

**Short Communication**

# Effect of Indole-3-acetic Acid on Membrane Potentials of Oat Coleoptile Cells<sup>1</sup>

Received for publication October 28, 1969

B. ETHERTON<sup>2</sup>

Argonne National Laboratory, Biological and Medical Research Division, Argonne, Illinois

This paper reports an effect of indole-3-acetic acid on cell membrane potentials. Solutions with increasing IAA concentrations (from  $10^{-9}$  to  $10^{-7}$  M) make membrane potentials of coleoptile cells more negative. This effect of IAA, which occurs at concentrations that also stimulate tissue elongation, was found inadvertently in the course of an experiment on the effect of IAA on cell wall potentials. In the latter case, no significant effects were found.

**MATERIALS AND METHODS**

Microelectrode techniques (1-3) were used for measuring membrane potentials of coleoptile cells immersed in solutions containing different concentrations of IAA. Growth rates were ascertained on the same tissues used for electrical potential measurements.

Coleoptiles were obtained from dark-grown oat (*Avena sativa* cv. Victory) seedlings. Coleoptile tips (5 mm in length) were removed, and the next 7 mm were excised and secured (apical end up) in a Lucite chamber. Coleoptile segments were immersed in a treatment solution the composition of which was maintained constant for the entire experimental period. Membrane potentials were measured after the segment had been exposed to a treatment solution for 2 to 8 hr. Segment lengths were ascertained immediately after excision and at the end of the experiment.

Treatment solutions had the following IAA concentrations: 0.0,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ , and  $10^{-6}$  M. The inorganic salt composition of all of the treatment solutions was KCl, 30;  $\text{Ca}(\text{NO}_3)_2$ , 10;  $\text{NaH}_2\text{PO}_4$ , 9.05;  $\text{Na}_2\text{HPO}_4$ , 0.48;  $\text{MgSO}_4$ , 2.5 mM. The pH was 5.3. Solutions containing IAA were always freshly prepared. A solution with the above concentrations of inorganic ions was also used to moisten the sand on which the seedlings were grown.

Membrane potentials of the parenchyma cells exposed on the cut surface of the apical end of the coleoptile were measured by slowly inserting a microelectrode into each cell and recording the change in potential (between the microelectrode and a reference electrode in the external solution) as the microelectrode passed through the cell wall and cytoplasm and into the vacuole.

Equipment used for measuring potentials was similar to that described previously (1, 2). It included a motor-driven micro-manipulator which was mounted on the stage of a compound microscope. Lucite chambers containing coleoptile segments were also attached to the stage of the microscope. A treatment solution flowed constantly through the chamber. Insertion of a microelectrode into cells was observed through a 40X long working distance objective. Ling and Gerard type microelectrodes (3) and similar

reference electrodes (both filled with 3 M KCl) were connected to a Keithley 603 electrometer amplifier via AgCl-coated Ag wires. The output of the electrometer was connected to a high speed recorder (Honeywell Visicorder).

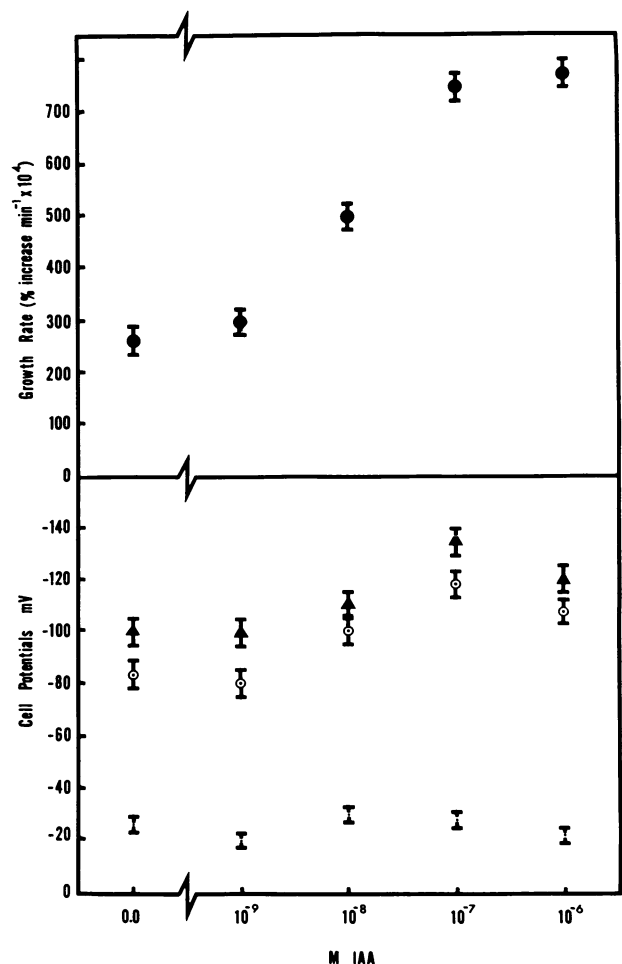


FIG. 1. Effect of IAA on oat coleoptile growth and cell potentials. Wall potential (•), cytoplasmic potential (○), vacuolar potential (▲), growth rate (●). The bars above and below each point show the standard error of the mean as determined by analysis of variance. Each point for cell potential (wall, cytoplasmic, vacuolar) is the mean of 16 measurements (four cells from each of four coleoptiles). Each point for growth rates is the mean of the growth rates of four coleoptiles used for cell potential measurements. Growth rate

$$= \frac{(\text{final length} - \text{initial length}) \times 100 \times 10^4}{\text{initial length} \times \text{min}}$$

<sup>1</sup> This work was supported by the United States Atomic Energy Commission and the National Aeronautics and Space Administration.

<sup>2</sup> Present address: Botany Department, University of Vermont, Burlington, Vermont 05401.

The temperature of the experiment was controlled at  $23.6 \pm 1$  C. Seedling growth was at  $25 \pm 1$  C.

Lighting conditions were as follows: seedlings were first grown in the dark, and then excised segments were continuously exposed to laboratory fluorescent lights. During the measurements of membrane potentials, portions of coleoptiles were exposed to relatively intense incandescent light used to illuminate the tissue for microscopic observations of cells.

### RESULTS AND DISCUSSION

In general, three distinct changes in the electrical potential difference (between the micro- and reference electrodes) occurred as a microelectrode penetrated a cell. I have named these wall ( $E_{\text{wall}} - E_{\text{medium}}$ ), cytoplasmic ( $E_{\text{cytoplasm}} - E_{\text{medium}}$ ), and vacuolar ( $E_{\text{vacuole}} - E_{\text{medium}}$ ) potentials. The names are tentative because it is difficult to measure wall potentials with microelectrodes and the location of the microelectrode tip during so-called vacuolar and cytoplasmic measurements has not been verified.

Effects of different concentrations of IAA on wall, cytoplasmic, and vacuolar potentials are shown in Figure 1. In general, solutions with IAA concentrations from  $10^{-8}$  to  $10^{-6}$  M made the cytoplasmic and vacuolar potentials more negative than solutions

with IAA concentrations of  $10^{-9}$  M or lower. No significant (5% level) differences in wall potentials could be found over the range of IAA concentrations used.

Figure 1 also shows the effect of different concentrations of IAA on growth rates of the tissues used for measuring cell potentials: Growth rates were greater in solutions containing  $10^{-8}$  to  $10^{-6}$  M IAA than they were in solutions with no added IAA.

It would be premature to speculate on the significance of the IAA effect on membrane potentials. More basic data should be added to these preliminary results before this is done. Also, more information is needed on the nature of plant cell membrane potentials which at present are incompletely understood (1).

*Acknowledgments*—Acknowledgements are due to Dr. S. A. Gordon for his support of this research and to Dr. R. R. Dedolph for his advice on the experimental design.

### LITERATURE CITED

1. ETHERTON, B. 1968. Vacuolar and cytoplasmic potassium concentrations in pea roots in relation to cell to medium electrical potentials. *Plant Physiol.* 43: 838-840.
2. ETHERTON, B. AND N. HIGINBOTHAM. 1960. Transmembrane potential measurements of cells of higher plants as related to salt uptake. *Science* 131: 409-410.
3. LING, G. AND R. W. GERARD. 1949. The normal membrane potential of frog sartorius fibers. *J. Cell. Comp. Physiol.* 45: 861-878.