A STUDY OF THE EFFECT OF EXUDATION OF WATER FROM PLANT CELLS BY FREEZING

A thesis

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by

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Analytical Table of Contents

History

Müller-Thurgau (1)* published the results of his investigations in 1886 concerning the effect of frost upon plants. His work was confirmed by that of Cavallero (1)* in 1888. These two men found that a cell did not suffer injury from the formation of ice crystals in the protoplasm. They also found that under the action of frost a rupture of the cell is very rare. Their results show that the formation of ice crystals occur in the intercellular spaces of the tissue of both hardy and delicate plants.

Abbe (1) summerizes the conclusions drawn by these two men as follows: Water exudes from the cell as the temperature is lowered. As a result, the contents become concentrated and injury is affected through chemical changes of the protoplasm. The water exuded from the cell is frozen in the intercellular spaces. If the temperature becomes very low, the protoplasm may freeze. The ice crystals formed in the protoplasm fill the space left by the exuded water. The cell wall is rarely broken for this reason. Some water may return to the cell but the majority is transpired or evaporates from the tissue as the ice crystals melt.

Müller-Thurgau concludes from his numerous experiments concerning freezing, that the exudation and freezing of water from a cell results in the death of that cell. Some few cases

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* Müller-Thurgau and Cavallero mentioned by Abbe in The Influence of Cold on Plants.

have been reported since the time of Müller-Thurgau in which cells have experienced an exudation of water caused by low temperature without the formation of ice crystals. This was found to be true by Greeley (3) who shows that if the animal Stentor is subjected to a temperature near 0° C., it will contract and become cyst like, probably with the exudation of water.

Livingston (5) has shown that when Spirogyra is mounted in oil and subjected to a temperature near 0° C. for six hours, the protoplasm will contract with the exudation of water drops into the oil. Molisch (10)* found that in some stamen hairs of Tradescantia subjected to a temperature of -5° C. for six hours the plasma membrane separated from the wall but no freezing took place until a temperature of -15° C. was reached. If subjected to a temperature at which crystals were formed immediately, the separation of the membrane from the wall occured rapidly.

Wiegland (10) believes that the extraction of water from a cell by freezing is a vital factor in the life of that cell.

Pfeffer (9) also supports the fact that dessication of a cell by freezing is a vital factor in the life of that cell. He states that owing to the progressive withdrawal of water with decreasing temperature, all plants which cannot withstand dessication must ultimately be killed by cold.

Harvey (4) states that the precipitation of proteins in

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* Molisch mentioned by Wiegland in The Passage of Water from Plant Cells During Freezing.

plant cells is a vital factor in connection with the death of cells by freezing. He states that the precipitation of proteins is due to an increase in the salt and hydrogen ion concentration of the protoplasm. Such an increase in the concentration of salts and hydrogen ions is caused by plasmolysis or dessication of the cells during freezing. The extraction of water from plant cells during freezing must be a vital factor in such a chemical change.

Meyer (6) has recently worked on the hardening process of Pitch Pine. He states that the seasonal variation in the relative proportion of bound water is the most important factor in the cellular physiology of the leaves of this species in relation to cold resistance. Meyer believes that the protoplasm of the cells in Pitch Pine leaves increases in colloidal content during the cold months. Such an increase in the colloidal content offers resistance to the extraction of water from the protoplasm of the cells.

Gail (2) worked on the osmotic pressure of the cell sap of the leaves of some non-deciduous trees and shrubs. He found higher osmotic pressure during the winter months and lower osmotic pressure during the summer months.

Newton (8) studied the effect of low temperature on winter wheat. He found that wheat which had been subjected to low temperature for some time was resistant to injury by freezing.

Introduction

Muller-Thurgau and Cavellaro found that ice crystals form in the intercellular spaces of plant tissue as the temperature is lowered. Their results are generally accepted today. Wiegland (10) states that there is no doubt but that cells exude water during freezing. There is, however, some doubt concerning the cause of death to cells as ice crystals are formed in the intercellular sapces of the tissue. It is desirable to know the most important of these in studying the resistance of plants to freezing.

A few cases are known where a contraction of protoplasm has been observed without the formation of ice crystals. This does not warrent us to conclude that all cells are affected in the same manner at the same temperature. The writer is unable to find any record of living cells whose protoplasm could not be contracted if the formation of ice crystals was sufficiently severe outside the cell.

Wiegland (10) states that water on the outer surface of the cell wall contains a relatively small amount of solute when compared to the protoplasm. As a result, ice crystals form on the outer surface of the cell wall when the tissue is subjected to a freezing temperature. He states that this formation of ice crystals causes water to be removed from the cell wall. The equilibrium in the water content of the wall and protoplasm is disturbed in this way. Water, which possibly contains some dissolved salts, is then drawn from the protoplasm by imbibition into the inner surface of the wall to re-establish the equilibrium between the water content of the wall and the protoplasm.

The process continues as ice crystals form outside the cell. The protoplasm is contracted as a result.

Method

There are other reactions apparent in plant tissue subjected to freezing which the writer has been unable to find in the literature. Several experiments are cited which may indicate the nature of these reactions and throw some light upon their importance in connection with the death of the tissue caused by freezing.

Filaments of Spirogyra were mounted on a slide in water in which the plant normally grew as a mounting medium. The filaments were examined under high power of a compound microscope to determine if the cells were in a normal condition. The slide was then placed in a large test tube, six inches long and two inches in diameter. A cork, through which a thermometer had been inserted, was placed in the test tube. This made it possible to acertain the temperature of the atmosphere in the test tube. Normal cells of Spirogyra mounted in this way were subjected to a gradually decreasing temperature by placing the test tube containing the slide in an ice salt bath.

Microscopic examinations of the same cell were made at different intervals. The cell remained normal until a temperature was reached at which ice crystals were formed in the water outside the cell wall. Plate 1 shows camera lucida drawings of two cells (1 and 4) which have been subjected to a temperature of -3° and -4° C. respectively. Figure 2 shows that the protoplasm of the cell experienced a slight con-

traction at a temperature of -3° C.. Numerous tests proved that no contraction of the protoplasm took place in the filaments studied until a temperature of -3° C. had been reached. Ice crystals were always evident outside the filament at this temperature. Cells subjected to very low temperatures produced by carbon dioxide gas were all severely plasmolized. The amount of solution exuded was found to vary directly as the temperature was lowered.

The slides containing the Spirogyra cells were now placed in room temperature and the crystals allowed to melt. The plasma membrane returned approximately to its normal position. Three definite reactions have occured in the cell as follows: (1) an exudation of solution from the protoplasm caused by freezing, (2) an absorption of solution as the ice crystals surrounding the cell melt, and (3) an absorption of solution by the plastids. Plate 1 Figure 3 and 6 show cells after these reactions have taken place. The plasma membrane is very slightly contracted and the chloroplastids have definitely changed their form. The cells were now placed in normal conditions for seventy-two hours. At the end of this time, the chloroplastids appeared as rounded bodies.

The first reaction caused by freezing in the cells of Spirogyra is an exudation of water and solute which has been described. It is evident from this experiment that a slight exudation of solution from the protoplasm occured at the temperature of -3° C.. Osterhout (7) has shown definitely that some dissolved substances pass into the protoplasm of living plant cells. This fact offers reason to believe that the

liquid exuded by a cell during freezing is not pure water but is more concentrated than the solution immediately surrounding the cell wall.

If the ice crystals are now allowed to melt outside the cell, water or a very dilute solution is reabsorbed into the protoplasm by osmosis. Plate 1 Figure 3 shows that the plasma membrane does not quite return to its normal position as a result of taking up this dilute solution. This fact may be explained in the following manner. The solution is withdrawn from the protoplasm of the cell during the process of freezing. The solution thus exuded is diluted by the water present on the outer surface of the cell as the ice crystals melt. Part of this dilute solution is then reabsorbed into the protoplasm of the cell by osmosis. The amount of solute present in the cytoplasm is lowered in this manner.

Such a reduction in the concentration of solute present in the cytoplasm may explain the behavior of the plastids in these cells following the freezing and thawing of the tissue. The chloroplasts decrease in length and increase in width following the melting of the ice crystals surrounding the cell. The nucleus, which is normally of a convexed lense shape, becomes spherical. Plate 1 Figures 3 and 6 show cells which contain plastids in such a condition. The following experiments are cited to show that a reduction in the concentration of the cytoplasm of cells of Spirogyra will produce such an effect upon the plastids.

Filaments of Spirogyra were mounted in water in which they naturally grew. A normal cell was then injured mechanically by

breaking a hole in the cell wall. Water was allowed to come in contact with the cytoplasm in this manner. Care was taken to avoid injuring the chloroplastids. Within two minutes the chloroplastids began to decrease in length and increase in width. The nucleus also became spherical in shape. Cytoplasm exuded through the hole in the cell wall in severe cases. The final condition of the plastids was similar to that produced by freezing which is shown in Plate 1 Figures 3 and 6.

The results of this experiment appear very reasonable if we consider that there is normally an osmotic equilibrium maintained between the chloroplastids and the cytoplasm in which they rest. This equilibrium may be varied by changing the concentration of the cytoplasm. The swelling of the cytoplasm of the cells used in the experiment just described shows that water had been taken up. The concentration of the cytoplasm is reduced in this way. The chloroplastids and nucleus then took up water to establish a new equilibrium. Their appearance resembles that produced in cells subjected to low temperature.

The same effect was produced in chloroplastids when they were placed in a solution of a known concentration which was lower than the protoplasm. The concentration of the protoplasm of the cells used was found to be 6.2660 atmospheres when determined by plasmolysis with sodium chloride solution. The concentration of the water in which the plants naturally grew was determined by the use of a Bechman thermometer, and found to be 0.2410 atmospheres. The normal cells of the plant were now mounted on slides in the water in which they naturally grew.

The chloroplastids were then forced outside the ruptured cell wall of several cells. This was accomplished by pressing on the coverslip. The chloroplastids enlarged due to absorbed water as they came in contact with the dilute solution outside the cell. They immediately decreased in length and increased in width. The effect on the plastids appeared to be the same as that produced when the wall of the cell was injured and the concentration of the cytoplasm was reduced.

Another experiment was conducted in which the concentration of the cytoplasm of cells of Spirogyra was definitely lowered. Normal cells of the plant were placed in distilled water from a metal still. The water contained two parts of solid matter per two million parts of liquid. Within twenty-four hours the chloroplastids assumed a position shown in Plate 2 Figure 1. The nucleus became spherical in shape. Plate 2 Figure 2 is a drawing of a cell having been in distilled water eighty-six hours. Plate 2 Figure 3 is a drawing of another cell after having been in distilled water thirty-two hours. This cell was then placed in water in which the plant naturally grew and kept in a normal condition. Plate 2 Figure 4 shows the same cell after having been in a normal condition for seventy-two hours. The cell did not recover from this condition.

It is known that the medium immediately surrounding the chloroplastids became less concentrated in the two experiments just described. The plastids took up water by osmosis. The shape of the plastids was definitely changed by this process. The change in the shape and size of the plastids due to this absorption of water appears similar to alterations in the shape

and size of plastids produced by freezing and thawing.

Cells in a filament of Moss protonema were found to contain chloroplastids having an average length of five microns. The average width of the chloroplastids was two and five tenths microns. The slides were subjected to a temperature low enough to freeze the water in which the filaments were mounted. The cells were slightly plasmolized in this way. It was found that as the ice crystals melted from the slide, the cells partly regained their normal turgidity. The chloroplastids took up water and became round. The average diameter of the plastids in this condition was found to be three and eight tenths microns.

These experiments show that solution was exuded from the cytoplasm of cells of Spirogyra and Moss protonema as freezing took place. The solution thus exuded became less concentrated when the ice crystals surrounding the cells were allowed to melt. The dilute solution thus formed was then partly reabsorbed by the protoplasm. The concentration of the cytoplasm was lowered as a result. The equilibrium between the plastids and the cytoplasm was disturbed. The nucleus and chloroplastids took up water as a result.

It has been found that cells of Moss protonema and Spirogyra are not injured by short exposures to freezing temperatures unless such cells experience a contraction of the protoplasm due to the formation of ice crystals. Very slight plasmolysis incurred by freezing in the cells of these plants was found to be fatal. The point at which there is a visible plasmolysis of the protoplasm of a cell subjected to a decreas-

ing temperature appeared to be a close index of the temperature at which the cell is injured.

It is evident that some plants are able to increase their resistance to low temperature during the winter months. Meyer (6) found that the leaves of Pitch Pine were frozen at a relatively high temperature during the summer. The same temperature produced a very slight effect upon the leaves during the winter months. Gail (2) found the leaves of various non-deciduous shrubs are able to resist lower temperature during the winter months than during the summer. Harvey (4) states that cabbage plants increase their resistance to cold when they are subjected to a moderately low temperature for a week. Newton (8) found winter wheat could be injured by a relatively high freezing temperature during the summer months. The same wheat was not injured during the winter when subjected to a theoretical temperature of -54.9° C..

Various suggestions are to be found in the literature concerning the factors which may cause such an increase in the resistance of plants to cold. Some of these factors are: an increase in colloidal content of the cell; an increase in the osmotic pressure of the cell sap; a decrease in the ease with which proteins are precipitated from the cell sap.

It has been found in unpublished work of Dr. F. W. Gail and the writer that the osmotic pressure of some plants increased as the concentration of alkalies in the soil in which the plants were growing was increased. The following experiments were conducted in an endeavor to determine if the resistance offered by the plants studied to freezing was in any appreciable

degree due to an increase in the osmotic pressure of the cell sap in these plants.

Flax seeds were planted in soil which was free from alkali and also in soil which contained a relatively large amount of alkali. The analyses of the soils used are given in Table 4. The external conditions were kept the same throughout the experiment since the plants were grown in the Department green house. The seedlings in the neutral soil were an inch and one half high at the end of three weeks, while those in the alkaline soil were only one inch high.

The osmotic pressures of the protoplasm of the cells of the plants in the neutral and alkaline soil were determined in the following manner. Thin sections were made through the mesophyll of the cotyledons. These sections were mounted in water solutions of sodium chloride of concentrations ranging from .20 M. to .51 M.. The concentrations of the sodium chloride solutions used varied as follows: .20 M., .21 M., .22 M. etc. to .51 M. solution.

A solution of sodium chloride was selected whose concentration would just slightly plasmolize the cells of the tissue which contained chloroplasts. The osmotic pressure of this isotonic solution was then determined by the freezing point depression. A standard Bechman thermometer was used to obtain the freezing point depression. The results are given in Table 4. It may be seen that the atmospheres of osmotic pressure of the protoplasm in cells from plants grown in alkaline soil is nearly two times greater than the atmospheres of pressure in cells of plants grown in neutral soil. An endeavor was now made

to determine if the increase in concentration would appreciably lower the freezing point of the plant tissue.

The actual freezing point of the tissue of the plants grown in the two different soils were determined in the following manner. Sections were made through the mesophyll of the cotyledons of plants grown in the respective soils. These sections were mounted in distilled water and were found to remain normal in this condition for thirty minutes. The time required to conduct the following experiments was never longer than fifteen minutes for each reading. The slides prepared in this manner were subjected to a gradually lowering temperature. A point was microscopically determined at which slight plasmolysis took place. The formation of ice crystals at this temperature was evidently sufficient to withdraw solution from the protoplasm of these cells.

Table 4 shows that the temperature at which injury took place in cells of plants grown in alkaline soil was -6 C.. The critical temperature of cells of plants grown in neutral soil was -5 C.. The table also shows that the difference in the osmotic pressure of the cell sap of plants grown in the respective soil is 9.549 atmospheres. It is evident from these results that an increase of 9.549 atmospheres in the concentration of the cell sap of flax lowered the temperature at which injury occured one degree. Such a slight resistance produced by increased osmotic pressure will only partially explain why the critical temperature of some plants is decidedly lower during the winter.

The osmotic pressure of Moss protonema was also studied in

connection with their resistance to low temperature. The concentration of the protoplasm of the cells was determined by the use of isotonic solutions. A balanced solution was prepared by adding 95 parts of 1 M. NaCl to 5 parts of 1 M. $CaCl_2$. The balanced solution was then diluted until a concentration was reached which would very slightly plasmolize the cells. Such a concentration was produced by adding 13.5 cc. of water and 5 cc. of the balanced solution. This solution was selected as representing the osmotic pressure of the protoplasm of the cells of Moss protonema grown in the laboratory. Moss protonema which was grown out of doors during the spring months and accustomed to low temperature was collected. The plants grown in the laboratory and those grown outside were placed side by side in various concentrations of the balanced solution. The plasmolysis produced by corresponding concentrations of the balanced solution was the same in both plants. A solution composed of 13.5 cc. of water and 5 cc. of balanced solution was found to represent the osmotic pressure of the protoplasm of protonema growing in the laboratory and that growing outside. An isotonic solution was also prepared from a 1 M. solution of NaCl and of a 1 M. solution of $CaCl_2$. The isotonic solutions of these pure salts also indicated that the osmotic pressure of protonema growing inside was the same as that of protonema accustomed to the lower temperatures.

Filaments of the plants were then mounted on slides. The water in which the Moss protonema was growing was used as a mounting medium. The slides were then subjected to low temperature by placing them in a large test tube previously described.

The cells of Moss protonema growing in the laboratory were plasmolized by a temperature of -5°C.. Cells of Moss protonema grown outside and accustomed to low temperature were treated in the same manner. The cells were not affected until a temperature of -10°C. was reached. Very slight plasmolysis produced by freezing was fatal to the life of the cells studied.

The results show that a temperature at which injury occured in the cells of plants accustomed to low temperatures was five degrees lower than the temperature at which injury occured in the cells of plants grown in the laboratory. The osmotic pressure of the protoplasm of the plants grown under the respective conditions was the same. We may conclude that Moss protonema are able to increase their resistance to freezing. This increase in resistance was not accompanied by an increase in the osmotic pressure of the protoplasm.

Conclusions

1. The protoplasm of cells of the plants studied may be plasmolized by the formation of ice crystals in the solution immediately surrounding the cells. Plasmolysis is not visible in these cells until ice crystals are formed outside of the cell wall.

2. The plants studied do not experience injury from a rapidly decreasing temperature until plasmolysis occurs.

3. A very slight plasmolysis caused by freezing proves fatal to the cells of Moss protonema and Spirogyra.

4. The temperature at which there is a visible plasmolysis of a cell appears to be a close index to the temperature at which the cell is injured.

5. Three reactions are evident in the cells studied during the process of freezing and thawing. First, there is an exudation of solution from the protoplasm due to the formation of ice crystals outside the cell walls. Second, the solution which is drawn out of the cell in this way, becomes less concentrated as the ice crystals melt and is partially reabsorbed into the protoplasm by osmosis. The concentration of the cytoplasm may be reduced in this manner. Third, the plastids now take up water to re-establish the equilibrium.

6. Increased osmotic pressure is only a slight factor in the resistance to freezing in these plants studied.

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Plate 1.

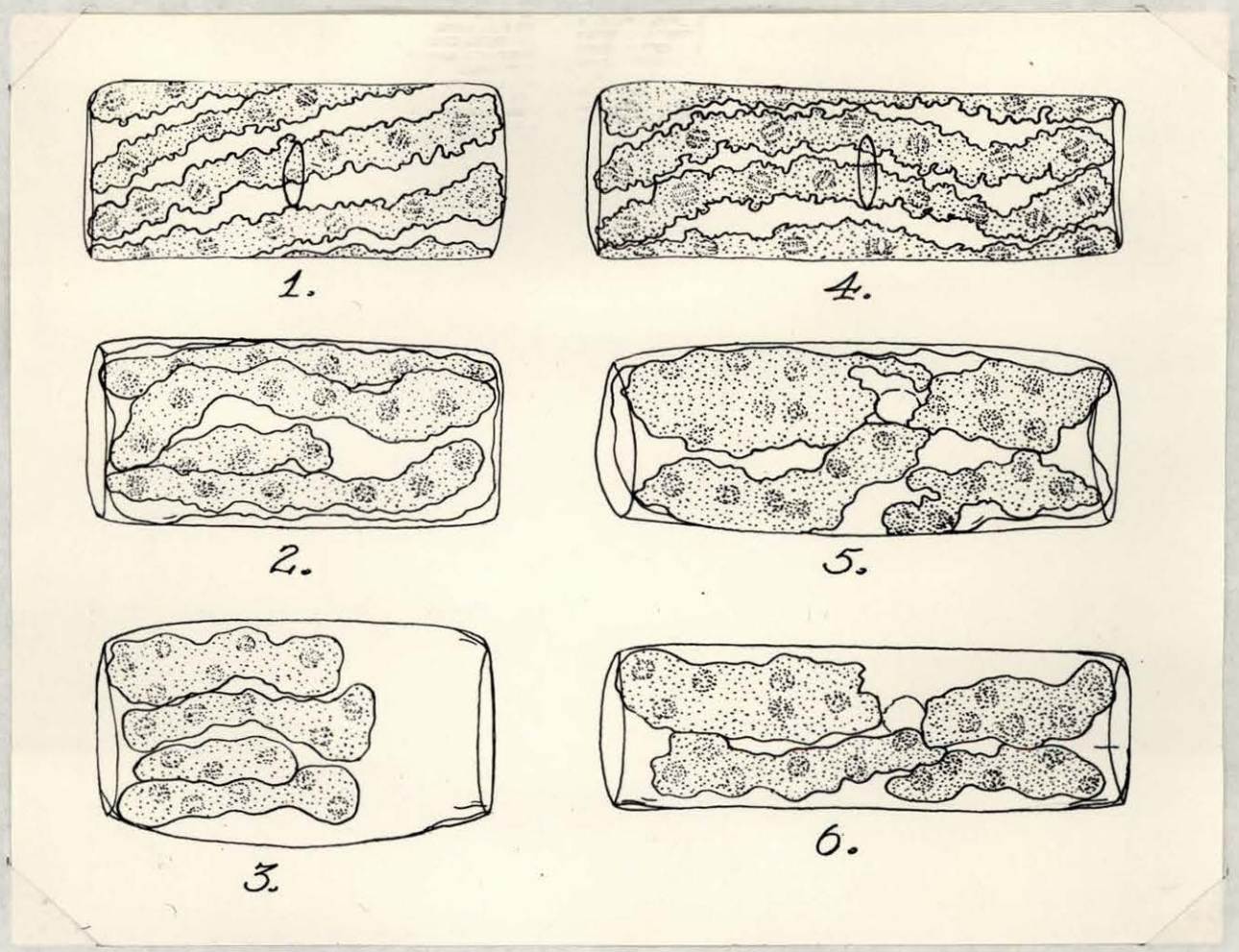
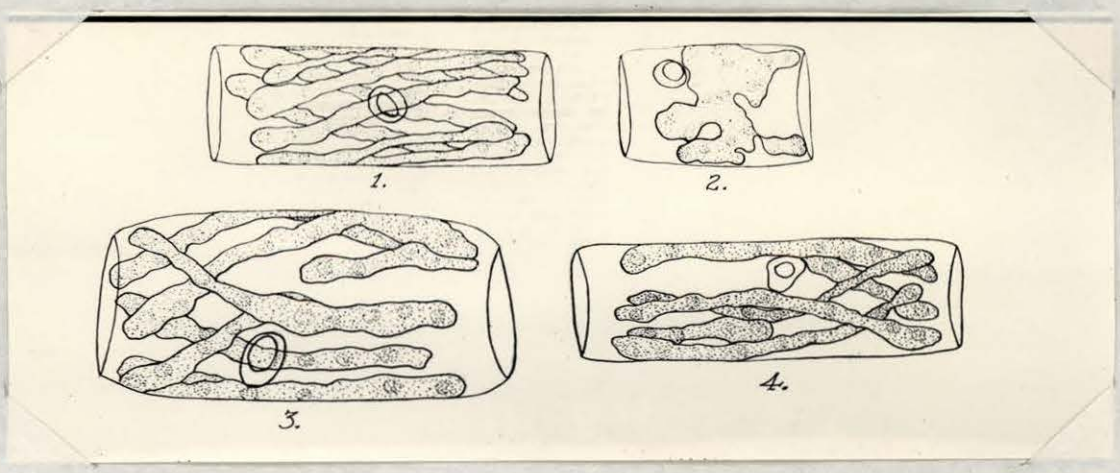


Figure 1 shows a normal cell of Spirogyra. Figure 2 shows the same cell after being subjected to a temperature of -3° C. Figure 3 shows the cell after ice crystals had melted from the slide and the temperature had returned to normal. Figures 4,5 and 6 show a cell subjected to a temperature of -4° C. and allowed to return to normal temperature in a similar manner.



Figures 1 and 2 show cells of Spirogyra after having been in distilled water twenty-four and eighty-six hours respectively. Figure 3 shows a cell after having been in distilled water thirty-two hours. Figure 4 shows the same cell after having been in normal conditions for seventy-two hours.

Table 4.

	Normal Soil	Alkali Soil
Alkali	Percent Present	Percent Present
CO_3	0.000	0.006
HCO_3	0.018	0.490
Cl	0.004	1.290
SO_4	0.006	1.950
	Atmospheres Osmotic Pressure of Isotonic Solution	Atmospheres Osmotic Pressure of Isotonic Solution
	11.312	20.816
	Temperature at which Plasmolysis Occured	Temperature at which Plasmolysis Occured
	-5° C.	-6° C.

This table shows the chemical analysis of alkali salts contained in the soils used and the atmospheres of osmotic pressure of isotonic sodium chloride solution representing the concentration of the protoplasm of the cells studied. It also shows the temperature at which plasmolysis occured in the cells of the Flax plants grown under the respective conditions. The analysis of Palouse silt loam given in the above table under Normal Soil was taken from the work of Neidig and Magnuson (11) published in Soil Science.

The writer wishes to acknowledge his gratitude to Dr. F. W. Gail, under whose supervision the work was carried on and to Professor H. P. Magnuson and Otto R. Brown, by whom many helpful suggestions were made.

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