# PLASMOLYSIS

RICHARD KING, Maryland

The word "plasmolysis" was first used by De Vries.[1] Certain cells, as those of the primary meristem of the root of *Vicia faba*, quickly die when plasmolyzed.[2] Other cells, however, when plasmolyzed with suitable solutions of sugar, may remain living from a few days to several weeks.[3] Again, the cells of some species of Spirogyra are killed in only a few hours when plasmolyzed. Thus, in the case of what we may term artificial plasmolysis, all degrees of differences in the length of life of a cell are noticeable after plasmolysis. But there are cells also in which plasmolysis is naturally produced.[4] An example of such natural plasmolysis is to be seen in the Conjugatae, as for example in Spirogyra, when conjugation is taking place.[4] In this case, each gamete contracts considerably, due to loss of water; and to such an extent that after the complete fusion of the gametes, the resulting body is about the size of one of the original gametes. Hence a comparatively large amount of water escapes in this process without causing the death, or even apparently of any visible change, in the character of the membrane. In *Spirogyra nitida*, as has been shown by De Bary, one cell may conjugate with two other cells; so that here also the resulting zygospore formation is preceded by the expulsion of a considerable quantity of water. The loss of water through a membrane in a natural way is also to be seen in those cases in which cell formation occurs, as in rejuvenescence (in *Stigeoclonium insigne*, for instance); and which is shown in those cases of rejuvenescence where the production of the zoogonidia in Oedogonium occurs. There are other instances of this phenomenon, but it is to be observed that such direct cell formation is restricted to reproductive cells of various kinds. There is a good deal of speculation concerning rejuvenescence which does not especially concern us here; but equally cloudy also is the reason for the escape of water through the living membranes mentioned, aside from space in Spirogyra, for example. Also interesting and unanswered are questions concerning the changes in the living membrane which allow for a time the expulsion of water, and hence the marked contraction which is so conspicuous, especially in Spirogyra. Our problem, however, does not concern itself directly with cases or instances of natural plasmolysis, so called; but only to cite these by way of comparison. It will also be noted in the case of natural plasmolysis that the cell or cells concerned are not killed or apparently injured by such a change, however great that may be, or however long or short the time involved. The length of time involved in a natural

[1] De Vries, Hugo—Untersuchugen über die mechanischen Ursachen des Zellstrechung 1877 p. 10.

[2] Pfeffer, W.—Druch-und-Arbeitsleistung durch wachsende Pfanzen 1883 p. 307.

[3] Klebs, G.—Unters, a.d. Bot. Inst. in Tübringen, 1886, Bd. 2, p. 504.

[4] Pfeffer, W.—Physiology of Plants, Trans. by Ewart 1897 vol. 1, p. 143.

"Proc. Ind. Acad. Sci., vol. 41, 1931 (1932)."

plasmolysis is much greater than in those experiments where artificial plasmolysis is produced, but other very decided differences are those as above stated, in which certain artificially plasmolyzed cells die quickly, others in a few hours, while still others may live for weeks under suitable conditions. Where, however, cells are plasmolyzed artificially, the death of such cells always occurs sooner or later.[5]

The object of this paper is to show not only the character of the solutions that may be used most successfully on the plants studied, but especially to ascertain the length of life of these plants when artificially plasmolyzed in the various solutions.

**Plants Used.** The plants used for the experiments were: *Elodea canadensis, Cladophora glomerata, Pinnularia viridis* and *Spirogyra crassa.*

**Solutions Used.** In this study, four solutions were used: namely, cane sugar, sodium chloride, potassium nitrate, and magnesium sulphate.

## EXPERIMENTS AND RESULTS WITH THE VARIOUS PLANTS

**Elodea canadensis** All of the four solutions above mentioned were used in the experiments on *Elodea canadensis.* In the first test, the cells of the leaves were able to recover after being plasmolyzed for twenty-five minutes in a twenty per cent solution of cane sugar; while the cells of one leaf recovered after being plasmolyzed for forty-five minutes. Observations of still another leaf (this one a leaf from a plant that had been obtained from the creek at a more recent time than the two preceding specimens) shows that it withstood the plasmolyzed state in the twenty per cent solution of cane sugar for one hour and fifteen minutes and was still able to deplasmolyze. These three results will show the variability of the different individual leaves of the specimens on hand and of the individual plants themselves.

Thus, after being plasmolyzed in a twenty per cent solution of cane sugar for one hour and forty-five minutes, the cells of the *Elodea canadensis* leaves experimented with were killed.

It was found that those cells in the midrib, those cells bordering the midrib in the upper half of the leaf, and those cells of the lower half of the leaf were the only cells to plasmolyze to any extent in the solution used; while the remaining cells in the leaf were unplasmolyzed, or were plasmolyzed only slightly. Thus, the O. G. of the cells of the *Elodea canadensis* experimented with equalled on the average a twelve per cent solution of cane sugar.

A similar test was conducted with a two per cent solution of sodium chloride. The specimen was left in the solution for five minutes, but failed to recover from the plasmolyzed state. A ten minutes exposure in a 1.6 per cent solution of sodium chloride, however, proved successful; but an additional five minutes exposure proved fatal to the cells, as they failed to resume the natural state.

When placed in an eight per cent solution of potassium nitrate for five minutes, the cells were plasmolyzed, but recovered. They failed to even show plasmolysis after an additional fifteen minutes in this same

[5] Pfeffer, W.—Physiology of Plants Trans. by Ewart 1897, vol. 2, p. 258.

solution. This, perhaps, may be explained by the fact that a thin film of water still adhered to the leaf when it was deplasmolyzed; and in the next exposure to the potassium nitrate, the solution was unable to penetrate the cells for a time. A third attempt of two hours in the potassium nitrate caused plasmolysis and death.

In testing with a ten per cent solution of magnesium sulphate, the cells of *Elodea canadensis* were able to recover after being in the solution for ten minutes, but were killed after an additional ten minutes.

**Cladophora glomerata.** The four solutions were also used in the tests on *Cladophora glomerata*, as in the tests on *Elodea canadensis*.

The first tests were made on specimens that had been kept in the laboratory for several days.

A thirty-four per cent solution of cane sugar was able only to cause a slight degree of plasmolysis, while a thirty-eight per cent solution brought on a good state of plasmolysis. In the latter solution, the cells were able to undergo plasmolysis for thirty minutes and recover, but were killed after two hours in the same solution.

A five per cent solution of sodium chloride was required to plasmolyze the *Cladophora glomerata* cells, but they continued to live after having been in the solution for one hour, but an additional ten minutes was sufficient to kill those that had been in the laboratory for some days.

The second set of tests on *Cladophora glomerata* was conducted on fresh specimens just brought into the laboratory in order that we might compare the response of the cells in fresh plants with those in plants which had been in the laboratory for several days.

The cells of the fresh *Cladophora glomerata* were able to recover from being in a six per cent solution of sodium chloride for two hours, but were killed after a four-hour exposure. In certain other specimens just brought into the laboratory, the plasmolyzed state was able to be brought about in a forty per cent solution of cane sugar, and it was found that the cells were still alive after exposure of one hour and of two hours and thirty minutes, but were killed when left for a longer period of time in the solution.

In testing with an eighteen per cent solution of magnesium sulphate, it was found that the cells could withstand the solution for three hours and still recover from the plasmolyzed state, but were killed when allowed to remain four hours.

Several tests had to be made when solutions of potassium nitrate were used. One test, however, was able to show that the cells deplasmolyzed slowly after an exposure of one hour and thirty minutes in a sixteen per cent solution, but were killed when left for twenty minutes longer. The degree of plasmolysis varied from cell to cell, and from extreme plasmolysis to none at all.

**Pinnularia viridis.** Only two of the solutions were used in the tests on *Pinnularia viridis*—cane sugar and potassium nitrate.

Much difficulty was encountered in obtaining plasmolysis and recovery, and it was possible throughout the experiments to observe only a very few plasmolyzed forms. These were obtained in a twenty per cent solution of cane sugar and in a five per cent solution of potassium nitrate.

When exposed to the cane sugar solution, the movement of the diatom was stopped during the plasmolyzed state, but was slowly resumed after recovery. It was further seen that movement was also stopped in higher concentrations than twenty per cent—as, for example, twenty-five per cent, which was the O. G. of the specimens experimented on. Isosmotic solutions stopped the movement.

The results of the tests with the solutions of potassium nitrate were similar to those obtained with the cane sugar solution. Movement was arrested during the plasmolyzed state in the five per cent solution and was resumed again after recovery.

**Spirogyra crassa.** The four solutions were used for the tests on *Spirogyra crassa* as for *Elodea canadensis* and *Cladophora glomerata.*

Of *Spirogyra crassa*, both fresh specimens (i. e., those which were just brought into the laboratory) and other specimens, which had been in the laboratory for several days, were used. The O. G. of the specimens tested equalled, on the average, eleven per cent of cane sugar. Individual specimens, or even certain cells, showed at times considerable variation from this per cent.

In cane sugar, the cells lived one hour and thirty-five minutes when plasmolyzed; when plasmolyzed in magnesium sulphate, they lived one hour and seven minutes; when potassium nitrate was used, they lived forty-five minutes; and when in the solution of sodium chloride, the cells were able to survive, on the average, about twenty-one minutes.

**Conclusions.** In summing up the results of these tests in which I have attempted to show the character of the solutions that may be used most successfully on the plants, and also to ascertain the length of life of the plants when artificially plasmolyzed in the solutions, I have found cane sugar[6] to be the least harmful of the four solutions used, since the specimens were able to undergo the plasmolyzed state for a longer period in the cane sugar and yet survive than in any of the other solutions. While magnesium sulphate was the next least harmful, with potassium nitrate a close third; since the solutions of magnesium sulphate and potassium nitrate averaged about the same in their effects on the plants. Sodium chloride was found to be the most harmful of the four solutions used.

As to the second point which I have attempted to bring out, I have found that *Spirogyra crassa* is the least resistant of the plants tested, and also, as was seen in *Elodea canadensis* and *Cladophora glomerata*, that fresh specimens which were just taken from the creek were more vigorous and resistant than specimens which had been kept in the laboratory for some time.

In order to arrive at the safe averages that I have obtained in this series of tests, it was necessary to test a great number of plants in the various solutions, so that I might overcome the difficulties encountered.

I wish to express my deep appreciation of the kind assistance and advice of Dr. F. M. Andrews, professor of plant physiology, Indiana University.

[6] Beck, W. A.—Cane sugar and potassium nitrate as plasmolyzing agents. Protoplasma, 1926, 1: 62.

(Beck found cane sugar to be superior to potassium nitrate for his work in plasmolysis.)