THE BEHAVIOR OF CHLORIDES IN THE CELL SAP OF NITELLA.

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(Received for publication, January 20, 1923.)

The writer has sought to develop direct methods for measuring penetration and exosmosis, since they are of fundamental importance in the study of permeability. In the case of *Nitella* direct determinations of chlorides can be made as follows: The end of a long cell, 4 or 5 inches in length, is cut off and the sap is gently pressed out on to a glass slide. When this is properly done a clear liquid free from chlorophyll granules is secured. This is taken up into a very fine capillary tube until it reaches a point marked on the surface. The sap is then blown out of the tube into a small white porcelain dish and titrated with a weak solution of $AgNO_3$ (0.002 to 0.0006 M) by counting the number of drops (delivered from a burette) which are required to change the color of the indicator (potassium monochromate). In a series of determinations of normal cells the probable error of the mean was less than 3 per cent of the mean.

The chloride content of the sap was ascertained by comparing it with NaCl 0.1 M which was treated in the same way as the cell sap. This was checked by collecting enough sap to fill a 1 cc. pipette and titrating this as described above. The average chloride content of the sap was 0.128 M.

Since the cells grow in water containing chlorides in very low concentration, the high concentration within the cell must be acquired during its growth. This accumulation of chloride can be followed by titration. The experiments will be reported in a later paper.

Attempts to increase the chloride content by placing cells for 2 days in balanced solutions (at pH 6.2) containing chlorides up to 0.128 M met with no success. Stronger solutions produced plasmolysis.

By placing cells in toxic solutions and titrating the cell sap at intervals the exosmosis of chlorides may be readily followed. It was found that no chloride came out unless the cells were injured, and the progress of injury could be followed by titration.

When a cell of *Nitella* (Woods Hole), 4 or 5 inches long, is placed in water with one end projecting above the surface, it is found that if this end be cut off a wave of injury progresses downward along the cell toward the uninjured end. This is accompanied by exosmosis of chlorides. In many cases if the cell is removed from the water within 1 or 2 minutes after the end is cut off, and if this portion is divided into several pieces and the sap from each is titrated separately, it is found that the lowest piece contains the most chloride and that the amount diminishes toward the cut end.

The experiments of the writer on chlorides and other inorganic substances, as well as on dyes, suggest that the entrance and accumulation of cations (except H) is favored by solutions of high pH value, and that of anions (except OH) by solutions of low pH value. The entrance of cations may involve simultaneous penetration of OH, while that of anions may be accompanied by penetration of H. It may also happen that the penetration of an ion involves exosmosis of another similarly charged ion.

This suggests that penetration and accumulation depend upon proteins, which, as shown by Loeb, react differently on the opposite sides of their isoelectric points. This assumption may also help to explain the exosmosis of substances from the cell into solutions of varying pH values.

SUMMARY.

1. A method is given for determining the chloride content in a drop (less than 0.03 cc.) of the cell sap of *Nitella*.

2. Chlorides accumulate in the sap to the extent of 0.128 M; this accumulation can be followed during the growth of the cell. The chloride content does not increase when the cell is placed for 2 days in solutions (at pH 6.2) containing chlorides up to 0.128 M.

3. The exosmosis of chlorides from injured cells can be followed quantitatively. When one end of the cell is cut off a wave of injury progresses toward the other end; this is accompanied by a progressive exosmosis of chlorides.