REGENERATION IN
SPLACHNUM AMPULLACEUM (L.) HEDW.

MARY MARGARET MACNUTT
CULTURAL DE DIFFERENTIATION AND REDIFFERENTIATION IN EUPHORBIA MILII (L.) MILL.

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This thesis is submitted in partial fulfilment of the requirements for the degree of Master of Science in Botany.

Dalhousie University
September, 1959.
The phenomena of dedifferentiation and redifferentiation in *Sphagnum aquilicaule* are discussed. A quantitative study of changes in the size and number of the main cellular constituents as well as changes in their position are investigated in mature leaf cells during regeneration. Cell division contributing to regeneration are studied by a comparison of apical and basal leaves.

The isolation of parts from the whole plant is necessary for cellular changes during dedifferentiation. These changes include increases in size and number of the various cell constituents, and cytoplasmic strand formation and syatropha. Syatropha is considered as an important event in cellular dedifferentiation. Isolation causes a loss of polarity, the reestablishment of which is necessary for redifferentiation.

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The isolation of parts from the whole plant is necessary for the initiation of cellular changes during dedifferentiation. These changes include increases in size and number of the various cell constituents, and cytoplasmic strand formation and systrophe. Systrophe is considered as an important event in cellular dedifferentiation. Isolation causes a loss of polarity, the reestablishment of which is necessary for redifferentiation.

It is concluded that wounding does not play an important role in dedifferentiation, but it is responsible, at least, in part for the reestablishment of polarity which leads to differential division and redifferentiation.
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The progressive development of the plant leads to an increase in the heterogeneity of its parts. This increased complexity of parts is due to differentiation at the cellular, tissue, and organ level. By definition, differentiation is the process by which the embryonic cell or cells of an organism are changed in a gradual manner to the mature tissues and organs characteristic of that organism. The study of differentiation is, in general, of basic importance for an understanding of plant development.

The process of differentiation which occurs during normal plant development is an orderly one. It does not follow a random course; it follows an almost unvarying sequence of events which result in visible patterns in the distribution of differentiated structures. But differentiation is not always a one way process. Once the mature state has been attained most cells still have the ability to regress to the undifferentiated, embryonic condition. The process which involves a return to the embryonic condition is called dedifferentiation or reembryonalization. The developmental state of the organism determines whether dedifferentiation will or will not occur. Linsbauer has
defined five separate developmental states in this connection:

(a) Indifferent (embryonic tissue)
(b) Plastic (differentiating)
(c) Differentiated (will develop if correlative inhibition is removed)
(d) Differentiated (capable of development only by dedifferentiation)
(e) Differentiated (irrevocably headed toward death)

The dedifferentiated cell may reach maturity again via the same sequence of events ending in the same mature structures or via another sequence ending in a different mature structure. This process is termed redifferentiation. Many examples of this may be cited - the appearance of new plants on a Begonia leaf, and the renewed vegetative growth of sex organs particularly in the lower plants. Therefore, it can be said that there is no loss of potentiality in developmental determination. During development only certain potentialities are realized while the others are masked.

The stability of developmental determination is governed by correlations between the various parts of the plant. These governing correlative factors inhibit the realization of the other potentialities. If, however, this system of correlations is interrupted by any unnatural means - such as the isolation of parts from the influence of the whole - the masked potentialities are allowed to
develop. This renewed development occurs by the processes of dedifferentiation and redifferentiation.

One of the best ways of recognizing and studying the developmental potentialities is the removal of correlative inhibitions. This allows renewed growth only by dedifferentiation and redifferentiation. These two processes together are known as regeneration. This phenomenon is only a part of the larger scheme of restorative processes called restitution (Driesch, 1929), which is the general tendency to restore the original form after a disturbance. Restitution may occur by any one of three processes: reparation, reactivation, and regeneration (MacQuarrie and von Maltzahn, 1959). Reparation includes only those processes which involve remoulding and growth toward the establishment of the original form. The release of embryonic cells from the effects of correlative inhibition is termed reactivation. This can be illustrated by the behaviour of axillary buds when the apical bud is removed. Finally, regeneration is that type of restitution which involves the dedifferentiation of mature cells and their redifferentiation toward maturity again. It is this final type with which this study is concerned.

Since dedifferentiation is characterized by a return to the embryonic condition it is necessary to define the characteristics of this condition. The distinctive feature of embryonic cells is their ability to undergo an unlimited
number of divisions. Visible embryonic characteristics usually include a large nucleus and nucleolus, a relatively great amount of cytoplasm, and a small vacuolar space. In the meristematic regions these cells are small in size and cuboidal in shape. If dedifferentiation represents a return to the embryonic condition then there must be some evidence of renewed synthetic activity. Increases in the size or number of the cytoplasmic components are indications of renewed activity (Caspersson, 1940).

Restitution does occur in many plants. In the Musci, for example, the gametophyte has two separate stages in development. There is both a juvenile stage, the protonema, and an adult stage, the gametophore. The protonema is the filamentous stage which is formed on the germination of the spore, or in the later stages of development, from the mature cells of the stem of the gametophore. When the correlative inhibition systems have been disturbed by isolation these protonemata may also be formed from other parts of the gametophore. Since these are mature structures, composed of mature cells, the protonemata must be formed by the processes of cellular dedifferentiation and redifferentiation. Since regeneration occurs very readily in the Musci, they are especially feasible material for a detailed study of regeneration as it occurs in plants.
DESCRIPTION OF THE MATERIAL

The genus *Splachnum* is a member of the Bryales or true mosses, which represent the highest degree of specialization in the Bryophytes. This order possesses a true alternation of generations, and the diploid sporophyte is largely parasitic on the gametophyte.

The gametophyte is an erect, branched, leafy shoot which at maturity is differentiated into a stem and leaves. These leaves are arranged spirally in three vertical rows with the exception of the leaves at the apex which grow on a much shortened stem and hence form a tuft at the apex. These are the perichaetial leaves which surround the sex organs and often may differ in shape from the foliage leaves. The leaves along the main stem show anisophylly in that the basal leaves are small and blunt, while the apical leaves are obovate with a slender apex. A mid-rib composed of several layers of elongated cells is present, while the remainder of the leaf is one cell layer in thickness.

The growth of the gametophyte is initiated by the cutting off of a three sided apical cell from a cell of a protonema. The first division of this apical cell is periclinal; the inner cell resulting from this division contributes to the developing stem. The outer cell then
divides perpendicular to the plane of the first division. The upper cell thus formed develops into a leaf, while the lower cell contributes to the cortical region of the stem. The initial cell of a leaf has two cutting faces which alternately cut off a cell to the left and to the right.

The sex organs of Splachnum are terminal and borne on separate gametophytes. They may form from the apical cell or from segments recently cut off from it. After fertilization, which occurs in the archegonia, the sporophyte begins development by enlargement and by a transverse division of the zygote. Both the upper and lower cell undergo two diagonal divisions to form apical cells with two cutting faces. Most of the capsule and seta of the sporophyte develop from the upper apical cell. The lower cell forms the foot and the lower portion of the seta.

Meiotic division occurs during the formation of the sporogenous tissue. The haploid spores are released when the capsule of the sporophyte dehisces. The germinating spore gives rise to a much-branched, filamentous primary protonema. Some authors have distinguished between two types of primary protonemata: those which grow downward into the soil or medium and possess few chloroplasts and diagonal cross walls, and those which grow along the surface or upright, containing more chloroplasts and having their cross walls at right angles to their long axis. However, this distinction is not apparent in Splachnum. After the initial of the
gametophyte has been cut off, the primary protonema usually die.

The secondary protonemata may arise from any part of either the mature gametophyte or sporophyte, although the former case is more usual than the latter. The appearance of protonemata at the base of the intact gametophore can be observed in the normal cycle of development. However, their appearance on the leaves and the upper part of the stem is inhibited. MacQuarrie and von Maltzahn (1959) report that the removal of the apex of the plant stimulates protonematal formation from the base, and that the isolation of either stem segments or leaves from the intact gametophore allows secondary protonemata formation.

Since the cells of the leaf or the stem of the gametophore are mature cells, their renewed growth as protonemata constitutes a coupling of the processes of dedifferentiation and redifferentiation. A study of the correlative systems active in the inhibition of reactivation and regeneration in the early stages in the development of *Splachnum ampullaceum* has already been performed (MacQuarrie and von Maltzahn, 1959).

This present study is an attempt to characterize certain aspects of the changes which occur during cellular dedifferentiation and redifferentiation.
MATERIALS AND METHODS

In this investigation the male strain of *Splachnum ampullaceum* was used. The original cultures were obtained from the Cambridge Collection in England. Since this strain possessed a marked ability for vegetative growth, the cultures were easily maintained in test tubes on Beijerinck's inorganic nutrient solution with the addition of 1% agar. The pH of the solution was adjusted to 5 before the solution was sterilized in an autoclave. The pH of the hardened medium was about 6.5. These cultures were grown under continuous fluorescent daylight lamps at a temperature between 24-28 degrees Centigrade. All plants used for experimental purposes were 20-21 days old.

In suitable habitats, mosses are propagated mainly by vegetative growth of the gametophyte. This is a method of asexual reproduction in which the sex organs, the archegonia and the antheridia, are not necessary. Vegetative propagation can occur by the isolation of leaves and branches or from protonemata. In this study the isolation of a stem segment containing three or four leaves was the usual method. This isolation allowed the development of the buds in the axils of the leaves, and therefore, the plants grew in clusters of three or four.

It has been reported (MacQuarrie and von Maltzahn, 1959)
that the isolation of a single plant from a cluster containing 4-6 plants has no effect on the regenerative behaviour of the gametophore. The isolation of the stem segments and the leaves was effected by the use of a small knife on 4% agar cutting plates in a sterile transfer room. The isolated segment was quickly transferred to a sterile test tube or petri dish containing the nutrient solution and 1% agar.

In any experiment where there was a large quantity of material it was necessary to kill and fix the leaves to prevent the occurrence of any further changes before they could be examined. After some preliminary work it was found that a 30 minute treatment with acetic Zenker's fixative was best-suited to the conditions present and caused little if any change in the cells. All measurements and count were made microscopically from temporary mounts at a magnification of 800 diameters. The angles were measured with a gonionometer eye-piece.

The time intervals of the measurements of the chloroplasts and nuclei varied with the experiment. In preliminary experiments measurements were taken every day for 4-5 days; this was later changed to intervals of 0, 6, 12, 24, 48, and 72 hours. In a bulky experiment it was found that a measurement 10 hours after the beginning of the experiment would sufficiently replace the two readings at 6 and 12 hours.
Slides of the leaf in cross section were prepared in the following manner: The leaves were killed in acetic Zenker's fixative and imbedded in paraffin via an ethyl alcohol series and xylene. Sections were cut on a rotary microtome at 14.1 μ and stained in Safranxin for 2 hours. A 5 to 10 second treatment with Fast Green was used as a counter-stain. The sections were mounted in permount.

RESULTS

General Characteristics of the Leaf Cells

Since it was shown in a previous study (von Maltzahn and MacNutt, 1957) that considerable differences exist in the regenerative potentialities of cells of leaves of different ages, only leaves from the middle region of the gametophore were used in the interest of uniformity. These leaves were of approximately the same physiological age. The structure of the leaf is relatively simple. The leaf is single-layered with the exception of a many-layered mid-rib. However, the single-layered portion does show some differentiation. There are evident differences in the size and shape of the component cells.
The size and shape of the cells were measured in the mature leaves of the middle region of the gametophore. The size gradient in the cells was expressed in terms of cell area, and is shown in Table 1 and Figure 1. The cells measured were in a line parallel to the mid-rib and several rows to the left or right of it. Measurements were taken only of every second cell.

**TABLE 1**

<table>
<thead>
<tr>
<th>Cell position</th>
<th>Area in mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base</td>
<td>1.85</td>
</tr>
<tr>
<td>1</td>
<td>1.40</td>
</tr>
<tr>
<td>2</td>
<td>1.32</td>
</tr>
<tr>
<td>3</td>
<td>1.01</td>
</tr>
<tr>
<td>4</td>
<td>1.03</td>
</tr>
<tr>
<td>5</td>
<td>1.05</td>
</tr>
<tr>
<td>6</td>
<td>1.07</td>
</tr>
<tr>
<td>7</td>
<td>1.39</td>
</tr>
<tr>
<td>Apex</td>
<td>8</td>
</tr>
</tbody>
</table>

This figure indicated a decrease in cell size to a minimum which was reached in the middle region of the leaf. Toward the tip there was again an increase in area but not to that attained at the base. Accompanying this there was a change in the cell shape. This is illustrated in Figure 2 by a
CELL AREA $10^{-3} \times \text{mm}^2$

CELL POSITION

BASE  APEX

FIGURE 1
comparison of the length:width ratios from different regions of the leaf. The basal cells were long and quite narrow; the cells in the middle region tended to be more square; and toward the tip of the leaf the cells were much larger and possessed almost equal lengths and widths.

In addition to these differences in the size and shape of the cells there were some physiological differences. The tooth cells showed different staining behaviour with fluorescence dyes than did the majority of leaf cells. The tooth cells fluoresced green almost instantaneously when placed in a 1:10,000 acridine orange solution. The remainder of the leaf cells showed only the bright red autofluorescence of the chloroplasts with no background fluorescence.

The present study was concerned only with cells in the single-layered section of the leaf. These cells, when mature, contained a large vacuole surrounded by a thin layer of cytoplasm. Their most conspicuous feature was the green chloroplasts. The nucleus was, in most cases, quite difficult to find.

The Behaviour of the Chloroplasts

To follow the behaviour of the chloroplasts during cellular dedifferentiation and redifferentiation, it was first necessary to describe their situation in the normal mature leaf cell. At the light intensities used in this
study the chloroplasts were equally distributed on the upper and lower surfaces of the cell, an arrangement of chloroplasts known as peristrophe (Senn, 1908). They were present in a single plane in which the chloroplasts were irregular in shape and packed closely together. (Figure 3). In fully differentiated cells there was little variation in the size of the chloroplasts. The question arose, however, of a possible correlation between chloroplast size and cell size and chloroplast number and cell size. Figure 4 showed that the size of the chloroplasts did not change with cell size, while Figure 5 indicated the relationship between chloroplast number and cell size. The correlation coefficient of these data was found to be 0.79 indicating a highly significant linear relation. Hence it was necessary that in any comparative study the observations should be taken from the same region of the leaves to eliminate errors due to this variation.

Figure 3. A surface view of leaf cells immediately after isolation X 780.
FIGURE 5

CHLOROPLASTS PER CELL

AREA $\times 10^{-3}$ mm$^2$
It has previously been shown that in isolated half leaf will regenerate, while a half leaf attached to a stem segment of a certain minimum length will not (MacQuarrie and von Maltzahn, 1958). Since regeneration is necessarily preceded by cellular dedifferentiation, chloroplast behaviour in dedifferentiating cells was investigated by means of a comparison of the following systems: (a) an isolated half leaf, (b) a half leaf attached to a stem segment, and (c) a whole leaf attached to a stem segment. It was thought that regeneration from an isolated leaf might be due to the presence of the wound surface incurred when a leaf is isolated from the stem. The attached half leaf showed no regeneration, however, even though it possessed a large wound surface. The attached whole leaf acted as a control, since it had been found in preliminary work that its behaviour paralleled that of a leaf attached to a whole gametophore.

(i) Changes in the number of chloroplasts in dedifferentiating cells.

The results of a typical experiment are shown in Table 2. Each figure represents an average of 30-40 cells with no more than four cells counted per leaf. The cells which were counted were selected from the middle region of the leaf where the variation in the cell size was small. Each experiment was repeated 2-3 times.
TABLE 2

The number of chloroplasts per cell

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated half leaf</td>
<td>65.0</td>
<td>66.4</td>
<td>98.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attached half leaf</td>
<td>73.2</td>
<td>69.2</td>
<td>74.6</td>
<td>75.4</td>
<td>79.4</td>
</tr>
<tr>
<td>Attached whole leaf</td>
<td>68.6</td>
<td>67.6</td>
<td>78.0</td>
<td>74.2</td>
<td>72.0</td>
</tr>
</tbody>
</table>

These results are shown graphically in Figure 6.

The number of chloroplasts in the isolated half leaf showed a definite increase during dedifferentiation. Within 24 hours after isolation the chloroplast number per cell had increased by approximately 50% over the initial number. After 24 hours it was almost impossible to determine the number of chloroplasts per cell in the isolated half leaf because of changes in the position of the chloroplasts. In earlier experiments when this was attempted it was found that the number of chloroplasts per cell in those cells which had already showed protonemata formation had decreased to about the same number as had been present at isolation. Table 3.

TABLE 3

The number of chloroplasts per cell

<table>
<thead>
<tr>
<th>Before cell division and protonemata formation</th>
<th>After cell division and protonemata formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time in hours</td>
<td></td>
</tr>
<tr>
<td>Isolated leaf</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>87</td>
</tr>
<tr>
<td>6</td>
<td>122</td>
</tr>
<tr>
<td>12</td>
<td>120</td>
</tr>
<tr>
<td>24</td>
<td>145</td>
</tr>
<tr>
<td>48</td>
<td>94</td>
</tr>
</tbody>
</table>
FIGURE 6

CHLOROPLASTS PER CELL

TIME IN HOURS

- - - O ISOL HALF
- - - [ ATT HALF
- - - [ ATT WHOLE
From the results there were no significant increases in the number of chloroplasts in the attached half leaf and the attached whole leaf.

(ii) Changes in the size of chloroplasts in dedifferentiating cells.

It was also noted that a decrease in the diameter of the chloroplasts occurred when the leaf was isolated from the stem. The behaviour of the chloroplasts in this respect was observed in the same three systems as was the chloroplast number. The results are shown in Table 4. Each figure in the table represents an average of 150 chloroplast diameters.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated half leaf</td>
<td>6.02</td>
<td>6.04</td>
<td>5.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attached half leaf</td>
<td>6.03</td>
<td>6.25</td>
<td>6.08</td>
<td>5.73</td>
<td>6.14</td>
</tr>
<tr>
<td>Attached whole leaf</td>
<td>6.05</td>
<td>5.97</td>
<td>6.01</td>
<td>6.06</td>
<td>6.04</td>
</tr>
</tbody>
</table>

The results are illustrated in a graph in Figure 7.

These results indicated a sharp decrease in the diameter of the chloroplast which occurred within the first 24 hours, and as can be seen from Figure 6, corresponded to an increase in the chloroplast number.
CHLOROPLAST DIAMETER \( \text{mm} \times 10^3 \)

TIME IN HOURS

FIGURE 7
Figure 8 illustrates the decreased chloroplast size in a camera lucida drawing (compare size with that in figure 3, p. 13). Although in the attached whole leaf and the attached half leaf there was some fluctuation in the diameter, the value at the end of the experiment was about the same as that at the beginning.

Many variations in chloroplast shape have been found in this experiment. There was a prevalence of "dumb-belled" shaped chloroplasts in the early stages after isolation (Figure 9). Since chloroplasts divide amitotically this would be indicative of dividing chloroplasts (Heitz, 1925a and 1925b). The chloroplasts tended to have a more regular oval or spherical shape about 24 hours after isolation when they have stopped dividing than they had at the beginning.
In long, narrow cells as, for example, in protonemata the chloroplasts were elongated and narrow. It should be emphasized that the chloroplasts were viewed in all cases in surface view. The chloroplasts have an elliptical shape in cross section.

Figure 9. A surface view of leaf cells 12 hours after isolation X 780.

The Behaviour of the Chief Cellular Components during Dedifferentiation

It was convenient to discuss the general topic of the behaviour of the chief cellular constituents during the process of dedifferentiation under three separate headings: the behaviour of the cytoplasm, the behaviour of the nucleus and nucleolus, and the movements of the chloroplasts. The correlation in the behaviour of these three constituents is discussed following
a detailed account of their individual behaviour.

(i) The cytoplasm in dedifferentiating cells

In a mature cell of an attached leaf the cytoplasm is restricted to a thin layer around the outer edge of the vacuole (see Figure 16, p. 24). This pattern is changed rapidly after isolation. The cytoplasmic behaviour was investigated in both attached and isolated leaves.

Within a few hours of isolation a strand of cytoplasm could be observed connecting the cytoplasm around the nucleus with the cytoplasm of some other part of the cell. As a rule this strand went directly across the vacuole of the cell to the side opposite the nucleus. Figure 10. In general also this strand migrated through the vacuole. It could not be determined whether it originated from the vicinity of the nucleus or whether it migrated toward the nucleus from the other side of the cell. After this primary strand formation other strands were formed from other parts of the cell, Figure 11. Chloroplasts moved along these strands (Figure 12) in the direction of the nucleus and eventually formed a tight cluster around it. Figure 13. This phenomenon is known as systrophe (Schimper, 1885). As strand formation continued one strand usually became more prominent than the others, and it was along this strand that the cell wall was formed. Figure 14.
Figure 10. A surface view of a leaf cell 6 hours after isolation X 780.

Figure 11. The numerous chloroplasts have been omitted to simplify this drawing of leaf cells 10 hours after isolation X 780.

Figure 12. A surface of a leaf cell showing plastid cystrophe clustering 30 hours after isolation X 780.

Figure 13. A surface view of plastid cystrophe after 48 hours X 780.
With the formation of the cell wall the cytoplasmic strands gradually disappeared. The remnants of these strands may be seen in figure 21, page 27. In the attached whole leaf and the attached half leaf there was no evidence of strand formation. The cytoplasm was, in both cases, limited to a thin layer in the peripheral region of the cell. This seems to indicate that strand formation is a characteristic peculiar to dedifferentiating cells.

Figure 14. A surface view of a leaf cell showing the formation of the phragmosome 48 hours after isolation X 780.

(ii). The nucleus and nucleolus in dedifferentiating cells.

The nucleus of the moss leaf cell was relatively large and spherical in shape. It contained a distinct nucleolus.
Its position in the cell varied to a certain extent, but it was most often found at the side walls of the cell (See Figure 16, page 24).

Examination of the nucleus in the living material proved to be quite difficult. Various stains - Unna's methyl-green pyronin solution, the Feulgan stain, aceto-carmine, and various vital stains were attempted in an effort to stain the nucleus but with no success. This failure was quite likely due to the presence in the moss plant of a relatively impermeable cell wall and cuticle. It was observed that the nuclei in the thin-walled protonemata stained very readily. The only method by which the nuclei could be seen with any degree of success was by the microscopic observations of the living or fixed material at a magnification of 800 diameters. In those cells in which no nuclei could be seen it must be assumed that the nuclei were situated at one of the four side walls.

The behaviour of the nuclei during dedifferentiation was studied in the cells of the isolated leaves, and their behaviour compared with that of the nuclei in the cells of an attached half leaf and an attached whole leaf. The nuclear diameter was measured at various time intervals after the isolation of the leaf from the stem, and in the two latter cases, after the isolation of the stem segment. The results are shown in Table 5.
TABLE 5

The diameter of the nucleus in mm. $X 10^{-3}$

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated half leaf</td>
<td>6.14</td>
<td>6.52</td>
<td>7.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attached half leaf</td>
<td>6.39</td>
<td>6.14</td>
<td>6.68</td>
<td>6.29</td>
<td>6.74</td>
</tr>
<tr>
<td>Attached whole leaf</td>
<td>6.42</td>
<td>6.23</td>
<td>6.77</td>
<td>6.70</td>
<td>6.66</td>
</tr>
</tbody>
</table>

The nuclei of the redifferentiated cells of the moss leaf increased in size. Figure 15 illustrates this in graph form. There was also a slight increase in the nuclei of the attached half leaf and the attached whole leaf. In the isolated half leaf it was impossible to obtain data for the nuclear size 48 hours after isolation because of changes in the nuclear position and especially because of changes in the chloroplast position which will be described in detail later.

Little information could be obtained about the behaviour of the nucleolus from a study of the living material because soon after isolation it was obscured by the clustering chloroplasts. Its presence was noted before this, but no attempt was made to ascertain its behaviour during dedifferentiation and redifferentiation. However, prepared slides of isolated leaves in cross section stained with safranin and fast green showed the nucleolus in most cells quite distinctly. Figures 16, 17, 18, 19, and 20 are from a series of camera lucidia drawings showing
Figure 15

NUCLEAR DIAMETER $\text{mm} \times 10^3$

TIME IN HOURS

- $\bigcirc-\bigcirc$ ISOL HALF
- $\square-\square$ ATT HALF
- $\triangle-\triangle$ ATT WHOLE
nuclear and nucleolar behaviour in the various stages in dedifferentiation and redifferentiation. During these stages there is an approximately four fold increase in the nucleolar volume. The greatest increase occurred early after the isolation of the leaf.

This method also yielded a clear picture of nuclear changes. From the initiation of dedifferentiation to the formation of the protonemata, the nucleus approximately doubled its original size. The observations from the prepared slides agree with those taken from the living material. The final measurements from the living material were taken at the stage shown in Figure 18. At this stage there was a certain increase but not to the extent which was reached after cell wall formation.

Figure 16. A cross section of leaf cells just after isolation X 780.

Figure 17. A cross section of a leaf cell showing beginning of strand formation after 48 hours X 780.
Figure 18. A cross section of a leaf cell showing plastid systrophe 48 hours after isolation X 780. Fixation has caused some plasmolysis.

Figure 19. As in figure 18 plasmolysis has occurred because of fixation. Phragmosome formation is commencing X 780.

Figure 20. A cross section of a leaf cell showing formation of the first protonema 48 hours after isolation. Chloroplasts have been omitted from the first protonema cell to simplify drawing X 780.
(iii) The movement of the chloroplasts

It has already been pointed out that the chloroplasts are present in a single plane along the surfaces of the mature cell. It has also been mentioned in the previous section that during cellular dedifferentiation changes occurred in the position of the chloroplasts. These are described more fully below. The chloroplast behaviour in the cells of isolated half leaves was again compared with that of the cells in attached half leaves and in attached whole leaves.

In normal leaves attached to intact gametophores movement of the chloroplasts was limited to the peripheral regions of the cell. The pattern of chloroplast movement changed rapidly after the isolation of the leaf. These movements led to a tight cluster of most of the chloroplasts in a particular region of the cell. There was always a small number of chloroplasts which remained scattered throughout the remainder of the cell. The nucleus almost certainly plays a definite role in cluster formation since it is always in the center of the cluster. (See Figure 13, P. 20.)

Within 6 hours of isolation protoplasmic strands were formed in the cell. When these strands had formed the chloroplasts moved along them in the direction of the nucleus. This movement continued from 6 hours after isolation to approximately 48 hours after isolation. During this time the cluster of the chloroplasts became
more tightly packed. The chloroplasts remained in this position until the nucleus had divided, and the cell wall had begun to form. The cluster was divided by the cell wall, and the chloroplasts were distributed to the first cell of the protonema and to the parent cell in apparently equal lots (see Table 3). Figure 21.

Figure 21. A surface view of a cell showing equal chloroplast division 48-60 hours after isolation X 780.

It was thought that this "systrophic" phenomenon might be caused by a loss of water from the leaf cells. Since these leaves were grown in Beijerinck solution, solidified with agar, it was possible that water would not diffuse quickly enough into the cell. To determine whether this was so leaves were isolated and placed in sterile liquid medium of 1% Beijerinck solution. They were examined after 48 hours, and it was found that strand formation and clustering also occurred in these
leaves. On this evidence it was concluded, therefore, that systrophe is not caused by a loss of water from the cell.

(iv) A Correlation in the Behaviour of the Chief Cellular Components during Dedifferentiation

The processes which led to the reestablishment of an embryonic state were not separate and distinct, but rather they formed an integrated series of related processes. The cells forming a semi-circle around the tip of the leaf first showed signs of cellular dedifferentiation. Only later was any activity observed in the remaining leaf cells, but after 24 to 48 hours almost all stages could be seen in a single leaf.

The normal cell at the moment of isolation has already been described (pages 10-12). The first evidence of cellular changes was in the appearance of the "dumb-belled" shaped chloroplasts. These chloroplasts were dividing amitotically. This process was usually completed 24 hours after isolation. Occurring almost simultaneously with this was the cytoplasmic strand formation. These strands radiated to all sides of the cell from the nucleus which had moved along the primary strand to a position near the center of the vacuole. When this had occurred, but while the chloroplasts were still dividing, the movement of the chloroplasts toward the nucleus began. In the interval between 24 and 48 hours
after isolation chloroplast number and strand formation was at a maximum. These strands facilitated the clustering of the chloroplasts around the nucleus. At the end of 48 hours one strand had become much thicker than the rest. During the period from 48 to 60 hours after isolation the nucleus, situated by now in the center of the cluster, divided, and the cell wall began to form along the most prominent strand. The cell wall divided the chloroplasts between the protonemal cell and the parent leaf cell. Simultaneously with nuclear division and cell wall formation a protuberance formed into which the chloroplast streamed, which enlarged, gradually, and which was finally cut off from the parent cell by the developing cell wall to become the first cell of the secondary protonema. (Figure 20, p. 25.). Figure 20 also illustrates the differential division which occurred in protonema formation. The parent leaf cell was, in most cases, divided into a smaller protonema-forming cell and a larger parent leaf cell.

The Position of the Clusters and the Factors Affecting It

As was noted in the foregoing section the movement of the chloroplasts after isolation led to the phenomenon of "systrophe", that is, the clustering of the chloroplasts in any part of the cell. Isolated leaves were examined after 48 hours to determine the orientation of the clusters in the cell.
In the first experiment leaves, isolated by excision at the base, were planted in small petri dishes on solid (1% agar) Beijerinck medium. The cells which were examined did not include the 3-4 rows of cells at the wounded edge because of possible wound influences. For convenience in tabulating the results, the cell was divided into 5 sections as shown in Figure 22. The areas of the cell in which the clusters were usually found were, for purposes of discussion, divided into rectangles of arbitrary but about equal size, with two sides parallel to the longitudinal axis of the leaf. Part A contained all those clusters which were oriented toward the tip of the leaf, part B those toward the base, part C those with the clusters in the center of the cell, while parts D and E showed orientation toward the outer leaf edge and toward the mid-rib respectively. The frequency of clustering in each part of the cell is shown in Table 6.

Figure 22.
The orientation of the clusters in the cell

<table>
<thead>
<tr>
<th>Region of the cell</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41</td>
</tr>
<tr>
<td>B</td>
<td>36</td>
</tr>
<tr>
<td>C</td>
<td>41</td>
</tr>
<tr>
<td>D</td>
<td>35</td>
</tr>
<tr>
<td>E</td>
<td>38</td>
</tr>
</tbody>
</table>

From the results it can be concluded that in the cells not in the neighbourhood of the wound surface there was no correlation between the position of the clusters of the chloroplasts in the cell and the alignment of the cells in the leaf. There was a completely random distribution.

The effect of wounding on the orientation of the clustering was also investigated. The position of the cluster was observed in those 3-4 rows of cells at the wound surface at the base of the leaf. The results are shown in Table 7.

The orientation of clusters at the wound surface

<table>
<thead>
<tr>
<th>Region of the cell</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>42</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
</tr>
<tr>
<td>D</td>
<td>9</td>
</tr>
<tr>
<td>E</td>
<td>12</td>
</tr>
</tbody>
</table>
These results indicated a definite basal orientation of the clusters in those cells near the wound edge.

As a means of checking these results, leaves were isolated at their base and wounds inflicted on other parts of the leaf; one parallel to the mid-rib by cutting off the outer edge of the lamina, and one at an angle of approximately 45 degrees from the mid-rib. The results are shown in Table 8 and 9 respectively.

**TABLE 8**

The orientation of clusters when the wound surface is parallel to the mid-rib.

<table>
<thead>
<tr>
<th>Region of the cell</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
</tr>
<tr>
<td>D</td>
<td>53</td>
</tr>
<tr>
<td>E</td>
<td>3</td>
</tr>
</tbody>
</table>

**TABLE 9**

The orientation of clusters when the wound surface is at an angle of 45 degrees to the mid-rib.

<table>
<thead>
<tr>
<th>Region of the cell</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>56</td>
</tr>
<tr>
<td>B</td>
<td>3</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>D</td>
<td>3</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
</tr>
</tbody>
</table>
From these data it can be seen that the clusters orientated toward the wound edge regardless of its relation to the biological axis of the leaf.

The Formation of the Cell Wall

The formation of the cell wall dividing the parent cell from the first cell of the protonema began about 48 hours after the isolation of the leaf. It was thought that there might be some relationship between the plane of the newly formed cell wall and the polar axis of the leaf. Leaves from the middle region of the gametophore were isolated and grown on Beijerinck and 1% agar in petri plates. Measurements were taken after 72 hours with the goninometer eyepiece. The results for a normal isolated leaf are shown in Table 10. Measurements were not taken near the wound edge.

TABLE 10

<table>
<thead>
<tr>
<th>Classes of angles</th>
<th>Frequency</th>
<th>Classes of angles</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 9</td>
<td>30</td>
<td>90 - 99</td>
<td>16</td>
</tr>
<tr>
<td>10 - 19</td>
<td>0</td>
<td>100 - 109</td>
<td>19</td>
</tr>
<tr>
<td>20 - 29</td>
<td>10</td>
<td>110 - 119</td>
<td>18</td>
</tr>
<tr>
<td>30 - 39</td>
<td>13</td>
<td>120 - 129</td>
<td>19</td>
</tr>
<tr>
<td>40 - 49</td>
<td>20</td>
<td>130 - 139</td>
<td>12</td>
</tr>
<tr>
<td>50 - 59</td>
<td>18</td>
<td>140 - 149</td>
<td>15</td>
</tr>
<tr>
<td>60 - 69</td>
<td>25</td>
<td>150 - 159</td>
<td>7</td>
</tr>
<tr>
<td>70 - 79</td>
<td>23</td>
<td>160 - 169</td>
<td>3</td>
</tr>
<tr>
<td>80 - 89</td>
<td>19</td>
<td>170 - 180</td>
<td>29</td>
</tr>
</tbody>
</table>
These results are shown in histogram form in Figure 23. From this figure it can be seen that the data falls naturally into two separate sets. First the two extreme values i.e. between 0 and 9 degrees and 169 and 180 degrees, represent 20% of the total population and are within a few degrees of being parallel to the polar axis. Considering the rest of the results the mean value is 88 degrees. This angle is at right angles to the polar axis. Therefore, it can be said that in the normal leaf there is a tendency for the new cell wall to be either perpendicular to or parallel to the polar axis of the leaf.

There is also a close relationship between the angle of the cell wall and the polar axis in the vicinity of the wound edge. This was true in the region of both the basal wound and other wound surfaces inflicted at the edge of the leaf. These results for a wound surface parallel to the mid-rib are shown in Table 11, and Figure 24.

<table>
<thead>
<tr>
<th>Classes of angles</th>
<th>Frequency</th>
<th>Classes of angles</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 9</td>
<td>19</td>
<td>60 - 69</td>
<td>6</td>
</tr>
<tr>
<td>10 - 19</td>
<td>12</td>
<td>70 - 79</td>
<td>5</td>
</tr>
<tr>
<td>20 - 29</td>
<td>15</td>
<td>80 - 89</td>
<td>4</td>
</tr>
<tr>
<td>30 - 39</td>
<td>10</td>
<td>90 - 99</td>
<td>1</td>
</tr>
<tr>
<td>40 - 49</td>
<td>11</td>
<td>100 - 109</td>
<td>0</td>
</tr>
<tr>
<td>50 - 59</td>
<td>6</td>
<td>110 - 119</td>
<td>1</td>
</tr>
</tbody>
</table>
Figure 23: Frequency of degrees from polar axis of leaf.
DEGREES FROM POLAR AXIS OF LEAF

FIGURE 24
The mean value of these data is 34.9 degrees. This indicates a tendency toward division approximately parallel to the wound surface.

The Effect of Age of the Leaf Cells on the Cellular Behaviour during Dedifferentiation

The effect of age on the dedifferentiating cells of the moss leaf was studied by the same methods as were employed in the first section of these results. An age gradient exists in the leaves of the gametophore, the first-formed or basal leaves of which are the oldest. The regenerative power of the leaves was found to decrease with age so that the basal leaves show only about one-third the number of protonemata the apical leaves did. A comparison was made between the apical and basal leaves with respect to chloroplast number. The results for the apical leaves are denoted by A, the basal leaves by B; and are shown in Table 12.

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>0</th>
<th>10</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated half leaf A</td>
<td>58.8</td>
<td>69.2</td>
<td>109.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>45.0</td>
<td>40.4</td>
<td>53.6</td>
<td>65.6</td>
<td>64.8</td>
</tr>
<tr>
<td>Attached half leaf A</td>
<td>68.0</td>
<td>68.2</td>
<td>81.8</td>
<td>68.8</td>
<td>66.8</td>
</tr>
<tr>
<td>B</td>
<td>40.2</td>
<td>38.0</td>
<td>37.0</td>
<td>39.2</td>
<td>37.2</td>
</tr>
<tr>
<td>Attached whole leaf A</td>
<td>65.8</td>
<td>67.8</td>
<td>65.8</td>
<td>60.6</td>
<td>61.8</td>
</tr>
<tr>
<td>B</td>
<td>38.0</td>
<td>37.6</td>
<td>38.2</td>
<td>38.2</td>
<td>40.4</td>
</tr>
</tbody>
</table>
A graph of the results is shown in Figure 25. Like the leaves from the middle region of the gametophore, the apical and basal leaves showed an increase in the chloroplast number per cell. However, this occurred only in the isolated leaves. The difference between the apical and basal leaves was in the magnitude of the reaction and in the time it took. The chloroplast number in the apical cell increased more quickly and to a greater extent than in the older basal cell. Initially there was no difference in the chloroplast diameter in the apical and basal leaves. Figure 26. Decreases in the diameter occurred in both apical and basal isolated leaves, but as with the increase in the chloroplast number, the older leaf reacted more slowly and to a lesser extent than the younger leaf. Figure 26. The attached half leaf and the attached whole leaf showed no significant increases or decreases in the chloroplast diameter during the experiment.

In preliminary experiments a comparison was made of nuclear diameters in the apical and basal isolated and attached whole leaves. The results are shown in Table 14.
CHLOROPLASTS PER CELL

TIME IN HOURS

FIGURE 25

○--○ ISOL HALF A
□--□ ATT HALF A
△--△ ATT WHOLE A
○--○ ISOL HALF B
□--□ ATT HALF B
△--△ ATT WHOLE B
In this case the results indicated an increase in the diameter of the nucleus in the isolated leaves of both the apex and base of the plant. However, more data is needed on the behaviour of the nucleus in these leaves.
DISCUSSION

In normal growth the course of development of the majority of the cells in the organism is from embryonic simplicity toward maturity and differentiation. The higher plants retain a few undifferentiated cells which are localized in the meristematic regions of the plant. In the non-vascular plants these regions are usually limited to a meristem composed of a few cells or often to only one actively dividing apical cell. As long as the developmental pattern is not interfered with, the mature plant cells are formed from the meristematic cells, are differentiated into specialized tissues or organs, and eventually die. This is not an inevitable process, however; correlative factors active in the organism as a whole have been found to control the potentialities realized in development. This is concluded from the fact that the developmental pattern can be reversed by the removal of certain plant tissues from these correlative influences. Isolation of parts will permit the renewed growth of their component cells, and visible changes in their pattern of development.

The fact that any type of plant tissue may by dedifferentiation give rise to any other type of plant tissue led Küster (1925) to suggest the non-specificity
of plant tissues as a possible consequence. His own results together with the work of Bunning (1956) and others and the modern work on tissue culture would appear to support this contention. It is possible to induce formation of completely new structures from the mature differentiated tissues and organs. However, there seems to be no direct path whereby mature cells of one tissue change into a tissue with different specialization. New growth must be preceded by a dedifferentiation of the mature tissue. Once dedifferentiation has occurred, the redifferentiation of new tissues may begin.

What are the main factors responsible for the initiation of dedifferentiation? Haberlandt (1914) reported that dedifferentiation was caused by the production of wound hormones which acted as a stimulus for cell division. The initiation of reactivity in the mature tissues of higher plants in response to injury has also been reported by Bloch (1941, 1952). His review articles on wound healing cover the available literature for higher plants thoroughly. However, wounding alone can not account for all reported cases of dedifferentiation. MacQuarrie and von Maltzahn (1959) report no regeneration from a half leaf attached to a short stem segment, while a great amount of regenerative growth was obtained from an isolated half leaf. Both of these leaves possessed
the same amount of wound surface. On this evidence the authors concluded that wounding was not a primary cause in the initiation of regeneration in *Splachnum*. These authors also report evidence of an active correlative system in this moss which controls, among other things, the distribution of regeneration over the gametophore. They concluded on the evidence obtained that the most important factor in the initiation of regeneration (and, therefore, of dedifferentiation) was the isolation of the part from the influence of the correlative inhibition present in the plant as a whole. These conclusions are also supported by the work of Meyer (1953a), Höfler (1954), and Heitz (1925). Meyer (1953a) isolated individual cells of *Asplenium adiatum-nigrum* by a fungal attack and found that all these cells formed new prothallia. He suggested that isolation and the removal of correlative inhibitions is a more feasible explanation of this phenomenon than wounding. This conclusion was also reached by Höfler to account for the regeneration which he found in some cells of the marine alga *Griffithsia Schousboei* after it had been transferred from salt water to fresh water for a short time. Heitz also concluded that wounding was not a satisfactory explanation for the initiation of cellular dedifferentiation in *Lophocolea bidentata* and other liverworts.
In an effort to characterize changes which occur when cells dedifferentiate a quantitative study was made of chloroplast number and size and nuclear and nucleolar size in both isolated and attached half leaves. This system also allowed an investigation of the effects of wounding. It was found that the chloroplast number increased rapidly after isolation, and at the same time, there was a decrease in their size. Both of these changes occurred only in the isolated leaves. There were no changes in either the attached half leaves or in the attached whole leaves. These changes, which occurred only in the isolated leaves, constitute a part of the process of cellular dedifferentiation. Heitz (1925) found a similar increase in chloroplast number and a decrease in chloroplast diameter in regenerating cells of the liverworts. He noted also the "dumb-bell" shaped chloroplasts predominated in those cells which would regenerate.

There was also an increase in the size of the nucleus in Splachnum, and preliminary observations indicated that this was accompanied by a great increase in nucleolar size. Stich (1951) reported that there was a close correlation between nuclear and nucleolar size on the one hand and cytoplasmic synthesis on the other for Acetabularia mediterranea. Caspersson (1940) has indicated that there is an intimate association between the nucleolus,
ribonucleoproteins, and protein synthesis. He said that any activity in the nucleolus is always accompanied by an intense synthesis of cytoplasmic ribonucleic acids. The nucleolus showed a rapid increase in size after isolation; thus suggesting that the cell is actively engaged in protein synthesis.

Further indications of the establishment of an embryonic type cell are the increases in the chloroplast number per cell and in nuclear size. However, Heitz (1925) found in Lophocolea and in Funaria that the nuclear volume increased in both the regenerating and non-regenerating cells in the isolated leaves. The nucleolus did not show any increase in size in the non-regenerating cells, while it did show a great increase in the regenerating cells in the leaves. In the present investigation no distinction could be made between regenerating and non-regenerating cells in the isolated leaves, since it appeared that all cells had the ability to dedifferentiate and under normal circumstances they all did. It was found that the nucleus in isolated half leaves of Splachnum showed a definite increase in volume. The results obtained for the nuclei of the attached half leaf and the attached whole leaf were not as clear because of variation in the size from cell to cell, but on the average there was no increase not attributable to variation. In any case wounding can not be the
cause of the increase in the isolated half leaves, because the wounded attached half leaf showed the same increase that the attached whole leaf did.

Wounding does, however, play a role in cellular dedifferentiation. The traumotactic behaviour of the nucleus has been described by many authors. Nestler (1898) described a positive traumotactic movement of the nucleus and cytoplasm toward a wound surface. He found that this occurred in monocotyledons, dicotyledons, and algae, and in all the organs of the plant with the exception of the guard cells. Ritter (1911), Miehe (1901), and Nestler (1898) have also described an increase in the nuclear size in the cells near the wound surface. This increase occurred during the positive traumotactic movement of the nucleus. The nucleus, however, returned to its original size when it resumed its initial position in the cell. Magda Ziegler (1955) found an initial decrease in the volume of the nucleus in the onion epidermis in response to wound stimulus. This was followed by the usual wound stimulus reaction of an increase in nuclear size, but the tendency is always to return to the original size for approximately 32 hours after wounding a decrease in size occurred. The nucleoli showed only an increase in volume.

It was found in the present study, however, that increase in nuclear volume in Splachnum is initiated mainly by the isolation of the leaf from the correlative influence
of the whole. This is in accord with Heitz's work (1925) in which he found that the nuclei in both regenerating and non-regenerating cells increased in size. He suggests that this might occur by the accumulation of sugars and not by the stimulus of the wound substances. On the basis of Miehe's (1901) discovery that mechanical isolation of the protoplasts, or isolation by plasmolysis leads to regeneration Schoser (1956) investigated regeneration in Cladophoraceen. He found an increase in the number of nuclear divisions at the basal pole of this coenocytic cell after isolation. This was accompanied by an increase in nuclear size. The D.N.A. content of the nucleus of a regenerating cell showed an increase over that of a resting cell. While this was not investigated in the present material it is quite probable that it does occur.

Other phenomena associated with the regenerating cells in Splachnum were the formation of cytoplasmic strands, cytoplasmic systrophe, and chloroplast systrophe. All these phenomena were observed only in the dedifferentiating cells of the isolated leaves. Heitz (1925) reported these events in the regenerating cells of Lophocolea bidentata leaves. He found that he was able to trace the origin of protonemata from these cells. A cytoplasmic strand migrated from the cytoplasm around the nucleus to the other side of the cell, and the nucleus migrated along it to the center region of
the cell. Other strands formed and radiated to all parts of the cell. The chloroplasts and other inclusions were carried along passively by protoplasmic streaming to the vicinity of the nucleus. Eventually these movements ended in a tight cluster of the cytoplasm, "plasma systrophe"; (Germ, 1931, 1933a and b) and a tight cluster of the plastids, "plastid systrophe"; (Schimper, 1885) around the nucleus. These phenomena have been reported by many authors. Germ (1931, 1933a, and 1933b) described similar occurrences in a variety of other plants after plasmolysis. He made the distinction between plasma systrophe and plastid systrophe, the latter occurring often without plasmolysis. Küster (1906) observed the same events after plasmolysis with sugar solutions in the orchid, Listera ovata. Sinnott and Bloch (1940, 1941) have described cell division in vacuolate cells in normal histogenesis. It would appear that during this process there was the formation of a typical "plasma systrophe". Günther (1957) reported plastid systrophe in cells of isolated leaves of Elodea. Systrophe did not lead to cell division but rather to death 3 to 4 weeks after the isolation of the leaves.

Various explanations have been given for the systrophic phenomena. Germ (1933) who has studied it extensively considered it to be a reaction of the living protoplasm to
plasmolysis. He was able to observe chloroplast systrophe in the Bryophytes after they had been subjected to intense illumination. Plasmolysis led to systrophe only in the sporophytic generation. Germ attributed the cause of systrophe to the irritation caused by the removal of the protoplast from the cell wall and not to a loss of water by plasmolysis with a hypertonic solution. The cellular dedifferentiation in vacuolate cells of Kalanchoe, Transcendania, and others and subsequent cytoplasmic systrophe were initiated by wounding (Sinnott and Bloch, 1940). The conclusion as to the cause of systrophe reached in this present study would, however, indicate that the isolation of cells either individually or as a tissue from the correlative influences of the whole plant would allow the cells to undergo dedifferentiation with the observed strand formation and systrophe being just a part of the whole process of cellular dedifferentiation. This would support Heitz's original observations and is in agreement with Bünning (1955). While Germ's work is not negated by this conclusion it is obvious that a new interpretation is needed. It is possible that plasmolysis acts as a means of isolation. The withdrawal of the protoplast from the cell wall with subsequent disruption of protoplastic connections between cells removes one cell from the influences of its neighbours. Thus the phenomena observed by Germ may be part
of the process of cellular dedifferentiation. In surface view there was a random distribution of clusters in cells not in the neighbourhood of the wound surface. In cross sectional view the position of the clusters was toward the center of the cell. The wound did effect the position of the clusters. Many authors have reported traumotactic movements of the nucleus (Ritter, 1911, Miehe, 1901, and Nestler, 1898). Since in Splachnum no cases were observed in which the nucleus was not in the cluster it is probable that the non-random distribution of the cluster can be accounted for by the positive traumotactic movement of the nucleus toward the wound surface.

The random distribution of the clusters in the cells away from the wound is an indication of a loss of cellular polarity. It must be concluded that, in this material, a loss of polarity accompanied dedifferentiation. Lack of polarity leads to undifferentiated growth of callose or tumor-like tissue. Meyer (1953b) reported the formation of tumor-like growths when the spores of a liverwort (possibly Cephalaziella) were treated with a hypertonic solution. Loss of polarity by the overcompensation of any normal gradient i.e. growth hormone will also cause undifferentiated growth. Von Wettstein (1953) found that Funaria spores after treatment with Vitamin B1 or Chloralhydrate formed a mass of apolar
undifferentiated cells which were maintained over 50 cell
generations. Therefore, it can be seen that the moss
spore is apolar and that any interference with the
reestablishment of polarity leads to undifferentiated
growth.

Bunning (1954) and Bloch (1943) stated that there
could be no differentiation without polarity. It was
noted in this present study that polarity reappeared
before redifferentiation occurred. This was particularly
apparent in the cell wall formation. Sinnott and Bloch
(1941) described the formation of the phragmosome which
is a diaphragm of anastomosing cytoplasmic strands in one
plane and which indicates the position of the new cell
wall. This phragmosome was clearly evident in Splachnum
in the later stages in systrophe. Since it was along the
phragmosome that the new cell wall formed, it is at this
stage that the reestablishment of polarity was again
observed. There was a definite relationship between the
plane of the newly formed cell wall and the polar axis of
the leaf. The plane of the wall showed a tendency to be
either parallel to or perpendicular to the axis of
symmetry of the leaf.

At the wound surface, however, all the newly formed
walls were parallel to the wound. Haberlandt (1914)
reported this correlation between cell wall formation and
wounding. Sinnott and Bloch (1941) have also reported cell wall formation parallel to the wound edge and perpendicular to any gradient in wound substances or hormones which have been formed in the wounded cells. Thus a new axis of polarity was formed. The reestablishment of polarity and differential division leads to redifferentiation (Bünning, 1954). In the present material it was found that the dedifferentiated cell was divided unequally between the first protonemal cell and the parent leaf cell. The inequality in this division was manifest in the great differences in the synthetic activities of the two cells. The protonemal cell immediately showed a great increase in nuclear and nucleolar size and in chloroplast number. As near as could be determined the chloroplast division was equal. Equal chloroplast division has also been described by Zepf (1952) for Sphagnum. It must be concluded that this differential activity between the protonemal cell and the parent cell was brought about by differential division of the cytoplasm or some component in it. Geitler (1955) found in a study of normal pollen development in Gasteria cheilophylla a differential division similar to that described here. However, he suggested that the displacement of the cytoplasm was the most important factor and questioned if there was initially any new synthesis of cytoplasm. This does not appear true for this present material if the basis
for judging the new synthesis of cytoplasm i.e. the increase in nucleolar size, is correct. The leaf of the moss, Sphagnum, is an excellent material for the study of differential division. Zepf (1952) has described the unequal divisions which led to the formation of an active chlorophyll cell and a highly specialized hyaline cell. Dedifferentiation has been described for the hyaline cell; it is not known whether the larger of the two daughter cell of Splachnum will dedifferentiate again. Ziegler (1955) has shown for Allium cepa epidermis that there is a change in the cytoplasmic density at the wound surface. The wound stimulated the displacement of the cytoplasm toward that side of the cell. This occurred before the positive traumotaxis of the nucleus and is in all probability the cause of the influence of the wound on the position of the clusters. Hence it seemed that for this present material polarity which is an important factor in the redifferentiation of the cell is established by some stimulus from the wound, at least, near the wound surface. Sinnott and Bloch (1941) results also support this statement since they have reported a close correlation between wound surface and cell wall formation. In the parts of the leaf away from the wound surface some other explanation for the establishment of polarity must be sought.

Polarity is known to be induced by many environmental factors. Light is probably the most effective of these.
In the spore of *Equisetium* the first wall which separates the basal rhizoidal cell from the apical prothallial cell is laid down perpendicular to the direction of the light rays (Mosebach, 1943). Here, as in *Splachnum*, the polarity is made visible before cell division by the polar displacement of the nucleus and chromatophores. This is also the case with the eggs of *Fucus*. They are apolar when they are liberated into the surrounding medium. Polarity in this case is induced by the environmental factor of light. As a rule environmental factors require only a short time to induce a polarity which is permanent for the life of the plant (Mosebach, 1943). It is possible that a gradient in some environmental factor - light, for example - is responsible for the reestablishment of polarity in the cells of the *Splachnum* leaf away from the wound surface; however, a more thorough investigation is needed before the induction factor can be determined for certain.

Von Maltzahn and MacNutt (1957) reported a great difference in the regenerative behaviour of the apical and basal leaves, with the younger apical leaves showing the greatest amount of regenerative growth. This is a strict age dependence in contrast to that reported by Bopp (1955) and Gemmell (1953). In *Funaria hygrometrica* Bopp found the least amount of regeneration in the middle region of the gametophore. Gemmell found for *Atrichum undulatum* that the position of the leaf was more important.
in determining the amount of regenerative growth than age.

It was found in preliminary experiments in this present study that the cellular behaviour during dedifferentiation is apparently the same, but the amount of the reaction, i.e. in the increase of chloroplast number and decrease in chloroplast size, is apparently less pronounced in the older basal leaves. A detailed study of the process of ageing is necessary before a more definite conclusion as to the causes of the differences in the reactions can be reached.

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APPENDIX A

Beijerinck Nutrient Solution:

\[
\begin{align*}
\text{NH}_4\text{NO}_3 & : & 0.5 \text{ gms.} \\
\text{MgSO}_4 \cdot 7 \text{H}_2\text{O} & : & 0.2 \text{ gms.} \\
\text{KH}_2\text{PO}_4 & : & 0.2 \text{ gms.} \\
\text{CaCl}_2 & : & 0.1 \text{ gms.} \\
\text{FeCl}_3 & : & \text{traces} \\
\text{H}_2\text{O} & : & \text{to 1 liter}
\end{align*}
\]
BIBLIOGRAPHY


