

5079

Studies on the Penetration of Dyes with the Glass Electrode.  
II. Penetration into *Nitella* from Solutions of Cresyl Blue,  
Azure B, and Methylene Blue Solution.

MARIAN IRWIN.

*From the Laboratories of The Rockefeller Institute for Medical Research.*

Two successive extractions of the sap were made from the vacuole of each living cell of *Nitella flexilis*. The first extraction, called for convenience "sap", was made without pressing on the cell wall, while the second extraction, called "sap mixture", was made by squeezing the cell wall vigorously. The pH value of the sap was found to be 0.6 pH lower than that of the sap mixture. This difference may be entirely due to the presence of the protoplasm in the latter, or partly due to the presence of fluid from the space between the cell wall and the protoplasm.

The pH values of the sap and the sap mixture remained unchanged during the period of extraction and of the subsequent measurement by the glass electrode.

*The sap.* (1) 0.07% cresyl blue, azure B, and methylene blue salt do not alter the pH value when dissolved in the sap *in vitro*, but 0.07% free base of cresyl blue and azure B cause it to increase about 0.5 pH with the former and 1 pH with the latter.

(2) *a.* When 0.07% dye has penetrated from the solution at pH 9.2 there is an increase in the pH value of the sap of 0.5 pH with cresyl blue, and 1 pH with the azure B or with the dye penetrating from methylene blue solution.

*b.* Spectrophotometric measurements show that cresyl blue and azure B penetrate as such while it is chiefly azure B that penetrates from methylene blue solution which always contains azure B as impurity.

(3) *a.* 0.07% cresyl blue penetrating in 15 minutes from the dye solution made up in buffer at pH 9.2 or at pH 6.8, or in tap water, raises the pH value of the sap equally (0.5 pH).

*b.* If cells are kept 15 minutes longer in the cresyl blue solution at pH 9.2, the dye continues to penetrate and raises the pH value still more, but if cells are transferred to the buffer solution at pH 9.2, containing no dye, there is no further penetration of dye and no further increase in the pH value of the sap.

*The sap mixture.* The sap mixture behaves toward these dyes like the sap when 0.07% dye salt or free base is added to it *in vitro*.

With penetration of cresyl blue there is practically no difference between these 2 extractions. But with the 0.07% dye penetrating from azure B and methylene blue solution at pH 9.2, there is only 0.6 pH increase in the sap mixture instead of 1 pH as in the case of sap. In the case of methylene blue, this difference is not due to the additional penetration of methylene blue salt into the protoplasm, because the dye in the sap mixture gives the same absorption curve as the dye in the sap, with the primary absorption maximum at 653 to 655  $m\mu$  (characteristic of a mixture of azure B and methylene blue, with the preponderance of the former).

It is concluded that cresyl blue penetrates chiefly as free base, and that azure B also penetrates chiefly as free base from azure B solution and from methylene blue solution at pH 9.2.

In these experiments contamination of the sap by the stained cell wall was avoided as previously described.

Impurity in the free base of dye when added to the sap *in vitro* does not alter the pH value.

## 5080

### Studies on the Penetration of Dyes With the Glass Electrode. III. Penetration into *Valonia* of Cresyl Blue and Azure B.

MARIAN IRWIN.

*From the Laboratories of The Rockefeller Institute for Medical Research.*

(1) The pH value of the sap extracted from the vacuoles of small living cells of *Valonia macrophysa* is found to be pH 5.4. The pH value remains unaltered for several hours, *i. e.*, much longer than the period required for the extraction of sap and subsequent measurement with the glass electrode.

(2) 0.01% cresyl blue penetrating (in about 20 minutes) from the sea water at pH 9.5, raises the pH value of the sap 0.5 pH. This increase is 0.2 pH less than the rise brought about by the addition of 0.01% free base of cresyl blue to sap *in vitro*.

(3) 0.03% cresyl blue penetrating (in about 1 hour) raises the pH value about 0.9 pH, which is about 0.4 pH less than the rise brought about by the addition of 0.03% cresyl blue free base to sap *in vitro*.

(4) If cells are kept 8 hours longer in this cresyl blue solution, they appear to be still in good condition, so that it is likely that the cells described under (2) and (3) are not at all injured.

(5) No alteration in the pH value of the sap occurs when cells