Reports

Transmembrane Potential Measurements of Cells of Higher Plants as Related to Salt Uptake

Abstract. Measurements of the difference in electropotential between the interior of the cell and the external solution have been made for the first time in cells of several crop plants (*1*). The interiors of cells of *Avena, Pisum,* and *Zea* seedling tissues all have potentials of about −80 to −115 mv relative to that of an external solution of 0.1 mmole of KCl per liter, bathing the tissue. The potential difference of *Avena* coleoptiles varies with the concentration of external KCl and is depressed by 2,4-dinitrophenol. The potential difference occurs between the cytoplasmic layer and the exterior; the potential of the vacuole does not appear to be significantly different from that of the cytoplasm. Obviously a relatively large cation accumulation ratio could be accounted for in plant cells by this large potential without invoking a chemical cation transport scheme.

In the literature on salt accumulation by plant cells, an implicit assumption appears to be that a higher concentration of an ion within the cell than outside means that a metabolic transport system for that ion must exist. However, cells of muscle (*2*), nerve (*3*), and giant algal cells—for example, *Nitella* (*4, 5*)—have a transmembrane potential which is sufficient to explain the accumulation of K^+ in the absence of a chemical transport system for K^+. In frog sartorius muscle under non-steady-state conditions, the potential difference is dependent on the ratio of internal and external K^+ concentrations, but the K^+ concentration gradient is not in itself the energy source maintaining the potential difference; rather, this appears to be the result of an active chemical transport system for Na^+ (*6*). No such transport system has yet been discovered in higher plants, but plant cells apparently do have steady membrane potentials (*1*). In *Nitella,* for example—as in muscle—the membrane potential difference closely approaches the relation expected of a K^+ diffusion cell in which

$$E = 58 \log (K^+)_{out}/(K^+)_{in}$$

where E is the potential difference and $(K^+)_{out}$ and $(K^+)_{in}$ are, respectively, the outer and inner K^+ concentrations (*5, 7*). This is the behavior expected if the membrane is impermeable (or relatively so) to all but K^+ ions (*5*)—that is, if the internal and external K^+ ion concentrations, though different, are in electrochemical equilibrium. Under this condition, it is a logical necessity that some other process—perhaps an anion pump—would be required to cause the potential difference. This point has been discussed briefly by MacRobbie and Dainty in connection with salt relations of the alga *Nitellopsis,* which appears to have active Na^+ efflux transport and Cl^- influx transport systems (*7*). However, in the case of the vascular plants, current ideas of ion uptake are predominantly based on the concept of specific ion carriers—a scheme in which no account has been taken of the cell transmembrane potential difference, its direction, and its location.

Coleoptiles or roots of *Avena sativa* var. Victory were used for most of the experiments, but some resting potentials were measured in epicotyl cells of *Pisum sativum* var. Alaska and coleoptile cells of *Zea mays* hybrid. Segments of root, coleoptile, or stem were excised and placed in the experimental solution for pretreatment of 1 to 3 hours. The tissue was held and kept under microscopic observation in a Lucite chamber in which it was possible to maintain a constantly flowing solution and to change the solution entirely without disturbing the preparation.

Resting potentials were determined by inserting a microcapillary electrode (tip diameter 0.5 to 1.0 μ) into the cell and measuring the change in potential between it and a similar electrode in the external solution (*2*). The microcapillaries were prepared with an automatic electrode puller (*8*). They were filled with electrolyte by boiling in 3*M* KCl for 15 minutes. Reference electrodes were similar to the measuring electrodes except that the tip diameters were 10 to 20 μ and the microcapillaries were filled with 3*M* KCl in agar. The electrolyte in the electrodes was connected to the measuring circuit with Ag-AgCl wires. The measuring circuit consisted of an electrometer tube cathode follower preamplifier connected to a calibrated oscilloscope with d-c amplification.

The resting potentials of all of the tissues measured were negative relative to the outside solution. The average potential of 12 cells of five different *Avena* coleoptiles in 0.1 m*M* KCl was −120 ± 23 mv (range −87 to −157 mv). The average potential in 20 cells of four pea epicotyls in 0.1 m*M* KCl was −132 ± 22 mv (range −74 to −157 mv). The average potential of eight readings on one corn coleoptile in 1.0 m*M* KCl was −96 ± 20 mv (range −75 to −128 mv). Several readings of potential were taken of the root-hair-forming cells of two different root tips bathed in 1.0 m*M* KCl. In one, the average potential of seven cells was −81 ± 7 mv; in the other, the average for six cells was −65 ± 7 mv.

The effect of the external KCl concentration on the cellular potential was

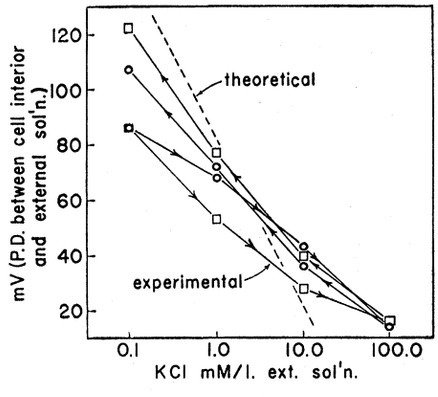

Fig. 1. Data from two experiments with *Avena* coleoptile showing the relation of the potential (in millivolts, negative) of the cell interior to the external KCl concentration. In each experiment KCl solutions of progressively higher concentration, up to 100 mmole/lit., were flushed through the tissue-holding chamber, and then the sequence was reversed to lower concentrations, as indicated by the arrows. The theoretical curve (dashed line) is based on a K^+ tissue content of 25 microequivalents per gram (fresh weight), as shown by assay.

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

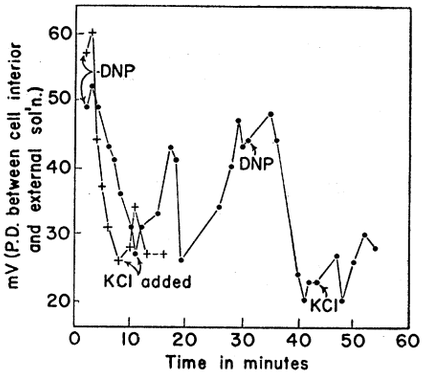

Fig. 2. Data from two experiments, showing depression of cell potential difference by dinitrophenol. In one experiment (solid points), reversal of DNP inhibition may be noted. (*Avena* coleoptile; KCl concentration, 25 mmole/lit.; DNP concentration, 0.2 mmole/lit.)

studied in the parenchyma cells of the coleoptiles. The tissue was excised and soaked in 0.2*M* mannitol (*9*) for 1 to 2 hours before the experiment, then mounted in the holder. With the electrode in a single cell, the tissue was successively perfused with 0.1, 1, 10, and 100 m*M* solutions of KCl, made up to 0.2*M* with mannitol. With the electrode still in the cell, the series was also run in reverse. Readings were recorded after each change in concentration when the potential had stabilized (a matter of seconds). As is shown in Fig. 1, the potential becomes less negative as the external concentration of KCl is increased and, conversely, more negative as the external concentration is subsequently decreased. The reversibility of the potential is a good indication that the seal around the electrode tip was maintained throughout the experiment. It is apparent that the slope is not the same as that expected of a K^+ diffusion cell see the theoretical line in Fig. 1). However, potential difference is obviously related to external KCl concentration and interpretation of the significance of slope must await further data on equilibrium conditions.

The effect of 2,4-dinitrophenol (DNP) on the cellular potential was also studied with parenchyma cells of *Avena* coleoptiles. In this case the tissue was excised and soaked in 25 m*M* KCl for 1 to 2 hours before it was mounted. Readings were taken on a single cell while the tissue was being perfused with 25 m*M* KCl and with 25 m*M* KCl plus 0.2 m*M* dinitrophenol. Figure 2 shows results of two experiments; in one the potential dropped to about one-half of its resting value in 6 minutes when dinitrophenol was added, and it did not recover when DNP-free KCl was added. The other curve is similar except that the potential jerkily returned to nearly its resting level when KCl was added. This potential was again depressed by dinitrophenol, but it did not recover a second time. It is known that dinitrophenol is a strong inhibitor of salt uptake by plant tissue (*10, 11*), and the magnitude of its effect appears to be correlated with metabolic activity (*11*). Therefore the depression of cell potential difference by dinitrophenol suggests that the potential may be directly or indirectly dependent on metabolic activity; however, an effect of dinitrophenol on permeability properties of cell membrane structure could possibly give a similar effect.

Attempts were made to ascertain the location within the cell of the potential difference. In root-hair cells of *Avena* at a stage of incipient hair elongation, a thick layer of cytoplasm is clearly visible, and it is thus possible to tell whether the electrode is in the cytoplasm or in the vacuole. The potential difference was measured when the electrode was inserted slowly into the cytoplasm and when it entered the vacuole. It was found that a major rise in potential occurred when the electrode entered the cytoplasm; the potential difference did not change significantly when the electrode entered the vacuole. Some cytoplasm-vacuole readings (in millivolts) in 10 m*M* KCl were 33/33, 30/39, 30/30, and 39/39; and in 1 m*M* KCl, 83/90, 87/84, 75/75, 78/60, and 71/71. These data, indicating that there is no difference in potential between cytoplasm and vacuole, indicate a situation similar to that in certain algae (*7*).

The existence of a constant cell potential in vascular plant tissue must be given important consideration in describing any scheme of salt absorption by plants. According to these data, the potential difference amounts to as much as 120 mv in *Avena* coleoptile cells—a difference which is sufficient to make the internal K^+ concentration more than 100 times greater than the external concentration. The location of the potential drop at the external cytoplasmic surface emphasizes the classical view of the cytoplasmic membrane as a barrier to ionic diffusion. It thus appears to definitely preclude the possibility that the vacuolar membrane is essentially the only ionic diffusion barrier and that the cytoplasm is, relatively speaking, "free space," as has been proposed (*12*).

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References and Notes

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Characteristics of Blood-Brain Barrier to Gamma-Aminobutyric Acid in Neonatal Cat

Abstract. Systemic γ-aminobutyric acid produces a rapid, sustained, but transiently reversible blockade of evoked axodendritic excitatory postsynaptic potentials in neonatal cortex when the "barrier" operating to restrict its passage has been altered by various experimental procedures. The data obtained under these conditions indicate the existence of well-developed synaptic pathways in the superficial neuropil of immature cortex.

The functional characteristics of the blood-brain barrier in newborn animals have been analyzed by comparing the relative uptake of systemically administered dyes (*1*), metabolites (*2, 3*), and ions (*4*) by the immature and mature brain. Conflicting opinions concerning the development of this "barrier" at birth derive largely from the use of different test substances, which presumably measure different aspects of blood-brain barrier activity (*5*).

The functional characteristics of the blood-brain barrier in newborn animals (kittens) were studied with a pharmacologically active metabolite, γ-aminobutyric acid. The latter, when applied directly to exposed cortex, rapidly