REGENERATION IN
SPLACHNUM AMPULLACEUM (L.) HEDW.

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MARY MARGARET MACNUTT

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CELLULAR

DEDIFFERENTIATION AND REDIFFERENTIATION

IN SPLACHNUM AMPULLACEUM (L.) HEDW.

Mary Margaret MacVutt

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

Dalhousie University.

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The phenomena of dedifferentiation and rediff-
erentiation in <u>Splachnum</u> <u>ampullaceum</u> 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 dedifferentiation. Cell
division, wall formation, and the factors affecting
them are considered. The effects of ageing on
regeneration are studied by a comparison of apical
and basal leaves.

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 reestablish-
ment of polarity which leads to differential division
and redifferentiation.

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GENERAL INTRODUCTION

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 <u>Splachnum</u> 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 an-isophylly 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 reacti-vation 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 <u>Splachnum</u> <u>ampullaceum</u> 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 measure-
ments 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 chloro-
plasts and nuclei varied with the experiment. In pre-
liminary 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 μ and stained in Safrannin for 2 hours. A 5 to 10 second treatment with Fast Green was used as a counter-stain. The sections were mounted in permount.

TABLE 1

The area of the cells

Cell position		Area in mm^2
Base	1	1.85
	2	1.49
	4	1.01
	5	1.01
	6	1.05
	7	1.07
Apex	8	1.32

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

The area of the cells

Cell position	Area in mm^2
Base 1	1.85
2	1.40
3	1.32
4	1.01
5	1.03
6	1.05
7	1.07
Apex 8	1.39

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
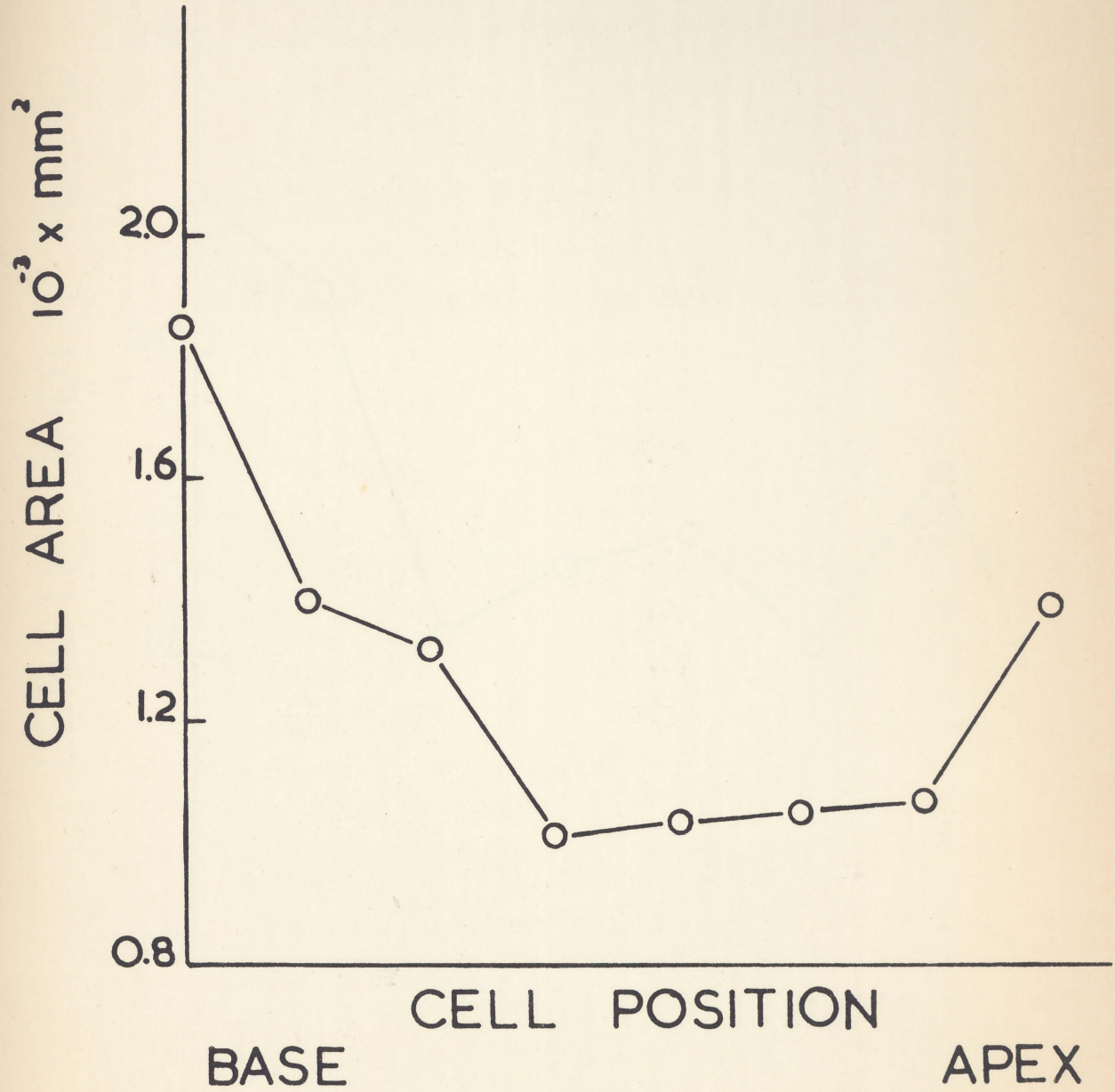in the cell shape. This is illustrated in Figure 2 by a
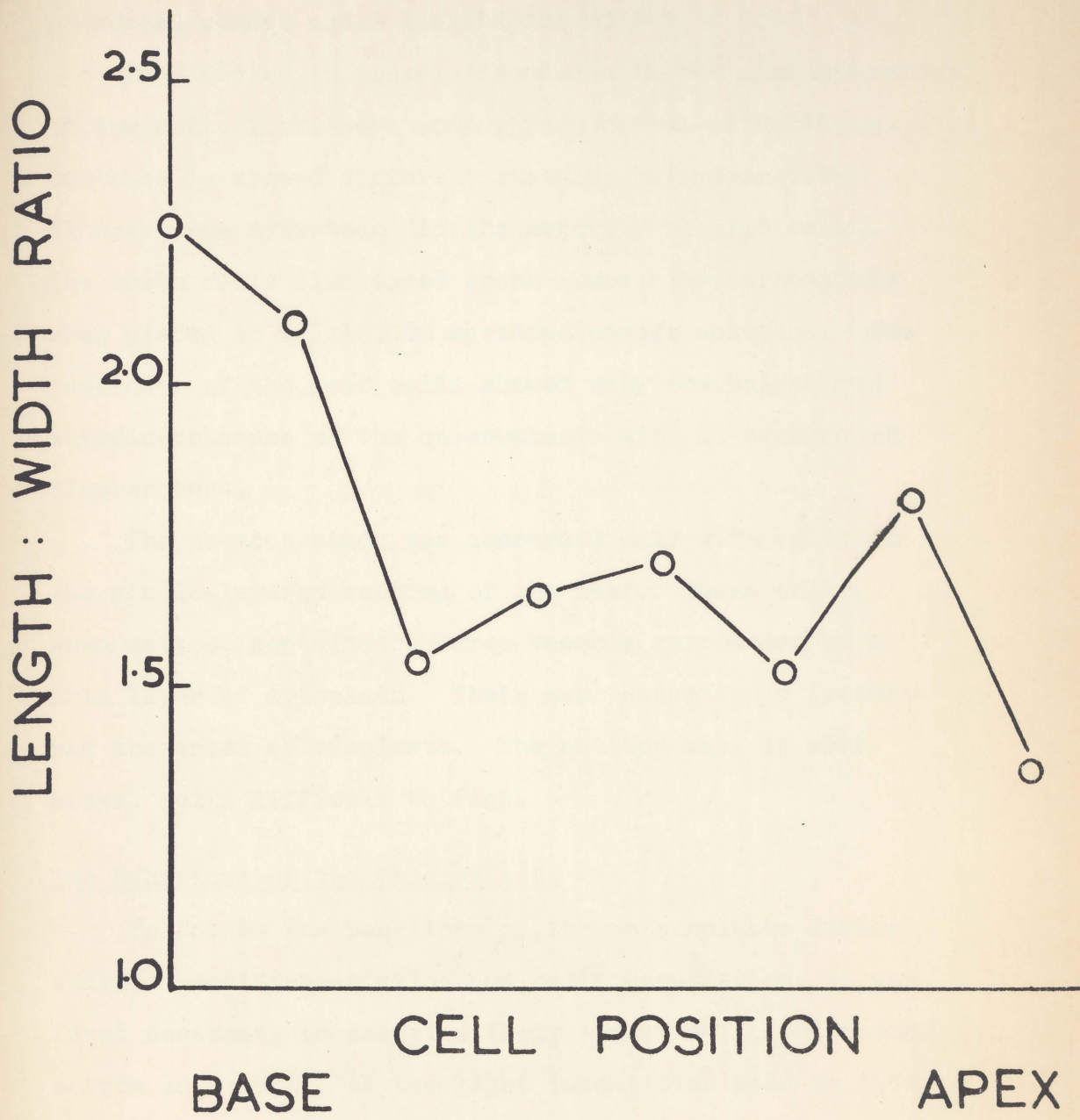
FIGURE 1

FIGURE 2

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

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

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
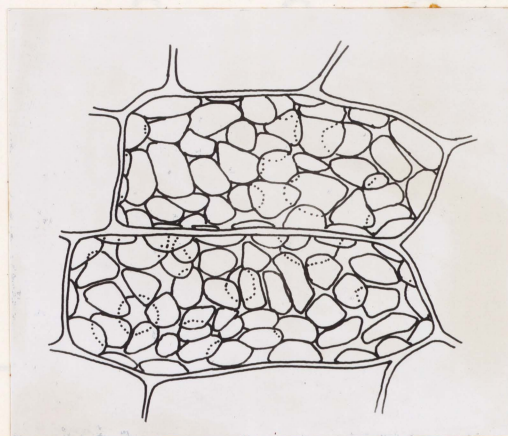eliminate errors due to this variation.

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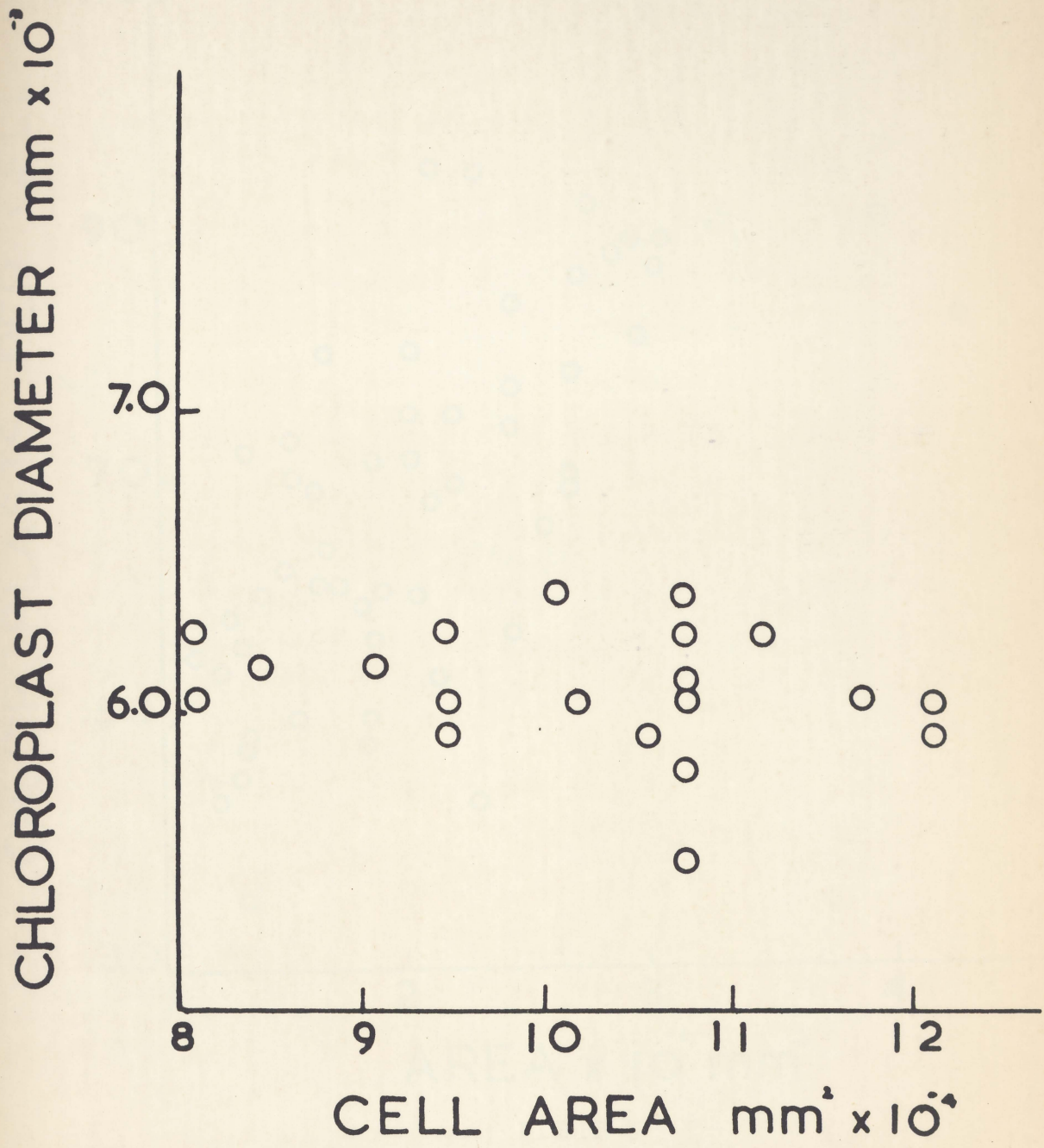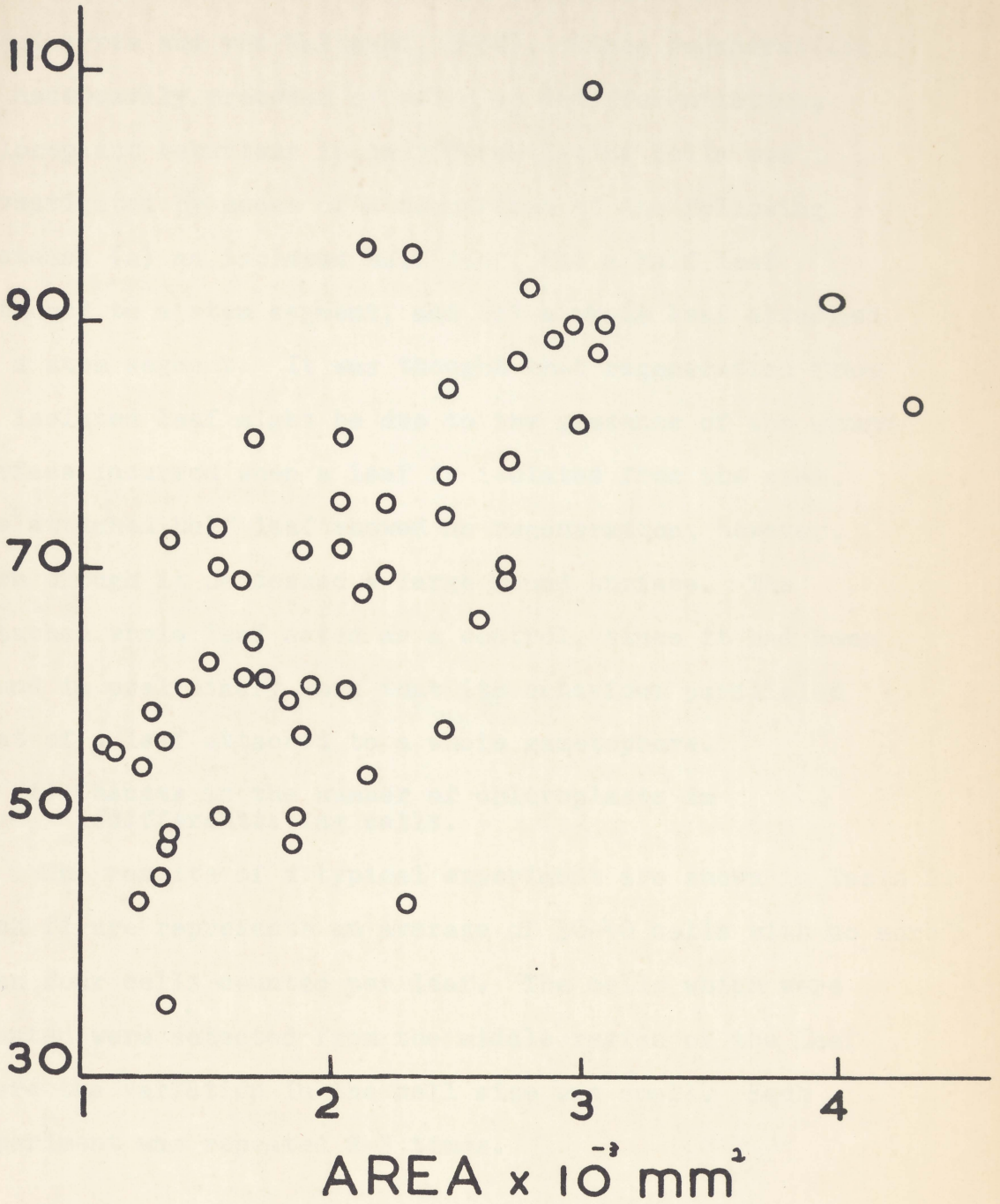after isolation X 780.

FIGURE 4

FIGURE 5

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

Time in hours	0	10	24	48	72
Isolated half leaf	65.0	66.4	98.6		
Attached half leaf	73.2	69.2	74.6	75.4	79.4
Attached whole leaf	68.6	67.6	78.0	74.2	72.0

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

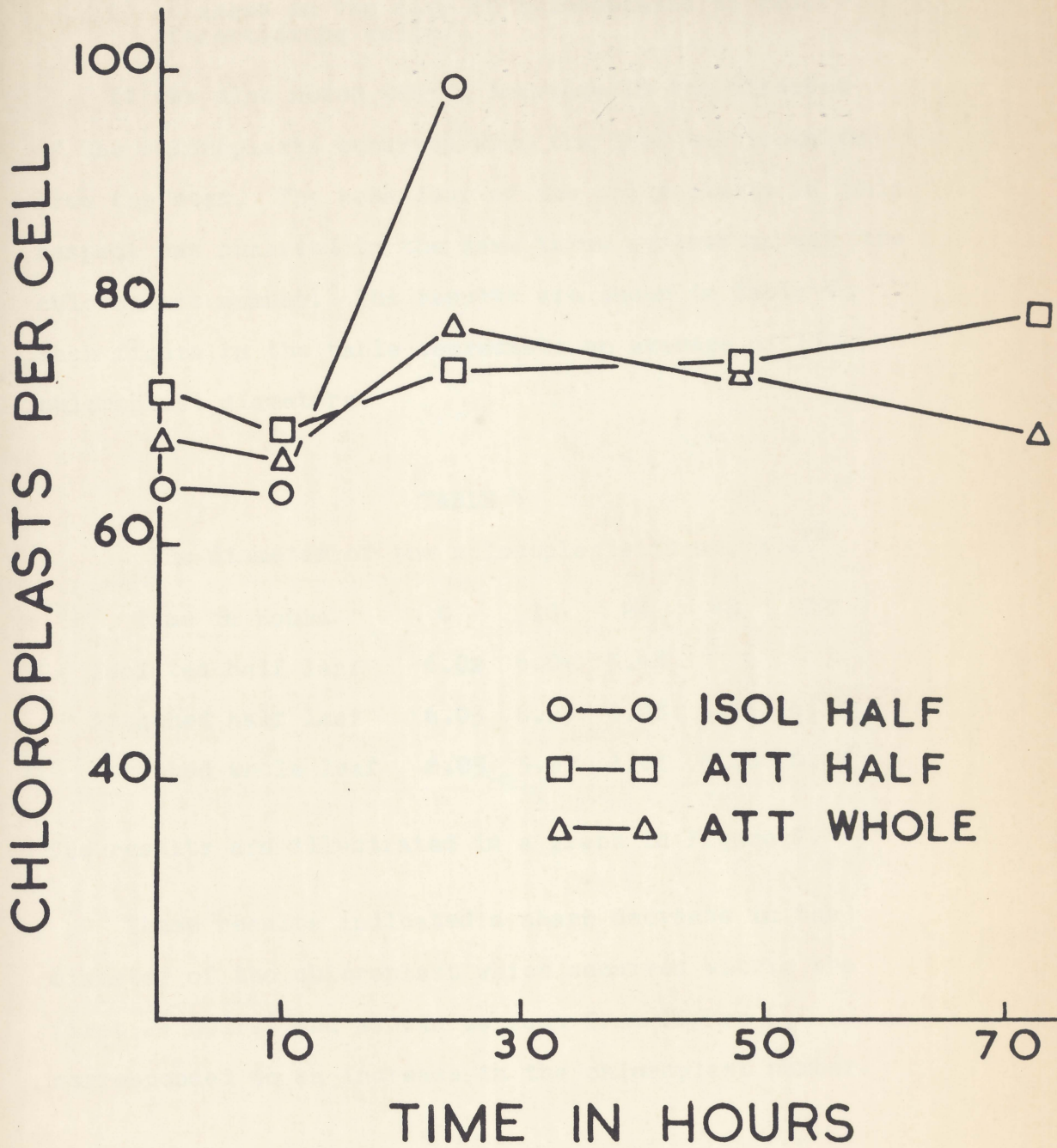| Before cell division and protonemata formation				After cell division and protonemata formation
Time in hours	0	6	12	24	48
Isolated leaf	87	122	120	145	94

FIGURE 6

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 dedif-
      ferentiating 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 4

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

Time in hours	0	10	24	48	72
Isolated half leaf	6.02	6.04	5.48		
Attached half leaf	6.03	6.25	6.08	5.73	6.14
Attached whole leaf	6.05	5.97	6.01	6.06	6.04

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,
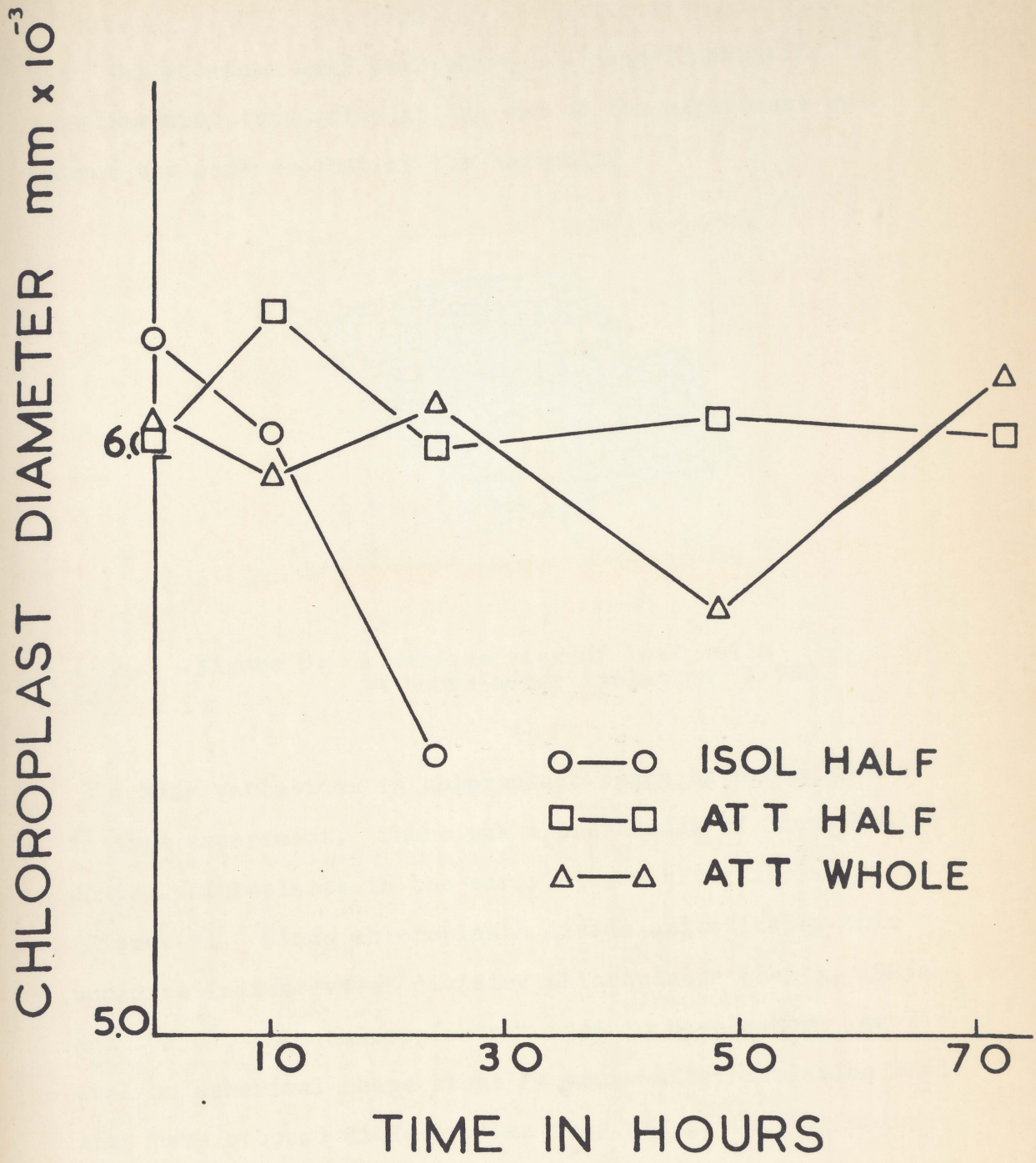corresponded to an increase in the chloroplast number.

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.
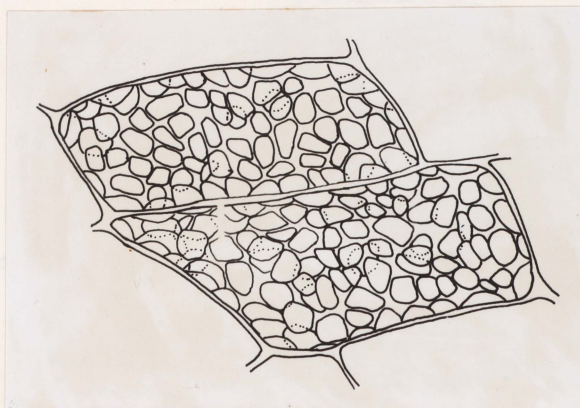

Figure 8. A surface view of leaf cells
24 hours after isolation X 780.

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 ellipitical
shape in cross section.
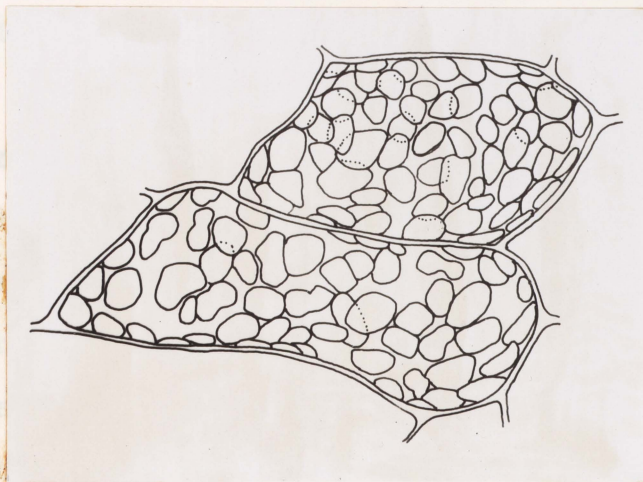
Figure 9. A surface view of leaf cells
12 hours after isolation X 780.

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
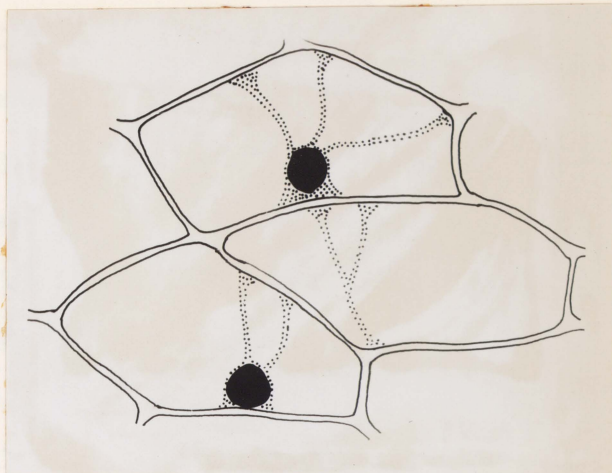　　　　　　X 780.

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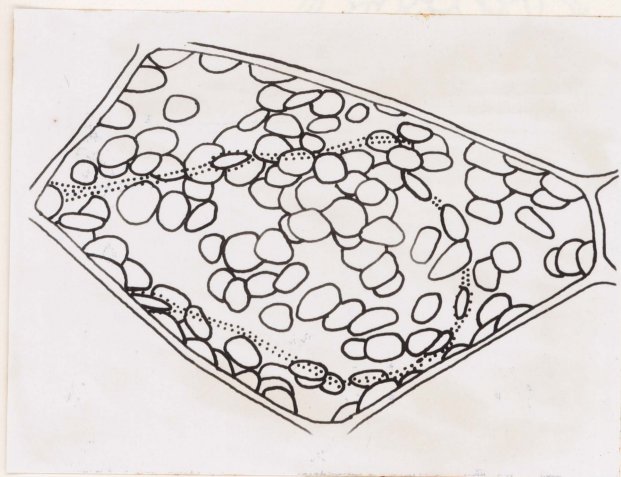　　　　　　cells 10 hours after
　　　　　　isolation X 780.

Figure 12.　A surface of a
　　　　　　leaf cell showing
　　　　　　clustering 30 hours
　　　　　　after isolation X 780.
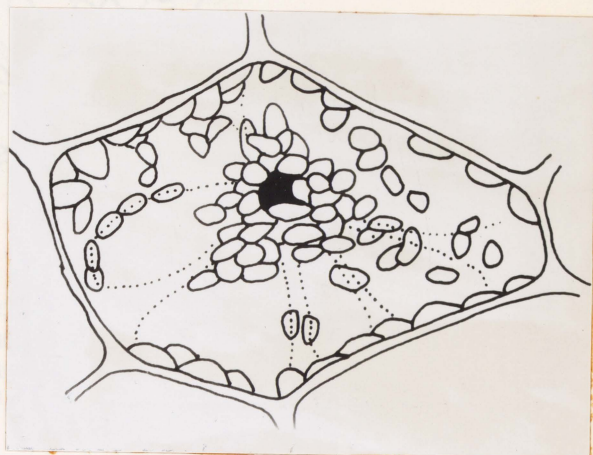
Figure 13.　A surface view of
　　　　　　plastid systrophe
　　　　　　after 48 hours.
　　　　　　X 780.

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 dedifferenting
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
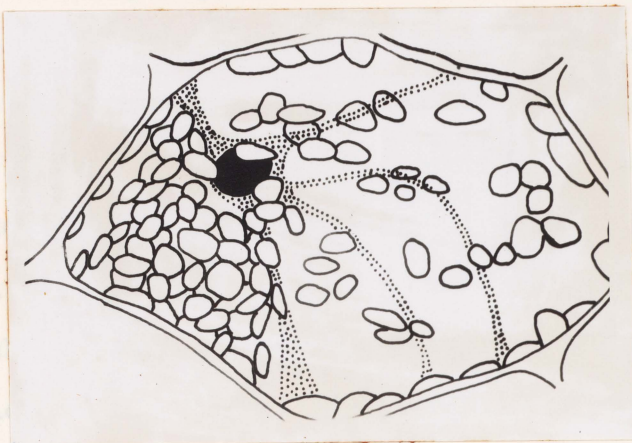proved to be quite difficult. Various stains - Unna's
methyl-green pyronin solution, the Feulgan stain, aceto-
carmine, and various vital stains were attempted in an
effort to stain the nucleus but with no success. This
failure was quite likely due to the presence in the moss
plant of a relatively impermeable 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}

Time in hours	0	10	24	48	72
Isolated half leaf	6.14	6.52	7.50		
Attached half leaf	6.39	6.14	6.68	6.29	6.74
Attached whole leaf	6.42	6.23	6.77	6.70	6.66

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 safrannin 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
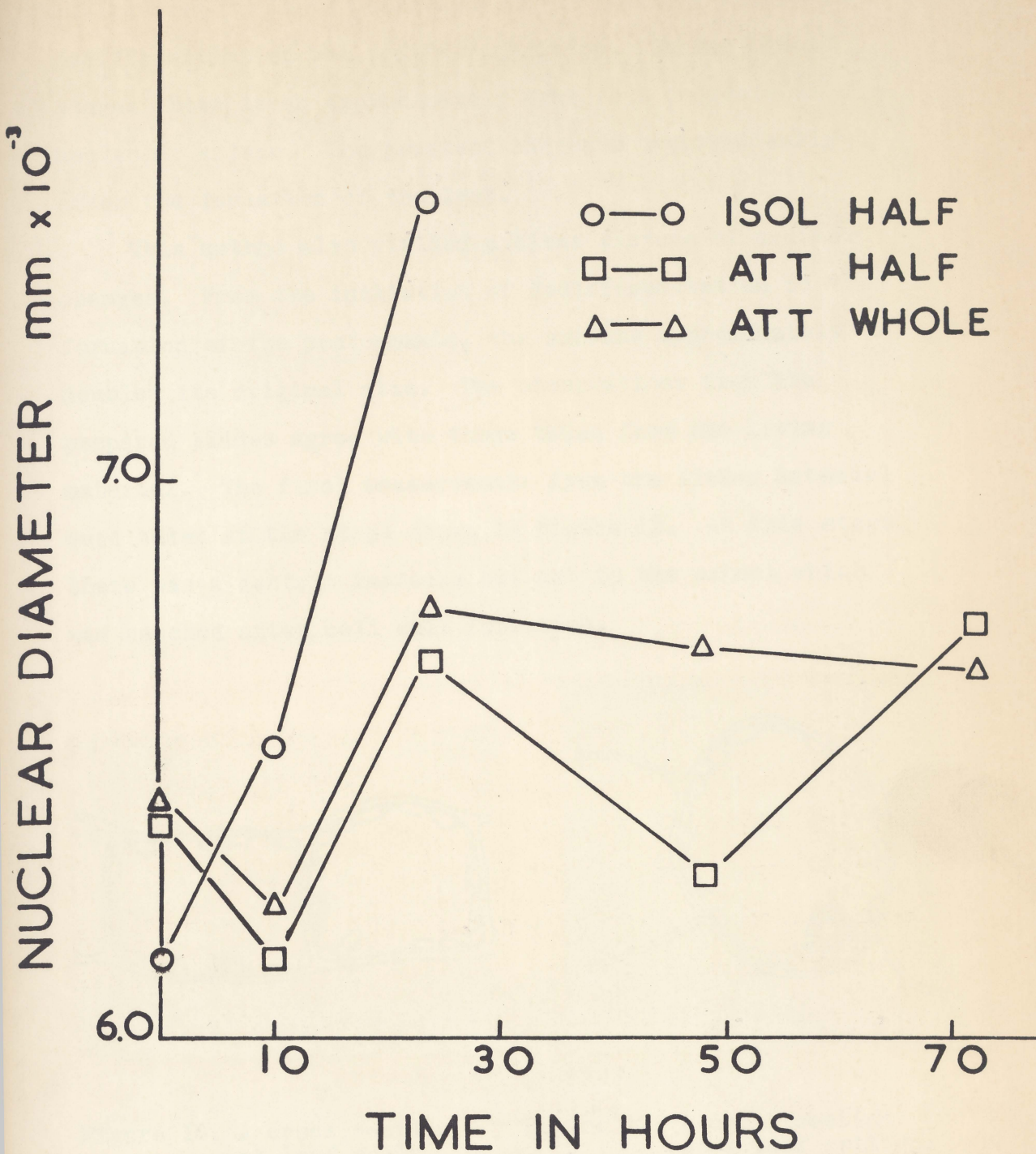
FIGURE 15

nuclear 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.

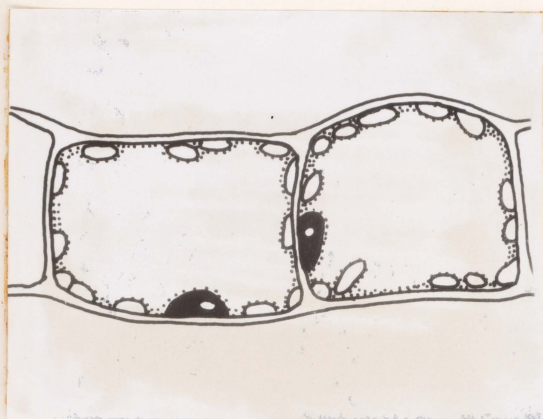Figure 16. A cross section
           of leaf cells
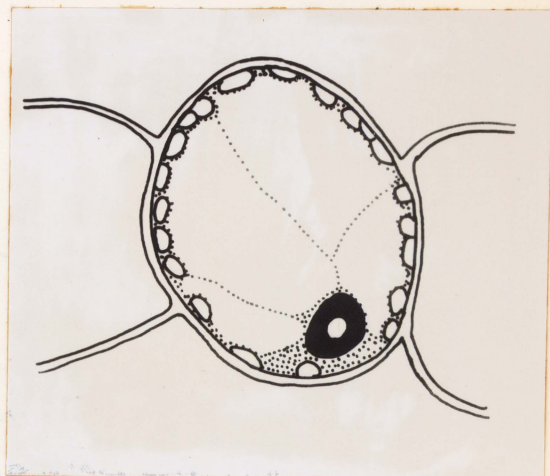   just after iso-
           lation X 780.

Figure 17. A cross section
           of a leaf cell
           showing beginning
           of strand formation
           after 48 hours X 780.

(iii) The movement of the chloroplasts

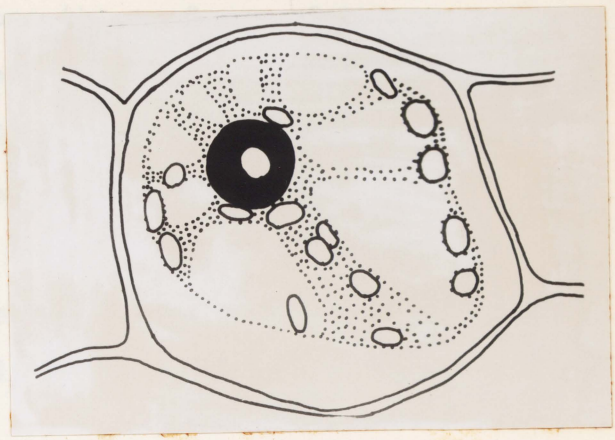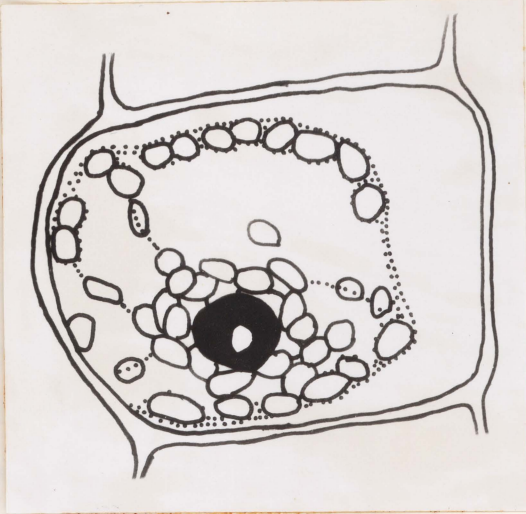It has already been pointed out that the chloroplasts

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.

In normal leaves attached to intact gametophores

movement of the chloroplasts was limited to the peripheral

regions of the cell. The return of chloroplast

changed rapidly after isolation of the leaf.

movements le   most of the chloro-

plasts in a particular region of the cell. There was

always a small number of chloroplasts which remained

scattered throughout   the

nucleus almost   cluster

formation since   cluster.

(See Figure 18.)

Within 6   ds were

formed in the c   the

chloroplasts mo   the

nucleus. This movement continued from 6 hours after

isolation to approximately 48 hours after isolation.

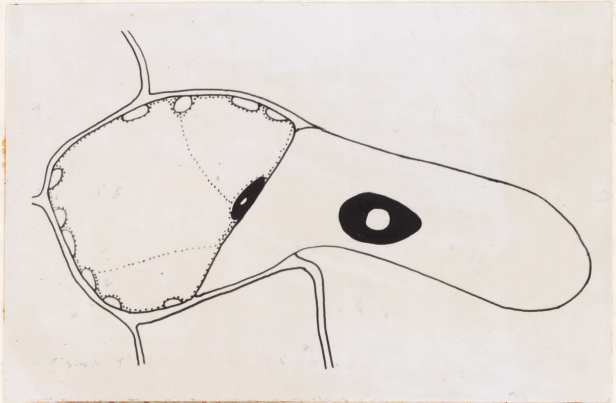During this time the   ma

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 chloro-
plasts 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
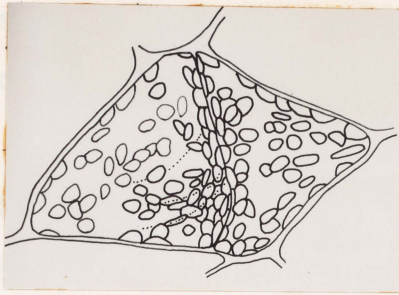equal lots (see Table 3). Figure 21.

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) <u>A Correlation in the Behaviour of the Chief</u>
<u>Cellular Components during Dedifferentiation</u>

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 forma-
tion. 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 arbitary but
about equal size, with two sides parallel to the longi-
tudinal 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
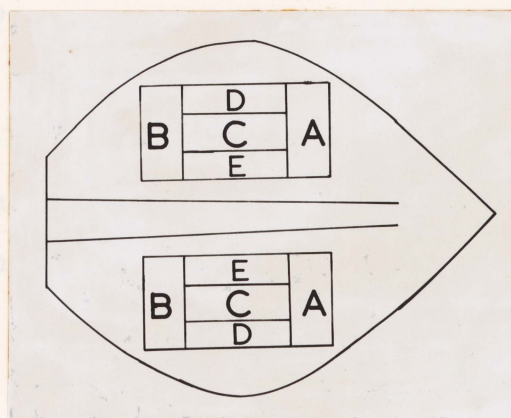each part of the cell is shown in Table 6.

Figure 22.

TABLE 6

The orientation of the clusters in the cell

Region of the cell	Frequency
A	41
B	36
C	41
D	35
E	38

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.

TABLE 7

The orientation of clusters at the wound surface

Region of the cell	Frequency
A	6
B	42
C	7
D	9
E	12

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.

Region of the cell	Frequency
A	3
B	1
C	1
D	53
E	3

TABLE 9

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

Region of the cell	Frequency
A	56
B	3
C	3
D	3
E	2

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

The angle between the polar axis of the leaf and the
newly formed cell wall

Classes of angles	Frequency	Classes of angles	Frequency
0 - 9	30	90 - 99	16
10 - 19	0	100 - 109	19
20 - 29	10	110 - 119	18
30 - 39	13	120 - 129	19
40 - 49	20	130 - 139	12
50 - 59	18	140 - 149	15
60 - 69	25	150 - 159	7
70 - 79	23	160 - 169	3
80 - 89	19	170 - 180	29

These results are shown in histrogram 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 perpen-
dicular 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 11

The wound surface parallel to the mid-rib

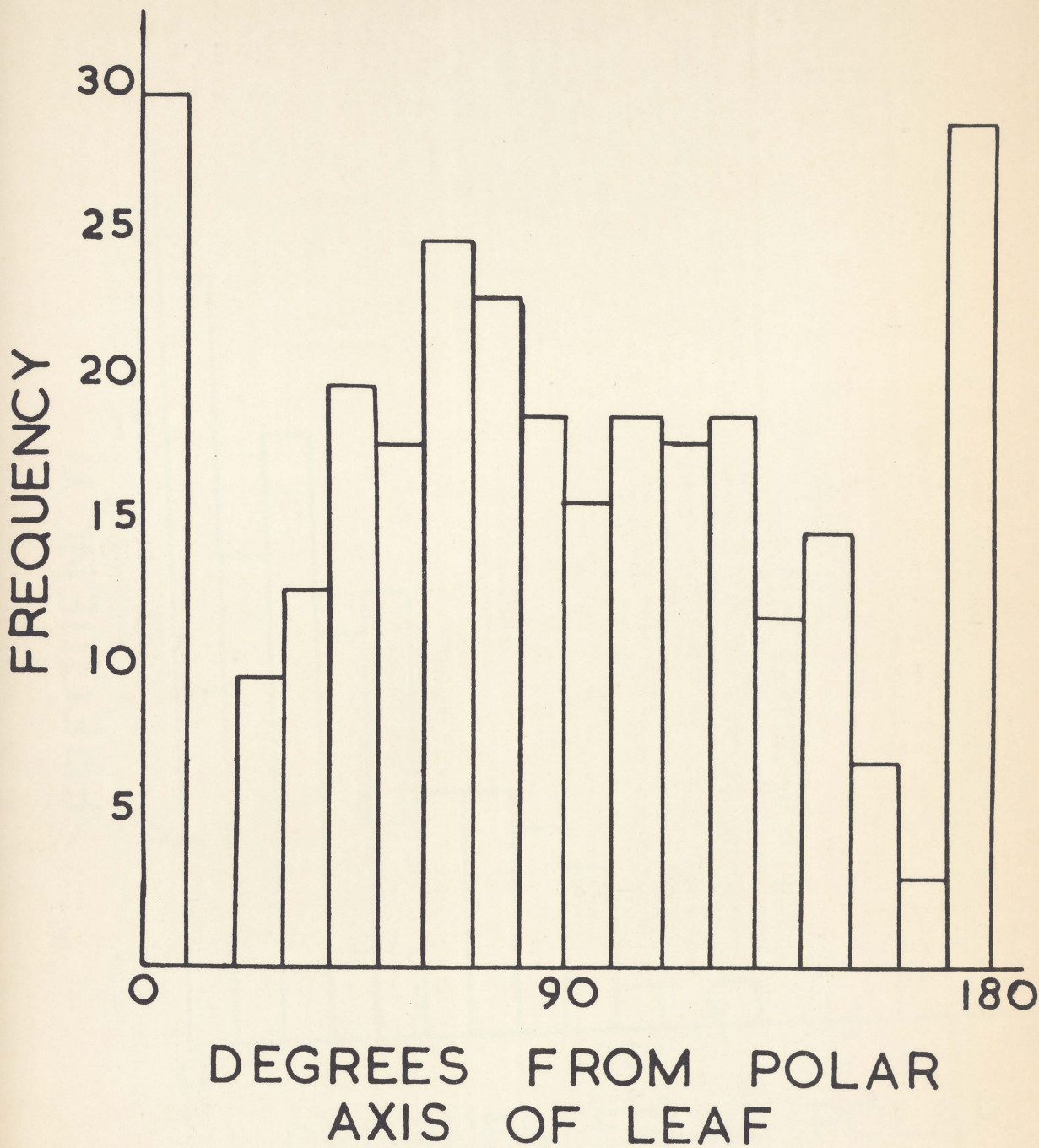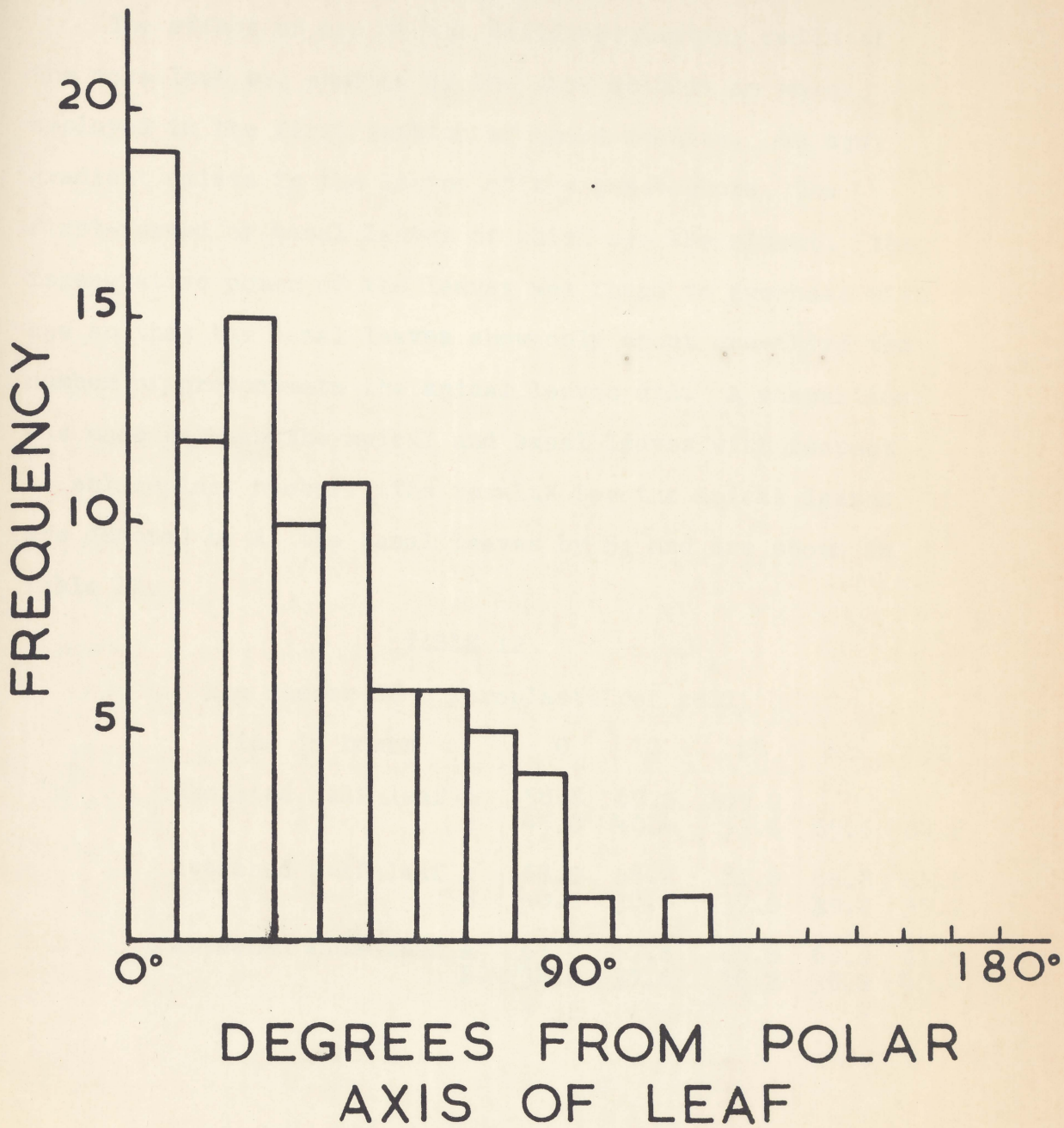| Classes of angles	Frequency	Classes of angles	Frequency
0 - 9	19	60 - 69	6
10 - 19	12	70 - 79	5
20 - 29	15	80 - 89	4
30 - 39	10	90 - 99	1
40 - 49	11	100 - 109	0
50 - 59	6	110 - 119	1

FIGURE 23

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 12

The number of chloroplasts per cell

Time in hours		0	10	24	48	72
Isolated half leaf	A	58.8	69.2	109.2		
	B	45.0	40.4	53.6	65.6	64.8
Attached half leaf	A	68.0	68.2	81.8	68.8	66.8
	B	40.2	38.0	37.0	39.2	37.2
Attached whole leaf	A	65.8	67.8	65.8	60.6	61.8
	B	38.0	37.6	38.2	38.2	40.4

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 chloro-
plast 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
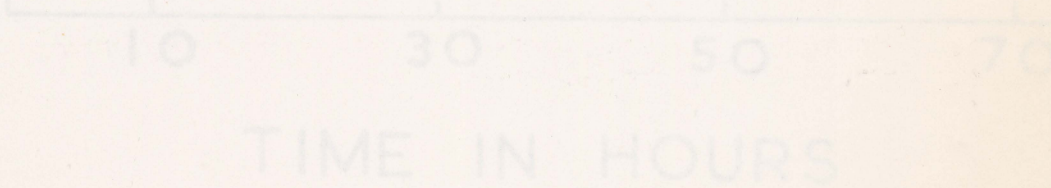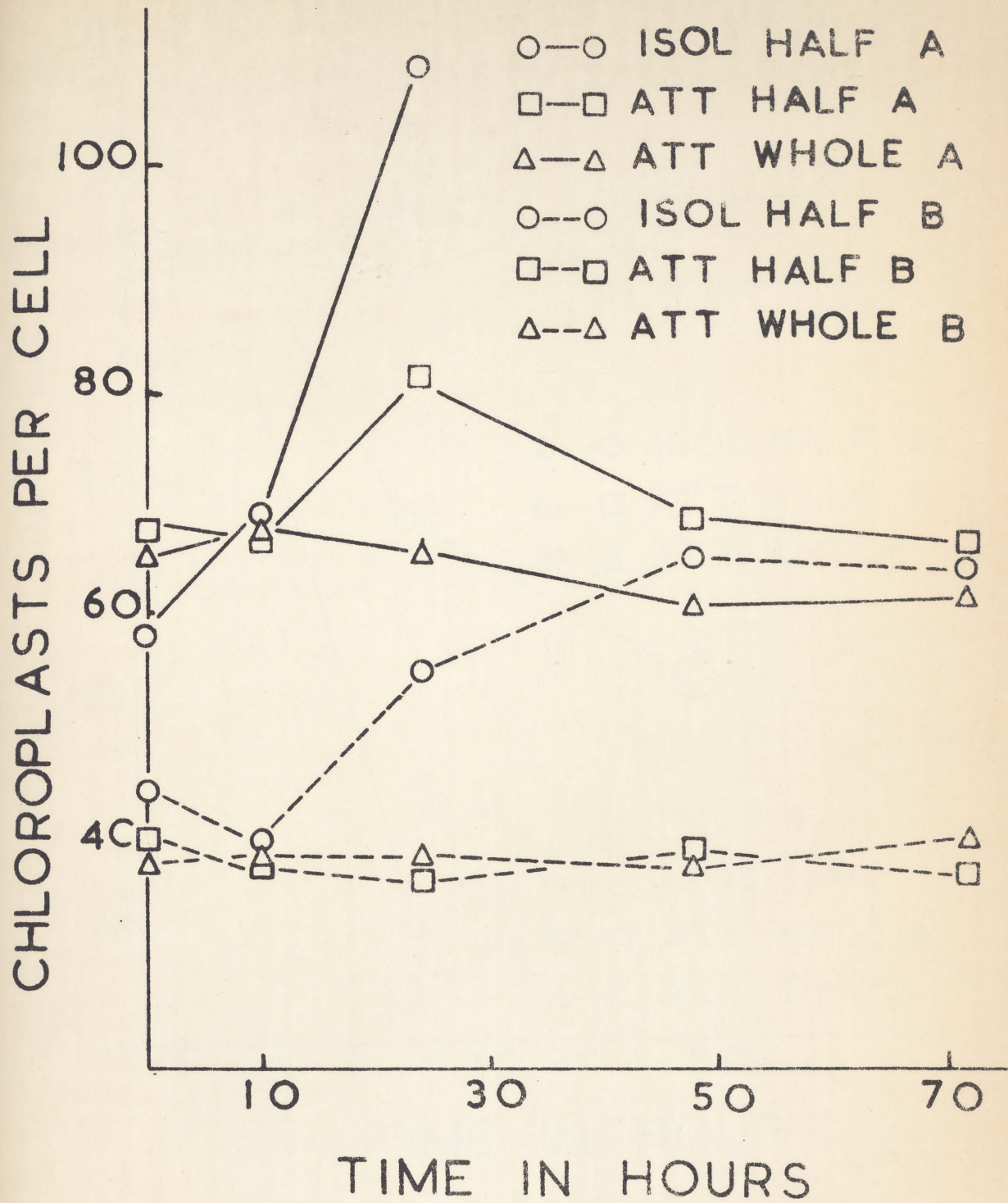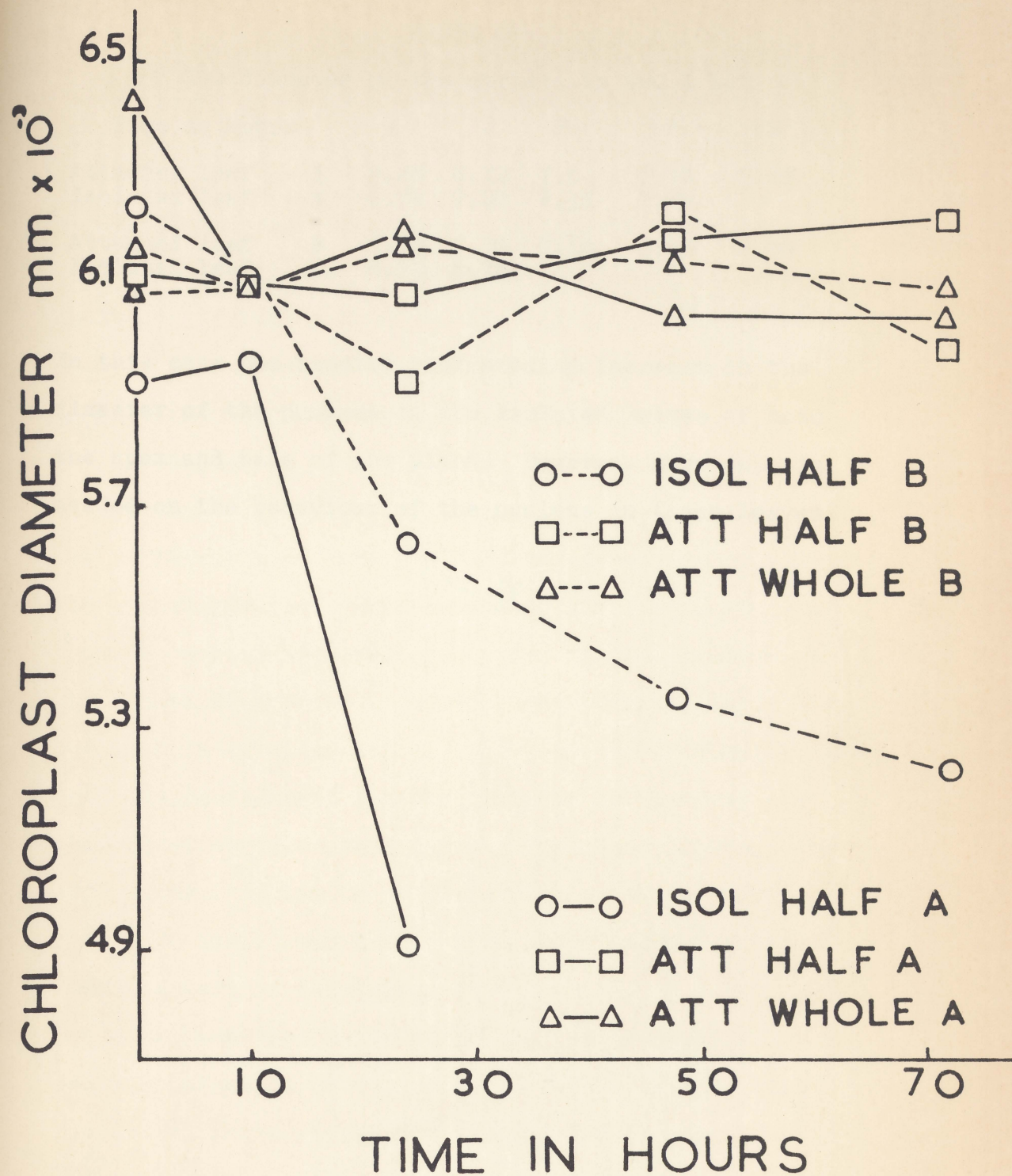and attached whole leaves. The results are shown in
Table 14.

FIGURE 25

FIGURE 26

DISCUSSION

TABLE 14

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

Time in hours		0	10	24	48	72
Attached leaf	A	7.88	8.12	7.0	7.48	7.07
Isolated leaf	A	6.74	7.02	8.35	7.94	
Attached leaf	B	5.80	7.10	7.10	6.95	6.95
Isolated leaf	B	6.23	8.16			

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 interferred 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 Bünning (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 initia-
tion 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 satis-
factory explanation for the initiation of cellular dedifferen-
tiation 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
mediterrenea. 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 attri-
butable 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 <u>Splachnum</u> 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, 1933 a 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, Transcedantia, 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 over-compensation of any normal gradient i.e. growth hormone will also cause undifferentiated growth. Von Wettstein (1953) found that Funaria spores after treatment with Vitamin B_1 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.

Bünning (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 establish-
ment 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 <u>Equisetium</u> 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 <u>Splachnum</u>, the polarity
is made visible before cell division by the polar displace-
ment of the nucleus and chromatophores. This is also the
case with the eggs of <u>Fucus</u>. 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 <u>Splachnum</u> 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 <u>Funaria</u> <u>hygrometrica</u>
Bopp found the least amount of regeneration in the middle
region of the gametophore. Gemmell found for <u>Atrichum</u>
<u>undulatum</u> 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:

NH_4NO_3	....	0.5 gms.
$MgSO_4 \cdot 7\ H_2O$	....	0.2 gms.
KH_2PO_4	....	0.2 gms.
$CaCl_2$	....	0.1 gms.
$FeCl_3$	....	traces
H_2O	....	to 1 liter

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