References and Notes

1. R. M. Elrick and R. J. Emrich, *Phys. Fluids* **9**, 28 (1966).
2. P. Bock, *Trans. N.Y. Acad. of Sci.* **25**, 902 (1963).
3. W. C. D. Whetham, *Phil. Trans. Roy. Soc. London* **181**, 559 (1890).
4. A. L. Copley, in *Flow Properties of Blood*, A. L. Copley and G. Stainsby, Eds. (Pergamon, New York, 1960), p. 104.
5. R. Fahraeus and T. Lindquist, *Amer. J. Physiol.* **96**, 562 (1931).
6. G. Bugliarello, C. Kapur, G. Hsiao, in *Symposium in Biorheology*, part 4, A. L. Copley, Ed. (Interscience, New York, 1965), p. 365.
7. R. K. Schofield and G. W. Scott Blair, *J. Phys. Chem.* **39**, 973 (1935).
8. V. Vand, *J. Phys. Coll. Chem.* **52**, 277 (1948).
9. A. D. Maude and R. L. Whitmore, *Brit. J. Appl. Phys.* **7**, 98 (1956).
10. H. W. Thomas, *J. Lab. Practice* **10**, 771 (1961).
11. L. E. Bayliss, in *Flow Properties of Blood*, A. L. Copley and G. Stainsby, Eds. (Pergamon, New York, 1960), p. 60.
12. A. A. Michelson, *Studies in Optics* (Univ. of Chicago Press, Chicago, 1927), p. 169.
13. E. H. Bloch, *Amer. J. Anat.* **110**, 142 (1962).
14. J. K. Emery, *Amer. Machinist* **110**, 18, 79 (1966).
15. Unitron model MEC, 660 Needham Street, Newton Highlands, Mass. Microscope choice is deliberate; metallurgical versions are designed for inspection of opaque objects under vertical incident illumination, a design easily modified for the generation of Fizeau bands.
16. J. Strong, *Concepts of Classical Optics* (Freeman, San Francisco, 1958), p. 227.
17. E. C. Albritton, Ed., *Standard Values in Blood* (USAF Tech. Rept. No. 6039, 1951), p. 7.
18. J. Strong, *ibid.*, p. 159.
19. A. C. Burton, *Physiology and Biophysics of the Circulation* (Year Book, Chicago, 1965), p. 52.
20. R. E. Wells, Jr., and E. W. Merrill, *J. Clin. Invest.* **41**, 1591 (1962).

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Fast Light-Evoked Potential from Leaves

Abstract. *When a leaf is illuminated with an intense flash of light, an electrical response with a time course in milliseconds can be recorded. This response was obtained between two wick electrodes placed at different positions on top of the leaf, with the entire leaf uniformly illuminated by the flash. During the first millisecond or so, the electrode nearer the apex of the leaf always became negative with respect to an electrode at the base, which indicates that the voltage-generating source is fixed longitudinally in the leaf.*

It was recently suggested (*1*) that fast electrical responses, such as those elicited from the vertebrate eye (*2, 3*) by a bright flash of light, may be a phenomenon widespread in all oriented biological pigment systems. This has now been confirmed by the discovery of similar fast light-evoked responses from the pigment epithelium-choroid complex (*4, 5*), from skin (*6*), and from the invertebrate eye of the *Limulus* (*7*). My report shows that such a fast light-evoked potential can also be recorded from leaves (*8*).

The techniques used to investigate the leaf potentials are similar to those used in recording the fast response from the vertebrate eye (*3, 9*). The light source was a Honeywell Strobonar P600 photographic flash housed in a soft steel box to reduce electromagnetic artifacts. The strobe has an input energy of 65 joules. Duration of the flash was 800 μsec, with the peak occurring at 250 μsec. Large-diameter Fresnel and conventional lenses were used to collect light from the strobe and focus it to give a uniform spot of light about 3 cm in diameter. This is sufficient to illuminate a considerable portion of the surface of the typical leaf used in these investigations. The incident energy on the leaf surface was approximately 0.05 joule/cm^2 in the visible region. Wick electrodes, well shielded from the light, were used for recording. A CR-4 low-noise differential amplifier (Princeton Applied Research) was used to amplify the signals, which were then displayed on a Tektronix type 502 oscilloscope.

A response was found in a number of different types of leaves, but the type chosen for detailed study was that of the bean plant, *Phaseolus vulgaris* var. Black Valentine, since this is a species that has been well studied in connection with photosynthesis. All plants used in my experiment were 3 to 4 weeks old and seemed to give large and reproducible responses.

In recording from the *Phaseolus* leaf, both electrodes were placed on top of the leaf [such as positions 1 and 2 on leaf diagram (Fig. 1)]. If electrodes were placed about 1 cm apart and the leaf uniformly illuminated with an intense flash of light, the response in Fig. 1A was recorded. The sign of the response was such that, for the first millisecond or so, the electrode at the apex of the leaf became negative with respect to the one close to the base; then a slow positive response was seen which often took as long as 1 second to decay. The fast response did not have a detectable latency, and its peak time was about 0.6 millisecond. The amplitude of this slow response varied from preparation to preparation with respect to the amplitude of the fast response, which suggests that these responses have different sources or pigment pools, or are brought about by different mechanisms.

Electrodes at positions 3 and 4 on the underside of the leaf gave a response with the same waveform as that shown in Fig. 1A, except that it was smaller. With the electrodes at positions 1 and 4 or 2 and 3, exactly opposite each other, there was no response.

Figure 1C shows that a response similar to that recorded with both electrodes on top of the leaf can also be recorded with one electrode on top and one underneath. This response is presumably the same as that recorded with both electrodes on top of the leaf, since no response was found with one electrode exactly above the other. If the leaf was turned over while the electrodes remain fixed and the electrodes were then placed along the midrib, but on the underside of the leaf, the response obtained was that shown in Fig. 1B. This response is similar to the responses obtained with illumination from the top, but it is much smaller, which suggests that the response comes primarily from what is normally the top surface of the leaf.

If the positions of the electrodes along the midrib on top of the leaf were interchanged, the waveform of the response was reversed, which shows that the effect is not one of "photoconduction" connected with the accidental polarization of one of the electrodes. In fact, the electrode near the apex of the leaf always became negative with respect to an electrode at the base, which shows that the voltage source producing the response must be longitudinally oriented. The amplitude of the response became larger as the electrodes were moved apart along the midrib of the leaf within the area of uniform illumination; this also indicates longitudinal polarization of the voltage source. Indeed, it is possible to make a "map" of equipotentials on the leaf surface. It is a little surprising to find the signal-generating structure fixed longitudinally in the leaf, in view of the perpendicular orientation of the signal generators in the vertebrate retina (*10*).

That a leaf should give a light-evoked potential at all is somewhat unexpected. Chloroplasts containing the pigments are not usually considered to be oriented as the receptors in the eye are. On the other hand, there is no evidence for a high degree of orientation in many of the other systems in which these fast potentials have now been observed, such as skin and the pigment epithelium-choroid complex. In many of these cases the effect may be

due only to a small departure from random orientation.

It is difficult to say at this time whether the fast light-evoked response from leaves, the early receptor potential of the vertebrate retina, and the other similar fast responses which have been discovered are all produced by similar mechanisms, although there are many similarities among these responses. All are electrical potentials with a millisecond component which are evoked by an intense flash of light (*2, 4, 6*). All the responses indicate some polarity in organization or structure; that is, in order for such responses to be seen, there must be oriented voltage sources (*4, 6, 10*). Experiments to be reported elsewhere show that the leaf response is linear with the flash energy absorbed (*11*); it shares this property in common with the early receptor potential (*3*) and the electrical responses from skin (*6*) and from the pigment epithelium-choroid complex (*5*).

However, there are some differences between the various responses. The amplitude and peak times of the leaf response appear to be almost independent of temperature from +60° to −5°C, although the response is abolished if the temperature of the leaf is lowered until the cells freeze and burst (*11*). Other differences include the nature of the light-absorbing pigments (*3, 5, 7*), the response thresholds, bleaching effects, and the saturation of the amplitude of the response at very high flash energies.

An order of magnitude calculation shows that at the highest flash energies used about 1 in 10 chlorophyll molecules absorbs a photon. A measurement of the magnitude of the current from the leaf passing through the wick electrode, which gives a lower limit on the total current, indicates that only about 1 electron is collected per 10^9 quanta absorbed. To turn the calculation around, if only a few quanta are needed to produce an electron, then only a small fraction of the electrons created are contributing to the observed voltage. If this is the case, the small current observed could be due either to inefficient collecting of the released electrons or to a very small asymmetry in current flow.

Pak and Ebrey (*9*) have shown that the fast light-evoked responses from the vertebrate eye can be related to the chemistry of the visual pigment rhodopsin, and recent experiments have carried these results considerably further (*12, 13*). Such techniques might also be used with the fast light-evoked responses of leaves and might give valuable clues to the chemical events associated with leaf pigments in the first millisecond after illumination.

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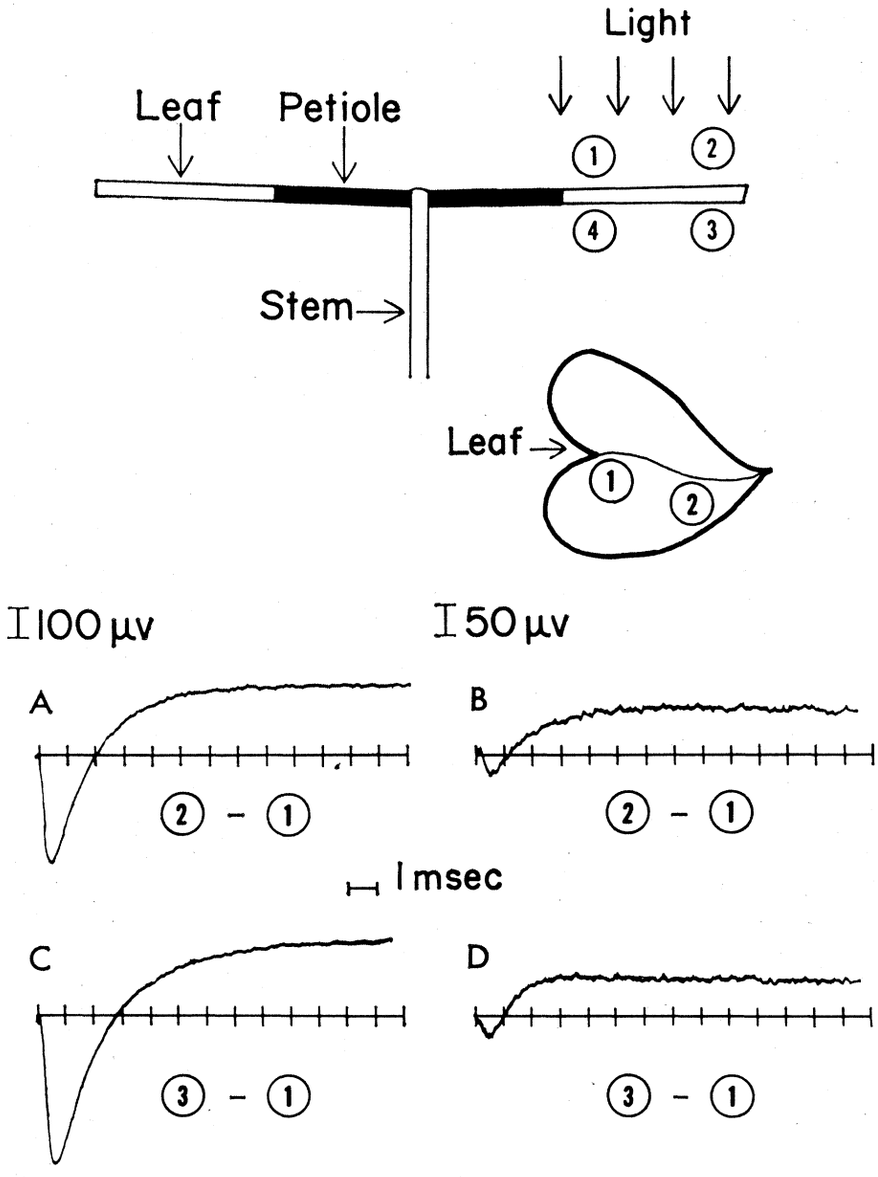

Fig. 1. Effect of the position of the electrodes on the waveform of the response. At the top of the figure is a schematic cross section of a bean plant showing the various possible positions for placing an electrode on the leaf. Below are typical responses observed with the electrodes at the positions indicated. (A) and (C), the light is incident on the top of the leaf; (B) and (D), the leaf is turned over while the electrodes remain fixed so the light is incident on the underside of the leaf. Numbering of the position of the electrodes is fixed with respect to the direction of the light. Bandwidth of amplifier: 1 hertz to 10 khertz.

References and Notes

1. J. R. Platt, G. Wald, K. T. Brown, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 501 (1965).
2. K. T. Brown and M. Murakami, *Nature* **201**, 626 (1964).
3. R. A. Cone, *ibid.* **204**, 736 (1964).
4. K. T. Brown, *ibid.* **207**, 1249 (1965).
5. T. G. Ebrey and R. A. Cone, *ibid.* **213**, 360 (1967).
6. H. E. Becker and R. A. Cone, *Science* **154**, 1052 (1966).
7. T. G. Smith, Jr., and J. E. Brown, *Quart. Progr. Rep. Res. Lab. Electron.* **51**, 249 (1966).
8. G. B. Arden, C. B. D. Bridges, H. Ikeda, *J. Physiol.* **186**, 123P (1966). Since my report was submitted, the work cited in the above abstract has been published [*Nature* **212**, 186 (1966)] and appears to differ on several points from the response that I report.
9. W. L. Pak and T. G. Ebrey, *Nature* **205**, 484 (1965).
10. G. S. Brindley and A. R. Gardner-Medwin, *J. Physiol.* **182**, 186 (1965).
11. T. G. Ebrey, in preparation.
12. R. A. Cone, *Science* **155**, 1128 (1967).
13. W. L. Pak and R. J. Boes, *ibid.*, p. 1131.
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