of deprivation did not have a significant effect; therefore, the data for the animals deprived of food and water were combined (Fig. 1). A feature of the data is that the animals trained on the table top exhibit a relatively high percentage of alternation at the outset, but alternate progressively less with trials. We interpret these results to mean that the effects of preliminary training were temporary (*5*).

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3 January 1961

Electron Microscopy and Autoradiography

Abstract. The combined techniques of electron microscopy and autoradiography were used for the purpose of differentiating radioactive from nonradioactive particles collected on membrane filters. Newer methods of processing the membrane filters and applying the nuclear emulsion have resulted in an improvement in the qualitative nature of the procedure.

A method for differentiation of radioactive and nonradioactive particles collected on membrane filters and examined with the electron microscope was reported in an earlier paper (*1*). Continual application of this method during the past year has resulted in a number of refinements and the development of a reliable and reproducible method for processing membrane filters used in the sampling of radioactive aerosols. The objective of the present report is to describe in detail the method of processing membrane filters for examination with the electron microscope, and to discuss those modifications of the technique which have improved its qualitative nature.

Previous workers have reported on application of the combined techniques of electron microscopy and autoradiography for various purposes (*2*). The technique described in the present report was developed for the purpose of obtaining a more accurate analysis of aerosols that contain radioactive particles. The aerosols in this particular case were used in a study on the effects of the inhalated radioactive particles. The same technique could be applied to a number of other studies involving the collection and analysis of air samples containing or potentially containing radioactive particles. Application of the present technique is limited to alpha-emitting particles. Further studies are presently being conducted to test its usefulness for beta-emitting particles.

In the technique under discussion, membrane filters (type AA; plain, Millipore Filter Corp., Bedford, Mass., 25-mm diameter), which are an integral part of closed chambers used to expose the lungs of animals to radioactive dusts, are processed for examination with the electron microscope in the following manner. The membrane filter containing the collected aerosol sample is positioned with the collecting side upward on a 2- by 2-in. piece of Teflon. While the filter is steadied with a dissecting needle or similar instrument, a small disk is cut out of the central portion of a membrane filter with a 3-mm brass cork bore. The small disk thus cut out of the filter is placed with the collecting side down on the surface of a Formvar and carbon-coated electron microscope grid. The grid(s) thus prepared are supported in a petri dish by a medium-pore, sintered-glass filter stick beneath which are three sheets of No. 41 Whatman filter paper. The filter solvent of choice (acetone in this case) is then carefully poured into the petri dish until the filter paper is completely saturated, but not covered, by the acetone. The petri dish is then covered with its lid and allowed to stand at room temperature until the acetone has evaporated and the specimen grid has dried. This usually requires approximately 16 hours.

Dissolving the filters in this manner is a distinct departure from previous methods, and in our experience it has resulted in a more uniform distribution pattern of the collected aerosol sample. The commonly used rapid method for dissolving samples of membrane filters (*1*, *3*) resulted in loss of the smaller particles and aggregation of the larger particles on or along the grid wires. After dissolution of the membrane filter, the dried specimen grids can be examined with the electron microscope and, if desired, selected areas can be photographed. The grids with the sample side up are then secured for application of the nuclear emulsion to one end of small glass pegs 3 mm in diameter by means of double-coated Scotch tape. The pegs, with the grids in an upright position, are then inserted into holes of slightly larger diameter drilled into a block of stainless steel. The depth of the drilled holes is equal

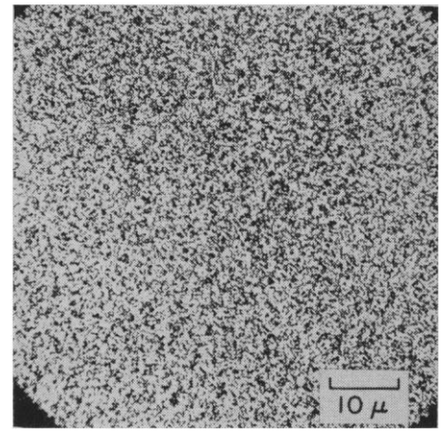

Fig. 1. Electron micrograph showing the distribution pattern of undeveloped silver grains over a specimen grid aperture.

to one-half the length of the glass pegs. All subsequent steps in the process of autoradiography are carried out in a darkroom under light filtered through a Wratten, Series OA, filter. Alpha-sensitive Eastman Kodak NTA emulsion in gel form (*4*) is heated at 45°C until fluid, and then 2 ml are diluted 1:1 with distilled water. One drop of a dilute solution (0.05 percent) of sodium lauryl sulfate is added to the diluted emulsion. In previous work the emulsion was more dilute (1:2) and the detergent was omitted. This resulted in an uneven distribution of silver grains over the specimen grid. The modification in the preparation of the emulsion has resulted in a uniform and even distribution of the silver grains over the entire specimen grid aperture. Figure 1 shows the distribution pattern of undeveloped silver grains applied by the above method.

The specimen grids secured to the glass pegs in the stainless steel block are then coated by means of a fine wire loop which is dipped into the emulsion

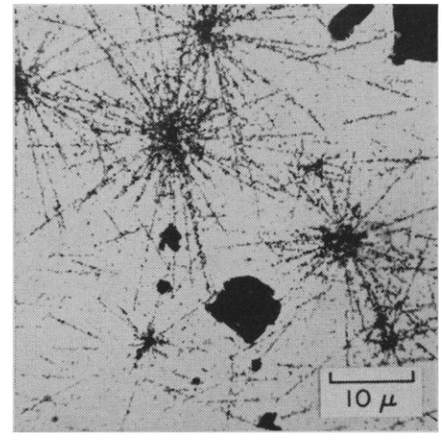

Fig. 2. Electron micrograph showing the differentiation of radioactive and nonradioactive particles.

and then slowly withdrawn, leaving a thin layer of the diluted emulsion in the loop. The emulsion is then placed on the specimen grid by passing the loop down over the stationary grid.

After coating, the secured grids are placed in a light-tight box and exposed for a suitable length of time at 4°C. With plutonium-239, the radioelement used in this study, 3 days of exposure were found to be sufficient. The autoradiograms are then developed by immersing the stainless steel block supporting the grids in Eastman Kodak D-19 developer for 5 minutes, rinsing twice in tap water, fixing in acid fixer for 15 minutes, and thoroughly rinsing in running tap water for 30 minutes. After drying, they are examined with an RCA EMU-2C electron microscope.

An example of the results obtained is shown in Fig. 2, which illustrates the differentiation of radioactive and nonradioactive particles (*5*).

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25 November 1960

Phototropic Inversion in Phycomyces

Abstract. Transient reversed bending follows an adequate step-up in light intensity given a bending cell from any direction around its axis, yet the plane of the response is specifically that in which prior phototropic bending occurred. The cell "remembers" what it was doing and reverses this in the ensuing light-growth response. Antecedent asymmetric growth itself built the pattern for reversal.

An upright sporangiophore of *Phycomyces* exposed to a single horizontal beam of visible light responds by bending toward the light source; this is its characteristic *positive* phototropism. Bending rate, measured as the change in angle between the cell's extremity and the vertical, is nearly constant over a period of 10 to 15 minutes, and over a wide range it is independent of the intensity (*1*). This implies that the differential growth underlying bending is primarily determined by the asymmetry of the illumination pattern around the cell's axis and not by the level of light intensity.

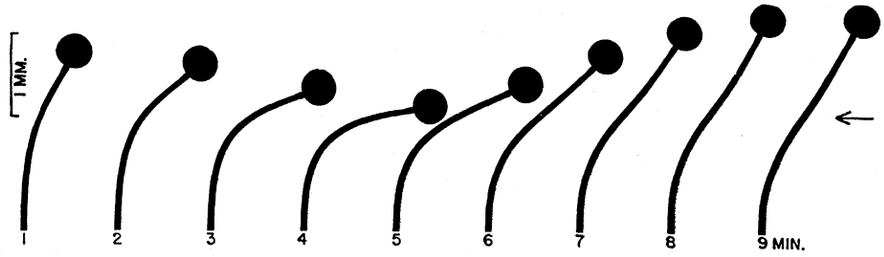

Fig. 1. Serial time-lapse photographs of *Phycomyces* showing normal bending interrupted by inversion. Abscissa, time; ordinate, vertical axis. Low intensity light source continuously illuminates cell from right (arrow); cell was straight when this commenced at −6 minutes. Normal positive bending is seen in pictures taken at 1 through 4 minutes. At 0 time the bending cell was given a 15-second flash of high intensity light from behind (perpendicular to plane of paper). Reversed bending is seen in pictures taken at 5 through 9 minutes; it takes place in the same vertical plane (that of the paper) as initial bending. Later (not shown) normal positive bending is restored.

Paradoxically, Reichardt and Varjú (*1*) showed that positive phototropic bending could be temporarily halted and reversed by a sudden sustained increase in the intensity of the horizontal stimulating beam. The established bending rate after a delay falls to zero and reverses its sign so that the cell for a time bends *away from* the light source; then this phase of *negative* phototropism ends, and the cell gradually regains its normal positive bending rate. This inversion is remarkable because it is caused simply by a properly timed increase in the intensity of the stimulating beam; the asymmetry of the illumination pattern remains unchanged.

I have studied inversion by photographic methods, previously described (*2*), that permit determination of both bending and cell elongation and have found that an acceleration of the cell's elongation rate indeed accompanies inversion. Reichardt and Varjú, however, always induced inversion by a step-up in intensity of the original stimulating beam, and their explanation assumes that an illumination pattern *within the cell* polarized in this direction and produced by the cell's lens action is necessary for inversion. This assumption is wrong. I have used a movable, supplementary light source to give the desired increase in light intensity along any chosen direction in the horizontal plane. I found that a sufficient step-up in intensity given even at right angles to the normal phototropic beam (or apparently at *any* angle to it) produces inversion (Fig. 1). So also does a 1-second flash of very high intensity light even though it is known to be phototropically ineffective and presumably saturating (*3*). Inversion is therefore caused by a step-up in light intensity *unspecific* as to both its direction and the cellular illumination pattern produced, even though the plane in which inversion occurs is *specifically* the plane of prior bending. Figuratively, the cell "remembers" what it was doing. This can only have a structural basis in the recent past history of the cell.

Growth requires supply systems and irreversibly forms the fibrils of the wall. Material so used comes from the substrate and more immediately from the cell's interior. The available local supply is the balance between rate of arrival and rate of use. I suppose that growth involves the presence of at least two materials, S and M. The supply of S is affected by light and tied to the adaptation system; M is used up in growth but is independent of light except as light affects its removal through use. Locally available M may be called growth *capacity*. The momentary rate of growth is considered proportional to the product $S \cdot M$. If more light is given, $S \cdot M$ temporarily rises, then declines in part because the growth capacity falls through the use of M. This is the light-growth response. With unilateral illumination $S \cdot M$ is kept slightly higher on the cell's far side by some consequence of the lens action. If subscripts n and f denote the near and far sides of the cell, the following conditions should apply during steady phototropic bending:

$$S_f \cdot M_f > S_n \cdot M_n$$
$$S_f > S_n$$
$$M_f < M_n$$

Thus local growth capacity, M, during steady bending is inversely related to the local growth rate, being high on the concave (near) side and low on the convex (far) side.

Such a bending cell is now given a step-up in light intensity: S rises rapidly wherever light acts and growth briefly accelerates, but then the ratio $S_f \cdot M_f / S_n \cdot M_n$ falls because M_f is now further depleted by fast growth; hence temporarily $S_n \cdot M_n$ can exceed $S_f \cdot M_f$ and *reversed* bending sets in. This is the inversion phenomenon, and it lasts until the level of M is restabilized in relation to S. The kinetics sought by Reichardt and Varjú involve the tem-