INTRACELLULAR ACIDITY IN VALONIA.

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The very large cells of the marine green alga *Valonia macrophysa* permit one to obtain several cubic centimeters of fluid from the central vacuole of a single cell (cf. Wodehouse, 1917), without involving appreciable alteration in the composition of the cell-sap through protoplasmic injury during the process of extraction (cf. Chambers, 1917). Estimations have been made of the acidity of this cell-sap. These measurements, although perhaps revealing no essentially new points of theoretic interest, are nevertheless valuable, since they bear so directly upon some questions regarding the reaction of the cell interior.

The cell-sap of *Valonia* is much more acid than the sea water in which the plant is living. The reaction of the sap from fifty cells as obtained immediately after their removal from the sea was found to vary in individual cases from pH 5.0 to 6.7, the mode being at 6.0, the average 5.9. In thus possessing an acid internal medium the *Valonia* cells resemble those of some flowering plants containing natural indicators (Haas, 1916).

*Valonia* grows in great masses at a depth of several feet beneath low-water level in certain mangrove "creeks," and also, less abundantly, among other seaweeds, on the reefs. The alkalinity of the sea water in the tidal creeks depends upon the state of the tide: at flood-tide, for example, in Fairyland Creek, the water most distant from the mouth of the creek was at pH = 8.2, while that half way toward the mouth of the creek was at 8.1, and in the adjacent regions of Great Sound, 8.07; at low-tide next morning, the alkalinity at the head of the creek was pH = 8.3. A similar rhythmic altera-
tion of alkalinity, conditioned by photosynthesis, and by the pulsatile supply of "outside" water to the creek at periods of high-tide, was regularly observed. Over the reefs a less extensive daily rhythm of variation in alkalinity was noted (cf. McClendon, 1918), the reaction of the ocean water being at pH = 8.1 to 8.2; the variation here concerned depended, in part, upon the ebbing of water from semi-enclosed sounds.

The acidity of the sap of Valonia under these differing external conditions was found, however, to be sensibly the same in all cases, even when the temperature varied from 18–28°C., at different times of the year.

No effect of darkness (i.e. cessation of photosynthesis) could be detected in the sap reaction, either in the field or when the cells were kept in darkened aquaria for some hours.

For the field measurements in particular, it was necessary to use various indicators in connection with buffer solutions. The sap to be tested was secured by first rinsing a selected cell rapidly in distilled water, drying it with filter paper or a soft cloth, then puncturing the cell-wall with a glass needle, thus allowing the sap to flow out into a test-tube. The addition of 1 to 2 cc. of sea water to 10 cc. of sap is sufficient to change the pH from 5.9 to 7.0,—hence contamination with sea water had to be avoided. Carbon dioxide, moreover, is readily lost by the Valonia sap, in which it exists at a considerable tension, so that squirting the sap through the air from a small puncture in the cell was not permissible.

In aquaria, Valonia quickly increases the alkalinity, through photosynthetic abstraction of CO2, and can in this way bring about an external alkalinity of pH = 9.5, or even greater (cf. Moore and others, 1914; Crozier, 1919). But the internal acidity seems to be constantly maintained, even under these extreme conditions, at a little less than pH = 6.0. The same was found in moderately hypoalkaline sea water (pH = 7.8 to 7.9). This finding indicates that in a method of studying photosynthesis, for example, such as that devised by Osterhout and Haas (1918), the varying external reaction forming the basis of the measurements does not itself materially affect the internal acidity, the latter presumably being the immediate regulator of respiratory and other protoplasmic processes.
At the same time, it seemed from my estimations highly probable that the reaction of the sap from different healthy cells was not always the same, even with careful precautions in taking the readings. No correlation could be seen between the size of a cell and the reaction of its fluid contents—the sap from young, actively growing cells was not more alkaline than that from much larger, healthy cells.

It was shown by Wodehouse (1917) that sap from dead Valonia cells contained SO₄, while that from healthy cells did not; occasional cells, otherwise healthy in appearance, showed a trace of SO₄ when tested with BaCl₂. In many instances I found an acidity of pH = 7.0 or less (7.0 to 8.0), always accompanied by the presence of SO₄. Cells obviously disorganized, though only to a slight degree, were always in this category. During 3 years several hundred cells in all were tested. As in some other plant cells (Haas, 1916), the acidity of the vacuole content decreases as natural death approaches; the reverse occurs in at least some plant cells, and in an animal, Chromodoris (Crozier, 1918 b). In Valonia it has been clear that natural death of a cell consumes some time; the change in the acidity of the cell-sap, paralleled by the change in permeability to SO₄, being a gradual process (cf. Osterhout, 1917).

BIBLIOGRAPHY.