, pH 6. K_{v_1} and K_{v_2} are estimates of vacuolar efflux rates and K_{c_1} and K_{c_2} are estimates of cytoplasmic efflux rates for control (●) and DTE (△) treatments, respectively.

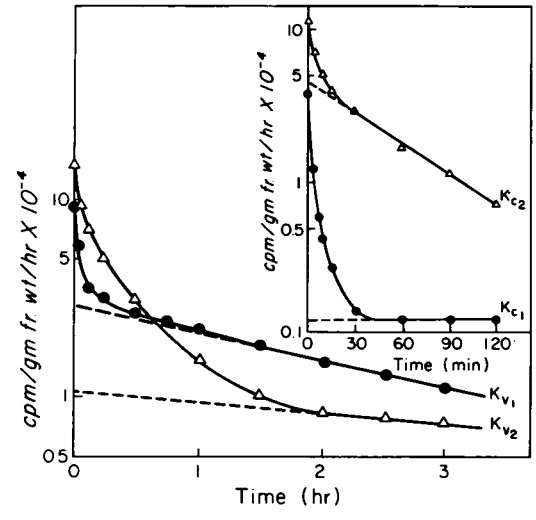

FIG. 6. Example of graphic compartment analysis for washed tissue, pH 7. Symbols as in Figure 5.

DTE produced a pronounced increase in K^+ efflux above pH 4, and there is good correspondence between DTE-activated net proton influx and DTE-activated K^+ efflux (*cf.* ΔH^+ for washed tissue, Table III, and ΔK^+ efflux, Table IVB). Within the limits of the efflux analysis, DTE appears to activate H^+/K^+ exchange across the plasmalemma.

Ion Absorption Rates. Although DTE inhibited K^+ absorption at pH 6, it promoted chloride and phosphate absorption rates by 12 to 25% from 0.2 mM solutions in both fresh and washed tissues (18). DTE increased the uptake of the slowly absorbed sulfate ion by washed tissue more dramatically, from 0.10 to 0.16 μmol/hr^{-1}·g fresh wt^{-1} using 0.2 mM concentrations of K_2SO_4.

Kinetic analysis was made of the effect of DTE on K phosphate uptake over two low concentration ranges, 0.05 to 0.25 mM and 0.1 to 1 mM (Table V). The depressing effect of DTE on K^+ absorption rates at low external K^+ concentration can be ascribed to an increase in apparent K_m; V_{max} is not significantly affected. Similarly, the increase in phosphate absorption from low concentrations caused by DTE can be attributed to a decrease in apparent K_m. As noted before (16), washing had little effect on K_m.

Table V. *Effect of DTE on K^+ and P_i^- Absorption Kinetics*

	K^+ Uptake				P_i^- Uptake			
	Km		Vmax		Km		Vmax	
	Control	DTE	Control	DTE	Control	DTE	Control	DTE
	mM		μmoles/g fr wt·hr		mM		μmoles/g fr wt·hr	
			I. 0.05-0.25 mM Concentration Range					
Fresh	0.028	0.038	0.85	0.86	0.017	0.014	0.25	0.26
4-hr washed	0.022	0.040	3.23	3.04	0.025	0.019	0.87	0.79
			II. 0.1-1.0 mM Concentration Range					
Fresh	0.040	0.044	0.91	0.90	0.209	0.132	0.45	0.41
4-hr washed	0.050	0.070	3.45	3.13	0.201	0.127	1.33	1.25

As a general conclusion, DTE does not have a pronounced effect on the metabolically linked ion absorption processes. There is a small increase in the affinity of the transport mechanism(s) for phosphate, and a small decrease in the affinity for K^+. Since V_{max} is not altered by DTE, it is unlikely that energy linkage of the transport mechanisms is affected.

H^+ and K^+ Transport Agents. If FCCP is added to facilitate H^+ transport across membranes (uncoupling ion transport and the electrogenic potential), subsequent addition of DTE produces an accelerated H^+ influx (Fig. 7). The same result can be obtained by reversing the order of addition.

Valinomycin is a K^+-binding ionophore which has the effect of increasing P_K, the K^+ permeability of the membranes. This is evident in the transient hyperpolarization it produces in cell potential (Fig. 8). Unfortunately, it is also an effective uncoupler of corn mitochondria (7, 9), which probably accounts for the subsequent depolarization which is very like that with FCCP (19), DNP (Fig. 2A), and CN^- (Fig. 2B). In conjunction with DTE, valinomycin produces responses like FCCP (Fig. 7).

It appears that DTE-activated net proton influx is independent of metabolic energy linkage, and that facilitating H^+ and K^+ fluxes serves to augment the DTE-activated H^+ influx.

PD and E_K. A series of PD determinations were made as a function of ion concentration externally, and E_K (the electrochemical equilibrium potential for K^+) was estimated from tissue

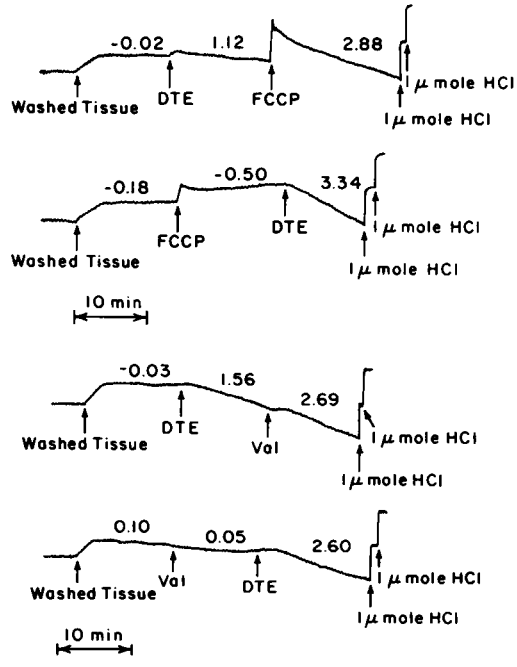

FIG. 7. Acceleration of DTE-induced proton influx in uncoupled tissue. See "Materials and Methods" for procedures. Concentrations: 0.5 mM DTE, 10 μM FCCP, and 1 μg/ml valinomycin.

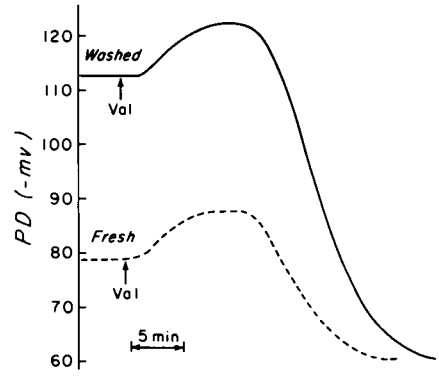

FIG. 8. Hyperpolarization and subsequent depolarization caused by valinomycin. Val = 1 μg/ml valinomycin.

K^+ concentrations (18). One experiment including DTE is shown here (Fig. 9). Phosphate was held constant at 0.2 mM, pH 6, with variation in Na^+ and K^+ as cation. Sodium produces a higher PD than does equivalent K^+ (18), but this has no effect on the hyperpolarization produced by DTE unless the tissue is uncoupled (Fig. 9). The relevant point for this study is that E_K, with or without DTE, is higher than PD at 0.2 mM K phosphate. This signifies active accumulation of K^+, and indicates a favorable electrochemical gradient for K^+ efflux as the resistance is lowered by DTE.

Mersalyl. As previously reported (19), mersalyl increases net H^+ efflux, presumably by binding to sulfhydryl groups and blocking the phosphate transport enzyme. It thus produces a result qualitatively—but not quantitatively—opposite to DTE. A study of the effect of mersalyl on PD was made.

The result was unexpected: mersalyl was almost as effective as FCCP in depolarizing the cell potential, and hence, by the same criterion, must be collapsing the electrogenic potential (Fig. 10). Although we have not yet examined the question in detail, the results indicate that there are sulfhydryl-sensitive enzymes associated with the electrogenic transport mechanism. ATP levels remain high (20). Simply adding a sulfhydryl-binding agent is not, therefore, a sufficient means of studying the DTE-activated H^+/K^+-exchanging enzyme; other enzymes which are already activated are affected.

DISCUSSION

The action of DTE in causing net proton influx is primarily on a passive mechanism. The mechanism is pH- and sulfhydryl-sensitive, and produces equivalent K^+ efflux from the cytoplasm (*cf.* ΔH^+ and ΔK^+, Tables III and IVB). DTE-activated proton influx is not blocked by uncoupling—rather, it is accelerated (Fig. 7). The inhibition by DTE of the metabolically driven K^+ influx is relatively small compared to the DTE-activated K^+ efflux (Table IV). Kinetic analysis shows no significant effect of

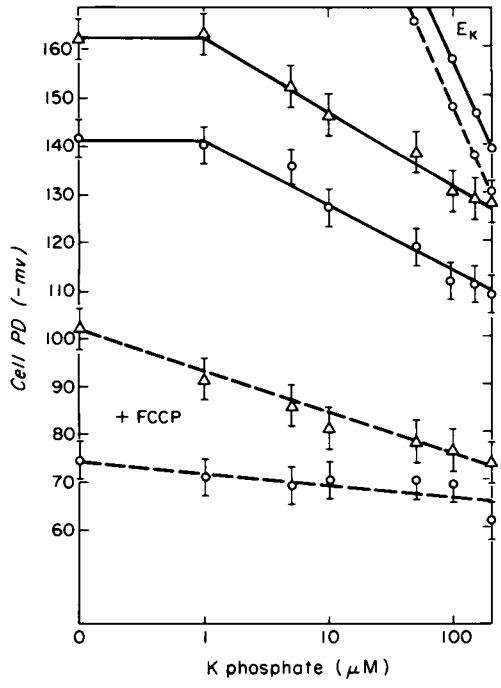

FIG. 9. Cell PD of washed tissue as a function of Na^+ to K^+ ratio and DTE. Phosphate concentration was constant at 0.2 mM, with substitution of K^+ for Na^+ as indicated. (△), plus 0.5 mM DTE; (●), control; (---), 10 μM FCCP present. E_K was calculated from K^+ content and displayed as Nernstian regressions for control and uncoupled tissue.

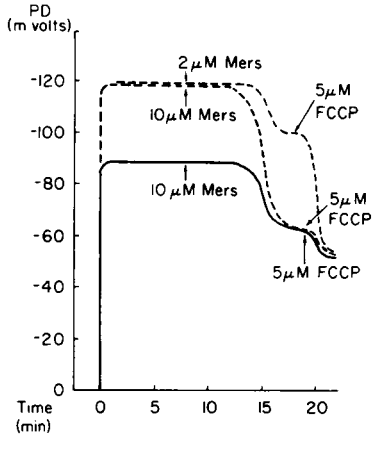

FIG. 10. Depolarization of cell PD with mersalyl. (——), fresh tissue; (---), washed tissue.

DTE on V_{max} (Table V). We can find no effect of DTE on energy metabolism.

The DTE-activated influx increases as the pH increases (Table III) and, assuming that the cytoplasm is about neutral, this means that the influx increases as ΔH^+ across the plasmalemma approaches zero and the electrochemical potential difference declines (PD is constant, Table II). Something else must be driving the H^+ influx, and this is indicated to be the DTE-activated K^+ efflux. DTE produces hyperpolarization of the diffusion potential (Fig. 1) and lowers membrane resistance (Fig. 3), which are characteristics of increasing P_K. Hence, DTE must act to increase plasmalemma permeability to K^+, and the increased efflux of K^+ down the cytoplasm to solution concentration gradient is somehow coupled to H^+ influx. Determinations of E_K show that an electrochemical potential is available for driving K^+ efflux (Fig. 9).

The exchange of H^+ for K^+ need not be directly coupled in the sense that H^+ enters the cell through the same DTE-sensitive enzyme responsible for K^+ efflux. It is conceivable that the hyperpolarization from increasing P_K is adequate to overcome the resistance to H^+ entry at another site. Thus, at a certain PD, the entry of H^+ would equal the exit of K^+, and the PD would stabilize at the one-for-one K^+/H^+ exchange indicated by the gross analyses. However, as a working hypothesis, we prefer the concept of a cation/H^+-exchanging enzyme, or antiport (23), with the qualification that one-for-one exchange is not obligatory to enzyme action. Increases in P_K by valinomycin should satisfy the requirements of the system postulated above, but it is obvious that DTE has a separate and distinct effect in promoting H^+ entry (Fig. 7). The action of uncouplers and ionophores in accelerating fluxes (Fig. 7) can best be explained as due to lowering the resistance of a lipid barrier to the H^+/K^+-exchanging enzyme, as done for corn mitochondria (9).

Under normal conditions of pH and ion concentration, the H^+/K^+ antiporter is not fully operative. The rapid efflux of K^+ at low pH (Control, Table IVB) suggests that an H^+ gradient from solution to cytoplasm may make it operative, but since there is no way of tracing H^+ influx discretely, this must remain a supposition. At higher pH, the antiporter must have only partial activity until treated with DTE or other sulfhydryl-protecting reagents. In principle, the antiporter should be able to function in K^+ influx/H^+ efflux as well as in the opposite direction, but this would require appropriate electrochemical gradients: that is, K^+ directed inward and/or H^+ directed outward. We have not yet made the appropriate investigations at high K^+ concentration and pH.

These investigations do not provide an explanation of the decline in net proton efflux or rise in electrogenic PD with washing. Washing does not alter membrane resistance (Fig. 3). With the exception of the unphysiologically low pH of 4, washing decreases net proton efflux at all pH tested (Table III). There is no obvious correspondence between net proton flux in washed tissue and the influx or efflux of K^+ (Control, Tables III and IV).

Perhaps this is because more than one system for transporting H^+ and K^+ exists. Metabolically driven proton efflux pumps are widely believed to exist, and are sometimes ascribed a primary role in the electrogenic PD (see references 10, 13, 28, 29, 31). Cation-sensitive ATPases could be the energy source (13). Hence, net proton fluxes as measured here probably represent the sum of the passive influxes from H^+/cation and OH^-/anion antiporters, and the electrogenic H^+ efflux driven by a plasmalemma ATPase. The basis for the rise in electrogenic PD and the decline in net proton efflux with washing must then lie with the sum of active and passive proton fluxes.

We have taken present evidence to create a tentative model of the proton fluxes in corn root cells (Fig. 11). It differs from the

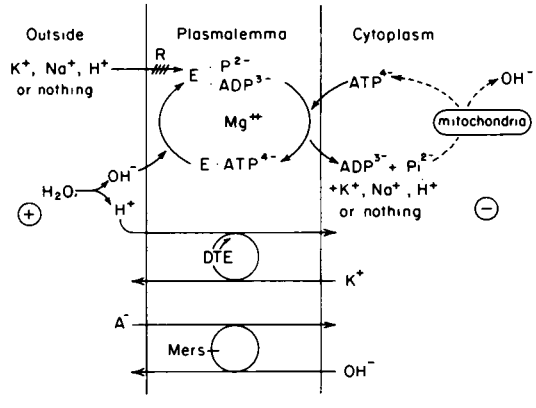

FIG. 11. Model of active and passive H^+/K^+ exchanging systems involved in creating a stable electrogenic potential.

previous model (20) in that it incorporates the DTE-activated H^+/K^+ antiport and adds detail to the functioning of the presumed ATPase which allows for the creation of a stable electrogenic PD.

We assume that the ATPase acts only to hydrolyze the terminal phosphate of ATP, and thus, the only way to achieve a proton gradient is to have a polarized alkaline hydrolysis (or its equivalent). This is accomplished by having the ATPase (indicated as E) bind ATP furnished from the cytoplasm. Bound adenine nucleotides are known for animal mitochondrial ATPase (8) and they may function in ATP formation (3); our laboratory has suggested a role for them in corn mitochondrial ATPase (2). Hydrolysis in the plasmalemma is from the solution side, producing bound ADP and P_i, and releasing H^+ to the solution (Fig. 11). The extra negative charge produces electrostatic binding and transport of a monovalent cation, with the net effectiveness conditioned by (*a*) coulombic forces; (*b*) the affinity of E·AdN for the cation; and (*c*) the nature of the lipid barrier governing access to E·AdN.

For an electrical potential to develop, the lipid barrier must impose a finite resistance to cation penetration. Thus, under conditions of low (or no) membrane potential, the hydrolysis can proceed through the cycle transporting no cation, yielding an electrical potential, negative inside. As the potential rises, however, there will be electrophoretic pull on the external cations, overcoming resistance to entry and producing influx transport. The actual PD established will reflect the resistance of the lipid barrier and the properties of the cation in penetrating it. The higher PD shown by Na^+ compared to K^+ (Fig. 9) can be explained by greater ionic hydration producing greater penetration resistance. It will be noticed that the model incorporates a high degree of PD stability: if PD falls, electrophoretic pull on the cation declines, and so does transport until PD is re-established. Of interest here is the observation that the only additive to the standard washing medium so far discovered which will significantly increase the washing-induced ion uptake is 1 μM linoleic acid (24; W. Lin, unpublished). With phospholipid membranes, increased fatty acid unsaturation increases permeability, apparently the result of increased surface area per phospholipid molecule and less effective sterol interaction with the acyl chains (4).

As indicated in Figure 11, we speculate that under conditions of low pH and high PD, H^+ may also be transported inward by the ATPase, effectively producing a neutral hydrolysis of ATP. This will help to explain the diminished net proton efflux and K^+ uptake as pH declines (Tables III and IV).

Continuous activity of the postulated ATPase would be inimical to ionic balance. For example, continued uptake of K^+ will occur as long as the ATPase remains functional and ATP is supplied, and experiments with corn root tissue suggest that these two conditions are met during washing (16, 20). However, we also know that after 4 hr of washing in 0.2 mM K phosphate, the K^+ content of the tissue has essentially reached a steady state at about 50 μmol/g fresh wt (18). Glass (5) has suggested that repression of carrier synthesis or allosteric inhibition can account for low rates of K^+ absorption into high K^+ roots. We have no evidence bearing on this point. We do suggest that the H^+/K^+ antiporter could play a role here by effectively reversing the active H^+/K^+ exchange of the ATPase. Rapid loss of $K^+(^{86}Rb)$ taken into the cytoplasm would ostensibly appear to be an inhibition of uptake. As noted above, the antiporter must normally be partially inhibited; indeed, it is this property that enables us to detect its presence with DTE. However, it may be activated by high internal K^+ concentrations.

For completeness in depicting avenues of proton flux, Figure 11 includes an OH^-/anion antiporter similar to that visualized for phosphate uptake by mitochondria, but with some reservations, since mersalyl is not very effective as an inhibitor of phosphate accumulation by corn root tissue (20). The principal point we wish to make in Figure 11 is that at least two systems, one active and one passive, may exist for H^+/K^+ transport, and that they may cooperate in maintaining K^+ balance without altering the PD and without direct involvement of the anion transport system.

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