

Fig. 1 Merocyanine dye.

light of wavelengths longer than 590 nm was measured with a photodiode at right angles to the incident beam. The stimulus current was passed between internal and external platinized platinum electrodes and the action potential was recorded with a second internal electrode.

Fig. 2 illustrates the simultaneous measurement of fluorescence intensity (*a*) and membrane potential (*b*), in a single oscilloscope sweep. The intensity increase during the spike represented a change in the resting fluorescence of about one part in  $10^3$ , five times larger than the largest change previously measured. Although the fluorescence increase appeared to be somewhat slower than the potential change, this mainly resulted from high frequency filtering of the optical signal. Measurements with better time resolution, using voltage-clamp steps and signal averaging, showed that this mero-

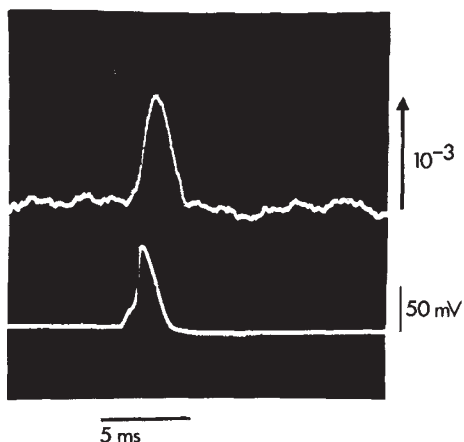


Fig. 2 Simultaneous measurement of fluorescent intensity (*a*) and membrane potential (*b*). The fluorescence increase during the action potential is quite large. The figure was made from a photograph of a single sweep on a dual beam oscilloscope. The response time constant of the light measuring system was 0.56 ms. The vertical arrow to the right of the trace represents the stated value of the change in intensity divided by the resting intensity. Axon diameter, 400  $\mu\text{m}$ . Temperature, 14° C.

cyanine fluorescence change followed the potential with a time lag of less than 0.15 ms. Combined voltage-clamp and intensity measurements were also used to show that the fluorescence change resulted from the change in membrane potential and not from the ionic currents or conductance increases that also occur during the action potential. The axon fluorescence was linearly dependent upon membrane potential. We did not detect any deleterious effect of the dye mixture on the axon.

It is clear from Fig. 2 that the fluorescence measurement was adequate for determining the occurrence of action potentials in the giant axon. We think this dye will also allow non-destructive monitoring of membrane potential in systems where electrodes cannot be used. It might be possible to measure changes in membrane potential of mammalian erythrocytes or the inward spread of potential in the transverse tubular system of muscle. A further application, involving invertebrate ganglia, has been suggested to us which we plan to pursue. An apparatus with a large number of photodiodes, arranged so that each detector would receive the light from an individual cell body, could, with a small computer, monitor the activity of, perhaps, a hundred cells at once. Such a large increase in the number of monitored

cells would facilitate the determination of functional connexions between cells, and ultimately lead to an understanding of the neuronal basis of behaviour.

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## Addendum to Welch *et al.*, page 143

*Note added in proof.* Recently, other investigators<sup>27-31</sup> have reported the use of urea and of anionic detergents as components of "banding cocktails". Simple aqueous detergent solutions, either anionic or cationic, have been used by some<sup>28,29</sup>, apparently unsuccessfully. Meanwhile, our further results show that cationic detergents also produce banding in human chromosomes, though less effectively than strongly anionic detergents.

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