THE STUDY OF CICATRIZATION IN PLANT TISSUES

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The purpose of this study was to observe the processes by means of which plants heal their wounds and protect the tissues in the vicinity of such wounds from harmful factors. The comparison was made of such processes in a small herbaceous land plant, *Oxalis,* and the water plant, *Elodea.* The leaves of the said plants were either cut with scissors or pierced with a needle so that the perforation passed entirely through the leaf. Then the operated leaves were cut off and fixed 10 minutes after the operation, 20 minutes, half an hour, one hour, two hours, and so on up to 24 hours, and then one day, two days, three days, and so on. The fixative used was almost exclusively acetic alcohol. However, in some cases the sublimate acetic was also used, as intended preparation to the Feulgen reaction. The stain used was exclusively the Harris Modification of Hematoxylin of Delafield. The leaves of both plants were thin enough to allow the total preparation, especially in the case of *Elodea.* In the case of *Oxalis,* which is less transparent, the method of microtome sections was sometimes used, the sections being prepared from 7 to 15 microns thick. These sections were also stained with Hematoxylin of Delafield, sometimes after sectioning, sometimes previously, staining the leaf in total and then preparing the sections. In all cases the stain was differentiated by means of alcohol acidulated with hydrochloric acid (2 drops per 100 cc.).

Another purpose of this study was to see if some kind of regeneration or new formation would take place at the operated points of the leaves as has been described in some cases, in *Begonia* and some other plants. During these experiments, no regeneration took place either in *Oxalis* or in *Elodea,* the process reducing itself to the formation of a protective layer. This process proved to be completely different in the land plant and in the water plant.

In the land plant, *Oxalis,* from the biological standpoint, showed nothing but rapid dying away of the cells surrounding the wounds owing to the rapid loss of water because the epidermis was destroyed on both sides of the leaf. The cells thus exposed to drying rapidly shrank, the protoplasm condensing while the central vacuole containing the cellular sap, and normally occupying the greater part of the cell, diminished and finally disappeared. The diminishing of protoplasm in volume caused the chlorophyll grains to be brought together and to become concentrated. Then the cell became an irregular body containing the nucleus, which also began to shrink in its central portion, but no process of any kind took place in them. Finally,

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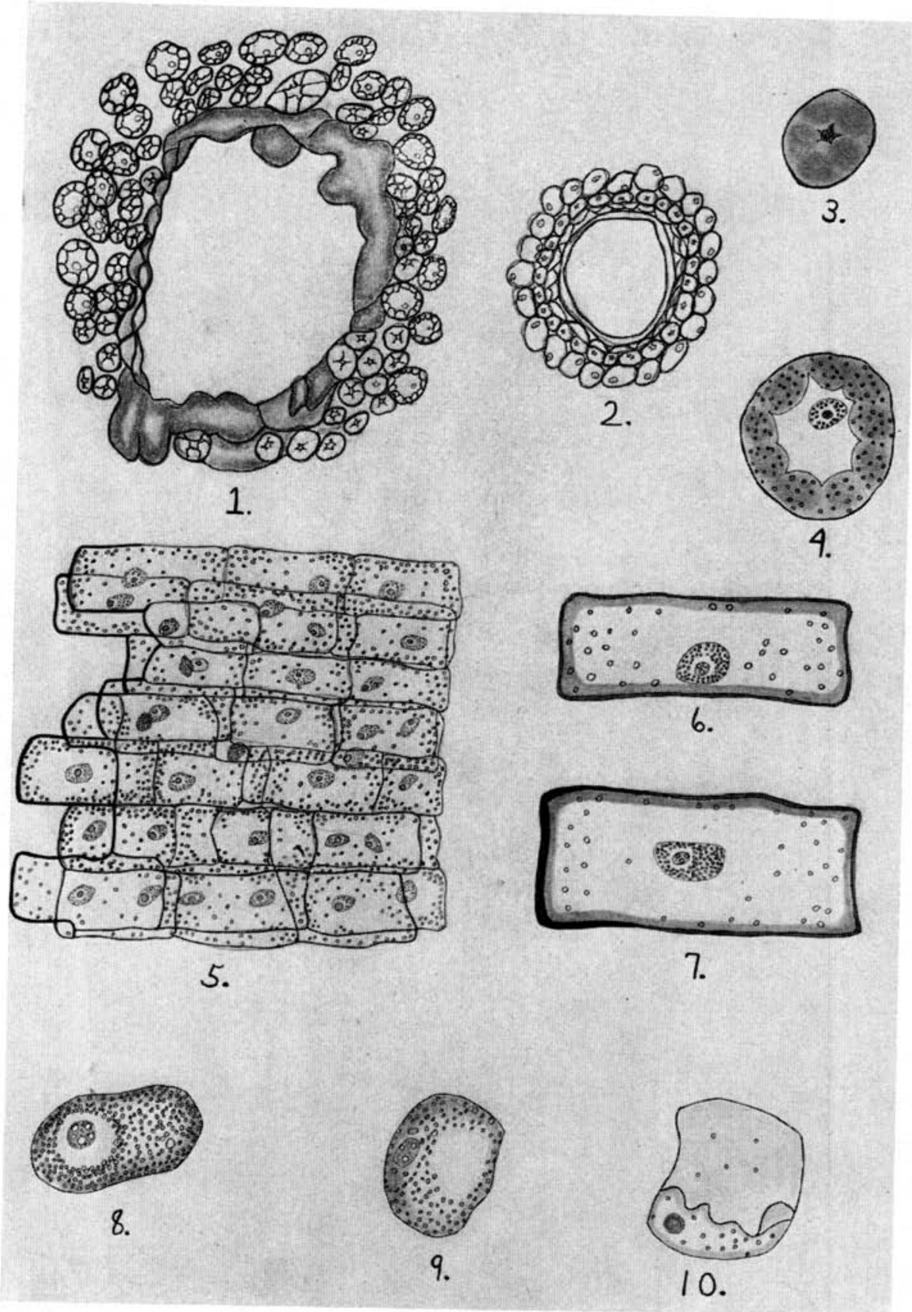

FIG. 1.—Partly schematized drawing of the perforated wound in a leaf of *Oxalis*.
FIG. 2.—Schematized drawing of the preceding.
FIGS. 3. and 4.—Parenchyma cells of Oxalis at different stages of contraction owing to evaporation through the wound.
FIG. 5.—Drawing of a portion of an incised edge of an *Elodea* leaf.
FIG. 6.—Normal cell of *Elodea*.
FIG. 7.—*Elodea* cell with part of membrane thickened because of contact with water.
FIG. 8.—Normal nucleus of parenchyma cell of *Elodea*.
FIG. 9.—Beginning of degeneration of same.
FIG. 10.—Nucleus in advanced stage of degeneration.

the nucleus was completely crushed by the hardening protoplasm and the whole cell became a small irregular clot. The adjacent cells, undergoing a similar change, fused with it and all together formed a continuous layer of substance in which the individual cells could no longer be distinguished. However, for some time the remainder of the nuclei were still seen in stained preparation as irregular granular masses.

This layer of substance, mostly proteic in nature, was nothing but coagulated protoplasm, which also contained cellular walls and therefore was partly cellulose. Then it dried up and formed a hard crust completely covering the surface of the wound. Nevertheless, this protective layer seemed to be not quite adequate to stop the evaporation until it became thick enough. The whole process was very rapid, taking only 10 to 20 minutes. A few days following the operation, the protective layer extended itself over a few more layers of cells which underwent the change described. Here the process stopped, because of the sufficient thickness of the protective layer which prevented the further evaporation of the water.

Figure 1 is a drawing of the wound as it appeared in the leaf of *Oxalis* when pierced with a thin needle. Figure 2 is a schematic representation of the same wound. The first layer of substance which can be seen surrounding the opening is simply a mass of cells crushed by the needle. Outside of this is a layer of cells which died because of the loss of water due to evaporation, and which form a protective layer. Adjacent to the latter layer appear the cells which have already shrunk but still can be distinguished individually. Each nucleus is seen as an irregular stellate body in the center. The outside layer contains living cells not yet affected by the process. The formation of the first protective layer was exceedingly fast, taking place in 10 to 20 minutes; then the only change observed during an interval of time varying from several hours to several days was growth of the layer. No reproduction of the surrounding cells was observed nor any tendency toward the regeneration.

As a conclusion it can be stated that the healing process of the wound in this small herbaceous plant is not biological in nature and cannot be termed as a true cicatrization process because it is simply a physical one consisting of loss of water due to evaporation through the wound, accompanied first by coagulation and then drying up of the protoplasm of the affected cells. However, the fact that these cells fused together allows the formation of a quite adequate protective crust which prevents further evaporation on one hand and on the other gives an adequate protection against the penetration of bacteria and fungi. The only biological phenomenon which takes place is of a necrotic nature. It is a rapid dying away of the cells directly or indirectly affected by the lesion. The extreme rapidity of the formation of the protective layer permits a comparison of it to a certain degree with blood coagulation rather than with cicatrization. However, the elements involved and the nature of the process are entirely different.

A control experiment was carried on by submerging and perforating the plant in water. No formation of a protective layer took place under these conditions, while the exposed cells suffered the process of gradual disintegration. Therefore, it may be concluded that the principal factor in the formation of the protective layer is the evaporation of water through the wound.

The second part of this work was directed to the study of the same process in a water plant, *Elodea*. The technique was essentially the same. The young leaves of *Elodea* were either cut or prickled and then fixed after regular intervals of time. The fixative and the stain was the same as for *Oxalis*. The process of the wound healing in this case proved to be entirely different than the one observed in the land plant, taking a considerably longer time, and being purely biological in nature and justifying the term "cicatrization." The cells adjoining the wound and exposed to direct contact with water did not die in most cases, but responded to the water stimulus by the thickening of that part of the cell wall which is exposed to such contact. In some cases, as can be seen on the illustrated figures, the cell wall became two or even three times thicker and also stained much more intensely than the ordinary cells. In some cases, it was also observed that the membrane became multilayered. This process of thickening or reinforcement of the membrane implies the local process of secretion, the whole response of the plant in this case being of a purely biological nature. Nevertheless, it must be observed that some cells apparently are unable to respond quickly enough and die away. Then the adjoining cells reinforce their walls and in this way stop the process of destruction. The nuclei of the cells which could not respond become more compact and refringent, later acquiring an irregular outline and finally being destroyed.