THE PERMEABILITY OF LIVING CELLS TO DYES AS AFFECTED BY HYDROGEN ION CONCENTRATION.

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In order to determine the influence of pH upon the penetration of dyes careful quantitative studies are necessary. In view of the fact that suitable methods are lacking (the only work known to the writer is an excellent paper by Harvey,[1] which, however, is purely qualitative in character) the writer has thought it desirable to develop one, and for this purpose has employed the cells of *Nitella*. These very long multinucleate cells contain a large, central vacuole filled with sap which is surrounded by a delicate layer of protoplasm. If the end of the cell is cut off, and gentle pressure applied, the clear sap flows out, free from protoplasm or chlorophyll granules. If the cell has been previously placed in a dye which has penetrated through the layer of protoplasm into the cell the concentration of the dye in the sap may be measured in a very simple manner. A drop of the sap is taken up into a capillary tube and the color is then compared with the colors of other tubes, of the same diameter, containing different concentrations of the same dye.[2]

For the most part dyes were employed which did not change color on being mixed with cell sap. One of the most useful is a basic dye, brilliant cresyl blue, which was employed in the experiments described in this paper. This dye penetrates rapidly and accumulates within the cell sap so that its concentration becomes much greater than in the outside solution.

[1] Harvey, E. N., *Science*, 1910, xxxii, 565.

[2] When this is done it at once becomes evident that the ordinary method of placing the cell in a dye and observing it under the microscope (without squeezing out the sap) is entirely misleading. In many cases the cell appears to be deeply stained (due to the absorption of the dye by the cell wall or the cell surface) where the sap is only slightly colored. This phenomenon is much more pronounced at certain pH values.

The rate of penetration of cresyl blue increases with increasing pH (the lowest pH employed was 6.5) until, in the neighborhood of pH 10, complications begin to appear which make the determinations doubtful. Thus the rate[3] at pH 7.38 was 5 while at pH 9 it was 350, and at pH 10 it was 910.

As the pH of the cell sap is about 5.6 it is clear that the dye is unable to penetrate rapidly unless the pH outside is decidedly greater than that inside.

Interesting results were obtained in studying the exosmosis of the dye. For this purpose cells were left in 0.002 per cent of cresyl blue at pH 8.98 until the concentration inside reached 0.04 per cent. The cells were then rinsed and placed in buffer solutions (containing no dye). It was found that exosmosis presents a striking contrast to endosmosis as regards the influence of the external pH. Penetration is very rapid at pH 9, while the reverse is true of exosmosis, but at pH 5.9 exosmosis is rapid and penetration is very slow.

Further experiments are in progress which will be described in detail in a later paper.

SUMMARY.

1. An accurate quantitative method of measuring the penetration of dye into the living cell is described.

2. Cresyl blue is unable to penetrate rapidly unless the pH outside the cell is decidedly greater than that inside. The rate of penetration increases with increasing pH.

3. Around pH 9 penetration of the dye is rapid while the reverse is true of exosmosis. At low pH values (5.9) exosmosis is rapid and penetration is very slow.

[3] In this case the rate was taken as a thousand times the reciprocal of the time required (at 20°C.) to raise the concentration in the sap to 0.01 per cent when the concentration of the outside solution was 0.002 per cent. The dye was dissolved in buffer solutions. There was no injury during the period of the experiment.