acid. This difference is due to the fact that cells were collected in two different localities. In the former case the cells, which were collected in New York, were more sensitive than in the latter (cells collected in Cambridge). This is shown by the fact that when sufficiently low concentrations of these acids were used (0.000025 M at pH 4.8) with New York *Nitella* (see footnote 4) the same result was obtained as with Cambridge *Nitella* (using acids at pH 4.)

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Exit of Dye from Nitella with Different Initial Concentrations of Dye in the Vacuole.

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Living cells of *Nitella* were placed in brilliant cresyl blue solution for different lengths of time, so that the concentration of dye[1] (DB plus DS) in the vacuole varied from 0.000035 M to 0.0028 M (cell wall only very slightly stained). When such cells were placed in the M/150 phosphate buffer solution at pH 5.4 (solution changed and stirred), the rate of exit of dye from the vacuole was found to be about the same between 0.000035 M and 0.00014 M. Above the latter concentration it decreased as the concentration increased. There is also a decrease in the velocity constant, k, calculated from the equation $dx/dt = k(a-x)$, where a equals the initial concentration of dye in the vacuole, and x equals the amount of dye that has disappeared from the vacuole at time t. The pH value of the sap was about the same as that of the normal (determined after the dye has come out of the vacuole).

It may be assumed that the conditions, either in the vacuole or in the protoplasm or both, change as the concentration of dye increases. This change may be due to the production of a substance which (1) combines with part of DB to form a non-penetrating compound (other than DS), of the same color as DB and DS, which breaks down slowly to form DB during the exit of the dye, or (2) otherwise changes the concentration of DB.

[1] It is assumed that the dye exists in two forms, free base (for convenience called DB) and salt (for convenience called DS). DB penetrates the living cell very rapidly while DS penetrates so slowly that its penetration in this case is negligible. As DB enters the vacuole a certain proportion of it changes to DS. Though we measure the amount of DB plus DS in the vacuole, this method is justified because there is always a definite ratio of DB to DS, and because both DB and DS are of the same color. (*Cf.* Irwin, M., *J. Gen. Physiol.*, 1926-27, x, 75.)