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# The Localization of Radiophosphate in Cells.

LORIN J. MULLINS.*

*From the Department of Zoology, University of California, Berkeley.*

The exact mode in which ions are distributed in protoplasm is at present unknown. Using radioactive isotopes, several authors have attempted, by placing photographic film in close contact with tissues and thus obtaining a radio-autograph, to record where added ions are localized in the cell. Unfortunately this procedure has not yielded any great amount of information on cellular localization, although excellent differentiation of different tissue masses has been obtained.[1] The difficulty in obtaining clear radio-autographs of cells is that the cell diameters are of the same order of magnitude as the distance that $\beta$-rays, not normal to the film, travel before striking the film. The net result is a general fog which obscures the desired detail. To obtain pictures with more detail it is necessary therefore either to collimate or to focus the electrons coming from the tissue to the film, or else to use cells of much larger dimensions. In this study the second alternative has been employed in that cells of *Nitella* or *Chara* sp., which have diameters of the order of 1-2 mm and lengths of about 40 mm, were used.

*Experimental.* Cells of *Chara* or *Nitella* were prepared for use as previously described.[2] The cells were removed from pond water, blotted to remove excess water and placed on end in a tube containing 0.001M radioactive sodium phosphate solution at 15° C and pH 7. In some experiments only enough phosphate solution was used to cover 1-2 mm of the cell as measured along its axis. The tube was then filled with washed paraffin oil. By this arrangement it was possible to expose only a minimal part of the surface of the cell to phosphate solution and hence obtain some idea of the rapidity of diffusion and possible circulation of the phosphate due to cyclosis. To be sure there must be a thin film of water extending up from the $PO_4$ solution along the cell surface but this method probably lets ion diffusion proceed as asymmetrically as possible. After various times of immersion the cells were removed from the tubes, washed thoroughly in running distilled water, placed on a glass slide and rapidly frozen and dried *in vacuo*. A piece of X-ray film was then placed over the dried cell and exposed for a suitable time.

*Results.* Some photographs resulting from these experiments are shown in Fig. 1. In photo "A" the cell *(Nitella)* was immersed in radioactive phosphate solution (P*) from its end marked by an arrow (↓) for a distance of 25 mm along its axis for a period of 25 minutes. As is to be expected, the P* concentration is highest in this region, but it can also be seen that the P* concentration at the node of the cell is quite high. This may be due to the greater mass of protoplasm here or to the more rapid metabolic turnover in this region, or to a combination of both. In photo "C" a cell which was immersed in P* solution for 25 min. to a depth of only 2 mm is shown. Directly below the radio-autograph (black background) is shown a photo of the cell taken by transmitted light. (The cell was fractured in placing photographic film upon it). It can be seen from this photo that although there is a slight amount of activity in the nodal region, the P* is mainly concentrated in rather discrete zones along the cell. This cell was *Chara*. The *Chara* cell generally has a rather heavy incrustation of salt deposits on its wall. It is possible that these localized zones of P* concentration represent the

* Fellow of the American-Scandinavian Foundation at the University Institute for Theoretical Physics, Copenhagen.

1 Eklundh-Ehrenberg, C., Euler, H. v., and Hevesy, G., *Arkiv f. Kemi.*, 1946, **23**A, 10.

2 Brooks, S. C., *Trans. Faraday Soc.*, 1937 **33**, 1002; PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 856.

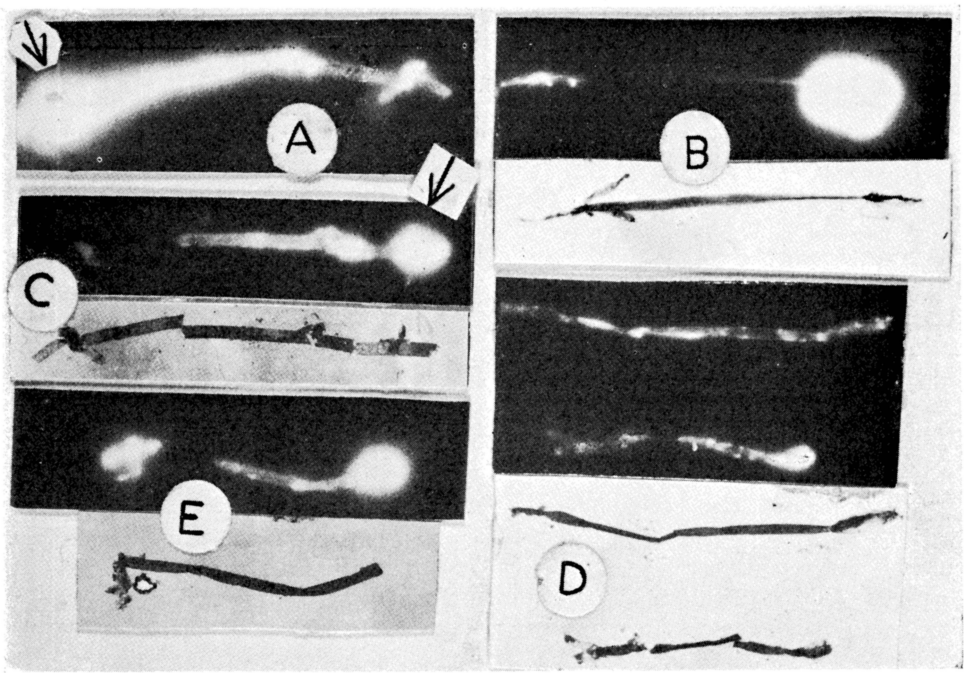


FIG. 1.
Showing the distribution of radiophosphate in *Nitella* and *Chara*. (See text.)

exchange of P* into such crystals, although the zones of concentration do not correspond with the visible areas of incrustation. (See transmitted light photo).

In photos "E" and "B" are shown radio-autographs taken after 60 and 90 minutes of immersion of cells to a depth of 2 mm. Both show concentration of P* in the node, and some slight localization of P* along the axis. Both cells are *Nitella*. Photo "D" is a radio-autograph of 2 *Nitella* cells together with transmitted light photographs taken after 660 min. immersion in P* solution to a depth of 2 mm as measured along the cell axis from the arrow. Here the P* has been distributed throughout the cell, but in discrete patches. One possible interpretation of such distribution is that in the course of vacuum drying of the cells, the protoplasm aggregated in clumps. This does not seem likely inasmuch as the transmitted light photos show that the chloroplasts must be rather evenly distributed throughout the cell as it is rather uniformly opaque. Microscopic observation confirms such a view. There is also the possibility that in the drying of the cell P* which has diffused throughout either or both cell wall and protoplasm crystallizes in quite large crystals. The distribution of P* in these photos, however, seems too sparse to render such a view likely. It would seem that P* in these algal cells is found in areas where there was already a high concentration of inactive $PO_4$ for exchange, and that the pictures obtained in this study represent the way P* is distributed as it diffuses along the cell.

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