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On Inhibiting Effect of Acetates and Acetic Acid on Living Cells of Nitella.

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When living cells of *Nitella* were exposed for 10 minutes (1) to M/150 acetate buffer mixture at pH 5.5 or at pH 4.8 (consisting of a mixture of acetic acid and sodium acetate), and (2) to acetic acid at pH 4.8 or at pH 4, the pH value of the sap in the vacuole was found to decrease (depending on the supply of acetic acid in the external solution). If such cells were now placed for ½ minute in the solution of brilliant cresyl blue, made up with M/150 borate buffer mixture at pH 7.85, the rate of penetration of dye into the vacuole was found to decrease, as compared to that of control cells which were placed directly from the tap water into the same dye solution. At pH 4.8, the acetate buffer mixture brought about much greater decrease in the rate of penetration of dye into the vacuole than acetic acid. This may be due to the presence of sodium and a greater supply of acetic acid in the case of the former.

When cells were placed in M/150 sodium acetate solution for 10 minutes the pH value of the sap was found to remain unchanged, but when such cells were placed in the dye solution, as before, the rate of penetration of dye into the vacuole was found to decrease considerably.

From these results we may assume that the inhibiting effect of acetate buffer mixture may be due partly (1) to the effect of acetic acid on the protoplasm (either due to a specific effect of the acid or to the entrance of acetic acid as undissociated molecules and its subsequent dissociation whereby lowering the pH value of the protoplasm corresponding to the lowering of the pH value of the sap in the vacuole); and (2) partly to the effect of sodium acetate on the protoplasm.

When living cells were exposed to these solutions as before and were placed in the dye solution made up with M/150 phosphate buffer mixture at pH 7.85, the rate of penetration of dye was found

to decrease in the case of the acetate buffer mixture and acetic acid, but it did not change from that of the control in the case of the sodium acetate. The inhibiting effect of acetic acid on the protoplasm might have persisted in this case because the acetic acid in the vacuole served as a reserve supply by diffusing into the protoplasm after the cells had been transferred from these solutions into the dye solution, while that of the sodium acetate did not persist because there was no storage of sodium acetate or acetic acid in the vacuole.

Some of these experiments seem to indicate that though a decrease in the rate of penetration of a basic dye into the vacuole may take place at the same time the pH value of the sap is decreased, such results do not necessarily discredit the theory that a basic dye enters the vacuole much more readily in form of free base than in form of salt. If we assume that the inhibiting effect of acetic acid on the protoplasm exceeds the accelerating effect on the vacuolar sap, and that the rate of penetration of dye into the vacuole is controlled in this case by the diffusion of dye in form of free base from protoplasm into the vacuole, then a decrease in the pH value or some other alteration in the protoplasm corresponding to a decrease in the pH value of the sap might very well bring about a decrease in the rate of penetration of the dye into the vacuole.

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A Method of Proteolytic Enzyme Titration.

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In 1906 Müller and Jochmann[1] proposed a simple method of determining the presence of proteolytic enzymes in biological fluids by erosion of the surface of a Loeffler serum plate under a drop of the fluid. Later this technique was modified by the use of a gelatin plate dyed with carmine, but at best it was a + or — method, probably because the substrate was not sufficiently uniform to permit quantitative estimates of enzyme action.

If a photographic plate, or film, is fogged on both sides by equal exposures, is developed, fixed, washed, and dried, it presents a gelatin surface and texture of sufficient uniformity for quantitative tests. Proteolytic enzymes erode the surface and free the included silver,