A Contribution to the Cell-physiologic Analysis of Growth and Morphogenesis in Fern Prothallia

By

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With 8 Text-Figures

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Fern prothallia have often been the object of investigations dealing with the problem of Morphogenesis in living systems, which is one of the most important problems of Biology. On the one hand these investigations were directed to ascertain the influence of certain external factors (light, gravitation, different chemical compounds etc.) on the development of the external form of the prothallia (Klebs 1893, 1917, Linsbauer 1926a, Orth 1934, 1937, Bussmann 1939, Kaufhold 1941, Hurel-Py 1946, 1947, Sossountzow 1950a, b etc.); on the other hand the problem of the regenerative capacity of different parts of the prothallia seemed to be of great interest (Nagai 1914, Linsbauer 1926b, Lawton 1932, 1936, Albaum 1938a, b). The results of these investigations led to the assumption that within the prothallia there exists a cellular continuity. The concept of this cellular continuity is now more clearly defined by the line of research chosen by Sinnott and Bloch 1945, 1946). Sinnott and Bloch introduced the term "Cytoplasmic pattern" and say the essence of histological differentiation both in the normal process of development and in regeneration is an establishment of such intercellular patterns or fields in the cytoplasm of groups of contiguous cells. As to the position of one single cell within this field of continuity it is given on the one hand by a system of genically determined cell polarities, on the other hand by a physiologic system which is mostly influenced by enviromental conditions. According to the literature three different explanations of this field of correlation could be given:

1. The investigations on regeneration carried out by Linsbauer (1926b) and Albaum (1938a, b) tend to assume that this field of correlation consists of chemical gradients which are determined by attraction centers according to the concept of Goebel (1902) and represents reciprocal action of substances, on the one hand formed by synthesis in the basic region, on the other hand by the growth substances of the apical region.

2. The electrophysiologic investigations of Lund (1947) carried out with different organs of higher plants would aim for an explanation of the correlation field within the prothallia of the ferns in the sense of a bioelectric field which is the result of the electric polarities of the single cells.

3. The cell-physiologic investigations within the scope of a protoplasmatic anatomy (Weber 1929, 1934, Reuter 1949) trend to a correlation field in a colloid-chemical sense, an assumption which is based on the observation of osmotic gradients within the prothallia of ferns (Gratzy-Wardengg 1928).

As to the growth conditions within the correlation field of a fern prothallium the quantitative measurements of Albaum (1938a) are of most importance. Albaum (1938a) was able to find that during the normal course of development a constant ratio between the increase in length and breadth of the prothallia occur. The size of the smallest and the biggest cell in a prothallium is nearly independent of the size of the whole prothallium. Big and small prothallia differ only in the number of cells. The highest cell division activity takes place in the apical region, the most marked cell elongation in the central parts; the longest cells are found in the basic region. The greatest amount of growth occurs in the border cells of the prothallium, as Doepp (1927) has already found by means of marks placed on the prothallia.

As to the development of adventitious prothallia, which grow from isolated basic parts of the prothallia their characteristic way of development shows a marked polarity, which is the result of the polarities of the cells within the normal texture of the prothallium.

The investigations to be described in this paper were conducted to give a better understanding of this correlation field of prothallia by trying to define it within the scope of the protoplasmatic anatomy. Prothallia with different rates of growth were used for these comparative investigations. The investigations were not limited to fully developed prothallia but were also intended to follow the unfolding of this correlation field during the process of development of the prothallia. Therefore the following program was set up for these investigations:

1. Germination and morphogenesis.
2. Observation of plasm-configuration and the qualities of the nucleus and the plastides.
3. Determination of the osmotic pressure.
4. Investigation of permeability.
5. Behavior of the cells against dyes.
6. Investigation of the resistence of the different cell types.

The investigations were carried out in 3 different series.

1. Investigations-series A: prothallia were grown in dispersed light.
2. Investigations-series B: prothallia were grown under the influence of a markedly reduced intensity of light.
3. Investigations-series C: prothallia were grown under the influence of an increased intensity of light.

Object and Method

The object of the investigations was *Dryopteris parasitica* grown in the greenhouse of the Botanical Department of the university of Pennsylvania in Philadelphia. The spores used for the cultures were taken from fern leaves with mature sporangia which were carefully scraped off the leaves. The material was squeezed through a finely porous lens paper applying only a very light pressure; by these means the spores were separated from the remainder of the sporangia. The culture of the spores was carried out on an agar cultur medium. Following the investigations of Linsbauer (1926a, b), Gratzy-Wardengg (1928) and Orth (1936) a 1.5% agar solution was used for this purpose. This agar solution was prepared by a short boiling of 1.5 g agar with 10 cc nutrition solution and 90 cc distilled water. The solution was poured into the petri discs destined for the cultures. The petri discs had a diameter of 9 cm and were filled with 30 cc each of the agar solution. As to its essential ingredients, the nutrition solution was prepared following the prescription of Hoagland and Shive (Seifriz 1938, p. 246) taking into consideration only the solution A and leaving out of consideration the solution B which contains important trace elements.

The composition of solution A was as follows:

Molar concentration	cc per liter of the solution
KH_2PO_4	1
KNO_3	5
$Ca(NO_3)_2$	5
$MgSO_4$	1
0.5% $FeCl_2$	1

The sowing of the spores was made through the fine lens paper to gain a homogeneous not too compact distribution of the spores all over the whole surface of the culture medium. Then the cultures were kept under the following conditions:

1. Series A: the petri discs were placed in the greenhouse exposed to dispersed light.

2. Series B: the intensity of light was markedly reduced all other conditions being constant.

3. Series C: the intensity of light was markedly increased all other conditions being constant.

The temperature for all three series was the same, 30° C.

1. Germination and Morphogenesis

According to Orth (1936) during the development of the prothallia the following stages were distinguished:

1. Germination. 2. The stage of the filamentous protonema, in which cell divisions take place only in one direction. 3. The flat prothallium which begins with the setting in of cell divisions in different directions and for which the formation of the vertex cell is of special importance.

Series A

Cultures in dispersed light

Under these conditions the spores of *Dryopteris parasitica* develop in the following way (Fig. 1):

1. Germination: About 10 days after the sowing on the culture medium the first cell grows out of the spore.

2. Stage of protonema: During the following 2 or 3 days the stage of protonema consisting of 3 or 4 cells develops.

3. Stage of prothallium: It begins with the formation of the vertex cell. This formation usually takes place in the following way: the last cell of the protonema (to this cell we must pay the most interest as the following cell-physiological investigations could show) is divided by a longitudinal cell wall and each of these two cells are divided by a transversal wall into two more cells. In one of these tip cells an oblique cell wall is now formed; by this means the descendants of the tip cell become the "side branch" ("Seitenast" following Orth 1936) and give origin to the vertex cell, whereas the descendants of the second tip cell go on continuing—so to speak—the protonema by forming cell walls only in a transversal direction. These cells represent the "main branch" following Orth (1936). The vertex cell now begins its activity and gives origin to cells alternately on the right and on the left. It is the tip cell of the protonema and its first descendants which are of special importance in forming the pattern of the prothallium. The directions in which the cell divisions occur one after another are not at all fixed and they may vary under the influence of external factors as we shall show below, describing the experiments made with both markedly reduced and also increased intensities of light. Even in cultures kept under the same normal external conditions we could often observe that after the formation of the 4 cells at the tip of the protonema in the above mentioned way through a certain period of cell divisions two equivalent cell filaments situated side by side were formed. The formation of an oblique cell wall giving origin to the vertex cell occurs only later.

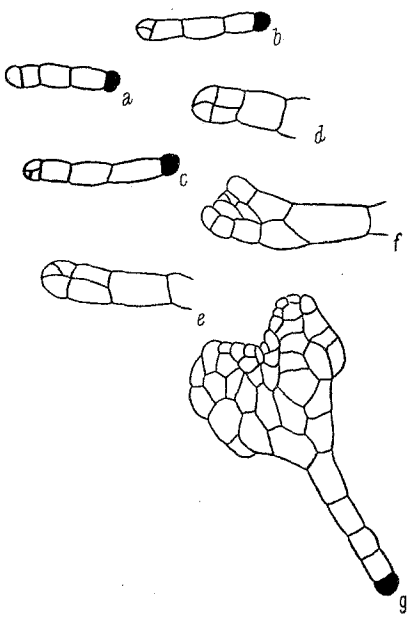

Fig. 1. Morphogenesis of the prothallia in series A. a) protonema, b) c) d) e) formation of the vertex cell, f) young prothallium, g) older prothallium.

For the cell-physiologic investigations it seemed to be advantageous to observe the following stages of development (Fig. 2):

1. The stage of the protonema with a segmentation in the cells 1, 2, 3 and 4[1].

2. The stage of a young prothallium in which we could distinguish besides the filamentous protonema within the flat part the zones I, II and III.

3. The stage of an older prothallium in which we distinguished, besides the filamentous protonema within the flat part, the zone I, II, III and IV.

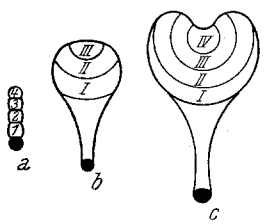

Fig. 2. Scheme of the different zones distinguished in the stages of development of series A. a) protonema, b) young prothallium, c) older prothallium.

Series B

Cultures under the influence of a markedly reduced intensity of light

Under these conditions the germination begins about one week or 10 days after the sowing (Fig. 3). The developing protonema consists of cells, progressively different as to their stage of development. These differences, however, are overcome in a short time by the quick elongation of the cells under the influence of the markedly reduced intensity of light. Within a short time the cells gain a much larger length than in series A. The ratio between length and breadth of these protonema cells is 5 : 1, or even more, whereas it is 2 : 1 in series A. The quick elongation stops soon, and the protonema consisting of very much elongated cells is kept for 5 till 6 weeks without any change. Only after this time does the development set in again. It begins by a cell division in a direction other than the usual direction. In the first of the examined protonemata a mode of division shown in Fig. 3c was observed. Afterwards this mode of division was changed by what perhaps may be explained as a consequence of the higher intensity of light given to the

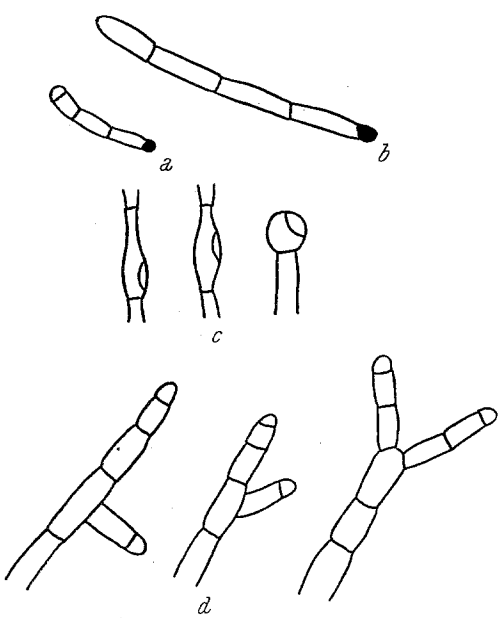

Fig. 3. Morphogenesis of series B. a) normal protonema, b) elongated protonema, c) ramification cell (elongated or spherical shaped), d) branched off cell-filaments (unequivalent and equivalent).

[1] Usually the protonema consists out of 4 cells, but in the cultures there could always be found protonemata consisting of a greater or smaller number of cells.

cultures in their daily examination under an illuminated microscope. Under these circumstances a cell of a nearly spherical shape is formed at the end of the protonema. The new membran formed within this cell can show a more or less oblique position. The following cell divisions occur again absolutely transverse to the longitudinal axis of the cells so that two filaments of cells result which meet in an angle of 30°—90°. Klebs (1917) was able to observe similar forms in his prothallia grown under markedly reduced intensity of light. A comparison of these prothallia with the prothallia grown under normal conditions enables us to identify the filaments of cells on the one hand with the main branch, and on the other hand with the side branch of the normally developed prothallia. Under the conditions of series B the second oblique cell wall, which gives origin to the vertex cell does not occur.

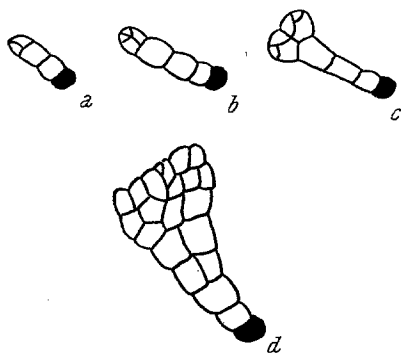

Fig. 4. Morphogenesis of series C. a) protonema, b) and c) formation of the vertex cell, d) prothallium.

For the cell-physiologic investigations the following stages of development were used:

1. Normal protonemata with the segmentation in cells 1, 2, 3 and 4, meaning the above defined early stage of the development of a protonema. On the basis of the morphologic qualities of the single cells we may assume that they are each in a different stage of development.

2. The elongated protonemata with the segmentation in the cells 1, 2, 3 and 4, meaning a protonema in which the process of elongation has stopped.

3. The stage of forming the ramification within the characteristic end-cell of the protonema; to this cell we must pay special attention showing the marked differences of its form.

4. The ramified cell filaments which are, according to the angle under which they meet, supposed to show differences in their stage of development. Within these cell filaments the cells following one after the other were marked with I, II, III, IV etc.

Series C

Cultures under the influence of markedly increased intensity of light

The germination begins under these conditions about one week after the sowing. The stage of protonema is characterized by very much shortened cells (usually the cells are more broad than long) (Fig. 4). The longitudinal cell division so important for the development of the flat prothallium occurs, under the conditions of series C, very often within the third cell of the protonema. In series A during the further development it was possible to make a distinction between a main-and a side branch. With series C, however, such a distinction is not so easy because after the longitudinal

division the cells behave absolutely equally as to the formation of transverse or oblique cell walls. The faint asymmetry of the prothallia of series A is not so marked here and is replaced by a nearly perfect bilateral symmetry.

According to series A the following stages of development were distinguished in the experiments:

1. The stage of protonema with the segmentation in the cells 1, 2 and 3.
2. The stage of a young prothallium with the same segmentation as with series A.
3. The stage of an older prothallium with the same segmentation as with series A.

2. Observation of the plasm configuration and the qualities of the nucleus and the plastides

One of the most striking facts during the development of the different cells within the prothallia of *Dryopteris parasitica* was the observation of a marked polarity of the plasm configuration of the different cells (Fig. 5). This is a behavior common to all cells within all 3 series. This polarity could be observed with untreated cells and becomes clearer after plasmolysis in the characteristic way in which the protoplast detaches from the cell walls ("Plasmolyseform und Plasmolyseort" Weber 1929b, c). This polarity may be observed particularly in the first cell coming out of the spore. When the process of elongation sets in within this cell, the protoplasm, the nucleus and the most of the plastides gather at the top of the cell. If such a cell is plasmolysed, we can detect at the apical end of the cell a negative spot of plasmolysis, which supports the assumption of an intimate contact between plasm and cell wall at the tip of the growing cell. If the cell afterwards is in the stage in which within a certain distance from the distal end of the cell a transverse cell wall is formed, the plasm moves away from the tip and gathers at the spot where the new cell wall is to be formed. With plasmolysis cells of this stage show a positive spot of plasmolysis at the tip. The phase following immediately the formation of the new membrane is characterized by the close contact of the plasm with both ends of the new formed membrane. This behavior is so common for all cells of the prothallia that it is possible to identify the most recent cell membrane within a cell without any difficulty by the occurrence of a negative spot of plasmolysis. If this newly formed cell wall has developed to a certain degree, then the plasm in the new formed cell gathers again at the apical end. At the same time the contact at the base gets looser. This loosening is in correlation with the behavior of the original cell. For—after having formed the new cell wall—in the original cell the plasm now detaches more easily from the distal

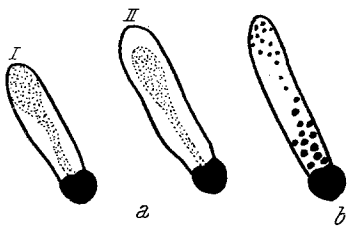

Fig. 5. First protonema cell. a) beginning of plasmolysis in 1 mol glucose, I younger and II older stage, b) cellular gradient.

membrane of the cell. In connection with this behavior the contact at the basic membrane of the cell gets closer and closer. In general during the development the following rule results for a single cell: in the early stages of development a close contact is evident between plasm and membrane at the distal end of the cell; in the late stages of development a close contact is evident between plasm and membrane at the base of the cell, and between these states we find a more or less indifferent stage with the contact nearly equal at both ends of the cell. In this respect the observations by Noll (1903) with Siphoneae, which were proved by others on different types of cells with apical growth, seem to be interesting. Noll (1903) states that the embryonic plasm characterized by certain morphologic qualities is always migrating to the tip. For an explanation of this observation Noll hat to assume the formation of an embryonic state within a part of the protoplast.

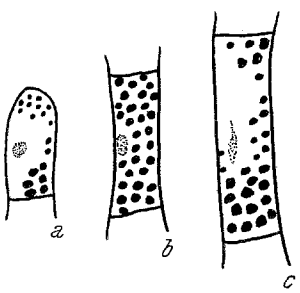

Fig. 6. Three characteristic cell stages. a) cell capable of dividing, b) cell plastic in differentiation, c) cell differentiated.

On the basis of many observations on the prothallia cells of *Dryopteris parasitica* we have to assume that such a differentiation between an apical embryonic part of the plasm and a basic somatic part which is further developed occurs in the cells of *Dryopteris parasitica* in the same way. Between these both extremes we find all kinds of transitional states. The observations on the qualities of the plastides seem especially to support this assumption. Very often we were able to detect fully developed plastides in the base—especially with elongated cells—while at the same time at the tip of the cell plastides of a still undeveloped stage could be found.

Besides the observation of the plasm configuration, the shape and the position of the nucleus and the qualities of the chloroplasts within the different types of cells were especially observed (Fig. 6). As to the shape of the nucleus there were found spherical, oval and in cells developed under certain conditions even spindle shaped forms. The plastides were either more or less oval or polygonal. There was either a uniform density of distribution in which case the chloroplasts do not show a great capacity for phototactic reaction, or the plastides were non uniformily arranged, a behavior which is always combined with a great mobility of the plastides under phototactic stimuli. These qualities of the nucleus and the plastides are in correlation with the configuration of plasm. In general, in young cells capable of division, spherical nuclei were found; within grown up cells, oval; and in elongated cells, spindle shaped ones. The arrangement of the plastides is correlated to the polarity of the cell. Only within cells of an intermediate stage of development could a more or less homogeneous arrangement of the plastides be observed. These cells are characterized, as we were able to show above, by no marked polarity. In this case the phototactic mobility of the plastides is very great. As to the content of amylum of the cells, it is not possible to give quantitative data only by staining the

amylum grains with Jodchloralhydrat because the volumes of the different cells differ too much. Therefore we could not get an exact proof as to wether there were gradients in the content of amylum within a prothallium or not. The assumption however that such gradients exist corresponding to the different stages of the development of the different cells seems to be founded.

In Fig. 7 the polarity of the cells of series A and B is shown schematically by arrows. To be able to mark the polarity conditions of the single cells within the correlation field of the whole prothallium more exactly, we distinguished the 3 following directions in normal prothallia:

1. radial polarity,
2. medial polarity,
3. tangential polarity .

With series A and C the polarity conditions shown schematically in Fig. 7 are valid. With series B we can only speak of a medial and a tangential polarity corresponding to the special form of the prothallia developed under these circumstances. Here cells with radial polarity are never formed.

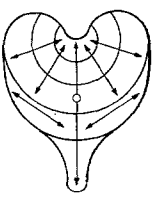

Fig. 7. Polarity of the different cells within the correlation field of the whole prothallium.
a) radial ←•→
b) medial ←⊖→
c) tangential ←→

3. Osmotic value

The first cell-physiologic gradients which were detected on fern prothallia are the osmotic gradients within the prothallia of *Nephrodium filix mas* and *Struthiopteris germanica* (Gratzy-Wardengg 1928). According to her investigations the osmotic gradient between the base and meristem is formed by the vertex cell by giving higher osmotic values to the later formed segments than to the earlier formed segments. For the investigations on the osmotic value within the prothallia of *Dryopteris parasitica* the method of threshold-plasmolysis in graduated solutions of glucose was used. In the tables the values gained for the different parts of the prothallia are given in mol glucose.

Series A

	protonema				young prothallium				older prothallium				
	1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
mol glucose .	0.25	0.30	0.35	0.40	0.25	0.30	0.35	0.40	0.25	0.30	0.40	0.45	0.50

In accordance with Gratzy-Wardengg (1929) osmotic gradients could be observed in the protonema and in the stage of prothallia as well in the sense that the youngest cell still capable of division shows the highest osmotic value whereas the oldest cell possess the lowest osmotic value. Comparing the different stages of development, we can see that the gradient within a young prothallium does not show essential differences. With older prothallia the gradient as a whole is a little bit higher.

From the results obtained we may conclude that corresponding to the progressing development and with the increase in the number of cells, the osmotic differences between 2 contiguous cells decrease more and more.

Series B

	normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
	1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
mol glucose	0.30	0.32	0.35	0.40	0.40	0.40	0.40	0.40	0.40	0.50	0.25	0.28	0.30	0.40	0.45

Under the circumstances of series B the developing protonema shows in the beginning an osmotic gradient too. Later, when the elongated protonemata are developed, as described above and growth has stopped, the osmotic gradients are compensated too. If under the influence of a small intensity of light a spherical ramification cell is formed, then this cell shows a higher osmotic value than the other cells. If the ramification however takes place in an elongated cell, then this cell does not differ very much from the other cells as to the osmotic value. As to the osmotic conditions of the ramified cell-filaments, they show a normal gradient from the tip to the base. With cell-filaments which seem to be equivalent as to their morphology these osmotic gradients were absolutely equal. With cell-filaments different as to their morphology the osmotic gradients show differences, and the shorter the filament, the greater is the gradient between apex cell and base cell.

Series C

	protonema			young prothallium				older prothallium				
	1	2	3	prot.	I	II	III	prot.	I	II	III	IV
mol glucose . .	0.35	0.40	0.45	0.35	0.37	0.40	0.45	0.35	0.37	0.40	0.42	0.45

With this series it was striking that in the stage of protonema as well as in young and older prothallia the base cell shows relatively high osmotic values. The cells in series C are, also assumed from the observation on the morphology of the cells, in a relatively early stage of development. The gradient within the whole prothallium then is smaller between apex-and base cell.

4. Permeability to Urea and Glycerin

It is a well known fact that there exists a clear correlation between the permeability qualities of a cell and its state of development (Weber 1931a, b, Marklund 1936, Reuter 1949). Besides investigations of whole permeability series, the permeability ratio between Urea and Glycerin was often used to characterize the permeability of a cell. In his critical investigations on permeability series Bogen (1949) has pointed out that the

differences in the permeability of Urea and Glycerin of a cell can not be explained without taking into consideration that there also exist differences between these two substances in the hydration of the surface of the plasm.

The only purpose of the investigations with *Dryopteris parasitica* was to find a possibility of characterizing the physiologic state of a cell. Preliminary experiments had shown that marked differences between the various types of cells exist in the time of deplasmolysis in Urea-and Glycerin solutions. So we tried at first to determine comparatively the permeability of the plasm for these two substances. The question as to wether these differences in the plasm are only caused by primary differences in the permeability or wether they are also influenced by differences in the behavior with hydration was, for the present investigation, not too important. Höfler (1930) and Huber and Höfler (1930) have shown that we can draw conclusions, as to the water permeability from the rate of plasmolysis also; the time necessary to reach the state of perfect plasmolysis seems to be in correlation with the viscosity of the plasm (Weber 1929b).

In the following tables the time of deplasmolysis of the cells for 1 molar Urea and for 1 molar Glycerin is given respectively. In table 3 the times of plasmolysis in 1 molar glucose is given. From these data we are able to draw conclusions as to the water permeability and the viscosity of the plasm.

Series A

Permeability to Urea

Time of deplasmolysis in 1 mol Urea

protonema				young prothallium				older prothallium				
1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
40—50 min.	30—40 min.	20—30 min.	15—20 min.	30—40 min.	20—30 min.	15—20 min.	10 - 15 min.	60—90 min.	30—60 min.	30—35 min.	25—30 min.	20—25 min.

Permeability to Glycerin

Time of deplasmolysis in 1 mol Glycerin

protonema				young prothallium				older prothallium				
1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
15—30 min.	10—15 min.	0	0	40—60 min.	20—40 min.	0	0	2—3 hrs.	1½—2 hrs.	10—15 min.	3—5 min.	2—3 min.

Permeability to Water

Time of plasmolysis in 1 mol glucose

protonema				young prothallium				older prothallium				
1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
5—10 min.	10—15 min.	15 min.	20 min.	5 - 10 min.	15 min.	20 min.	20—30 min.	5—10 min.	10 min.	10 min.	10—20 min.	20—30 min.

As to the permeability of Urea and Glycerin the results show that the youngest cell possess the highest permeability to Urea and Glycerin whereas,

with the progressing development, the permeability for both substances decreases markedly. A comparison of both substances shows that the degree of this change in the permeability with progressing development varies markedly for both substances. While the youngest cells are more permeable to Glycerin than to Urea, the fully developed cells are more permeable to Urea than to Glycerin. It is this difference in the degree of permeability which gives us the possibility of easily determining the state of development of a cell by finding the ratio of the permeability of Urea to that of Glycerin. The experiments with 1 molar glucose showed that longer times of plasmolysis exist in younger cells, a fact which supports the assumption that there is in younger cells a closer contact between plasm and membrane and a smaller water-permeability.

Series B

Permeability to Urea

Time of deplasmolysis in 1 mol Urea

normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
15—20 min.	10—15 min.	7—10 min.	5—7 min.	15—20 min.	15—20 min.	15—20 min.	15—20 min.	15 min.	5 min.	15—20 min.	15—20 min.	10—15 min.	7—10 min.	5—7 min.

Permeability to Glycerin

Time of deplasmolysis in 1 mol Glycerin

normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
7—10 min.	5—7 min.	2—3 min.	2—3 min.	15—20 min.	15—20 min.	15—20 min.	15—20 min.	15 min.	0	15—20 min.	5—7 min.	5—7 min.	2—3 min.	2—3 min.

A comparison of the results of series B to the results of series A is difficult, because as a whole the dark cultures show, as compared to the light cultures, a great increase in the permeability of both Glycerin and Urea. Because of this great increase the differences in permeability between the different types of cells become less and less pronounced. In spite of this fact weak gradients could be observed in normally developed protonemata for Urea as well for Glycerin. These gradients were cancelled in the state of the elongated protonemata by a general decrease of the permeability. This observation fits well in the results of the experiments on the osmotic values of the cells. The ramification cell has, if it is an elongated cell, a permeability of the same order of magnitude as the other protonema cells, but generally speaking its permeability seems to be a little bit higher. If the ramification cell is spherical shaped it has a special high permeability. The branched off cell-filaments again show normal gradients of permeability. In this respect, according to the investigations on the osmotic value, equal gradients were found within morphologically equal cell-filaments, and

different gradients in morphologically different cell-filaments. This indicates that the shorter the cell-filament is, the greater the gradient. As to the observations on the water permeability and on the viscosity of the cells in series B, no detailed data may be given. Under the circumstances of series B and in consequence of the very close contact between plasm and membrane plasmolysis may produce serious injury to the surface of the plasm. Therefore well defined differences in the time of plasmolysis between the different cells could not be found.

Series C

Permeability to Urea

Time of deplasmolysis in 1 mol Glycerin

protonema			young prothallium				older prothallium				
1	2	3	prot.	I	II	III	prot.	I	II	III	IV
40–50 min.	30–40 min.	20–30 min.	30–40 min.	20–30 min.	15–20 min.	10–15 min.	60–90 min.	30–60 min.	30–35 min.	25–30 min.	20–25 min.

Permeability to Glycerin

Time of deplasmolysis in 1 mol Glycerin

protonema			young prothallium				older prothallium				
1	2	3	prot.	I	II	III	prot.	I	II	III	IV
0	0	0	20 min.	5 min.	0	0	20 min.	10 min.	5 min.	0	0

Under these circumstances the results are similar to the results of series A. Indeed the total gradient is not as great as in series A. This supports the concept that the cells of the whole prothallium under the circumstances of series C are in a relatively less developed stage than the cells of series A. The results of the experiments on the behavior of plasmolysis in 1 molar glucose agree almost completely with the results of series A so that here giving the accumulated data for this case is not necessary.

Summarizing the experiments on permeability we can say that a comparison of the results is in a certain way difficult because of the fact that the cells whose permeabilities are to be compared show differences in their osmotic value and in their size. So, in the state of perfect plasmolysis, differences as to the degree of plasmolysis result in the different types of cells. We have, however, a good reason to assume that the differences of time of deplasmolysis cannot be caused only by the differences in the osmotic value. With normally developed prothallia such marked differences, especially as to permeability of Glycerin, were found between the meristematic cells and the cells of the base that they could not be explained only by the relatively small differences in the osmotic value. For a more detailed investigation of this question future experiments are planned with differently concentrated solutions of Urea and Glycerin, the concentrations

of which solutions are to correspond with the osmotic conditions of each single cell.

5. Behavior of the cells to dyes

The reaction to dyes was early used for characterizing cells and parts of cells; the creation of the new staining methods using graduated p_H series we owe to Strugger (1937, 1949). By choosing different p_H values it is possible to let the dye act on the one hand as an ion, on the other hand as a molecule. With living material and with preserved material differences in the possibilities of staining result. Without paying attention to the different attempts of finding a satisfactory explanation for the different types of staining, we can state that for the staining of living cells the permeability of the plasm and the presence of certain of the dye staining substances within the cell are first of all essential; whereas with preserved material the electric charge of the various parts of the cell is at first of great importance. For investigations dealing with the analysis of tissues as they were planned for the prothallia of *Dryopteris* both investigations with living cells as well as with killed ones seemed to be necessary.

Strugger (1937, 1949) was able to show for living cells that when using a basic dye, as for instance neutral red, within the acid range when the dye is present in the form of a dye cation, a staining of the negatively charged membrane is gained by electro-adsorption. In the neutral range, when the dye is not dissociated, the molecule of the dye may penetrate into the interior of the cell and be the cause of a staining of the cell sap. To what an extent the gained staining is dependent of the physiologic state of each single cell could be shown with the leaf of *Helodea* (Strugger 1949, p. 146). By staining with neutral red it was found that young growing cells are not able to take up and stain with the dye, whereas grown up cells are capable of an intensive staining. These differences in the behavior cause characteristic staining gradients which correspond to the zones of cells in different stages of their development.

With fixed and preserved *Helodea*-leaves Drawert (1938) could also prove the existence of gradients of stainability. Drawert (1937a, b) elaborated a method for determining the IEP, that is the isoelectric zone of plasm, nucleus and plastides. This method consists of using two different dyes, an acid and a basic one, in graduated p_H ranges. At the p_H where the isoelectric zone of the cell-organ concerned is reached, the dye cannot be retained further by electro-adsorption and therefore no staining occurs. With the leaf of *Helodea* Drawert (1938) was able to show that this isoelectric zone of the different parts of the cells is correlated with the degree of differentiation to the cell. As to cell membranes, Drawert (1938) found that the lower the isoelectric zone the younger the membrane was. This may be explained by the high protein content of young membranes. As to the other parts of a cell as a whole the IEP seems to be higher in young cells than in grown up cells; in this respect of course differences in permeability of cells in different stages of development may be of great importance in these investigations.

For the staining experiments with the prothallia of *Dryopteris parasitica* the method described by Strugger (1937, 1949) was used.

For characterizing the single cells within the prothallia of *Dryopteris parasitica* on the basis of preliminary experiments the following kinds of staining seemed to be advantageous:

1. Vital staining of the cell sap with neutral red.
2. Staining of the cell content of cells preserved in 70% alcohol with toluidinblue.
3. Staining of the cell content of cells preserved in 70% alcohol with acid fuchsin.
4. Staining of the content of preserved cells with methylgreen acetic acid.

For the preparation of the graduated p_H ranges the following standard solutions were used:

1. Neutral red solution in a concentration of 1 : 1000 and acid fuchsin and toluidinblue solutions in a concentration of 1 : 2000.
2. *n*/10 HCl.
3. Standard Buffer Solution I: 1/15 mol KH_2PO_4.
4. Standard Buffer Solution II: 1/15 mol Na_2HPO_4.
5. Standard Buffer Solution III: 1/15 mol K_3PO_4.

The composition of the p_H ranges used is given in the following table:

Quantity of the standard solution in cc

p_H	n/10 HCl	I	II	III	H_2O + Stain
2.0— 2.2	9.5	0.5	—	—	80 + 10
3.4— 3.9	0.5	9.5	—	—	80 + 10
4.6— 4.9	—	10	—	—	80 + 10
5.6— 5.7	—	9.5	0.5	—	80 + 10
5.8— 6.1	—	9	1	—	80 + 10
6.3— 6.5	—	8	2	—	80 + 10
7.0— 7.1	—	5	5	—	80 + 10
7.5— 7.6	—	2	8	—	80 + 10
8.0— 8.3	—	4.5	—	5.5	80 + 10
9.8—10.1	—	5	—	5	80 + 10
10.7—10.8	—	3	—	7	80 + 10

Vital staining of the cell sap with neutral red

Neutral red is dissociated as far as about p_H 6.3. Within the range of p_H 6.3 to 7 it occurs as a molecule of the dye base. For preliminary investigations the whole p_H series shown above was used. By this means we could observe the special behavior of the rhizoids and the papillae occurring only in old prothallia. These show an extremely high permeability to neutral red both in the form of an ion and in the form of a molecule as well; for within all p_H ranges an intensive staining of the dye within the cell sap was

observed. This rapidly appearing diffused staining of the cell sap lasts only a few minutes, changing soon to a non-homogeneous coloration ("Krümmelfärbung") which is, especially in strongly acid p_H ranges, brought to an end after few minutes by lethal injuries. The younger the cells are the more serious are these injuries.

Series A

Neutral red

p_H	protonema				young prothallium				older prothallium				
	1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
5.6—5.7													
6.3—6.5					+ +				+ +	+ +			
7.0—7.1					+ + +				+ + +	+ + +			

Corresponding with the above results with *Elodea* we could show within the prothallia of *Dryopteris parasitica* that only those cells which are far advanced in their development are capable of staining with the dye. In the stage of protonema none of the cells have already reached this stage of development. Therefore, with neutral red at different p_H ranges no vital staining was gained. Within young prothallia the cells of the protonema are nearly absolutely grown up, whereas within older prothallia the cells of the protonema and the cells of zone I are in this advanced state of growth. On the basis of these results it is possible to use vital staining with neutral red within a p_H range of 6.3 to 7.1 to identify the cells with finished growth within the different forms of prothallia.

Series B

Neutral red

p_H	normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
	1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
5.6—5.7															
6.3—6.5	++	++			+++	++	++	++	++	–	++	++			
7.0—7.1	++	++			+++	+++	+++	++	++	–	++	++			

In series B the results of the experiments on vital staining with neutral red showed that within both normal developed protonemata and the ramified cell filaments there exist gradients. These gradients are removed in elongated protonemata. The ramification cell shows, corresponding to its shape, a different behavior. If it is elongated a staining of the dye occurs which is not the case if it is spherical.

A special behavior common to all cells formed under the conditions of series B is the fact that the diffused staining of the cell sap changes very quickly into a non-homogeneous staining of the cell sap which may be explained by the low resistence of the prothallia grown under the influence of a markedly reduced intensity of light.

Series C

Neutral red

p_H	protonema				young prothallium				older prothallium				
	1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
5.6—6.7													
6.3—6.5													
7.0—7.1	+ +				+ + +				+ + +				

The staining of the cell sap of the cells developed under the conditions of series C occurs very seldom. The results of the experiments on vital staining support very well the concept that the cells of the prothallia of series C are as a whole in a relatively early stage of their development. This was already presumed on the basis of the results of the experiments on the osmotic value and on permeability.

Staining of the cell content of cells preserved in 70% alcohol with toluidinblue

Toluidinblue is dissociated far within the basic range to p_H 10. Similar to the behavior of the cells against vital staining with neutral red, the rhizoids and the papillae of old prothallia also show a special high stainability for toluidinblue within all p_H ranges. Within the other prothallium cells, with toluidinblue, a clear staining of the membrane, the nucleus, and the plastides can be gained within certain p_H ranges. A delicate distinction between the occurrence of a staining of the nucleus and of the plastides was sometimes not easy with the cells of *Dryopteris parasitica*—especially within very young cells because of their high plastides content. Therefore we decided not to give more details of our experiments with the differential staining of the different parts of the cell with toluidinblue. We restrict ourselves to speak in the following description only about the staining of the whole cell content without distinguishing in each case between the occurrence of a staining of the nucleus, the plastides and the plasm.

As to this staining of the whole content of the cells we observed generally speaking in the prothallium cells of *Dryopteris parasitica* that the younger cells stain the dye in a lower p_H range than the older ones, which does not agree with the results Drawert (1937a, b) gained with the leaves of Helodea. Besides these general statements it was striking that

within certain parts of the prothallium a staining of the cell content never occurs regardless of the applied p_H range (Fig. 8). This result agrees with the results gained by Drawert (1937a, b) with the leaf of *Helodea*.

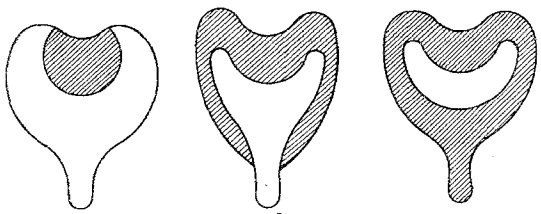

Fig. 8. Gradients in a normally developed prothallium by staining with toluidinblue. a) with p_H 6, b) with p_H 7, c) with p_H 8.

Therefore besides the degree of the development of a cell other factors seems to be of importance in the staining of the cell content with toluidinblue. Similar results were gained with acid fuchsin, as will be described later. Because the occurence of a staining is caused by different factors, the results gained with the cells of *Dryopteris parasitica* by treating them with toluidinblue or acid fuchsin do not show the uniformity we found with our experiments on the osmotic value and on permeability. These qualities seem to be only caused by the degree of development of the cells concerned.

Series A

Toluidinblue

p_H	protonema				young prothallium				older prothallium				
	1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
2.0— 2.2								++					+++
3.4— 3.9								++					+++
4.6— 4.9								++				+	+++
5.6— 5.7				+				++				++	+++
5.8— 6.1			+	+				++				++	+++
6.3— 6.5		+	+	++		+		++				+++	+++
7.0— 7.1	+	+	++	++	+	++		++		+		+++	+++
7.5— 7.6	+	++	++	+++	++	++		++	+	+		++	++
8.0— 8.3	++	+++	+++	+++	+++	+++		+++	+++	+++		+	+
9.8—10.1	++	++	++	++	+++	++		+++	+++	+++		++	+
10.7—10.8	+	+	+	+	+++	++		+++	+++	+++		++	+

In the stage of protonema within low p_H ranges only the tip cell is stained; with increasing p_H ranges, from p_H 7 up, the whole filament is stained. In the stage of a prothallium in low p_H ranges only the meristem on the front end of the prothallium is stained; with increasing p_H the stained border zone increases more and more in area, until (this value depends on the age of the examined prothallium) it closes almost to a circle.

Within the protonema cells of the prothallium a staining occurs only at a relatively high p_H. Within a certain area in the center of the prothallium a staining could not be observed at any p_H. This unstained area decreases more and more, however, with increasing p_H.

Series B

Toluidinblue

p_H	normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
	1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
6.3– 6.5				+	–	–	–	–	–	+					+
7.0– 7.1			+	++	–	–	–	–	–	++				+	++
7.5 – 7.6		+	++	+++	–	–	–	–	–	++			+	++	++
8.0 – 8.3	+	++	++	+++	–	–	–	–	–	+++		+	++	++	++
9.8–10.1	++	++	+++	+++	–	–	–	–	–	+++	+	++	++	++	++
10.7–10.8	+++	++	–	+++	–	–	–	–	–	++	++	++	++	++	++

The results of series B showed staining gradients for normal protonemata and for the ramified cell-filament; the elongated protonemata do not show a staining within any of the examined p_H ranges. The ramification cell is not stained if it shows the form of an elongated cell; if it is spherical its content is stained within a wide p_H range, as usual for meristematic cells. It was however striking that in the special form of prothallia grown under the conditions of series B we never could find an equivalent for the unstained zone within the prothallia of series A. This fact supports the assumption that this unstained part of the normal prothallium is not caused by certain physiologic qualities of the cells dependent of their state of development, but rather by certain qualities of the cuticula of the cells of the central part of a normally developed prothallium.

Series C

For series C the results were very similar to series A. Therefore we need not give the data. The p_H ranges were shifted to a lower p_H value, which can be explained in this case too by a relatively undeveloped state of the single types of cells.

Staining of the cell content of cells preserved in 70% alcohol with acid fuchsin

Acid fuchsin as an acid dye never shows a staining of the negative membrane, therefore in this case the staining of the cell content was also observed. Preliminary investigations had shown that staining only occurs in strongly acid ranges, therefore only p_H ranges between 2.0 to 6.0 were used.

Series A

Acid fuchsin

p_H	protonema				young prothallium				older prothallium				
	1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
2.0—2.2	—	—	—	+	+	+	—	+	—	—	—	—	—
3.4—3.9	—	—	—	++	++	++	—	++	+++	+++	—	++	++
4.6—4.9	+	—	—	++	++	++	—	++	—	—	—	—	—
5.6—5.7	++	—	—	++	—	—	—	—	—	—	—	—	—

The results are similar to the results gained with toluidinblue. There can be proved on the one hand differences in the behavior of young and old cells as especially shown in the stage of protonema and, on the other hand, for the fully developed prothallia there is characteristic the appearance of an unstained part within the center of the prothallium. In this case there seems, however, to exist a more clear correlation between this unstained zone and the physiologic state of the cells. For in the state of protonema some cells, apparently in a certain stage of their elongation, do not show a staining.

Series B

Acid fuchsin

p_H	normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
	1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
2.0—3.2	+	—	—	+	—	—	—	—	—	+	—	+	—	—	+
3.4—3.9	++	++	—	++	—	—	—	—	—	+	++	++	++	—	++
4.6—4.9	+	+	—	+	—	—	—	—	—	+	++	++	++	—	++
5.6—5.7	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

For series B, in the same way, the appearance of unstained center cells is valid both in the state of a normally developed protonema and in the ramified cell-filaments. In the elongated protonema cells a staining does not occur, whereas the ramification cell may show a staining or not dependent of its physiologic state.

Series C

The results of series C are nearly just the same as in series A, therefore we do not give the data. The p_H values are shifted a little bit to the more acid range than in series A.

Staining of the content of preserved cells with methylgreen acetic acid

Series A

Methylgreen acetic acid

protonema				young prothallium				older prothallium				
1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
−	−	+	++	−	−	+	+	−	−	−	+	+

Series B

Methylgreen acetic acid

normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
−	−	+	+	−	−	−	−	−	+	−	−	−	+	+

Series C

Methylgreen acetic acid

protonema			young prothallium				older prothallium				
1	2	3	prot.	I	II	III	prot.	I	II	III	IV
−	−	+	−	−	−	+	−	−	−	+	+

For all three investigations series it could be shown in the same way that—neglecting the rhizoids and papillae which take up and stain the dye quickly—only the young cells capable of division stain the dye. We were able, by neutral red, to characterize cells in the last state of their development, and with methylgreen to identify cells in the earliest stage of their development.

6. Investigation of the resistence of the different cell types

The investigations with graduated p_H series showed great differences in the resistence of the single cells according to their degree of differentiation. The younger the cells are the quicker they show lethal injuries in strongly acid buffer solutions. The resistence qualities of the single cells could also be proven by experiments with 20% ethylalcohol. The prothallia were put into the ethylalcohol for 2 to 20 minutes and afterwards the stage of injury was determined by plasmolysis in 1 mol glucose. In the following tables for the different types of cells the duration of lethal action is given. For all three of the investigation series it was shown that there exist marked differences of resistence which vary with the state of development of the cells. The younger the cells are the more sensitive they are to the action

Series A

Resistence to 20% ethylalcohol
Duration of lethal action in minutes

protonema				young prothallium				older prothallium				
1	2	3	4	prot.	I	II	III	prot.	I	II	III	IV
5–7	5	2	2	10	7	2	1	10–15	7–10	3–4	2–3	1–2

Series B

Resistence to 20% ethylalcohol
Duration of lethal action in minutes

normal protonema				elongated protonema				ramification cell		equivalent branched off cell-filaments				
1	2	3	4	1	2	3	4	elong.	spheric.	prot.	I	II	III	IV
5	5	2–3	1–2	2–3	2–3	2–3	2–3	2–3	2–3	7	5–7	5	2	2

Series C

Resistence to 20% ethylalcohol
Duration of lethal action in minutes

protonema			young prothallium				older prothallium				
1	2	3	prot.	I	II	III	prot.	I	II	III	IV
5	5	3	10	8	5	3	15	10	8	6	4

of injuring substances. An exception is represented by the elongated cells of the protonemata grown under the circumstances of series B as they show a special high sensitivity to this destructive action though they are in a very highly progressed state of their development. As to the ramification cell, in the experiments on resistence there were found no marked differences between the two characteristic forms, the elongated ramification cell or the spherical ramification cell. In both cases mentioned the ramification cell is characterized by a high sensitivity. In this case, however, the assumption is obvious, on the basis of the above mentioned cell-physiologic observations, that in the first case it is a decrease of the resistence as is characteristic for cells grown in the dark and in the second case it is the small resistence as is characteristic for young cells still capable of division.

Discussion

It seems advantageous to discuss the results of the cell-physiologic investigations with the prothallia of *Dryopteris parasitica* in three different parts:

I. Results with prothallia grown under conditions which are supposed to be normal.

II. Comparison of these results with the results obtained with prothallia under changed conditions.

III. Attempt to refer all the obtained results to the results of the experiments on regeneration with fern prothallia described in the literature.

I. Paying attention at first only to the results of the investigations on fern prothallia, which were grown under normal conditions, we can summarize them into two points of view:

1. Within the prothallia there are marked cell-physiologic gradients from the top to the base, which are a manifestation of the differently well progressed degree of differentiation of the single cells and therefore are to be interpreted first of all as growth gradients.

2. Within each single cell there exist gradients, too, which manifest themselves in the polarity of the cells. This polarity is especially evident in young growing cells and exists here in a differentiation in an embryonic plasm at the apical end of the cell and a somatic plasm at the base of the cell. The plasm in the embryonic stage is considered to be more or less indifferent while the somatic plasm is considered to be adapted to one or several particular functions both qualitatively and quantitatively.

Goebel (1902) tried to characterize the essence of progressing differentiation by his concept, that the somatic cells are embryonic cells which became in a certain way "incrusted," meaning an additional characteristic which gave them their characteristic "stamp." The decrease of the development potential is according to this concept of Goebel, not based on its total cessation, but only on the fact that the possibility for further development becomes latent or obscure, an idea which is, as Linsbauer (1926b) pointed out, especially important for the understanding of the process of regeneration. In the case of the prothallia cells of *Dryopteris parasitica* the process of differentiation is characterized by the fact that the cellular gradients become gradually less when certain cell physiologic qualities which are characteristic for differentiated cells, are acquired.

For the further understanding of the process of development of the fern prothallia the concept, which was already pointed out by Linsbauer (1925), seemed to be of importance that within the ontogenetic development of a multicellular organism we have to distinguish between a cytologic or cellular development and a histogenic development. If we try to apply these ideas to the process of development of the prothallia of *Dryopteris parasitica* it follows that only in the first protonema-cell can the process of the cytologic development be observed without any restriction. If by the formation of the new membrane within this cell the segmenting off of a second cell occurred, then these two cells are not, subjected only to the law of cytologic development, but also at the same time to the law of the histogenic development. The cause of this histogenic development is to be seen in the fact that the original cell, as its differentiation increases after having segmented off the embryonic part of its plasm, becomes a functioning cell. The correlation of the cells which is of such an importance for the histogenic development is to be understood first of all as a correlation of chemical sub-

stances, that is that the differentiated cells take over the preparation of the nutritive substances while the cells capable of division produce the growth substances. The manifestation of this correlation as can be seen from the experiments on regeneration after plasmolysis by Nagai (1914), might occur by means of the plasmodesmata. The assumption is obvious that with the prothallium cells of *Dryopteris parasitica* this correlation between cytologic and histogenic development (the results of which the formation of the gradients is to be regarded) receives its manifestation by the manner in which with the increasing number of cells, the rhythm between the elongation of the cell and the formation of the new transverse membrane is changed. Under normal conditions the divisions occur in shorter and shorter periods so that the ratio between the length and the breadth of the cell approaches 1. If this point is reached then the development of the protonema stops and the divisions occur which are characteristic for the formation of the flat shaped prothallium and orientated in another than in a transverse direction to the longitudinal axis of the cell.

II. While up to now we paid attention only to the results with prothallia grown under normal conditions we shall try next to bring these results in harmony with the results obtained with prothallia grown under changed external conditions. Klebs (1917) has already pointed out in an account of his extended investigations on the morphogenesis with fern prothallia as in other publications too, that they are the quantitative changes in the external general and essential factors of life which realize the variety of morphogenesis within plants. Sinnott, Dunn and Dobzhansky (1950) emphasize, dealing with the question "genetics and development," that one of the most important problems is the question of how the genotype is related to the developed character; and they accentuate, in discussing the development of form differences, that it is first of all the ratio between length and breadth of an organ which is genically controlled: "Experiments indicate that it is this ratio rather than any particular ratio between dimensions that is genically controlled... Such differences in dimensional growth occur primarily during the period of cell division and are related to constant differences in the plane in which the cells divide. This in turn is evidently one manifestation of cell polarity. The genic control of shape in such cases therefore seems to be exercised through a control of cell polarity. The genic mechanism by which they are controlled are far from clear, hormone actions, differences in electrical potential and various other means for producing inequalities in growth have been suggested but without proof." On the basis of these data we must regard for the morphogenesis of an organism or an organ first of all as essential:

1. the ratio of the increase of length to the increase of breadth;

2. the polarity of the cell in its correlation to the plane in which the cell divides, or to the direction in which the new cell wall is formed.

By changing especially the conditions of light with the experiments on *Dryopteris parasitica* it was possible to influence the growth conditions of the single cells in a different way. With markedly reduced intensity of light

the prothallia show a distinct tendency to "étiolement" which manifests itself in markedly elongated cells, while with high intensity of light the disturbance of the correlation between the formed nutritive substances and the growth promoting substances produce short, broad, more or less isodiametric cells. The question, so important for an explanation of the results obtained with the prothallia of *Dryopteris parasitica*, was wether there exists a correlation between the observed polarity of the single cells and the growth ratio. The results obtained with prothallia grown under normal conditions had already shown that a change in the direction in which the new membrane is formed occurs only if the dividing cell has a nearly isodiametric form. With series B in which during the first couple of weeks only long cell filaments develop, never a cell membrane was formed in another direction than vertically to the longitudinal axis of the cell. If these cell-filaments were exposed to light, the above mentioned spherical shaped ramification cell was formed. Within this cell we were able to detect by a great deal of observations that there exists undoubtedly such a correlation between the polarity in the plane in which the new cell membrane is formed and the ratio between the length and the breadth. The more the ramification cell approached in its form an isodiametric shape, the more oblique was the position of the new cell membrane; the more the elongation of the ramification cell predominated the increase in the breadth, the more vertically is the new cell wall orientated to the longitudinal axis of the cell.

The observations with the cells of series C fit absolutely in this assumption. Under the conditions of light of series C the elongation is markedly reduced. Therefore within the 3rd protonema cell the characteristic ratio between length and breadth is often reached which makes the occurrence of a membrane in another direction than transverse to the longitudinal axis of the protonema possible. The prothallia formed under the conditions of series C show a marked tendency to forming oblique membranes. This tendency does not only manifest itself in the way in which the stage of protonema turns earlier to the stage of prothallium but also in the fact that a distinction between a main- and a side branch is not so easily made as within series A because of the occurrence of oblique cell membranes in both branches and not only in the side branch as in series A. As to the shape of the newly formed prothallium this change in the developmental tendency of the cells becomes evident by compensating, under the conditions of series C, the fair asymmetry which occurs in series A by the differentiation in a main- and a side branch. Instead of this we find nearly a perfect bilateral symmetry.

III. A comparison of these results with the results of the experiments on regeneration of fern prothallia described in the literature seems to be interesting. Both Linsbauer (1926b) and Albaum (1938a, b) were able to show that there is a marked difference in the process of regeneration, the question being whether the regenerating part of the prothallium includes a part of the actively growing meristem, or whether it only consists of the basic part of the prothallium. In the first case the marginal meristem continues growing and the differentiated cells of the prothallium do not

become embryonic. In the second case a perfect regeneration takes place because the already differentiated cells become embryonic. If we try now to bring in harmony these results with the results obtained with *Dryopteris parasitica* we have to assume that the cytologic and the histogenic tendency of the development are in a certain correlation. If we regard as the manifestation of the cytologic differentiation the formation of the gradients within a single cell or the degree of its polarity, and as the manifestation of the histogenic differentiation the formation of the gradient within the whole prothallium, we can say that the more pronounced the cytologic differentiation of a cell the more actively it intervenes in the process of histogenic differentiation. In the same manner as the polarity of the cell is more or less cancelled out it becomes more influenced by the process of histogenic development. If a cell is isolated from the correlation field of the whole prothallium, that is from the histogenic process of development, by means of certain treatments (injury, plasmolysis etc.), then even differentiated cells possess the capacity of again forming cellular gradients which are the prerequisite of a regenerative process.

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Summary

The cell physiologic investigations on the analysis of growth and morphogenesis of the prothallia of *Dryopteris parasitica* brought the following results:

1. Within a normally developed prothallium there are pronounced gradients. These gradients are to be regarded first of all as gradients of growth. For characterizing them the data for three special states of development given in table p. 27 may be used.

2. With prothallia grown under changed conditions of light reacting to this change with a form different from the normal one (Fig. 3), we could show that morphologically equivalent cell-filaments possess also cell physiologically equivalent gradients while morphologically unequivalent cell-filaments show also cell physiologically unequivalent gradients.

3. On the basis on the one hand of the described investigations and on the other hand of the results on growth in fern prothallia cited in the bibliography, the opinion is pointed out that in the process of morphogenesis there is a correlation between a cytologic and a histogenic tendency of development. A manifestation of the cytologic tendency of development is the formation of cellular gradients, while a manifestation of the histogenic tendency is to be regarded as the formation of the gradients within the whole prothallium.

Table

	cell capable of dividing	cell plastic in the process of differentiation	cell differentiated
polarity	pronounced polarity, differentiation in an embryonic plasm at the tip and a somatic at the base of the cell, close contact between plasm and membrane at the distal end of the cell	weak polarity, the contact between plasm and membrane is nearly the same at both ends of the cell	polarity pronounced, close contact of the plasm at the base of the cell
nucleus	round	oval	spindle shaped
plastides	non-homogeneous arrangement, small phototactic mobility	homogeneous arrangement, great phototactic mobility	non-homogeneous arrangement, small phototactic mobility
behavior with plasmolysis	long time of plasmolysis, therefore high viscosity and low permeability to water	intermediate time of plasmolysis, therefore intermediate viscosity and intermediate permeability to water	short time of plasmolysis, therefore low viscosity and high permeability to water
osmotic value	0.45—0.50 mol glucose	0.35—0.40 mol glucose	0.25—0.30 mol glucose
permeability	glycerin > urea	glycerin ≒ urea	glycerin < urea
vital staining with neutral red	0	0	with p_H 6—7 staining of the cell sap
staining with methylgreen acetic acid	nucleus and plastides stained	0	0
staining with acid fuchsin	with p_H 2—4 nucleus and plastides stained	0	within p_H 3—4 nucleus and plastides stained
resistence	low	intermediate	high

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