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BAND STRUCTURE OF SURFACE ELECTRIC POTENTIAL IN GROWING ROOTS

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For growing roots of azuki bean (*Phaseolus chrysanthos*), an electric potential is measured minutely along the surface of the root, together with the surface pH. It was found that the root begins to display a band-type pattern of potential with a spatial period of about 2 cm in a mature region as soon as it grows to about 10 cm in root length, while the surface potential shows only one convex peak around a position 5–20 mm behind a root tip and a succeeding concave peak around 20–35 mm, providing the length of root is shorter than about 10 cm. Since the surface potential takes a relatively positive value on average at the side of the root base compared with that in an elongation zone near the tip, electric current is supposed to flow into the elongation zone, accompanied by some local current loops in the mature region. The present band-type pattern observed first in multi-cell systems seems to be a kind of dissipative structure appearing far from equilibrium, and hence its relationship to growth is discussed.

1. Introduction

Many studies of surface potential in seed roots have been made in the last few decades. The first work in this field was provided by Lund [1], and later by Scott [2], where self-sustained oscillations of the surface potential were found in an elongation zone behind the root tip. Recently, an electric current was measured by the use of an extracellular vibrating electrode in growing barley roots of a few centimeters length [3]. Although it was suggested that protons flow into the elongation zone, the effects of the electric pattern on growth parameters such as growth speed were not examined in detail. Furthermore, Mulkey et al. [4] investigated correlations between pH and growth patterns during geotropism and phototropism in seedlings, and stressed an H^+ growth mechanism [5] from an observation of acidification in the growing side. However, detailed measurements of the electric-potential pattern were not performed.

In the present situation, therefore, much systematic and detailed work is necessary before we can obtain the whole profile on the surface potential of seed roots. In the present work, the following three points are taken into account: (1) A spatial pattern of surface potential is specially investigated from the viewpoint of nonequilibrium thermodynamics, whereas Scott mainly concentrated on self-oscillations only. (2) For this purpose, bean roots up to about 15 cm were systematically investigated here, while only roots of a few to several centimeters in length had been used in many previous works. (3) To pursue the relation between the electric pattern and growth, we performed an electric isolation of the elongation zone from a mature region in a pH measurement.

A spatial pattern of electric current has also

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been observed in growing and regenerating cells [6–8]. Among them, eggs of the brown alga *Fucus* have been investigated thoroughly for over a century, and the formation of an electric-current pattern around and within the egg is suggested as an essential step for rhizoid formation. In fact, a theory based on nonequilibrium thermodynamics [9] has shown that rhizoid formation is one of the typical self-organization processes appearing in far-from-equilibrium conditions, which is strongly coupled with the formation of an electric-current pattern. Spontaneous formation of macroscopic patterns such as a spatial distribution of ion flux on the membrane, therefore, may mediate between successive necessary steps in growth. These macroscopic patterns can be regarded as dissipative structures appearing far from equilibrium [9,10]. For multi-cell systems, however, no report has been presented on the band pattern of the electric current.

In the present paper, we shall report the observed results that a band-type pattern of surface potential appears in a mature region of long bean roots of about 10 cm length, and that the potential declines as a whole from the root base to the root tip. When the elongation zone was electrically isolated from the mature region, the acidification near the elongation zone appearing under the usual conditions [4] was inhibited so as to diminish the growth speed. From these facts, it is suggested that the H^+ current forms local current loops as a band-type pattern in the mature region to result in a net flow for acidifying the elongation zone, thus maintaining the continuous growth of root. A role of the formation of a dissipative structure as the present band pattern in the surface potential is discussed in relation to growth.

2. Materials and methods

2.1. Materials

Seeds of azuki bean, *Phaseolus chrysanthos*, were soaked for 3 h and were sown on three layers of filter paper, moistened with 0.01 mM KCl. These were kept in darkness in a temperature-controlled room at 30°C. After a while a root appeared and grew linearly with remarkable elongation velocity. Plants with root length from a few centimeters to 15 cm were used.

2.2. Potential measurement

Fig. 1 shows the experimental arrangement used for measuring the potential at points in the liquid medium (0.01 mM KCl) surrounding the bean root. This method is similar to that employed by Walker and Smith [11] to measure the surface potential of *Chara*.

In each case, dark-grown roots were laid on the axis of the chamber and the medium was added to submerge the root. The experiment was started about 1 h after setting up the plant to avoid artifacts arising from handling, etc. A potential close to the surface of the root at various points along its length was detected with Ag/AgCl wire embedded in a pipette electrode, filled with 1 mM KCl plus 1% agar with a tip diameter of about 100 μm. The electrode tip was located 0.5–1.0 mm from the surface of the root with a micromanipulator, in order to avoid direct contact with the root which may cause a random disturbance on the surface potential distribution [12].

2.3. Determination of elongation zone

To confirm the location of the elongation zone, a series of india ink dots were made at 1 mm intervals along the length of the roots. Roots were placed on wetted filter paper and kept in darkness. 5 h later, the change in the width between dots was measured using a low power (×10) microscope. Relative growth (RG) during 5 h was calculated

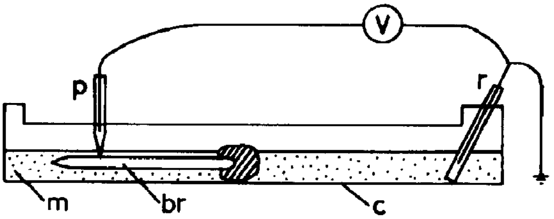

Fig. 1. Schematic diagram of experimental setup. p, pipette electrode; r, reference electrode; br, bean root; c, chamber; m, medium.

using the following equation:

$$\mathrm{RG} = (D/T) \times 100,$$

where D is the elongation of each region and T the total elongation.

2.4. *Electrical isolation*

An elongation zone of the root was isolated electrically and mechanically from a mature part by employing a special chamber with thin walls a and a′ which separate the medium into two compartments A and B (fig. 2i). Roots were gently laid perpendicularly to the walls through an appropriate hole in such a way that a few millimeters of the apical end, containing the elongation zone, dipped into compartment A and the basal end into compartment B. They were carefully dried in the wall space (b). Vaseline was applied around the root at the wall space to isolate electrically the neighboring compartment. The medium in both sides of the walls was 0.01 mM KCl.

Fig. 2ii shows cross-sections of control (left) and isolation (right) experiments. The partial shield of the former was made in order to allow an electrical connection between compartments A and B. To estimate the growth rate the root length of compartment A was measured, and the growth rate was plotted vs. the root length using the following equations:

$$\text{Root length} = (x + y)/2,$$

$$\text{Growth rate} = (y - x)/(Y - X),$$

where x and y (mm) are the root lengths at times X and Y (h), respectively, which elapsed from the start of experiment.

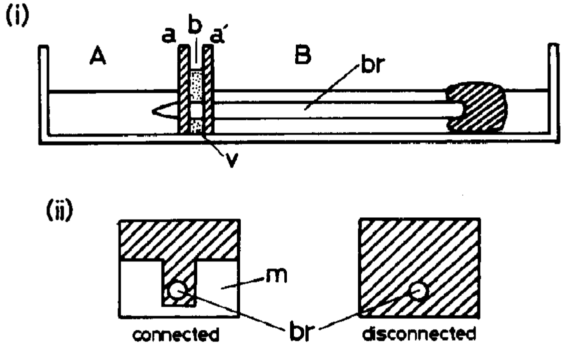

Fig. 2. Electrical isolation of the elongation zone of root from the mature region. (i) A longitudinal section through the experimental chamber and through a root. The space (b) is empty, allowing for the introduction of vaseline (v), which isolates compartment A from compartment B electrically. (ii) Cross-sections of shield: left, connected as a control series, where the plastic shield was partially made so as to allow an electrical connection between A and B: right, disconnected as an experimental series. For further explanation see text.

2.5. *pH measurement*

The participation of H^+ was investigated with a pH indicator dye and pH meter. To determine roughly the location of the pH change along the length of bean primary roots, seedlings were placed in a medium containing 0.01 mM KCl and 0.2 mM bromocresol purple, adjusted to pH 6.8. Under this condition, the zone of different pH was readily discerned.

More accurate measurement was conducted with a pH meter (Corning, model 125) equipped with a pH electrode (Iwaki Glass, model IW202). The tip diameter of the electrode was smaller than 1 mm. The medium used in pH measurements was 0.01 mM KCl, which was also adjusted to pH 6.8.

3. Results

3.1. *Band structure of surface potential*

Bean roots growing at a rate of about 1.5 mm/h were surrounded by a natural electric potential. Some observed examples of surface potential generated by these roots are shown in fig. 3. These data were obtained from the same specimen at different root lengths of 4.0, 8.0 and 13.3 cm. In many previous works [2–4], roots of several centimeters were mainly used, and there was no successive measurement which followed each growth stage. The value of the surface potential was usually about 10 mV. The potential near the tip sometimes oscillated as described by Scott [2], and hence the average value was recorded in those regions.

With regard to oscillations, an interesting phe-

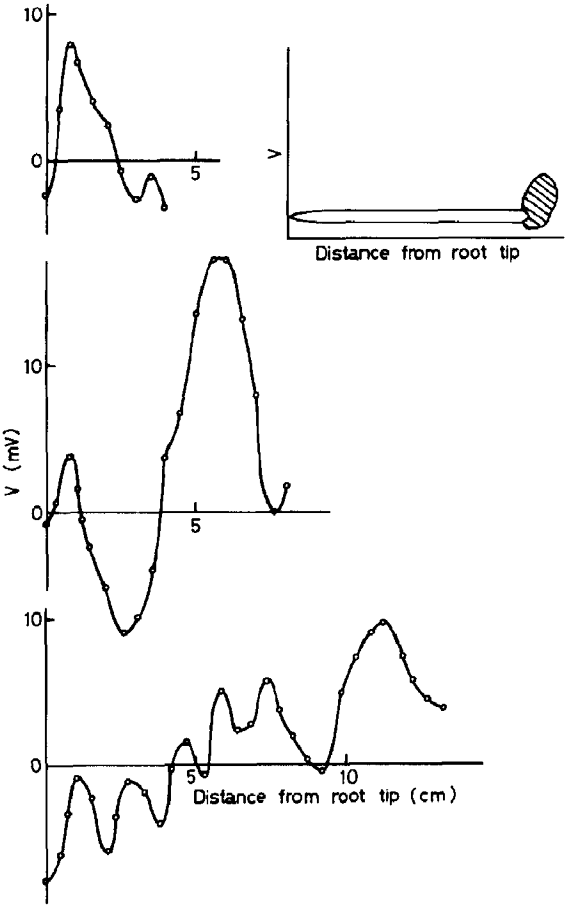

Fig. 3. Change in surface-potential patterns with the growth of roots. Potentials were measured in the medium close to the surface of the bean root under dim light. These patterns were obtained with the same specimen at intervals of about 1 day. The root was placed in the medium throughout the experiment. The surface potential is denoted by V.

nomenon was found besides Scott's results: The periods of oscillation amounted to 6, 12 and 18 min, and the amplitudes were also 1.5, 3.0 and 4.5 mV, respectively, where the integer ratio existed and they changed mutually with time. What cellular situation is reflected by such an integer ratio change in the surface potential is not clear at the present stage. Some nonlinear coupling between many biochemical steps seems to play a main role. While a mathematical description by coupled oscillators may be possible, further detailed experiments may be necessary for the explanation of this phenomenon.

In general, the surface potential was relatively negative at the tip and positive at about 1 cm behind the tip. This pattern was in accord with that obtained earlier [2]. Hitherto, the measurement of surface potential in longer roots reaching about 15 cm has not been reported. It is worthy of note that the profile of the potential alters remarkably with the length of roots. Around a root of 8 cm, a positive peak 2 cm from a bean became large. Many peaks can be seen with a root of 13.3 cm. They are arranged in a space period of 1.5 cm except for the large one near the base of the root.

Surface potential measurements were made on two opposite sides of the root. As shown in fig. 4, the location of the peaks matches quite well, while their amplitudes differ slightly. This indicates that the spatial potential pattern forms a band structure surrounding the roots.

Potential patterns varied between specimens naturally even at the same root length. However, they were fairly stable in each root, because similar patterns were obtained as shown in fig. 5, when the measurements were repeated within short time intervals, or less. Generally, the location of a peak was more stable than its amplitude, which altered

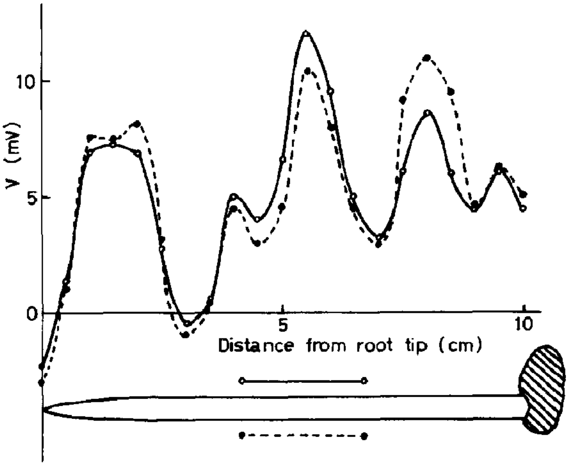

Fig. 4. Symmetry of surface potential on both sides of a root. The root was scanned on two opposite sides with a pipette electrode. (O——O) One side, (O------O) another side, measured 20 min later.

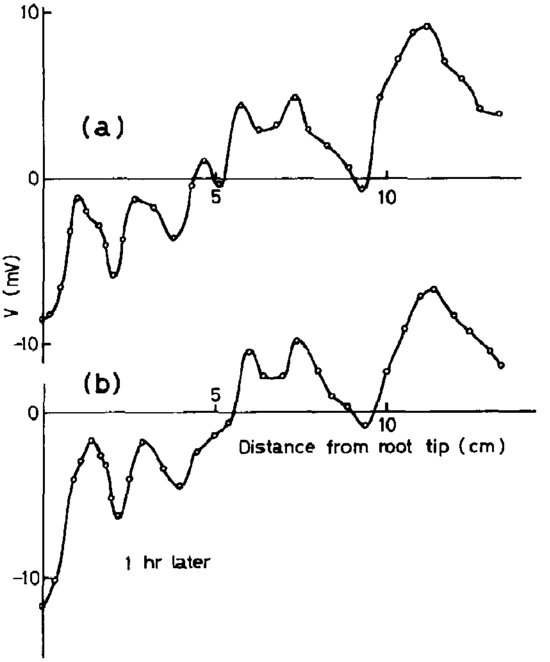

Fig. 5. Stability of surface potential. (a) Surface potential of a root. (b) Surface potential of the same root at the same side, measured 1 h later.

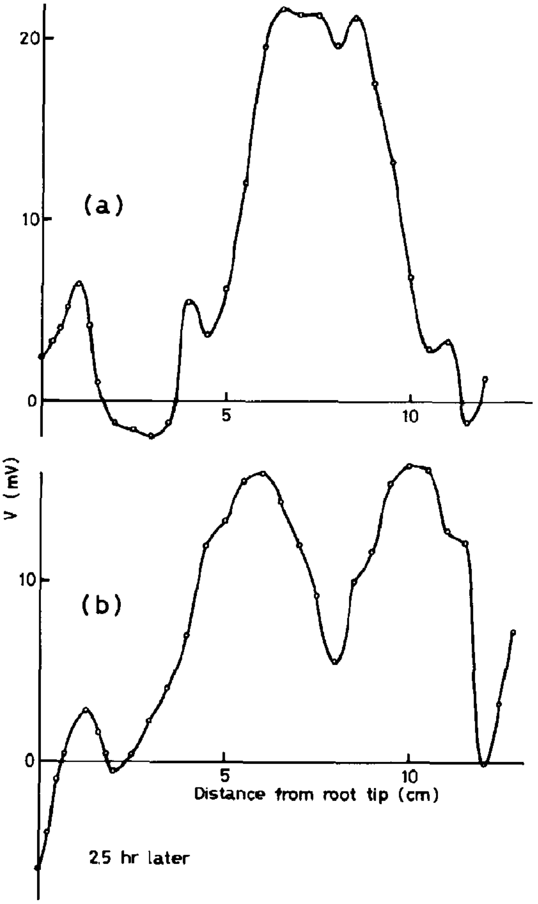

Fig. 6. Change in surface potential. (a) Surface potential of a root; (b) surface potential of the same root at the same side, measured 2.5 h later.

gradually. As an extreme instance, the amplitude of a certain positive peak changed from 15 to 7 mV in 1 h, but the location remained the same.

The band structure of a surface potential was stable over a short run. However, roots grew with remarkable elongation velocity. In the long run, a surface potential changed slowly accompanied by the elongation of roots. The shift of the band pattern is shown in fig. 6, where one peak is split into two. While at times the combination of some neighboring peaks into one was observed, the main change of the band structure seems to originate from the splitting of the peak.

3.2. Surface potential and root elongation

Subsequently, the relation between the surface potential and the growth of roots was investigated. An elongation zone of roots is known to be usually located near the apex [13,14]. This was checked with an india ink method on the moistened filter paper. Soon after, the roots were transferred into liquid medium and the surface potential was measured (fig. 7).

The elongation zone was located 1–2 mm behind the tip, where the surface potential was relatively negative. In addition, a relatively positive peak existed in a small region at the rear. This indicates that positive ions flow from the positive peak into a negative region, the elongation zone of roots, or that negative ions flow from a negative region into the positive peak. Moreover, the surface potential was relatively positive on average (figs. 4, 5 and 7) at the side of the root base compared with

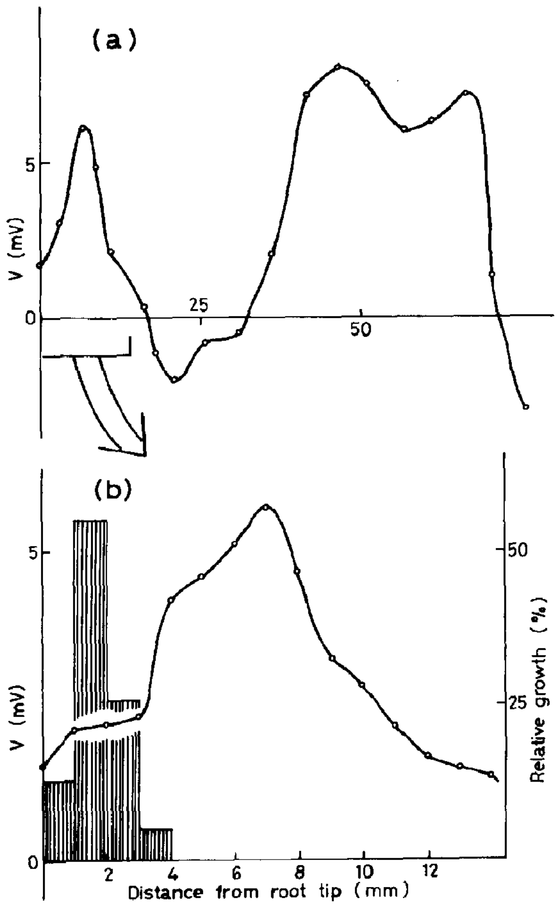

Fig. 7. Surface potential and relative growth-rate distribution. India ink was dotted at 1 mm intervals on the root and 5 h later the length of each region was measured. Immediately after the growth test, the root was transferred into a liquid medium and the surface potential was measured (a). Panel b shows that only the tip region expanded.

the elongation zone near the tip, so the electric current as a whole is supposed to flow into the elongation zone.

3.3. Electrical isolation and acid growth

The above-mentioned results suggest that the mature region mediates the elongation of roots with such an electrical method. To confirm this, electrical isolation of the elongation zone from the mature region of roots was performed and the effect of the electrical isolation on growth was investigated.

Fig. 8 shows that the electrical isolation effectively represses the growth of roots. About 60 roots of different lengths were divided randomly into two groups, control and test, and were used in the experiment. The surface of a root was wetted even if its moisture was wiped off with filter papers, so that complete isolation with vaseline was difficult.

While complete repression of growth was not anticipated with this electrical isolation method, the effect of the treatment was clear. In the test group, the current flowing from the mature region into the elongation zone was suppressed but not blocked completely, therefore roots grew at a lower rate than the control. Later, the mature region reached the opposite side where the elongation zone had existed and then the roots grew with the usual velocity.

An acid-growth mechanism of plants has been

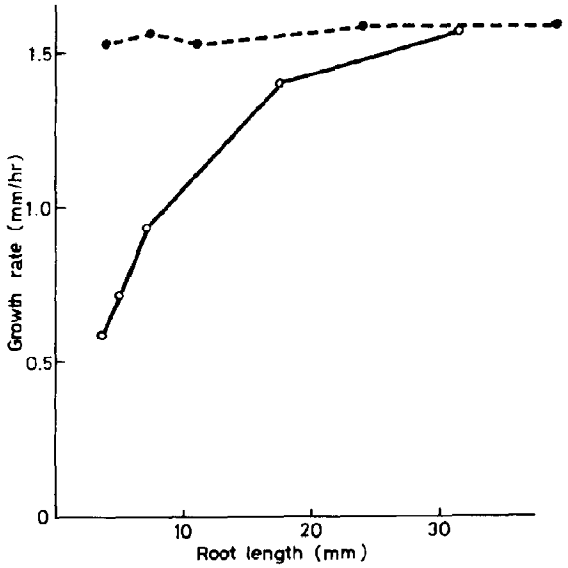

Fig. 8. Suppression of growth by electrical isolation. The elongation zone of roots was electrically isolated from the mature region. The length of roots at the side of the elongation zone, which lies in compartment A (fig. 2) was measured. Each value is the average of 30 specimens. (○———○) Electrically isolated, (●-----●) control.

proposed [5]. When bean roots were placed in the pH indicator dye, bromocresol purple (pH 6.8, purple), the medium near the root tip became yellow at first. The change in color of the indicator from purple to yellow suggests an acidification near the apex of the root. As for the surface potential, two opposite regions existed near the root apex. At the elongation zone, the surface potential was negative and at the mature region positive. Therefore, it was difficult to determine precisely from which point protons flow out by the use of the pH indicator dye.

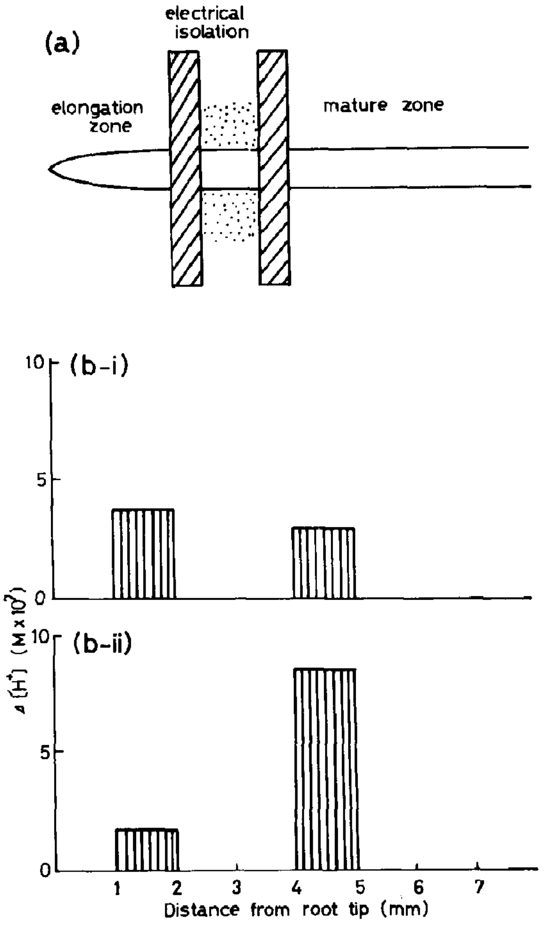

Fig. 9. Change in proton concentration near the tip of a root. (a) Schematic representation of experimental setup. (b) Change in proton concentration at both sides of the electrical isolation. (i) Control, (ii) electrical isolation. Each value is the average of 20 individuals. The scale given for distance in panel b is also valid for panel a. $\Delta[\mathrm{H}^+]$, difference between proton concentration at the measuring point and at a place far from the root. The latter was usually 1.58×10^{-7} M (= pH 6.8).

Therefore, electrical isolation with the surface pH measurement was applied to determine the proton efflux region. About 40 roots were divided into two groups, control and test. As shown in fig. 9, the pH values of both sides of the shield, elongation zone and mature region were measured. When the connection of both sides was blocked, the mature region became more acidic than the elongation zone. Thus, protons can be supposed to flow out from the mature region where the surface potential was relatively positive. In control experiments, the elongation region became a little more acidic than the mature region. These results indicate that protons flow out from the mature region into the elongation zone.

4. Discussion

In the present experiments, the band-type pattern of surface electric potential was found in roots of particular lengths. The surface potential near the root base was shown to be more positive than the potential near the root tip on the whole. The electric isolation of the elongation zone from the mature region inhibited the acidification near the tip and furthermore diminished remarkably the growth velocity. These facts may suggest that the H^+ current forms local current loops such as the band-type pattern in the mature region so as to enter the elongation zone on average.

Let us now discuss the relationship between band formation and growth. Fig. 10 illustrates two kinds of flow of protons from the region near the root base to the elongation zone; the situation on the assumption of no band is shown in panel a, and the case of band formation in panel b. Since the electric potential presumably originating from the pump activity is higher near the root base, protons may tend to flow down to the elongation zone with the lower electric potential. However, if the bands are not formed, there is a greater possibility of the protons flowing away to the surrounding aqueous solution, where the electric potential

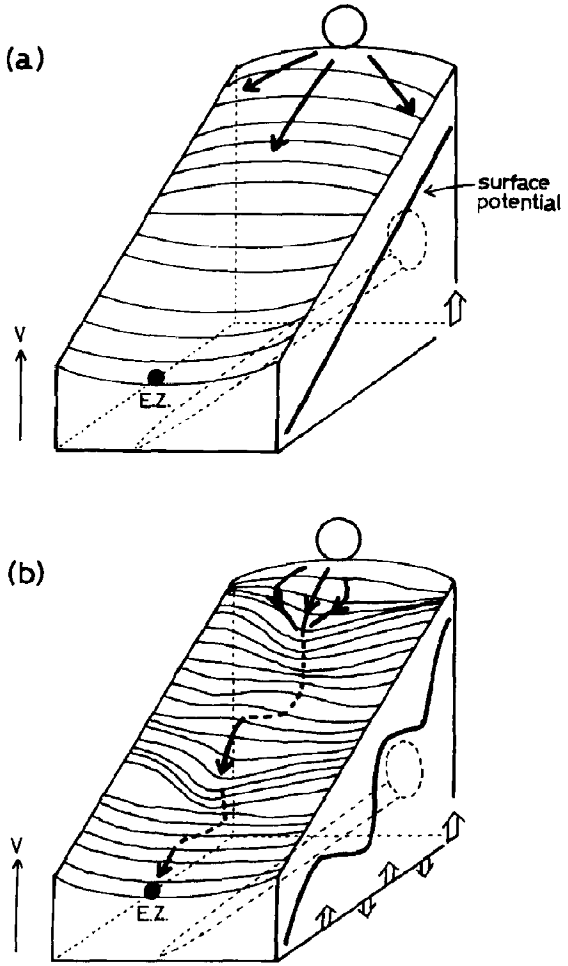

Fig. 10. Schematic illustration of proton flow from the region near the root base to the elongation zone. (a) No band, (b) band-forming situation. A proton is represented by the large circle with E.Z. denoting the elongation zone; (⇑) proton extrusion, (⇓) proton intrusion. The surface potential of the root is shown by a solid line on the plane of one side.

is relatively low compared with the base region as seen from fig. 3. All protons cannot reach the elongation zone, and hence this method for transporting protons does not seem very effective in the continuous growth of roots.

When the bands are formed, on the other hand, protons are taken up once into the root and again extruded from the region nearer to the elongation zone, since the band-type pattern of electric potential may be considered to reflect the alternating extrusion and intrusion of H^+ in a similar way to Characean cells [11]. The extruded protons may be absorbed again in the region much nearer to the elongation zone. As a result of the repetition of this process, it can be expected that all protons first extruded near the base reach the elongation zone precisely. The bands can, therefore, be supposed to facilitate the transport of protons to the elongation zone so that the root can maintain continuous growth through the acid-growth mechanism [5]. The band-type pattern does not appear while the root is short. This may be because the effective transport is possible due to the short distance even without the help of bands.

In this way, band formation is purposeful for root growth. A detailed mechanism of the spontaneous band formation, however, cannot be put forward at the present stage owing to a lack of theoretical analyses of this phenomenon. According to a suggestion from the case of Characean cells [15], the bands of bean roots can also be considered as one of the dissipative structures appearing far from equilibrium [10]. In fact, the bands in bean roots may resemble spatial patterns observed in the Belousov-Zhabotinsky reaction [10] and the Liesegang ring [16] appearing under nonequilibrium conditions. Further biochemical experiments seem to be required for the understanding of the mechanism of band formation on the basis of nonequilibrium thermodynamics.

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