EFFECT OF LIGHT ON THE BIOELECTRIC POTENTIALS OF ISOLATED *ELODEA* LEAVES

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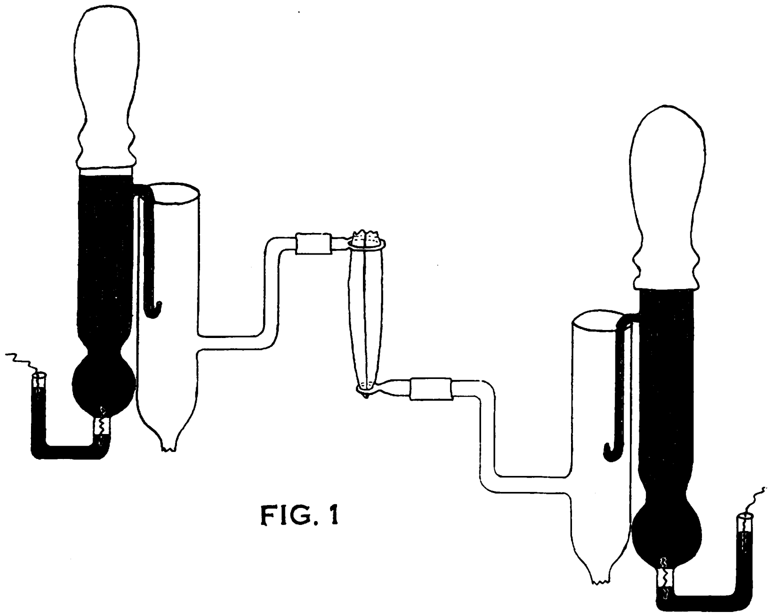
(WITH NINE FIGURES)

Introduction

The theory that the electric polarities of cells and tissues of living organisms are probably due to oxidation-reduction systems has been advanced by LUND and his associates (**2**). They have further suggested that such potentials constitute a mechanism of correlation between the cells or parts of the organism (**2**). The effect of light upon such systems seems therefore worthy of investigation. In 1892 OTTO HAAKE showed that green leaves increase their E.M.F.s in a positive direction when illuminated, and that other plant tissues, if chlorophyll-free, are not so affected (**1**). Since that time methods for the detection of electrical phenomena have been greatly improved, but the question of the effect of radiant energy upon bioelectric phenomena in plants has remained practically unexplored. A summary of the work done up to the present upon these problems is to be found in the paper by WALLER (**7**). In the present paper the results of a study of the responses made by single isolated leaves of *Elodea* are reported. This material was selected because of its simplicity of structure, each leaf having only two similar layers of large rectangular cells, and only one vein, the midrib; and because the absence of a cuticle facilitates the measurement of the potentials.

Method

The potentials between the tips and basal ends of detached leaves were measured with a Compton quadrant electrometer, the electrical circuit being identical with the one described by LUND (**3**). Isoelectric saturated zinc sulphate electrodes were used, and contact with the leaves was made through glass contact cups and arms, containing tap water (fig. 1). The leaves were placed in a moist chamber covered by a hood to exclude the light. They were supported on the contact arms in an upright position, so that the basal end was connected to the grounded quadrants, and the apex to the opposite pair (fig. 1). Since diffusion through the capillary arms of the contacts was very slow, and the water in them was changed every day, there was no danger of zinc sulphate solution reaching the leaf and injuring it. The contact cups passed through holes in the base of the moist chamber. These openings were sealed with flexible rubber joints, permitting movement of the contacts from outside the chamber while maintaining its hermetic sealing. When the walls of the moist chamber were covered with wet filter paper and the chamber was saturated with moisture, a leaf could be kept alive and in a normal state in it for many hours. The source of illumination was an electric globe with a linear filament, the beam being focused, after passing through a water chamber, by a microscope objective and ocular, then passing through a window into the moist chamber, and finally through the aperture of an iris diaphragm so as to fall upon the leaf



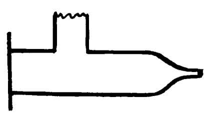


FIG. 1. Diagram of zinc sulphate electrodes, contact cups containing tap water, and contacts, with a leaf in position.

FIG. 1A. Special capillary contact for leading off at various points along surface of leaf.

in a small round spot 3 mm. in diameter, with an intensity of illumination of approximately 18,000 foot-candles. The light source was mounted upon a heavy base arranged to provide micro-manipulatory movement in three planes. All experiments were repeated at least three times, and more in the case of divergence. No two leaves react in precisely the same way, yet the general agreement is very good. Readings were plotted directly in graph form from the scale at 12-second intervals.

Absence of effect of light on electric polarity of onion root

Preliminary readings were made on onion roots, 4 to 6 cm. in length. These remained intact upon the bulb in a perfectly normal state, and continued growth following the experiments. The usual large inherent potentials were observed, which fluctuated when the roots were mechanically stimulated; but absolutely no effect followed illumination. This result confirms the observations of HAAKE (**1**) and WALLER (**7**), and apparently definitely confirms the relation of the phenomena to be described in *Elodea* to the presence of the chloroplasts.

Effect of light on electric polarity of *Elodea* leaf

The isolated leaves of *Elodea* were always dark-adapted, and allowed to remain in the moist chamber for a period varying from 40 minutes to 2 hours before illumination was begun. This was necessary in order that the effect of mechanical stimulation might subside, and that the potential might reach a stable level. This basal level of the electric polarity in a dark-adapted leaf was low, generally amounting to less than 10 millivolts between apex and base.

Experiments were conducted using both apical and basal leaves. By the former term is meant those leaves taken from a region within 2 inches of the growing tip, and of a light yellowish green color; by the latter term is meant one taken 10 or more inches from the tip, of a dark green color, and from a region where the whorls are spaced far apart.

Either apical or basal leaves, when placed in the moist chamber, showed that initially the apex was positive to the base. This E.M.F. was always found to be falling rapidly, as soon as observations could be made, and soon reached a level close to zero potential, either slightly above or below, depending upon the original condition of the leaf. If the hood was not

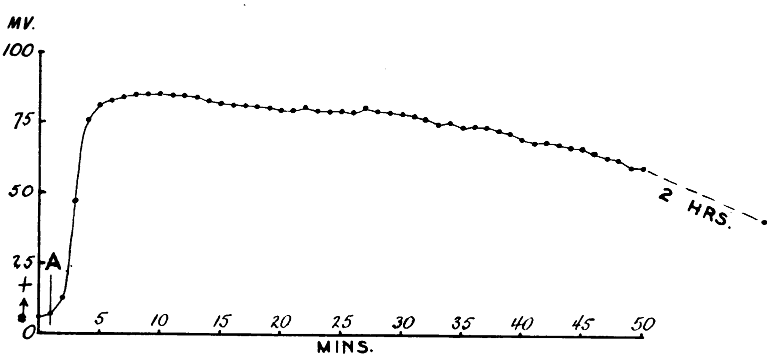


FIG. 2. Change of potential in an apical *Elodea* leaf under constant illumination of an apical spot; *A*, beginning of illumination.

placed over the leaf, it recovered to such a state that the apex again was positive to the base after a period of 1–2 hours, by an amount approximately equal to the originally observed potential. But when the leaf was placed in the dark, it found a new level of equilibrium, close to zero potential.

When a dark-adapted apical leaf of *Elodea* was illuminated by a round spot focused on the apex of the leaf, within a period of 5–10 minutes the potential between apex and base rose from nearly zero to a level between 75 and 100 millivolts (fig. 2). Under continuous illumination after the maximum response was attained, there was always found to be a slow

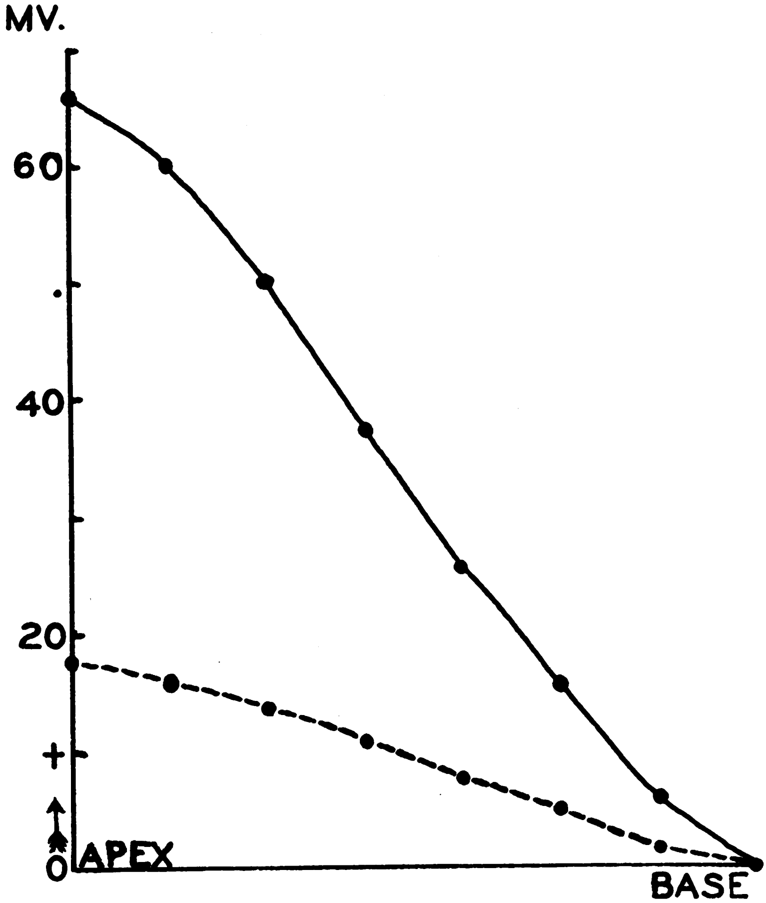


FIG. 3. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unillumined;

solid line, when illuminated at an apical spot.

decrease in the magnitude of the potential. After several hours, it was only about half as great as at first. When more than one reading was made upon the same leaf, the successive ones were always of decreasing magnitude. WALLER (5, 6) has reported some evidence of "fatigue" in his experiments upon the effects of light on the potentials of green leaves. This seems to be confirmatory, therefore, but should not be regarded as conclusive evidence, since the observations were only incidental to the main purpose in view, and the conditions were therefore not sufficiently controlled to that end.

It has been stated that the apex is positive to the base before illumination. But often both apex and base are positive to the middle portion of the leaf (fig. 4). Yet the decrease of E.M.F.s along the leaf may be uniform, and the base then the point of lowest potential (fig. 3). Illumination of the apex causes its potential to be greatly increased over that of the base; and an increase of potential also occurs along the entire leaf, diminishing with distance from the point of illumination. The decrease of E.M.F.s along the leaf is uniform, and appears as practically a straight line in the graph (fig. 3). This occurred in all cases, regardless of the original orientation of E.M.F.s along the leaf. When the leaf was illuminated at the basal end, the base became markedly positive to the apex, and again the transmission of the effect of non-illuminated parts of the leaf was observed.

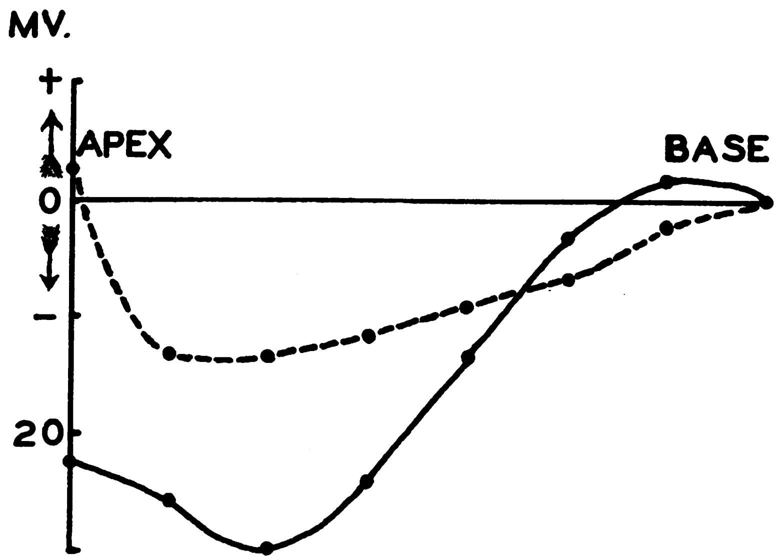


FIG. 4. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unillumined;

solid line, when illuminated at a basal spot.

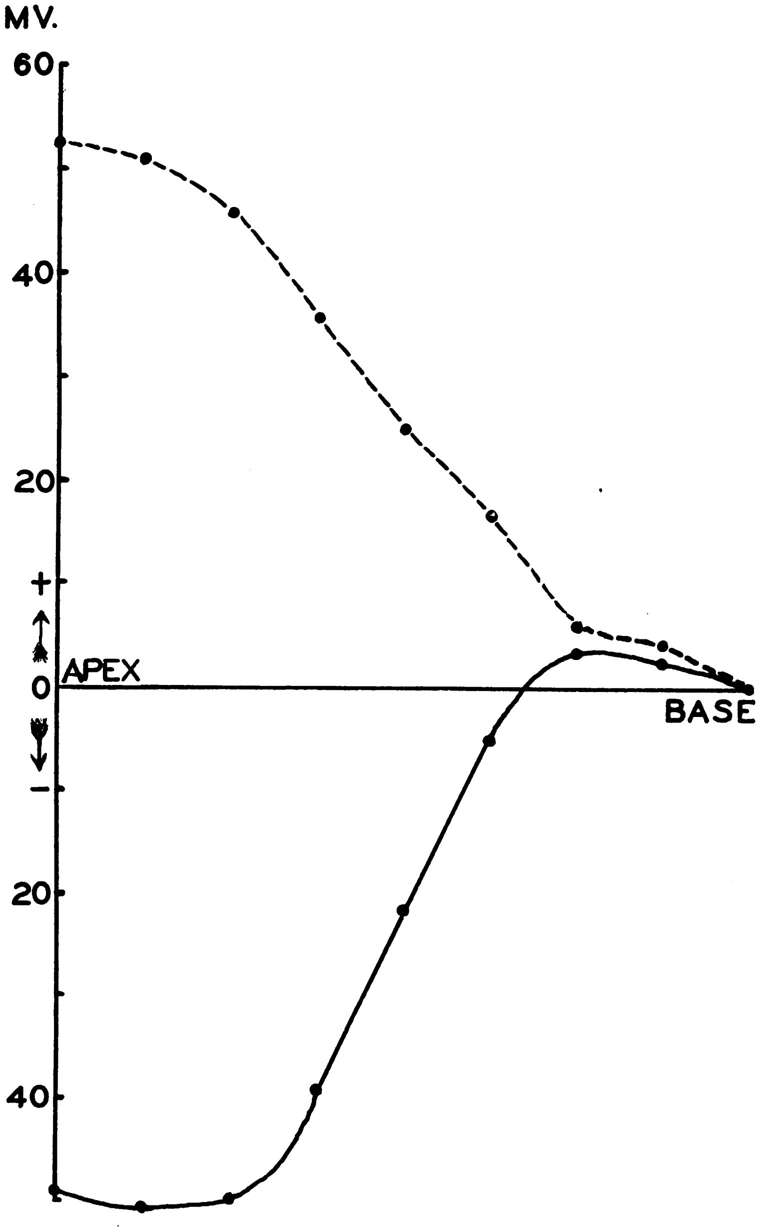


FIG. 5. Same as in figure 4.

This too occurred regardless of the original orientation of the E.M.F.s (figs. 4, 5). The solid lines on both these figures show, however, that there is a short region close to the tip of the leaf where the original orientation is preserved. It is further to be observed in these two figures that the E.M.F. is at a maximum at the point of application of the light, 2 to 4 mm. from the base. Again, when a point outside the positions of the contacts was illuminated, the region between the contacts being maintained in darkness, transmission of the effect of light to the region between the contacts was observed in the same manner. In plotting the magnitudes of the E.M.F.s along the leaf, the contact at the base was connected with the grounded quadrants, as before; while a new capillary tip contact was made which could be moved up or down at will, and also moved back from the leaf surface, thus avoiding stimulation of the leaf by motion of the contact along it; actual contact was made only through the drop of tap water at the tip (fig. 1A). Figures 4 and 5 show that although the base of the leaf was illuminated and the apex was maintained in darkness, yet apparently the potential at the apex has been greatly altered while that at the base has been maintained constant. It must be emphasized that this effect is only apparent, owing to the conditions of the experimental technique. It is due to the fact that, as in the experiment graphed in figure 3, the base of the leaf was connected with the grounded quadrants. What actually occurs is that, as all other evidence shows, the potential of the base is greatly increased when it is illuminated, while that of the apex, remaining in darkness, is relatively constant.

Apical leaves react to illumination in the typical manner shown in figure 6. Curve I represents the change of potential under constant illumination of a spot at the apex of the leaf; curve II represents the same change when a spot in the center of the leaf is illuminated; and curve III similarly for a spot at the base. The time at which the light was turned on is marked by the vertical line at *A*, and the time at which it was turned off by the arrow at *B* upon each curve. In each case the response is rapid, reaching the maximum in 7 to 10 minutes. As soon as the illumination ceases, the potentials promptly drop. The response when the apex is illuminated is always positive, *i.e.*, the potential of the apex as compared with that of the base is increased. It is large in magnitude. When the central portion is illuminated, the response is always positive but is much less in magnitude; when the base is stimulated the response is again large, but is in a negative direction, *i.e.*, the potential of the base with respect to that of the apex is increased. This response is sometimes even larger than the positive response when the apex is illuminated. It was also noted that in the second of the three cases the response varied considerably, being sometimes large and sometimes small. Twice positive responses were obtained, although of slight magnitude, when the basal end was illuminated. These exceptions to the general case were probably due to poor material.

Basal leaves were also similarly illuminated (fig. 7). The magnitude of the responses, other conditions being the same, appear to be smaller than the responses in apical leaves.

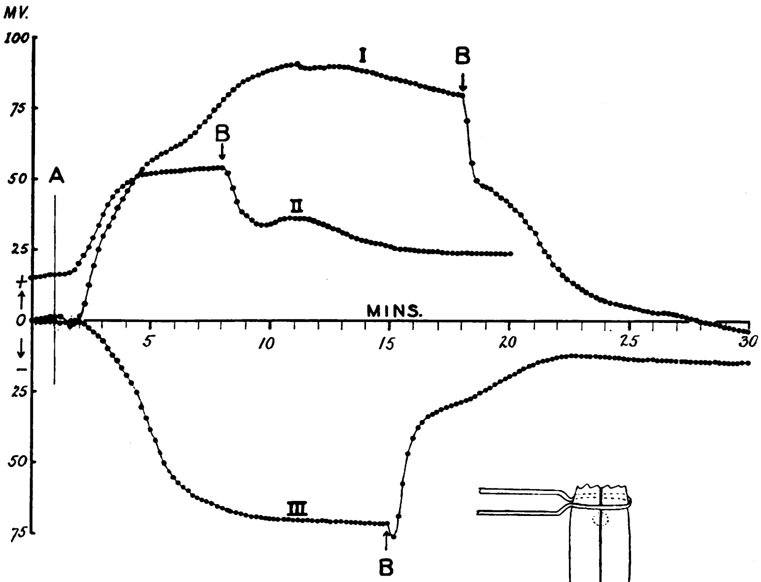


FIG. 6

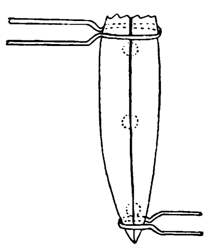


FIG. 6A

FIG. 6. Change of potenial in an apical *Elodea* leaf under illumination:
I, at apical spot (see fig. 6A);
II, at spot in center of leaf;
III, at basal spot.

A marks the instant at which illumination began; *B* the one at which, in each case, it ceased.

FIG. 6A. Diagram of an *Elodea* leaf in position on contacts. The circles mark various positions illuminated.

Examination of the individual curves (figs. 6, 7) shows certain constant features. There is a slight lag of about a minute after the illumination begins before any response can be observed. The initial response is sometimes a change in the opposite direction to that in which the succeeding main response occurs. The steady decrease after maximum response is attained has already been mentioned. When illumination ceases, there is a

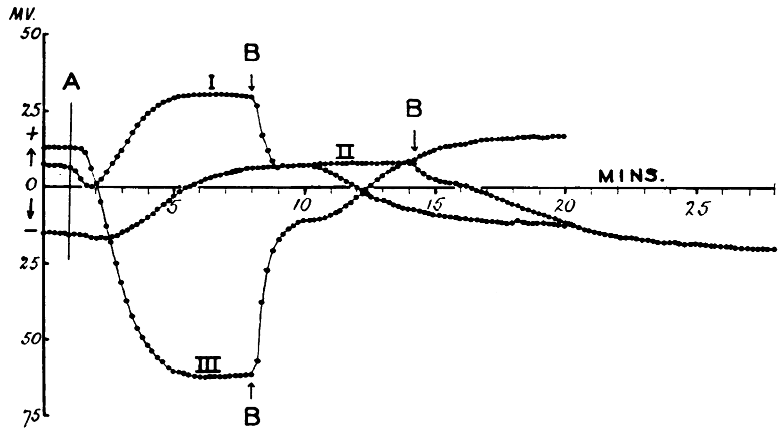


FIG. 7. Change of potential in basal *Elodea* leaf; notation as in figure 6.

sharp drop immediately, then a slowing down of the rate of decrease of potential, causing a flattening of the curve which may sometimes even take the form of a slight rise; this is then followed by a further rapid fall to approximately the original level of the potential between apex and base. There is no "over-shooting," that is, the potential reaches its final level slowly, not passing by it and then returning to it. In about 20 per cent. of the cases the double character of the descending curve cannot be discerned, perhaps because it is too slight.

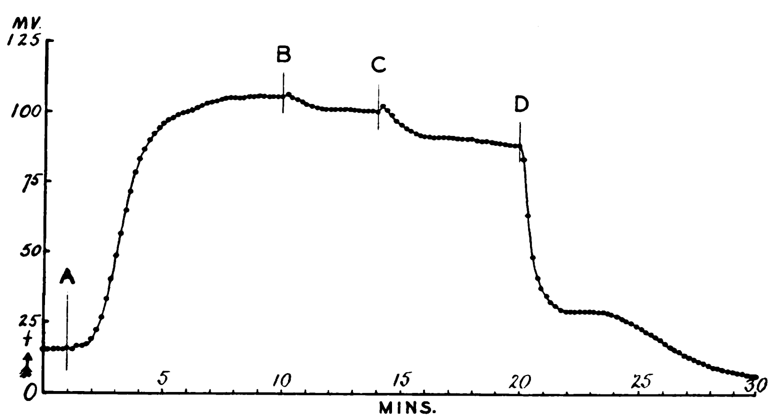


FIG. 8. Effect of decreasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

A, illumination began; intensity 18,000 foot-candles;
B, intensity decreased to 9000 foot-candles;
C, intensity decreased to 1700 foot-candles;
D, illumination ended.

Preliminary experiments were also conducted upon the effect of varying the intensity of the incident light. This was done by means of a resistance inserted into the lighting circuit, capable of cutting down the intensity of the illumination of the spot upon the leaf from 18,000 to approximately 1700 foot-candles. It was found that when the intensity of the light was first applied at this maximum and then diminished, a corresponding diminution of potential was to be observed (fig. 8); but when the intensity of the light was first applied at 1700 foot-candles, the response observed was the maximum, further increase of the intensity failing to raise the potential (fig. 9). Evidently the intensity of the light is not the only limiting factor concerned here. Further work is being planned to determine this point fully.

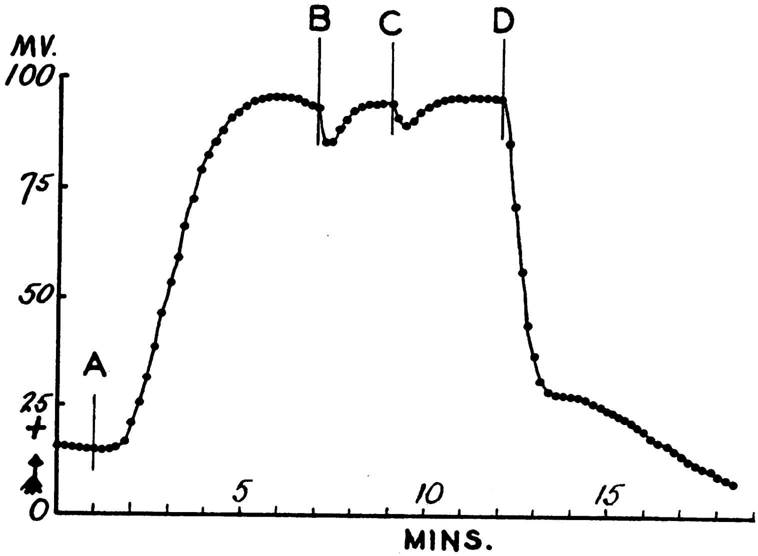


FIG. 9. Effect of increasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

A, illumination began; intensity 1700 foot-candles;
B, intensity increased to 9000 foot-candles;
C, intensity increased to 18,000 foot-candles;
D, illumination ended.

Discussion

WALLER (8) reported potentials from many plants. The highest potential he observed amounted to only 15 millivolts under illumination. SHEARD (4) reported 0.3 volt in sunflower leaves under illumination; even this high potential, in relation to the area of leaf surface, is small in comparison with the potentials reported in the present paper for *Elodea*. The leaf of the latter measures only 10–12 mm. in length and 3–5 mm. in width, yet potentials up to 100 millivolts have been observed under illumination between its apex and base. It should be evident, both from the size of the potentials observed and from their great constancy of behavior, that we are here concerned with something connected with the fundamental energy processes of the organism. It can hardly be believed that any expenditure of electrical energy by a plant in which the potential of a single leaf may vary by as much as 100 millivolts can be either insignificant or of secondary importance.

The relation of photosynthesis to these phenomena should prove of decided interest. The peculiarities of the curves found should throw further light on the question, particularly as to the chemical and physical processes involved, which give rise to them.

Transmission of the effect of illumination to non-illuminated regions is obvious. Illumination of a point on the leaf outside the region between the contacts has also been found to change the magnitudes of the E.M.F.s between the contacts. Figure 3 is particularly clear in showing such transmission. It should be observed that, since the decrement of E.M.F. along the leaf is practically a straight line, this means that the effect of illumination is felt not only at non-illuminated regions, but that the effect extends throughout the leaf, regardless of the distance from the point illuminated. This makes it very probable that the effect of light upon the potential of the leaf is not a direct photoelectric effect, but that it reacts first upon the chloroplasts, which in some way set up changes in the state of the leaf, giving rise in turn to the electric phenomena.

Summary

1. Apical and basal leaves of *Elodea* respond to illumination of the apex of the leaf by a large increase of the potential of the apex with respect to that of the base. The decrease in the magnitudes of the E.M.F.s along the leaf from apex to base in such a case is uniform.

2. Apical and basal leaves illuminated in the middle show a positive response, but to a less extent than when illuminated at the tip.

3. Apical and basal leaves illuminated at the base show a reversal of the electrical polarity found in the two preceding cases. The base becomes positive to the apex.

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