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Natural H^+ Currents Traverse Growing Roots and Root Hairs of Barley (*Hordeum vulgare* L.)[1]

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ABSTRACT

With the aid of an extracellular vibrating electrode, natural electric fields were detected and measured in the medium near growing roots and root hairs of barley seedlings. An exploration of these fields indicates that both the root as a whole, as well as individual root hairs, drive large steady currents through themselves. Current consistently enters both the main elongation zone of the root as well as the growing tips of elongating root hairs; it leaves the surface of the root beneath the root hairs. These currents enter with a density of about 2 microamperes per square centimeter, leave with a density of about 0.5 to 1 microampere per square centimeter, and total about 30 nanoamperes.

Responses of the natural fields to changes in the ionic composition of the medium as well as observations of the pH pattern in the medium near the roots (made with bromocresol purple) together indicate that much of the current consists of hydrogen ions. Altogether, H^+ ions seem to leak into growing cells or cell parts and to be pumped out of nongrowing ones.

Natural electric currents seem to play a major role in the differentiation and growth of cells and tissues (8). For example, in the zygotes of the fucoid alga *Pelvetia* (9, 17, 23) and in the pollen grains of *Lilium longiflorum* (6, 32, 33), steady self-generated currents of about 1 μamp cm^{-2} (in zygotes) and up to 4 μamp cm^{-2} (in pollen grains) enter the sites of future or actual growth and leave the opposite, nongrowing parts of the cells. The ions that carry these natural currents are Na^+, K^+, Ca^{2+}, and Cl^- in *Pelvetia*, and K^+, Ca^{2+}, and H^+ in *Lilium*.

If natural currents are an essential factor that controls cell differentiation and growth, currents should traverse other developing cells and tissues, too. To test this conclusion we have investigated the growing root hair and the root. Root hairs and roots were selected for three main reasons: (*a*) Root hairs take up water and salts from the soil and are therefore of great importance for the mineral nutrition of plants, a fact that calls for a better understanding of their development and growth. (*b*) Root hairs grow at their very tips; they therefore seem to need some mechanism to control this very localized growth, perhaps a self-generated current. (*c*) There are some older reports on natural electric fields around growing roots (25). We thought that it would be valuable to reinvestigate these natural electric fields with our small vibrating electrode and to link the fields to the flow of particular ions.

Roots sprout from the seeds of barley within 2 days after wetting; grow fairly rapidly, straight, and in low salt media of simple composition. This latter property is an advantage for determining the ionic species responsible for the electric currents. Barley roots have been widely used for studies of ion absorption (10, 20). A considerable literature therefore exists about their properties with respect to salt uptake. Moreover, phytochrome-induced changes in their surface charge have been reported (30). This fact makes the barley root an object of interest to photobiologists. The results from investigations of natural currents might provide clues as to the mechanism of action of phytochrome.

MATERIALS AND METHODS

Growth Conditions. Ten to 15 barley seeds were sown in dishes on three layers of filter paper, wetted with (APW),[3] a medium containing 1.0 mM NaCl, 0.1 mM KCl, and 0.1 mM $CaCl_2$, at pH 5.2 to 5.6. The dishes were kept in darkness in a temperature-controlled room at 20 C. On the 3rd day after sowing, most of the seeds had grown three to five roots of 10- to 30-mm length and a short coleoptile.

Root Morphology. Young barley roots showed four different regions (see Figs. 1 and 2): a clearly discernible cap of about 0.3-mm length, protecting the meristematic tissue; a meristematic tissue with a length of about 0.5 mm; a main elongation zone which ended with the beginning of the root hair zone about 2.3 mm behind the tip; and a root hair zone that extended almost up to the seed. In the elongation zone and at the beginning of the root hair zone the diameter of a typical barley root was about 0.45 mm or 450 μm. Root hairs had a diameter of 10 to 12 μm with the exception of a short wider basal part. They grew to a length of 400 to 600 μm under our growth conditions.

Measurements of Natural Currents. One or two seedlings with straight roots of 15- to 25-mm length were placed in small dishes and fastened by covering the grain with a few drops of a 40 C solution of 2% agar in distilled water. After the agar had gelled the dish was filled with the experimental air-saturated medium to cover the roots with a layer of medium about 2 mm deep. Measurements near the growing roots began 1 to 2 h after fastening. This delay was necessary because root hairs (particularly older ones) stopped growing as a result of handling and/or submersion, but resumed growth after about 1 h. During all measurements the roots were horizontal and observed from below with an inverted microscope.

The magnitude and pattern of natural currents through growing root hairs and roots were investigated by measuring the electric fields caused by passage of these currents through the external medium. To measure these fields, a highly sensitive vibrating electrode was employed (7). This electrode consisted of a metal-filled micropipette with a platinum-black tip of 20 μm in diameter and a concentric reference electrode a few mm behind the tip. The

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[3] Abbreviations: APW: artificial pond water; SEM: standard error of the mean (all errors are given as SEM).

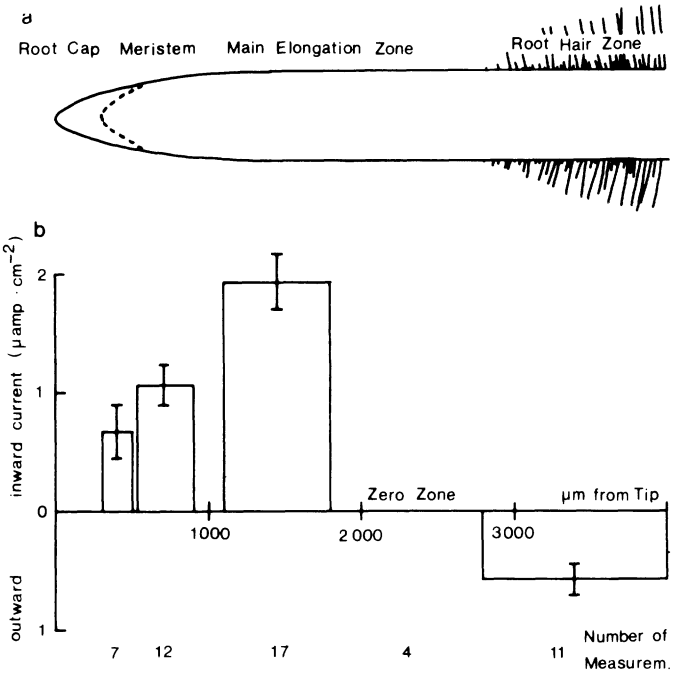

FIG. 1. Pattern and density of the natural current traversing barley roots growing horizontally in the low salt medium APW or in 0.5 mM $CaCl_2$ (pH 5.2–5.7). a: Schematic representation of the morphology of an average barley root. b: Average current densities (±SEM) entering or leaving normal to the root surface at different sites along the root. These current densities were calculated according to equation 1 from measurements at one to two root radii off the surface. The width of the bars represents the spread of the measuring positions. "Zero zone" means that in this zone almost all of the current is flowing parallel to the surface. The roots grew at an average rate of 5.1 ± 0.7 μm min^{-1} during measurements. The scale given for distance in (b) is also valid for (a).

electrode was vibrated horizontally with a frequency of about 400 Hz and an amplitude of 30 μm. This vibration transformed any DC voltage between the end positions of the electrode into a sinusoidal AC voltage whose amplitude was measured with the aid of a lock-in amplifier tuned to the vibration frequency and set to a time constant of 1 s. The current density at the site of the vibrating tip was then calculated by multiplying the magnitude of the electric field by the conductivity of the medium.

During measurements the vibrating electrode was moved to various positions along the root hair or along the root. The electric field at each position was repeatedly recorded for 0.5 to several min. For measuremets on root hairs the vibrating electrode was alternatingly placed in front of and behind the tip, so that the center of vibration lay 30 μm in front of or 30 μm behind the tip (see Fig. 3b). Special care was taken to vibrate the probe parallel to the axis of the root hair. As a reference position we chose one 2 to 3 mm away from the surface of the root. No changes in growth of the root hairs were observed when the electrode was vibrated as close as a few μm from the tip as long as the electrode did not touch the tip. To investigate natural currents of the root as a whole the vibrating electrode was placed on either side of, and roughly in the midplane of the root. In these positions the electrode was vibrated either normal or parallel to the surface, or consecutively in both directions. All measuring positions, except those at the cap, lay within two root radii of the root surface. As with the measurements on root hairs the reference position was 2 to 3 mm away from the surface.

To estimate the current density traversing the surface of the growing root, the root was considered to be a cylindrical current source or current sink. This assumption was verified in several experiments where the decline of the field with distance from the root was studied in the elongation zone. Within a distance of two to three radii from the surface the field strength was found to decrease inversely with the distance. The current density (i_s) at the surface is therefore given by the equation:

$$i_s = i\left(\frac{r'}{a'}\right) \quad (1)$$

where a′ is the radius of the root at the site of measurement and r′ is the distance of the measuring position from the center of the root.

From the difference in measurements of the electric field just in front of and just behind the tip of growing root hairs the current density entering the tip could be estimated. For this estimate we assumed that the tip of the root hair is a small current sink with a diameter of 10 to 20 μm. The current density at the surface of the root hair (i_s) can then be estimated from the equation:

$$i_s = i\left(\frac{r}{a}\right)^2 \simeq 10\,i \quad (2)$$

where i is the current density at the site of measurement, r is the distance of the measuring position from the center of the sink, and a is the radius of the sink. The assumption of an isolated sink seems justified by our measurements which show that current enters only at the tip and leaves the surface of the root.

Ionic Composition of Currents. To find the ions that were involved in the natural currents, the response of the electric field to a change in the bathing medium was recorded. During these experiments the medium of the dish was exchanged within 1 to 2 min for a medium which differed in the concentration of only one ion species at a time. With this method the effects of changes in H^+, K^+, Ca^{2+}, and Cl^- were investigated.

Besides these electrophysiological measurements we tried to demonstrate the participation of H^+ with pH indicators. Two- to 3-day-old seedlings were placed on the surface of 3-mm-thick plates that were prepared from APW, 0.5 or 1.0% agar, and 0.4 g l^{-1} (0.71 mM) bromocresol purple, adjusted to pH 5.0 with 0.1 N HCl. Bromocresol purple appears yellow at pH 5.2 and becomes purple at pH 6.8 (3). At the concentration employed, the pH indicator bromocresol purple had no harmful effects on the growing roots. Other pH indicators like methyl red and bromothymol blue were found to inhibit growth in barley roots.

During most experiments with bromocresol purple the barley roots grew horizontally and were illuminated with 300 lux white fluorescent light from above and with 1,000 lux from below. In some experiments the plates were oriented vertically. In some experiments they were placed in the dark. The pH-bands along the roots were recorded on color film (Agfachrom professional 50L) and the length and location of the bands were measured on the projected images of these slides.

RESULTS

Natural Current through Growing Roots. Barley roots grew with rates of 1 to 20 μm min^{-1}. In roots submerged in media the growth rate was always smaller than in roots growing on the surface of moist filter paper or agar medium. Good growth of the roots was observed in media of pH 5 to 7.5.

Growing barley roots were found to be surrounded by natural electric fields. From the pattern of these fields three zones with respect to the direction of current could be distinguished (Fig. 1). (*a*) A zone with strong inward current: this zone begins at the meristematic tissue and ends near the root hair zone. The strongest inward current was always found to enter the middle of the elongation zone. (In a few roots small patches with an inward current were also found within the root hair zone.) (*b*) A zone with outward current; this zone begins with the root hair zone and probably extends up to the seed in young seedlings. (*c*) A zone which had either inward or outward current; this zone is the root

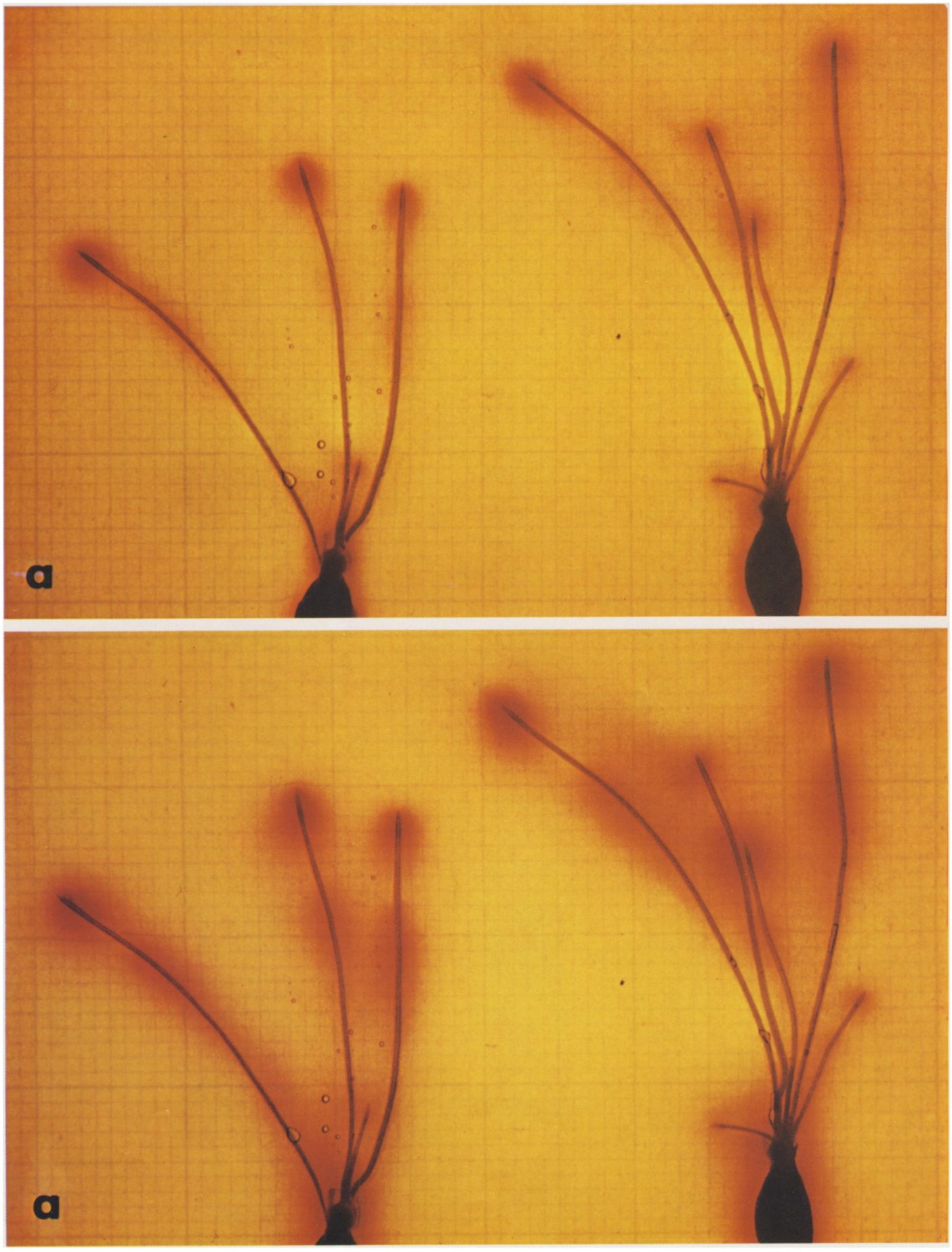

FIG. 2a

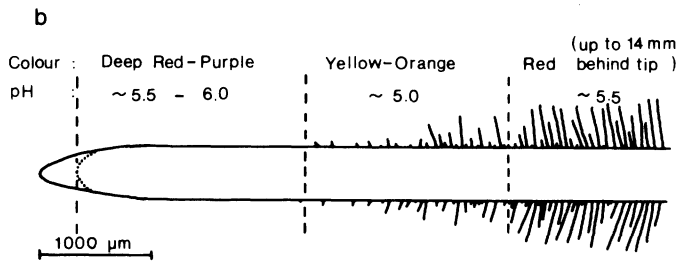

FIG. 2. Representative examples and the average pattern of pH bands along barley roots growing on the surface of 3-mm-thick agar medium containing the pH indicator bromocresol purple. The medium was made up of APW + 0.5% agar + dye, titrated to pH 5.0. In (a), the photograph on top shows two 3-day-old seedlings after 10 min on the plates; the photograph at the bottom shows the same seedlings 80 min later. The small squares of the background grids are 1 × 1 mm. (The bottom grid was accidentally shifted upward as may be seen from the positions of the small air bubbles trapped in the agar medium.)

In (b) the results from 51 roots are compiled to show the average length and location of the different pH bands, their typical color appearance in translucent light and their approximate pH as suggested by the color and coarse pH measurements. The roots grew at an average rate of 17.3 ± 1.7 µm min^{-1} on the plates.

cap. In five roots we found current entering the cap, in nine roots we saw current leaving the cap. At present we do not know if a cap can have first an outward, then an inward current, or vice versa, or only one type of current all of the time. Since we observed outward current mainly in freshly submerged roots, a change from outward to inward current with time seems possible.

These zones were found on both sides of the horizontally growing root. (Measurements above and below the root could not be made with our present measuring set-up.) Slight shifts of the sites with strongest inward, or outward current, which were respectively closer to or farther away from the root apex, were observed during measurements. After fixation of the roots in glutaraldehyde all currents had disappeared.

The density of the inward current at the surface of the elongation zone was about 1 to 3 µamp cm^{-2}; that of the outward current at the beginning of the root hair zone, about 0.5 to 1 µamp cm^{-2}. At every measuring position the current densities were not very stable but fluctuated by a few tenths of a µamp cm^{-2} within 0.5 to 1 h.

Information about the ions involved in the natural current traversing growing roots was obtained by changing the ionic composition of the medium. When the medium was replaced by a medium containing either no Cl^- or no Ca^{2+}, or when the roots were submerged in a medium containing no K^+ and Na^+ but only $CaCl_2$ no significant differences in the current densities were found compared with APW. However, increasing the pH of the medium seemed to cause a small but rapid change of the current density (and in some cases also a substantial shift of the main site of inward current toward the root apex). In 10 roots bathed in a medium of pH 5.5 the inward current at the surface of the elongation zone was 2.6 ± 0.4 µamp cm^{-2}. Increasing the pH to 7.1 decreased the inward current to 1.9 ± 0.3 µamp cm^{-2} within a few minutes. The average growth rate of these roots did not change significantly, going from 5.3 ± 1.2 µm min^{-1} at pH 5.5 to 4.6 ± 1.2 µm min^{-1} at pH 7.1. In contrast to the inward current, the outward current in the root hair zone did not respond to the pH change; in the three roots tested it remained at 0.9 µamp cm^{-2}.

The involvement of H^+ ions in the root current, as suggested by the electrical measurements, was further tested with pH indicators. Barley roots growing on the surface of an agar medium containing bromocresol purple generated clearly visible bands in the medium (Fig. 2). Within a few minutes after placing the seedlings on the surface of this medium the vicinity of the elongation zone became red to purple, *i.e.* the pH of the medium increased. Coarse measurements with a micro-pH electrode (Microelectrodes Inc., Londonderry, New Hampshire, type MI-410) showed a pH increase of about 0.5 pH units. In some cases the medium around the root caps appeared less alkaline. The vicinity of the first 2 mm of the root hair zone always remained relatively yellow, indicating that this region was more acidic than the elongation zone. The remainder of the root hair zone turned reddish within 0.5 to 2 h of growth. This indicated a slow increase of the pH of the medium in this region. These bands of different pH moved along the surface of the medium with the growing roots. The same pattern of pH bands developed along roots growing on vertically oriented plates. The same pattern also developed along roots growing on a medium made up of only distilled water, bromocresol purple, and dialyzed agar.

The results with bromocresol purple support the suggestion drawn from the electrical measurements that an important component of the natural currents in growing roots consists of H^+ ions. Furthermore they indicate that these currents are neither an artifact due to the horizontal position of the root nor an artifact due to submersion.

Natural Current through Growing Root Hairs. In APW, root hairs grew at a rate of 0.4 to 1.2 µm min^{-1}. Young root hairs (20–50 µm long) grew less rapidly than root hairs of medium length (200–400 µm); in still longer root hairs (>500 µm) the growth rate again declined.

Figure 3a shows a representative recording of the current densities near a growing root hair. This recording shows that current (*i.e.* a flow of positive ions) leaves the surface of the root. This outward current was always strongest close to the root hair and at the surface of the root. But current was also seen flowing from the surface of the root at some distance from any root hairs. It therefore seems likely that one source of the outward current is the basal parts of each root hair, but that other cells also give off current. The example shows further that in front of the growing root hair the outward current is greatly reduced or even reversed. This decrease in outward current is far larger than would be expected from the increase in distance to the root surface between measuring positions 2 and 1. In other cases, when the "comparison" position 2 of the electrode was not behind the tip, as in Figure 3a, but in front of and 50 to 100 µm to either side of the tip, significantly less outward current was also recorded in front of the tip. Figure 3b tries to illustrate the general pattern of the natural current traversing growing root hairs as implied by measurements of about 100 different root hairs.

However, the outward current from the root was not greatly reduced or reversed in front of three anomalous root hairs. In all of these three cases growth measurements showed that the growth of the root hairs had ceased. In some other cases both the inward current and growth stopped when a root hair's tip was touched deliberately with the vibrating electrode.

From the differences in outward current in front of and behind the tip, the current entering growing root hairs was calculated according to equation 2. In our standard medium, APW (pH 5.2–5.6) the average inward current was 2.0 ± 0.2 µamp cm^{-2}. The root hairs investigated were between 80 and 520 µm long and grew with an average rate of 0.86 ± 0.09 µm min^{-1} during measure-

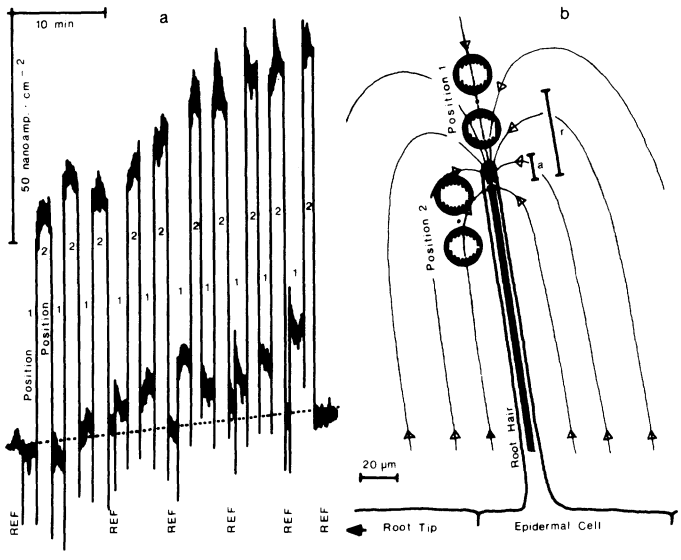

FIG. 3. Natural current through growing root hairs. a: Representative example of recordings of electric fields near growing root hairs. An electrode with a tip of 20 μm in diameter was vibrated with 30-μm amplitude in front of (position 1) or behind the tip (position 2) or at a distance of 2.5 mm from the root surface (position REF). An upward deflection of the recorder indicates current leaving the surface of the root. b: Scale drawings of a short root hair, the vibrating electrode, its typical measuring positions, and the current pattern as suggested by the results of field measurements. a and r are the distances referred to in equation 2.

ments. Similar current densities as in APW were measured in two other media, namely 0.6 mM $CaCl_2$ (pH 5.3–5.9) and 0.6 mM $CaSO_4$ (pH 5.6–5.8) (Fig. 4). In the $CaCl_2$ medium the average current density of 20 growing root hairs was 2.8 ± 0.24 μamp cm^{-2}. In the $CaSO_4$ medium eight growing root hairs showed an average inward current of 3.2 ± 0.52 μamp cm^{-2}. In both media the growth rates were quite similar, namely 0.96 ± 0.07 μm min^{-1} in $CaCl_2$ and 0.92 ± 0.09 μm min^{-1} in $CaSO_4$.

The latter two media lacked both Na^+ and K^+ ions. Nevertheless, the incurrent densities were about 50% higher than in the standard medium and the rates of elongation scarcely changed. Hence, neither Na^+ nor K^+ ions seem to be important components of current which enters growing root hair tips. Nor do Ca^{2+} ions seem to be a major component: when Ca^{2+} was partially or completely replaced with Mg^{2+}, no significant changes in either inward current density or growth rate were detected.

In contrast to its response to changes in the concentration of the ions Na^+, K^+, Ca^{2+}, and Cl^- the current did clearly respond when the pH of the medium was changed (Fig. 4). Upon reducing the H^+ concentration of the medium 30-fold, from $10^{-5.6}$ to $10^{-7.1}$ M, the inward current density fell in half. Moreover, when the data from all of the measurements in media of pH 5.2 to 5.9 (46 root hairs) and in media of pH 6.5 to 7.5 (43 root hairs) are pooled, irrespective of the other components of the media, a similar pH dependence of the natural current becomes obvious. At a pH of 5.2 to 5.9 the average current density was 2.6 ± 0.2 μamp cm^{-2}; at a pH of 6.5 to 7.5 it was 1.4 ± 0.1 μamp cm^{-2}.

However, we were somewhat surprised to find that the elongation rate of root hairs did not fall with increasing pH and the concomitant fall in tip current. In media of pH 6.5 to 7.5 it was 1.05 ± 0.05 μm min^{-1}. This might be interpreted as indicating that beyond a certain magnitude of current, the current does not function as a limiting factor for growth. A similar phenomenon was observed in growing pollen tubes by Weisenseel and Jaffe (32).

DISCUSSION

CURRENT PATTERN AND SIZE

The pattern of natural electric fields near growing barley roots and root hairs shows that endogenous currents enter those regions of the root that grow, *i.e.* the root's elongation zone and the tips of root hairs. The main sources of these currents are cells of the root hair zone. The net, measured, steady current which enters the root can be estimated from Figure 1 to be about 30 namp (1 μamp/cm^2 × 0.2 cm × π × 0.05 cm ≅ 30 namp). The net measured current which leaves the root is evidently smaller. However, since the outward current was not explored farther up the root, we believe that this smaller figure is relatively unreliable, and that 30 namp is the best estimate of the total current traversing the root which can be obtained from these data.

There is a considerable literature on the natural electrical fields around growing roots. These papers—particularly those of Lund and Kenyon (11, 12) on onion roots and of Scott *et al.* (25–27) on bean roots—agree with ours in indicating a flow of current into the growth zone, and in indicating a net current of the order of 100 namp. Moreover, Scott *et al.* agreed with our finding that the chief source of this current lies basal to the growth zone, as well as with our finding that peak surface current densities reach 1 to 2 μamp cm^{-2}.

In their remarkable, pioneering study, Lund and Kenyon (12) carefully measured the potentials along the surface of onion roots growing in water-saturated air. (They had been growing in tap water before the measurements.)

The curves in their Figures 2 and 3 indicate considerable variability from root to root, but generally agree in showing a deep potential trough (which indicates a region of current entry) from about 4 to 11 mm behind the tip. This is described in the

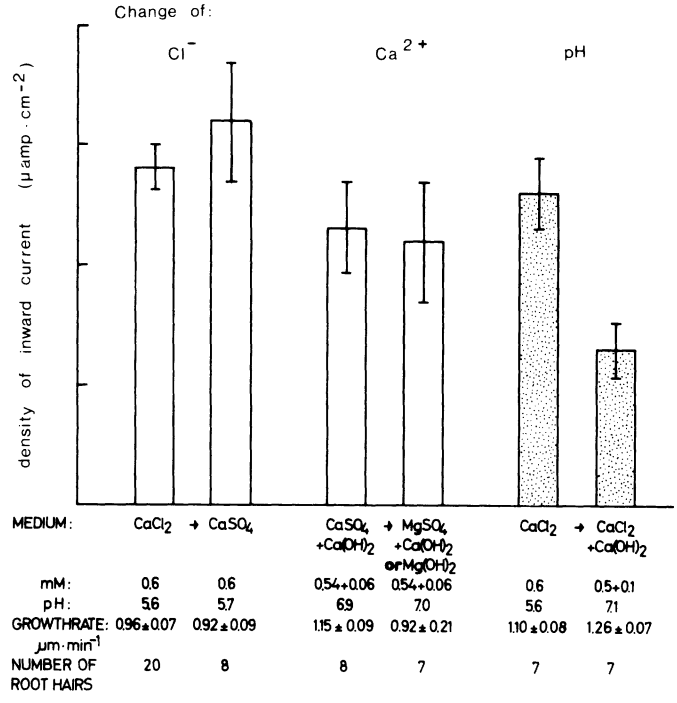

FIG. 4. Average current densities of natural current entering tips of growing root hairs. Each bar represents the current density (±SEM) of root hairs bathed in the medium indicated below. Right column of each couple represents current densities of root hairs from experiments in which the medium had been exchanged during measurements. In the left column of each couple all measurements with root hairs in the same medium were pooled.

text as "the region of beginning enlargement of cells and differentiation of meristematic tissue." Potential peaks, which indicate current sources, occur in the first 4 mm, which includes the root cap and the mitotic region. There are also some indications of current sources well behind the elongation zone. Lund and Kenyon argued that measurements of bioelectric currents (as opposed to voltages) are hard to interpret, and give no information on the size of the currents through growing onion roots. However, in a later reinvestigation of onion root potentials, Lund (11) inferred that onion roots drive currents of the order of 100 to 200 namp.

Scott *et al.* (27) measured the fields in the vicinity of bean roots growing in 0.1 mM KCl. This is an unnatural and ultimately injurious medium; static, 500-μm-diameter probes were used which greatly limited the resolution obtainable; moreover, the published data are too crude to yield clear inferences as to the current paths and sizes. Nevertheless, it is stated that "more detailed experiments have been made from which the current density and current direction in the neighborhood of the root can be deduced." On the basis of these unreported data, they inferred that current enters the growing root "in the region which is elongating most rapidly (2–7 mm from the tip)"; that the chief source of current is basal to the elongation zone (while the root cap is a secondary source); that the net current is about 50 namp; and that current densities through the root surface can be as high as 0.2 μamp cm^{-2}. However, in a later paper by Scott and Martin (26), peak surface densities of 1 to 2 μamp cm^{-2} were inferred.

This is the first report of electric currents through growing root hair cells. However, currents have been measured, or at least detected through a number of other growing plant cells. This list includes currents through *Pithophora* filaments, *Neurospora* hyphae (?), *Acetabularia* stem segments (?), fucoid eggs, and lily pollen (Table I of ref. 8). Like these others, barley root hair currents enter the growing end of the cell and do so with densities of the order of 1 to 10 μamp cm^{-2}.

IONIC COMPONENTS OF CURRENTS

Our measurements indicate that H^+ ions are the main component of the current which enters the root's elongation zone.[4] However, seven possibilities were considered *a priori*. These included influxes of K^+, Na^+, Ca^{2+}, and H^+ ions and effluxes of Cl^-, HCO_3^-, and OH^- ions.

Hydroxyl ion efflux is practically equivalent to H^+ influx because of the water dissociation equilibrium. Bicarbonate ion efflux is also nearly equivalent to H^+ influx (at least at the external pH values of 5.0 to 5.5 used in most of our experiments) because of the carbonic acid reactions: assuming a cytoplasmic pH of 7.0 to 7.5, and a pK of 6.3 (4), almost every HCO_3^- ion which leaves the root will be replaced by the dissociation of carbonic acid in the cytoplasm of the root cells:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+ \quad (3)$$

while almost every HCO_3^- ion which enters the medium, at pH 5.0 to 5.5, will combine with H^+ to generate CO_2 in a reversal of reaction (3). The net result is an influx of almost one H^+ ion per bicarbonate ion.[5] We will consider effluxes of OH^- and of HCO_3^- to be nearly equivalent to, and in a sense mechanisms of, H^+ influx. Moreover, chloride ion efflux seems quite unlikely since roots always take up net chloride. We are therefore left with four possibilities: namely K^+, Na^+, Ca^{2+}, and H^+ influx.

[4] Neither Lund nor Scott considered this possibility. Lund did not seem to believe that any ion movements were responsible for bioelectric potentials, whereas Scott and Martin (26) only considered Na^+ and K^+ ions as possible components of the entry currents.

[5] A CO_2 molecule will also be moved, but CO_2 should cross the membrane so freely by direct means that this should be of little consequence.

The failure of the inward current density to respond to K^+ and Na^+ deletion or to Ca^{2+} deletion suggests that these three main ions are not major components of the inward current. On the other hand, reduction of $[H^+]$ by 40-fold caused a small (~25%) but rapid and statistically significant fall in inward current density. This response may well have been muted by the bicarbonate mediated transport of H^+ ions, for this indirect mechanism (as opposed to direct H^+ movement) should be relatively unaffected by a fall in external $[H^+]$. This indirect mechanism would be limited by the rate of net bicarbonate efflux. Net bicarbonate efflux, in turn, should be markedly reduced by reductions in external $[H^+]$ only if the rate of bicarbonate influx became comparable to its efflux. This should not happen through the plasma membranes of barley root epidermal cells which are externally positive by about 100 mv (15).

Strong direct evidence of H^+ ion entry into the growth zone is furnished by the sharp rise in pH seen near it. No plausible alternative mechanisms for this alkalinization are apparent to us. The density of this H^+ ion influx can be estimated from the amount of H^+ ion needed to overcome the buffering power or capacity of the dye itself, as well as from the amount of H^+ ion needed to overbalance the H^+ ion efflux produced by CO_2 production. Both of these calculations indicate that the density of H^+ ion influx is comparable to the density of the electrical incurrent.

1. Calculation of H^+ Ion Influx from Bromocresol Purple Capacity

$$J_H = VC/At$$

where J_H is the H^+ ion influx (mol cm^{-2} s^{-1}), V is the volume of color change near the growth zone (cm^3), A is the area of contact of gel with the growth zone (cm^2), C is the specific capacity of the dye (mol cm^{-3}), and t is the time needed (s).

$$V/A \cong \Delta$$

where Δ is the thickness of the volume changed.

$$\therefore J_H \cong \Delta C/t$$

Within a few minutes, a sharp change in dye color was seen near the growth zone. So we may take Δ as ~ 0.5 mm; t as ~ 200 s, and C as ~ 1/10 of 0.71 mM or 7.10^{-8} mol cm^{-3}.

$$\therefore J_H \cong 5.10^{-2} \text{ cm} \times 7.10^{-8} \text{ mol cm}^{-3} \times (200 \text{ s})^{-1}$$
$$\cong 20 \text{ pmol cm}^{-2} \text{ s}^{-1} = 2 \text{ μamp cm}^{-2}$$

2. Calculation of Minimum H^+ Ion Influx Needed to Overcome CO_2 Efflux. Pitman has reported that growing barley roots produced about 10 to 20 μE $CO_2/g \cdot h$ (18, 19). Taking a root which is 0.5 mm thick and assuming a roughly uniform rate of respiration along the root, this corresponds to about 50 pmol cm^{-2} s^{-1} of CO_2 being emitted from the growth zone. At the pH values (5.0–5.5) of our bromocresol purple experiment, the order of one-tenth of this would dissociate into $HCO_3^- + H^+$. So to overbalance this, rather more H^+, say the order of 10 pmol cm^{-2} s^{-1} must be taken up. This corresponds to about 1 μamp cm^{-2}.

Our measurements also suggest that H^+ ions are the mean component of the current which enters growing root hair tips, since the measured entry densities actually rose (by 50%) when external Na^+ and K^+ ions were replaced by Ca^{2+} ions, were unaffected when Ca^{2+} ions were replaced by Mg^{2+} ions, but fell in half when the H^+ ion concentration was reduced 30-fold.

The available facts also suggest that hydrogen ions are the main component of the current which leaves the growing barley root. Again there are seven main possibilities to be considered *a priori*, namely effluxes of K^+, Na^+, Mg^{2+}, Ca^{2+}, or H^+ ion and influxes of Cl^- or HCO_3^- ions. Effluxes of K^+, Mg^{2+}, or Ca^{2+} ions or an influx of HCO_3^- ions are all unlikely for the simple reason that they would contradict the main function of the root and lack any precedent. An efflux of Na^+ ions is unlikely, because as far as is

known the efflux of each Na^+ ion is in exchange for one or more K^+ or H^+ ions (10). An influx of Cl^- ions is unlikely since the inward current densities we measured were unaffected when external Cl^- ions were replaced by SO_4^{2-}. Moreover, there appears to be no known precedent for the electrogenic uptake of Cl^- ion by "glycophytes," *i.e.* plants which live in low salt media (22).

This process of elimination leaves an efflux of H^+ ions as the most probable main component of the electrical currents which leave the root. Our observations of the pH changes near the main current source are consistent with this inference, although it should be noted that we have not yet distinguished between local acidification due to a direct efflux of H^+ ions from one due to an efflux of CO_2. An efflux of H^+ ions would certainly take work to drive it. In other words, H^+ ions would have to be pumped out; but there is ample precedent for this. There is growing evidence that a wide variety of glycophyte cells, including barley root cells, can pump out a large current of H^+ ions (1, 21, 22). Individual internodal cells of *Nitella* seem to drive large H^+ ion currents through themselves (29, 31). Finally, it is interesting to note here that as long ago as 1928, Marsh (13) reported an observation on onion roots that would now be taken as good evidence that the current which leaves the end of this root is driven by an electrogenic ion pump: his Figure 3B shows the response to local cyanide poisoning of the surface potential near the root tip. It repeatedly and reversibly falls by 30 to 40 mv with a half-time of about two min.

What might be the biological function of these natural currents traversing growing root hairs and roots? Our results suggest a relationship between currents and growth. It seems possible that the currents create and maintain gradients of charged cytoplasmic and membrane components which support the localized growth of cells and tissues.

In particular, we have inferred that H^+ ions flow into the epidermal cells of the zone of root elongation and leave the epidermal cells in the root hair zone. Relatively little is known of the pathways for ion flow in between (28). It seems reasonable to guess that there is a substantial basipetal flow of H^+ ions through the epidermal cells in between. Young epidermal cells seem likely to grow rather uniformly, as judged by their apolar appearance, as well as the difficulties in coordination that could accompany their tip growth. Older epidermal cells of *Hordeum jubatum* (and presumably epidermal cells of barley) become highly polarized as they initiate root hair development (24). They undergo a highly unequal division so as to form a short dense cell at their apical ends, and it is these classical "meristemoid" cells (22) which in turn initiate growth at their apical ends so as to form root hairs. Since there is evidence that local cation (actually Ca^{2+} ion) entry helps initiate as well as maintain local growth in fucoid and pollen grains (16, 17, 32, 33) it seems reasonable to speculate that a flow of H^+ ions into the apical ends of these epidermal cells helps to initiate tip growth there while a continuing flow of H^+ ions into the hair tips helps maintain growth there.

Finally, it seems to us that the root hair might be an excellent system to study the effects of auxin on a cellular level. Root hairs are known to be very sensitive to auxin (5). Auxin seems to effect H^+ secretion (*e.g.* 14). Measurements of the effects of auxin on the natural currents through growing root hairs might provide further insights into the relationships between auxin, H^+ pumps, and growth.

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